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Whey management based on bioreactor and membrane processes: clean technology gaining valuable components of whey

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ABSTRACT

Whey, the product after cheese production, accounts for 50%–60% of the dry milk matter. On average, 9 L of whey are generated from 10 L of processed milk. Since it contains significant amounts of sugar and protein, it is unfriendly to the environment because it causes excessive growth of microorganisms in water reservoirs. COD from the initial value of 80 g O_2/L must be lowered to 150 mg O_2/L before whey is discharged to the environment. Therefore, the purpose of the work was to isolate and concentrate those whey components that are the most valuable, that is, protein and lactose. A multi-stage membrane separation was used for this purpose. The first step was microfiltration (MF) for protein concentration at their retention coefficient of 0.88. In the permeate, there were mainly glycomacropeptides (GMPs). Further the concentration of whey proteins and elution of GMPs was done by an ultrafiltration (UF) process associated with diafiltration. The permeates obtained during MF and UF were directed to a nanofiltration process in the aim of lactose concentration. A *Bacillus licheniformis* strain degraded organic matter not recovered in concentrates was used during the final step of the technology. At a residence time of 30 h, the concentrations of an organic matter in the exit stream met the standards. This time could be significantly shortened (e.g., to 5–6 h) by application of a membrane bioreactor.

Keywords: Whey; Membrane filtration; Whey protein concentrate; Biodegradation; Lactose; *Bacillus licheniformis*

1. Introduction

Whey is a by-product from the dairy industry. Its world production based on dry weight is approximately 1.6×10^8 ton/y [1]. It is a liquid that contains a high amount of lactose and many different proteins. Therefore, whey requires management or utilization because as an effluent, it harms the balance in the aquatic environment. The organic matter concentration in wastewater should be reduced to approximately 1% w/v to meet the environmental standards in accordance with the Polish Regulation of the Ministry of Maritime Economy and Inland Navigation of July 12, 2019 [2]. Deep treatment generates costs, so much better is to processes whey into valuable products [3]. One of the main directions is the concentration of proteins. Their diversity, shown in Table 1 [4,5], affects the variety of their properties (Table 2). The function of some of them is well recognized, for example, β-lactoglobulin is a transporter of retinol [6], α-lactoalbumin can be used in the cancer prevention [7], serum albumin has an anti-mutagenic function [8]. Glycomacropeptides (GMP) are not typical whey proteins but peptide fragments (up to 105 amino acids) coming from κ-casein treatment during cheesemaking. Additionally, whey protein concentrate (WPC) is widely used as a nutritive supplement in the diet of athletes [9], as it influences muscle building.

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Table 1
Main proteins present in goat whey [4,5,10,11]

Protein	Average concentration (g L^{-1})	Molecular mass (kDa)
β-Lactoglobulin	4.0	18.3
α-Lactoalbumin	1.5	14.1
Serum albumin	0.6	66.3
Immunoglobulins	0.9	150–1,000
Lactoferrin	0.1	80
Glycomacropeptide	>2.0	<10

Whey proteins can be isolated using micro- or ultrafiltration membranes [22–25]. Sanmartin et al. [23] used a ceramic 10 kDa membrane to whey proteins concentrate 20 times. In the next step, vacuum drying was applied to obtain a protein powder. However, this procedure did not affect the quality of the whey proteins (in powder lactose and GMP were also present).

Kukucka and Kukucka [24] used the polysulfone membranes at cut-off 50–100 kDa that is much bigger than cut-off the membranes used in Sanmartin et al.'s [23] research. Application of investigated UF membranes has given WPC with 5–6 times excess amount of protein content in regards to starting one. At the same time, the prevalent content of lactose has been removed in permeate.

Ilchenco et al. [25] tested different membranes at cut-off in the range 10–100 kDa. The best results were obtained with the membrane of 50 and 10 kDa. With the membrane of 50 kDa, the protein retention was about three times higher than the membrane of 100 kDa. The concentrate obtained by UF membrane (10 kDa, 10°C and 2 bar) in laboratory scale showed a mean protein retention of 80%, greater protein solubility, emulsion stability and the identification of β-lactoglobulins (18.3 kDa) and α-lactalbumin fractions (14.2 kDa).

