indicators developed in the CIS process and applied at river basin level. Such targets should address all the main water using sectors (industry, energy production, agriculture, households, etc.) and should be closely linked to the objective of good status. They should be relied upon together with the above mentioned incentive water pricing and they could become part of the water allocation process and objective setting in the RBMPs.

Particularly in the field of agriculture, the Commission's proposals for reforming the CAP provide scope for funding to improve irrigation efficiency in ways that are consistent with the WFD objectives. This includes minimum water use reductions, but ought to include the use of treated wastewater for irrigation too. This is important as agriculture accounts for 24 % of water abstraction in Europe, although that might not sound like much compared to the 44 % abstracted for cooling water in energy production.

The stakeholder consultations leading to the Blueprint showed that water re-use for irrigation or industrial purposes as an emergent issue, as it is considered to have a lower environmental impact than other alternative water supplies (e.g. water transfers or desalinisation), it is only used to a limited extent in the EU. Subsequently, the EU is already taking some steps towards the inclusion of water reuse within the Blueprint policy options: as water reuse is only used to a limited extent in the EU and this appears to be due to the lack of common EU environmental-health standards for re-used water and the potential obstacles to the free movement of agricultural products irrigated with re-used water, the Commission is looking into the most suitable EU-level instrument to encourage water re-use, including a regulation establishing common standards. In 2015, it will make a proposal, subject to an appropriate impact assessment, to ensure the maintenance of a high level of public health and environmental protection in the EU.

EUROPEAN COMMISSION - A Blueprint to Safeguard Europe's Water Resources.
EUROPEAN COMMISSION – Water Reuse System Management Manual AQUAREC. Ed. Davide Bixio and Thomas Wintgens, Directorate-General for Research, Brussels, 2006.
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[1176] APPLICATION OF THE SEQUENCE ULTRA/NANOFILTRATION FOR VALORIZATION AND REDUCTION OF THE ENVIRONMENTAL IMPACT OF OVINE CHEESE WHEY

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ABSTRACT. The by-products of agro-industries, due to its high content of nutritive substances, in particular proteins and carbohydrates, have been the subject of intense investigation with a view to their recovery/reuse, towards sustainability. One of these by-products is ovine cheese whey, that is mainly produced in Southern European countries, as a result of the production of ovine cheeses of Protected Designation of Origin. In most cases, that whey is disposed off into public sewage, causing problems in conventional treatment plants, due to its high organic load. In some countries, e.g. Portugal, Spain and Italy, part of the ovine cheese whey is further processed to obtain whey cheeses, designated by different names, such as *requeijão*, *requesón* and *ricotta*. However, not all of these cheese whey can be transformed due to the high volumes generated. Membrane technology has emerged as a significant innovation for recovery and treatment, because it is more economical than other alternatives, require much less land area than competing technologies and may produce water suitable for multiple proposes. In this work, an example of using membrane processes in the recovery of ovine cheese whey is presented. The operations of ultrafiltration (UF) and nanofiltration (NF) of ovine cheese whey were investigated with the objective of producing added-value products, such as protein concentrates by UF, lactose concentrates by NF and final permeates with a very low organic load. UF experiments were performed with skimmed cheese whey, both in total recirculation and concentration modes. The equipment used was a plate-and-frame unit (Lab Unit M20). Ultrafiltration was performed with organic membranes ETNA 10PP with a surface area of 0,072 m2 and a cut-off of 10 kDa. Ultrafiltration allowed a clear separation between the protein fraction and a fraction rich in lactose and minerals. About 40% of organic matter, expressed as COD was retained. Nanofiltration of UF permeates was done with membranes NFT50 with a membrane surface area of 0,072 m^2..This operation allowed a high retention of lactose (98.8%) and the production of a permeate with a very low organic load. The retention of organic matter, in terms of COD was about 93%. The sequence of operations UF/NF allowed to produce two added-value products, reducing at the same time the organic pollution of the final stream. Nevertheless, the quality of the final water should be assessed, according with the intended use.

Keywords: Nanofiltration; ovine cheese whey; ultrafiltration.

