PRODUCTION OF ACYLGLYCEROL CATALYSED BY RICE BRAN LIPASE IN A PACKED BED REACTOR

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ABSTRACT

Abstract A 20 litre packed bed reactor (PBR) with heating and water removal system was designed and fabricated for the esterification of palm oil fatty acid distillate (PFAD) catalyzed by immobilized rice bran lipase (RBL). The PBR was designed based on the characteristics of immobilised RBL and the optimized esterification conditions obtained from method scouting performed in shaked flask. The optimal ratios of immobilised RBL and water removal agent (silica gel) to PFAD for the shaked flask esterification process were 5:1 and 1:2, respectively. The intensified esterification reaction of PBR was operated by circulating the reaction mixture (PFAD and glycerol) in hexane through a packed bed column filled with immobilised RBL. The water generated from esterification reaction was absorbed by silica gel filled in the water removal vessel. The maximum degree of esterification achieved in this developed PBR was 61%. The reaction time required to achieve the maximum degree of esterification was 25% faster than that in the shaked flask.

Keywords: *Acylglycerol, Esterification, Packed Bed Reactor, PFAD, Rice Bran Lipase*

1.0 INTRODUCTION

Mixtures of mono- and diacylglycerol (MAG and DAG) are widely used as emulsifiers in food, pharmaceutical, and cosmetic industries [1]. Furthermore, DAG oil was reported to play an important role in the management of obesity when it is consumed as part of a sensible diet [2]. However, the amount of natural DAG in most of the vegetable oil is very low (1-5% w/w) [3]. Therefore, it is important to develop an efficient esterification process to increase its content.

In this study, palm oil fatty acid distillate (PFAD) was used as raw material for the esterification process. PFAD is a byproduct from the physical refining of crude palm oil [4, 5], which are abundantly available in Malaysia. It is a potential source of free fatty acid for the production of acylglycerol due to its high free fatty acids content [6, 7]. On the other hand, rice bran, a byproduct of rice processing industries is rich with lipid enzyme such as phospholipases, glycolipases, and esterases [8]. The application of rice bran lipase (RBL) in the esterification of free fatty acid has been previously demonstrated [9].

The esterification of acylglycerols using immobilised lipase can be performed in various type of bioreactor such as shake flask [7], stirred-tank bioreactor (STR) [3] and packed bed reactor (PBR) [10]. The continuous packed bed column reactors was reported to be the most suitable type of reactor for the process involving immobilised biocatalyst particles [10,11,12]. PBR is generally preferable for large scale process because of its relatively higher efficiency, lower capital and operating costs, and ease to operate and maintain [10, 12]. In PBR, the immobilised enzyme can be loaded into the reactor at a higher density, and meanwhile it is safe from the breakage caused by the shear stress due to the intensive agitation of STR.

Water generated during the esterification reaction can lead to the undesired hydrolysis of esterified product. It was usually overcome by the addition of water removal agent [13, 14] or the reduction of reactions pressure [10]. The former method is easier to design compared to the later, which requires complicated instrumentation.

The aim of the present study is to develop a suitable bioreactor for the esterification process of PFAD using immobilised RBL as biocatalyst. Since the immobilised RBL is mechanically fragile, packed bed reactor, which has lower hydrodynamic shear stress is preferred. The PBR was designed and fabricated based upon on preliminary data obtained from the methodology scouting performed in shaked flask, including the optimum ratios of substrate to immobilised RBL, and water removal agent and other optimised process parameters (reaction time, reaction temperature and type of water removal agent). The performance of the fabricated PBR was evaluated and compared with the shaked flask process.

2.0 MATERIALS AND METHODS

2.1 Materials

Materials such as glycerol, monobasic natrium phosphate, dibasic natrium phosphate, silica gel and iodine were supplied by Sigma-Aldrich Inc. (USA). The silica gels with molecular size of 230-400 mesh were used to remove the water produced from the esterification reaction. The removal of reaction water is to prevent the hydrolysis of esterification product (acylglycerols) (Figure 1). Technical grade of n-hexane was obtained from Kofa Chemical Co. (Malaysia). The palm oil fatty acid distillate (PFAD) was obtained from a local edible oil refinery, Golden Jomalina Food Industries Sdn. Bhd (Malaysia). The content of free fatty acid (FFA) of palm oil distillate (PFAD) was about 80% according to

alkaline titration method [15]. The rice bran was obtained from a local rice mill, Bernas Tanjung Karang (Malaysia). The rice bran lipase was immobilised as previously described in Chong *et al.* [9]. The characteristics of the immobilised rice bran were analysed and tabulated in Table 1.

