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PERVAPORATION-ASSISTED ENZYMATIC ESTERIFICATION OF OLEIC ACID AND ETHANOL

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บุญอนันต์ สำราญวงศ์ : เพอร์แวพอเรชันร่วมกับปฏิกิริยาเอสเทอริฟิเคชันด้วยเอนไซม์ของกรดโอเลอิกและเอทานอล (PERVAPORATION-ASSISTED ENZYMATIC ESTERIFICATION OF OLEIC ACID AND ETHANOL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.เหมือนเดือน พิศาลพงศ์, 89 หน้า.

การศึกษากระบวนการแบบกะและแบบต่อเนื่องของการทำงานร่วมกันระหว่าง ปฏิกิริยาเอสเทอริฟิเคชันจากกรดโอเลอิกกับเอทานอลโดยอาศัยเอนไซม์เป็นตัวเร่งปฏิกิริยา และ กระบวนการเพอร์แวพอเรชันได้ถูกพัฒนาจนสำเร็จ ในงานวิจัยนี้แผ่นฟิลม์แบคทีเรียเซลลูโลสที่ถูกอัดแอลจิเนตไว้ได้ถูกใช้เป็นเยื่อเลือกผ่านเพื่อดึงน้ำออกจากของผสมในปฏิกิริยา ผลของรูปแบบการทำงานของเพอร์แวพอเรชัน, ปริมานเอมไซม์ (โนโวไซม์ 435) และลักษณะการไหลภายในถังปฏิกรณ์ได้ถูกศึกษา โดยปฏิกิริยาเอสเทอริฟิเคชันจากกรดโอเลอิกกับเอทานอลโดยอาศัยเอนไซม์เป็นตัวเร่งปฏิกิริยาได้ทำการศึกษาภายใต้สภาวะดังต่อไปนี้: อัตราส่วนโดยโมลของกรดโอเลอิกกับเอทานอล 1 ต่อ 2, อุณหภูมิ 45 องศาเซลเซียส, ความเร็วใบพัดเท่ากับ 250 รอบต่อนาที, เอนไซม์ 5% โดยน้ำหนักเทียบกับกรดโอเลอิก และความดันเพอร์มิเอทเท่ากับ 10 มิลลิเมตรปรอท ผลการทดลองแสดงให้เห็นว่าเยื่อเลือกผ่าน บีซีเอ มีความสามารถสูงในการเลือกให้น้ำผ่านโดยพบว่าฝั่งเพอร์มิเอทที่ถูกดึงออกมาจากของผสมในปฏิกิริยามีองค์ประกอบของน้ำอยู่ถึง 95 เปอร์เซนต์ ที่อัตราการไหลผ่านน้ำ 140 ถึง 270 กรัมต่อตารางเมตรต่อชั่วโมง จากผลการทดลองแสดงให้เห็นว่าการเริ่มต้นการทำงานของเพอร์แวพอเรชันหลังจากเข้าสู่จุดสมดุลของปฏิกิริยาเพื่อที่จะขยับสมดุลของปฏิกิริยาไปทางผลิตภัณฑ์ไบโอดีเซลเป็นรูปแบบการทำงานที่เหมาะสมที่สุดเมื่อพิจรณาถึงการสิ้นเปลืองพลังงานน้อยที่สุด โดยที่ค่าการเปลี่ยนแปลงของกรดไขมันสูงขึ้นจาก 84.37 % ไปที่ 88.50 % โดยอาศัยระบบที่ทำงานร่วมกับเพอร์แวพอเรชัน จากการศึกษารูปแบบการไหลผ่านภายในถังปฏิกรณ์แสดงให้เห็นว่าการใช้เบดแบบขยายให้ อัตราการเกิดปฏิกิริยา และ ค่าการเปลี่ยนแปลงของกรดไขมัน ที่สูงกว่าเบดแบบนิ่งเมื่อเปรียบเทียบที่ปริมาตรของถังปฏิกรณ์คงที่ ในการประเมินการทำงานแบบต่อเนื่องของปฏิกิริยาเอสเทอริฟิเคชันในถังปฏิกรณ์ที่ใช้เบดแบบขยายโดยมีเวลาในการทำปฏิกิริยา 50 นาที แสดงให้เห็นว่าสามารถเพิ่มค่าการเปลี่ยนแปลงของกรดไขมันสูงขึ้นจาก 65.88 % ไปที่ 69.73 % โดยอาศัยระบบที่ทำงานร่วมกับเพอร์แวพอเรชัน จากการสังเกตลักษณะโครงสร้างพื้นผิวของโนโวไซม์ 435 ที่ถูกใช้ในปฏิกิริยาเอสเทอริฟิเคชันด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดแสดงให้เห็นว่า การทำงานร่วมกันของกระบวนการแบบ กะ และแบบต่อเนื่อง กับระบบเพอร์แวพอเรชันส่งผลให้โนโวไซม์ 435 มีระดับการบวมที่น้อยกว่าเมื่อเปรียบเทียบกับกระบวนการที่ไม่ได้ทำงานร่วมกับระบบเพอร์แวพอเรชันซึ่งมีประโยชน์สำหรับการใช้ตัวเร่งปฏิกิริยาชีวภาพในระยะยาว

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2559

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KEYWORDS: PERVAPORATION / ENZYMATIC ESTERIFICATION

BOONANUN SUMRANWONG: PERVAPORATION-ASSISTED ENZYMATIC ESTERIFICATION OF OLEIC ACID AND ETHANOL. ADVISOR: ASSOC. PROF. MUENDUEN PHISALAPHONG, Ph.D., 89 pp.

The batch and continuous processes of enzymatic esterification of oleic acid and ethanol coupled pervaporation (PV) unit were successfully developed in this study. Bacterial cellulose (BC) impacted with alginate film was used as selectively-permeable membrane to remove water from the reaction mixture. The effects of PV operating modes, amount of enzyme (Novozym 435) loading, flow pattern in the reactor were investigated. The enzymatic esterification of oleic acid and ethanol were carried out under the operating conditions as follows: molar ratio of oleic acid to ethanol of 1:2, temperature of 45^{o}C, turbine rate at 250 rpm, enzyme loading at 5% (w/w oleic acid) and pressure permeate side at 10 mmHg. The result shows that the BCA membrane was high selectivity to water, in which the permeate containing about 95 %(w/w) water could be removed from the reaction mixture with the water flux of 140 – 270 $gm^{-2}h^{-1}$. Considering less energy consumption, the start operation of pervaporation at the end of the reaction (late pervaporation) to move equilibrium toward biodiesel product is suggested, in which the FFA conversion was increased from 84.37 % to 88.50 % by using the system coupled with the PV unit. On the study of the flow pattern in the reactor, it was shown that the higher initial rate and FFA conversion were obtained by using the expanded bed, as compared to the fixed bed at the same reactor volume. The evaluation of the continuous esterification process in the expanded bed reactor with the retention time of 50 min shows the improved of the FFA conversions from 65.88% to 69.73% by using the system coupled with the PV unit. From the observation of surface morphology of Novozym 435 being used in the esterification by SEM, it was demonstrated that the degrees of swelling of Novozym 435 in the batch and continuous processed coupled with pervaporation were less compared to those from the systems without the PV unit, which could be benefit for long term use of the biocatalyst.

Department:	Chemical Engineering	Student's Signature	.....
Field of Study:	Chemical Engineering	Advisor's Signature	.....
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CHAPTER I

INTRODUCTION

1.1 Motivation

In recent years, problems of environment such as climate change, global warming are becoming hot issues. Since the uses of fossil fuels such as gasoline and diesel can cause significant negative environmental impacts, so that alternative fuels were produced to replace fossil fuels (1). Biodiesel, which is mono-alkyl esters of long chain fatty acids, is produced from trans-esterification or esterification of vegetable oil or animal fat with short chain alcohol catalyzed by alkali, acid, or enzyme catalysts (2, 3). Nowadays, most of biodiesel productions use trans-esterification with alkali catalyst, but the main problems involved in this process are the separation of glycerol from biodiesel, washing step and acid value of the product. Biodiesel can be produced by esterification of carboxylic acid which is a major content of vegetable oil and animal fat with short chain alcohol such as methanol, ethanol and catalyzed with enzyme. The advantages of using immobilized enzymes for biodiesel production were include the mild reaction conditions, less waste water and reusability (4). Because the esterification is a reversible reaction, the conversion is generally low due to limited by the thermodynamic equilibrium. Therefore, to achieve a high ester yield, it is usual to drive forward the equilibrium position by either excess of cheap reactant or removing byproducts.

The potential application of membrane to couple with bio-reaction is becoming more attractive. Research studies for the combination of bio-reaction and separation into a single process unit for improved process performance have been established. Pervaporation (PV) is a one of separation membrane units. PV membrane allows selective permeation of a component in a mixture, which can enhance the conversion of thermodynamic equilibrium reaction by the removal of product component from reaction mixtures. In this system, the mass transport that passes through the membrane is induced by reduced pressure (vacuum) on the permeate side. In general, PV can be effective used to dehydrate organic solvent which contains water in the feed mixture not to high(5).

Water is a byproduct from the esterification. PV could be used for removing water during the esterification process in order to improve the biodiesel productivity. PV has many benefits in terms of energy efficient, high selectivity, mild operating conditions and environmental acceptable separation technique. The separation mainly depends on the properties of a pervaporation membrane, membrane area and difference in partial pressures between feed and permeation side to remove water or some solvents from mixtures. The variables that show efficiency of PV system are flux and selectivity of fluid that pass through membrane(6, 7). Recently, bacterial cellulose-alginate (BCA) nano composite membrane has been successfully developed as an effective membrane to separate water from biodiesel synthetic–water mixtures. The BCA membrane has many advantages including high chemical stability, heat resistance, good mechanical properties and high affinity toward water molecules(8).

In this work, the esterification of oleic acid and ethanol using the enzyme catalyst (Novozym 435) coupled with PV unit using BCA membrane for removing water from the reaction mixture was investigated. The effects of the PV system on the reaction rate and equilibrium conversion were investigated

1.2 The objectives of this research

To investigate pervaporation-assisted enzymatic esterification by using BCA as a selective membrane for water removal from ethyl oleate– oleic acid- ethanol mixtures during the esterification process.

1.3 The scope of this research

1.3.1 Batch process

i. Enzymatic esterification is carried out in 1-L agitation tank.

ii. BCA is used as a selective membrane for water removal from ethyl oleate–oleic acid-ethanol mixtures.

iii. Oleic acid and hydrous ethanol (95 vol. % ETOH + 5 vol. % H_2O) were used as substrates.

iv. The operating conditions were as follows: temperature of 45°C, the molar ratio of oleic acid: ethanol at 1:2, the stirrer speed at 250 rpm, the enzyme (Novozym435) loading at 5 and 10 % w/w of FFA, the permeate pressure at 10 mmHg.

v. Effect of volume ratio of bed column to catalyst volume was examined.

1.3.2 Continuous process

i. Enzymatic esterification is carried out in 380 mL expanded bed reactor integration with PV unit.

ii. The operating conditions follow the optimal condition in the previously work (9).

1.3.3 Characterization & analysis:

i. Scanning Electron Microscopy (SEM) was used to determine the surface morphology of Novozym 435 before and after the reaction. SEM was also used to investigate the structure morphology of BC and BCA.

ii. Karl Fischer analyzer was used to determine the water content in the permeates.

iii. Titration method was used to determine the conversion of oleic acid to ethyl oleate.

1.4 Expected Benefit

The expected benefit of this research is to improve the process for enzymatic esterification of oleic acid and ethanol by the integration of PV unit, using BCA as a selective membrane.

CHAPTER II

THEORIES AND LITERATURE REVIEWS

2.1 Biodiesel

Biodiesel is a type of ester- based oxygenated fuels that was produced from renewable biological sources. It can be made from processed organic oils and fats. In theory, biodiesel is a mono alkyl esters of long chain fatty acid which is derived from vegetable oils or animal fats (10). Biodiesel properties have been developed for more than 2 decade years. Nowadays, the property of biodiesel product is quiet similar to petroleum diesel, so that it is compatible to blend with petroleum diesel at any proportion to produce a stable biodiesel blend. This makes biodiesel to be a common biofuel as transport fuels in the world (11).

The physical and fuel properties of biodiesel are similar to petroleum fuel. As the main fuel properties are flash point, density, higher heating value and viscosity. Moreover, the advantage of biodiesel combustion better than petroleum diesel was confirmed (12). Biodiesel is considered as a pure fuel because it has no sulphur, no aromatic and can complete burn with 10% oxygen. Its higher cetane number helps to increase the ignition quality (13, 14).

The feed stocks of biodiesel are depending on availability and cost economy that are the major factors affecting production of the biodiesels. Normally, the feed stocks are vegetable oils such as castor, grape seed, maize, camelina, pumpkin seed, beech nut, rapeseed, lupine, pea, poppy seed, peanut, hemp, linseed, chestnut, sunflower seed, palm, olive, soybean, cotton seed and shea butter (15). Moreover, animal fats such as beef tallow and used cooking oil can also be used as biodiesel after refining (10).

2.2 Biodiesel process

2.2.1 Trans-esterification reaction

The most reaction that is used to produce biodiesel is transesterification reaction as shown in figure 1. Vegetable oil/animal fat and short-chain alcohol such as methanol and ethanol are used as reactants for the reaction (14).

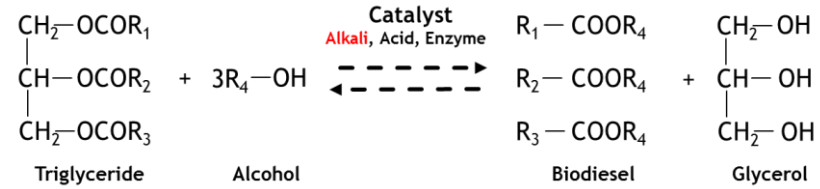


Figure 1 Transesterification reaction of triglyceride with alcohol

Figure 2 Saponification reaction of free fatty acid with alkali catalyst

To increase rate of reaction and yield, catalyst was used. The conventional catalysts that are used in commercial are alkaline catalyst such as NaOH, KOH, CH_3ONa and CH_3OK; and acid catalyst such as H_2SO_4, HCl, and H_3PO_4. However, the conversion is not only depending on the catalyst. The factor such as immiscible of fat/oils with short chain alcohols can cause of low conversion of triglyceride to biodiesel. The other problems are such as side reaction and acid value of product were considerable. The side reaction as saponification, the reaction between FFA in raw material and alkaline catalyst, can generate soap. This will cause miscible mixture of biodiesel-glycerol and soap, which is very difficult to separate. This problem results in higher cost for installing another separation in the process. Next problem is the acid value of product. From the reaction that use alkali or acid as a catalyst, the product will be non- neutral; therefore, the process must require a washing step (14).

2.2.2 Esterification reaction

The esterification reaction can also use to generate biodiesel. It used fatty acid and short chain alcohol as reactants with the supply of heat to reaction.

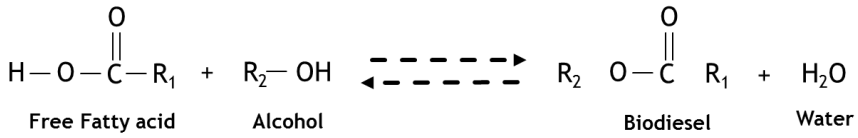


Figure 3 Esterification reaction of fatty acid with alcohol

As principle of Le Chatelier, the yield and selectivity of the product are increased by the removal of the byproducts of the reaction. The esterification reaction is a typical example of an equilibrium-limited reaction (Figure 3) that produces by product of water, which can reduce the catalytic activity of catalysts and the equilibrium conversion.

Kusdiana D. and Saka S. (2014) found that the presence of water could deactivate the catalyst activity, which slowed down the reaction rate of esterification reaction(16). To shift equilibrium to the products for enhance fatty acid conversion, alcohol should be used in excess, water should be removed from the reaction and the reaction rate should be accelerated by a suitable catalyst (17).

2.3 Fatty acid

Biodiesel properties are depended on the fatty acid composition of the vegetable oil/animal fat. The fatty acid profile of various sources of biodiesel is shown in Table 1. Oleic acid is mono unsaturated fatty acid, which means that it is stable to thermal oxidation than other unsaturated fatty acid components. It is a major component in many low cost raw materials such as canola oil, palm oil, jatropha oil, rapeseed oil, soybean oil, tallow and yellow grease(18).

2.4 Ethanol

Many researchers may prefer methanol as alcohol reactant in esterification because nowadays it is less costly and easily obtainable (12, 14, 19, 20). However, almost of methanol is derived from fossil resource, so biodiesel that was produced could not be completely renewable energy. On the other hand, ethanol or ethyl alcohol (C_2H_6O) can be produced from both petrochemical and biological processes. Ethanol, which is renewable

and environmentally friendly alcohol, is considered to be one of the best alternatives transport fuel (18). Especially in Thailand, a major bulk of ethanol was produced by fermentation technologies, using sugar molasses and starch as bio feedstock, which environmentally friendly process(12) . The physical properties of absolute ethanol compare with various primary alcohols; gasoline and diesel are given in Table 2. Ethanol properties are quite similar to methanol but it is less toxic, less corrosive and high energy efficiency more than methanol. The azeotrope point of ethanol and water is at ≈ 95% ethanol and 5% water, thus it is not possible to completely dehydrate ethanol by normal distillation. Normally 95% ethanol is usually used in industries because it is considerable cheaper than absolute ethanol.

