Filter Membrane Separation and Purification of Jatropha Protein Extraction Solution

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***Abstract*—Jatropha curcas is a multiple purpose plant and its seeds contain about 30%-40% oil. The oil can be directly used as fuel or to be the raw material for biochemical industry. The protein is usually extracted from the pressed cake. As noted that oil content in the pressed cake will affect the protein extraction efficiency. After de-oil pretreatment of the pressed cake, the oil content can be reduced from 23% to 0.581%. The Response Surface Methodology (RSM) was used to get the optimal operating parameters for protein extraction from de-oil cake and further separation by the multistage membrane filtration process. The highest yield of protein was obtained at 30 ℃, with 0.1 M sodium hydroxide solution, and solid to liquid ratio of 1:12. The protein solution was further passed through a series of tubular membrane in different connections, which can get the purity of raw protein powders in 78.44% after drying.**

***Index Terms*—membrane, filtration, Jatropha, protein**

I. Introduction

Jatropha curcas belongs to the family of Euphorbiaceae and its seeds contain about 30 to 40 wt% of oil. This oil is non-edible and has good oil quality and low temperature performance in engine. Jatropha curcas has potential to become one of the most competitive biomass resources [1]. Jatropha curcas oil is a promising fuel as it is non-edible and does not pose food security problem [2], [3]. It can be cultivated on marginal and degraded land and could be produced easily in rural areas [4]. Several studies have indicated that Jatropha curcas show promise for use as an oil crop for biodiesel. Use of Jatropha curcas oil and its blends with diesel has been carried out successfully by various researchers in diesel engines [5]. Some authors tried to reduce viscosity of Jatropha curcas oil by heating and/or blending in order to improve its performance in diesel engines [6], [7].

After the oil press, huge amount of pressed cake will be produced which had high protein content (about 40-50 wt% in the press cake). However, due to the toxicity of phorbol ester and toxins in the pressed cake [8], heat and other pretreatment were needed for the extracted protein to make animal feed. However, it can be used to replace the industrial protein (e.g. to make bio-based adhesive) without any detoxification.

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Separation and purification of protein from the extraction solution with membrane filtration seems a promising process, because it could remove the impurities simply according to the difference of molecular weight. Furthermore, membrane filtration process provides low energy consumption [9], [10]. Unfortunately, low permeate flux is the main problem of the membrane filtration, leading to the decrease in the filtration efficiency. The main reason is the existence of impurities resulted in a layer formed on the membrane surface during the filtration process [11], [12]. Part of the problem can be solved by increasing the operational temperature and pressure to improve the permeate flux [13]-[15], but the cost of energy in the process will be increased.

Extraction of protein from the press cake of seeds, e.g. Jatropha curcus, can be one of the important protein sources for food industry. It is rare to use membrane filtration to purify the extraction solution in industry because the fouling on membrane surface is usually happened. Therefore, the multistage membrane process was designed to overcome the fouling phenomena and used for further separation and purification of the protein solution in this study. The RSM and the analysis of variances (ANOVA) were applied in the experimental design and the data discussion. The effect of variables and the interaction between variables in this protein extraction and multistage membrane filtration processes were conducted and can be extended to the protein extraction and purification of other oil seeds. Two kinds of the multistage membrane series connections were designed and compared in this work. The result and suggestion about the multistage membrane filtration process in this study can be applied to most of the purification of protein extraction solution in industry. This work opens the door for further research on the feasibility of an industrial-scale protein extraction and purification process by using multistage filter membranes.

II. Experimental Section

A. Pressed Cake from Jatropha curcas Seeds

The Jatropha curcas seeds (from Indonesia) were heated at 80 ℃ about 15 minutes. Then put the seeds into screw oil press machine to separate the oil and the pressed cake. Then grinding machine was used to get the cake powder.

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B. De-Oil Pretreatment for the Pressed Cake

The pressed cake powders still have oil residue and the de-oil process was needed. First, the powders will be placed in the solvent of n-hexane for 15 minutes and the solid to liquid ratio (w/v) is 1g:4.5mL. In order to ensure the oil content can be decrease to the minimum, the procedure will be repeated again and use a rotary vacuum evaporator to remove the n-hexane. Finally put the de-oil pressed cake powders in vacuum oven for 12 hours at 60 ℃ until the weight to be constant.

C. Protein Extraction form De-Oil Pressed Cake

Place the powders of de-oil pressed cake in the NaOH solution and the solid to liquid ratio (w/v) is 1g:8mL. The NaOH concentration is selected in 0.1M and the temperature is controlled at 30 ℃ for 4 hours. After the extraction process, let the solution pass through the sieves to remove the fiber (the mesh screens are 30 mesh and 60 mesh).

