Food Research 7 (6) : 306 - 313 (December 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Mass transfer studies on the solvent-free lipase-catalyzed partial hydrolysis of palm oil in packed-bed reactor

[1,*]Phuah, E.T., [2]Lai, O.M., [3]Tang, T.K., [4,5]Lee, Y.Y., [6]Choong T.S.Y., [7]Tan, C.P. and [1]Hong, S.P.

[1]*Department of Food Science and Technology, School of Applied Sciences and Mathematics, Universiti Teknologi Brunei, BE1410 Bandar Seri Begawan, Brunei Darussalam*
[2]*Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[3]*Malaysian Palm Oil Board, 43000 Kajang, Selangor, Malaysia*
[4]*School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia*
[5]*Monash Industry Palm Oil Research Platform, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor, Malaysia*
[6]*Department of Chemical Engineering, Faculty of Engineering, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[7]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

Article history:
Received: 16 December 2021
Received in revised form: 26 January 2022
Accepted: 22 June 2022
Available Online: 29 December 2023

Keywords:
Mass transfer model,
Packed bed reactor,
Partial hydrolysis,
Lipase,
Palm oil

DOI:
https://doi.org/10.26656/fr.2017.7(6).1005

Abstract

Packed-bed reactor (PBR) is widely employed for enzyme-mediated reactions at an industrial scale because the reactor configuration enables reusability of the biocatalyst with ease, thereby reducing the overall operating expenses. However, mass transfer limitation remains a key challenge in fixed-bed column systems, especially at large scale. Therefore, the present study investigated the external mass transfer effects on solvent-free lipase-catalyzed partial hydrolysis reaction and aimed to develop a mass transfer model for the operation. The influence of volumetric flow rate, one of the crucial operating parameters affecting the external mass transfer resistance, was examined. Results demonstrated increasing the flow rate led to an increase in hydrolysis reaction with the highest reaction rate observed at 5 ml min^{-1}. A dimensionless mathematical mass transfer model of Colburn factor, $J_D = K(Re)^{n-1}$ which is a function of Reynolds and Schmidt numbers, was then proposed to simulate the enzymatic partial hydrolysis reaction of palm oil in PBR. The mass transfer model was analyzed with different n values and results revealed that the mass transfer correlation of $J_D=0.92(Re)^{-0.2}$ was able to predict the experimental data accurately. The model developed could describe the dominance of mass transfer effects besides providing useful information for the scale-up design of industrial reactor.

1. Introduction

The epidemic of obesity has reached an alarming stage with more than 1.9 billion adults being overweight. The current figure is projected to continue on an upward trend for the next couple of decades as revealed by the World Health Organization (WHO, 2021). Robust evidence indicates a significant positive correlation between obesity and chronic illnesses such as heart disease, cancer, diabetes mellitus, hypertension and physical discomfort which reduce the quality of life (Louie *et al.*, 2013; Schetz *et al.*, 2019). Excessive consumption of dietary fat is suggested to be the main culprit that leads to the prevalence of obesity. Consequently, considerable strategies have been proposed to alleviate the unresolved global issue in which the inclusion of fat substitutes or fat mimetics into food products has been given much emphasis. Despite their low caloric value, fat mimetics fail to replicate similar sensory properties attributed to conventional edible oil whereas fat substitutes may lead to fatty stools coupled with poor absorption of fat-soluble vitamins (O'Sullivan, 2016). The recent discovery of diacylglycerol (DAG)-enriched oil is gaining attention as an attractive functional oil due to its scientifically proven ability to reduce the accumulation of body fat thereby potentially inhibiting the onset of obesity (Flickinger and Matsuo, 2003).

*Corresponding author.
Email: *engtong.phuah@utb.edu.bn*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

Chemical glycerolysis is the most common industrial practice used to manufacture partial acylglycerols containing both DAG and monoacylglycerol (MAG). However, the chemical method is an energy-intensive process and a large amount of undesired by-products are generated during the reaction, not to mention relatively low DAG yield (Arpi *et al.*, 2016). On the other hand, enzymatic approach is shown to be a better option owing to its milder operating conditions, high specificity as well as production yield (Phuah *et al.*, 2015; Lee *et al.*, 2020). The disclosure of lipase-catalyzed partial hydrolysis of triacylglycerol (TAG) has been an attractive processing method as the process involves substantial amounts and inexpensive main feedstocks namely edible oil and water (Lai *et al.*, 2007). Nevertheless, the high cost of commercial lipases remains a major hindrance to the widespread application of lipase-mediated reactions on an industrial scale. In addition, the solid enzyme granules are susceptible to mechanical inactivation in a stirred tank reactor system, prohibiting the reusability of the enzyme particles which translates into up-surging in production costs. Therefore, the development of efficient processes and effective reactor systems will become the preliminary requirements prior to the commercialization of the products.

