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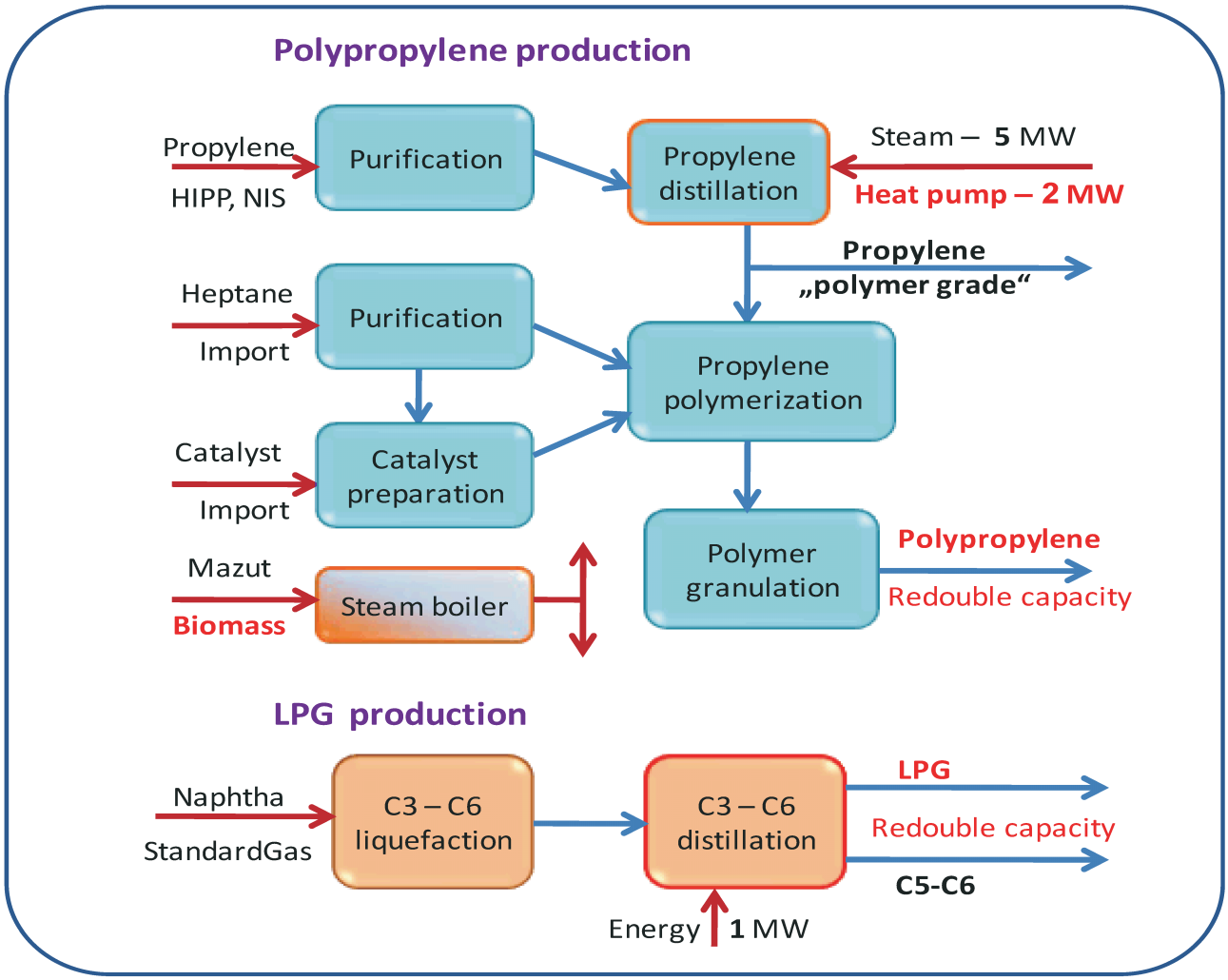
Hemijska industrija

Vol. 70

Časopis Saveza hemijskih inženjera Srbije

Chemical Industry



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Hemijska industrija

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Konvencionalna i napredna tečna biogoriva

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Izvod

Krajem 20. veka intenzivirana su istraživanja i razvoj tehnologija proizvodnje goriva iz biomase, kao jedinog izvora obnovljive energije koji se može prevesti u tečna goriva. Postoje dobro razvijeni, konvencionalni procesi konverzije prvenstveno gajenih biljaka do tečnih goriva, kao što su bioetanol i biodizel (alkoholni estri masnih kiselina). Međutim, korišćenje ovakvih sirovina za biogoriva ima uticaj na rast cena hrane, te najnovija istraživanja biogoriva isključivo se odnose na konverziju nejestivih biljaka, otpadne organske materije i vodenih organizama u biogoriva. U radu je dat pregled osnovnih pojmova vezanih za biomasu i biogoriva, mogućih sirovina za dobijanje tečnih biogoriva, sumarni prikaz puteva njihove konverzije, kao i samih konvencionalnih i naprednih tečnih biogoriva uporedivih po osobinama fosilnom benzinu i dizelu.

Ključne reči: biomasa, konverzija, bioetanol, biodizel, celulozni etanol, sintetički dizel, zeleni dizel.

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Energija je dostupna u različitim oblicima, kao što je toplotna, hemijska, električna, mehanička, itd. a može se prevoditi iz jednog oblika u drugi različitim procesima konverzije. Izvori, tj. nosioci energije, mogu se podeliti na obnovljive i neobnovljive. Neobnovljivim izvorima se smatraju fosilna goriva (ugalj, nafta i prirodni gas) i nuklearna goriva, s obzirom da su brzine njihovog nastajanja znatno manje od brzine trošenja, te se utrošene zalihe ne mogu obnoviti u kraćim vremenskim periodima (merenim ljudskim vekom). Najveći deo danas komercijalno dostupnih ležišta ugljovodonika su nastala pre mnogo miliona godina (u doba krede i tercijara [1]; ona obezbeđuje preko 80% svetskih potreba za energijom [2], a predviđa se da će pri postojećoj brzini korišćenja, ukupne svetske rezerve nafte trajati bar još oko 50 godina [3], ne uzimajući u obzir moguće, za sada, neotkrivene izvore koji bi mogli produžiti ovaj period dodatno za 20–40 godina [4]. Dakle, proizvodnja i korišćenje fosilnih goriva ne smatraju se održivim [5], s obzirom na to da održivi razvoj podrazumeva zadovoljavanje potreba sadašnje populacije ne ugrožavajući potrebe budućih generacija [6].

Obnovljivi izvori energije predstavljaju izvore energije, koji se nalaze u prirodi i obnavljaju se u celosti ili delimično, jer brzina njihovog trošenja ne premašuje brzinu nastajanja u prirodi. Neki od obnovljivih izvora energije su poznati vekovima, kao što je energija vode i vetra (korišćena u mlinovima) i energija biljne mase (bioenergija). Do 19. veka, drvo je predstavljalo osnovno gorivo za zagrevanje, pripremu hrane, pravljenje grnčarskih i metalnih predmeta, itd. a biljna ulja za osvetljavanje. Upravo je tokom 20. veka – veka intenzivne industrijalizacije i modernizacije čovečanstva u čijoj osnovi se nalazi korišćenje fosilnih goriva, prepoznat veliki značaj obnovljivih izvora energije, kada i započinje istraživanje mogućnosti efikasnog iskorišćenja obnovljivih izvora energije, a vezano za ubrzano iskorišćenje rezervi fosilnih goriva, uočene negativne efekte njihovog korišćenja, i pojave prvih energetskih kriza. U obnovljive izvore energije (OIE) spadaju: energija vetra, sunčeva energija, biomasa, energija vodene mase i geotermalna energija. Samo oko 16% globalne potrošnje energije obezbeđuje se iz obnovljivih izvora [7]. Na slici 1 prikazana je klasifikacija izvora energije i njihov udeo u ukupnoj svetskoj potrošnji energije.

Od svih oblika OIE, očekuje se da biomasa odigra veoma značajnu ulogu u sve većim energetskim potrebama u svetu u narednom periodu, upravo zbog činjenica da se jedino biomasa od svih obnovljivih izvora može koristiti za dobijanje tečnih goriva, uporedivih sa postojećim fosilnim tečnim gorivima, za kojima postoji ogromna potražnja u svetu prvenstveno za potrebe saobraćaja. Naime, potrošnja tečnih goriva dominira u odnosu na druge nosioce energije, a očekuje se da tako ostane i posle 2030. godine [8]. Danas postoji više od jedne milijarde različitih vrsta prevoznih sredstava u okviru drumskog saobraćaja, a očekuje se da će se u sledećih 10–20 godina ovaj broj udvostručiti [9]; kao primer, može se navesti da postoji preko 600 miliona putničkih automobila (ne uključujući motore, autobuse, kamione, i druge vrste drumskih vozila), koji dnevno troše oko $3{,}5\times10^9$ litara benzina [10]. Prema predviđanjima očekuje se rast potražnje za sirovom naftom od 1% godišnje u naredne dve decenije, i to prvenstveno usled povećanja energetskih potreba u Kini, Indiji i drugim zemljama Južne Azije, koje nisu članice

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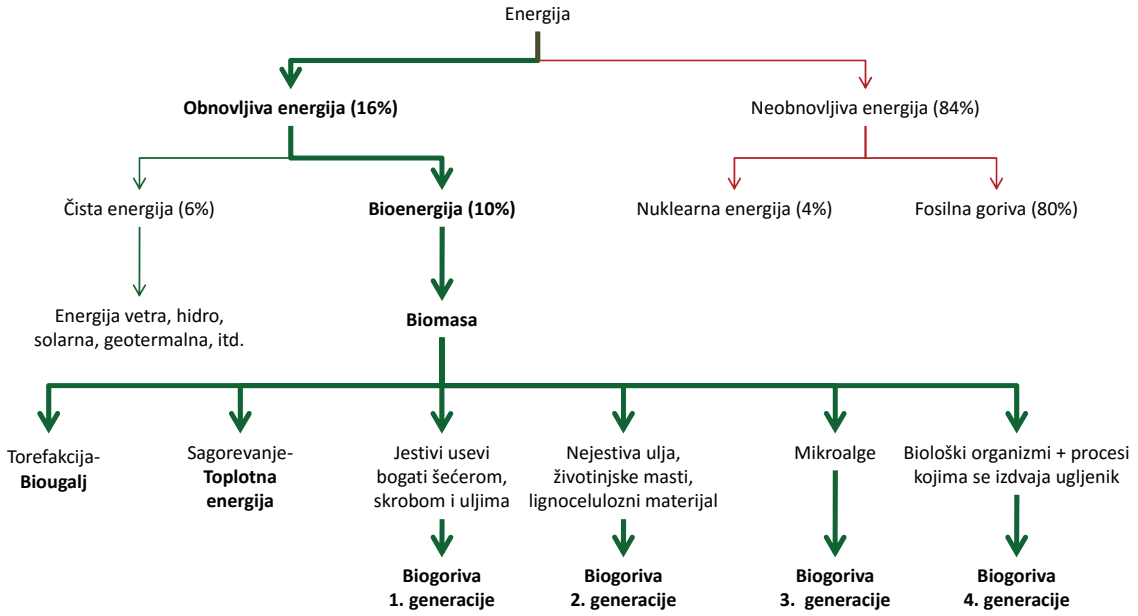


Slika 1. Klasifikacija izvora energije i njihovo učešće u ukupnoj svetskoj potrošnji energije [7].
Figure 1. Classification of energy sources with the contribution to the global energy consumption [7].

Organizacije zemalja izvoznica nafte (OPEC). Pri tome, očekuje se da u ovom porastu čak 97% se odnosi na potražnju za gorivima u transportnom sektoru [8]. Dakle, da bi se odgovorilo na stalno povećanje potreba za tečnim gorivima, naročito u transportu, postoji neodložna potreba za razvojem obnovljivih izvora goriva, i to prvenstveno supstituenata benzina i dizela, koji će po svojim karakteristikama odgovarati postojećim sistemima snabdevanja i korišćenja naročito u vidu smeša sa tradicionalnim gorivima (tzv. „drop-in" goriva) [11]. Zanimljivo je spomenuti da su za pokretanje prvih automobila korišćena upravo biogoriva, a ne fosilna goriva: prvi motori sa unutrašnjim sagorevanjem pokretani su gorivom smešom sa etanolom, dok je 1900. god. na Prvoj svetskoj izložbi demonstriran rad Otto motora koji je radio sa uljem kikirikija [10]. I pored uspešne promocije korišćenja biljnih ulja u prvim automobilima, ova vrsta goriva nije našla dalju primenu zbog niza nedostataka (na primer, velika viskoznost, nestabilnost usled prisustva nezasićenih masnih kiselina), koji su se pojavili u toku njihove primene, a i zbog ekspanzije primene naftnih derivata.

Obnovljeni interes za biogoriva, prvenstveno za etanolom, a zatim i za biodizelom, pojavio se sa energetskom krizom tokom sedamdesetih godina 20. veka, da bi u prvoj deceniji 21. veka industrija biogoriva dobila na značaju. Svetska proizvodnja biogoriva, i to bioetanola (proizvedenog iz kultura na bazi šećera ili skroba), koji se koristi za namešavanje sa benzinom, i biodizela (proizvedenog od biljnih ulja kao što su sojino, uljane repice, suncokretovo, i sl.), najčešće namešavanog sa dizelom, povećena je za 37% samo u periodu između 2006. i 2007. god. predstavljajući oko 1,5% od ukupno potrošenih goriva za prevoz u 2008. god. Očekuje se i dalji intenzivni rast proizvodnje biogoriva, prvenstveno pod uticajem novih regulativa, državnih subvencija, itd. Direktivom Evropske komisije 2009/28/EC [12], postavljen je cilj da udeo OIE u energetskim potrebama do 2020. god. bude 20%, od toga 10% potreba za energijom u transportnom sektoru bi treba da se obezbedi iz OIE, što znači da će 50 milijardi litara fosilnih goriva u saobraćaju u zemljama Evropske unije (EU) biti zamenjeno biogorivima [13].

U SAD, Akt o energiji donet 2005. god. [14] i Akt o energetskoj nezavisnosti i bezbednosti iz 2007. god. [15] promovišu OIE uključujući i biomasu prvenstveno kroz tečna biogoriva, postavljajući za cilj proizvodnju 136 milijardi litara biogoriva za sektor transporta u 2022. god. Po nekim prognozama, do 2030. god. očekuje se da će više od 5% goriva u sektoru drumskog i 1% u vazdušnom saobraćaju u svetu biti zamenjeno biogorivima [8].

S obzirom na veliki značaj koja imaju, kao i prognoze za većom potražnjom za biogorivima u sektoru transporta, cilj rada je pregled postojećih, u ovom momentu konvencionalnih tečnih biogoriva uporedivih po osobinama fosilnom benzinu i dizelu, i naprednih tečnih biogoriva, uz prikaz odgovarajućih sirovina i puteva njihove konverzije. Rad dodatno doprinosi definisanju i terminološkom razlikovanju osnovnih pojmova vezanih za biomasu i klasifikaciju biogoriva.

Biomasa i procesi njene konverzije

Pojam „biomasa" može se definisati na različite načine. U najširem smislu, biomasa predstavlja materiju biološkog porekla ili organsku materiju (sačinjenu od

prirodnih jedinjenja ugljenika), koja se može koristiti na razne načine (slika 1) i prevesti u različite oblike energije (toplotnu, mehaničku ili električnu), gasovita, tečna ili čvrsta goriva (i/ili u tzv. „zelene“ hemikalije ili biohemikalije [16]).

O značajnom energetskom potencijalu biomase govore i sledeći podaci: u svetu se godišnje proizvede 120×10^{15} g suve biljne mase, čiji energetski sadržaj iznosi $2{,}2\times10^{21}$ J. Radi poređenja, svetska potražnja za energijom u 2010. god. iznosila je $5{,}5\times10^{20}$ J, dok se predviđa da će u 2020. god. iznositi $6{,}6\times10^{20}$ J, a u 2040. god. $8{,}6\times10^{20}$ J [10]. Dakle, energija koja se nalazi u biljakama je tri do četiri puta veća od godišnjih energetskih potreba u celom svetu. Ukoliko se uzme u obzir ukupna površina zemljišta u svetu, izuzimajući obradivo zemljište, zemljište iskorišćeno za infrastrukturne objekte, zemljište pokriveno divljim predelima i gustim šumama, procenjeni ukupni bioenergetski potencijal iznosi $1{,}90\times10^{20}$ J/god, tj. 35% od trenutne svetske energetske potražnje [10].

Definicija pojma „biomase“ nalaze se u mnogim pravnim odredbama i programima različitih zemalja, koji podržavaju razvoj, istraživanje i promociju biomase kao alternativnog izvora energije. U SAD, pojam „biomasa“ prvi put je uveden od strane Kongresa u Aktu o termoelektranama i industrijskom gorivo iz 1978. god., u kome je navedeno da je to „organska materija, koja je dostupna na obnovljiv način, uključujuči poljoprivredni otpad i ostatke, drvo i drvni otpad i ostatke, životinjski otpad, gradski otpad i vodene biljke“ [17]. Prema direktivi Evropske unije 2003/30/EC [18], koja je poznata kao direktiva o biogorivima, biomasa predstavlja biorazgradive delove proizvoda, otpada ili ostataka iz poljoprivrede (biljnog i životinjskog porekla), šumarstva i srodnih industrija, kao i biorazgradive delove industrijskog i gradskog otpada. Ova direktiva zamenjena je direktivom o obnovljivim izvorima 2009/28/EC [12], koja na veoma sličan način daje opis biomase kao biorazgradivih delova proizvoda, otpada ili ostataka iz poljoprivrede (biljnog i životinjskog porekla), šumarstva i srodnih industrija uključujući ribarstvo i akvakulturu, kao i biorazgradivih delova industrijskog i gradskog otpada.

Zakon o energetici Republike Srbije [19] definiše biomasu u skladu sa Direktivom 2003/30/EC, dok se Nacionalni akcioni plan za korišćenje obnovljivih izvora energije Republike Srbije usvojen 2013. god. [20], oslanja na evropsku Direktivu 2009/28/EC, te razmatra biomasu iz šumarstva, poljoprivrede i ribarstva, i biomasu iz otpada (u koju se ubraja: 1. biorazgradivi gradski čvrsti otpad (biorazgradivi otpad iz dvorišta i parkova, hrana i kuhinjski otpad iz domaćinstava, restorana, pripreme hrane i maloprodajnih objekata i sličan otpad iz postrojenja za preradu hrane) i deponijski gas; 2. biorazgradiva frakcija industrijskog otpada (uključujući papir, karton, palete, i sl.) i 3. kanalizacioni mulj).

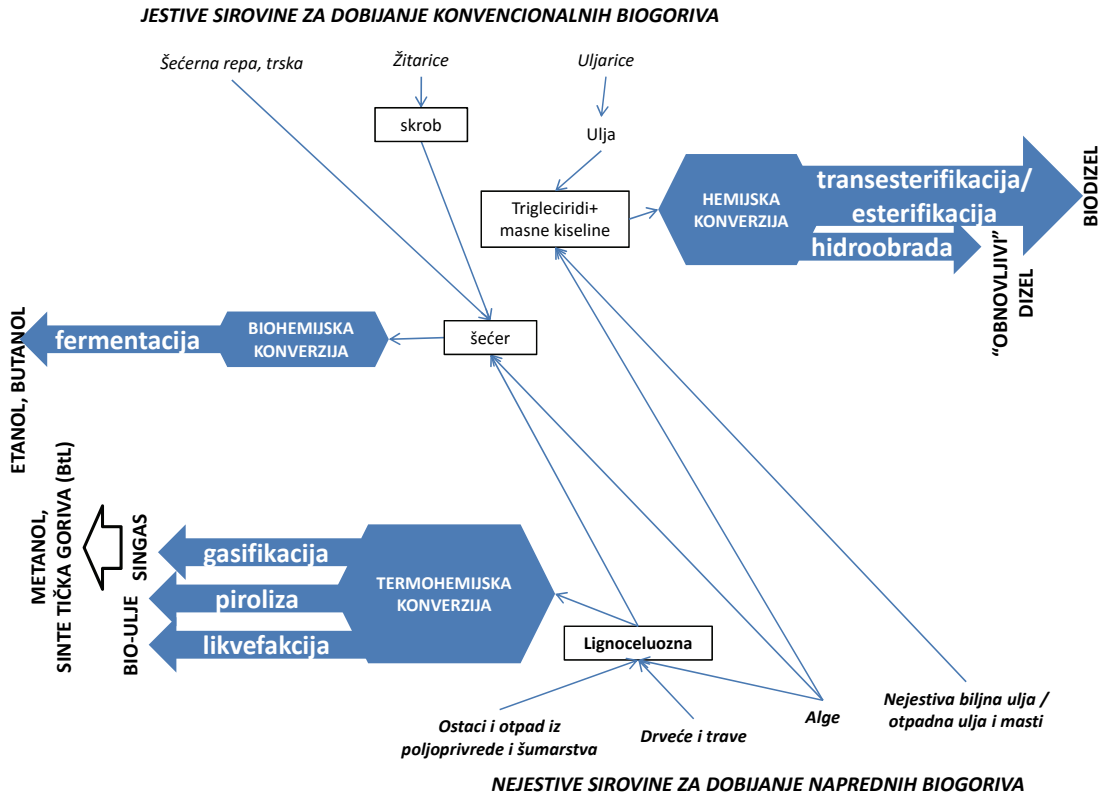
Osnovni sastojci, tj. konstituenti biomase, koji utiču i na sam izbor procesa konverzije su ugljeni hidrati različite složenosti molekula (mono-, oligo- ili polisaharidi) i lipidi. U skladu sa tim, biomasa kao sirovina za proizvodnju biogoriva može se podeliti na: a) bogatu skrobom (visokomolekulski ugljeni hidrat, koji spada u tzv. rezervne polisaharide biljaka), b) šećerom (na primer, saharozom, koja spada u oligosaharide – ugljene hidrate sastavljene od 2–10 molekula prostih šećera), c) lignocelulozu biomasu (u kojoj preovlađuje celuloza i hemiceluloza - strukturni polisaharidi u biljakama, koje prati lignin – makromolekulsko jedinjenje izgrađeno od fenilpropanskih jedinica) i d) biomasu bogatu lipidima (trigliceridima-estrima trohidroksilnog alkohola glicerola sa višim masnim kiselinama).

Energetski sadržaj ovih osnovnih jedinjenja biomase, važnih sa stanovišta njene konverzije u biogoriva, je različit i raste sa smanjenjem sadržaja kiseonika, kao i sa porastom odnosa vodonika i ugljenika u njihovim molekulima, tako da energetski sadržaj po jedinici mase opada redom od lipida, preko lignina do šećera, koji imaju najmanji sadržaj energije [8]. Iako se po ovome čini da su ulja i masti idealne polazne sirovine za proizvodnju biogoriva, smatra se da će buduća proizvodnja biogoriva većih razmera biti zasnovana na nejestivoj lignocelulozoj biomasi prvenstveno zbog velikih raspoloživih količina i široke rasprostranjenosti, a bez uticaja na cenu hrane u svetu.

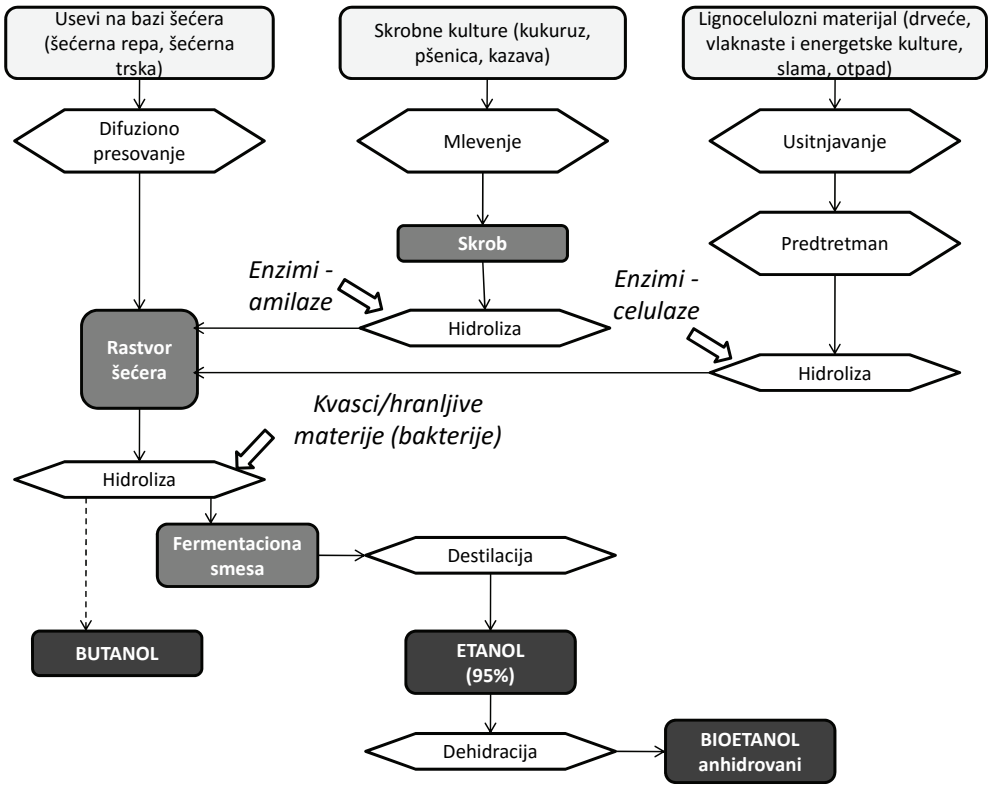
Najznačajniji procesi prevođenje (konverzije) biomase u biogoriva se mogu svrstati u termohemijske, biohemijske i hemijske procese. Termohemijski procesi podrazumevaju hemijske reakcije transformacije polaznih jedinjenja biomase pod uticajem povišene ili visoke temperature bilo pod atmosferskim ili povišenim pritiskom; biohemijski procesi podrazumevaju transformaciju pod dejstvom organizama, isključivo mikroorganizama (kvasci, bakterije, i dr.), dok hemijska konverzija podrazumeva transformaciju u prisustvu određenih reaktanata i najčešće katalizatora.

Uzimajući u obzir osnovne sastojke različitih izvora biomase, mogući procesi konverzije do tečnih biogoriva mogu se sumirati kao što je prikazano na slici 2. Osnove procesa biohemijske, hemijske i termohemijske konverzije različitih izvora biomase do tečnih goriva ilustrovani su na slikama 3–5.

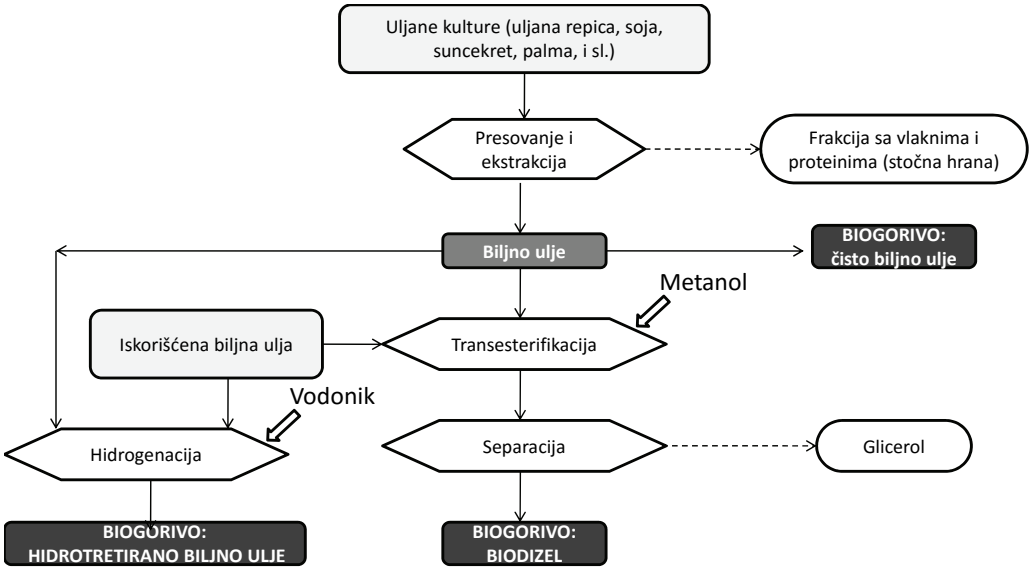
U slučaju biomase bogate šećerom, kao što je šećerna repa ili trska, ili nakon prevođenja skroba kukuruza u jednostavnije ugljene hidrate prethodnim tretmanom (hidrolizom) same sirovine, dobijanje etanola kao biogoriva vrši se prvenstveno biohemijskim procesom – fermentacijom (slike 2 i 3). Konverzija triglicerida jestivih biljnih ulja, otpadnih i nejestivih ulja i masnoća ili algi u biogoriva prvenstveno se odvija he-



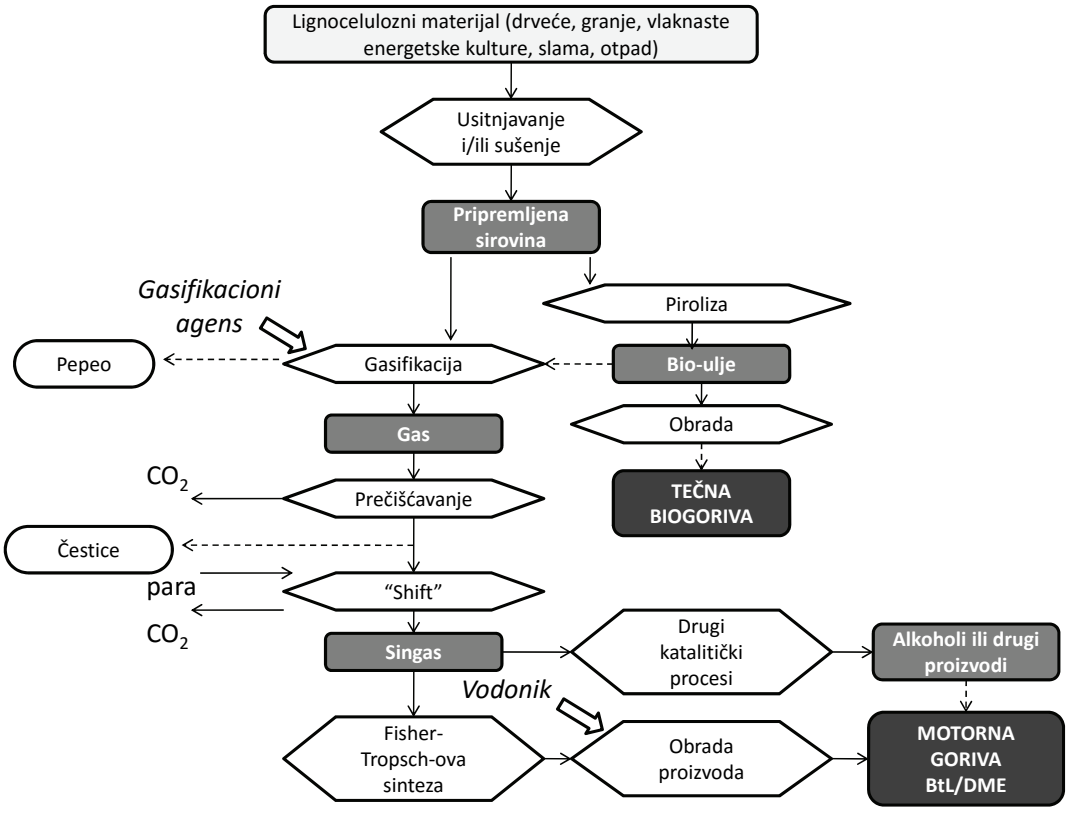
Slika 2. Osnovni putevi konverzije biomase do tečnih biogoriva.
Figure 2. Main routes of biomass conversion to liquid biofuels.



Slika 3. Biohemijski putevi konverzije biomase na bazi šećera, skroba i lignoceluloze do tečnih biogoriva (bio-alkohola) [23].
Figure 3. Biochemical conversion of sugar-based, starch-based and lignocelulosic biomass to liquid biofuels [23].



Slika 4. Hemijski putevi konverzije lipidne biomase do tečnih biogoriva [23].
Figure 4. Chemical conversion of lipid biomass to liquid biofuels [23].



Slika 5. Termohemijski putevi konverzije lignoceluloze biomase do tečnih biogoriva [23].
Figure 5. Thermochemical conversion of the lignocellulosic biomass to liquid biofuels [23].

mijskim reakcijama, i to transesterifikacijom uz dobijanje biodizela, slike 2 i 4; kao predtretman lipidnih sirovina sa visokim sadržajem slobodnih masnih kiselina može se primeniti esterifikacija sa kiselim katalizatorima [21,22]. Fertmentacija i transesterifikacija su dva najčešća konvencionalna načina dobijanja biogoriva, i to prvenstveno od gajenih (u najvećoj meri jestivih) biljnih kultura.

Konverzija lipidne biomase u biogoriva može se izvršiti i naprednim hemijskim procesima (slike 2 i 4), kao što je hidrotretman ulja (hidrogenizacija i hidrodeoksigenizacija) i dobijanje hidrotretiranih biljnih ulja

(HVO – hydrotreated vegetable oils) ili tzv. „obnovljivog“ dizela [24], koji spada u napredna biogoriva ili biogoriva 2. generacije.

Kao što se vidi na slikama 2 i 5, lignocelulozna masa se kroz termohemijske i biohemijske procese može prevesti u tečna biogoriva. Mogući termohemijski procesi su (slika 2): gasifikacija uz dobijanje singasa (gasovitog proizvoda koji se sastoji od vodonika, ugljen monoksida, metana, ugljen dioksida i tragova viših ugljovodonika), piroliza i likvefakcija čiji je osnovni proizvod bio-ulje (ili bio-nafta, tamno braon viskozna, korozivna i kisela tečnost, u čiji složeni sastava ulaze: alifatični alkoholi/aldehidi, furanoidi, piranoidi, benzenoidi, masne kiseline, visokomolekulski ugljovodonici i voda (25-45%)). Singas se može koristiti direktno za dobijanje energije, a takođe se kroz dalje hemijske procese može prevesti u metanol, sintetički (supstituisani) prirodni gas (bioSNG), vodonik [25] ili sintetička biogoriva (tzv. BtL goriva od engleske fraze „Biomass-to-Liquid“, tj. „od-biomase-do-tečnosti“) po osobinama veoma slična fosilnim gorivima (benzinu ili dizelu). Bio-ulje iz procesa pirolize i likvefakcije se ne može direktno koristiti kao transportno gorivo zbog visokog sadržaja kiseonika i vode, te je potrebna njegova obrada, najčešće u prisustvu vodonika i katalizatora, pri čemu se dobija tzv. HTU dizel. Lignoceluloznu biomasu moguće je takođe odgovarajućim procesima pripreme [26] prevesti u jednostavnije šećere (pri čemu zaostaje lignin) i dalje kroz biohemijske procese fermentacije prevesti u etanol, koji se naziva celulozni etanol, radi razlikovanja od bioetanola dobijenog konvencionalnim načinima fermentacije skroba iz kukuruza ili šećera iz šećerne trske ili repe.

Tečna biogoriva i njihova podela

Prema evropskoj Direktivi 2009/28/EC [12], na koju se oslanja Nacionalni akcioni plan za korišćenje obnovljivih izvora energije Republike Srbije [20], biogoriva predstavljaju tečna ili gasovita goriva koja se koriste u saobraćaju, proizvedena iz biomase. Prema ovoj Direktivi, biogoriva se razlikuju od biotečnosti, koje predstavljaju tečna goriva proizvedena iz biomase sa primenom za grejanje, hlađenje, proizvodnju električne energije, ali ne i kao goriva za transport.

Postoje različiti pristupi klasifikacije biogoriva zbog velike raznolikosti sirovina, kao i raznolikosti samih procesa konverzije, koji se razvijaju u pravcu održivosti i zahtevanih standarda kvaliteta goriva.

U literaturi [5,27,28] najčešće korišćena podela biogoriva je prema generacijama, i to na:

– biogoriva 1. generacije (1G): bioetanol, biodizel, bio-etil terc-butil etar (bioETBE), biljna ulja (engl. *“Straight Vegetable Oils”* – *SVOs* ili *“Pure Plant Oil”* – *PPO*) i biogas/deponijski gas,

– biogoriva 2. generacije (2G): bioalkoholi (celulozni etanol, biobutanol, biometanol), BtL goriva (sintetička tečna goriva koja se dobijaju termohemijskom konverzijom biomase, na primer FT dizel i FT kerozin), biodizel od otpadnih sirovina, HTU dizel, bio-dimetilfuran (bioDMF), hidrotretirana biljna ulja (obnovljivi dizel), bio-dimetiletar (bioDME), bio-sintetički gas (bioSNG) i bio-vodonik i:

– biogoriva 3. generacije (3G): algalna biogoriva (na primer, algalni bioalkoholi, biodizel i BtL goriva).

U osnovi ovakve podele je poreklo sirovine, koja se koristi za dobijanje biogoriva (slika 1). Sirovine za dobijanje tečnih biogoriva su sledeće:

- biogoriva 1. generacije se dobijaju od šećera izdvojenog iz gajenih biljaka sa visokim sadržajem šećera (šećerna repa i šećerna trska), skroba izdvojenog iz biljaka kao što su na primer žitarice (pšenica) ili krtolaste biljke (krompir), ili ulja izdvojenih iz uljarica (na primer, palmino, repičino ulje). U ovu grupu tečnih biogoriva spadaju bioetanol, biodizel, bioETBE, biljna ulja), od kojih su bioetanol i biodizel prva biogoriva čija proizvodnja je komercijalizovana u 20. veku;
- biogoriva 2. generacije (kao što su celulozni etanol, sintetička BtL goriva, obnovljivi dizel, HTU dizel, i dr.) se prvenstveno povezuju za lignoceluloznu sirovinu, kao što su poljoprivredni ili šumski otpad ili ostaci, kao i ciljano gajene nejestive kulture (na primer, energetske trave i sl.); ponekad se u ovu grupu sirovina za biogoriva 2. generacije eksplicitno svrstavaju i otpadna i druga nejestiva ulja i masnoće [28], mada neki autori biodizel dobijen od ovakvih lipidnih sirovina [29] svrstavaju u biogoriva 1. generacije, s obzirom da se dobija konvencionalnim procesom. Ipak, kako nejestiva ulja i masnoće dele zajedničku osobinu sa lignoceluloznom materijom da su nejestive, te ne utiču na sirovinsku osnovu za proizvodnju hrane, čini se da je svrstavanje biodizela dobijenog iz ovih sirovina u biogoriva 2. generacije opravdanije nego svrstavati ga u 1. generaciju;
- biogoriva 3. generacije se dobijaju od organizama mikroskopskih veličina, kao što su mikroalge i mikroorganizmi poput kvasaca i plesni i [27]; u zavisnosti da li se koristi lipidni ili ugljenohidratni deo algi, one se mogu prevesti u bioetanol, biodizel i druga biogoriva po karakteristikama slična onim 1. i/ili 2. generacije. U starijoj literaturi može se naći da se pojam biogoriva 3. generacije primenjuje na vodonik i njegovu primenu u gorivim ćelijama [30], ali ovo je usamljen primer.

Kao što je naznačeno na slici 2, za proizvodnju 1G biogoriva koriste se biohemijski procesi konverzije šećera u alkohol i alkalna tranesterifikacija lipida u biodizel [31]. Termohemijski procesi preovlađuju pri dobijanju 2G biogoriva. Biomasa algi se može podvrći termohemijskih procesima, a ulje izdvojeno iz algi se kroz procese transesterifikacije može prevesti u biodizel.

U poslednje vreme u literaturi [28,32] se pojavljuje i pojam „biogoriva 4. generacije“ pod kojima se podrazumeva korišćenje genetski modifikovanih organizama, kao što su fotosintetske mikroalge sa većim udelom lipida i sposobnošću korišćenja većih količina ugljen dioksida od uobičajenih za procese fotosinteze, učestvujući tako i u procesima sekvestracije ugljenika (uklanjanjem ugljen-dioksida iz atmosfere) i posledičnog smanjenja efekta staklene bašte [33]. Inače, sama proizvodnja biogoriva iz ovakvih organizama slična je procesima proizvodnje biogoriva 3. generacije (slika 2).

Uporedni pregled sirovina, procesa konverzije, prednosti i nedostataka tečnih biogoriva od prve do četvrte generacije prikazan je u tabeli 1; u tabeli su dati i izabrani pregledni radovi u kojima se nalazi više relevantnih detalja za određenu vrstu biogoriva, koji prevazilaze obim ovog rada.

Međunarodna agencija za energiju (IAE-International Agency for Energy) u svojoj tzv. tehnološkoj mapi za biogoriva u transportu [51], ne deli sama biogoriva, već tehnologije dobijanja biogoriva na konvencionalne i napredne. U konvencionalne tehnologije spadaju raz-

Tabela 1. Uporedni pregled sirovina, procesa konverzije, prednosti i nedostataka tečnih biogoriva 1., 2., 3. i 4.generacije
Table 1. Comparison of the 1st, 2nd, 3rd and 4th generation liquid biofuels, their feedstocks and conversion processes

Generacija	Sirovina	Procesi konverzije	Biogorivo	Prednost	Nedostatak	Primeri preglednih radova
Prva	Gajene kulture: uljarice i biljke sa visokom koncetracijom šećera i skroba	Transesterifikacija ulja Fermentacija šećera	Biodizel Bioetanol BioETBE Biljna ulja	Smanjenje efekta staklene bašte, konvencionalni procesi konverzije	Mali prinosi, ugrožavanje proizvodnje hrane, nemaju neutralni ILUC[a] faktor niti su u potpunosti neutralni u odnosu na ugljenik	[31,34]
Druga	Lignocelulozna sirovina, nejestiva ulja i masnoće, gradski otpad	Termohemijski procesi konverzije lignocelulozne sirovine do singasa ili bio-ulja koji se dalje hemijskim procesima prevode u biogoriva. Fermentacija lignocelulozne sirovine nakon njene prethodne pripreme. Hidroobrada ulja i masnoća. Esterifikacija/transesterifikacija nejestivog i otpadng ulja.	BtL goriva, Bioalkoholi, Obnovljivi dizel, Biodizel, HTU dizel, BioDMF	Smanjenje efekta staklene bašte, korišćenje otpada, nema konkurencije sa hranljivim usevima, korišćenje marginalnog ili neobradivog zemljišta, neutralni u odnosu na ugljenik, diverzifikacija sirovina. U slučaju BtL goriva moguće je fino „podešavanje“ sastava goriva prema specifičnim zahtevima („dizajnirana“ goriva) i tako uticati i na smanjenje emisije štetnih gasova.	Skup predtretman sirovina, napredne i skupe tehnologije proizvodnje, mogućnost erozije obradivog zemljišta u slučaju nekontrolisanog uklanjanja poljoprivrednih ostataka	[26,35-42]
Treća	Alge	Kultivacija, ubiranje, fermentacija ili ekstrakcija ulja, transesterifikacija ili termohemijska konverzija	Biodizel, Bioalkoholi, BtL goriva	Lako uzgajanje u svim vodenim sistemima, velika brzina rasta i veliki prinos, nema konkurencije sa hranljivim usevima niti se koristi zemljište.	Potrebna energija za uzgoj algi, mali sadržaj lipida u biomasi	[43–50]
Četvrta	Genetski modifikovani mikroorganizmi i alge	Genetski inženjering, kultivacija, ubiranje, fermentacija ili ekstrakcija ulja, transesterifikacija ili termohemijska konverzija	Biodizel, bioalkoholi, BtL goriva	Veliki prinos, intenzivirano vezivanje ugljen dioksida u fotosintezi tako da su negativni u odnosu na ugljenik.	Skupa početna istraživanja	[28]

[a]Indirect Land Use Change

vijeni procesi pomoću kojih se već komercijalno proizvode biogoriva, koja se uobičajeno svrstavaju u biogoriva 1. generacije: etanol dobijen od šećera i skroba, biodizel dobijen iz uljarica i biljnih ulja, kao i biogas dobijen anaerobnom digestijom. Prema IAE, napredne tehnologije za proizvodnju biogoriva su još uvek u fazi razvoja, pilot ili demonstracionih postrojenja. Dobijena biogoriva se označavaju kao 2. ili 3. generacije, a uključuju hidrotretirana biljna ulja, biogoriva dobijena iz lignocelulozne biomase, kao što su celulozni etanol, BtL goriva (na primer FT dizel i FT avionsko gorivo) i bio-sintetički gas (bio-SNG), algalna biogoriva, kao i ona dobijena konverzijom šećera u biogoriva slična dizelu korišćenjem enzima ili hemijskih katalizatora [51]. Šematski prikaz stepena komercijalizacije osnovnih tečnih i gasovitih biogoriva dat je na slici 6.

Slično podeli tehnologija na konvencionalne i napredne, postoji i podela samih biogoriva na [23]:

- konvencionalna biogoriva – biogoriva proizvedena od jestivih kultura na bazi šećera, skroba ili biljnih ulja, koja se koriste i za ishranu ljudi i stoke.
- napredna biogoriva ili biogoriva sledeće generacije, koja se dobijaju od nejestivih sirovina.

Pri tome, pojam napredna biogoriva se često koristi u širem smislu da bi se opisala [23]:

– biogoriva dobijena naprednim procesima iz nejestivih sirovina, pri čemu dobijena biogoriva mogu biti goriva ekvivalentna konvencionalnim biogorivima (kao što su bioetanol i biodizel), ali i biogoriva nove vrste (kao na primer biokerozin), ili

– biogoriva sa poboljšanim osobinama, kao što su „obnovljivi“ dizel, bio-avionsko gorivo, biobutanol, itd.; ovi proizvodi mogu biti čak kompatibilniji sa strukturom postojećih goriva ili imaju određene tehničke prednosti u odnosu na konvencionalna biogoriva (kao što je slučaj sa biobutanolom u odnosu na bioetanol).

Evropska inicijativa za industrijsku bioenergiju (EIBI – European Industrial Bioenergy Initiative) pokrenuta u novembru 2010. god. kao nastavak predloga Evropske tehnološke platforme za biogoriva (EBTP – European Biofuels Technology Platform) i Evropske komisije [23], prepoznaje napredna biogoriva kao goriva proizvedena od lignocelulozne sirovine (tj. otpada iz poljoprivrede, šumarstva, kao što su slama, kukuruzne stabljike, šumska biomasa), nejestivih kultura (trave, alge i dr.), ili (industrijskog) otpada, koja imaju malu emisiju CO_2 ili utiču na značajno smanjenje gasova staklene bašte, i imaju nulti ili veoma mali ILUC (Indirect Land Use Change). ILUC je faktor tzv. indirektne promene namene zemljišta, koji govori u kojoj meri korišćenje obradivog zemljišta za proizvodnju biogoriva izaziva potrebu iznalaženja novih obradivih površina na račun iskorišćenja nepoljoprivrednog zemljišta u drugim delovima regiona (ili čak sveta) kako bi se održala proizvodnja potrebnih količina hrane, te kao jedan od rezultata dovodi i do deforestacije zemljišta (krčenja šuma) [53].

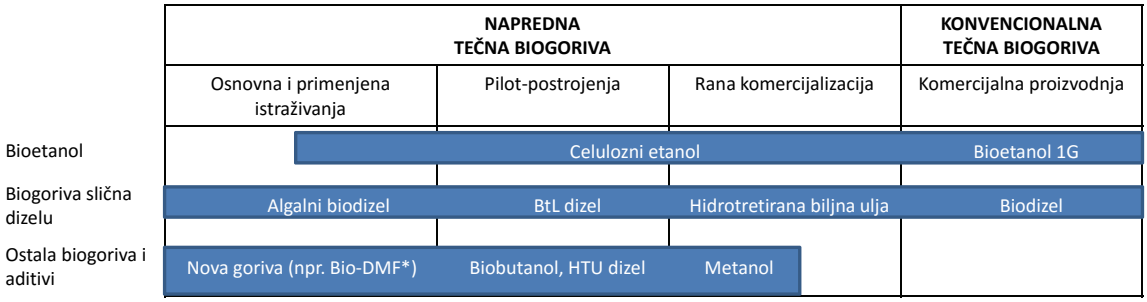
Prema spomenutim definicijama naprednih biogoriva, biodizel dobijen od ulja nejestivih kultura, gajenih na marginalnom zemljištu, ili od korišćenih, otpadnih ulja ili masnoća iz domaćinstava i restorana, primenom tehnologije prve generacije ne može se nazvati „naprednim“ biogorivom, već u tom slučaju se koriste pojmovi kao što je gorivo „sledeće generacije“ ili održivo gorivo, s obzirom da se dobija od obnovljivih sirovina.

Sirovine za tečna biogoriva 1. generacije

Tečnim biogorivima 1. generacije se smatraju goriva proizvedena od (delova) poljoprivrednih kultura visokog energetskog sadržaja, proizvedena konvencionalnim procesima i sa definisanim parametrima kvaliteta na osnovu odgovarajućih standarda o kvalitetu. Od tečnih biogoriva 1. generacije, primarnu ulogu u sektoru transporta imaju bioetanol i biodizel.

Sirovine za bioetanol. Sirovine za dobijanje bioetanola procesom fermentacije su gajene biljke sa visokim sadržajem šećera i skroba.

Hemijska struktura sirovine na bazi šećera sastoji se od monosaharide sa 5 C-atoma (pentoze $C_5H_{10}O_5$; najznačajniji su ksiloza i arabinoza) i 6 C-atoma (heksoza



* Bio-DMF – nova klasa biogoriva, nazvana po furanu koji se nalazi u osnovi molekula ovog biogoriva; dobija se korišćenjem ugljenih hidrata biomase, tj. njihovom razgradnjom do fruktoze, koja se deoksigenacijom, dehidratacijom i hidrogenolizom prevodi u dimetilfuran (DMF). Tehnologija proizvodnje ovog goriva razvijena je od strane Avantium Technologies iz Holandije.

Slika 6. Stepen komercijalizacije tečnih biogoriva [52].
Figure 6. Commercialization level of different biofuels [52].

$C_6H_{12}O_6$; najznačajniji su glukoza, fuktoza, manoza), kao i oligosaharide sastavljenih od 2 ili više monosaharida (najnačajniji su disaharidi $C_{12}H_{22}O_{11}$ u koje spadaju saharoza, maltoza, laktoza). Ove sirovine ne zahtevaju skupe postupke predtretmana, jer sadrže šećere koji se direktno fermentišu biohemijskih postupkom prerade [31], tako da se priprema šećerom bogatih sirovina svodi na njihovo usitnjavanje u cilju ekstrakcije šećera, razblaživanje i usklađivanje sa potrebama kvasaca, tj. mikroorganizama pomoću kojih se izvodi alkoholna fermentacija, kao i parametrima tehnološkog procesa. Najznačajnije poljoprivredne kulture sa velikim sadržajem šećera, koje se koriste za proizvodnju bioetanola su: šećerna repa, šećerna trska, topinambur [31].

Sirovine na bazi skroba su krtolaste kulture (krompir i kasava) i žitarice (pšenica, kukuruz, ječam, sirak) [31]. Skrob je polisaharid sastavljen od monomera D-glukoze tako da je neophodno razgradnjom veza u skrobu osloboditi monomerne jedinice glukoze, koje se kroz procese fermentacije prevode u bioetanol. Razgradnja veza u skrobu se postiže procesom hidrolize, i to najčešće enzimske, tokom koje se skrobna sirovina meša sa vodom na povišenoj temperaturi i tretira se enzimima – amilazama u dva stepena, tokom kojih dolazi do razlaganja polimera skroba do dekstrina i oligosaharida, i dalje hidrolize dekstrina i oligosaharida do fermentabilnih šećera, koje mikroorganizmi mogu fermentisati do bioetanola [5]. Posle procesa fermentacije, vrši se izdvajanje bioetanola najčešće procesom destilacije; dobijeni proizvod sa vrha destilacione kolone je smeša sa 37% etanola, koja se dalje odvodi u rektifikacionu kolonu iz koje se kao proizvod izdvaja rafinisani bioetanol koncentracije do 96%. Proizvod dna rektifikacione kolone odvodi se u kolonu za stripovanje radi izdvajanja vode i dobijanja anhidrovanog etanola sa 99,6% alkohola i 0,4% vode, koji se koristi kao gorivo odnosno komponenta za namešavanje sa fosilnim benzinom [5,31,54].

Rasprostranjenost sirovina od kojih se proizvodi bioetanol, zavisi od klimatskih uslova pogodnih za gajenje odgovarajuće biljne kulture. Šećerna repa se gaji u Evropi, SAD i Rusiji, šećerna trska se gaji u Brazilu koji je najveći svetski proizvođač ove kulture. U Brazilu 40% proizvedenog bioetanola potiće od šećerne trske. Od skrobnih sirovina, kukuruz predstavlja osnovnu sirovinu za proizvodnju bioetanola u SAD. Cena bioetanola proizvedenog od šećerne repe još uvek je nekonkurentna ceni bioetanola proizvedenog iz šećerne repe ili kukuruza [31].

U Srbiji ne postoji organizovana proizvodnja i potrošnja etanola kao motornog goriva uprkos značajnom potencijalu. Proizvodnja bioetanola se odvija u 10 postrojenja ukupnog kapaciteta 40 miliona L apsolutnog etanola, a bazira se na melasi (50%) i žitaricama (50%) [55]. Ovaj bioetanol se prvenstveno koristi u industriji alkoholnih pića, medicinskih i farmaceutskih proizvoda. Potrebno je izgraditi nova postrojenja za proizvodnju bioetanola kako bi se proizvelo dovoljno bioetanola kao alternativnog goriva. Na osnovu trenutne poljoprivredne proizvodnje u Srbiji, sirovine na bazi skroba imaju najbolju perspektivu za proizvodnju bioetanola, i to kukuruz: tokom 2009. godine ukupan prinos kukuruza iznosio je 7 miliona t, a procenjene domaće potrebe ne prelaze 4–4,5 miliona t, što znači da se proizvodi dovoljno kukurza i za druge namene, uključujući i za bioetanol kao motorno gorivo [55]. Međutim, cena kukuruza na svetskom tržištu raste, zbog čega se mogućnost primene jeftinijih sirovina sve više ispituje, kao što su oštećeni i usevi slabijeg kvaliteta (kukuruz, pšenica i krompir) i usevi koji se gaje na marginalnom zemljištu (sirak, čičoka i tritikale) [55].

Sirovine za biodizel. Reakcijom transesterifikacije ili alkoholize između alkohola (najčešće metanola) i triglicerida dobijaju se alkil estri masnih kiselina, tj. biodizel (u slučaju metanola – metil estri masnih kiselina) i glicerol kao sporedni proizvod. Reakcija transesterifikacije može se odvijati u prisustvu katalizatora ili bez prisustva katalizatora u natkritičnim uslovima metanola. Katalizatori se prema prirodi dele na hemijske (kiseli, bazni) i biološke (enzimi), a prema rastvorljivosti u reakcionoj smeši na homogene (rastvorne u reakcionoj smeši) i heterogene (nerastvorne u reakcionoj smeši). U industrijskim uslovima najviše se primenjuje proces homogene bazno-katalizovane transesterifikacije zbog visoke katalitičke aktivnosti homogenih baznih katalizatora pri blagim reakcionim uslovima i visokog prinosa metil estara masnih kiselina za relativno kratko vreme trajanja reakcije [56]. Međutim, primena baznih katalizatora je ograničena kvalitetom sirovina i podrazumeva da su reaktanti anhidrovani, a sadržaj masnih kiselina u ulju ispod 1 mas.%. Pored toga, homogeni katalizatori se ne mogu reciklirati, a za njihovo uklanjanje iz estarske faze se najčešće primenjuje neutralizacija kiselinom i višestruko ispiranje metil estarskog sloja vodom, što dodatno otvara problem otpadnih voda i povećava ukupne troškove proizvodnje [56]. Heterogena, enzimska i natkritična metoda se smatraju novim, unapređenijim tehnologijama dobijanja biodizela [56].

Izvori triglicerida u slučaju proizvodnje biodizela 1. generacije su prvenstveno (preko 95%) ulja gajenih biljaka: oko 84% svetske proizvodnje biodizela se zasniva na repičinom ulju, zatim na suncokretovom (13%), palminom (1%) i ulju soje (2%). Kao i u slučaju sirovina za dobijanje bioetanola, rasporstranjenost sirovina za proizvodnju biodizela, zavisi od klimatskih uslova pogodnih za gajenje odgovarajuće biljne kulture, tako da su sirovine najznačajnije za proizvodnju biodizela u Evropi uljana repica, suncokret i soja, u Americi soja, a u zemljama Azije uljana palma [54,57].

Dakle, zajedničko za sirovine biogoriva 1. generacije je da imaju primenu i u proizvodnji hrane i to je upravo razlog zašto je razvoj biogoriva 1. generacije praćen velikom pažnjom medija, šire i naučne javnosti, kao i raznim kontraverzama i političkim debatama oko opravdanosti korišćenja jestivih kultura za proizvodnju goriva, uticaja proizvodnje i korišćenja biogoriva na životnu sredinu, uključujući i nepoznanice oko uloge biogoriva u smanjenju efekta staklene bašte, te su pokrenute mnoge kampanje radi skretanja pažnje na ekološke i sociološke uticaje biogoriva od jestivih kultura. Održiva i ekonomska proizvodnja biogoriva 1. generacije je ograničena usled [58]: visokih troškova proizvodnje i prerade koji često zahtevaju državne subvencije kako bi bili konkurentni ceni goriva iz fosilnih sirovina; visoka cena proizvodnje i prerade je posledica visoke cene polaznih sirovina koje u velikom udelu učestvuju u krajnjoj ceni proizvoda; potreba za obradivim zemljištem i vodom, resursima koji su potrebni i za proizvodnju hrane [59], što je i dovelo do konkurencije u korišćenju zemljišta, iako gajenje useva za biomase zauzima manje od 2% obradivog zemljišta u svetu [58], povećanja cene hrane zbog istovremenog korišćenja sirovina za proizvodnju biogoriva i u ishrani ljudi i životinja, i koje se procenjuje na 15–25% u odnosu na ukupnu cenu hrane [58].

Sirovine za tečna biogoriva sledećih generacija

S obzirom na brojna ograničenja u proizvodnji goriva 1. generacije, sve veći značaj u istraživanjima posvećenih proizvodnji biogoriva dobijaju sirovine, koje se ne koriste u proizvodnji hrane, kao što su: lignocelolluzna biomasa, nejestiva i otpadna ulja i životinjske masti [43] i vodeni organizmi, prvenstveno mikroalge.

Lignocelulozne sirovine. Za napredna biogoriva mogu se grupisati na [58]:

- namenski gajene energetske biljke (brzo-rastuće drvenaste biljke, višegodišnje trave),
- primarne ostatke iz poljoprivrede i šumarstva,
- sekundardne ostatke iz procesa korišćenja biljnih sirovina u različitim industrijima (otpaci od šećerne repe, voća, ostaci od drveta, piljevina),
- tercijarne ostatke (otpaci gotovih proizvoda, komunalni otpad).

Procesi dobijanja biogoriva iz lignocelulozne sirovine mogu se podeliti na termohemijske i biohemijske procese (slike 2, 3 i 5).

Prednosti korišćenja lignocelulozne sirovine u proizvodnji biogoriva su:

- rasprostranjenost i dostupnost same sirovine: neki podaci govore da bi 10% ostataka iz poljoprivrede i šuma mogli da obezbedi 4,2-6,0% trenutne potražnje goriva u saobraćaju [60];
- veći energetski prinos (GJ/ha) u odnosu na sirovine 1. generacije iz koje se dobijaju biogoriva pri gajenju na istom zemljištu [56];
- mogućnost gajenja na zemljištu lošijeg kvaliteta, manji zahtevi za vodom i manja agrotehnička ulaganja,
- niža cena [61] u odnosu na sirovine za proizvodnju biogoriva 1. generacije;
- nulti ili veoma mali uticaj na promenu namene zemljišta, tj. nulti ili mali ILUC faktor, što ukazuje da ova proizvodnja nije povezana sa velikim promenama namene zemljišta, uključujući i krčenje šuma.

S ovim je povezana i sledeća prednost korišćenja lignocelulozne sirovine, a to je da su biogoriva od lignocelulozne sirovine neutralna ili čak negativna u odnosu na ciklus ugljenika [5], jer sav ugljenik u njima, koji se kroz procese sagorevanja emituje u atmosferu u vidu CO_2 prethodno je bio "apsorbovan" u procesu fotosinteze i razvoja biljake (koje čine lignocelluloznu sirovinu) tek nekoliko meseci ili godina ranije. S druge strane, kada se predviđa dalji porast proizvodnje biogoriva 1. generacije, mora se uzeti u obzir i potreba za povećanjem obradivih površina, koje bi bile namenjene uzgoju odgovarajućih kultura za proizvodnju biogoriva i to na račun krčenja šuma. Zbog toga, CO_2, koji bi inače bio apsorbovan od strane drveća, u slučaju intenzivnih seča šuma bi zaostajao u atmosferi i uticao na povećanje ukupne količine CO_2 u atmosferi.

Nejestiva ulja. Postoji veliki broj biljnih vrsta (više od 350) čiji se razni delovi (plodovi, semenke, koštice plodova, i sl.) bogati lipidima, mogu smatrati izvorom nejestivih biljnih ulja kao alternativnom sirovinom za dobijanje biogoriva. Za gajenje ovih vrsta može se koristiti zemlja lošijeg kvaliteta sa manjim agrotehničkim ulaganjima, a s obzirom da dobijena ulja često sadrže opasne (toksične) sastojke, ne koriste se u ishrani ljudi i životinja [35]. Sve to utiče da je njihova cena niska, što utiče i na smanjenje cene biodizela, koji se iz njih dobija.

Međutim, periodičnost kultivacije, zavisnost od klimatskih uslova, neponovljivi, ponekad mali prinos ulja na marginalnom zemljištu, otežano prikupljanje sa različitih lokacija, glavni su nedostaci korišćenja nejestivih ulja kao sirovine za dobijanje biodizela. Najveći deo biljaka kao izvor nejestivih ulja može se naći u Aziji, Južnoj Americi i Africi [38].

Otpadna ulja i masti. Proizvodnja biodizela iz otpadnih (iskorišćenih) ulja [40–42] i masti [39] pored toga što predstavlja alternativu postojećim sirovinama za održivu proizvodnju biodizela, istovremeno predstavlja i način rešavanja problema odlaganja ogromnih količina otpadnog materijala sa značajnom energetskom vrednošću, koji se često, naročito u domaćinstvima, nepravilno i opasno po životnu sredinu odlažu ispuštanjem u kanalizaciju ili bacanjem na deponije. Do tačnih podataka o količinama otpadnog ulja širom sveta teško je doći, ali se pretpostavlja da se radi oko 5 miliona tona svake godine, i to: 0,7–1 miliona tona u Evropi [62], 1,5 miliona tona u SAD [63] i 2–3 miliona tona u Kini [64]. U

našoj zemlji ne postoje pouzdani podaci o količini otpadnog ulja, ali se pretpostavlja da se radi oko 10000 L otpadnog ulja godišnje [65]. Sistematskim rešenjem sakupljanja otpadanih ulja i korišćenjem za proizvodnju biodizela 2. generacije rešili bi se problemi usled njihovog odlaganja; takođe bi se smanjila količina obradivog zemljišta koja bi se koristila za proizvodnju goriva. Ipak, preko 80% otpadnog ulja nastaje u domaćinstvima [42], i glavni problem iskorišćenja ovih količina je odsustvo sistemskih metoda sakupljanja. Takođe, da bi se otpadna ulja koristila za biodizel konvencionalnim postupkom (homogena alkalna transesterifikacija), neophodna je priprema takve sirovine na odgovarajući način kako bi se dobio zadovoljavajući prinos biodizela zahtevanog kvaliteta.

Alge. Predstavljaju jednu od obećavajućih alternativnih sirovinu za dobijanje etanola fermentacijom i biodizela transesterifikacijom [43–50]. Faktori koji utiču na rast algi mogu se podeliti na abiotičke faktore u koje spadaju: svetlost (intenzitet sunčeve energije), temperatura, voda (njen salinitet, pH, hemijski sastav, toksičnost), aeracija odnosno potrebna koncentracija O_2 i CO_2; i biotičke faktore u koje spadaju patogeni mikroorganizmi koji usporavaju rast – kao što su bakterije, gljive, virusi, prisustvo drugih algi konkurentskih i inhibitorskih za datu vrstu algi.

Brojna istraživanja su pokazala da korišćenje mikroalgi kao sirovine za dobijanje bioetanola i biodizela ima brojne prednosti: [43,47,66]:

- gaje se u različitim vodenim sistemima odnosno u sredinama koje su neprikladne za gajenje konvencionalnih poljoprivrednih kultura, tako da za njihov uzgoj ne postoje potrebe za zemljištem [67], i time ne utiču na smanjenje površine zemljišta, koje se koriste za proizvodnju hrane, stočne hrane i drugih proizvoda [43];
- gaje se u različitim vodenim sredinama kao što su slatke, slane vode ili jezera, (kao otvoreni sistemi za gajenje) ili u bioreaktoru (kao zatvoreni sistemi); za gajenje mogu se koristiti i otpadne vode, jer za svoj rast koriste NH_4^+, NO_3^- i PO_4^{3-}, i time predstavljaju organizme koji se mogu koristiti za prečišćavanje otpadnih voda;
- njihovo gajenje nije uslovljeno sezonskim uslovima, i mogu se gajiti u toku cele godine
- u odnosu na biljne kulture imaju kratak vegetativni period pa u toku jedne proizvodne godine mogu se ostvariti i nekoliko „žetvi“ [47];
- 1 hektar površine na kojoj se uzgajaju alge može dati 10 do 100 puta više ulja u poređenju sa tradicionalnim kulturama za proizvodnju biodizela. Najčešće, mikroalge imaju nivo ulja u rasponu od 20 do 70% po masi suve biomase. Na primer, prinos biodizela iz algi (58700 L/ha), koje sadrži samo 30% ulja po masi, je mnogo veći u poređenju sa uljanom repicom iz koje se može dobiti do 1190 L/ha) [28];
- korišćenjem tehnologija dobijanja energije iz algi doprinosi se smanjenju emitovanja gasova staklene bašte, koji se najviše emituju iz rafinerija i drugih industrijskih procesa. [68];
- posle ekstrakcije ulja, alge se mogu koristiti za dobijanje etanola, metana, kao organsko đubrivo zbog visokog sadržaja azota i fosfora ili kao gorivo u postrojenjima za dobijanje električne i toplotne energije;
- preradom mikroalgi dobijaju se sporedni proizvodi kao što su biopolimeri, proteini, ugljeni hidrati i ostaci biomase, koji mogu da se iskoriste kao hrana ili đubriva. Pored toga, uzgoj mikroalgi ne zahteva herbicide ili pesticide [43];
- mikroalge mogu čak uticati i na smanjenje nivoa atmosferskog CO_2: za nastajanje 1 kg suve materije, algama je potrebno 1,8 kg CO_2 [43].

„Konvencionalni“ i „napredni“ bioetanol

Kada su u pitanju razlike između bioetanola i celuloznog etanola, prvenstveno se misli na razlike u sirovinama i procesima njihove konverzije, tj. o značajnim razlikama u ceni dobijanja samog biogoriva. Inače, hemijski se radi o istom jedinjenju: bezbojnoj prozirnoj tečnosti karakterističnog mirisa, lako mešljive sa vodom, lako zapaljive. Anhidrovani etanol (sadržaj vode <1%) može se koristiti kao komponenta za nameša-vanje sa benzinom i sa dizelom u različitim odnosima ili kao čisto gorivo kod motora sa unutrašnjim sagorevanjem [31]. Prednost etanola kao goriva je što doprinosi smanjenju emisije štetnih gasova pri sagorevanju u motorima, a takođe je i biorazgradljiv u znatno većoj meri nego što su fosilni benzin i dizel podložni degradaciji pod uticajem mikroorganizama [31]. U upotrebi su različite mešavine etanola i benzina, koje se označavaju sa „EXX“, pri čemu je E oznaka za bezvodni etanol, a XX broj koji iskazuje procentualni udeo etanola u gorivu; najčešće su to E5, E10, E85 i E95 [69]. Zemlje u kojima najviše koriste smeše etanola i benzina su SAD, Brazil i zemlje EU (Nemačka, Švedska, Francuska, Španija); u ovim zemljama su odgovarajućim standardima (na primer u zemljama EU, EN 15736 [70] i EN 228 [71]) propisane karakteristike etanola koji se namešava sa benzinom, kao i kvalitet same mešavine.

Proizvodnja bioetanola (1G) je široko komercijalizovana i njegova proizvodnja u svetu iznosi oko 200 miliona litara dnevno [72]. Najveći proizvođači su SAD i Brazil sa preko 80% svetske proizvodnje, dok je Evropa na trećem mestu sa oko 5–6% [73]. Proizvodnja celuloznog etanola još uvek je u ranoj fazi komercijalizacije. Prvo komercijalno postrojenje za proizvodnju celuloznog etanola pušteno je u rad oktobra 2013. god. u Italiji (Crescentino Bio-refinery [10]) sa očekivanim godišnjim kapacitetom od 10 miliona galona (1 galon ≈ 4,546 L) etanola dobijenog od pšenične slame i bambusa

Arundo donax, dok je nekoliko sličnih postrojenja u izgradnji kako u Evropi tako i u SAD, te se procenjuje da će komercijalna proizvodnja celuloznog etanola značajno doprineti svetskoj proizvodnji bioetanola u skorije vreme.

Biodizel, obnovljivi, BtL i HTU dizel

Biodizel. Konvencionalnom (fosilnom) gorivu može dodati u manjoj (1,5–5%) ili većoj (5–30%) količini; ove mešavine nose oznaku „BXX“, gde B označava biodizel, a XX njegov procentualni sadržaj. Na tržištu se nalaze B5, B7, B20, i B100 [31]. Proizvodni kapaciteti u Evropskoj uniji u 2012. god. iznosili su 23,5 miliona tona biodizela, pri čemu najveće kapacitete imaju Nemačka, Španija, Francuska, Italija i Holandija; u 2011. god. ukupna proizvodnja biodizela u EU iznosila je 8,6 miliona tona, od toga u Nemačkoj je proizvedeno 2,8 miliona tona, a u Francuskoj 1,8 miliona tona [74]. Biodizel je prvo alternativno gorivo s karakteristikama definisanim odgovarajućim standardom, a prvi nacionalni standard za biodizel donela je Austrija ÖN C1191, u kojoj je izgrađeno i prvo pilot postrojenje 1985. [31]. Evropski standard EN14214 usvojen prvi put 2003. god. definiše zahteve i metode ispitivanja metilestara masnih kiselina za korišćenje u dizel-motorima i zagrevanje, s tim da je do sada više puta izvršena njegova revizija i dopuna (aktuelna je verzija iz 2012. [70]). Standard EN 590 [71], koji definiše karakteristike i metode ispitivanja dizel goriva, predviđa da gorivo za vozila sa dizel motorom sadrže do 7 zapr.% metilestara masnih kiselina.

Karakteristike triglicerida, koje utiču i na kvalitet samog biodizela (na primer, viskoznost, gustinu, niskotemperaturne osobine, oksidativnu stabilnost i dr.), zavise od viših masnih kiselina koje se nalaze u njihovom sastavu. Više masne kiseline, koje često preovlađuju su: palmitinska (C16:0), stearinska (C18:0), oleinska (C18:1), linoleinska (C18:2), i linolenska (C18:3). Postoje i druge kiseline koje se mogu naći u sastavu triglicerida, na primer palmitoleinska (C16:1), arahidonska (C20:0), i dr., ali u znatno manjim količinama [38]. Povećanje ugljeničnog lanca u estrima masnih kiselina povećava toplotnu moć goriva i smanjuje vreme paljenja, viskoznost se povećava sa povećanjem ugljeničnog niza, a smanjuje sa povećanjem broja dvostrukih veza (tj. sa povećanjem nezasićenosti); povećanjem udela estara nezasićenih masnih kiselina smanjuje se oksidativna stabilnost goriva; biodizel sa velikim udelom estara zasićenih masnih kiselina ima loše niskotemperaturne osobine, ali i veliku oksidativnu stabilnost. Sastav masnih kiselina u trigliceridima biljnog porekla zavisi od same biljne vrste i donekle od uslova njenog uzgoja.

Međutim, u slučaju algi, sastav masnih kiselina značajno se menja pod uticajem abiotičkih faktora sredine u kojima se uzgajaju, kao što je temperatura, osvetljenost, količina hranljivih materija, itd. [75]. U odnosu na osnovne masne kiseline, sva ulja se mogu podeliti na ona sa velikim udelom zasićenih masnih kiselina, sa velikim udelom mononezasićenih masnih kiselina (sa jednom nezasićenom vezom, na primer C18:1) i sa velikim udelom polinezasićenih kiselina (sa dve ili tri nezasićene veze, na primer, C18:2 i C18:3). Sa aspekta proizvodnje biodizela najpoželjinija su ulja sa većim udelom mononezasićenih kiselina u kombinaciji sa malim procentom zasićenih, polinezasićenih (naročito onih sa tri dvostruke veze) i kiselina dugih lanaca (sa 20 i više atoma C), čime je obezbeđen kompromis između niskotemperaturnih osobina i oksidativne stabilnosti, kao i između kinematske viskoznosti i cetanskog broja [76]. Pregled jestivih i nejestivih biljnih ulja u odnosu na sastav masnih kiselina i osnovne karakteristike biodizela koji bi se dobio od takvih ulja može se naći u radu Škrbić i sar. [38]. Pregled sastava masnih kiselina algalnih lipida dat je u radu Knothe [75]. Da bi se iskoristio širok spektar ulja alternativnih sirovina za proizvodnju biodizela, moguće je korišćenje smeša ulja u odnosima kojima se superponiraju udeli određenih masnih kiselina u pravcu postizanja poželjnih osobina biodizela [77].

Obnovljivi („zeleni“) dizel. Osnovna sirovina za dobijanje obnovljivog dizela su različita biljna ulja i životinjske masnoće. Dobija se katalitičkim hidroprocesom, a ne transesterifikacijom i za razliku od biodizela, njegove osobine ne zavise od porekla sirovine. S obzirom na različite procese dobijanja, postoji i razlika u hemijskom sastavu biodizela i obnovljivog dizela, koji se prvenstveno sastoji od alkana, a ne sadrži aromate, jedinjenja kiseonika i sumpora. Osnovne razlike između obnovljivog dizel i biodizela su sledeće (tabela 2): obnovljivi dizel ima veći cetanski broj i toplotnu vrednost od biodizela, dok mali sadržaj aromata (<0,1 mas.%) utiče na smanjenje čađenja i emisiju čestične materije [5,78]. Oksidativna stabilnost obnovljivog dizela je znatno veća nego biodizela. Ponekad može da ima lošije niskotemperaturene karaktristike u odnosu na biodizel (tabela 2). Po svojim osobinama, obnovljivi dizel u potpunosti je kompatibilan za namešavanje sa dizelom.

U literaturi se mogu naći i drugi nazivi za obnovljivi dizel kao što su: zeleni dizel, hidrotretirana biljna ulja (HVO – hydrotreated vegetable oil). Knothe [24] ističe da bi trebalo koristiti naziv „obnovljivi dizel“, koji ukazuje da se radi o obnovljivom poreklu goriva, bez aluzije o samoj prirodi goriva, dok prefiks „zeleni“ iako asocira na obnovljivo poreklo sirovine i pozitivniji uticaj samog goriva na smanjeni efekat staklene bašte, može i pogrešno da navede da se radi o biorazgradljivom gorivu, što nije slučaj, s obzirom na sastav i potpunu mešljivost sa dizelom.

Tabela 2. Karakteristike različitih biogoriva sličnih dizelu i njihovo poređenje sa dizelom i standardizovanim karakteristikama dizela [78]
Table 2. Comparison of properties of different biofuel types similar to fossil diesel [78]

Parametar	Merna jedinica	FAME biodizel	BtL dizel	Obnovljivi dizel	Dizel	Standard za dizel, min/max
Gustina	g/ml	0,855–0,9	0,72–0,82	0,77–0,83	0,85	0,8/0,845
Sumpor	mg/kg	–0,012	<10	<10	12	/10
Cetanski indeks	–	58,3	70	50–105	54,57	46/
Cetanski broj	–	45–72,7	55–99	80–99	50	51/
Temperatura paljenja	°C	98–188	55–78	68–120	52–136	60/170
voda	mg/kg	28,5–500	19	42–95	0,5	/200
Koksni ostatak	mas.%	0,02–0,3	0,02–4,5			/0,3
Viskoznost na 40 °C	cSt	3,89–7,9	2,1–3,5	2,5–4,15	2,71	2/4,5
Korozija bakarne trake	3 h na 50 °C	1	–	–	<3	K lasa 1
Indukciono vreme na 110 °C	h	0,9–10,9	>22	>22		6/
Temperatura destilacije pri 90 zapr.% kondenzata	°C	–	295–335	298–342	341	85/360
Toplotna vrednost	MJ/kg	37,1–40,4	43–45	42–44	34,97	35/
CFPP filtrabilnost	°C	–13–15	–22–0	>20	–6	–5/5
Tačka zamućenja	°C	–3–17	–25–0	–25–30	–5	–5/12
Tačka tečenja	°C	–15–16	–	–3–29	–21	–13/10

Takođe, postoji i tzv. „beli“ dizel [78,79] koji se kao i obnovljivi dizel, dobija katalitičkim hidroprocesima, ali se kao sirovina koriste 100% otpadna (iskorišćena) biljna ulja.

Proizvodnja obnovljivog dizela nije komercijalizovana u većem obimu; 2007. god. počelo je sa radom prvo postrojenje Neste Oil's Porvoo rafinerija u Finskoj godišnjeg kapaciteta 170000 t; komercijalni naziv tehnološkog postupka razvijenog u ovom postrojenju je NExBTL [78].

BtL dizel (ili Fiscer–Tropsch, tj. FT dizel). Sintetičko dizel gorivo dobijeno Fischer–Tropsch procesom. U Fischer–Tropsch sintezi, ugljen-monoksid i vodonik prisutni u singasu iz procesa gasifikacije (na primer, lignocelulozne sirovine) u prisustvu katalizatora (kobalt ili gvožđe) grade ugljovodonični, parafinski lanac (obrazujući niz metilenskih grupa, $–CH_2–$, konstitutivnu jedinicu ovog lanca).

