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Full paper

Effect of Operating Parameters on Performance of Ultrafiltration (UF) to Fractionate Catfish Protein Hydrolysate

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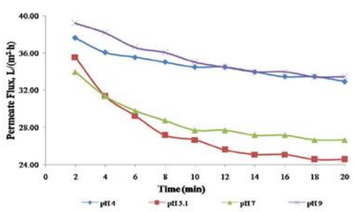
Graphical abstract



Abstract

The effect of pH, ionic strength and feed concentration on performance of ultrafiltration (UF) to fractionate Catfish protein hydrolysate (CFPH) through 5kDa regenerated cellulose (RC) membrane was studied. The highest and lowest permeate flux belonged respectively to pH 9 and isoelectric point (IEP) with flux reduction of 5.75 L/m^2.h at pH 9 and 10.98 L/m^2.h at pH isoelectric through operating time. Further, by adding the salt, the highest permeate flux and transmission obtained at highest ionic strength of 0.15 M NaCl with 52.96% of transmission (in average). Then, the transmission reached to 54.18% by increasing feed concentration up to 1.5 mg/ml.

Keywords: pH; ionic strength and feed concentration; ultrafiltration (UF); fish protein hydrolysate

1.0 INTRODUCTION

Numerous applications of proteins and protein hydrolysates in biomedical and food industries [1] have attracted the interest of many researchers to fractionate, separate, concentrate, purify and recover these valuable products in last decades. The high functional properties of fish protein hydrolysates (antioxidant, antimicrobial, ACE inhibition and etc.) prepared by enzymatic hydrolysis, has led to use of these value added products in different industries such as fermentation industry as a nitrogenous substrate in microbial medias [2] or as preservatives in food and cosmetic industries [3].

In most studies, membrane technology including ultrafiltration and nanofiltration has been considered as a tool to separate, fractionate and improve their functional and biological properties [3]. Only in a few works, separation of fish protein hydrolysates by ultrafiltration (UF) has been mentioned [3-4]. The membrane filtration technology due to the higher yield and easy to scale- up than other separation methods (chromatography methods) [5] is known as an effective method in mentioned industries.

Many works have shown the effect of pH and ionic strength of protein solutions on yield of separation. For instance, Lin *et al*. [5] could get more yield of hemoglobin (Hb) and bovine serum albumin (BSA) separation at pH higher than IEP of Hb (> 7.1) and at low ionic strength. Das *et al*., [6] studied the effect of pH on sesame protein hydrolysate flux and permeate concentration (Cp) and achieved higher Cp at IEP (pH 4.9). Mathew *et al*. [7] have reported the increase in transmission and flux of BSA, ovalbumin and lysozyme with increase in salt concentration from 0 to 1M and achieved all BSA in permeate by 0.1 M NaCl in BSA solution. The aim of this research work was to evaluate the influence of pH and ionic strength on behavior of flux and transmission of catfish protein hydrolysate (CFPH) to enrich the concentration of CFPH in permeate flux which is affected by fouling as an effective factor on yield of membrane filtration process.

2.0 EXPERIMENTAL

2.1 Materials

Fish protein hydrolysate was prepared from fresh Channel catfish (Ictalurus punctatus) by adding Alcalase (purchased from Sigma-Aldrich, St. Louis, MO) to substrate in Tris-HCl buffer in shaking water bath for 5 hours. Filtration was done through 5 kDa flat sheet regenerated cellulose (RC) membrane in 200 ml Amicon stirred ultrafiltration cell (model 8200) purchased from Millipore Corp., Bedford, MA. All solutions prepared by ultrapure water, obtained from a water purification system with a resistivity of 18.2 MΩ cm. The filtration was carried out in dead end mode and permeates were collected for further analysis. In each step same amount of sample dissolved in various buffer solutions and then, three different ionic strength values of NaCl were tested.

2.2 Characterizations

The molecular weight of CFPH (less than 6.5 kDa) which has most effect on yield of flux and transmission, was determined with SDS-PAGE electrophoresis according to the method of Schägger *et al*. [8] Zeta sizer Nano from Malvern Instrument used to measure isoelectric point of sample. Peptides content in feed, permeate and retentate was measured by following the method of Church *et al*. [9] using o-phthaldialdehyde (OPA) reagent.

■3.0 RESULTS AND DISCUSSION

3.1 Effect of pH on Permeate Flux and Transmission

The effect of pH on flux and transmission was evaluated at various pHs (4, IEP, 7, 9). The highest fluxes belonged to pH 4 and 9 away from isoelectric point and gradually decreased at pH7 and reached to lower value at IEP. Generally, at isoelectric point due to the zero charged protein hydrolysate molecules and minimized intermolecular electrostatic repulsion, the aggregation of protein hydrolysate on membrane surface are expected to be maximum [6-10-11] which results to the reduction of permeate flux and, consequently fouling would be the most at pH near CFPH isoelectric point (Figure 1) [12].

