Semi-continuous Production of 2-Ethyl Hexyl Ester in a Packed Bed Reactor: Optimization and Economic Evaluation

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Abstract: The aim of this work was to investigate the technical as well as the economic feasibility of producing 2-ethyl hexyl oleate (2-EHO), a non-phthalate plasticizer in a solvent free medium. The esterification reaction between oleic acid and 2-ethyl hexyl alcohol was carried out in a packed bed reactor (PBR) using Candida antarctica lipase B (Novozym 435; Novozymes; Copenhagen-Denmark) as biocatalyst. RSM was employed to optimize the esterification reaction conditions. The optimum reaction conditions were found to be flow rate of 1.5 mL/min, No. of cycles of 12 and molar ratio of 4:1 2-ethyl hexanol to oleic acid. The maximum experimental and predicated conversions were found to be 95.8% and 95.61% respectively. Formation of 2-EHO was approved by FTIR, $^1$HNMR and $^1$CNMR. From the economic prospective, PBR was capable of producing 2-EHO with a purity of more than 94% over 480 h without remarkable reduction of enzyme activity. This revealed an economic production of 2-EHO at a yield of 2 tons kg$^{-1}$ lipase. The manufacturing cost was found to be $1.88 /kg 2-EHO, this contributed to a profit of about 30% compared to the commercial price of 2-EHO. Such results approve the technical and economic feasibility for this sustainable method in esters production.

Key words: esterification, oleic acid, Novozym 435, packed bed reactor, manufacturing cost

1 Introduction

Plasticizers are used in polymer processing to increase the flexibility and physical standards of polymers; they represent about 80% of polymer additives market share$^1$. Most of the plasticizers present as phthalate esters which are petroleum-based products such as diocyl phthalate (DOP) and di-isodecylphthalate (DIDP)$^2$. Due to the instability of phthalate esters in the polymer chain and the migration feature from polymer matrix, they are recognized as toxic compounds and could have a negative effect on human reproduction system$^3,4$. As a result of these impacts and risks, the European Union (EU) considered the DOP as a reprotoxic compound and obligated to find a safer alternative, particularly for those used in medical apparatus and tools$^5$. Additional action was raised from France, which developed a law that prohibited the usage of DOP in medical equipment’s in all hospitals$^6$. In consequence, suggesting environmental harmless and valid alternative to phthalate esters is strongly recommended. Thus, the synthesis of bio-compatible plasticizers is getting a considerable attention in recent decades since they are produced from green resources, biodegradable and safer$^7,8$. Fatty esters such as 2-ethyl hexyl oleate and epoxidised fatty esters are among the most compatible plasticizers with Polyvinyl Chloride (PVC), this is due to their high influences in improving the tensile strength, elongation and modulus$^9,10$. Also, it is common to use epoxidised soybean, epoxidised sunflower oil and epoxidised linseed oil as non-DOP plasticizer, which are from green resources$^7$. Currently natural non-DOP plasticizers are produced industrially using the chemical catalyzed technology which is not considered as green technology. This process occurs at elevated temperature, typically between 160°C and 220°C. When these extreme acidic conditions are employed, some undesirable by-products are formed resulting in colored outputs; leading to further separation and purification steps$^{11,12}$. Therefore, various researches’ efforts focused on suggesting cleaner catalytic processes to avoid such dis-
advantages. The usage of lipases to catalyze various processes such as esterification, transesterification, alcoholysis, and hydrolysis has become of interest. The main goal of using lipases as biocatalyst is established on their selectivity and biodegradability. There are many other advantages behind utilizing lipases in producing bio-based products; the lipase catalyzed reactions are carried out under milder temperatures which saves energy, improves the quality of the end-products, and offers an environmentally friendly production process. There are many published papers in the area of lipase catalyzed reactions, all papers agree about the technical feasibility of using lipases catalyzed process instead of chemicals catalyzed process. However, as far as the authors’ knowledge there are no published papers in the literature propose a complete economic study for lipase catalyzed esterification reactions. Mustafa et al. reported that the high cost of commercial available enzymes is the main obstacle along the path to their commercialization, which in turn hinders the industry to invest in this green process and push the investments toward the conventional chemical process instead. Therefore, integrated researches that propose producing improved green products based on enzymatic method with proven economic feasibility is needed. In the present work, a semi-continuous enzymatic process was proposed to produce 2-EHO in a packed-bed reactor. Novozym 435 was used to biocatalyze the esterification reaction of oleic acid and 2-ethyl hexanol in a solvent free system. The proposed process was assessed technically by optimizing the conditions using RSM and economically by conducting a detailed economic assessment. To further prove the feasibility of proposed process in large scale, a plant design based on complete process flow diagram was proposed.

