ORIGINAL ARTICLE

Potential use of nanofiltration membrane in treatment of wastewater from fish and surimi industries

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Abstract

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This study was carried out to determine the potential use of nanofiltration (NF) membranes in treating the wastewater, generated from the fish and surimi industries. The possibility of recovering the protein from the wastewater was also investigated, since these effluents contain a large amount of protein, which could be concentrated by means of NF and recycled into the fishmeal process. The protein could also be traded as fertilizer or animal feed by-products. In this study, fish and surimi washing wastewater was generated in the laboratory. Then, the wastewater was subjected to pre-treatment by using a filter paper (due to the high concentration of suspended matter in these effluents) before it was treated/separated by using a polyamide NF membrane of 500 Da. Permeation experiments showed that NF was capable of reducing COD and TSS up to 93 % and 87 %, respectively. Study on long-term flux decline indicated that polyamide NF membrane fouled much more slowly.

Key words : nanofiltration, wastewater, recovery, protein

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Washing is one of the most important steps in surimi manufacture. The cyclic washing and rinsing of the raw fish and fish mince with water are the central process in surimi production. The washing process improves gel-forming ability due to leaching of a considerable amount of fat and sarcoplasmic proteins (Suzuki, 1981; Wu *et al.*, 1991; Lin *et al.*, 1995; Huidobro *et al.*, 1998). Washing also separates myofibrillar proteins (Huidobro *et al.*, 1998), enzymes (proteases), pigments/blood and haem compounds causing lipid oxidation leading to protein denaturation (Hall and Ahmad, 1992). According to classical protein chemistry, NaCl (0.3-0.6 M) is needed to solubilize myofibrillar protein (Huidobro *et al.*, 1998) and it is also essential for the conversion of surimi into the final products and in the removal of the sarcoplasmic proteins in surimi manufacture (Hall and Ahmad, 1992). However, salt can also be added to a final wash to remove the last traces and improve water removal (Hall and Ahmad, 1992).

For a two-step industrial washing process, it has been estimated that more than 70 % of protein losses occurred during the first washing operation in surimi manufacture. This washing water represents 60 % of the total effluent volume (Jaouen and Quemeneur, 1992). In addition, the large volumes of water (9 to 15 l/kg of surimi, 3 to 5 l/kg of raw fish) (Jaouen and Quemeneur, 1992) used in every stage of surimi production pose problems in terms of the pre-treatment of hard water, if necessary, and as a pollution hazard (Hall and Ahmad, 1992) especially to the wastewater which carries the worst contamination with, for example, blood (Lee, 1986). The direct discharge of these effluents from the fishmeal factories can cause important environmental problems, as found in some areas like Galicia (northwest of Spain) and region VIII of Chile (Roeckel *et al.*, 1996; Guerrero *et al.*, 1998).

The wastewater contains an average of 6 g of soluble protein per litre, until now regarded as pollutant, but actually representing an annual loss of more than 50000 metric tons (Jaouen and Quemeneur, 1992). Ultrafiltration and nanofiltration offer considerable potential to be an integral part of a clean technology process (Afonso and Borquez, 2002). However, processing of the wastewater by ultrafiltration or nanofiltration without pre-treatment is not practicable.

The aim of the present work was to determine the potential use of nanofiltration membrane (polyamide membrane of 500 Da) in treating the wastewater, generated from fish and surimi industry, as well as to examine the possibility of recovering the proteins from the wastewater effectively. In this study, fish and surimi washing wastewater was generated in the laboratory. Due to the high amount of suspended matter present in the fish meal wastewater, pre-treatment by filtration had to be applied first to the raw wastewater.