5.2 Lipase Catalyst and its immobilization

The development of enzyme technology has provided lipases as an alternative choice for the use in biodiesel production with focus on esterification (12, 20, 21). Lipases are able to catalyze the esterification of fatty acid to ester at lower temperature than alkaline and acid catalyst. Moreover, Lipases also have high catalytic activity and stability for esterification while the purification of biodiesel is easier and cheaper than alkaline and acid catalyst (20, 22). Other advantages include its reusability, low energy cost and environmental friendly (23). "Immobilized enzymes" is defined as enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities which can be used repeatedly and continuously (24). Novozym (R) 435 is the commercial immobilized *Candida antarctica* lipase B (CALB) produced by submerged fermentation of a genetically modified Aspergillus microorganism. The lipase is adsorbed on a macro porous resin called Lewatit VP OC 1600, according to the information given by the Novozymes Co. in their website. This macro porous resin is a polymer of metacrylic acid cross-linked with divinylbenzene (DVB) and possesses certain hydrophobic nature (25).

Table 1 Fatty acid composition of various sources of biodiesel (18).

Common name	Abbrev.	Fatty acid composition											
		Camelina	Canola	Coconut	Corn	Jatropha	Palm	Rapeseed	Safflower	Soy	Sunflower	Tallow	Yellow Grease
Capriotic	6:0			0.59								0.1	
Capprylic	8:0			6.74			0.77						
Capric	10:0		0.09	5.35			0.48	0.56				0.1	
Lauric	12:0	0.36		47.26		0.09	0.29	0.09		0.09	0.1	0.19	0.19
Tridecylic	13:0										0.1		
Myristic	14:0	2.56		18.33		0.028	1.05		0.1	0.09		2.55	0.78
Myristoleic	14:1											0.29	
Pentadanoic	15:0											0.58	0.09
Pentadecenoic	15:1											0.1	
Palmic	16:0	5.79	4.05	9.02	11.43	14.36	41.59	4.04	8.14	11.07	6.31	23.85	16.03
Palmitoleic	16:1		0.28	0.1	0.2	0.93	0.19	0.09	0.1	0.18	0.1	2.55	0.85
Hexade cadienoic	16:2												

Hexade catrienoic	16:3												
Heptadecanoic	17:0		0.09			0.09	0.1	0.09		0.09	0.1	1.35	0.09
Heptadecenoic	17:1		0.09		0.1						0.1	0.58	0.09
Stearic	18:0	2.66	1.93	2.65	1.88	5.88	4.11	1.54	2.48	3.72	3.55	17.86	6.9
Oleic	18:1	15.96	58.3	6.74	26.45	38.94	40.41	57.26	14.15	22.61	21.39	41.42	43.34
Linoleic	18:2	16.15	20.46	2.06	58.36	34.89	9.3	20.69	74.14	51.33	65.35	4.32	24.39
Linolenic	18:3	33.81	9.27	0.1	0.59	0.28	0.29	8.08	0.1	5.63	1.46	0.88	1.07
Stearidonic	18:4											0.39	0.47
Archidic	20:0	1.33	0.65	0.1	0.3	0.19	0.29	0.37	0.1	0.27	0.29	0.2	0.28
Gondoic	20:1	13.68	1.45		0.1	0.09	0.1	2.025		0.27	0.19	0.59	0.47
Elcosadensic	20:2	1.42	0.09					0.09					
Elcosatrienoic	20:3	0.72											
Elcosatr tetraenoic	20:4												
Elcosapen taenoic	20:5												

Behenic	22:0	0.81	0.28		0.1	0.19	0.1	0.28		0.27	0.58	0.1	0.38
Erucic	22:1	2.94	0.47		0.1	0.09		0.46		0.09	0.1	0.1	0.09
Docosa tetraenoic	22:4												
Docosa pentaenoic	22:5												
Docosa hexaneoic	22:6												
Ugnoceric	24:0	0.66	0.19		0.1	2.51	0.1	0.09		0.09	0.19		0.19
Nervonic	24:1	0.18	0.19	0.98		0.09		0.09		0.27			4.28
Other/Unknow	?	0.95	2.12		0.3	1.11	0.86	4.14	0.79	3.91	0.1	1.92	
Total		100	100	100	100	100	100	100	100	100	100	100	100
Total Satureates	SFA	14.18	7.29	90.03	13.81	23.58	48.85	7.06	10.82	15.7	11.22	46.87	24.94
Total Unsatureates	UFA	84.87	90.59	9.97	85.9	75.31	50.28	88.8	88.39	80.39	88.69	51.21	75.06

Table 2 Fuel and physicochemical characteristics of petroleum-derived fuels and its potential substitutes (26).

	Energy content (Mj/L)	Solubility (g/L)	Centane number	Lubricity (μm corrected wear scar)	Viscosity cST	Density	Auto ignition temperature (°C)	Boiling point (°C)	Flash point (°C)	Vapour pressure (mmHg)	Freezing point (°C)
Methanol	16	Miscible	2	1100	0.6 @40°C	0.79	463	65	11	127	-98
Ethanol	19.6	Miscible	11	603	1.1 @40°C	0.79	420	78	17	55	-114
1-Butanol	29.2	77	17	623	1.7 @40°C	0.81	343	117	29	7	-90
1-Hexanol	31.7	7.9	23	534	2.9 @40°C	0.81	285	158	59	1	-45
1-Octanol	33.7	0.59	39	404	4.4 @40°C	0.83	270	195	81	0.08	-16
1-Decanol	34.6	≈0.04	50	406	6.5 @40°C	0.83	255	233	108	< 0.1	6
1-Dodecanol	35.3	≈0.004	64	345	9.0 @40°C	0.83	275	261	119	< 0.1	24
Hydrogenated bisabolene	≈37	Immiscible	42	Unknow	2.91	0.82	Unknow	267	111	< 0.01	< -78
Biodiesel	32.1	Immiscible	60	314	4-6 @40°C	0.87(avg)	177-330	315-350	100-170	< 1	-3 to -5
Petrodiesel	40.3	Immiscible	45-50	315	1.8-5.8 @40°C	0.84(avg)	210	150-350	52-96	0.4	-12
Petroleum	32.1	Immiscible	13-17	711-1064	0.4-0.8 @20°C	0.82(avg)	246-280	27-225	-40	275-475	-60

2.6 Pervaporation application

Because esterification reaction is a reversible reaction, the reaction conversion is limited by the thermodynamic equilibrium. Water is a byproduct in esterification reactions. To shift the equilibrium to products, an excess of one reactant or removing water has been considered. Moreover, if a water phase is formed in the environment of lipase enzymes, it should cause a very low pH value due to the concentration of acid and water, resulting in some disadvantages on enzyme activity/stability (27). From both reasons, water should be removed during esterification reactions. Water can be removed by using 3 conventional methods: adsorption by molecular sieve (27, 28), distillation (17) and pervaporation (17, 29). Nevertheless, molecular sieve such as silica gel might not be convenience to be used in continuous process because it must be regenerated by removing water after pores are entirely filled with water. Distillation is also not a suitable method to couple enzymatic esterification reaction. Since the esterification medium (reagents and products) is a non-ideal mixture so that it might not fit for vapor equilibrium-based technology (17). In addition, the enzymatic esterification should be operated at mild condition, thus it is not suitable to be coupled with a distillation unit which has to be

operated at high temperature. Moreover, high energy consumption of distillation unit can cause high operating cost (25). Therefore, pervaporation technique is selected to couple esterification reaction for water removal in this study.

Previously, pervaporation has been used for separation of water from organic liquid mixture by partial vaporization through hydrophilic membrane. The hydrophilic membrane acts as a selective barrier between liquid phase feed side and vapor phase permeate side. Selective water component of liquid phase feed side could transfer pass through the membrane by vaporization. The separation of pervaporation is independent from vapor liquid equilibrium because the transport resistance is dependent on sorption equilibrium and mobility of water permeates that pass through the membrane. The driving force of this process is different of partial pressure between two sides (feed and permeate). The permeate side is kept at vacuum (to control pressure lower than equilibrium vapor pressure) and feed side is controlled at atmospheric pressure. Pervaporation is a mild operating system that has advantages such as low energy consumption, no entrain required in process; therefore, the system is not contaminated with another component. It is also independent from azeotrope point of mixtures (30). The pervaporation hybrid system has been separated in two categories separation process and reaction process, respectively. This system can be operated in two type modes (R1 and R2) as shown in figure 5. In case of R1-type, the main product is removed as the permeate, whereas R2-type, the byproduct is removed as the permeate (17).

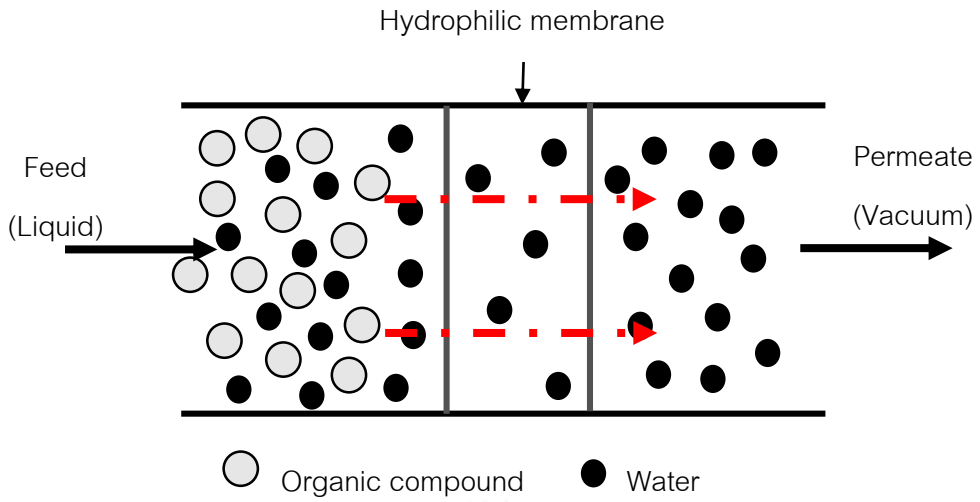


Figure 4 Overview of the pervaporation process for aqueous organic mixtures (30).

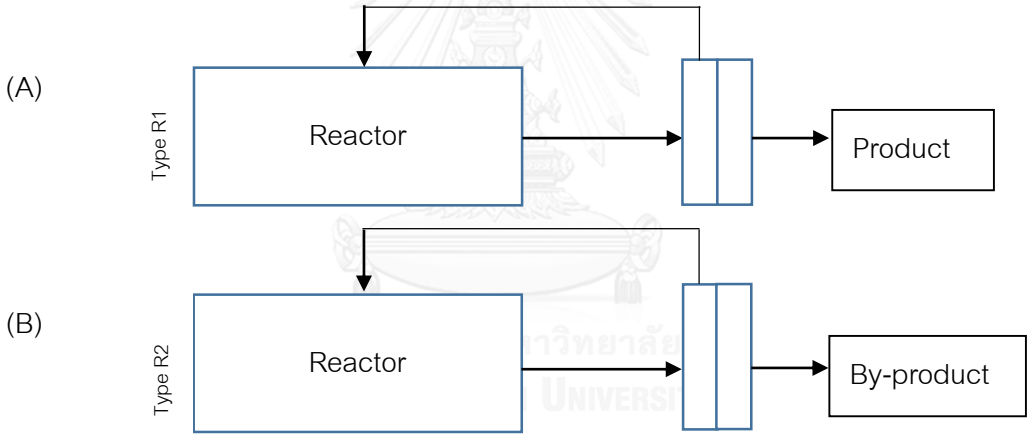


Figure 5 Reaction-type hybrid pervaporation configuration in (A) type R1 (the main product permeates through membrane) and (B) type R2 (the byproduct is selectively removed from the reaction medium) (23).

The integration of a pervaporation process with enzymatic esterification process can give a chance to continuous shift the conversion upper the thermodynamic equilibrium by removal water from the reaction.

Figueiredo et al., (2008) investigated the pervaporation which using hydrophilic PV membranes integrated with esterification of oleic acid and ethanol. The hydrophilic

membrane was able to remove water from the reaction mixture, thus increasing ester yield(31).

Sevinc et al., (2009) studied effects of catalyst loading, membrane thickness and catalyst type in batch pervaporation membrane reactor (PVMR) coupled with esterification of acetic acid and isobutanol. The reaction was catalyzed by homogeneous (sulphuric acid) and heterogeneous (Dowex 50W-X8) by using polydimethylsiloxane (PDMS) membrane which was selective to removal ester product. The researcher observed that the PDMS membrane can be used to selective remove the isobutyl acetate with acceptable conversions and pervaporation fluxes more than other component in mixture. Moreover, they have found that conversion of acetic acid increased with catalyst concentration, and using thinner membranes. Regarding type of catalyst, the reaction rate in heterogeneous catalyzed with PVMR was slower than using homogeneous, but the duration of the conversion was longer (6).

Weixing et al., (2013) studied the esterification of acetic acid and n-propanol with vapor permeation (VP) by using NaA zeolite membrane. Its morphology of membrane was characterized properties of membrane by using SEM. The resulted showed that the zeolite powders were compacted one by one together as dense membrane with a thickness of about 15 μm. The application of the membrane in VP system with water/n-propanol mixtures. The result showed that the NaA membrane had good selectivity for water removal. The final conversion of acetic was significantly increased from 78.2% to 98.6% by using VP at molar ratio of n-propanol to acetic acid of 2:1, catalyst loading at 6wt%, reaction temperature of 100.0^{o}C and reaction time of 420 minutes of operation (7).

Zhang et al., (2014) studied enzymatic reaction coupled with pervaporation membrane unit for synthesis lauryl stearate by using a composite catalytically membrane, which immobilized Candida rugosa by immersion phase inversion technique. The excess water was removed by a pervaporation membrane unit, which helped to improve activity of the immobilized lipase and the conversion of stearic was increased by approximately 40%, comparing to the equilibrium conversion obtained from a batch reactor (32).

Ying et al., (2015) studied properties of NaA zeolite membrane and optimum

operating conditions of coupling NaA zeolite membrane pervaporation with a fixed bed reactor for esterification of oleic acid and ethanol. The NaA zeolite membrane was found to have good separating property in removing water from the organic mixture, and still had good performance after successive runs for 8 times. The final conversion of oleic acid was increased from 84.23% to 87.18% by PV, using the NaA zeolite membrane at the optimal conditions: ethanol to oleic acid molar ratio of 15:1, feedstock flow rate of 1.0 ml/min, reaction temperature of 80.0°C, catalyst bed height of 132 mm and reaction time of 24 h of operation (33).

Koszorz et al., (2003) investigated enzymatic esterification of oleic acid and i-amyl-alcohol coupled with pervaporation using PERVAP1005 as selective membrane. The operating condition was set at 500 rpm of shaking incubator, temperature of 40°C and volume of 25 cm^3. They suggested the math model of water concentration profile from reaction mechanism for removal water of pervaporative (eqn. 1) and water generate from esterification (eqn. 2). It was found that the absence of water at the beginning would strongly decrease enzyme activity. The relationship of membrane area ratio to mass of the reaction mixture and the initial molar ratio of alcohol to acid could limit the reaction, especially when the operation was performed at high concentration of alcohol and using small membrane area as show in figure 6 (6).

$$E + B + A \begin{array}{l} \overset{K_A'}{\leftrightarrow} EA + B \overset{K_B}{\leftrightarrow} \\ \underset{K_B'}{\leftrightarrow} EB + A \underset{K_A}{\leftrightarrow} \end{array} EAB \overset{k}{\rightarrow} E + P + Q \quad \ldots\ldots\ldots\ldots (1)$$

$$\left(\frac{dX_w}{d\tau}\right)_P = \left[\frac{A_m P_w X_w (X_w - 1)}{N}\right]_1 + \left[\frac{k \cdot E \cdot X_A X_B}{K_A' K_B + K_B X_A + K_A X_B + X_A X_B}\right]_2 \quad \ldots\ldots\ldots\ldots (2)$$

where Am is membrane area

E is Total enzyme concentration

k is reaction rate constant

K_A, K_A', K_B is Equilibrium constants

N is total amount of mol

X is molar ratio

subscription A is alcohol

B is acid

W is water

P is integrated process

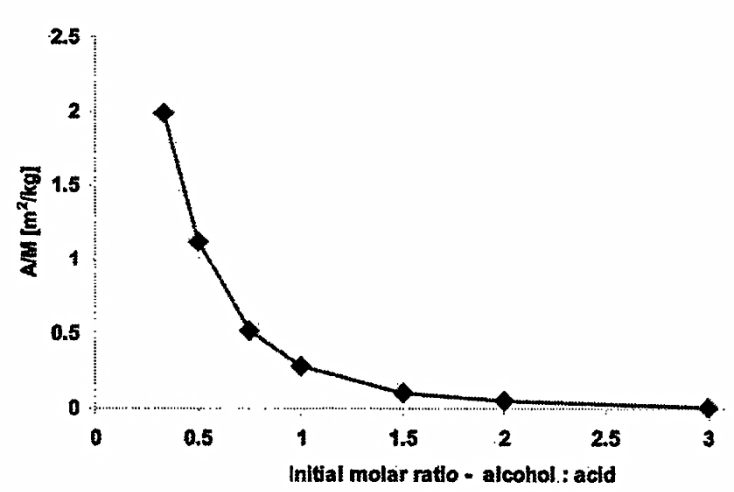


Figure 6 'Border' parameters for enzymatic esterification coupled with pervaporation.

