The membrane separation mechanism in protein concentration from the extract of waste press cake in biofuel manufacturing process of *Jatropha* seeds

T W Chung[1, 2], C K Chen[1] and S H Hsu[1]

[1]Department of Chemical Engineering, Chung Yuan Christian University, Taoyuan 32023, Taiwan

E-mail: twchung@cycu.edu.tw

Abstract. Protein concentration process using filter membrane has a significant advantage on energy saving compared to the traditional drying processes. However, fouling on large membrane area and frequent membrane cleaning will increase the energy consumption and operation cost for the protein concentration process with filter membrane. In this study, the membrane filtration for protein concentration will be conducted and compared with the recent protein concentration technology. The analysis of operating factors for protein concentration process using filter membrane was discussed. The separation mechanism of membrane filtration was developed according to the size difference between the pore of membrane and the particle of filter material. The Darcy's Law was applied to discuss the interaction on flux, TMP (transmembrane pressure) and resistance in this study. The effect of membrane pore size, pH value and TMP on the steady-state flux (J_{st}) and protein rejection (R) were studied. It is observed that the J_{st} increases with decreasing membrane pore size, the J_{st} increases with increasing TMP, and R increased with decreasing solution pH value. Compare to other variables, the pH value is the most significant variable for separation between protein and water.

1. Introduction

Jatropha curcas oil is gradually considered important to be the biofuel feedstock [1]. A large amount residue of pressed cake is generated after oil extraction. Both organic solvent and enzyme were used in various extractions [2-4]. The refined plant oil can be produced as raw material for biodiesel to replace traditional diesel fuel. However, the pressed cake has toxic substance of phorbol esters, resulting in the pressed cake is not suitable to be the animal feed. The pressed cake of *Jatropha curcas* contains high protein. The high protein meal can be used as fertilizer, biogas and adhesives to reduce the overall cost of biomass [5,6]. The pressed cake of *Jatropha curcas* is a mixture which containing fiber, protein and oil, therefore the separation process which include centrifugal [7-9], membrane and chromatography technologies were necessary to elevate usability of protein products [10,11]. The purpose of protein concentration is for the protein product preserving and transportation conveniently. The protein concentration was mostly carried out by using freeze-drying, which was a high energy-consumption process [12]. Therefore, membrane technology was applied as an improving method that was a simple operation and energy-saving separation process [13,14].

The separation mechanism of membrane filtration was the sieving, which is in accordance with the size difference between the membrane pore and the particle of filter material. The micro- and ultra-

filter membranes were chosen for protein concentration usually [15, 16]. The pH value is a major factor for the protein concentration by membrane [17, 18]. The aggregation phenomenon of protein molecules was by electric interaction at specific range of pH value, resulting in the flux sharply fluctuation. In addition, it was found that the flux was influenced by transmembrane pressure (TMP) according to the Darcy's Law, which was usually applied to discuss the interaction on flux, TMP and resistance [19,20]. In this study, the membrane filtration for protein concentration will be conducted and the analysis of operating factors for protein concentration process using filter membrane was discussed. The separation mechanism of membrane filtration was developed according to the size difference between the pore of membrane and the particle of filter material.

2. Experimental section

2.1. Chemicals and raw materials
The materials and reagents used in this study include *Jatropha curcas* pressed cake, hydrochloric acid (HCl, 35 vol%, Showa), ammonium hydroxide (NaOH, ACS, Macron) and cellulose acetate filter membrane (CA, 0.2, 0.5 and 0.8 μm, Advance).

