Investigation of whey protein concentration by ultrafiltration elements designed for water treatment

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Abstract

The suitability of polysulfone ultrafiltration membranes (UFM) designed for commercial water treatment has been investigated for separation of protein (PR) from sweet whey. Ultrafiltration (UF) of whey originated from dairy has been realized by a self-made pilot plant, which has been in service for about one year. The influence of two whey temperatures (9 and 30 °C) on the efficiency of protein concentration has been examined. Application of investigated UF elements gave whey protein concentrate (WPC) with 5 to 6 times excess amount of protein content compared to the initial. At the same time, the prevalent content of lactose was removed to permeate. Better results were obtained with cold whey filtration. Besides the fact that the molecular weight cut-off (MWCO) of the investigated membranes was 50–100 kDa, the results showed very successful concentrating of whey proteins of dominantly lower molar weights than 50–100 kDa. Investigated membranes are beneficial for design and construction of UF plants for exploitation in small dairies.

Keywords: Ultrafiltration membranes for water treatment, whey protein concentration, temperature influence.

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Cheese whey contains significant amount of proteins, lactose (L) and minerals that can be extracted and reused in food industry and biotechnologies. Water also presents great percentage of cheese whey, which can also be separated and reused in a function of dairy wastewater purification hence leading to environmental protection [1]. Purifying dairy wastewater has been investigated using membrane processes such as reverse osmosis [2], nanofiltration and ultrafiltration [3,4] as well as coagulation and adsorption [5].

The β-lactaglobuline (β-LG) and α-lactalbumin (α-LA) are the two biggest protein fractions in cheese whey. These fractions, together, account for 70 to 80% of total proteins in whey, but concentration of β-LG is twice that of α-LA. The molecular weight (MW) of α-LA is 14 kDa and MW of β-LG (as dimer) is 36.8 kDa [6]. The rest of the whey proteins are serum albumin in percentage of ≈10% with MW of 69 kDa and immuneglobulin (MW 160–1000 kDa) with share of ≈10% [7].

Extracting proteins from whey by membrane filtration has been investigated in previous studies using different types of membranes, such as ceramic microfilters combined with polyetersulfone UFM [8–10] and tangential flow filtration modules [11,12]. The fractionation of whey into lactose enriched and protein-enriched streams using UFM of regenerated cellulose materials has also been investigated [13]. More research was done using commercial UFM designed for the separation of protein from milk [14–18]. The contribution of tubular ceramic membrane research [19,20] to study the mechanism of separation of protein from whey is also very significant. There have also been recent investigations on the removal of whey proteins using ultrafiltration membranes with molecular weight cut-off (MWCO) of 50–100 kDa [6].

Whey protein is concentrated by ultrafiltration on a daily basis in many industrial plants in the world. The goal of WPC separation from whey is to obtain a solution rich in protein that can be used to get various types of cheese [21], such as ricotta [22], cheddar [23] and white cheese [24]. By drying of WPC is obtained whey powder, which is an excellent additive in food products [25]. Also, whey protein can be disassociated to building components that are used for sophisticated applications [10]. UF permeate is practically used to obtain the crystalline lactose [26], or enzymatic digestion of lactose to monosaccharides gets a sweet solution to substitute the water and sucrose and is a great base for manufacturing the entire range of soft drinks rich in natural minerals derived from milk [27].

Industrial ultrafiltration takes place on specially designed flat-sheet membranes at relatively high transmembrane pressure (from 2.5 to 5 bar). Special UF membranes for filtration of milk and whey are differ from UF ultrafiltration membranes designed for water, in that they do not contain anti-telescoping caps and that their diameters and lengths are made according to special standards. These standards include the most common sizes of Ø 3.8"×38" length and Ø 8"×38" and

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require special housing. Since the production of UFM for treatment of whey is in significantly smaller amounts than those for the ultrafiltration of water, the cost of making devices for filtration of UF whey special membranes are significantly higher. In addition, it is quite complicated (complex) to obtain special UFM with housings, but the world's leading manufacturers insist on a purchase a complete UF device.