Embine S. and Ferhan S. studied the esterification of acrylic acid and n-butanol catalyzed by acid catalyst (Amberlyst 131) coupled with Pervap 2201 as membrane. The effects of temperature, initial molar ratio of n-butanol to acrylic acid, catalyst loading and ratio of membrane area to volume were investigated. The maximum conversion of acrylic acid was 96.3%, which was obtained under the conditions of 358 K, initial molar ratio of n-butanol to acrylic acid of 8:1, catalyst loading of 10 g/L and ratio of membrane area to volume of 70 m^{-1}(34)

Delgado P. et al. investigated the esterification of lactic acid and ethanol catalyzed by acid resin Amberlyst 15 integrated with the pervaporation unit, using Pervap 2201 as membrane to synthesis ethyl lactate in a batch system. The model to predict the final ethyl lactate concentration from the initial reactant molar ratio was suggested. From the integration of the pervaporation unit, a higher conversion than the equilibrium limited conversions in a conventional reactor was achieved by the removal water. The pervaporation and initial reaction rates increased with the operating temperature. Moreover, the higher ester a conversion was obtained related to increasing ratio membrane area to initial reaction volume. (35)

2.7 Bacterial cellulose –alginate membrane

Cellulose is basic material of all plant substances, which has been found in nature as polysaccharide. Cellulose obtained from plant is unpurified cellulose which almost contains other kinds of natural fibers such as lignin and hemicellulose. On the other hand, cellulose which obtained from bacterial cellulose (BC) is nearly purified cellulose (36). BC membrane was produced by the bacteria *Acetobacter xylinum* that use glucose as a common substrate. BC membrane has unique properties such as high water absorption capacity, high mechanical strength, high crystallinity and an ultra-fine and highly pure fiber network structure (37-39). Sodium alginate (SA) is biopolymers derived from natural substance. It is a famous water soluble polysaccharide found in brown seaweed. Alginate has been applied to form polymer network membranes. Alginate has a number of advantageous properties, including excellent biocompatibility, non- toxicity, non-immunogenicity, biodegradability, relatively low costs and easy combination with divalent cations (40). It has very high water absorption capacity which can absorb 200-300 times of its own weight due to its good membrane forming property and high activity of carbonyl group and hydroxyl group on its chains. On the contrary, it has poor mechanical strength (8, 40).

Suratago et al., (2016) studied the dehydration of biodiesel mixtures composed of methanol, water and biodiesel by using pervaporation unit. BCA membrane prepared by BC film immersed in 3% w/v alginate solution and cross-linked with $CaCl_2$ was used as a selective membrane. It was shown that the BCA membrane had good potential for removing water from the biodiesel-methanol mixtures. The permeation contained about 95% (w/w) water and 5% (w/w) methanol. Methyl ester was completely rejected by the BCA membrane. Concentration of water in the biodiesel- methanol mixtures and temperature of the process significantly affect the separation performance. It was demonstrated that increasing of water concentration and operating temperature in the mixtures resulted in an increasing the permeate flux but lowered the selectivity(41).

CHAPTER III

EXPERIMENTS

3.1 Chemicals

1. Oleic acid
2. Ethanol
3. Novozym 435 (lipase B from C. antarctica, EC 3.1.1.3), a nonspecific lipase immobilized on macro porous acrylic resin was purchased from S.M.Chemical suppliers Co., Ltd, Bangkok, Thailand. The diameters of the particle beads are in a range of 0.3–0.9 mm with approximate density of 0.4 $g \cdot ml^{-1}$. The catalytic activity was 10000 $PLU \cdot g^{-1}$.
4. BC (98-99% water content in wet weight) was kindly provided by Pramote Thamarat (Institute of Research and Development of Food Product, Kasetsart University).
5. Sucrose
6. Ammonia sulfate
7. Sodium hydroxide
8. Sodium alginate
9. Phenolphthalein
10. Deionized water
11. Calcium dichloride
12. Liquid Nitrogen
13. Acetic acid

3.2 Equipment

1. Hotplate with magnetic stirrer set
2. Beaker, flasks, plate
3. Silicone tube
4. Scanning electron microscope (FESEM-EDS 7610F, INSPECT S50)

5. Burette

6. Thermometer, thermo couple

7. Graduated cylinder

8. Cylinder Reactor with pack bed set

9. Vacuum pump (Model RV5 Edwards, England)

10. Karl Fischer analyzer (C20 Compact.KF.Coulometer, Germany)

3.3 Preparation of bacterial cellulose (BC membrane)

The culture medium was coconut-water added with 5.0%wt of sucrose, 0.5%wt of ammonium sulfate ,and 1.0%(v/v) of acetic acid. This medium was sterilized at 110^{o}C for 5 minutes. Next , A. xylinum (preculture) of 4 mL was added to the medium of 75 mL in a sterile petri dish (diameter 14 cm) and statically incubated at 30±2^{o}C for 7 days. The pellicles were washed by running water for 30 minutes, treated with 1% (w/v) of sodium hydroxide solution for 24 hours, and rinsed with deionized (DI) water until they became neutral. Lastly, the pellicles were soaked in DI water again and stored in refrigerator at 4^{o}C until use.

3.4 Preparation of bacterial cellulose impact with 3% (w/v) alginate and cross-linked with $CaCl_2$ (BCA membrane)

The BCA membranes were prepared by immersing BC membrane in sodium alginate solutions with concentration of 3% (w/v) at 50±2 ^{o}C for 5 days. The BCA pellicles were rinsed with DI water, then cross-linked with 5% (v/v) of calcium chloride ($CaCl_2$) for 3 hours, and then rinsed again with DI water. Lastly, BCA membrane was dried in ambient air for 3 days (7).

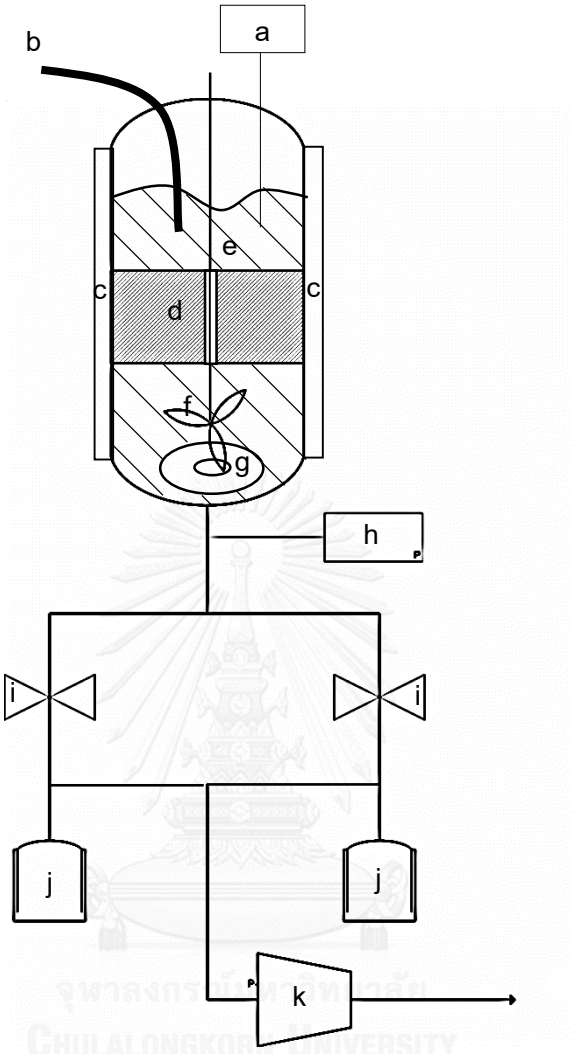
3.5 Batch enzymatic esterification reaction and pervaporation

3.5.1 Esterification processes

The batch esterification process was performed in a 1 L-reactor equipped with temperature sensor and four flat- blade disk- turbine impellers at atmospheric pressure. The reactants mixture at molar ratio of oleic acid to ethanol of 1:2 which mixing and pre-heated to 45°C for 30 minutes was added. The amount of Novozym 435 loading was varied at 5 and 10 % w/w of oleic acid. It was packed in bed support that made by aluminum net covered with cotton fabric. Then the biocatalyst bed was placed in the middle of the reactor. Next the liquid mixture in the vessel was circulated at 250 rpm and the reaction temperature was controlled at 45°C by using an electric heating pad wrapped outside the reactor. One milliliter of samples was collected from the reaction mixture every 15 minutes for the first hour. Then every 1 h until 20 h. Water, which was a reaction byproduct and residual alcohols, was removed from the samples via thermal evaporation. The purified product was analyzed by the titration method to determine the FFA conversion.

3.5.2 Esterification process coupled with pervaporation unit

A schematic diagram of the esterification process coupled with pervaporation unit is shown in figure 7. The reactor holds the reactants mixture at atmospheric pressure similar as batch process, while the pressure at the permeate side of the pervaporation unit was set at 10 mmHg by using a high-vacuum pump. The BCA membrane with the effective area of 19.6 cm^2 was mounted at the bottom of the reactor. The permeate samples were condensed and collected from the liquid nitrogen trapped every 1 hour until 20 hours. The permeation rate were determined by weight of the collected samples and water content of permeate which determined by Karl Fischer analyzer

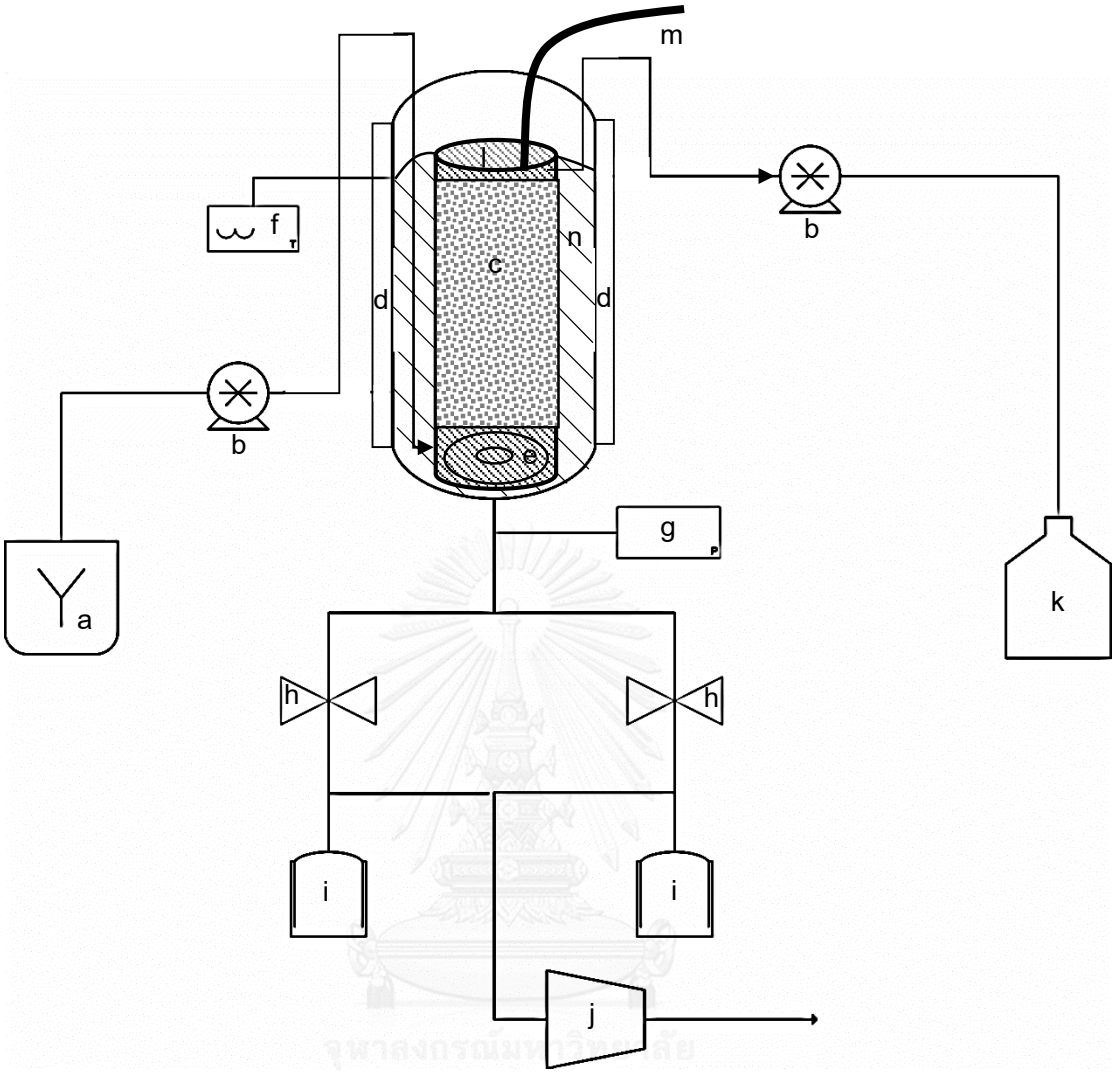


Note: a = thermocouple; b = sampling silicone tube; c = heating jacket; d = expanded bed; e =mixture; f = turbine; g = membrane; h = pressure gauge; i = valve; j = trap of liquid nitrogen; k = vacuum pump and h = reaction mixture.

Figure 7 Schematic diagram of the pervaporation-assisted enzymatic esterification in batch process.

3.6 Continuous enzymatic esterification reaction and pervaporation

The continuous enzymatic esterification process was performed in a 380 ml-reactor which surface area of 24. 64 cm^2 equipped with temperature sensor at atmospheric pressure and cover with water heating jacket as shown in figure 8. The reactants mixture at molar ratio of oleic acid to ethanol of 1:2 which mixing and pre-heated to 45^{o}C for 30 minutes was feed at flow rate 5 cm^3/min. The Novozym 435 was packed as single expand bed support that made by aluminum net covered with cotton fabric with the volume ratio of bed column to catalyst volume of 2:1. Then the biocatalyst bed was placed in the middle of the reactor. The reaction temperature was controlled at 45°C by using a water heating jacket. One milliliter of samples was collected from the reaction mixture every 1 h until 8 h. Water, which was a reaction byproduct and residual alcohols, was removed from the samples via thermal evaporation. The purified product was analyzed by the titration method to determine the FFA conversion. A schematic diagram of the esterification continuous process coupled with pervaporation unit is shown in figure 8. The reactor holds the reactants mixture at atmospheric pressure while the pressure at the permeate side of the pervaporation unit was set at 10 mmHg by using a high-vacuum pump. The BCA membrane with the effective area of 19.6 cm^2 was mounted at the bottom of the reactor. The permeate samples were condensed and collected from the liquid nitrogen trapped every 1 hour until 8 hours. The permeation rate were determined by weight of the collected samples and water content of permeate which determined by Karl Fischer analyzer



Note: a = mixer tank; b = peristaltic pump; c = expanded bed; d = heating jacket; e = membrane; f = thermocouple; g = pressure gauge; h = valve; i = trap of liquid nitrogen; j = vacuum pump; k = product tank; l = reaction mixture; m = sampling silicone tube; n = water jacket.

Figure 8 Schematic diagram of the pervaporation-assisted enzymatic esterification in continuous process.

3.7 The character of enzyme pack bed in batch and continuous process

In case of batch process of enzymatic esterification reaction coupled with pervaporation, the size of hollow cylinder pack bed was already set at 110 cm^3. So that, when it was packed by Novozym 435 at 5%wt of oleic acid in reaction mixture 650 mL, during the operation, the packed bed was in form like expanded bed at volume ratio of bed column to catalyst volume of 2:1. On the other hand, by loading Novozym at 10%wt of oleic acid in reaction mixture 650 mL, the packed bed was in form of fixed bed at volume ratio of bed column to catalyst volume of 1:1. The size of cylinder pack bed in continuous process of enzymatic esterification reaction coupled with pervaporation was already set at 250 mL. It was pack by Novozym 435 at volume ratio of bed column to catalyst volume of 2:1 as expanded bed reactor. Table 3 illustrates the character of Novozym 435 that was pack in batch process at 5 and 10%wt of oleic acid and continuous process at volume ratio of bed column to catalyst volume of 2:1.

3.8 Characterization of sample

3.8.1 FFA conversion analysis

The percentage of oleic acid conversion was determined by titration with 0.1 M of KOH solution and using phenolphthalein as an indicator. The conversion was calculated from titration volume of KOH solution. The report values were the average values of each duplicate set.

3.8.2 Amount of water permeate flux of esterification with PV

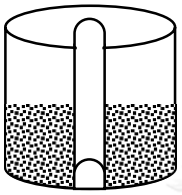
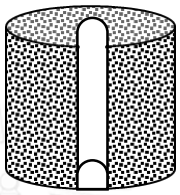
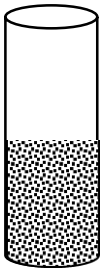
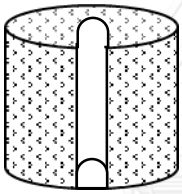
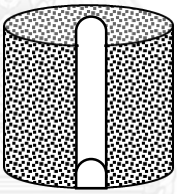
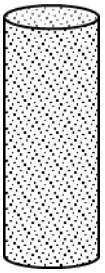
The permeate vapor of the esterification process coupled with pervaporation unit that pass through the BCA membrane was collected in a cold trap immersed in liquid nitrogen. The proportion of water was determined by a Karl Fischer analyzer.

The permeate flux (J) was calculated as follows:

$$J = \frac{Q}{A \times t}$$

Where Q is the mass of permeate collected in time (t), A is the effective membrane area.

Table 3 The character of enzyme in the packed bed reactors under batch and continuous processes.

	Batch process		Continuous process
	5 % (w/w) Novozym 435 to oleic acid	10% (w/w) Novozym 435 to oleic acid	Expanded bed : volume ratio of bed column to catalyst volume of 2:1
Before the operations			
During the operations			

3.9 Characterization of Membrane

3.9.1 Scanning electron microscopy (SEM)

The cross- sectional and surface morphologies of BC and BCA were analyzed by scanning electron microscopy (SEM). Scanning electron micrographs were taken with microscope (FESEM- EDS 7610F) at Scientific and technological research equipment center, Chulalongkorn University. The BC membranes were prepared by critical point drying method. SEM was obtained at 2 kV which was considered to be a suitable condition since too high energy can burn the samples.