The described research aims to develop a coherent process of whey management. It includes micro-, ultra- and nanofiltration, partially supported by diafiltration for the concentration of organic matter. Two main products are obtained: the concentrated solution of protein (WPC) and the concentrated solution of lactose (LC). The separation of particular proteins from whey is not economically efficient because of their relatively low concentration. Much better is to obtain them from colostrum [22]. The final step is biodegradation of the side streams. Water recovery and the lack of the waste makes the technology environmentally friendly and can be called pure technology.

2. Materials and methods

2.1. Pretreatment

The subject of the conducted experiments was the whey obtained after goat cheese production (Kozia Laka, Poland). The concentrations of proteins/peptides and lactose in the crude medium vary seasonally in the ranges of 9–14 and 39–45 g L^{-1}, respectively.

To eliminate residues of casein clots and fat, in the first step the whey was centrifuged (Hettich Zentrifugen Universal 320R, Germany) at 9,000 rpm, 4°C for 20 min. Then, $CaCl_2$ was added to the whey, according to the procedure described in the literature [26]. Next, a pH of 7.3 was set using 6 M NaOH. Then, the medium was heated to 55°C and held at this temperature for 8 min. After being cooled, the suspension was centrifuged at 9,000 rpm for 20 min.

2.2. Two steps membrane filtration: micro- and ultrafiltration

The membrane units were manufactured by PolyMem Tech (Warsaw, Poland). In microfiltration, the steel membrane module was fitted with the symmetric, seven-channels tabular, ceramic membrane of 0.14 μm pore size, surface 0.75 m^2 and diameter 10 mm (Filtanium, Tami Industries, France). The ultrafiltration unit was equipped with the spiral coiled polyethersulfone membrane (PolyMemTech, Warsaw, Poland). Its cut-off was 10 kDa and surface 0.8 m^2. The temperature of both processes (MF and UF) was kept at 25°C; thanks to the use of coolers and thermostated feed tanks.

The defatted whey (30 L) was dosed by a gear pump (P1) (Zuwa Combistar 2000, Germany) at 300 L h^{-1} to the lumen side of the membrane module with the ceramic membranes. The velocity of the feed along the membrane was approximately 2 m s^{-1}. The initial transmembrane pressure during microfiltration was set to 1.4×10^5 Pa that allowed to obtain the permeate stream of 0.91 L h^{-1}. Every 7 min, a membrane surface was renovated by a back-pulse. The microfiltration process was carried out with retentate recirculation until its volume was reduced to 40% volume of feed. Then the retentate stream was directed to the ultrafiltration (UF) unit.

The transmembrane pressure during UF was kept at 2.5×10^5 Pa. The feed volume was 12 L, and the retentate stream was average 270 L h^{-1}. During the process, a single-step diafiltration was carried out. When the retentate volume was reduced to 50% initial value, the amount of water equal to the obtained volume of the permeate was added. The process was carried out till 83% reduction of the retentate volume.

Protein and lactose concentrations in the permeate and retentate streams were monitored in real-time. The protein compounds concentration was determined by the Lowry method [27] using a spectrophotometer (Hitachi U-1900, Japan) and a standard curve prepared for whey protein concentrate (Olimp Laboratories, Poland); $C_{protein}$ (g L^{-1}) = 0.296 · A(750). The lactose content was determined by the DNS method [28] using a standard curve for lactose (Avantor, Poland); $C_{lactose}$ (g L^{-1}) = 2.182 · A(550). All analyses were performed in duplicates.

The scheme of the proposed technology of whey management is presented in Fig. 1.