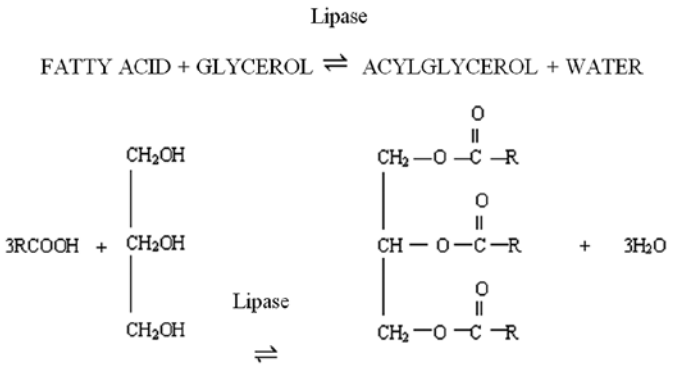

Figure 1: The reaction mechanism for the lipase catalysed esterification of fatty acid with glycerol

Table 1: Characteristics of the immobilised rice bran lipase

Characteristics of the immobilised rice bran lipase, obtained from the shaked flask experiments:

i. Sieved particle size: 300-1000μm
ii. Wet bulk density (in oil): 0.82 g/ml
iii. True density: 0.5 g/ml
iv. Heat capacity: 0.94 kJ/kg.ºC
v. Stable at temperatures from 45 to 65ºC
vi. Stable in hexane
vii. Mechanical stability in oil is fine and not deformed during operation

3.0 ESTERIFICATION OF PFAD CATALYSED BY IMMOBILISED RBL

The optimissed conditions for the esterification of PFAD catalysed by RBL were investigated in shaked flask. A typical production mixture for the esterification consists of 20 g of PFAD, 3.35 g of glycerol and 40 g of n-hexane. Various amount of immobilised RBL (2, 20, 40, 100, 200 g) was used to catalyse the esterification reaction. The reaction was performed at 65ºC and agitated at 100 rpm in a water bath shaker. The water generated from the esterification reaction was removed by the addition of silica gels (2, 4, 10, 20 g). Each reaction was carried out in triplicate. A reaction mixture without any lipases was used as control.

In the performance test of the self fabricated PBR, 10 kg of the immobilised rice bran lipase was loaded into the packed bed vessel. The reaction mixture consists of 2.0 kg of PFAD, 0.335 kg of glycerol and 4.0 kg of hexane was premixed in the feeding tank before it was sprayed over the immobilised RBL bed. In order to increase the level of esterification, the partial esterified reaction mixture was recycled back to the immobilised RBL vessel through the water removal vessel packed with 1 kg of silica gels.

4.0 ANALYTICAL METHODS

Reaction products (MAG, DAG and TAG) were identified qualitatively by thin-layer chromatography (TLC) method as previously described in Siew *et al.* [16] and Medina *et al.* [17]. TLC plates (4 cm x 14 cm) precoated with silica-gel (Sigma-Aldrich Inc., USA) were activated by heating at 105°C for 30 min. The samples were then applied onto the starting line on the bottom of the plate. The plate was heated briefly (10-15 s) on a hot plate at 90°C to evaporate the water. The samples were spotted directly on the plate with authentic standards of mono-, di- and triolein (Nu-Check-Prep, USA). The spotted plate was then developed in a solvent mixture containing chloroform: acetone: methanol (90:8:2 v/v/v). Spots of each glycerides were visualised by staining with iodine vapour.