The morphology change of enzyme (Novozym 435) after used in enzymatic esterification coupled with pervaporation were analyzed by scanning electron microscopy

(SEM). Scanning electron micrographs were taken with microscope (INSPECT S50) at Department of Mechanical Faculty of Engineering, Chulalongkorn University. Excess oil and residual around sample were washed by Kimwipes paper. SEM was obtained at 2-5 kV which was considered to be a suitable condition since too high energy can burn the samples.

CHAPTER IV

Result and Discussion

In this part, results and discussions were individually described into 4 parts of batch and continuous systems as follows;

4.1 Characterization of Bacterial cellulose impact with alginate membrane in various concentrations of ethanol and water.

4.2 Pervaporation – assisted enzymatic esterification of oleic acid and ethanol in batch system.

4.2.1 Effect of operating mode of PV (continuous and late) on esterification of oleic acid and ethanol using Novozym 435.

4.2.2 Influences of Novozym 435 loading at 5 and 10 %wt of oleic acid.

4.3 Pervaporation – assisted enzymatic esterification of oleic acid and ethanol in continuous system.

4.4 Effects of operating condition to morphology of Novozym 435 in batch and continuous systems.

4.1 Characterization of Bacterial cellulose impact with alginate membrane in various concentrations of ethanol and water

The performance of bacterial cellulose impacted with alginate (BCA) membrane was investigated. Suratago et al., (2016) studied the dehydration of biodiesel-methanol mixture by using BCA membrane under operating condition at temperature 30°C, pressure at permeate side of 10 mmHg, weight ratio of methyl ester(C10:0): methanol: water at 42.3: 52.7: 5. He observed that the permeate was contained 94.5% w/w water and methyl ester could not pass through the BCA membrane (41). In this work, BC and BCA membrane was refer to BC membrane without and with addition alginate respectively. BCA membranes were prepared following the previous work (41) by immersing BC in 3%(w/v) of alginate aqueous solution and then crossing with $CaCl_2$ solution. The surface structure and cross section of the membrane were analyzed by scanning electron microscopy (SEM). It was expected that the BCA membrane was effective to be used as a PV membrane for water removal from the reaction mixture. The SEM images of BC, BCA, BCA having been immersed in 95%(v/v) ethanol, 100%(v/v) ethanol and deionized water were shown in figure 9-14. The surface views in figure 11 indicated that the dried BC was fibrous, porous and composed of dense network nano fibers. The membranes were thicker and more compact after the integration of alginate. The surface and cross section views in figure 11 and 14, respectively show that BCA membrane which had been immersed in water exhibits high swelling degrees as compared with that immersed in ethanol. BCA membrane immersed in 95% shows more membrane swelling as compared to that immersed in 100% ethanol. The results illustrated that increasing amount of water in the system resulted in rising degree of swelling of BCA membrane. The membrane swelling would cause an increase in the membrane pore size, leading to an increase in permeability of ethanol and water and a decrease in rejection of ethanol. The pervaporation performance of the enzymatic esterification of oleic acid and ethanol coupled with BCA membrane in batch process at the conditions of molar ratio of oleic acid to ethanol 1:2, Novozym 435 of 5wt% of oleic acid, reaction time of 8 hour, turbine rate at 250 rpm, pressure at permeate side 10 mmHg and temperature at 45°C

was summarized in Table 4. The result indicated that the percentage of water in permeate of the permeation side of PV were approximately 95 % and the water permeate flux and total permeate flux were 230.03 and 218.37 $gm^{-2}h^{-1}$ respectively. The permeate solution contained only water and ethanol. Either ethly-oleate or oleic acid was not found in the permeate solution.

Table 4 The pervaporation performance using BCA membrane coupled enzymatic esterification of oleic acid and ethanol at 45 ^{o}C and permeate pressure of 10 mmHg. Values are the means ± standard derivation (SD).

Operation mode of pervaporation	Total permeate flux ($gm^{-2}h^{-1}$)	Water permeate flux ($gm^{-2}h^{-1}$)	Water in permeate (%w/w)
Continuous	230.03 ± 24.69	218.37 ± 29.29	94.75 ± 4.15

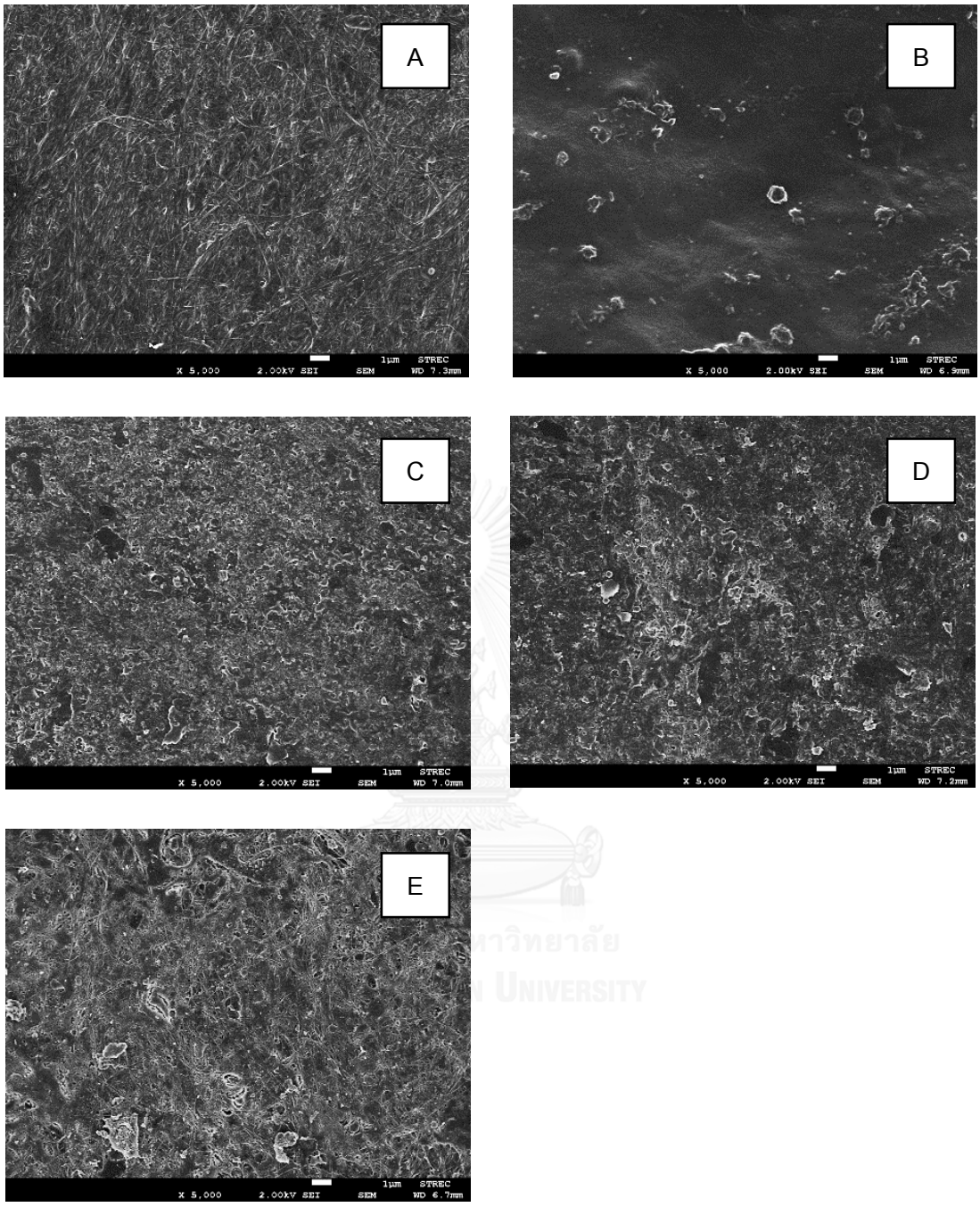


Figure 9 SEM images of surface structure of BC (A), BCA (B), BCA immersed in 95% ethanol (C), BCA immersed in 100% ethanol(D) and BCA immersed in deionized water(E) at magnification of 5000x.

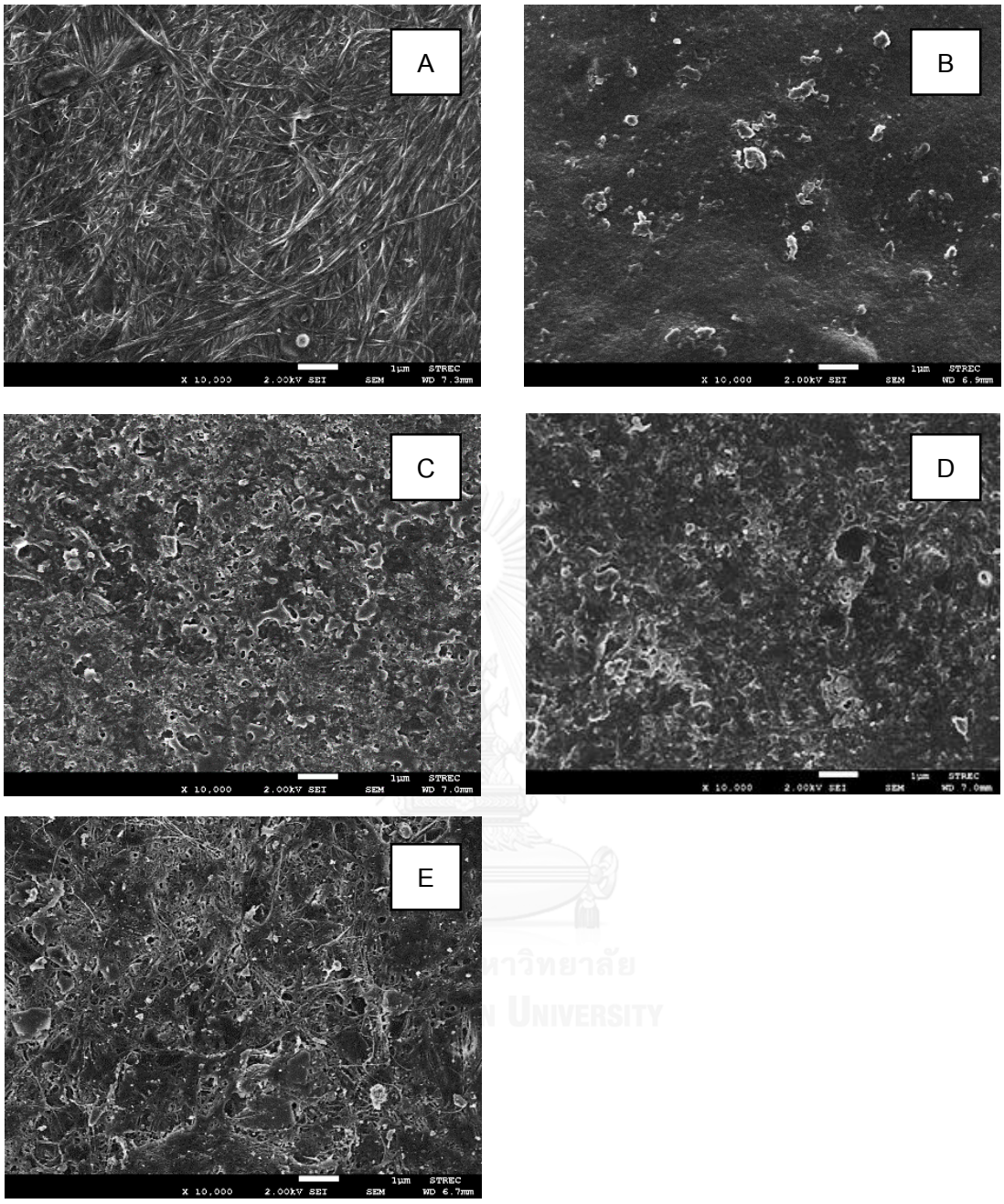


Figure 10 SEM images of surface structure of BC (A), BCA (B), BCA immersed in 95% ethanol (C), BCA immersed in 100% ethanol(D) and BCA immersed in deionized water (E) at magnification of 10000x.

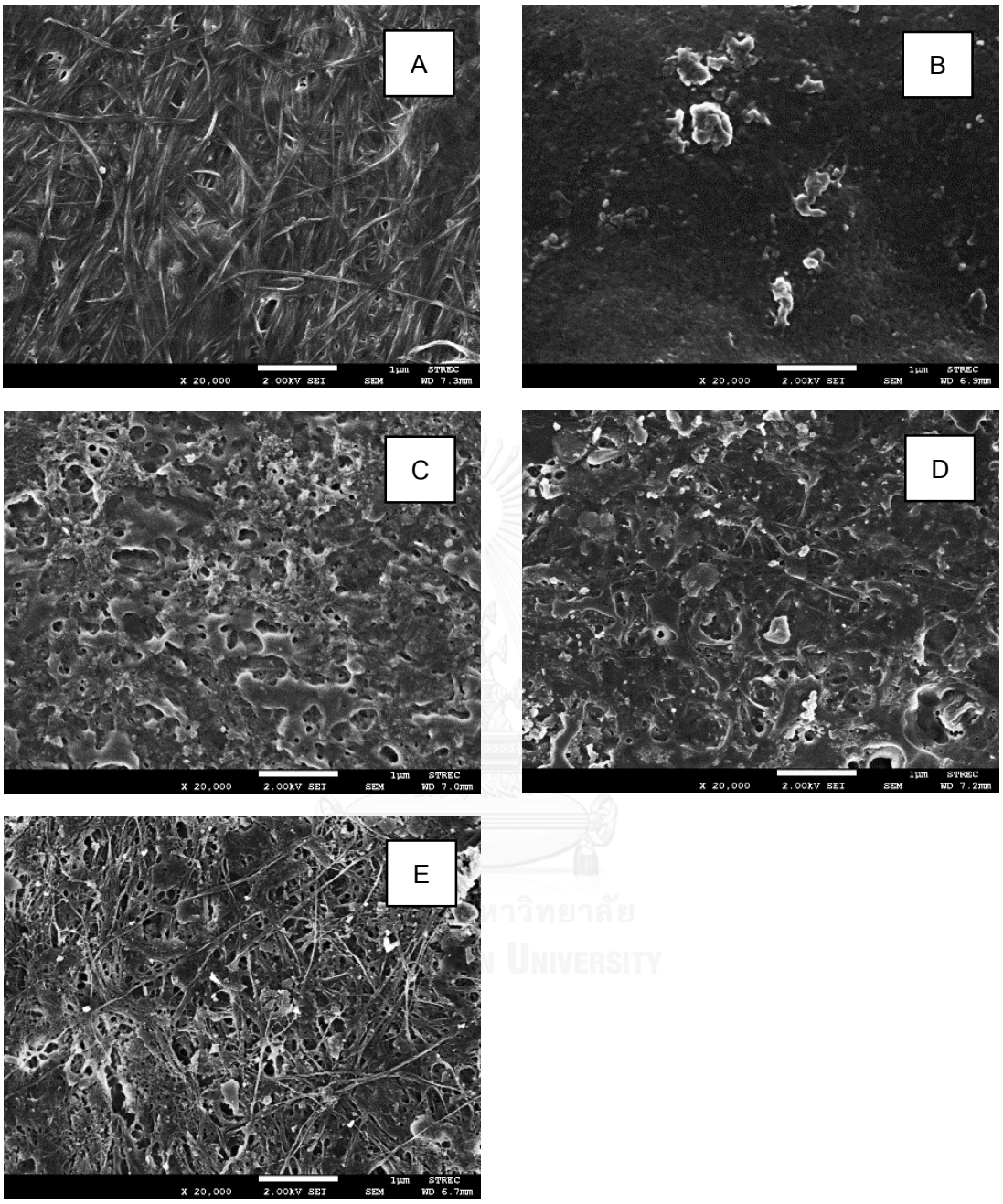


Figure 11 SEM images of surface structure of BC (A), BCA (B), BCA immersed in 95% ethanol (C), BCA immersed in 100% ethanol(D) and BCA immersed in deionized water(E) at magnification of 20000x.