2 Materials and Methods

2.1 Materials

Novozym 435, Candida antarctica lipase B, in the form of dried powder (10,000 U/g; propylarate units PLU), physically immobilised by a macroporous support (Lewatit VPOC Fig. 1 Experimental setup of semi-continuous operation using biocatalyst.
2.2 Experimental Set-up

Lipase-catalyzed esterification of 2-ethyl hexyl alcohol and oleic acid was carried out in a packed bed reactor system (PBR). Figure 1 shows an experimental setup figure for the utilized PBR. The reactor composed of two water jacketed glass columns with a length of 300 mm and an inner diameter of 12 mm. Immobilized enzymes, pieces of loofa sponge and molecular sieves were packed and fixed inside the columns, whereas, 30% of reactor volume was left free. Two glass sieves of 50 meshes were fixed in the bottom of reactors to hold the enzymes and molecular sieves. Fatty acid and alcohol were mixed in a solvent free system and oleic acid was carried out in a packed bed reactor (PBR). Temperature of PBR and reactants receiver were kept at 300 rpm. Before starting the esterification reaction, the temperature of PBR and reactants receiver were kept at 60 °C using water bath circulator. Afterwards, the reaction mixture was fed upward to the reactor by using peristaltic pump. All conversions results were analysed in triplicate and the conversion values determined as the mean value of the three measurements.

To calculate the reaction progress, the percentage of fatty acids conversion to ester was carried out by a titration method, AOCS Official Method Ca5a-40. Periodically, 200 µL of aliquots were withdrawn and dissolved in 10 mL of ethanol. Then, titration with NaOH (0.1 M) to the endpoint using phenolphthalein as an indicator was performed. Control experiments were performed without addition of enzyme. The reaction progress was indicated by conversion percentage calculated by Equation 1\textsuperscript{(1)}:

\[
\text{Conversion of ester (\%)} = \frac{N - N'}{N} \times 100
\]  

Where:

N: NaOH volume depleted before the reaction, \(N'\): NaOH volume depleted after the reaction

2.3 Product characterization

The synthesized 2-EHO was characterized by functional group analysis using FTIR and \(^1\)H NMR spectroscopy. FTIR spectra of synthesized 2-EHO were carried out on a Nicolet IS10(Nicolet Instrument Corp., USA) Fourier transform infrared spectrophotometer. The product was located between two plates of KBr. The spectra were obtained in the range of 4000 to 500 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). \(^1\)H NMR spectra of 2-EHO obtained were recorded on an AV-300 NMR spectrometer (Bruker, Germany) at a frequency 300 MHz with tetramethylsilane as an internal standard and CDCl\(_3\) as solvent.

2.4 Experimental design

To optimize the reaction conditions for 2-EHO production, RSM was used to study the interactive effects of operating variables on the conversion. Full factorial central composite design (CCD) consisting of 4 axial points, 8 factorial points and 6 centre points was performed in this study. Three independent variables were selected: \(X_1\), alcohol/acid molar ratio; \(X_2\), No. of cycles; and \(X_3\), flow rate mL min\(^{-1}\), while the response Y is the fatty acid conversion percentage into esters. In order to calculate standard deviation and pure error of experiments, centre points were repeated for six times. Table 1 shows the coded variables and the corresponding value for each coded level.

2.5 Statistical analysis

Based on the data of the responses obtained after doing the set of the experimental runs, a regression equation that links between the obtained responses and variables was generated. The values of F-ratio test and coefficient of determination were used to examine the proposed regression equation validity. The data obtained were fitted to a second-order polynomial equation (Equation 2):

\[
Y = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=1}^{3} \sum_{j=i+1}^{3} b_{ij} x_i x_j
\]  

The term Y represents conversion percentage, \(b_0, b_i, b_{ij}\) are constant coefficients and \(x_i, x_j\) are the uncoded independent variables. Design Expert Software (Version 10) from Stat-Ease Inc. (USA) was used to perform the response surfaces, analysis of variance and regression analysis. The optimum conditions that give the maximum response were obtained by solving equations 2 and 3.