APLICAÇÃO DA SEQUÊNCIA ULTRA/NANOFILTRAÇÃO PARA VALORIZAÇÃO E REDUÇÃO DO IMPACTO AMBIENTAL DE LACTOSSORO DE QUEIJO DE OVELHA

RESUMO. Os subprodutos das agro-indústrias, devido ao seu elevado teor em substâncias nutritivas, em particular proteínas e hidratos de carbono, têm sido objecto de uma intensa investigação com objectivos de recuperação/reutilização, tendo em vista um desenvolvimento sustentável. Um destes subprodutos é o soro de queijo de ovelha, produzido principalmente nos países do sul da Europa, como resultado da produção de queijos de ovelha de Denominação de Origem Protegida. Na maioria dos casos, o soro produzido é lançado nos esgotos municipais, causando problemas em estações de tratamento convencionais, devido à sua elevada carga orgânica. Em alguns países, como Portugal, Espanha e Itália, parte do soro é processado para obtenção de queijos de soro, designados por nomes diferentes, como *requeijão, requesón e ricota*. No entanto, nem todo o soro produzido pode ser transformado, devido aos elevados volumes gerados. As tecnologias de membranas surgiram como uma inovação significativa para a recuperação/tratamento de vários produtos, dado que relativamente a outros processos alternativos, são mais económicas, podendo produzir água de qualidade adequada para diversos fins. Neste trabalho, é apresentado um exemplo de utilização de processos de membrana na recuperação de soro de queijo de ovelha. As operações de ultrafiltração (UF) e nanofiltração (NF) foram investigadas com o objetivo de produzir produtos de valor acrescentado, como concentrados proteicos por UF, concentrados de lactose por NF e permeados finais, com cargas orgânicas baixas. Os ensaios de UF foram realizados com soro desnatado, em recirculação total e em concentração. O equipamento utilizado foi um módulo de pratos planos (Lab Unidade M20). Na ultrafiltração, foram usadas membranas orgânicas ETNA 10PP, de área superficial 0.072 m^2 e com um peso molecular de corte de 10 kDa. A ultrafiltração permitiu uma nítida separação entre a fração proteica e uma fração rica em lactose e sais minerais. Cerca de 40% da matéria orgânica, expressa como CQO, foi retida. Na nanofiltração dos permeados da UF utilizaram-se membranas NFT50, com uma área de superfície de 0.072 m^2. Esta operação permitiu uma elevada retenção de lactose (98.8%) e a obtenção de um permeado com uma carga orgânica bastante reduzida. O factor de retenção da matéria orgânica, em termos de CQO, foi cerca de 93%. A sequência de separações UF / NF permitiu produzir dois produtos de valor acrescentado, reduzindo ao mesmo tempo a poluição orgânica da corrente final. No entanto, a qualidade da água final deve ser avaliada, de acordo com o uso pretendido.
Palavras-chave: *Nanofiltração; Soro de queijo de ovelha; ultrafiltração.*

1. INTRODUCTION

The by-products of agro-industries, due to its high content of nutritive substances, in particular proteins and carbohydrates, have been the subject of intense investigation with a view to their recovery/reuse, towards sustainability. One of these by-products is ovine cheese whey, that is mainly produced in Southern European countries, as a result of the production of ovine cheeses of Protected Designation of Origin. In most cases, that whey is disposed off into public sewage, causing problems in conventional treatment plants, due to its high organic load. In some countries (e.g. Portugal, Spain and Italy), part of the ovine cheese whey is further processed to obtain whey cheeses, designated by different names, such as *requeijão*, *requesón* and *ricotta* (Pereira *et al.*, 2007). However, not all of these cheese whey can be transformed due to the high volumes generated. In addition, the production of *requeijão* is not very efficient because it still results a second cheese whey, called "sorelho", that contains almost all the lactose and about an half of the total nitrogen of the original cheese whey (Pereira, Díaz & Cobos, 2002; Macedo *et al*., 2005). Sorelho has no use being released directly into drains and causing problems in conventional water treatment plants, due to its high content of organic matter. A recent review about the biological processes used in treatment of cheese whey wastewaters, with different physicochemical pretreatments, is presented in Carvalho *et al*. (2013).

This research is focused in the application of membrane technology for recovery and treatment of ovine cheese whey. Membrane technology has emerged as a significant innovation, because it is more economical than other alternatives, require much less land area than competing technologies and may produce water suitable for multiple proposes. Bovine cheese whey, produced in greatest volume throughout the world has been used mainly in the food industry for the production of whey protein concentrates (WPC) by ultrafiltration (Bordenave-Juchereau *et al*., 2005). These WPC´s have many applications, such as additives in cooked foods in the dairy products (like yogurt, cheese, etc.), meats, beverages and baby food (Zydney, 1998), or to increase yields in cheese making processes (Maubois & Ollivier, 1997; Hinrichs, 2001). However, the manufacture of WPC´s cannot solve the problem of whey utilization because the permeates from ultrafiltration still contain large amounts of lactose, salts, peptides, non-protein nitrogen (NPN), forming its high COD. The recovery of lactose is still the major problem since it represents about 75% of the total solids (Macedo A., 2011) and so, a further treatment of the reutilization of the permeate is needed. Nanofiltration

is very important to recover lactose and those compounds, not only due to its lower cut-off, but also because separation can be achieved by electrochemical effects, through the use of charged membranes.
The aims of this work were:
(i) the valorization of ovine cheese whey through the production of two added-value products, a protein fraction and a lactose fraction, using membrane technologies, namely ultrafiltration (UF) and nanofiltration (NF);
(ii) minimization of the environmental impact of this by-product, through the production of a final permeate with a very low organic load.