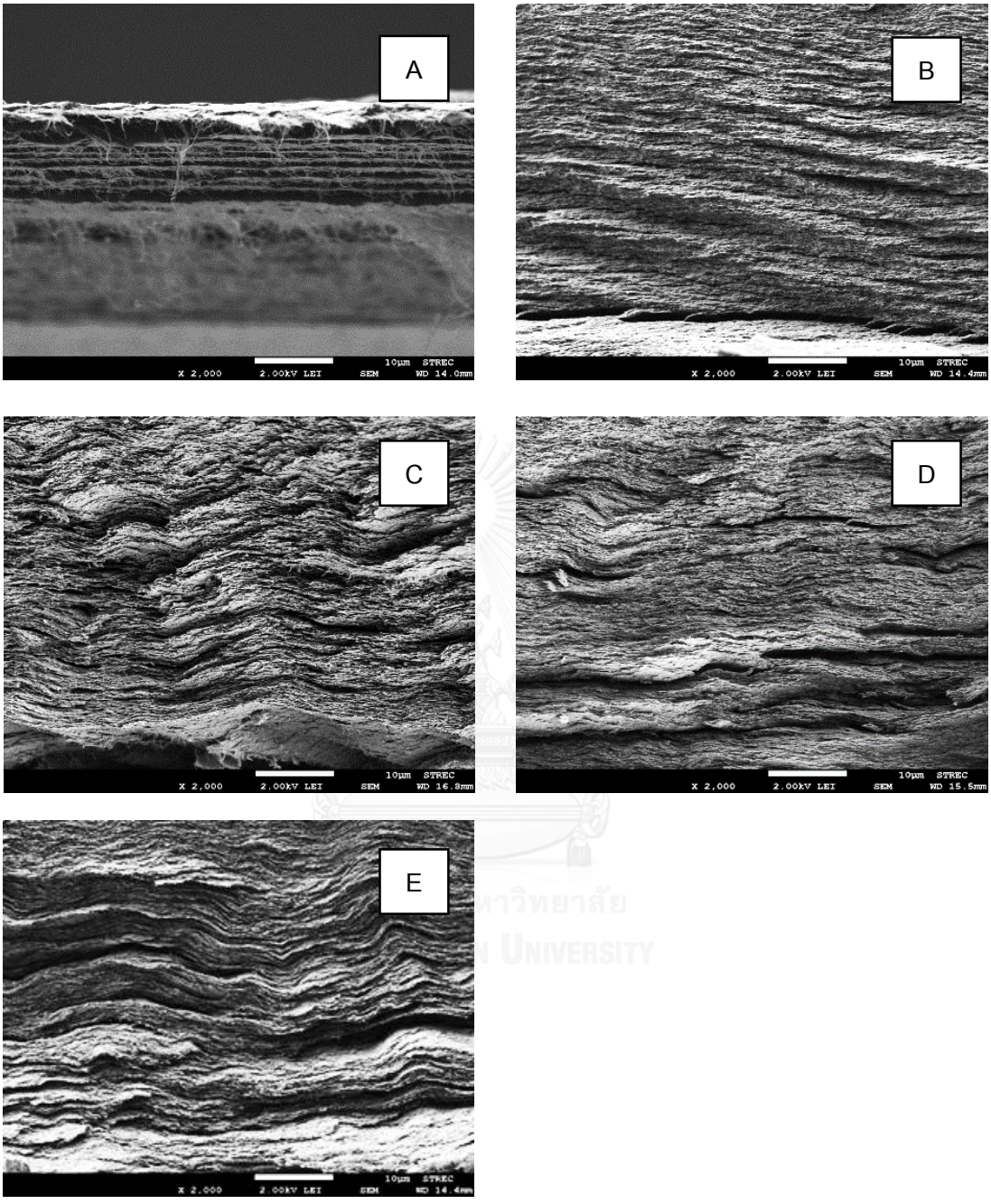


Figure 12 SEM images of cross section of BC (A), BCA (B), BCA immersed in 95% ethanol (C), BCA immersed in 100% ethanol(D) and BCA immersed in deionized water(E) at magnification of 2000x.

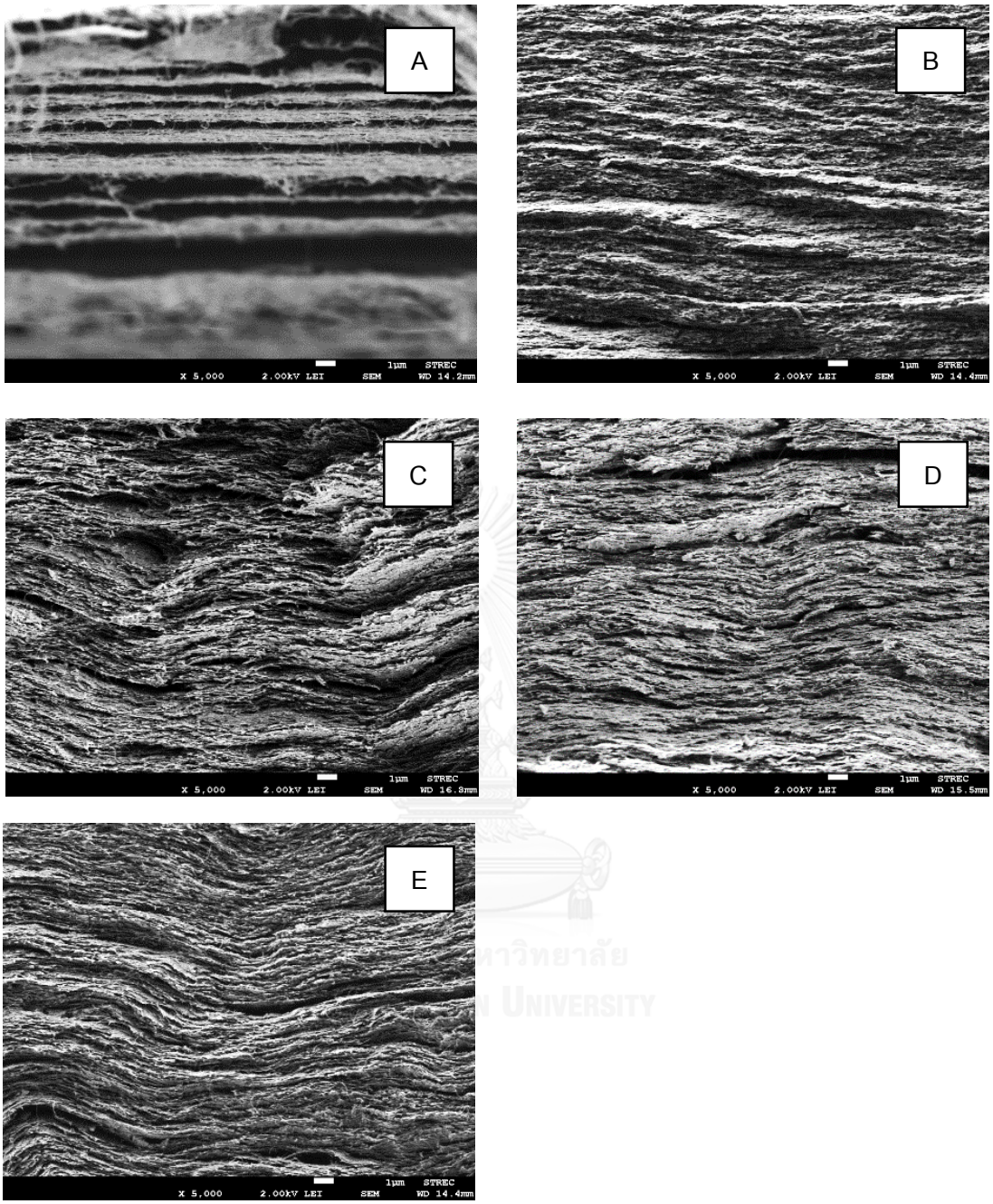


Figure 13 SEM images of cross section of BC (A), BCA (B), BCA immersed in 95% ethanol (C), BCA immersed in 100% ethanol(D) and BCA immersed in deionized water(E) at magnification of 5000x.

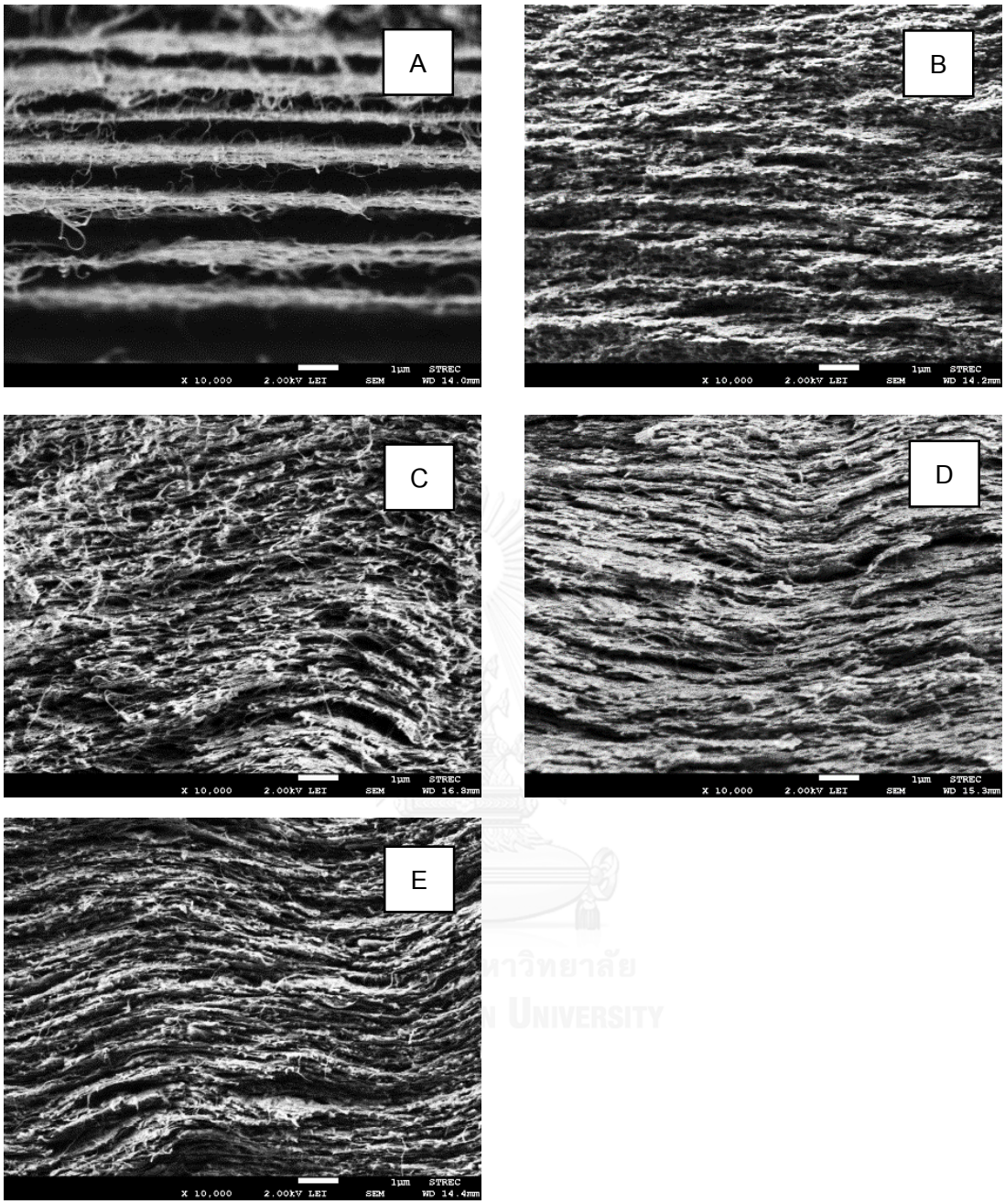


Figure 14 SEM images of cross section SEM images of cross section of BC (A), BCA (B), BCA immersed in 95% ethanol (C), BCA immersed in 100% ethanol(D) and BCA immersed in deionized water(E) at magnification of 10,000x.

4.2 Pervaporation – assisted enzymatic esterification of oleic acid and ethanol in batch system

4.2.1 Effect of operating mode of PV (continuous and late) on esterification of oleic acid and ethanol using Novozym 435

This part of experiment was to investigate the type of pervaporation (non-pervaporation, continuous pervaporation and late pervaporation) that affected to the equilibrium shift of the esterification of oleic acid with ethanol catalyzed by Novozym 435 at controlled conditions: molar ratio of oleic acid to ethanol of 1:2, Novozym 435 loading at 5wt% of oleic acid, reaction time of 20 h, turbine rate at 250 rpm, pressure at permeate side of 10 mmHg and temperature at 45°C. The most effective type of pervaporation was late pervaporation as shown in figure 15. As shown in figure 15, the oleic acid conversion of all type rapidly increased in first hour. Then it gradually increased and achieved the equilibrium at 11 h. The final conversion of oleic acid of non pervaporation and continuous pervaporation were 84.37% and 88.49% respectively. For the late pervaporation, the conversion was constant at equilibrium in period at the 11th to 16th h. During the first period (the pervaporation system was off), the conversion of late pervaporation was similar to that of the non-pervaporation system. After the pervaporation system was on, the equilibrium shift of the reaction moved toward the biodiesel product. It took 3 h for the reaction to reach the maximum conversion at 88.77%, which was very close to the maximum conversion obtained from the continuous pervaporation (88.99%). The results indicated that the removal of water by PV system could enhance the conversion of oleic acid. The main reason was from the equilibrium shift of the reaction due to the removal of water, one of the reaction products. As the reaction was performed for only a single batch process (not a repeated batch process); the effect of water on the enzyme (Novozym 435) deactivation was not observed. Under consideration of short term energy consumption, the late pervaporation was therefore suggested for the operation for the pervaporation - assisted enzymatic esterification of oleic acid and ethanol in a single - batch system. However, for the apply in a repeated batch process, because the removal of water by the pervaporation system could also help to decrease the swelling of Novozym 435, which in

turn should benefit to the structural stability and catalytic activity of the used immobilized enzyme (Novozym 435); accordingly, the effects of the operating procedure on catalytic activity and stability of immobilized enzyme for long-term repeated-batch process should be further studied and optimized. The performances of BCA membrane in continuous pervaporation and late pervaporation were shown in figure 16, 17 and Table 6, 7 respectively. It was shown that with the use of either late pervaporation or continuous pervaporation - assisted enzymatic esterification, the percentage of water in the permeate was approximately 95%. The total permeate fluxes of continuous pervaporation and late pervaporation were approximately 219.28 and 247.98 $gm^{-2}h^{-1}$, respectively. The water permeate fluxes of the continuous pervaporation and late pervaporation systems were approximately 220.12 and 237.96 $gm^{-2}h^{-1}$, respectively. According to the overall results from this Section (4.2.1), the operating mode of late pervaporation-assisted enzymatic esterification of oleic acid and ethanol was selected for the next study.

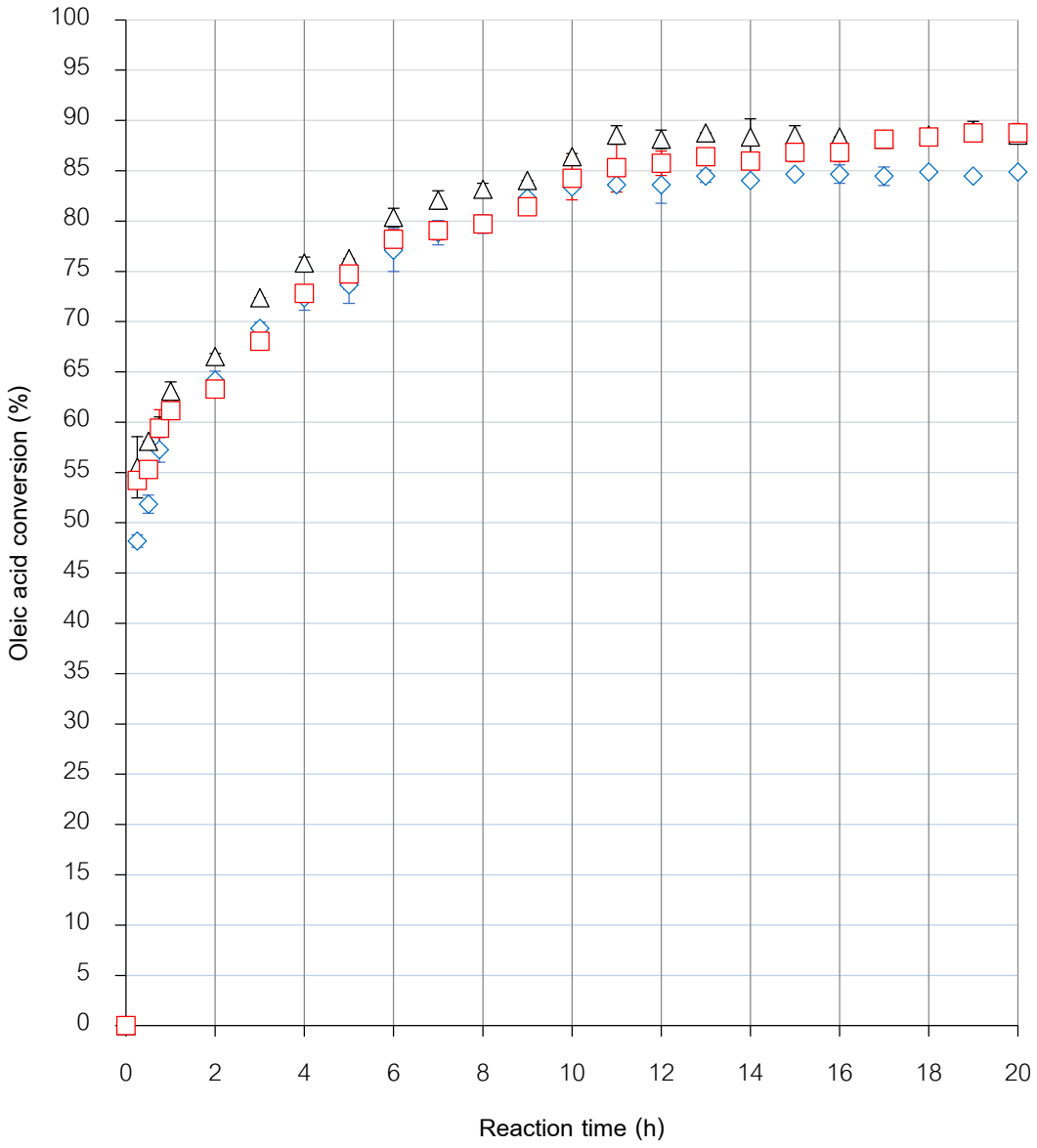


Figure 15 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with pervaporation in a single batch system under controlled reaction conditions: Oleic acid to ethanol molar ratio = 1:2, Novozym 435 = 5wt% of oleic acid, Temperature 45°C, Turbine speed rate = 250 rpm, Reaction time 20 h, Pressure at permeate side 10 mmHg. Type of pervaporation: Non-pervaporation(◊), Continuous pervaporation(Δ) and Late pervaporation (□)

Table 5 Data sheet of oleic acid conversion at operating mode of PV (non, continuous and late) assisted esterification of oleic acid and ethanol using 5 (%w/w) of Novozym 435 to oleic acid. Values are the means ± standard derivation (SD).