2.2. Experimental set up
An experimental schematic illustration, cross-flow microfiltration system is shown in figure 1.

E-1 Tank
E-2 Pump
E-3 Membrane Module
E-4 Electronic balance
E-5 Flowmeter
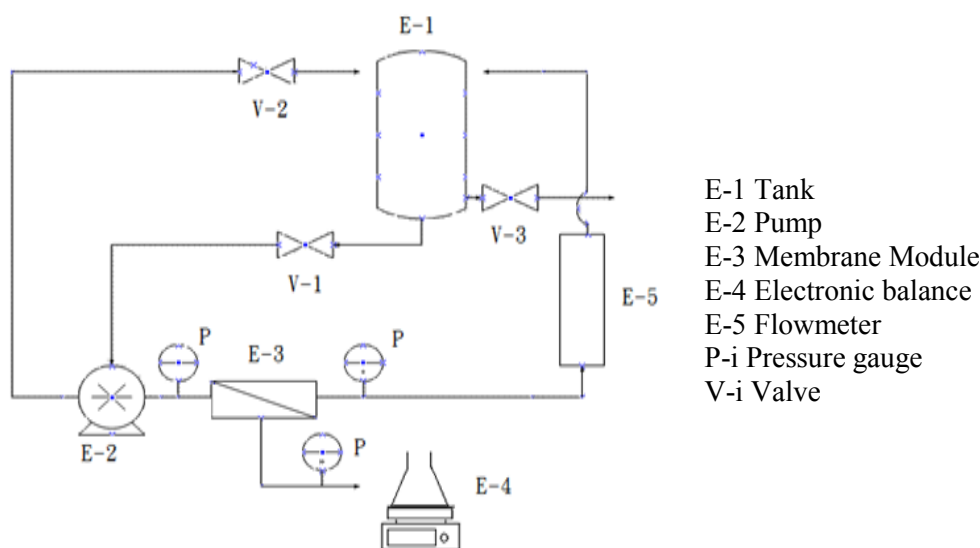P-i Pressure gauge
V-i Valve

Figure 1. Experimental setup in this work.

The initial concentration of *Jatropha curcas* protein solution was 8.92 mg/mL. The pH values of protein solution were adjusted by HCl to 2, 5 and 8. Different pore sizes of flat-sheet cellulose acetate (CA) filter membrane were selected with 0.2, 0.5 and 0.8 μm. The effective filter area is $9.62 \times 10^{-4} \, m^2$. The transmembrane pressure (TMP) was controlled by V-2 in figure 1. The steady-state flux (J_{st}) and protein rejection (R) were defined as usual and expressed as following equation:

$$J_{st} = \frac{V_p}{A \times t} \tag{1}$$

where J_{st} means the steady-state flux, which is a constant value at final stage of filtration using $L/m^2 h$ as unit, A means an effective filter area, which is $9.62 \times 10^{-4} \, m^2$ in this study, t means an operation time, which control at 3 hours at a steady-state in this study, V_p means the accumulative permeation volume.

$$R = 1 - \frac{C_p}{C_F} \tag{2}$$

where R means the protein rejection, which is used to discuss the degree of separation using % as unit. ELISA technology [21, 22] was used to measure the concentration of protein in the solution. C_F and C_p express the protein concentration in the feed and permeation solution, using mg/mL as unit.

2.3. Theory

The filtration resistance was calculated by Darcy's law as following equation [23]:

$$J = \frac{\Delta P}{\mu R t} \tag{3}$$

where J (m/s) means permeation flux, ΔP (Pa) means the transmembrane pressure (TMP), μ (Pa•S) means the dynamic viscosity of the solution in filtration process, and R_t (1/m) means the overall filtration resistance. The overall filtration resistance (R_t) can be expressed as the combination of various resistances as follow.

$$R_t = R_m + R_c + R_f \tag{4}$$

where R_m (1/m) means the resistance of membrane itself by measuring the steady-state DI water flux, R_c (1/m) means the resistance of cake formation onto the membrane surface at filtration process, R_f (1/m) means the resistance of fouling inside membrane pore.

