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Chemical Industry & Chemical Engineering Quarterly 15 (4) 211–226 (2009)
CI&CEQ

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REVIEW PAPER

UDC 547.262:664.2:663.142/.143

DOI: 10.2298/CICEQ0904211M

PROGRESS IN THE PRODUCTION OF BIOETHANOL ON STARCH-BASED FEEDSTOCKS*

Bioethanol produced from renewable biomass, such as sugar, starch, or lignocellulosic materials, is one of the alternative energy resources, which is both renewable and environmentally friendly. Although, the priority in global future ethanol production is put on lignocellulosic processing, which is considered as one of the most promising second-generation biofuel technologies, the utilizetion of lignocellulosic material for fuel ethanol is still under improvement. Sugar-based (molasses, sugar cane, sugar beet) and starch-based (corn, wheat, triticale, potato, rice, etc.) feedstock are still currently predominant at the industrial level and they are, so far, economically favorable compared to lingocelluloses. Currently, approx. 80 % of total world ethanol production is obtained from the fermentation of simple sugars by yeast. In Serbia, one of the most suitable and available agricultural raw material for the industrial ethanol production are cereals such as corn, wheat and triticale. In addition, surpluses of this feedstock are being produced in our country constantly. In this paper, a brief review of the state of the art in bioethanol production and biomass availability is given, pointing out the progress possibilities on starch-based production. The progress possibilities are discussed in the domain of feedstock choice and pretreatment, optimization of fermentation, process integration and utilization of the process byproducts.

Key words: bioethanol; starch-based feedstock; fermentation; hydrolysis; pretreatment; byproducts.

As a consequence of the industrial development and population growth, there is an increase of energy consumption in the world. The world-wide energy consumption has increased 17-fold in the last century [1]. However, conventional energy resources, like fossil fuels, cannot meet the increasing energy demand. The quantities of non-renewable (conventional) energy resources are limited and they have a considerable negative environment impact *e.g.* increased greenhouse gas emissions. Therefore, one of the challenges for the society is to meet the growing demand for energy for transportation, heating and industrial processes; also to provide raw materials for the industry in a sustainable way and to reduce greenhouse gas emissions. Our energy systems will need to be renewable and sustainable, efficient and cost-effective, convenient and safe.

These problems make it urgent to develop alternative energy resources that are both renewable and environmentally friendly. Bioethanol produced from renewable biomass such as starch, sugar or lingocellulosic materials, is believed to be one of these alternatives. It is expected to be one of the dominating renewable biofuels in the transport sector within the twenty years to come. The transport sector itself is considered as one of the largest energy consumers as well as environmental pollutant. According to International Energy Agency statistics [2], the transporttation sector accounts for about 60% of the world's total oil consumption. It is responsible for about one fifth of CO_2 emission on a global scale [3]. According to Goldemberg [4], motor vehicles account for more than 70% of global CO emissions and 19% of global CO_2 emissions. This could be alleviated by using biofuels and it is expected that by 2030, one third of the EU and US need of energy for road transportation can be met by converting biomass to biofules [5,6].

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Paper received: 14 July, 2009
Paper accepted: 31 July, 2009
*A part of this study was presented as a section lecture at the 8th Simposium „Novel Technologies and Economics Development“, University of Niš, Faculty of Technology, Leskovac, October 21-24, 2009.

Ethanol can be blended with petrol or used as neat alcohol in dedicated engines, taking advantage of the higher octane number, low cetane number and higher heat of vaporization, and also it is an excellent fuel for future advanced flexi-fuel hybrid vehicles. Using ethanol fuel (E85 – with 85% of bioethanol) in a midsize passenger vehicle can reduce greenhouse gas emissions by 41-61% km^{-1} driven, compared to gasoline-fueled vehicles [7]. In addition, the ethanol is an oxygenated fuel containing 35% oxygen, which reduces particulate and NO_x emissions from combustion, it is biodegradable and contributes to sustainability [8].

Bioethanol has already been introduced on a large scale in Brazil, the US and European countries [8-16]. The production has increased remarkably because many countries look for reducing oil imports, boosting rural economies and improving the air quality. The world ethyl alcohol production has reached about 51000 milliion liters in 2007 [17], the USA and Brazil being the first producers and they together account for about 70% of the world bioethanol production (see Table 1). On average, 73% of produced ethanol worldwide corresponds to fuel ethanol, 17% to beverage ethanol and 10% to industrial ethanol. In 2007, the bioethanol production represented about 4% of the 1300 billion liters of gasoline consumed globally [8]. Furthermore, the world and European production of bioethanol for fuel is constantly expanding with the possibility to reach 120000 million liters per year until 2025 [16-18]. Bioethanol currently accounts for more than 94% of global biofuel production, with the majority coming from sugar cane [19]. About 60% of global bioethanol production comes from sugar cane which is predominant in Brazil and 40% from other crops [20]. Currently, nearly all bioethanol fuel is produced by fermentation of corn glucose in the United States or sucrose in Brazil.

The European Commission plans to substitute progressively 20% of conventional fossil fuels with alternative fuels in the transport sector by 2020, with an intermittent goal set at 5.75% in 2010 [21,22]. This indicative target has been adopted by most Member States in their national biofuel objectives. Some member states like Finland, Sweden or Germany have already fulfilled the set quota [23]. However, although the amount of biofuels produced in the EU is growing, the quantities in general remain small compared to the total volume of mineral-based transport fuel sold, it was approximately 0.3% of all EU petrol and diesel fuel in 2003 [24,25]. Figure 1 presents the amounts of bioethanol produced in EU in 2005 and 2007 and the amounts needed to be produced in order to fulfill the aims of the directive 2003/30/EC [22,26]. The potential demand for bioethanol as fuel for transportation in EU countries, calculated on the basis of Directive 2003/30/EC, is estimated at about 6 billion liters in 2006 and 12.7 billion liters in 2010. This is in market disproportion with the current level of EU production capacity of about 2 billion liters per year [27]. In Europe, the feedstock used for bioethanol is predominately wheat, sugar beet, corn and waste from the

Table 1. World production of ethanol in million liters

Country	2006	2005
1. USA	18376	16139
2. Brazil	16998	15999
3. China	3849	3800
4. India	1900	1699
5. France	950	908
6. Germany	765	431
7. Russia	647	749
8. Canada	579	231
9. Spain	462	352
10. South Africa	386	390
11. Thailand	352	299
12. United Kingdom	280	348
13. Ukraine	269	246
14. Colombia[a]	269	27
15. Poland	250	220
Total	51056	45988

[a] These data correspond to the fuel ethanol produced in new distilleries the construction of which started in 2005; industrial and beverage ethanol are not included, although their share is significantly lower. Modified from renewable Fuels Association, 2007 [17]