2.3. Analysis by size-exclusion high-performance liquid chromatography

Qualitatively particular fractions were analysed by size-exclusion high-performance liquid chromatography (SE-HPLC) using Prominence Module System (Shimadzu, USA). The analyses were performed under isocratic conditions

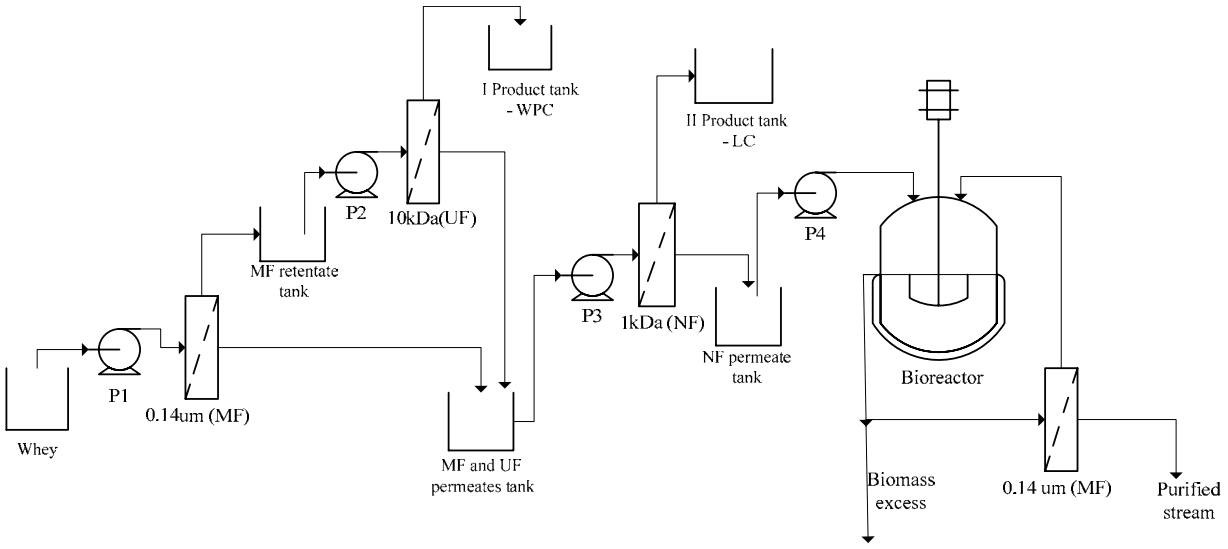

Fig. 1. Proposed whey management as a clean technology.

using two columns connected in series: a 300 × 7.8 mm BioSep-SEC-s2000 column (Phenomenex, USA) and a 300 × 7.8 mm Yarra-SEC-2000 column (Phenomenex, USA). The samples were first filtered through a 0.22 μm syringe filter and then eluted with 0.1 M phosphate buffer at pH 6.8 and 25°C for 60 min. The flow rate was 0.036 L h^{-1}, and the peak absorbance was monitored at 214 nm. Bovine serum albumin (66.0 kDa), ovalbumin (44.3 kDa), carbonic anhydrase (29.0 kDa), α-lactalbumin (14.2 kDa), cytochrome C (12.4 kDa), aprotinin (6.5 kDa) and vitamin B12 (1.3 kDa) were run as standards. The molecular weights of the compounds were found using the standard curve (log (Mw) = –0.1164 × time retention (min) + 4.6813). The area under the peak of the determined molecular weight fractions (proportional to the fraction mass) was derived from HPLC software (LabSolutions LC/GC version 5.51, Shimadzu Corporation, Kyoto, Japan). The concentration of each particular fraction ($C_{f,i}$) was calculated as:

$$C_{f,i}\left(\text{mol L}^{-1}\right)=\frac{A_{f,i}\cdot C_t}{M_{f,i}\cdot\sum A_{f,i}} \quad (1)$$

where $A_{f,i}$ – area under peak in the HPLC chromatogram for the i fraction, C_t – total concentration of proteins and peptides, $M_{f,i}$ – molar mass of i fraction.

The retention coefficient of each particular fraction ($R_{f,i}$) was calculated according to the below equation:

$$R_{f,i}=1-\frac{A_{f,i,\text{per}}}{A_{f,i,\text{ret}}} \quad (2)$$

where $A_{f,i,\text{per}}$ and $A_{f,i,\text{ret}}$ are the area under peak in HPLC chromatogram for i fraction in permeate and retentate, respectively.