U poređenju sa drugim gorivima prikazanim u tabeli 2, BtL dizel je sličan fosilnom dizelu u odnosu na gustinu, viskoznost i krajnju temperaturu destilacije, koje su u granicama predviđenim standardom o kvalitetu fosilnog dizela EN 590, te predstavlja atraktivno gorivo – supstituenta dizela. Ima veću toplotnu vrednost od biodizela i od dizela, kao i visok cetanski broj što ukazuje na dobre karakteristike paljenja (tabela 2) [5,78]. Kao i obnovljivi dizel, BtL dizel ima veoma mali sadržaj aromata i jedinjenja sumpora, ali i ponekad loše niskotemperaturne karakteristike (tabela 2). Nedostatak je i niska tačka paljenja, što može biti uzrok samopaljenja (tabela 2).

U literaturi se pored naziva FT dizel (FT ulje) i BtL dizel, javlja i zeleno motorno gorivo [5].

Iako su faze pri proizvodnji BtL dizela dobro poznate i uspešno demonstrirane na industrijskom nivou, integracija različitih tehnologija (priprema sirovine, gasifikacija, prečišćavanje singasa, Fisher–Tropsch sinteza) radi komercijalne proizvodnje ovog goriva još uvek predstavlja tehnološki izazov. Izgradnja prvog komercijalnog postrojenje za BtL dizel u svetu na bazi Choren Carbo-V® procesa, koje se gradilo u Frajbrugu, Nemačka, nije zaživelo s obzirom da je kompanija Choren Industries otišla u stečaj u 2011. Očekuje se da novi vlasnik Linde Engineering Dresden nastavi sa razvojem Choren Carbo-V® tehnologije [80].

HTU dizel. Zbog velikog sadržaja vode i jedinjenja sa kiseonikom, bio-ulje dobijeno u procesu likvefakcije ili pirolize biomase se mora dodatno obraditi kako bi se dobilo odgovarajuće motorno gorivo. U osnovi hidrotoplotne (hidrotermalne) obrade bio-ulja (engl. Hydro-Thermal Upgrading, skraćeno HTU) nalazi se katalitička deoksigenacija – proces u kojem se u prisustvu vodonika i katalizatora izdvaja kiseonik iz molekula i prevodi u vodu, a ostatak prevodi u ugljovodonike. Nakon uklanjanja vode, karakteristike dobijenog proizvoda, tzv. HTU dizela, omogućavaju njegovo namešavanje (10-20%) sa konvencionalnim, fosilnim dizelom i korišćenje ovakve smeše u standardnim dizel motorima bez potreba za njihovom modifikacijom [81]. Za razliku od tečnih biogoriva upoređenih sa standardizovanim karakteristikama dizela (tabela 2), u literaturi nisu pronađene brojčane vrednosti ovih karakteristika za HTU dizel. Prema nekim podacima [81], cetanski broj ovog goriva je veći od cetanskog broja dizela, gustina odgovara manjim vrednostima u opsegu gustina definisanih standardom za kvalitet dizel goriva EN590

(tabela 2). Karakteristike HTU dizela koje ne zadovoljavaju standard EN590 su mazivost i sadržaj vode, zbog čega je potrebna njegova dalja obrada [81].

ZAKLJUČAK

Sve veće energetske potrebe u svetu, potrebe država za energetskom sigurnošću i nezavisnošću, povećanje i promenljivost cena nafte, iskorišćenje ležišta ugljovodonika (nafte i gasa), uticaj fosilnih goriva na efekat staklene bašte i globalne klimatske promene, predstavljaju osnovne izazove sa kojima se današnja društva susreću i koji će biti sve izraženiji u predstojećim decenijama. Očekuje se da biogoriva bar donekle smanje ove probleme, predstavljajući istovremeno i osnovu održivog razvoja društva.

Dosadašnji razvoj tehnologija za proizvodnju biogoriva pratio je potrebe prevazilaženja nedostataka postojećih biogoriva i njihovih sirovina, te su tako i razvijene različite nove vrste biogoriva. Tehnološki procesi dobijanja biogoriva mogu se podeliti na konvencionalne, danas široko primenjivane tehnologije, i napredne procese, koji su u ovom momentu u fazi rane komercijalizacije ili se nalaze u različitim fazama razvoja ili demonstracije.

Proizvodnja biogoriva prve generacije primenom konvencionalnih tehnologija uticala je na povećanje cena hrane, a s obzirom na očekivani stalni porast broja stanovnika na Zemlji, pokrenula je i mnoge polemike i rasprave na temu „hrana ili gorivo“. Kao odgovor na ovo, započeo je razvoj biogoriva druge generacije zasnovanih prvenstveno na lignocelulloznoj biomasi (gajene nejestive biljke ili otpadna organska materija) i primeni naprednih tehnologija.

S obzirom na to da i lignocelulozna biomasa donekle zavisi od raspoloživog zemljišta, kao sledeći korak u razvoju biogoriva pojavila su se biogoriva treće generacije dobijena od biomase vodenih organizama (algi) i mikroorganizama, sa krajnjim ciljem smanjenja opterećenja zemljišta.

Dalji napredak (razvoj četvrte generacije biogoriva) ide u pravcu korišćenja novih saznanja iz oblasti metaboličkog inženjeringa radi modifikacije metabolizma algi ili dobijanja poboljšanih sojeva algi sa aspekta njihovog efikasnijeg iskorišćenja u proizvodnji biogoriva, i istovremeno da u procesu fotosinteze vezuju veće količine ugljen dioksida iz vazduha, doprinoseći smanjenju gasova staklene bašte.

Osnovna prednost konvencionalnih biogoriva je primena razvijenih tehnoloških procesa, međutim, njihovo dalje korišćenje zavisi od netehničkih faktora, kao što su politička i ekonomska pitanja.

Zajedničko za napredna tečna goriva razmatrana u ovom radu je izražena raznolikost (nejestivih) jeftinih sirovina, za razliku od konvencionalnih tečnih biogoriva, koja se prvenstveno dobijaju od nekoliko specifičnih, gajenih (uglavnom jestivih) vrsta biljaka. Napredna biogoriva se još uvek ne proizvode komercijalno u velikim količinama. Zbog visoke cene proizvodnje ova goriva trenutno nisu konkurentna na tržištu, ali se očekuje da sa unapređenjem tehnologija njihovog dobijanja ona preuzmu važnu ulogu u budućnosti.

Prednost konvencionalnih biogoriva, naročito biodizela, je što se mogu proizvoditi u decentralizovanim i manjim pogonima, predstavljajući prednost za razvoj ruralnih sredina. Suprotno, proizvodnja naprednih biogoriva je uglavnom vezana za velike pogone, kako bi se postigli bolji rezultati na ekonomskom planu. To istovremeno znači da su potrebne i velike investicije, koje mogu da priušte samo velike kompanije. Zbog toga, ovakve kompanije će i dominirati tržištem naprednih biogoriva i u velikoj meri kontrolisati cene sirovina te tako ograničiti dobrobit koju nosi proizvodnja biogoriva za ruralne sredine. Međutim, mora se napomenuti da iako nude veliki potencijal za budućnost, još uvek nije potvrđeno da će napredna biogoriva imati bolje ekonomske karakteristike, kao i uticaj na ciklus ugljenika u prirodi, u odnosu na proizvodnju konvencionalnih biogoriva.

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LITERATURA

[1] B.P. Tissot, D.H. Welte, Petroleum Formation and Occurence, Springer-Verlag, Berlin, 1984.

[2] IEA World energy outlook 2013. Paris, France: International Energy Agency.

[3] J.G. Speight, Chapter 1 - Occurrence and Formation of Crude Oil and Natural Gas. Subsea and Deepwater Oil and Gas Science and Technology, 2015, pp. 1–43.

[4] D. Russo, M. Dassisti, V. Lawlor, A.G. Olabi, State of the art of biofuels from pure plant oil, Renew. Sust. Energ. Rev. **16** (2012) 4056–4070.

[5] S.N. Naik, V.V.Goud, P.K. Rout, A.K. Dalai, Production of first and second generation biofuels: A comprehensive review, Renew. Sust. Energ. Rev. **14** (2010) 578–597.

[6] Sustainable production and consumption, https://ec.europa.eu/jrc/en/research-topic/sustainable-production-and-consumption

[7] A.K. Azad, M.G. Rasul, M.M.K. Khan, S.C. Sharma, M.A. Hazrat, Prospect of biofuels as an alternative transport fuel in Australia, Renew. Sust. Energ. Rev. **43** (2015) 331–351.

[8] M. Crocker, Thermochemical conversion of biomass to liquid fuels and chemicals, Royal Society of Chemistry, London, 2010.

[9] D. Sperling, D. Gordon, Two Billion Cars: Driving Toward Sustainability, Oxford University Press, Oxford, 2009.

[10] M. Guo, W. Song, J. Buhain, Bioenergy and biofuels: History, status, and perspective. Renew. Sust. Energ. Rev. **42** (2015) 712–725.

[11] L. Zhang, G. Hu, Supply chain design and operational planning models for biomass to drop-in fuel production. Biomass Bioenerg **58** (2013) 238–250.

[12] Directive 2009/28/EC of the European Parliament and of the Council, 23 April 2009, on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC, Official Journal of the European Union, L 140/16, 2009.

[13] N.S. Bentsen, C. Felby, Biomass for energy in the European Union–a review of bioenergy resource assessments, Biotechnol. Biofuels 5/25 (2012).

[14] Congress US Energy Policy Act of 2005, Washington DC, USA, 2005.

[15] Congress US Energy Indepedence and Security Act of 2007, Washington DC, USA, 2007.

[16] Y. Xu, M.A. Hanna, L. Isom, „Green" chemicals from renewable agricultural biomass-a mini review, Open Agr. J. **2** (2008) 54–61.

[17] K. Bracmort, Biomass: Comparison of Definitions in Legislation, Congressional Research Service Report 7-5700, R40529, www.crs.gov, 2015.

[18] Directive 2003/30/EC of the European Parliament and of the Council, 8 May 2003, on the promotion of the use of biofuels or other renewable fuels for transport, Official Journal of the European Union, L 123/42, 2003.

[19] Zakon o energetici, „Službeni glasnik RS", br. 57/2011 od 1. avgusta 2011.

[20] Nacionalni akcioni plan za korišćenje obnovljivih izvora energije Republike Srbije-2013 (U skladu sa obrascem predviđenim Direktivom 2009/28/E3 – odluka 2009/ /548/E3).

[21] Z. Predojević, B. Škrbić, N. Đurišić-Mladenović, J. Cvejanov, Esterification pretreatment process to reduction of high content of free fatty acid in feedstockes for biodiesel production, Proceedings of 15th Danube-Kris-Mures-Tisa (DKMT) Euroregion Conference on Environment and Health with satellite event LACREMED Conference "Sustainable agricultural production: restoration of agricultural soil quality by remediation", Novi Sad, Serbia, 16–17 May 2013, pp. 135–138.

[22] Z. Predojević, B. Škrbić, N. Đurišić-Mladenović, Waste oil valorization – production of biodiesel from feedstocks with high free fatty acid contents, Book of Abstracts, XXIII Congress of Chemists and Technologists of Macedonia, p.146, Ohrid, Macedonia, 8-11 October, 2014.

[23] European Biofuels Technology Platform, Advanced Biofuels in Europe, http://www.biofuelstp.eu/advancedbiofuels.htm

[24] G. Knothe, Biodiesel and renewable diesel: A comparison, Prog. Energ. Combust. **36** (2010) 364–373.

[25] B. Škrbić, Distribucija i skladištenje vodonika, Gas **19** (2014) 5–10.

[26] Z. Predojević, Pretreatments of lignocellulosic feedstock for bioethanol production, Hem. Ind. **64** (4) (2010) 283––293.

[27] P.S. Nigam, A. Singh, Production of liquid biofuels from renewable resources, Prog. Energ. Combust. **37** (2011) 52–68.

[28] K. Dutta, A. Daverey, J-G. Lin, Evolution retrospective for alternative fuels: First to fourth generation, Renew. Energ. **69** (2014) 114–122.

[29] K. Ullah, M. Ahmad, S.V.K. Sharma, P. Lu, A. Harvey, M. Zafar, Sh. Sultana, C.N. Anyanwu, Algal biomass as a global source of transport fuels: Overview and development perspectives, Prog. Nat. Sci. Mater. Int. **24** (2014) 329–339.

[30] P. Girrard, A. Fallot, Review of existing and emerging technologies for the production of biofuels in developing countries, Energy Sustain. Dev. **10** (2) (2006) 92–108.

[31] Z. Predojević, Goriva iz biomase-bioetanol i biodizel, Univerzitet u Novom Sadu, Tehnološki fakultet, 2010.

[32] A. Lali, Next generation biofuels-India Scenario and areas for cooperation, India-EU and Member State Partnership for a Strategic Roadmap in Research and Innovation, New Delhi, 12 November 2010, http://ec.europa.eu/research/iscp/pdf/india-eu-conference-2010/lali_en.pdf

[33] B. Škrbić, N.Đurišić-Mladenović, Gasovi staklene bašte, Gas **12** (2007) 11–15.

[34] Z. Predojević, B. Škrbić, N. Đurišić-Mladenović, Transesterification of linoleic and oleic sunflower oils to biodiesel using CaO as a solid base catalyst, J. Serb. Chem. Soc. **77** (2012) 815–832.

[35] A.E. Attabani, A.S. Silitonga, H.C. Ong, T.M.I. Mahlia, H.H. Masjuki, Irfan Anjum Badruddin, H. Fayaz, Non-edible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production, Renew. Sust. Energy Rev. **18** (2013) 211–245.

[36] M. Kelbert, A. Romaní, E. Coelho, F. B. Pereira, J.A. Teixeira, L. Domingues, Lignocellulosic bioethanol production with revalorization of low-cost agroindustrial by-products as nutritional supplements, Ind. Crop. Prod. **64** (2015) 16–24.

[37] S-Y. No, Application of bio-oils from lignocellulosic biomass to transportation, heat and power generation - A review, Renew. Sust. Energy Rev. **40** (2014) 1108–1125.

[38] B. Škrbić, J. Cvejanov, N. Đurišić-Mladenović, Chemometric characterization of vegetable oils based on the fatty acid profiles for selection of potential feedstocks for biodiesel production, J. Biobased Mater Bioenerg. (2015), accepted article

[39] I.B. Banković-Ilić, I.J. Stojković, O.S. Stamenković, V.B. Veljković, Y-T. Hung, Waste animal fats as feedstocks for biodiesel production, Renew. Sust. Energy Rev. **32** (2014) 238–254.

[40] Z. Predojević, The production of biodiesel from waste frying oils: a comparison of different purification steps, Fuel **87** (2008) 3522–3528.

[41] Z. Predojević, B. Škrbić, Alkali-catalyzed production of biodiesel from waste frying oils, J. Serb. Chem. Soc. **74** (2009) 993–1007.

[42] A. Talebian-Kiakalaieh, N.A:S. Amin, H. Mazaheri, A review on novel processes of biodiesel production from waste cooking oil, Appl. Energ. **104** (2013) 683–710.

[43] A.L. Ahmad, N.H. Mat Yasin, C.J.C. Derek, J.K. Lim, Microalgae as a sustainable energy source for biodiesel production: A review Renew. Sust. Energy Rev. **15** (2011) 584–593.

[44] L. Brennan, Ph. Owende, Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products, Renew. Sust. Energy Rev. **14** (2010) 557–577.

[45] M.F. Demirbas, Biofuels from algae for sustainable development, Appl. Energy **88** (2011) 3473–3480.

[46] A. Demirbas, M.F. Demirbas, Algae Energy-Algae as a New Source of Biodiesel, Springer-Verlag, London 2010.

[47] I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux, Biodiesel from microalgae: A critical evaluation from laboratory to large scale production, Appl. Energ. **103** (2013) 444––467.

[48] C. Sambusiti, M. Bellucci, A. Zabaniotou, L. Beneduce, F. Monlau, Algae aspromising feedstocks for fermentative biohydrogen production according to a biorefinery approach:A comprehensive review, Renew. Sust. Energy Rev. **44** (2015) 20–36.

[49] B.R. Danilović, J.M. Avramović, J.T. Ćirić, D.S. Savić, V.B. Veljković, Proizvodnja biodizela iz ulja mikroalgi, Hem. Ind. **68** (2014) 213–232.

[50] T.P.T. Pham, R. Kaushik, G.K. Parshetti, R. Mahmood, R. Balasubramanian, Food-waste-to-energy conversion technologies: Current status and future directions, Waste Manag. (2014) (Article in Press) http:////dx.doi.org/10.1016/j.wasman.2014.12.004

[51] International Agency for Energy, Technology Roadmap „Biofuels for transport“, 2011, http://www.iea.org/publications/freepublications/publication/Biofuels_Roadmap_WEB.pdf

[52] The Energy Technology Roadmap – Bifuels: the next generation. How innovation can brighten Europe's energy future. Report of Science/Business symposium, Science Business Publishing Ltd., 2011, www.sciencebusiness.net

[53] E. Gawel, G. Ludwig, The ILUC dilemma: How to deal with indirect land use changes when governing energy crops? Land Use Policy **28** (2011) 846–848.

[54] A. Pandey, Handbook of Plant-Based Biofuels, CRC Press, Boca Raton, FL, 2009.

[55] V. Semenčenko, Ispitivanje različitih hibrida kukuruza kao sirovine za proizvodnju bioetanola, skroba i hrane za životinje, Doktorska disertacija, Univerzitet u Beogradu, Tehnološko–metalurški fakultet, Beograd, 2013.

[56] V. Veljković, O. Stamenković, Perspektivne tehnologije dobijanja biodizela, Zaštita materijala, **53** (2012) 281––291.

[57] A. Kurki, A. Hill, M. Morris. Biodiesel: The Sustainability Dimensions. National, Sustainable Agriculture Information Service, 2006. http://www.attra.ncat.org/attrapub/PDF/biodiesel_sustainable.pdf

[58] R.E.H. Sims, W. Mabee, J.N. Saddler, M. Taylor, An overview of second generation biofuel technologies, Bioresour. Technol. **101** (2010) 1570–1580.

[59] A. Ajanović, Biofuels versus food production: Does biofuels production increase food prices?, Energy **36** (2011) 2070–2076.

[60] IEA-2010 Sustainable Production of Second-Generation Biofuels; http://www.iea.org/publications/freepublications/publication/second_generation_biofuels

[61] Greenergy perspectives-2010-Second Generation Biofuels,updated June 2010, http://www.greenergy.com/Environment/perspectives/02-second_generation.pdf

[62] M.G. Kulkarni, A.K Dalai, Waste Cooking Oil – An Economical Source for biodiesel: A review. Ind. Eng. Chem. Res. **45** (2006) 2901–2913.

[63] D.J. Tiffany, The growth of alternative fuels: Minesota and U,S perspectives, Submitted for a joint conference of University of Minnesota, University of Padova, University of Bologna, 2002, www.ddgs.umn.edu/prod//groups/cfans/@pub/@cfans/@ansci/documents/asset/cfans_asset_413801.pdf

[64] C.S. Ki Lin, L.A. Pfaltzgraff, L. Herrero-Davila, E.B. Mubofu, S. Abderrahim, J.H. Clark, A.A. Koutinas, N. Kopsahelis, K. Stamatelatou, F. Dickson, S. Thankappan, Z. Mohamed, R. Brocklesby, R. Luque, Food waste as a valuable resource for the production of chemicals, materials and fuels, Current situation and global perspective, Energy Environ. Sci. **6** (2013) 426–464.

[65] M. Tešić, F. Kiss, V. Janković, Mogućnost proizvodnje i korišćenja biodizela u AP Vojvodini, Novi Sad, Vojvođanska akademija nauka i umetnosti, 2008.

[66] M.T. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: A review, Renew. Sust. Energy Rev. **14** (2010) 217–232.

[67] M. Daroch, S. Geng, G. Wang, Recent advances in liquid biofuel production from algal feedstocks, Appl. Energ. **102** (2013) 1371–1381.

[68] A. Demirbas, Energy priorities and new energy strategies. Energy Educ. Sci. Technol. **16** (2006) 53–109.

[69] Z. Predojević, B. Škrbić, N. Đurišić-Mladenović, Effects of ethanol and MTBE additives on properties at blends with base gasoline, Fresen. Environ. Bull. **20** (2011) 1401–1405.

[70] SRPS EN 14214:2012 - Tečni naftni proizvodi – Metilestri masnih kiselina (MEMK) za korišćenje za dizel-motore i zagrevanje – Zahtevi i metode ispitivanja.

[71] SRPS EN 590:2014 – Goriva za motorna vozila – Dizel--gorivo – Zahtevi i metode ispitivanja.

[72] Bio fuels production worlwide http://en.globometer.com/energy-bioethanol-world.php

[73] Renewable Fuel Association, http://ethanolrfa.org//pages/World-Fuel-Ethanol-Production

[74] Statistics-The EU biodiesel industry. European Biodiesel Board. www.ebb-eu.org/stats.php

[75] G. Knothe, A technical evaluation of biodiesel from vegetable oils *vs.* algae. Will algae-derived biodiesel perform?, Green. Chem. **13** (2011) 3048–3065.

[76] B.R. Moser, Impact of fatty ester composition on low temperature properties of biodiesel–petroleum diesel blends, Fuel **115** (2014) 500.
[77] G. Martınez, N. Sanchez, J.M. Encinar, and J.F. Gonzalez. Biomass Bioenerg. (2014) http://dx.doi.org/10.1016/ /j.biombioe.2014.01.034
[78] S. Bezergianni, A. Dimitriadis, Comparison between different types of renewable diesel, Renew. Sust. Energy Rev. **21** (2013) 110–116.
[79] S. Bezergianni, L.P. Chrysikou, Oxidative stability of waste cooking oil and white diesel upon storage at room temperature, Bioresour. Technol. **126** (2012) 341–344.
[80] European Biofuels Technology Platform, Biomass to Liquids (BtL), http://www.biofuelstp.eu/btl.html
[81] Biofuels production via HTU and *via* pyrolysis, Report 2GAVE-05.07, SenterNovem, Utrecht, The Netherlands, 2005.

SUMMARY

CONVENTIONAL AND ADVANCED LIQUID BIOFUELS

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(Review paper)

Energy security and independence, increase and fluctuation of the oil price, fossil fuel resources depletion and global climate change are some of the greatest challenges facing societies today and in incoming decades. Sustainable economic and industrial growth of every country and the world in general requires safe and renewable resources of energy. It has been expected that re-arrangement of economies towards biofuels would mitigate at least partially problems arised from fossil fuel consumption and create more sustainable development. Of the renewable energy sources, bioenergy draws major and particular development endeavors, primarily due to the extensive availability of biomass, already-existence of biomass production technologies and infrastructure, and biomass being the sole feedstock for liquid fuels. The evolution of biofuels is classified into four generations (from 1st to 4th) in accordance to the feedstock origin; if the technologies of feedstock processing are taken into account, than there are two classes of biofuels – conventional and advanced. The conventional biofuels, also known as the 1st generation biofuels, are those produced currently in large quantities using well-known, commercially-practiced technologies. The major feedstocks for these biofuels are cereals or oleaginous plants, used also in the food or feed production. Thus, viability of the 1st generation biofuels is questionable due to the conflict with food supply and high feedstocks' cost. This limitation favoured the search for non-edible biomass for the production of the advanced biofuels. In a general and comparative way, this paper discusses various definitions of biomass, classification of biofuels, and gives brief overview of the biomass conversion routes to liquid biofuels depending on the main constituents of the biomass. Liquid biofuels covered by this paper are those compatible with existing infrastructure for gasoline and diesel and ready to be used in mixture with them as „drop-in" fuels: bioethanol, cellulosic ethanol, biodiesel, renewable diesel and BtL diesel; their major advantages and drawbacks are compared.

Keywords: Biomass • Conversion • Bioethanol • Biodiesel • Cellulosic ethanol • Synthetic diesel • Green diesel

Biosorpcioni potencijal otpadne biomase mladog ploda oraha za jone olova: Kinetička i ravnotežna ispitivanja

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Izvod

U radu je ispitivana mogućnost primene otpadne biomase iz procesa proizvodnje orahovog likera, kao jeftinog biosorbenta, za uklanjanje Pb(II) jona iz vodenog rastvora u stacionarnim uslovima. Strukturna svojstva biosorbenta okarakterisana su pomoću FTIR spektroskopije. Biosorpcioni potencijal otpadne biomase mladog ploda oraha proučavan je u funkciji: pH (2–6), kontaknog vremena (0–120 min), količine biosorbenta (2–20 g) i početne koncentracije Pb(II) jona (10–120 mg dm^{-3}), pri temperaturi od 25 °C, uz mešanje (120 rpm) i konstantnoj jonskoj jačini (0,02 mol dm^{-3}). Ravnotežna biosorpcija Pb(II) jona je postignuta nakon 50 min, u opsegu pH 4–5. Eefikasnost uklanjanja Pb(II) jona od 84% postiže se pri početnoj koncentraciji sorbata od 15 mg dm^{-3} i dozi biosorbenta od 6 g po dm^3 sorbata. Biosorpciju najbolje opisuje Langmuir model ($R^2 \geq 0{,}990$). Maksimalni biosorpcioni kapacitet otpadne biomase mladog ploda oraha za Pb(II) jone na 25 °C i pH 4,5 iznosi 19,23 mg g^{-1}. Najbolje slaganje sa eksperimentalnim rezultatima, u temperaturnom opsegu 25–40 °C, pokazuje kinetički model pseudo-drugog reda.

Ključne reči: biosorpcija, otpadna biomasa, plod oraha, joni olova, kinetika, izoterme.

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Zbog nedostatka pitke vode u svetu, kvalitet površinskih i podzemnih voda danas je veoma aktuelan problem. Glavni izvori zagađenja prirodnih voda su industrijske i urbane otpadne vode, kišnica, kao i vode iz deponija. Prisustvo teških metala (olovo, kadmijum, živa i bakar) u prirodnim vodama je naročito opasno za žive organizme. Metali imaju toksično dejstvo na nervni, imuni, hematopoetski i probavni sistem, a narušavaju i funkcionisanje bubrega [1]. Iz tog razloga, veoma je važno svako rešenje problema uklanjanja jonskog oblika metala iz vodenih rastvora.

Olovo je jedan od najpoznatijih štetnih teških metala. Brojni su primeri zagađivanja voda ili zemljišta olovom [2]. Najčešće, emisije olova potiču iz hemijske industrije koja se bavi proizvodnjom predmeta od olova, procesima štampanja, bojenja i proizvodnjom insekticida. Jedan od izvora zagađenja životne sredine olovom je i tetraetil-olovo, koje se dodaje benzinu kao aditiv [2]. Koncentracija olova u atmosferskim vodama se kreće od 1 do 50 μg dm^{-3}, a u gusto naseljenim i industrijskim zonama može dostići i 1000 μg dm^{-3} [3]. Olovo može zagaditi vodu za piće i kada je ta voda u kontaktu sa bojama koje sadrže ovaj metal. Tako, u područjima gde je pretežno zastupljena tvrda voda i gde pH vrednost ne opada ispod 7, protočne cevi od olova su stabilne. U ovim uslovima, Ca i Mg iz vode, kao i formirani karbonati olova, ponašaju se kao zaštitni sloj koji sprečava rastvaranje olova. Međutim, u područjima gde preovladava meka voda, a pH vrednost može biti smanjena do pH 5, dolazi do veće rastvorljivosti olova. Pod ovim uslovima, kada je u kontaktu sa olovnim cevima, voda može da sadrži i više od 1 mg dm^{-3} olova. Svetska zdravstvena organizacija preporučuje smanjenje maksimalne koncentracije do 0,01 mg dm^{-3} olova [3].

U naučnoj literaturi se mogu naći brojne studije i fizičko–hemijske metode koje se koriste za eliminaciju teških metala iz otpadnih voda [4]. Poslednjih godina, uglavnom su popularni radovi koji se odnose na upotrebu organskog otpada (biomase) kao biosorbenta za uklanjanje jona teških metala (Pb, Cu, Cd, Cr, Zn i Ni) ili njihovih kompleksa [5–7]. Prečišćavanje vode biosorpcijom može se uspešno primeniti za širok spektar zagađujućih materija, u vrlo različitom opsegu koncentracija, koje se drugim postupcima ne mogu ukloniti, ili kada je uklanjanje drugim metodama ekonomski necelishodno. Biosorbenti su od izuzetnog značaja u postupcima prečišćavanja vode zbog njihove niske cene, dobrih performansi i dostupnosti u velikim količinama. Ovi materijali, kao jeftini i efikasni sorbenti, predstavljaju dobro rešenje sa ekološkog aspekta, naročito za uklanjanje jona teških metala iz kontaminiranih voda. Biosorbenti imaju sposobnost da selektivno koncentrišu ciljni sorbat (molekule, atome, jone ili čestice) na svojoj površini [8]. Postojanje ovog fenomena je zabeleženo

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kod mnogih biljnih materijala, koji predstavljaju lako dotupne vrste biomasa, a kojih u prirodi ima u izobilju. Dobre sorpcione karakteristike pokazali su naročito prirodni materijali koji sadrže celulozu, kao što su: drvenasti delovi biljaka (piljevina i šećerna trska) [9,10], kore biljnih plodova (tikva, narandža i banana) [7,11], ljuske (lešnik, orah, badem, pistaći, kikiriki i pirinač) [6,12–14], koštice (kajsija, masline, breskva i šljiva) [6,15–17], list paprata [18], pšenična slama [19] i drugi. Brojna istraživanja su pokazala da se kao biosorbenti mogu koristiti i morske alge, mikroorganizmi (bakterije, gljive, kvasci), aktivni mulj i drugo [20,21]. Osim toga, prirodne biomase su veoma pogodna sirovina i za proizvodnju aktivnog uglja, koji se pokazao izuzetno dobrim sorbentom organskih polutanata i njihovih kompleksa [6,10,22].

Međutim, i pored brojnih istraživanja u ovoj oblasti, mehanizam vezivanja i koncentrisanja teških metala pomoću sorbenta prirodnog porekla još uvek nije u potpunosti razjašnjen. Uklanjanje teških metala se može posmatrati kroz proces jonske izmene zahvaljujući prisustvu funkcionalnih grupa, kao što su: karboksilne, fenil i hidroksilne grupe [5–8]. Pored toga, moguće su i reakcije kompleksiranja, a od posebnog značaja su hemisorpcija, fizička adsorpcija, mikro precipitacija, kao i redoks reakcije [23]. Generalno, lignin i celuloza su glavni konstituenti biomase. Druge komponente su hemiceluloza, lipidi, proteini, šećeri, tj. jedinjenja koja sadrže različite funkcionalne grupe (poput karboksilne, karbonilne, fenolne, hidroksilne, amido, amino, sulfohidrilne, acetamido grupe), koje pokazuju različiti afinitet prema uklanjanju metala [24]. Učešće ovih grupa u hemijskim reakcijama je odgovorno za kapacitet razmene katjona kod upotrebe biomase agro-otpadnog materijala. Za tumačenje mehanizma vezivanja jona teških metala od posebne važnosti su informacije koje se odnose na: oblik metala u rastvoru, njegovu koncentraciju i uslove procesa sredine. Sorpciona svojstva biomase su takođe važna, naročito ukoliko postoji mogućnost modifikacije njene površine fizičkim ili hemijskim metodama [7,25].

Mogućnost primena ljuske oraha ili aktivnog uglja na bazi ljuske oraha kao biosorbenata teških metala (Cu, Cr, Pb, Cd, Cs i Mn) [12,22,26–29], fosfora [30], organske boje Rodamin B [31], kiselih boja [32] ili ulja [33] iz industrijskih otpadnih voda ili vodenih rastvora je široko opisana u literaturi. Međutim, u literaturi nisu poznati podaci o ispitivanju biosorbenta na bazi biomase mladog, nedozrelog celog ploda oraha.

Poznato je da nedozreli plod oraha *Juglans regia* L. sadrži flavonoide, karotenoide, hlorofil, vitamine, mineralne soli i fenolna jedinjenja [34]. Pomoću RP-HPLC metode, identifikovano je više jedinjenja (galna, hlorogenska, kafeinska, protokatehinska, elaginska, sinapinska, ferulna, vanilinska, kumarinska i siringinska kiselina, miricetin, katehin, epikatehin, regiolon, juglon, 1,4-naftohinon), kao i elagitanin pedunkulagin [35,36]. Njihov sadržaj zavisi od uslova životne sredine, kao i genotipa različitih sorti [36]. Zbog lekovitih svojstava ovih materija, nedozreli plodovi oraha se u narodnoj medicini tradicionalno koristite za proizvodnju raznih ekstrakata [37,38]. Posebno je cenjen lekoviti liker, kako za unutrašnju, tako i za spoljašnju primenu. Efikasnost izolovanja fenolnih jedinjenja zavisi od rastvarača i primenjene metode. Kvalitet likera je već ispitivan i naučno potvrđen [39]. Nakon izdvajanja lekovitih materija određenim tretmanom nedozrelog ploda oraha (Slika 1), kao otpad zaostaje lignocelulozna biomasa koju čini celuloza (38–42%), hemiceluloza (22–26%) i lignin (18–21%) [34,36]. Ova tri jedinjenja obiluju uglavnom hidroksilnim grupama koje su odgovorne za uklanjanje metala iz rastvora [40]. Otpadna biomasa može biti interesantna sa aspekta iskorišćenja kao sekundarne sirovine u svojstvu biosorbenta za teške matale ili druge organske zagađujuće materije.

Zato je predmet ovog rada ispitivanje mogućnosti primene otpadne biomase mladog ploda oraha (MPO) *Juglans regia* L. iz procesa proizvodnje likera, kao biosorbenta teških metala iz voda kontaminiranih olovom. Cilj istraživanja je utvrđivanje i poređenje biosorpcionih svojstava usitnjenog MPO za Pb(II) jone iz vodenog rastvora, u stacionarnim uslovima. U tom smislu, u radu je vršeno ispitivanje i definisanje uticaja odabranih faktora na proces biosorpcije jona, kao što su: koncentracija biosorbenta i sorbata, pH rastvora i temperatura biosorpcije. Eksperimenti su izvođeni pod različitim uslovima u cilju optimizacije efikasnosti procesa biosorpcije. Za bolje razumevanje procesa biosorpcije vršena su ravnotežna i kinetička ispitivanja.

Slika 1. Postupak dobijanja sorbenta na bazi biomase ploda oraha (maceracija 30 dana na sobnoj temperaturi i izdvajanje likera).
Figure 1. The process of obtaining sorbent based on fruit walnuts biomass (maceration 30 days at room temperature, and liquors extraction).

EKSPERIMENTALNI DEO

Priprema i karakterizacija biosorbenta

Kao biosorbent u ovim ispitivanjima korišćen je mladi plod oraha *Juglans regia* L., dobijen kao otpadni materijal iz procesa proizvodnje orahovog likera. Materijal, ispiran više puta dejonizovanom vodom i osušen na 50±0,5 °C do konstantne mase, usitnjen je na komade veličine 2 do 3 cm, a potom samleven čeličnim laboratorijskim blenderom i prosejan kroz standardna čelična sita veličine 0,5–0,8 mm. Pre biosorpcije, zrna su tretirana azotnom kiselinom (0,001 mol dm^{-3}) radi uklanjanja eventualnih nečistoća, a zatim ispirana redestilovanom vodom do neutralne reakcije na nitrate i sušena na temperaturi od 50±0,5 °C do konstantne mase. Karakterizacija pripremljene i osušene biomase MPO pre i posle biosorpcije vršena je IR spektroskopskom analizom (FTIR spejtroskop Bomem MB-100, Canada) sa ciljem utvrđivanja prisutnih funkcionalnih grupa koje mogu imati aktivnu ulogu u procesu biosorpcije. Za pripremanje uzorka primenjena je KBr tehnika (0,5 mg biosorbenta u 150 mg KBr). IR spektri su snimani u oblasti talasnih brojeva 4000–400 cm^{-1}, pri rezoluciji 0,2 cm^{-1}. Za obradu IR spektara korišćen je WIN Bomem Easy softver.

Biosorpcioni eksperimenti

Biosorpcioni eksperimenti su izvođeni u staklenim posudama (100 cm^3) na magnetnoj mešalici, sa odgovarajućom masom biosorbenta (2,0 do 20,0±0,05 g) i rastvorima sorbata početne koncentracije 10,0 do 120,0 mg dm^{-3}. Polazni rastvor olova (1000 mg dm^{-3}) je pripremljen rastvaranjem odgovarajuće količine $Pb(NO_3)_2$ (Merck) u dejonizovanoj vodi, a rastvori željene koncentracije su dobijani odgovarajućim razblaženjem. Ispitivanje uticaja pH vrednosti rastvora na proces biosorpcije jona vršeno je u opsegu pH 2,0 do 6,0 pri konstantnoj jonskoj jačini 0,02 mol dm^{-3}. Vrednost pH je podešavana rastvorima HNO_3 (0,02 mol dm^{-3}) i KOH (0,02 mol dm^{-3}), a jonska jačina kontrolisana rastvorom KNO_3 (0,04 mol dm^{-3}). Momenat dodavanja biosorbenta u pripremljeni rastvor Pb(II) jona predstavljao je početak tretmana. Sadržaj je kontinuirano mešan magnetnom mešalicom (120 rpm) pri konstantnoj temperaturi od 25,0±0,5 °C.

Efekat temperature i kinetika biosorpcije Pb(II) jona na biomasi MPO analizirani su u temperaturnom opsegu od 25,0 do 40,0±0,5 °C, pri pH vrednosti 4,5±0,1, početnoj koncentraciji Pb(II) jona od 15 mg dm^{-3} i koncentraciji biomase MPO od 6,0 g dm^{-3} sorbata, u toku 60 min.

Analiza sadržaja jona i ravnotežna ispitivanja

U određenim vremenskim intervalima (0 do 120 min), iz reakcionog suda su uzimani uzorci za analizu sadržaja rezidualnih jona i filtrirani pomoću 0,45 μm membranskog filtera (Agilent, USA) radi uklanjanja čestica biomase MPO. Sadržaj jona olova u rastvorima pre, tokom i posle sorpcije određivan je na emisionom spektrofotometru sa indukovanom argonskom plazmom ICP-OES Analyzer (Arcos Spectro), na 261,42 nm. Za svaki ispitivani uzorak su izvršena po 3 merenja. Količina sorbovanih Pb(II) jona određivana je iz formule:

$$Q = \frac{V\left(c_{0-}c_{\mathrm{k}}\right)}{m} \quad (1)$$

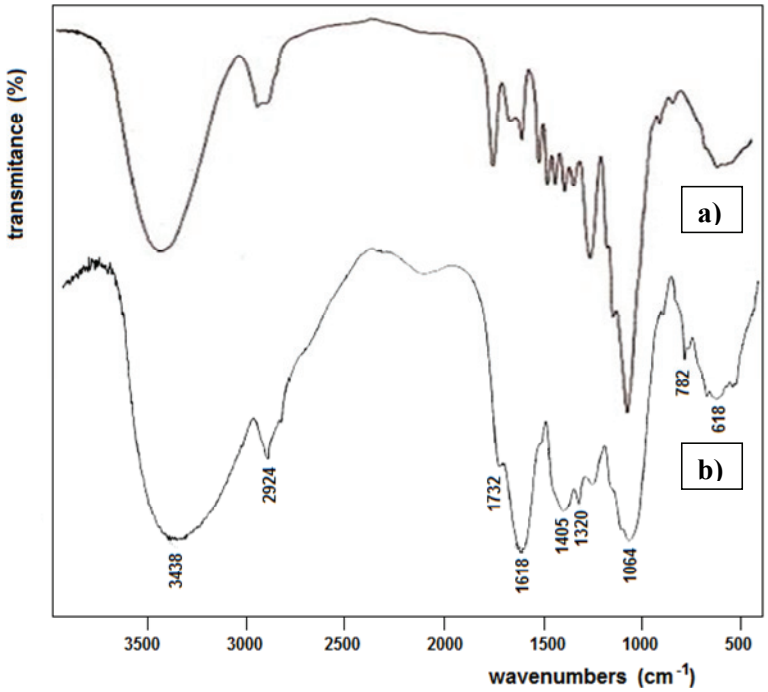
gde je: Q (mg g^{-1}) – količina metalnih jona po gramu biosorbenta, V (dm^3) – zapremina rastvora, c_0 i c_k (mg dm^{-3}) – početna i finalna koncentracija jona, m (g) – masa suvog biosorbenta. Eksperimentalno dobijeni rezultati biosorpcije Pb(II) jona na ispitivanoj biomasi MPO su upoređivani sa odgovarajućim teorijskim modelima Langmuir, Freundlich i Temkin izoterme, koji se uobičajeno koriste za opisivanje sorpcione ravnoteže.

REZULTATI I DISKUSIJA

FTIR karakterizacija biosorbenta

Spektroskopska FTIR analiza može dati informacije o hemijskom sastavu korišćene biomase MPO i ukazati na eventualne razlike ispitivane biomase u poređenju sa netretiranom zelenom korom oraha. Iz tog razloga, na slici 2 su prikazani FTIR spektri zelene kore oraha i biomase MPO, koja je korišćena kao biosorbent za jone olova. Poređenjem datih spektara može se uočiti velika sličnost, koja ukazuje na istu lignoceluloznu biljnu materiju. Naime, u IR spektru zelene kore oraha (slika 2a) se mogu uočiti karakteristične trake na oko 3440 i 1425 cm^{-1} koje potiču od vibracija O–H i N–H veza i ukazuju na prisustvo ovih grupa na površini ispitivanog materijala. IR traka na 1744 cm^{-1} potvrđuje prisustvo C=O grupe, dok trake u oblasti 1620–1430 cm^{-1} ukazuju na prisustvo aromatičnih veza. Spektar pokazuje traku na oko 1580 cm^{-1} zbog valentine C=C vibracije aromatičnog prstena, koja je pomerena usled interakcije sa polarnim grupama. Pik na 1662 cm^{-1} odgovara COO ili N–H vibracijama. Traka na 1380 cm^{-1} potiče od vibracije karboksilatne COO^- grupe. Trake u oblasti 1300–1000 cm^{-1} se mogu asignirati generalno kao C–O valentine vibracije karboksilnih kiselina i alkohola. Traka na oko 1240 cm^{-1}, kao i njen partner u deformacionoj oblasti na 559 cm^{-1}, potiče od vibracije S=O veze. Prisustvo navedenih funkcionalnih grupa na površini biosorbenta potvrđuju i drugi literaturni podaci [40–43].

Izvesne spektroskopske razlike, u smislu pomeranja traka ili promene u intenzitetu traka, usled uklanjanja nekih flavonoida i fenolnih kiselina iz kore oraha tokom tretmana u procesu proizvodnje likera, mogu se uočiti u IR spektru biomase MPO na slici 2b. U slučaju IR spektra korišćene biomase MPO kao biosorbenta, može



Slika 2. FTIR spektri zelene ljuske oraha (a) i biomase MPO (b).
Figure 2. FTIR spectra of green walnuts crust (a) and biomass YFW (b).

se uočiti vrlo široka traka u opsegu 3500–3000 cm^{-1} koja se odnosi na valentnu O–H vibraciju hidroksilne grupe biosorbenta i adsorbovane vode sa jedne strane [5,6], kao i na preklopljenu traku od N–H valentne vibracije [41]. Položaj, oblik i asimetrija ove kompleksna IR trake u nižoj oblasti talasnih brojeva ukazuje na prisustvo jakih vodoničnih veza. Prisustvo kristalohidrata potvrđuju IR trake u deformacionim oblastima spektra na oko 1620 (u ravni) i 620 cm^{-1} (van ravni). Pikovi u valentnoj oblasti spektra na 2924 i 2870 cm^{-1}, kao i njihovi partneri u deformacionim oblastima na oko 1400 i 900 cm^{-1}, mogu se pripisati C–H vibracijama celulozne mase. Apsorpciona traka slabijeg intenziteta sa maksimumom na 1732 cm^{-1} se može pripisati C=O valentnoj vibraciji karbonilne grupe biosorbenta, koja potvrđuje prisustvo lignoceluloznog biljnog materijala u strukturi MPO nakon tretmana. Traka slabog intenziteta na 1243 cm^{-1} potiče od vibracije $–SO_3$ grupe, a složena traka na 1064 cm^{-1} odgovara valentnoj C–O vibraciji kiselina, alkohola, fenola ili estra [40–43].

FTIR spektroskopska analiza biomase MPO pre i posle biosorpcije Pb(II) jona prezentovana je u Tabeli 1. Rezultati analize ukazuju na činjenicu da se na ćelijskom zidu površine biomase MPO nalaze različite funkcionalne grupe (O–H alkoholna, C–O alkoholna, fenolna ili estarska, C=O lignocelulozna, C–H i SO_3 grupa) koje imaju mogućnost da učestvuju u procesu biosorpcije Pb(II) jona iz vodenog rastvora.

Analiza efekta procesa biosorpcije

Uticaj koncentracije biosorbenta

Efekat koncentracije biomase MPO na proces biosorpcije Pb(II) jona iz vodenog rastvora prikazan je na

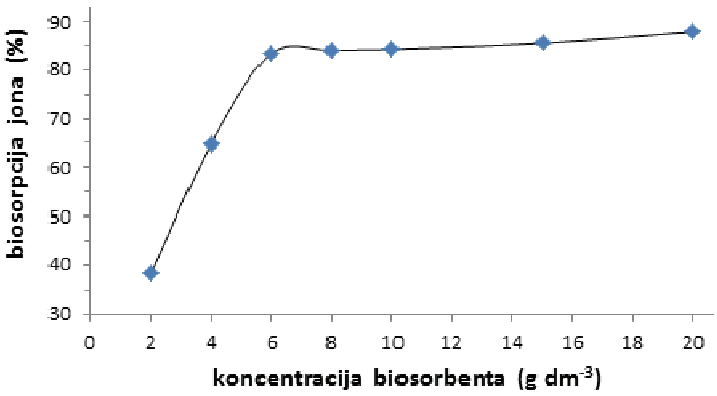
Tabela 1. FTIR spektralne karakteristike biomase MPO pre i posle biosorpcije Pb(II) jona
Table 1. The FTIR spectral characteristics of biomass YFW before and after biosorption of Pb(II) ions

IR oblast (cm^{-1})	Položaj IR trake, cm^{-1}			Poreklo
	Pre sorpcije	Posle sorpcije	Pomeranje	
3500–3000	3438	3392	–46	Valentna O–H
2900–2800	2924	2915	–9	Valentna C–H
1740–1680	1732	1725	–7	Valentna C=O lignin
1640–1580	1618	1620	+2	Deformaciona H_2O
1500–1400	1436	1420	–16	Valentna COO^- i deformaciona NH
1270–1240	1248	1243	–5	Valentna SO_3
1150–970	1064	1036	–28	Valentna C–O
650–480	618	627	+9	Deformaciona O–H

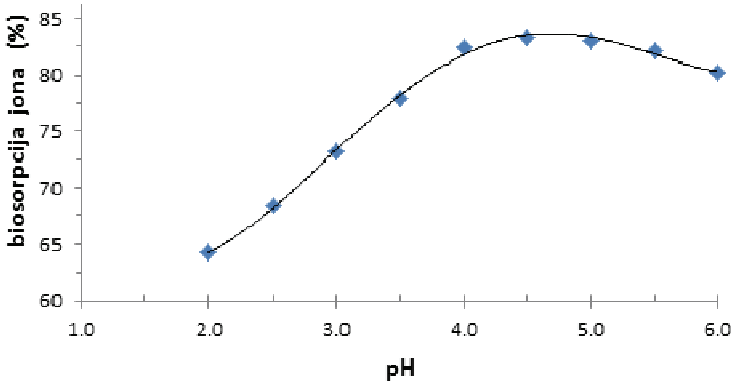
slici 3. Na osnovu preliminarnih ispitivanja, za eksperiment su odabrani sledeći parametri biosorpcije: početna koncentracija Pb(II) jona od 15 mg dm^{-3}, pH vrednost rastvora 4,5±0,1 i jonska jačina 0,02 mol dm^{-3}, temperatura od 25,0±0,5 °C, veličina čestica biosorbenta 0,5–0,8 mm. Ispitivanje efekta biosorpcije vršeno je variranjem koncentracije biomase MPO u opsegu 2,0 do 20,0 g dm^{-3}, pri kontaktnom vremenu od 50 min. Kriva zavisnosti efekta biosorpcije (Slika 3) pokazuje da se stepen uklanjanja Pb(II) jona iz vodenih rastvora povećava sa porastom koncentracije biosorbenta, pri čemu se ravnotežna biosorpcija Pb(II) jona od 83,34± ±0,25% postiže pri koncentraciji biosorbenta od 6,0 g dm^{-3} sorbata i koncentraciji sorbata od 15 mg dm^{-3}. Dobijeni rezultati pokazuju da povećanje koncentracije biosorbenta od 2,0 do 6,0 g dm^{-3} sorbata znatno povećava efikasnost uklanjanja Pb(II) jona iz rastvora (40–80 %). Dalje povećanje koncentracije biosorbenta u rastvoru preko 8,0 g dm^{-3} ne utiče značajno na efikasnost biosorpcije jona. Početno povećanje kapaciteta biosorpcije može se pripisati povećanju površine biosorbenta i boljoj pristupačnosti jona ka sorpcionim centrima. Blagi porast efikasnosti uklanjanja jona (84–88%) pri koncentracijama biosorbenta većim od 6,0 g dm^{-3} može se objasniti prisustvom većeg broja aktivnih centara na površini biosorbenta dostupnih za vezivanje jona metala, u pogledu iste količine Pb(II) jona pri primenjenoj početnoj koncentraciji od 15 mg dm^{-3}. Sa druge strane, pri većim koncentracijama biosorbenta verovatno postoji mogućnost agregacije čestica biosorbenta u rastvoru, te na taj način može biti ograničen pristup jona metala ka aktivnim centrima (funkcionalnim grupama) u masi biosorbenta. Zbog toga je za dalje biosorpcione eksperimente, kao optimalna koncentracija biosorbenta, odabrana koncentracija biomase MPO od 6,0 g dm^{-3} sorbata za početnu koncentraciju Pb(II) jona od 15 mg dm^{-3}. Ova zapažanja su u saglasnosti sa drugim ispitivanjima poznatim u literaturi za biosorpciju različitih metalnih jona (Cu, Cr, Pb, Cd, Cs i Mn) pomoću ljuske oraha i sličnim biološkim materijalima [6,12,22,26–29]. U poređenju sa literaturnim podacima, u slučaju biosorpcije Pb(II) jona ljuskom oraha kao biosorbentom, može se uočiti da su dobijeni slični rezultati, pri čemu je maksimalno iscrpljivanje Pb(II) jona (oko 80%) postignuto je pri koncentraciji biosorbenta od 5 g dm^{-3} sorbata [17].

Uticaj pH rastvora

Jedan od najvažnijih faktora koji kontroliše uklanjanje jona teških metala iz vodenih rastvora je pH vrednost rastvora, koja je značajna i sa aspekta utvrđivanja kvaliteta i koncentracije jonskog oblika u rastvoru. Naime, metali u jako kiselom rastvoru postoje uglavnom kao katjoni, pa će postepeno povećanje pH vrednosti dovesti do formiranja kompleksnih jona i taloženja metalnih jona u obliku hidroksida. Imajući u vidu da koeficijent rastvorljivosti $Pb(OH)_2$ iznosi 20 [44], može se konstatovati da će u ispitivanom rastvoru, pri početnoj koncentraciji Pb(II) jona od 15,0 mg dm^{-3}, doći do potpunog taloženja $Pb(OH)_2$ pri pH > 6,0. Zato je uticaj ovog faktora na biosorpciju Pb(II) jona ispitivan u kiselim rastvorima, u opsegu pH 2,0–6,0 (±0,1). Na osnovu preliminarnih ispitivanja, za eksperiment su odabrani sledeći parametri biosorpcije: početna koncentracija Pb(II) jona 15,0 mg dm^{-3}, koncentracija biosorbenta 6,0 g dm^{-3} sorbata, veličina čestica biosorbenta 0,5–0,8 mm, jonska jačina 0,02 mol dm^{-3}, temperatura 25,0±0,5 °C, vreme biosorpcije 50 min. Rezultati ispitivanja uticaja pH na proces biosorpcije Pb(II) jona prikazani su na slici 4. Kao što se može videti, dobijeni rezultati potvrđuju uticaj pH rastvora na efikasnost uklanjanja Pb(II) jona ispitivanim biosorbentom. U ispitivanom opsegu pH vrednosti od 2,0 do 4,0 može se uočiti povećanje stepena biosorpcije (60–80%). Biosorpcioni maksimum od 84% postiže se pri pH vrednosti 4,5±0,1. Sa daljim povećanjem pH vrednosti iznad 5,0 dolazi do smanjenja biosorpcije Pb(II) jona. Manji stepen biosorpcije jona u jako kiselim (pH < 4,0), ili alkal-



Slika 3. Uticaj koncentracije biomase MPO na biosorpciju Pb(II) jona (pH 4,5±0,1, T = 25,0±0,5 °C, vreme kontakta: 50 min, c_0 Pb(II) = 15 mg dm^{-3}).
Figure 3. Effects of biomass YFW concentration on Pb(II) biosorption (pH 4.5±0.1, T = 25.0±0.5 °C, contact time: 50 min, c_0 Pb(II) = 15 mg dm^{-3}).



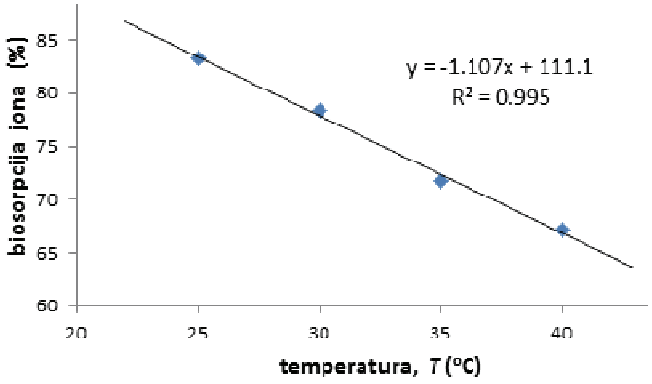
Slika 4. Efekat pH vrednosti na biosorpciju Pb(II) jona iz vodenog rastvora (T = 25,0±0,5 °C, vreme kontakta: 50 min, c_0 Pb(II) = 15 mg dm^{-3}, koncentracija biosorbenta 6,0 g dm^{-3}).
Figure 4. Effects of pH on Pb(II) biosorption from water solution (T = 25.0±0.5 °C, contact time: 50 min, c_0 Pb(II) = 15 mg dm^{-3}, biosorbent concentration 6 g dm^{-3}).

nim rastvorima, verovatno je povezan sa različitim naelektrisanjima na površini biosorbenta. Poznato je da vrsta i veličina naelektrisanja zavise od pH vrednosti i funkcionalnih grupa (aktivnih centara sorpcije) na površini sorbenta [16]. Prisustvo funkcionalnih grupa je, u ovom slučaju, potvrđeno FTIR analizom.

Naime, kao što je poznato [16], uklanjanje katjona, u ovom slučaju Pb(II) jona, favorizovano je iznad vrednost nultog naelektrisanja (pHpzc). Pri pH vrednostima ispod pH_{pzc} površina sorbenta je pozitivno naelektrisana, dok pri pH većim od pHpzc površina sorbenta ima negativno naelektrisanje. Pozitivno naelektrisanje na površini sorbenta pri nižim pH može se objasniti prisustvom alkalnih funkcionalnih grupa i prekomernom površinskom protonacijom [16]. Ovakva pozitivno naelektrisana barijera na površini sorbenta ograničava pristup metalnim katjonima ka površini sorbenta. Pored toga, istovremeno sa smanjenjem pH vrednosti, povećava se i konkurencija između jona vodonika i metalnih katjona za aktivna mesta na površini sorbenta. Efekat ove pojave je smanjenje kapaciteta sorpcije jona. Sa druge strane, pri višim pH površina sorbenta ima negativno naelektrisanje kao rezultat jonizacije kiselih grupa (uglavnom hidroksilne). Tada se javlja elektrostatičko privlačenje između jona metala i površine sorbenta, čime se povećava i stepen sorpcije jona. Ovaj fenomen se može uočiti i na posmatranom primeru biosorpcije jona Pb(II) u ispitivanom opsegu pH vrednosti (slika 4). Kada pH vrednost raste, rastvor sadrži manje vodonikovih jona koji konkurišu ispitivanim Pb(II) jonima u procesu biosorpcije na biomasi MPO, čime se i povećava efikasnost biosorpcije.

Uticaj temperature na biosorpciju

Sa ciljem utvrđivanja uticaja temperature na biosorpciju Pb(II) jona vršena su ispitivanja u temperaturnom opsegu od 25,0±0,5 do 40±0,5 °C, pri istim uslovima biosorpcije kao u prethodnim eksperimentima. Za otpadnu biomasu MPO kao biosorbenta ispitivanih jona konstatovano je smanjenje stepena biosorpcije (84–67%) sa povećanjem temperature u navedenom opsegu (slika 5). Dobijeni rezultati ispitivanja potvrđuju egzotermnu prirodu biosorpcije Pb(II) jona na MPO. Smanjenje biosorpcionih svojstava biosorbenta u ispitivanom temperaturnom opsegu može biti



Slika 5. Efekat temperature na biosorpciju Pb(II) jona iz vodenog rastvora (pH 4,5±0,1, vreme kontakta: 50 min, c_0 Pb(II) = 15 mg dm^{-3}, koncentracija biosorbenta 6,0 g dm^{-3}).
Figure 5. Effects of temperature on Pb(II) biosorption from water solution (pH 4.5±0.1, contact time: 50 min, c_0 Pb(II) = 15 mg dm^{-3}, biosorbent concentration 6 g dm^{-3}).

posledica narušene površine biosorbenta pa time i aktivnih mesta, ili posledica pomeranja ravnoteže procesa u smeru desorpcije metalnih jona od površine biosorbenta ka rastvoru [27].

Kinetičke studije procesa biosorpcije

Kinetičke studije procesa sorpcije određuju brzinu kojom se zagađujuće materije uklanjaju iz vodene sredine. U tom smislu, predloženi su brojni kinetički modeli kojima se može opisati mehanizam odvijanja procesa sorpcije. Ovaj mehanizam je, u većini slučajeva, kompleksan i može biti razlog hemijske reakcije između funkcionalnih grupa sorbenta i metalnih jona, jonske izmene ili formiranja kompleksa. Pored toga, moraju se uzeti u obzir i procesi transfera mase, poput transporta materije u tečnoj fazi, difuzije iz tečne faze na površinu čvrste, kao i difuzije u unutrašnjosti makropora i mikropora [45].

Biosorbent–sorbat kontaktno vreme

Biosorpciona kinetika je ispitivana u cilju boljeg razumevanja dinamike biosorpcije Pb(II) jona na biomasi MPO kao biosorbentu, što omogućava procenu efikasnosti biosorpcije tokom vremena kontakta i pruža informacije o mehanizmu biosorpcije. Ova informacija je posebno važna za scale-up većih sistema [45]. Uticaj kontaktnog vremena je ispitivan na rastvoru početne koncentracije Pb(II) jona od 15 mg dm^{-3}, tretiran sa 6,0 g biosorbenta, pri pH 4,5 i temperaturi 25,0±0,5 °C. Uzorci za analizu su uzimani tokom vremena u opsegu 2 do 120 min, kako bi se utvrdila rezidualna koncentracija metalnih jona. Rezultati analize su prikazani na slici 6.

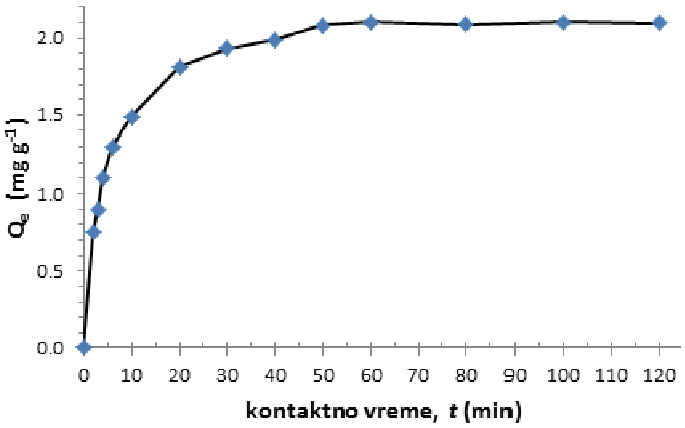
Može se uočiti da se biosorpcija jona olova, kao funkcija kontaktnog vremena, odvija u dve faze. Prva faza uključuje brže vezivanje metalnih jona na biosorbentu tokom prvih 30 min biosorbent-sorbat kontakta, a potom sledi sporija faza uklanjanja jona iz rastvora do postizanja ravnoteže. Biosorpcija metalnih jona tokom prvog perioda kontakta iznosi oko 77%, dok je za postizanje ravnoteže potrebno 50 min, pri čemu je sorbovano oko 84% jona olova. Ovakav primer biosorpcije jona olova, kao i drugih metalnih jona, gde je prva faza brža, karakterističan je za brojne biosorbente opisane u literaturi [6,7,12,26–29]. Bržu fazu karakteriše verovatno veći broj aktivnih centara na biosorbentu, dok sa postepenim zauzimanjem ovih mesta biosorpcija postaje manje efikasna u sporijoj fazi. Iz praktičnih razloga, za primenu biosorpcije u realnim i većim sistemima tokom prečišćavanja otpadnih voda od jona olova, preporučuje se kontaktno vreme od 30 min. Za dalju analizu u radu, obzirom na ravnotežni proces biosorpcije, kao optimalna vrednost odabrano je vreme kontakta biosorbent-sorbat od 50 min.

Model pseudo-prvog reda

Kinetički model pseudo-prvog reda opisuje brzinu sorpcije koja je proporcionalna broju nezauzetih mesta vezivanja na sorbentu, a može biti izražen na osnovu Lagergren-ove jednačine [46], čiji je linearni oblik:

$$ln\,(Q_e - Q_t) = ln\,Q_e - k_1 t \qquad (2)$$

gde je: Q_e (mg g^{-1}) – količina metalnih jona sorbovanih u ravnoteži po g sorbenta; Q_t (mg g^{-1}) – količina metalnih jona sorbovanih u vremenu t (min) po g sorbenta; k_1 (min^{-1}) – konstanta brzine pseudo-prvog reda. Ukupna konstanta brzine k_1 je izračunata iz nagiba krive sa grafika zavisnosti ln (Q_e–Q_t) u odnosu na t, a ravnotežna količina sorbovanih jona, Q_e, je određena iz odsečka krive grafičke zavisnosti $y = -0{,}085x + 0{,}434$. Dobijeni rezultati kinetičkih parametara su sumirani u Tabeli 2. Analizom parametara se može uočiti da koeficijent korelacije dobijen korišćenjem modela pseudo-prvog reda ima nižu vrednost (R^2 = 0,938). Takođe, izračunata Q_e vrednost po ovom modelu nije dala razumnu vrednost, obzir da je suviše niska u poređenju sa ekspe-



Slika 6. Uticaj kontaktnog vremena na efekat biosorpcije Pb(II) jona na biomasi MPO (Q_e – količina (mg g^{-1}) sorbovanog jona, c_0 Pb(II) jona 15 mg dm^{-3}, koncentracija biosorbenta 6 g dm^{-3}, T = 25,0±0,5 °C, pH 4,5).
Figure 6. The effect of contact time on Pb(II) biosorption onto biomass YFW (Q_e – the amount (mg g^{-1}) of the sorbed ions, c_0 Pb(II) = 15 mg dm^{-3}, biosorbent concentration 6 g dm^{-3}, T = 25.0±0.5 °C, pH 4.5).

Tabela 2. Kinetički modeli i parametri biosorpcije Pb(II) jona na biomasi MPO
Table 2. Kinetic models and parameters of Pb(II) ions biosorption by the biomass YFW

Pseudo-prvi red (y = –0,085x + 0,434)				
Q_{exp} / mg g^{-1}	Q_e / mg g^{-1}	dQ / %	k_1 / min^{-1}	R^2
2,09	1,54	26,3	0,085	0,938
Pseudo-drugi red (y = 0,447x + 1,981)				
Q_{exp} / mg g^{-1}	Q_e / mg g^{-1}	dQ / %	k_2 / g mg^{-1} min^{-1}	R^2
2,09	2,23	6,69	0,102	0,999

rimentalnim (Q_{exp}) rezultatom. Očigledno je da se kinetika biosorpcije Pb(II) jona na biomasi MPO ne podudara sa modelom pseudo-prvog reda, verovatno zbog ograničenja površinskog sloja koji kontroliše proces biosorpcije. Dobijeni rezultati su u skladu sa drugim studijama biosorpcije Pb(II) jona na slične biosorbente [27,47], koje takođe ukazuju na loše slaganje sa modelom pseudo-prvog reda.

Model pseudo-drugog reda

Imajući u vidu da model pseudo-prvog reda može pogodno opisati kinetiku sorpcije samo kada se ona veoma brzo odvija, razvijen je model pseudo-drugog reda [48], koji može opisati ceo sorpcioni period i može se primeniti na većinu biosorbenata. Model pseudo-drugog reda podrazumeva da je brzina sorpcije jona na aktivnim mestima sorbenta proporcionalna kvadratu broja slobodnih i zauzetih mesta. Linearni oblik kinetičkog modela pseudo-drugog reda predstavljen je jednačinom:

$$\frac{t}{Qt} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e} t \qquad (3)$$

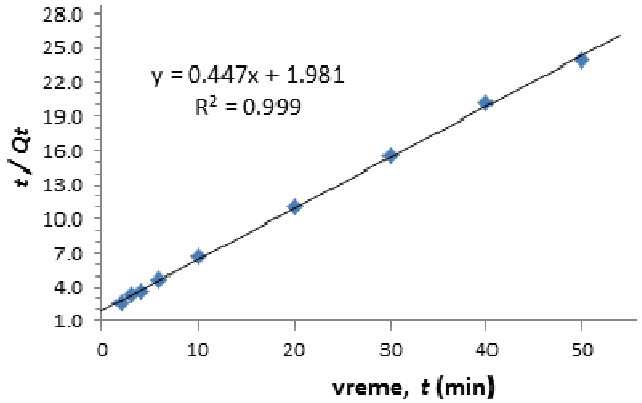
gde je: Q_e (mg g^{-1}) – količina metalnih jona sorbovanih u ravnoteži po g sorbenta; Q_t (mg g^{-1}) – količina metalnih jona sorbovanih u vremenu t (min) po g sorbenta; k_2 (g mg^{-1} min^{-1}) – konstanta brzine pseudo-drugog reda. Iz nagiba linearne zavisnosti t/Q_t u odnosu na t (slika 7) može se odrediti ravnotežno Q_e, a konstanta brzine k_2 iz vrednosti odsečka sa istog grafika. Dobijeni rezultati kinetičkih parametara su sumirani u tabeli 2.

Rezultati ispitivanja biosorpcije Pb(II) jona pokazuju da model pseudo-drugog reda veoma dobro fituje eksperimentalne podatke. Kao što se može videti, korelacioni koeficijent za linearnu zavisnost po ovom modelu je veoma visok (R^2 = 0,999) za kontaktno vreme od 60 min i znatno bolji nego u prethodnom slučaju. Osim toga, teorijska Q_e vrednost predviđena iz modela drugog reda je veoma približna eksperimentalnoj (Q_{exp}) vrednosti. Ova zapažanja ukazuju na to da biosorpcija Pb(II) jona na biomasi MPO prati drugi red reakcije, sugerišući da je proces vezivanja katjona metala verovatno kontrolisan hemijskom reakcijom. Činjenica je da je model pseudo-drugog reda već uspešno primenjen za opisivanje biosorpcije i drugih teških metala na sličnim biosorbentima [9,18,27,47]. Navedena ispitivanja ukazuju da je hemijska reakcija značajni korak koji kontroliše brzinu, a da kinetika reakcije pseudo-drugog reda obezbeđuje najbolju korelaciju eksperimentalnih podataka. Sa druge strane, predloženi model pseudo-prvog reda dobro fituje eksperimentalne podatke samo za početni period reakcije.

Ravnotežna ispitivanja procesa biosorpcije

Uticaj početne koncentracije sorbata

Početna koncentracija metalnih jona obezbeđuje važnu pokretačku silu za savladavanje otpora prenosa mase metalnih jona između vodene i čvrste faze. Efekat



Slika 7. Kinetički model pseudo-drugog reda biosorpcije Pb(II) jona na biomasi MPO.
Figure 7. Pseudo-second order biosorption kinetics of Pb(II) onto biomass YFW.

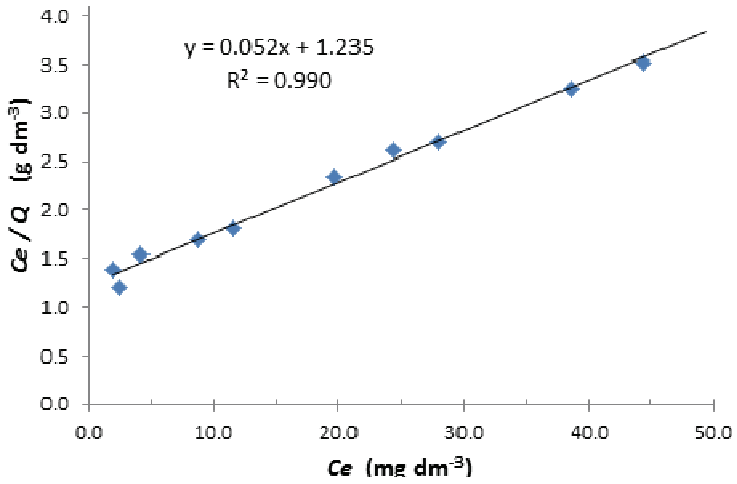
početnih koncentracija Pb(II) jona ispitivan je u opsegu od 10 do 120 mg dm^{-3}, na 25±0,5 °C i pH 4,5 u toku 50 min. Rezultati ispitivanja su analogni rezultatima prikazanim na slici 8. Dobijeni rezultati pokazuju da se apsolutni iznos sorbovanih jona metala povećava sa porastom početne koncentracije Pb(II) jona u rastvoru. Karakteristično je da biosorpcionu ravnotežu na 25±0,5 °C vrlo brzo postižu rastvori koji sadrže manju koncentraciju Pb(II) jona. Ovo se verovatno dešava zbog toga što se joni metala sorbuju prvo na neometanim lokacijama biosorbenta. U slučaju koncentrovanijih rastvora, joni metala u početku popunjavaju najpre ove neometane lokacije biosorbenta, a potom i prikrivena mesta na biosorbentu. Ovakvu konstataciju potvrđuje činjenica da se vreme potrebno za biosorpcionu ravnotežu povećava sa početnom koncentracijom metalnih jona. Efekat početne koncentracije jona metala može se objasniti povećanjem broja istih jona koji su konkurenti za stalno dostupna aktivna mesta u biomasi, kao i nedostatkom mesta za vezivanje metalnih jona pri višim početnim koncentracionim nivoima. Takođe, zbog zauzetosti površinskih mesta vezivanja, joni metala treba da difunduju unutar pora biomase. Međutim, činjenica je da biosorpcioni kapacitet biomase MPO za Pb(II) jone raste sa porastom početne koncentracije Pb(II) jona, tako da biosorbent postaje zasićen sa maksimalnom biosorpcijom jona od oko 12 mg g^{-1}, pri rezidualnoj koncentraciji jona od 40 mg dm^{-3}, što odgovara početnoj koncentraciji Pb(II) jona od 110 mg dm^{-3}.

Sorpcione izoterme

Sorpcione izoterme su važni kriterijumi za optimizaciju korišćenog sorbenta jer opisuju odnos između mase adsorbata po masi sorbenta kao funkciju njihove koncentracije u rastvoru, kao i prirodu interakcije između adsorbata i sorbenta [45]. Postoje brojni izrazi koji opisuju sorpcione izoterme. Za ova ispitivanja biosorpcije Pb(II) jona na biomasi MPO odabrane su tri poznate izoterme: Langmuir, Freundlich i Temkin model. Početna koncentracija Pb(II) jona menjana je u opsegu 10 do 120 mg dm^{-3}. Karakteristike ovih modela, kao i vrednosti koeficijenata linearnih jednačina izotermi određenih metodom regresije, prikazani su u tabeli 3. Svi parametri, kao i njihove nesigurnosti, dobijeni su korišćenjem Microsoft Excel programa.

Langmuir adsorpciona izoterma

Langmuir izoterma podrazumeva monoslojnu adsorpciju adsorbata na površini, koja se sastoji od



Slika 8. Langmuir adsorpciona izoterma za biosorpciju Pb(II) jona na biomasi MPO (T = 25,0±0,5 °C, pH 4,5).
Figure 8. Langmuir adsorption isoterm for biosorption of Pb(II) by biomass YFW (T = 25.0±0.5 °C, pH 4.5).

Tabela 3. Karakteristični parametri adsorpcionih izotermi za biosorpciju Pb(II) jona na biomasi MPO na 25,0±0,5 °C i pH 4,5
Table 3. Characteristic parameters of adsorption isotherms for Pb(II) biosorption on biomass YFW at 25.0±0.5 °C and pH 4.5

Langmuir (y = 0,052x + 1,235)			
Q_{exp} / mg g^{-1}	Q_{max} / mg g^{-1}	K_L / dm^3 mg^{-1}	R^2
12,68	19,23	0,042	0,990
Freundlich (y = 0,684x + 0,046)			
n	K_F / mg g^{-1}	b_F (1/n)	R^2
1,462	1,047	0,684	0,985
Temkin (y = 3,580x – 1,754)			
b	B / J mol^{-1}	K_t / dm^3 g^{-1}	R^2
692,1	3,580	0,613	0,974

konačnog broja identičnih mesta homogene adsorpcione energije, gde nema interakcije između sorbovanih molekula na susednim lokacijama. Ne postoji dalja adsorbcija koja se može odvijati na tom mestu, pa je adsorpciona energija konstantna i ne zavisi od stepena zauzetosti aktivnih centara sorbenta. Model Langmuir sorpcije se može izraziti u linearnoj formi jednačinom:

$$\frac{c_e}{Q_e} = \frac{1}{Q_{\max} K_L} + \frac{1}{Q_{\max}} c_e \quad (4)$$

gde je: Q_e (mg g^{-1}) – količina sorbovanih jona metala u ravnoteži po gramu sorbenta; c_e (mg dm^{-3}) – ravnotežna koncentracija Pb(II) jona u rastvoru; Q_{max} (mg g^{-1}) – Langmuir konstanta, kapacitet monoslojnog zasićenja sorbenta; K_L (dm^3 mg^{-1}) – Langmuir konstanta, odnosi se na slobodnu energiju adsorpcije. Langmuir parametri adsorpcije Pb(II) jona na biomasi MPO su dobijeni iz grafika Langmuir izoterme (slika 8), koji pokazuje zavisnost c_e/Q_e u odnosu na c_e, gde je c_e preostala koncentracija jona Pb(II) u ravnoteži. Teorijska vrednost konstante Q_{max}, koja predstavlja kapacitet monoslojnog zasićenja sorbenta, izračunava se iz nagiba krive linearne zavisnosti, a vrednost K_L iz odsečka sa grafika. Konstanta K_L, predstavlja energiju adsorpcije, tako da niža vrednost konstante ukazuje na veći afinitet sorbenta prema metalnim jonima. Generalno, dobar sorbent treba da karakteriše niža vrednost konstante K_L i visoka vrednost konstante Q_{max} [49]. Langmuir parametri i korelacioni koeficijent za biosorpciju Pb(II) jona dati su u Tabeli 3.

Freundlich adsorpciona izoterma

Freundlich izoterma pretpostavlja heterogenu površinu sa neuniformnom raspodelom toplote adsorpcije preko površine. Tako, Freundlich model opisuje adsorpciju na energetski heterogenoj površini, na kojoj su sorbovani molekuli interaktivni. Linearna forma Freundlich izoterme je data jednačinom:

$$\ln Q_e = \ln K_F + b_F \ln c_e \quad (5)$$

gde je: K_F (mg g^{-1}) – Freundlich konstanta proporcionalna sorpcionom kapacitetu; b_F – Freundlich konstanta koja odgovara intenzitetu adsorpcije n, a određuje se kao $b_F = 1/n$. Freundlich parametri adsorpcije Pb(II) jona na biomasi MPO su dobijeni iz grafika Freundlich izoterme (y = 0,684x + 0,046; R^2 = 0,985), koji pokazuje zavisnost ln Q_e u odnosu na ln c_e. Konstante u Freundlich izotermi pružaju dragocene informacije o sorpcionom procesu [21]. Koeficijent b_F izračunava se iz nagiba krive linearne zavisnosti, a konstanta K_F iz odsečka sa grafika. Iz koeficijenta b_F se, kao recipročna vrednost, može izračunati konstanta n, koja se obično nalazi u u intervalu 1–10, što pokazuje prednost adsorpcije na ispitivanom sorbentu. Što su niže b_F (ili $1/n$) vrednosti, to je veća uniformnost u pogledu energije adsorbcionog sistema. Freundlich parametri i korelacioni koeficijent za biosorpciju Pb(II) jona dati su u Tabeli 3. Za razliku od Langmuir-ovog modela, adsorpcija opisana Freundlich izotermom nije ograničena na monosloj.

Temkin adsorpciona izoterma

Temkin izoterma [50] pretpostavlja da se toplota adsorpcije svih sorbovanih molekula u sloju na biosorbentu smanjuje linearno sa pokrivanjem usled sorbent-sorbat interakcije, a da je adsorpcija okarakterisana uniformnom raspodelom energije vezivanja, do neke maksimalne energije. Temkin izoterma se može izraziti u linearnoj formi pomoću jednačine:

$$Q_e = B \ln K_t + B \ln c_e \quad (6)$$

gde je: Q_e (mg g^{-1}) – količina sorbovanih jona metala u ravnoteži po gramu sorbenta; c_e (mg dm^{-3}) – ravnotežna koncentracija Pb(II) jona u rastvoru; K_t (dm^3 g^{-1}) – Temkin konstanta; B (J mol^{-1}) – Temkin koeficijent. Koeficijent B se odnosi na toplotu adsorpcije, a izračunava se iz nagiba krive linearne zavisnosti $Q_e = f$ ($ln\ c_e$). S obzirom na to da je ovaj koeficijent $B = RT/b$, gde je R univerzalna gasna konstanta (J mol^{-1} K^{-1}) a T temperatura (K), može se izračunati faktor b koji predstavlja varijaciju adsorpcione energije. Temkin parametri adsorpcije Pb(II) jona na biomasi MPO su dobijeni iz grafika Temkin-ove izoterme (y = 3,580x – 1,754; R^2 = = 0,974), koji pokazuje zavisnost Q_e u odnosu na $ln\ c_e$. Ravnotežna konstanta K_t odgovara maksimalnoj energiji vezivanja i dobija se iz odsečka sa grafika [45]. Temkin parametri i koeficijent korelacije za biosorpciju Pb(II) jona dati su u Tabeli 3.