The concentration of free fatty acid in the sample of reaction product was quantified by titration with 0.5 M NaOH. All the samples analysis was performed in triplicate. The degree of esterification was calculated based on the following equation:

$$\text{Amount of esterified free fatty acid, } \mu\text{ mol} = (V_c - V_s) \times 1000 \quad (1)$$

$$ED = \frac{\textit{Amount of esterified free fatty acid, } \mu \textit{ mol}}{\textit{Amount of free fatty acid in control reaction, } \mu \textit{ mol}} \times 100\% \quad (2)$$

where V_c = volume of NaOH used for the control, ml
V_s = volume of NaOH used for the sample, ml
M = molarity of NaOH solution
ED = degree of esterification, %

5.0 RESULTS AND DISCUSSION

5.1 Method Scouting in Shaked Flask

The shaked flask experiments were conducted to determine the optimum ratio of RBL and water removal agent to RBL for the esterification process. These optimal ratios were used for the sizing of packed bed vessel and water removal column. Figure 2 shows that 2 g of immobilised RBL was insufficient to catalyse the esterification reaction. The degree of esterification was increased as the amount of RBL was increased from 20 to 100 g. However, there was no significant improvement in degree of esterification as the amount of RBL further increased from 100 to 120 g.

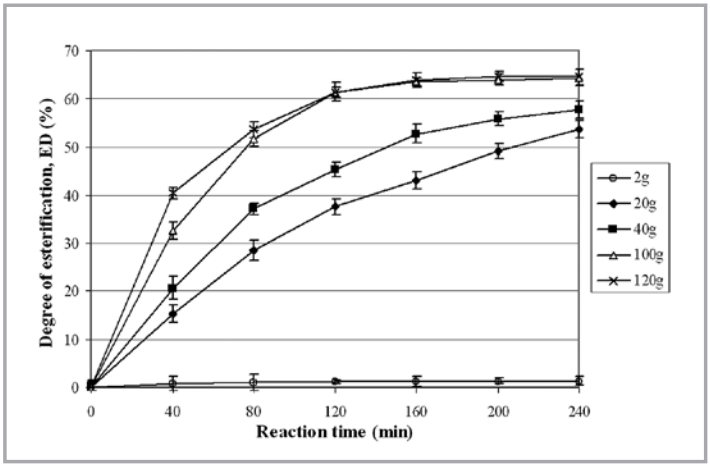

Figure 2: The effect of amount of immobilised RBL on the degree of esterification of PFAD

This result was agreed with that observed by Lo *et al.* [7] and Kaewthong *et al.* [18] in the esterification reactions using immobilised microbial lipases. Thus, 100 g of immobilised RBL was an optimal amount of lipase for the esterification process using 20 g of PFAD. This ration of RBL to PFAD has resulted a 64% degree of esterification after 4 h reaction. Thus, a ratio of immobilised RBL to PFAD of 5:1 was used for the sizing of packed bed vessel of PBR.

The effect of amount of silica gels on the degree of esterification was depicted in Figure 3. The highest degree of esterification (69.8%) was achieved in the reaction mixture containing 10 g of silica gel. Further increased of amount of silica gel to 20 g has caused a reduction in the degree of esterification. The excess amount of silica gels may strip off the essential water needed to maintain the enzyme activity of immobilised lipase [19]. Hence, a ratio of silica gel to PFAD of 1:2 was used for the design

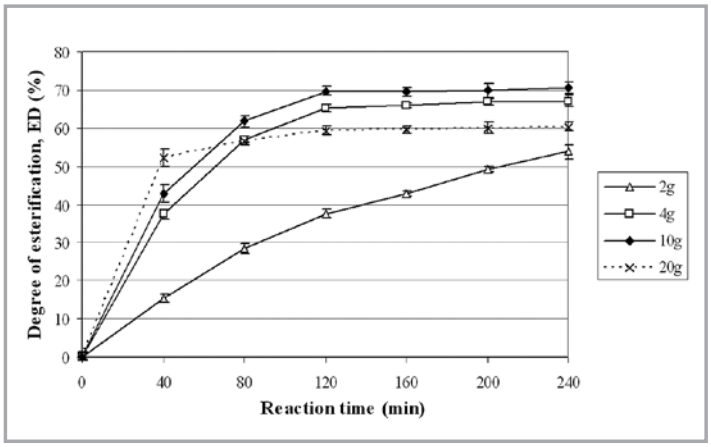

Figure 3: Effect of amount of silica gels on the degree of esterification of PFAD

of water removal column of PBR.