Due to their whey content, dairy wastewaters are significant polluters with BOD5 content of several thousand to tens of thousands mg O_2/L. Small dairies processing up to 50000 L of milk a day, due to the high cost of equipment for ultrafiltration, are not able to obtain specialized equipment designed to extract the proteins from whey. Currently, the unprocessed whey is being discharged as waste in most dairies in Serbia, directly affecting the environment. Thus, one of the goals of this work was to design cheaper equipment that can be used in small dairies across Serbia.

The aim of the investigation was to determine the ability of whey protein concentration using commercial UFM provided for ultrafiltration of water. A UF pilot plant was designed and built in the company "Envirotech", Kikinda, Serbia, and installed in the dairy "Kikinda industry of milk", part of the French Bongrain group in Kikinda, Serbia. This dairy processed daily up to 50000 L of milk to semi-hard cheeses (Gouda, Edam and Trappe). The formed part of the sweet whey was used in experiments at the pilot unit. Defining the technical design of pilot devices and basic investigation of the potential concentration of whey protein lasted for two years. In the third year, a series of studies were conducted with hot and cold ultrafiltration of whey. The obtained WPC was used daily in a period of six months in dairy production of ricotta and mixed with the milk for the production of semi-hard cheeses.

MATERIALS AND METHODS

Design of the pilot plant and operating conditions

The pretreatment of whey before entering the collection tank (WM, Figure 1) consisted of centrifugal separation of milk fat and dispersed particles, as well as temperature settings. The operations were carried out by existing equipment of the dairy. In accordance with the experimental procedure, the whey temperature was 9 °C – cold whey (CW) or 30 °C – hot whey (WW). The average concentration of milk fat in whey after the milk fat centrifugal separation and the average pH was 0.04% and 6.7, respectively. Commercial spiral wound UFM designed for ultrafiltration of water (Woongjin Chemical Co., Ltd., Korea) was investigated. The UFM characteristics are shown in Table 1. The pilot plant contained two serial connected UF membranes.

The operating regime of the pilot unit was run by a programmable controller ZEN (Omron, Japan). Before the start of whey UF, back-flushing of UFM using demineralized water automatically took place for 2 min. Then the process of cross-flow filtration of whey was initiated using pumps CP1 and CP2 and MF (Figure 1).