On the other hand, the membrane filtration behavior can be expressed by the law of filtration blocking mode, which the cake layer heap on membrane surface at the last stage of filtration [24]. In the figure 2(a), the schematic diagram of cake blocking mode can be expressed as a function of time as following:

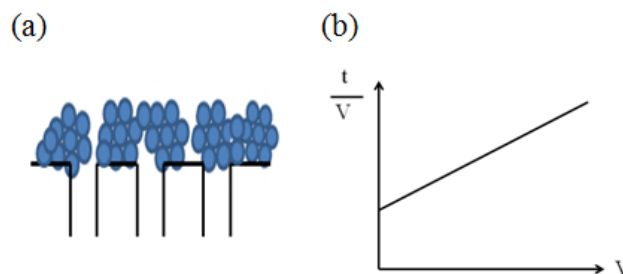$$\frac{t}{V} = \frac{1}{Q_0} + \frac{K_c}{2} \tag{5}$$

Figure 2. (a) The diagram of cake blocking mode; (b) Expressed as a function of time.

Figure 2(b) can be used to find out the range of cake blocking according to equation (5). It was found that the value, which the filtered time (t) multiplied by inverse permeation volume (1/V) linearly increased with permeation volume (V) where V was a volume per area.

3. Results and discussion

The effect of membrane pore size, pH value and transmembrane pressure on the steady-state flux (J_{st}) and protein rejection (R) were studied. It is observed that the steady-state flux (J_{st}) increases with decreasing membrane pore size. The membrane resistance (R_m) was smaller for the membrane with larger pore size, the protein molecules could pass easily through larger pore size membrane. However, the larger J_{st} was appeared on the membrane with smaller pore size. The filtration blocking law and Darcy's law were used to explain the relationship between J_{st} and membrane pore size.

In order to figure out this phenomenon, the cake formation time of filtration blocking stage (t) varied with membrane pore size of 0.2, 0.5 and 0.8 μm under 0.5 kg/m² TMP was observed first. It was found that a linear relationship (figure 3) between the t multiplied by inverse permeation volume

(1/V) and permeation volume (V) by graphing method of equation (5). It is integrated that the t increases with decreasing membrane pore size (table 1). It means the cake was formed slowly on the larger pore size of membrane surface, resulting in the smaller protein molecules easily adsorb onto the membrane to generate a larger cake resistance layer (figure 4). As a consequence, it was found that the cake resistance (R_c+R_f) increased with the larger membrane pore size operation, which given a poor result of J_{st}.

Table 1. The effect of membrane resistance (R_m), cake resistance (R_c+R_f) and the time of filtration blocking stage (t) on J_{st} varying membrane pore size.

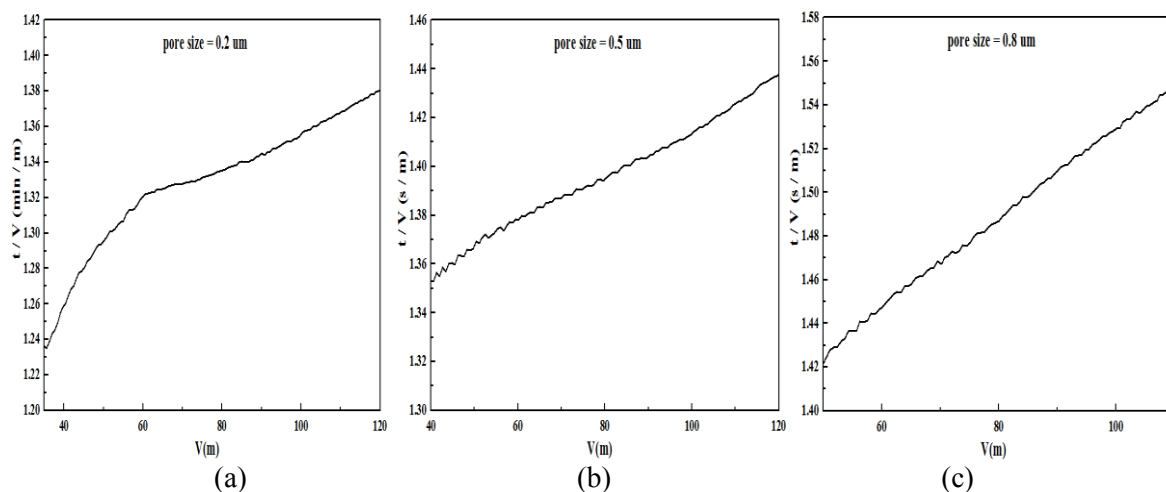| pore size (um)	0.2	0.5	0.8
J_{st} (L/m^2-h)	43.36	41.76	38.75
R_mx10^{-9} (1/m)	14.22	7.44	3.79
(R_c+R_f)x10^{-12} (1/m)	6.84	7.13	7.68
t (min)	40-180	50-180	70-180