6.0 THE DESIGN OF PACKED BED REACTOR

The optimal ratios of PFAD to immobilised RBL and silica gel obtained from the shaked flask experiments were used to calculate the size of packed bed vessel (Table 2) and water removal column

Table 2: Summary of design criteria and dimension of packed bed vessel

Parameter	Value
Ratio of immobilised RBL to PFAD (w/w)	5:1
Amount of PFAD to be esterified (kg)	2
Amount of immobilised RBL needed (kg)	10
Density of immobilised RBL (kg/l)	0.5
Volume of immobilised RBL (l)	20
Volume of product compartment (l)	10
Volume of head space (l)	10
Total volume of packed bed vessel needed (l)	40
Ratio of tank height to tank diameter	2:1
Diameter of tank (m)	0.3
Height of tank (m)	0.6
Total volume of tank provided (l)	***42.4***

Table 3: Summary of the design criteria and dimension of water removal column

Parameter	Value
Ratio of silica gel to PFAD	1:2
Amount of PFAD to be esterified (kg)	2
Amount of silica gel required (kg)	1
Bulk density of silica gel (kg/l)	0.5
Volume of silica gel (l)	2
Ratio of tank height to tank diameter	3:1
Tank height (m)	0.3
Tank diameter (m)	0.1
Volume of provided tank (l)	2.4

(Table 3). The optimal ratio of amount of immobilised RBL to PFAD obtained is 5:1. Thus, 10 kg of immobilised RBL is needed to process 2 kg of PFAD. Since the density of immobilised RBL is 0.5 kg/l; hence the volume of packed bed required is 20 l. The packed bed vessel has a cone base, which is used as compartment to store the esterified product, while waiting to be recycled. The volume of this compartment is 10 l, similar to that of the volume of reaction mixture. Beside, a head space about the same volume of the reaction mixture is provided to prevent the overflow caused by the blockage of packed bed.

In the packed bed vessel (Figure 4), the reaction temperature was maintained by the heating element installed in the immobilised enzyme bed and water jacket around the packed bed vessel. The PBR heating elements consist of 5 pieces of SUS 316-cartridge immersion heater, 33 cm in length with 3 cm for cold zone and 15.8 mm tubing diameter. The heating system is designed to heat up 10 kg of the immobilised rice bran lipase (c_p = 0.94 kJ/kg.K) from 277 K (4ºC) to 338 K (65ºC) in 2 min. The summary of the design criteria and calculation of the heating system installed in the immobilised RBL are tabulated in Table 4. The 5 pieces of immersion heater are arranging in a '+' order to improve the rate of conduction through the solid particles of the immobilised RBL

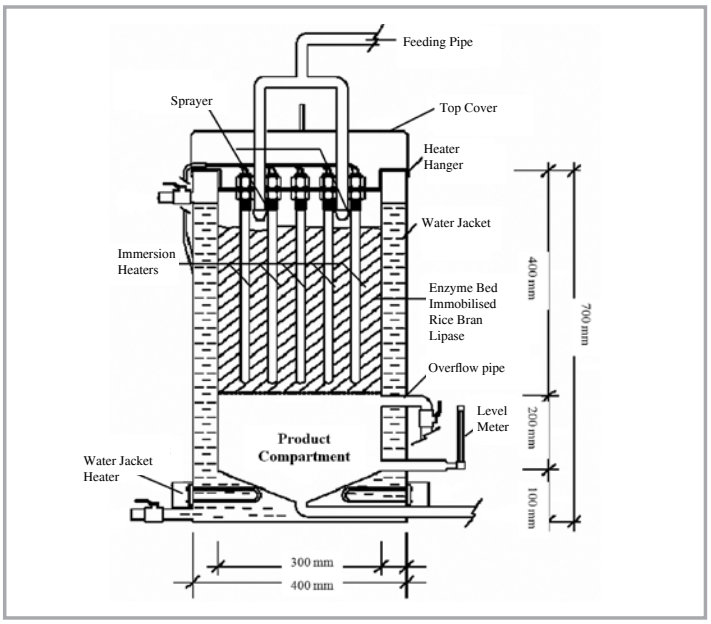

Figure 4: Detail drawing of the packed bed vessel filled with the immobilised RBL