Materials and Methods

Wastewater preparation

The fish used in these experiments were sardine obtained from the local fish market. In order to stimulate the actual wastewater within the fish industries, five different types of wastewater were generated corresponding to the different level of washing. The first type of fish wastewater was generated after the raw fish were being washed three times (10 minutes for each cycle) by sodium chloride solution (0.3 % M) at 4 $^{\circ}$C. Individual fish were then headed, gutted and washed three times (10 minutes for each cycle) in the same concentration of sodium chloride solution to generate the second type of fish wastewater. For each type of fish wastewater, the volume of salt solution used should follow the ratio of salt solution : fish = 3:1. Then, the skin and bones were removed to transform the raw fish into fish mince, which was the immediate raw material for the surimi process. Three cycles of 10 minutes contact with cold water (4 $^{\circ}$C): mince ratio of 3:1 was applied in these experiments, stirring constantly to generate three types of surimi wastewater. All the wastewaters were subjected to pretreatment under vacuum through a Whatman No. 4 paper to remove suspended particles before they were treated or separated by NF. All

the pre-filtered samples were kept in the refrigerator at 4 °C and were brought to ambient temperature (25 °C) before being analyzed and processed by NF.

Effect of pressure system, membrane characterization and NF permeation experiments

The membrane used in these experiments was polyamide NF membrane of 500 Da obtained from Berghof Filtration (Germany). The experiments were carried out using an SEPA stirred membrane cell (maximum volume = 300 ml), from Osmonics Inc. (USA). The effective membrane area was 19.33 cm^2 (1.933×10^{-3} m^2).

At the beginning, the membrane was wetted out by circulating distilled water at 16 bar for 30 minutes so that the excess of chemicals attached to the membrane surface could be released. The procedure also prevented the membrane compaction throughout the permeation or separation experiments (Afonso and Borquez, 2002).

After this conditioning step, about 250 ml distilled water, sodium chloride solution (0.3 % M) as well as different types of fish and surimi wastewater were treated separately at pressures of 4, 8, 12, 16 and 20 bar in order to measure the corresponding permeate fluxes, J_v. The time (in seconds) to obtain 2 ml of permeate volume of each samples would be taken. Each of the samples was tested for 3 runs at 25 °C (ambient temperatrure).

Flux behavior and fouling

To observe the effects of protein (in the wastewater) adsorption on polyamide NF membrane, NF permeation experiments for five types of fish and surimi wastewater were carried out for 270 minutes. For each wastewater, the flux was taken every 30 minutes. In this study, the only pressure considered was 16 bar. Each of the samples was tested for 3 runs at 25 °C (ambient temperature). Obviously, the methodology used in order to quantify this adsorption was only an approach, for neither pressure nor kinetics was taken into account, whereas both parameters were involved in protein NF. Nevertheless, these results may be used as a working guide in order to choose membranes on which a more detailed analysis of fouling mechanisms may be carried out under real NF conditions. Pore size reduction or $\Delta r/r$ allowed quantification of the adsorption effect by using formulations derived from Poiseuille's (Zeman, 1983),

$$\Delta r / r = 1 - \left(\frac{J_a}{J_o}\right)^{0.25} \quad (1)$$

where J_o is the pure water flux measurement of a new membrane and J_a is the pure water flux measurement of the absorbed (saturated) membrane.

Analytical techniques

Samples from the pre-filtered effluents and from the permeate streams of the NF experiments were collected and analyzed. Total suspended solids (TSS) were analyzed according to Standard Methods (APHA-AWWA-WEF, 1992). The Hach Method of 8000 (Reactor Digestion Method) was used to determine accurately the value of COD from the samples. Water-soluble proteins were analyzed by using Lowry's method (Lowry *et al*., 1951) whereby the total proteins were analyzed by using Kjeldahl Distillation Method (APHA-AWWA-WEF, 1992). pH and conductivity were determined by using electronic probe. The protein recovery, R_p could be calculated as

$$R_p = \frac{C_a - C_b}{C_a} \times 100\% \quad (2)$$

where R_p (%) is the protein recovery, C_a (mg/ml) is the concentration of the protein in the wastewater before the membrane separation and C_b (mg/ml) is the concentration of the protein in the permeate after the membrane separation.

