PROTEIN SEPARATION FROM ALKALINE PROCESS WATER OF SHRIMP WASTE PROCESSING INDUSTRY USING MEMBRANE FILTRATION

Le Quynh Thu Nguyen[1*], Heiko Lippich[1], Martin Wagner[1], Peter Cornel[1], Robert Lutze[2], Markus Engelhart[2]

[1]*Chair of Wastewater Technology and Water Reuse, Institute IWAR, TU Darmstadt, Germany*

[2]*EnviroChemie GmbH, Rossdorf, Germany*

Received 26 August 2012

Abstract

The recovery of protein and caustic soda solution from shrimp waste alkaline process water was experimentally investigated by a two-step filtration process with polymer membranes of different molecular weight cut-offs. Experiments for nine combinations of microfiltration/ultrafiltration and nanofiltration membranes were conducted in batch-mode using a stainless steel stirred cell with a membrane area of 38.5 cm². It could be shown that this process concept is capable of separating protein and dissolved organic carbon from the alkaline hydrolysis of shrimp waste. Totally, more than 30 % of protein was fractionated by the first step filtration while the second step reached a higher protein rejection of more than 88 %. The protein concentrate could be reused as animal feeding and the recovered sodium hydroxide solution couldbe recycled to the production process.

Keywords: Membrane filtration, protein recovery, alkaline process water.

1. INTRODUCTION

More than 50 % of Vietnamese revenue comes from agriculture [1, 2]. Rice and seafood are major exports of the Mekong Delta while farmers in the North earntheir livingmostly from livestock. Industrial farming (livestock and aquatic farms) leads to a rising demand for animal feed and the respective raw materials and ingredients as main inputs in the feed industry of which currently 60 % are imported [2]. In recent years, seafood processing has become an important economicsector in the Mekong Delta generating a considerable and growing amount of shrimp by-products as wastes. The reuse of shrimp wastes for the productionof chitin, chitosan, and the pharmaceutical product glucosamine is realized by a Vietnamese biochemical company and appears to be a promising way to turn "wastes into products". In this process, proteins are extracted from the shrimp wastes by an alkaline treatment. As a byproduct of this extraction, an alkaline process stream is generated (shrimp waste alkaline hydrolysis) that contains a high concentration of proteins and requires further treatment before discharge.

In addition, proteins of the currently discharged process stream are a potential valuable resource that could be recovered and reused as animal feed.

Due to its selectivity, membrane filtration is a suitable treatment option for the separation of wastewater constituents without changing their properties. In this context the treatment of shrimp waste alkaline hydrolysis by membrane technology under local conditions with the aim to recover proteins and to reuse the purified alkaline solution in the production process has been investigated in this study.

A two-step filtration with micro-/ultrafiltration (MF/UF) membranes coupled with different types of nanofiltration (NF) membranes was investigated in lab-scale. The investigation aimed to characterize the process water properties as well as the membranes themselves. The combined membranes were evaluated in regard to retention and flux performance during the filtration. The filtration was performced at transmembrane pressures of 3-5 bar for MF and UF and 15 bar for NF membranes. This paper reports on experimental lab results for this process.

2. MATERIAL AND METHODS

2.1. Shrimp waste alkaline process water used for experiments

Alkaline process water which is generatedduring the first treatment step of chitin processing (deproteination) was produced in the following way: Shrimp by-products (heads, shells and tails) were manually removed from defrosted Black Tiger prawn (penaeusmonodon) and heated at 100 °C for 2 hours with 4 % sodium hydroxide at a weight-volume ratio of 1:6 (shrimp waste mass to caustic solution volume). The solid parts were subsequently separated from the alkaline solution by a sieve with a pore size of 1 mm. For the whole experiments 22 L of process waterweregenerated in several batches. To ensure the same inflow characteristic for every filtration experiment, liquid batches have been mixed thoroughly and stored at 8 °C during the experimental period. The process water composition can be found in table 2.

2.2. Membranes

All tested membranes were flat sheets. MF and UF (Microdyn Nadir, Germany) were used as the first step filtration while three NF membranes(Dow Filmtec and Koch Membrane Systems, USA) with different salt retention ratios have been investigated as the second filtration step. The selected membranes were resistant to the high pH andtemperature up to 50 °C.

Before each filtration test, the permeability of the membranes was determined with pure water. Additionally, NF membranes were characterized in terms of ionic selectivity with 2 % magnesium sulfate solution at varying transmembrane pressures. Table 1 shows the membrane cut-off (manufacturer's information) and measured values of the membrane permeability and salt retention rates of the NF membranes.