bed. The temperature of the heater is provided with temperature control by thermostat installed. A 45 l of water is needed to fill up the water jacket of the packed bed vessel (Figure 5). The water, having a c_p = 4.187 kJ/kg.K is being preheated from 300 K (27ºC) to 338 K (65ºC) in the jacketed packed bed vessel. This water jacket is acting as a water bath to maintain the temperature of the immobilised enzyme bed by preventing the heat loss through the vessel wall. The summary of the design criteria and calculation of

Table 4: Summary of heating system in the packed bed of immobilised RBL

Parameter	Value
Amount of immobilised RBL	10
Heat capacity, c_p of RBL (kJ/kg.K)	0.94
Initial temperature of RBL (ºC)	4
Reaction temperature (ºC)	65
Heating time (min)	2
Power required (kW) *	5
No of heater provided	5
Power of each heater (kW)	1

the heater installed in the water jacket are tabulated in Table 5. The detail dimension of the packed bed vessel is presented in Figure 4.

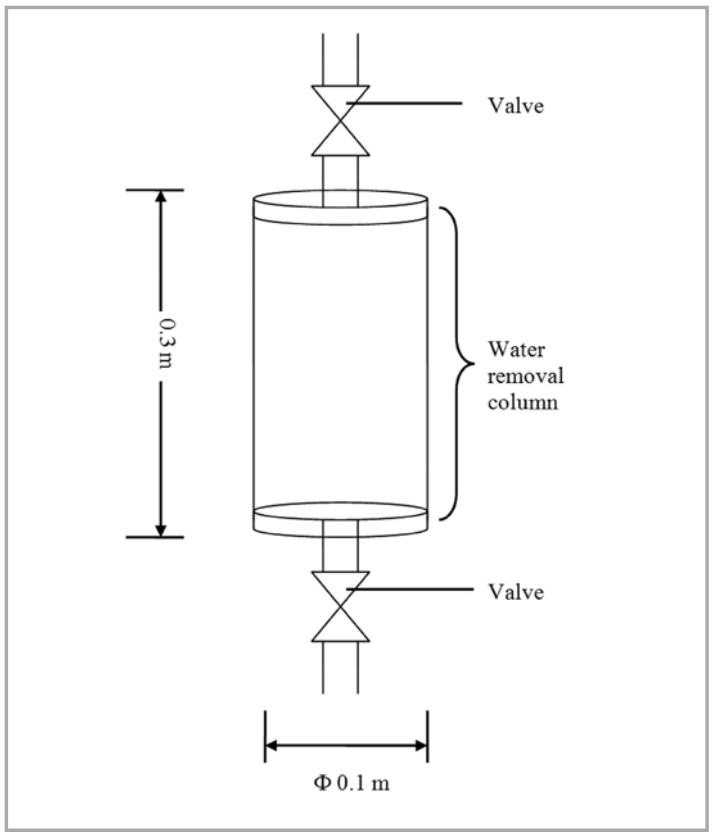

Figure 5: Detail drawing of the water removal column

$$*P = mc_p \frac{d\theta}{dt}$$

The volume of water removal column is determined by the ratio of water removal agent to PFAD. The optimal ratio of water removal agent (silica gel) to PFAD is 1:2. Therefore, 1 kg of silica gel is needed to process 2 kg of PFAD. Since the density of silica gel is 0.5 kg/l, hence the volume of water removal column needed

Table 5: Summary of heating system in the water jacket

Parameter	Value
Volume of water (l)	45
Density of water (kg/l)	1
Heat capacity of water (kJ/kg.K)	4.187
Initial temperature of water (ºC)	27
Temperature of water needed to be maintained (ºC)	65
Heating time (h)	1
Total power required (kW) *	2
No of heater provided	2
Power of each heater provided (kW)	1

is 2 l. A summary of the design criteria and the dimension of the water removal column are tabulated in Table 5. The detail drawing of the water removal column is presented in Figure 5.