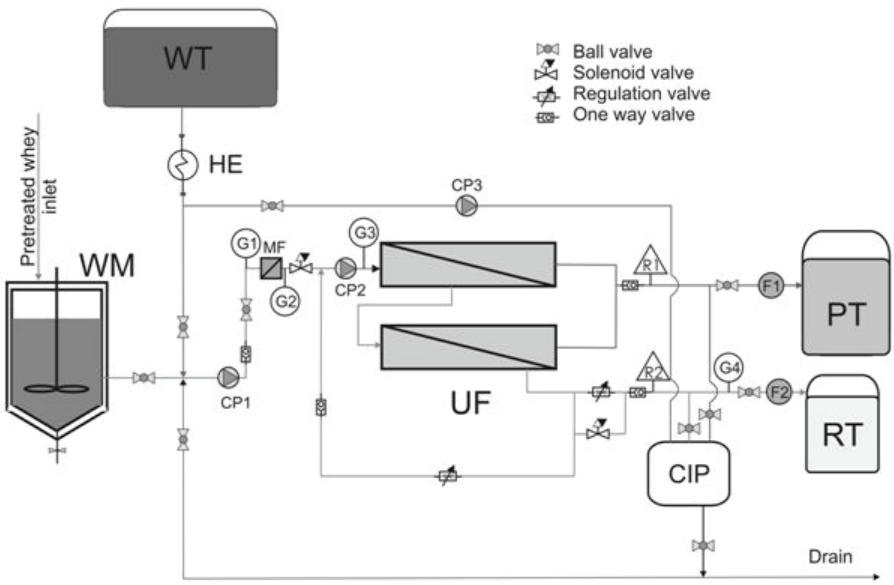

Figure 1. The flow sheet of an ultrafiltration pilot plant; WM – thermally insulated tank with a mixer for whey; WT – tank with demineralized water; HE – heat exchanger; MF – polypropylene microfilter of 5 µm; UF – ultrafiltration modules; PT –tank of permeate; RT – tank of retentate; CIP – vessel for the storage of the solutions for UF membrane cleaning; CP1 – feed pump; CP2 – booster pump of UF modules; CP3 – pump for demineralized water transport; R1 – continuous flow meter of permeate, R2 – continuous flow meter of retentate; G1/2 – pressure gauges before and after MF ; G3/4 – pressure gauges before and after the membranes; F1 – cumulative permeate volume meter; F2 – cumulative retentate volume meter.

Table 1. Characteristics of UF membrane

Type	Material	Configuration	Dimensions Ø/mm×H/mm	Molecular weight cut off, kDa	Membrane area, m^2	Permeate flow rate, m^3/h	Average flux L/(h m^2)
Homogenous asymmetric flat sheet	Polysulfone (PSF)	Spiral-wound, FRP wrapping	203×1,016	50–100	33.9	2.2	64.90

Upon termination of service modes, the membranes were again back-flushed with water for 2 min. Permeate from the UF unit was collected in the tank, PT, and retentate was stored in the reservoir, RT. Part of the retentate through the valve inlet mixed with whey.

During each test series, the following operating conditions (OC) of the pilot device were measured: pressure before and after MF and UF, permeate and retentate flow rates.

Sampling of whey from tank WM was performed at the beginning of each investigated batch. After each series with CW and WW, permeate and retentate volumes were measured and permeate and retentate aggregate samples were taken from the tanks PT and RT. In all samples, the contents of chemical composition parameters (CP) of whey, permeate and retentate: temperature (T), protein, lactose and total solids (TS) were measured (Table 2).

The first 10 series of WPC separation were carried out by filtration of CW. The following 10 series were dedicated to concentration of proteins from WW. For the duration of the filtration every series contained 10 check points of operating parameters measurements and sampling of permeate and retentate.

Processes of UFM washing and cleaning with water and chemicals were manually operated. Centrifugal pump CP3 supplied water for solutions preparation from the tank WT. In the CIP container different cleaning solutions were formed, which were circulated in a closed cycle using centrifugal pump CP1 through the membranes back into the CIP container, at a pressure of 2.5 bar and flow rate of 2000 L/h. The membranes were washed in the first phase with hot water (at 50 °C) for 20 min. Then came the enzymatic cleaning solution by combining of Ultrasil 67 and 69 [28], at a temperature of 55 °C for 20 min. The membranes were again washed with hot water (at 50 °C) for 10 min and then treated using Ultrasil 11 [29], at 50 °C for 60 min. The final washing of the membranes was done with cold water at 18 °C for 30 min.

Physicochemical methods and analytical instruments used in the sample analyses

For the investigated whey, permeate and retentate, general quality parameters, such as fat, protein, lactose and TS were measured on a MilkoScan Minor instrument (Foss, Denmark) by photometric analytical methods [30]. Control values of these parameters were obtained by analysis of ten random samples of whey, permeate and retentate in an accredited laboratory Sojaprotein (Bečej, Serbia). The measured values using MilkoScan differed from values obtained in the accredited laboratory by ±1.1%.

Calculation of the cross-flow filtration parameters

Normalized differential pressure NDP (bar) as a function of permeate and retentate flow rates was calculated by the following equation:

$$NDP = \Delta p \frac{\left(2Q_{R0} + Q_{P0}\right)^{1.5}}{\left(2Q_R + Q_P\right)^{1.5}} \tag{1}$$

where Δp – differential pressure on UF membranes; Q_{R0} – initial retentate flow rate (L/h); Q_{P0} – initial permeate flow (L/h), Q_R – retentate flow rate (L/h), Q_P – flow of permeate (L/h).

The flux - flow of filtrate per unit area of membrane was calculated as follows:

$$J = \frac{Q_p}{A_m} \tag{2}$$

where J (L/h/m^2) – flux, $\mathbf{A}_m$ (m^2) – surface of the UFM.