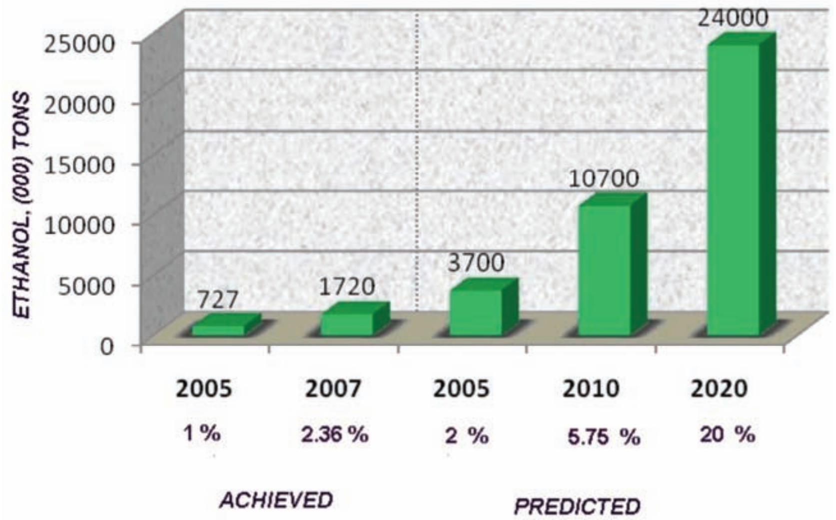

Figure 1. Amounts of bioethanol produced in EU in 2005 and 2007 and the amounts needed to be produced in order to fulfill the aims of the directive 2003/30/EC [22,26].

wine industry. It is estimated that between 4 and 13% of total agricultural land in the EU would be needed to produce the biofuels needed to fulfill the directive [9].

Current production of bioethanol in Serbia is based on molasses (50%) and cereals (50%) [13]. Today in Serbia, the bioethanol production is performed in 10 plants with a total production capacity of 40 million hL° [13]. This production scale, which is now even lower than the production scale in 1996 (see Figure 2), is not enough to fulfill the country's ethanol needs just for beverages, medical and pharmaceutical purposes. Our recent analysis revealed that Serbia will need to build new bioethanol plants in order to produce enough bioethanol for use as a fuel and thus to follow the aims of the directive 2003/30/EC. In this context, it is estimated that about 80000 tons of bioethanol will be needed in Serbia in 2010 for 5.75% substitution of motor oil [13].

An important benefit regarding bioethanol utilization as a biofuel is a fact that it can create economic growth in rural areas and industry. Several reports published in recent years have tried to assess the effects of bioethanol production on job creation. One of them claims that the ethanol industry has created 700000 jobs in rural areas of Brazil [28] while others predict the creation of 200000 jobs in the biofuel industry in US by 2017, 100000 jobs in EU by 2020 and 600000 jobs in China by 2020 [5,29]. Most developing countries today are dependent on imported oil, and recent global energy price increases have been economically detrimental for these nations. According to UN-Energy, no country in modern times has substantially reduced poverty in the absence of access to energy [30].

However, bioethanol production presents risks as well as opportunities. There are three main areas of concern: 1) increased food prices; 2) the environ-

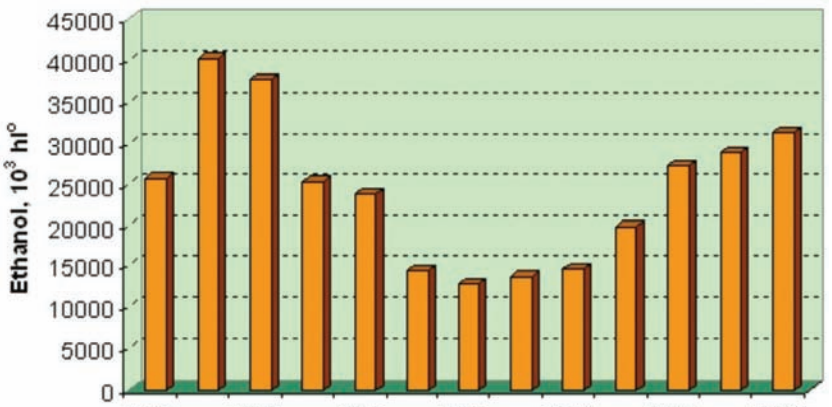

Figure 2. Production of ethanol in Serbia from 1985-2006 [13].

mental impact of increased agriculture; 3) changes in land use [5]. Therefore, there is no doubt that it is important to monitor these risks closely, but one should also be aware that food prices depend on many other factors than demand for crops for biofuels. Corn is the most debated crop when it comes to increase in prices in relation to bioethanol. However, it must be noted that on a global scale only around 8% of all corn currently produced (2006/07) is used for bioethanol [31]. In addition, the World Watch Institute states that higher agricultural prices could suit well to the world's rural poor, since due to recent price increases they can sell their crops at a profitable price. Another area of concern is the environmental consequences of the increased agricultural production, which may lead to the increased consumption of fertilizers and water. This, in turn, may cause the increased release of nutrients into the aquatic environment and of greenhouse gases into the atmosphere. Finally, the third area of concern is that the increasing demand for bioethanol may lead to changes in land use. Natural vegetation such as rainforests may be replaced by agricultural crops, a development that could cause loss of biodiversity and a reduction in the carbon-binding capacity of the land. However, the vast majority of the bioethanol produced today comes from places where bioethanol crops are grown on traditional agricultural land and does not lead directly to deforestation. The pointed concerns suggest the need of a sustainable production of bioethanol which comprise that the high-biodiversity and high-carbon stock land should be protected and the use of sophisticated agricultural practices and new biofuel feedstocks.

In this paper, a brief review of the state of the art in bioethanol production and biomass availability will be given, pointing out the progress possibilities in this area. Generally, a significant progress and enhancement of the efficiency of bioethanol production can be obtained by proper feedstock choice, optimization of feedstock pretreatment and ethanol fermentation itself, and by an adequate utilization of the process byproducts. For these reasons, the possibilities of the enhancement of the ethanol production on starch-based feedstock, which are predominant in Serbian agricultural production, by applying various treatments such as microwaves and ultrasound, before or during the common enzymatic hydrolytic treatment, will be considered. In the domain of fermentation, the issues such as the choice of the production microorganism, media optimization by addition of various grow factors, and the choice of the most appropriate process flow sheet (simultaneous saccharification and fermentation, utilization of immobilized yeasts, etc.) will be discussed. In the domain of the utilization of byproducts, possibilities for thin stillage recirculation or its use for the production of pure lactic acid and utilization of the distiller's grains for the production of high value feed with a probiotic activity (obtained by lactic acid fermentation with selected bacterial strains) will be evaluated.