2. MATERIALS AND METHODS

2.1. Ovine cheese whey preparation and characterization

Ovine cheese whey was collected at "Ovelheira, Casa Agrícola de la Féria, Lda", a portuguese cheese and "*requeijão*" factory, located at the Protected Geographical Region of Serpa cheese. Immediately after reception, ovine whey was filtered and afterwards skimmed by means of a Westfalia separator. This pretreatment was done with the aim of minimizing the long term fouling of membranes, extending its life time and improving their efficiency. Samples of the initial cheese whey and pretreated cheese whey were object of the following determinations: pH (Metrohm pH meter); specific conductivity (Methrom AG CH-9100 Herisau); viscosity (Viscotester VT 550); total solids, according to the gravimetric procedure; total suspended solids; chemical oxygen demand (COD); protein by the Kjeldahl method; lactose, according to Munson and Walker process; fat content in the whey was determined using Gerber´s butyrometric determination and in the pretreated whey by the Soxhlet extraction method, sodium and potassium by flame photometry (Corning photometer 410), calcium and magnesium by atomic absorption spectrometry (Thermo Jarrell Ash); chloride by Charpentier-Volhard method and phosphate, by vanadomolibdophosphoric acid method.

2.2. Ultrafiltration and nanofiltration experiments

Ultrafiltration experiments were realized in a plate & frame filtration unit (Lab Unit M20 from Alfa Laval, Denmark), with three different flat sheet membranes with a total membrane surface area of 0.072 m^2. Before testing the sample, the membranes were subjected to a cleaning and disinfection cycle, described by the manufacturer, and after they were subjected to a compression of 5.0 bar for 3 hours. After this procedure, the hydraulic permeability to pure water was determined for all the membranes, by measuring the permeation fluxes at different transmembrane pressures. Pretreated cheese whey was ultrafiltered in total recirculation mode with the different membranes and operating conditions in order to select the best membrane and experimental conditions (Macedo *et al.,* 2011). In concentration mode, the essays were performed with the preselected membranes, ETNA10PP with a cut-off of 10 kDa and in the following operating conditions: transmembrane pressure of 2.0 x10^5 Pa, cross-flow velocity of 0.94 m s^{-1} and T = 30 ºC, till a volume concentration factor of 3.0.
Whey permeate fractions obtained after ultrafiltration were nanofiltered through NFT50 membranes. The same filtration set-up was used for ultrafiltration and nanofiltration experiments. The ultrafiltration permeates were previously nanofiltered in total recirculation mode at different transmembrane pressures and feed recirculation flow rates, to select the best operating conditions. Based on the analysis of the results obtained in these preliminary tests, the concentration experiments were performed at a transmembrane pressure of 30 x10^5 Pa and a cross-flow velocity of 1.42 m s^{-1}, till a volume concentration factor of 2.5. The pH was kept constant at 6.0 and the temperature at 25ºC, in order to avoid the precipitation of salts which is favored under conditions of high pH and temperature (Marshall & Daufin, 1995).
Samples of feed, concentrates and permeates were taken for analyses and the retention factors, r_F, defined as:

$$r_F = \frac{(C_f - C_p)}{C_f} \qquad (1)$$

where C_f is the concentration of a solute in the feed and C_p is the concentration of the solute in permeate, were calculated.

The selectivity of both operations was expressed in terms of the retention factors.
Figure 1 shows a diagram of the process carried out in this work.

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Figure 1: Diagram of the experimental work realized

3. RESULTS

3.1. Characterization of cheese whey and pretreated cheese whey

Table 1 presents the results of the characterization of cheese whey and pretreated cheese whey (feed). The results shown are the mean values obtained for the various parameters and their confidence intervals of 95%, calculated on the basis of Student's t distribution, since the sample size, n, is less than 30 (Montgomery 1994) . For each parameter the sample size (n) is indicated.

The sample used is classified as sweet cheese whey because its pH is about 6.0 and it is produced from milk clotting through enzymatic hydrolysis of casein by chymosin at a pH not lower than 5.6 (Morr, 1989).

As can be seen in Table 1, the pretreatment carried out to the sample was efficient, because it allowed the removal of about 99% of fat, 87% of the total suspended solids and 25% of the total solids.