Reaction Time (h)	Oleic acid conversion (%)		
	Non Pervaporation	Continuous Pervaporation	Late Pervaporation at (16^{th}-20^{th} h)
0	0	0	0
0.25	48.18 ± 0.61	55.52 ± 3.05	54.23 ± 0.00
0.50	51.85 ± 0.92	58.11 ± 0.00	55.30 ± 0.31
0.75	57.25 ± 1.22	59.62 ± 0.92	59.41 ± 1.83
1	61.57 ± 1.22	63.08 ± 0.92	61.13 ± 0.00
2	64.16 ± 0.92	66.53 ± 0.31	63.29 ± 0.61
3	69.34 ± 0.61	72.36 ± 0.00	68.04 ± 0.61
4	72.36 ± 1.22	75.82 ± 0.61	72.79 ± 0.61
5	73.66 ± 1.83	76.25 ± 0.00	74.74 ± 1.53
6	77.11 ± 2.14	80.35 ± 0.92	78.19 ± 0.92
7	78.84 ± 1.22	82.08 ± 0.92	79.06 ± 0.92
8	79.90 ± 0.92	83.16 ± 0.61	79.70 ± 0.61
9	82.29 ± 0.00	84.02 ± 0.00	81.43 ± 0.61
10	83.37 ± 0.31	86.40 ± 0.31	84.24 ± 2.14
11	83.59 ± 0.00	88.56 ± 0.92	85.32 ± 2.44
12	83.59 ± 1.83	88.12 ± 0.92	85.75 ± 1.22
13	84.45 ± 0.61	88.77 ± 0.00	86.40 ± 0.31
14	84.05 ± 0.29	88.34 ± 1.83	85.96 ± 0.31
15	84.67 ± 0.31	88.56 ± 0.92	86.83 ± 0.92
16	84.67 ± 0.92	88.34 ± 0.00	86.83 ± 0.92
17	84.45 ± 0.92	88.12 ± 0.31	**88.12 ± 0.31**
18	84.89 ± 0.00	88.56 ± 0.31	**88.34 ± 0.00**
19	84.45 ± 0.31	88.99 ± 0.92	**88.77 ± 0.61**
20	84.89 ± 0.00	88.56 ± 0.31	**88.77 ± 0.92**

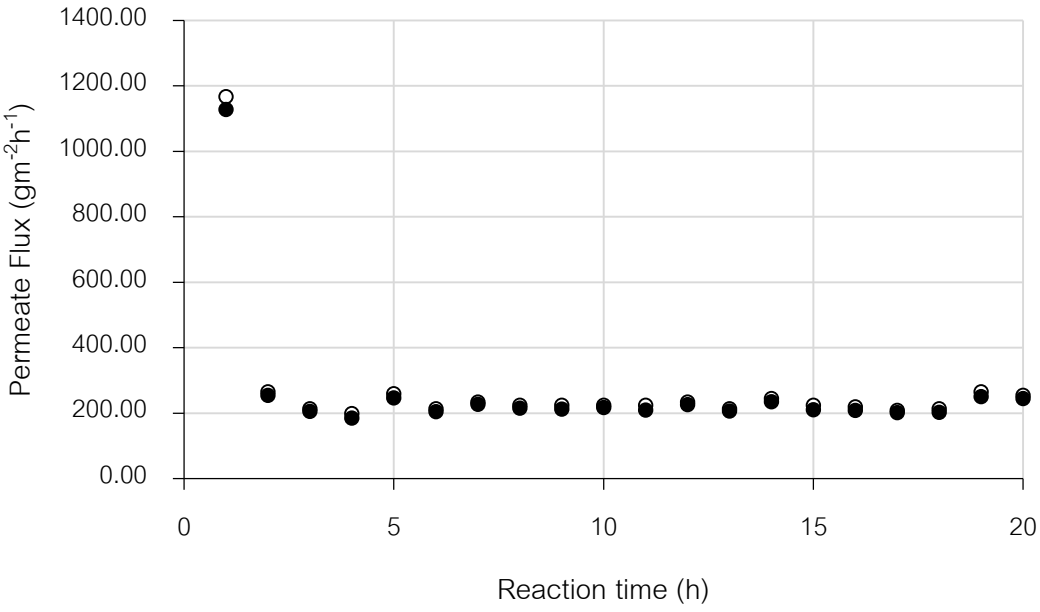


Figure 16 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with continuous pervaporation in a single batch system under controlled reaction conditions: Oleic acid to ethanol molar ratio = 1:2, Novozym 435 = 5wt% of oleic acid, Temperature 45°C, Turbine speed rate = 250 rpm, Reaction time 20 h, Pressure at permeate side 10 mmHg. The permeate flux: Total permeate flux (**O**) and Water permeate flux (●)

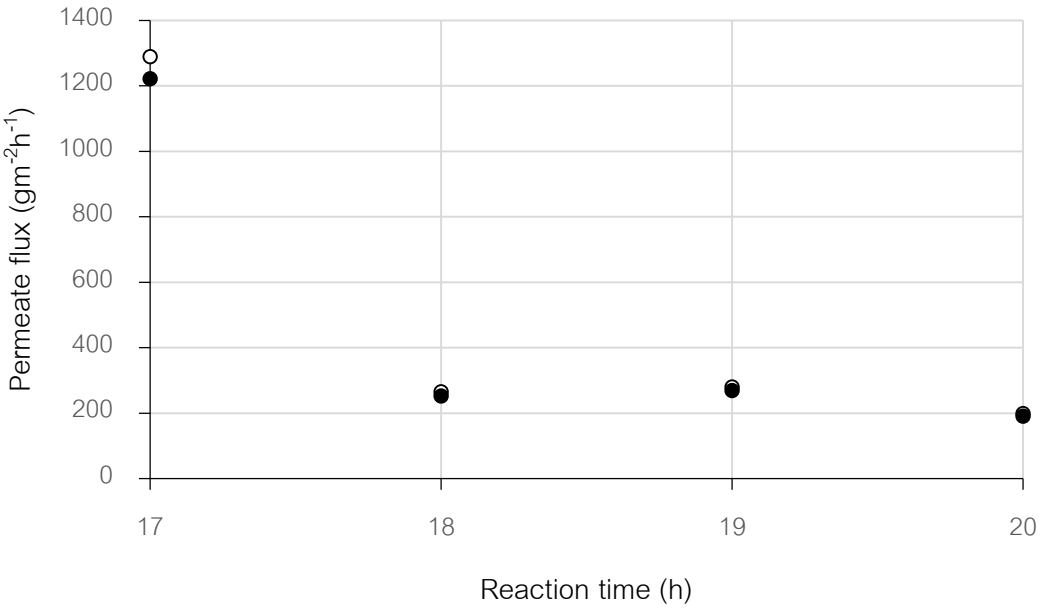


Figure 17 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with late pervaporation in a single batch system under controlled reaction conditions: Oleic acid to ethanol molar ratio = 1:2, Novozym 435 = 5wt% of oleic acid, Temperature 45°C, Turbine speed rate = 250 rpm, Reaction time 20 h, Pressure at permeate side 10 mmHg. The permeate flux: Total permeate flux (**O**) and Water permeate flux (●)

Table 6 Data sheet percentage of water in permeate of continuous PV – assisted esterification of oleic acid and ethanol using 5 (%w/w) of Novozym 435 to oleic acid. Values are the means ± standard derivation (SD).

Time (h)	percentage of water in permeate	Time (h)	percentage of water in permeate
1	96.67 ± 2.96	11	93.74 ± 5.43
2	95.99 ± 5.17	12	96.65 ± 2.93
3	96.19 ± 4.37	13	96.51 ± 1.90
4	93.35 ± 5.81	14	96.31 ± 3.40
5	94.88 ± 5.31	15	93.87 ± 2.98
6	95.57 ± 5.52	16	95.32 ± 4.50
7	96.85 ± 2.77	17	96.43 ± 3.73
8	96.01 ± 4.36	18	94.86 ± 4.88
9	94.74 ± 4.60	19	94.57 ± 3.87
10	96.90 ± 3.47	20	95.94 ± 3.57

Table 7 Data sheet percentage of water in permeate of late PV – assisted esterification of oleic acid and ethanol using 5 (%w/w) of Novozym 435 to oleic acid. Values are the means ± standard derivation (SD).

Time (h)	percentage of water in permeate
17	94.79 ± 2.96
18	95.53 ± 2.96
19	96.12 ± 2.15
20	96.31 ± 1.59

4.2.2 Influences of Novozym 435 loading at 5 and 10 %wt of oleic acid

In this section, we focus on the influence of Novozym 435 loading, which was an important factor for enzymatic esterification. According to the previous results in the section 4.2.1, the late pervaporation was selected for the operation mode in this section. The influences of Novozym 435 loading at 5 and 10%wt of oleic acid on the initial rate and conversion of oleic acid were studied for the reaction with and without applying late PV. The controlled reaction conditions were as follows, molar ratio of oleic to ethanol of 1:2, reaction time for 20 h, turbine rate at 250 rpm, pressure at permeate side of 10 mmHg and temperature at 45°C. The conversions of oleic acid in case of Novozym 435 loading 10%wt and 5% wt of oleic with and without applying late PV are shown in figure 18 and 20, respectively. The oleic acid conversion of both two types rapidly increased in first hour. Afterward, under the system with Novozym 435 loading at 10% wt, the conversions was gradually increased and achieved the equilibrium at around 81-82% within 16 h (for both systems). Then, after prolonging for another 4 h for PV operation, the final conversion of oleic acid of non PV and late PV were 80.86% and 84.94% (figure 18) respectively.

In general, the reaction rate should increase with the increase of enzyme concentration. However, the opposite results were observed in this study. As compared to the result from Novozym 435 loading at 5%wt (figure 20), the increasing twice amount of Novozym435 from 5 to 10%wt of oleic acid did not enhance the conversion of oleic acid. It was consistent with the result of initial rate that the initial rate in case of Novozym 435 loading 10%wt of oleic acid was not greater than that of the system using Novozym 435 loading at 5%wt of oleic acid. Conversely, it was found that the reaction rate and maximum conversion obtain from the reaction by Novozym 435 loading at 10%wt were lower than those by loading at 5%wt of oleic acid. The difference in the flow pattern of both systems should be the main reason for the decrease of the reaction rate and conversion with the increase of enzyme loading from 5 to 10%wt. As informed in Table 3, with the control of reactor volume, the characters of enzyme in the packed bed reactor under batch with enzyme loading at 5 to 10%wt are different. By using enzyme loading at 5%wt of oleic acid in reaction mixture 650 mL, during the operation, the packed bed was

in form of expanded bed at volume ratio of bed column to catalyst volume of 2:1. On the other hand, by loading Novozym at 10%wt of oleic acid in reaction mixture 650 mL, the packed bed was in form of fixed bed at volume ratio of bed column to catalyst volume of 1:1. It was shown that the reaction rate and FFA conversion in the expanded bed were higher than those in the packed bed, which should be due to the more contact surface area of the independently moving catalysts and the less external mass transfer resistance in the expanded bed as compared to the fixed bed. The moving catalysts might help to increase mixing of reactant and enzyme and reduce the external mass and heat transfer effects (9). However, the operating mode of late PV was still effective to shift the equilibrium conversion of the reaction. By using pervaporation - assisted enzymatic esterification of oleic acid and ethanol in batch system, after the reaction reached equilibrium, the final conversion of oleic acid in case of 5% loading enzyme and 10% loading enzyme were 88.50% and 84.94% respectively.

The performance of BCA membrane was shown in figure 19 and Table 9. It indicated that to the use of late PV-assisted enzymatic esterification with Novozym 435 at 10%wt of oleic acid, the percentage of water in the permeate was approximately 95% and the total permeate flux and water permeate flux were approximately 146.07 and 138.69 $gm^{-2}h^{-1}$ respectively. The results for the comparison of the performance of BCA membrane by increasing Novozym435 loading from 5 to 10% wt are shown in figure 21 and table 11. No significant different in the water content of the permeate was observed from the systems using late pervaporative – assisted enzymatic esterification either by using Novozym 435 loading at 5 % or 10% wt. The percentage of water in permeate from both results were approximately 95%. However, the water permeate fluxes of the systems with 10% loading enzyme was approximately 138.69 gm^{-2} h^{-1}, which was considerably less as compared to that of the expanded bed (237.96 gm^{-2} h^{-1}). According to the lower reaction rate of the fixed bed reactor as compared to the expanded bed, the generation of water was also less in the fixed bed reactor. Thus, the water permeate rate and the total permeate flux of the system with 10% loading enzyme were lower than those with 5% loading enzyme. Owing to the higher reaction rate and FFA conversion of the expanded

bed, PV - assisted enzymatic esterification of oleic acid and ethanol in expand bed was selected for the further study of continuous process.

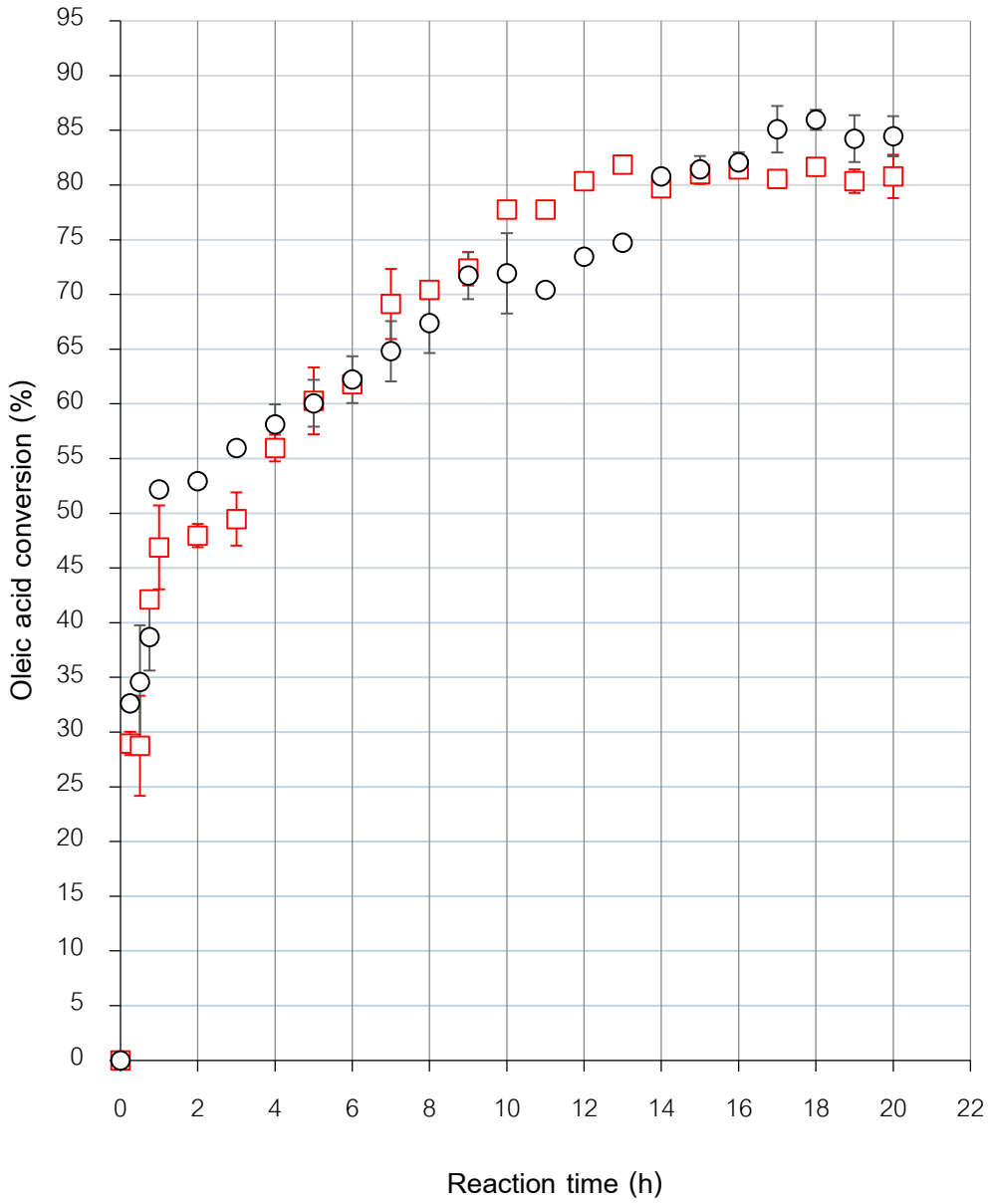


Figure 18 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with pervaporation system at reaction conditions: Oleic acid to ethanol molar ratio = 1:2, Turbine speed rate = 250 rpm, Novozym 435 = 10wt% of oleic acid, Temperature at 45°C, Reaction time of 20 h, Pressure at permeate side 10 mmHg. Type of pervaporation: Non pervaporation (□), Late pervaporation (**O**)

Table 8 Data sheet of oleic acid conversion at operating mode of PV (non and late) assisted esterification of oleic acid and ethanol using 10 (%w/w) of Novozym 435 to oleic acid. Values are the means ± standard derivation (SD).