Biomass for the production of bioethanol

Biological feedstocks that contain appreciable amounts of sugar or materials that can be converted into sugar, such as starch or cellulose can be fermented to produce bioethanol to be used in gasoline engines [8,13,15]. Molasses is a traditional raw material for the production of ethanol. It is obtained as a byproduct in the production of sugar from sugar beet (beet molasses) or from sugarcane (cane molasses). For a long time, molasses was practically the only raw material for the production of ethanol. However, since molasses can also be used for the production of other important biotechnological products such as baker's yeast, organic acids, amino acids, enzymes, etc., it is no more considered to be a cheap and widely available raw material, and because of that, there is a growing interest worldwide to find out new, abundant and economically more favorable carbohydrate sources for the production of bioethanol [32]. Currently, a focus is on bioethanol production from crops, such as corn, wheat, sugar cane, as well as on highly abundant agricultural wastes. The availability of feedstock for bioethanol can vary considerably from season to season and depends on geographic locations. Locally available agricultural biomass will be used for the bioethanol production. For a given production line, the comparison and choice of the feedstock includes several issues [33]: 1) chemical composition of the biomass, 2) cultivation practices, 3) availability of land and land use practices, 4) use of resources, 5) energy balance, 6) emission of greenhouse gases, acidifying gases and ozone depletion gases, 7) absorption of minerals to water and soil, 8) injection of pesticides, 9) soil erosion, 10) contribution to biodiversity and landscape value losses, 11) farm-gate price of the biomass, 12) logistic cost (transport and storage of the biomass), 13) direct economic value of the feedstock taking into account the coproducts, 14) creation or maintain of employment and 15) water requirements and water availability.

Bioethanol feedstocks can be conveniently classified into three types: *i*) sucrose-containing feedstock (*e.g.* molasses, sugar beet, sweet sorghum and sugar cane), *ii*) starch-containing feedstock (*e.g.* wheat, corn, rice, triticale potato, and barley) and *iii*) lignocellulosic biomass (*e.g.* wood, straw, and grasses). Different feedstocks that can be utilized for bioethanol production and their comparative production potential are given in Table 2 [10,13,34]. As it can be seen, Brazilian bioethanol is less expensive than that produced in the United States from corn or in Europe from sugar beet, because of shorter processing times, lower labor costs, lower transport costs and input costs [8].

The priority in future ethanol production is put on lignocellulosic processing, which is considered as one of the most promising second-generation biofuel technologies [35]. There are many reports on utilization of various lignocellulosic waste materials such as rice straw [36], corn stover [37,38], recycled paper sludge [39], mahula (*Madhuca latifolia* L.) flowers [40], alfa alfa fibers [41], switch grass, coffee husks, sunflower hulls [42], etc. However, utilization of lignocellulosic material for fuel ethanol is still under improvement. Sugar-based and starch-based feedstocks are currently predominant at the industrial level and they are still economically favorable compared to lignocelluloses. Conversion technologies for producing bioethanol from lignocellulosic biomass such as forest materials, agricultural residues and urban wastes are under development and have not yet been demonstrated commercially on a larger scale [8,13].

Starch-based materials are currently most utilized for the ethanol production in North America and Europe. Corn and wheat are mainly employed for these purposes [11]. Corn, which is currently used to make about 90% of all US bioethanol, is expected to remain the predominant feedstock, although its share likely will decline modestly by 2015. A combination of improved corn yields and land shifts from other crops will enable the US corn sector to supply the bioethanol industry without significant increases in prices that would adversely affect bioethanol profitability or the livestock and poultry industry [9].

An important issue in the biomass evaluation for the bioethanol production is land requirement. The other considerable fact is global and local land availability. In 2007, approximately 11.4 million hectares were used to provide bioethanol feedstock in five major producing countries. This would account for about 2.2% of arable land in these countries [9]. Because of that, many researchers predict greater involvement of genetic engineering in modifying the biomass properties to better suit biofuel production and also in order to improve the biomass yield [43,44].

In Serbia, according to current agricultural production, starch- based raw materials for the bioethanol production are generally the most prospective since close to 70% of arable land is planted with

Table 2. Parameters for the assessment of the suitability of various feedstocks for bioethanol production [10,13,34]

Type of feedsock	Annual yield t/ha	Specific conversion rate to ethanol, l/t	Annual ethanol yield, l/ha·	Output/input ratio	Cost, US$/kg	Cost of production of anhidrous ethanol US$/l
Sugar cane (Brazil)	70-122	68-70	5345-9381	2.5-10.2	0.0100	0.1980
Sugar beet	66-78	80-100	5000-6600	1.9	0.170	0.4910
Corn (USA)	6-10	350-460	6600	1.34-1.53	0.076	0.2325
Wheat	1.5-3.0	340-370	1020-3214	2.24-2.84	0.188	0.402
Potato	17-20	100	1700-2000	-	0.020	1.330
Sorghum	1-6	340	340-2040	-	0.149	0.386
Sweet sorghum	25-35	68-86	1700-9030	-	-	-
Cassava	20	180	3600	-	-	-
Straw	1.93-3.86	170-261	-	-	-	0.651

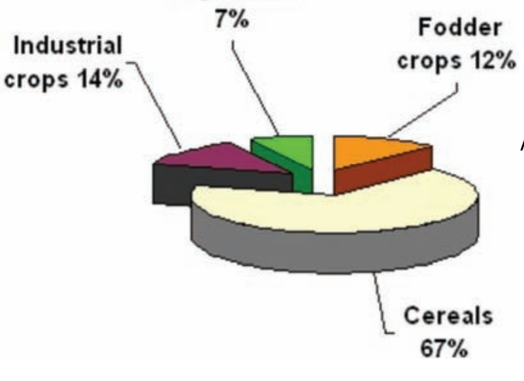

Figure 3. Arable land planted in Serbia in 2006 [45].

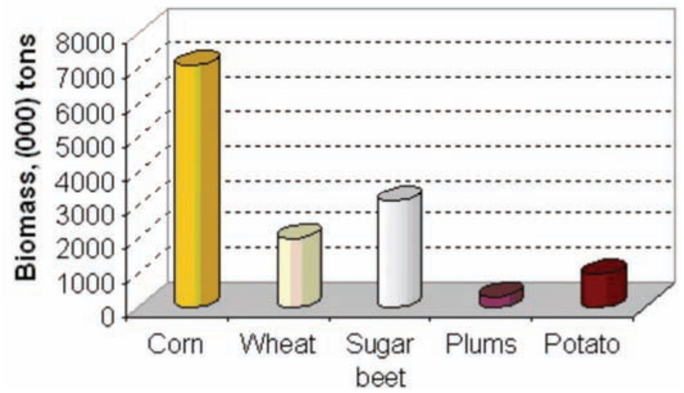

Figure 4. Production of biomass which could be used for bioethanol production in Serbia in 2006 [45,46].

cereals (Figure 3). Among biomass that could be used for bioethanol production (Figure 4) the corn production is the largest. It has been reported in 2008 that the average corn yield in Serbia was ~6-7 million ton, while estimated domestic needs for corn are only 4-4.5 million ton [47,48]. This means that there is enough corn for other purposes besides the food; therefore significant amounts can be used for the bioethanol production. Other prospective starch-based raw materials in Serbia are wheat and triticale.