D. Membrane Filtration Process

The membrane filtration device was shown in Fig. 1. Tubular polypropylene (PP) membranes were used for the filtration and the order of membrane pore sizes is 200μm, 100μm, 50μm, and (10, 5 or 1μm). In order to prevent the membrane fouling and get better quality of the final product, two lower pore size in 0.5μm and 0.05μm of PP tubular membranes were used. This tubular membrane series connection will be divided into two kinds of design to test. One is one-step filtration (as shown in Fig. 2), the other one is two-step filtration (as shown in Fig. 3).

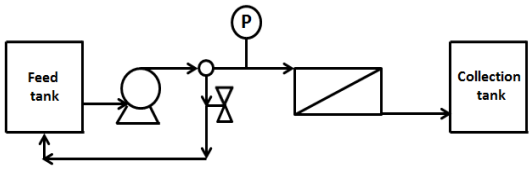

Figure 1. The flowchart of the typical membrane filtration process.

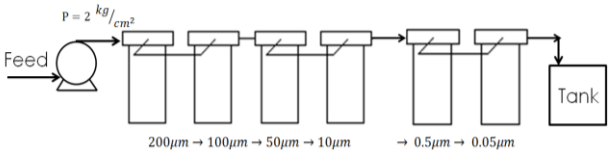

Figure 2. The one-step filtration process with tubular membrane modules.

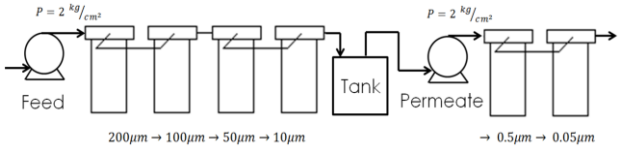

Figure 3. The two-step filtration process with tubular membrane modules.

III. Results and Discussion

A. De-Oil Pretreatment

In this study, the pressed cake still has oil content and it will affect the protein extraction efficiency. The solvent of n-hexane was used to remove the residual oil form the pressed cake. The process parameters include temperature, heating time, and replace numbers of n-hexane solvent. In Fig. 4, the data showed the oil content already decrease to 10.57% in one hour. The oil content was kept almost constant in the next one hour. Therefore, the de-oil time would be selected as one hour. The temperature was chosen at 25 ℃ (room temperature), 30 ℃ and 40 ℃. The data was shown in Table I. Even though the highest temperature (40 ℃) had the lowest residual oil content. It didn't change significantly when temperature changing. Therefore, the temperature would be chosen at the room temperature (25 ℃) in the following experiments. In Fig. 5 and Fig. 6 show that the oil content kept at around 10% when the n-hexane wasn't replaced during the process, However, the oil content decrease to 0.581% when the n-hexane was replaced one time during the process (after operating 15 minutes). The result indicates that the replacement of n-Hexane will increase the oil extraction efficiency in the pressed cake significantly.

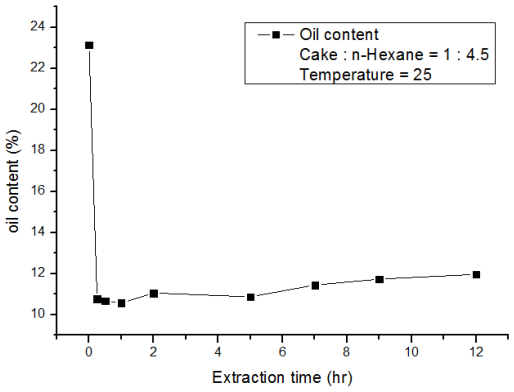

Figure 4. The oil content in the de-oil process during a long time operation.

TABLE I. The Residual Oil Content in the Press Cake

Number of the replacement of n-hexane	0	0	0
Temperature(℃)	25	30	40
Heating time (min)	1	1	1
Cake to solvent ratio (w/v)	1:4.5	1:4.5	1:4.5
Oil content (wt%)	11.37	10.58	8.767

B. Protein Extraction Process

The Response Surface Methodology (RSM) was used to design the experimental runs and to simulate the optimum value. Solid to liquid ratio (X_1), extraction temperature (X_2), and concentration of NaOH (X_3) will be the selected as the experimental parameters (i.e. factors). The responses will be the concentration of protein (Y_1) and the yield of protein (Y_2). The experimental parameters were shown in Table II. Table III shows the design of the experimental runs and the data of each run. On the basis of the experimental data, the optimum operating variables can be X_1=12 (w/v), X_2=30 ℃, and X_3=0.1M and the corresponding yield will be 96.19%. If we choose 40 ℃ for the above optimum operating variables, the yield is

still kept in 96.19%. For energy consideration, we will choose 30℃ for the optimum operation in continuous study. Fig. 7 shows that the effect of the variable factors on the responses. The effect of the factor of seed cake to solvent ratio on the response of protein concentration or yield is more significant than the other two factors.