Temperature compensated specific flux TCSF defines the permeability of the membrane depending on

Table 2. The results of measurements of whey and UF effluents parameters

Sample	*T* / °C	Protein, %	Lactose, %	*TS* / %	Volume, L
Whey	9	1.03	4.51	5.99	11,657
Retentate		5.22	4.41	11.79	1,199
Permeate		0.21	3.79	5.78	10,458
Whey	30	0.99	4.12	5.33	4,460
Retentate		5.24	4.68	12.03	424
Permeate		0.21	3.65	5.67	4,036

the transmembrane pressure TMP and whey temperature (°C). TCSF indicates the chemical degradation or membrane fouling:

$$TCSF = \frac{J}{TMP} e^{\left(-0.031(T-20)\right)} \quad (3)$$

The yield of protein, PY, in the retentate was calculated from the ratio of protein content in the retentate, C_r (%), and whey protein content, C_0 (%), by the expression:

$$PY = \frac{C_r}{C_0} \quad (4)$$

Rate changes of NDP – RC_{NDP} (bar/min), during the UF process is calculated as follows:

$$RC_{NDP} = \frac{\left(NDP_f - NDP_s\right)}{t} \quad (5)$$

where NDP_f (bar) – NDP in the final point of UF; NDP_S (bar) – NDP in the starting point of UF; t (min) – lasting time of UF.

Rate change of TMP – RC_{TMP} (bar/min) during the UF is calculated as:

$$RC_{TMP} = \frac{\left(TMP_f - TMP_s\right)}{t} \quad (6)$$

where TMP_f (bar) – TMP in the final point of UF; TMP_S (bar) – TMP in the starting point of UF; t (min) – duration of UF.

Membrane efficiency, η (%), was calculated as:

$$\eta = 100\left(1 - \frac{C_p}{C_0}\right) \quad (7)$$

RESULTS AND DISCUSSION

During the course of the cold filtration of whey, microfilter MF differential pressures were equably about 1 bar. The range of inlet and outlet pressures was from 7 to 6 bar. Inlet and outlet pressures of membrane elements were in the range of 9.5 to 9.8 bar and 8.5 to 8.8 bar, respectively. UFM differential pressures were constant of 1 bar. The average duration of CW ultrafiltration series was 630 min.

UF testing of hot whey have played at inlet pressures of pump CP1 of 6.9 bar at the beginning to 5.8 bar in the end of process. MF pressure drops were from 0.9 to 1.4 bar. Cross flow filtration processes have carried out under booster pump CP2 pressures of 9.1 bar in the start to 7.9 bar at the final check point. Membrane elements differential pressures were in the range 0.6 to 1.1 bar. Separation of WW protein lasted 250 min in average.

Mean values of changes NDP, TMP and WPC depending on the duration of UF process are shown in Figures 2 and 3. Figure 4 presents the membrane efficiency for the removal of proteins with regard to flux change, while Figure 5 describes the L/Pr ratio during the ultrafiltration of CW and WW. Figure 6 shows the dependence of the mean values of flux and TCSF during the lasting time of the ultrafiltration process.

Figures 2, 3 and 6 show that the ultrafiltration of CW successfully took place in about 2.5 times longer period of WW cross-flow filtration.

All ultrafiltration experiments took place until the appearance of fouling. The beginning of fouling was followed by the decrease in flux with increasing of differential pressure (Figures 2 and 6). Fouling was manifested in significantly reduction of protein yield (PY) in the retentate (Figure 3). Using the above-described procedure, membranes were chemically and enzymatically cleaned 154 times during the six months. After each cleaning, the UFMs have renewed their flux, and the initial permeate flow. After last cleaning the total flux decline was 2.4% compared to the pre-flux of new membranes.