Table 1: Properties of tested membranes

Membrane	Cut-Off / Retention	Permeability [L/(m²·h·bar)]
MF	0.05 µm	300-330
UF 1	150 Da	300-350
UF 2	50 Da	90-120
NF 1	R_{MgSO4} = 92 %	7-8
NF 2	R_{MgSO4} = 10 %	15-18
NF 3	R_{MgSO4} = 96 %	2-3

2.3. Experimental set-up

The filtration experiments were conducted in a stainless steel stirred membrane test cell (Berghof, Germany). This test device basically consists of a cylindrical vessel (400 mL) with an active membrane area of 38.5 cm². The membrane pressure was generated by compressed nitrogen from a gas bottle which was adjusted by a valve and controlled by a manometer on the cell top. The stirring rate was 300 min^{-1}. The permeate flux was measured with a balance (Acculab ALC-3100.2, Sartorius Group, Germany) connected to a laptop for data recording.

2.4. Experimental performance

Each test run was conducted as batch filtration (dead-end filtration) so that the process watervolume is reduced as the filtration advances. The filtration was investigated at ambient temperature (20-22 °C) as follows: After two repeated pure water tests, the filtration with alkaline process water was carried out with the MF and UF membranes. The permeate of the various MF/UF membranes from the first filtration was collected and used as influent for the second NF filtration step. Transmembrane pressures of 3, 5 and 15 bar were applied for the MF, UF and NF membranes, respectively.The retention rate describes the membrane capability and can be calculated as follows.

$$R_{i,M} = \frac{c_{Fi,M} - c_{Pi,M}}{c_{Fi,M}} \quad (1)$$

$$R_{i,B} = \frac{c_{Fi,B} - c_{Pi}}{c_{Fi,B}} \quad (2)$$

There are two different kinds of retention rates of a component *i*; membrane and system retention. Membrane retention $R_{i,M}$ (equation 1) refers to the retention rate at a given point of time during the dead end filtration process and is calculated using the measured permeate concentration of i$c_{Pi,M}$ and the feed concentration $c_{Fi,M}$ at this moment. The system retention $R_{i,B}$ (equation 2) is calculated using the unified permeate concentration c_{Pi} after the end of the dead end filtration and the concentration of the influent at the beginning of the filtration $c_{Fi,B}$. In batch-mode experiments the volumetric permeate recovery rate *VPR* is defined as the ratio between the filtered volume V_P to the starting volume V_F (equation 3).

$$VPR = \frac{V_P}{V_F} \quad (3)$$

2.5. Analytical methods

Dissolved chemical oxygen demand (COD) and total nitrogen bound (TN_b) weredetermined in mixed samples by Hach Lange Cuvette Tests (Dr. Lange LCK 514, LCK 314, LCK 238, LCK 338). Crude protein was calculated by multiplying total nitrogen with a factor of 6.25 [3].

3. RESULTS AND DISCUSSION

3.1. Properties of the alkaline process water

The COD and TN_b concentration of the alkaline process water in the different batches ranged from 36 to 60 g/L and 3 to 5.2 g/L, respectively. The variations in the concentrations of dissolved COD and TN_b can be explained by the manual processing. However, the ratio of COD to TN_b remained constant around 11.5. The composition of the unifiedprocess water is given in table 2.

Table 2: Process water composition

Parameter	Unit	Mean
pH	-	12
Temperature	°C	95
COD	g/L	43
TN_b	g/L	3.8
Protein (TN_b*6.25)	g/L	24
COD/TN_b	-	11.5

3.2. Ultrafiltration

UF membranes are widely used for protein recovery in the food industry, especially in dairy processing industry. As a result of the filtration process, proteins can be separated from salt and lactose [4]. In this study, porous MF and UF membranes were used to separate particles and macromolecular substances contained in the process water.

Table 3 shows that the COD and TN_b retention of the first filtration step ranged between 21-31% and 14-24 %, respectively. This corresponds to a protein retention of 14-24 %.

It can be expected that the retention of COD and TN_bincreases with decrease of membrane cut-off. Additionally, during the dead end filtration acake layer is forming on the membrane surface whichactsas a second barrier that booststhe retention.

The membrane screening results revealed the different size-fractions of the protein in the hydrolysis solution. Table 3 shows that 14 % of the proteins is larger than 0.05 µm and nearly 7 % of them has molecular size between 50 and 150 Dalton. Almost 24 % of nitrogen containing substances have been retained with a pore size of 50 Dalton (UF 2). It can be concluded thatpossible protein fractionation would be improved by a subsequent filtration step using tighter membranes.