TABLE II. VARIABLE FACTORS AND THEIR LEVELS IN THE PROTEIN EXTRACTION PROCESS

Parameter	Symbol	Low (-)	Center (0)	High (+)
Seed cake to solvent ratio (w/v)	x_1	6	9	12
Temperature of extraction (℃)	x_2	30	40	50
Concentration of NaOH(M)	x_3	0.05	0.1	0.15

TABLE III. THE RESPONSES OF PROTEIN EXTRACTION IN EACH OPERATING RUN

No.	Pattern			X_1	X_2	X_3	Y_1 (Protein conc.)	Y_2 (Yield)
1	+	0	+	12	40	0.15	10.8	96.19
2	-	+	0	6	50	0.1	19.9	88.62
3	0	-	+	9	30	0.15	13.5	90.18
4	0	0	0	9	40	0.1	13.9	92.85
5	+	-	0	12	30	0.1	10.8	96.19
6	-	-	0	6	30	0.1	17.2	76.6
7	0	0	0	9	40	0.1	14.3	95.52
8	0	0	0	9	40	0.1	14	93.52
9	0	+	-	9	50	0.05	13.6	90.85
10	-	0	+	6	40	0.15	18.5	84.17
11	0	-	-	9	30	0.05	10.4	69.47
12	-	0	-	6	40	0.05	17.9	79.71
13	0	0	0	12	50	0.1	11	97.97
14	0	+	+	9	50	0.15	14.1	94.19
15	+	0	-	12	40	0.05	10.1	89.96

C. *Membrane Filtration Process*

The Response Surface Methodology (RSM) was used again to simulate and to discuss the membrane filtration process. Two factors were chosen including feed pressure (X_1) and permeate pore size (X_2). The response are transmission of protein (Y_1) and turbidity of protein solution (Y_2). The selected operating parameters' range was shown in Table IV. Table V shows the design of the experimental runs and the data of each run. To keep the reasonable transmission of protein and the moderate turbidity of protein solution, the variable factors were chosen as the pressure of 2kg/cm^2 and permeate pore size of 10μm from Table V.

TABLE IV. VARIABLE FACTORS AND THEIR LEVELS IN THE MEMBRANE FILTRATION PROCESS

Parameter	Symbol	Low (-)	Center (0)	High (+)
Pressure of feed (kg/cm^2)	x_1	2	3	4
Membrane pore size (μm)	x_2	1	5.5	10

TABLE V. THE RESPONSE OF MEMBRANE FILTRATION IN EACH OPERATING RUN

No.	Pattern		X_1	X_2	Y_1 (Transmission)	Y_2 (Turbidity)
1	-	-	2	1	74.73	273.6
2	+	+	4	10	94.59	961.2
3	0	a	*3*	*1*	74.52	315.8
4	0	A	3	10	84.08	730
5	-	+	2	10	82.8	443.2
6	0	0	3	5.5	79.19	466.8
7	a	0	2	5.5	76.96	414.4
8	0	0	3	5.5	75.45	496.8
9	A	0	4	5.5	90.34	629.6
10	+	-	4	1	86.94	359.6

After a series of membrane filtration from 200μm, 100μm, 50μm to 10μm, the turbidity is still high according to the choice of variable factors in the above discussion. Therefore, adding two more tubular membranes with smaller pore size are necessary for the turbidity of protein solution and protein powder purity. Two pore sizes in 0.5μm and 0.05μm were chosen to connect after the 10μm tubular membrane. In this study two different connections of the tubular membrane series were discussed. First one is a series connection for all six tubular membranes in one-step as shown in Fig. 2. Second one is a two-step process as shown in Fig. 3. The result was shown in Fig. 8. For one-step process, the permeate flux always keeps in lower value which may due to the small pore size of membrane. The fluid passed through the small pore size of membrane which needed higher pressure. Most particles would be blocked during 200μm to 50μm, and raised the resistance in whole series. For the two-step process, the filtration flux would higher than one step. After the first tank, the pump would supply new driving force for the solution to filtrate and the filtration flux can be kept in a higher value.