2.6 Economic Assessment

2.6.1 Manufacturing cost

The economic study aimed to calculate the economic viability of the proposed process for producing 2-EHO. The

| Code | X1 | X2 | X3 |
|------|----|----|----|
| +1   | 4  | 12 | 2.5 |
| 0    | 2.885 | 9 | 2  |
| −1   | 1.77 | 6  | 1.5 |
| +α   | 4.7602 | 14.045 | 2.8409 |
| −α   | 1.0098 | 3.9546 | 1.159 |
economic viability could be judged by calculating the manufacturing cost of 2-EHO. The manufacturing cost was estimated after conducting a process mass balance. The cost of raw materials, energy and other indirect costs were taken into consideration to calculate the total manufacturing cost. Official prices of raw materials including oleic acid and 2-ethyl alcohol and 2-EHO were provided after contacting Malaysian and Chinese suppliers. Novozym 435 price was given from Novozymes, Denmark. Energy consumptions and cost were calculated based on Mustafa et al.16. To calculate the consumption of Novozym 435 for producing a unit mass of product, an operational stability based on semi-continuous production of 2-EHO was performed.

2.6.2 Operational stability of Novozym 435

The operational stability study was performed by leaving the PBR works in semi-continuous process for producing 2-EHO over 480 h. The reaction was carried out in a packed bed reactor operated under the optimum conditions and at a temperature of 60°C. Enzymes with loofa were loaded inside the reactor’s bed and 30% of reactor volume was left free. At the end of every batch (12 hours), the enzyme and molecular sieves columns were washed with 2-ethyl hexanol effectively to remove any residues of substrates or product. Then, another substrates mixture was charged to the clean reactor for the next batch in the next day.

3 Results and Discussion

3.1 Analysis of Variance

Table 2 shows the predicted and experimental values obtained for 2-EHO production utilizing Novozym 435. The results showed normal distribution for errors and good correlation between experimental and predicted conversions resulted from low obtained residuals’ values of not more than ±5%. Table 3 shows the model F value for the proposed model. A value of 48.73 indicated that the proposed model is significant and only a chance of 0.01% that the F value was affected by noise. The high value of 0.9777 for the coefficient of determination, $R^2$ indicates a confident statistical correspondence between the chosen variables and the response at 97.77% level. This means that a low percentage of 2.23% of the total model variations is not explained. Consequently, the responses values obtained from the reaction between 2-ethyl hexanol and oleic acid may be slightly affected by other associated variables.

3.2 Regression Analysis

Model equations were developed based on the regression analysis of datasets. Also a set of coefficients were generated. In order to evaluate the signal to noise ratio for the proposed model, the adequate precision coefficient was measured. A value of more than 4 is appropriate for a desirable model. In this study an obtained value of 24 indicates that the proposed model is appropriate and it could be used to navigate the design space. Also a low coefficient of variation value of 6.89% reveals precise and reliable performed runs.
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Table 3 shows the Prob>F values for the independent variables, when values are less than 0.05 indicate that this model variable is significant. In the proposed model, the "Prob>F" for variable A (Alcohol to oleic acid molar ratio) and variable B (cycle pass) are less than 0.0001, followed by 0.0002 for variable C (flow rate). Accordingly, the molar ratio of alcohol to oleic acid and No. of cycle pass values are the most noteworthy factors inflicting the conversion percentage. Based on the aforementioned values, an empirical equation was obtained as shown in equation 3. As shown, the value of response could be estimated by substituting the values of variables between coded levels +1 (high level) and −1 (low level) at any point:

\[ Y = 91.94 + 7.69A + 7.74B - 3.72C - 2.07AB + 1.46AC + 3.01BC - 4.24A^2 - 4.34B^2 - 0.34C^2 \]  

(3)

Where Y is the response, and A, B and C are alcohol to oleic acid molar ratio, cycle pass and flow rate, respectively. Equation 3 was used to facilitate the response surfaces plot.

3.3 plots of Response surface

3.3.1 Interactive effect of flow rate and molar ratio on 2-EHO synthesis

Figure 2a shows the interactive relation between flow rates and alcohol to oleic acid molar ratios variations on 2-EHO production. The interactive plot was produced based on a cycle pass of 9 cycles, which is the middle cycle pass value in the investigated range. As shown in the figure, high conversions are obtained at high molar ratios and at high or low flow rates. Also lower conversions are obtained at low molar ratio and at any value of flow rates.