Analiza adsorpcionih izotermi

Vrednosti linearnih koeficijenata korelacije za tri primenjena modela adsorpcionih izotermi (Tabela 3) ukazuju na to da Langmuir-ova izoterma (R^2 = 0,990) najbolje fituje eksperimentalne podatke i pruža odgovarajući model za opisivanje ravnotežne biosorpcije Pb(II) jona na biomasi MPO, u ispitivanom opsegu koncentracija. Nasuprot tome, Freundlich i Temkin model pokazuju niže vrednosti koeficijenata korelacije. Takođe, na osnovu vrednosti maksimalnog kapaciteta biosorpcije (tj. maksimalne količine metalnih jona potrebnih za stvaranje kompletnog monosloja), a koji se može proceniti korišćenjem Langmuir modela, izvršena je kvantitativna procena procesa biosorpcije putem koeficijenta, Q_{max}. Iz Tabele 3 se može videti da je eksperimentalno dobijena vrednost kapaciteta biosorpcije u ravnoteži (Q_{exp}) za biomasu MPO i ispitivane Pb(II) jone najpribližnija maksimalnom kapacitetu biosorpcije (Q_{max}) izračunatom iz Langmuir-ovog modela. U skladu sa Langmuir modelom, biosorpcija prati monoslojno pokrivanje površine biosorbenta ispitivanim metalnim

jonima, pri čemu je interakciju između dva susedna jona zanemarljiva. Drugi parametar Langmuir izoterme, konstanta K_L, koja se odnosi na energiju adsorpcije, ukazuje na umereni afinitet biomase MPO prema ispitivanim Pb(II) jonima. Model Langmuir izoterme ukazuje na hemijski uravnotežen i zasićen mehanizam biosorpcije, koji je u ovom slučaju karakterističan za ispitivanu biomasu MPO.

Slično ovim konstatacijama, mnoge druge studije [9,18,27,47] koje se bave sličnim biosorbentima, pokazuju da je od svih ispitivanih modela Langmuir izoterma u prilično dobroj saglasnosti sa eksperimentalnim podacima. Ove studije ukazuju na činjenicu da je prisustvo kiseoničnih funkcionalnih grupa na površini biosorbenta (naročito hidroksilne) od presudnog značaja za sorpciju teških metala, s obzirom na to da imaju sposobnost vezivanja teških metala putem doniranja elektronskog para kiseonika metalnim jonima u rastvoru. Relativno visoka koncentracija kiseoničnih funkcionalnih grupa na površini biosorbenta, koje su odgovorne za vezivanje metala, uključuje različite mehanizme kao što su: jonska izmena, helatizacija, kompleksiranje i drugo. Upoređivanje vrednosti biosorpcionog kapaciteta i drugih parametara ispitivanog biosorbenta (biomase MPO) sa sličnim biosorbentima drugih studija nije bilo moguće zbog različitih eksperimentalnih uslova i korišćenih modela. Ipak, na osnovu prikazanih rezultata može se zaključiti da biomasa MPO ima značajan potencijal za uklanjanje jona olova iz vodenih rastvora.

ZAKLJUČAK

Navedena ispitivanja, koja se odnose na mogućnost iskorišćenja sekundarnog otpada, pokazuju da se otpadna biomasa MPO može koristiti kao biosorbent za uklanjanje Pb(II) jona iz vodenih rastvora, a u cilju prečišćavanja vode kontaminirane olovom. Karakterizacija biosorbenta FTIR spektroskopijom ukazala je na prisustvo različitih funkcionalnih grupa, koje mogu biti odgovorne za uklanjanje Pb(II) jona iz rastvora. Za proces biosorpcije Pb(II) jona utvrđeni su i definisani uticaji raznih parametara, kao što su: kontaktno vreme biosorbent-sorbat (0–120 min), pH sredine (2–6), temperatura biosorpcije (25– 40 °C), koncentracija biomase (2–20 mg dm^{-3}) i početna koncentracija metalnih jona (10–120 mg dm^{-3}). Kinetička ispitivanja biosorpcije Pb(II) jona na biomasi MPO pokazala su maksimalnu efikasnost biosorpcije od 84% u toku 50 min, pri pH 4,5 i temperaturi od 25 °C, a da proces biosorpcije najbolje prati kinetički model pseudo-drugog reda. Ispitivanja su pokazala da se porastom temperature od 25–40 °C biosorpcija postepeno smanjuje. Povećanje koncentracije biosorbenta od 2,0 do 8,0 mg dm^{-3} dovodi do značajnog povećanja efikasnosti uklanjanja Pb(II) jona, kao rezultat povećanja površine biosorbenta i veće dostupnosti biosorpcionih centara. Sa tehnološko-ekonomskog aspekta, kao optimalni parametri, odabrani su koncentracija biosorbenta od 6,0 g dm^{-3} sorbata i početna koncentracija metalnih jona 15 mg dm^{-3}. Ravnotežna biosorpcija Pb(II) jona na biomasi MPO opisana je pomoću modela Langmuir, Freundlich i Temkin izoterme, u koncentracionom opsegu jona od 10–120 mg dm^{-3}. Utvrđeno je da se kapacitet biosorpcije Pb(II) jona povećava sa povećanjem početne koncentracije metalnih jona, a da je maksimalni teorijski kapacitet biosorpcije otpadne biomase MPO 19,23 mg g^{-1}. Langmuir model sorpcije je pokazao najbolje fitovanje eksperimentalnih podataka, sa koeficijentom korelacije $R^2 \geq 0{,}990$. Na osnovu dobijenih rezultata ispitivanja, može se zaključiti da biomasa MPO, nakon iskorišćenja iz procesa proizvodnje orahovog likera, ima potencijal da se koristi kao efikasan i ekonomičan alternativni biosorbent za uklanjanje Pb(II) jona iz vodenih rastvora.

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LITERATURA

[1] G. Nordberg, M. Nordberg, B. Fowler, L. Friberg, Handbook on the toxicology of metals, Academic Press, 2007.

[2] J.W. Moore, S. Ramamoorthy, Heavy metals in natural waters – Applied monitoring and impact assessment, Springer Series on Environmental Management, Springer, 1984.

[3] World Health Organization, Guidelines for drinking water quality, Vol. 1, 3rd ed., 2008.

[4] J.W. Patterson, Industrial wastewater treatment technology, 2nd ed., Butterworth Publisher, Stoneham, MA, 1985.

[5] A. Demirbas, Heavy metal adsorption onto agro-based waste materials: a review, J. Hazard. Mater. **157** (2008) 220–229.

[6] M. Kazemipour, M. Ansari, S. Tajrobehkar, M. Majdzadeh, H.R. Kermani, Removal of lead, cadmium, zinc, and copper from industrial wastewater by carbon developed from walnut, hazelnut, almond, pistachio shell, and apricot stone, J. Hazard. Mater. **150** (2008) 322–327.

[7] D. Mitić-Stojanović, D. Bojić, J. Mitrović, T. Andjelković, M. Radović, A. Bojić, Equilibrium and kinetic studies of Pb(II), Cd(II) and Zn(II) sorption by *Lagenaria vulgaris* shell, Chem. Ind. Chem. Eng. Q. **18** (2012) 563–576.

[8] N. Kuyucak, B. Volesky, Biosorbents for recovery of metals from industrial solutions, Biotechnol. Lett. **10** (1988) 137–142.

[9] M. Rafatullah, O. Sulaiman, R. Hashim, A. Ahmad, Adsorption of copper(II), chromium(III), nickel(II) and lead(II) ions from aqueous solutions by meranti sawdust, J. Hazard. Mater. **170**(2) (2009) 969–977.

[10] D. Mohan, K.P. Singh, Single- and multi-component adsorption of cadmium and zinc using activated carbon

derived from bagasse - an agricultural waste, Water Res. **36** (2002) 2304–2318.

[11] G. Annadurai, R.S. Juang, D.L. Lee, Adsorption of heavy metals from water using banana and orange peels, Water Sci. Technol. **47** (2002) 185–190.

[12] T. Altun, E. Pehlivan, Removal of copper(II) ions from aqueous solutions by walnut, hazelnut and almond shells, Clean **35** (2007) 601–606.

[13] C.S. Zhu, L.P. Wang, W.B. Chen, Removal of Cu(II) from aqueous solution by agricultural by-product: peanut hull, J. Hazard. Mater. **168** (2009) 739–746.

[14] M.M. Zulkali, A.L. Ahmad, N.H. Norulakmal, *Oryza sativa* L. husk as heavy metal adsorbent: optimization with lead as model solution, Bioresour. Technol. **97** (2006) 21–25.

[15] M. Calero, F. Hernainz, G. Bazquez, M.A. Martin-Lara, G. Tenorio, Biosorption kinetics of Cd(II), Cr(III) and Pb(II) in aqueous solutions by olive stone, Braz. J. Chem. Eng. **26** (2009) 265–273.

[16] Z.R. Lopičić, J.V. Milojković, T.D. Šoštarić, M.S. Petrović, M.L. Mihajlović, Č.M. Lačnjevac, M.D. Stojanović, Uticaj pHvrednosti na biosorpciju jona bakra otpadnom lingo-celuloznom masom koštice breskve, Hem. Ind. **67** (2013) 1007–1015.

[17] A. Gala, S. Sanak-Rydlewska, A comparison of Pb^{2+} sorption from aqueous solutions on walnut shells and plum stones, Polish J. Environ. Stud. **20** (2011) 877–883.

[18] Y.S. Ho, W.T. Chiu, C.S. Hsu, C.T. Huang, Sorption of lead ions from aqueous solution using tree fern as a sorbent, Hydrometallurgy **73** (2004) 55–61.

[19] V.B.H. Dang, H.D. Doan, T. Dang-Vu, A. Lohi, Equilibrium and kinetics of biosorption of cadmium(II) and copper(II) ions by wheat straw, Bioresour. Technol. **100** (2009) 211–219.

[20] P. Lodeiro, J.L. Barriada, R. Herrero, M.E. Sastre de Vicente, The marine macroalga *Cystoseira baccata* as biosorbent for cadmium(II) and lead(II) removal: kinetic and equilibrium studies, Environ. Pollut. **142** (2006) 264–273.

[21] R. Say, A. Denizli, M. Arica, Biosorption of cadmium(II), lead(II) and copper(II) with filamentous fungus *Phanerochaete chrysosporium*, Bioresour. Technol. **76** (2001) 67–70.

[22] W. Bae, J. Kim, J. Chung, Production of granular activated carbon from food-processing wastes (walnut shells and jujube seeds) and its adsorptive properties, J. Air Waste Manag. Assoc. **64** (2014) 879–886.

[23] K. Edzwald, Water quality and treatment, 6^{th} ed., McGraw-Hill, New York,. 2011.

[24] D. Sud, G. Mahajan, M.P. Kaur, Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions – a review, Bioresour. Technol. **99** (2008) 6017–6027.

[25] A.A. Abia, O.B. Didi, E.D. Asuquo, Modelling of Cd^{2+} sorption kinetics from aqueous solutions onto some thiolated agricultural waste adsorbents, J. Appl. Sci. **6** (2006) 2549–2556.

[26] T. Altun, E. Pehlivan, Removal of Cr(VI) from aqueous solutions by modified walnut shells, Food Chem. **132**(2) (2012) 693–700.

[27] A. Almasi, M. Omidi, M. Khodadadian, R. Khamutian, M.B. Gholivand, Lead(II) and cadmium(II) removal from aqueous solution using processed walnut shell: kinetic and equilibrium study, Toxicol. Environ. Chem. **94** (2012) 660–671.

[28] D. Ding, Y. Zhao, S. Yang, W. Shi, Z. Zhang, Z. Lei, Y. Yang, Adsorption of cesium from aqueous solution using agricultural residue-walnut shell: equilibrium, kinetic and thermodynamic modeling studies, Water Res. **47** (2013) 2563–2571.

[29] F. Zhang, Z. Liu, J. Zhao, M. Yanan, W. Ding, The sorption of manganese ion in water onto walnut shell, Sci. Technol. Rev. **30** (2012) 49–52.

[30] M. Feizi, M. Jalali, Sorption of aquatic phosphorus onto native and chemically-modified plant residues: modeling the isotherm and kinetics of sorption process, Desalin. Water Treat. **52** (2014) 7401–7413.

[31] J. Shah, M. Jan, A. Haq, Y. Khan, Removal of rhodamine B from aqueous solutions and wastewater by walnut shells: kinetics, equilibrium and thermodynamics studies, Front. Chem. Sci. Eng. **7** (2013) 428–436.

[32] H. Aydin, G. Baysal, Y. Bulut, Utilization of walnut shells (*Juglans regia*) as an adsorbent for the removal of acid dyes, Desalin. Water Treat. **2** (2009) 141–150.

[33] A. Srinivasan, T. Viraraghavan, Removal of oil by walnut shell media, Bioresour. Technol. **99** (2008) 8217–8220.

[34] R.B.N. Prasad, Walnuts and pecans, in: B. Caballero, L. Trugo, P. Finglas (eds.), Encyclopaedia of food sciences and nutrition, Academic Press, London, 2003, pp. 6071––6079.

[35] J. Jakopič, R. Veberič, F. Štampar, Extraction of phenolic compounds from green walnut fruits in different solvents, Acta Agric. Slov. **93** (2009) 11–15.

[36] S. Cosmulescu, I. Trandafir, G. Achim, M. Botu, A. Baciu, M. Gruia, Phenolics of green husk in mature walnut fruits, Not. Bot. Hort. Agrobot. Cluj **38** (2010) 53–56.

[37] F. Štampar, A. Solar, M. Hudina, R. Veberič, M. Colarič, Traditional walnut liqueur – cocktail of phenolics, Food Chem. **95** (2006) 627–631.

[38] M. Veličković, N. Nikićević, T. Vulić, V. Tešević, Č. Oparnica, D. Radivojević, B. Đorđević, I. Vučković, Proizvodnja likera od plodova mladog oraha (*Juglans regia* L.) različitog stepena zrelosti i njegova antioksidativna aktivnost, J. Sci. Agric. Res. **68**(1) (2007) 31–38.

[39] C. Alamprese, C. Pompei, Influence of processing variables on some characteristics of nocino liqueur, Food Chem. **92** (2005) 203–209.

[40] J. Heredia-Guerrero, J. Benitez, E. Dominguez, I. Bayer, R. Cingolani, A. Athanassiou, A. Heredia, Infrared and Raman spectroscopic features of plant cuticles: a review, Front. Plant Sci. **305** (2014) 1–14.

[41] S. Ebrahim, F. Rasheed, Removal of copper ions onto walnut shells by using batch and continuous fluidized bed, J. Eng. **19** (2013) 978–991.

[42] C. Lal, A. Raja, P. Pareek, D. Shakyawar, K. Sharma, M. Sharma, Juglans nigra: Chemical constitution and its

application on Pashmina (Cashmere) fabric as a dye, J. Nat. Prod. Plant Resour. **1** (2011) 13–19.

[43] A.M. Farhan, N.M. Salem, A.H. Al-Dujaili, A.M. Awwad, Biosorption studies of Cr(VI) ions from electroplating wastewater by walnut shell powder, Amer. J. Environ. Eng. **2** (2012) 188–195.

[44] W. Mizerski, Chemical Tables, Wyd. Adamantan, Warszawa, 2008.

[45] J. Febrianto, A.N.J.K. Sunarso, Y.H. Ju, N. Indraswati, S. Ismadji, Equilibrium and kinetic studies in adsorbtion of heavy metals using biosorbent: a summary of recent studies, J. Hazard. Mater. **162** (2009) 616-–645.

[46] S. Lagergren, K. Sven, Zur theorie der sogenannten adsorption gel-ster stoffe, Kungliga Svenska Vetenskapsakad. Handl. **24** (1898) 1–39.

[47] M.N.M. Ibrahim, W.S.W. Ngah, M.S. Norliyana, W.R.W. Daud, M. Rafatullah, O. Sulaiman, R. Hashim, A novel agricultural waste adsorbent for the removal of lead(II) ions from aqueous solutions, J. Hazard. Mater. **182** (2010) 377–385.

[48] Y.S. Ho, G. McKay, Pseudo-second order model for sorption processes, Process Biochem. **34** (1999) 451–-465.

[49] S. Gupta, D. Kumar, J.P. Gaur, Kinetic and isotherm modeling of lead(II) sorption onto some waste plant materials, Chem. Eng. J. **148** (2009) 226–235.

[50] M.I. Temkin, V. Pyzhev, Kinetic of ammonia synthesis on promoted iron catalyst, Acta Physicochim. URSS **12** (1940) 327–356.

SUMMARY

THE BIOSORPTION POTENTIAL OF WASTE BIOMASS YOUNG FRUIT WALNUTS FOR LEAD IONS: KINETIC AND EQUILIBRIUM STUDY

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(Scientific paper)

The biosorption potential of waste biomass young fruit walnuts (YFW) as a low-cost biosorbent, processed from liqueur industry, for Pb(II) ions from aqueous solution was explored. The structural features of the biosorbent were characterized by FTIR spectroscopy, which indicates the possibility that the different functional groups may be responsible for the binding of Pb(II) ions from aqueous solution. The effects of relevant parameters such as pH (2–6), contact time (0–120 min), biosorbent dosage (2–20 g), initial metal ion concentration (10–120 mg dm^{-3}), at a temperature of 25 °C with stirring (120 rpm) and a constant ionic strength of 0,02 mol dm^{-3} were evaluated in batch experiments. The sorption equilibrium of Pb(II) ion (when 84% of metal ions were sorbed at an initial concentration of 15 mg dm^{-3}) was achieved within the pH range 4–5 after 50 min. Kinetic data were best described by the pseudo-second order model. Removal efficiency of Pb(II) ion rapidly increased with increasing biosorbent dose from 2.0 to 8.0 g per dm^{-3} of sorbate. Optimal biosorbent dose was set to 6.0 g per dm^3 of sorbate. An increase in the initial metal concentration increases the biosorption capacity. The sorption data of investigated metal ion are fitted to Langmuir, Freundlich and Temkin isotherm models. The equilibrium data were well fitted by the Langmuir isotherm model ($R^2 \geq 0.990$). The maximum monolayer biosorption capacity of waste biomass YFW for Pb(II) ion, at 25.0±0.5 °C and pH 4.5, was found to be 19.23 mg g^{-1}. This available waste biomass is efficient in the uptake of Pb(II) ions from aqueous solution and could be used as a low-cost and an alternative biosorbent for the treatment of wastewater streams bearing these metal ions.

Keywords: Biosorption • Waste biomass • Fruit walnut • Lead ions • Kinetic • Isotherms

Visual, instrumental, mycological and mycotoxicological characterization of wheat inoculated with and protected against *Alternaria* spp.

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Abstract

The aim of this work was to characterize visual properties, instrumentally measured colour properties, field fungi presence and *Alternaria* toxins levels in wheat samples grown under conditions aimed at inhibition and stimulation of wheat infection with fungi from the *Alternaria* genus. Experiment was carried out on the wheat treated by fungicide and wheat inoculated by *Alternaria* spp., while non treated wheat was used as a control. Statistically significant difference was observed between all three treatments using visual scale. Protected wheat samples were significantly different from other samples in terms of all measured color parameters while inoculated and control wheat samples were significantly different in terms of lightness and dominant wavelength. Identification of field fungi in the all examined wheat samples showed that the dominant mycotoxigenic fungus was *Alternaria* spp., followed by *Fusarium* spp. The content of *Alternaria* toxins in samples of wheat hulls and dehulled kernels indicated higher concentrations of *Alternaria* toxins in hulls than in dehulled kernels.

Keywords: colour, wheat ears, wheat kernels, field fungi, *Alternaria* toxins.

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Cereal grains are the primary source of energy in the human diet, with wheat being the third most produced grain worldwide. Recent studies have shown that besides *Fusarium* spp. fungi of the genus *Alternaria* became the dominant source of wheat kernels contamination [1]. The two major features of *Alternaria* species are the production of melanin, especially in the spores, and the production of host-specific toxins in the case of pathogenic species [2]. Apart from a role in conidial development [3], melanin appears to have an indirect as well as a direct function in virulence. It acts as body armor, protecting fungi against environmental stress or unfavorable conditions like extreme temperatures, UV-radiation and compounds secreted by microbial antagonists, thus adding to longevity and survival [3,4]. In addition, the melanin rapidly reacts with free oxygen radicals which are components of the host defense system against the penetration of the pathogen, thus increasing the susceptibility of the host [5]. The usual symptom of infection with *Alternaria* spp. is darkening of the cereal ears (Figure 1a) before harvest [6]. There are various types of discolouration that can affect common (*Triticum aestivum* L.) wheat kernels. Black point and dark smudge, mostly associated with *Alternaria alternata* (Fr.) Keissl., and *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dast. (anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker) [7] are common discolourations of cereal seed, which occur in most regions where these crop species are grown. The condition in wheat or barley known as black point is a dark discoloration at the embryo end of the kernel (Figure 1b), resulting in downgrading of the grain quality. In severe cases, the discoloration occurs in the outer pericarp and inner seed coat tissue, and may extend along the groove on the ventral side of the grain [8,9].

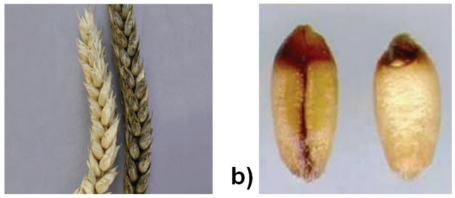

Figure 1. Symptoms of infection with Alternaria spp. on wheat ear (right) (a, taken from URL: http://www.hgca.com/, accessed 06/05/2015) and on grain (b, taken from URL: http:// //agropedia.iitk.ac.in/content/black-point-disease-wheat/, accessed 06/05/2015).

These types of kernel discoloration vary significantly in incidence and severity depending on environmental conditions during kernel maturation. Numerous other studies indicate that black point may be a result of abiotic stresses, as symptoms are more likely to occur after extreme environmental conditions such as heavy rain, high humidity and extremes of temperature [7,10–15]. However, a recent study showed that

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although abiotic factors, such as high humidity levels, can promote the occasional development of black point or dark smudge on durum wheat kernels under controlled-environment conditions, fungal infection by *C. sativus* or *A. alternata* was the main factor associated with their development [16]. Besides pathogenicity and reduction of quality of kernels, several *Alternaria* spp. are known producers of toxic secondary metabolites, *Alternaria* mycotoxins (tenuazonic acid, alternariol, alternariol monomethyl ether (Figure 2a–c), altenuen and altertoxins), which might be harmful for human and animal health [1].

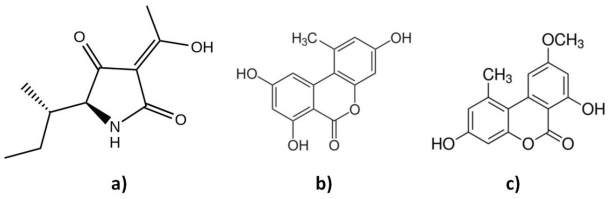

Figure 2. Structures of examined Alternaria toxins: tenuazonic acid (a), alternariol (b) and alternariol monomethyl ether (c).

In this context, the aim of this work was to explore the possibility of determining the intensity of field fungi infestation by visual scale application and instrumental measurement of wheat ears and kernels color and to determine field fungi presence and *Alternaria* toxins levels in wheat samples grown under conditions aimed at inhibition and stimulation of infection of wheat with fungi from the *Alternaria* genus.

MATERIAL AND METHODS

Material and climate conditions

Experiment was carried out in the 2012/2013 season in the region of Vojvodina, north Serbia, on the wheat (*Triticum aestivum* cv. Sirtaki) protected by fungicide and wheat inoculated by *Alternaria* spp., while non treated wheat was used as a control. May 2013 was characterized by weather fluctuations, warmer and more humid weather conditions than multiannual average, with surplus precipitation (ΣPrec. 125 mm). According to Standardized Precipitation Index (SPI), *Z* index, and Palmer Drought Severity Index (*PDSI*) values (1.9, 4.8 and 4.0, respectively), this month had extreme humid weather conditions.

Fungus culture and inoculation

The isolates of *A. tenuissima* were multiplied in 8 L Chapek medium at room temperature (25 °C) with daylight cycles for 14 days. Medium was aerated during the isolate multiplication. For the purpose of inoculation, a conidial suspension was sprayed on the plants with a hand atomizer. Before spraying, flasks were shaken vigorously and 1 mL of suspension was poured in to a haemocytometer and the number of infective particles was counted in ten replications. The average concentration of *A. tenuissima* conidia was 0.13025×10^6 infective particles/mL. At the full flowering stage inoculation was performed with 8 L of aqueous suspension of fungal isolates. Inoculated spikes were immediately covered with plant protection cover (Stocker, Italy) for 48 h. Spikes were used as control. In the full ripeness, stage spikes from each plot were cut by hands and used for next analysis.

Chemicals and reagents

Alternariol (AOH, purity 99.0%), alternariol monomethyl ether (AME) (purity 99.5%) and tenuazonic acid (TeA, purity 99.5%) standards were purchased from Sigma-Aldrich (St Louis, MO, USA). Stock solutions of AOH (2500 μg mL^{-1}), AME (2500 μg mL^{-1}) and TeA (10000 μg mL^{-1}) were prepared in methanol and stored at –20 °C. The following solvents were used: methanol (MeOH, J.T.Baker, Deventer, Netherlands) and ethyl acetate (EtOAc, Sigma-Aldrich (Saint Louis, USA), all LC–MS grade, formic acid (FA, purity 99.9%, Carlo Erba, Italy), and fuming HCl (37%, pa, Merck, Darmstadt, Germany). Deionized water was sourced from a Millipore Simplicity UV water purification system (Bedford, MA, USA).

Visual scale establishing and colour measurement

Visual scale (1–6) of wheat ear color was established, where 1 represented the lightest sample and 6 represented the darkest sample. These samples were also measured instrumentally in order to explore the correlations between these two methods. The color of wheat kernels was only instrumentally measured.

The color of all samples was measured with Konica Minolta Chroma Meter CR-400, using different attachments; for the measurement of wheat ears color, Light Protection Tube CR-A33f was used, while the color of wheat kernels and hulls was measured using Granular Attachment CR-A50. The CIE L* (lightness), CIE a* (red–green) and CIE b* (yellow–blue), and dominant wavelength (DWL) were read using a D_{65} light source and the observer angle at 2°. The tristimulus values of CIE L*, a* and b* readings were calibrated against a standard white plate (Y = 84.8; x = 0.3199; y = 0.3377). Each wheat ear sample was divided in four subgroups, and color of one hundred ears from each subgroup (400 ears from one sample) was measured on 6–8 locations, depending on the ear size. Each wheat kernel sample was also divided in four subgroups, and ten replications were measured from each subgroup (40 replications per sample in total).

Percent of kernel infection

According to the method proposed by Pitt and Hocking [17], 100 wheat kernels were randomly sel-

ected from each treatment. The samples were disinfected with 0.4% NaClO, rinsed with water for 2 min and placed on Petri plates in four repetitions (25 kernels per plate). Incubation was conducted at 25 °C and after seven days intensity of infection was assessed. Confirmation of fungi genera was carried out by microscopic examination on potato dextrose agar (PDA) and malt extract agar (MEA) media after 7 days of incubation at 25 °C.

LC–MS/MS analysis

The Agilent 1200 series liquid chromatography, consisting of vacuum degasser, binary pump, autosampler and thermostated column compartment was used for separation of analytes, whose detection was carried out by means of Agilent series 6410A triple-quad mass spectrometer with electrospray ionization (ESI). MassHunter ver. B.03.01 software (Agilent Technologies Inc., USA) was used for instruments control and data analysis. The separation was achieved using a Zorbax Eclipse XDB-C18 column (50 mm×4.6 mm i.d., 1.8 µm) (Agilent Technologies) with a column compartment temperature of 50 °C. The binary mobile phase consisted of 0.05% aqueous formic acid (A) and methanol (B) and was delivered at a flow rate of 1 mL/min. Components were eluted in gradient mode, starting with 30% B, followed by a linear gradient reaching 70% B in 6.0 min, then by a linear gradient reaching 100% B in 9.0 min and holding for 3.0 min, with post-time of 3.0 min. The entire effluent was transferred to mass spectrometer, without flow splitting. The injection volume for all samples was 15 µL. ESI parameters were as follows: drying gas (N_2) temperature 350 °C, flow 10 L/min, nebulizer gas pressure 50 psi, and capillary voltage 4 kV. Compounds were quantified in negative ionization dynamic selected reactions monitoring mode. Each compound was monitored at determined retention time ±1.5 min. Fragmentor voltage and collision energies were optimized for each analyte during infusion of the pure standard, and the most abundant fragment ions were chosen for the selected reaction monitoring. The utilized MRM transitions (*m*/*z* mother ion→quantifier/qualifier) were *m*/*z* 196.2→139.0 (fragmentor 170 V, collision energy 15V)/112.0 (fragmentor 170 V, collision energy 20 V) for TeA, *m*/*z* 257.2→213.0 (fragmentor 180 V, collision energy 20 V) /215.0 (fragmentor 180 V, collision energy 25 V) for AOH and *m*/*z* 271.3→256.0 (fragmentor 130 V, collision energy 20 V)/228.0 (fragmentor 160 V, collision energy 30 V) for AME. The mean retention times (n = 20) were 4.14 min for TeA, 5.03 min for AOH and 7.01 min for AME.

Sample preparation

Method of sample preparation by Siegel *et al*. [18] was slightly modified. Samples (500 g) were ground to a 1 mm particle size using laboratory mill (KnifetecTM 1095 mill, Foss, Hoganas, Sweden). After that, approximately 1 g (exact weights known) of homogenized samples were mixed with 7 mL water. Subsequently, 2 mL of 2 mol/L aq. HCl and 5 mL of EtOAc were added. The resulting ternary phase systems were shaken on shaker (Griffin and George, Wembley, England) for 45 min, ultrasonicated for 10 min (ATM40-3LCD, Madrid, Spain) and shaken again for 45 min. Then, the extracts were transferred into glass cuvettes and centrifuged (Tehtnica, Železniki, Yugoslavia) at 3200*g* for 15 min to achieve complete phase separation. Thereafter, 2 mL of the upper EtOAc layers were transferred into another glass cuvette, and evaporated under a stream of nitrogen (Reacti-Therm I #18821, Thermo Scientific, USA). The dry residue was dissolved in 1 mL of LC/MC grade MeOH, and transferred to an HPLC vial through the Econofilter PTFE (13 mm, 0.2 µm) syringe filter (Agilent Technologies, China) and stored at –20 °C until analysis.

Validation of method

The developed method was validated by in-house quality control procedure following the guidelines of Commission Decision 2002/657/EC [19]. Method validation was performed in terms of matrix effects, linearity, trueness, precision (repeatability), limit of detection (LOD) and limit of quantification (LOQ). The calibration curves for all of the compounds in pure solvent and in matrix were obtained by plotting the peak areas against the concentrations of the corresponding calibration standards at five calibration levels ranging from 25.0 to 100.0 µg/kg for TeA and 2.5 to 10.0 µg/kg for AOH and AME. The linearity of calibration curves was expressed by the correlation coefficient (r^2). For the matrix-matched calibration curves (MMC), the blank wheat samples were enriched with working standard solutions at the final reconstitution step providing linearity over the range from the 25.0 to 100.0 µg/kg for TeA and from the 2.5 to 10.0 µg/kg for AOH and AME (five-point MMC). For overall method recovery, the blank wheat samples were spiked prior to sample preparation, providing linearity also over the range from the 25.0 to 100.0 µg/kg for TeA and from 2.5 to 10.0 µg/kg for AOH and AME (five-point R_A) in three replicates. Spiked samples were left overnight at room temperature to allow solvent evaporation and equilibration between analytes and matrix. To differentiate between extraction efficiency and matrix-induced signal suppression/enhancement, the slope ratios of the linear calibration functions were calculated to yield the apparent recovery (R_A), *i.e*., the overall method recovery and the signal suppression/enhancement (SSE) due to matrix effects. The recovery of the extraction step (R_E), *i.e*., sample preparation recovery was calculated by dividing the overall recovery by the matrix effect as follows (modified after Matuszewski *et al*. [20]):

R_A(%) =
= 100×slope spiked sample/slope liquid standard (1)

SSE(%) =100×slope matrix-matched standard/slope liquid standard (2)

R_E(%) = 100×slope spiked sample/slope matrix-matched standard (3)

The precision of the method was expressed in terms of repeatability, *i.e*., as relative standard deviation (*%RSD*) of 6 replicates at three concentration levels (25.0, 50.0 and 100.0 μg/kg for TeA and 2.5, 5.0 and 10.0 μg/kg for AOH and AME) using the spiked blank wheat samples prior to analysis using the MMC curve.

The limit of detection (*LOD*) and limit of quantification (*LOQ*) were estimated by injecting decreasing concentrations of matrix-matched standards and measuring the response at a signal-to-noise ratio (*S/N*) of ≥3 and ≥10 for the *LOD* and *LOQ*, respectively.

Statistical analysis

Pearson correlation coefficients on different significance levels (5, 1 and 0.1%) between visual scale and measured parameters were calculated. Analysis of variance (ANOVA) and Duncan's multiple range tests were applied to compare means at 5% significance level. Principal Component Analysis (PCA) was applied to explore the relationships among the colour parameters and to group the wheat ears used for visual scale establishing. Data analysis was performed using the statistical data analysis software system Statistica, version 12.0.

RESULTS AND DISCUSSION

Obtained results are discussed in the scope of the color properties of wheat ears, kernel color properties, presence and distribution of the field fungi infecting wheat and presence and concentration of *Alternaria* toxins.

Visual and instrumental assessment of wheat ear color

The results obtained by application of visual evaluation of wheat ears and their instrumental colour measurement (Table 1) indicate that all three treatments differed significantly among each other by scores obtained using visual scale, with protected wheat sample assessed with the lowest scores, and inoculated wheat sample with the highest scores. Protected wheat samples were significantly different from other samples in terms of all measured color parameters (L*, a*, b* and *DWL*). Inoculated and control wheat samples were significantly different in terms of lightness and dominant wavelength.

Regarding the correlations between methods of visual and instrumental assessment of color, values of Pearson correlation coefficients calculated between visual scale and instrumental measurement showed that L* values were in the highest negative correlation (–0.97, $p < 0.001$) with the visual scale. Other colour parameters (b*and *DWL*) were also in high correlation ($p < 0.001$) with the visual scale (–0.72 and +0.74, respectively). Color parameter a* was also significantly positively correlated with the visual scale at 5% significance level. Six wheat ears used for visual scale establishing differed significantly among each other only by L* (lightness) values.

PCA was performed for the color values the wheat samples used for establishment of visual scale, and the results showed that high percentage of total variance is explained by the first two components (95.66%, Figure 3a).

Lightness (L*) and yellow tone (b*) were close to the circle line and close to each other, which indicated high contribution of these two parameter to the total variance as well as high correlation (*r* close to +1) between them. Red tone (a*) and dominant wavelength (*DWL*) were also close to the circle line which pointed out their importance in explanation of variance between the wheat samples.

Wheat ears that comprised the visual scale were completely separated by color parameters (Figure 3b). Wheat ears assessed as 1, 2 and 3 were distinguished mostly by lightness and yellow tone. Wheat ear assessed as 6 was almost in opposite to the wheat ear marked as 3, indicating dark color of this sample. Higher values of *DWL* and a* caused differentiation of wheat ears marked as 4 and 5, which could be explained by more prominent red hue of these samples. Measured color parameters were in most cases in accordance with appearance of wheat ears used for visual scale establishing.

Table 1. Colour parameters of differently treated wheat ears; 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample. Results are presented as mean±standard deviation (n ≈ 2400). Values with the different superscript within the same column are statistically different (p < 0.05)

Sample	L*	a*	b*	*DWL* / nm	Visual scale
0	$60.91±3.05^b$	$2.89±0.70^a$	$24.07±3.89^b$	$578.33±0.52^a$	$2.90±0.50^b$
1	$63.16±3.46^a$	$2.49±0.69^c$	$25.41±3.32^a$	$577.94±0.49^c$	$2.35±0.74^c$
2	$60.09±3.87^c$	$2.81±0.71^b$	$24.11±4.01^b$	$578.29±0.59^b$	$3.01±0.75^a$

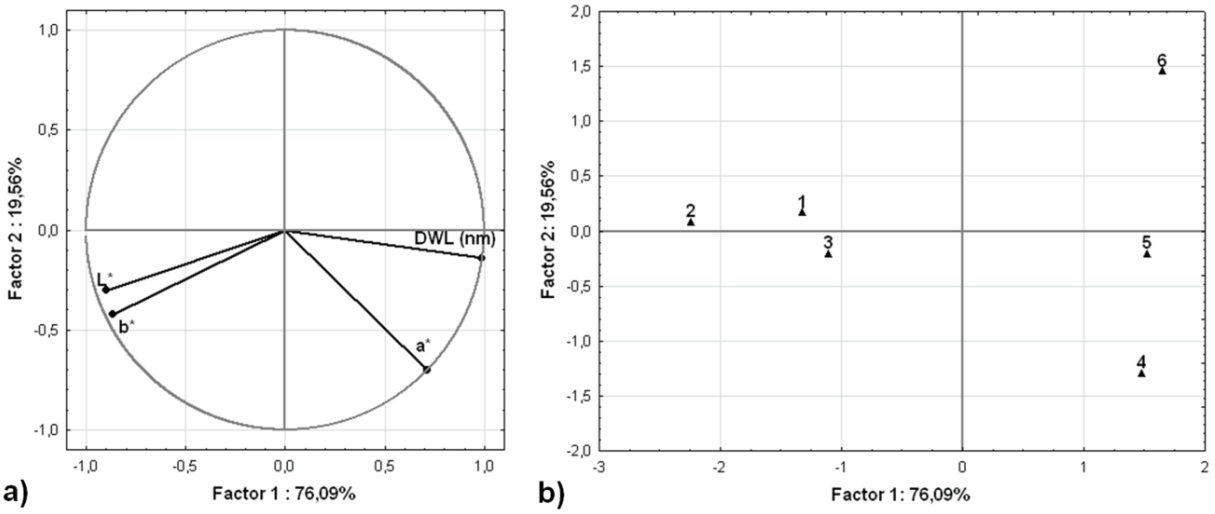

Figure 3. Projection of variables (a) and cases (b) on the factor plane.

Instrumental assessment of wheat kernel colour

Results of kernel color measurement were slightly different: kernels of inoculated wheat differed significantly from other samples in terms of L*, a* and b* parameters (Table 2), whereas all samples belonged to the same homogenous group by dominant wavelength values.

Table 2. Color parameters of differently treated wheat kernels ; 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample. Results are presented as mean± ±standard deviation (n = 40). Values with the different superscript within the same column are statistically different (p < 0.05)

Sample	L*	a*	b*	*DWL* / nm
0	53.05±1.63^a	8.73±0.91^a	23.81±1.60^a	582.64±0.41^a
1	53.34±1.88^a	8.87±0.88^a	24.09±1.84^a	582.68±0.37^a
2	52.20±1.53^b	8.30±0.96^b	22.25±1.84^b	582.67±0.40^a

Considering all mentioned above, it can be concluded that infection entered the kernel in greater extent in inoculated wheat samples, while in non-treated samples it was less prominent. Protected wheat samples were generally characterized by higher lightness and more prominent yellow tone. Results obtained by instrumental color measurement are in accordance with the visual scale assessment of treated wheat samples.

Field fungi distribution

Identification of field fungi in all examined wheat samples showed (Table 3) that the dominant field fungi was *Rhizopus* spp., while of mycotoxigenic fungus *Alternaria* spp. was dominant, followed by *Fusarium* spp.

Percent of infection by *Alternaria* spp. was the highest for inoculated samples as expected. This caused the differentiation of this wheat sample by its dark color as determined both by application of visual scale and instrumental measurement, which was expected due to the fact that *Alternaria* spp. produce melanin pigments.

Presence of *Alternaria* toxins

Presence of *Alternaria* toxins in examined hulls and dehulled wheat samples for the applied treatments was analyzed by high performance liquid chromategraphy with electrospray ionization triple quadrupole mass spectrometry (HPLC-ESI–MS/MS). The validation data of the employed analytical method for the determination of selected *Alternaria* toxins are given in Table 4, while precision, expressed as repeatability gave *RSD* values of 15.0, 10.2 and 9.3% for AOH, 19.9, 10.4 and 15.8% for AME and 10.7, 8.3 and 8.9% for TeA. Linearity, gave values of correlation coefficients (r^2) above 0.9903 for all three *Alternaria* toxins for standard and matix-matched calibration curves and calibration curves of spiked samples in the concentration

Table 3. Presence of certain genera of fungi in wheat samples; 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample

Sample	Fungi genus, %				
	Fusarium	*Alternaria*	*Cladosporium*	*Rhizopus*	*Penicillium*
0	2	31.5	1	65.5	–
1	2	29	–	69	–
2	4	44.5	–	50	1.5

range from 25.0 to 100.0 μg/kg for TeA and from 2.5 to 10.0 μg/kg for AOH and AME.

Table 4. Limit of detection, limit of quantification, matrix effects, overall method recovery and sample preparation recovery data of the employed analytical method; LOD – limit of detection; LOQ – limit of quantification ; SSE – slope ratio of matrix-mached calibration curve and standard calibration curve for selected analytes; R_A – overall method recovery (slope ratio of calibration curve of spiked samples and standard calibration curve for selected analytes ; R_E – sample preparation recovery (slope ratio of calibration curve of spiked samples and matrix-mached calibration curve for selected analytes)

Alternaria toxin	*LOD/LOQ* μg/kg	SSE %	R_A %	R_E %
AOH	0.75/2.5	99.6	71.4	71.7
AME	0.1/0.3	92.7	70.7	76.2
TeA	2.5/7.5	125.3	87.7	70.0

Based on obtained validation parameters, the developed method was successfully validated according to the criteria specified in Commission Decision 2002/657/EC for quantitative confirmation method [19].

The results on presence of *Alternaria* toxins in examined samples are given in Table 5.

Table 5. Content of alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) in hulls and dehulled wheat samples for the applied treatments (μg/kg); 0 – non-treated wheat sample; 1 – protected wheat sample; 2 – inoculated wheat sample

Sample	AOH	AME	TeA
0 – hulls	10.6	0.99	189
1 – hulls	–	0.54	105
2 – hulls	21.1	1.25	227
0 – dehulled wheat	–	–	59.5
1 – dehulled wheat	–	–	30.7
2 – dehulled wheat	–	0.46	96.6

It should be noted that the *Alternaria* toxins were quantified by external matrix-matched calibration procedure. Also, the presented results are corrected with samples preparation recovery and it was recalculated on the dry matter.

TeA was the predominant toxin quantified in all analysed cases. In the hulls of treated and non-treated wheat samples all three examined *Alternaria* toxins were detected, while in hulls of protected wheat sample AOH was not detected. Also, it can be seen that of dehulled wheat kernel samples, the highest concentration of TeA was detected in inoculated wheat kernels. The detected levels of TeA were about three times higher in hulls compared to kernels from all treatments. It should be noted that concentration of AOH was doubled in hulls of inoculated wheat sample compared to hulls of non-treated wheat sample, while AOH was not detected in dehulled wheat samples in any of analysed cases. The detected level of AME was about three times higher in hulls compared to dehulled kernels in inoculated wheat sample. Hulls might be considered as physical barriers with a protective effect from pathogens on kernels, which has been proved in hulled barley and oat varieties [21,22]. There are several studies dealing with the protective role of hulls against mycotoxins produced by *Fusarium* species [21–24], but data on the ability of hulls to protect wheat kernels from the *Alternaria* toxins is still scarce. Similarly to our findings, Vučković *et al*. [25] showed the protective effect of hulls on the occurrence of *Alternaria* toxins in spelt wheat.

On the basis of obtained results, visual scale or instrumental measurement of wheat ear color could be applied for the fast determination of infection degree by *Alternaria* spp. in the field. Anyhow, these methods should be validated with larger number of samples, cultivars and production years. Methodology of sampling should be also further elaborated in order to obtain representative results. One of the further directions of research should be examination of possible relation between color and content of *Alternaria* toxins as well as prediction of toxin content by colour measurements performed in field. In addition, possible protective effect of hulls could be important for organic farming.

CONCLUSIONS

Results obtained for the non-treated, protected and inoculated wheat samples showed that instrumentally measured color parameters are in accordance with the visual scale assessment. Pearson correlation coefficients between visual scale and instrumental measurement showed that L* values were in the highest negative correlation with the visual scale; moreover, samples used for visual scale establishing differed significantly among each other only by L* values. Identification of field fungi in the all examined wheat samples showed that the dominant mycotoxigenic fungus was *Alternaria* spp., followed by *Fusarium* spp. It can be concluded that higher degree of infection by *Alternaria* spp., higher score on visual scale and lower L* (lightness) values were directly related due to the production of melanin pigments by this genus of fungi. Obtained results indicate the higher concentrations of *Alternaria* toxins in hulls than in dehulled kernels which implicate the possible protective effect of wheat hulls.

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REFERENCES

[1] EFSA on Contaminants in the Food Chain (CONTAM), Scientific opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food, EFSA J. **9**(10) (2011) 2407 (http:// //www.efsa.europa.eu/efsajournal).

[2] B. Thomma, A*lternaria* spp.: from general saprophyte to specific parasite, Mol. Plant Pathol. **4** (2003) 226–236.

[3] C. Kawamura, T. Tsujimoto, T. Tsuge, Targeted disruption of a melanin biosynthesis gene affects conidial development and UV tolerance in the Japanese pear pathotype of *Alternaria alternata*, Mol. Plant Microbe Interact. **12** (1999) 59–63.

[4] A.L. Rehnstrom, S.J. Free, The isolation and characterization of melanin-deficient mutants of *Monilinia fructicola*, Physiol. Mol. Plant P. **49** (1996) 321–330.

[5] E. Jacobson, E. Hove, H. Emery, Antioxidant Function of Melanin in Black Fungi, Infection Immun. **63** (1995) 4944–4945.

[6] HGCA The Encyclopaedia of Cereal Diseases [online] Available from URL: http://www.hgca.com/, access date 06/05/2015.

[7] M.R. Fernandez, J.M. Clarke, R.M. DePauw, R.B. Irvine and R.E. Knox, Black point and red smudge in irrigated durum wheat in southern Saskatchewan in 1990–1992, Can. J. Plant Pathol. **16** (1994) 221–227.

[8] C. Mónaco, M. Sisterna, A. Perelló, G.D. Bello, Preliminary studies on biological control of the black point complex of wheat in Argentina, World J. Microb. Biot. **20** (2004) 285–290.

[9] P.M. Williamson, Black point of wheat: in vitro production of symptoms, enzymes involved, and association with *Alternaria alternata*, Aust. J. Agr Res. **48** (1997) 13–19.

[10] M.P. Clarke, M.J. Gooding and S.A. Jones, The effects of irrigation, nitrogen fertilizer and grain size on Hagberg falling number, specific weight and black point of winter wheat, J. Sci. Food Agr. **84** (2004) 227–236.

[11] R.L. Conner, Influence of irrigation and precipitation on incidence of black point in soft white spring wheat, Can. J. Plant Pathol. **11** (1989) 388–392.

[12] R.L. Conner, J.G.N. Davidson, Resistance in wheat to black point caused by *Alternaria alternata* and *Cochliobolus sativus*, Can. J. Plant Pathol. **68** (1988) 351––359.

[13] J. Kumar, P. Schafer, R. Huckelhoven, G. Langen, H. Baltruschat, E. Stein, S. Nagarajan, K.-H. Kogel, *Bipolaris sorokiniana*, a cereal pathogen of global concern: Cytological and molecular approaches towards better control, Mol. Plant Pathol. **3** (2002) 185–195.

[14] R.S. Sadasivaiah, S.M. Perkovic, D.C. Pearson, B. Postman, B.L. Beres, Registration of 'AC Andrew' wheat, Crop Sci. **44** (2004) 696–697.

[15] R.S. Sadasivaiah, S.M. Perkovic, D.C. Pearson, B. Postman, B.L. Beres, Registration of 'AC Meena' wheat, Crop Sci. **44** (2004) 697–698.

[16] M.R. Fernandez, M. Sissons, R. L. Conner, H. Wang, J.M. Clarke, Influence of biotic and abiotic factors on dark discoloration of durum wheat kernels, Crop Sci. **51** (2011) 1205–1214.

[17] J.I. Pitt, A.D. Hocking, Fungi and Foog Spoilage, CSIRO Division of Food Research, Academic press, Sydney, 1985.

[18] D. Siegel, M. Feist, M Proske, M Koch, I. Nehls, Degradation of the Alternaria mycotoxins alternariol, alternariol monomethyl ether, and altenuene upon bread baking. J. Agric. Food Chem. **58** (2010) 9622–9630.

[19] European Commission, Commission Regulation 2002/ /657/EC of 14 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities L **221** (2002) 8–36.

[20] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng., Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. Anal. Chem. **75** (2003) 3019–3030.

[21] A. Adler, H. Lew, J. Moudry, Z. Sterba, K. Vratilova, W. Edinger, W. Brodacz, E. Kiendler, Microbiological and mycotoxicological quality parameters of naked and covered oats with regard to the production of bran and flakes, Bodenkultur **54** (2003) 41–48.

[22] P.A. Khatibi, G. Berger, S. Liu, W.S. Brooks, C.A. Griffey, D.G. Schmale, Resistance to *Fusarium* head blight and deoxynivalenol accumulation in Virginia barley, Plant Dis. **96** (2012) 279–284.

[23] M. Wiwart, J. Perkowski, H. Jackowiak, D. Packa, A. Borusiewicz, M. Buoeko, Response of some cultivars of spring spelt (*Triticum spelta*) to *Fusarium culmorum* infection, Bodenkultur **55** (2004) 29–36.

[24] M. Wiwart, J. Perkowski, W. Budzy´ nski, E. Suchowilska, M. Bu´sko, A. Matysiak, Concentrations of ergosterol and trichothecenes in the grains of three *Triticum* species. Czech J. Food Sci. **29** (2011) 430–440.

[25] J. Vučković, M. Bodroža-Solarov, Đ. Vujić, A. Bočarov-Stančić, F. Bagi, The protective effect of hulls on the occurrence of *Alternaria mycotoxins* in spelt wheat. J. Sci. Food Agric. **93** (2013) 1996–2001.

IZVOD

VIZUELNA, INSTRUMENTALNA, MIKOLOŠKA I MIKOTOKSIKOLOŠKA KARAKTERIZACIJA PŠENICE GAJENE U USLOVIMA STIMULACIJE I INHIBICIJE ZARAŽENOSTI SA *Alternaria spp.*

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Žita predstavljaju primarni izvor energije u ljudskoj ishrani, pri čemu u svetskoj proizvodnji pšenica zauzima treće mesto po proizvedenoj količini. Skorašnja istraživanja su pokazala da su pored *Fusarium* spp. gljive iz roda *Alternaria* postale dominantni zagađivači pšeničnog zrna. Pored patogenosti i narušavanja kvaliteta pšeničnog zrna, pojedine vrste roda *Alternaria* su poznate kao proizvođači sekundarnih toksičnih metabolita, *Alternaria* toksina, koji mogu biti štetni po zdravlje ljudi i životinja. Cilj ovog rada je bio karakterizacija vizuelno i instrumentalno merenih svojstava boje, prisustva i zastupljenosti poljskih fitopatogenih gljiva i *Alternaria* toksina u uzorcima pšenice gajenih u uslovima usmerenim na inhibiciju i stimulaciju razvoja gljiva iz roda *Alternaria*. Eksperiment je izveden na uzorcima pšenice tretirane fungicidom i na uzorcima pšenice inokulisane sa *Alternaria* spp., dok je netretirana pšenica korišćena kao kontrola. Boja klasova pšenice koji su predstavljali vizuelnu skalu je instrumentalno izmerena radi utvrđivanja korelacije između ove dve metode. Grupisanje pšeničnih klasova po vrednostima svetloće (L*) bilo je u skladu sa korišćenom skalom za vizuelnu ocenu boje. Štaviše, vrednosti svetloće su bile u najvećoj negativnoj korelaciji (-0,97, $p < 0,001$) sa vizuelnom skalom. Pri vizuelnoj oceni boje pšeničnih klasova utvrđena je statistički značajna razlika između tri tretmana. Uzorci pšenice zaštićene fungicidom su se statistički značajno razlikovali od ostalih uzoraka u pogledu svih izmerenih parametara boje (L*, a*, b* i dominantna talasna dužina). Uzorci inokulisane i kontrolne pšenice su se međusobno statistički značajno razlikovali u pogledu svetloće klasova i dominantne talasne dužine. Boja pšeničnog zrna je ispitana samo primenom instrumentalne ocene boje. Boja inokulisanog pšeničnog zrna se statistički značajno razlikovala od boje zrna ostalih uzoraka u pogledu L*, a* i b* parametara boje. Identifikacija poljskih gljiva u svim ispitivanim uzorcima pšenice je pokazala da je dominantna gljiva koja produkuje mikotoksine upravo *Alternaria* spp., a zatim sledi *Fusarium* spp. Dodatno je ispitan sadržaj *Alternaria* toksina u uzorcima pšenične plevice i u pšeničnom zrnu za primenjene tretmane primenom visoko performansne tečne hromatografije spregnute sa masenim detektorom. Dobijeni rezultati ukazuju na veće koncentracije *Alternaria* toksina u pšeničnoj plevici u odnosu na pšenično zrno bez plevice ukazujući na mogući zaštitni efekat plevice pšenice.

Ključne reči: Boja • Pšenični klasovi • Pšenično zrno • Fitopatogene gljive • *Alternaria* toksini

Antioxidant activity and polyphenol profile of Vranac red wines from Balkan region

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Abstract

The objective of the present study was to investigate the correlation between the radical--scavenging properties (measured by evaluating the quenching of the stable 2,2-diphenyl--1-picrylhydrazil radical) of Serbian, Macedonian and Montenegrin red wine Vranac of different geographical origins, and their contents of total phenolics, total flavonoids and polyphenol profile. All tested Vranac wines samples showed a high antioxidant activity ranging from 13.00 to 15.02 mmol/L, while the total polyphenolic content was between 3478.70 and 3935.19 mg/L. The predominant anthocyanin was malvidin-3-glucoside (179.04–281.31 mg/L), predominant flavonol was quercetin-3-glucoronide (5.88–11.78 mg/L), predominant flavan-3-ol was catechin (24.43–76.78 mg/L) and predominant hydroxycinnamic acid was *t*-caftaric acid (13.46–38.56 mg/L). Generally, red wines Vranac produced from Balkan regions are rich source of phenolics, which the evident antioxidant capacity showed.

Keywords: red wine, Vranac, phenolic profile, antioxidant activity, HPLC-DAD.

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Phenolic compounds play a very important role in the quality of red wine, owing to their contribution to the wine sensory properties, mainly colour, astringency and bitterness [1].

Wine phenolics can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavonols). Anthocyanins are the main phenolic compounds involved in the colour of the red wine. Flavan-3-ols are a large family of polyphenolic compounds which are mainly responsible for the astringency, bitterness and structure of wines, and also play an important role in the stabilisation of colour during aging. Flavonols, which also contribute to bitterness, display antioxidant activity and affect red wine colour acting as co-pigments of anthocyanins [2]. The polyphenolic contents of wine depend on the grape variety, vineyard location, cultivation system, climate, soil type, vine cultivation practices, harvesting time, production process and ageing [3].

In recent years an increasing number of studies have demonstrated the role of phenolic compounds in the antioxidant activity of many food products [4]. Wine is an excellent source of various classes of polyphenols, including benzoic and cinnamic acid derivatives, flavan-3-ols, flavonols and anthocyanins. A lot of effort has been put into the analysis of red wine polyphenols, and the relationship between polyphenolic content and antioxidant activity. The antioxidant properties of red wines have been correlated with their content in total polyphenols [5,6], anthocyanins [7] and hydroxicinnametes [8]. Red wines contain significantly higher amounts of total polyphenols compared to white wines.

Vranac is a variety of red grapes cultivated in Serbia, Montenegro and Macedonia used in the production of high quality wines. In this study, we evaluated the antioxidant activity of 9 Vranac wines produced from different agronomical and winemaking regions, and their correlation with the total phenolics and flavonoids.

MATERIALS AND METHODS

Chemicals

Standards of catechin, epicatechin, quercetin, myricetin and the phenolic acid standards, such as gallic, ferulic, *p*-coumaric and caffeic acids, were purchased from Sigma Chemicals Co. (St. Louis, Mo). Malvidin-3--glucoside was purchased from Extrasynthèse (e, France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich (Steinheim, Germany). 6-Hydroxy-2,5,7,8-tetramethylchromancarboxylic acid (Trolox) and Folin–Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). Other chemicals and solvents were of analytical grade.

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Wine samples

Nine Vranac wines, produced from three different Balkan countries (Serbia, Macedonia and Montenegro) originating from different wine regions were taken after production from the wineries (Table 1).

Determination of total phenolic content (TPC)

The total phenolic content of wines was determined by the Folin–Ciocalteau method [9] using gallic acid as the standard. One milliliter (diluted 1:100 with methanol) of red wines was added to a 25 mL volumetric flask filled with 9 mL deionised water. A reagent blank using deionized water sample was prepared, too. Folin––Ciocalteau phenol reagent (0.5 mL) and 5 mL of 5% sodium carbonate solution was immediately diluted to 25 mL with deionized water and mixed thoroughly. The absorbance was measured at 765 nm by a UV–Vis spectrophotometer (Agilent, Santa Clara, CA USA) after incubation for 1 h in dark at room temperature. The quantification was based on the calibration curve generated by the gallic acid standard solutions, and the content was expressed as mg gallic acid equivalent (GAE)/L of wine. All samples were analyzed in triplicate.

Determination of total flavonoid content (TFC)

The measurement of the total flavonoid content in the investigated wines has been determined spectrometrically [10], using a method based on the formation of complex flavonoid-aluminium. 0.5 mL (diluted 1:50 with methanol) of wine has been placed in 10 mL volumetric flask, and 5 mL of deionised water and 0.3 mL of 5% $NaNO_2$ have been added and mixed. After 5 min, 0.6 mL of 10% $AlCl_3{\cdot}6H_2O$ was added. Two milliliters of 1 mol/L NaOH were added 5 min later, and the volume was then made up to 10 mL with deionised water. The solution was mixed well and the absorbance was measured immediately at 510 nm. The flavonoid contents were calculated using a standard calibration curve, prepared from (+)-catechin.

Free radical scavenging activity

The DPPH radical scavenging method [11] has been based on the reduction of DPPH radicals in the presence of a hydrogen donating antioxidants in methanol solution. DPPH radical solution showed an absorption band at 515 nm and was of intensively violet color. The absorption and color intensity decreased when DPPH was reduced by an antioxidant compound. The remaining DPPH radical corresponded inversely to the radical scavenging activity of the antioxidant. Each wine was diluted 1:10 with methanol immediately before the analysis. In the test tubes, 0.2 mL of samples was added to 4.8 mL of DPPH solution (5.2×10^{-6} mol/L in methanol) and the mixture was well mixed. The absorbance at 515 nm was measured at 30 min against a blank (0.2 mL methanol and 4.8 mL DPPH solution in methanol). All determinations were performed in triplicate.

The radical scavenging capacity (*RSC*) expressed in percentage was calculated by the following equation [12]:

$$RSC\ (\%) = 100(A_{\text{blanc}} - A_{\text{sample}})/A_{\text{blanc}} \quad (1)$$

The chart of the remaining DPPH concentration against the concentration of Trolox in the standard samples was used to calculate the total antioxidant activity (TAA) of the wines.

Performance liquid chromatography (HPLC-DAD) analysis of individual phenolic compounds

The concentration of individual anthocyanins, flavonols, flavan-3-ols and hydroxycinnamic acid was determined by HPLC, employing a direct-injection method [13,14]. The equipment used was an HPLC Agilent-1200 series with UV–Vis DAD for multi wavelength detection and fluorescence detection for acquisition of the emission response. The column was thermostated at 30 °C. After injecting 5 μL of sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6·150 mm column. Two solvents were used for the gradient elution: A – (H_2O+5%HCOOH) and B – (80% CAN + 5% HCOOH + H_2O). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gra-

Table 1. Studied Vranac wine samples

No.	Wine and vintage	Wine producer
1	Vranac, 2009.	Vinoprodukt Čoka, Subotica, Serbia
2	Vranac, 2009.	Rubin Kruševac, Serbia
3	Vranac, 2009.	Vino Župa, Aleksandrovac, Serbia
4	Crnogorski vranac, 2009	Plantaže 13. jul, Montenegro
5	Vranac-Pro corde, 2009.	Plantaže 13. jul, Montenegro
6	Crmnički Vranac-Barrique, 2009.	Vinarija Mašanović-Virpazar, Montenegro
7	Vranac, 2009.	Povardarie, Negotino, Macedonia
8	Tga za jug, 2009.	Tikveš, Macedonia
9	Vranac, 2009.	Skovin, Macedonia

dually increases 0–25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25-50% B, from 35 to 40 min gradually increases 50–80% B, and finally for the last 5 min gradually decreases 80–0% B. All identifications of individual compounds were based on the retention times of the original standards where available, and spectral data [15–18]. Monitoring of the evaluation was performed at 520 nm for the identification of anthocyanins, 360 nm for the identification of flavonols and 320 nm for the identification of hydroxycinnamic acids. Three flavan-3-ols including catechin, procyanidin B2 (dimer of proanthocyanidins epicatechin–epicatechin), and epicatechin were monitored at 275/322 nm ($\lambda_{ex}/\lambda_{em}$) with fluorescence detector. The results were expressed as mg/L of wine.

Statistical analysis

The experiments were reported at least 3 times, and the data were analyzed statistically. All results were given as mean ±standard deviation (*SD*). Statistical analysis was done by one-way analysis of variance (ANOVA), and significant differences between the results were determined by Duncan's multiple range test. The differences were considered significant at $p < 0.05$. Relationships between phenolic compound contents and antiradical efficiency were established using the Pearson correlation test ($p < 0.05$).

RESULTS AND DISCUSSION

Spectrophotometric analysis the content of polyphenols and flavonoids in Vranac wine samples

The results of the determination of total phenolic content in Vranac wine samples from different Balkan wine-producing regions by Folin–Ciocalteu method are presented in Table 2. The total phenolic content varied from 3478.70 to 3935.19 mg GAE/L (averaging 3729.90 mg GAE/L). There is a significant difference in the total phenolic content between the wines made of Vranac grape from different wine-producing regions of the Balkans. Wines from Montenegro have the highest level of total polyphenols (3830.32 mg GAE/L), as opposed to Macedonian (3808.20 mg GAE/L) and Serbian ones (3551.43 mg GAE/L). The total phenolic content of Vranac (Subotica, Serbia) and Vranac (Kruševac, Serbia) was significantly lower from the others ($p < 0.05$). Significant differences were found in the total phenolic content in comparison between "Crnogorski vranac" and "Crmnički vranac-Barrique" ($p < 0.05$); however, significant differences in total phenolic content were not found between Crnogorski vranac and "Vranac-Pro corde". Also, significant differences were found in the total phenolic content in comparison between "Tga za jug" (Macedonia) and Vranec (Negotino, Macedonia); however, significant differences in the total phenolic content were not found between Vranec (Negotino, Macedonia) and Vranec (Skovin, Macedonia). It is well known that genetic and agronomic or environmental factors play important roles in the phenolic composition and thus nutritional quality of crops. The mean concentration of the phenolic content of wine Vranac from the Balkan region was 3729.9 mg GAE/L. Our values for red wine are in the range of values determined by other authors. Majo *et al*. [19] measured 2360–3730 mg GAE/L for Sicilian red wines. Lucena *et al*. [4] measured 3200–5900 mg GAE/L for Brazilian red wines. But, Anli and Vural [20] measured lower values, 1070–2410 mg GAE/L, for Turkish red wines.

Total flavonoids of the Vranac wines were measured (Table 2). The Vranec (Negotino, Macedonia) showed the highest flavonoid content (2630.22 mg CE/L), followed by Vranac-Pro Corde, Crnogorski vranac and Crmnicki vranac-Barrique. The flavonoid content of wine samples 4, 5 and 6 (Macedonia) was significantly different from samples 1 and 2 (Serbia), $p < 0.05$. However, significant differences in the total flavonoid content were not found in comparison among sample 3 (Serbia) and samples 4–9. Only 1.14-fold difference in the in total flavonoid content was found between the highest and the lowest ranked wine samples, Vranec (Negotino, Macedonia) and Vranac (Kruševac, Serbia).

Table 2. Total phenol content (TP), total flavonoid content (TF), radical scavenging capacity (RSC) and total antioxidant activity (TAA) of wines; data are reported as mean±SD (n = 3); bars with no letters in common are significantly different (p < 0.05) in the same column

No. of sample	pH	Polyphenols, mg GAE/L	Flavonoids, mg CE/L	*RSC* / %	*TAA* / mmol TE L^{-1}
1	3.42	3528.04±28.53$^{fg1)}$	2328.53±8.35^{c}	75.62±0.36^{e}	13.22±0.20^{c}
2	3.45	3478.70±23.23^{g}	2297.32±8.56^{c}	74.40±0.36^{f}	13.00±0.26^{c}
3	3.42	3647.52±9.49^{ef}	2553.27±13.35^{ab}	78.91±0.26^{d}	13.79±0.26^{cb}
4	3.50	3935.19±22.68^{ab}	2597.14±12.50^{a}	86.12±0.26^{a}	15.02±0.35^{a}
5	3.48	3850.50±21.65^{cd}	2618.03±6.50^{a}	84.23±0.26^{b}	14.79±0.20^{a}
6	3.52	3705.22±18.59^{edc}	2593.54±14.76^{a}	79.09±0.20^{d}	13.82±0.20^{cb}
7	3.45	3868.54±19.17^{b}	2630.22±15.51^{a}	82.51±0.26^{c}	14.41±0.26^{ab}
8	3.51	3693.81±15.79^{ed}	2470.24±14.70^{b}	77.89±0,26^{d}	13.63±0.17^{c}
9	3.54	3862.23±17.51^{b}	2548.91±5.18^{a}	83.12±0.36^{c}	14.50±0.26^{ab}

Total antioxidant activity of Vranac wines using the DPPH scavenging assays

The radical scavenging activity was evaluated by measuring the scavenging activity of the examined red wine samples on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. All results of antioxidant analysis are summarised in Table 2. The investigated wines showed antioxidant behavior in the range from 74.40 to 86.12%. The percentage for Cabernet Sauvignon wines from the Balkan region was 71.30–83.53% [21]. The percentage for Croatian red wines was 54.6–82.6% [22]. Significant differences were found in the radical scavenging activity when comparing all samples (except for sample 3 and samples 6 and 8, and between samples 7 and 9)

Total antioxidant activity (*TAA*) of the Vranac wines, expressed in mmol of Trolox equivalent per L of red wine is shown in Table 2. Crnogorski vranac had the highest antioxidant activity (15.02 mmol TE/L), followed by Vranac-Pro Corde (14.79 mmol TE/L) and Vranec, Skovin, Macedonia (14.50 mmol TE/L).

TAA values of the Vranac wines from the Balkan region analyzed in this study were in the range obtained from red wines from other wine-producing countries such as Italy (7.8–19.8 mmol TE/L, [23]), Spain (4.65–17.41 mmol TE/L, [24]) and South Africa (9.51–12.30 mmol TE/L, [25]).

The wines containing high total phenolic contents had higher antioxidant activities. The present study reveals a very strong correlation between the total antioxidant activity and total phenolics (R^2 = 0.97). Also, a very good correlation between the total flavonoid content, and antioxidant activity (R^2 = 0.85) of the tested Vranac wine samples was confirmed.

HPLC Analysis

For a better description of Vranac red wine from the Balkan region, the profile of individual polyphenolic compounds were studied. In order to separate and determine individual phenolic compounds present in 9 wine samples from different geographical regions, HPLC method was applied.

The content and the distribution of anthocyanins in Vranac wine samples

The total of 12 phenolics compounds was identified as anthocyanins due to the information provided by their UV–Vis spectra. This primary information differentiated 5 glucosylated anthocyanins (*λ* maximum at 520 nm): delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and malvidin-3-*O*-glucoside: and 7 acylglucosylated anthocyanins: cyanidin-3-acetylglucoside, petunidin-3-acetylglucoside, peonidin-3-acetylglucoside, malvidin-3-acetylglucoside, petunidin-3-*p*-coumaroylglucoside, peonidin-3-*p*-coumaroylglucoside and malvidin-3-*p*--coumaroylglucoside.

Certain quantitative differences in anthocyanins between wine samples were presented in Table 3. The total content of anthocyanins varied from 298.39 (Vranac, Kruševac, Serbia) to 448.82 mg/L (Crnogorski vranac*,* Montenegro). The wines from Montenegro have the highest level of total anthocyanins (mean 408.38 mg/L), as opposed to Macedonian (mean 381.69 mg/L) and Serbian ones (mean 334.14 mg/L). Malvidin-3-*O*--glucoside, which is responsible for the blue-red color of wines, was the most abundant anthocyanin and its concentration was dependent on winemaking, whereas low concentrations (10 to 20 time lower that malvidin-3-*O*-glucoside) of delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and peonidin-3-*O*-glucoside were detected. Malvidin-3-*O*-glucoside content ranged from 179.04 to 281.31 mg/L. Cyanidin-3-*O*-glucoside was the anthocyanin present in the lowest amounts (2.98–9.15 mg/L). The values are within the range reported for red wines by other researchers [26,14,27].

Quantitatively, the content of glucosided anthocyanins was predominant in the investigated Vranac wines (mean amount of content was 310.82 mg/L – from 246.74 to 378.07 mg/L), followed by the content of acetylated ones (mean amount was 45.70 mg/L – from 37.47 to 53.69 mg/L) and the content od *p*-coumarylated ones (mean amount was 18.21 mg/L – from 14.18 to 22.14 mg/L).

The acetylated forms were mainly detected in wines produced through cryo-macetation, delestage, prolonged maceration and traditional technology [27]. Acelylated malvidin-3-glucoside was the main compound. In our case, malvidin-3-acetylglucoside varied from 67.6 to 92.2% with respect to total acetyl derivatives.

The coumarolated anthocyanin fraction in Vranac wines was composed of only three anthocyanin coumarates (malvidin-3-*p*-coumaroylglucoside, peonodin-3-p-coumaroylglucoside and petunidin-3-*p*-coumaroylglucoside). The most predominant p-coumaroyl derivative was malvidin-3-*p*-coumaroylglucoside which accounted for 77.5% (from 53.8 to 84.8%) of total *p*-coumaroyl derivative content. The importance of coumarylated forms is related to sensory analysis.

Another point worth mentioning is that the order of abundances based on average value of distribution for each anthocyanin was the following (Table 3):

Mvgl > Mvgl-ac > Ptgl > Pngl >Mvgl-p-coum >Dpgl, for Vranac wines produced in Serbia;

Mvgl > Mvgl-ac > Ptgl ≈ Pngl > Dpgl > Mvgl-p-coum, for Vranac wines produced in Montenegro;

Mvgl > Mvgl-ac > Pngl > Ptgl >Dpgl >Mvgl-p-coum, for Vranac wines produced in Macedonia.

Table 3. Anthocyanin composition of wine samples; (1) delphinidin-3-O-glucoside; (2) cyanidin-3-O-glucoside; (3) petunidin-3-O-glucoside; (4) peonidin-3-O-glucoside; (5) malvidin-3-O-glucoside; (6) cyanidin-3-acetylglucoside; (7) petunidin-3-acetylglucoside; (8) peonidin-3-acetylglucoside; (9) malvidin-3-acetylglucoside; (10) petunidin-3-p-coumaroylglucoside; (11) peonidin-3-p-coumaroylglucoside; (12) malvidin-3-p-coumaroylglucoside; TA, total anthocyanins; TA-G, total anthocyanin-glucosides, TA-A, total anthocyanin-acetylglucosides, TA-C, total anthocyanin-coumaroylglucosides. Results expressed as mg/L of wines and are presented with mean (n = 3)

Cmpd.	Sample No.								
	1	2	3	4	5	6	7	8	9
1	10.95±0.29[2]	8.56±1.14	12.03±2.36	18.83±2.15	12.32±1.14	14.18±0.09	20.18±2.11	17.94±1.54	19.30±2.09
2	3.25±0.01	2.98±0.01	9.15±1.10	4.88±0.03	6.05±0.01	2.87±0.21	2.85±0.21	5.23±0.29	5.80±0.00
3	29.05±2.01	28.55±2.15	29.32±3.00	36.51±3.14	27.78±1.14	20.08±3.00	25.62±3.01	28.78±0.24	30.72±4.54
4	28.53±2.42	27.61±3.12	27.98±3.14	36.54±2.15	27.69±2.01	19.92±1.14	30.89±5.00	32.12±1.14	36.23±2.54
5	*205.38*±6.38	*179.04*±4.06	*227.93*±8.01	*281.31*±5.14	*278.52*±8.62	*235.32*±6.67	*238.72*±9.01	*197.34*±2.77	*252.63*±6.67
6	1.93±0.29	1.97±0.03	4.05±0.04	2.26±0.01	1.22±0.29	1.79±0.04	2.58±0.01	2.29±0.01	1.93±0.00
7	5.07±0.31	4.47±0.78	6.08±0.11	4.69±0.07	2.00±0.21	3.17±0.01	3.26±0.04	3.08±0.06	2.85±0.04
8	2.85±0.22	1.98±0.21	4.25±0.23	2.38±0.04	0.88±0.45	2.38±0.01	3.02±0.32	2.67±0.11	1.57±0.00
9	32.73±3.04	20.05±2.66	30.02±3.18	44.36±4.12	48.28±3.14	34.58±2.15	36.58±2.96	35.78±4.17	45.28±5.24
10	1.83±0.21	1.59±0.21	1.92±0.11	1.65±0.29	3.90±0.06	2.85±0.01	2.13±0.11	1.44±0.00	2.18±0.11
11	1.12±0.14	1.47±0.14	1.88±0.01	1.38±0.21	4.88±0.07	2.38±0.01	1.49±0.00	1.29±0.00	1.40±0.00
12	13.52±0.36	11.12±0.24	13.22±0.45	14.03±1.14	10.24±1.14	13.05±0.45	18.52±0.45	15.34±1.04	18.05±1.14
TA	**336.21**	**298.39**	**367.83**	**448.82**	**423.76**	**352.57**	**385.84**	**341.30**	**417.94**
TA-G	277.16	246.74	306.41	378.07	352.36	292.37	318.26	281.41	344.68
TA-A	42.58	37.47	44.40	53.69	52.38	41.92	45.44	41.82	51.63
TA-C	16.47	14.18	17.02	17.06	19.02	18.28	22.14	18.07	21.63

According to the wine sample results, it is obvious that the content of anthocyanin constituents of single-cultivar wines coming from different viticulture regions and wine producers were different, which may be related to the thickness of the grape skin, the climate in which the grape was grown, the degree of ripeness of the grape, the application of different vinification techniques and the wines ages [21,28].

The content of flavonols, flavan-3-ols and hydroxycinnamic acids in Vranac wine sample

The concentrations of flavonols, flavan-3-ols and hydroxycinnamic acids determined by HPLC in the investigated wine samples are shown in Table 4.

The main flavonol was quercetin-3-glucoronide, followed quercetin in second and third place, respectively,

Table 4. Amount of flavonols (1–5), hydroxycinnamic acids (6–11) and flavan-3-ols (12–14) in wine samples; (1) myricetin-3-glucoside; (2) quercetin-3-glucoside; (3) quercetin-3-glucoronide; (4) myricetin; (5) quercetin; (6) t-caftaric acid; (7) GRP; (8) t-coutaric acid; (9) caffeic acid; (10) p-coumaric acid; (11) ferrulic acid; (12) catechin; (13) procyanidin dimer B2; (14) epicatechin. Results are presented with mean (n = 3) and expressed in mg/L of wine

Cmpd.	Sample No.								
	1	2	3	4	5	6	7	8	9
1	2.05±0.22[2]	1.85±0.02	2.26±0.01	1.85±0.04	1.22±0.00	0.95±0.00	2.18±0.01	2.03±0.08	1.89±0.00
2	2.95±0.11	3.17±0.37	3.08±0.01	3.15±0.07	2.98±0.02	2.65±0.01	4.79±0.10	4.65±0.14	3.08±0.04
3	*7.23*±1.00	5.*88*±0.07	*8.53*±0.03	*9.18*±1.08	*11.78*±0.96	*10.08*±0.84	*8.28*±1.00	*6.03*±0.23	*7.92*±0.33
4	2.65±0.12	1.99±0.00	1.76±0.00	1.68±0.00	1.00±0.00	1.12±0.03	3.39±0.22	0.89±0.00	1.85±0.02
5	3.23±0.11	2.02±0.09	3.65±0.01	4.25±0.16	5.32±0.21	3.62±0.11	5.96±0.25	2.83±0.01	6.32±0.29
Total	**18.13**	**14.93**	**19.28**	**20.60**	**22.30**	**18.42**	**23.60**	**16.43**	**21.06**
6	27.34±3.01[2]	13.46±0.78	23.58±1.24	15.34±0.77	17.89±1.16	16.93±0.95	32.52±3.54	17.74±1.10	38.56±2.96
7	2.97±0.02	1.86±0.01	1.52±0.01	4.18±1.11	1.22±0.00	2.11±0.01	4.69±0.22	3.97±0.09	1.35±0.00
8	17.56±2.31	12.85±1.67	17.62±2.81	2.45±0.09	12.58±1.47	10.86±0.87	9.52±0.45	4.80±0.04	14.32±2.78
9	3.12±0.14	1.55±0.00	2.96±0.12	6.95±2.01	4.72±0.02	3.28±0.04	1.52±0.01	6.02±0.03	3.05±0.02
10	3.96±0.01	3.08±0.03	3.28±0.17	2.81±0.12	1.92±0.01	1.32±0.00	1.03±0.00	2.39±0.04	1.85±0.01
11	0.98±0.00	2.11±0.01	0.62±0.00	3.62±0.14	1.89±0.00	1.18±0.00	10.43±1.12	4.28±0.32	1.33±0.00
Total	**55.83**	**34.91**	**49.58**	**35.35**	**40.22**	**35.68**	**59.71**	**39.20**	**60.46**

Table 4. Continued

Cmpd.	Sample No.								
	1	2	3	4	5	6	7	8	9
12	*62.05*±9.57[2]	*24.43*±1.74	*58.32*±4.12	*55.38*±6.01	*60.82*±5.67	*50.98*±4.65	*76.78*±6.13	24.50±2.10	*68.30*±5.63
13	23.78±0.44	12.39±1.12	19.90±1.40	30.28±2.63	38.09±2.14	37.80±3.00	14.50±2.61	12.37±1.04	17.82±1.74
14	19.53±1.23	15.98±0.99	18.98±1.22	39.70±3.11	29.31±1.74	17.30±2.51	14.74±0.57	*30.15*±2.94	19.20±2.11
Total	**105.36**	**52.80**	**97.20**	**125.36**	**128.22**	**105.38**	**109.02**	**67.02**	**105.32**

by quercetin-3-glucoside. The content in myricetin and its derivatives accounts for approximately 15.0–38.4% of total flavonols in Vranac variety wines. Kaempferol are not detected in all wine samples.