2.4. Lactose separation in nanofiltration process

Permeates from the MF and UF units (34 L) were directed to nanofiltration (NF) process. The NF unit was manufactured by PolyMemTech (Warsaw, Poland) and was equipped with spiral wounded (A = 1.02 m^2) polyethersulfone membrane (PolyMemTech, Warsaw, Poland) at the cut-off coefficient of 1 kDa. The feed was dosed by a gear pump (P3) (Zuwa Combistar 2000, Germany) at 200 L h^{-1}. The transmembrane pressure was set to 2.0 × 10^6 Pa. The process was carried out at different pH value, with or without addition of NaCl at its final concentration of 0.01 M. The process was carried out with retentate recirculation until a 90% volume reduction was achieved. The retentate stream was the final stream rich in lactose. The permeate stream with some amount of organic compounds was subjected to a biodegradation process.

2.5. Lactose biodegradation in CSTR

Bacillus licheniformis (PCM-1849, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wroclaw, Poland) was used in the experiment. The feed (the permeate from the NF was rich in lactose [4.90 g L^{-1}] and contained a small amount of short peptides [2.39 g L^{-1}]) was dosed by gear pump P4 (Gear Pump, Cole-Parmer Instrument Company, USA) into a thermostatic (37°C) reactor (New Brunswick BioFlo, USA), with a working volume of 3.0 L. The flow rate of the dosed stream was equal to that of the outlet, 76.9 × 10^{-3} L h^{-1}. The intensity of stirring was 70 rpm. The culture was aerated by a compressor (HL275/50 Specair, Netherlands), and the flow rate of the air was 90.0 L h^{-1}. The culture purity was monitored by the daily inoculation of agar plates (N9405, Fluka Analytical, USA).

It was established (on the base of measurements in time) that a steady-state emerged after four-volume exchanges. The biomass concentration was calculated based on the standard curve C_{biomass} (g L^{-1}) = 0.389 · A(550 nm), which had been based on a dry mass content determined on weight method. Then, samples were centrifuged at 6,000 rpm for 10 min (Hettich Zentrifugen Eba 20, Germany) before performing the Lowry and DNS assays.

3. Results and discussion

3.1. Two steps membrane filtration: micro- and ultrafiltration

As it is recommended [29–31] before membrane processes, the feed stream should be pretreatment to fouling limitation. Thus, the residues of casein clots and fat were centrifuged. A high concentration of proteins in whey causes undesirable fouling, and therefore, a back-pulse was applied every 7 min of microfiltration process. The concentrations of proteins/peptides and lactose in the feed was 12.51 ± 0.63 and 41.22 ± 1.11 g L^{-1}, respectively.

During the microfiltration process, the initial transmembrane pressure was set to 1.4 × 10^5 Pa. Under this driving force, the permeate stream was 0.91 L h^{-1}. Despite the use of back-pulse, protein deposition on the membrane caused an increase in flow resistance. By keeping the stable permeate stream, an increase in the transmembrane pressure was observed (Fig. 2).

The tested ceramic membrane with a 0.14 μm pore size retained almost all proteins present in the whey (Fig. 3). The retention coefficient was calculated based on Eq. (2). Only the retention coefficient of GMP, with a molecular mass smaller than 10 kDa, was lower than 0.8. These data challenged the theory of the particle size retained on the microfiltration membrane. Probably, a key factor influencing these results is the protein layer formed in the first seconds of the filtration process. Based on the mass balance, it was estimated that after 160 min, the protein surface concentration was 79.1 g m^{-2}, that corresponds to 15.81% of the mass of proteins present in feed. Similar data were also obtained in other experiments [32]. The microfiltration process was carried out with retentate recirculation until its volume was reduced to 40% volume of feed. In the laboratory test, it corresponded to 24.2 h. Then the retentate stream was directed to the ultrafiltration unit. The concentration of proteins and lactose in this stream was 22.58 ± 1.16 and 40.6 ± 1.76 g L^{-1}, respectively.

The concentrated solution of whey proteins depleted of GMP and shorter peptides was directed to the ultrafiltration unit equipped with a polymeric membrane with pores at a cut-off equal to 10 kDa. The ultrafiltration process was very stable. The permeate stream did not change significantly and at the transmembrane pressure of 2.5 × 10^5 Pa was 0.21 L h^{-1}. Based on the mass balance after 30 h of the UF, the protein surface concentration was calculated to be 19.8 g m^{-2} that corresponds to approximately 7.4% of the initial amount of proteins in feed.