Results and Discussion

Figure 1 (a) and 1 (b) show the plot of permeate flux versus applied pressure (4-20 bar) for various samples. As Figure 1 (a) shows, the plot for the distilled water was a linear profile (J_v α

ΔP). The result agrees with the Hagen-Poiseuille equation (Bowen and Mohammad, 1998). Figure 1 (a) and (b) also show that the permeability values for sodium chloride solutions of 0.3 % M and the five types of wastewater were lower than the distilled water. This phenomenon could be explained by the presence of solutes.

Figures 2 (a) and (b) show effects of protein adsorption towards the permeate flux on nanofiltration polyamide membrane for the duration of 270 minutes and pressure of 16 bar. The permeate flux for all of the wastewater was remained almost stable after 120 minutes. As according to Jaouen and Quemeneur (1992), the type of the membrane would determine the extent to which the phenomenon of fish protein adsorption contributed to membrane fouling. For an example, sulphonated polysulphone (Tech-Sep Iris 3026, 100 kDa) had a strong affinity for fish proteins until the pure water flux could be changed from J_o = 925 l/hm^2 to J_a = 50 l/hm^2 within 16 hours (the experiment was done at T = 30^{o}C and ΔP = 10^5 Pa). The pore size reduction (Δr/r) for this kind of membrane was 0.52. This meant that the 100 kDa membrane once fouled (a few seconds or minutes were enough), behaved like a 10 kDa cut-off membrane. Therefore, sulphonated polysulphone membrane was not suitable to be used in treating the fish and surimi wastewater that contained a lot of proteins. By using the data from Figures 2 (a) and (b) (time duration 4.5 hours), the values of Δr/r (the pure water flux was replaced by permeate wastewater flux) for the polyamide membrane were 0.11 (first type of fish wastewater), 0.081 (second type of fish wastewater), 0.11 (first type of surimi wastewater), 0.15 (second type of surimi wastewater) and 0.14 (third type of surmi wastewater). The values of Δr/r would be expected to be lower if the pure water flux was used in the calculations. The results might indicate that nanofiltration polyamide membrane (500 Da) was suitable to be used in treating the fish and surimi wastewater. Further research should be engaged to investigate the effect of proteins on fouling and flux behavior of the polyamide membrane.

Flux patterns for the different samples were qualitatively similar. Figures 2 (a) and (b) show that there was a substantial decrease in initial flux after which apparent steady-state equilibrium was achieved. Huang *et al*. (1997) hypothesized that this rapid decline in initial permeate flux of wastewater was a result of pore blocking. Although Figures 2 (a) and (b) show that the permeate flux was almost stable after 120 minutes, permeate flux for all the wastewater actually declined slowly. The subsequent decline was related to the boundary layer near the membrane surface and the cake layer deposited on the membrane surface (Huang *et al*., 1997). The other reason of the subsequent decline might be that the experiments that were carried out at room temperature (≈ 25 ^{o}C) although the original temperature of the wastewater was around 4 ^{o}C. These findings agree with Jaouen and Quemeneur (1992) whereby with soluble fish proteins a temperature 15 ^{o}C or less should be recommended to carry out a separation in order to avoid protein

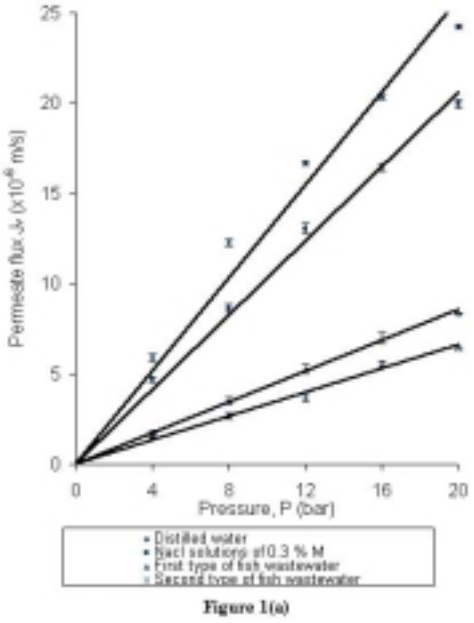

Figure 1(a). Average permeate flux versus pressure for distilled water, NaCl solutions of 0.3 % M, first and second type of fish wastewater. The data were fitted to a linear Hagen-Poiseuille equation.