Table 1: Physicochemical characterization of cheese whey and pretreated cheese whey, indicating the confidence intervals at 95% for the mean and sample size (n) (Macedo, 2011).

Parameter	*Cheese whey*	*n*	*Pretreated cheese whey*	*n*
pH (25ºC)	5.62 ± 0.29	25	5.58 ± 0.28	25
K ($S\ m^{-1}$)	2.09 ± 0.07	23	2.10 ± 0.07	23
ρ ($kg\ m^{-3}$)	1035 ± 0.7	22	1037 ± 0.6	22
$\mu^{25ºC}$ (mPa s)	1.99 ± 0.11	5	1.37 ± 0.17	5
ST ($kg\ m^{-3}$)	108.34 ± 4.74	25	87.34 ± 2.36	25
SST ($kg\ m^{-3}$)	30.20 ± 3.87	21	4.00 ± 0.71	22
Nitrogen Kjeldahl ($kg\ m^{-3}$)	2.777 ± 0.121	24	2.682 ± 0.109	22
Total protein ($kg\ m^{-3}$)(1)	17.74 ± 0.77	22	17.10 ± 0.70	22
Lactose ($kg\ m^{-3}$)	52.0 ± 0.9	22	52.1 ± 1.0	22
Fat ($kg\ m^{-3}$)	20.79 ± 4.12	22	0.23 ± 0.04	22
COD (mg O_2 L^{-1})	76941.7 ± 52.3	19	76064 ± 56.6	17
Minerals				
Sodium ($kg\ m^{-3}$)	7.138 ± 0.260	22	7.142 ± 0.260	22
Potassium ($kg\ m^{-3}$)	0.993 ± 0.045	22	0.991 ± 0.042	22
Calcium ($kg\ m^{-3}$)	0.492 ± 0.023	24	0.474 ± 0.004	24
Magnesium ($kg\ m^{-3}$)	0.089 ± 0.005	20	0.087 ± 0.005	20
Chloride ($kg\ m^{-3}$)	7.44 ± 0.44	22	7.54 ± 0.47	22
Phosphate ($kg\ m^{-3}$)	1.43 ± 0.16	18	1.46 ± 0.15	18

(1) Total protein = Nitrogen Kjeldahl x 6.38.

3.2. Ultrafiltration and nanofiltration experiments

Based on the results achieved in physicochemical characterization of the permeates of ultrafiltration and nanofiltration, we have calculated the retention factors for each of the components analyzed, both for ultrafiltration and nanofiltration operations (Table 2). The ultrafiltration permeates were those obtained for a volume concentration factor of 3.0 and the ones of nanofiltration permeates were produced from a volume concentration factor of 2.5. The retention factors were calculated according with equation (1).

The results in Table 2 show that, under the experimental conditions used, it was possible a clear separation by ultrafiltration of two main fractions: a concentrate rich in nitrogen compounds (possible mainly composed of proteins), and a permeate rich in lactose and minerals. Indeed, there is a great difference between the retention factor of nitrogen compounds and those of the other components (mainly lactose and minerals) with the exception of phosphate, which also has a high retention. Ultrafiltration allowed a reduction of about 40% of the organic load, measured by the COD.

The nanofiltration of permeates led to a concentrate mainly composed of lactose and bivalent cations (the retention factors of these components were very high) and some nitrogen compounds. In spite of the sequence of the membrane operations UF/NF has resulted in a final permeate with a low organic load (the retention factor for COD after NF was about 93%), it still contains mostly sodium and chloride and so its future use should be reflected.

Table 2: Retention factors obtained from ultrafiltration and nanofiltration of cheese whey (Macedo, 2011)

Parameters	Retention factor, r_F, of ultrafiltration (%)	Retention factor, r_F, of nanofiltration (%)
Total solids (kg m^{-3})	34.9	96.9
Nitrogen Kjeldahl (kg m^{-3})	76.6	41.7
Lactose (kg m^{-3})	4.4	98.8
COD (mg O_2 L^{-1})	39.7	92.9
Sodium (kg m^{-3})	5.7	6.1
Potassium (kg m^{-3})	1.8	7.6
Calcium (kg m^{-3})	5.8	97.6
Magnesium (kg m^{-3})	2.9	97.4
Chloride (kg m^{-3})	3.4	8.7
Phosphate (kg m^{-3})	73.0	-

4. CONCLUSION

The application of membranes technologies is a very important tool for valorizing by-products of the food industry. The sequence of membrane operations proposed in this research allow the production of concentrates and permeates that still can have several possible uses. Besides, it avoids the release for the environment of sorelho, so contributing for a sustainable development.

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