A single step of diafiltration supported the ultrafiltration process. It allowed obtaining protein concentrate ($C_{protein}$ = 99.64 ± 2.16 g L^{-1}) with a reduced content of GMP, peptides and lactose ($C_{lactose}$ = 2.87 ± 0.17 g L^{-1}). The proteins with a molecular mass higher than 21 kDa accounted for more than 70% proteins present in the final concentrate – Fig. 4.

3.2. Lactose separation in nanofiltration process

The lactose sufficiently permeated through the micro- and ultrafiltration membranes. Finally, its concentration in the pooled permeates from these processes was 34.70 ± 0.26 g L^{-1} while the protein concentration was 2.97 ± 0.07 g L^{-1}.

To increase lactose concentration, the nanofiltration polymeric membrane at the cut-off coefficient 1 kDa was used. The lactose retention coefficient was in range 0.18–0.55 and strongly depended on pH – Fig. 5. Addition of NaCl at its final concentration of 0.01 M influenced the retention coefficient (increased up to 0.69 at pH 4.0). Thus, the synergistic effect of the solution acidity and the ionic strength on the impact of the nanofiltration process was observed. This observation is consistent with the literature [33,34]. Simultaneously, with this membrane the retention coefficient of 0.8 was obtained for protein compounds at a molecular mass of 7 kDa, while for the peptides at 1 kDa retention coefficient was 0.44 [35].

At reducing the volume to 30% of the initial volume and the application pH 4.0 and NaCl (at the final concentration 0.01 M), the lactose was concentrated three times. The final retentate contains mainly lactose (its concentration was 106.20 ± 2.16 g L^{-1}) while GMP concentration was below 4.41 ± 0.38 g L^{-1}. A powder of these products can be obtained using, for example, spray-drying.

The permeate did not meet the Regulation of the Ministry of Maritime Economy and Inland Navigation, the

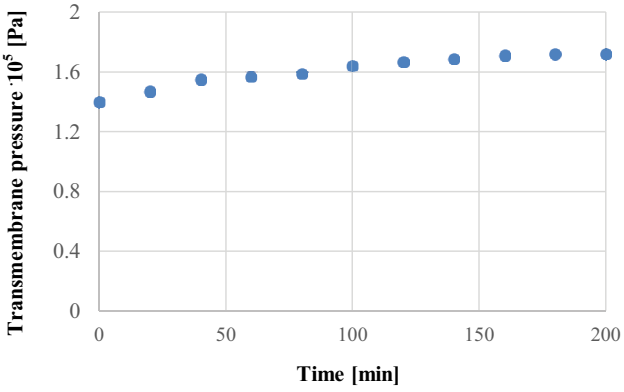

Fig. 2. Microfiltration of the defatted whey on the ceramic membrane with a 0.14 μm pore size – the changes over time of transmembrane pressure (A = 0.75 m^2, $Q_{permeate}$ = 0.91 L h^{-1}).

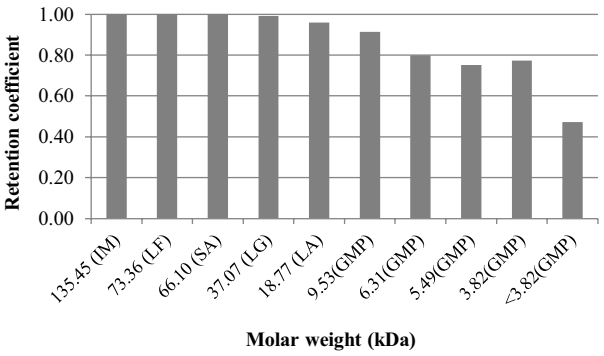

Fig. 3. Microfiltration of defatted whey on the ceramic membrane with a 0.14 μm pore size – the retention coefficient value after 160 min filtration for main whey proteins. Lactose passed freely through the pores.