This indicates that molar ratio is more significant than flow rate in terms of effecting the conversion. For example the conversion value obtained at 4:1 alcohol to oleic acid molar ratio and 2.5 mL/min flow rate is close to that obtained at the same molar ratio and 1.5 mL/min flow rate. Such results could be due to the fact that at high flow rate, the negative impact of the low contact time between the substrate mixture and enzyme bed was offset by high 2-ethyl hexanol to oleic acid molar ratio that facilitate the product synthesis because of decreased viscosities. On the other hand, conditions of low 2-ethyl hexanol to oleic acid molar ratio and high flow rate appear not to be favourable conditions for 2-EHO synthesis, even with increased contact time of the substrate mixture with the enzyme bed via ap-

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plying low flow rates. This is due to the mass transfer limitations inside the reaction medium, which occur due to the high viscosity of the oleic acid phase that resist the diffusion of alcohol and fatty acid molecules to reach the enzyme active sites and complete the reaction. To overcome this problem higher alcohol quantities should be used which can reduce the medium viscosity and facilitate the transport and collision of molecules inside the reaction medium.

3.3.2 Interactive effect of cycle pass and flow rate on 2-EHO synthesis

Figure 2b shows the interactive response surface between cycles pass and flow rate on 2-EHO production using CALB. The interactive plot was produced based on a molar ratio of 2.885:1 alcohol to fatty acid. As can be obviously seen from the figure, conversion values are increasing with increasing cycle pass at any flow rate in the tested range. A maximum (in the range investigated) cycle pass of 12 cycles with the lowest flow rate of 1.5 mL/min seem to be favourable conditions to achieve high product yield. The results shown on Fig. 2b revealed that the cycle pass is more significant variable than the flow rate on ester yield. The more cycle pass for the substrate mixture over the enzyme bed will allow for more contact time between the mixture and biocatalyst resulting in high conversions. Varying the value of flow rate at fixed No. of cycles seems to have no significant effect on conversion.

3.3.3 Interactive effect of cycle pass and molar ratio on 2-EHO synthesis

Figure 2c shows the interactive response surface between cycle pass and alcohol to fatty acid molar ratio. The interactive plot was produced based on a fixed value of flow rate. It is obvious from the figure that an increase in the conversion was obtained with increasing cycle pass and molar ratio. A high conversion of more than 94% was obtained at high molar ratio of 4 and high cycle pass of 12. On the other hand, drastic decrease in the conversion of less than 50% was observed with low molar ration of 1 and low cycle pass of 6. Not surprisingly, at low molar ratio the viscosity of reaction mixture increases which hinders the ability of lipases to catalyze the esterification reaction between oleic and 2-ethyl hexanol. Moreover at low No. of cycles of 6, the reaction time was not satisfactory leading to incomplete esterification reaction. This would further emphasize that molar ratio and cycle pass are significant variables with a "Prob>F" values of less than 0.0001.

As concussion, the optimum conditions obtained from RSM study were 12 cycle passes, 4:1 2-ethyl hexanol to oleic acid molar ratio and 1.5 mL/min flow rate. These conditions resulted in the highest conversion of 95.8%. Waskitoaji et al. has produced the same product proposed herein but with using conventional chemical catalysed process at 160°C. The oleic acid conversion into 2-ethyl hexyl oleate was 94.86% at 1% w/w sulfuric acid as a catalyst and at 1:1 alcohol to fatty acid molar ratio. Whereas, the high viscosity of the reaction mixture in such molar ratio could be offset by the elevated reaction temperature of 160°C.

3.4 Product (2-EHO) Characterization

The infrared spectral analysis was performed as shown in Fig. 3. It showed a strong band at 1738 cm⁻¹ corresponds to the carbonyl group (C=O) of ester. This confirms the ester formation since the C=O of the carboxylic acid (COOH) appears at 1710 cm⁻¹. As shown in Fig. 4, the 1H NMR spectral data showed a triplet signal at 2.28 ppm corresponds to CH₂C=O protons (blue) of the ester group. Most importantly, C=OOCH₃ protons (yellow) appeared at 3.96 ppm. Also, the ethylene protons (CH=CH) (red) appeared at 5.33 ppm. These chemical shifts confirmed the formation of ester form the corresponding oleic acid. Both
FTIR and $^1$HNMR results come into agreement with Waskitoaji et al.\textsuperscript{10}. Also $^{13}$CNMR spectrum confirmed the esterification process as shown in Fig. 5. The carbonyl group signal appeared at 174 ppm. At 130.16 ppm another signal appeared which is corresponding to the CH=CH group. Another signal assured the ester formation (66.62 ppm) corresponds to C=OOCH.