Reaction Time (h)	Oleic acid conversion (%)	
	Non Pervaporation	Late Pervaporation at (16^{th}-20^{th} h)
0	0	0
0.25	28.96 ± 1.07	32.63 ± 0.61
0.50	28.75 ± 4.58	34.58 ± 5.19
0.75	42.13 ± 0.00	38.68 ± 3.05
1	46.88 ± 3.83	52.18 ± 0.17
2	47.96 ± 1.07	52.93 ± 0.00
3	49.47 ± 2.44	55.95 ± 0.00
4	55.95 ± 1.22	58.11 ± 1.83
5	60.27 ± 3.05	60.05 ± 2.14
6	61.78 ± 0.15	62.21 ± 2.14
7	69.12 ± 3.21	64.80 ± 2.75
8	70.42 ± 0.46	67.40 ± 2.75
9	72.36 ± 1.53	71.71 ± 2.14
10	77.76 ± 0.46	71.93 ± 3.66
11	77.76 ± 0.15	70.42 ± 0.30
12	80.35 ± 0.46	73.44 ± 0.30
13	81.86 ± 0.00	74.74 ± 0.30
14	79.70 ± 0.61	80.78 ± 0.30
15	81.00 ± 0.92	81.43 ± 1.22
16	81.43 ± 0.30	82.08 ± 0.92
17	80.57 ± 0.00	**85.10 ± 2.14**
18	81.65 ± 0.15	**85.96 ± 0.92**
19	80.35 ± 1.07	**84.24 ± 2.14**
20	80.78 ± 1.98	**84.45 ± 1.83**

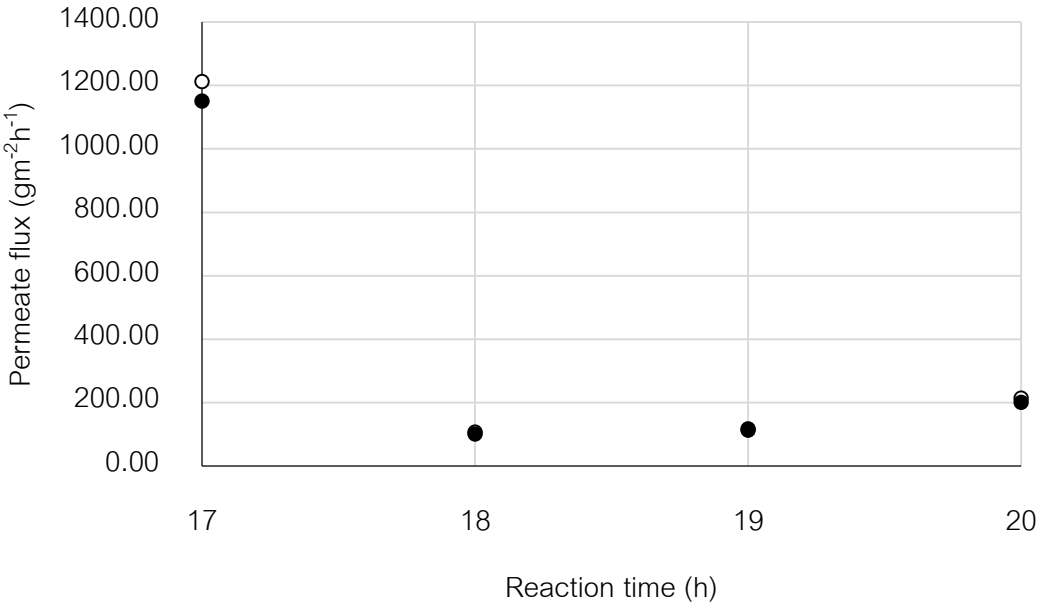


Figure 19 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with late pervaporation system in batch system at reaction conditions: Oleic acid to ethanol molar ratio = 1:2, Novozym 435 = 10wt% of oleic acid, Temperature 45°C, Turbine speed rate = 250 rpm, Reaction time 20 h, Pressure at permeate side 10 mmHg. The permeate flux: Total permeate flux (**O**) and Water permeate flux (●)

Table 9 Data sheet for percentage of water in permeate of late PV on esterification of oleic acid and ethanol using 10 (%w/w) of Novozym 435 to oleic acid. Values are the means ± standard derivation (SD).

Time (h)	Percentage of water in permeate
17	94.88±5.00
18	94.59±1.75
19	96.80±3.95
20	94.11±2.97

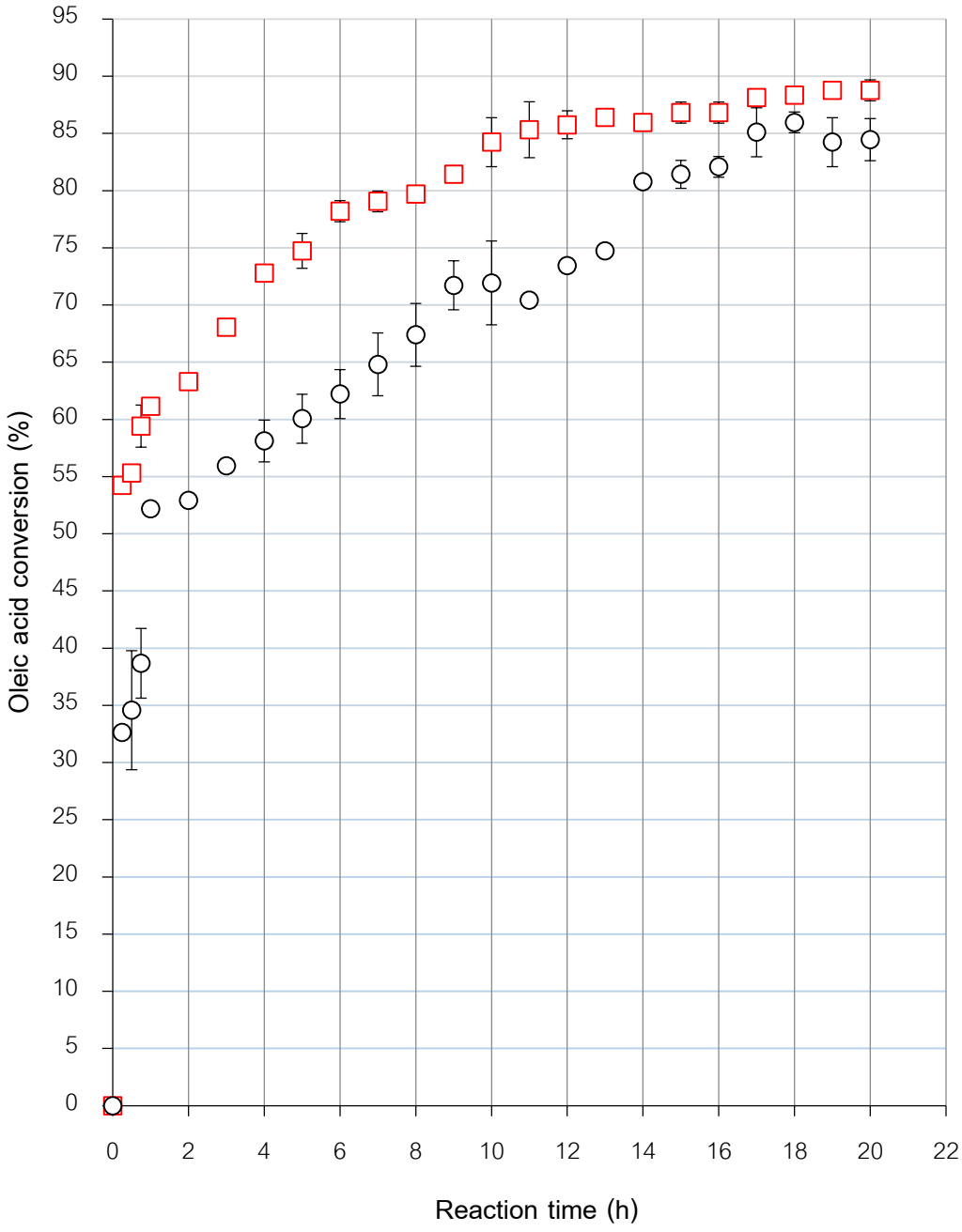


Figure 20 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with pervaporation system at reaction conditions: Oleic acid to ethanol molar ratio = 1:2, Operating mode of pervaporation is late PV, Turbine speed rate = 250 rpm, Temperature 45°C, Reaction time 20 h, Pressure at permeate side 10 mmHg. Amount of enzyme loading: 5 (%w/w) of Novozym 435 to oleic acid (□), 10 (%w/w) of Novozym 435 to oleic acid (**O**)

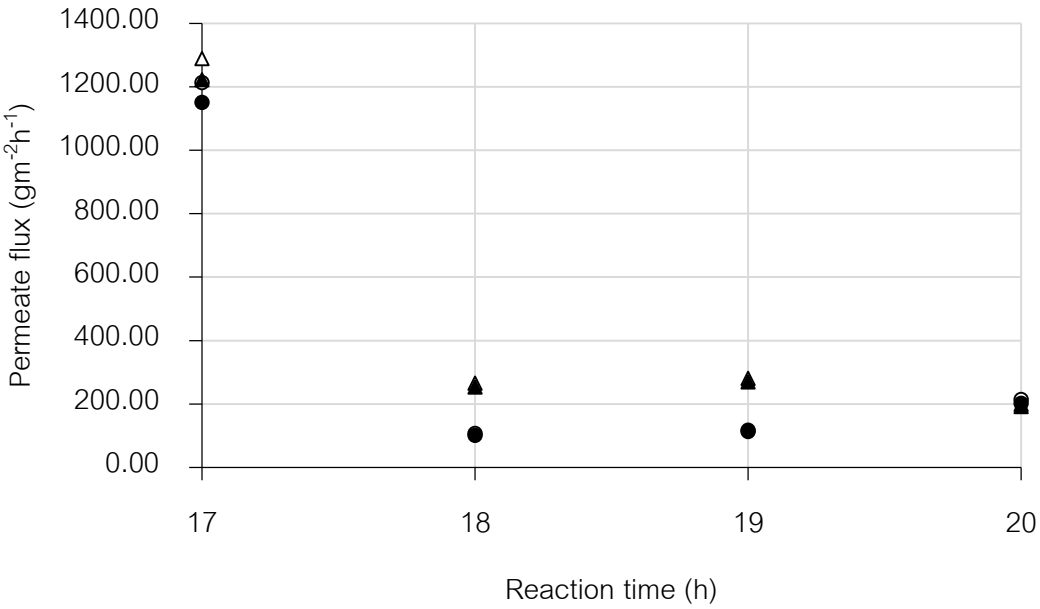


Figure 21 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with late pervaporation system in batch system at reaction conditions: Oleic acid to ethanol molar ratio = 1:2, Novozym 435 = 5 and 10wt% of oleic acid, Temperature 45°C, Turbine speed rate = 250 rpm, Reaction time 20 h, Pressure at permeate side 10 mmHg. The permeate flux: Total permeate flux of Novozym 435 10wt% (O), Water permeate flux of Novozym 435 10wt% (●),Total permeate flux of Novozym 435 5wt% (Δ), Water permeate flux of Novozym 435 5wt% (▲)

Table 10 Data sheet of oleic acid conversion at operating mode of late PV on esterification of oleic acid and ethanol using 5 and 10 (%w/w) of Novozym 435 to oleic acid. Values are the means ± standard derivation (SD).

Reaction Time (h)	Oleic acid conversion (%)	
	5 (%w/w) of Novozym 435 to oleic acid	10 (%w/w) of Novozym 435 to oleic acid
0	0	0
0.25	54.23 ± 0.00	32.63 ± 0.61
0.50	55.30 ± 0.31	34.58 ± 5.19
0.75	59.41 ± 1.83	38.68 ± 3.05
1	61.13 ± 0.00	52.18 ± 0.17
2	63.29 ± 0.61	52.93 ± 0.00
3	68.04 ± 0.61	55.95 ± 0.00
4	72.79 ± 0.61	58.11 ± 1.83
5	74.74 ± 1.53	60.05 ± 2.14
6	78.19 ± 0.92	62.21 ± 2.14
7	79.06 ± 0.92	64.80 ± 2.75
8	79.70 ± 0.61	67.40 ± 2.75
9	81.43 ± 0.61	71.71 ± 2.14
10	84.24 ± 2.14	71.93 ± 3.66
11	85.32 ± 2.44	70.42 ± 0.30
12	85.75 ± 1.22	73.44 ± 0.30
13	86.40 ± 0.31	74.74 ± 0.30
14	85.96 ± 0.31	80.78 ± 0.30
15	86.83 ± 0.92	81.43 ± 1.22
16	86.83 ± 0.92	82.08 ± 0.92
17	88.12 ± 0.31	85.10 ± 2.14
18	88.34 ± 0.00	85.96 ± 0.92
19	88.77 ± 0.61	84.24 ± 2.14
20	88.77 ± 0.92	84.45 ± 1.83

Table 11 Data sheet percentage of water in permeate of late PV- assisted esterification of oleic acid and ethanol using 5 and 10 (%w/w) of Novozym 435 to oleic acid. Values are the means ± standard derivation (SD).

Time (h)	Percentage of water in permeate	
	5 (%w/w) of Novozym 435 to oleic acid	10 (%w/w) of Novozym 435 to oleic acid
17	94.79±2.96	94.88±5.00
18	95.53±2.96	94.59±1.75
19	96.12±2.15	96.80±3.95
20	96.31±1.59	94.11±2.97

4.3 Pervaporation assisted- enzymatic esterification in continuous system

In this section, the previous batch process of enzymatic esterification with expanded bed coupled with the pervaporation unit was developed into continuous process in order to improve ethyl oleate productivity. The instrument from batch process was adapted to be able to operate for continuous processes of enzymatic esterification as shown in figure 7. Most of operating conditions for the reaction followed the optimal conditions obtained from the previous work (9). The operating conditions were as follows: 380 mL of reaction volume, at the volume ratio of bed column to catalyst volume of 2:1 (expanded bed reactor), temperature at 45^{o}C, oleic acid to ethanol molar ratio of 1:2, flow rate mixing of reactant of 5 cm^3min^{-1}, height of bed column of 10 cm, effective area of membrane of 19.625 cm^2 and pressure at permeate side of 10 mmHg. The oleic acid conversion profiles of non and continuous pervaporation were shown in figure 22. It was shown that conversions rapidly increased in first hour and the system approached the steady state conditions at 4 h. Under the retention time of 50 minutes, the average FFA conversion of non and continuous pervaporation were 65.52% and 70.27%, respectively. The overall performance of BCA membrane was shown in figure 23 and Table 13 . It indicated that with the use of pervaporation assisted - enzymatic esterification in the

continuous process, the percentage of water in the permeate of both systems was approximately 95% , where the total permeate flux and water permeate flux were approximately 249.68 and 244.05 $gm^{-2}h^{-1}$, respectively. The summary of conversion and productivity of pervaporation – assisted enzymatic esterification of oleic acid and ethanol was shown in Table 14. It was shown that the productivity of continuous process was higher than the batch process due to the retention time of reaction in continuous process was only 50 min, whereas the reaction time in the batch process was 11-16 h. The productivity of enzymatic esterification in continuous system was increased from 1180.42 to 1221.48 $gL^{-1}h^{-1}$ by using continuous pervaporation. The summary performance of BCA membrane on pervaporation-assisted enzymatic esterification of oleic acid and ethanol at permeate pressure of 10 mmHg was shown in Table 15. It was indicated that the BCA membrane could selectively remove water from the reaction mixture, resulting in the high water content at approximately 95 %(w/w) in the permeate, where the total flux permeate was ≥ than 200 $gm^{-2}h^{-1}$.

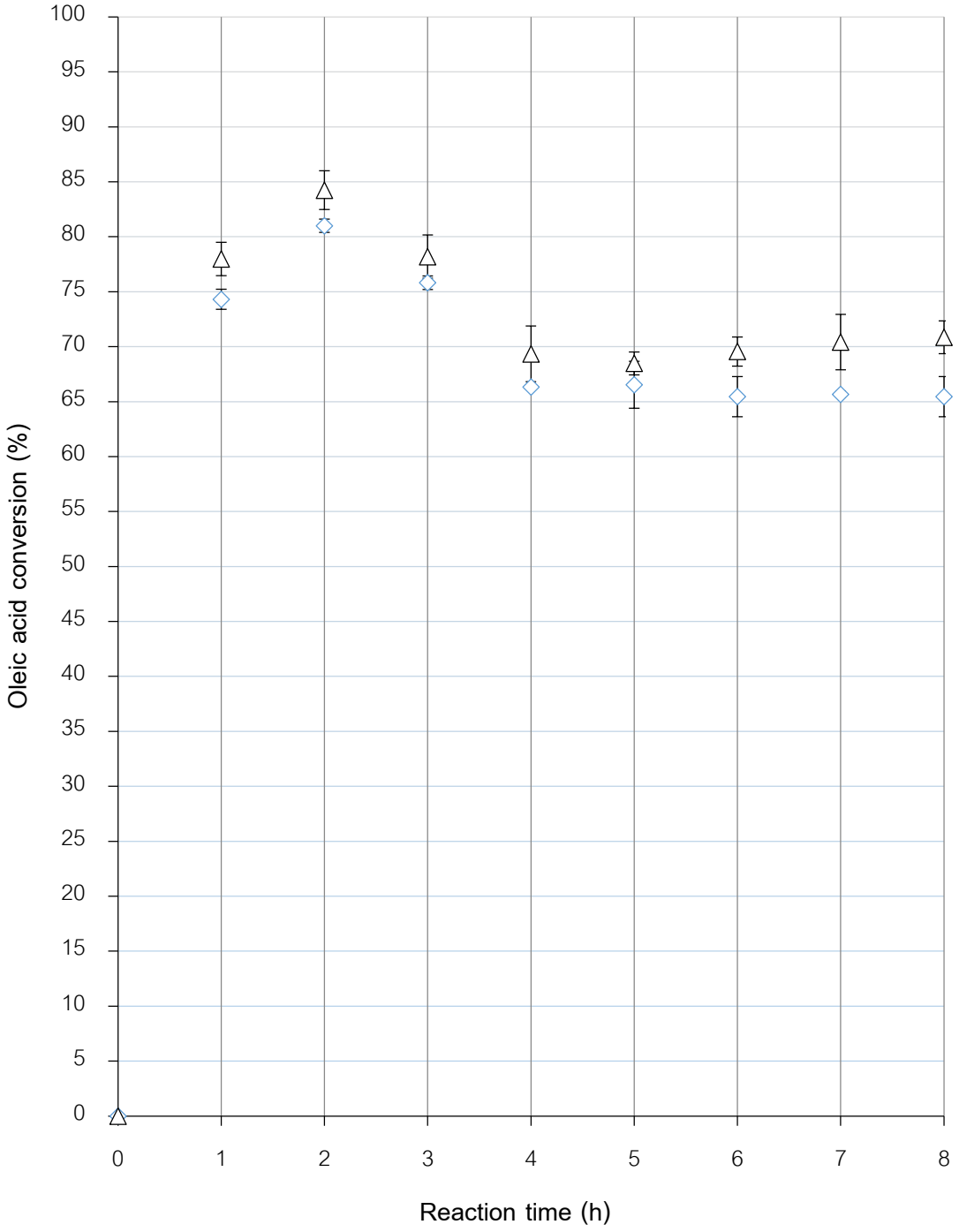


Figure 22 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with pervaporation in continuous process at reaction conditions: Oleic acid to ethanol molar ratio = 1:2, volume ratio of bed column to catalyst volume of 2:1, Temperature 45°C, flow rate = 5 cm^3min^{-1}, Retention time 50 minutes, Pressure at permeate side 10 mmHg. Type of pervaporation: Non pervaporation (◊), Continuous pervaporation (Δ)

Table 12 Data sheet of oleic acid conversion at operating mode of continuous PV- assisted enzymatic esterification of oleic acid and ethanol in continuous process. Values are the means ± standard derivation (SD).