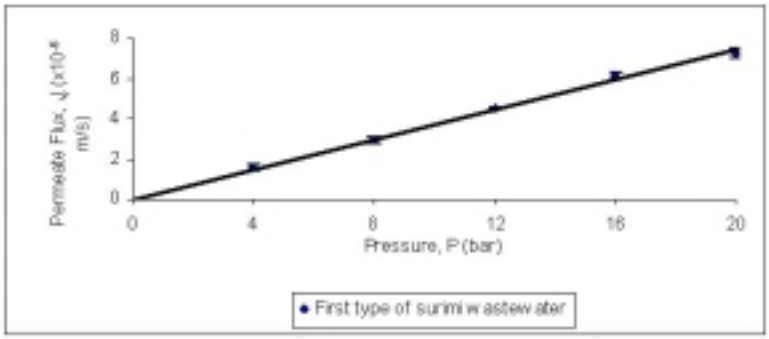

Figure 1 (b, i)

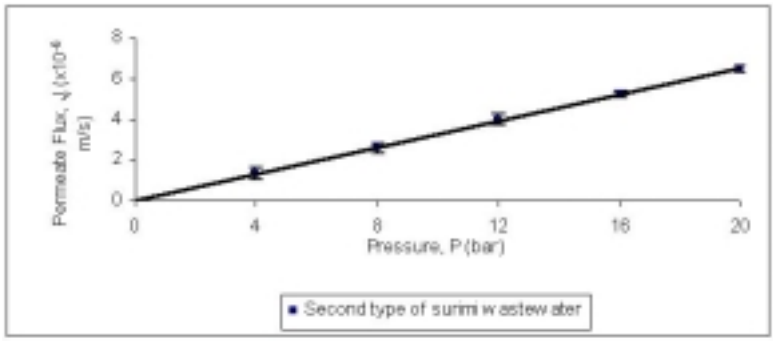

Figure 1 (b, ii)

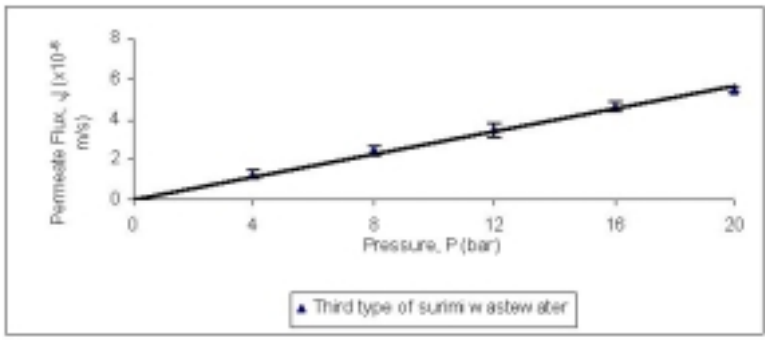

Figure 1 (b, iii)

Figure 1(b). Average permeate flux versus pressure for first, second and third type of surimi wastewater. The data were fitted to a linear Hagen-Poiseuille equation.

denaturation, as protein aggregates would both accelerate and increase membrane fouling. These findings also indirectly agree with Lee (1984) who gave a detailed account of these factors whereby the water temperature for the washing process was usually 5-10 ^{o}C to prevent muscle protein denaturation. It should be noted that the permeate flux was not always declined with the increasing of the sample's temperature. Therefore, it was suggested and would have been better to carry out the experiments at constant temperature. Further research should be engaged to investigate the effect of temperature on fouling and flux behavior of the polyamide membrane.