4 Economic Assessment

4.1 Manufacturing Cost

Manufacturing cost was calculated based on the quantities of raw materials and utilities needed for producing one ton of 2-EHO. Quantities of raw materials was calculated after performing mass balance based on the esterification reaction of oleic acid and 2-ethyl hexanol to produce 2-EHO and water as shown in Table 4. Novozym 435 consumption was calculated based on the operational stability study conducted in this work, where every 1 kg of enzyme produces 2 tons of 2-EHO. The up-to-date free of board (FOB) prices of raw materials were obtained from official bulk suppliers. The prices were $1.1/kg, $1.25/kg, $2.69/kg and $700/kg for oleic acid, 2-ethyl hexanol, 2-EHO and Novozym 435, respectively. The energy consumption and electric requirement as well as their costs were calculated based on\textsuperscript{16}. The indirect costs were assumed to be 9%, 3% and 3% of manufacturing cost for depreciation, interest and tax and repair, respectively. As shown in Table 4 the total manufacturing cost for producing one ton of 2-EHO by the proposed enzymatic method was $1878.5/ton. As per the commercial price of 2-EHO is $2690/ton, therefore the profit can be calculated as about 30%, which is a reliable profit. The proposed technical and economical investigations prove the validity of this green method in practical application. The proposed semi continuous enzymatic
process is an improvement for our previous research published before\textsuperscript{16}. Mustafa et al. have performed an ester production using enzymatic process in a batch stirred reactor\textsuperscript{16}. The authors reported that 52\% of manufacturing cost was due to lipase cost. While, in this research cost of enzymes contributed for a parentage of only 18.6\% of manufacturing cost. The lower enzyme cost obtained in this research reveals the reliability of fixed bed reactor instead of batch stirred reactor in esters production. Unlike packed bed reactor, in the stirred tank reactor, the effect of shear stresses resulting from the mechanical agitator causes damage to the enzymes immobilization system thus inhibiting the activity of enzymes.

It should be mentioned that there are no previous investigations in the literature similar to what proposed here related to 2-EHO production economics or even for esters, except our previous work\textsuperscript{16}. Therefore, up to the authors best of knowledge this is the first paper addresses both the technical and economic feasibility of 2-EHO. However there are many previous economic studies that calculated the manufacturing cost of biodiesel using Novozym 435 as biocatalyst. Jegannathan et al. obtained a manufacturing cost of 10.84 $/L.

Table 4 Total Manufacturing cost of 2-EHO.

| Raw Materials                   | Price ($) | Quantity (kg) for producing ton of product | Total Price/ton product |
|---------------------------------|-----------|--------------------------------------------|------------------------|
| Oleic Acid                      | 1.1/kg    | 715.23                                     | 786.77                 |
| 2-Ethyl hexanol                 | 1.25/kg   | 330.44                                     | 413                    |
| Novozym 435                     | 700/kg    | 0.5                                        | 350                    |
| Energy                          |           |                                            |                        |
| Steam                           | 0.0227/MJ | 68.5 MJ/ton                                | 1.56                   |
| Electric power                  | 0.136/kWh | 5 kWh/ton                                  | 0.68                   |
| Heat Losses from insulation     | 0.0227/MJ | 9 MJ/ton                                   | 0.2                    |
| Total                           |           |                                            | 1,552                  |
| Depreciation                    | 9\%       |                                            | 140                    |
| Repair                          | 3\%       |                                            | 46.5                   |
| Interest and tax                | 3\%       |                                            | 140                    |
| Total                           |           |                                            | 1878.5 $/ton           |

Fig. 5 $^{13}$C NMR spectrum of 2-EHO.
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4.2 Operation Stability of Novozym 435

Commercial enzymes especially Novozym 435 is expensive, this fact hinders the replacement of the conventional chemical catalysed method with the enzymatic method until now. To have a profitable process the total manufacturing cost must be less than the commercial price of the product by a reasonable percentage. In this research the operational stability of Novozym 435 was studied in order to have an economic wise process. A fixed amount of enzymes was placed in the reactor and reused several times for 480 h without significant enzyme deactivation. Novozym 435 showed high operational stability by maintaining more than 90% of its original activity (with product yield > 94%) during the 480 h of operation. The activity then dropped to 80% by passing 600 h of operation. The activity dropped further to 75% by passing 720 h of operation. After that the run was stopped. From the proposed investigation, it could be concluded that the 1 kg of Novozym 435 can produce up to 2 tons of 2-EHO.