The levels of quercetin and its derivatives (11.07––20.08 mg/L) and myricetin and its derivative (2.07––5.57 mg/L) are highest compared to Croatian wines (from Central and Southern Dalmatia, 3.6–10.4 mg/L and 0.5–3.3 mg/L, respectively [29]) and are in agreement with values obtained from Italian red wines (2.8––28.5 mg/L and 0.6–9.6 mg/L, respectively [23]). Variations in flavonol content of individual wines may be explained by several factors. It has long been known that the increased biosynthesis of polyphenols, especially flavonols, is greatly influenced by sunlight exposure and temperature, so it would be normally expected that the wines made from grapes, which are grown in warmer, sunnier areas, have a higher level of flavonols. Wines from Montenegro and Macedonia have the highest average level of total flavonols (20.44 and 20.36 mg/L, respectively) as opposed to Serbian ones (17.45 mg/L).

Hydroxycinnamic acids and their tartaric acid derivatives have been monitored at 320 nm, since is their characteristic wavelength, together with GRP (grape reaction product or 2-5-glutathionyl-*t*-caftaric acid). GRP is the product of the reaction between caftaric acid and glutation, which, as can be observed, is only detectable in the wine and not in the grape skins [14].

The predominant hydroxycinnamic acid was *t*-caftaric (13.46–38.56 mg/L), followed by *t*-coutaric (2.45––17.62 mg/L), caffeic (1.55–6.95 mg/L) and *p*-coumaric (1.32–3.96 mg/L). Ferulic acid was the hydroxycinnamic acid present in the lowest amounts (0.62–3.62 mg/L) in the Vranac wines from Serbia and Montenegro. *p*-Coumaric acid was the hydroxycinnamic acid present in the lowest amount (1.03–2.39 mg/L) in Vranac wines from Macedonia. There is a significant difference in the total hydroxycinnamic acid content between wines made of Vranac grape from different wine-producing regions of the Balkans. Wines from Macedonia have the highest average level of total hydroxycinnamic acids (53.12 mg/L), as opposed to Serbian (46.77 mg/L) and Montenegran ones (37.08 mg/L).

Many studies on the health benefits studies of red wine have been linked to the catechin content. Catechins possess antioxidant properties [20] and exert a more potent antioxidant effect than flavonols and polymeric anthocyanidins [30]. During vinification, only a portion of catechins and procyanidins is extracted from seeds and is diffused to wine [31]. The use of fluorescence detector has allowed increasing selectivity and sensitivity for the determination of the concentration of catechin and epicatechin in addition to other phenolic compounds [32]. Catechin, epicatechin, and procyanidin dimer (B2) were identified by fluorescence detector in wine samples (Fig. 4). The relative amounts of catechin, epicatechin and procyanidin B2 (three flavan-3-ols compounds) were the highest in Vranac wines from Montenegro (means, 55.73, 35.39 and 28.77 mg/L, respectively). Similar values for the determination of catechin, epicatechin and procyanidin B2 have been reported by others (55.2, 48.5 and 10.8 mg/L, [26]; 31.01, 12.78 and 8.96 mg/L [14]).

PCA Analysis

The large number of the data which are the results of anthocyanin, flavanols, hydroxycinnamic and flavan-3-ols analysis of vranac wine samples, was reduced using PCA analysis regarding to make easily interpretation of the experimental data. According to such a plot, it is possible to classify Vranac wine samples by their geographical origin.

One of the main objectives of PCA is to identify factors that are substantially meaningful. Experimental data were set in matrix with dimensions 9×26. Using latent root (eigenvalue) criteria [32], as a result of PCA calculations, 6 new variables were obtained which were characterized by consecutive eigenvalues 7.71 (29.6%), 6.16 (23.69), 4.34 (16.68%), 3.38 (13.01%), 1.74 (6.71%), 1.37 (5.28%). Two first PCs account 53.30% of the total variance. Taking into account that the first two components show high percentage of the total variance (50%), the distribution of wine samples is illustrated in two-dimensional plot of PC1 versus PC2.

As illustrated in Fig. 1, it is possible to separate three groups of samples what is according to their geographical origin. They are differenced because of their content of individual phenolic compounds. Vranac wine samples with low concentrated of the analyzed phenolic compounds are located on the left side of the

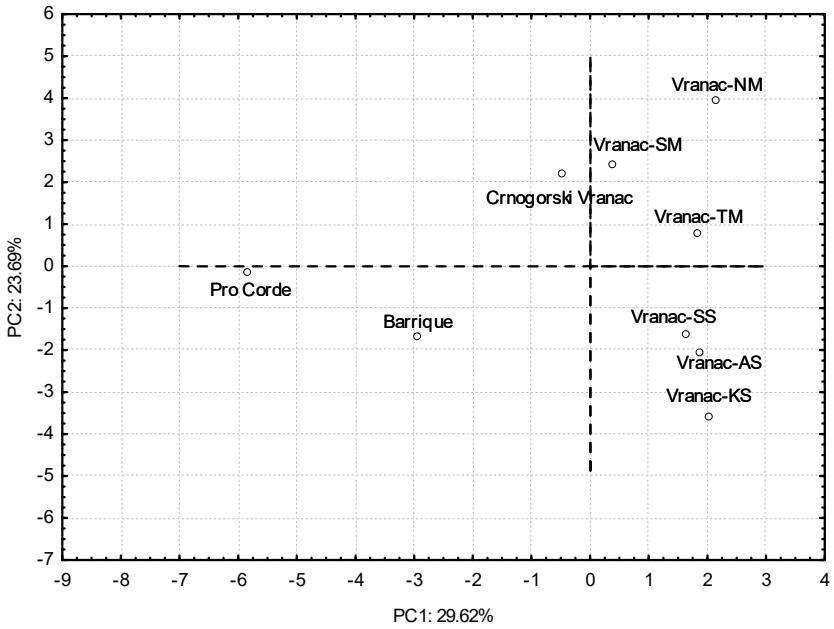

Figure 1. Scatterplots of the first two principal components (PC1 vs. PC2) for Vranac wine samples.

plot, but samples rich in phenolic compounds, can be found on the opposite side.

The behavior of variables on the PC1 and PC2 is shown in Figure 2. Also, the loadings and communality of each phenolic compound are given in Tables 5 and 6. The loading were large for cyanidin-3-acetylglucoside (6), petunidin-3-acetylglucoside (7), peonidin-3-acetylglucoside (8), myricetin-3-glucoside (13) and myricetin (16) on the first component, for delphinidin-3-*O*-glucoside (1), peonidin-3-*O*-glucoside (4), malvidin-3-*O*-glucoside (5), malvidin-3-acetylglucoside (9), malvidin-3-*p*-coumaroylglucoside (12), quercetin-3-glucoside (14), quercetin (17), *t*-caftaric acid (18), GRP (19), ferrulic acid (23) and catechin (24) on the second component, for myricetin (16), t-caftaric acid (18), t-coutaric acid (20) and catechin (24) on the thrd component, for cyanidin-3-*O*-glucoside (2), *t*-coutaric acid (20) and catechin (24) on the forth component, for cyanidin-3-*O*--glucoside (2) on the sixth component. Table 6 gives the score values for each principal component for each vranac wine sample. From the scores on the first principal component it can be interpreted that the concentrations of cyanidin-3-acetylglucoside (6), petunidin-3--acetylglucoside (7), peonidin-3-acetylglucoside (8), myr-

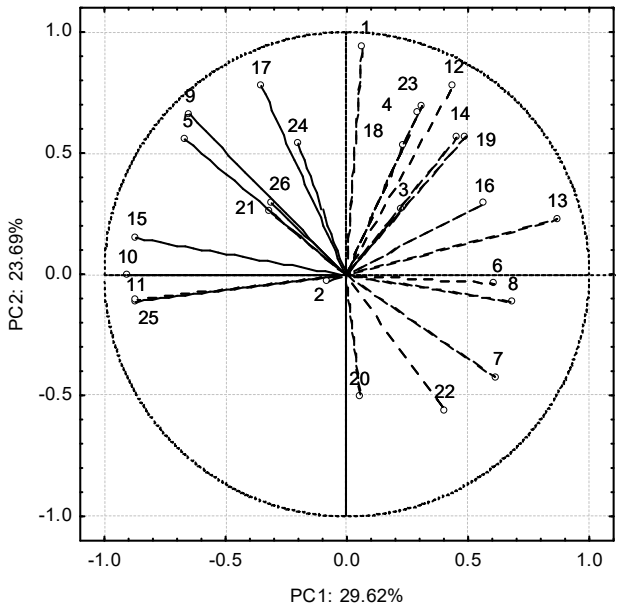

Figure 2. The score plot of the phenolic compounds in the space of the two first PCs (PC1 vs. PC2).

Table 5. The loadings of the first six principal components; (1) myricetin-3-glucoside; (2) quercetin-3-glucoside; (3) quercetin-3-glucoronide; (4) myricetin; (5) quercetin; (6) t-caftaric acid; (7) GRP; (8) t-coutaric acid; (9) caffeic acid; (10) p-coumaric acid; (11) ferrulic acid; (12) catechin; (13) procyanidin dimer B2; (14) epicatechin. Results are presented with mean (n = 3) and expressed in mg/L of wine

Phenolic compound	PC1	PC2	PC3	PC4	PC5	PC6
1	0.065	0.939	–0.091	–0.072	–0.078	0.127
2	–0.076	–0.033	–0.247	0.758	–0.188	0.557
3	0.222	0.271	–0.679	0.477	0.312	–0.177
4	0.289	0.666	–0.433	0.305	0.412	0.021
5	–0.669	0.557	–0.113	0.385	–0.153	–0.168
6	0.609	–0.041	0.003	0.494	–0.546	0.257
7	0.612	–0.424	–0.175	0.491	–0.264	–0.302
8	0.686	–0.115	0.056	0.293	–0.627	0.020
9	–0.646	0.660	–0.186	0.259	0.109	0.018
10	–0.905	–0.003	0.351	0.040	–0.091	0.057
11	–0.869	–0.118	0.098	0.043	–0.132	0.163
12	0.440	0.781	0.276	0.013	0.075	0.127
13	0.868	0.228	–0.074	0.343	0.041	0.0599
14	0.492	0.568	–0.014	–0.429	–0.185	0.295
15	–0.867	0.152	0.128	0.246	–0.354	–0.134
16	0.567	0.290	0.530	0.099	0.0297	–0.501
17	–0.354	0.776	0.397	0.311	0.096	0.046
18	0.235	0.533	0.595	0.394	0.301	0.079
19	0.453	0.565	–0.267	–0.401	–0.315	–0.339
20	0.052	–0.506	0.634	0.510	0.195	0.037
21	–0.315	0.257	–0.872	0.034	–0.103	0.021
22	0.403	–0.562	–0.412	0.421	0.191	–0.268
23	0.311	0.694	0.181	–0.426	–0.279	–0.087
24	–0.202	0.537	0.530	0.518	–0.118	–0.317
25	–0.869	–0.110	–0.059	0.112	–0.264	–0.333
26	–0.310	0.291	–0.890	0.075	–0.0518	–0.090

Table 6. The scores of the first six principal components; Vranac-SS (Vinoprodukt Čoka, Subotica, Serbia); Vranac-KS (Rubin Kruševac, Serbia); Crnogorski Vranac (Plantaže 13. jul, Montenegro); Pro Corde (Plantaže 13. jul, Montenegro); Barrique (Vinarija Mašanović-Virpazar, Montenegro); Vranac NM (Povardarie, Negotino, Macedonia); Vranac-TM (Tikveš, Macedonia); Vranac-SM (Skovin, Macedonia)

Wine sample	PC1	PC2	PC3	PC4	PC5	PC6
Vranac-SS	0.596	–0.772	–0.902	0.455	0.642	–1.520
Vranac-KS	0.729	–1.457	–0.851	–0.930	0.961	–0.110
Vranac-AS	0.681	–0.828	–0.825	1.808	–1.325	0.916
Crnogorski Vranac	–0.160	0.867	–1.349	0.399	–0.349	–1.312
Pro Corde	–2.102	–0.057	–0.015	0.181	0.147	0.287
Barrique	–1.058	–0.696	0.706	–0.976	–0.960	–0.164
Vranac-NM	0.785	1.577	1.401	–0.665	–0.827	–0.386
Vranac-TM	0.670	0.308	–0.827	–1.146	–0.066	1.490
Vranac-SM	–0.141	0.958	0.610	0.872	1.776	0.798

icetin-3-glucoside (13) and myricetin (16) on the first principal component loadings are higher for Vranac-SS, Vranac-KS, Vranac-As, Vranac-NM and Vranac-TM than the other wine samples and are lower for Pro Corde, Barrique and Crnogorski Vranac. When the second principal component is interpreted delphinidin-3-*O*--glucoside (1), peonidin-3-*O*-glucoside (4), malvidin--3-*O*-glucoside (5), malvidin-3-acetylglucoside (9), malvidin-3-*p*-coumaroylglucoside (12), quercetin-3-glucoside (14), quercetin (17), t-caftaric acid (18), GRP (19),

ferrulic acid (23) and catechin (24) are higher for Crnogorski Vranac, Vranac-NM and Vranac-SM and are lower for Vranac-KS and Vranac-AS than the other wine samples investigated. On the third principal component myricetin (16), *t*-caftaric acid (18), *t*-coutaric acid (20) and catechin (24) are higher for Vanac-NM, Barrique and Vranac-SM and are lower for Crnogorski Vranac, Vranac-KS and Vranac-TM than for the other samples. Cyanidin-3-*O*-glucoside (2), *t*-coutaric acid (20) and catechin (24) concentrations in the fourth principal component are higher for Vranac-AS and Vranac-SM and lower for Vranac-TM, Barrique and Vranac-KS. Finally, cyanidin-3-*O*-glucoside (2) concentration on the sixth principal component are higher for Vranac-TM, Vranac-AS and Vranac-SM and lower for Vranac-SS and Crnogorski Vranac.

The classification of the Vranac wine samples from the view point of phenolic compounds was also made using two (Fig. 3) and three way PC score graphs (Fig. 4). The PC 1-2 and PC 1-2-3 graph shows the highest percentage of total variance of 53.30 and 69.99%, respectively. It can be seen from the PC 1-2 and PC 1-2-3 graph that the vranac wine sample can be classified into three groups. These groups include: Vranac-SS, Vranac-KS and Vranac-AS (group 1), Pro Corde and Barrique (group 2) and Vranac-NM, Vranac-TM, Vranac-SM and Crnogorski Vranac (group 3).

CONCLUSIONS

In recent years, phytochemicals, especially phenolics, have attracted increasing attention for their anti-

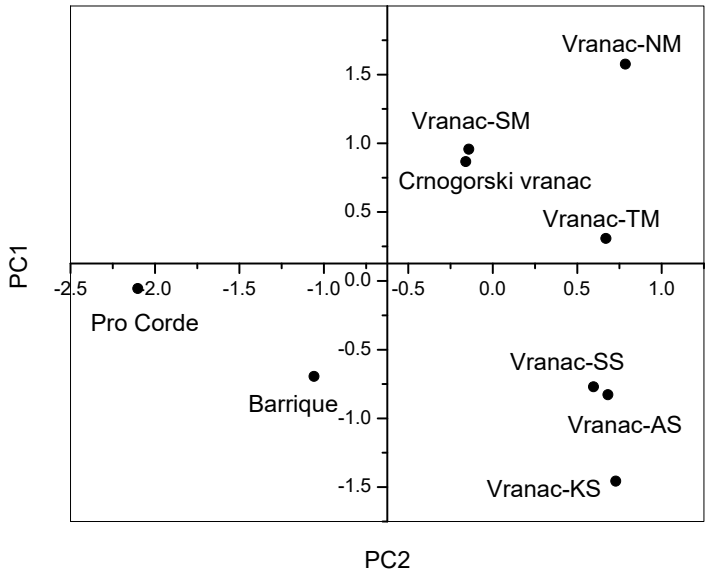

Figure 3. Two way PCA score plot (PC1 vs. PC2).

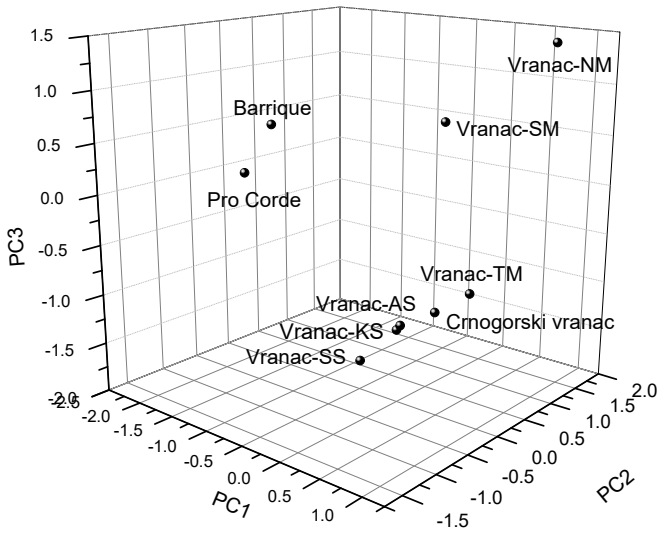

Figure 4. Three way PCA score plot (PC 1-2-3).

oxidant activities. Wines provide phenolic antioxidant, which contribute to their potential health benefits. This work has shown that the phytochemicals present in wines produced in the Balkan region have potent antioxidant activities and that the antioxidant activity in wines is positively correlated with total phenolic content. Red wines produced in Montenegro were found high in anthocyanins and flavonols. Also, red wines from Serbia and Macedonia were good source of hydroxycinnamic acids. From the obtained results, we can conclude that the content of phenolic compounds in the single-cultivar investigated wines (Vranac) depends most of all on agroclimatic factors and genological practices of the particular Balkan vineyard producing wine.

Acknowledgments

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REFERENCES

[1] R.B. Boulton, The copigmentation of anthocyanins and its role in the colour of red wine: A critical review. Am. J. Enol. Vit. **55** (2001) 67–87.

[2] E. Puértolas, G. Saldaña, S. Condón, I. Álvarez, J. Raso, Evolution of polyphenolic compounds in red wine from Cabernet Sauvignon grapes processed by pulsed electric fields during aging in bottle, Food Chem. **119** (2010) 1063–1070.

[3] D.D. Majo, M. Guardia, S. Giammanco, L. Neve, M. Giammanco, The antioxidant capacity of red wine in relationship with its polyphenolic constituents, Food Chem. **111** (2008) 45–49.

[4] A.P S. Luceana, R.J.B. Nascimento, J.A.C Maciel, J.X. Tavares, J.M. Barbasa-Filho, E.J. Oliviera, Antioxidant activity and phenolics content of selected Brazillian wines, J. Food Compos. Anal. **23** (2010) 30–36.

[5] K. Höner, R. Cervellati, C. Neddens, Measurements of the *in vitro* antioxidant activity of German white wines using a novel method, Eur. Food Res. Technol. **214** (2002) 356–360.

[6] E. Maletić, J.K. Kontić, D. Preiner, A. Jeromel, C.D. Patz, H. Dietrich, Anthocyanin profile and antioxidant capacity of some autochthonous Croatian red wines, J. Food Agric. Environ. **7** (2009) 48–51.

[7] N. Pellegrini, P. Simonetti, C. Gordana, O. Brenna, F. Brighenti, P. Pietta, Polyphenol content and total antioxidant activity of Vini Novveli (young red wines), J. Agric. Food Chem. **48** (2000) 732–735.

[8] D.P. Markis, E. Psarra, S. Kallithraka, P. Kefalas, The effect of polyphenolic composition as related to antioxidant capacity in white wines, Food Res. Int. **36** (2003) 805–814.

[9] V.L. Singleton, C.F. Timberlake, A.G.H. Lea, The phenolic cinnamates of white grapes and wine, Method in Enzymol. **299** (1999) 152–178.

[10] Z. Jia, T. Tang, J. Wu, The determination of flavonoids content in mulberry and scavenging effect on superoxide radicals, Food Chem. **64** (1999) 555–559.

[11] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, Lebensm. Wiss. Technol. 28 (1999) 25–30.

[12] G.C. Yen, P.D. Duh, Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species, J. Agric. Food Chem. **42** (1994) 629–632.

[13] R.M. Lamuela-Raventós, A.L. Waterhouse, A direct HPLC separation of wine phenolics, Am. J. Enol. Viticult. **45** (1994) 1–5.

[14] S. Gomez-Alonso, E. Garcia-Romero, E. Hermovin-Guiteirrez, HPLC analysis of diferse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence, J. Food Compos. Anal. **20** (2006) 618–626.

[15] M. Gomez-Miguez, L.M. Gonzalez-Miret, F.J. Heredia, Evolution of colour and anthocyanin composition of syrah wines elaborate with prefermentative cold maceration, J. Food Eng. **79** (2007) 271–278.

[16] E. Revilla, J.M. Ryan, Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography-photodiode array detection without sample preparation, J. Chromatogr., A **881** (2000) 461–469.

[17] M. Monagas, C. Gomez-Cordoves, B. Bartolome, O. Laureano, J.M.R. Silva, Monomeric, Oligomeric, and Polymeric Flavan-3-ol Composition of Wines and Grapes from Vitis Vinifera L. Cv. Graciano, Tempranillo and Cabernet Sauvignon, J. Agr. Food Chem. **51** (2003) 6475–6481.

[18] M.N. Mitić, J.M. Souquet, M.V. Obradović, S.S. Mitić, Phytochemical Profiles and Antioxidant Activities of Serbian Table and wine Grapes, Food Sci. Biotechnol. **21** (2012) 1619–1626.

[19] D. Di Majo, M. La Guardia, S. Giammanco, L. La Neve, M. Giammanco, The antioxidant capacity of red wine in relationship with its polyphenolic constituents, Food Chem. **111** (2008) 45–49.

[20] R.E. Anli, N. Vural, Antioxidant Phenolic Substances of Turkish Red Wines from Different Wine Regions, Molecules **14** (2009) 289–279.

[21] B. Radovanović, A. Radovanović, Free Radical Scavenging Activity and Anthocyanin Profile of Cabernet sauvignon Wines from the Balcan Region, Molecules **15** (2010) 4213–4226.

[22] V. Katalinić, M. Miloš, D. Modun, I. Musić, M. Boban, Antioxidant effectiveness of selected wines in comparison with (+)-catechin, Food Chem. **86** (2004) 593–600.

[23] P. Simonetti, P. Pietta, G. Testolin, G. Polyphenol Content and Total Antioxidant Potential of Selected Italian Wines, J. Agr. Food Chem. **45** (1997) 1152–1155.

[24] M.S. Fernandez-Pachon, D. Villano, M.C. Garcia-Parrila, A.M. Troncoso, Antioxidant activity of wines and relation with their polyphenolic composition, Anal. Chim. Acta **513** (2004) 113–118.

[25] D. De Beer, E. Joubert, W.C.A. Gelderblom, M. Manley, Antioxidant Activity of south African Red and White Cul-

tivar Wines: Free Radical Scavenging, J. Agr. Food Chem. **51** (2003) 902–909.

[26] S. Kallithraka, E. Tsoutsouras, E. Tzourou, P. Lanaridis, Principal phenolic compounds in Greek red wines, Food Chem. **99** (2006) 784–793.

[27] A. Baiano, C. Terracone, G. Gambacorta, E.L. Notte, Phenolic Content and Antioxidant Activity of Primitivo Wine: Comparison Among Winemaking Technologies, J. Food Sci. **74** (2009) 258–267.

[28] S. Kallithraka, A. A. Mohdaly, D. P. Makris, P. Kefalas, Determination of major anthocyanin pigment in Hellenic native grape varieties (vitis vinifera sp.): Association with antiradical activity, J. Food Compos. Anal. **18** (2007) 375–386.

[29] V. Rastija, G. Srečnik, M. Medić–Šarić, Polyphenolic composition of Croatian wines with different geographical origins, Food Chem. **115** (2009) 54–60.

[30] D.M. Blackhurst, A.D. Marais, Concomitant consumption of red wine and polyunsaturated fatty acids in edible oil does not influence the peroxidation status of chylomicron lipids despite increasing plasma catechin concentration, Nutr. Metab. Cardiovasc. Dis. **16** (2006) 550––558.

[31] S. Gonzalez-Manzano, J.C. Rivas-Gonzalo, C. Santos--Buelga, Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration, Anal. Chim. Acta **513** (2004) 283–289.

[32] F.S. Dias, M.P. Lovillo, C.G. Barroso, J.M. David, Optimization and validation of a method for the direct determination of catechin and epicatechin in red wines by HPLC/fluorescence, Microchem. J. **96** (2010) 17–20.

[33] I.T. Jolliffe, Principal Component Analysis. Second ed. Springer Series in Statistics. New York: Springer-Verlag New York, 2002.

IZVOD

ANTIOKSIDATIVNA AKTIVNOST I POLIFENOLNI SASTAV CRVENOG VINA VRANAC SA PODRUČJA BALKANA

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(Naučni rad)

Poslednjih godina mnoga istraživanja su dokazala da fenolna jedinjenja imaju presudnu ulogu u antioksidativnoj aktivnosti mnogih prehrambenih proizvoda. Vina su odličan izvor polifenola, uključujući derivate benzoeve i cimetne kiseline, flavan-3-ole, flavonole i antocijane. Glavni cilj našeg istraživanja je bio da se ispita korelacija između radikal-hvatačkog kapaciteta srpskih, makedonskih i crnogorskih vina Vranac sa različitih geografskih područja u odnosu na sadržaj fenola, flavonoida i individualnih polifenolnih jedinjenja. Sva analizirana vina su pokazala visoku antioksidativnu aktivnost od 13.00 do 15.02 mmol/L, dok je sadržaj fenolnih jedinjenja bio između 3478.70 i 3935.19 mg/L. U cilju razdvajanja i određivanja individualnih fenolnih jedinjenja (flavan-3-ola, flavonola, hidroksicimetnih kiselina i antocijana) upotrbljena je HPLC metoda, metoda direktnog injektiranja. Identifikacija je vršena na osnovu retencionih vremena i spektralnih osobina određenih komercijalnih standarda. Nakon analize je utvrđeno da je najzastupljeniji antocijanin malvidin-3-glukozid (179,04–281,31 mg/L), najzastupljeniji flavonol je kvercetin-3-glukoronid (5,88–11,78 mg/L), najzastupljeniji flavan-3-ol je katehin (24,43–76,78 mg/L), dok *t*-kaftarna kiselina (13,46–38,56 mg/L) spada u red najzastupljenijih hidroksicimetnih kiselina. Na osnovu dobijenih rezultata, spektrofotometrijskom i HPLC metodom, mozemo pouzdano tvrditi da vina sa područja Balkana pokazuju visoku antioksidativnu aktivnost i da je ona u pozitivnoj korelaciji sa fenolnim sadržajem. Takođe se može zaključiti da sadržaj fenolnih jedinjenja u vinima proizvedenim od iste sorte grožđa pre svega zavisi od agroklimatskih faktora, uslova gajenja grožđa i procesa proizvodnje vina.

Ključne reči: Crveno vino • Vranac • Fenolni profil • Antioksidativna aktivnost • HPLC-DAD

The effect of ethoxylated oleyl-cetyl alcohol on metabolism of some fungi and their potential application in mycoremediation

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Abstract

The effect of ethoxylated oleyl-cetyl alcohol at a concentration of 1% on the growth and metabolism of *A. tenuis* Nees and *P. verrucosum* Dierckx was examined in this paper. The fungal growth was investigated by monitoring the diameter of colonies on solid media and dry weight biomass in liquid media. *A. tenuis* had better response to applied pollutant in solid medium, whereas *P. verrucosum* had better response in liquid medium. During exponential fungal growth in liquid media with and without pollutant (control), the following physico-chemical and biochemical parameters were carried out: pH, quantity of free and total organic acids, proteins, carbohydrates, proteolytic activity. The ethoxylated oleyl--cetyl alcohol had influence on decrease in pH value and increase in free organic acids of both fungi. Furthermore, it has influenced production in way that lower amount of total organic acids, proteins, glucose and fructose were gained in fermentation broth of *P. verrucosum* compared to *A. tenuis*. The proteolityc activity of fungi was partially (*A. tenuis*) or fully inhibited (*P. verrucosum*) by presence of pollutant in liquid medium. Based on the obtained results, these fungal species act as potential candidates for mycoremediation of alcohol ethoxylated contaminated environments and biotechnology.

Keywords: colony diameter, dry weight biomass, monosaccharides, organic acids, proteins, proteolytic activity.

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Ethoxylated oleyl-cetyl alcohol (Fig. 1) is surfactant from the group of fatty alcohol ethoxylates (FAEs) that are representing the most important group of non-ionic surfactants from economical point of view. FAEs are widely used in domestic and commercial detergents, household cleaners and personal care products. They are employed as wetting and washing agents in the cosmetics, agriculture, paper, oil and other sectors of processing industry. Constant increase in production volume of these non-ionic surfactants in the world over the past 20 years, especially in Europe, is conditioned by many FAEs desired characteristics, such as rapid biodegradation, low-to-moderate foaming ability, superior cleaning of man-made fibers, tolerance of water hardness and ability to perform in cold water [1].

On the other hand, rapid growth of production alcohol ethoxylates (AEs) in the world points to the possibility of an increased quantity of this pollutant in aquatic ecosystems at concentrations above expectations. After usage, residual surfactants and their deg-

Name	Structure	Formula
		$R\text{-}O(CH_2CH_2O)n\text{-}H$
Ethoxylated oleyl-	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CH_2O\text{-}$	R-blend of oleyl and cetyl
cetyl alcohol (AOC)	$CH_2CH_2OCH_2(CH_2)_{14}CH_3$	alcohol
		n-number of ethylene oxide

Figure 1. Chemical structure surfactant.

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radation products are discharged to sewage treatment plants or directly to surface water sand sediments [2]. Experimental results from many biodegradation studies in laboratory and field conditions suggest that there is a high quantity of primary and ultimate biodegradation

of these surfactants in the environment [3]. AEs are degraded biologically by wastewater treatment plant (WWTP) in excess 95–99% [4,5]. Concentration of total AEs in WWTP effluents is in the range 1.0–23 μg/l in Europe, Canada and USA [6,7]. From the middle of '70s until today, several environmental risk assessments were carried out on AEs [8,9]. These surfactants have a strong affinity for sorption to solids such as activated sludge, river water solids and, ultimately, sediments [10–12]. Level of toxicity to aquatic organisms, measured by EC_{50}, ranges from very toxic (< 1 mg/l) to harmful (between 10–100 mg/l). Also, during the mention time period, studies related to understanding the biodegradation mechanisms of AEs in the presence of complex microbial communities were carried out using different methods. Several AEs degrading bacteria were isolated under aerobic [13] and anaerobic conditions [14,15]. In the last two decades, bacteria were in the focus of bioremediation studies, unlike fungi which were studied much less. Mycoremediation is an innovative biotechnology that uses living fungus for *in situ* and *ex situ* cleanup and management of contaminated sites [16]. Filamentous fungi have ability to grow on wide spectrum of substrates by secreting extracellular hydrolytic enzymes, even capable of growing under ambient environment. Moreover, due to the low substrate specificity of their degradative enzyme machinery, fungi are able to perform the breakdown of a wide range of organic and xenobiotic pollutants: petroleum hydrocarbons, chlorophenols, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dioxins and furans, pesticides, herbicides and nitroaromatic explosives [17,18]. These fungal properties are utilized in a variety of processes (biological control agent, biobleaching, bioremediation, waste treatment).

Based on our previous researches [19–22], it was identified that several species of fungi such as *A. niger*, *T. roseum*, *F. oxysporum, etc.*, can grow and metabolize ethoxylated oleyl-cetyl alcohol at a wide concentration range 0.01–1%. For this reason, current study was conceptualized in order to investigate the effect of ethoxylated oleyl-cetyl alcohol at a concentration of 1% on the growth of selected fungi and changes of their metabolic activity. Isolation and identification of fungi from aquatic ecosystems that are resistant to the presence of high concentrations of this pollutant on the one hand, and the effect of a pollutant on their metabolism on the other hand, are crucial parameters for application of fungi in mycoremediation.

EXPERIMENTAL

Isolation and identification of fungi from wastewater

The fungi applied in this study were isolated from the sample of wastewater river basin of Lepenica, Kragujevac (the place of wastewater flood, sewage). The sample of water was taken in a sterile container. The sample was transferred to the microbiology laboratory and was afterwards inoculated onto Petri plate's nutrient malt agar with streptomycin (in duplicates). The Petri plates were then incubated for 5–7 days at standard temperature 28±2 °C. Pure cultures were obtained by the method of exhausting on poor malt agar plates and potato dextrose agar (PDA) plates. Identification of the fungi was based primarily on the macroscopic and microscopic morphology and was carried out by Systematic keys at the Faculty of Biology, University of Belgrade, Serbia. The fungi selected as test organisms in this study were: *Alternaria tenuis* Nees (1817) and *Penicillium verrucosum* Dierckx (1913). The fungi were maintained on PDA plates, stored at 4±0.5 °C and subcultured monthly in sterile conditions.

Inoculums preparation

Inoculums suspensions were prepared from fresh, mature (from 3- to 5-day-old) cultures grown on PDA plates. The colonies were covered with 5 ml of distilled sterile water. The inoculums were achieved by carefully rubbing the colonies with a sterile loop; the isolates were shaken vigorously for 15 s with a Vortex mixer and then transferred to a sterile tube. The inoculums sizes were adjusted to 1.0×10^6 spores/ml by microscopic enumeration with a cell-counting hematocytometer (Neubauer chamber).

Cultivation of fungi on solid media and culture condition

The Czapek Dox's solid media was prepared according to the formulation shown in Figure 2, with addition of 20 g agar-agar and autoclaved at 121 °C for 20 min (autoclave pressure, 0.14 MPa). After cooling to 45 °C, culture media were dispensed into sterile Petri dishes

Growth medium	Mark	*c* (g/l)					
		$NaNO_3$	K_2HPO_4	$MgSO_4 x7H_2O$	$FeSO_4 x7H_2O$	Sucrose	AOC[a]
Control	C	3	1	0.5	0.01	30	
C + 1 % AOC	AOC	3	1	0.5	0.01	30	10

[a]AOC - Ethoxylated oleyl-cetyl alcohol (Henkel, Krusevac)

Figure 2. Composition of growth media in 1000 ml distillated water.

for solidification. The tested fungi were inoculated at the center of the agar plates. The plates were incubated at room temperature over 8 days, in order to examine the exponential growth of fungi.

Cultivation of fungi in liquid media and culture condition

The Czapek Dox's liquid growth media (100 ml) was prepared in 250 ml Erlenmeyer flask, according to procedure mentioned above, but without addition of agar. One ml spore suspension of both fungi was inoculated in liquid media. Following inoculation, Erlenmeyer flasks were placed on an orbital shaker (Kinetor-m, Ljubljana) thus enabling uniform and constant mixing. All Erlenmeyer flasks were incubated at room temperature, under alternate light and dark for 8 days. Sampling has begun on day 4 and repeated daily until the end of experiment. All experiments were conducted in triplicate.

Measurement of pH values

A pH value of the fermentation broth (initial pH value about 5.0) in the experiment was measured by a pH meter (type MA-5705, the product "Iskra", Kranj) during fungal growth from day 4 to day 8.

Determination of colony diameters

Colony diameters (*CD*) were measured with a ruler at intervals of 24 h from inoculation until day 8. The growth curves were constructed from the diameter of the colonies (cm) *versus* incubation time (day). From the growth curves, the exponential growth phases of fungi were determined in the period of cultivation from day 4 to day 8. This period was selected for further study of physicochemical and biochemical parameters of fungi in liquid growth media.

Determination of dry weight biomass

The 8-day-old mycelia was separated from the fermentation broth by filtration through pre-weighed filter paper. The mycelia was washed with distilled water several times. Filter papers with mycelia were dried in an oven at 80 °C to constant weight. The dry weight of the mycelia was calculated by subtracting the initial weight of the filter paper from the weight of mycelia and filter paper. The results are presented as g/l.

Determination of concentrations total and free organic acids

The concentration of free and total organic acids (FOA and TOA) was determined by ion exchange chromatography according to method by Bullen *et al.* [23], as described in greater detail in our previous work [20]. The results are presented as percentages (%).

Determination of monosaccharides quantity

The quantity of monosaccharides, glucose and fructose, were also determined by ion exchange chromatography according to procedure which is discribed in our previous work [21]. The results are presented as percentages (%).

Determination of protein concentration

Protein concentration in fermentation broth of fungi was determined according to the method by Kjeldahl [24]. A sample was digested with a strong acid so that it releases nitrogen, which was determined by a suitable titration technique. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used for calculating the quantity of protein, according to the Eq. (1):

$$\text{The quantity of proteins (mg)} = 6.25 \times \text{quantity of nitrogen} \quad (1)$$

Assays of alkaline protease activity (EC 3.4.21-24)

The assay of alkaline protease was carried out by Anson's method [25]. Reaction mixture, which contained 5 ml of casein and 1 ml fermentation broth, was incubated at 37 °C for 30 min. The reaction was stopped by adding 1 ml of 5% trichloroacetic acid (TCA). The mixture was centrifuged at 4.000 rpm/min and then 5 ml of 6% Na_2CO_3 and 1 ml diluted Folin–Ciocalteu's phenol reagent were added to supernatant. The solution was kept at room temperature for 30 min and absorbance was read at 660 nm using tyrosine standard. One unite of alkaline protease activity was defined as the amount of enzyme capable of producing 1μg of tyrosine from casein in a minute under assay condition.

Statistical analysis

The results were expressed as mean ± standard deviation of data obtained from three independent measurements. The database was analyzed using the Software Package for Social Science for Windows 14.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISSCUSION

Our previous studies emphasize that some fungi species which originated from wastewater can metabolize the detergent components (*e.g.*, ethoxyled oleyl-cetyl alcohol and sodium tripolyphosphate) for growth and biomass accumulation [19–22]. Having this in mind, this study was designated in order to investigate the influence of ethoxyled oleyl-cetyl alcohol, which is added to nutrient medium in a high concentration (1%) on the growth, development and metabolic activity of *A. tenuis* Nees and *P. verrucosum* Dierckx. The obtained results should serve as a theoretical basis for practical

application of tested fungi in mycoremediation of environment. Research results of this study are presented in Figures 3–5.

The effect of surfactant on fungal growth on solid medium

Chemical composition of Czapek Dox's nutrient medium has optimal properties for growth and high biomass production of numerous fungi [19–22,26]. In addition, the presence of various pollutants (*e.g.* dye, heavy metals, pesticides, surfactants) in the culture medium may have an inhibitory or stimulatory effect on the fungal growth depending on type and applied concentration of pollutant, and the fungal species. For testing of fungal growth, the fungi were grown previously on solid media with (AOC) and without pollutant (C) over a period of 8 days. The colonies diameters were measured daily and growth curves were constructed. For both fungi, the exponential growth phase occurred in the period of cultivation from day 4 to day 8 (Figure 3). As Figure 3 shows, *CD* of *A. tenuis* had gradually increased on C medium, from day 4 to day 8, whereas *P. verrucosum* had a lower growth rate compared to *A. tenuis.* Different growth rates between the tested fungi in C medium can be explained by their morphophysiological differences that affect different response for the adoption and nutrient transport.

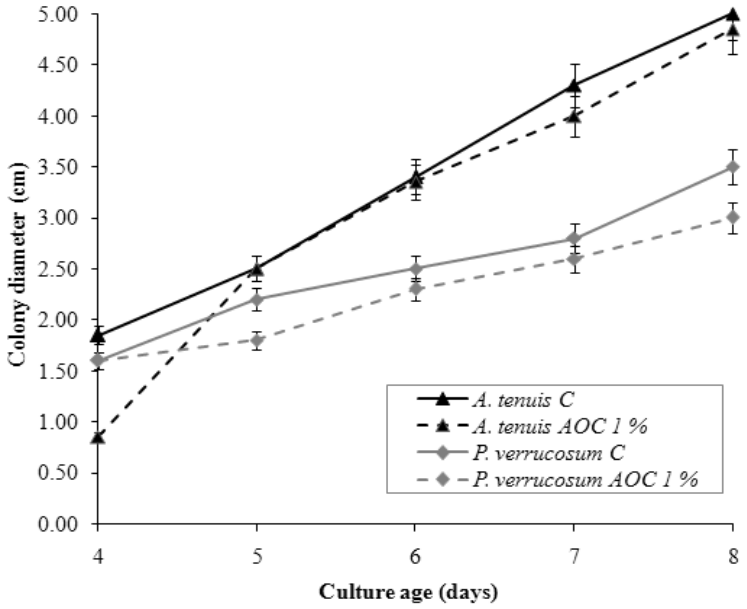

Figure 3. The colonies diameter of A. tenuis and P. verrucosum during exponential growth on solid media (C-control, AOC-medium with 1% ethoxylated oleyl-cetyl alcohol).

Fungi	Medium / Days	Dry weight biomass (g/l) C	Dry weight biomass (g/l) AOC	pH C	pH AOC	Proteolytic activity (μg/ml) C	Proteolytic activity (μg/ml) AOC
Alternaria tenuis	4	0	0	7.14±0.01	7.03±0.06	0	0.19±0.07
	5	nd	nd	7.16±0.02	7.04±0.12	0.28±0.04	0
	6	nd	nd	7.18±0.02	7.05±0.05	0.63±0.10	0.02±0.01
	7	nd	nd	7.16±0.04	7.06±0.05	0.31±0.08	0.32±0.14
	8	0.19±0.09	0.55±0.12	7.15±0.01	7.05±0.01	0.19±0.05	0.40±0.08
Penicillium verrucosum	4	0	0	7.12±0.05	7.25±0.02	0.18±0.09	0
	5	nd	nd	7.05±0.04	7.08±0.05	0.20±0.10	0
	6	nd	nd	6.88±0.02	6.84±0.09	0.35±0.12	0
	7	nd	nd	6.73±0.07	6.82±0.06	0.18±0.05	0
	8	0.14±0.05	0.17±0.06	6.75±0.01	6.66±0.01	0.15±0.05	0.05±0.01

Each value is expressed as mean ± standard deviation ($n = 3$), nd-not determined

Figure 4. Total dry weight of biomass, pH value and proteolitic activity of fungi in liquid media (C – control, AOC – medium with 1% ethoxylated oleyl-cetyl alcohol).

		Proteins (mg/ml)		Free org. acids (%)		Total org. acids (%)		Glucose (%)		Fructose (%)	
Fungi	Medium / Days	C	AOC	C	AOC	C	AOC	C	AOC	C	AOC
Alternaria tenuis	4	0.61±0.03	0.76±0.21	0.67±0.04	0.17±0.05	7.07±1.02	1.67±0.35	0.98±0.16	0.03±0.01	0.49±0.12	0.03±0.01
	5	0.68±0.09	0.62±0.26	nd	nd	nd	nd	nd	nd	nd	nd
	6	0.72±0.10	0.63±0.42	nd	nd	nd	nd	nd	nd	nd	nd
	7	0.68±0.12	0.68±0.32	nd	nd	nd	nd	nd	nd	nd	nd
	8	0.70±0.05	0.76±0.25	0.33±0.06	2.33±0.55	5.67±1.26	7.67±1.48	0.32±0.24	0.73±0.15	0.32±0.09	0.44±0.10
Penicillium verrucosum	4	0.10±0.02	0.08±0.04	0.28±0.02	0.28±0.08	0.56±0.10	2.22±0.65	0.10±0.06	0.07±0.05	0.05±0.02	0.07±0.02
	5	0.20±0.07	0.10±0.05	nd	nd	nd	nd	nd	nd	nd	nd
	6	0.24±0.04	0.11±0.05	nd	nd	nd	nd	nd	nd	nd	nd
	7	0.97±0.19	0.21±0.07	nd	nd	nd	nd	nd	nd	nd	nd
	8	1.80±0.62	0.05±0.01	1.94±0.09	3.33±0.05	8.89±1.32	7.78±1.28	0.78±0.09	0.21±0.04	0.71±0.09	0.37±0.16

Each value is expressed as mean ± standard deviation ($n = 3$), nd-not determined

Figure 5. Quantity of proteins, free and total organic acids and monosaccharides (glucose and fructose) in liquid media (C – control, AOC – medium with 1% ethoxylated oleyl-cetyl alcohol).

When fungi were grown on solid medium with addition of AOC, they had lower CD compared to the control. The growth of *A. tenuis* on AOC medium was significantly ($p < 0.01$) or slightly inhibited on day 4 and from day 7 to day 8, respectively. These results can be explained by a period of fungal adaptation to the presence of AOC in medium, during the initial stage of growth, as well as the creation of some degradation products that slow down fungal growth. Ethoxylated alcohol showed an inhibitory effect on *CD* of *P. verrucosum* over a period of three days (from day 5 to day 8), but had no influence on fungal growth on day 4. Obviously, the fungus *P. verrucosum* is more tolerant to a high concentration of the AOC in medium than *A. tenuis*, considering its CD was two times higher that CD of *A. tenuis* on day 4. However, the AOC degradation products had a higher intoxicating effect on the growth of *P. verrucosum* than parent molecule. Consequently, CD of *P. verrucosum* was significantly lower (14.29%) on day 8, whereas CD of *A. tenuis* was lower only by 3% compared to the control.

The effect of surfactant on fungal growth in liquid medium

The DW biomass of tested fungi grown in the liquid Czapek Dox's media was measured on day 8 (at the end of exponential growth) and results are presented in Figure 4. The fungus *A. tenuis* had higher DW biomass (0.55 g/l) than fungus *P. verrucosum* (0.17 g/l) in C medium. On the other hand, AOC added in liquid medium showed strong inhibitory effect (67%) on DW biomass of *A. tenuis* and mildly stimulating effect (13.5%) on DW biomass of *P. verrucosum,* compared to control. The finding that fungi could survive and grow in solid and liquid Czapek Dox's media with AOC at a high concentration (1%) provided evidence for the fungal resistance to this pollutant. The different response of fungi to growth on solid and liquid media with AOC was also confirmed in this study. Therefore, *A. tenuis* had better response to presence of AOC in solid medium, whereas *P. verrucosum* had better response to presence of AOC in liquid medium. These characteristics of fungi make them utilizable in bioremediation of solid and liquid environments. The obtained results are consistent with the results of our previous studies, which revealed the influence of AOC on the DW biomass of fungi *T. roseum*, *F. oxysporum* and *A. niger*. Therefore, this pollutant has a mild stimulating effect on the biomass of *T. roseum* and *F. oxysporum* but has a very strong inhibitory effect on the biomass of *A. niger*, under the same experimental conditions [21,22].

The influence of surfactant on pH media

Transport of nutrients through the cell membranes and growth of microorganisms are closely related with ambient pHs. Although, most fungal species live in a wide range of external pH, they proliferate more rapidly at acidic pH. When grown in an unbuffered medium, filamentous fungi often rapidly acidify their environment to very low and even sometimes detrimental pH values [27]. The major mechanism behind this acidification is controversially discussed, although it is most often attributed to either organic acid excretion [27,28] or proton release by the plasma membrane H-ATPase [29]. The addition of some organic molecules (*e.g.*, AOC, sodium tripolyphosphate or commercial detergent) in nutrient medium influences the change of pH values towards an alkaline environment that can be considered as a stress condition. According to litera-

ture, fungal response to alkaline pH is based on two possible mechanisms. First is the proteolytic activation of PacC transcription factors (*A. nidulans, C. albicans, S. cerevisiae,* Y. *lipolytica*) [30] and second mechanism is existence of the calcium-mediated pathway [31].

This study has evaluated the changes of pH value of fermentation broth during fungal growth from day 4 to day 8, as Table 3 shows. The pH values of liquid control media (C) were closely related and in neutral range (*P. verrucosum* 7.12 units, *A. tenuis* 7.14 units) on day 4. During the growth of fungi, the pH values of C media were changed to different intensity, depending on the fungal species. Therefore, during those four days, the pH value of C medium of *P. verrucosum* was gradually decreasing and the largest decrease in pH value (0.17 units) was measured from day 5 to day 6. On the contrary, the changes of pH value of C medium of *A. tenuis* were slightly increasing from day 4 to day 6. From that point on, the pH value was then decreasing with the almost same intensity until day 8. The presence of AOC at a concentration of 1% in a liquid media resulted in pH changes of media. The pH value of AOC medium of *A. tenuis* was insignificantly lower on day 4, whereas the pH value of *P. verrucosum* was significantly higher as compared to control. During the growth of *P. verrucosum*, pH value of AOC medium was decreasing, and the largest decrease in pH value (0.24 units) was observed from day 5 to day 6. On the contrary, the pH values of AOC medium of *A. tenuis* changed in the opposite manner, but the changes were very small. Decreasing of media pH is widespread phenomenon observed during extensive mycelium development of many fungal species such as *A. niger*, *F. oxysporum*, *P. chrysogenum*, *etc*. The results obtained in this study evidently suggest that fungi probably have different mechanism regulation of external pH, which depends on numerous factors (*e.g.*, pH value, chemical composition of medium, fungal morphology, *etc.*).

Activity of alkaline protease (EC 3.4.21-24) in liquid media

Proteases are degradative enzymes, which catalyze the cleavage of peptide bonds in proteins. They have wide-ranging applications in industrial products and processes such as detergent, food, pharmaceuticals, tannery, waste treatments, *etc*. In literature, several microbial strains including fungi (*Aspergillus flavus, Fusarium graminarum, Penicillium griseofulvin, etc.)* and bacteria *(Bacillus licheniformis, B. firmus, B. subtilis, etc.*), are reported to produce protease. Due to these facts, the alkaline protease activity of *A. tenuis* and *P. verrucosum* was evaluated in this paper.

Data presented in Figure 4 has shown that tested fungi produced extracellular protease when grown in C medium more effectively than in AOC medium. The proteolytic activity of fungi was increased parallel with the fungal growth (*A. tenuis* and *P. verrucosum*) in C medium, from day 4 to day 6. The maximum proteolytic activity was measured in the fermentation broth of *A. tenuis* (0.63 mg/ml) on day 6. Fungus *P. verrucosum* had two times lower proteolytic activity in same medium, with its maximum (0.35 mg/ml) achieved on day 6. Addition of ethoxylated oleyl-cetyl alcohol, in liquid nutrient medium at a concentration of 1%, contributed to a partial (*A. tenuis*) or complete inhibition (*P. verrucosum*) of proteolytic activity, during fungal growth. Proteolytic activity of *A. tenuis* in AOC medium was expressed on day 4 and from day 6 to day 8, with its maximum (0.400 mg/ml) achieved on day 8. In the presence of AOC, fungal proteolytic activity was inhibited about 37% (or remained about 63% activity) in relation to control. Obviously, various morpho-physiological characteristics of the fungi and some degradation products of AOC in medium have caused differences in effects of this pollutant on proteolytic activity. These results are also supported by the findings of Stojanović *et al.* [22] who reported that AOC at concentration of 1% has a strong inhibitory effect on the activity of proteolytic enzymes the fungus *T. roseum*. However, the same authors also reported that the AOC at concentration of 1% has a strong stimulating effect on the proteolytic activity of *A. niger*, under the same experimental conditions [19]. According to Evans and Abdullahi [32], surfactants may have improved the permeability of the cell membrane through disruption of lipid bilayer thereby increasing the uptake of nutrient into the organism and the secretion of enzyme into the culture medium. Non-ionic surfactants type of ethylene oxides, bind to active site of enzymes through hydrogen bonds in order to enhance conformation flexibility [33]. Zeng *et al.* [34] revealed that incorporation of Tween-80 into fermentation medium have shown to enhance production and secretion of protease. Li *et al.* [35] demonstrated that Tween-80 and acetonitril increased the yield of protease activity of *Serratia sp.* SYBC H by 5.0 and 4.3 folds, respectively. Maruthiah *et al.* [36] has reported enhanced protease activity of *Bacillus flexus* by non-ionic surfactant Tween-20, Tween-40, Tween-60 and Triton X-100. According to Barberis *et al.* [37], proteolytic activity of *araujiain* increased or remained constant while non-ionic surfactant concentration was being increased (0.1, 0.4 and 1%). Enzyme stability in the presence of detergent ingredients, such as surfactants, builders and activated bleach, *etc.*; optimum activity at alkaline pH; effectiveness at low wash temperatures of 20–40 °C; are very important properties for its use in detergent formulations [38]. Based on presented results and facts mentioned above, performances of *A. tenuis* alkaline protease are suitable for its potential application as an additive in laundry detergent formulations ethoxylated oleyl-cetyl alcohol type.

Production of protein in liquid media

Numerous studies of protein secretion has been made with filamentous fungi, but the molecular basis for the protein secretion in fungi is still lacking. Bearing that in mind, the fungi were referred as "a highly productive black box" by Peberdy [39]. Therefore, the examination of protein secretion of each fungal species in various media is very important. The tested fungi had produced a different amount of protein in C medium on day 4, and it was ranged from 0.10 (*A. tenuis*) to 0.67 mg/ml (*P. verrucosum*), Figure 5. The amount of proteins secreted in this medium was increased parallel with the fungal growth. Fungus *A. tenuis* has secreted the highest amount of protein (0.72 mg/ml) on day 6, and *P. verrucosum* (1.80 mg/ml) on day 8. Ethoxylated oleyl-cetyl alcohol added in the culture medium seems to have slightly stimulated the proteins secretion of *A. tenuis* (0.76 mg/ml), whereas it strongly inhibited the proteins secretion of *P. verrucosum* (0.21 mg/ml). The least deviation in the secretion of proteins between the media was found by *A. tenuis*. Data that was found in the literature confirmed inhibitory/stimulatory effect of AOC at a concentration of 1% on protein production of *T. roseum* [22], and *A. niger* and *F. oxysporum* [19,21]. The results of this study as well as results of mentioned authors are evidently indicating that fungal morphology is also directly correlated with protein production.

The influence of surfactant on organic acids excretion

The tested fungi excreted different amount of FOAs and TOAs depending on the type of medium and culture age (Figure 5). The amount of FOAs measured in C medium was significantly higher (*P. verrucosum*) or low (*A. tenuis*) on day 8, compared to day 4. During the same cultivation period, AOC at a concentration of 1% showed strong stimulatory effect on FOAs excretion of both fungi, in relation to control. To summarize, the fungus *P. verrucosum* excreted about 1.5-fold larger amount of FOAs and *A. tenuis* excreted about 7-fold larger amount of FOAs in AOC medium in relation to control. The amount of TOAs measured in fermentation broth of C medium of *A. tenuis* was significantly lower or, as in the case of *P. verrucosum*, significantly higher on day 8 than on day 4. The AOC added in medium with a concentration of 1% has influenced TOAs amount in both fungi by increasing it significantly on day 8 compared to day 4. Generally, *A. tenuis* excreted higher amount of TOAs in both culture media than *P. verrucosum*. These results provide evidence that significant differences exist between the tested fungi in organic acids excretion in both media. Decreasing of pH media of *P. verrucosum* is in positive correlation with increasing of organic acids excretion. In contrast, increasing of pH control medium of *A. tenuis* is in negative correlation with amount of organic acids excreted, which could be explained with a reuptake of organic acids. Therefore, organic acids excreted in media are serving another purpose (charge balance, energy spilling or chelation of trace elements) besides acidification of external medium.

The influence of surfactant on amount of monosaccharides

Monosaccharides, glucose and fructose, are the reducing sugars produced by the action of invertase on sucrose. Generally, when sucrose is used as an only carbon source, the fungus utilizes rather glucose than fructose for its metabolism [40]. Sucrose is necessary in medium with AOC for fungal biodegradation of pollutant. Taking this into consideration, the effect of AOC on amount of monosaccharides was investigated in this study. The concentration of glucose and fructose in the fermentation broth of fungi was determined at the beginning (day 4) and at the end of exponential growth phase (day 8), as Figure 5 shows. On day 4, very small amount of glucose and fructose was measured in C medium of *P. verrucosum* but significant amount of monosaccharides was measured in C medium of *A. tenuis*. Regardless of these differences, both fungi produced lower amount of fructose than glucose, which means that fungi metabolized fructose rather than glucose in medium with sucrose as only carbon source. These results are opposite from results of above-mentioned authors. Obviously, parameters such as fungal morphology and experimental conditions (pH medium, chemical composition of medium, aeration, *etc.*), have influenced monosaccharides uptake rates. At the same time, very low amount of glucose and fructose was also measured in both AOC media. In this medium, fungi metabolized equal amount of monosaccharides. On day 8, the amount of glucose and fructose measured in C medium of *P. verrucosum* was significantly higher than on day 4. Nevertheless, the amount of glucose and fructose in same medium of *A. tenuis* was lower, especially glucose. In both AOC media, amount of monosaccharides was higher on day 8 compared to day 4. Accordingly, *A. tenuis* produced about 0.71% glucose and 0.44% fructose, whereas *P. verrucosum* produced about 0.18% glucose and 0.35% fructose. These results indicate that tested fungi have different flux for monosaccharides in presence of AOC. Therefore, *A. tenuis* utilizes fructose rather than glucose, whereas *P. verrucosum* rather utilizes glucose in medium with AOC. Results obtained in this study are in accordance with report by Stojanović *et al.* [22] who found that ethoxylated alcohol stimulates the production of glucose of *F. oxysporum* and fructose of *T. roseum* and *F. oxysporum*. However, the same authors confirmed the inhibitory effect of this pollutant on production of monosaccharides in experiment with *A. niger* [21]. The differences in the fungal utilization of

glucose and fructose observed in this study could be caused either by differences in the transport systems or by the subsequent intracellular metabolism of the sugars. Also, AOC could influence synthesis of inducible enzymes involved in regulation of carbohydrates metabolism or some of its degradation products have a role of competitive inhibitor of these enzymes.

CONCLUSIONS

Based on obtained results, ethoxylated oleyl-cetyl alcohol at a concentration of 1% had different effect on the growth, development and metabolic activity of the tested fungi, depending on the species of fungi. Both fungi species could survive and grow in solid and liquid Capek Dox's medium with addition of AOC at a high concentration (1%). The *A. tenuis* has better response to AOC on solid medium whereas *P. verrucosum* has better response to AOC in liquid medium. Ethoxylated oleyl-cetyl alcohol has influenced metabolic activity of fungi in direction of production of significant amount of organic acids, proteins, fructose (*P. verrucosum*) and glucose (*A. tenuis*). The alkaline protease activity of fungus *A. tenuis* had retained about 63.50% activity in the presence of AOC, so it could have a potential application in detergent formulation. The results presented in this study undoubtedly indicate the possible application of the tested fungi in both mycoremediation of contaminated solid and liquid environments and in different areas of industries.

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REFERENCES

[1] HERA, Human & Environmental Risk Assessment on ingredients of European household cleaning products, Alcohol Ethoxylates, 2009. http://www.heraproject.com

[2] G.G. Ying, Fate, behavior and effects of surfactants and their degradation products in environment, Environ. Int. **32** (2006) 417–431.

[3] Danish EPA, Environmental and Health Assessment of Substances in Household Detergents and Cosmetic Detergent Products. Environmental Project No. 615, 2001. http://www.mst.dk/udgiv/ Publications/2001/87-7944--596-9 /html/default_eng.htm

[4] T. Wind, R.J. Stephenson, C.V. Eadsforth, A. Sherren, R. Toy, Determination of the fate of alcohol ethoxylate homologues in a laboratory continuous activated-sludge unit study, Ecotoxicol. Environ. Saf. **64** (2006) 42–60.

[5] T.W. Federle, N.R. Itrich, Fate of free and linear alcohol-ethoxylate-derived fatty alcohols in activated sludge, Ecotoxicol. Environ. Saf. **64** (2006) 30–41.

[6] S.W. Morrall, W.S. Eckhoff, A. Evans, M.L. Cano, J.C. Dunphy, D.C. McAvoy, Removal and environmental exposure of alcohol ethoxylates in US sewage treatment, Ecotoxicol. Environ. Saf. **64** (2006) 3–13.

[7] C.V. Eadsforth, A.J. Sherren, M.A. Selby, R. Toy, W.S. Eckhoff, D.C. McAvoy, E. Matthijs, Monitoring of environmental fingerprints of alcohol ethoxylates in Europe and Canada, Ecotoxicol. Environ. Saf. **64** (2006) 14–29.

[8] S.E. Belanger, P.B. Dorn, R. Toy, G. Boeije, S.J. Marshall, T. Wind, R.Van Compernolle, D. Zeller, Aquatic risk assessment of alcohol ethoxylates in North America and Europe, Ecotoxicol. Environ. Saf. **64** (2006) 85–99.

[9] L. Bin-Le, Y. Meng, Y. Ishikawa, A. Tokai, A new approach to estimate concentrations of alcohol ethoxylate in rivers in Japan for screening-level risk assessment, J. Risk Res. **14** (2011) 1109–1126.

[10] D.C. McAvoy, K.M. Kerr, In: C.E. Clapp, M.H.B. Hayes, N. Senesi, P.R. Bloom, P.M. Jardine (Eds.), Association of alcohol ethoxylates with a dissolved humic substance. Humic substances and chemical contaminants, Madison, WI: Soil Science Society of America, 2001, pp. 177–186.

[11] L. Zhu, K. Yang, B. Lou, B. Yuan, A multi-component statistic analysis for the influence of sediment/soil composition on the sorption of a nonionic surfactant (Triton X-100) onto sediments/soils, Water Res. **37** (2003) 4792–4800.

[12] S.M. Mudge, P.C. De Leo, S.D. Dyer, Quantifying the anthropogenic fraction of fatty alcohols in a terrestrial environment, Environ. Toxicol. Chem. **31** (2012) 1209––1222.

[13] E.C. Tidswell, N.J. Russell, G.F. White, Ether-bond scission in the biodegradation of alcohol ethoxylate surfactants by *Pseudomonas* sp. strain SC25A, Microbiology **142** (1996) 1123–1131.

[14] M.A. Grant, W.J. Payne, Anaerobic growth of *Alcaligenes faecalis* var. *denitrificans* at the expense of ether glycols and nonionic detergents, Biotechnol. Bioeng.**25** (1983) 627–630.

[15] S. Wagener, B. Schink, Fermentative degradation of non-ionic surfactants and polyethylene glycol by enrichment cultures and by pure cultures of homoacetogenic and propionate-forming bacteria, Appl. Environ. Microbiol. **54** (1988) 561–565.

[16] R. Ramachandran, J.J. Gnanadoss, Mycoremediation for the treatment of dye containing effluents, IJCOA **2** (2013) 286–293.

[17] A.L. Juhasz, R. Naidu, Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[*a*]pyrene, Int. Biodeter. Biodegr. **45** (2000) 57–88.

[18] M.L. Rabinovich, A.V. Bolobova, L.G. Vasilchenko, Decomposition of natural aromatic structures and xenobiotics by fungi, Appl. Biochem. Microbiol. **40** (2004) 5––23.

[19] J. Stojanović, M. Grbavcić, A. Cosović, M. Stojanović, The influence of detergent, the active component of detergent and sodium tripolyphosphate on the biochemical process of some fungi, Riv. Biol. **97** (2004) 329–339.

[20] J. Stojanović, V. Jakovljević, I. Matović, Z. Mijušković, T. Nedeljković, The influence of detergent, sodium tripolyphosphates and ethoxyled oleyl-cetyl alcohol on metabolism of the fungi *Penicillium verrucosum* Peyronel, Acta Vet-Beograd **60** (2010) 67–77.

[21] J. Stojanović, V. Jakovljević, I. Matović, O. Gajović, Z. Mijušković, T. Nedeljković, Influence of detergent on metabolic activity of fungi *Aspergillus niger*, Natural Science **3** (2011) 466–470.

[22] J. Stojanović, J. Milićević, O. Gajović, V. Jakovljević, I. Matović, Z. Mijušković, T. Nedeljković, The effects of detergent, sodium tripoly-phosphate and ethoxyled oleyl-cetyl alcohol on metabolic parameters of the fungus *Trichothecium roseum* Link, Arch. Biol. Sci. **63** (2011) 1001–1006.

[23] W.A. Bulen, J.E. Varner, R.C. Burrell, Separation of Organic Acids from Plant Tissues, Anal. Chem. **24** (1952) 187–190.

[24] J.T. Kjeldahl, Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern, Z. Anal. Chem. **22** (1883) 366–382.

[25] M.L. Anson, The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin, J. Gen. Physiol. **22** (1938) 79–89.

[26] V.D. Jakovljević, J.M. Milićević, J.D. Stojanović, S.R. Solujić, M.M. Vrvić, Influence of detergent and its components on metabolism of *Fusarium oxysporum* in submerged fermentation. Hem. Ind. **68** (2014) 465–473.

[27] J.K. Magnuson, L.L. Lasure, In: J.S. Tkacz, L. Lange (Eds.), Organic acid production by filamentous fungi. Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine, Kluwer Academic/Plenum Publishers, New York, 2004, pp. 307–340.

[28] M.R. Andersen, L. Lehmann, J. Nielsen, Systemic analysis of the response of *Aspergillus niger* to ambient pH, Genome Biol. **10** (2009) R47.

[29] K. Jernejc, M. Legisa, Activation of plasma membrane H^+-ATPase by ammonium ions in *Aspergillus niger*, Appl. Microbiol. Biotechnol. **57** (2001) 368–373.

[30] W. Li, A.P. Mitchell, Proteolytic activation of Rim1p; a positive regulator of yeast sporulation and invasive growth, Genetics **145** (1997) 63–73.

[31] R. Serrano, A. Ruiz, D. Bernal, J.R. Chambers, J. Ariño, The transcriptional response to alkaline pH in Saccharomyces cerevisiae: evidence for calcium-mediated signaling, Mol. Microbiol. **46** (2002) 1319–1333.

[32] E.C. Evans, A. Abdullahi, Effect of surfactant inclusions on the yield and characteristics of protease from *Bacillus subtilis*, Proc. Rom. Acad. Ser. B **2** (2012) 108––112.

[33] S.Ž. Grbavčić, D.I. Bezbradica, I.M. Karadžic, Z.D. Knežević-Jugovic, Lipases and proteases produced by indigenous *Pseudomonas aeruginosa* strain as potential detergent additives, Hem. Ind. **63** (2009) 331–335.

[34] G.-M. Zeng, J.-G. Shi, X.-Z. Yuan, J. Liu, Z.-B. Zhang, G.-H. Huang, J.-B. Li, B.-D. Xi, H.-L. Liu, Effects of Tween 80 and rhamnolipid on the extracellular enzymes of Penicillium simplicissimum isolated from compost, Enzyme Microb. Technol. **39** (2006) 1451–1456.

[35] G.Y. Li, Y.J. Cai, X.R. Liao, A novel nonionic surfactant- and solvent-stable alkaline serine protease from *Serratia sp.* SYBC H with duckweed as nitrogen source: production, purification, characteristics and application, J. Ind. Microbiol. Biotechnol. **38** (2011) 845–853.

[36] T. Maruthiah, P. Esakkiraj, G. Immanuel, A. Palavesam, Alkaline serine protease from marine *Bacillus flexus* APCMST-RS2P: purification and characterization, Curr. Biotechnol. **3** (2014) 238–243.

[37] S. Barberis, E. Quiroga, C. Barcia, C. Liggieri, Effect of laundry detergent formulation on the performance of alkaline phytoproteases, Electron. J. Biotechn. **16** (2013) 1-8. http://dx.doi.org/10.2225/vol16-issue3-fulltext-1

[38] E. Smulders, W. Rähse, W. Von Rybinski, J. Steber, E. Sung, F. Wiebel, In: E. Smulders (Ed.), Detergent ingredient. Laundry detergent, Wiley-VCH, Verlag GmbH & CoKGaA, Germani, 2002, pp. 38–98.

[39] J.F. Peberdy, Protein secretion in filamentous fungi – trying to understand a highly productive black box, Trends Biotechnol. **12** (1994) 50–57.

[40] E. Peynaud, S. Domercq, Sur les espbces de levures fermentant selectivement le fructose, Annales de l'Institut Pasteur, **89** (1955) 346–351.

IZVOD

UTICAJ ETOKSILOVANOG OLEIL-CETIL ALKOHOLA NA METABOLIZAM NEKIH GLJIVA I NJIHOVA POTENCIJALNA PRIMENA U MIKOREMEDIJACIJI

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(Naučni rad)

Uticaj etoksilovanog oleil-cetil alkohola 1% koncentracije na metabolizam gljiva *A. tenuis* Nees and *P. verrucosum* Dierckx, koje su izolovane iz otpadnih kanalizacionih voda, bio je predmet istraživanja ove studije. Dejstvo 1% etoksilovanog oleil-cetil alkohola na rast gljiva ispitivano je praćenjem prečnika kolonija na čvrstoj i merenjem suve biomase micelija u tečnoj Čapekovoj podlozi. Gljiva *A. tenuis* imala je bolji odgovor na prisustvo polutanta u čvrstoj podlozi dok je *P. verrucosum* ispoljila bolji odgovor na polutant u tečnoj podlozi. Tokom eksponencijalnog rasta gljiva u tečnoj podlozi sa i bez navedenog polutanta (kontrola), praćene su promene sledećih fizičko-hemijskih i biohemijskih parametara: pH, količina: slobodnih i ukupnih organskih kiselina, proteina i ugljenih hidrata, proteolitička aktivnost. Etoksilovani oleil-cetil alkohol uticao je na smanjenje pH vrednosti podloge i povećanje količine slobodnih organskih kiselina obe gljive. Pomenuti polutant uticao je na produkciju manje količine ukupnih organskih kiseline, proteina, glukoze i fruktoze, u fermentacionoj tečnosti gljive *P. verrucosum* u odnosu na *A. tenuis.* Proteolitička aktivnost gljiva bila je delimično (*A. tenuis*) ili potpuno inhibirana (*P. verrucosum*) prisustvom polutanta u tečnoj hranljivoj podlozi. U prisustvu 1% etoksilovanog oleilcetil alkohola alkalna proteaza *A. tenuis* zadržala je oko 67% aktivnosti tako da bi se mogla koristiti kao aditiv u formulaciji deterdženta. Na osnovu dobijenih rezultata može se zaključiti da se testirane gljive mogu smatrati potencijalnim kandidatima za mikoremedijaciju životne sredine (zemljišta, voda) kontaminirane alkoholnim etoksilatima.

Ključne reči: Monosaharidi • Organske kiseline • Prečnik kolonije • Proteini • Proteolitička aktivnost • Suva biomasa

The combustion of biomass – The impact of its types and combustion technologies on the emission of nitrogen oxide

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Abstract

Harmonization of environmental protection and the growing energy needs of modern society promote the biomass application as a replacement for fossil fuels and a viable option to mitigate the greenhouse gas emissions. For domestic conditions this is particularly important as more than 60% of renewables belongs to biomass. Beside numerous benefits of using biomass for energy purposes, there are certain drawbacks, one of which is a possible high emission of NO_x during the combustion of these fuels. The paper presents the results of the experiments with multiple biomass types (soybean straw, cornstalk, grain biomass, sunflower oil, glycerin and paper sludge), using different combustion technologies (fluidized bed and cigarette combustion), with emphasis on the emission of NO_x in the exhaust gas. A presentation of the experimental installations is given, as well as an evaluation of the effects of the fuel composition, combustion regimes and technology on the NO_x emissions. As the biomass combustion took place at temperatures low enough that thermal and prompt NO_x can be neglected, the conclusion is the emissions of nitrogen oxides primarily depend on the biomass composition – it is increasing with the increase of the nitrogen content, and decreases with the increase of the char content which provides catalytic surface for NO_x reduction by CO.

Keywords: fluidized bed, cigarette combustion, biomass, NO_x.

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Since biomass is the only CO_2-neutral-carbon--based renewable energy source its application becomes more and more important for climate protection, wherein the biomass combustion is the most important and proven thermochemical conversion technology for heat and power production. However, biomass combustion is related with potential problems concerned with the environmental pollution, even though they are less pronounced in comparison with the coal. The emission of nitrogen oxides (NO_x) is one of the most important challenges in the field.

Nitrogen oxides play a significant role in pollution problems such as formation of photochemical smog, ground level ozone and acid rain, visibility impairment, causing damage to natural ecosystems and crops. Over 90% of nitrogen oxides emitted due to the combustion process makes NO, while the rest is NO_2. In the atmosphere NO is converted to NO_2, so the regulations in the field of environmental protection treat all nitrogen oxides as NO_2. Nitrous oxide (N_2O) is also important because of its greenhouse effect, but its emitted quantity is significantly less than of the previous two. NO_x is formed both from atmospheric nitrogen, N_2, and from nitrogen contained in fuel, by the following fundamentally different mechanisms [1,2]:

1. Thermal – NO_x, high temperature (>1200 °C) oxidation of atmospheric nitrogen by oxygen in combustion air;
2. Fuel – NO_x, oxidation of fuel-bound nitrogen;
3. Prompt – NO_x, combustion of atmospheric nitrogen and hydrocarbons in the rich mixture conditions/very low air–fuel ratios.

- During the combustion of biomass, the oxidation of fuel-bound nitrogen is the dominant mechanism of forming NO_x. The amount of thermal and prompt NO_x is negligible due to relatively low combustion temperatures conditioned with low melting temperature of biomass [1–6].
- The formation of NO_x from fuel bound N takes place predominantly in the gas phase oxidation of the nitrogenous species released with the volatiles (66––75%) and less through the heterogeneously catalysed oxidation of the nitrogen retained in the char (< 25%) [1].
- N released with the volatiles from the biomass fuels generally ends up as NH_3 rather than as HCN (HCN

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is the most important precursor for N_2O formation). Both of them can be oxidized to NO during subsequent combustion. At the same time, the two precursors (especially NH_3) can also serve as reducing agents for NO reduction, as follows:

$NH_3 + O_2 \rightarrow NO + H_2O + H$

$NH_3 + NO \rightarrow N_2 + H_2O + H$

- Quite a low N_2O concentration was detected during large-scale biomass combustion [3,5]. This phenomenon has been attributed to the N functional groups present in biomass fuels, mainly amino groups, which are usually form NH_3 as the main N-product during pyrolysis [1,3–6]. Furthermore, NH_3 tends to be oxidized to NO and N_2 instead of N_2O at temperatures of 800–900 °C [7], so N_2O emission is not the subject of this paper.
- Another point of consideration with respect to NO_x emissions during the biomass combustion is the catalytic effect of the char and ash on NO_x formation and reduction [6].
  - Char provides a catalytic surface for the gas phase NO reduction by CO.
  - The catalytic effect of the ash, especially the presence of CaO, MgO and Fe_2O_3, may be important because it can catalyse the reduction of NO and NO_2.
- Exceeding the emission limits can be expected at fuel-N concentrations above 0.6 wt.% on dry basis [8].

Controlling NO_x emissions is becoming a considerable technical challenge as increasingly strict emission limits are being imposed. The NO_x control technologies can broadly be classified into:

1. pre-combustion which involve the use of low nitrogen fuels,
2. combustion control or primary measures – modifying design and operating features of the combustion unit and
3. post-combustion techniques (end-of-pipe treatment) or secondary measures – flue gas treatment (FGT) after the combustion process.

The *pre-combustion* measures implied an informed choice of biomass (*e.g.*, knowledge of fertilizer treatment, length of storage and harvest time because natural senescence decreases N content as the N is remobilized to the roots or rhizomes) and/or pretreatments with a target of minimizing heterocyclic N-compounds*. The pre-combustion measures include also modification of the fuel composition by usage of fuel additives, fuel blending and co-combustion, *e.g.*, the use of biomass/coal cofiring that decreases NO_x and SO_x emissions [10].

Primary and secondary measures of NO_x reduction are given in Figure 1.

Knowledge of the nitrogen oxides emission, in addition to the need for developing better biomass

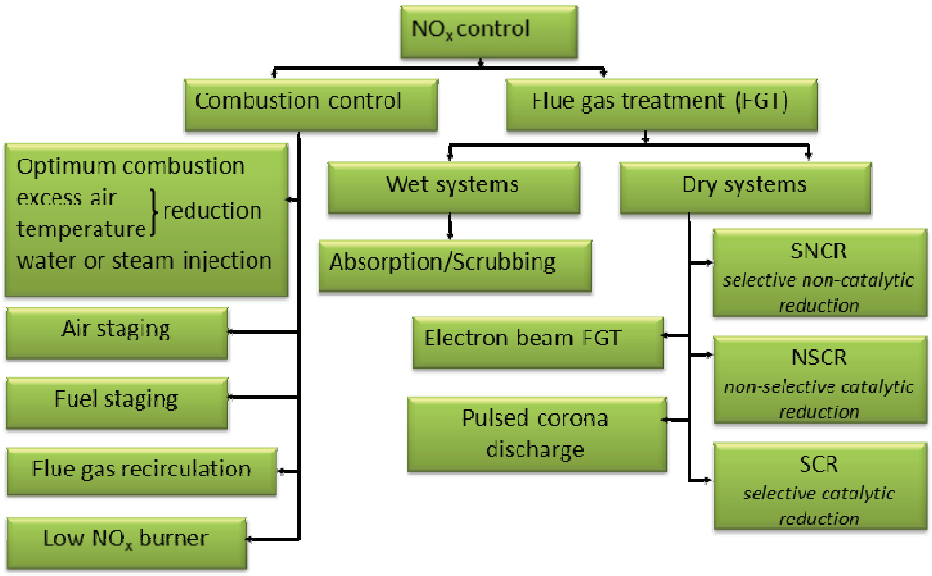


Figure 1. Overview of deNO$_x$ techniques.

- It is difficult to reduce CO and NO_x simultaneously –- decreasing one may result in an increase of the other [9]. And finally, it is important to note that the influences of different fuel properties on NO_x generation are very much inter-related, and as such, it is difficult to examine the influence of an individual property in isolation.

combustion technologies and consequently NO_x techniques, is also necessary when setting emission regulations. It should be noted that the Regulation on

*Heterocyclic N compounds seem to decompose mostly through HCN, while amino acids and proteinic nitrogen appear to produce mostly ammonia, NH_3.

limit values for emissions of air pollutants [11] has not defined NO_x emission limit for combustion of biomass that is not the wood nature, which can be considered as a failure, especially when taken into account that of the available amount of biomass in Serbia, 63% is the biomass from agricultural production, and a crop biomass, which accounts the largest part of agricultural biomass, is characterized by a very high N content due to intensive fertilization of crops.