Wheat is a very good raw material for the bioethanol production and is considered as a primary commodity for the bioethanol production in Europe and Australia [49,50]. Although the production of bioethanol on corn is economically more favorable than on wheat (see Table 2), the advantage is that wheat kernels contain native amylolytic enzymes able to degrade starch contained in the grains and thus could enable an easier pretreatment of the raw material during bioethanol processing and decrease the consumption of technical enzymes [51,52]. Even higher autoamylolytical quotient is noticed for triticale, which is a hybrid of wheat and rye. It is an appropriate plant for Serbian climate which can grow on a land of quite low quality [52-56]. When considering potential biomass for bioethanol production, a special attention should be paid on biomass which could be produced on marginal land. It is estimated that there are about 100 thousands hectares of low quality lands in Serbia which are not appropriate for conventional agricultural cultures, but could be used to cultivate alternative feedstock for bioethanol production such as sorghum, Jerusalem artichoke or triticale [15]. Another issue that should be explored is the utilization of wasted crops, damaged cereals or that of lower quality which do not meet the food requirements. According to one global analysis [57], there are about 73.9 Tg of dry wasted crops in the world that could potentially produce 49.1 GL of bioethanol annually. In that context, but on the local level, in 2005 Pejin *et al.* [58] explored the possibilities of using a domestic wheat type Kantata for the bioethanol production. This wheat type, obtained from the localities: Kovin, Zrenjanin, Pančevo and Vrbas, was shown as inappropriate for use in bakeries for bread production. However, bioethanol yields higher or close to 40% of the theoretical yields were achieved using this feedstock, depending on the temperature of the pretreatment (see Table 3).

Table 3. Ethanol yield obtained on wheat meal from hybrid Kantata from various localities at different pretreatment temperatures (duration of thermal and enzymatic treatment was 30 min) [58]

Temperature, °C	Ethanol yield, %			
	Kovin	Zrenjanin	Pančevo	Vrbas
70	40.9	40.6	39.4	41.2
80	41.4	40.8	41.2	41.0
85	42.1	40.6	43.4	40.3
90	41.4	40.6	41.5	40.5

Bioethanol production on starch-based feedstock and progress possibilities

Bioethanol is produced by fermentation of simple sugars present in biomass and the sugars obtained by prior chemical or enzymatic treatment of the biomass. The fermentation is performed by microorganisms, traditionally by yeasts, although some types of bacteria such as *Zymomonas mobilis* [51] could also be used. After the fermentation, the ethanol is being separated from the fermentation broth, conventionally by means of distillation and rectifycation or by using more efficient separation technologies such as pervaporation, membrane filtration or molecular sieves [11,13,51]. A schematic of bioethanol production on biomass is presented in Figure 5 [13].

Hydrolysis of starch

The hydrolysis of starch may be considered as a key step in the processing of starch-based feedstock

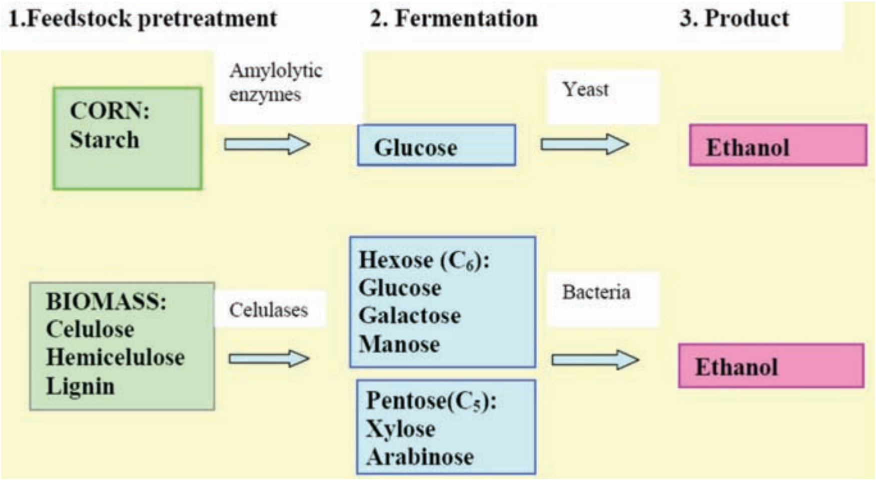

Figure 5. Schematic presentation of bioethanol production on biomass [13].

for the bioethanol production. The main role of this step is to effectively provide the conversion of two major starch polymer components: amylose, a mostly linear α-D-(1-4)-glucan and branched amylopectin, a α-D-(1-4)-glucan, which has α-D-(1-6) linkages at the branch points, to fermentable sugars that could subsequently be converted to ethanol by yeasts or bacteria. The hydrolysis may be performed by acids, an older process which is now mainly abandon and replaced by more efficient enzymatic process. The starch-based bioethanol industry has been comercially viable for about 30 years; in that time, tremendous improvements have been made in enzyme efficiency, reducing process costs and time, and increasing hydrolysis and bioethanol yields [59]. Recent advances in developing of termostable α-amylases, the starch liquefying enzymes which catalyze the hydrolysis of internal α-D-(1-4)-glucosidal linkages in starch in a random manner [60-63] and effective glucoamylases [64,65], the starch saccharifying enzymes which catalyze the hydrolysis of α-D-(1-4) and α-D-(1-6)-glucosidal bonds of starch from the non-reducing ends giving glucose as the final product, have led to commercial establishment of the so called "the two-step enzymatic cold process" [15]. The main advantages of this process are lower energy consumption and a lower content of non-glucosidal impurities, and thus much better suitability for the ethanol production. The mode of action of α-amylase and glucoamylase is presented in Figure 6.

The amount of endogenous enzymes for the hydrolysis of starch-based feedstock and the parameters of hydrolysis such as pH, temperature, substrate concentration, process time etc. depend on the type of the feedstock; its chemical composition, presence of the native autoamylolytic potential as well as on the origin of endogenous enzymes and their activity. The employment of additional, mainly physical treatments, such as grinding [66,67], micronization [68], cooking and steaming [23,69], microwave [70-73], ultrasound [74-78] etc., improves the starch gelatinization process, the substrate susceptibility to enzymes and can greatly influence and improve the effects of hydrolysis and subsequent ethanol fermentation.

Fermentation

Efficient bioethanol production requires a rapid fermentation leading to high ethanol concentrations,

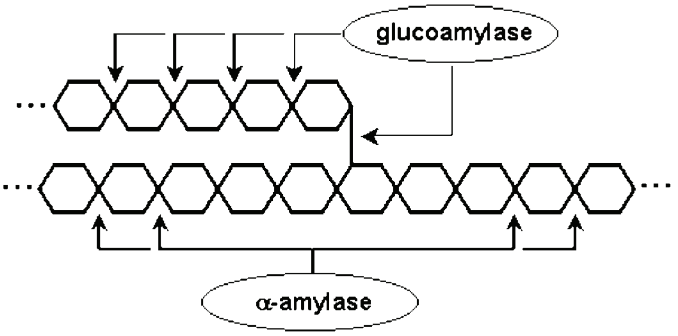

Figure 6. Mode of action of α-amylase and glucoamylase on α-D-(1-4) and α-D-(1-6) -glucosidal bonds of starch.

therefore, a yeast strain must have a good specific growth rate and specific ethanol production rate at high osmotic pressure and ethanol concentration [79,80]. An important issue for the efficient ethanol production is to optimize the fermentation step regarding following main parameters: temperature, pH, media composition, mixing, aeration, elimination of infection etc. [13,80].