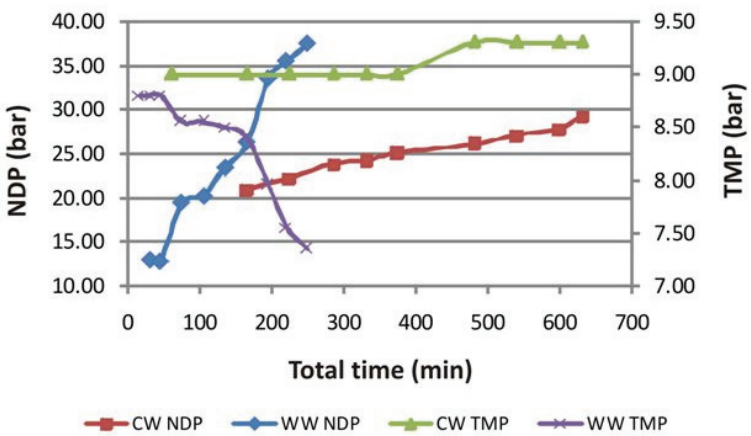

Figure 2. The differences between the changes of the NDP and TMP during the filtration of hot and cold whey.

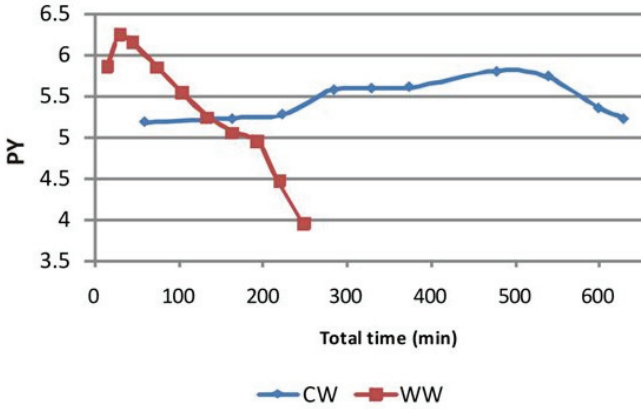

Figure 3. Changes of protein yield during the lasting time of filtration.

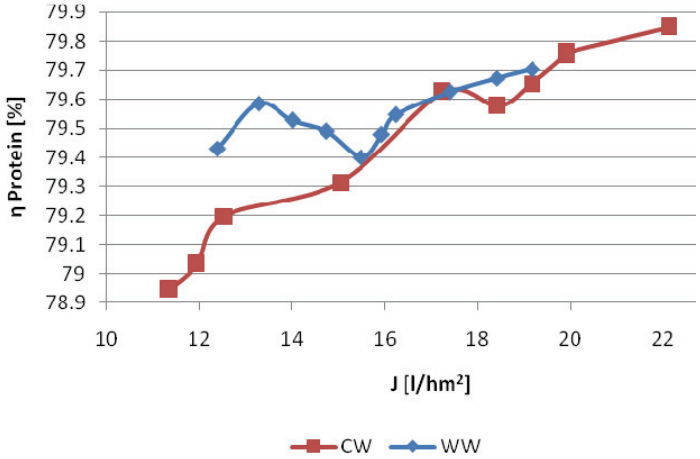

Figure 4. Protein removal membrane efficiency vs. flux during the the CW and WW ultrafiltration.

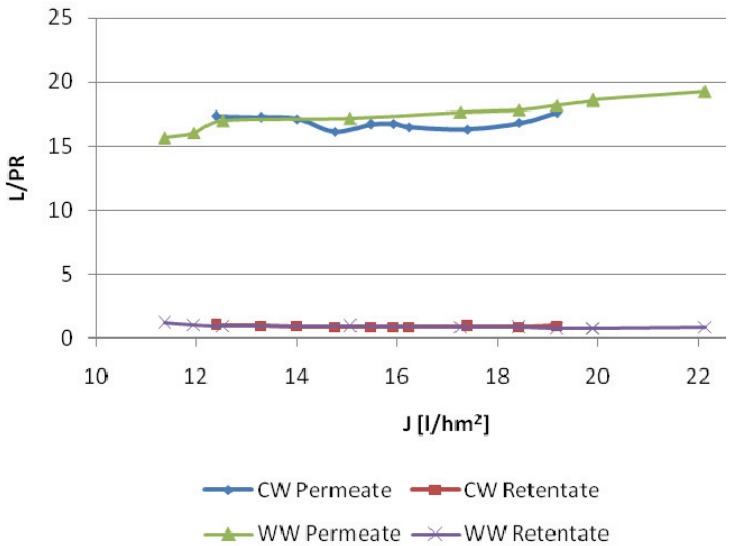

Figure 5. Lactose and protein ratio with regard to flux change.