DESCRIPTION OF THE EXPERIMENTAL FACILITIES

In order to improve knowledge of the nitrogen oxides emission and its influencing variables investigations were conducted with multiple biomass types, using different combustion technologies (fluidized bed and cigarette combustion). Thus, this paper presents the results of combustion of sunflower oil and glycerin on a semi-industrial experimental fluidized bed facility, FB1 (100 kW_{th} capacity), as well as of combustion of paper sludge and corn kernel on the experimental hot water FB boiler, FB2 (500 kW_{th} capacity). The fluidized bed combustion (FBC) technology was selected because of its low operating temperatures of about 850 °C that suppress the formation of thermal and prompt NO_x. Further, experiments were performed on the industrial-scale hot water boiler (1.5 MW_{th} capacity) for combustion of large soya straw bales (0.7 m×1.2 m×2.0 m) and on the two experimental facilities: the boiler (75 kW_{th}) burning small soya straw bales (0.8 m×0.5 m×0.4 m) and the furnace (50 kW_{th}) burning small corn stalk bales (0.45 m×0.35 m×0.80 m). The latter three use cigar burner combustion system (CBCS). The cigarette type combustion was chosen because it is cost-effective and energy conscious way of utilizing the baled biomass.

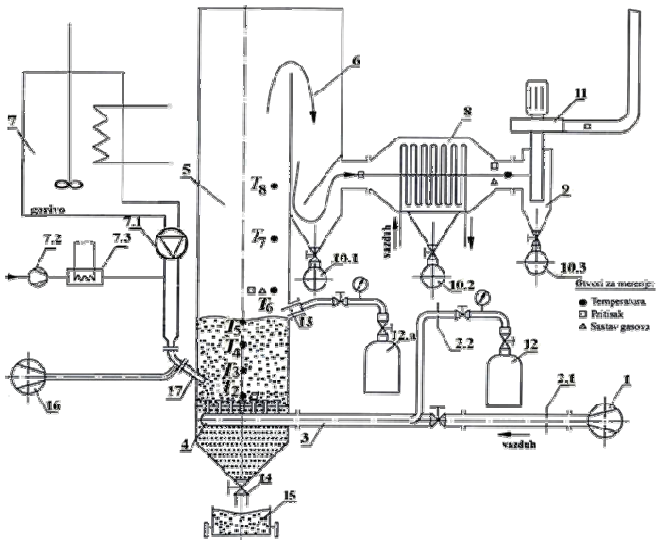
Simplified schemes of facilities with both combustion technologies (FBC and CBCS) where the combustion experiments were carried out are presented in Figures 2–6. More about the experimental FB facility shown in Figure 2 and demonstration FB boilers in Figure 3 can be found in [12–16].

The principle of operation of facilities with cigarette burner system (CBS) can be found in papers [17–19]. An industrial-scale hot water boiler at Fig. 4 was installed in the Agricultural Corporation Belgrade [17,20] and it is used for heating 1 ha of greenhouses.

The experimental boiler at Figure 5 is installed on an individual farm where is used for residential heating.

Fuel characterization

The fuels selected for this investigation are mainly biowastes considered at the moment as ballast. Namely, data of the Ministry of Energy, Development and Environmental Protection of the Republic of Serbia indicate that consumption of edible oil in Serbia is around 16 per capita per year; which means that about 10,000



Legend:

1. Primary air blower
2. (2.1 - 2.2) Measuring orifices
3. Electric heater
4. Air chamber with the air-distributor
5. Fluidized bed furnace (1st draft)
6. Particle separation chamber (2nd draft)
7. Liquid fuel feeding - 7.1 dosing pump, 7.2 air blower and 7.3 heater of carrier air;
8. Flue gases cooler
9. Cyclone for particle separation
10. (10.1 – 10.3) Vessels for particle disposal
11. Flue gas fan
12. (12.a) Propane-butane flask
13. Start-up burner
14. Furnace material removal valve
15. Furnace material collector
16. Air blower for jet cooling
17. Nozzle

Figure 2. The scheme of semi-industrial experimental FB1 facility (100 kW capacity) [12–14].

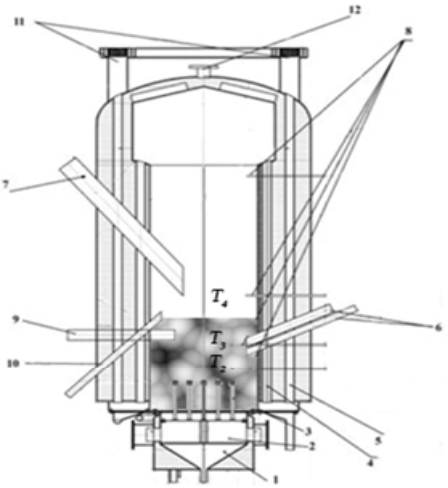


Figure 3. The scheme of demonstrative experimental hot water FB boiler (500 kW), FB2 [15,16].

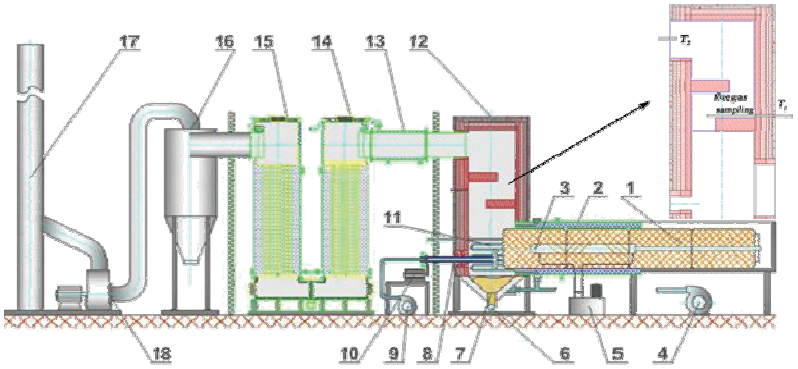


Figure 4. The scheme of the demonstrating hot water CBS boiler with thermal power of 1.5 MW, CBS1: 1. fuel feeding, 2. feeding channel, 3. hydraulic feeder, 4. primary air fan, 5. motor driven VSD controlled conveyor, 6. ash transporter, 7. ash, 8. movable cross and primary air supply, 9. secondary air fan, 10. secondary air driver,11. water cooled grate, 12. furnace isolation, 13. flue gas exit, 14, 15. first and second section of the gas-water heat exchanger, 16. multi-stage cyclone, 17. stack, 18. flue gas fan.

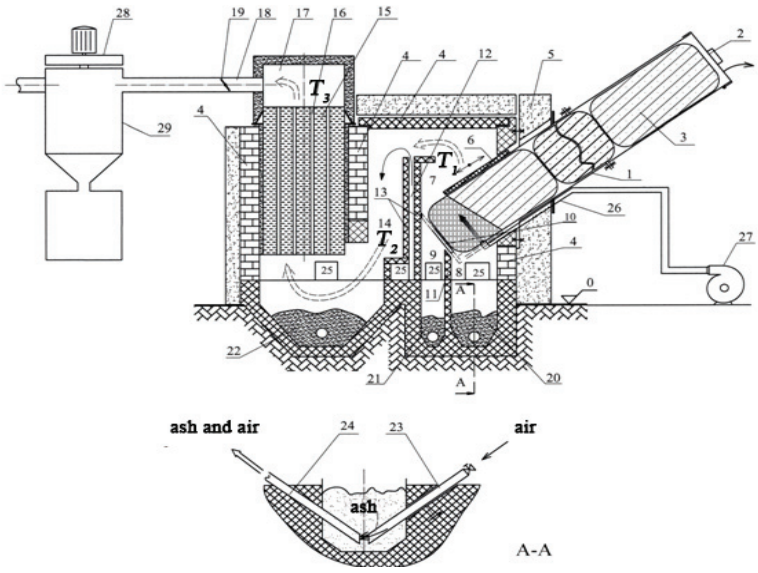


Figure 5. The scheme of the experimental CBS boiler (75 kW), CBS2: 1. fuel feeding, 2. cover, 3. baled biomass, 4., 5. heat insulation, 6. regulation of combustion zone, 7. primary combustion chamber, 8. primary air supply, 9. secondary air supply, 10. grate, 11. compartment between primary and secondary air, 12. tertiary air introduction, 13. tertiary air channels, 14. burnout zone, 15. heat exchanger, 16. flue gas channel, 17. flue gases collector, 18. smoke stack, 19. flap, 20.,21.,22. ash collector, 23. air tube for ash removal, 24. ash removal tube, 25. revision opening, 26. air distributor, 27. air fan, 28. flue gases fan, 29. cyclone separator with bunker.

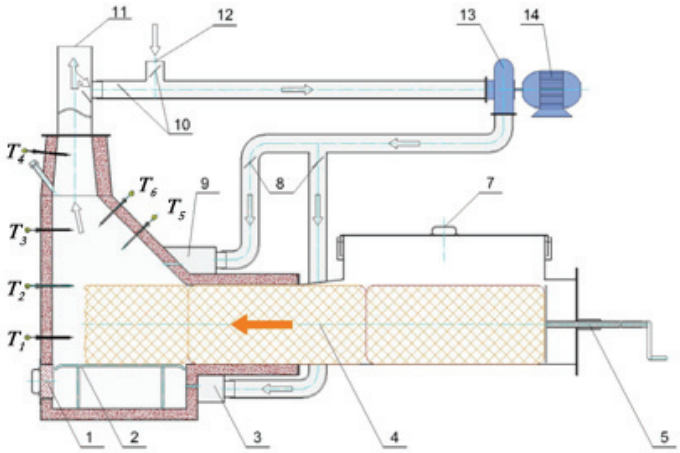


Figure 6. The scheme of the experimental CBS furnace (50 kW), CBS3: 1. ignition and ash removal, 2. furnace grid, 3, 9. combustion air distributive chamber, 4. baled biomass, 5. fuel introducing ball screw, 7. cover, 8. flaps, 10. control valves for recirculation rate, 11. stack, 13, 14. electric fan for combustion fluid transport.

tons of used cooking oil per year could be collected and could be used as a fuel. Glycerine is the main byproduct of biodiesel production. Increasing demand for biodiesel may lead to glycerine oversupply so it could be treated as waste and used as fuel. The paper sludge is the byproduct of the paper industry (Cardboard factory Umka, Avala Ada, Paper Factory Belgrade). Because of its high content of moisture and a non-uniform composition it is not suitable for further processing, but sutable for combustion in FB with support of a higher calorific fuel. As the paper is made of a net of very thin celluloid fibers obtained mainly from trees, the paper sludge from processing is treated as biomass. Although the cereals are primarily important for human and animal nutrition, they can also be used as a fuel for production of heat and hot water at farms and small-scale district-heating plants, if they're used as non-food-second-rate cereals (waste from the mills and cornflakes factories, unused or contaminated seed grain, etc.). This is paticularly important, bearing in mind the problem from 2012 associated with aflatoxin contamination of maize, when only 32% of the domestic genus was safe to use. In the total potential of renewable energy sources in Serbia biomass accounts 63% of which biomass from agricultural production accounts for about 60% of this potential, while the share of agricultural crop residues is about 37%.

Prior to the experimental investigation the characterization of the fuels was performed (Table 1), in order to calculate the adiabatic combustion temperature – the base for the facility adjustment (defining the fuel and air flow, in order to obtain steady state on the designed combustion temperature).

As seen in Table 1, biomass is highly oxygenated with respect to conventional fossil fuels. Due to carbohydrate structure, the principal constituent of biomass is carbon, making up from 30–60 wt.% on dry basis depending of ash content. Hydrogen is the third major constituent comprising 5–6 wt.% on dry matter. Nitrogen is a macronutrient for plants and critical for their

Table 1. Characterization of fuel – a partial proximate and ultimate analysis of fuels used in the tests (fuel as received, %)

Component	Sunflower oil		Glycerine	Paper sludge + gas		Corn kernel	Soybean straw bale		Corn stalk
	I[a]	II[b]	I	I	Equivalent fuel[c]	I	Large	Small	
Moisture	0.1	23.04	–	46.09	35.56	11.9	11.35	18.80	8.38
Ash	0	0	–	13.94	10.76	1.9	7.05	5.66	4.36
Volatile	99.17	76.13	99	39.35	53.6	76.71	60.73	59.08	69.76
Char	–	–	–	14.56	10.9	11.39	27.92	22.12	21.86
C	77.52	59.72	39.1	15.99	31.16	38.52	36.72	33.99	42.5
H	11.49	8.85	8.7	2.68	6.08	6.32	5.71	5.29	5.58
O	10.88	8.39	52.2	20.46	15.79	40.28	38.60	35.73	38.48
N	0.11	0.07	0	0.73	0.56	1.07	0.41	0.39	0.61
S	0	0	0	0.12	0.09	0.01	0.16	0.15	0.09
NCV [MJ/kg]	37	27.9	17	4.8	14.26	17.3	13.98	13.69	13.98

[a]Combustion of fuel combusted in the fluidized bed; [b]combustion of fuel mixed with water combusted in the fluidized bed; [c]equivalent fuel composition calculated on basis of mass fraction of gas and paper sludge and their elemental compositions

growth, it is mainly bound in proteins, amino acids and sugars [7]. As this paper is primarily concerned with NO_x emission from biomass combustion it is very important to note that the N-fuel content, although it is strongly influential, should not be used alone as a mean of predicting NO_x emission. The tendency of the fuels to forming certain types of pollutants, like NO_x is, is also indicated by the combustion environment. So the air supply, the combustion temperature and the applied type of combustion technology need to be taken into account as influencing variables for NO_x formation.

RESULTS OF MEASUREMENTS IN STEADY REGIMES OF OPERATION

Experimental tests on the FB installations

After starting the installation by combustion of liquid gas and reaching temperatures of FB required for beginning of the examined fuel combustion, it is dosed with the increasing of operating FB temperature to the desired value. By adjusting the flows of fuel and air, stationary operation of installation with pre-defined performance parameters was achieved. Then measurements of flue gas composition and flow rates of fuel, primary and secondary air were taken (Table 2).

The combustion process in the experiments performed on FB1 was at approximately adiabatic conditions (no heat exchange in the bed), and in the demo-industrial boiler FB2 there was a certain heat exchange between the bed and the combustion chamber walls. The combustion chamber of FB2 is bordered by water-cooled cylindrical sheath lined with refractory bricks in the area of fluidized bed. The firebricks were hindering the heat transfer from bed to the combustion chamber sheath, so combustion in FB2 was also close to the adiabatic conditions. Therefore, the measured excess air λ in all experiments at both FB installations are approximately corresponded to λ at the theoretical combustion temperature of fuel.

Table 2 shows efficient combustion of granular biomass and paper sludge fed on the fluidized bed of FB2, with higher temperatures in the bed than above it, which is not the case during the combustion of high-volatile liquid fuel where the zone with the highest combustion temperature is located above the fluidized bed (FB1). Temperatures T_3–T_5 in Table 2 corresponds to measuring points of the scheme in Fig. 2. Mixing the oil and water facilitates the feeding process, and results in a shift of the intensive combustion zone deeper in the bed, with a simultaneous reduction of CO and a slightly reduction of NO_x emission (see row II, Table 2). In all the tests the measured concentration of CO in the flue gases are much lower than the legally allowed limits, so the losses due unburned in the gaseous products of combustion are negligible. The combustion efficiency also was favorable from the standpoint of satisfying environmental regulations regarding the emissions of SO_2, as was expected considering the elemental composition of the tested fuels (Table 1).

Regarding the emissions of nitrogen oxides (NO and NO_2), expressed as NO_x, during the combustion of sunflower oil and glycerin in install FB1, and co-combustion of paper sludge and gas on the installation FB2, legal norms were not exceeded, but they were at the combustion of the granular biomass (FB2). Besides, in all the experiments, NO is the dominant compound with NO_2 in most cases being less than 5%, which is in accordance with other authors [1]**.** The values of law defined emission limits are given in Tables 2–4 in the column $NO_{x,per}$ [11]. The corn kernel has the highest fuel N content (Table 1). As the combustion experiments with this fuel did not exceed 820 °C, a temperature unfavorable for the formation of thermal and prompt NO_x, the higher emissions of nitrogen oxides is not a consequence of the combustion organization, but a consequence of the high fuel bound N. Similar might be expected during the combustion of paper sludge, which also have the high fuel bound N. The paper sludge is combusted with support of propane/butane mixture. The energy share of the gas was 73% in the mixture, which is practically an incineration of the paper sludge. In this case, the equivalent fuel (the mixture of paper sludge and liquid gas) had a nitrogen content of 0.55% as received, *i.e*., 1.04% dry ash free [15]. The high N-content in the equivalent fuel in this case did not lead to higher emissions of NO_x.

Experimental tests of baled biomass combustion

Experimental tests of soybean straw combustion (Table 3) were carried out at a furnace temperature 850–900 °C, which is high enough for complete combustion of straw, and safe from the point of ash melting. The stationary measurement regime was remained for several hours, enough to perform the necessary conclusions about the quality of combustion.

Temperatures T_1–T_3 in Table 3 corresponds to measuring points of the scheme in Figures 4 and 5. Power of both CBCS facilities was regulated by adjusting the flows of fuel, primary and secondary air, maintaining approximately the same flue gases temperature. Average power during the test were 1.55 MW and 64 kW, respectively, at the average excess air of 1.5. Temperature of the output (flue) gases in stationary regime was 160, *i.e*., 200 °C. Under these conditions carbon monoxide concentration (Table 3) in the combustion products (except in extreme cases, of collapse of the part of the burned bales in a fluidized bed of its own ashes) was 66 and 150 ppm, *i.e*., 99 mg/m^3 (reduced to 11% O_2) and 173 mg/m^3 (reduced to 13% O_2). These values were below the emission limit value of 150 mg/m^3 for the CBC installation for large bales which

Table 2. Operating parameters of FB installation 1 and 2

Regime		Fuel flow kg/h	Temperature of the active FB			The composition of the gas									λ	Air flow, L/h		N	H_{exp} mm	P_{FURma} kW_{th}
			T_3	T_4	T_5	CO_2	O_2	CO	SO_2	NO	NO_2	NO_x[a]	$NO_{x,ref}$[b]	$NO_{x,per}$		Primary	Secondary			
			°C			%		ppm				mg/m^3								
Sunflower oil	I	3.7	898	899	907	5.4	14.5	14	0	12	0	24.64	68.2[c]	100/280	3	116500	2410	4.8	459	38
	II	4.7	871	871	884	5.3	14	2	0	10	0	20.54	52.8[c]	100/280	3	116220	2300	4.6	451	38
Glycerin		8	811	813	893	5.9	14.1	14	0	5	0	12.32	27.3[c]	100/280	3	114720	2630	4.1	498	38
Paper sludge+gas		58,1 p.s.+17.2 gas	866	832	–	7.2	11.7	87	78	42	2.4	90.36	77.7[d]	250	2.4	565063	–	4.6	489	300
Corn kernel		41.2	817	769	–	6.1	14.5	22	0	246	5	515.45	634.4[d]	250	3	513722	–	4	457	170

[a]NO_x [mg/m^3] = $NO_{x,measured}$ [mg/m^3] = (NO_2+NO) [ppm]×M_{NO2}/22.4; [b]$NO_{x,ref}$ [mg/m^3] = (21–O_{2ref})/(21–$O_{2,measured}$)×$NO_{x,measured}$ [mg/m^3] [11]; [c]emission limit for small combustion plants using liquid/gaseous fuels refers to the volume fraction of O in the exhaust gas of $O_{2,ref}$ = 3%; [d]emission limit values for small combustion plants using solid fuels, which are not coal, briquettes of coal and coke, refers to the volume fraction of oxygen in the exhaust gas of $O_{2,ref}$ = 13%

Table 3. Operating parameters of CBCS installations for large and small soya straw bales

Soya straw bale	Fuel flow kg/h	Combustion temperature			Gas composition									λ	Air flow kg/h	$P_{LOŽmax}$ kW_{th}
		T_1	T_2	T_3	CO_2	O_2	CO	SO_2	NO	NO_2	NO_x	$NO_{x,ref}$	$NO_{x,per}$			
		°C			%		ppm				mg/m^3					
Large	400	850	892	160	12.42	7.88	66	-	160	6	340.8	259.8[a]	250	1.5	4100	1553
Small	17	800	710	200	13	7.35	150	-	138	7	299.8	175.7[b]	–[c]	1.5	175	64

[a]NO_x emission limit for medium combustion plants on wood refers to the volume fraction of O in the exhaust gas of $O_{2,ref}$ = 11%; [b]NO_x emission limit values for small combustion plants using solid fuels, which are not coal, briquettes of coal and coke, refers to the volume fraction of oxygen in the exhaust gas of $O_{2,ref}$ = 13%; [c]capacity of this facility is less than 100 kW so it is not subject of legislative restrictions [11]

Table 4. Oper ating parameters of CBCS furnaces for combustion corn stalk bales

Fuel flow, kg/h	Combustion temperature, T / °C	Gas composition									λ	Air flow, kg/h		$P_{LOŽmax}$ kW_{th}
		CO_2	O_2	CO	SO_2	NO	NO_2	NO_x	$NO_{x,ref}$	$NO_{x,per}$		Upper inlet	Lower inlet	
		%		ppm				mg/m^3						
9	780	9.05	11.9	45	–	165	8	355.3	312.3[a]	–	2.3	36	52	50

[a]NO_x emission limit values for small combustion plants using solid fuels, which are not coal, briquettes of coal and coke, refers to the volume fraction of oxygen in the exhaust gas of O_{2ref} = 13%

belongs to the medium scale facilities; and 4000 mg/m^3 for the installation of combustion of small bales which belongs to the low scale facilities [11]. Emissions of NO_x during the combustion of large soya straw bales in installation of power of 1.5 MW_{th}, was slightly higher than the permitted emission limit values, while at combustion of small soya straw bales, NO_x was within acceptable limits, although it is not subject of legislative restrictions [3].

Experimental test of baled cornstalk combustion (Table 4) in the selected stationary regime also showed a high quality combustion process. Good isolation and high combustion temperatures of 780 °C caused the low carbon monoxide content 40–50 ppm. Also, the carbon dioxide and oxygen was within the expected range for adiabatic conditions, so the excess air was λ = 2.3. Registered emission of NO_x during the baled cornstalk combustion was high, but it is not subject of legislative restrictions [3]. As well as in the FB combustion experiments, also in experiments with baled soybean straw and cornstalk the combustion can be considered complete under the given conditions. This is important because the measurements of fuel-N originating NO_x are representative for what may be expected in an industrial plant under nominal running conditions.

DISCUSSION

Based on the results of the measured NO_x emissions in biomass combustion experiments and the facts from previous chapters the following conclusions can be drawn:

1. Temperature has the least influence on NO_x formation due to narrow temperature range in all the experiments of biomass combustion (800900 °C).

2. Liquid biofuels combusted in the FB did not exceed the NO_x emission limits, which was logical to expect because they comprise a negligible amount of N.

3. Paper sludge co-combusted with the gas in FB2, also does not exceed the legal norms of NO_x emissions, despite the high content of N in the equivalent fuel (Tables 1 and 5 – above 0.6 wt.% on dry basis). This suggests that most likely a bigger impact on the measured NO_x emission had the char and ash content in the equivalent fuel and their catalytic effect on the NO_x reduction. In addition, this fuel contains the maximal moisture content of all tested fuels, and it is known that water reduces the NO_x emission (Figure 1). In favor of it, slightly reduce of the NO_x was recorded at the combustion of sunflower oil mixed with water.

4. At the corn kernel combustion in FB2, the highest NO_x emission was measured, which is a direct consequence of the fuel composition. The influence of the combustion conditions can be disregarded, because the operating experiment parameters prove a good organization of combustion. The high N content originates from the high protein and the other amino group content in the corn kernel. Further, this fuel has the lowest content of char of all the examined fuels that emit significant amounts of NO_x (see Table 5).

5. Trade-off between NO_x emissions and CO is best illustrated by the following two diagrams (Figure 7). In both cases**,** decreasing CO result in increased NO*,* which is especially pronounced for the incineration of paper sludge- a fuel that has a larger content of char of these two.

6. At combustion of large soya straw bales a somewhat higher emission of NO_x recorded which slightly surpass the limits, but accompanied by very low CO emission (Table 3). Low CO emission and achieved excess air of λ = 1.5 indicates efficient operating conditions.

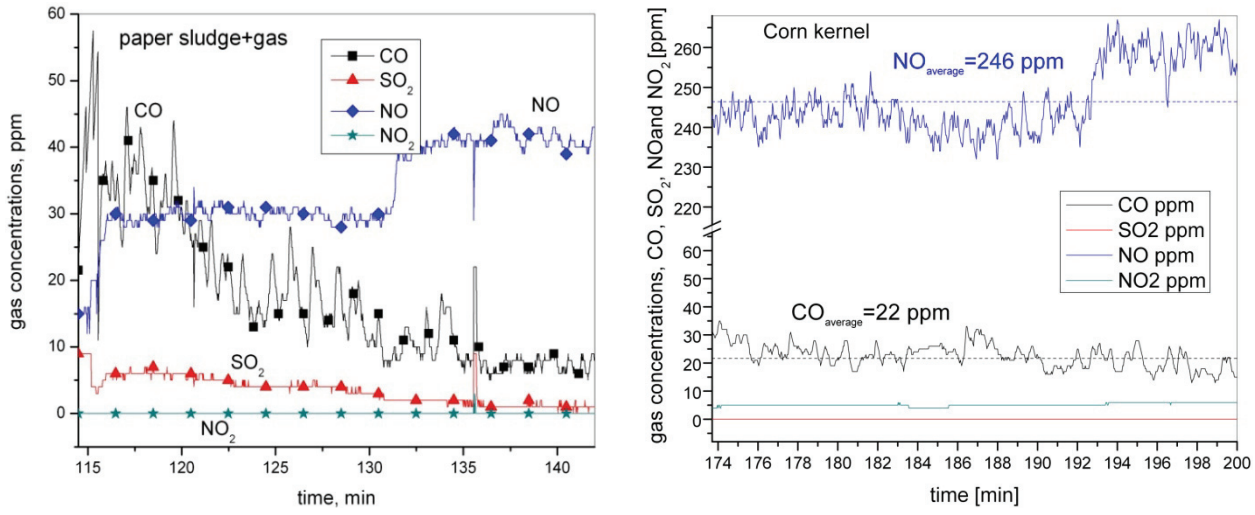
7. Recorded emissions from small facility for combustion of small soya straw bales and from a small furnace for combustion of baled corn stalks are also interesting, although both facilities are not subject of legislative restrictions. Having more nitrogen and less char in its composition (Tables 1 and 5) cornstalks emits higher amounts of nitrogen oxides then soybean straw, while it emits significantly less carbon monoxide. This fact also confirms the reducing CO and char catalytic effect on the NO_x reduction.

Analysis of denox tecniqes applicable in the presented biomass facilities

Increased environmental performance at a modest cost is one of the drivers for the use of biomass for energy purposes and that is the concept that should be taken in the selection of DeNOx techniques. Therefore, for small installations up to 100 kW, which include both systems with FB and CBS installations with small soyabean and cornstalks bales, proper choice is implementation of primary measures of NO_x reduction. The

Table 5. Char and nitrogen content calculated on fuel dry ash free base

Dry ash free, %	Paper sludge +gas	Corn kernel	Soybean straw bale		Corn stalk
			Large	Small	
	FB1	FB2	CBS1	CBS2	CBS3
Char	20.19	13.21	34.22	29.28	25.05
N	1.04	1.24	0.50	0.52	0.70



(a) (b)

Figure 7. Gas concentration (expressed in ppm) in the flue gas a) gas and the paper sludge, b) corn kernel combustion experiments.

following measures have been implemented in these installations.

Staging the introduction of combustion air is a measure of splitting the combustion air stream which creates a fuel-rich primary and fuel-lean secondary zone. It has been applied in combustion experiments with sunflower oil and glycerin (primary and secondary air supply) as well as in experiments with combustion of soybean straw and cornstalks bales (primary, secondary and the tertiary air supply).

Fuel staging (reburning) involves the injection of a proportion of fuel above the combustion zone, creating a fuel-rich secondary combustion zone where NO_x formed from the primary combustion zone is reduced through decomposition. It has been applied in experiment of paper sludge and gas co-combustion in FB 2 (liquefied gas is brought through air distributor in a bed and paper sludge is dosed on the bed) and in experiments with combustion of soybean straw bales in CBS (bale forehead burns in the porous layer, while the remaining amount withering and post-combustioning in a fluidized bed of its own ashes). This technique is of particular importance in the case of the combustion of paper sludge in FB2, where despite of the high nitrogen content of the fuel (Table 5), high NO_x emissions is not recorded.

Flue gas recirculation (FGR) technique can significantly reduce primarily thermal NO_x production by reducing flame temperatures and overall excess air. Thereby the recirculated flue gas acts as an inert gas containing mainly CO_2, CO, H_2O, with a low level of O_2 and N_2. The high concentration of CO_2 and CO caused by FGR could play a significant role in NO_x emissions. It is therefore worthwhile to examine the effect of combined staged-air combustion and FGR on NO_x formation at combustion of soya straw bales, as it was, in a way, done at combustion of corn stalks in furnace from Figure 4. Namely, mathematical model that simulates the recirculation of cold flue gases has been developed [19], primarily to reduce the maximum combustion temperature in order to solve the biomass ash sintering problem. Conducted a parametric analysis showed that the flue gas recirculation has a positive effect on this issue and, at the same time, a lower content of nitrogen oxides in the flue gas is achieved in a way that recirculation of 17% of the flue gas lowers the emission of NO for 2.5%, and FGR of 50%, reducing NO for 29.5%. It is necessary to bear in mind that the implementation of FGR reduces flue gas temperatures, as well as the boiler output.

When combustion modifications alone is insufficient to meet with the emission standards or cannot be applied in existing combustors, required additional reduction can be achieved by using of end-of-pipe flue gas treatment (FGT) technologies. The most proven FGT technologies are selective catalytic reduction (SCR) and selective non-catalytic reduction (SNCR).

SCR is the most advanced and effective method for NO_x reduction and can do so by up to 60–90%. SCR implies the reaction of NO_x with NH_3 (as a reducing agent) within a heterogeneous catalytic bed in the presence of O_2 at the temperature range of 250–400 °C. The predominant reactions are [21]:

$$4NO + 4NH_3 + O_2 \rightarrow 4N_2 + 6H_2O$$

$$6NO_2 + 8NH_3 \rightarrow 7N_2 + 12H_2O$$

The most common and successfully commercialized NH_3-SCR catalysts is vanadium pentoxide (V_2O_5), supported on titanium dioxide (TiO_2) with or without the addition of either tungsten trioxide (WO_3) or molybdenum trioxide (MoO_3). No catalysts have been active at temperatures above 600 or below 250 °C. SCR is also possible by using hydrocarbons as a reducing agent

(HC-SCR). However, at temperatures above 500 °C all of the hydrocarbons are consumed by combustion reactions. Unfortunately, the SCR process can be problematic due to high risks of catalyst poisoning by vapours of volatile metals and sulfur oxides, catalyst bed erosion and its rapidly fouling and plugging by dust and the potential occurrence of an ammonia slip-stream in the exhaust. In addition of high capital costs, there are some concerns asociated with anhydrous ammonia storage.

Selective non-catalytic reduction (SNCR) is a process of reduction of NO_x to N_2 in the presence of O_2 by reaction with amine-based reagents, either ammonia (NH_3) or urea ($CO(NH_2)_2$) at the temperature window of 800–1000 °C, the higher temperature being needed for urea. SNCR systems can reduce NO_x emmision by 30–70%, which is highly variable for different applications. Taking NH_3 as the reagent the predominant reactions are [21]:

$4NH_3 + 6NO \rightarrow 5N_2 + 6H_2O$

$4NH_3 + 4NO + O_2 \rightarrow 4N_2 + 6H_2O$

$8NH_3 + 6NO_2 \rightarrow 7N_2 + 12H_2O$

If urea is the reagent the reaction scheme is:

$H_2NCONH_2 + 2NO + ½O_2 \rightarrow 2N_2 + CO_2 + H_2O$

The reagent ammonia or urea can be injected directly into combustion chamber. When the reaction temperature increases over 1000 °C, the NO_x removal rate decreases due to thermal decomposition of NH_3 ie $CO(NH_2)_2$. It is important to bear in mind that ammonia slip from SNCR systems could occurs either from injection at temperatures too low for effective reaction with NO_x or from over-injection of reagent leading to uneven distribution, because NO_x distribution varies within the cross section. Thus, more NH_3 must find its way to the center, where more NO_x will form, and less near the walls (which are cooler so less NO_x will form), otherwise NO in the center meets insufficient ammonia for reduction and excess NH_3 near the walls slips through. This can be especially acute in larger boilers. A typical SNCR system involves reagent storage and handling systems (similar to those for SCR systems), multi-level reagent-injection equipment, and associated control instrumentation. Because of higher stoichiometric ratios required at equivalent efficiency, both NH_3 and urea SNCR processes require larger quantities of reagent than SCR systems to achieve similar NO_x reductions [21]. The technology is attractive due to its relative simplicity, catalyst-free system (hence free of associated problems), ease of installation on existing plants, applicability to all types of stationary-fired equipments, lower capital and operating cost, the fact that it is largely unaffected by fly ash and usability with other NO_x emission control technologies.

The end-of-pipe techniques are economically justified only in the installation with the cigarette combustion of large bales. It should be noted that plants of capacity less than 100 kW for the time being are not subject of legal restrictions with regard to NO_x emissions.

Facility design for combustion of large soya straw bales anticipated places for the installation of SCR (between the two heat exchangers, where the corresponding temperature window would be regulated by closure of an adequate number of gas pipes) and for SNCR (on the gases exit from the boiler), as shown in Figure 8.

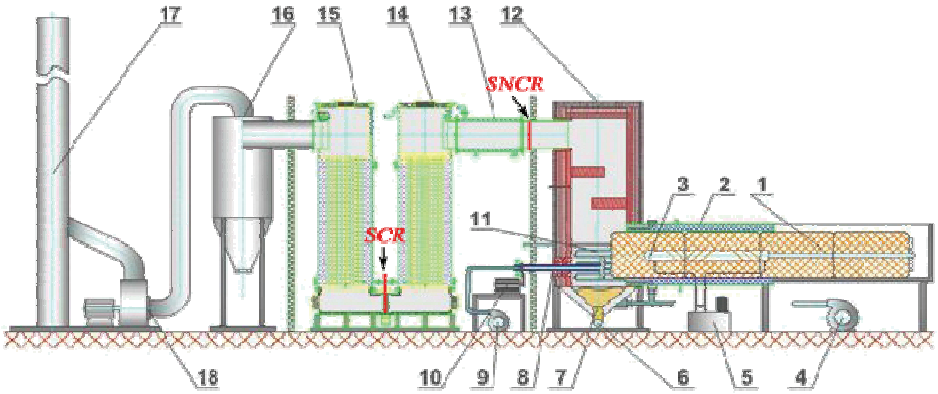


Figure 8. Places provided for testing applicability of SNCR and SCR at the facility for burning large bales.

The problem of high NO_x emissions from granular biomass combustion in large FB plants could be solved in a similar way, by installing SCR or SNCR, depending on the economic analysis for such a plant, or by conducting of co-combustion of biomass and coal or other fossil fuels, with a higher content of carbon/char (C-fixed).

CONCLUSION

The chemical properties of the different kinds of biomass affect their thermal utilisation and thus the combustion and flue gas cleaning technologies needed.

The fuel N content is responsible for NO_x formation and NO_x emissions belong to the main environmental impact factors of biomas combustion. An experimental investigation was carried out to study the NO_x emission by combustion of six types of biomass (sunflower oil, glycerin, paper sludge, granulated bimass – corn kernel, large and small soya straw and cornstalks bales) using different combustion technologies (fluidized bed and cigarette combustion). Liquid fuels (sunflower oil, glycerin) almost not containing nitrogen in their composition emit negligible nitrogen oxides at their combustion. Corn kernel has the highest nitrogen and the lowest char contents that result in a high NO_x emission level. Baled cornstalks containing high nitrogen had also high NO_x emission, although the cigarete burner combustion facility at its capacity is not subject to legal restrictions. Experiments have shown that there is some trade-off between NO_x emissions and carbon monoxide – decreasing one result in increases in the other. During the co-combustion of high N content paper sludge and propane butane, a high NO_x emission was not recorded, which is merit to high char and moisture content and due in part to fuel staging combustion. Also, during the combustion of soybean straw bales (of various sizes) on two different installations with same CBCS technology at similar combustion parameters different NO_x emissions were recorded, and in the case of combustion of large bales a slightly exceeding of the limits.

The paper presents existing and proposes possible measures for reducing NO_x emissions in the investigated installations.

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REFERENCES

[1] A. Khan, W. De Jong, P.J. Jansens, H. Spliethoff, Biomass combustion in fluidized bed boilers: Potential problems and remedies, Fuel Process. Technol. **90** (2009) 21–50.

[2] EPA Technical Bullletein, Nitrogen Oxides (NO_x),Why and How They Are Controlled, EPA-456/F-99-006R, November 1999, http://www.epa.gov/ttncatc1/dir1/ /fnoxdoc.pdf

[3] B.Leckner, M.Karlsson, Gaseous emissions from circulating fluidized bed combustion of wood, Biomass Bioenerg. **4** (1993) 379−389.

[4] C.S. Chyang, F.P. Qian, Y.C. Lin, S.H. Yang, NO and N_2O emission characteristics from a pilot scale vortexing fluidized bed combustor firing different fuels, Energ. Fuel. **22** (2008) 1004−1011.

[5] L.A.C. Tarelho, D.S.F. Neves, M.A.A. Matos, Forest biomass waste combustion in a pilot-scale bubbling fluidised bed combustor, Biomass Bioenerg. **35** (2011) 1511−1523.

[6] J. Werther, M. Saenger, E.-U. Hartge, T. Ogada, Z. Siagi, Combustion of agricultural residues, Prog. Energy Combust. Sci. **26** (2000) 1−27.

[7] J. Bai, C. Yu, L. Li , P. Wu, Z. Luo, M. Ni, Experimental Study on the NO and N_2O Formation Characteristics during Biomass Combustion, Energ. Fuel. **27** (2013) 515−522.

[8] I. Obernberger, T. Brunner, G. Bärnthaler, Chemical properties of solid biofuels – significance and impact, Biomass Bioenerg. **30** (2006) 973–982.

[9] E. Houshfar, T. Løvås, Ø. Skreiberg, Experimental Investigation on NO_x Reduction by Primary Measures in Biomass Combustion: Straw, Peat, Sewage Sludge, Forest Residues and Wood Pellets, Energies **5** (2012) 270––290

[10] L. Baxter, Biomass-coal co-combustion: opportunity for affordable renewable energy, Fuel **84** (2005) 1295––1302.

[11] Uredba o graničnim vrednostima emisije zagađujućih materija u vazduhu, Službeni glasnik RS, broj 71/2010, 6/2011 - ispr, 2010/11 (in Serbian)

[12] S. Nemoda, M. Mladenović, S. Beloševic, R. Mladenović, D. Dakić, Numerical model of gaseous fuel jet injection into a fluidized furnace, Int. J. Heat Mass Transfer **52** (2009) 3427–3438.

[13] M. Mladenović, S. Nemoda, D. Dakić, B. Repić, A. Erić, D. Đurović, M. Komatina, The Experiments of Fluidized Bed Combustion of Diversely Liquid Fuel, Termotehnika **36** (2010) 133–142.

[14] M.R. Mladenović, D. V.Dakić, S.Dj. Nemoda, R.V. Mladenović, A.M. Erić, B.S. Repić, M.S. Komatina, Combustion of low grade fractions of Lubnica coal in fluidized bed, Therm. Sci. **16** (2012) 295–309.

[15] M. Mladenović, D. Dakić, S. Nemoda, A. Erić, D. Đurović, M. Paprika, A paper waste combustion in a demo-industrial facility with FB, International Conference Power Plants 2012, Zlatibor, Serbia, Proceedings on CD-ROM, pp. 1220–1236.

[16] M. Mladenović, S. Nemoda, D. Dakić, P. Milijana, D. Đurović, B. Repić, Mogućnosti sagorevanja kontaminirane zrnaste biomase i drugih ostataka poljoprivredne proizvodnje u fluidizovanom sloju, Savremena poljoprivredna tehnika **39** (2013) 213–220 (in Serbian).

[17] V. Turanjanin, D. Đurović, D. Dakić, A. Erić, B. Repić., Development of the boiler for combustion of agricultural biomass by products, Therm. Sci. **14** (2010) 707––714.

[18] B.S. Repić, D.V. Dakić, A.M. Erić, D.M. Đurović, S.Dj. Nemoda, M.R. Mladenović, Development of the technology for combustion of large bales using local biomass, In: Sustainable Energy-Recent Studies, A. Gebremedhin (Ed.) , InTech, Rijeka, 2012, pp. 55–87

[19] A.M. Erić, S.Đ. Nemoda, D.V. Dakić, Experimental and Numerical Study of Biomass Combustion in 50 kW Cigar Furnace, in Proceedings of the 16th Symposium on Thermal Science and Engineering of Serbia, Sokobanja, Serbia, October 22–25, 2013, pp. 377–385.

[20] D. Dakić, A. Erić, D. Đurović, M. Erić, G. Živković, B. Repić, M. Mladenović, S. Nemoda, N. Mirkov, A. Stojanović, One approach of using the agricultural biomass for heating. In: Proceedings of the 18th European Biomass Conference "From research to industry and markets", 2010 May 3–7, Lion, France, 2010.

[21] S. Mahmoudi, J. Baeyens, J.P.K. Seville, NO_x formation and selective non-catalytic reduction (SNCR) in a fluidized bed combustor of biomass, Biomass Bioenerg. **34** (2010) 1393–1409.

IZVOD

SAGOREVANJE BIOMASE – UTICAJ NJENE VRSTE I PRIMENJENE TEHNOLOGIJE SAGOREVANJA NA EMISIJU AZOTOVIH OKSIDA

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(Naučni rad)

Usklađivanje potreba očuvanja životne sredine i rastućih energetskih potreba savremenog društva promoviše primenu biomase kao zamene za fosilna goriva i održivu opciju za ublažavanje emisije gasova staklene bašte. Za domaće prilike ovo je od posebnog značaja stoga što više od 60% obnovljivih izvora pripada biomasi. Pored niza prednosti upotrebe biomase u energetske svrhe, postoje i izvesni nedostaci, od kojih je i moguća relativno visoka emisija NO_x prilikom sagorevanje ove vrste goriva. Stoga su u radu prezentovani i analizirani rezultati eksperimenta sagorevanja više vrsta biomase (balirane sojine slame i kukuruzne stabljike, zrnaste biomase, suncokretovog ulja, glicerina i papirnog mulja), primenom različitih tehnologija sagorevanja (fluidizovan sloj i cigaretno sagorevanje), sa akcentom na emisiju NO_x u dimnim gasovima. Dat je prikaz eksperimetalno-demonstracionih instalacija, kao i analiziran uticaj sastava predmetnog goriva, režima i tehnologije sagorevanja na emisiju NO_x. Kako su se svi režimi sagorevanja biomase odvijali na temperaturama dovoljno niskim da se emisije termičkog i promptnog NO_x mogu zanemariti, zaključak je da emisija azotovih oksida prvenstveno zavisi od sastava biomase i raste sa sadržajem azota, a opada sa sadržajem koksnog ostatka koji obezbeđuje katalitičku površinu za redukciju azotovih oksida ugljen- - monoksidom. U radu su prezentovane primenjene i predložene mere/tehnike za smanjenje emisije azotnih oksida pri sagorevanju biomase.

Ključne reči: fluidizovan sloj • cigaretno sagorevanje • biomasa • NO_x

Benzo[*a*]piren, benzo[*a*]antracen, benzo[*b*]fluoranten i hrizen u dimljenom mesu i dimljenim proizvodima od mesa – Validacija metode

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Izvod

U ovom radu prikazani su rezultati validacije metode za određivanje benzo[*a*]pirena, benzo[*a*]antracena, benzo[*b*]fluorantena i hrizena, tj. PAH4 jedinjenja (Polycyclic Aromatic Hydrocarbons, PAH) u dimljenom mesu i dimljenim proizvodima od mesa. Metoda je zasnovana na ekstakciji lipida i lipofilnih jedinjenja ubrzanom ekstrakcijom rastvaračima, prečišćavanju dobijenog ekstakta i kvalitativnom i kvantitativnom određivanju PAH4 jedinjenja, HPLC tehnikom sa fuorescentnim detektorom. Tokom validacionog procesa, ispunjeni su kriterijumi propisani Regulativom Komisije EU br. 836/2011, kao sto su LOD, LOQ, ponovljivost, preciznost u uslovima ponovljivosti, specificnost i prinos. Metoda se uspešno može primeniti za svakodnevnu analizu PAH4 jedinjenja u u dimljenom mesu i dimljenim proizvodima od mesa.

Ključne reči: PAH4 jedinjenja, benzo[*a*]antracen, hrizen, benzo[*b*]fluoranten, benzo[*a*]piren, dimljeno meso i dimljeni proizvodi od mesa, validacija.

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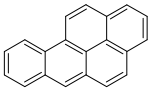
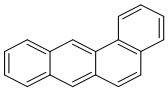
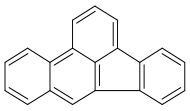
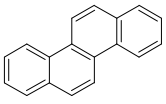
Benzo[*a*]piren, benzo[*a*]antracen, benzo[*b*]fluoranten i hrizen su policiklični aromatični zagađivači, tj. PAH jedinjenja, koja u meso i proizvode od mesa, najčešće dospevaju procesom sagorevanja drveta tokom dimljenja. Dimljenje mesa i proizvoda od mesa jedna je od najstarijih tehnologija konzervisanja, koja se primenjuje od davnina. Dimljenje se definiše kao proces prodiranja isparljivih komponenti, nastalih termalnom razgradnjom drveta, u meso i proizvode od mesa [1]. Dim koji nastaje sagorevanjem drveta sadrži veliki broj hemijskih jedinjenja, od kojih mnoga negativno utiču na ljudsko zdravlje [2,3]. Činjenica da neka PAH jedinjenja imaju mutagene i karcinogene osobine, podstakla su mnoge naučnike u svetu da se bave raznovrsnim proučavanjima policikličnih aromatičnih ugljovodonika [4–9].

Internacionalna agencija za istraživanje raka [10] klasifikuje benzo[a]piren kao karcinogeno jedinjenje (Grupa 1), a benzo[*a*]antracen, benzo[*b*]fluoranten i hrizen kao moguće karcinogena jedinjenja (Grupa 2B), (slika 1). Ova četiri jedinjenja (PAH4 jedinjenja), su u izveštaju evropske komisije o zagađivačima u lancu ishrane, predložena za markere prisustva drugih policikličnih aromatičnih ugljovodonika u hrani [11]. Prvobitno je Evropska Unija [12,13] predložila zemljama

Benzo[a]piren, **BaP** Grupa 1 	Benzo[a]antracen, **BaA** Grupa 2B 
Benzo[b]fluoranten, **BbF** Grupa 2B 	Hrizen, **CHR** Grupa 2B 

Slika 1. Nazivi, strukturne formule i skraćenice PAH4 jedinjenja.
Figure 1. Titles, chemical structures and abbreviations of the PAH4 compounds.

članicama, da se u različitim namirnicama, ispita sadržaj takozvanih 16 EU prioritetnih PAH jedinjenja (benzo[*c*]fluoren, benzo[*a*]antracen, ciklopenta[*c*,*d*]piren, hrizen, 5-metilhrizen, benzo[*b*]fluoranten, benzo[*j*]fluoranten, benzo[*k*]fluoranten, benzo[*a*]piren, benzo[*g*,*h*,*i*]perilen, dibenzo[*a*,*h*]antracen, indeno[1,2,3-*cd*]pi-

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ren, dibenzo[*a*,*e*]piren, dibenzo[*a*,*h*]piren, dibenzo[*a*,*i*]-piren, dibenzo[*a*,*l*]piren). Benzo[*a*]piren je, takođe, prvobitno bio korišćen kao marker i indikator prisustva i karcinogenosti PAH jedinjenja u analiziranim uzorcima.

Regulativom komisije Evropske Unije od 19. avgusta 2011 [14] odlučeno je da se benzo[a]piren više ne može smatrati pogodnim markerom i indikatorom PAH jedinjenja, a u tu svrhu, umesto benzo[a]pirena, koristi se suma sadržaja PAH4 jedinjenja (benzo[*a*]piren, benzo-[*a*]antracen, benzo[*b*]fluoranten i hrizen). Takođe, Komisija je propisala i maksimalno dozvoljene količine (MDK) za benzo[*a*]piren i sumu PAH4 u različitim namirnicama i one su i sastavni deo propisa Republike Srbije (tabela 1) [15].

Cilj ovog rada bio je validacija metode za kvalitativno i kvantitativno određivanje benzo[a]antracena, hrizena, benzo[*b*]fluorantena i benzo[*a*]pirena u dimljenom mesu i dimljenim proizvodima od mesa u skladu sa zahtevima Pravilnika Republike Srbije [15] koji je stupio na snagu 13. marta 2014. Ovaj rad predstavlja nastavak našeg prethodnog istraživanja [16].

EKSPERIMENTALNI DEO

Plan validacije metode

Validacija metode urađena je po postupku koji je zasnovan na odluci EU 657/2002 [17]. S obzirom da su PAH jedinjenja lipofilna, kao matriks na kome će vršiti validacija izabrana je svinjska mast, za koju je analizom utvrđeno da ne sadrži ostatke jedinjenja koja podležu validacionom procesu (blanko uzorak masti). Smeša standarda PAH4 jedinjenja kojom su obogaćivani blanko uzorci masti pripremljena je tako da suma PAH4 jedinjenja, kao i sadržaj BaP budu jednaki MDK vrednostima propisane ovim pravilnikom, koja važe od 1. septembra 2014. godine (tabela 1). U tabeli 2 dati su teoretski sadržaji BaA, CHR, BbF i BaP u dimljenom mesu i dimljenim proizvodima od mesa u μg/kg na različitim MDK nivoima obogaćenosti blanko uzoraka masti. Tokom validacije metode, određivani parametri bili su specifičnost, ponovljivost, intra-laboratorijska reproducibilnost, limit detekcije, limit kvanifikacije i prinos, u skladu sa EU Regulativom br. 836/2011 [18].

Tabela 2. Teoretski sadržaji pojedinačnih PAH4 jedinjenja (μg/kg) na različitim MDK nivoima
Table 2. Theoretical content of the PAH4 compounds (μg/kg) on different MRL levels

MDK / μg kg^{-1}	Jedinjenje				
	BaA	CHR	BbF	BaP	suma PAH4
0,25	0,75	0,75	1	0,5	3
0,5	1,5	1,5	2	1	6
1	3	3	4	2	12
1,5	4,5	4,5	6	3	18

Reagensi i ostali materijali

Tokom procesa validacije metode korišćeni su: acetonitril (Sigma Aldrich, Germany), metilen-hlorid (J.T.

Tabela 1. MDK (maksimalno dozvoljene količine) za PAH jedinjenja
Table 1. MRL (maximum residue level) for the PAH4 compounds

Tačka	Proizvod	Benzo[a]piren *MDK* / μg kg^{-1}	Suma benzo[*a*]pirena, benzo[*a*]-antracena, benzo[*b*]fluorantena i hrizena, *MDK* / μg kg^{-1}
6.1.1.	Ulja i masti (isključujući kakao puter i kokosovo ulje) namenjena za neposrednu ljudsku potrošnju ili kao sastojak u hrani	2,0	10,0
6.1.2.	Kakao u zrnu ili proizvodi od kakao zrna	5,0	35,0 do 31.3.2015. godine; 30,0 od 1.4.2015. godine.
6.1.3.	Kokosovo ulje namenjeno za neposrednu ishranu ljudi ili kao sastojak u hrani	2,0	20,0
6.1.4.	Dimljeno meso i dimljeni proizvodi od mesa	5,0 do 31.8.2014. godine; 2,0 od 1.9.2014. godine	30,0 do 31.8.2014. godine; 12,0 od 1.9.2014. godine
6.1.5.	Meso dimljene ribe i dimljeni proizvodi ribarstva, osim proizvoda iz tačke 6.1.6. i 6.1.7. Maksimalne količine za dimljene rakove važe za mišićno meso sa dodacima i grudi, a u slučaju dimljenih kraba i rakova sličnim krabama (*Brachyura* i *Anomura*) se odnosi na mišićno meso iz dodataka	5,0 do 31.8.2014. godine; 2,0 od 1.9.2014. godine	30,0 do 31.8.2014. godine; 12,0 od 1.9.2014. godine
6.1.6.	Dimljene papaline i konzervirane dimljene papaline (*Sprattus sprattus*); školjke (sveže, ohlađene ili zamrznute); termički obrađeno meso i termički obrađeni proizvodi od mesa namenjeni za neposrednu ishranu ljudi	5,0	30,0
6.1.7.	Školjke (dimljene)	6,0	35,0

Baker, USA), n-heksan (Sigma Aldrich, Germany), voda HPLC čistoće (Sigma Aldrich, Germany), smeša za sušenje (poly(acrylic acid), partial sodium salt-*graft*-poly-(ethylene oxide), cross-linked) (Sigma Aldrich, Germany), blanko mast (Oma's SCHMALZ, Schachinger, Germany), glass Fiber Filter _ Cellulose (Dionex, 19.8 mm, 100 PCS), mega SPE kolonice (punjene sa 5 g silika faze, 20 mL) (Phenomenex, USA), PTFE filter (Whatman) veličine pora 1 μm. Svi korišćeni rastvarači bili su HPLC čistoće. Standardi PAH4 jedinjenja, bili su analitičke čistoće proizvođača Dr. Ehrenstorfer, Germany.

Priprema uzoraka za HPLC analizu i HPLC analiza

Ubrzana ekstrakcija pomoću rastvarača (accelerated solvent extraction, ASE)

Blanko uzorak pripremljen je tako, što je ekstrakciona ASE ćelija od 33 ml, napunjenja smešom za sušenje. Matriksi obogaćeni PAH4 jedinjenjima pripremani su na sledeći način: 2,00 g blanko masti pomešano je sa istom ili većom masom smeše za sušenje. Tako pripremljen uzorak stavljen je u ASE ćeliju, i u nju je dodata određena zapremina PAH4 smeše, poznate koncentracije. Obogaćivanje je vršeno na nivou od 0,5 MDK, 1 MDK ili 1,5 MDK. Nakon obogaćivanja, ASE ćelije su dopunjavane smešom za sušenje. Ekstrakcija uzorka rađena je na aparatu ASE 200 Dionex (Sunnyvale, USA), uz korišćenje n-heksana kao rastvarača, na temperaturi od 100 °C i pritisku od 10 MPa. Nakon ekstrakcije rastvarač je uparen u struji azota, u N-uparivaču sa kabinetom na 50 °C. Nakon uparavanja, ASE viale su ostavljane u eksikator preko noći, a zatim su obogaćeni uzorci matriksa iz ASE viale preneti n-heksanom u normalni sud od 10 ml.

Ekstrakcija na čvrstoj fazi (solid phase extraction, SPE)

Ekstrakcija na čvrstoj fazi je korišćena kao postupak za uklanjanje molekula lipida iz ispitivanih uzoraka. Prečišćavanje je vršeno pomoću mega SPE kolonica. Od pripremljenog uzorka nakon ASE ekstarkcije uzimano je 1 ml i nanošeno na kolonu, nakon ispiranja i kondicioniranja mega SPE kolonice [19].

HPLC analiza

HPLC ispitivanja izvođena su korišćenjem visokoefikasne tečne hromatografije sa fluorescentnim detektorom, korišćenjem HPLC uređaja (Shimadzu, Japan), koji se sastoji od pumpe LC-20AB, autosamplera SIL-20A, degazera DGU-20A5, peći CTO-20A i fluorescentnog detektora RF-10AXL. Uslovi HPLC analize utvrđeni su u prethodnom istraživanju [16], kolona: Phenomenex, Envirosep PP 5 μm PP, LC column 125 mm×4.6 mm; predkolona: Security Guord Cortridges C 18 4 mm×3,0 mm ID; vreme trajanja analize: 35 min; injektovana zapremina: 50 μl; protok mobilne faze: 1,2ml/min: pritisak u koloni: 0,0–35,0 MPa; talasne dužine (ekscitacija/emisija): BaA i CHR – 275/385 nm, BbF – 256/446 nm, BaP – 260/410 nm; T peći 25–85 °C; mobilna faza: ACN/H_2O=70/30).

Statistička obrada podataka

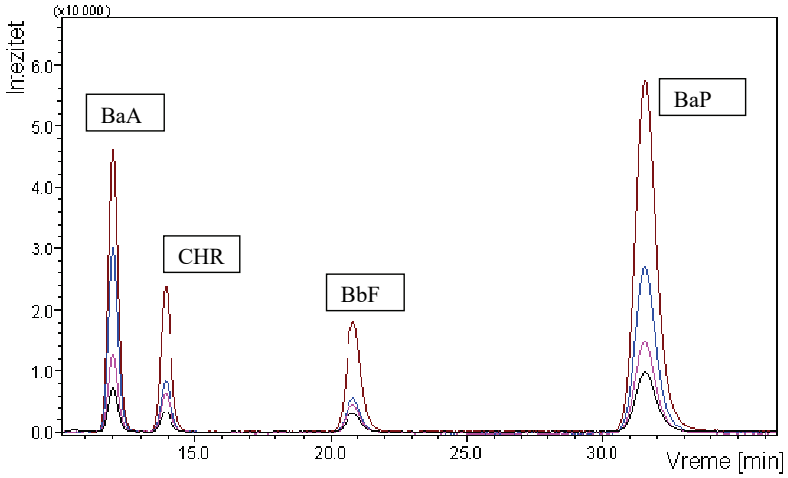
Statistička evaluacija dobijenih rezultata urađena je korišćenjem statističkog programa Minitab 16, primenom analize varijansi (ANOVA One-Way) i Tukey HSD post hoc testa za određivanje statistički značajnih razlika (p < 0,05) između srednjih vrednosti prinosa PAH4 jedinjenja, za odgovarajuće merene vrednosti.

REZULTATI I DISKUSIJA

Ispitani su blanko uzoraci masti u cilju potvrde specifičnosti metode (odsustva interferenci). 20 blanko uzoraka masti pripremljeno je za HPLC analizu kao što je opisano u eksperimentalnom delu. Na osnovu dobijenih hromatograma ispitanih uzoraka utvrđeno je da je metoda specifična, tj. na retencionim vremenima analiziranih jedinjenja ne pojavljuju se interference koje bi onemogućavale identifikaciju i kvantifikaciju BaA, CHR, BbF i BaP. Na slici 2 prikazani su hromatogrami standardne smeše PAH4 jedinjenja na nivoima obogaćenosti blanko uzorka masti od 0,25, 0,5, 1 i 1,5 MDK. Na osnovu ovih hromatograma određena je linearnost u pet tačaka, uključujući i nulu, za svako ispitano PAH jedinjenje (r^2_{BaA} = 0,997; r^2_{CHR} = 0,997; r^2_{BbF} = 0,994; r^2_{BaP} = 0,993; p < 0,05-za sve korelacije). Kalibracija se izvodila pre svakog validacionog eksperimenta.

Blanko uzorci masti (n = 20), obogaćeni su PAH4 smešom na nivou 1 MDK. Na osnovu dobijenih rezultata nakon HPLC analize izračunata je granica odluke – *Ccα*. Granica odluke je granična vrednost na i iznad koje se, sa α verovatnoćom greške (α – procenat lažno pozitivnih rezultata), može zaključiti da uzorak nije u skladu sa propisima [17]. Prinosi analiziranih jedinjenja bili su u opsegu od 76,6 do 100,0% za BaA, od 98,4 do 102,3% za CHR, od 98,5 do 100,0% za BbF i od 98,2 do 103,3% za BaP. Granica odluke se računa tako što se za ispitanih 20 obogaćenih uzoraka blanko masti izračuna standardna devijacija (Sd) sadržaja pojedinačnog jedinjenja u uzorku, pomnoži sa 1,64 i doda vrednosti MDK. Izračunata vrednost za Ccα iznosi 12,46 μg/kg.

Sposobnost detekcije određena je na sledeći način. Blanko uzorci masti (n = 20) obogaćeni su PAH4 smešom na nivou *Ccα*, a zatim je na osnovu dobijenih rezultata nakon HPLC analize izračunata je vrednost za *Cc*β, tj. sposobnost detekcije. Sposobnost detekcije je najmanja količina supstance koju je moguće detektovati, identifikovati ili kvantifikovati u uzorku, sa β verovatnoćom greške (β – procenat lažno negativnih rezultata). U slučaju supstanci za koje je utvrđena maksimalno dozvoljena količina (*MDK* vrednost), sposobnost detekcije je koncentracija na kojoj se metodom mogu, sa statističkom sigurnošću od 1–β, detektovati koncentracije na *MDK* vrednosti. Na osnovu dobijenih



Slika 2. Hromatogrami standarda PAH4 jedinjenja na nivou 0,25, 0,5, 1 i 1,5 MDK.
Figure 2. Chromatograms of standard solutions of the PAH4 compounds at the level of 0.25, 0.5, 1 and 1.5 MRL.

rezultata utvrđeno je da se prinosi analiziranih jedinjenja kreću u opsegu od 86,7 do 100,1% za BaA, od 99,0 do 100,5% za CHR, od 98,8 do 100,5% za BbF i od 99,7 do 100,3% za BaP. Sposobnost detekcije – *Cc*β, se računa tako što se za ispitanih 20 obogaćenih uzoraka blanko masti, tokom trećeg dana validacije, izračuna standardna devijacija (*Sd*) sadržaja pojedinačnog jedinjenja u uzorku, pomnoži sa 1,64 i doda vrednost *Cc*α. Izračunata vrednost za *Cc*β iznosi 12,76 µg/kg.

U cilju ispitivanja ponovljivosti, tri dana je po 6 blanko uzoraka masti obogaćeno PAH4 smešom na nivoima od 0,5, 1 i 1,5 *MDK*. U tabeli 3 date su izračunate vrednosti prinosa nakon HPLC analize. Na osnovu dobijenih rezultata izračunata je vrednost za koeficijent varijacije (*Cv*) i ispitana je ponovljivost. Uobičajeno je da se teorijska vrednost koeficijenta varijacije izračunava iz Horovicove jednačine [20]. Međutim, za niske sadržaje (npr. 10 ili 1 µg/kg) Horovicova jednačina daje neprihvatljivo visoke vrednosti za *Cv*, pa se u tim slučajevima koristi Tompsonov model izračunavanja [21]. Koeficijent varijacije prinosa, za niske sadržaje, prema Tompsonovoj jednačini treba da bude manji od 22%.

Korišćenjem dobijenih podataka izračunat je koefi-

Tabela 3. Prinosi obogaćenih blanko uzoraka masti (n = 6) tokom tri dana validacije metode u cilju ispitivanja ponovljivosti
Table 3. Recovery of spiked fat blank samples (n = 6) during three days of validation of the method in order to test the repeatability

Proračun	Obogaćenost matriksa	PAH4	Prinos, (SV±SD)[a] / %		
			Ia	IIa	IIIa
Ia, IIa, IIIa – dani	0,5 *MDK*	BaA	100,1±0,7	100,7±1,4	99,8±0,3
		CHR	102,4±3,0[b]	99,4±0,8[b]	99,5±0,4[c]
		BbF	100,5±2,9	97,6±2,7	99,7±0,8
		BaP	91,0±8,9	96,4±4,4	97,3±5,0
	1 *MDK*	BaA	108,6±5,1[b]	100,0±0,1[c]	99,9±0,4[c]
		CHR	97,1±1,9[b]	99,7±0,4[c]	99,9±0,1[c]
		BbF	96,3±2,3[b]	99,6±0,5[c]	99,9±0,1[c]
		BaP	104,2±5,0	103,3±6,1	100,0±0,3
	1,5 *MDK*	BaA	99,8±0,4	99,8±0,1	100,1±0,3
		CHR	99,6±0,5	99,9±0,5	99,8±0,3
		BbF	99,6±0,2	99,7±0,7	99,8±0,2
		BaP	96,3±4,1	96,9±4,8	97,7±3,3
		BaA	CHR	BbF	BaP
$Cv_{srednje}$ / %		3,17	1,72	1,94	6,16
$HORRAT_r$ definisan regulativom 836/2011		< 2	< 2	< 2	< 2
$HORRAT_r$ izračunat	0,5 *MDK*	1,000	0,999	1,001	1,008
	1 *MDK*	0,996	1,002	1,002	0,996
	1,5 *MDK*	1,000	1,000	1,000	1,005

[a]srednja vrednost ± standardna devijacija]; [a,b]vrednosti u istom redu koje su označene različitim slovima se statistički značajno razlikuju (p < 0,05). Vrednosti koje se statistički značajno ne razlikuju nisu obeležene slovnim oznakama

cijent varijacije prinosa (tabela 3), za svako ispitano jedinjenje, na svim nivoima obogaćenosti uzoraka. Na osnovu dobijenih vrednosti, koje su znatno ispod 22%, može se zaključiti da je postignuta dobra ponovljivost. U skladu sa Regulativom Komisije EU br. 836/2011 [18], a u cilju ispitivanja ponovljivosti izračunata je $HORRAT_r$ vrednost (tabela 3). Izračunate $HORRAT_r$ vrednosti za sva četiri ispitana jedinjenja ispunjavaju propisane uslove.

Preciznost u uslovima ponovljivosti ispitana je na isti način kao i ponovljivost. U toku tri dana, po 6 blanko uzoraka masti obogaćeno je PAH4 smešom, na nivoima od 0,5, 1 i 1,5 *MDK*. U tabeli 4 date su izračunate vrednosti prinosa nakon HPLC analize tako pripremljenih uzoraka. Cilj je bio da se izračuna koeficijent varijacije (*Cv*) svih rezultata, koji, prema Tompsonovoj jednačini treba da bude manji od 22%, i na taj način ispita preciznost u uslovima ponovljivosti.

Na osnovu dobijenih vrednosti za koeficijent varijacije prinosa, može se zaključiti da je postignuta dobra itralaboratorijska reproducibilnost. U skladu sa Regulativom Komisije EU br. 836/2011 [18], dobra intra-laboratorijska reproducibilnost je postignuta ukoliko je izračunata $HORRAT_R$ vrednost (tabela 4) manja od 2. Izračunate $HORRAT_R$ vrednosti za ispitana PAH4 jedinjenja ispunjavaju propisane uslove.

Statističkom obradom rezultata, koji su dobijeni u cilju ispitivanja ponovljivosti (tabela 3), kao i u cilju ispitivanja preciznosti u uslovima ponovljivosti (tabela 4), ustanovljeno je da postoji statistički zanačajna razlika (p < 0,05) između srednjih vrednosti prinosa za određena PAH jedinjenja na istom nivou obogaćenosti matriksa (tabele 3 i 4).

Nakon završene in-house validacije, izračunate su vrednosti za limit detekcije (Limit of detection, *LOD*) i limit kvantifikacije (Limit of quantification, *LOQ*) za PAH4 jedinjenja. *LOD* je izračunat kao trostruka vrednost šuma koji se javlja na retencionom vremenu traženog jedinjenja, u blanko uzorku masti, preračunat na sadržaj jedinjenja, izražen u µg/kg . *LOQ* predstavlja osam puta veću vrednost od vrednosti šuma na retencionom vremenu analiziranog PAH4 jedinjenja, u blanko uzorku masti, izražen u µg/kg. *LOD* i *LOQ* vrednosti dobijene za PAH4 jedinjenja (BaA, CHR, BbF i BaP) prikazane su u tabeli 5.

Tabela 5. Limit detekcije (LOD) i limit kvantifikacije (LOQ) PAH4 jedinjenja
Table 5. Limit of detection (LOD) and limit of quantification (LOQ) of the PAH4 compounds

PAH4	*LOD* / µg kg^{-1}	*LOQ* / µg kg^{-1}
BaA	0,23	0,87
CHR	0,25	0,82
BbF	0,19	0,50
BaP	0,03	0,07

ZAKLJUČAK

Tabela 4. Prinosi obogaćenih blanko uzoraka masti (n = 6) tokom tri dana validacije matode u cilju ispitivanja preciznosti u uslovima ponovljivosti
Table 4. Recovery of spiked fat blank samples (n = 6) during three days of validation of the method in order to test the within-laboratory reproducibility

Propračun	Obogaćenost matriksa	PAH4	Prinos, (SV±SD)[a] / %		
			Ib	IIb	IIIb
Ib, IIb, IIIb – dani	0,5 *MDK*	BaA	100,0±0,3	99,7±0,4	99,7±0,3
		CHR	99,8±0,5	99,7±0,3	99,8±0,5
		BbF	99,6±0,2[b]	99,5±0,3[b]	99,0±0,3[c]
		BaP	90,9±10,8	94,6±5,2	96,0±5,9
	1 *MDK*	BaA	100,0±0,2[b]	99,6±0,4[c]	99,7 ±0,2[b,c]
		CHR	99,8±0,1	99,7±0,3	99,7±0,1
		BbF	99,3±1,2	99,0±1,6	99,9±0,2
		BaP	99,8±0,3	99,7±0,1	99,9±0,2
	1,5 *MDK*	BaA	99,9±0,2	99,5±0,3	99,5±0,3
		CHR	99,6±0,4	99,6±0,2	99,8±0,3
		BbF	99,8±0,3	99,7±0,2	99,8±0,4
		BaP	96,6±5,1	96,6±5,2	97,4±4,0
		BaA	CHR	BbF	BaP
$Cv_{srednje}$ / %		0,33	0,32	0,72	5,74
$HORRAT_r$ definisan regulativom 836/2011		< 2	< 2	< 2	< 2
$HORRAT_r$ izračunat	0,5 MDK	1,000	1,000	1,001	1,010
	1 MDK	1,000	1,000	1,001	1,000
	1,5 MDK	1,001	1,000	1,000	1,005

[a]Srednja vrednost ± standardna devijacija]; [a,b]vrednosti u istom redu koje su označene različitim slovima se statistički značajno razlikuju (p < 0,05). Vrednosti koje se statistički značajno ne razlikuju nisu obeležene slovnim oznakama

Na osnovu rezultata in-house validacije metode za kvalitativno i kvantitativno određivanje PAH4 jedinjenja (benzo[a]piren, benzo[a]antracen, benzo[b]fluoranten, hrizen) u dimljenom mesu i dimljenim proizvodima od mesa potvrđeni su zahtevi (specifičnost, ponovljivost, preciznost u uslovima ponovljivosti, prinos, LOD i LOQ) definisani Regulativom Komisije EU br. 836/2011. U svakodnevnoj analitičkoj praksi metoda se uspešno može primeniti za analizu PAH4 jedinjenja u u dimljenom mesu i dimljenim proizvodima od mesa.

Napomena

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LITERATURA

[1] L. Tóth, Chemie der Riiucherung, Verlag Chemie, Berlin, 1983.

[2] D. Castro, K. Slezakova, C. Delerue-Matos, M.C. Alvim-Ferraz, S. Morais, M.C. Pereira, Polycyclic aromatic hydrocarbons in gas and particulate phases of indoor environments influenced by tobacco smoke: Levels, phase distributions, and health risks, Atmos. Environ. **45** (2011) 1799–1808.

[3] O. Oguntoke, A. Adebulehin, H. Annegarn, Biomass Energy Utilisation, Air Quality and the Health of Rural Women and Children in Ido LGA, South-Western Nigeria, Ind. Built Environ. **22** (2013) 528–534.

[4] G.D. Charles, M.J. Bartels, T.R. Zacharewski, B.B. Gollapudi, N.L. Freshour, E.W. Carney, Activity of benzo*(a)*pyrene and its hydroxylated metabolites in an estrogen receptor-α receptor gene assay, Toxicol. Sci. **55** (2000) 320–326.

[5] P. Šimko, Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives, J. Chromatogr., B **770** (2002) 3–18.

[6] M. Lodovici, M. Venturini, E. Marini, D. Grechi, P. Dolara, Polycyclic aromatic hydrocarbons air levels in Florence, Italy, and their correlation with other air pollutants, Chemosphere **50** (2003) 377–382.

[7] K. Straif, R. Baan, Y. Grosse, B. Secretan, F. El Ghissassi, V. Cogliano, Carcinogenicity of polycyclic aromatic hydrocarbons (on behalf of the WHO International Agency for Research on Cancer Monograph Working Group), Lancet Oncol. **6** (2005) 931–932.

[8] W. Jira, K. Ziegenhals, K. Speer, PAH in smoked meat products according to EU standards, Fleischwirtschaft Int. **4** (2006) 11–17.

[9] J. Djinovic-Stojanovic, A. Popovic, A. Spiric, W. Jira, Emission of Polycyclic Aromatic Hydrocarbons from Beech Wood Combustion, Energ. Sources Part A **35** (2013) 328–336.

[10] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, Vol. 92, Lyon, 2010.

[11] European Food Safety Authority (EFSA), Polycyclic Aromatic Hydrocarbons in Food Scientific Opinion of the Panel on Contaminants in the Food Chain, EFSA J. **724** (2008) 1–114.

[12] European Commission, Commission Recommendation 2005/108/EC of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods, O. J. EU L **34** (2005) 43–45.

[13] Joint FAO/WHO Expert Committee on Food Additives (JECFA).Summary and Conclusion. Sixty-Fourth meeting, Rome, 8-17 February, 2005, JECFA/64/SC, http:// //www.who.int/ipcs/food/jecfa/summaries/en/

[14] European Commision,Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs, O. J. EU L **215** (2011) 4–8.

[15] Službeni glasnik Republike Srbije, br. 29/2014, Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja.

[16] J. Đinović-Stojanović, J. Stišović, A. Popović, D. Nikolić, S. Janković, Određivanje PAH4 jedinjenja u dimljenom mesu i dimljenim proizvodima od mesa – razrada metode, Tehnologija mesa **55** (2014) 184–198.

[17] European Commision, Commission Decision 2002/ /657/EC of 12 August 2002 implementing Council Directive 96/23/EC establishes criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories, O. J. EU L **221** (2002) 8–36.

[18] European Commision, Commission Regulation (EU) 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo-(*a*)pyrene in foodstuffs Text with EEA relevance, O. J. EU L **215** (2011) 9–16.

[19] S. Moret, L.S. Conte, A rapid method for polycyclic aromatic hydrocarbon determination in vegetable oils, J. Sep. Sci. **25** (2002) 96–100.

[20] W. Horwitz, L.R. Kamps, K.W. Boyer, Quality assurance in the analysis of foods and trace constituents, J. AOAC Int. **63** (1980) 1344–1354.

[21] M. Thomposon, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Anal. Commun. **125** (2000) 385–386.

SUMMARY

Benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and chrysene in smoked meat and smoked meat products - validation of the method

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(Scientific paper)

Polycyclic aromatic hydrocarbons (PAHs) are products of the incomplete combustion or pyrolysis of organic material and they are among the most harmful compounds. During process of meat smoking, wood combustion is one of the most important sources of PAH compounds, which can be adsorbed by the surface of meat. The EFSA (European Food Safety Authority) Panel on Contaminants in the Food Chain (CONTAM Panel) recommended to the member states of European Union to use the sum of benzo[*a*]pyrene (BaP), benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF) and chrysene (CHR), (PAH4 compounds), as a marker for the occurrence and impact of carcinogenic PAHs in food, instead of benzo[*a*]-pyrene. The maximum content of BaP and sum of all four compounds (PAH4) has been established by European Commission Regulation No. 835/2011. For smoked foods, from 1st September 2014, the maximum BaP content was lowered to 2 µg/kg, while the content of PAH4 is allowed to 12 µg/kg. The new maximum residue limits (MRL) both for BaP and sum of PAH4 compounds in smoked meat and meat products were defined by the legislation of Serbia, as well, and it is in accordance with EU regulation. The aim of this paper was the validation of the method for identification and determination of benzo[*a*]pyrene, chrysene, benz-[*a*]anthracene and benzo[*b*]fluoranthene in smoked meat and smoked meat products. Accelerated solvent extraction (ASE) was used for extraction of lipids and lipophilic compounds. Solid Phase Extraction (SPE) was used in order to remove lipids from analysed samples. High-performance liquid chromatographic with fluorescence detection *(*HPLC-FL) was applied for identification and quantification of PAH4 compounds. Fluorescence detector operated at excitation/emission wavelength 275/385 nm for BaA and CHR, 256/446 nm for BbF and 260/410 nm for BaP, respectively. On the base of experimental results, it is possible to conclude as follows: The in-house validation procedure of the method meets all criteria (applicability, specificity, repeatability, reproducibility, recovery, LOD and LOQ) set out by EU Regulation No. 836/2011. The method is suitable for operative control of PAH4 content in smoked meat products.

Keywords: PAH4 compounds • Benz[*a*]-anthracene • Chrysene • Benzo[*b*]fluor-anthene • Benzo[*a*]pyrene • Smoked meat and smoked meat products • Validation

Whether integrating refining and petrochemical business can provide opportunities for development of petrochemical industry in Serbia

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Abstract

Since the beginning of 90s of last century both the petroleum industry and petrochemical industry have operated in difficult circumstances. In particularly, margins of petroleum and petrochemical industry were exacerbated during global economic crisis in 2008–2009 years. At that time, as one option that could be the solution, the global analysts had started to more intense investigate the benefits of Refining-Petrochemical Integration. Shortly afterwards, more and more petroleum refineries and petrochemical manufacturers began to see the future in this kind of operational, managerial, marketing and commercial connection. This paper evaluates, in particular, the achieved level of integration of refinery and petrochemical businesses in Central and South-Eastern Europe. Specifically, the paper identifies current capabilities and future chances of linking this kind of integration between Serbian refining and petrochemical players. The viability of integration between possible actors and benefits of every single refining-petrochemical interface in Serbia depend on many factors, and therefore each integrated system is unique and requires prior serious cost benefit analysis.

Keywords: refining-petrochemical integration, serbian petrochemistry, development goals, cost-benefit analysis.

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Already for a decade and more there is not prognostic study on development of the petroleum industry and/or petrochemical sector (or related published paper, presentation at the conference, etc.) that did not take into consideration the integration of refining and petrochemical business as a crucial global trend. There are already 4–5 years how this theme is subject of discussions in Serbia also, practically from the moment of recognizing the additional potential for business activities between petroleum refinery in Pančevo of JSC "NIS" and neighboring petrochemical enterprise JSC "HIP-Petrohemija". Slowly, as time went on, the professional community has begun to notice the integrative potential that possesses some other petrochemical plants in Serbia.