The choice and development of the efficient production microorganism is of crucial importance [13,81]. As a result, a lot of research is currently going on aiming to produce a microorganism with a resistance to higher substrate and ethanol concentrations [82–85]. The research has revealed that the ability of yeast strains to achieve a high level of ethanol strongly depends on the nutritional conditions and protective functions that some nutrients can provide [79,81].

The microorganism with higher resistance to ethanol may be used in a very high gravity (VHG) ethanol fermentation process which utilizes the medium containing sugar in excess of 250 g l^{-1} to achieve over 15% (by volume) ethanol. This process was proposed in the1990s [86,87]. However, up to now, it is not widely applied at the industrial level.

The immobilization of yeast or the fermenting organism for the bioethanol production has been greatly explored as a strategy to overcome the substrate and product inhibition and to improve the ethanol tolerance. Among this approach, the most explored are immobilization of yeasts in/on adequate matrices such as calcium alginate, k-carragenan gel, polyacrylamide-alumina [40,48,79,80,88], wooden chips [89], PVA gel [90], orange peel [91], etc. Bai *et al.* [81] are giving a priority to simple adsorptive and self flocculation immobilization techniques due to the possibility to remove the slow growing cells from the system. The immobilization is often combined with the choice of an appropriate process mode, such as continuous or semi-continuous fermentation [92] and enables easier biomass separation and recirculation or its repeated use.

Very challenging research in this area is to obtain the microorganism with such a metabolism which would allow utilization of wider sugar spectra, especially xyloses and arabinoses mainly present in a hemicelluloses' part of the plant, and thus enable more complete substrate utilization [93-95]. Genetic engineering technologies are mostly applied for these purposes.

Process integration

Besides the optimization of individual process steps of the ethanol production, an overall process design and integration is also of great importance and may vastly influence the production efficiency and economy. Various process dynamics and fermentation regimes such as batch, fed-batch and continuous could be chosen and certain production steps could be integrated in order to minimize the production costs [8-14].

It is generally accepted that the integration of the enzymatic saccharification step and fermentation step which are carried out in one vessel in so called simultaneous saccharification and fermentation (SSF) process could reduce the production cost and process time compared to conventional separate hydrolysis and fermentation (SHF) process [96-98]. The presence of yeast or bacteria along with enzymes minimizes the sugar accumulation in the vessel because the fermenting organism immediately consumes the released sugars. Since the sugar produced during starch breakdown slows down α-amylase action, higher rates, yields and concentrations of ethanol are possible using SSF rather than SHF, at lower enzyme loading. Additionally, the presence of ethanol makes the mixture less vulnerable to contamination by unwanted microorganisms, which is a frequent burden in case of industrial processes [81]. Also, capital investments are lower in this process as the total reactor volume is decreased due to higher productivity. On the other hand, the critical problem with SSF is that it operates at non-optimal hydrolysis temperature since optimal temperatures for the yeast and the enzymes differ [97]. SHF and SSF process schemes are presented in Figures 7 and 8, respectively.

Other promising integration alternative for starchy and lignocellulosic feedstock is the inclusion of pentose fermentation in the SSF, process known as simultaneous saccharification and co-fermentation (SSCF) [81]. In this configuration, it is necessary that both fermenting microorganisms are compatible in terms of operating pH and temperature. A combination of *Candida shehatae* and *S. cerevisiae* was reported as suitable for the SSCF process [11].

Bioethanol economy

The cost of bioethanol as a fuel is also an important issue. In order to ensure the market, bioethanol must be competitive with other biofuels and with mineral fuels such as petrol and diesel. Currently, the cost of bioethanol is still higher than the cost of fossil gasoline supply. Because of that, national governments have to enact special policies such as agricultural subsidies and taxation free polices in order to encourage the production and use of bioethanol in the transportation sector [13,8]. Nevertheless, at sustained high oil prices and with a steady progression of more efficient and cheaper technology, bio-

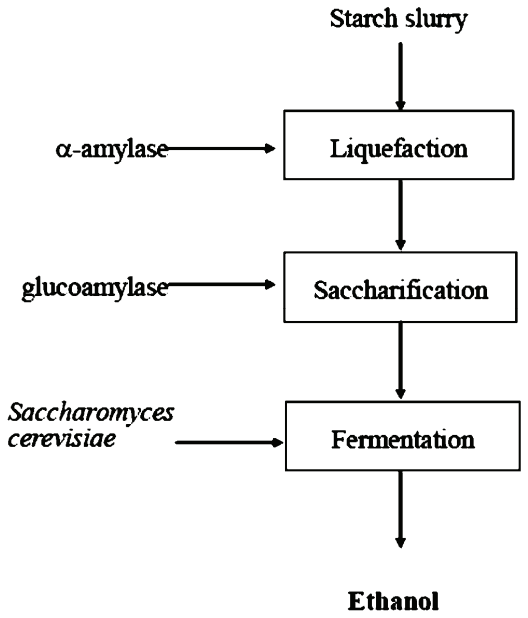

Figure 7. Scheme of SHF process for bioethanol production on starch based biomass.

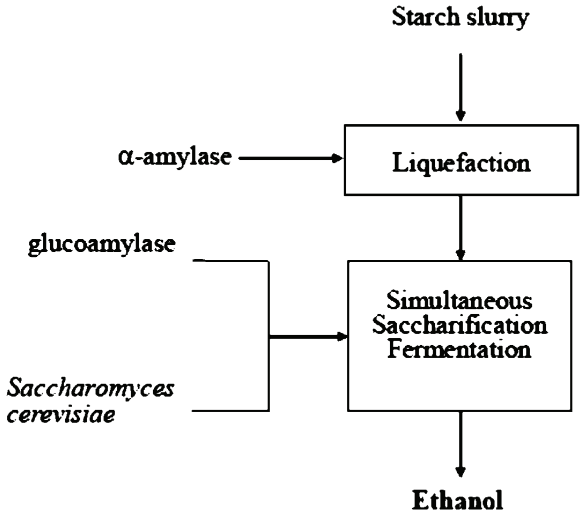

Figure 8. Scheme of SSF process for bioethanol production on starch based biomass.

ethanol could be a cost-effective alternative in the near future in many countries. Estimates show that bioethanol in the EU becomes competitive when the oil price reaches US$70 a barrel while in the United States it becomes competitive at US$50-60 a barrel. For Brazil the threshold is much lower–between US$ 25 and 30 a barrel [9].

The price of raw material used for the bioethanol production plays a major role on the total production costs. It represents 60-75% of the total bio-ethanol production cost [13,8,99]. The seasonal production pattern due to the harvest period of various agricultural feedstock used for bioethanol production is another important factor on the final price of the fuel on the market. The estimates of the costs of the bioethanol production from different feedstocks are shown in Table 2 [10,13,34]. Table 4 presents average costs of the production of bioethanol from wheat and sugar beet in Europe (EU-25) in 2004 [99]. It can be seen from Table 4 that utilization of the byproducts on wheat decreases the production costs for about 25%. It points out a necessity for the proper valorization of byproducts from the bioethanol production on cereals.