During the CW and WW ultrafiltrations, the volume of obtained retentate was 10.29 and 9.51%, respectively, compared to the volume of incoming whey.

For the duration of the process of protein separation from the CW, *TMP* increased very slightly with a mean rate of RC_{TMP} = 5.3×10^{-4} bar/min. In CW filtration *NDP* also grew over the time at a mean rate of RC_{NDP} =

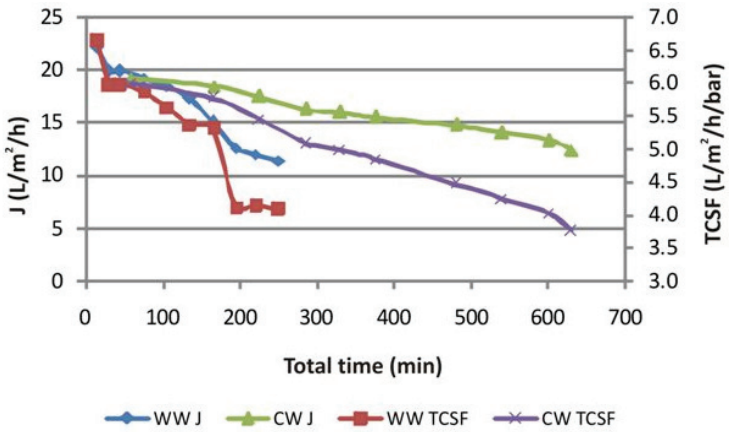

Figure 6. Comparison of flux and TCSF for the duration of the CW and WW ultrafiltrations.

= 0.015 bar/min. UFM differential pressure was constant during the course of UF. *TMP* was almost constant and the difference between the *NDP* from the beginning and end of the process was 8.28 bar.

During the filtration of hot whey, *NDP* sharply increased in a short period at an average rate of RC_{NDP} = = 0.11 bar/min, while reducing the *TMP* of approximately $RC_{TMP} = -7\times10^{-3}$ bar/min.

Test series with WW were characterized by anomalies that with increase of the *NDP* the *TMP* decreased. The difference in differential pressure of UFM during WW filtration of 0.5 bar produced a large *NDP* difference of 24.6 bar, which originated as a consequence of decrease of the flow of permeate and retentate (Eq. (1)). These results mean that the UFM are more permeable at 30 °C than at the temperature of 9 °C. Increased permeability at the hot process of UF caused faster flux reduction followed by growth of the *NDP*, which are a consequence of fouling.

The protein content in the retentate during the ultrafiltration of CW through the cycle was 5 to 5.8 times higher than in the starting whey. In CW ultrafiltration WPC reaches a maximum value of the *NDP* of 26 bar at 480 min of filtration. At the beginning and end of the process yield of protein is approximately equal. During CW filtration mean protein content in the retentate is 5.32 times higher than in whey.

Concentration of protein from WW characterized by a rapid decrease in protein yield decreased in direct proportion to *TMP*. With an increase of the *NDP*, *PY* for 250 min reduced 1.6 times compared to the start of UF process.

As can be seen from Figure 4, protein removal membrane efficiency was extremely high and constant which was documented by average η values of 79.47 and 79.54% in WW and CW, respectively. Obtained results testify that investigated membranes can be used, with great efficiency, for removal of proteins from whey. The protein removal membrane efficiency curve, during the experiment with CW, exhibits great linear correlation (R^2 = 0.964) while η protein curve in experiment with WW has an uneven trend during the flux change (R^2 = 0.506).