During last several months, the possible deeper involvement of "NIS" in "HIP-Petrohemija" has been discussed intensively by newspaper articles and Internet blogs expecting/willing/proposing/supporting that it happens upon strong interest of minority "NIS" shareholder, which is Republic of Serbia. Instead of that, "NIS" clearly declared about not having interest for taking over "HIP-Petrohemija" but supporting its development and "softening" commercial relationship.

In this paper we do not have pretension to support an unconditional merge of petroleum refining and petrochemical businesses in Serbia, but we wish to identify in one place and in the most concise form all of the elements that a serious Cost-Benefit Analysis (CBA) which treats integration of refinery and petrochemical facilities has to take into account. We believe that including and quantifying of all those elements in CBA might be a significant shift from the previous arbitrary estimates. Besides, it should be mentioned that in Serbia there are two other petrochemical enterprises with the potential for economically efficient integration with existing and planned production activities of the "NIS" - these are the companies JSC "MSK" Kikinda and JSC "Hipol" Odžaci.

Drivers for refining and petrochemical integration

Petroleum industry works in difficult circumstances since the beginning of 90s of last century, what is exacerbated during global economic crisis in 2008––2009 years. Factors that determine or direct the development of refining in Europe are: stagnation or decline in consumption of motor fuels, trend in motor fuel consumption which favours diesel over gasoline (which means surplus of FCC, Fluid Catalytic Cracking, gasoline), market fragmentation, environmental regulations, global competition (especially from regions with

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high rates of economic growth, such as Asia and Middle East), and in comparison with the rest of the World (in average) [1]:

- older production facilities (higher maintenance costs, lower energy efficiency),
- smaller dimensions of installed process plants (higher fixed operating costs), and
- higher labour costs.

Significant increase of the consumption of diesel fuel at the expense of gasoline, as well as a broader substitution of heavy fuel oil as an energy resource in petroleum refineries with natural gas (driven by European directives related to reduction of sulphur emissions), have been the main drivers of structural changes that occurred during last decade in the European demand of petroleum products. The question of surplus gasoline fractions partly have been solved through cooperation with petrochemical industries where naphtha is used as a raw material (mostly in Europe), or through export (mainly to the North American market, where there is a continuous shortage of gasoline). Using of natural gas as a fuel for use in petroleum refineries releases a solid amount of a naphtha's waste gases, which can be then used as raw material for the production of hydrogen and a series of basic petrochemicals (ethylene, propylene, para-xylene ...). The survival of the European petroleum refineries is conditioned by raising the efficiency and reaching the competitiveness in comparison to the modern refineries that are located in regions with high rates of economic growth. Approach includes technological and organizational-managerial optimizations of the existing production processes and commercial activities.

The specificity of the petrochemical sector is a highly cyclical character of this business influencing its profitability, with cycles that capture phase of prosperity (period with high margins impacting investment decisions and business development) and phase of recession (period of low, even negative, margins impacting closure of the least efficient capacities). The height and length of the amplitudes in these cycles primarily depends on the imbalances of supply and demand of petrochemical commodities, as well as on the occasional disharmonies in vertical integration starting from primary feedstocks (naphtha, ethane, natural gas, coal, and from recently also a shale gas and bio-feedstocks), *via* basic petrochemicals and petrochemical intermediates up to the final products (polymers, elastomers, synthetic resins, chemical fibers, surfactants,...), usually lasting 4–7 years. However, increasingly important factor is also unpredictable impacts of global or regional economic crisis, armed conflicts and other geopolitical disturbances, as well as natural disasters (unfortunately, more and more frequently), and consequently the dynamics of cycles of prosperity and recession is less predictable [2].

All analysts agree that global demand of petrochemical commodities will continue to grow, but very differently by regions. Leadership in demand and production has already shifted to China and other Asian countries and most new projects are developing in this region (using economy of scale and high growth of regional demand).

In Europe, some spectacular development of petrochemical industry is not expected, mainly due to stagnation of Western European economy. Within the global petrochemical industry, the capacities for production of naphtha-based olefins will continue to be in the most unfavourable economic position, and unfortunately the facilities of this type are characteristic for Europe. Manufacturers will strive to maximize production from other advanced resources, such as condensate of natural gas, coal derived liquid hydrocarbons and shale gas.

Overall, petrochemical companies throughout the world are restructuring, consolidating and implementing domestic and global strategies to improve profitability and contend with forecast economic changes, particularly in Europe where growth is lagging.

The last 5–6 years, starting with the outbreak of the global economic crisis, the manufacturers of petroleum products and petrochemicals in Europe were operating with low margins and modest rates of capacity utilization. The competition of non-European manufacturers is growing, mainly due to economy of scale, lower labor cost and access to cheaper raw materials. In the last two years there has been a certain recovery of profitability culminating in the beginning of 2015.

One option for a possible “ironing” the amplitudes of profitability in the petrochemical industry, and the specific weaknesses that characterize both refining and petrochemical sector in Europe, is integration of business activities in the field of petroleum products and petrochemicals manufacturing (called usually “Refining-Petrochemical Integration” or “Refining-Petrochemical Interface Optimization”).

Other drivers for refining and petrochemical integration include [2]:

- Reliability and security of feedstock supply with less transport cost;
- Higher possibilities for processing and re-processing of hydrocarbon streams both from refinery and petrochemical plant;
- Increased use of natural gas and own fuel gases as fuel in petroleum refineries and petrochemical manufacturing plants (substitution of heavy fuel oil);
- Lowering the costs per unit of final product (mainly fixed costs, but very often variable costs also);

- More flexibility in storing/transporting off core products and by-products;
- More outlets for high-value by-products;
- Energy savings in well-integrated hydrocarbon processes;
- Reduced needs for inventories, and therefore a significant savings in storage requirement;
- Significant reduction in shared utilities system, less variable costs;
- Supply chain optimization resulting in faster delivery of products and optimum distribution;
- Centralized support services, engineering, maintenance, laboratory, security, finance, human resources, etc., less fixed costs;
- Possibility to reach higher cash margin.

Indeed, the primary integration driver is competitiveness. Desire for higher profitability in an increasingly competitive market pushes petroleum refining and petrochemical companies to innovate ways to squeeze out costs and capture new opportunities.

The refining-petrochemical interface is primarily centered in the European refineries to take advantage of proximity to feedstock source and to get cost benefits by a joint infrastructure.

There are many examples that indicate that the refinery-petrochemical integration will continue to be crucial trend in the future. Management of the major Italian oil company "Eni" sees [9] the future of refining business in Europe through a focus on four activities: refining and petrochemical interface, increase of flexibility, achievement of technological superiority and an increase in efficiency. Sibylle Tinhof, spokesman of the major Austrian oil company "OMV", said [10] that the company will continue to be interested only in those refineries that are integrated with petrochemical business. Aslam Moola, New Business Development Manager in „Shell Chemicals", says [6] that most of the integration value comes from directing hydrocarbons to the highest-value application, irrespective of traditional refining–chemical boundaries. He says also that secondary or by-product streams from refining units may have their highest value as feedstock for chemical units, likewise, by-products from chemical units may be most cost-effective as refinery feeds or fuel blending components.

Benefits of Refining and Petrochemical Integration

Benefits of refining and petrochemical integration (RPI) are measurable both on the costs side and on the revenues side:

a) RPI means operating the activities under the same management with common objectives to optimize operations in the business or geographical areas which are in the phase of saturation or declining and also those which are in the prosperous phase, and develop new projects in the fast-growing areas. Putting together refining and chemicals teams creates synergies and generates new ideas, which brings a lot of innovation.* Main synergetic benefits that come through increased flexibility in operations and innovative technological development are:

- Increased production of light olefins (particularly propylene) and BTX (Benzene-Toluene-Xylene fraction) range aromatics from vacuum gas oil in an integrated petroleum refinery for use as petrochemical feedstock relative to maximum gasoline;
- Opportunity to convert this lower-value intermediate product from light cycle oil from the FCC unit in an integrated petroleum refinery into high-value BTX aromatics for petrochemical production (particularly para-xylene and benzene);
- Conversion of heavy distillate and vacuum gas oil feeds in an integrated petroleum refinery into maximum Naphtha for subsequent reforming, or a mixture of Naphtha and premium quality jet and diesel streams for transportation fuel blending;
- Opportunity to increase olefin production in an integrated petrochemical plant by improving the quality of the cracker feed and improving the ethylene/propylene selectivity of the product slate (these improvements are achieved in an integrated refining-petrochemical complex pre-processing Naphtha separating a n-paraffin rich cracker feed stream from an iso-paraffin rich, high octane stream for gasoline blending);

b) RPI provides additionally a number of key synergistic opportunities through increased integration of streams and flexibility in operations. These possibilities in an integrated refining-petrochemical complex are diverse and include:

- Stabile supply of low-cost feedstocks through advanced adaptation of an Ethylene Plant to allow valorization not only Naphtha but also Refinery gases, LPG (Liquid Petroleum Gases, usually Propane-Butane) and some heavier refinery streams;
- Economics of kerosene's fraction could be significantly increased by the extraction of normal paraffin and production of their derivatives within integrated petrochemical complex;
- By-products from integrated petrochemical plants could provide streams for fuels blending or cheaper chemical components to be used in economically justified production of motor fuels under the new standards;
- Recovery and re-use of Hydrogen through the integrated complex to reduce net Hydrogen production costs;

c) The substitution of heavy fuel oil as refinery fuel by natural gas increases the efficiency of the integrated

*Although, as aforesaid, there are significant differences in business philosophy between these two professions, that occasionally are causing the negative consequences.

refining-petrochemical system in a several ways. Use of natural gas as a refinery fuel [7]:

- releases a solid quantity of naphtha, which is used as a raw material and fuel in steam reforming (hydrogen production) and as fuel in gas turbines – the released naphtha is then valorized in a much more profitable manner through production of light olefins or aromatics;
- provides the opportunity for recovering valuable components from waste gases, such as hydrogen, ethane, ethylene, propylene and propane;
- allows the complete vacuum residue to be processed by Bottom-of-the-Barrel Technology, for example in the "delayed coker", and thus to increase the yield of distillate (diesel fuel + naphtha) at the expense of heavy fuel oil for 10–12%. Integrated system sends generated naphtha in the petrochemical pyrolysis. Moreover, the waste gases from "delayed coker" contain a solid amount of propylene;
- provides the following resulting bonuses: reduced production of heavy fuel oil, increased production of diesel fuel, increased availability of feedstock to produce olefins and aromatics, and reduced CO_2 emissions [8].

d) In today's competitive and volatile business environment, an integrated refining and petrochemical complex offers considerable opportunities for enhancing operational efficiencies and unique synergies that reduce not only variable material operating costs, but also the variable costs of energy and other utilities, then many items of the fixed costs, and also capital costs and working capital expenditures:

- savings in logistics costs are particularly significant and important. Some analyzes have shown that the reduction of logistic costs by 10% within the supply chain of raw materials/intermediates in chemical production increases profitability by 6–8% (for example, reduce of administration costs by 10% increases profitability by only 2–4%).
- shared utilities systems and storage capacities, and opportunities for hardware integration, results in savings of capital expenses. Reduced needs for inventories reduces amount of required working capital.

e) Finally, RPI generates opportunity for increasing the overall revenues through the production of high quality transportation fuels and high-value petrochemical intermediates and/or end-products.

Should be mentioned that a previous list of benefits does not contain item „re-processing of the by-products from petrochemical complex to the various petrochemical derivatives of higher-value". Why is that? This kind of investment activity undoubtedly increases overall revenue and therefore is practically mandatory for every modern petrochemical complex, whether it is integrated or not. Except in Serbia, as will be seen later in this paper.

Does refining-petrochemical integration guarantees improved economic performance?

Still, not everything is so simple and unambiguous.

Some conservative analysts of petroleum industry still dispute the benefits of refining-petrochemical integration by the fact that periodic recessions very often pushed petrochemistry into such a profound losses that are unimaginable for the petroleum refining industry. But just in significant cyclicality of the petrochemical business lies a competitive advantage of refining-petrochemical integrations. To put it simply, if the joint "benchmarking" is applied on a single integrated system of type "oil refinery + petrochemical complex", then in the prosperous periods of petrochemical business the management of an integrated system physically maximizes production of petrochemicals. Those petrochemicals are then marketed while achieving high profit rates that are unimaginable for refined petroleum products. Likewise, in recessionary periods of petrochemical businesses the management of an integrated system physically maximizes production of refined petroleum products whose placement is characterized by stable rentability.

On the other hand, some petrochemical analysts are of the opinion that availability of petrochemical production facilities of world-scale dimension is much more important for economic performance then backwards integration with a petroleum refinery.

Finally, the analysts from both sides agree that almost each individual refining-petrochemical integration was accompanied by some problems of objective or subjective nature, that might be considered as inevitable. The integration challenges that are noticed by the global analysts or managerial staff of petroleum refineries and petrochemical enterprises include:

1) increased complexity of integrated system;
2) limited operational flexibility;
3) conflicting planning objectives;
4) difference in business philosophies.

It is not justified to claim in advance that certain refining and petrochemical interface is sustainable. Each refinery–petrochemical integrated system is a case for itself and the estimation of its economic viability requires prior conducting of the very detailed cost-benefit analysis.

Status of refining-petrochemical interface in gravitating region

Petroleum refining and the petrochemical industry account for a major share of the world's energy and industrial markets. In many situations, these industrial sectors represent the economic backbone of national economy.

With exception of Romania, all countries of Central and South-Eastern Europe are dependent on imports of crude oil and natural gas. On the other side, this region has a significant number of process plants to produce basic petrochemicals – finished commodities that are not profitable enough to withstand the high costs of transport to distant destinations, limited regional demand and relatively small installed capacities.

In the last two decades this region have responded on aforementioned weakness just through activities on integrating refining and petrochemical business, as it can be seen in Table 1 [3]. This kind of integration had allowed the conversion of the various available hydrocarbon streams from petroleum refineries and petrochemical plants into petrochemical derivatives of higher order derivatives – consequently, the domestic sales and profits had become larger.

Refining-Petrochemical Integration Index (RPII) defines the percentage of refinery's outputs that are delivered to further processing in petrochemical plants. For the refineries that operate in the region of Central and South-Eastern Europe this percentage ranges from 6% in the refinery company „SLOVNAFT“ at the location Bratislava (Slovakia) to almost 30% in the „UNIPETROL GROUP“ Litvinov/Kralupy (Czech Republic). Virtually all the more serious petrochemical complexes in the region of Central and South-Eastern Europe are already integrated with refineries. The only non-integrated petrochemical capacities in region had the Croatian company DIOKI at locations in Zagreb and on the island Krk, but this company went bankrupt at the end of 2013.

Refining-petrochemical integrated systems are today successful companies in Poland, Slovakia, Czech Republic, Austria, Hungary and Romania. In this countries the goals on recovery of petroleum refining and petrochemical sectors were initially defined at the state level. Over time, the state was continuously reducing its stake in already profitable companies.

Viability of Refining-Petrochemical Integration in Serbia

Possible actors of refining-petrochemical interface

Record production of petrochemical commodities in Serbia was achieved 25 years ago, when all factories were still in social ownership (see Figure 1).

The privatization processes in chemical industry of Serbia have started in 1995, upon lifting of UN sanctions. These processes were interrupted by the NATO bombing campaign in 1999, during which the large facility for production of VCM (vinyl chloride monomer) in Pančevo was destroyed, and therefore two domestic PVC (Polyvinyl Chloride) plants, in Pančevo and Šabac, were left without the raw material.

Table 1. Refining-petrochemical Integration in Central and South-Eastern Europe

Olefine/polyolefine production plants in CE & SEE Region					Remarks on petrochemical plants and percentage of petrochemical products from integrated capacities
Company	Location	Country	Polyolefines integrated with naphtha pyrolysis	Olefines integrated with streams of oil refinery	
OMV AG	Schwechat	Austria	Yes	Yes	*RPII* = 9%
Lukoil Neftochim Burgas	Burgas	Bulgaria	No	Yes	Olefin plant is idle since 2009, but PP plant works integrated to refinery's streams
Dioki	Zagreb	Croatia	Yes	No	Bankrupted in 2013
Dioki	Omišalj	Croatia	No	No	Bankrupted in 2013
Unipetrol Group, incl. Česká Rafinerská	Litvínov/Kralupy	Czech R.	Yes	Yes	*RPII* = 29%
Mol Group – TVK	Szazhalombatha	Hungary	Yes	Yes	*RPII* = 11%
PKN Orlen / Basell Orlen Polyolefins	Plock	Poland	Yes	Yes	*RPII* = 14%
Rompetrol Rafinare SA PETROMIDIA Navodari	Navodari	Romania	Yes	Yes	–
Petrom Petrochemical Arges (earlier Petrom SA Arpechim)	Pitesti	Romania	Yes	Yes	Working status unclear since 2008
Petrom SA PETROBRAZI	Brazi	Romania	Yes	Yes	Not working since 2006
NIS – HIP Petrohemija	Pancevo	Serbia	Yes	No	*RPII* = 17%
Mol Group - SLOVNAFT	Bratislava	Slovakia	Yes	Yes	*RPII* = 6%
PETKIM Petrokimya SA	Aliaga	Turkey	Yes	Yes	–

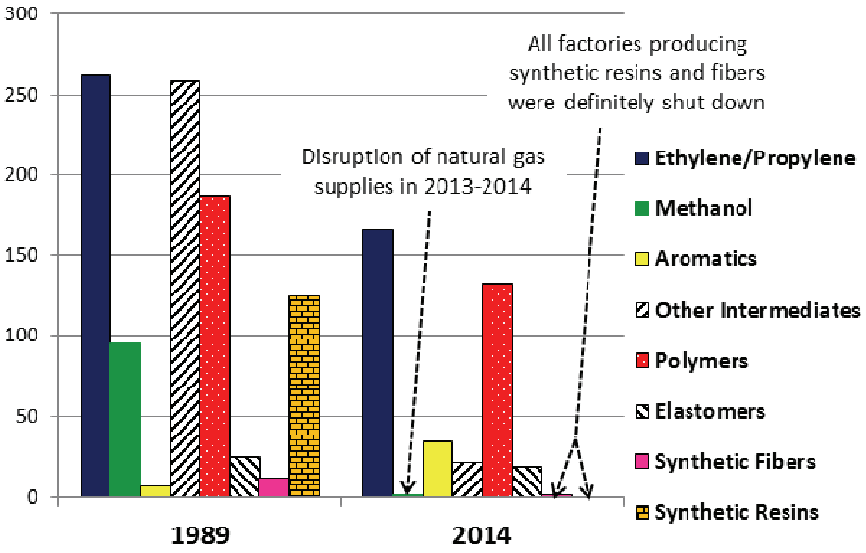

Figure 1. Production of petrochemical products in Serbia.

The transition processes in Serbian economy were accelerated since 2001, as the part of changes in Serbia after October 2000, but activities on privatization were carried out on such a disastrous way that practically complete basic chemical industry had been devastated. Let us mention here only several chemical companies that once were big and known even in frame of European scale, and nowadays even do not exist, such as "Zorka" Šabac, "Viskoza" Loznica, "Prvi Maj" Čacak, "Duga" Belgrade or "IHP" Prahovo, or a couple of them in bankruptcy and without promising future, such as "Župa" Kruševac or "Poliester" Priboj. In fact, many believe that corrupt or unprofessionally implemented privatizations have caused greater damage to Serbian chemical industry than UN sanctions and NATO bombing together [4].

Therefore, the possible actors in the eventual integration of refining and petrochemical business in Serbia are:

- JSC „NIS" Novi Sad (or abbreviated: NIS), formerly national oil company and today a joint stock company majority owned by "Gazprom Neft". Its basic business activities include the exploration, production and processing of crude oil and gas, as well as the supply of a wide portfolio of petroleum products. It owns 2 refineries: Novi Sad and Pančevo. Petroleum refinery near the town of Pančevo has capacity to process 4.8 million tonnes per year of crude oil. In late 2012, NIS completed the first stage of Pančevo petroleum refinery modernization, which enabled the transition to the production of European-quality fuel, increased production of „white derivatives", and optimization of energy consumption. NIS also has activities in up-stream executing exploration and production of natural gas and crude oil in north-Serbian region (having advanced gas process plant located in Elemir).

The only three petrochemical companies that "survived" privatization "Made in Serbia" are:

- JSC "HIP-Petrohemija" Pančevo (or abbreviated: HIPP) is the largest petrochemical company in Serbia and organized as a joint stock company majority owned by the state of Serbia. Production complex in Pancevo was put into operation in 1979. It owns capacity to produce around 250,000–350,000 t per year of petrochemical products: Ethylene as excess to needs of captive polyethylenes production, Propylene – "chemical grade", 1,3-butadiene as excess to SBR (styrene–butadiene rubber) needs, MTBE (methyl-tert-butyl-ether), HDPE (high-density polyethylene), LDPE (low-density polyethylene), SBR, and around 100,000–200,000 tpy of by-products (pyrolytic gasoline, pyrolytic fuel oil, raffinate II) at production sites in Pančevo (southern Vojvodina) and Elemir (central Vojvodina). HIPP also operates two plants to produce HDPE pipes and fittings at location Luka Dunav, and HDPE granules near town of Crepaja.

Major feedstock: naphtha, supplied by the NIS Refinery Pančevo and/or imported. Major fuel: natural gas.

Current HIPP configuration and main interlinks with NIS are described on Figure 2.

Development programs:

a) De-methanolisation of Raffinate II for further upgrade within HIPP (or possibly also in refining sector); b) Co-cracking naphtha with LPG/*n*-butane; c) extraction of pentane and naphthalene from pyrogas and PFO, the by-products of ethylene Plant; d) Increasing the production capacity of HDPE from 90,000 to 110,000 tpy; e) Increasing the production capacity of LDPE from 54,000 to 70,000 tpy (through eliminating of bottlenecks); f) valorization of available ethylene and propylene (and benzene produced in the the NIS Refinery Pančevo) to another derivatives.

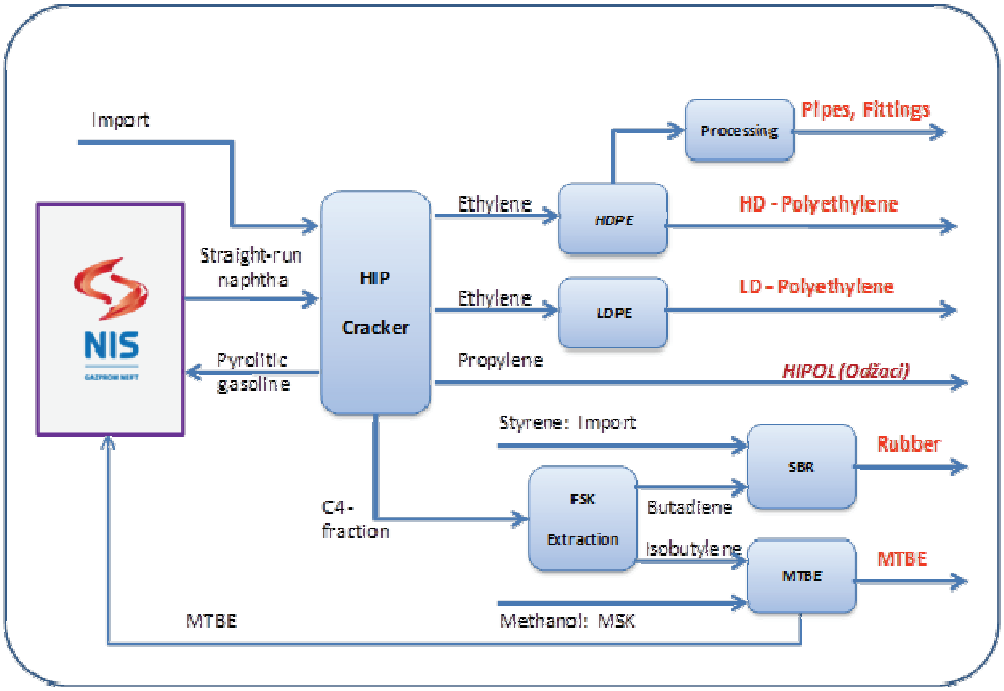

Figure 2. Current interface between NIS and HIP-Petrohemija.

- JSC "Hipol" Odžaci, a joint stock company majority owned by the state of Serbia. It is located on production site near town of Odžaci (western Vojvodina). Production complex was put into operation in 1983. It has capacity to produce 33,000 tpy polypropylene, 300–400 tpy atactic polypropylene (APP) and 20,000–30,000 tpy propylene – "polymer grade" (as excess to polypropylene plant's needs). Company also operates two distillation columns to process light naphtha (C_3–C_6 fraction) and produce 50,000 tpy LPG and C_5–C_6 residue for its partner.
- Major feedstocks: propylene - "chemical grade" supplied by HIPP, propylene - "refinery grade" supplied by NIS and/or imported and Light Naphtha supplied by the company "Standard Gas", from its production and distribution center in the town of Odžaci. Major fuels: fuel oil and wood pellets.

Development programs:

a) Revamp of a third column in the Light Naphtha Distillation Plant (investment activity in progress which will enlarge output capacity to around 80,000 tpy LPG); b) Revamp of polypropylene plant, with enlarging of the capacity to 50,000 tpy (by adding a third reactor and a new 5 tph extruder); c) debottlenecking of propylene splitter; d) construction of special column for distillation of C_5–C_6 fraction in order to separate pentane and C_{6+} fraction; e) construction of facility to produce oxidized APP.

Current "Hipol" configuration and proposed development are described on Figure 3.

- JSC "Methanol–Acetic Acid Complex" Kikinda (or abbreviated: MSK) is a joint stock company majority owned by the Public Company "Srbijagas". It is located in the vicinity of the town of Kikinda (6 km to the south of the Hungarian border and 25 km to the west of the Romanian border). Production complex was put into operation in 1987. It has capacity to produce 200,000 tpy methanol, 100,000 tpy acetic acid (glacial), and 220,000 tpy oxygen.

Major feedstocks: petrochemical complex in Kikinda is originally designed to use domestic natural gas from the neighboring gas-fields, but in practice it was always consumed only imported Russian gas delivered by the company "Gasprom Neft". Major fuel: natural gas.

Development programs:

a) Polymeric emulsions plant to produce 20,000 tpy homo-polymeric and co-polymeric emulsions of vinyl acetate monomer (polyvinyl-acetates) and acrylates; b) Partial replacement of imported natural gas* with cheaper "sour gas"**; c) construction of the Cogeneration Plant for the combined production of steam, heat and electricity; d) plant to produce 25,000 tpy ethyl acetate and *n*-butyl acetate/isobutyl acetate; e) multipurpose process unit with capacity of 2,500 tpy for production of low-tonnage acetate esters, and service recovery of other oxygenated solvents; f) construction of dimethyl ether (DME) Plant.

*Natural gas is used in "MSK" Kikinda both as a feedstock and fuel.

**"Sour gas" is produced on gas-fields nearby Kikinda which are operated by NIS. It is reach in CO_2, and therefore has low caloric value (not suitable for use as a fuel, but suitable for acetic acid production).

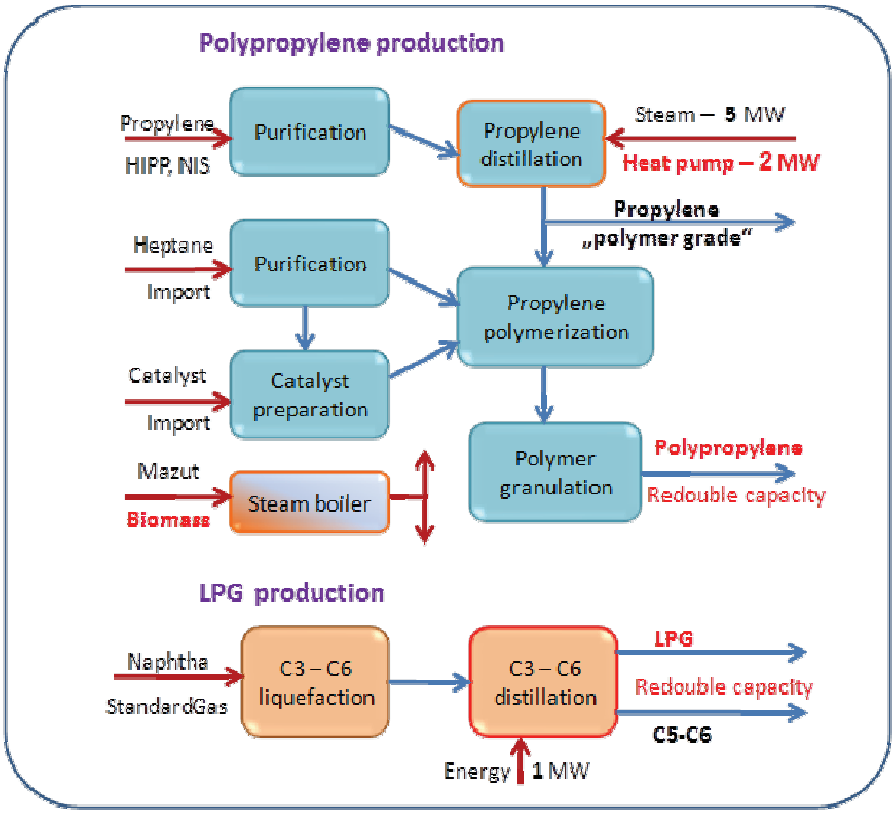

Figure 3. Current scheme of production processes in Hipol.

Current MSK configuration and proposed development are described on Figure 4.

According to European standards, the petrochemical companies HIPP and MSK belong to a category of medium-sized enterprises. These two manufacturers were lucky not to enter up to now into privatization processes „Made in Serbia". When HIPP and MSK operate continuously and with a high utilization of available production capacities, and this will happen if stable supply of naphtha (HIPP) and natural gas (MSK) is provided, these two petrochemical manufacturers are generally ranked among the top five domestic exporters.

The company "Hipol" could be considered as relatively small manufacturer of basic chemical commodities. What this company makes exceptional is the fact that it is the only Serbian company in the field of base chemistry that has passed the process of privatization and still exists. And how difficult it was the best describes the business „Odyssey" which had lasted almost a decade including one cleavage of the company on its base and the processing part, two sales, two terminations of the privatization contract due to buyers' failure to fulfill contractual obligations, and two transformations into state ownership.

The only world-scale petrochemical capacity in Serbia is facility to produce 100,000 tpy of glacial acetic acid in MSK. The existing capacities to produce olefins, aromatics, methanol, polyolefins (LDPE, HDPE and PP) and elastomers (SBR) in Serbia are of small size according to the current economic standards. But maybe the main weakness is a fact that the domestic petrochemical manufacturers have not translated into industrial practice any development initiative in the last 30 years and have not introduced any significant capacity improvement (only HDPE capacity was modified from 60,000 to 90,000 tpy). Therefore, the base petrochemicals produced in Serbia today are internally converted into more profitable derivatives at low percentage (excl. ethylene and butadiene), almost as it was in the late seventies of the previous century. A smaller part of basic petrochemicals is sold in the country (as low profitable semi-finished goods), and the rest is exported (very often to very remote destinations, which implies a high share of transport costs in the selling price).

From the other side, an in accordance with European standards, the NIS-Petroleum Refinery Pančevo is an industrial facility of medium-size. The percentage of capacity utilization in this refinery is rather low, which influences the cost of refined products, esp. fixed costs. The process of recent modernization, significantly improved the competitive position of oil refinery in Pančevo. The structure of output streams was changed in the direction of adjusting the structure of assortment to market demands, and increase the share of higher-value derivatives along with achieving quality in accordance with European standards. On the other hand,

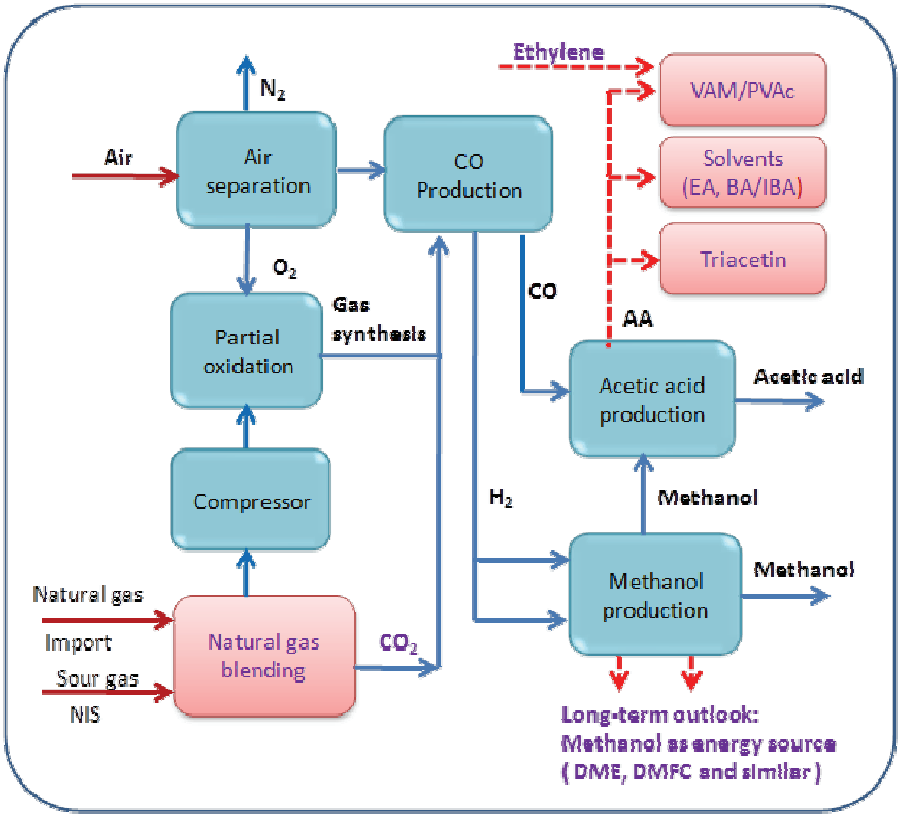

Figure 4. Current products scheme of MSK and its development projects.

however, still remains some disadvantages, or better say unused comparative advantages. Given the topic of this paper, here we primarily meant the economically inefficient use of some refinery's by-products or the non-optimal use of synergies in the exchange (already existing or technologically possible) of hydrocarbon streams with neighboring petrochemical complex HIPP.

Current opporunities and future chances for refining-petrochemical interface

All global analysts agree that today in Europe a petrochemistry based on the pyrolysis of naphtha economically cannot "survive" without valorizing all the core products and by-products in an optimal way. "An optimal way" is internal conversion of primary petrochemicals into profitable derivatives (polymers, elastomers, resins, fibers, speciality chemicals,...) to the highest possible degree. Unfortunately, the largest domestic petrochemical company, HIPP is just a rare example of an European petrochemical manufacturer which economically inefficiently uses a series of output streams of its production system (propylene, pyrolysis fuel oil, raffinate II). All of these output streams can be optimally valorized just within an integrated refinery––petrochemical system.

To these general weaknesses of the Serbian petrochemical industry, we can certainly add the low energy efficiency and surplus of employees.

There are some weaknesses that are specific to an individual company, such as unexplained fact that MSK for twenty years as by rule had not worked in the prosperous phases for the markets of methanol and acetic acid, and had worked mainly during periods of recession, when even the global leaders in production of these two petrochemicals were running their plants at a loss.

Almost all aforementioned weakness of refining and petrochemical sector in Serbia, however, might be in a way considered as drivers for the integration of refining and petrochemical business at national level.

However, according to the *ChemSystems* [5] categorization of the refining-petrochemical integrations achieved worldwide, the Serbian refining and petrochemical operators are currently integrated at level of 1st generation, *e.g.*, through only the simplest commercial relationships (sale or purchase of products and by-products, and certain integration of utilities between refinery and HIPP in very early stage), and without any additional joint considerations regarding finding the most optimal valorization of by-products or integration of available hydrocarbon streams, and introduction of new production processes based on that synergetic potential.

The specificity of integration between refining and petrochemical facilities in Serbia might be that not only there is a significant number of existing and potentially

possible links between hydrocarbon streams of the NIS-Oil Refinery Pančevo, on the one hand, and the three Serbian petrochemical companies, on the other hand, but as well a fact that exchange of material flows exist also between the individual chemical companies. This applies not only to current state in exchanging of hydrocarbon streams (propylene, methanol, MTBE, C_5–C_6 residuum) between Serbian petrochemical plants, but also to the matrix of all possible links between the material streams being generated by all the potential actors of R-P Interface in Serbia and which in the future might represent comparative advantage for the implementation of the several planned development programs.*

The main exchanged streams (or in future potentially could be) are shown on Figure 5:

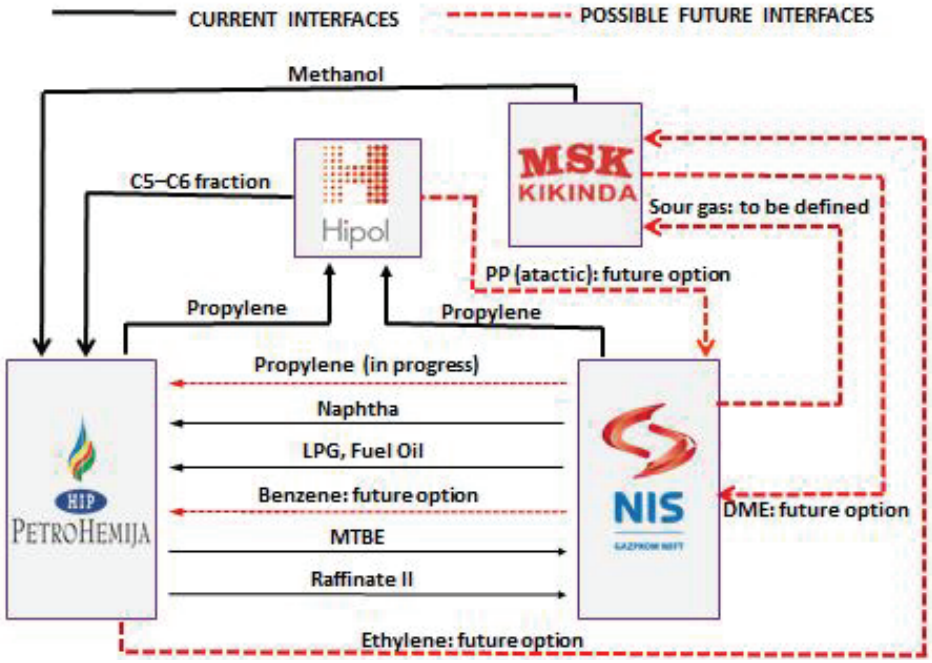

Figure 5. Refining–petrochemical Interface in Serbia: current and possible future integrations of hydrocarbon streams.

NIS → HIPP:

- Naphtha (preferably 70–105 °C fraction), LPG (preferably *n*-butane), fuel oil.
- Benzene (possible future option upon eventual construction of the facility to produce ethylbenzene/ /styrene within HIPP, or some other derivative to be defined).
- Propylene – „refinery grade“ (upon eventual construction of C3-Splitter within HIPP to produce propylene - „polymer grade“, which is an option in planning phase).

HIPP → NIS:

- Py-gas (source of benzene and toluene).
- MTBE (possibly converted into ethyl-tert-butyl ether) in future.
- De-methanolised raffinate II (for further upgrade in refining sector).

HIPP → NIS:

- Utilities and infrastructural services: existing HIPP Energy Plant capacity utilisation and NIS CAPEX optimisation; Waste Water Treatment Plant.

NIS → HIPOL:

- Propylene – „chemical grade“.

HIPP → HIPOL:

- Propylene – „chemical grade“.

HIPOL → NIS:

- Atactic Polypropylene (possible future use in production of polymer modified bitumenes or abbreviated PMBs, to be used in manufacturing of roofing membranes.

HIPOL → HIPP:

- C_5–C_6 residue (co-feedstock for pyrolysis in Pančevo).

HIPP – HIPOL – NIS:

- Optimisation of propylene balance and market value (through construction of the facility to produce „polymer grade“ propylene in HIPP or enlarging the PP production capacity in „HIPOL“ or a construction a new PP plant in Pančevo.

NIS → MSK:

- „Sour gas“ as feedstock for production of methanol/acetic acid (future option for substitution of imp-

*The viability of these development programs is already elaborated, or will be in the near future.

orted natural gas by gas rich in CO_2 and produced by NIS on the gas-fields nearby Kikinda).

MSK → NIS:

• DME for blending into LPG (possible future option upon eventual construction of the facility to produce DME from methanol within MSK).

MSK → HIPP:

• Methanol (for MTBE production in Elemir).

HIPP → MSK:

• Ethylene (possible future option upon eventual construction of the facility to produce Vinyl Acetate Monomer within „MSK“).

It should be mentioned that on the long-term basis there are a number of additional development concepts based on RPI to be analyzed and evaluated.

Anyhow, what is stated in this paper clearly shows that there is potential for achievement of positive synergies between Serbian actors in refining-petrochemical integrations. Based on this potential and conducting all necessary cost-benefit analysis, the current not so shiny situation regarding profitability of petroleum refining and petrochemical business in Serbia might be converted into positive direction. This goal requires closer cooperation between all entities and their major shareholders (keeping fair market related conditions which takes into account product quatation and their import/export parity and considering further increase of installed capacities for selected products).

CONCLUSIONS

As it is today in almost all spheres of life, the main motive for refining and petrochemical interface is possibility to enhance profit. Since refining-petrochemical integration is no longer just a theoretical postulate, but as business operation already has a serious history in industrial practice and the corresponding results in terms of enlarged profits, the critics are less and less. Actually, they no longer have a question of whether integration should be implemented, but only which degree of integration is economically optimal.

It is a fact that each integrated system is the story for itself, and it is also a fact that the domestic petrochemical companies have a number of objective and subjective weakness, but it is also a fact that so far in Serbia up to now no one has conducted a comprehensive CBA of synergies generated throughout integrating refining and petrochemical business. The complexity of task is increased by the fact that the viability of the RPI in the specific case of Serbia must be calculated as a mixture of economic and social (national) interests. Namely, it is necessary to define the lowest common denominator:

- for the purely economic interest of the majority owner of the refinery business in Serbia, the Russian company "Gazprom Neft",
- for the interests of foreign companies that are seen as potential strategic partners (willing to extend existing domestic petrochemical capacities, to develop the brand new facilities to produce more sophisticated petrochemical derivatives, and possibly to participate on deeper implementation of Refining-Petrochemical interface optimization), and
- for the interest of Serbian Government to keep operational two economic backbones of national economy.

It is a task that requires urgent implementation, especially because all the predictions say that in the period after the year 2016 the European petrochemical industry is entering into a more prosperous phase.

REFERENCES

[1] I. Souček, Z. Popović, O. Ocić, Refinery and Petrochemical Interface, Refining and Petrochemical Round Table at 15th Central and Eastern European Refining and Petrochemical Conference - WRA, Bucharest-Romania, October 23–25, 2012.

[2] Z. Popović, I. Souček, O. Ocić, N. Ostrovskii, S. Adžić, Refining and Petrochemical Interface. Case study: HIP Petrohemija – NIS, Energija, Ekonomija, Ekologija **16** (2014) 237–242.

[3] I. Souček, Meeting experts, Global Report on Refining and Petrochemical Integration, World Refining Association (2014) 12–13.

[4] Z. Popović, D. Marinković, Revitalization and development of production of highly processed derivatives: A precondition for survival Serbian petrochemical industry, Istraživanje i projektovanje za privredu **7** (2009) 33–43 (in Serbian).

[5] ChemSystems' Refinery Petrochemical Interface, 1997 (http://www.chemsystems.com).

[6] A. Moola, Exploiting Refinery and Petrochemical Integration, 2014 (http://www.shell.com).

[7] T. Taraphadar, P. Yadav, M.K.E Prasad, Natural gas fuels the integration of refining and petrochemicals, Petrol. Tech. Q. **17** (2012) 1–9.

[8] S. Ratan, R.van Uffelen, Curtailing refinery CO_2 through H_2 plant, P.T.Q. Gas (2008) 19–23.

[9] M. Maugeri, The Future of Refining in Europe, 2014 (http://www.eni.com).

[10] S. Tinhof, Austria's OMV in negotiations to divest Bayernoil stake, August 21, 2013. (http://www.icis.com/ /resources/news/2013/08/21/9699409).

IZVOD

DA LI INTEGRISANJE RAFINERIJSKOG I PETROHEMIJSKOG BIZNISA PRUŽA MOGUĆNOST ZA RAZVOJ PETROHEMIJSKE INDUSTRIJE U SRBIJI

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(Stručni rad)

Industrija prerade nafte i petrohemijska industrija posluju u otežanim uslovima još od početka devedesetih godina prošlog veka. Posebno veliku eroziju profitnih stopa su ova dva industrijska sektora doživela tokom eskalacije globalne ekonomske krize u 2008. i 2009. godini. Upravo u tom periodu, kao jednu od mogućih solucija oporavka globalni analitičari počinju da intenzivnije elaboriraju benefite integrisanja rafinerijskog i petrohemijskog biznisa. Nedugo zatim svoju budućnost u ovoj vrsti proizvodno-poslovnog povezivanja počinje da vidi sve više i više naftnih rafinerija i petrohemijskih kompleksa. Ovaj rad evaluira dostignuti nivo integrisanja rafinerijskog i petrohemijskog biznisa u Centralnoj i Jugoistočnoj Evropi. Potom se u radu identifikuju aktuelne mogućnosti i buduće šanse za povezivanje ovakvog tipa u Srbiji. Održivost integrisanja među potencijalnim domaćim akterima i isplativost svakog pojedinačnog povezivanja rafinerijskog i petrohemijskog biznisa zavisi od mnogo faktora, te realizacija svakog integrisanog sistema predstavlja slučaj za sebe i zahteva prethodno sprovođenje veoma ozbiljnih tehnoekonomskih analiza.

Ključne reči: Integrisanje rafinerijskog i petrohemijskog biznisa • Srpska petrohemija • Razvojni ciljevi • Tehnoekonomska valorizacija

Uticaj operacije končanja pređe na UV zaštitni faktor pletenina od konoplje

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Izvod

Cilj ovog rada bio je analiza uticaja operacije končanja pređe na UV zaštitna svojstva pletenina. U tu svrhu su, polazeći od jednožične i končane pređe od konoplje, u kontrolisanim uslovima proizvedene glatke DL pletenine. Budući da su ove pletenine najpodložnije relaksacionim promenama, sproveden je postupak njihove mokre relaksacije. Eksperimentalno ili računski su određena konstrukciona i fizička svojstva pletenina, kao i promene nastale posle njihove pune relaksacije. UV zaštitna sposobnost suvo i mokro relaksiranih pletenina je kvantitativno ocenjena pomoću parametra UV zaštitni faktor (UPF, *Ultraviolet Protection Factor*) pletenina standardnim *in vitro* postupkom koji podrazumeva spektrofotometrijsko ispitivanje transmisije UV radijacije. Činjenica da se relaksiranoj pletenini izrađenoj od končane konopljine pređe pripisuju odlična UV zaštitna svojstva, uz prednost u pogledu propustljivosti vazduha, ukazuje na potencijal koji operacija končanja pređa ima u oblasti komforne i zdrave letnje odeće.

Ključne reči: UV zaštitni faktor, UV transmisija, pletenina, pređa, končanje, konoplja.

Dostupno na Internetu sa adrese časopisa: http://www.ache.org.rs/HI/

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Poslednjih godina u javnosti se intenzivno govori o pojavi ozonskih rupa i pojačanom sunčevom UV zračenju. Zbog toga, iako je dobro poznat pozitivan učinak čovekovog izlaganja sunčevim zracima kao što je podsticanje stvaranja vitamina D i drugi terapeutski efekti, pokazalo se da dugotrajno i nekontrolisano profesionalno ili rekreativno izlaganje suncu izaziva izvesne efekte štetne po zdravlje. Najočigledniji kratkoročni efekat prekomernog izlaganja sunčevim zracima su opekotine ili crvenilo na koži (erythema). Hronično oštećenje kože izazvano UV zracima izaziva prerano starenje kože, benigne i maligne tumore na koži. S obzirom da u Istočnoj i Južnoj Evropi u letnjim mesecima UV indeks može biti na nivou vrednosti indeksa zračenja u Australiji, zaštita od UV zračenja je od velike važnosti za stanovništvo, posebno za radnike na otvorenom prostoru, decu i omladinu. Pored prirodnih izvora UV zračenja u svakodnevnom životu su prisutni i veštački izvori UV zračenja kao što su razna osvetljenja radnih prostora, industrijska električna pražnjenja, svetleće reklame i slično.

Smatra se da odeća obezbeđuje najefikasniju UV zaštitu. Kolika će biti zaštita koju odeća pruža zavisi od mnogih parametara. U toku poslednje decenije rađena su brojna istraživanja u pravcu definisanja odgovarajućih performansi odeće kao barijere za UV radijaciju (UVR) i utvrđeni su ključni parametri odgovorni za efikasnost tekstilnih materijala u pogledu UVR zaštite. Rezultati ovih istraživanja pokazali su da, pored dizajna odeće [1,2], sirovinski sastav, parametri pređe, prepletaj, površinska masa i debljina tekstilnih materijala uslovljavaju nivo UV zaštitne sposobnosti odevnog predmeta [3–11]. Pored toga, pokazalo se da je sposobnost odeće da blokira UVR uslovljena bojom, primenjenim postupcima dorade (beljenje, upotreba UV apsorbera i sl.) [12,13], i parametrima koji se odnose na upotrebu odevnih predmeta kao što su količina vlage u materijalu, istezanje materijala i postupci nege odevnih predmeta [14–17].

Opšte je prihvaćeno da sintetička vlakna pružaju bolju UV zaštitu, međutim, treba imati u vidu činjenicu da tekstilni materijali izrađeni od sintetičkih vlakana nisu komforni za nošenje u uslovima visokih spoljnih temperatura (kada su visoke vrednosti UVR). Sa druge strane, zahvaljujući odličnim higijenskim svojstvima tekstilni materijali na bazi celuloze su izuzetno komforni za nošenje, posebno u letnjim mesecima. Generalno se smatra da celulozni tekstilni materijali ne pružaju visoku UV zaštitu jer celulozna vlakna (pamuk, lan, konoplja, viskoza) imaju mali kapacitet UV apsorpcije. Ipak, potencijal ovih vlakana u pogledu UV zaštite leži u činjenici da ona u sebi sadrže i do 30% pratećih materija (voskovi, pektin, lignin i pigmenti) koje se ponašaju kao odlični apsorberi UVR. U tom smislu, upotreba sirovih (nemodifikovanih) celuloznih vlakana za izradu odevnih tekstilnih materijala dobija na značaju. Dosadašnja istraživanja UV zaštitnih svojstava celuloznih tekstilnih materijala uglavnom se odnose na pamuk [18–20], što je donekle i razumljivo kada se ima u vidu

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zastupljenost ovog vlakna na tržištu. Sa porastom interesovanja za revitalizaciju vlakana konoplje u oblasti visokokvalitetnih odevnih tekstilnih proizvoda, zahvaljujući njihovim dobrim predispozicijama sa aspekta toplotnog komfora [21], i imajući u vidu ozbiljan nedostatak rezultata vezanih za UV zaštitna svojstva tekstilnih materijala od konoplje [22], nameće se potreba analize potencijala vlakana konoplje u pogledu sposobnosti UV zaštite.

Končanjem pređe (stručenjem i naknadnim upredenjem dve ili više jednožičnih pređa) modifikuje se čitav niz svojstava pređe koja su značajna za njenu dalju upotrebu. Promene se najpre odnose na poboljšanje mehaničkih karakteristika, povećanje ravnomernosti, voluminoznosti i abrazione otpornosti uz modifikovanje geometrije površine pređe. S obzirom na to da je najčešće smer upredanja končane pređe suprotan smeru upredanja polaznih komponenata, jačina končane pređe ne zavisi više od migracije vlakana i njihovog upredanja, već samo od međusobnog upredanja komponenata jedne oko druge. Pored toga, skoro paralelan položaj vlakana u končanoj pređi doprinosi boljem iskorišćenju njihove jačine, čime se poboljšavaju specifična jačina dobijenih složenih pređa, njihova istegljivost i sposobnost oporavka od mehaničkih naprezanja. Končane pređe imaju otvoreniju strukturu i bolju pokrivnu sposobnost u tkaninama. Takođe, redukcijom jednosmernog torzionog napona smanjuje se neuravnoteženost pređa. Usled ublažavanja varijacije elastičnih svojstava jednožičnih komponenata i varijacije neravnomernosti debljine kombinovanjem više niti povećava se ravnomernost končanih pređa uz modifikovanje njihovih estetskih svojstava [23].

Pored oskudnog broja istraživanja efekata koje struktura i svojstva pređe imaju na UV zaštitna svojstva tekstilnih materijala [4,24–26], u naučnoj literaturi nema podataka o potencijalu operacije končanja pređa u tom smislu. Polazeći od navedenih činjenica, u okviru ovog rada analizirana su UV zaštitna svojstva glatkih DL pletenina izrađenih od jednožične ili končane pređe od konoplje. Kao mera efikasnosti ispitivanih pletenina u pogledu UV zaštite poslužio je parametar UV zaštitni faktor ili UPF (*Ultraviolet Protection Factor*), određen *in vitro* metodom.

Ispitivanje fizičkih svojstava i UV zaštitne sposobnosti suvo relaksiranih DL pletenina od konoplje (bez mehaničkih i hemijskih tretmana koji bi promenili njihovu strukturu ili strukturu pređa) pojednostavljuje ocenu efekta končanja pređe na svojstva pletenine. Ipak, imajući u vidu činjenicu da se tekstilni materijali podvrgavaju mokrim obradama prilikom finalizacije proizvoda, u okviru ovog istraživanja pristupilo se postupku kvašenja pletenina i oceni UV zaštitnih svojstava nakon njihove mokre relaksacije.

EKSPERIMENTALNI DEO

Materijal

U okviru ovog istraživanja korišćena je pređa od konoplje (Linificio Canapificio Nazionale, Italy) sa nominalnom finoćom od 50 tex i nominalnom upredenošću od 400 uvoja po metru (*Z* pravac). Postupkom končanja tj. upredanjem dve jednožične pređe dobijena je končana pređa od konoplje (konoplja/konoplja) nominalne finoće 100 tex i nominalne upredenosti 310 uvoja po metru (*S* pravac). Po završetku končanja, končana pređa je podvrgnuta postupku relaksacije parenjem u autoklavu u trajanju od 20 min na 80 °C i odležavanju najmanje 72 h u kondicioniranom uslovima (vlažnost vazduha 65±2% i temperatura 20±2 °C), čime se končana pređa oslobađa od zaostalog torzionog napona. Strukturne karakteristike jednožične i končane pređe od konoplje (konoplja/konoplja) prikazane su u tabeli 1. Faktičke vrednosti finoće i upredenosti upotrebljenih pređa ispitivane su prema važećim SRPS standardima [27,28], dok je prečnik obe pređe određen upotrebom mikroskopa Nikon SMZ800, i na osnovu 50 očitavanja određena je srednja vrednost prečnika. Pomoću prečnika i finoće pređe izračunata je gustina pređa. Faktor pakovanja pređa određen je kao količnik gustine pređa i gustine vlakana.

Tabela 1. Karakteristike pređa od konoplje
Table 1. Characteristics of the hemp yarn

Svojstvo	Konoplja	Konoplja/Konoplja
Finoća, tex	47,8	95,6
Upredenost, m^{-1}	370	297
Prečnik, mm	0,22	0,41
Gustina, $g \cdot cm^{-3}$	1,258	0,721
Faktor pakovanja	0,84	0,48

Imajući u vidu rastući trend upotrebe pletenina za izradu letnje odeće, za potrebe ovog istraživanja su od jednožične i končane konopljine pređe proizvedene dve varijante glatkih DL (desno-levih) pletenina. Prvi uzorak pletenine je proizveden od dublirane jednožične pređe od konoplje (konoplja+konoplja, 2Cs), a drugi od končane pređe od konoplje (konoplja/konoplja, KCs). Na taj način su dobijene pletenine istih konstrukcionih karakteristika, čime je omogućeno njihovo poređenje. Nakon izrade pletenine su podvrgnute obaveznoj tzv. suvoj relaksaciji, odnosno odležavanju u nezategnutom stanju na ravnoj površini u kondicioniranim uslovima (vlažnost vazduha 65±2% i temperatura 20±2 °C) u trajanju od najmanje 72 h. Konstrukcione karakteristike ovih glatkih pletenina prikazane su u tabeli 2.

S obzirom na to da su obe pletenine proizvedene na istoj mašini uz kontrolisane tehnološke parametre, konstrukcione karakteristike ovih pletenina su veoma slič-

ne. Uočene male razlike, kako će kasnije biti objašnjeno, pripisuju se izvesnim razlikama u strukturi upotrebljenih pređa. Debljina pletenina određena je prema standardu SRPS EN ISO 5084 [29] a ostale konstrukcione karakteristike pletenina ispitivane su prema uobičajenim procedurama [30]. Šema eksperimentalnog materijala prikazana je na slici 1.

Tabela 2. Konstrukcione karakteristike suvo relaksiranih glatkih pletenina od konoplje
Table 2. Construction characteristics of the dry relaxed plain hemp knitted fabrics

Svojstvo		Konoplja+Konoplja[a], 2Cs	Konoplja/Konoplja[b], KCs
Gustina cm^{-1}	D_v	13,7	12,0
	D_h	5,5	5,5
Površinska gustina cm^{-2}		75,5	66,0
Dužina petlje, mm		5,0	5,3
Debljina, mm		0,916	0,926
Površinska masa g m^{-2}		360,4	334,4

[a]Dublirana pređa od konoplje; [b]končana pređa od konoplje

Slika 1. Šema eksperimentalnog materijala.
Figure 1. Design of the experimental material.

Metode ispitivanja

UV zaštitna svojstva pletenina od konoplje, kvantitativno iskazana kroz parametar UPF (UV zaštitni faktor) ispitivana su *in vitro* metodom prema evropskom standardu EN 13758-1 [31]. Ova široko rasprostranjena laboratorijska metoda podrazumeva merenje direktne i difuzione UV transmisije upotrebom UV–Vis spektrofotometra u intervalu talasnih dužina od 290 do 400 nm (UVB oblast od 290 do 315 nm i UVA oblast od 315 do 400 nm) u koracima od 5 nm. UPF se izračunava kao odnos srednje vrednosti količine UV zračenja emitovanog UV izvorom i količine UV zračenja transmitovane kroz uzorak tekstilnog materijala uz korekciju koja uzima u obzir različitu biološku delotvornost različitih talasnih dužina u okviru intervala UV radijacije. Ova korekcija je neophodna s obzirom da biološka aktivnost kraćih talasnih dužina u okviru UVB intervala znatno prevazilazi aktivnost UVA spektra. Korišćen je spektrofotometar UV/Vis/NIR Perkin-Elmer Lambda 9 (Perkin-Elmer, Boston, MA, USA). Ovaj uređaj je opremljen integrišućom sferom u kojoj se prikupljaju UV zraci koji kroz uzorak prolaze direktno ili difuzijom. Da bi se umanjile greške pri merenju aktivira se odgovarajući UV transmisioni filter. Pomoću spektrofotometra su registrovane talasne dužine od 290–400 nm u koracima od 5 nm. Merenje transmisije je vršeno na uzorcima pletenina koji su normalno i u nezategnutom stanju postavljeni na izvor UV zraka, tako da je njihova prednja strana okrenuta ka UV izvoru. Za svaku pleteninu je vršeno četiri merenja na osnovu kojih su izračunate srednje vrednosti UVB, UVA i UVR transmisije, kao i vrednosti UPF-a prema relaciji (1):

$$UPF = \frac{\sum_{\lambda=290}^{\lambda=400} E(\lambda)\varepsilon(\lambda)\Delta\lambda}{\sum_{\lambda=290}^{\lambda=400} E(\lambda)T(\lambda)\varepsilon(\lambda)\Delta\lambda} \quad (1)$$

gde je: $E(\lambda)$ – solarno zračenje (količina radijacije na određenoj površini, W m^{-2} nm^{-1}), $\varepsilon(\lambda)$ – relativna eritemalna spektralna efikasnost, $\Delta\lambda$ – primenjeni korak merenja u UV intervalu talasnih dužina (nm) i $T(\lambda)$ – izmerena transmisija za talasnu dužinu λ.

U svrhu analize UV zaštitnih svojstava pletenina od konoplje pristupilo se merenju propustljivosti vazduha i izračunavanju gustine i poroznosti pletenina. Propustljivost vazduha pletenina je ispitivana pomoću digitalnog uređaja NBFY (Ningbo Textile Instrument Factory, China), prema standardu ASTM D737 [32]. Uzorak pletenine se postavlja licem na gore na okrugli otvor (površine 20 cm^2) usisne glave aparata, i uz konstantni gradijent pritiska (100 Pa) registruje se količina propuštenog vazduha u m^3 m^{-2} min^{-1}. Propustljivost vazduha pletenina od konoplje određena je kao srednja vrednost pet merenja po uzorku pletenine. Gustina pletenine (zapreminska masa) δ (kg m^{-3}) određena je odnosom njene površinske mase i debljine. Ukupna poroznost pletenine P (%), definisana kao ukupna količina vazduha u pletenini (pore između pređa i pore unutar pređe), izračunata je prema relaciji (2) [30]:

$$P = 100 - \frac{\delta}{\rho}100 \quad (2)$$

gde je ρ (kg·m^{-3}) gustina vlakana. Gustina vlakana konoplje iznosi 1500 kg·m^{-3}.

Delimično relaksirane (suva relaksacija) pletenine od konoplje, čija su strukturna i fizička svojstva prethodno bila definisana, podvrgnute su postupku mokre relaksacije prema nešto izmenjenom standardnom postupku (SRPS F. S2.020, metoda E) [33]. Postupak relaksacije je sproveden kvašenjem uzoraka pletenine u hladnoj vodi. Uzorak smešten između dve staklene ploče potapa se u destilovanu vodu temperature do 20 °C. Posle 2 h statičke relaksacije uzorak se cedi između upijajuće hartije da bi se odstranila suvišna voda i ostavi da se suši na sobnoj temperaturi i vlažnosti, rašireno na tankoj žičanoj mreži. Posle perioda kondicioniranja izračunava se procenat skupljanja S (%) pletenine prema relaciji (3) [34]:

$$S = 100\frac{L_{H0}L_{V0} - L_{H1}L_{V1}}{L_{H0}L_{V0}} \quad (3)$$

gde su L_{H0} i L_{V0} – dužina epruvete (uzorka) u pravcu redova i nizova pletenine pre kvašenja, a L_{H1} i L_{V1} - dužina epruvete (uzorka) u pravcu redova i nizova pletenine posle mokre relaksacije.

Prethodno opisane procedure ispitivanja pletenina od konoplje (konstrukcione karakteristike, UPF, propustljivost vazduha, gustina i poroznost) ponovljene su na uzorcima pletenina podvrgnutim mokroj relaksaciji. Pored toga, definisana je geometrija (prečnik, gustina i faktor pakovanja) jednožične i končane pređe od konoplje nakon mokre relaksacije pletenina.

Za statističku analizu dobijenih rezultata korišćen je Sudent *t*-test, prema kome se u slučaju da je vrednost statistike (*p*) manja od praga značajnosti (α = 0,05) odbacuje nulta hipoteza o jednakosti aritmetičkih sredina (srednjih vrednosti). Efekat mokre relaksacije pletenina na UVR transmisiju statistički je analiziran upotrebom *t*-testa uparenih uzoraka. Ovim testom se porede srednje vrednosti dva uparena uzorka (transmisija pre i posle kvašenja pletenine). Efekat je statistički potvrđen u slučaju kada je vrednost statistike (*p*) manja od zadatog praga značajnosti (α = 0,05).

Makroporoznost (otvorena slobodna površina) pletenina je kvalitativno ocenjena pomoću SEM (*Scaning Electron Misroscopy*) mikrofotografija (Jeol JSM-840A). Epruvete pletenina su prethodno bile prevučene slojem zlata.

REZULTATI I DISKUSIJA

Polazeći od činjenice da je idealnu gustinu pakovanja pređe (0,907) teško dostići, gustina pakovanja jednožične konopljine pređe od 0,84 ukazuje na njenu kompaktnu strukturu, što je potvrđeno i vrednošću gustine pređe (1,258 g·cm^{-3}) koja je za samo 16% manja od gustine vlakna konoplje (1,5 g·cm^{-3}). Kompaktna struktura ove pređe objašnjava se smanjenom elastičnošću i gipkošću vlakana konoplje usled čega je usporena njihova migracija prilikom formiranja pređe. Upredanjem dve jednožične pređe od konoplje formira se nova složena (končana) pređa kod koje je neminovno došlo do promene orijentacije vlakana usled sekundarnog upredanja (končanja) u smeru suprotnom od smera upredanja jednožične komponente. Kao rezultat, vlakna konoplje se delimično raspredaju, odnosno dolazi do „otvaranja“ strukture končane pređe, što se manifestuje kroz smanjenje faktora pakovanja i gustine končane pređe za oko 57% u odnosu na jednožičnu komponentu (tabela 1). Kao posledica razlika u internoj strukturi jednožične i končane pređe od konoplje, kod DL pletenina izrađenih od ovih pređa uočene su izvesne male razlike u površinskoj gustini (broj petlji na jedinici površine pletenine), površinskoj masi, debljini i dužini petlje. Manja površinska gustina petlji kod pletenine izrađene od končane konopljine pređe (KCs) posledica je manje vertikalne gustine KCs pletenine (tabela 2). Budući da su obe pletenine proizvedene uz konstantne parametre mašine, različita vertikalna gustina pletenina može se jedino pripisati različitoj savitljvosti jednožične i končane konopljine pređe. Kako prilikom končanja dolazi do delimičnog raspredanja jednožičnih komponenata, pokretljivost vlakana u novonastaloj složenoj pređi raste, što vodi ka povećanju savitljivosti pređe. Otuda je kod KCs pletenine došlo do povećanja dužine petlje i debljine pletenine, i smanjenja vertikalne gustine petlji. Usled većeg broja petlji na jedinici površine 2Cs pletenine, veća je i njena površinska masa u poređenju sa KCs pleteninom.

Ispitivanje fizičkih svojstava i UV zaštitne sposobnosti suvo relaksiranih DL pletenina od konoplje imalo je za cilj da da pouzdanu ocenu efekta končanja pređe na svojstva pletenine. Svaka mehanička ili hemijska dorada pletenina promenila bi strukturu i pređa i pletenina i eventualno maskirala efekat operacije končanja pređe. Ipak, treba imati u vidu da se tzv. suvom relaksacijom pletenina samo delimično relaksira, odnosno oslobađa se samo deo prisutnog napona u pletenini i zaostalih torzionih sila u pređi, dok se mokrom relaksacijom struktura pletenine gotovo u potpunosti relaksira uz veliku sklonost ka promeni svojih dimenzija. Zbog toga se u okviru ovog istraživanja pristupilo postupku kvašenja pletenina od konoplje, i praćenju promene njihove strukture i fizičkih svojstava u svrhu ocene uticaja relaksacionih procesa na UV zaštitna svojstva pletenina. Izračunate vrednosti skupljanja pletenina (relacija (3)) nakon mokre relaksacije iznose 11,9% za 2Cs pleteninu i 10,6% za KCs pleteninu. Treba ipak reći da uočena razlika u procentu skupljanja ove dve plete-

nine nije statistički potvrđena [p(0,498) > α(0,05)]. Dimenzionalne promene pletenina ukazuju na činjenicu da je u procesu mokre relaksacije došlo do skupljanja kako jednožične tako i končane konopljine pređe. To se pripisuje velikom afinitetu vlakana konoplje prema molekulima vode, odnosno njihovim hidrofilnim svojstvima. Promene u vlaknu koje su nastale prilikom apsorpcije vode dovele su do promene u gustini pakovanja vlakana u pređi. Geometrija obe pređe se promenila, što se manifestovalo blagim povećanjem prečnika i smanjenjem gustine pređe (tabela 3).

Tabela 3. Karakteristike pređa od konoplje posle mokre relaksacije
Table 3. Characteristics of the hemp yarns after wet relaxation

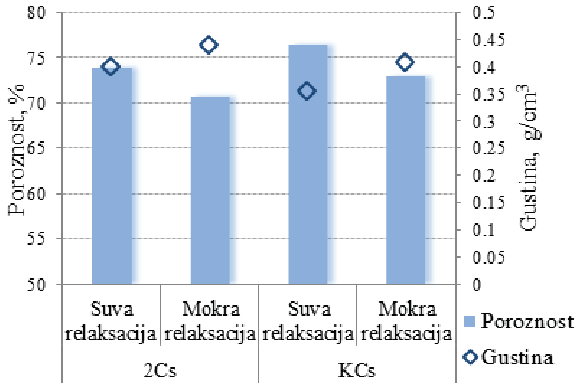
Svojstvo	Konoplja	Konoplja/Konoplja
Prečnik, mm	0,23	0,45
Gustina, g·cm^{-3}	1,151	0,601
Faktor pakovanja	0,77	0,40

Usled skupljanja pređe došlo je do povećanja horizontalne gustine petlji, odnosno došlo je do promene oblika petlji, što se objašnjava distorzijom i izvijanjem petlji u pravcu treće dimenzije usled njihove težnje da zauzmu ravnotežni oblik. To je dovelo do povećanja površinske gustine petlji i debljine pletenina, zbog čega se povećava i površinska masa pletenina (tabela 4). Promena oblika petlji u toku procesa relaksacije pletenine kvašenjem, omogućena je dejstvom tečnosti koja predstavlja određeno sredstvo "podmazivanja" tačaka preplitanja pređe. Time se smanjuje površinski koeficijent trenja segmenata pređe, koja lakše klize u tačkama međusobnog kontakta.

Tabela 4. Konstrukcione karakteristike mokro relaksiranih glatkih pletenina od konoplje
Table 4. Construction characteristics of the wet relaxed plain hemp knitted fabrics

Svojstvo		Konoplja+Konoplja, 2Cs	Konoplja/Konoplja, KCs
Gustina cm^{-1}	D_v	13,6	12,0
	D_h	6,2	6,0
Površinska gustina cm^{-2}		84,3	72,0
Dužina petlje, mm		5,2	5,2
Debljina, mm		0,929	0,933
Površinska masa g m^{-2}		419,0	378,6

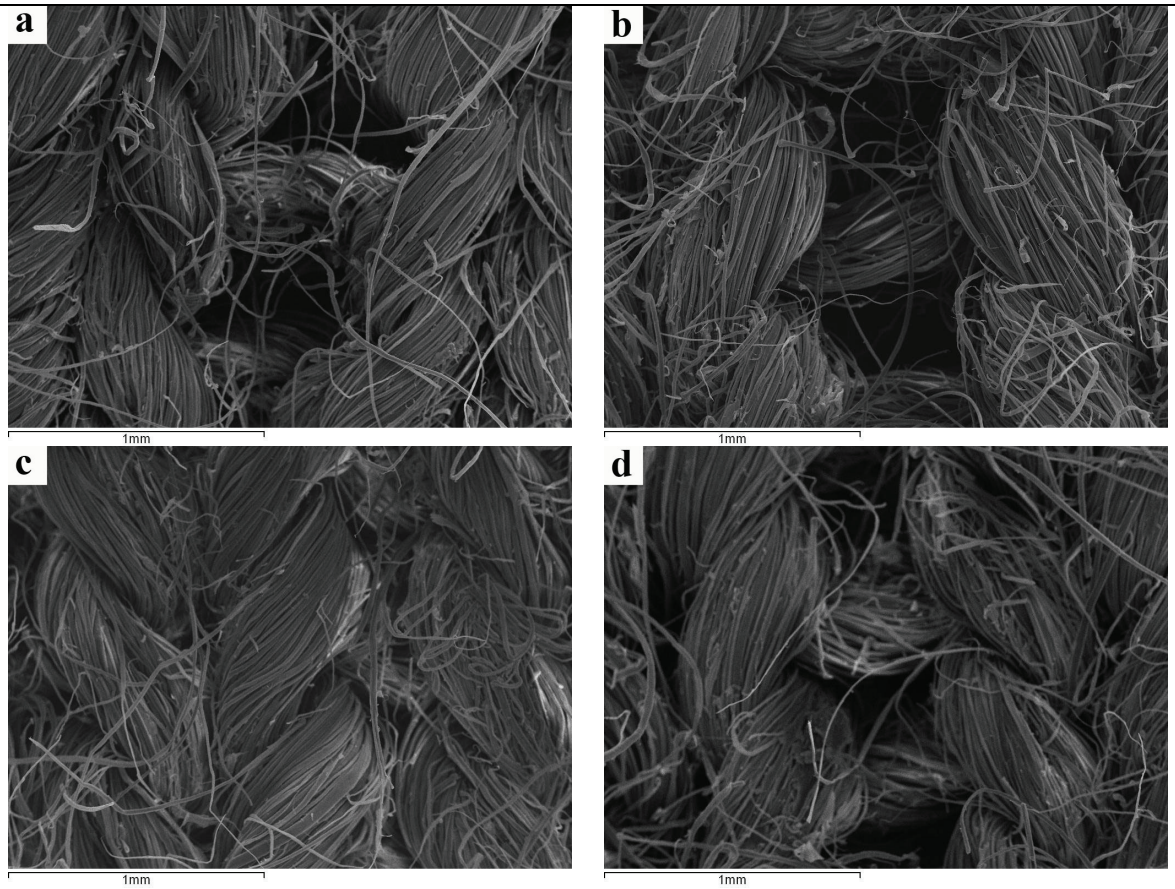
Promene u strukturi pletenina izazvane relaksacionim procesima odrazile su se i na njihova fizička svojstva (gustina, poroznost i propustljivost vazduha). Poroznost i gustina glatkih pletenina od konoplje posle suve i mokre relaksacije prikazane su na slici 2. Kao posledica manje gustine petlji, suvo relaksirana KCs pletenina je okarakterisana većim sadržajem vazduha (većom poroznošću i manjom gustinom) u odnosu na 2Cs pleteninu. Daljom relaksacijom pletenina kvašenjem došlo je do povećanja gustine kod obe pletenine usled povećanja njihove površinske mase, a kao posledica povećanja gustine, smanjena je poroznost pletenina. Ipak, i posle mokre relaksacije KCs pletenina se odlikuje većom poroznošću i manjom gustinom.



Slika 2. Gustina i poroznost glatkih pletenina od konoplje (2Cs – pletenina izrađena od jednožične pređe; KCs – pletenina izrađena od končane pređe)
Figure 2. Density and porosity of the plain hemp knitted fabrics (2Cs – knitted fabric made from two assembled yarn; KCs - knitted fabric made from two-folded yarn)

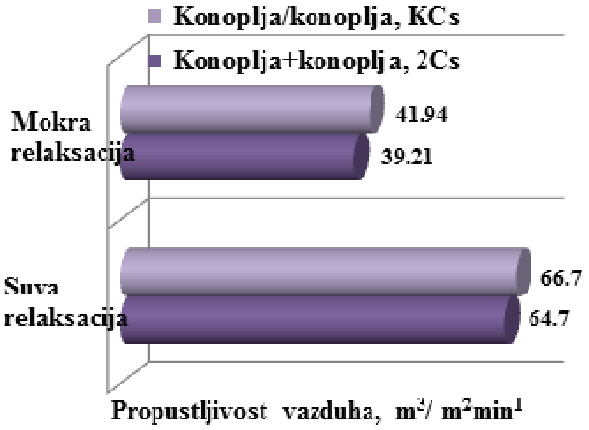
Vrednosti poroznosti glatkih pletenina od konoplje date na slici 2 predstavljaju ukupnu poroznost, odnosno sadržaj vazduha između vlakana u pređi i između segmenata pređe u pletenini. Zbog toga se ovaj parametar ne može uzeti kao pokazatelj distribucije vazduha, odnosno pora u pleteninama, a što je od velike važnosti prilikom ocene potencijala pletenina u pogledu UV zaštitnih svojstava. Naime, poznato je da se transmisija UV zračenja kroz tekstilne materijale dešava u najvećoj meri kroz otvorene pore između pređa (nastale preplitanjem dva sistema žica kod tkanina ili jednog sistema žica kod pletenina) ili makropore. SEM mikrofotografije glatkih pletenina od konoplje poslužile su za kvalitativnu ocenu njihove makroporoznosti pre (slika 3a i b) i posle (slika 3c i d) mokre relaksacije. Lako se uočava veća „otvorenost" pletenine izrađene od končane konopljine pređe, odnosno prisustvo većih otvorenih površina između nizova petlji, kako u slučaju suve relaksacije (slika 3b) tako i u slučaju mokre relaksacije (slika 3d). „Zatvorenija" struktura suvo relaksirane 2Cs pletenine (slika 3a) postaje još očiglednija posle mokre relaksacije kada se makropore teško uočavaju, a susedni nizovi se dodiruju stranicama petlji (slika 3c).

Budući da je propustljivost vazduha tekstilnih materijala, pored debljine, u najvećoj meri uslovljena poroznošću, odnosno kako je dokazano, makroporoznošću,



Slika 3. SEM mikrofotografije suvo relaksiranih 2Cs (a) i KCs (b), i mokro relaksiranih 2Cs (c) i KCs (d) glatkih pletenina od konoplje.
Figure 3. SEM microphotographs of the dry relaxed 2Cs (a) and KCs (b), and wet relaxed 2Cs (c) and KCs (d) plain hemp knitted fabrics.

rezultati propustljivosti vazduha konopljinih pletenina u okviru ovog rada su u određenoj meri posmatrani kao kvantitativna ocena njihove makroporoznosti. Takođe, imajući u vidu činjenicu da propustljivost vazduha predstavlja bitnu komponentu termofiziološkog komfora tekstilnih materijala, dobijeni rezultati ukazali su na potencijal pletenina u tom pogledu. Rezultati propustljivosti vazduha prikazani su na slici 4. Pletenina izrađena od končane konopljine pređe okarakterisana je većom propustljivošću vazduha u poređenju sa pleteninom izrađenom od jednožične konopljine pređe, što je potvrđeno Student *t*-testom ($p(0{,}016) < \alpha(0{,}05)$). Veća površinska gustina petlji 2CS pletenine podrazumeva veći broj makropora na jedinici površine pletenine koje su, zbog toga, manjih dimenzija u odnosu na KCs pleteninu (slika 3a i 3b). S obzirom da sa smanjenjem veličine pora raste otpor strujanju vazduha, opada sposobnost propuštanja vazduha 2CS pletenine. Strukturne promene do kojih dolazi u toku mokre relaksacije pletenina izazivaju smanjenje propustljivosti vazduha. Sa povećanjem površinske gustine petlji i povećanjem prečnika jednožične i končane konopljine pređe, redukovna je veličina makropora pa opada sposobnost propuštanja vazduha. Pri tome se KCs pletenina i dalje odlikuje većom propustljivošću vazduha [$p(0{,}031) < \alpha(0{,}05)$], što joj daje prednost nad 2Cs pleteninom u pogledu termofiziološkog komfora.