Bioethanol production from corn

As already mentioned, the corn is one of the most utilized feedstock for ethanol production globally and also the most abundant and prospective in Serbia.

The authors of this paper have thoroughly studied the utilization of corn and other cereals such as

Table. 4. Average costs of the production of bioethanol from wheat and sugar beet in Europe (EU-25) in 2004 [99]

Cost type	Wheat			Sugar beet		
	€/L	€/GJ	€/ten	€/L	€/GJ	€/ten
Feedstock	0.40	18.9	790	0.26	12.3	513
Gain from byproducts	0.15	7.1	296	0.03	1.4	59
Total feedstock costs	0.25	11.8	493	0.23	10.9	454
Conversion	0.28	13.3	553	0.22	10.4	434
Blending with gasoline	0.05	2.4	99	0.05	2.4	99
Distribution costs	0.01	0.5	20	0.1	4.7	197
Total costs	0.59	27.9	1165	0.6	28.4	1184

wheat and triticale for bioethanol production in recent several years. The research has been conducted through TR 18002 project supported by Serbian Ministry of Science and Technological Development. The research encompassed the most of the relevant parameters and progress trends of this paper such as: a) the choice of suitable domestic corn hybrids, b) choice of the yeast strain for bioethanol production on corn, c) optimization of the pretreatment, hydrolysis and fermentation steps, d) use of immobilized yeasts, e) choice of suitable process flow sheet, f) adequate valorization of the stillage for the production of functional animal feed with a probiotic activity, etc.

Among 5 tested domestic corn hybrids (ZP-341, ZP-434, ZP-505, ZP-544 and ZP-704wx) which were produced in the Maize Institute - Zemun Polje, the highest ethanol yield of 90.2% was obtained after 34 fermentation hours for ZP-434 (Figure 9) [100]. Maximum ethanol concentration on this hybrid of 9.56% (corresponding to 95.6% of the theoretical yield) was achieved after 34 fermentation hours. The starch content of this hybrid was about 70% and compared to other hybrids it had the lowest oil content, density, flotation index and milling response. This ethanol content was achieved in a batch SHF process and further improvements could be expected in SSF process. Mojović *et al.* [98] attained higher process productivity, reduced overall processing time and lower energy consumption in SSF process on commercially available corn meal compared to the parameters achieved in SHF process.

Since many reports confirmed lower productivity of bacteria *Z. mobilis* in ethanol fermentations on corn and pointed out that the yeasts are still remaining the major ethanol producers, Rakin *et al.* [101] tested several yeast strains from *Sacccharomyces* genera: *S. cerevisiae, S. cerevisiae var. ellipsoideus, S. carlsbergensis* and *Schizosaccharomyces pombe*. The best results regarding ethanol yield were obtained using *S. cerevisiae var. ellipsoideus* (Figure 10). Similarly, Okunowo and Osuntoki achieved superior ethanol production by using this yeast for wine fermentation compared to the production obtained with *S. cerevisiae* and *S. carlsbergensis* [102]. The *S. cerevisiae var. ellipsoideus* yeast was immobilized in order to reach higher ethanol tolerance and even-

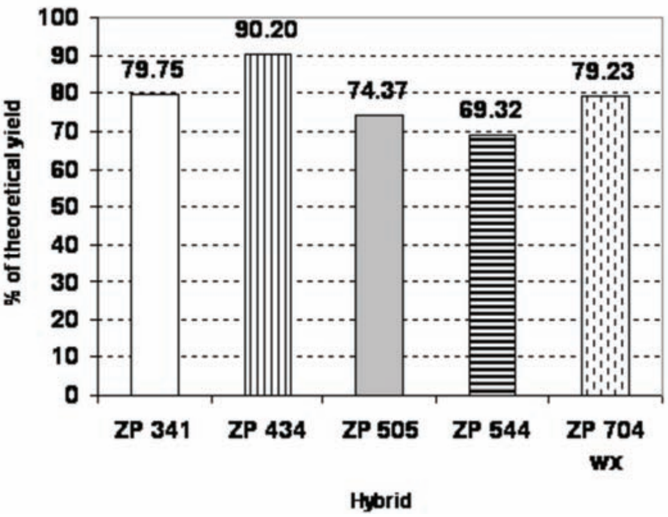

Figure 9. Ethanol yield (in % of the theoretical yield) achieved after 36 h of the fermentation of samples of corn meal hydrolysates [100].

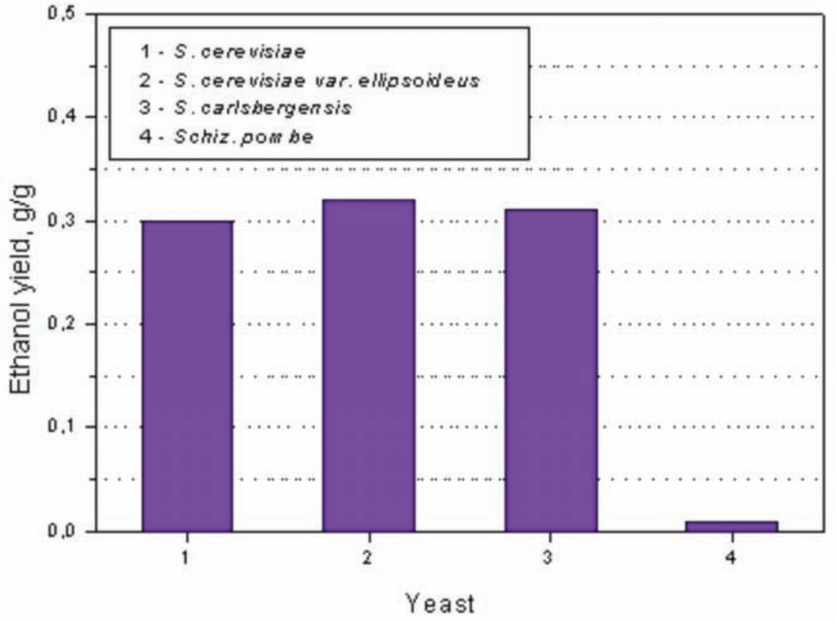

Figure 10. Ethanol yield obtained by fermentation of corn meal hydrolysates with different yeasts. Process conditions: Hidromodul 1:3 pH=5.0, 30 °C, τ=48 h , mixing rate 100 rpm, initial glucose concentration ~150 g/L, initial viable cell number ~2.5×10^7 CFU/mL provided by 2% (by volume) of yeast [101].

tually enable reuse [48,79–90]. For this purpose Rakin *et al.* applied immobilization in two biocompatible polymers such as: PVA and Ca–alginate [90] demonstrating that the PVA exhibited better mechanical properties and stability in repeated use, while Ca-alginate immobilized yeast gave higher ethanol yields, most probably due to lower mass transport restricttions. The best results regarding ethanol yield and productivity were achieved by combining the effect of media supplementation, yeast immobilization in Ca–alginate and utilization of the SSF process [48,79]. The most appropriate yeast activators for immobilized cell system were mineral salts (10 mM of Mg^{2+} and 1 mM of Zn^{2+}) which caused an increase in ethanol concentration for 8.60% over the control samples without the supplementation [48]. In this case, ethanol concentration of 10.23% (w/w) was achieved after 48 h of the process. Addition of magnesium and zinc contributed to the achievement of high productivity of the batch SSF of corn meal, while still preserving a physical and chemical stability of Ca-alginate gel beads.