Tables 1 and 2 show the concentration of water-soluble proteins contained in the waste-

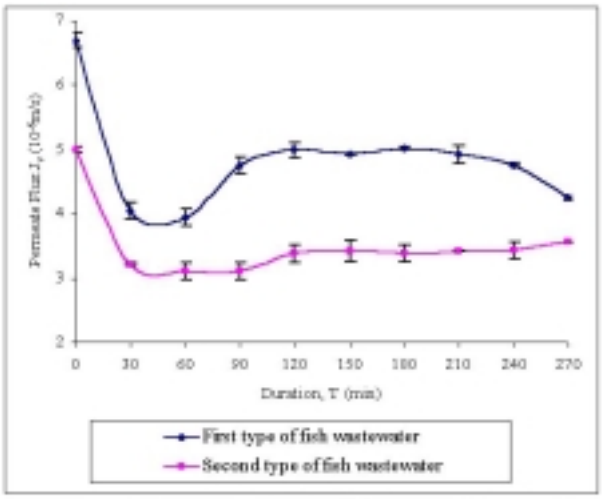

Figure 2 (a)

Figure 2(a). Average permeate flux versus time duration of 270 minutes for first and second type of fish wastewater. The operating pressure was 16 bar at ambient temperature.

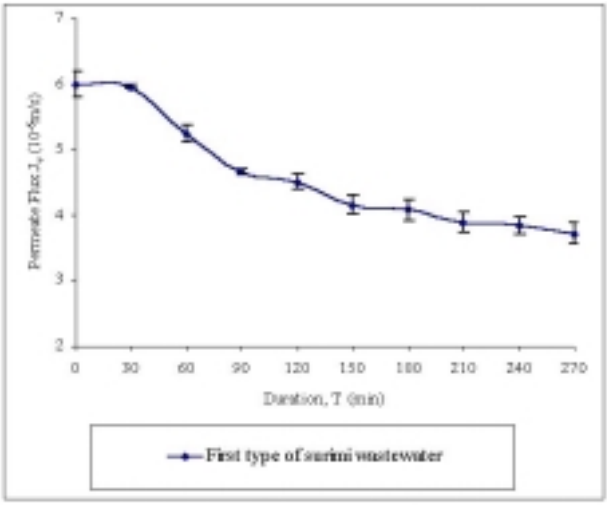

Figure 2 (b, i)

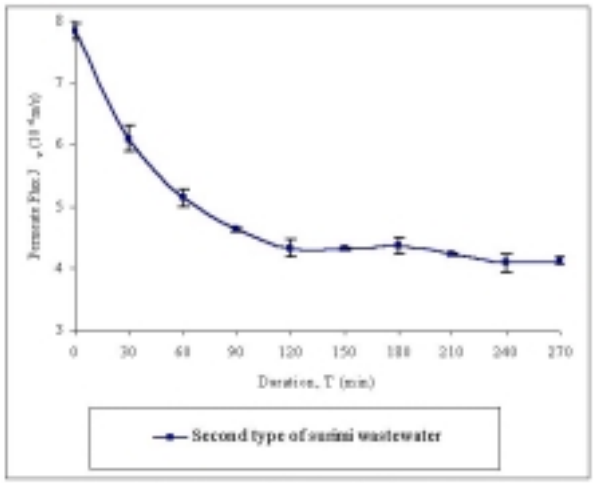

Figure 2 (b, ii)

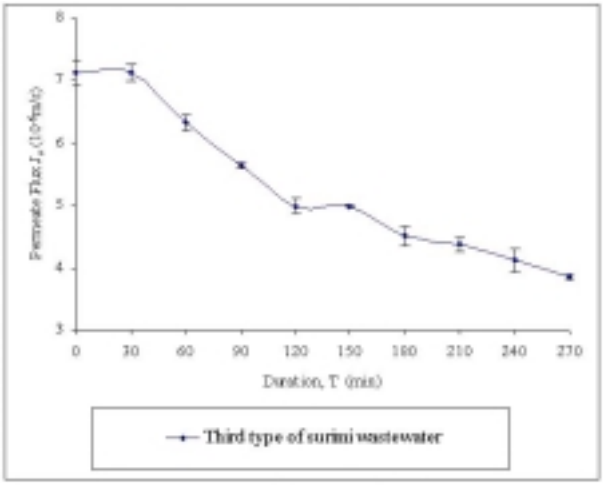

Figure 2 (b, iii)