Slika 4. Propustljivost vazduha glatkih pletenina od konoplje.
Figure 4. Air permeability of the plain hemp knitted fabrics.

Procenat transmisije UVR kroz delimično (suvo) relaksirane 2Cs i KCs pletenine i izračunate vrednosti UPF dati su u tabeli 5. Imajući u vidu interval debljine ispi-

tivanih pletenina, odnosno vrednosti prečnika pređa od kojih su pletenine proizvedene, u okviru ovog istraživanja pošlo se od pretpostavke da upotrebljene pređe ne propuštaju UVR. Zbog toga, uočene razlike u vrednostima UV transmisije pletenina od konoplje, koje su statistički potvrđene ($p(2{,}58\times10^{-58}) < \alpha(0{,}05)$), mogu se pripisati njihovoj različitoj „otvorenosti" strukture tj. makroporoznosti. KCs pletenina, koja je okarakterisana većom makroporoznošću (manifestovano kroz veću propustljivost vazduha) u poređenju sa 2Cs pleteninom, odlikuje se i većom UVR transmisijom, odnosno nižom vrednošću UPF koja joj ne obezbeđuju zadovoljavajuća UV zaštitna svojstva. Naime, prema standardu EN 13758-2 [35] tekstilni materijali sa UVR transmisijom većom od 5%, odnosno sa UPF vrednošću manjom od 20, ne obezbeđuju dovoljnu UV zaštitu. Prema vrednosti UV zaštitnog faktora 2Cs pletenine, odnosno prema vrednosti UVR transmisije (3,4–5,0%) [35], ova pletenina se kategoriše kao materijal sa dobrim UV zaštitnim svojstvima.

Tabela 5. UV zaštitna svojstva suvo relaksiranih glatkih pletenina od konoplje
Table 5. UV protection properties of the dry relaxed plain hemp knitted fabrics

Parametar		Konoplja+Konoplja, 2Cs	Konoplja/Konoplja, KCs
Transmisija %	UVA	4,123	5,936
	UVB	4,370	5,965
	UVR	4,251	5,950
UPF		21,10	15,02
UPF klasifikacija		Dobar	–

Snižavanje sposobnosti propuštanja vazduha posle mokre relaksacije pletenina ukazalo je na smanjenje njihove makroporoznosti, i potencijalno smanjenje UVA i UVB transmisije, što su rezultati i potvrdili (tabela 6). Uticaj mokre relaksacije pletenina na UVR transmisiju potvrđen je statističkom analizom (*t*-test uparenih uzoraka) za 2Cs ($p(1{,}74\times10^{-42}) < \alpha(0{,}05)$) i KCs ($p(8{,}85\times10^{-42}) < \alpha(0{,}05)$) pleteninu. Pored toga, pokazalo se da se posle mokre relaksacije UPF vrednost povećala za 2,8 puta kod obe pletenine. Tako je 2Cs pletenina i dalje okarakterisana većom vrednošću UPF u poređenju sa KCs pleteninom zahvaljujući većoj površinskoj gustini petlji (tabela 4). To je potvrđeno testiranjem značajnosti razlike između srednjih vrednosti UVR transmisije ove dve pletenine ($p(6{,}7\times10^{-49}) < \alpha(0{,}05)$). Imajući u vidu nisku vrednost gustine pakovanja konopljinih vlakana u končanoj pređi (0,4), ne treba zanemariti mogućnost da se UVR transmisija dešava u određenoj meri i kroz končanu pređu. Ipak, najbitnija činjenica je da se strukturnom relaksacijom obe pletenine UVA i UVB transmisija snižava ispod 2,5%, odnosno povećava se njihova UPF vrednost i dostiže prag 40. Time 2Cs i KCs pletenina stiču oznaku 40+, i svrstavaju se u kategoriju materijala sa odličnim UV zaštitnim svojstvima. Ova činjenica potvrđuje potencijal koji operacija končanja pređe ima u pogledu UV zaštitnih svojstava, posebno ako se ima u vidu pozitivan efekat končanja na komfor tekstilnih materijala od konoplje [36].

Tabela 6. UV zaštitna svojstva mokro relaksiranih glatkih pletenina od konoplje
Table 6. UV protection properties of the wet relaxed plain hemp knitted fabrics

Parametar		Konoplja+Konoplja, 2Cs	Konoplja/Konoplja, KCs
Transmisija %	UVA	1,722	2,390
	UVB	1,719	2,386
	UVR	1,721	2,388
UPF		59,77	59,77
UPF klasifikacija		40+ (odličan)	42,05

ZAKLJUČAK

U okviru ovog istraživanja izvršeno je poređenje UVR transmisije i UV zaštitnog faktora (UPF) glatkih pletenina izrađenih od jednožične ili končane konopljine pređe u cilju utvrđivanja potencijala operacije končanja pređe na UV zaštitna svojstva pletenina. Analiziran je uticaj operacije končanja konopljine pređe na strukturu i fizička svojstva (gustina, poroznost, propustljivost vazduha) pletenina. Efekat koji operacija končanja pređe ima na konstrukcione karakteristike pletenina objašnjava se povećanom savitljivošću končane pređe, koja je posledica smanjenja gustine pakovanja vlakana u novonastaloj složenoj pređi. Razlike u konstrukcionim karakteristikama glatkih pletenina od konoplje uticale su na različitu raspodelu pora, što je uslovilo njihova različita „transportna svojstva". Pored veće poroznosti, pletenina izrađena od končane konopljine pređe odlikuje se makroporama većih dimenzija koje su izazvale više vrednosti transmisije i niže vrednosti UV zaštitnog faktora u poređenju sa pleteninom izrađenom od jednožične konopljine pređe. Strukturne promene nastale tokom mokre relaksacije pletenina, uz smanjenje ukupne poroznosti i makroporoznosti, povećavaju efikasnost UV zaštite obe pletenine. Međutim, pored činjenice da se pletenina izrađena od končane pređe, kada je relaksirana od unutrašnjih napona, odlikuje odličnom UV zaštitnom sposobnošću, treba imati u vidu njenu veću propustljivost vazduha koja joj daje prednost sa aspekta termofiziološkog komfora. Može se reći da su preliminarni rezultati ukazali na potencijal koji operacija končanja pređe ima u pogledu UV zaštitnih svojstava pletenina. Ipak, kako bi se dobile preciznije smernice u tom smislu, neophodna su dalja istraživanja uz proširenje eksperimentalnog materijala u

pravcu primene šireg intervala sekundarnog upredanja (končanja) pređa i konstrukcionih karakteristika pletenina.

LITERATURA

[1] C.A. Wilson, A.V. Parisi, Protection from solar erythemal ultraviolet radiation – simulated wear and laboratory testing, Textile Res. J. **76** (2006) 216–225.

[2] M. Khazova, J.B. O'Hagan, K.J.-L. Grainger, Assessment of sun protection for children's summer 2005 clothing collection, Radiat. Prot. Dosimetry **123** (2007) 288–294.

[3] I. Algaba, A. Riva, P.C. Crews, Influence of fiber type and fabric porosity on the UPF of summer fabrics, AATCC Review **4** (2004) 26–31.

[4] S.B. Stankovic, D. Popovic, G.B. Poparic, M. Bizjak, Ultraviolet protection factors of gray-state plain cotton knitted fabrics, Textile Res. J. **79** (2009) 1034–1042.

[5] K. Dimitrovski, F. Sluga, R. Urbas, Evaluation of the Structure of Monofilament PET Woven Fabrics and their UV Protection Properties, Textile Res. J. **80** (2010) 1027–1037.

[6] S. Davids, L. Capjack, N. Kerr, Clothing as protection from ultraviolet radiation: which fabric is most effective? Int. J. Dermatol. **36** (1997) 374–379.

[7] A. Grancarić, Ž. Penava, A. Tarbuk, UV protection of cotton – the influence of weaving structure, Hem. Ind. **59** (2005) 230–234.

[8] M. Duleba-Mejak, Transmission of UV radiation through woven fabrics in dependence on the inter-thread spaces, Fibers Text. East. Eur. **17** (2009), 34–38.

[9] A. Majumdar, V.K. Kothari, A.K. Mondal, P. Hatua, Effect of weave, structural parameters and ultraviolet absorbers on in vitro protection factor of bleached cotton woven fabrics, Photodermatol. Photoimmunol. Photomed. **28** (2012) 58–67.

[10] W.-Y. Wong, J.K.-C. Lam, C.-W. Kan, R. Postle, Influence of knitted fabric construction on the ultraviolet protection factor of greige and blended cotton fabrics, Textile Res. J. **83** (2013) 683–699.

[11] C.W. Kan, A study on ultraviolet protection of 100% cotton knitted fabrics: effect of fabric parameters, Sci. World J. (2014) 506049.

[12] P.D. Dubrovski, The influence of color and woven fabric construction on ultraviolet protection factor, in Proceedings of the 6th World Textile Conference AUTEX 2006, NC State University, USA, 2006.

[13] U. Osterwalder, W. Schlenker, H. Rohwer, E. Martin, S. Schuh, Facts and fiction on ultraviolet protection by clothing, Radiat. Prot. Dosimetry **91** (2000) 255–260.

[14] D.G. Stanford, K.E. Georgouras, M.T. Pailthorpe, The effect of laundering on the sun protection afforded by a summer weight garment, J. Eur. Acad. Dermatol. Venereol. **5** (1995) 28–30.

[15] S.Q. Wang, A.W. Kopf, J. Marx, A. Bogdan, D. Polsky, R.S. Bart, Reduction of ultraviolet transmission through cotton T-shirt fabrics with low ultraviolet protection by various laundering methods and dyeing: clinical implications, J. Am. Acad. Dermatol. **44** (2001) 767–774.

[16] T. Gambichler, K.L. Hatch, A. Avermaete, P. Altmeyer, K. Hoffman, Influence of wetness on the ultraviolet protection factor (UPF) of textiles: *in vitro* and *in vivo* measurements, Photodermatol. Photoimmunol. Photomed. **18** (2002) 29–35.

[17] A. Kocić, Uticaj dimenzijalne stabilnosti glatkih pletenina na njihova UV zaštitna svojstva, završni master rad, Tehnološko–metalurški fakultet, Beograd, 2013.

[18] K. Hoffmann, K. Kaspar, T. Gambichler, P. Altmeyer, In vitro and in vivo determination of the UV protection factor for lightweight cotton and viscose summer fabrics: a preliminary study, J. Am. Acad. Dermatol. **43** (2000) 1009–1016.

[19] J.H. Xin, W.A. Daoud, Y.Y. Kong, A new approach to UV--blocking treatment for cotton fabrics, Textile Res. J. **74** (2004) 97–100.

[20] G. Hustvedt, P. Cox Crews, The ultraviolet protection factor of naturally-pigmented cotton, J. Cotton Sci. **9** (2005) 47–55.

[21] S. Stanković, Uticaj strukture pređa na bazi agroceluloznih vlakana na njihovu dalju tekstilnu transformaciju i upotrebna svojstva, Doktorska disertacija, Tehnološko––metalurški fakultet, Beograd, 2009.

[22] D. Grifoni, L. Bacci, G. Zipoli G. Carreras, S. Baronti, F. Sabatini, Laboratory and outdoor assessment of UV protection, offered by flax and hemp fabrics dyed with natural dyes, Photochem. Photobiol. **85** (2009) 313–320.

[23] S. Milosavljevic, T. Tadic, S. Stankovic, A new twist on plied yarn properties, Textile Month September (1998) 26–29.

[24] S.A. Sinclair, B.L. Diffey, Sun protection provided by ladies stocking, Br. J. Dermatol. **136** (1997) 239–241.

[25] A.V. Parisi, M.G. Kimlin, L.R. Meldrum, C.M. Relf, Field measurement on protection by stocking from solar erythemal ultraviolet radiation, Radiat. Prot. Dosimtery **86** (1999) 69–72.

[26] A. Tarbuk, A.M. Grancaric, Z. Penava, A. Zampetakis, The influence of yarn linear dnesity on UV protection of woven cotton fabrics, in Proceedings of the 3rd International Textile, Clothing & Design Conference – Magic World of Textiles", Dubrovnik, Croatia, 2006, pp. 739––744.

[27] SRPS ISO 2060: Pređa u namotajima - Određivanje linearne gustine - Metoda povesma, 1995.

[28] SRPS ISO 2061: Tekstil - Određivanje uvoja u pređi – Metoda direktnog brojanja, 1994.

[29] SRPS EN ISO 5084: Tekstil – Određivanje debljine tekstila i tekstilnih proizvoda, 2013.

[30] A. Koblyakov, in A. Koblyakov (Ed.), Laboratory Practice in the Study of Textile Materials, Mir Publisher, Moscow, 1989, pp. 254–262.

[31] EN 13758-1:2002 Textiles – Solar Ultraviolet Protective Properties – Part I: Methods of test for Apparel Fabrics.

[32] ASTM D737 – 96: Standard Test Method for Air Permeability of Textile Fabrics.

[33] SRPS F. S2.020: 1958, Fizikalna ispitivanja tekstila – Određivanje skupljanja tkanine.

[34] J.E. Booth, Principles of Textile Testing, Butterworths, London, 1968.

[35] EN 13758-12:2003 Fabrics – Solar UV Protective Properties – Part 2: Classification and Marking of Apparel.

[36] S.B. Stankovic, M. Bizjak, Effect of yarn folding on comfort properties of hemp knitted fabrics, Cloth. Text. Res. J. **32** (2014) 202–214.

SUMMARY

INFLUENCE OF YARN FOLDING ON UV PROTECTION PROPERTIES OF HEMP KNITTED FABRICS

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(Scientific paper)

In the last years the media have highlighted the damage of the ozone layer and the resulting increase of ultraviolet radiation (UVR) reaching the Earth's surface. Prolonged and repeated, both occupational and recreational, sun exposure of the population causes some detrimental effects. Clothing is considered to be one of the most important tools for UV protection. It is generally accepted that synthetic fibres provide a high UV protection capability of textiles, while cellulose fibres (cotton, linen, hemp, viscose) have a low UV absorption capacity. However, natural pigments, pectin and waxes in natural cellulose fibers, and lignin in hemp fibers, act as UV absorbers having a favorable effect on UPF of grey-state fabrics. Bearing in mind the trend of reintroduction of hemp fibers as a source of eco-friendly textiles, there is a serious lack of study about the potential of hemp materials in terms of UV protection. Folded yarn is a complex yarn composed of two or more component yarns arranged parallel and twisted together to make a "new quality" yarn. Folding of yarns is an operation undertaken in order to modify single-yarn properties to an appreciable degree. There are very few investigations concerning the relationship between the yarn properties and UV protection effectiveness of the fabric made therefrom. In addition, there is no any result in the scientific literature about the influence of yarn folding on UV protection properties of textile materials. Having this in mind, for our research the idea was to evaluate the effect of yarn folding in this regard. The plain knitted fabrics composed of single or two-folded hemp yarn were compared in terms of UV protection properties. The Ultraviolet Protection Factor (UPF), as the quantitative measurement of the material effectiveness to protect the human skin against UVR, was determined for the textile materials by *in vitro* test method according to the European standard EN 13758. The knitted fabrics construction and physical properties were also determined. Bearing in mind that plain knitted fabrics are particularly susceptible to relaxation, they were subjected to relaxation and shrinkage by wetting process, and testing procedure was repeated on the water-treated samples. The results obtained indicated that the folding operation influences UV protection properties of knitted fabrics through an influence on a loop configuration, i.e. the fabric openness. Relaxation and shrinkage of the knitted fabrics due to wet relaxation caused the reduction of macro-porosity increasing the UPF of the knitted fabrics. Although the knitted fabric produced from single hemp yarn was characterised by higher UPF, the UVR transmittance of the folded hemp yarn knitted fabric after wet relaxation placed it in the "excellent UV protection category" (according to European Standard EN 13758-2). This fact together with the better thermal comfort manifested itself in higher air permeability, confirmed the potential of folding operation in terms of UV protection properties of textile materials.

Keywords: UPF • UV Transmission • Knitted fabric • Yarn • Folding • Hemp

Carboxymethyl cellulase production from a *Paenibacillus* sp.

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Abstract

Cellulases are industrially important enzymes with a potential to convert cellulose into fermentable sugars. Novel bacterial isolate *Paenibacillus* sp. CKS1 was tested for cellulase activity and the optimal conditions for carboxymethyl cellulase (CMCase) production were determined. Maximum CMCase activity was obtained in the third passage of the bacterial culture after 3 days of incubation at 30 °C. Cellobiose and yeast extract was the optimal source of carbon and nitrogen for induction of CMCase activity. In addition, with initial pH 7 of the medium and 40 ml of working volume in 500 ml culture flasks with shaking at 150 rpm, the maximum CMCase activity in a crude culture supernatant reached value of 0.532±0.006 U/ml. For crude CMCase, optimal temperature was 50 °C and optimal pH 4.8, respectively. HPLC analysis confirmed the bacterium is capable to hydrolise CMC to glucose and other soluble sugars.

Keywords: *Paenibacillus* sp., cellulose, CMCase production, optimal conditions.

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Cellulose is the most abundant natural biopolymer on Earth and the most dominant component of agricultural waste [1]. Cellulosic biomass is a renewable and an abundant resource that can be used for production of biofuels and animal feed [1,2]. Because of its potential applications in industry, microbial conversion of cellulose into simple sugars or bioethanol has received excessive attention in the past decades [3,4]. Active research on cellulases began in the early 1950s and lead to an increasing application of cellulases in biotechnological processes in various industries including food, brewery and wine, animal feed, textile and laundry, pulp and paper, and agriculture [5]. The major source of cellulases are microorganisms that produce these enzymes while growing on cellulosic materials [5,6]. Cellulase is a family of at least 3 groups of enzymes, endo-(1,4)-β-D-glucanase (EC 3.2.1.4), exo-(1,4)--β-D-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21), that hydrolyze cellulose while the mechanism of enzymatic activity differs between the different enzyme classes: endoglucanases (carboxymethyl cellulases), exoglucanases (avicelases) and β-glucosidases [5–7]. Endoglucanases cut the amorphous cellulose polysaccharide chain at random internal sites and generate oligosaccharides of various lengths [1]. Exoglucanases are active on the reducing or non-reducing ends of the cellulose polysaccharide chains and liberate either glucose or cellobiose as major products [1]. β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose and act on the non-reducing ends [1]. Most cellulases are secreted by microbial strains belonging to fungi, or bacterial strains within the *Bacilli* and *Actinomycetes* classes although fungal cellulases have been studied the most [3]. Nevertheless, the studying of bacterial ceullulases is very promising since isolation, screening and selection have enabled the discovery of numerous novel cellulase-producing bacteria from a wide variety of environments [8]. The aim of this study was to select a cellulolytic strain from a culture collection of bacterial soil isolates. A *Paenibacillus* strain has been identified as a potent producer of cellulases as it has shown a notable cellulolytic activity on carboxymethyl cellulose (CMC) agar plate. The conditions that enabled maximal carboxymethyl cellulase (CMCase) production were optimized with emphasis of the following parameters: passaging of the culture, incubation time, carbon and nitrogen source, medium pH, medium volume/surface ratio and aeration. Finally, the crude CMCase was characterized for the optimal temperature and pH.

MATERIAL AND METHODS

Microorganism and chemicals

The microorganism was isolated from the soil sample and screened for cellulase production and identified based on the following characteristics: Gram stain, aerobic growth, morphological characteristics of colonies and bacterial cells, spore formation, appearance and shape. Identification of the strain was done

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by 16S rRNA encoding gene (ARB database, gene accession number KP715850).

Chemicals used for preparation of cultivation media were purchased from Torlak Institute of Immunology and Virology (Belgrade, Serbia) except for the casein hydrolisate that was purchased from Fluka. CMC and cellobiose was purchased from Sigma, Avicel from Merck and Croscarmellose-Na from J. Rettenmaier & Sohne.

Screening for cellulase producing bacteria

Screening for cellulase producing bacteria was performed by growing bacterial strains on CMC agar plates (per liter: CMC 1 g, yeast extract 3 g, K_2HPO_4 3 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g and agar 6 g). An overnight bacterial culture was performed in a liquid CMC medium (the same composition as CMC agar medium without addition of agar). A 4% of bacterial culture was inoculated into fresh CMC liquid medium and incubated for 24 h on 30 °C, with shaking at 150 rpm. After overnight growth, 5 µl of liquid bacterial culture was spot plated on CMC agar plates. After incubation for 24–48 h at 30 °C, plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 min. Clear zones appearing around growing bacterial colonies indicated cellulose hydrolysis [9].

Enzyme assay for CMCase

Carboxymethyl cellulase (CMCase) activity was measured by reduction of 3,5-dinitrosalicylic acid (DNS) in the presence of glucose released by enzymatic hydrolysis of cellulose according to the method of Müller [10]. CMCase activity was determined as follows: 500 µl of enzyme solution (crude bacterial supernatant) was added in test tubes with 500 µl of 1 % CMC in 0.1 M acetate buffer at pH 4.80 and incubated at 50 °C for 30 min in a rotary shaker with rotation speed of 150 rpm. After incubation, 1 ml DNS reagent was added. The reaction mixture was boiled for 5 min in a water bath. After cooling at room temperature 5 ml of distilled water was added to each tube and absorbance of the solution was measured at 540 nm on spectrophotometer (Ultrospec 3300 *pro* Amersham Bioscience). CMCase activity was determined by using a calibration curve for glucose. One unit of CMCase activity was defined as the amount of enzyme that released 1 µmol of glucose equivalent per minute. All assays were carried out in triplicate, while the results are presented as mean value with given standard deviation.

Effect of temperature and pH on CMCase activity

The optimal temperature and pH of crude enzyme was determined using the following procedure: the crude enzyme (bacterial supernatant) was incubated with 1 % CMC in 0.1 M acetate buffer pH 4.80 for 30 min at temperatures between 30 and 70 °C with steps of 10 °C. After incubation, the CMCase activity was measured using the method of Müller [10]. To determine the optimum pH of the enzyme, the crude enzyme was mixed with substrates prepared in following buffer solutions: 100 mM citrate buffer (pH 3.0, 4.0 and 4.8), 100 mM sodium phosphate buffer (pH 6.0 and 7.0), 100 mM Tris–HCl (pH 8.0 and 9.0) and incubated at the optimum temperature of 50 °C for 30 min. CMCase activity was measured according to the method of Müller [10]. The maximum CMCase activity obtained at different temperatures and pH was considered to be 100%.

Influence of various factors on CMCase production

CMCase production was measured in liquid non-optimized medium composed by mixing CMC 5.0 g/l, yeast extract 3.0 g/l, KH_2PO_4 4.0g/l, Na_2HPO_4 4.0g/l, $MgSO_4 \cdot 7H_2O$ 0.2g/l, $CaCl_2 \cdot 2H_2O$ 0.001g/l and $FeSO_4 \cdot 7H_2O$ 0.004 g/l. The pH of the medium was adjusted to 7 before autoclaving. After sterilization at 121 °C for 20 min, a 4% of an overnight bacterial culture was inoculated into fresh medium in a rotary shaker with mixing speed of 150 rpm at 30 °C. The culture medium was centrifuged at 6000*g* for 15 min to remove the cells. The crude cell-free supernatant (pH 5.15) was analyzed for CMCase activity.

The influence of the following parameters on the crude enzyme activty were tested: bacterial passaging, incubation time, pH of the growth medium, various carbon sources, concentration of the optimal carbon source, various nitrogen sources, concentration of the optimal nitrogen source and different medium volumes and agitation speeds.

Influence of the bacterial passaging on CMCase production

The effect of the bacterial culture passaging on CMCase production was examinated by measuring CMCase activity after transfering the bacterial culture every 24 h into fresh medium (passaging) which contained CMC as an inducer for CMCase production. Each passage was monitored for CMCase activity for 4 days.

Influence of the incubation time on CMCase production

The effect of incubation time on CMCase production was investigated by taking samples every 24 h, for five days, and CMCase activity was determinated.

Influence of the pH of the growth medium on CMCase production

The effect of initial pH of the growth medium was examinated by adjusting the pH of the medium to 4, 5, 6, 7, 8, 9 and 10 with 1 M NaOH or 1 M HCl before

autoclaving. CMCase activity was determinated on a third day of incubation.

Influence of the different carbon sources on CMCase production

Different carbon sources including CMC, Avicel, cellobiose and crosscarmellose were added to the growth medium in concentration of 2.5 g/l to evaluate the effect on the CMCase production. For the optimal carbon source (cellobiose), influence of different concentrations (1.5, 2.5, 5.0, 6.0 and 7.0 g/l) on CMCase production was examined.

Influence of the different nitrogen sources on CMCase production

Different nitrogen sources: yeast extract, tripton, meat extract and NH_4NO_3 were added to the growth medium in concentration of 3 g/l. The influence of different cocentrations(2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 g/l) of the optimal nitrogen source (yeast extract) on CMCase production was measured.

Influence of different medium volumes (surface/ /volume ratio) and agitation speeds on CMCase production

The effect of different working medium volumes 40, 100 and 150 ml in 500 ml flask (surface/volume ratio 78.5/40; 78.5/100 and 78.5/150) with shaking speed at 150 rpm during fermentation was investigated for CMCase production. Finally, the effect of different agitation speeds 100, 120 and 150 rpm on CMC-ase production at a constant working volume of 40 ml in 500 ml flask was investigated.

HPLC analyses of the CMC hydrolysis

The CMC hydrolysis product, obtained from optimized medium with maximum CMCase activity, was analyzed by high performance liquid chromatography (HPLC). 5.0 ml of enzyme solution (crude bacterial supernatant) was incubated at 50 °C with 5.0 ml of 1% (*w*/*V*) CMC in 0.1 M acetate buffer (pH 4.80) in a rotary shaker with rotation speed of 150 rpm. After 30 min, hydrolysis was stopped by boiling the sample for 5 min. The sample was then filtered through a 0.22 μm membrane filter.

For quantitative analysis of the obtained sample, the Dionex Ultimate 3000 Thermo Scientific (Waltham, USA) HPLC system was used. A carbohydrate column (Hyper REZ XP Carbohydrate Ca^{2+}, 300 mm×7.7 mm, 8μm) on 80 °C was employed. Water (HPLC grade, JT Baker (USA)) was used as sole mobile phase with an elution rate 0.6 ml/min during the analysis. Detection was performed by RI detector (RefractoMax 520, ERC, Germany). All data acquisition and processing was done using Chromeleon Software. The separated hydrolysis products were identified by comparison with glucose standard.

Statistical analysis

Mean values of various experiments were compared by the analysis of variance. All statistical analyses were performed using the Origin Pro 8 software.

RESULTS AND DISCUSSION

Screening for cellulase producing microorganism

Screening for cellulase production was tested on CMC agar plate by the appearance of a halo around the bacterial colony. Based on physiological characteristics, optimal temperature for growth of the strain was 30 °C, thus this temperature was used for incubation. The strain with cellulolytic activity was identified as member of the *Paenibacillus* genus based on the following characteristics: Gram positive reaction, aerobic growth, spore formation with characteristic ellipsoidal spores that were larger than the viable cells. The bacterial strain with cellulolytic activity designated as *Paenibacillus* sp. CKS1 was identified as *Paenibacillus chitinolyticus* based on the almost full-length 16S rRNA gene sequence. The sequence was deposited to the GeneBank database under accession number KP715850.

CMCase production

Cellulase systems consist of endoglucanase, exoglucanase, and β-glucosidase and the synergy of all these enzymes makes hydrolysis of cellulose to glucose possible [11]. CMC is an example of an amorphous cellulose and is generally used as a substrate for the study of endoglucanases or CMCases [12]. In our study, the most potent cellulolytic isolate strain *Paenibacillus* sp. CKS1 was grown on amorphous cellulose (CMC). The strain *P.chitinolyticus* CKS1 showed greater catalytic affinity for CMC, so the cellulases secreted by this isolate could be categorized as endoglucanase or CMCases.

Subculturing (passaging) of an microorganism in a medium of essentially the same composition as that employed for final culture showed to be an effective way to enhance a desired property [13]. Particularly for large enzyme complexes, such as cellulases, such adaptation of a microorganism is expected to enhance enzyme synthesis. In contrast to this expectation, Beckord *et al*. [14] found that one subculture to a medium similar to the final culture medium had a beneficial effect, while subculturing more than once had no significant influence on the production of enzyme. In order to define if the adaptation of the microorganism to the specific cultivation medium had an impact on CMCase activity, the influence of passaging of bacterial culture was examined. The results showed that CMCase activity increased with culture passaging and

with the incubation time (Figure 1). The highest CMCase activity was detected at the third passage and at the third day of incubation (0.197±0.019 U/ml). CMCase activity increased with culture passaging and the highest CMCase activity was detected at the third passage, thus third passage was applied to all further experiments. In further tests the second passage was used as inoculum for further investigation of CMCase production. With regard to CMCase activity, which increased with culture passaging, the highest CMCase activity was detected at the third passage, thus third passage was applied to all further experiments.

When testing the influence of incubation time on the CMCase activity it was determined that the highest CMCase production was obtained after three days of incubation (Figure 2). After this period CMCase production decreased. The decrease of CMCase activity could be a consequence of changed conditions in the medium (pH change, production of inhibiting byproducts), or due to the depletion of nutrients in the fermentation medium as seen for other bacterial strains [15]. The timing of the optimal CMCase production is a strain dependent characteristic. Some cellulolytic *Paenibacillus* sp. had the optimal CMCase production as shorter as 24 h (*Paenibacillus* sp. P118 [16]) or similar to our strain *P.chitinolyticus* CKS1 of about 72 h (*Paenibacillus tarimensis* L88 [17] and *Paenibacillus* sp. ME-271 60 h [18]). In general, the increase in the enzyme activity during the incubation period depends on the culture characteristics and growth rate of selected microorganism. *Paenibacillus* sp. CKS1 produced CMCase during the earlier stage of fermentations, in a late exponential phase (data not shown), while the maximum CMCase production was achieved in the late stationary phase.

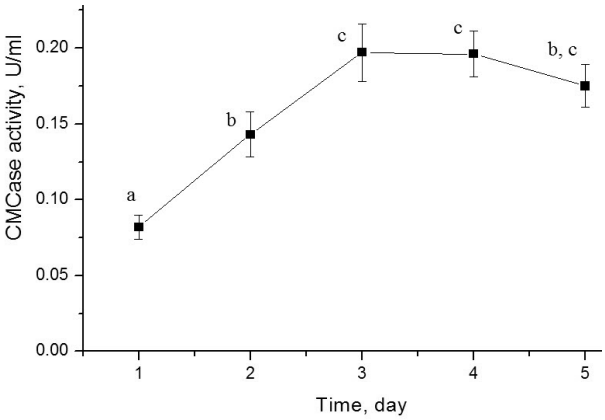

Figure 2. CMCase activity for the strain CKS1 during 5 days of incubation on 30 °C at 150 rpm with 40 ml of working volume in 500 ml flask.

It is well established that initial pH of the bacterial growth medium has an effect on the availability of certain metabolites and ions and influences the permeability of the cell membrane [4]. Our results also showed that the pH of the growth medium was an important factor affecting the CMCase activities. The optimum pH of the growth medium for maximum production of CMCase for the strain CKS1 was 7.0 (Figure 3).

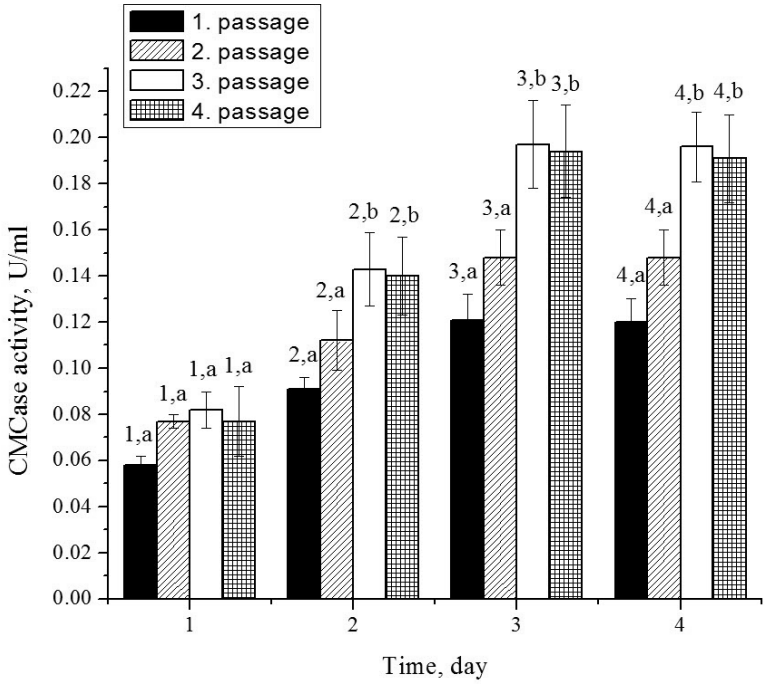

Figure 1. The influence of passaging culture on CMCase activity. A 4% of bacterial culture was inoculated in each passage for four days at 30 °C at 150 rpm.

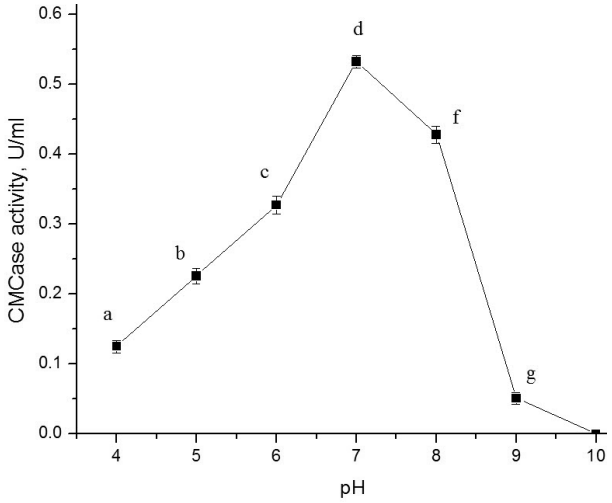

Figure 3. Effect of initial pH of the growth medium on CMCase production for the strain CKS1 on 30 ℃ at 150 rpm with 40 ml of working volume in 500 ml flask.

Similar to other parameters, various *Paenibacillus sp.* strains show the optimal CMCase activity under different medium acidity conditions. Some other species produce the highest levels of CMCase at neutral pH including *P. curdlanolyticus* B-6 (isolate from an anaerobic digester fed with pineapple wastes) and *P. cam-pinasensis* BL-11 (isolate from black liquor) [19,20], while others perform the best under alkali conditions such as *P. terrae* ME27-1 (pH 8) (isolate from soil sample from the subtropical region of China) [18], or acidic conditions such as *Paenibacillus polymyxa* an isolate from degrading citrus peel (pH 5.5) [21].

Generally, production of cellulases was shown to be inducible and was affected by the nature of the carbohydrate used in fermentation as a carbon source. Different commercial substrates have been used as inducers of CMCase production by *Penibacillus* sp. CKS1 (Figure 4a). According to this results it appeared that *Paenibacillus* sp. CKS1 produced the largest amount of CMCase (0.326±0.011 U/ml) while growing on cellobiose, although it produced CMCase on all tested substrates (Figure 4a). Mandels [22] and Paul [23] also reported that cellobiose is a good cellulase inducer for fungi and *Bacillus* sp. [24–26] but for some other *Paenibacillus* sp. the most potent inducer of the CMCase activity was CMC [18,27,28]. The tested *Paenibacillus* sp. CKS1 is the first reported *Paenibacillus* sp. for which cellobise is better inducer of CMCase production than CMC itself.

Cellobiose had the inducing effects in the concentration range of 1.5–7 g/l (Figure 4b). With cellobiose

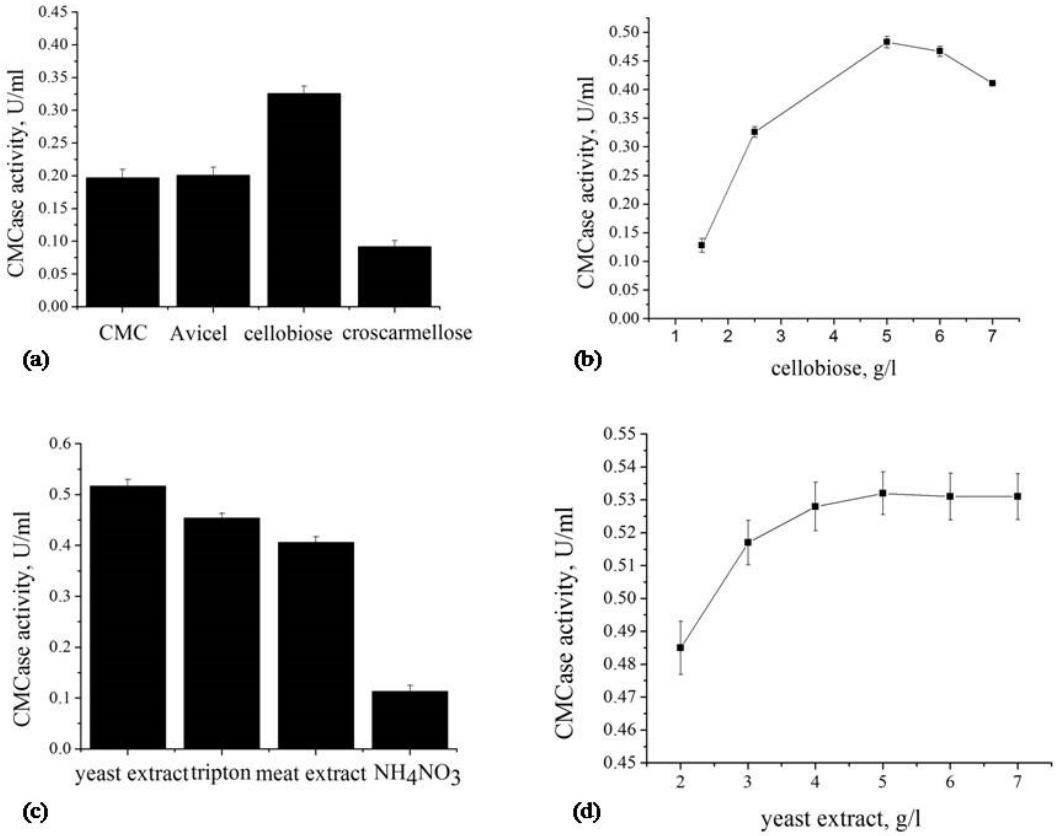

Figure 4. Effect of carbon and nitrogen sources on CMCase production by the strain CKS1 for 3 days of incubation. a) Different carbon sources: CMC, Avicel, cellobiose and croscarmellose, respectively. b) The concentration of cellobiose. c) Different nitrogen sources: yeast extract, tripton, meat extract and NH_4NO_3, respectively. d) The concentration of yeast extract.

concentration of 5 g/l the maximum CMCase activity (0.483±0.010 U/ml) was achieved. Further increase of the concentration leads to a slight decrease in CMCase activity.

Regarding the influence of the different nitrogen sources, it was determined that yeast extract is the most stimulative for CMCase production as growth of the strain on the medium containing yeast extract led to CMCase activity of up to 0.517±0.006 U/ml (Figure 4c). The optimal concentration of the yeast extract in the medium was 5 g/l (Figure 4d) thereby providing a 0.532±0.006 U/ml of CMCase activity. Yeast extract is the main nutritional supplement which serves as a rich source of amino acids, vitamins, nitrogen and carbon for bacterial growth [29]. Based on our results, it is evident that *Paenibacillus* sp. CKS1 prefers organic nitrogen sources for growth and enzyme production. When growing on medium that contained inorganic nitrogen source, NH_4NO_3, *Paenibacillus* sp. CKS1 strain produced notably lower amount of CMCase compared to the used organic sources of nitrogen (Figure 4c). Our results are in line with previous reports for the majority of cellulolytic *Paenibacillus* strains [17,20,28] although some strains are able to produce CMCases in large quantities while growing in presence of inorganic nitrogen [19,27,30].

Oxygen in the medium may have great influence on the production of metabolites and enzymes, including extracellular enzymes [31]. CMCase production was significantly affected by medium volume since all tests were done in the same type of flasks and the medium volume had a direct effect on the surface/volume ratio. Given that surface/volume ration and the aeration area is relatively decreased when larger volumes are used for bacterial growth, we evaluated the influence of medium volume on CMCase production for our strain *Paenibacillus* sp. CKS1. The highest CMCase production was achieved when the culture medium volume was 40 ml in 500 ml volume flasks. Increasing the volume of media leads to significant decrease of the enzyme activity (Figure 5). The strain CKS1 grows under strictly aerobic conditions and any reduction in oxygen level leads to a decrease of the CMCase production.

Furthermore, agitation had a significant influence on the CMCase production. When the strain was grown under stationary conditions, the CMCase activity was under the detection level of the applied methodology (data not shown). The CMCase activity did not change significantly when agitation speed changed in the range from 120 to 150rpm.

The literature data available on the production of cellulase by *Paenibacillus* sp. are difficult to compare with each other due to different growing conditions of microorganisms, different substrates, ways the results of enzymatic activity is interpreted. Also, CMCase from the strain CKS1 are from crude, not purified culture supernatant which may have influence on a lower value of enzyme activity. Nevertheless, our results indicated that relatively higher aeration surface had a stimulative effect on CMCase activity of *Paenibacillus* sp. CKS1 indicating the positive effect of oxygen on the CMCase production and activity.

Effect of temperature and pH on CMCase activity

The influence of temperature on CMCase activity produced by the *Paenibacillus* sp. CKS1 was examined at various temperatures ranging from 30 to 70 °C at pH 4.8. Optimal temperature for CMCase activity was 50 °C at pH 4.8 (Figure 6a).

The optimal temperature for other CMCases produced by *Paenibacillus* strains is close to or slightly higher than the one determined for our strain as it values 50 °C for *Paenibacillus terrae* ME27-1 [18], 55 °C for *P. cookii* SS-24 [32], 60 °C for *Paenibacillus* sp. B39A

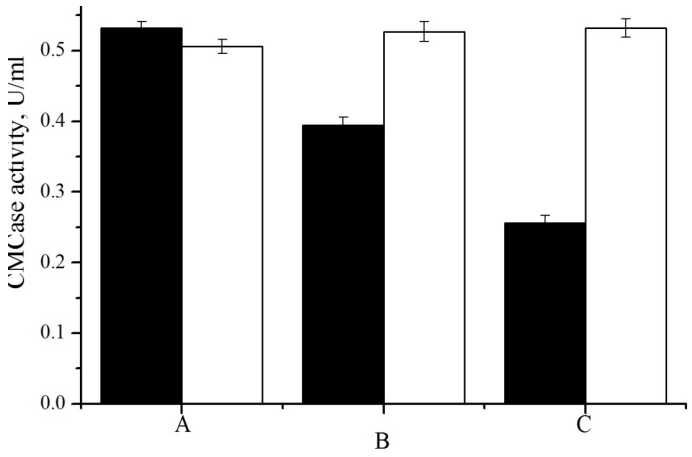

Figure 5. Effects of different medium volume (filled square) and agitation speeds (opensquare) on CMCase production from Paenibacillus sp. CKS1. The cultures were carried out at different working volumes in 500 ml flasks: A) 40; B) 100 and C) 150ml; with shaking at 150 rpm. The effect of agitation speeds: A) 100; B) 120 and C) 150 rpm on CMCase production was performed in a constant working volume (40 ml).

and *Paenibacillus elgii* [30,33] and 65 °C for *P. barcinonensis* CMCase [28].

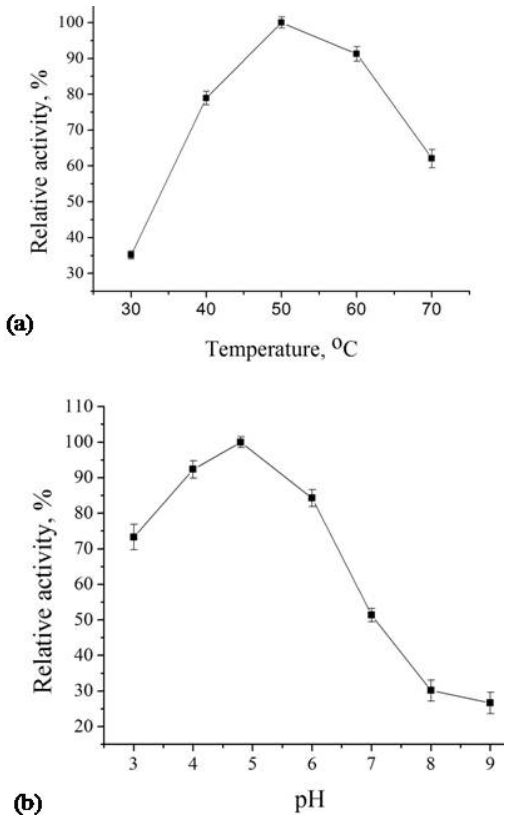

Figure 6. Effect of a) temperature and b) pH on CMCase activity.

The optimal pH for CMCase activity of *Paenibacillus* CKS 1 was 4.8 (Figure 6b) therefore the tested CMCase can be considered as acidophilic enzyme. This feature is in contrast to the majority of CMCases reported in the literature, since these enzymes typically favor neutral or alkaline conditions [18,20,33,34] although there are exception like *P. polymyxa* that shows optimal activity at slightly acidic conditions of pH 5.5 [21].

Hydrolysis products of CMCase

Paenibacillus sp. CKS1 CMCase hydrolyzed CMC to form glucose and a number of oligosaccharides assuming cellobiose, cellotriose, cellotetraose (Figure 7). In addition to these small molecules, some complex components, most likely larger oligosaccharide residues seem to be present in the hydrolysate. The presence of glucose and other olighosaharides indicates the existence of the enzyme complex of *Paenibacillus* sp. CKS1 which is responsible for degradation of CMC. The formation of glucose and cellobiose suggests the sequential action of endoglucanase and exoglucanase [35]. Many cellulases have been reported to show both endo- and exo-glucanase activities [12,20,30,36]. *Paenibacillus* sp. CKS1 could hydrolyse cellulose, amorphous-CMC and microcrystalline cellulose-Avicel (data not shown) suggesting that it produces both endo- and exo-glucanase.

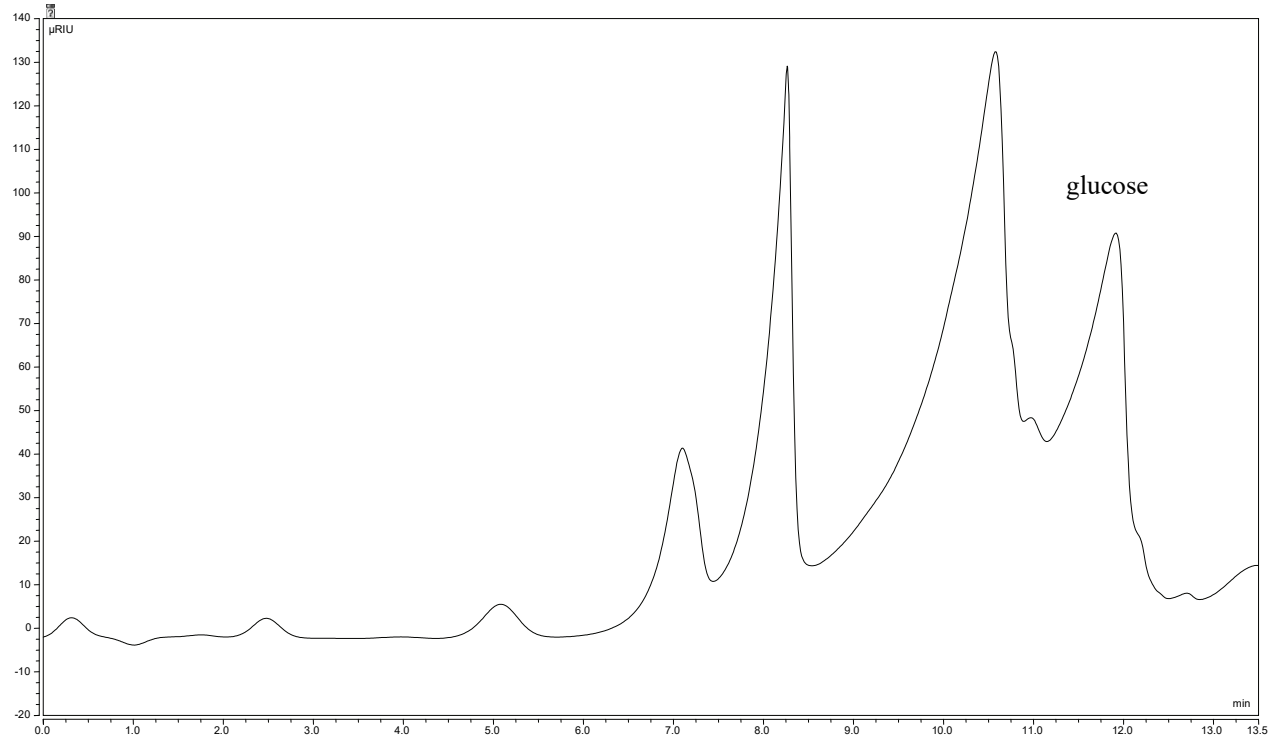

Figure 7. HPLC analysis of hydrolysis CMC.

CONCLUSION

In this study, we have identified three novel *Paenibacillus* strains as potent cellulolytic bacteria. Among them, *Paenibacillus* sp. CKS1 showed the highest cellulolytic potential. Culture conditions were optimized to enable the highest CMCase production by this strain. HPLC analysis confirmed the degradation of CMC into glucose and other oligosaccharides. *Paenibacillus* sp. CKS1 is a novel candidate for the CMCase production facilitating its potential use in industrial applications. Due to its acidophilic nature (pH 4.8) and relatively good tolerance to high temperatures, our CMCase might be a useful enzyme for industrial applications such as animal feed industry, clarification of fruit juices, biofuels production or in stonewashing and biopolishing in jeans industry.

Acknowledgements

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REFERENCES

[1] S. Sadhu, T.K. Maiti, Cellulase production by Bacteria: A Review, Br. Microbio. Res. J. **3** (2013) 235–258.

[2] M. Karmakar, R. Ray, Current trends in research and application of microbial cellulases, Res J. Microbiol. **6** (2011) 41–53.

[3] Y. Feng, J.-X. Jiang, L.-W. Zhu, Recent developments in activities, utilization and sources of cellulase, For. Stud. China **11** (2009) 202–207.

[4] Y. Liang, J. Yesuf, S. Schmitt, K. Bender, J. Bozzola, Study of cellulases from a newly isolated thermophilic and cellulolytic *Brevibacillus* sp. strain JXL, J. Ind. Microbiol. Biot. **36** (2009) 961–970.

[5] M. Bhat, Cellulases and related enzymes in biotechnology, Biotechnol. Adv. **18** (2000) 355–383.

[6] B.-K. Kim, B.-H. Lee, Y.-J. Lee, I.-H. Jin, C.-H. Chung, J.-W. Lee, Purification and characterization of carboxymethylcellulase isolated from a marine bacterium,< i> *Bacillus subtilis*</i> subsp.< i> s*ubtilis*</i> A-53, Enzyme Microb. Tech. **44** (2009) 411–416.

[7] R.C. Kuhad, R. Gupta, A. Singh, Microbial cellulases and their industrial applications, Enzyme Res. **2011** (2011) 1––10.

[8] M. Maki, K.T. Leung, W. Qin, The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass, Int. J. Bio. Sci. **5** (2009) 500–516.

[9] R.C. Kasana, R. Salwan, H. Dhar, S. Dutt, A. Gulati, A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine, Curr. Microbiol. **57** (2008) 503–507.

[10] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. **31** (1959) 426––428.

[11] L.R. Lynd, P.J. Weimer, W.H. Van Zyl, I.S. Pretorius, Microbial cellulose utilization: fundamentals and biotechnology, Microbiol. Mol. Biol. R. **66** (2002) 506–577.

[12] G. Rastogi, A. Bhalla, A. Adhikari, K.M. Bischoff, S.R. Hughes, L.P. Christopher, R.K. Sani, Characterization of thermostable cellulases produced by< i> *Bacillus*</i> and< i> *Geobacillus*</i> strains, Bioresource Technol. **101** (2010) 8798–8806.

[13] A. Boidin, J. Effront, Process for treating amylaceous substances, United States Patent Office (1917) 1,227,374.

[14] L. Beckord, E. Kneen, K. Lewis, Bacterial Amylases, Ind. Eng. Chem. **37** (1945) 692–696.

[15] A. Karim, M.A. Nawaz, A. Aman, S.A.U. Qader, Hyper production of cellulose degrading endo (1, 4) β-D-glucanase from< i> *Bacillus licheniformis*</i> KIBGE-IB2, J. Radiat. Res. Appl. Sci. (2014) 1–6.

[16] A.L. de Castro, R.E. Vollú, R.S. Peixoto, A.L. Grigorevski-Lima, R.R. Coelho, E.P. Bon, A.S. Rosado, L. Seldin, Cellulolytic potential of a novel strain of *Paenibacillus* sp. isolated from the armored catfish *Parotocinclus maculicauda gut*, Braz. J. Microbiol. **42** (2011) 1608––1615.

[17] N. Raddadi, A. Cherif, D. Daffonchio, F. Fava, Halo-alkalitolerant and thermostable cellulases with improved tolerance to ionic liquids and organic solvents from< i> *Paenibacillus tarimensis*</i> isolated from the Chott El Fejej, Sahara desert, Tunisia, Bioresource Technol. **150** (2013) 121–128.

[18] Y.-L. Liang, Z. Zhang, M. Wu, Y. Wu, J.-X. Feng, Isolation, Screening, and Identification of Cellulolytic Bacteria from Natural Reserves in the Subtropical Region of China and Optimization of Cellulase Production by *Paenibacillus terrae* ME27-1, BioMed Res. Int. **2014** (2014) 1–13.

[19] R. Waeonukul, K.L. Kyu, K. Sakka, K. Ratanakhanokchai, Isolation and characterization of a multienzyme complex (cellulosome) of the< i> *Paenibacillus curdlanolyticus*</i> B-6 grown on Avicel under aerobic conditions, J. Biosci. Bioeng. **107** (2009) 610–614.

[20] C.-H. Ko, W.-L. Chen, C.-H. Tsai, W.-N. Jane, C.-C. Liu, J. Tu, < i> *Paenibacillus campinasensis*</i> BL11: A wood material-utilizing bacterial strain isolated from black liquor, Bioresource Technol. **98** (2007) 2727–2733.

[21] D. Kumar, M. Ashfaque, M. Muthukumar, M. Singh, N. Garg, Production and characterization of carboxymethyl cellulase from *Paenibacillus polymyxa* using mango peel as substrate, J. Environ. Biol. **33** (2012) 81–84.

[22] M. Mandels, E.T. Reese, Induction of cellulase in fungi by cellobiose, J. Bacteriol. **79** (1960) 816–826.

[23] J. Paul, A. Varma, Influence of sugars on endoglucanase and β-xylanase activities of a *Bacillus strain*, Biotechnol. Lett. **12** (1990) 61–64.

[24] J. Singh, N. Batra, R. Sobti, A highly thermostable, alkaline CMCase produced by a newly isolated *Bacillus* sp. VG1, World. J. Microb. Biot. **17** (2001) 761–765.

[25] N. Annamalai, R. Thavasi, S. Vijayalakshmi, T. Balasubramanian, A novel thermostable and halostable carboxymethylcellulase from marine bacterium *Bacillus licheni-*

formis AU01, World. J. Microb. Biot. **27** (2011) 2111––2115.

[26] S. Sadhu, P. Saha, S.K. Sen, S. Mayilraj, T.K. Maiti, Production, purification and characterization of a novel thermotolerant endoglucanase (CMCase) from *Bacillus* strain isolated from cow dung, Springer Plus **2** (2013) 1––10.

[27] R. Waeonukul, K.L. Kyu, K. Sakka, K. Ratanakhanokchai, Effect of carbon sources on the induction of xylanolytic-cellulolytic multienzyme complexes in *Paenibacillus curdlanolyticus* strain B-6, Biosci. Biotechnol. Biochem. **72** (2008) 321–328.

[28] B.M. Asha, M. Revathi, A. Yadav, N. Sakthivel, Purification and characterization of a thermophilic cellulase from a novel cellulolytic strain, *Paenibacillus barcinonensis*, J. Microbiol. Biotechnol. **22** (2012) 1501–1509.

[29] H. Tanguler, H. Erten, The effect of different temperatures on autolysis of baker's yeast for the production of yeast extract, Turk. J. Agric. For. **33** (2009) 149–154.

[30] C.M. Wang, C.L. Shyu, S.P. Ho, S.H. Chiou, Characterization of a novel thermophilic, cellulose-degrading bacterium *Paenibacillus* sp. strain B39, Lett. App. Microbiol. **47** (2008) 46–53.

[31] A.-X. Li, L.-Z. Guo, W.-D. Lu, Alkaline inulinase production by a newly isolated bacterium *Marinimicrobium* sp. LS–A18 and inulin hydrolysis by the enzyme, World. J. Microb. Biot. **28** (2012) 81–89.

[32] S. Shinoda, S. Kanamasa, M. Arai, Cloning of an endoglycanase gene from *Paenibacillus cookii* and characterization of the recombinant enzyme, Biotechnol. Lett. **34** (2012) 281–286.

[33] P. Lisdiyanti, E. Suyanto, N. Gusmawati, W. Rahayu, Isolation and characterization of cellulose produced by cellulolytic bacteria from peat soil of Ogan Komering Ilir, South Sumatera, Int. Environ. Bioenergy. **3** (2012) 145––153.

[34] P. Pason, K.L. Kyu, K. Ratanakhanokchai, *Paenibacillus curdlanolyticus* strain B-6 xylanolytic-cellulolytic enzyme system that degrades insoluble polysaccharides, App. Environ. Microb. **72** (2006) 2483–2490.

[35] Y.-C. Lo, G.D. Saratale, W.-M. Chen, M.-D. Bai, J.-S. Chang, Isolation of cellulose-hydrolytic bacteria and applications of the cellulolytic enzymes for cellulosic biohydrogen production, Enzyme. Microb. Tech. **44** (2009) 417–425.

[36] Y.-K. Kim, S.-C. Lee, Y.-Y. Cho, H.-J. Oh, Y.H. Ko, Isolation of cellulolytic Bacillus subtilis strains from agricultural environments, ISRN Microbiol. **2012** (2012) 1–9.

IZVOD

PRODUKCIJA KARBOKSIMETIL CELULAZA POMOĆU SOJA *Paenibacillus* SP.

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(Naučni rad)

Celulaze su enzimi koji katalizuju hidrolizu β-1,4-glikozidne veze u molekulu celuloze. Spadaju u veoma važnu grupu enzima koje se koriste u različitim sferama industrije. Najvažniji enzimski proces je hidroliza biljne biomase pomoću celulaza u cilju dobijanja glukoze koja se može koristiti direktno kao proizvod za životinjsku i humanu primenu ili kao polazna sirovina za proizvodnju alkohola, aminokiselina, organskih kiselina, biogoriva i mnogih drugih korisnih proizvoda. U prehrambenoj industriji se celulaze uglavnom koriste u pekarstvu, u proizvodnji voća i povrća, piva i sokova. Takođe, se koriste u industriji deterdženata. *Paenibacillus* sp. predstavlja Gram pozitivne, aerobne i endosporoformirajuće bakterije. Značajan broj *Paenibacillus* vrsta su od industrijskog kao i poljoprivrednog značaja zbog sposobnosti degradacije složenih ugljovodonika i produkcije različitih enzima celulaza, amilaza, hitinaza. S tim u vezi, ispitivana je mogućnost produkcije enzima celulaze iz soja *Paenibacillus* sp. Ispitivanje celulolitičke aktivnosti vršeno ja kvalitativno i kvantitativno. Tokom kvalitativnog ispitivanja na karboksimetil celuloznoj agarnoj podlozi, kao najbolji producent celulaza, medju ispitivanim sojevim, pokazao se soj CKS1. U radu su određeni optimalni uslovi za produkciju karboksimetil celulaza uključujući efekat pasažiranja ispitivane kulture, vreme inkubacije, izvori ugljenika i azota, uticaj početnog pH, kao i uticaj zapremiene mediuma i brzine mešanja na produkciju karboksimetil celulaza. Najveća aktivnost karboksiemtil celulaze dobijena je u trećem pasažu nakon 72 h pri temperaturi od 30 °C. Kao jedini izvor ugljenika korišćena je celobioza (5 g/l) a kao izvor azota kvaščev ekstrakt (5 g/l). Takođe, podešavanjem početnog pH podloge na 7 i korišćenjem 40 ml radne zapremine podloge u erlenmajeru od 500 ml, na tresilici sa 150 rpm, postiže se maksimalna karboksimetil celulazna aktivnost od 0.532±0.006 U/ml. Na ovakav način dobijena sirova karboksimetil celulaza pokazuje maksimum svoje aktivnosti pri temperature od 50 °C i pH 4.8. HPLC analizom krajnjih produkta hidrolize CMC utvrđeno je prisustvo glukoze i ostalih oligosaharida. Soj CKS1 može naći primenu u industrijskoj proizvodnji karboksimetil celulaza kao i u proizvodnji bioetanola.

Ključne reči: *Paenibacillus* sp. • Celuloza • Karboksimetil celulaza • Produkcija • Optimalni uslovi

Ispitivanje olopatadin-hidrohlorida pod stres uslovima metodom tečne hromatografije hidrofilnih interakcija

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Izvod

U savremenoj farmaceutskoj analizi neke farmaceutski aktivne supstance studije forsirane degradacije imaju veliki značaj jer se iz njih može dobiti veliki broj značajnih posataka o stabilnosti leka. U ovom radu, opisano je ispitivane stabilnosti olopatadin-hidrohlorida pod utcajem stres agenasa a u cilju dobijanja podataka o njegovoj stabilnosti. Za praćenje ponašanja olopatadin-hidrohlorida primenjena je metoda tečne hromatografije hidrofilnih interakcija (eng. Hidrophilic Interaction Liquid Chromatography – HILIC). Olopatadin-hidrohlorid izložen je dejstvu kiseline, baze, povišene temperature i oksidacionog sredstva, a stepen degradacije praćen je u definisanim vremenskim intervalima. Takođe, određena je i kinetika degradacije u cilju sticanja boljeg uvida u stabilnost supstance i njen mehanizam razgradnje. Definisani su red reakcije, konstanta brzine reakcije i poluvreme degradacije nakon izlaganja dejstvu kiseline, baze i oksidacionog sredstva.

Ključne reči: olopatadin-hidrohlorid; studije forsirane degradacije; kinetika degradacije; tečna hromatografija hidrofilnih interakcija.

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Studije forsirane degradacije ili stres studije sprovode se u cilju ispitivanja osnovne hemijske stabilnosti aktivne farmaceutske supstance i farmaceutskog doziranog oblika [1]. Ove studije izvode se pod uslovima koji su drastičniji od uslova propisanih za ubrzane studije stabilnosti (temperatura 40 °C, relativna vlažnost 75%). Na osnovu studija forsirane degradacije dobijaju se informacije o mogućim degradacionim putevima i mehanizmu degradacije aktivne supstance u kratkom vremenskom intervalu, kao i o predloženom degradacionom profilu [2]. Takođe, omogućavaju identifikaciju i karakterizaciju degradacionih proizvoda koji se spontano mogu javiti tokom sinteze, proizvodnje, upotrebe i čuvanja leka [3]. Nastali proizvod može biti srodna supstanca aktivnoj supstanci ili ekscipijensima, ili može nastati kao rezultat interakcije između aktivne supstance ili ekscipijenasa.

Osnovni cilj studija forsirane degradacije je dobijanje uzoraka koji sadrže degradacione proizvode u koncentraciji od 5 do 20% [4]. Degradacija ispod 5% ne uzima se u razmatranje, jer se smatra da veoma male količine degradacionih proizvoda nastalih pod stres uslovima, ne bi nastale pod preporučenim uslovima čuvanja aktivne supstance ili gotovog proizvoda. S druge strane, degradacija preko 20% ne dovodi do pouzdanog definisanja degradacionog profila. Takođe, korisne su i za potvrdu specifičnosti metode tokom razvoja, optimizacije i validacije analitičkih metoda za praćenje stabilnosti (eng. Stability Indicating Method) [5–8].

Uprkos značaju studija forsirane degradacije u svim fazama farmaceutskog razvoja, važeće smernice koje se odnose na ove studije veoma su uopštene. U Q1A (R2) smernici Međunarodne konferencije za harmonizaciju (eng. International Conference on Harmonisation – ICH), navodi se da se studije sprovode na jednoj seriji lekovite supstance ili gotovog proizvoda, u čvrstom obliku, odnosno, u obliku rastvora ili suspenzije. Preporuka je da se izvode pod sledećim uslovima: povišena temperatura (za oko 10 °C viša od temperature kod ubrzanih studija stabilnosti, tj. 50 °C, odnosno 60 °C), povećana relativna vlažnost (75% i više), hidroliza u širokom opsegu pH vrednosti, fotoliza i oksidacija [9]. Studije fotostabilnosti integralni su deo stres testova i detaljno su opisane u ICH Q1B smernici. Fotoliza se izvodi izlaganjem leka kombinaciji bele svetlosti, UV i fluorescentnih lampi ili korišćenjem halogenih ili ksenon lampi [10]. Međutim, pomenute smernice ne obezbeđuju dovoljno informacija o strategiji i principima sprovođenja stres testova, odnosno, ne specificiraju opseg pH vrednosti, kao ni temperaturni opseg i specifična oksidaciona sredstva [11].

Cilj ovog rada je izvođenje studija forsirane degradacije na olopatadin-hidrohloridu i praćenje nastalih proizvoda degradacije metodom tečne hromatografije hidrofilnih interakcija (eng. Hidrophilic Interaction

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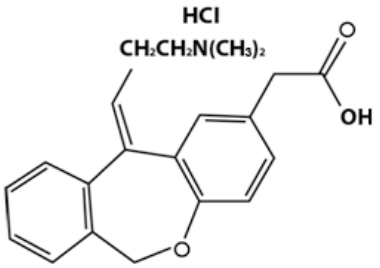
Liquid Chromatography – HILIC) opisanom u radu [12]. Pored toga, cilj je bio i određivanje kinetika degradacije kako bi se izračunale odgovarajuće konstante brzine i poluvremena razgradnje u kiseloj, baznoj sredini, kao i nakon izlaganja oksidacionom sredstvu. Tumačenje parametara kinetike reakcije degradacije olakšava razumevanje mehanizma degradacije i sticanje boljeg uvida u stabilnost ispitivane supstance [13,14].

Olopatadin-hidrohlorid ((11*Z*)-11-(3-(dimetilamino)-propiliden]-6,11-dihidrodibenzo[*b,e*]oksepin-2-il)sirćetna kiselina-hidrohlorid je antialergijski lek sa bifaznim dejstvom (selektivni inhibitor histaminskih H_1 receptora i stabilizator mastocita), koji se koristi u terapiji alergijskog konjuktivitisa [15]. Struktura analiziranog jedinjenja prikazana je na slici 1.



Slika 1. Hemijska struktura analizirane supstance.
Figure 1. Chemical structure of the analyzed substance.

Olopatadin-hidrohlorid je oficinalan u USP/NF farmakopeji, koja za određivanje aktivne farmaceutske supstance i srodnih supstanci propisuje metodu reversno-fazne tečne hromatografije (eng. Reverse Phase High Performance Liquid Chromatography – RP-HPLC) [16].

Olopatadine *in bulk* i u kapima za oči određivan je UV–Vis spektroskopskom metodom (eng. Ultraviolet-Visible Spectroscopy) [17] ili jon-par HPLC metodom [18]. Dalje, derivativna spektroskopska metoda opisana je za određivanje supstance u farmaceutskim doziranim oblicima [19]. U tabletama, određivanje je vršeno izokratskom RP-HPLC i HPTLC metodom (eng. High Performance Thin Layer Chromatography) [20,21]. Kvantitativna analiza aktivne supstance u formulaciji i studije forsirane degradacije izvedene su i primenom RP-HPLC metode za praćenje stabilnosti [22]. Identifikacija i karakterizacija četiri degradaciona proizvoda ((11*E*)-11-(3-(dimetilamino)propiliden)-6,11-dihidrodibenz[*b*,*e*]-oksepin-2-il)sirćetna kiselina, ((9*Z*)-9-(3-(dimetilamino)-propiliden)-9H-fluoren-7-il)sirćetna kiselina metil--((11*Z*)-11-(3-(dimetilamino)propiliden)-6,11-dihidrodibenz[*b*,*e*]oksepin-2-il)acetat i metil-((11*E*)-11-(3-(dimetilamino)propiliden]-6,11-dihidrodibenz[*b*,*e*]oksepin-2--il)acetat dobijena pod *stres* uslovima postignuta je LC--MS/TOF (eng. Liquid Chromatography-Mass Spectrometry/ Time-of-Flight) tehnikom [23]. *N*-oksid, kao proizvod termalne i oksidativne degradacije, identifikovan je UHPLC metodom (eng. Ultra High Performance Liguid Chromatography) nakon sterilizacije kapi za oči toplotom i filtracijom [24]. U humanoj plazmi određivanje koncentracije leka i njegovih metabolita vršeno je LC metodom sa masenom detekcijom [25,26].

Pregled literature pokazao je da stabilnost olopatadin-hidrohlorida nije još uvek dovoljno proučena, kao i da u dosadašnjoj literaturi nije opisana primena HILIC metode za praćenje stepena degradacije ispitivanog jedinjenja pod *stres* uslovima.

EKSPERIMENTALNI DEO

Hromatografski uslovi

Eksperimenti su izvedeni na hromatografskom sistemu Waters Breeze, koji se sastoji od Waters 1525 binarne HPLC pumpe, Waters 2487 UV/Vis DAD detektora i Breeze Software Windows XP za prikupljanje i obradu podataka. Hromatografska analiza urađena je na Betasil Cyano (100 mm×4,6 mm, 5 µm veličine čestica) koloni, sa mobilnom fazom koja se sastoji od acetonitrila i vodenog rastvora amonijum-acetata 5 mM (čija je pH vrednost podešena na 4,50 glacijalnom sirćetnom kiselinom), u odnosu 85:15 *V*/*V*. Pripremljena mobilna faza je deaerizovana i profiltrirana kroz membranski filter Alltec (Loceren, Belgium), veličine pora 0,45 µm. Temperatura kolone podešena je na 30 °C, a brzina protoka mobilne faze na 1 mL min^{-1}. Volumen injektovanja bio je 20 µL, a talasna dužina detekcije 257 nm [12].

Reagensi

Za pripremu mobilne faze i rastvora korišćeni su reagensi HPLC čistoće:acetonitril (SIGMA, St.Louis, MO, USA), amonijum-acetat (J.T.Baker, Holandija), glacijalna sirćetna kiselina (Zorka Pharma, Srbija) i voda HPLC kvaliteta (Simplicity 185 sistem, Milipore, Nemačka).

Priprema rastvora za izvođenje studija forisrane degradacije

Za hromatografsku analizu korišćen je radni standard olopatadin-hidrohlorida (Enaltec Labs, Indija). Osnovni rastvor (c = 1 mg mL^{-1}) pripremljen je u mobilnoj fazi navedenoj kod eksperimentalnih uslova. Radni rastvori (c = 100 µg mL^{-1}) pripremljeni su razblaživanjem odgovarajućim *stres* agensima. Kao stres agensi korišćeni su 0,5 i 1 M natrijum-hidroksid, 0,5, 0,1 i 0,01 M hlorovodonična kiselina, kao i 3, 15 i 30% rastvori vodonik-peroksida.

Degradacija na povišenoj temperaturi praćena je zagrevanjem vodenog rastvora na temperaturi od 60 °C. Neposredno nakon dodatka stres agenasa, odnosno u nultom minutu pod opisanim hromatografskim uslovima radjena je analiza. Degradacija svih uzoraka pra-

ćena je u definisanim vremenskim intervalima a koncentracija olopatadin-hidrohlorida nakon izlaganja stres agensima određenja je poređenjem sa rastvorom standarda olopatadin-hidrohlorida koncentracije 100 μg mL^{-1}.

REZULTATI I DISKUSIJA

U ovom radu opisano je ispitivanje stabilnosti olopatadin-hidrohlorida nakon izlaganja rastvora aktivne supstance stres agensima prema propisu ICH Q1 (R2) smernice [9]. Olopatadin-hidrohlorid tretitan je različitim stres agensima, kao što su kiselina, baza, oksidaciono sredstvo i povišena temperatura. Stepen degradacije pod uticajem različitih *stres* agenasa praćen je HILIC metodom [12].

HILIC metoda predstavlja alternativni tip tečne hromatografije pod visokim pritiskom koja primenjuje polarne stacionarne faze (kolone od čiste silike i hemijski modifikovane derivatizovane silika kolone), kao i mobilne faze sa visokim procentom organskom rastvarača (najčešće acetonitril u koncentraciji > 70%) i malim udelom polarnog rastvarača (>2,5%) [27]. Vodena faza uglavnom uključuje dodatak pufera, pre svega amonijum-acetata i amonijum-formijata, koji smanjuju elektrostatičku interakciju između naelektrisanog analita i deprotonovanih silanolnih grupa na površini stacionarne faze. HILIC se smatra tehnikom izbora za analizu nenaelektrisanih visoko polarnih hidrofilnih i amfifilnih supstanci, kao i baznih lekova, ugljenih hidrata i brojnih naelektrisanih i neutralnih supstanci [28]. Mehanizmi razdvajanja u HILIC su veoma kompleksni i podrazumevaju kombinaciju particije i adsorpcije analita, kao i jonske interakcije analita sa negativno naelektrisanim silanilnim grupama [29]. Kako je ponašanje olopatadin-hidrohlorida u HILIC-u opisano u referenci [12], očekivano je da se u posmatranom sistemu mogu pratiti degradacioni proizvodi različite polarnosti i time dobiti novi podaci o stabilnosti olopatadin-hidrohlorida. U ovom istraživanju, olopatadin-hidrohlorid najpre je tretiran bazom, odnosno 0,5 i 1 M NaOH. Tokom 72 sata tretiranje olopatadin-hidrohlorida sa 0,5 M NaOH nije dovelo do značajne degradacije.Dodatak jače baze, 1 M NaOH, nije uticao je na povećanje degradacije. Dobijeni rezultati pokazuju da se olopatadin-hidrohlorid u nultom minutu razgradio 2,07%, nakon 24 h 2,72%, nakon 48 h 3,55% u odnosu početnu koncentraciju. Smatra se da degradacija manja od 5% nije reprezentativna i da ovi degradacioni proizvodi neće nastati u redovnim studijama stabilnosti pri preporučenim uslovima čuvanja [4]. Može se zaključiti da olopatadin-hidrohlorid stabilan u baznoj sredini.

U sledećoj fazi ispitan je uticaj povećanja temperature na stabilnost olopatadin-hidrohlorida. Zagrevanjem vodenog rastvora na 60 °C pokazano je da ne dolazi do razgradnje pod dejstvom povišene temperature, odnosno da je ispitivana supstanca termostabilna. U definisanim vremenskim intervalima (0 h, 15 min, 1 , 2 i 3 h), nije došlo do promene, odnosno, smanjenja koncentracija analita.

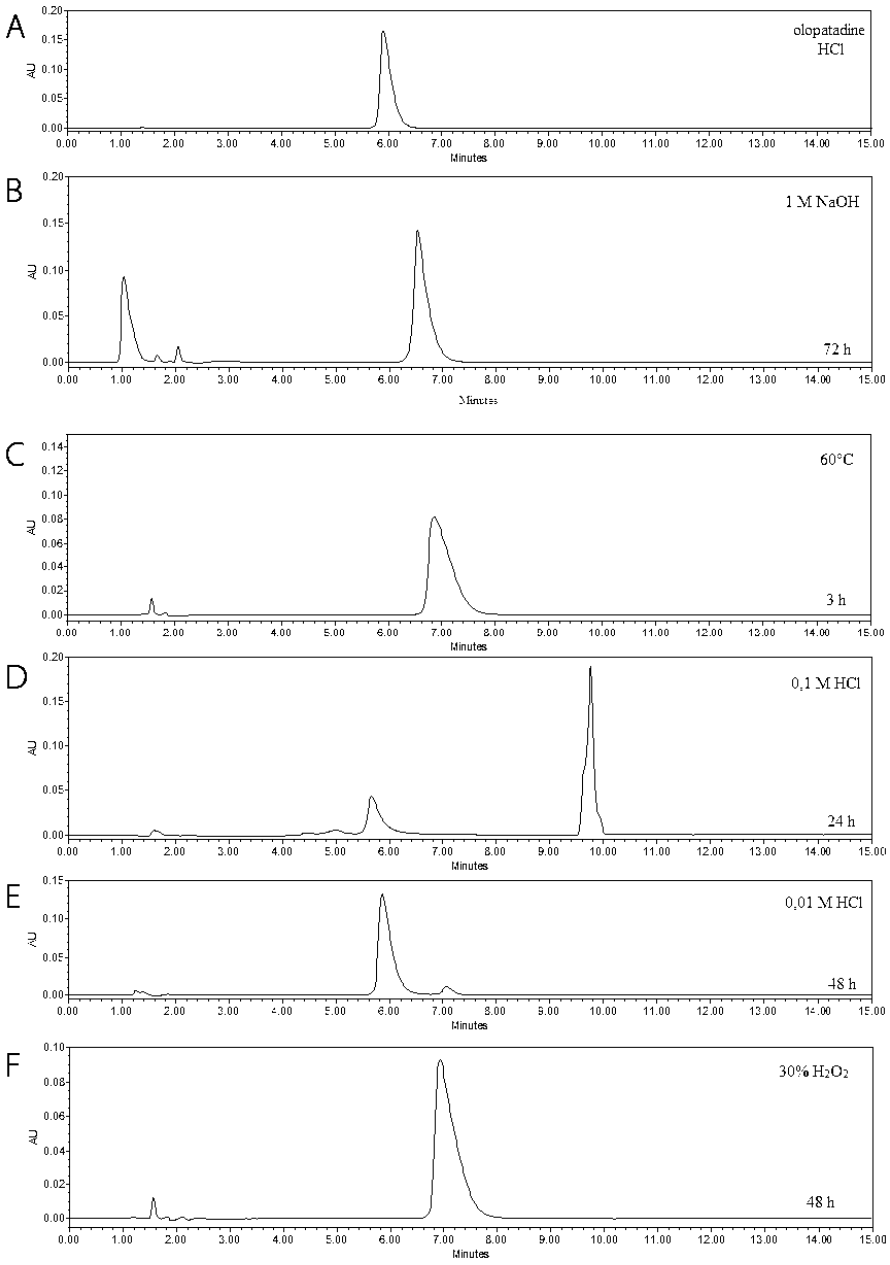
Hidroliza u kiseloj sredini sa 0,5 M HCl pokazala je potpunu degradaciju u nultom minutu, pa je analiza ponovljena sa manjim koncentracijama ove kiseline. Tretiranje sa slabijom 0,1 M HCl dovelo je do značajnog smanjenja koncentracije analita, te se može pretpostaviti izrazita nestabilnost analita u kiseloj sredini. Koncentracija se trenutno smanjila za 34%, nakon 15 min za 39%, 1 h za 46,6%, a nakon 2 h za 48,3%. Nakon 24 h procenat degradacije bio je 61,3% u odnosu na inicijalnu koncentraciju. Tretiranje sa slabijom kiselinom, 0,01 M HCl, dovelo je do reprezentativne i manje intenzivne degradacije ispitivanog analita. U definisanim vremenskim intervalima (0 h, 15 min, 1 i 2 h) smanjenje koncentracije olopatadin-hidrohlorida bilo je manje od 10% u odnosu na inicijalnu koncentraciju. Nakon 24 i 48 h procenat degradacije nije se značajno povećavao.