Many recent studies have shown that pretreatments such as microwave heating [72,103] and ultrasound [74,77,103] influence the process of swelling and gelatinization of corn starch granules and thus could be very efficient in destroying the corn starch crystalline arrangement and obtaining a soft gel. These phenomena could also enhance the enzyme susceptibility needed for the efficient hydrolysis, which may later on improve the outcome of ethanol fermentation. The important parameters that should be optimized when applying these treatments are temperature, power, the length of treatment and its dynamics. Since these treatments may be energy consuming, the obtained benefits should be considered and compared with the increased production costs. Table 5 compares the effects of the microwave and ultrasound treatment achieved in ethanol fermentation of corn meal hydrolyzates under optimized conditions [103]. It can be seen that higher ethanol concentrations during the fermentation were realized and superior process productivities (P) and feedstock utilizations measured by ethanol yield on starch as a substrate ($Y_{P/S}$). When compared to the control without pretreatment, the ethanol concentration was increased for 13.4% by microwave treatment and for 8.8% by ultrasound (Table 5). SEM photographs of the suspension of corn meal before and after microwave and ultrasound treatment are presented in Figure 11 (Mojovic *et al.*, unpublished data).

Bioethanol production from wheat and triticale

Wheat and triticale are a good raw material for the bioethanol production. However, especially wheat is traditionally utilized for food purposes. Recently, Pejin *at al.* have tested four wheat (NS 40S, Dragana, Rapsodija and Renesansa) and triticale (Oganj, Jutro, Odisej and NST 21/06) varieties from the Institute for Crops and Vegetables (Rimski Sancevi locality), Novi Sad (Serbia) [52,56]. All of these varieties displayed significant autoamylolytical quotient which ranged

Table 5. Comparison of significant process parameters obtained after 32 hours of SSF of corn meal hydrolyzates with S. cerevisiae var. ellipsoideus yeast under optimized conditions of microwave and ultrasonic treatment [103]

SSF of corn meal hydrolyzates with *S. cerevisiae* var. *ellipsoideus*	Ethanol, mass%	Theoretical ethanol yield[a], %	$Y_{P/S}$ g/g	P g /L·h
With microwave treatment	9.91	92.27	0.52	3.01
With ultrasonic treatment	9.51	87.48	0,50	2.97
Control sample (without treatment)	8.74	81.38	0.46	2.73

[a]The yields were calculated based on starch content: 76.75% (w/w)

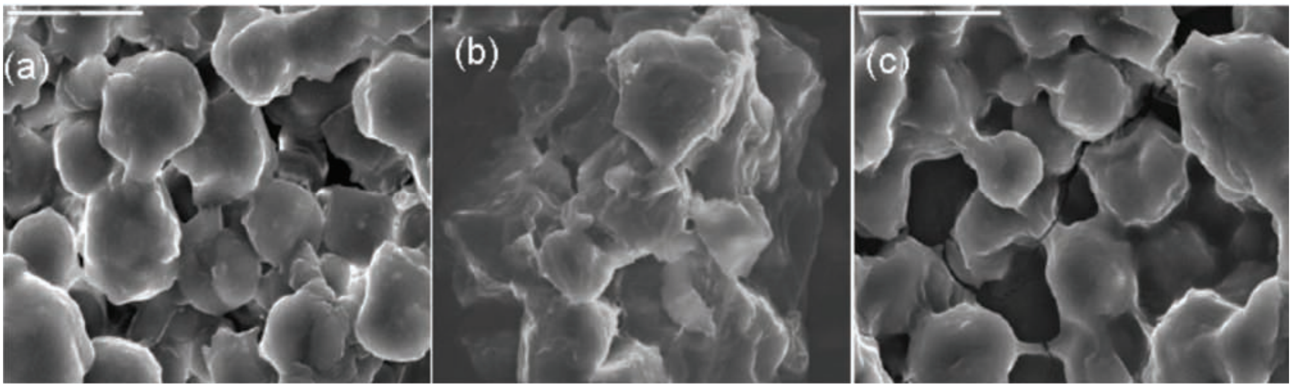

Figure 11. SEM photographs of corn meal suspension: Control sample without treatment (a); the sample treated with microwaves of 80W for 2.5 min prior enzyme addition (b); the sample treated with ultrasound of 40 kHz frequency for 2.5 min before enzyme addition (c). The SEM magnification was 500× (Mojovic et al., unpublished data).

from 62.15 to 81.46% for wheat and 94.24-99.30% for triticale varieties. Among wheat the most suitable was found to be a wheat variety Renesansa since it could degrade 84.1% its starch content .The most suitable triticale varieties for ethanol production were Odisej and Oganj, also due to high autoamylolytical quotient (99.55 and 99.30%, respectively) implying that these varieties have a sufficient amount of amylolytic enzymes to degrade native starch. It should also be emphasized that the triticale varieties required preparation at 60 °C whereas the wheat varieties required somewhat higher temperature of 65 °C (which is still lower than the temperatures employed in corn pretreatment). Lower temperatures during the preparation and the absence of need to utilize technical enzymes make the use of triticale as a substrate for bioethanol production more advantageous. The savings in energy and technical enzymes has been reported by several authors [52,104].

In addition to low pretreatment requirements, triticale shows a number of advantages for the grower. The main distinguishing features are as follows: higher grain yield even in unfavorable conditions, higher test weight, resistance to soil-climatic conditions, tolerance to dryness, tolerance to more acid soils and a lower requirement of nutrient substances. Also, it does not need as much fertilizer when compared to types and varieties providing the same yields [53]. Triticale has a lower susceptibility to diseases and pests which attack rye and wheat and this reduces the necessity of chemical protection against harmful agents [105].