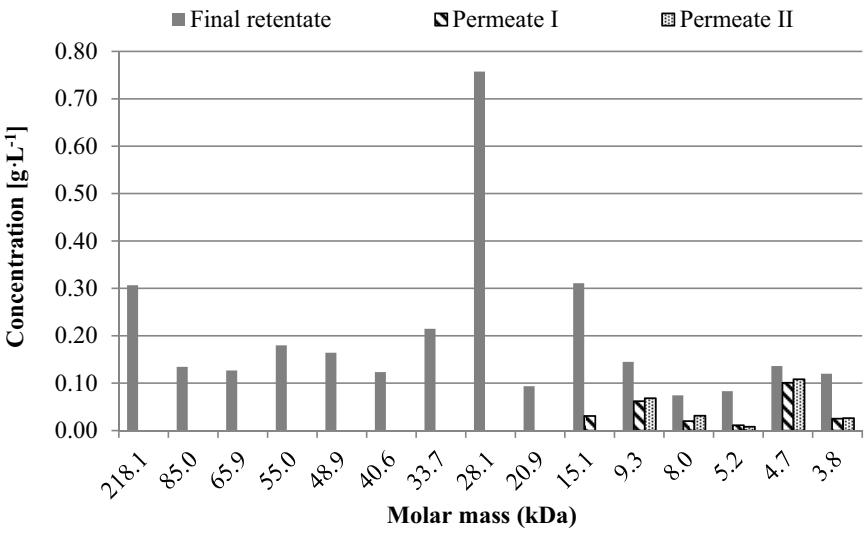

Fig. 4. Concentration of a particular protein in first (before diafiltration) and second permeate and in the final retentate and during ultrafiltration on the polymeric membrane (A = 0.8 m^2, cut-off 10 kDa, permeate stream 0.21 L h^{-1}).

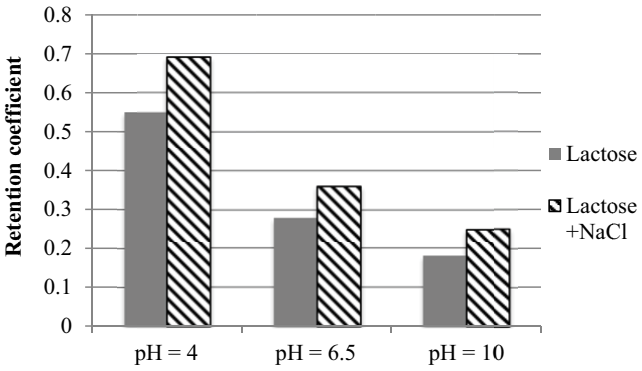

Fig. 5. Retention coefficient of lactose at different pH and with and without the presence of NaCl (polyethersulfone membrane at the cut-off coefficient 1 kDa, $\Delta P = 2.0 \times 10^6$ Pa).

Table 2
Biological function of whey proteins [12–21]

Biological function	References
Prevention of cancer	[12–14]
• Breast and intestinal cancer	
• Chemically induced cancer	
Increment of glutathione levels	[15–17]
• Increase of tumour cell vulnerability	
• Treatment of HIV patients	
Antiviral activities	[18]
Antimicrobial activities	[19]
Increment of satiety response	[20]
• Increment in plasma amino acids, cholecystokinin and glucagon-like peptide	
Immunomodulatory effect	[21]

Republic of Poland [2], that is why it was subjected to a biodegradation process.

3.3. Lactose biodegradation in CSTR

In our previous paper [36], we presented a detailed study on whey biodegradation by *Bacillus licheniformis* (PCM-1849). We provided evidence that the residence time in a continuous stirred tank reactor (CSTR) using this strain should be approximately 30 h to meet the Regulation of the Minister of Maritime Economy and Inland Navigation, the Republic of Poland [2]. The organic matter concentration in the discharge stream should be less than 1 g L^{-1}.

As it was expected, at the residence time 30 h, the lactose concentration in the outlet stream was 0.80 ± 0.09 g L^{-1}. Simultaneously, thanks to the ability of *B. licheniformis* to respire nitrate, the protein concentration was also slightly reduced to 1.93 ± 0.12 g L^{-1}. The concentration of biomass was on average at a constant level – 1.09 ± 0.13 g L^{-1}.