Dodatkom oksidacionog sredstva H_2O_2 u nižim koncentracijama (3 i 15%), olopatadin-hidrohlorid je ostao relativno stabilan. Međutim, veća koncentracija vodonik-peroksida od 30% dovela je do intenzivnije degradacije. Supstanca se u nultom minutu razgradila 0,76%, nakon 1 h nije došlo do značajne razgradnje, ali nakon 48 h procenat razgradnje bio je 7,55. Prikaz rezultata studija forsirane degradacije dat je u tabeli 1.

Rezultati stres studija pokazuju da je olopatadin-hidrohlorid stabilan u baznoj sredini, kao i pod dejstvom povišene temperature (60 °C). S druge strane, hidroliza u kiseloj sredini i oksidacija u prisustvu visoke koncentracije vodonik peroksida ukazuju na relativnu nestabilnost supstance u prisustvu pomenutih *stres* agenasa. Odabrani hromatogrami olopatadin-hidrohlorida dobijeni nakon izlaganja različitim *stres* agensima prikazani su na slici 2.

Tabela 1. Pregled rezultata studija forsirane degradacije (stepen degradacije, %)
Table 1. Summary of results of stress degradation studies (degradation degree, %)

Agens	Vreme degradacije, h						
	0	0,25	1	2	3	24	48
1 M NaOH	2,07	2,26	2,49	2,65	–	2,72	3,55
0,1 M HCl	34,0	39,0	46,6	48,3	–	61,3	–
0,01 M HCl	4,97	6,34	7,22	9,50	–	10,16	10,23
30% H_2O_2	0,76	1,39	3,08	5,07	5,41	5,52	7,55



Slika 2. Odabrani hromatogrami olopatadin-hidrohlorida dobijeni nakon izlaganja različitim stres agensima; A. Hromatogram standarda olopatadin-hidrohlorida (c = 100 µg mL^{-1}); B. Hromatogram uzorka nakon tretitanja olopatadin-hidrohlorida sa 1 M NaOH; C. Hromatogram uzorka nakon zagrevanja vodenog rastvora olopatadin-hidrohlorida na 60 °C; D. Hromatogram uzorka nakon tretitanja olopatadin-hidrohlorida sa 0,1 M HCl; E. Hromatogram uzorka nakon tretitanja olopatadin-hidrohlorida sa 0,01 M HCl; F. Hromatogram uzorka nakon tretitanja olopatadin-hidrohlorida sa 30% H_2O_2.
Figure 2. Selected chromatograms of the olopatadine HCl after exposure to a different stress agents; A. Chromatogram of olopatadine HCl standard (c = 100 µg mL^{-1}); B. Chromatogram of the sample after treatment olopatadine HCl with 1 M NaOH; C. Chromatogram of the sample after heating an aqueous solution olopatadine HCl at 60 °C; D. Chromatogram of the sample after treatment olopatadine hydrochloride with 0,1 M HCl; E. Chromatogram of the sample after treatment olopatadine hydrochloride with 0,01 M HCl; F. Chromatogram of the sample after treatment olopatadine hydrochloride with 30% H_2O_2.

Na osnovu priloženih hromatograma može se zaključiti da je HILIC metoda uspešno primenjena za praćenje stabilnosti olopatadin-hidrohlorida pod uticajem različitih stres agenasa. Takođe, može se zaključiti da se pod uticajem 0,1 M HCl (slika 2D) dobija degradacioni proizvod (retenciono vreme ≈ 10 min) koji ne nastaje pod ostalim stres uslovima. Pozicija pika na hromatogramu (eluiranje nakon olopatadin-hidrohlorida) ukazuje da je degradacioni proizvod veće polarnosti jer u HILIC povećanje polarnosti dovodi do dužeg zadržavanja analita u koloni.

Naime, u sistemu reverznih faza kada je analit veće polarnosti kraće se zadržava u koloni i eluira se ranije što u zavisnosti od prirode analita može voditi neretencionom ponašanju čime se značajno otežava identifikovanje novog pika. Upravo u ovome se i ogleda prednost primenjene HILIC metode jer zbog različitih mehanizama u odnosu na najčešće primenjivani reverzno-fazni sistem omogućava kasnije eluiranje polarnih struktura te se nedvosmisleno može ukazati na postojanje novog entiteta u hromatografskom sistemu što je od posebnog značaja u izvođenju *stres* studija.

U nastavku istraživanja određena je kinetika reakcije degradacije kako bi se doneo konačan zaključak o ponašanju i degradacionom profilu olopatadin-hidrohlorida. Cilj je bio razumevanje mehanizma degradacije, kao i predviđanje brzine reakcije degradacije i poluvremena degradacije. Tok reakcija degradacije zavisi od rastvarača, koncentracije reaktanata, temperature i pH vrednosti. Red reakcije opisuje zavisnost brzine reakcije od koncentracije reaktanata. Lekovite supstance uglavnom se razlažu po principu reakcija nultog, prvog ili pseudo-prvog reda. Međutim, mogu biti zastupljeni i složeniji mehanizmi degradacije, odnosno reakcije višeg reda [30]. Brzina reakcije određuje se na osnovu brzine smanjenja koncentracije reaktanata ili brzine porasta koncentracije proizvoda reakcije. Za opisivanje reakcija koristi se zakon brzine reakcije, koji može imati diferencijalni i integralni oblik što je opisano u literaturi [14,30], a način određivanja reda reakcije dat je u radovima [31,32]. Kao konačan podatak izračunava se konstanta brzine reakcije [33–35].

U ovom radu, kinetika degradacije olopatadin-hidrohlorida određena je u kiseloj i baznoj sredini, kao i nakon izlaganja oksidacionom sredstvu. Definisani vremenski intervali i dobijeni rezultati prikazani su u tabelama od 2 do 5.

Tabela 2. Kinetika degradacije olopatadin-hidrohlorida nakon izlaganja 1 M NaOH
Table 2. Degradation kinetics of the olopatadine hydrochloride degradation after exposure to a 1 M NaOH

Vreme, h	*c* / mM	ln *c*	1/*c*
0	0,289141	–1,24084	3,458520
0,25	0,281805	–1,26654	3,548553
1	0,289836	–1,23844	3,450227
3	0,286052	–1,25158	3,495868
24	0,281232	–1,26857	3,555783
48	0,278904	–1,27689	3,585463
Koeficijent korelacije (*r*)	0,763281	0,7656	0,7680

Na osnovu najviših vrednosti koeficijenata korelacije, pokazano je da su kisela, bazna i oksidativna degradacija reakcije drugog reda.

Iako promena koncentracije sa vremenom pruža detaljan opis brzine reakcije, poželjno je definisati i jednostavnu meru brzine reakcije, odnosno poluvreme reakcije. Poluvreme reakcije predstavlja ono vreme za koje se početna koncentracija reagujuće supstance smanji na polovinu, i što je reakcija brža, poluvreme reakcije je kraće [30]. Za reakciju prvog reda poluvreme reakcije ne zavisi od koncentracije, dok je za reakciju drugog reda obrnuto proporcionalno početnoj koncentraciji reaktanta.Vrednosti konstanti brzina degradacije i poluvremena reakcija, izračunatih na osnovu prikazanih jednačina, predstavljeni su u tabeli 6.

Tabela 3. Kinetika degradacije olopatadin-hidrohlorida nakon izlaganja 0,1 M HCl
Table 3. Degradation kinetics of the olopatadine hydrochloride degradation after exposure to a 0.1 M HCl

Vreme, h	*c* / mM	ln *c*	1/*c*
0	0,172523	–1,75722	5,796329
0,25	0,154454	–1,86786	6,47442
1	0,139529	–1,96948	7,166969
2	0,135062	–2,00202	7,404007
24	0,101241	–2,29025	9,877421
Koeficijent korelacije (*r*)	0,8665	0,8228	0,9391

Tabela 4. Kinetika degradacije olopatadin-hidrohlorida nakon izlaganja 0,01 M HCl
Table 4. Degradation kinetics of the olopatadine hydrochloride degradation after exposure to a 0.01 M HCl

Vreme, h	*c* / mM	ln *c*	1/*c*
0	0,252369	–1,37054	3,977488
0,25	0,250506	–1,38427	3,991920
1	0,248151	–1,39372	4,029804
2	0,242071	–1,41852	4,131019
24	0,240298	–1,42587	4,161499
48	0,240097	–1,42671	4,164983
Koeficijent korelacije (*r*)	0,7172	0,7200	0,7228

Tabela 5. Kinetika degradacije olopatadin-hidrohlorida nakon izlaganja 30% H_2O_2
Table 5. Degradation kinetics of the olopatadine hydrochloride degradation after exposure to a 30% H_2O_2

Vreme, h	*c* / mM	ln *c*	1/*c*
0	0,293641	–1,2254	3,405519
0,25	0,28956	–1,23939	3,453516
1	0,284596	–1,25668	3,513753
2	0,27875	–1,27744	3,587443
3	0,277757	–1,28101	3,600269
24	0,277426	–1,2822	3,604565
48	0,271469	–1,30391	3,683662
Koeficijent korelacije (*r*)	0,7287	0,7351	0,7415

Na osnovu dobijenih poluvremena reakcije, zaključeno je da je degradacija u kiseloj sredini sa 0,1 M HCl najbrža reakcija ($t_{1/2}$ = 41,4 h), dok je degradacija u baznoj sredini najsporija reakcija ($t_{1/2}$ = 1572 h). Na ovaj

Tabela 6. Vrednosti konstanti brzina degradacije i poluvremena degradacije; [A_0] – početna koncentracija
Table 6. The values of degradation rate constants and half-lives

Parametar	1 M NaOH	0,1 M HCl	0,01 M HCl	30% H_2O_2
Konstanta brzine degradacije, k / mM^{-1} h^{-1}	0,0022	0,1399	0,0035	0,0039
Poluvreme degradacije $t_{1/2} = 1/k[A_0]$ (h)	1572	41,4	1132.1	873,2

način, potvrđena je velika stabilnost olopatadin-hidrohlorida u baznoj sredini. S druge strane, olopatadin-hidrohlorid najosetljiviji je na prisustvo kiseline 0,1 M HCl. Kisela degradacija sa jačom kiselinom 0,1 M HCl znatno je brža reakcija od kisele degradacije sa slabijom kiselinom 0,01 M HCl. Takođe, hidroliza u kiseloj sredini sa 0,1 M HCl značajno je brža reakcija i od reakcije oksidacije u prisustvu 30% H_2O_2, odnosno analit je znatno stabilniji na oksidaciju nego na prisustvo kiseline.

ZAKLJUČAK

U ovom radu opisano je praćenje degradacije olopatadin-hidrohlorida pod uticajem različitih stres agenasa HILIC metodom. Na osnovu navedenih ispitivanja i dobijenih rezultata, pokazano je da je olopatadin-hidrohlorid stabilan u baznoj sredini tj da tretiranje sa 1 M natrijum-hidroksidom u trajanju od 48 h daje degradaciju od svega 3,55%. Takođe, studije forsirane degradacije potvrdile su da je olopatadin-hidrohlorid stabilan na temperaturi od 60 °C, kao i pod dejstvom nižih koncentracija oksidacionog sredstva dok se pri visokim (30% vodonik-peroksida) razlaže za 7,55% ako je izložen 48 h. Nasuprot tome, olopatadin-hidrohlorid je nestabilan pod dejstvom kiseline (više od 60 %za 24 h sa 0,1 M HCl). Pokazano je da se HILIC metodom uspešno može pratiti degradacija olopatadin-hidrohlorida. Takođe, određena je kinetika degradacije idobijeno je da su kisela, bazna i oksidativna degradacija reakcije drugog reda. Na osnovu izračunatih poluvremena degradacije zaključeno je da je kisela degradacija najbrža reakcija, dok je bazna degradacija najsporija reakcija. Takođe, definisanje parametara kinetike reakcije pruža dragocene informacije o njegovom degradacionom profilu i omogućava predviđanje mehanizma degradacije. Ovakvo ponašanje olopatadin-hidrohlorida pruža mogućnost za dodatna i opsežnija ispitivanja njegove stabilnosti, kao i identifikaciju degradacionih proizvoda i puteva degradacije.

Zahvalnica

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LITERATURA

[1] S. Singh, M. Junwal, G. Modhe, H. Tiwari, M. Kurmi, N. Parashar, P. Sidduri, Forsed degradation studies to assess the stability of drugs and products, Trends. Anal. Chem. **49** (2013) 71–88.

[2] K.M. Alsante, A. Ando, R. Brown, J. Ensing, T.D. Hatajik, W. Kong, Y. Tsuda, The role of degradant profiling in active pharmaceutical ingridients and drug products, Adv. Drug Del. Rev. **59** (2007) 29–37.

[3] D.W. Reynolds, K.L. Facchine, J.F. Mullaney, K.M. Alsante, T.D. Hatajik, M.G. Motto, Available guidance and best practices for conducting forced degradation studies, Pharm. Technol. (2002) 48–56.

[4] S.W. Baertschi, K.M. Alsante, R.A. Reed, Pharmaceutical Stress Testing: Predicting Drug Degradation, 2nd ed., CRC Press, Taylor & Francis Group, 2011.

[5] M. Bakshi, S. Singh, Development of validated stability-indicating assay methods-critical review, J. Pharm. Biomed. Anal. **28** (2002) 1011–1040.

[6] S.P. Bhardwaj, S. Singh, Study of forced degradation behavior of enalapril maleate by LC and LC-MS and development of a validated stability-indicating assay method, J. Pharm. Biomed. Anal. **46** (2008) 113–120.

[7] M. Bakshi, S. Singh, ICH guidance in practice: establishment of inherent stability of secnidazole and development of a validated stability-indicating high-performance liquid chromatographic assay method, J. Pharm. Biomed. Anal. **36** (2004) 769–775.

[8] R. Maggio, S.E. Vignaduzzo, T.S. Kaufman, Practical and regulatory considerations for stability-indicating methods for the assay of bulk drugs and drug formulations, Trends, Anal. Chem. **49** (2013) 57–70.

[9] ICH Harmonized Tripartite Guideline, Stability Testing of New Drug Substances and Drug Products, ICH Q1A (R2), Geneva, 2003.

[10] ICH Harmonized Tripartite Guideline, Stability Testing: Photostability Testing of New Drug Substances and Products, ICH Q1B, Geneva, 1996.

[11] K. Huynh-Ba, Handbook of Stability Testing in Pharmaceutical Development, Springer Science, New York, 2009.

[12] J. Maksić, M. Jovanović, T. Rakić, I. Popović, D. Ivanović, B. Jančić Stojanović, Chromatographic analysis of olopatadine in hydrophilic interaction liquid chromatography, J. Chromatogr. Sci. **53** (2014) 680–686.

[13] R.M. Bianchini, P.M. Castellano, T.S. Kautman, Validated stability-indicating HPLC method for the determination of pridinol mesylate. Kinetics study of its degradation in acid medium, J. Pharm. Biomed. Anal. **48** (2008) 1151––1160.

[14] J. Espenson, Chemical Kinetics and Reaction Mechanism, 2nd ed., McGraw-Hill Companies, 2002.

[15] H.P. Rang, M.M. Dale, J.M. Ritter, P.K. Moore, Farmakologija, peto izdanje, prvo srpsko izdanje, Data Status, Beograd, 2005.

[16] The United States Pharmacopeia and The National Formulary (USP35/NF30), Rockville, MD, USA, 2012.

[17] S. Dey, Y.V. Reddy, B. Swetha, S.D. Kumar, P.N. Murthy, S.K. Sahoo, D. Kumar, S.S. Patro, S. Mohapatra, Method development and validation for the estimation of olopatadine in bulk and pharmaceutical dosage forms and its stress degradation studies using UV-VIS spectrophotometric method, Inter. J. Pharm. Pharmaceut. Sci. **2** (2010) 212–218.

[18] R.V. Rele, C.B. Warkar, Application of high performance liquid chromatographic technique for olopatadine hydrochloride and its impurity in ophtalmic solution, Int. J. Chem. Sci. **2** (2011) 601–614.

[19] M.M. Annapurta, G.H. Bindu, I. Divya, Derivative spectrophotometric method for thedetermination of olopatadine in pharmaceutical dosage form, Drug Invent. Today **10** (2012) 540–542.

[20] S.J. Varghese, A. Kumar, A. Manikanta, T.K. Ravi, Stability-indicating high-performance column liquid chromatography and high performance thin-layer chromatography for the determination of olopatadine hydrochloride in tablet dosage form, J. AOAC Int. **94** (2011) 1815–1820.

[21] A. Mahajan, P.S. Gandhi, N. Pandita, S.V. Gandhi, P.B. Deshpande, Validated high performance thin layer chromatographic method for estimation of olopatadine hydrochloride as bulk drug and in ophthalmic solutions, Int. J. Chem.Tech. Res. **3** (2010) 1372–1375.

[22] P.D. Bhatt, J. Akhtar , Development and validation of stability-indicating RP-HPLC method for estimation of olopatadine hydrochloride in bulk drug and it's formulations, Int J. Pharmaeut. Sci. Rev. Res. **2** (2011) 153–158.

[23] A.A. Mahajan, K. Mohanraj, S. Kale, A.K. Thaker, Study of olopatadine hydrochloride under ICH recommended stress conditions by LC, LC-MS/TOF for identification, and characterization of degradation products, J. Liq. Chromatogr. Rel. Technol. **13** (2013) 1881–1898.

[24] I.M. Jurkić, M. Buratović, I. Valentić, D. Stanfel, UHPLC study of the degradation profiles of olopatadine hydrochloride in eye drops subjected to heat and filtration method of sterilisation, Chromatographia **77** (2014) 1067–1080.

[25] K. Fujita, H. Magara, H. Kobayashi, Determination of olopatadine, a new antiallergic agent, and its metabolites in human plasma by high-performance liquid chromatography with electro sprey ionization tandem mass spectrometry, J. Chromatogr., B: Biomed. Sci. Appl. **731** (1999) 345–352.

[26] P. Zhu, Y.G. Wen, X.P. Fan, Z.L. Zhou, R.X. Fan, J.M. Chen, K.L. Huang, X.L. Zhu, Y.F. Chen, J. Zguang, A rapid and sensitive liquid chromatography-tandem mass spectrometry method for determination of olopatadine concentration in human plasma, J. Anal. Technol. **35** (2011) 113–118.

[27] B. Buszowski, S. Noga, Hydrophilic interaction liquid chromatography (HILIC)-a powerful separation technique, Anal. Bioanal. Chem. **402** (2012) 231–247.

[28] A.J. Alpert, Hydrophilic interaction chromatography for the separation of peptides, nucleic acids and other polar compounds, J. Chromatogr. **499** (1990) 177–196.

[29] D.V. McCalley, Study of the selectivity, retention mechanisms and performance of alternative silica-based stationary phases for separation of ionised solutes in hydrophilic interaction chromatography, J. Chromatogr., A **1217** (2010) 3408–3417.

[30] A.C. Kenneth, Chemical kinetics: the study of reaction rates in solution, 1st ed., John Wiley & Sons, 1990.

[31] E. Souri, A.A. Negahban, N. Adib, A stability indicating HPLC method for determination of mebeverine in the presence of its degradation products and kinetic study of its degradation in oxidative conditions, Res. Pharm. Sci. **9** (2014) 199–206.

[32] M.A. Korany, R.S. Haggag, M.A.A. Ragab, O.A. Elmallah, Kinetic investigation of pentoxifylline based on nonparametric linear regression of derivative and convoluted derivative chromatographic and spectrophotometric responses, J. Liq. Chromatogr. Rel. Technol. **37** (2014) 475–497.

[33] U. Hubicka, B.W. Zumorska, D. Knapczyk, J. Krzek, Determination of danofloxacin and its photodegradation products by thin-layer chromatography: Kinetic evaluation of degradation process, J. Liq. Chromatogr. Rel. Technol. **37** (2014) 2915–2928.

[34] H. Alaani, Y. Alnukkary, I. Alashkar, Stability and kinetic studies for the estimation of shelf life of chloramphenicol, dexamethasone sodium phosphate, and tetrahydrozoline hydrochloride opthalmic solution, Int.. J. Pharm. Sci. Rev. Res. **30** (2015) 327–330.

[35] A.H. Akarabi, D.R. Shah, S.A. Shah, B.N. Suhagia, Kinetic degradations of pitavastatin calcium by stability indicating HPTLC method, J. Liq. Chromatogr. Rel. Technol. **38** (2015) 521–531.

SUMMARY

INVESTIGATION OF OLOPATADINE HYDROCHLORIDE UNDER STRESS CONDITIONS BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

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(Scientific paper)

The purpose of the present research was to conduct stress degradation studies on the olopatadine hydrochloride, an antiallergic drug, using the hydrophilic interaction liquid chromatography (HILIC). HILIC requires the utilization of polar and moderately polar stationary phases and aqueous-organic mobile phase usually containing more than 70% of organic solvent. In this study, olopatadine hydrochloride was subjected to acid and base hydrolysis, oxidation and thermolytic degradation in order to estimate its stability under different *stress* conditions recommended by ICHQ1A (R2) guideline. Degree of degradation was followed by HILIC method. The chromatographic conditions were: column Betasil Cyano (100 mm×4.6 mm, 5 µm particle size), mobile phase consisted of acetonitrile and ammonium acetate 5 mM (pH adjusted to 4.50) in ratio 85:15 *V*/*V*, flow rate was 1 mL min^{-1}, column temperature was set at 30 °C and detection was performed at 257 nm. Results obtained for *stress* studies indicated that olopatadine hydrochloride underwent transformation under acidic and oxidative (30% hydrogen peroxyde) conditions showing high degree of degradation. Furthermore, it was found that olopatadine hydrochloride is relatively stable when exposed to thermal (60 °C) and basic (1 M NaOH) conditions. Therewith, kinetics of degradation reaction was determined with an aim to define the corresponding reaction rate constants and half-lives. Firstly, the order of the reaction was evaluated experimentally using the integral method. Based on the calculated values of the correlation coefficients, it was shown that the acidic, basic and oxidative degradation are the second-order reaction. High stability under basic conditions was achieved on the basis of the great degradation half-life values. Also, it has been verified that acidic degradation is the fastest reaction.

Keywords: Olopatadine hydrochloride • Forced degradation studies • Degradation kinetics • Hydrophilic interaction liquid chromatography

Hydrogeochemical approach to estimate the quality of bottled waters in Serbia

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Abstract

Bottled waters were analyzed for different chemical parameters and activity concentrations of radionuclides. The hydrocarbonate ion was dominant in all samples, while the major cation composition was a combination of Ca–Mg–Na ions. Physicochemical properties of bottled water samples are influenced by underlying geology. The sum of trace element concentrations varied from 79.7 to 9349.7 μg/l. The dietary reference intake (DRI) system was applied and contributions of some essential elements were calculated according to age group and gender. Hierarchical cluster analysis (HCA) grouped bottled water samples into four clusters based on the similarities of the groundwater quality and essential elements concentrations. The origin of radioactivity is natural and could be traced to minerals in felsic igneous rocks. Two brands exhibited elevated beta activity (1.087±0.134 Bq/l; 1.242±0.146 Bq/l). Effective doses were found to be below the reference level of 0.1 mSv/yr.

Keywords: bottled water, dietary reference intake, essential elements, natural radioactivity, hierarchical cluster analysis, Serbia, water quality.

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According to new data, the production of bottled water in Serbia is threatened by the economic crisis as well as the good quality of the tap water in the country [1]. However, data available from the past years showed that the consumption of bottled water was increasing rapidly [2,3]. Due to the importance of drinking water for human health, their quality must be carefully and systematically controlled [4].

Fourteen mineral elements have been established as essential for good health; these elements in combined form affect bone and membrane structure (Ca, P, Mg and F), water and electrolyte balance (Na, K and Cl), metabolic catalysis (Zn, Cu, Se, Mg, Mn and Mo), oxygen binding (Fe), and hormone functions (I and Cr) [5]. The chemical composition of natural mineral water depends on many factors, including the mineralogy/ /lithology of the aquifer, residence time of the water, amount of solids and trace elements which can be soluble under appropriate pH and redox conditions [6–9].

In the last few years many studies have been focused on the hydrogeochemical properties of bottled waters [2,10–15], on the chemical composition of bottled waters and its health effects [16–18] and also on the radiation dose estimations in various water samples [19–21]. In Serbia, the quality of bottled waters was the subject of only several studies [2,4,14,22,23].

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This study covered a wide range of major and trace elements, and also investigated the natural radioactivity of bottled waters. The goal of the research was to examine the potential contribution of bottled waters to essential elements intake and exposure to ionizing radiation.

METHODS

Study area

The study area was the Republic of Serbia which is located in South-eastern Europe, occupying an area of 88.361 km^2. Serbia is consisted of very complex geological units, as part of the Central Balkan Peninsula. The geological framework comprises several geotectonic units [24]: Carpatho–Balkanides composite terrane (CBCT), Serbo–Macedonian composite terrane (SMCT), Vardar zone (VZ), Jadar Block terrane (JBT), Drina–Ivanjica terrane (DIT), Dinaridic ophiolite belt terrane (DOBT) and External Dinarides (ED), Fig. 1.

CBCT extends through eastern Serbia and represents lower Palaeozoic units which are merged before the Upper Permian [25]. Mesozoic limestone and dolomite are the most important aquifer, in this region, with more than 1000 m thickness. SMCT is a crystalline basement which occupies the central part of the territory of Serbia. It is composed of very thick Proterozoic metamorphic rocks: gneiss, micaceous shale, various types of schist, marble, quartzite, granitoid rocks, and igneous rocks. Deep reverse faults constitute the boundary with other geotectonic units [14]. VZ is represented by a composite assemblage of continental and

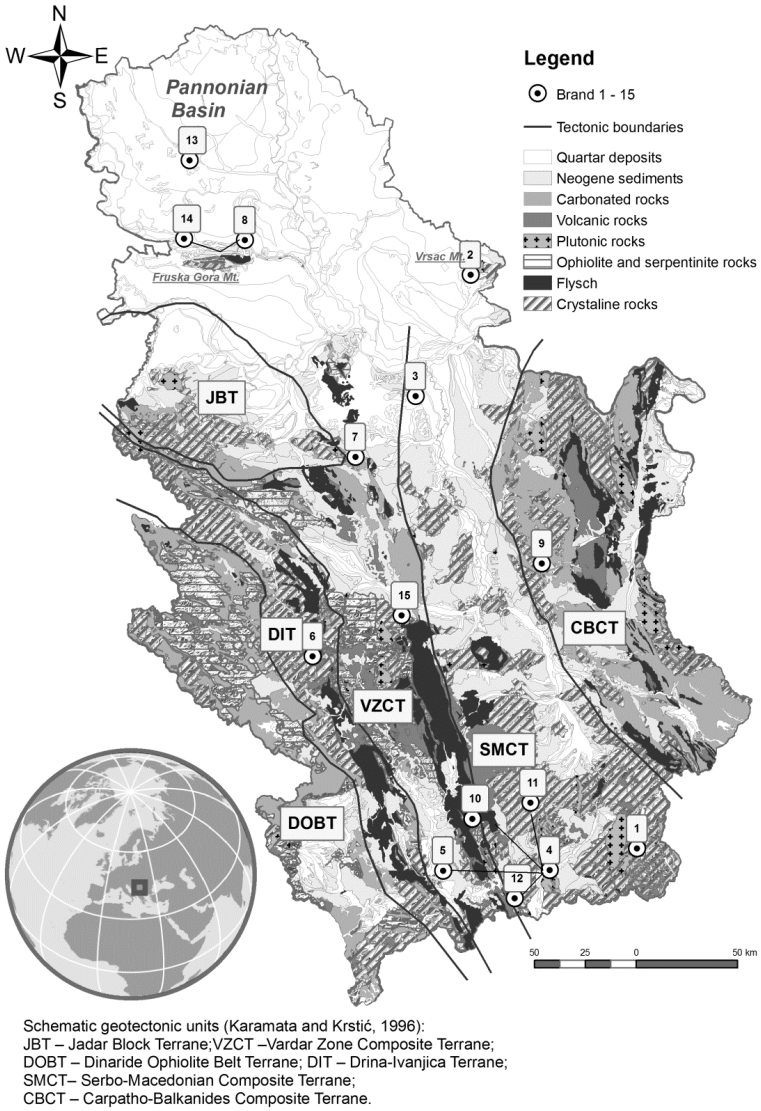

Figure 1. Simplified geological map of Serbia showing the distribution of major rock types.

oceanic units, intruded by Oligocene to Pliocene calc--alkaline magmatic rocks [26]. The continental units are characterized by a Paleozoic basement covered by Middle to Late Triassic, mainly carbonate, sequences, whereas the oceanic units are represented by Jurassic ophiolites. In addition, slices of Late Jurassic melange and Late Cretaceous turbidite are also recognized. On the whole, the Vardar zone is regarded as a suture zone developed after the collision between Eurasia and Adria [27]. JBT is an exotic block of the Earth's crust, thought to be derived from further west [28] and is highly correlative with the Dinaride – South Alpine belt. This unit is bounded by deep fault zones and tectonic melanges. DIT occupies the western part of territory of Serbia, where there are large masses of ultrabasic rocks and serpentinites. At the south–east, it has a boundary with the Ophiolite Belt which is mostly covered by Triassic limestone. The north–west part of the terrane is covered by Eocene deposits [24]. DOBT is characterized by ophiolites ranging in age from Triassic to Jurassic, which are regarded as representative of the oceanic basin. This nappe includes a stack of ophiolitic units overlying a sub-ophiolitic melange [29]. Ophiolite includes terrigenous sedimentary rocks, minor cherts and limestones, together with basalts, diabases, various gabbros and ultramafic rocks [24,30,31]. ED is large tectono–stratigraphic unit, composed of Jurassic to Late Cretaceous clastic and carbonate sequences, up to 4000–5000 m thickness [32]. The Pannonian basin (PB) rests on thrust sheets of the Inner Carpathian foldbelt in northern and central areas and, to the south, on those of the Dinarides and Vardar Zone. During the Tertiary period, largely clay and sand sediments were deposited within the basin, whose thickness in the north–east is greater than 2500 m [33]. Neotectonic movements formed the horsts of Fruška Gora Mt. (a part of the VZ) and Vršac Mountains constructed of crystalline schist and granite (part of the SMCT).

Sampling and laboratory methods

During 2012, 15 different bottled water samples were purchased from local markets. The selected brands are the most commonly produced. Locations of groundwater sources are presented in Fig. 1. Major cations (Ca, K, Mg and Na) in the groundwater samples were measured by inductively-coupled plasma optical emission spectrometry (ICP-OES). Major anions were determined by ion chromatography (Dionex ICS–3000 DC). Trace elements were analyzed by HR–ICP/MS high resolution magnetic sector ICP/MS using a Finnegan Mat Element 2 instrument for 57 elements. To test the accuracy and precision of the method, NIST® 1643e "Trace Elements in Water" SRM was analysed and compared to the certificate values.

Gross alpha and beta activities were examined with the procedures recommended by iso 9696 water quality – measurement of gross alpha activity in non--saline water, thick source method, and ISO 9697 water quality – measurement of gross activity in non-saline water [34,35]. Gamma spectrometry analysis was undertaken following the procedure of ISO 10703 water quality – determination of the activity concentration of radionuclides by high resolution gamma-ray spectrometry [36]. Gross alpha/beta activity measurements were made on a low level α,β-proportional counter PIC-WPC-9550 (Protean Instrument Corporation), featuring efficiencies of 31% for alpha radiation and 44% for beta radiation, using the reference materials ^{241}Am and ^{90}Sr. Gamma activity was determined by gamma-spectrometry measurements using a HP Ge detector with relative efficiency of 25% and energy resolution of 1.85 keV (1332.5 keV ^{60}Co). The calibration was made using an AMERSHAM standard in a Marinelli beaker.

Calculation of dietary reference intake

The dietary reference intakes (*DRI*) are a set of reference values for vitamins, minerals, and other nutrients important to human health established by the Institute of Medicine (IOM) of the U.S. National Academy of Sciences. DRI system includes the recommended dietary allowance (RDA), adequate intake (AI) and tolerable upper intake level (UL), and it is used to calculate contributions of the elements essential for human health according to age group and gender [37].

Calculations were done according to the formula:

$$DRI = \frac{100MV}{RDA} \quad (1)$$

where M is element concentration (mg/l); V is water consumption according to age group and gender (L/d) (Table 1); *RDA* – *RDA*/AI/UL (mg/d), Table 1. An *RDA* is an average daily dietary intake level; sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. If sufficient scientific evidence is not available to establish an *RDA*, an *AI* is usually developed. *UL* is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population [37].

Hierarchical cluster analysis (HCA)

Cluster analysis is a multivariate method which aims to classify a sample of objects on the basis of a set of measured variables into a number of different groups such that similar subjects are placed in the same group. Squared Euclidean distances were chosen to measure similarity/dissimilarity among the variables while Ward's linkage method was chosen to link initial clusters resulting from the initial clustering steps. Finally, the result of this statistical method is a graphical representation of individual groups (dendrogram) [38,39].

Estimation of annual effective doses

The annual radiation doses from ^{226}Ra, ^{228}Ra and ^{238}U in the bottled water samples have been calculated using the Recommended Adequate Intakes for water (Table 1) [40], together with the measured radioactivity concentrations and the radiation dose coefficients for ingestion [41].

The formula used for this purpose is:

$$D = V\times A^{226}\text{Ra}\times h(\text{g})^{226}\text{Ra} + V\times A^{228}\text{Ra}\times h(\text{g})^{228}\text{Ra} + V\times A^{238}\text{U}\times h(\text{g})^{238}\text{U} \quad (2)$$

where V is water consumption according to age group and gender (Table 1); A^{226}Ra, A^{228}Ra and A^{238}U are activity concentrations of radionuclides (Bq/l); $h(\text{g})^{226}\text{Ra} = 4.9\times10^{-7}$ Sv/Bq, $h(\text{g})^{228}\text{Ra} = 6.9\times10^{-7}$ Sv/Bq, $h(\text{g})^{238}\text{U} = 4.5\times10^{-8}$ Sv/Bq are dose coefficients for radionuclides.

RESULTS AND DISCUSSION

Water quality: major hydrochemistry, trace elements, compliance with regulations

The major chemical composition of bottled water samples is shown in Table 2. Total dissolved solids (TDS) ranged from 56 to 3400 mg/l with a median of 464 mg/l. pH values ranged from 6.37 to 7.93, while CO_2 concentrations ranged from 7.48 to 1621 mg/l.

The Schoeller diagram was used for a comparative view of bottled water quality (Fig. 2). The hydrocarbonate ion was dominant in all the samples. Low–mineralized water samples (*TDS* < 1000 mg/l) had a preponderance of Ca and Mg ions, while CO_2 rich waters (CO_2 > 250 mg/l) had a preponderance of Na ions. CO_2 rich waters are associated with different regional geological-structural features and related to granite intrusions and volcanic rocks [42]. Bottled water samples 7, 10, 11, 12 and 15 had the highest TDS levels and CO_2 con-

Table 1. Recommended dietary allowances/adequate intakes/tolerable upper intake levels (mg/d) for children, adolescents, adults, pregnant and lactating females (IOM 2004)

Age group		Water obtained from drinks per day (L)	Ca	Cu	Fe	Mg	Mo	Se	Zn	Cr	Mn	K	Na	Cl	B
Children	1–3 y	0.9	700	0.34	7	80	0.017	0.02	3	0.011	1.2	3000	1000	1500	3
Children	4–8 y	1.2	1000	0.44	10	130	0.022	0.03	5	0.015	1.2	3800	1200	1900	6
Boys	9–13 y	1.8	1300	0.7	8	240	0.034	0.04	8	0.025	1.9	4500	1500	2300	11
Girls	9–13 y	1.6	1300	0.7	8	240	0.034	0.04	8	0.021	1.6	4500	1500	2300	11
Boys	14–18	2.6	1300	0.89	11	410	0.043	0.055	11	0.035	2.2	4700	1500	2300	17
Girls	14–18 y	1.8	1300	0.89	15	360	0.043	0.055	9	0.024	1.6	4700	1500	2300	17
Adults	Men > 19 y	3	1000	0.9	8	410	0.045	0.055	11	0.033	2.3	4700	1375	2100	20
	Women >19 y	2.2	1000	0.9	13	320	0.045	0.055	8	0.022	1.8	4700	1375	2100	20
Females	Pregnant 19–50 y	2.3	1000	1	27	355	0.05	0.06	11	0.03	2	4700	1500	2300	20
	Lactating 19–50 y	3.1	1000	1.3	9	315	0.05	0.07	12	0.045	2.6	5100	1500	2300	20

Table 2. Basic chemical composition (mg/l) and aquifer lithology of bottled waters in Serbia

Brand	Aquifer lithology	pH	Ca	Mg	Na	K	HCO_3	SO_4	Cl	*TDS*	CO_2
1	Deluvial deposits	7.93	10	0.91	2.7	1	42.7	5.4	1	56	7.48
2	Crystalline rocks dated Precambrian or Lower Paleozoic	7.65	22.26	8.37	10.91	0.753	109	12	8.2	146	76.56
3	Neogene sediments	7.2	86	50	33.11	1.19	561	9	10	420	36
4	Sandstones and granite sand	7.1	50.46	6	19.32	1.84	189	25	13	221	98
5	Alluvial aquifers comprised of gravels and sands	7.06	37.24	8.75	23	2	165	21	14.9	232	33
6	Serpentinite and limestone/dolomitic limestones	7.4	66.7	42.8	4.1	0.877	398.3	17.9	2.1	319	30.8
7	Dolomitic limestones	7	144	36.4	286	28	1329	14	7.2	1175	1499.52
8	Neogene sediments	7.25	72	28.75	130.7	2.5	578.3	0.35	87.67	603	219
9	Limestones	7.4	70	15	10	1.5	300	20	6	422.5	20
10	Granitoid rocks	6.5	85.4	20.6	1216	52	3290	173	54.1	3400.8	1621
11	Granitoid rocks	6.94	55	11.56	1230	52.8	3100	181	55	3100	1496
12	Granitoid rocks	6.37	84.1	23	930	20.5	3050	174	32	2705	1296
13	Neogene sediments	7.2	26.8	27.63	99.59	0.7	470	5.3	1.2	631	208
14	Neogene sediments	7.85	24.31	22.98	400.3	4.33	774.6	0.35	282.9	1123	203
15	Serpentinite and Paleozoic shales	7.62	72.14	58.36	324.2	3.56	1232	31.6	18.16	1200	599.28

centrations, thus pH values of these brands are slightly acidic to nearly neutral (pH 6.37–6.94). These waters are found in areas of large tectonic faults, in SMCT and VZ units, while low-mineralized waters originated from aquifers present in different geological units (Table 2). Groundwaters enriched in Na and Cl, captured from Neogene sediments in the Pannonian Basin, with a long residence time in aquifer are marked as "mature groundwater" (Fig. 2).

To evaluate the quality of the bottled waters in Serbia, 66 parameters are summarized in Table 3 with the appropriate regulation standards. The sum of trace element concentrations varied from 79.7 to 9349.7 μg/l with few elements significantly below 1 μg/l, namely some of elements of the rare earth group, Th, Ta and Ag. The widest range in trace element concentrations was found for Cs, Ge, Rb, Tl, Cr, B, Mn, Zn, Zr, Nd, La and Ce.

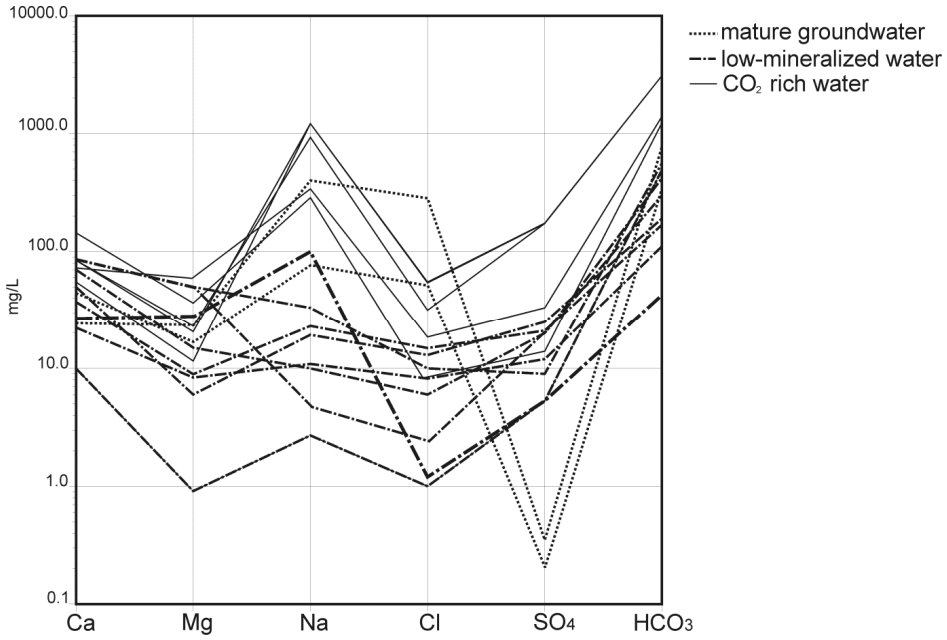

Figure 2. Schoeller diagram of major chemical composition.

Table 3 shows that most samples comply with guidelines. Exceptions include three samples that exceeded national requirements for bottled water. Brand 12 had an elevated concentration of As (10.9 μg/l), Brand 7 an elevated concentration of Fe (238.77 μg/l), and Brand 5 showed an elevated Se concentration (13.97 μg/l).

Toxic elements, such as Cd, Cr, Hg and Pb occur at very low concentrations, mostly below the detection limits in the sampled bottled waters.

Applying the dietary reference intakes

The calculated values of *DRI* for bottled water samples according to age group and gender are presented in Table 4. With regard to mineral intake for children and adults, some waters contribute significantly to the DRIs for Ca (Brand 7: 28.8% for children and 44.64% for adults), Mg (Brand 15: 65.66 and 58.36%), Na (Brand 11: 147 and 254.2 %), B (Brand 12: 142 and 109.9 %), Cr (Brand 3: 99.8 and 106.1 %), Se (Brand 5: 62.8 and 61.87 %), Mo (Brand 7: 21.5 and 22 %) and Cl (Brand 14: 32 and 38.13 %).

Ingestion of Brands 10 and 11 exceeds the maximum recommended daily intake of Na in all groups (up to 246%) and Brand 12 for boys, girls (14–18 years) and adults. Ingestion of Brand 12 exceeds the tolerable upper intake level for B for children (up to 213%), men (106%) and lactating females (109.8%). Cr may have a significant contribution especially in Brand 3 (106.7% of daily intake of Cr for women). Other essential microcomponent elements (Cu, K, Mn, Fe and Zn) have a contribution to *DRI* less than 5.1%, in all considered groups.

Hierarchical cluster analysis (HCA)

HCA was used to identify natural groupings in a dataset according to chemical similarity of the samples. Water quality parameters (pH, CO_2 content, major cations – Ca, Mg, Na and K, major anions – HCO_3, Cl, SO_4 and other essential elements – Cr, Cu, B, Mn, Mo, Fe and Zn) are selected as variables for HCA. Bottled waters are subdivided into four clusters (C1–C4, Fig. 3). Cluster 1 is characterized by low-mineralized water samples (*TDS*: 56–631 mg/l), with pH values greater than 7. Ca and Mg are dominant cations in this group, which make the greatest contribution to DRIs: 2.67% Ca for children (4–8 years) to 21.7% Ca for lactating females and 3% Mg for girls (14–18 years) to 31.08% Mg for children (1–3 years). Other essential elements in C1 have a contribution of less than 5%. Cluster 2 includes Brands 8 and 14, originating from PB, which are enriched with Na and Cl ions. This composition is a consequence of water filtration through sediments formed in marine or lake marine conditions. DRI contribution of Na in C2 is 11.76–82.73% and Cl is 5.2–38.1% (lower value refers to children of 1–3 years, and upper value to lactating females).

In Cluster 3 (Brands 3, 6, 7 and 15) Mg concentrations have a significant contribution to the *DRI*s: 18.2% Mg (in girls 14–18 years group) – 65.66% Mg (in children 1–3 years group). Ca ingestion from these waters is also important: 8% (for children 4–8 years of age) to 46.4% (lactating females). Mg concentrations are related to water circulation through an aquifer formed at the contact of serpentinite and Triassic limestone/dolomitic limestones or Paleozoic shales. Elevated CO_2 concentrations enhance the solubility of Ca and Mg carbo-

Table 3. Summary of the 66 parameters measured in the bottled waters in Serbia

Parameter	Unit	Detection limit	Min	Max	Median	Official Gazette of Serbia and Montenegro 53/05	EU Directive 2003/40/EC	Brand above national standards
Ca	mg/l	0.05	10	144	66.7			
Mg	mg/l	0.05	0.91	58.36	22.98			
Na	mg/l	0.05	2.7	1230	99.59			
K	mg/l	0.05	0.7	52.8	2.5			
HCO_3	mg/l	0.05	42.7	3290	561			
SO_4	mg/l	0.05	0.35	181	14.4			
Cl	mg/l	0.05	1	282.9	13			
pH		–	6.37	7.93	7.2			
TDS	mg/l	–	56	3400	420			
Ag	µg/l	0.002	<0.002	0.0038	0.0038			
Al	µg/l	0.5	<0.5	8.47	3.69	200		
As	µg/l	0.02	<0.1	10.91	0.75	10	10	Brand 12
B	µg/l	0.1	3.32	7087.75	149.45			
Ba	µg/l	0.01	0.86	191.25	55.66		1000	
Be	µg/l	0.001	<0.001	0.013	0.0038			
Bi	µg/l	0.001	<0.001	0.031	0.0075			
Cd	µg/l	0.001	<0.005	0.26	0.029	3	3	
Ce	µg/l	0.001	<0.001	0.59	0.015			
Co	µg/l	0.001	<0.001	0.081	0.02			
Cr	µg/l	0.01	<0.01	12.05	0.075	50	50	
Cs	µg/l	0.001	<0.001	118.04	0.27			
Cu	µg/l	0.05	<0.05	12.06	0.86	2000	1000	
Dy	µg/l	0.0005	0.0005	0.017	0.002			
Er	µg/l	0.00005	0.00032	0.011	0.00096			
Eu	µg/l	0.00005	0.00253	0.079	0.023			
Fe	µg/l	1	<1	238.77	7.47	200		Brand 7
Ga	µg/l	0.001	<0.001	0.012	0.0025			
Gd	µg/l	0.00005	<0.00005	0.016	0.0002			
Ge	µg/l	0.001	<0.001	16.2	0.21			
Hf	µg/l	0.00005	<0.00005	0.007	0.0002			
Hg	µg/l	0.05	<0.05	<3	–	1	1	
Ho	µg/l	0.00001	0.00001	0.002	0.0003			
In	µg/l	0.0001	0.0034	<0.0185	–			
La	µg/l	0.001	<0.001	0.66	0.012			
Li	µg/l	0.05	1.51	985	13.82			
Lu	µg/l	0.00005	<0.0005	0.003	0.001			
Mn	µg/l	0.05	<0.05	39.5	1.5	50	500	
Mo	µg/l	0.005	<0.005	3.55	0.26			
Nb	µg/l	0.0001	<0.0001	0.005	0.00034			
Nd	µg/l	0.0001	<0.0001	0.086	0.003			
Ni	µg/l	0.05	< 0.05	1.49	0.23	20	20	
Pb	µg/l	0.005	<0.005	0.78	0.19	10	10	
Pr	µg/l	0.00005	<0.00005	0.025	0.00087			
Rb	µg/l	0.005	<0.005	253.98	1.22			
Re	µg/l	0.0001	0.0004	0.16	0.026			
Sb	µg/l	0.001	0.029	2.2	0.59	5	5	
Sc	µg/l	0.01	<0.01	0.35	0.038			
Se	µg/l	5	<5	13.97	9.6	10	10	Brand 5

Table 3. Continued

Parameter	Unit	Detection limit	Min	Max	Median	Official Gazette of Serbia and Montenegro 53/05	EU Directive 2003/40/EC	Brand above national standards
Si	mg/l	0.03	0.3	41.5	13			
Sm	µg/l	0.0005	<0.0005	0.017	0.002			
Sn	µg/l	0.01	<0.01	0.35	0.14			
Sr	µg/l	0.01	20.45	1490	475.03			
Ta	µg/l	0.0005	<0.0005	0.0025	0.0023			
Tb	µg/l	0.00002	<0.00002	0.0045	0.00075			
Te	µg/l	0.001	<0.001	0.23	0.0075			
Th	µg/l	0.00002	<0.00002	0.0014	0.000075			
Ti	µg/l	0.01	<0.01	0.64	0.038			
Tl	µg/l	0.0001	<0.0001	0.31	0.0008			
Tm	µg/l	0.0001	<0.0001	0.0014	0.00038			
U	µg/l	0.0001	0.0072	3.45	0.1			
V	µg/l	0.001	0.0065	4.45	0.20			
W	µg/l	0.001	<0.001	0.49	0.1			
Y	µg/l	0.0005	<0.0005	0.093	0.013			
Yb	µg/l	0.00005	<0.00005	0.009	0.00075			
Zn	µg/l	0.5	<0.5	360.8	15.2			
Zr	µg/l	0.001	<0.001	1.11	0.0375			

Table 4. Dietary reference intakes (DRI): essential elements from bottled water samples

Age group			*RDA* / %							*AI* / %		*UL* / %			
			Ca	Mg	Cu	Fe	Mo	Se	Zn	Cr	Mn	Na	K	Cl	B
Children	1–3 y	Min	1.29	1.02	0.08	0.00	0.07	23.54	0.01	1.44	0.12	0.24	0.02	0.06	0.10
		Max	18.51	65.66	3.19	0.16	18.80	62.87	0.36	98.62	2.91	110.70	1.58	16.97	212.63
		Median	7.07	21.36	0.32	0.02	2.02	43.20	0.04	4.17	0.50	6.64	0.06	0.69	4.03
	4–8 y	Min	1.2	0.84	0.08	0.00	0.07	20.92	0.01	1.40	0.13	0.27	0.02	0.06	0.07
		Max	17.28	53.87	3.29	0.14	19.37	55.89	0.29	96.43	3.10	123.00	1.67	17.87	141.76
		Median	6.60	17.53	0.33	0.01	2.09	38.40	0.03	4.08	0.53	7.38	0.06	0.73	2.69
	Boys 9–13 y	Min	1.38	0.68	0.07	0.01	0.07	23.54	0.01	1.26	0.16	0.32	0.02	0.08	0.05
		Max	19.94	43.77	3.10	0.27	18.80	62.87	0.27	86.79	3.67	147.60	2.11	22.14	115.98
		Median	7.62	14.24	0.31	0.03	2.02	43.20	0.03	3.67	0.63	8.86	0.08	0.90	2.20
	Girls 9–13 y	Min	1.23	0.61	0.07	0.01	0.06	20.92	0.01	1.34	0.16	0.29	0.02	0.07	0.05
		Max	17.72	38.91	2.76	0.24	16.71	55.89	0.24	91.84	3.88	131.20	1.88	19.68	115.98
		Median	6.77	12.66	0.27	0.02	1.80	38.40	0.02	3.89	0.66	7.87	0.07	0.80	2.20
	Boys 14–18 y	Min	2.00	0.58	0.08	0.01	0.08	24.72	0.01	1.45	0.22	0.47	0.03	0.11	0.05
		Max	28.80	37.01	3.52	0.29	21.47	66.05	0.29	99.88	5.11	213.20	2.92	31.98	108.40
		Median	11.00	12.04	0.35	0.03	2.31	45.39	0.03	4.23	0.87	8.32	0.11	1.30	2.05
	Girls 14–18 y	Min	1.38	0.46	0.06	0.00	0.05	17.12	0.01	1.32	0.19	0.32	0.02	0.08	0.05
		Max	19.94	29.18	2.44	0.14	14.87	45.73	0.24	90.40	4.36	147.60	2.02	22.14	108.40
		Median	7.62	9.50	0.24	0.01	1.60	31.42	0.02	3.83	0.74	8.86	0.07	0.90	2.05
Adults	Men	Min	3.00	0.65	0.10	0.01	0.08	28.53	0.01	1.50	0.22	0.54	0.03	0.13	0.05
		Max	43.20	41.69	4.02	0.45	23.68	76.21	0.33	103.32	5.06	246.00	3.37	36.90	106.32
		Median	16.50	13.56	0.40	0.05	2.55	52.37	0.03	4.37	0.86	14.76	0.12	1.50	2.01
	Women	Min	2.20	0.63	0.07	0.00	0.06	20.92	0.01	1.54	0.20	0.40	0.02	0.10	0.04
		Max	31.68	40.12	2.95	0.15	17.36	55.89	0.33	106.07	4.74	180.40	2.47	27.06	77.97
		Median	12.10	13.06	0.29	0.01	1.87	38.40	0.03	4.49	0.81	10.82	0.09	1.10	1.48
	Pregnant	Min	2.30	0.60	0.07	0.00	0.06	20.05	0.01	1.34	0.19	0.41	0.02	0.10	0.04
		Max	33.12	38.35	2.77	0.10	16.34	53.56	0.25	92.41	4.46	188.60	2.58	28.29	81.51

Table 4. Continued

Age group			*RDA* / %							*AI* / %		*UL* / %			
			Ca	Mg	Cu	Fe	Mo	Se	Zn	Cr	Mn	Na	K	Cl	B
Adults	Pregnant	Median	12.65	12.48	0.28	0.01	1.76	36.80	0.03	3.91	0.76	11.32	0.09	1.15	1.54
	Lactating	Min	3.10	0.91	0.07	0.01	0.08	23.16	0.01	1.21	0.20	0.56	0.03	0.13	0.05
		Max	44.64	58.36	2.88	0.42	22.02	61.87	0.31	83.04	4.62	254.20	3.21	38.13	109.86
		Median	17.05	18.99	0.29	0.04	2.37	42.52	0.03	3.51	0.79	15.25	0.12	1.55	2.08

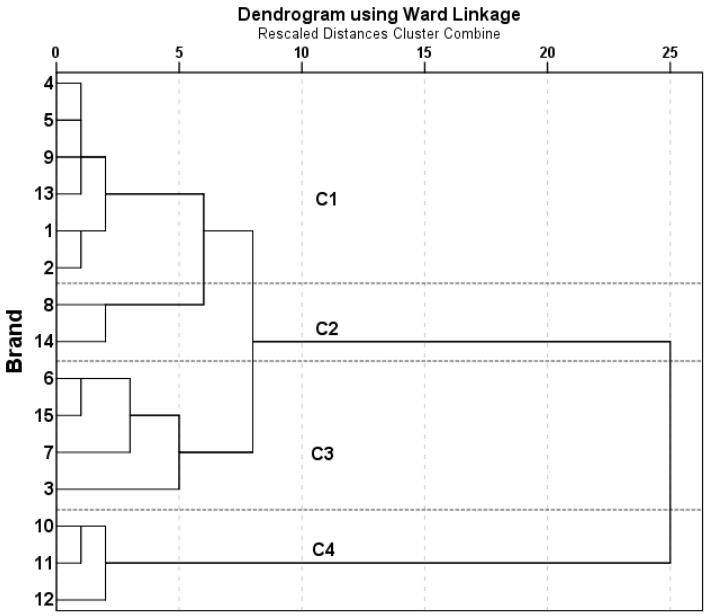

Figure 3. HCA dendrogram of bottled waters.

nates, and consequently, increase Ca/Mg concentrations in the groundwater, in the case of Brands 7 and 15. Brand 3 is specific by a high contribution to the Cr intake (83% in lactating females – 106% for women) and Mg concentrations (25% for girls 14–18 years of age to 56.26% for children 1–3 years of age). Cluster 4 included Brands 10, 11 and 12, which belong to $NaHCO_3$-water type and which originated from aquifers in granitoid rocks. They are characterized by elevated concentrations of dissolved solids and CO_2 concentrations. The contribution of Na in C4 is 83.7% for children (1–3 years) to 192.2% in lactating females, while the contribution of B is 78% for women to 212.6% for children (1–3 years of age).

Radioactivity of bottled waters and annual effective doses

Exposure to ionizing radiation is expressed by the effective dose which is estimated for human beings to be, on average, 2.5 mSv/year from natural radiation [41,43]. In general, water consumption is a minor source of radionuclide intake compared to food [44]. The guideline value for drinking water requires that the total estimated dose per year from all radionuclides, excluding the dose from ^{40}K should not exceed 0.1 mSv (K is an essential element, absorbed mainly from ingested food and ^{40}K does not accumulate in the body) [43,45].

The radioactivity in groundwater comes mainly from radionuclides of the natural decay chains ^{238}U and ^{232}Th, and ^{40}K in soil and bedrock. Some radionuclides can dissolve easily in water, depending on the mineralogical and geochemical composition of rock, redox conditions and the residence time of ground water in bedrock, as a result of the reaction of the ground water with soil and bedrock [46]. To gain insight into the radioactive properties of bottled waters of Serbia, radionuclide activity concentrations of ^{238}U, ^{228}Ra, ^{226}Ra and ^{40}K were examined.

Summary statistics for gross alpha and beta activities and activity concentrations of ^{238}U, ^{226}Ra, ^{228}Ra and ^{40}K are presented in Table 5. With respect to water quality standards [43,47] bottled waters showed that no brand exceeded the guidance level for alpha activity. Two brands exhibited elevated beta activity (Brand 10: 1.087±0.134 Bq/l; Brand 11: 1.242±0.146 Bq/l), measured in samples whose composition was formed in contact with granitoid rocks or circulation through the granites.

Table 5. Gross alpha and beta activities and gamma spectrometry findings in 15 bottled water samples (Bq/l)

Value	α	β	^{40}K	^{228}Ra	^{238}U	^{226}Ra
Min	0.018±0.01	0.018±0.003	0.025±0.001	<0.01	<0.05	<0.01
Max	0.3±0.037	1.242±0.146	1.27±0.07	<0.1	<0.8	0.12
Median	0.04	0.103	0.15	0.04	0.1	0.02
Guidance level (Official Gazette of RS, 2011.)	0.5	1	–	0.2	3	0.49
Guidance level (WHO, 2011.)	0.5	1	–	1	10	1

Alpha and beta activities correlated very well with the *TDS* (R^2 = 0.945 and 0.629, respectively), significant at $P < 0.01$. ^{40}K was also significantly correlated with gross beta activity (R^2 = 0.982, Fig. 4), which may indicate that β-radioactivity originates from ^{40}K which is present in felsic igneous rocks.

Calculations of the effective doses based on Eq. (2) for the 15 bottled water samples showed that all effective doses were below the recommended limit of 0.1 mSv/yr (Fig. 5).

Consequently, all samples of bottled water complied with quality standards for bottled water, such that these waters may be consumed on a daily basis. The largest doses are observed in CO_2 rich waters, especially for lactating females and men, groups that have the highest water intake.

CONCLUSION

The results obtained in this study show that Serbian bottled waters are rich in various trace and ultra-trace elements. All samples comply with European standards for bottled waters, while three samples exceeded national requirements (Fe, As and Se). The majority of bottled waters have a preponderance of a combination of Ca–Mg–Na–HCO_3, while more mature mineral waters of the Na–Cl–HCO_3 type are found in sediments formed in marine or lake marine conditions. A wide range of TDS values (56–3400 mg/l) were found in bottled water samples.

Several conclusions can be made from the methods applied:

– Ca, Mg, Na, B, Cr, Se, Mo and Cl intake from selected commercially available bottled waters may be appreciably high, while other essential elements intake (Cu, K, Mn, Fe, Zn) contributes less than 5.1% to DRIs in all considered groups.

– The highest potential contributions of low-mineralized bottled waters to RDA is for Ca (up to 21.7%) and Mg (up to 31.08%) ingestion, which are major components of the given bottled waters (TDS: 56–631 mg/l, pH > 7).

– Mature groundwaters enriched with Na and Cl concentrations, have a DRI contribution of Na 11.76––82.73% and Cl 5.2–38.1% (the lower value refers to children 1–3 years of age, while the upper value refers to lactating females).

– Mg intake from waters related to serpentinite rocks or limestone/dolomitic limestones amounts 18.2% Mg for girls (14–18 years of age) to 65.66% Ca for children (1–3 years of age).

– CO_2 rich waters from granitic rock aquifers (Brands 10–12) were particularly enriched with Na and B ions. Consumption of such water (according to recommended adequate water intake) exceeds the maximum RDA or UL of these elements.

– Alpha and beta activities correlated very well with the TDS. Brands 10 and 11 (from granitoid rocks) with elevated concentrations of dissolved solids, registered the highest beta activity.

– The calculated effective doses were below the recommended limit of 0.1 mSv/yr, therefore these waters may be consumed on a daily basis.

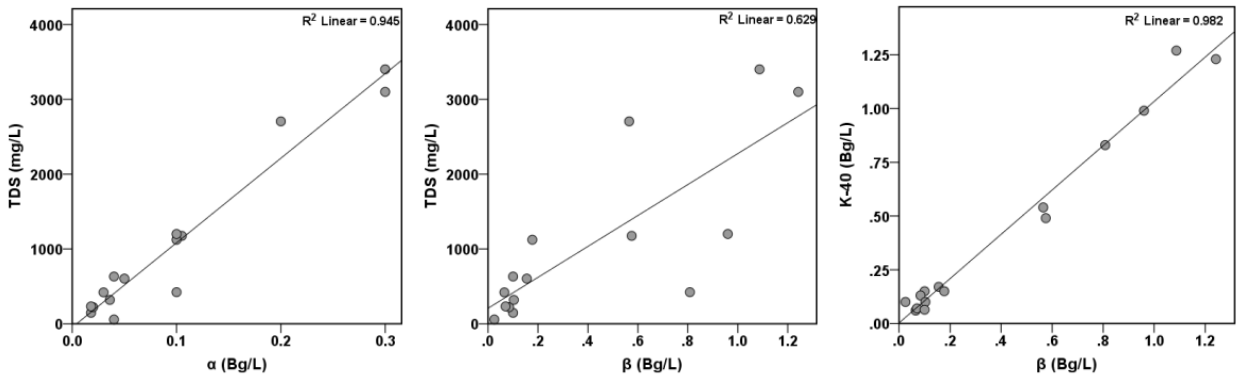

Figure 4. Correlation diagrams of the alpha activity vs. TDS, beta activity vs. TDS, and beta activity vs. ^{40}K.

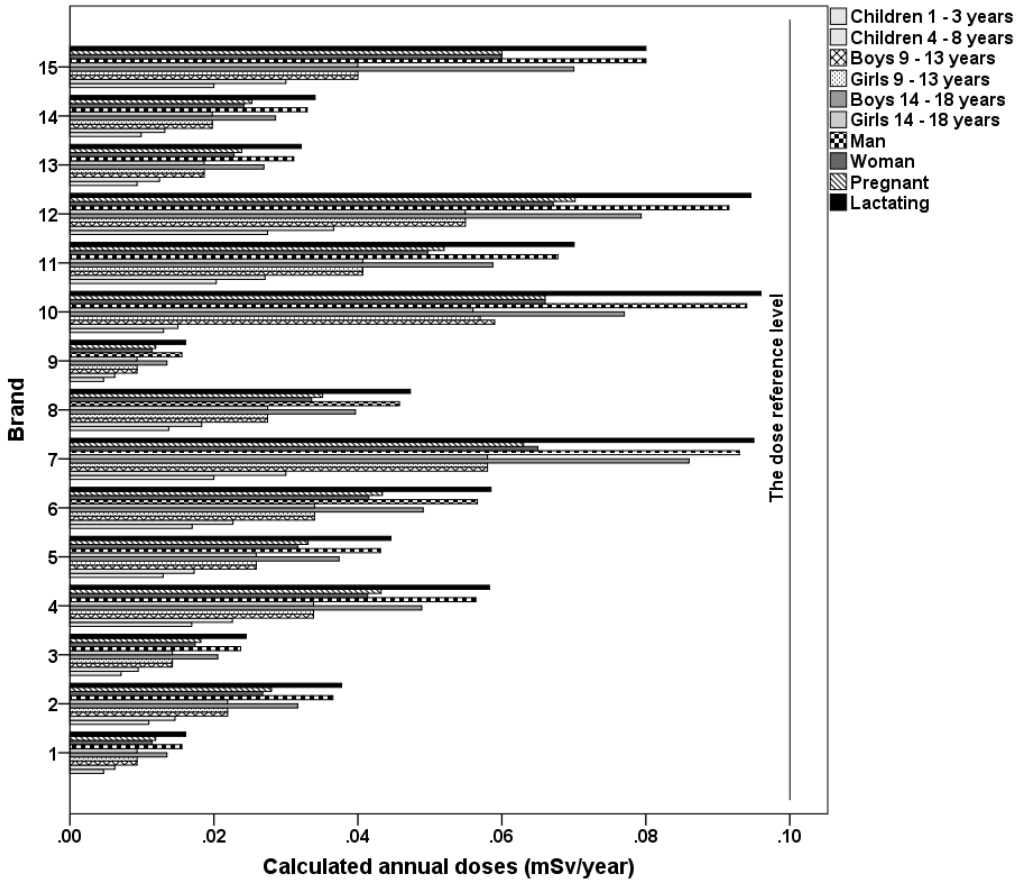

Figure 5. Sum of calculated annual effective doses (mSv/year) by age and gender groups.

Taking all these facts into consideration, mineral waters can be a significant source of essential elements for human health. In the case of waters with high TDS levels, reduced amounts of water intake are preferred because daily intake of certain elements can be above the recommended levels.

Acknowledgement

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REFERENCES

[1] EUI (Euromonitor International) 2014 http://www.euromonitor.com/bottled– water– in– serbia/report (last accessed October 28, 2014).

[2] T. Petrović, M. Zlokolica Mandić, N. Veljković, P. Papić, J. Stojković, in: F.F. Quercia, D. Vidojević (Eds), Clean Soil and Safe Water: Geochemistry of Bottled Waters in Serbia, NATO Science for Peace and Security Series C: Environmental Security, Springer, 2010, pp. 247–266.

[3] MWIA 2014 (Mineral Water Industries Association, Business Association) http://www.mineralwater.rs (last accessed October 28, 2014).

[4] J. Joksić, M. Radenković, M. Milanković, Natural radioactivity of some spring and bottled mineral waters from several central Balkan sites, as a way of their characterization, J. Serb. Chem. Soc. **72** (2007) 621–628.

[5] WHO, Nutritients in Drinking Water. Geneva: Water, Sanitation and Health. World Health Organization Library Cataloguing– in– Publication Data, 2005.

[6] D. Banks, C. Reimann, O. Røyset, H. Skarphagen, O.M. Sæther, Natural concentrations of major and trace elements in some Norwegian bedrock groundwaters, Appl. Geochem. **10** (1995) 1–16.

[7] A. Misund, B. Frengstad, U. Siewers, C. Reimann, Variation of 66 elements in European bottled mineral waters, Sci. Total. Environ. **243**/**244** (1999) 21–41.

[8] B. Frengstad, D. Banks, A. Skrede Midtgard, J.R. Krog, U. Siewers, T. Strand, The hydrochemistry of crystalline bedrock groundwatter in Norway, NGU bull. **439** (2002) 87 – 97.

[9] N. Grošelj, G. Van der Veer, M. Tušar, M. Vračko, M. Novič, Verification of the geological origin of bottled mineral water using artificial neural networks, Food Chem. **118** (2010) 941–947.

[10] A. Demetriades, General ground water geochemistry of Hellas using bottled water samples. J. Geochem. Explor. **107** (2010) 283–298.

[11] E. Dinelli, A. Lima, B. De Vivo, S. Albanese, D. Cicchella, P. Valera, Hydrogeochemical analysis on Italian bottled mineral waters: Effects of geology. J. Geochem. Explor. **107** (2010) 317–335.

[12] U. Fugedi, L. Kuti, G. Jordan, B. Kerek, Investigation of the hydrogeochemistry of some bottled mineral waters in Hungary. J. Geochem. Explor. **107** (2010) 305–316.

[13] Z. Peh, A. Šorša, J. Halamić, Composition and variation of major and trace elements in Croatian bottled waters, J. Geochem. Explor. **107** (2010) 227–237.

[14] T. Petrović, M. Zlokolica-Mandić, N. Veljković, D. Vidojević, Hydrogeological conditions for the forming and quality of mineral waters in Serbia, J. Geochem. Explor. **107** (2010) 373–381.

[15] S.A. Oyebog, A.A. Ako, G.E. Nkeng, E.C. Suh, Hydrogeochemical characteristics of some Cameroon bottled waters, investigated by multivariate statistical analyses, J. Geochem. Explor. **112** (2012) 118–130.

[16] C. Güler, M. Alpaslan, Mineral content of 70 bottled water brands sold on the Turkish market: Assessment of their compliance with current regulations, J. Food Compos. Anal. **22** (2009) 728–737.

[17] D. Cicchella, D. Albanese, B. De Vivo, E. Dinelli, L. Giaccio, A. Lima, P. Valera, Trace elements and ions in Italian bottled mineral waters: Identification of anomalous values and human health related effects, J. Geochem. Explor. **107** (2010) 336–349.

[18] A.F. Dijkstra, A.M. de Roda Husman, in: Y. Motarjemi, H. Lelieveld (Eds), Bottled and Drinking Water. Food Safety Management. A Practical Guide for the Food Industry, Academic Press, Elsevier Inc, Netherlands, 2014, pp. 347–377.

[19] B. Porntepkasemsan, K. Srisuksawad, Assessment of ^{226}Ra age-dependent dose from water intake, Appl. Radiat. Isot. **66** (2008) 1654–1656.

[20] Ş. Turhan, E. Özçıtak, H. Taşkın, A. Varinlioğlu, Determination of natural radioactivity by gross alpha and beta measurements in ground water samples, Water. Res. **47** (2013) 3108–3103.

[21] F. Korkmaz Gorur, H. Camgoz, Natural radioactivity in various water samples and radiation dose estimations in Bolu province, Turkey, Chemosphere **112** (2014) 134––140.

[22] T. Petrović, M. Zlokolica– Mandić, N. Veljković, P. Papić, M. Poznanović, J. Stojković, S. Magazinović, Macro- and micro-elements in bottled and tap waters of Serbia, Hem. Ind. **66** (2012) 107–122.

[23] M. Todorović, P. Papić, M. Ćuk, J. Stojković, Rare earth elements in some bottled waters of Serbia, Annales Géologiques de la Péninsule Balkanique **74** (2013) 71––81.

[24] S. Karamata, B. Krstić, in: V. Knežević-Đorđević, B. Krstić (Eds.), Terranes of Serbia and neighboring areas, Faculty of Mining and Geology, Belgrade, 1996, pp. 25–44.

[25] V. Cvetković, D. Prelević, H. Downes, M. Jovanović, O. Vaselli, Z. Pecskay, Origin and geodynamic significance of Tertiary post-collisional basaltic magmatism in Serbia (Central Balkan Peninsula). Lithos **73** (2004)161–186.

[26] B. Lugovic, B. Aegvic, R. Altherr, Petrology and tectonic significance of greenschists from the Medvednica Mts. (Sava Unit, NW Croatia), Ofioliti **31** (2006) 39–50.

[27] M.D. Dimitrijević, Geology of Yugoslavia. Barex, Special Publications, Belgrade, 1997.

[28] V. Cvetković, P. Giampiero, G. Christofides, A. Koroneos, Z. Pécskay, K. Resimić–Šarić, V. Erić, The Miocene granitoid rocks of Mt Bukulja central Serbia; evidence for Pannonian extension–related granitoid magmatism in the northern Dinarides, Eur. J. Mineral. **19** (2007) 513–532.

[29] V. Bortolotti, M. Marroni, L. Pandolfi, G. Principi, Mesozoic to Tertiary tectonic history of the Mirdita ophiolites, Northern Albania, The Island Arc. **14** (2005) 471–493.

[30] M.N. Dimitrijević, M.D. Dimitrijević, J. Olujić, In: S. Karamata, S. Janković (Eds.), Dinaridic ophiolite belt. Proceedings of the International Symposium Geology and metallogeny of the Dinarides and the Vardar Zone, Academia of sciences and arts of the Republic of Srpska, Banja Luka, 2000, pp.161–164.

[31] J. Pamić, B. Tomljenović, D. Balen, D. Geodynamic and petrogenetic evolution of Alpine ophiolites from the central and NW Dinarides: an overview, Lithos. **65** (2002) 113–142.

[32] J. Pamić, I. Gušić, V. Jelaska, Geodynamic evolution of the Central Dinarides. Tectonophysics **297** (1998) 251––268.

[33] L. Royden, F. Horvath, The Pannonian basin: A case study in basin evolution, AAPG Memoir. **45** (1988) 1–394.

[34] ISO 9696, Water Quality – Measurement of Gross Alpha Activity in Non-saline Water-thick Source Method (ISO/TC 147), 1992.

[35] ISO 9697, Water Quality-Measurement of Gross Beta Activity in Non-saline Water, (ISO/TC 147), 1992.

[36] ISO 10703, Water Quality-Determination of the activity concentration of radionuclides by high resolution gamma-ray spectrometry (ISO TC 147/WG4), 1997.

[37] IOM Institute of Medicine of the National Academies, in: J.J. Otten, J.P. Hellwig, L.D. Meyers (Eds.), Dietary Reference Intakes. The Essential Guide to Nutrient Requirements, The National Academies Press, Washington DC, 2006.

[38] C. Güler, G.D. Thyne, J.E. McCray, A.K. Turner, Evaluation of graphical and multivariate statistical methods for classification of water chemistry data, Hydrogeology J. **10** (2002) 455–474.

[39] E. Wanda, M. Monjerezi, J. Mwatseteza, L. Kazembe, Hydro-geochemical appraisal of groundwater quality from weathered basement aquifers in Northern Malaw, Physics and Chemistry of the Earth. Parts A/B/C 36 (2011) 1197–1207.

[40] IOM Institute of Medicine of the National Academies: Dietary Reference Intakes for Water, Potassium, Sodium, Chloride and Sulphate. Food and Nutrition Board. The National Academies Press, Washington DC, 2004.

[41] ICRP. Compendium of Dose Coefficients based on ICRP Publication 60. ICRP Publication 119. Ann. ICRP 41(Suppl.), 2012.

[42] G. Marinković, P. Papić, J. Stojković, V. Živanović, J. Andrijašević, Lithostratigraphic CO_2 substrata and the depth of carbonated mineral water systems in the lithosphere of Serbia, TTEM **8** (2013) 550–558.

[43] WHO, Guidelines for Drinking-water Quality. 4th ed., World Health Organization, Geneva, 2011.
[44] C. Kralik, M. Friedrich, F. Vojir, Natural radionuclides in bottled water in Austria, J Environ. Radioact. **65** (2003) 233–241.
[45] European Commission 1998. Council Directive 98/83/EC on the quality of water intended for human consumption.
[46] P. Vesterbacka, Natural radioactivity in drinking water in Finland, Boreal. Environ. research, **12** (2007) 11–16.
[47] Official Gazette of RS. 86/ 2011. Regulation on limits of radionuclides in drinking water, food, fodder, drugs, objects of general use, building materials and other commodity intended for commercial use.

IZVOD

HIDROGEOHEMIJSKI PRISTUP PROCENE KVALITETA ODABRANIH FLAŠIRANIH VODA U SRBIJI

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(Naučni rad)

Za potrebe izrade ovog rada odabrano je 15 uzoraka flaširanih voda sa domaćeg tržišta u kojima su određene koncentracije makro i mikro elemenata i aktivne koncentracije radionuklida. Cilj rada je bio da se ispita kvalitet voda i da se izračuna potencijalni unos esencijalnih elemenata konzumiranjem flaširanih voda. Hidrokarbonantni jon je dominantan u svim uzorcima, dok katjonski sastav čini kombinacija Ca–Mg–Na jona. Analizirane vode pripadaju slabo kiselim do blago alkalnim vodama (vrednost pH indeksa 6,37–7,93). Suma koncentracija mikroelementa u uzorcima varira od 79,7 do 9349,7 μg/l. Korišćenjem dijetetskog referentnog unosa (*DRI*) izračunat je doprinos određenih esencijalnih elemenata prema starosnim grupama, na osnovu konzumiranja preporučenog dnevnog unosa flaširanih voda. Unos Ca, Mg, Na, B, Cr, Se, Mo i Cl iz flaširanih voda može da bude značajno visok, dok ostali elementi (Cu, K, Mn, Fe and Zn) imaju niske dijetetske unose u svim razmatranim grupama. Kao statistička metoda korišćena je hijerarhijska klaster analiza (HCA) kako bi se izvršilo grupisanje većeg broja podataka u manje grupe prema hemijskoj sličnosti uzoraka. Korišćeni su parametri kvaliteta vode (pH, sadržaj CO_2, glavni katjoni – Ca, Mg, Na, K, glavni anjoni – HCO_3, Cl, SO_4 i drugi esencijalni elementi – Cr, Cu, B, Mn, Mo, Fe, Zn) kao varijable za ovu analizu. HCA je grupisala uzorke flaširanih voda u četiri klastera na osnovu čega je razmatrana veza esencijalnih elemenata sa geološkim uslovima. Dva uzorka flaširanih voda su pokazala povišenu beta aktivnost (1.087±0.134 Bq/l i 1.242±0.146 Bq/l), međutim utvrđeno je da su sve efektivne doze ispod referentnog nivoa od 0,1 mSv/god.

Ključne reči: Flaširane vode • Dijetetski referentni unos • Esencijalni elementi • Hijerarhijska klaster analiza • Prirodna radioaktivnost • Srbija • Kvalitet voda