The authors of this paper have recently studied the possibilities of degradation triticale starch by ultrasonic pretreatment which could possibly allow utilization of even lower temperatures than 60 °C [106]. For these purposes the Odisej variety, previously proven as suitable for ethanol fermentation, was chosen. Triticale was first ground and mixed with water at 1:4 hidromodul. The influence of ultrasound on triticale starch was analyzed by using a 2.5 min ultrasound treatment followed by a thermal treatment at various temperatures. After the ultrasound treatment, the suspension was kept in a water bath at temperatures of 40, 50 and 60 °C for one hour, cooled to 20 °C and analyzed for sugar content. The results are presented in Table 6. It can be seen that the amount of fermentable sugars such as glucose, maltose and maltotriose increased by ultrasonic treatment compared to untreated control sample, especially with the temperature increase. The highest content of individual sugars and total fermentable sugars were achieved in ultrasound treated samples at 60 °C. However, it can be seen that the ultrasound treated samples which were subsequently held at 50 °C superseded the total fermentable sugar content and individual sugar con-

Table 6. Content of sugars and amylolytic quotient obtained in triticale suspensions (hidromodul 1:4) treated by ultrasound (2.5 min) ano kept at various temperatures for 1 h (Pejin et al., 2009, unpublished data)

Treatment conditions		Sugars, % on dry matter			Fermentable sugars, % on dry matter	Amylolytic quotient, %
		Maltotriose	Maltose	Glucose		
30 °C	With ultrasound	2.11	32.46	3.51	51.82	86.21
50 °C	With ultrasound	3.05	35.11	2.80	52.20	86.84
60 °C	No ultrasound	2.74	33.65	1.51	51.50	85.67
	With ultrasound	2.89	35.72	2.65	52.70	87.67

tents (maltose, trioses and glucose) of the untreated control held at 60 °C. Further advantages of this treatment are expected to be proven by improved ethanol fermentation in the experiments which are currently being performed.

Utilization of byproducts from bioethanol processing on cereals

Generally, the amounts and types of byproducts which could be obtained from bioetahnol production on cereals depend on the type of the process employed. Basically, two different processes can be used to produce ethanol from starch crops: dry milling and wet milling. The wet milling process has the capability to produce various end products and considerable higher process flexibility, compared to the dry milling [107]. Despite that fact, currently, about 65% of the ethanol in the US is produced from dry milling corn processing plants [108].

Major byproducts of bioethanol production from dry milling process are carbon dioxide and stillage. An average stillage amount produced in the bioethanol process is approximately 13 hL per hL of bioethanol [13,109]. In the USA, around 85% of the liquid stillage has been dried together with spent grains to produce dry distiller's grains with solubles (DDGS) which are being used as animal feed. In Europe the most of the stillage for animal feed is used in wet form because the drying itself is a costly process which requires a lot of energy [110]. In the majority of industrial facilities in Serbia, the bioethanol byproducts have not been utilized posing therefore a hardly solvable environmental problem. The complex composition of stillage causes high BOD_5 values which range from 15–340 g/L. [109].

There are many possibilities for valorization of stillage from bioethanol processing. Some of them are the stillage recirculation and reuse [13,110,111], production of soil fertilizers [110], anaerobic fermentations for the production of lactic acid or butanol [112,113] and the production of various types of animal feed [13,113,114].

Pejin *et al.* [109] investigated a possibility of thin stillage recirculation in the mashing process in order to decrease the amount of stillage and water used in the production of ethanol from maize and evaluated various process parameters such as a fermentation rate, a bioethanol yield, and the content of solids in stillage after distillation. It was shown that as the amount of recirculated stillage increased (from 10 to 30%) higher bioethanol yields and starch utilization efficiencies were observed. The ethanol yield was increased from 97 to more than 100%, which could be explained by the fact that stillage enriched the slurry with amino acids, vitamins and the products of yeast cells degradation. The dry matter content in the slurry after the fermentation also increased with the increasing amount of recirculated stillage. The highest dry matter content (9.40%) was determined in the slurry after the fermentation obtained in the sixth cycle with 30% stillage recirculation. The dry matter remained after filtration of the slurry could be used as a cattle feed because of its high total protein content.

Rakin *et al.* [113] analyzed a chemical composition of the stillage obtained as a byproduct from ethanol fermentation of corn meal and corn flour hydrolyzates and compared their quality with animal feed mixes available on the market. By following the chemical compositions of corn meal and corn flour samples before the hydrolysis, after hydrolysis and after the fermentation, it was concluded that nutritive values of the samples were improved after the fermentation compared to the values of the initial raw materials. The most evident was an increase in the protein content from 6.35% in the initial corn meal to 35% in corn meal after fermentation, while enrichment in cellulose, phosphor and some minerals was also noticed. Generally, the stillage obtained from corn meal was of better quality than that of corn flour; it had slightly higher protein content and the content of minerals such as Cu, Zn, Fe and Na.

A higher added-value could be obtained by the production of lactic acid or functional animal feed on the stillage. The lactic acid obtained by anaerobic fermentation of the liquid stillage could be used for the production of biodegradable polymers (poly-L-lactic acid) or for food preservation. By enrichment of the stillage remained after fermentation of cereals with different strains of probiotic lactic acid bacteria (LAB) high quality feed may be obtained [111,115]. This kind of feeding may enhance the health of cattle and eliminate using antibiotic as additives. Another approach to obtain high quality feed is the stillage enrichment with yeasts with probiotic activity, or carrying out the ethanol fermentation with such yeast types [116]. The selection of LAB suitable for feed production should be done according to the following criteria: a) inhibition of growth of pathogens; b) survival of low pH values; c) survival in the presence of 0.3% bile salts. The latest two criteria are the conditions which are present in the intestinal tract of ruminants. According to these criteria, Vukašinović *et al.* [111] selected the strains *Lb. fermentum* PL-1, *Lb. pentosus* NRRL B-2217 and *Lb. plantarum* PL-4 for the production of feed with probiotic activity among twelve tested strains.

CONCLUSIONS

Bioethanol produced from renewable biomass such as sugar, starch, or lignocellulosic materials, is expected to be one of the dominating renewable biofuels in the transport sector within the coming twenty years. Although the priority in global future in the ethanol production is put on lignocellulosic processing, which is considered as one of the most promising second-generation biofuel technologies, the utilization of lignocellulosic material for fuel ethanol is still under improvement. Sugar-based and starch-based feedstocks are still currently predominant at the industrial level and they are so far economically favorable compared to lignocelluloses. In Serbia, one of the most suitable and available agricultural raw material for the industrial ethanol production are cereals such as corn, wheat and triticale. In addition, surpluses of these feedstocks are being constantly produced in our country. The choice of suitable and abundant raw material is of great importance since the feedstock cost represents a major part of the production cost.

A significant progress and enhancement of the economy of the bioethanol production on starch-based raw materials may be obtained by the optimazation of feedstock pretreatment and ethanol fermentation itself, and by an adequate utilization of the process byproducts. The introduction of new pretreatments such as microwave and ultrasound can improve the starch gelatinization process, the substrate susceptibility to enzymes and greatly influence and improve the effects of hydrolysis and subsequent ethanol fermentation. In the domain of fermentation, the choice of the production microorganism, media optimization, and the choice of the most appropriate process flow sheet (simultaneous saccharification and fermentation, utilization of immobilized yeasts, etc.) are significant for the development of an efficient production process.

In the domain of the utilization of byproducts, thin stillage recirculation or its fermentation to produce lactic acid, and the stillage utilization for the production of high value feed with probiotic activity are the ways which could substantially decrease the production costs.

Acknowledgements

This work was funded by the Serbian Ministry of Science and Technological Development (TR 18002).

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