Xu et al. proposed two stage system to produce biodiesel using Novozym 435 in a PBR\(^{24}\). The productivity obtained was 1.56 kg kg\(^{-1}\) enzyme which is a comparable productivity compared to our result but for biodiesel synthesis. Chen et al. obtained 80% oil conversion to biodiesel during an operation over 30 days\(^{25}\). Watanabe et al. obtained a conversion of 90% after using Novozym 435 for 100 days in a PBR for biodiesel production\(^{26}\).

However all these previous works have not estimated the profit margins. In other words, utilizing Novozym 435 for many days does not guarantee that the process is profitable. In the proposed work a guaranteed profit of 30% was calculated based on 2-EHO production in a PBR using Novozym 435 in a solvent free process.

5 Process Description

To further develop the proposed enzymatic method, a plant design was proposed for producing 2-ethyl hexyl oleate (2-EHO). Figure 6 shows a suggested semi continuous process for 2-EHO production through reacting oleic acid with 2-ethyl hexanol in a solvent free medium. The proposed plant is divided into two sections namely reaction section and downstream section. In the reaction section: fatty acids and alcohol are fed to the reactants mixing re-
receiver where molar ratio is adjusted and heating to the reaction temperature is performed. After arriving the desired temperature, homogeneous mixture of fatty acid and alcohol are pumped to the main esterification vertical packed bed reactor (PBR) followed by molecular sieves (MS) column. The mixture is recycled through the reactor and column several cycles until achieving the desired conversion. In the esterification PBR, enzymes are fixed inside the bed and the reactants are being passed through the enzymes which catalyze the esterification reaction and produce 2-EHO and water, then mixture is allowed to pass through molecular sieves column to remove water by product resulted from the esterification reaction. The MS step is essential to protect enzymes from inhibition during the cycle. The three equipment PBR, MS column and mixing tank are working in a closed loop until desired conversion is achieved, conversion is being checked through sampling point attached with the recycle pipeline. After reaction completion, mixture of 2-EHO, unreacted fatty acid and excess alcohol are fed to a product receiving tank then to the downstream processes section.

In the downstream section: product mixture is fed to a single effect evaporator that works under vacuum where excess alcohol is removed. The vaporized alcohol is condensed and sent again to the reactants mixing receiver. The resulting mixture of 2-EHO with free fatty acids is sent to neutralization receiver where sodium hydroxide solution is added to neutralize free fatty acids forming sodium soap of fatty acid. The semi purified product is sent to centrifuge to separate soap from 2-EHO.

6 Conclusion

PBR, packed with sponge loofa pieces and Novozym 435 was successfully developed to produce 2-EHO in a solvent free system. RSM was used to optimize the esterification reaction conditions. The optimum conditions were found to be flow rate of 2.5 mL/min, No. of cycles of 12 and molar ratio of 4:1 alcohol to fatty acid. A product purity of more than 95% was obtained at the optimum conditions. The operational stability of the Novozym 435 was studied by operating the PBR for 480 h. This revealed a feasible production of 2-ethyl oleate with a productivity equivalent to 2 tons per 1 kg of Novozym 435. A detailed economic assessment based on manufacturing cost estimation revealed that a cost of $1.88 is needed to produce one kilogram of 2-EHO. Considering the commercial price of 2-EHO costs $2.69/kg, so this yields a 30% profit. Despite the environmental and economic advantages of the proposed enzymatic process, the cost of commercial enzyme amounted for about 18.6% of manufacturing cost, which is relatively high compared to the chemical catalysts. One way to reduce the cost of enzyme is to locally produce active immobilized microbial enzymes by using economic substrates. Also industry can immobilize the commercial free diffusing enzymes locally which would tremendously reduce the cost compared to the commercial immobilized.

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