Figure 2(b). Average permeate flux versus time duration of 270 minutes for first, second and third type of surimi wastewater. The operating pressure was 16 bar at ambient temperature.

water before and after nanofiltration (only permeate of the sample would be considered), which was operated at 16 bar. The concentrations of proteins in both of the fish wastewater were higher than those in any of the types of the surimi wastewater. This was because the fish washing process involved salt solutions that might extract the water-soluble proteins and enzymes more easily from the fish (Hall and Ahmad, 1992). The concentration of proteins for the first type of fish wastewater (0.94 mg/ml) was lower than that for the second type of fish wastewater (2.02 mg/ml) because the first washing process involved only the external washing of the fish whereas the second washing process involved the external as well as the internal washing of the fish after the

Table 1. Water-soluble and total protein concentration (mg/ml), water-soluble and total protein recovery (%), COD and TSS values (mg/l), percentage reduction (%) of COD and TSS, conductivity value (mS/cm) as well as pH value for the fish wastewater before and after nanofiltration (refer to the permeate only). The operating pressure was 16 bar at ambient temperature.

Analysis	Fish Wastewaters							
	1st type of fish wastewater				2nd type of fish wastewater			
	Raw wastewater (inlet)	Standard deviation	Permeate (outlet)	Standard deviation	Raw wastewater (inlet)	Standard deviation	Permeate (outlet)	Standard deviation
Water-soluble protein concentration (mg/ml)	0.94	0.046	0.51	0.050	2.02	0.092	0.85	0.096
Water-soluble protein recovery (%)	45.7 (No standard deviation will be indicated here because only the min values are used in the calculation)				57.9 (No standard deviation will be indicated here because only the min values are used in the calculation)			
Total protein concentration (mg/ml)	8.1	1.23	0.20	0.078	17.9	1.10	0.38	0.070
Total protein recovery (%)	97.5 (No standard deviation will be indicated here because only the min values are used in the calculation)				97.9 (No standard deviation will be indicated here because only the min values are used in the calculation)			
COD (mg/l)	2840	52.9	690	65.6	11350	137.0	2750	132.3
Percentage reduction of COD (%)	75.7 (No standard deviation will be indicated here because only the min values are used in the calculation)				75.77 (No standard deviation will be indicated here because only the min values are used in the calculation)			
TSS (mg/l)	1012	79.1	225	27.4	2228	228.0	359	56.7
Percentage reduction of TSS (%)	77.8 (No standard deviation will be indicated here because only the min values are used in the calculation)				83.9 (No standard deviation will be indicated here because only the min values are used in the calculation)			
Conductivity (mS/cm)	13.95	0.036	13.07	0.062	20.80	0.075	18.16	0.046
pH	6.45	0.060	7.12	0.082	7.10	0.26	7.51	0.12

Table 2. Water-soluble and total protein concentration (mg/ml), water-soluble and total protein recovery (%), COD and TSS values (mg/l), percentage reduction (%) of COD and TSS, conductivity value (mS/cm) as well as pH value for the surimi wastewater before and after nanofiltration (refer to the permeate only). The operating pressure was 16 bar at ambient temperature.