$$*P = mc_p \frac{d\theta}{dt}$$

Two additional tanks with a volume of 28 l are provided for the storage of reaction mixture and final esterified product. A 3 kW heater is equipped in the feeding tank for heating up the reaction mixture to reaction temperature prior to the esterification reaction. The summary of the design criteria and calculation of the heater is tabulated in Table 6. A platform stand of 92 cm x 101 cm dimension complete with wheels is provided to support the vessel and piping. All the vessel is interconnected with 15 mm diameter stainless steel (SUS 304) piping and the fluid flow is controlled by valves installed. Two magnetic pumps with a flow rate of 20 l/min are installed to transfer the reaction mixture to the packed bed vessel and to circulate the partial esterified product through the water removal column before it

Table 6: Summary of heating system in the feed tank

Parameter	Value
Volume of reaction mixture (l)	10
Density of reaction mixture (kg/l)	0.6335
Heat capacity of reaction mixture (kJ/kg.K)	3.95
Initial temperature of reaction mixture (ºC)	29
Reaction temperature (ºC)	65
Heating time required (min)	5
Total power required (kW) *	3
No of heater provided	1
Power of each heater (kW)	3

was recycled back to the packed bed vessel. The pump was also used to pump the complete esterified product to the product storage tank. The three dimensional diagram of the developed

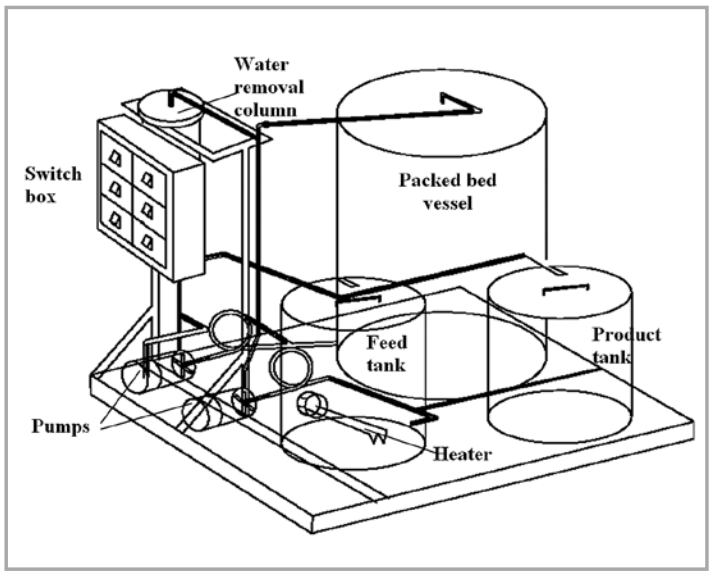

Figure 6: The three dimension view of the developed packed bed bioreactor

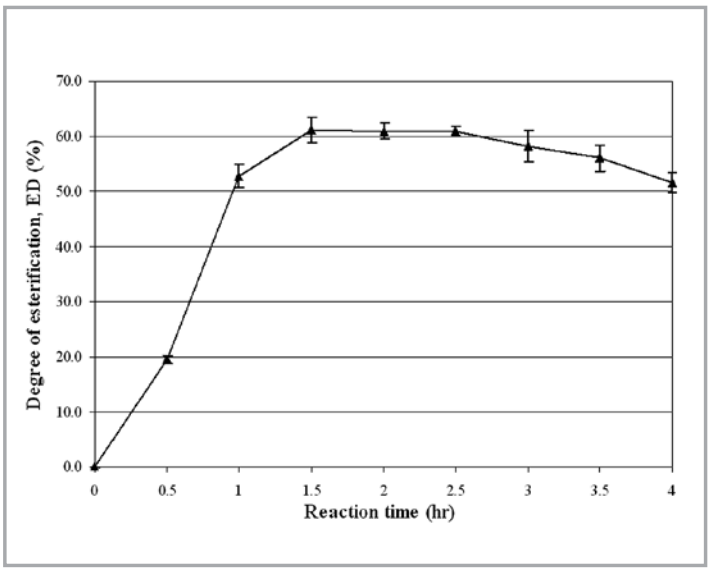

Figure 7: Esterification of PFAD and glycerol in hexane catalysed by immobilised RBL in a self fabricated PBR