Table 3: System retention of the first step filtration

	R_{COD}, %	R_{TNb}, %
MF	21.1	14.0
UF 1	22.7	17.1
UF 2	31.4	23.8

Figure 1 depicts the performanceof the permeate flux during the batch-filtration. As a result of the dead-end operation the permeate recovery increased with the filtration time while the remained volume decreased accordingly. No differences in flux performance could be observed for the tested membranes. A steady flux decline has been found from the beginning until the end of the experiments. The UF 1 and UF 2 membranes started at the same flux (13.1 L/(m²·h)) which was higher than the one of the MF membrane (11.6 L/(m²·h)). A flux decline of about 27 % and 44 % was observed at a permeate recovery rate of 50 % and 80 %, respectively. The MF membrane maintained a stable flux decrease up to a permeate recovery of 100 %. In case of the UF membranes a fouling layer built up which led to a sharp decline of the flux performance starting from a permeate recovery of 80 %.

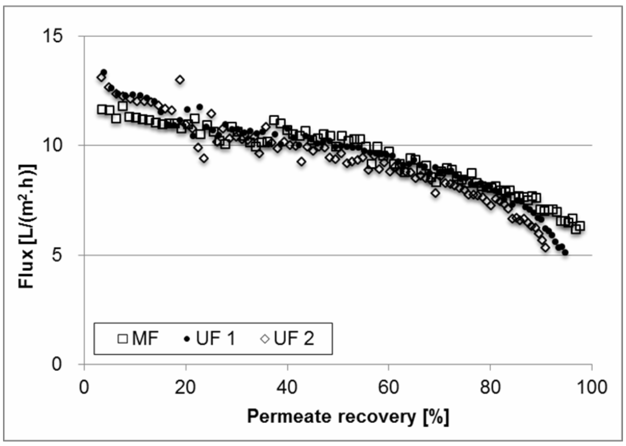

Fig. 1: First step filtration - membrane performance

The experiments also showed that the MF membrane achieved the same results as the UF membranes with a lower transmembrane pressure of 3 bar compared to 5 bar. The permeate flux that was achieved during the filtration of shrimp waste alkaline process water was considerably lower compared to the pure water flux for both, MF and UF membranes. Probably, under pressure a fouling layer builds up immediately on the active membrane surface due to protein absorption in the pore wall [5].

3.3. Nanofiltration

In this concept a second filtration step with NF membranes is used with the aim to increase the recovery rate of proteins. Thesepressure-driven NF membranes can reject multivalent ions and are commonly used for desalination and rejection of hardness in drinking water processing [4].

All nine possible combinations of this two-step filtration process have been tested. The results of the system retention are shown in table 4.

Table 4: System retentionof total nitrogen bound of the two-step filtration

R_{TNb}		Second step		
		NF 1, %	*NF 2, %*	*NF 3, %*
First step	*MF*	65.8	57.0	88.7
	UF 1	69.0	76.9	88.5
	UF 2	75.7	90.7	87.8

It can be seen that two different ranges of protein retention were obtained: below 77 % and over 88 %. With the exception of the combination MF-NF 2, all combinations achieved recovery rates of > 60 %. Despite a good salt rejection, NF 1 showed the lowest protein retention rate of all NF membranes (< 75.7 %). The combination of the tightest porous membrane with a low rejection NF membrane (UF2-NF 2) achieved the best system retention of 90.7 %. NF 3 showed a stable retention rate of > 88 % which did not depend on the first filtration step.

With regard to the flux performance, all NF membranes could reach a permeate recovery of 80 %. The flux capability matched the pure water flux which was determined under the same testing condition. NF 2 appeared to obtain the best permeate flux 47.42 L/(m²·h) at a permeate recovery of 50 % while NF 1 and NF 3 showed a considerable flux decline as well as the NF 3's flux varied widely. Figure 2 shows the permeate flux as a function of the volumetric recovery rate exemplified for NF 2.