Analysis	Surimi Wastewaters											
	1st type of surimi wastewater				2nd type of surimi wastewater				3rd type of surimi wastewater			
	Raw wastewater (inlet)	Standard deviation	Permeate (outlet)	Standard deviation	Raw wastewater (inlet)	Standard deviation	Permeate (outlet)	Standard deviation	Raw wastewater (inlet)	Standard deviation	Permeate (outlet)	Standard deviation
Water-soluble protein concentration (mg/ml)	0.46	0.056	0.33	0.070	0.39	0.072	0.31	0.040	0.63	0.079	0.37	0.061
Water-soluble protein recovery (%)	28.3 (No standard deviation will be indicated here because only the min values are used in the calculation)				20.5 (No standard deviation will be indicated here because only the min values are used in the calculation)				41.3 (No standard deviation will be indicated here because only the min values are used in the calculation)			
Total protein concentration (mg/ml)	4.1	0.56	0.2	0.069	3.3	1.01	0.15	0.056	5.4	0.87	0.15	0.05
Total protein recovery (%)	95.1 (No standard deviation will be indicated here because only the min values are used in the calculation)				95.5 (No standard deviation will be indicated here because only the min values are used in the calculation)				97.2 (No standard deviation will be indicated here because only the min values are used in the calculation)			
COD (mg/l)	240	45.8	43	8.54	100	10.1	35	9.54	780	26.5	54	13.5
Percentage reduction of COD (%)	82.1 (No standard deviation will be indicated here because only the min values are used in the calculation)				65.0 (No standard deviation will be indicated here because only the min values are used in the calculation)				93.1 (No standard deviation will be indicated here because only the min values are used in the calculation)			
TSS (mg/l)	212	28.7	30	13.1	188	28.0	44	9.54	689	68.9	89	13.5
Percentage reduction of TSS (%)	85.9 (No standard deviation will be indicated here because only the min values are used in the calculation)				76.6 (No standard deviation will be indicated here because only the min values are used in the calculation)				87.1 (No standard deviation will be indicated here because only the min values are used in the calculation)			
Conductivity (mS/cm)	0.834	0.010	0.372	0.0070	1.172	0.062	0.460	0.087	1.250	0.087	0.657	0.018
pH	7.53	0.075	7.58	0.070	6.52	0.087	7.53	0.10	6.45	0.10	7.57	0.10

fish had been headed and gutted.

Tables 1 and 2 also show the percentage of protein recovery in the wastewater with the use of the nanofiltration system, which was operated at 16 bar. The water-soluble protein recovery rates for all the tests were very low compared to the other previous works, such as Miyata (1984) (protein recovery rate = 90 %; ultrafiltration system), Green *et al*. (1984) (protein recovery rate = 73 %; ultrafiltration system, 50 kDa), and Ninomiya *et al*. (1985) (protein recovery rate = 90 %; ultrafiltration system, 20 kDa.

It is postulated that the low value of protein recovery found in this study was due to the inaccurate analysis of the protein content using Lowry's method of analysis. These inaccuracies are due to the following reasons:

1. Lowry's method could only be used to measure the concentration of water-soluble proteins. However myofibrillar proteins (insoluble proteins) also appeared in the surimi processing wastewater (Lin *et al*. 1995).
2. As according to Peterson (1979), the presence of detergents, lipids and some salts in the samples could interfere the efficiency of Lowry's method since these substances might cause precipitation of the Folin-phenol reagent. However, lipids and salts were the two important elements that appeared in the fish and surimi wastewater (Hall and Ahmad, 1992).
3. The Lowry procedure is highly empirical. Increasing reaction/incubation temperatures might reduce differences in colour development between different proteins, with little effect on sensitivity (Salerno, 1985). In addition, precise timing of reagent addition and mixing are necessary for accurate and reproducible results (Yada *et al*., 1996).

Kjeldahl Distillation Method should be used to obtain more accurate total proteins analysis.

The total protein concentration and the total recovery of protein are shown in Tables 1 and 2. From the results obtained, the total protein recovery was as high as 97.9 % (Kjeldahl Distillation Method), compared to the highest protein recovery of 57.9 %, which was done by using Lowry's method of analysis. As expected earlier, nanofiltration should recover protein (>95 %) much better than the ultrafiltration, which had been done by Miyata (1984), Ninomiya *et al*. (1985), etc.