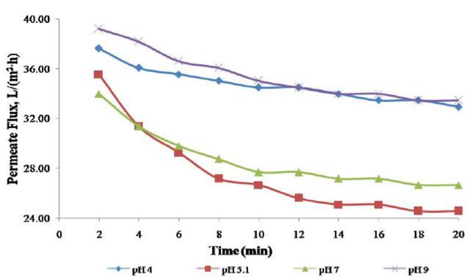


Figure 1 Effect of pH on permeate flux through 5 kDa membrane. Other experimental conditions: 0.5 mg/ml CFPH, 2 bar

Far from the IEP, the increase in intermolecular electrostatic repulsion as a reducer factor of membrane fouling causes less flux reduction than IEP [13-14]. From the results the highest reduction of flux occurred at isoelectric point and it varied from initial amount of 35.54 L/(m^2.h) to the final permeate flux of 24.56 L/(m^2.h). By increasing the pH up to pH7 the flux slightly increased and it changed from 33.97 L/(m^2.h) to 26.66 L/(m^2.h) in 20 minutes. At pH 4 and 9 reduction was slowly and changed from 37.63 L/(m^2.h) to 32.93 L/(m^2.h) and from 39.20 L/(m^2.h) to 33.45 L/(m^2.h) in 10th collection respectively and showed 34.70 and 35.43 (L/m^2.h) of permeate flux in average respectively (Table 1).

Table 1 The results of permeate flux and percentage of transmission at four different pHs (The results are shown in average in operating time of filtration)

pH	4	IEP	7	9
Permeate Flux (L/m^2.h)	34.7	27.49	28.69	35.43
Transmission %	40.50	48.81	40.97	39.3

The transmission of CFPH in different pHs are shown at Figure 2. The maximum transmission was expected at IEP due to the absence of electrostatic repulsion as an effective factor on reduction of protein hydrolysate molecules transmission [10-12]. The increase in transmission at this pH also can be explained by influence of concentration polarization which is in conjunction with the aggregation of molecules on membrane surface [10-11]. By varying pH to pH 7 transmission slightly reduced since the protein hydrolysate molecules are relatively larger [6]. At acidic and alkaline pH, the transmission decreased due to the high repulsion between protein molecules themselves and membrane surface. The results of transmission in average showed 39.30 % at pH 9 (lowest transmission), 40.5% at pH 4, 40.97% for pH 7 and by changing the pH to isoelectric point it reached to the highest value of 48.81% in average.

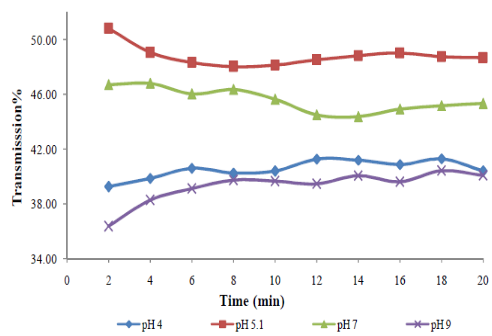


Figure 2 Effect of pH on transmission of 5 kDa membrane for 5 h CFPH. Other experimental conditions: 0.5 mg/ml CFPH, 2 bar

3.2 The Effect of Ionic Strength on Permeate Flux and Transmission

The effect of this parameter was studied at three different values (0.05, 0.1 and 0.15 molar) of NaCl at IEP which was selected based on the highest transmission than other examined pHs (Figure 3 and 4). Without adding NaCl due to the high aggregation and fouling, the flux was at its lowest value. At IEP in absence of ions, the protein molecules have zero charge and they are in their smallest size which causes the least permeability of deposited layer on membrane surface. By adding salt at this pH, the net charge and the size of protein hydrolysate molecules increased due to the anion binding which led to increase in permeate flux by increasing ionic strength.15 The flux at 0.05 M of NaCl showed 3.39 (L/m^2.h) increase than solution without salt addition and flux increased to 3.97 and 5.8 (L/m^2.h) respectively at 0.1 and 0.15 M of NaCl in average. The initial flux at 0.05 M changed from 38.15 L/(m^2.h) to 27.18 L/(m^2.h) in 20 minutes. By increasing the ionic strength to 0.1 M the flux did not show significant increment and changed from 37.63 L/(m^2.h) to 28.22 L/(m^2.h). By adding NaCl up to 0.15 M the flux showed higher values with reduction from 39.20

L/(m^2.h) to 30.31 L/(m^2.h) in 20 minutes. The average permeate flux and transmission are shown in Table 2.

Formation of dense layer compacted with small molecules on membrane surface 16-17 plays a significant role on yield of transmission. Molecular enlargement as a result of anion binding, directed to formation more open structure of adsorbed molecules on membrane surface and finally improvement in transmission.18 The results showed that by increasing the ionic strength up to 0.15 M, the transmission changed from 50.83% (in solution without NaCl) to maximum value of 55.08% (in 0.15 M) at 2 minutes. It improved from 48.67% to 51.73% at 20 minutes in protein solution without NaCl and 0.15 M solution respectively.