Figure 3. The cake blocking mode on varying membrane pore size with (a) 0.2 (b) 0.5 (c) 0.8 μm.
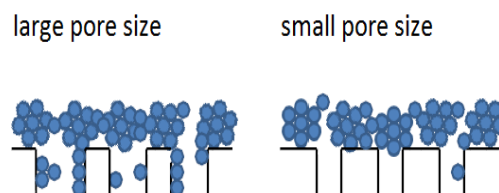

Figure 4. The schematic diagram of blocking mode on varying membrane pore size.

The protein aggregation phenomenon means the protein molecules aggregated to become the group of protein by electrostatic attraction (figure 5). For the smaller particle, non-aggregation protein would easily adsorb onto the membrane, resulting in the cake resistance (R_c+R_f) was larger. Therefore, it was observed that the larger value of J_{st} at lower pH of solution.

Similarly, it was found that R increased with decreasing solution pH value. Compared to other variables, the pH value is the most significant variable for separation between protein and water. The condition of non-aggregation protein has a lot of smaller protein molecules, resulting in these

molecules can pass easily through the membrane to increase the protein concentration of permeation (figure 5). According to equation (2), C_p is smaller at the condition of protein aggregation, resulting in the R is higher than the condition of non-aggregation protein. Therefore, R increased with decreasing solution pH value.
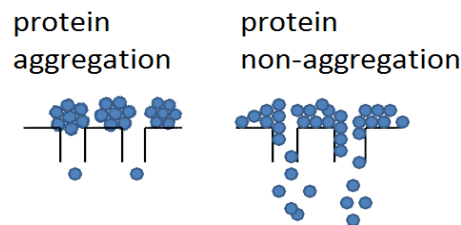

Figure 5. The schematic diagram of blocking mode on varying solution pH value.

It was found that J_{st} increases with increasing TMP which follows the Darcy's law. In the figure 6, it is observed that J_{st} increases linearly with increasing TMP due to the overall filtration resistance is quite small when the TMP below 0.5 kg/cm^2, and then the overall resistance sharply rise when the TMP above 0.5 kg/cm^2, resulting in the increasing of J_{st} is limited. On the other hand, it was observed that the protein rejection (R) was strongly dependent on the solution pH value. But, it was important that the R was independent on the interaction of membrane pore size and transmembrane pressure.
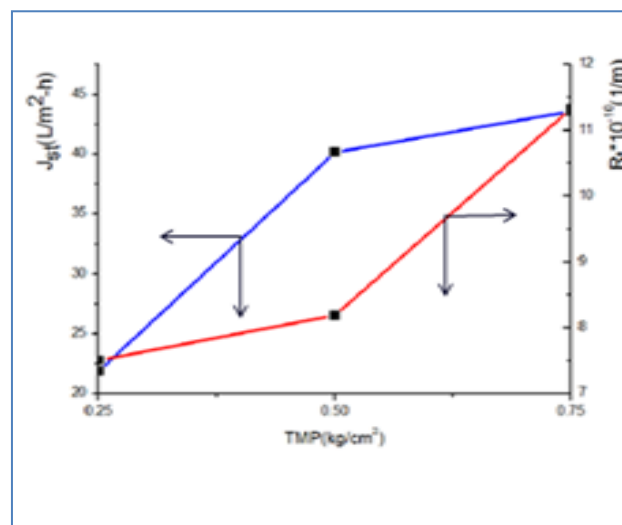

Figure 6. The effect of TMP on J_{st} and overall resistance.