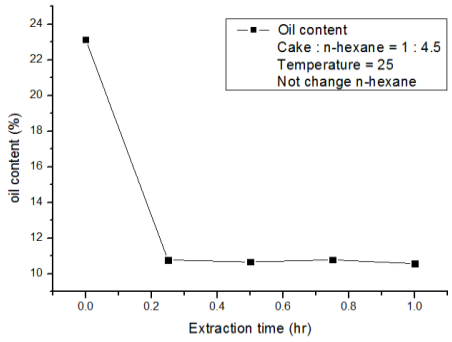

Figure 5. The oil content in the de-oil process without the replacement of n-hexane.

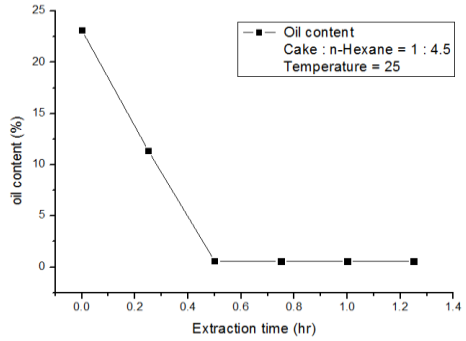

Figure 6. The oil content in the de-oil process with the replacement of n-hexane.

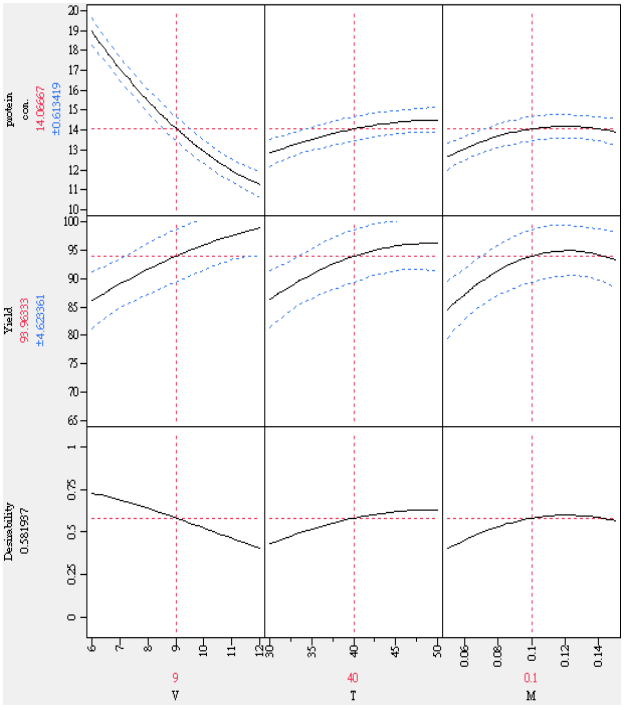

Figure 7. The effect of the solid to liquid ratio (V), extraction temperature (T) and concentration of NaOH (M) on the responses.

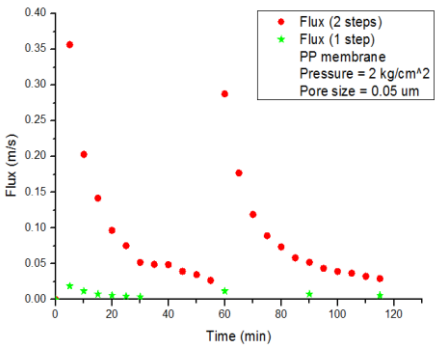

Figure 8. The permeate flux of one-step and two-step membrane filtration process.

IV. Conclusion

Protein extraction form the pressed cake of Jatorpha curcas seeds was conducted in this study. The protein extraction solution was further purified by the membrane filtration process. The Response Surface Methodology (RSM) and the analysis of variances (ANOVA) were applied in the experimental design and the data discussion. The optimum operating conditions in the protein extraction are solid to liquid ratio of 12, temperature at 30 ℃ and NaOH concentration in 0.1M. In the membrane filtration process, the feed pressure was selected at 2kg/cm^2 and permeation pore size was 10μm for the consideration of higher protein transmission and lower turbidity of the protein solution. Two kinds of the tubular membrane series connections were designed and compared in this work. After filtration process, the permeate solution was conducted to the freeze-drying process to get the raw protein powders. The results show that the two-steps process was better and the purity of raw protein powders was increased from 38.45% to 78.44%.

Future study will be conducted on the membrane concentration of the purified protein solution to remove some water before sending to the freeze-drying process. This will result in the energy saving in the drying process and reduce the drying time for producing the protein powder. However, the fouling should be overcome in the design of the membrane concentration process.

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