4. Conclusions

The aim of the research was to develop a complete whey management process, as presented in Fig. 1. First, it includes a membrane separation of whey proteins using micro- and ultrafiltration supported by diafiltration. In retentate of UF there are mostly concentrated whey proteins (WPC) that present unique properties (Table 2). The retentate is the main product, but both permeates (of MF and UF) could also be utilised. The separation of short peptides and lactose present in these solutions is possible by chromatographic techniques [22], but on the industrial scale it is expensive. Therefore, we propose a nanofiltration process for lactose separation and concentration. A significant amount of lactose was maintained in the retentate.

The permeate, from NF consisted mainly of lactose, and small amounts of protein compounds, was directed

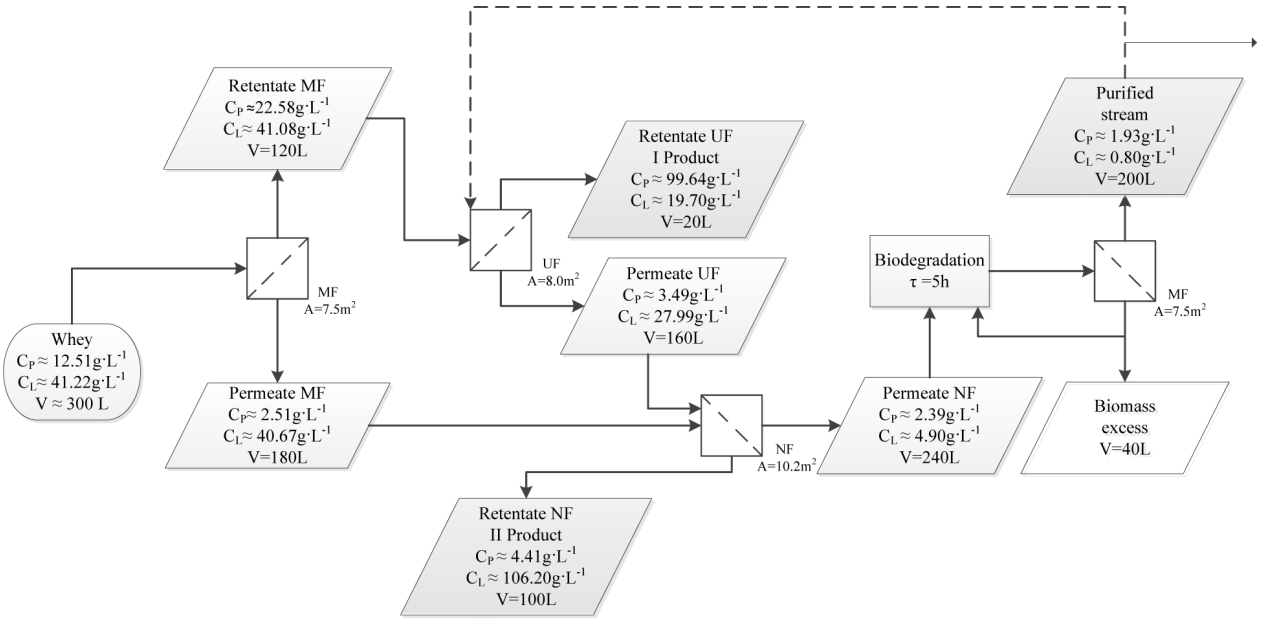

Fig. 6. Integrated process of whey management – the values of streams and concentrations.

to biodegradation. Both types of organic matter could be degraded by *B. licheniformis* strain to the concentrations required by standards. Because the biodegradation process is relatively long, CSTR could be replaced by membrane bioreactor. According to the presented previous model [37], the same concentration of organic matter will be obtained at residence time 5 h at $\psi = 6$. The purified stream can be used partially during the diafiltration accompanying ultrafiltration process.

The project of industrial-scale of whey management based on bioreactor and membrane processes is presented in Fig. 6. It allows for whey protein isolation from a mixture of protein compounds (recovery approximately 53%) and pure lactose separation (recovery about 86%).

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