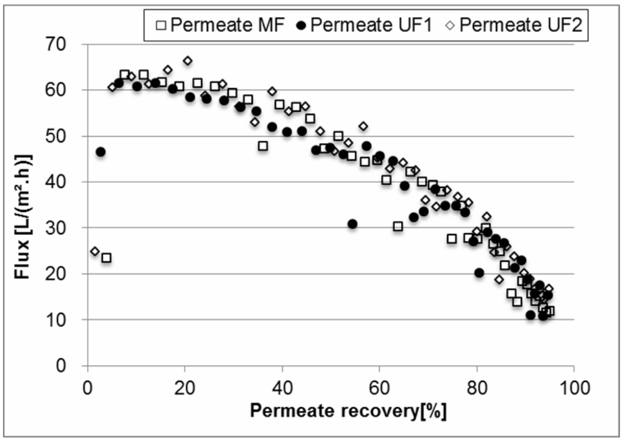

Fig. 2: Flux performance of NF 2 with permeates from different MF/UF membranes

Batch-filtration is rarely applied in practice due to the poor surface control which leads to a loss of permeability and rapid blockage of the membranes. However, the results of this study allow to compare the different tested membranes and to define the membrane performance under the tested conditions. In order to estimate the mass of retained proteins the concentration of total nitrogen was analyzed at eachrecovery rate step of 10 % during the filtration period of the combination UF 1-NF 3. Figure 3 shows the concentrations of TN and proteins at different permeate recovery rates. It can be seen that more than 88 % of protein retention is attainable at a permeate recovery of 80 %. Thus it can be concluded that protein separation from alkaline process waterbymembrane filtration is feasible.

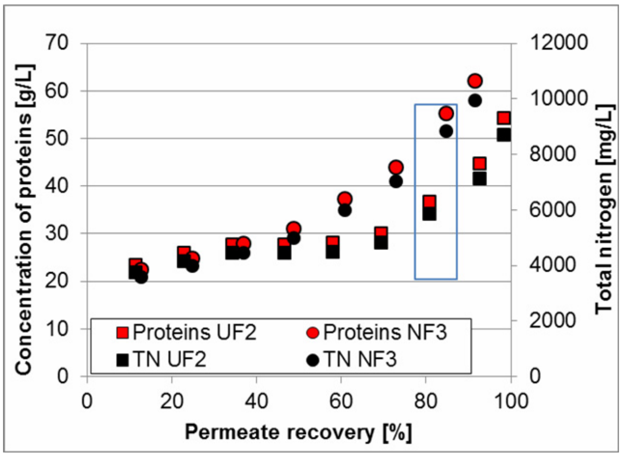

Fig. 3: Concentration elevation as a function of permeate recovery, two-step filtration UF 2-NF 3

4. CONCLUSIONS

This study showed that a two step membrane filtration is feasible to recover proteins contained in alkaline process water(more than 88 % with UF 1-NF 3 as well as 90.5 % with UF 2-NF 2). Moreoverpurified alkaline solution could be reused in the process.

This way, the organic load of the discharged wastewateras well aschemical, water and energy consumption can be reducedresulting in significant savings of operational costs.

This approach will be tested in pilot scale experiments in order to further investigate the membrane behaviour, the flux performance, the achievable recovery and retention rate as well as the optimal operating conditions and cleaning strategy under local conditions. Also operating cost must be calculated taking the pilot scale results into account. Additionally, the quality of proteins has to be considered to meet the market requirements and possible application.

Acknowledgement: *This work has been financially supported by the German Federal Ministry of Education and Research (BMBF) within the research grant02WA1066. The authors would like to thankcolleagues of the Institute IWAR–TU Darmstadt and colleagues of the industrial partner EnviroChemie GmbH for the kind cooperation and support.*

REFERENCES

1. GFA Consulting Group. *Agrar-und Ernährungswirtschaft in Vietnam, Sektorüberblick, Märkte und Investitionsbedingungen*, Schlussbericht gefördert von Bundesministerium für Ernahrung, Landwirtschaft und Verbraucherschutz (BMELV), Bonn, Germany (2009).
2. Osec Business Network Switzerland. *Vietnam Feed Industry*, compiled by Swiss Business Hub ASEAN/Vietnam Office (2012).
3. L. D. Ngoan, J. E. Lindberg, B. Ogle, S. Thomke. *Anatomical proportions and chemical and amino acid composition of common shrimp species in central Vietnam*, Asian-Australasian Journal of animal sciences, **13(13)**, 1422-1428 (2000).
4. T. Melin, R. Rautenbach. *Membranverfahren – Grundlagen der Modul- und Anlagenauslegung*, Springer-Verlag, Berlin Heidelberg (2007).
5. A. Saxena, B. P. Tripathi, M. Kumar, V. K. Shadi. *Membrane-based techniques for the separation and purification of proteins: An overview*. Advances in Colloid and Interface Science, **145**, 1-22 (2009).

Corresponding author: **Le Quynh Thu Nguyen**
TechnischeUniversitaet Darmstadt
Institute IWAR – Chair of Wastewater Technology and Water Reuse
Franziska-Braun-Str. 7
54267 Darmstadt, Germany
Email: t.nguyen@iwar.tu-darmstadt.de.