3.3 Effect of Feed Concentration on Permeate Flux and Transmission

The higher flux at lower feed concentration is expected due to the less accumulation of molecules on membrane surface [6]. The flux reduction as a result of increase in feed concentration (increase in mass transfer) leads to accumulation of more protein hydrolysates on membrane [3-6-19]. The values varied from 39.20 to 30.31 L/(m^2.h) with 0.5 mg/ml of sample and from 34.49 to 23.52 L/(m^2.h) with 1.5 mg/ml of hydrolysate (Figure 5). The average amount of permeate flux and transmission are shown in Table 3.

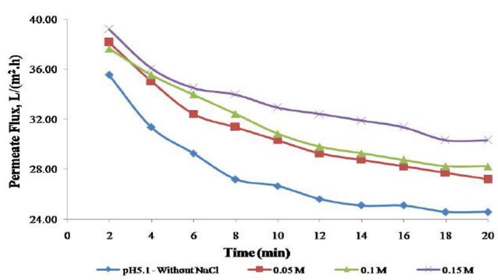


Figure 3 Effect of ionic strength on permeate flux through 5 kDa membrane. Other experimental conditions: 0.5 mg/ml CFPH, pH: 5.1, 2 bar

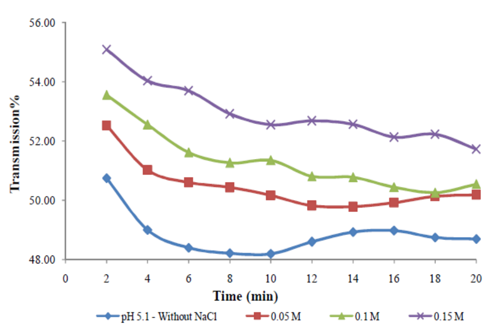


Figure 4 Effect of ionic strength on transmission of 5 kDa membrane for 5 h CFPH. Other experimental conditions: 0.5 mg/ml CFPH, pH: 5.1, 2 bar

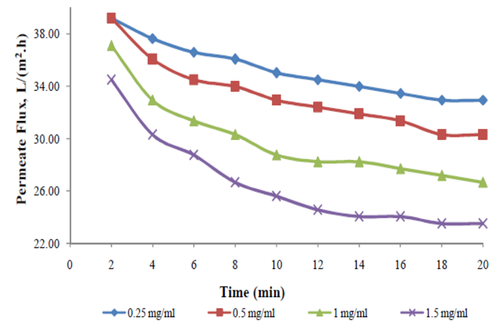


Figure 5 Effect of feed concentration on permeate flux of 5 kDa membrane for 5 h CFPH. Other experimental conditions: 2 bar, pH: 5.1, 0.15 M

Table 2 The results of permeate flux and percentage of transmission at three different ionic strength (The results are shown in average in operating time of filtration)

Ionic Strength	No Salt	0.05 M	0.1 M	0.15 M
Permeate Flux (L/m^2.h)	27.49	30.88	31.46	33.29
Transmission %	48.81	50.47	51.31	52.96

Although increase in feed concentration causes more fouling, still due to the more solute transport through the membrane pores, transmission is enhancing. The behaviour of 5 h protein hydrolysate was in the same way with above explanation and the maximum value was achieved at highest tested concentration of 1.5 mg/ml, as evidenced from Figure 6. The variation of transmission was from 56.37% to 52.96% at highest concentration in 20 minutes.

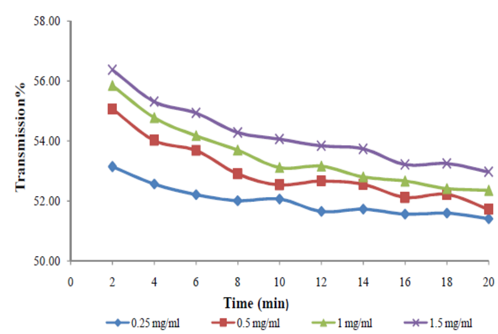


Figure 6 Effect of feed concentration on transmission of 5 kDa membrane for 5 h CFPH. Other experimental conditions: 2 bar, pH: 5.13, 0.15 M

Table 3 The results of permeate flux and of transmission at three different feed concentrations (The results are shown in average in operating time of filtration)

Feed Concentration	0.5 mg/ml	1 mg/ml	1.5 mg/ml
Permeate Flux (L/m^2.h)	33.32	29.84	26.55
Transmission %	52.93	53.49	54.18

■4.0 CONCLUSION

The influence of pH, ionic strength and feed concentration on permeate flux and transmission of catfish protein hydrolysate (CFPH) in dead-end mode ultrafiltration (UF) were studied in current study. The data showed that by manipulating these parameters, the yield of filtration could improve up to 54.18 % in average and it made possible to optimize the filtration process. Based on the results, the use of ultrafiltration to produce more concentrated and enriched solution of protein hydrolysate in permeate could be suggested as an easy method considering controlling the operating parameters.

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