Packed bed reactor (PBR) is undoubtedly considered to be an effective multiphase bioreactor design for enzymatic applications by virtue of its cost-effectiveness, ease of product separation and recovery. Moreover, this reactor configuration is simple and a satisfactory reaction rate can be attained under optimal operating conditions owing to the high enzyme concentration in the bed column. Most importantly, due to the entrapment of enzyme particles in column tube, the reusability of biocatalysts is made feasible and shear inactivation of immobilized enzyme is greatly reduced (Chew *et al.*, 2008; Cozentino *et al.*, 2020). Nevertheless, the external mass transfer limitation is the major drawback of heterogeneous catalysis in this reactor configuration especially on an industrial-scale. The external mass transfer phenomenon involves the diffusion of the reactant molecules from the substrate liquid phase to the external surface of the catalyst particles through a fictitious stagnant boundary layer of a certain thickness enclosing the solid enzyme particles *via* molecular diffusion. External mass transfer resistance will develop when the rate of diffusional transport through the stagnant film is rate-determining step which results in the concentration gradient across the film layer (Macabe *et al.*, 2005; Chew *et al.*, 2008). The evaluation of the mass transfer coefficient in a fixed-bed system is therefore of great importance because the substrate diffusion velocity affects the overall reaction performance in PBR.

In the present study, a quantitative analysis of external mass transfer combined with biochemical reaction was performed and a mass transfer correlation model was then developed to simulate the lipase-catalyzed partial hydrolysis reaction in PBR under a solvent-free environment. The developed mathematical model can be applied to estimate the mass transfer coefficient at different scales and operating parameters, to predict reactor performance and to aid scaling up besides overcoming other engineering constraints. Additionally, the effect of substrate flow rate on the partial hydrolysis reaction in PBR for DAG synthesis was also analyzed.

2. Materials and methods

2.1 Materials

Refined, bleached and deodorized palm oil (RBDPO) was obtained from Golden Jomalina Food Industries Sdn. Bhd. (Malaysia). Commercial lipase *Rhizomucor miehei* (Lipozyme RMIM) was purchased from Novozymes A/S (Bagsvaerd, Denmark). The enzyme is immobilized on macroporous anionic exchange resin with particle size and bulk density ranging from 0.2 – 0.6 mm and 350 – 450 kg/m^3, respectively. Both acetone and acetonitrile purchased from Merck (Germany) were of HPLC grade. All other chemicals obtained were analytical grade.

2.2 Lipase-catalyzed partial hydrolysis in packed bed reactor

The partial hydrolysis of palm oil for DAG production was conducted in lab scale packed bed reactor using a jacketed glass column with dimensions of 2.4 cm internal diameter × 16.8 cm length. Table 1 gives the optimum conditions obtained from the experimental work that was performed in a batch system (Phuah *et al.*, 2012; Phuah *et al.*, 2016) and were used as a basis to conduct our present PBR studies. The substrate mixture which comprised both water and palm oil was first mixed thoroughly at 500 rpm in a conical flask before pumping downwards through immobilized enzyme-packed column using a peristaltic pump. The upper and lower end of the column was layered with cotton wool.

Table 1. Summary from experimental studies conducted in batch system.

Parameter	Optimum condition
Enzyme	Lipozyme RM IM
Water to palm oil weight ratio	1:20
Temperature	55^{o}C
Packed bed height	10 cm (13.8-wt% based on oil weight)
System	Solvent free

https://doi.org/10.26656/fr.2017.7(6).1005

Samples were taken periodically for acylglycerol components analysis. Experiments were repeated for five different flow rates (1, 3, 5, 10 and 15 mL/min).