PBR is showed in Figure 6.

$$*P = mc_p \frac{d\theta}{dt}$$

7.0 PERFORMANCE TEST OF PBR

The reactor was designed for down flow operation in semi-continuous mode. Stainless-steel sieve was placed at the bottom of the column to support the rice bran and to prevent the escape of bran particles into the product tank (Figure 4). The reaction mixture (PFAD and glycerol) in hexane was first pumped from feed tank through the top of packed bed vessel and was sprayed homogenously over the bed of immobilised RBL. The esterified product (acylglycerols) was trickling through the enzyme bed and collected at the product compartment, situated at the bottom of the packed bed vessel. The partial esterified product collected at the product compartment was pumped through the water removal column and recycled back to the immobilised RBL bed. Sampling was performed every 30 min to estimate the degree of esterification, by drawing samples from the product compartment. When the process was completed, the esterified product was transferred to the product tank.

An esterification process of PFAD and glycerol catalysed by the immobilised RBL for the production of acylglycerols was performed to test the performance of the newly fabricated PBR. The packed bed vessel was first filled with 10 kg of immobilised RBL. The immobilised RBL was then sprayed with 10 L of reaction mixture consist of PFAD, glycerol and hexane. The partial esterified reaction mixture was recycled back to the immobilised RBL vessel through the water removal vessel packed with 2 kg of silica gels until a maximum degree of esterification was achieved. Figure 7 shows that the degree of esterification increased steadily with the reaction time and achieved maximum degree of esterification after 1.5 h of reaction, which was 25% faster than that in the shaked flask. The maximum degree of esterification achieved in this developed PBR was 61%, a value comparable to that reported by Wanatabe *et al.* [10]. Watanabe *et al.* [10] reported a 69% yield after a 2 h reaction in a PBR using reduced pressure to remove the reaction water. The TLC chromatogram showed that the fatty acids were successfully esterified into acylglycerols (majority as DAG) catalysed by immobilised RBL in this self fabricated PBR (Figure 8).

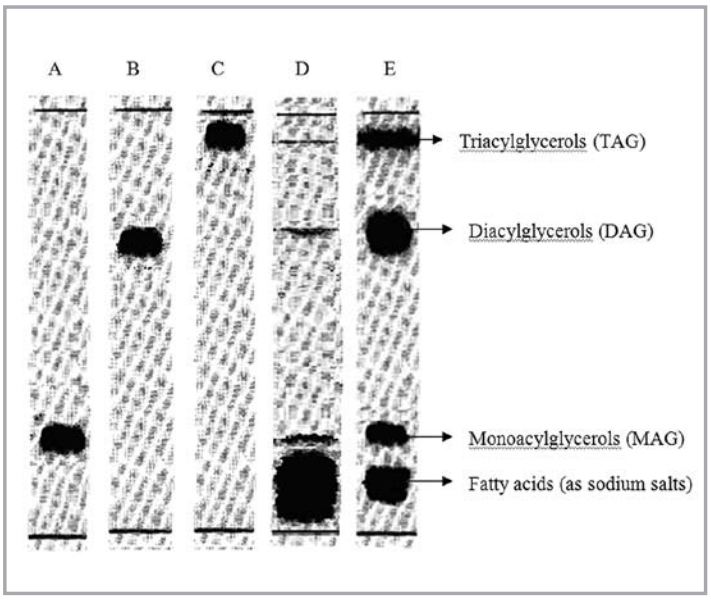

Figure 8: Thin layer chromatograms. Lane A, Lane B, Lane C: Standards of mono-, di- and triolein. Lane D: Reaction mixture before esterification. Lane E: Esterification product from the PBR

8.0 CONCLUSION

The reaction time needed to achieve maximum degree of esterification in PBR was 25% shorter than that in the shaked flask. Nevertheless, the maximum degree of esterification achieved in PBR (61%) was comparable to that in shaked flask (69.8%). The primary advantage of the PBR system compared to the batch system is that a large amount of substrate could be processed in a shorter reaction time. The performance of the PBR was comparable to that reported in literature [10].

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