4. Conclusion
On the basis of the energy-saving consideration, the membrane filtration process was chosen to replace the traditional heating of protein solution. In this study, the protein solution form extraction of *Jatropha curcus* pressed cake was conducted by the cellulose acetate filter membrane to concentrate. The effect of selected variables on the steady-state flux and protein rejection on the membrane were investigated. The higher steady-state flux was found at a condition of lower solution pH and higher transmembrane pressure. The higher protein rejection was found at a condition of lower solution pH and lager membrane pore size. The membrane filtration for protein concentration was conducted and compared with the recent protein concentration technology. The separation mechanism of membrane filtration was developed according to the size difference between the pore of membrane and the particle of filter material. The membrane filtration behavior can be expressed by the law of filtration

blocking mode, which the cake layer heap on membrane surface at the last stage of filtration. The cake blocking mode was discussed with the varying membrane pore size, pH value, and TMP successfully.

References
[1]   Hamarneh A, Heeres H, Broekhuis A and Picchioni F 2010 *Intl J. Adhesion and Adhesives* **30** 615-25
[2]   Gofferje G, Klingele S, Stabler A, Weisz U and Floter E 2013 *Waste Biomass* **29** 301-8
[3]   Lestari D, Mulder W and Sanders J 2010 *Biochemical Eng. J.* **50** 16-23
[4]   Russin T, Arcand Y and Boye J 2007 *Biomass* **13** 308-19
[5]   Giibitz G, Mittelbach M and Trabi M 1999 *Bioresource Tech.* **67** 73-82
[6]   Deeba F, Kumar V, Gautam K, Saxena R and Sharma D 2012 *Biomass and Bioenergy* **40** 13-8
[7]   Naderi N, House J and Pouliot Y 2014 *J. Food Eng.* **141** 85-92
[8]   Lindner J, Menzel K and Nirschl H 2013 *Chemical Eng. Sci.* **97** 385-93
[9]   Strixner T and Kulozik U 2013 *J. Food Eng.* **117** 89-98
[10]   Zobel S, Helling C, Ditz R and Strube J 2014 *Ind. and Eng. Chem. Res.* **53** 9169-85
[11]   Liu Y, Feng Z, Shao Z and Chen X 2012 *Materials Sci. Eng.* C **32** 1669-973
[12]   Fan L, Ding S, Liu Y and Ai L 2012 *Intl J. Biological Macromolecules* **50** 1008-10
[13]   Pouliot Y 2008 *Intl Dairy J.* **18** 735-40
[14]   Daufin G, Escudier J, Carrere H, Berot S, Fillaudeau L and Decloux M 2001 *Trans IChemE* **79** 89-102
[15]   Onics Inc. 2004 *Filtration & Separation* **41** 32-33
[16]   Narsaiah K and Agarwal G P 2007 *J. Membrane Sci.* **287** 9-18
[17]   Huisman H, Prádanos P and Hernández A 2000 *J.* Membrane *Sci.* **179** 79-90
[18]   Ibanez R, Almecija M, Guadix A and Guadix E 2007 *Sepa. Purif. Tech.* **57** 314-320
[19]   Ouammou M, Tijani N, Calvo J, Velasco C, Martin A, Martinez F, Tejerina F and Hernandez A 2007 *Colloids and Surfaces A: Physicochem. Eng. Aspects* **298** 267-73
[20]   Ripperger S and Altmann J 2002 *Sepa. Purif. Tech.* **26** 19-31
[21]   Chen V, Fane A, Madaeni S and Wenten I 1997 *J. Membrane Sci.* **125** 109-22
[22]   Jiang W, Liu X, Wu D, Wang H, Wang Y, Chen H and Yuan L 2014 *Biotechnology and Applied Biochemistry* **20** 126-31
[23]   Ecker C, Ertl A, Pulverer W, Nemes A, Szekely P, Petrasch A, Martin G and Markl M 2013 *Food Chem.* **141** 407-18
[24]   Dizge N, Soydemir G, Karagunduz A and Keskinler B 2011 *J. Membrane Sci.* **366** 278-285