Reaction Time (h)	Oleic acid conversion (%)	
	Non-Pervaporation	Continuous Pervaporation
0	0	0
1	74.31 ± 0.92	77.98 ± 1.51
2	81.00 ± 0.61	84.24 ± 1.77
3	75.82 ± 0.61	78.19 ± 1.97
4	66.32 ± 3.18	69.34 ± 2.53
5	66.53 ± 2.14	68.48 ± 1.04
6	65.45 ± 1.83	69.56 ± 1.32
7	65.67 ± 0.30	70.42 ± 2.51
8	65.45 ± 1.83	70.85 ± 1.49

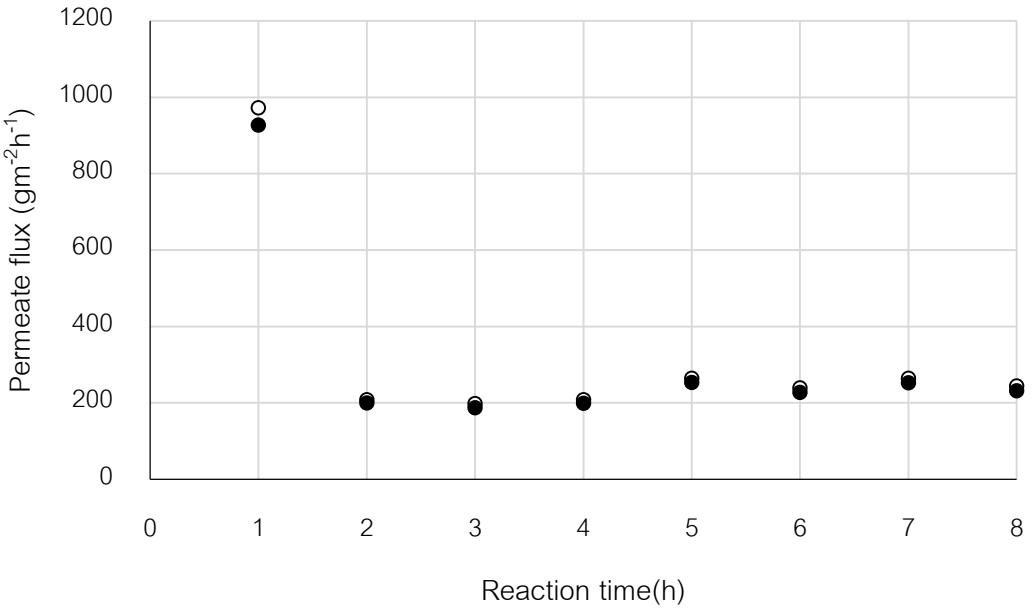


Figure 23 Esterification of oleic acid with ethanol catalyzed by Novozym 435 coupled with pervaporation in continuous process at reaction conditions: Oleic acid to ethanol molar ratio = 1:2, volume ratio of bed column to catalyst volume of 2:1, Temperature 45°C, flow

rate = 5 cm^3min^{-1}, Retention time 50 minutes, Pressure at permeate side 10 mmHg. The permeate flux: Total permeate flux (**O**) and Water permeate flux (●)

Table 13 Data sheet for percentages of water in permeate of continuous PV- assisted enzymatic esterification of oleic acid and ethanol in continuous process. Values are the means ± standard derivation (SD).

Time (h)	percentage of water in permeate
1	95.35±0.89
2	95.66±1.32
3	94.48±1.11
4	95.41±1.85
5	95.77±1.57
6	95.15±0.75
7	95.61±3.47
8	94.72±1.27

Table 14 Summary of concentration of ethyl-oleate, conversion and productivity of pervaporation assisted with enzymatic esterification of oleic acid and ethanol.

Process	Type	Conversion	Productivity (g/Lh)
Batch*	Non pervaporation with Novozym 435 5%wt of oleic acid	84.89	31.12
	Continuous pervaporation with Novozym 435 5%wt of oleic acid	88.99	34.20
	Late pervaporation with Novozym 435 5%wt of oleic acid	88.77	32.40
	Non pervaporation with Novozym 435 10%wt of oleic acid	81.65	33.35
	Late pervaporation with Novozym 435 10%wt of oleic acid	84.45	31.12
Continuous**	Non continuous pervaporation with expanded bed column	65.52	1180.42
	Continuous pervaporation with expanded bed column	70.27	1221.48

* The report value are at the maximum conversion.

** The report value are average value after steady state conversion.

Table 15 Summary performance of BCA membrane on pervaporation assisted with enzymatic esterification of oleic acid and ethanol at permeate pressure of 10 mmHg. Values are the means ± standard derivation (SD).

Process	Type	Total permeate flux ($gm^{-2}h^{-1}$)	Water permeate flux ($gm^{-2}h^{-1}$)	Water in permeate (%w/w)
Batch	Continuous PV with Novozym 435 5%wt of oleic acid	219.28 ± 19.12	220.12 ± 21.99	95.82 ± 3.53
	Late PV with Novozym 435 5%wt of oleic acid	247.98 ± 43.34	237.96 ± 41.14	95.98 ± 0.41
	Late PV with Novozym 435 10%wt of oleic acid	146.07 ± 59.1	138.69 ± 54.66	95.17 ± 1.4
Continuous	Continuous PV with expanded bed column	249.68 ± 13.48	244.05 ± 13.73	95.26 ± 0.5

4.4 Effects of operating condition on morphology of Novozym 435 in batch and continuous system

In this section, we tried to investigate the effects of operating condition to morphology of Novozym 435 in batch and continuous system. Novozym 435 catalyzed the reaction with high activity under highly water-deficient conditions. The presence of water within the system might flood the enzyme pores, causing decreases in the catalytic activity. Previous work by Mulalee et al., (2014) was reported for the deactivation of Novozym causing by the presence of water in the system. The reduction in catalytic activity or the degree of deactivation of Novozym 435 was related to the swelling degree of the catalyst surface (9). It was demonstrated that the presence of water at 4-5% in ethanol could reduce the reusability of Novozym 435 (9). In this work, the surface morphology of Novozym 435 of fresh enzyme and used enzyme at various conditions were observed by SEM (INSPECT S50). It was noticed that the degrees of swelling of the Novozym 435 catalysts used in the esterification of oleic acid with ethanol catalyzed coupled with/without pervaporation were to some extent different. As shown in figure 24, surface morphology of fresh enzyme was relatively flat and smooth. Some swelling of the catalyst surface was observed after being used in the batch enzymatic esterification without PV unit (figure 24 B), whereas surface swelling was not observed in the single batch system coupled with PV unit (either continuous (figure 24 C) or late operation (figure 24 D)). With higher generation rate of water, higher degrees of surface swelling were observed in the continuous system as compared to the batch system. Moreover, it was shown that the swelling degree on the catalyst surface in the continuous system without PV unit (figure 24 E) was higher as compared to that of the continuous system coupled with PV unit (figure 24 F). These observed effects should be according to the presence of water in the system. The integration of the PV unit could lower water concentration in the system by removal of water, thus it helps to decrease degree of swelling of enzyme. From the preliminary examination of the effects of operating condition on morphology of Novozym 435 in batch and continuous system, it is expected that the effective removal of water from the reaction mixture in the reactor with the PV unit should enhance the

reusability of Novozym 435 in repeated batch process and improve stability in continuous process by prolonging the catalytic activity of Novozym 435 for long-term use.

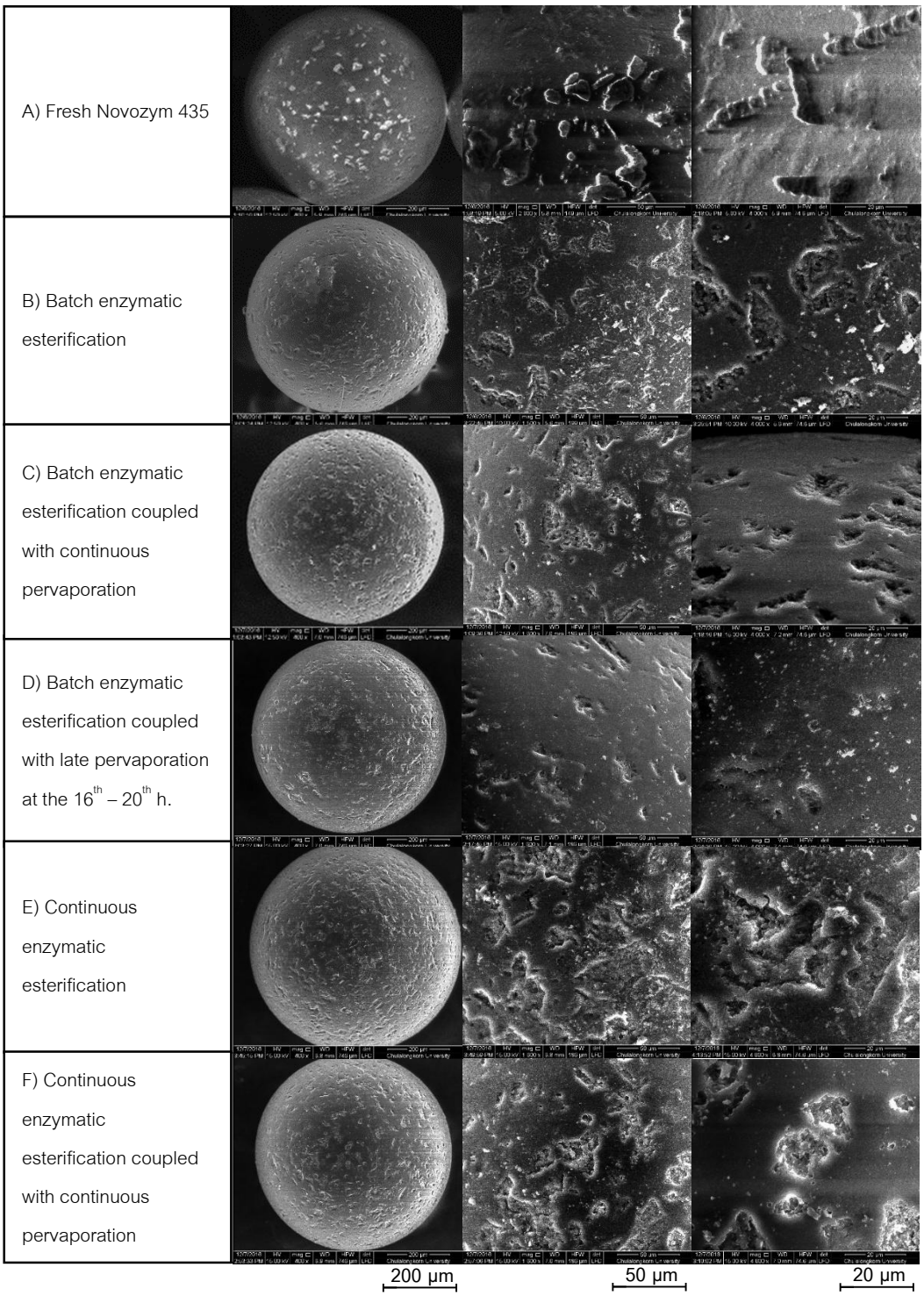


Figure 24 SEM morphology of Novozym 435 of enzymatic esterification of oleic acid and ethanol coupled with pervaporation in batch and continuous system.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

In this work, in order to improve the FFA conversion, the batch and continuous processes of enzymatic esterification of oleic acid and ethanol were coupled pervaporation unit. Bacterial cellulose (BC) impacted with alginate film was used as selectively-permeable membrane to remove water from the reaction mixture. The enhanced equilibrium conversions in the system coupled with PV unit were achieved. The batch experiments of enzymatic esterification of oleic acid and ethanol were carried out under the operating conditions as follows: molar ratio of oleic acid to ethanol of 1:2, temperature of 45°C, turbine rate at 250 rpm, enzyme loading at 5% (w/w oleic acid) and pressure permeate side at 10 mmHg. Considering less energy consumption, the start operation of pervaporation at the end of the reaction (late pervaporation) to move equilibrium toward biodiesel product is suggested, in which the FFA conversion at 88.50% could be obtained (or about 4% greater than that of the system without the PV unit).

On the study of the flow pattern in the reactor, it was shown that the higher initial rate and FFA conversion were obtained by using the expanded bed, as compared to the fixed bed at the same reactor volume, which should be due to the more contact surface area of the independently moving catalysts and the less external mass transfer resistance in the expanded bed.

The modification of pervaporation coupled with enzymatic esterification in expanded bed reactor from the batch process to the continuous process was carried out. It was shown that the final conversion of oleic acid was increased from 65.88% (obtained from the system without the PV unit) to 69.73% by the integration of the PV unit.

From the observation of surface morphology of Novozym 435 being used in the esterification in comparison to the fresh one, the degree of swelling of Novozym 435 was related significantly to the presence of water in the system. It was demonstrated that the degrees of swelling of Novozym 435 in the batch and continuous processed coupled with pervaporation were less compared to those from the systems without the PV unit.

Therefore, it is expected that the integration of PV unit should enhance the reusability of Novozym 435 in repeated batch process and improve stability in continuous process by prolonging the catalytic activity of Novozym 435 for long-term use.

Recommendation

- The study of effects of water that related to reusability of enzyme when use BCA membrane as selective removal water for long period experiment.
- The study of effect of reduced pressure on the permeate side of PV unit on the removal efficiency of water from the reaction mixture in esterification processes.
- The study of effect of ratio of effective surface area of BCA membrane to volume of reactor on the conversion in esterification processes.

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APPENDIX

Appendix A

A1 Data sheet of novozym435

Novozym® 435

Valid from **2011-09-14**

Product Characteristics:

Declared enzyme	Lipase
Declared activity	10000 PLU/g
Colour	Off-white Colour can vary from batch to batch. Colour intensity is not an indication of enzyme activity.
Physical form	Immobilized Granulate
Approximate density (g/ml)	0.40
Carriers	Acrylic resin
Production organism	Aspergillus niger
Production method	Produced by submerged fermentation of a genetically modified micro organism. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Product Specification:

	Lower Limit	Upper Limit	Unit
Propyl Laurate Unit PLU	10000		/g
Loss on Drying 105 C	-	3	%

Packaging: See the standard packaging list for more information.

Appendix B

B1 Productivity calculation of ethyl oleate production from oleic acid and ethanol catalyzed by Novozym 435 in expand bed continuous system coupled with continuous pervaporation

Information data

- Chemical property

Name	Formula	MW ($gmol^{-1}$)	Density (g/cm^3)
Oleic acid	$C_{18}H_{34}O_2$	282.47	0.89
Ethanol	C_2H_6O	46.07	0.81
water	H_2O	18.02	0.99
Ethyl oleate	$C_{20}H_{38}O_2$	310.51	0.87

The productivity was calculated at the average value after steady state conversion. The reaction was steady state at the 6^{th} to 8^{th} h

- Substrate ratio

Molar ratio: Oleic acid: ethanol (mol) 1 : 2

Mass ratio: Oleic acid : ethanol (g) 282.47 : 92.14

- Operating conditions: Mixture feed flowrate of 5 $cm^3 min^{-1}$

  Retention time 50 minutes

  Volume of reactor 380 cm^3

Therefore, productivity of ethyl oleate

The productivity at 6^{th} h

$$= \frac{\text{weight of ethyl-oleate (g)}}{\text{retention time (h)} \times \text{volume reactor (L)}}$$

$$= \frac{384.8308 \times 1000}{380 \times \left(\frac{50}{60}\right)}$$

$$= 1215.255 \left(\frac{g}{hL}\right)$$

The productivity at 7^{th} h

$$= \frac{387.1946 \times 1000}{380 \times \left(\frac{50}{60}\right)}$$

$$= 1222.72 \left(\frac{g}{hL}\right)$$

The productivity at 8^{th} h

$$= \frac{388.3765 \times 1000}{380 \times \left(\frac{50}{60}\right)}$$

$$= 1226.45 \left(\frac{g}{hL}\right)$$

$$\text{Average productivity at steady state} = \left(\frac{1215.255 + 1222.72 + 1226.45}{3} \right)$$

So that the productivity ethyl oleate production from oleic acid and ethanol catalyzed by Novozym 435 in expand bed continuous system coupled with continuous pervaporation in unit of $gh^{-1}L^{-1}$ was at 1221.48 $gh^{-1}L^{-1}$

VITA

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