Tables 1 and 2 show the COD value for various types of wastewater before and after nanofiltration, which was operated at 16 bar. In general, COD values for both of the fish wastewater were much higher than the surimi wastewater. This was mainly because the fish wastewater carried the worst contamination with, for example, blood (Lee, 1986). Both of the COD values for the fish wastewater were very high. These findings agree with Lin *et al*. (1995), who reported extremely high COD (6000-27000 mg/l) in the wastewater, especially in the first discharged water. According to Jaouen and Quémeneur (1992), soluble proteins were regarded as pollutant in the wastewater. Therefore, it was not surprising to find that the highest concentration of protein in the wastewater gave the highest value of COD too. The COD values for the fish wastewater were still very high after nanofiltration (690 mg/l for first type of fish wastewater and 2750 mg/l for second type of fish wastewater). The results were within the expectations because as according to Rautenbach and Janisch (1987), in treating the protein-rich process water of the fish industry, it was recommended that a combination of membrane and conventional processes such as evaporation would give optimal results. The recommendation was also supported by Jaouen and Quémeneur (1992) and Afonso and Borquez (2002) who said that membrane separation processes were often associated with other separation methods as an intermediate step for both adding value of by- or co-products, and for process wastewater treatment.

Tables 1 and 2 also show the TSS value for various types of wastewater before and after nanofiltration, which was operated at 16 bar. Fish wastewater contained a lot of suspended

matter compared to the surimi wastewater. These findings agree with Jaouen and Quemeneur (1992), who reported that surimi raw wash water contained important quantities of insoluble materials (e.g. fibres, fats and denatured proteins). As Table 1 shows, the TSS value for the second type of fish wastewater was higher than that for the first type of fish wastewater. This finding agrees with Lin *et al*. (1995), who reported considerable quantities of myofibrillar proteins (myosin and actin) when the muscle was washed with water several times in succession, myosin and actin in the forms of suspended solids appearing from the second wash on.

The results of the NF experiments for the percentages reduction of COD were in the range of 75 % - 93 % (Tables 1 and 2). The results agree with Jaouen *et al*. (1989) who reported that the effluent polluting load was reduced by more than 75 % (expressed in COD and BOD_5) by using 10 kDa membrane. Therefore, percentage reduction of COD was expected to be higher than 75 % since 500 Da membrane was used in the experiments.

The highest percentage reduction of TSS was 87 % (Table 2). The percentage reduction of TSS was expected to be higher because their dimension was far bigger than the pore size of the membrane. The situation may be due to the biological fouling to form denatured proteins. As the filtered permeate solutions had been kept in the refrigerator for a few days before being analyzed, the soluble proteins contained in the sample were likely to be denatured (insoluble materials) (Jaouen and Quemeneur 1992).

The conductivity values (refer to the sodium chloride in the wastewater) for the permeate (Tables 1 and 2) were not greatly reduced after the nanofiltration process. This was because nanofiltration was only able to hold some of the NaCl in the retentate and most of the NaCl was able to pass through the membrane. The results agree with Nystrom *et al*. (1995), who reported that the retention of NaCl for most of the NF membranes was only around 0-50 %.

The results of the NF experiments (Tables 1 and 2) for measuring the pH of the wastewater were almost same (pH ≈ 7) before or after the nanofiltration process. This was mainly because NF membrane was unable to hold H^+ from passing through the membrane. Therefore, the concentration of H^+ was same for the feed mixture (before nanofiltration) and permeate stream (after nanofiltration).

Conclusions

The NF of the fish and surimi wastewater, after pre-treatment under vacuum through a Whatman No. 4 paper was investigated. Polyamide membranes of 500 Da MWCO were tested in this work. The permeate flux of the wastewater remained almost stable for about 270 minutes, which was a very promising finding since polyamide membrane was suitable to be used in the fish and surimi industries to treat the wastewater. The low protein recoveries observed (19-58 %) clearly pointed out that other analysis of protein measurements (such as Kjeldahl Distillation Method) should be used in order to obtain more accurate protein concentrations, seeing that Lowry's method was not that suitable to be used in the wastewater that contained lipids and salt. To reduce the value of COD and TSS in the fish and surimi wastewater more effectively, membrane separation process itself could not accomplish the task but membrane separation process was recommended in association with other separation methods as an intermediate step for both adding value of by- or co-products and for process wastewater treatment.

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