Ratio of lactose and protein contents in the whey amounted 4.38. In the course of ultrafiltration this ratio has been remarkably changed. The L/PR ratios have been calculated to obtain the results of transition effects of lactose and protein during the ultrafiltration. Figure 5 shows low L/PR ratios for retentates and high L/PR ratios for permeates. The average L/PR ratios in permeate and retentate were found to be 3.82 and 4.01 times higher and 4.87 and 4.92 times lower in CW and WW, respectively, than that in the inlet whey. Thus, lactose was prevalent in permeate and protein was dominant in retentate.

During the time course of protein separations with increasing NDP, TCSF and flux declined at both operating temperatures, with almost linear decrease in the separation of proteins from the CW. Reduction in flux and TCSF in WW investigation was in relation to the duration of the filtration 4 and 2.78 times larger, respectively.

CONCLUSION

Ultrafiltration membranes designed for water filtration have been successfully applied to whey protein concentrating. The average fluxes of CW and WW ultrafiltration were 16.77 and 15.71 L/hm^2, respectively. These *J* values were 3.87 and 4.13 times lower than the average flux of investigated UF membranes in the case of water ultrafiltration. The average volume of obtained retentate was 10 times smaller than the initial volume of whey. Ultrafiltration process is successfully taking place at relatively low trans-membrane pressures in relation to TMP that are necessary for the functioning of special membranes designed for ultrafiltration of whey. Cross-flow filtration of whey cooled to 9 °C is more efficient with obtained equal yield of protein in the retentate 5.5 to 6 times higher than the protein

content in whey. At the same time, the prevalent content of lactose was removed to permeate. The duration of the ultrafiltration of more than 10 h before fouling appearance and the downtime for membrane cleaning is practically very acceptable.

In order for the process of protein concentration to last at least 10 h with a steady yield of protein in the retentate, it is necessary to provide a constant differential pressure on the UFM and almost equal *TMP* in the process. The slight decrease of permeate and retentate contributes to a small difference in the *NDP* during UF.

Ultrafiltration of hot whey at 30 °C was able to run at most 4.5 h before the emergence of fouling and rapid decline in the yield of protein.

Besides the fact that MWCO of investigated membranes were 50–100 kDa, the results showed very successfully concentrating of whey proteins of dominantly lower molar weights than 50–100 kDa. These results are similar to the recently published results [6]. It is known that there are many factors affecting membrane-based process, like type of membrane and its *MWCO*, *TMP*, temperature of operation, feed pH and so on, which are important operating variables that influence process efficiency. The scope of the further investigations will be focused to finding explanations for the obtained phenomena.

Acknowledgments

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IZVOD

ISPITIVANJE KONCENTRISANJA PROTEINA SURUTKE ULTRAFILTRACIONIM ELEMENTIMA DIZAJNIRANIM ZA FILTRACIJU VODE

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Ispitivana je pogodnost ultrafiltracionih membrana (UFM) od polisulfona koje se komercijalno proizvode za tretman vode, za izdvajanje proteina iz slatke surutke. Ultrafiltracija (UF) surutke poreklom iz mlekare je izvedena pomoću pilot uređaja sopstvene konstrukcije u trajanju od oko jedne godine. Određivan je uticaj temperature surutke (9 i 30 °C) na efikasnost koncentrovanja proteina. Primenom ispitivanih UFM je dobijen koncentrat proteina surutke koji je sadržao 5–6 puta više proteina u odnosu na polaznu surutku. Istovremeno je veći deo laktoze izdvojen u permeatu. Bolji rezultati su dobijeni filtracijom hladne surutke. Pored činjenice da su ispitivane membrane imale MWCO od 50–100 kDa, dobijeni rezultati ukazuju na uspešno koncentrisanje proteina surutke čija je molarna masa najvećim delom manja od 50–100 kDa. Ispitivane membrane su pogodne za projektovanje i izradu UF postrojenja za koncentrovanje susrutke u malim mlekarama.

Ključne reči: Ultrafiltracione membrane za tretman vode • Koncentrovanje proteina surutke • Uticaj temperature