2.3 Analysis of diacylglycerol content

Reverse-phase high performance liquid chromatography (RP-HPLC) was used to determine the acylglycerol composition in this study. Samples were first prepared by dissolving 5% of reactant into an appropriate amount of acetone which were then filtered through a 0.45 µm PTFE membrane filter before injecting samples into Merck KGaA (Darmstadt, Germany) LiChrospher® 100 RP-18e 5 µm (250 mm x 4 mm) column using a Waters e2695 HPLC equipped with Waters 2414 RI detector (London, United Kingdom) under isocratic conditions which comprised of acetone (solvent A) and acetonitrile (solvent B) in ratio of 62.5:37.5 at constant flow rate of 2 ml/min. Reference standards of commonly found fatty acids and acylglycerols moieties in palm oil (palmitoyl, oleoyl, linoleoyl and stearoyl) were used to determine the range of retention times at which these compounds were eluted. The retention time of MAG and free fatty acid (FFA), DAG and TAG ranged from 3 to 4.5 min, 4.5 to 9 min and 12 to 29 min, respectively. For quantification of each group, reference standards such as palmitic acid, monopalmitin, dipalmitin and tripalmitin were used as a representative for group FFA, MAG, DAG and TAG, respectively and external calibration curves were constructed based on the specific area.

2.4 External mass transfer model description

The external mass transfer correlation model proposed in the present study is in accordance with the approach suggested by Rovito and Kittrell (1973) with several following assumptions being made (Rovito and Kittrell, 1973).

a) The reaction obeys pseudo first order kinetics.
b) Steady state plug flow condition in fixed bed column with negligible axial dispersion.
c) The immobilized enzyme particles are spherical in shape.
d) Uniform enzyme activity throughout the particle.
e) Isothermal reaction.

2.4.1 Apparent reaction rate

In steady state condition, the corresponding material balance for substrate palm oil or TAG in the bed column is related to the equation as shown below.

$$\left(\frac{HQ}{W}\right)\left(\frac{dC}{dz}\right) \times (6 \times 10^{-2}) = -r \quad (1)$$

Where r is the substrate consumption rate (mg g^{-1} h^{-1}); Q is the volumetric flow rate (ml min^{-1}); H is the height of the column (cm); W is the weight of the immobilized biocatalyst (g); and dC/dz the concentration gradient along the column length (mg l^{-1} cm^{-1})

Considering first order reaction rate, the relation between the apparent reaction rate and bulk palm oil or TAG substrate concentration in the column is given as

$$R = k_p C \quad (2)$$

The kinetic parameter k_p represents the apparent first -order reaction rate constant (l g^{-1} h^{-1}) and C is the concentration of bulk substrate in reactant fluid (mg l^{-1}).

Substituting Eq. (2) into Eq. (1) gives

$$\left(\frac{HQ}{W}\right)\left(\frac{dC}{dz}\right) \times (6 \times 10^{-2}) = -k_p C \quad (3)$$

By integrating Eq. (3) using boundary conditions at z = 0 of C = C_{in} and at z = H of C = C_{out}, Eq. (4) is obtained as given below.

$$\ln\left(\frac{C_{In}}{C_{Out}}\right) = \frac{W}{Q}k_p \times \left(\frac{10^3}{60}\right) \quad (4)$$

Where C_{in} and C_{out} show the concentration of substrate palm oil or TAG (mg l^{-1}) at the column inlet and outlet, respectively. The substrate concentration at the outlet of the bed column is therefore given by

$$C_{Out} = C_{In} e^{-N} \quad (5)$$

With N defined as

$$N = \frac{W}{Q}k_p \times \left(\frac{10^3}{60}\right) \quad (6)$$

Eq. (5) only gives the relation between the inlet and outlet substrate concentration of palm oil or TAG in the packed-bed column every time the fluid flows through the column. Since a recycling system is involved, the inlet concentration to the column changes for every cycle. Assuming that the reservoir is a perfectly mixed tank, the total mass balance gives

$$\frac{dv}{dt} = 0 \quad (7)$$

Where is V the volume of the reacting solution in the reservoir (mL).

The component balance in the reservoir gives

$$\frac{dc_A}{dt} = -\frac{1}{\tau}(c_B - c_A) \quad (8)$$

Where τ is the residence time (min) in the reservoir (V/Q); Both c_A and c_B represent the concentration of substrate palm oil or TAG (mg l^{-1}) in the reservoir and at the outlet of the bed column to be circulated back to the reservoir. Based on Eq. (5), c_B can be defined as follows

https://doi.org/10.26656/fr.2017.7(6).1005

$$c_B = c_A e^{-N} \quad (9)$$

Combining both Eq. (8) and Eq. (9) generates

$$\frac{dc_A}{dt} = -\frac{1}{\tau}(c_A e^{-N} - c_A) \quad (10)$$

Integrating Eq. (10) using boundary conditions of V = V_{Res} and $c_A = c_o$ when t = 0 gives the change of substrate concentration in the reservoir with time as shown below.

$$c_A = c_o \exp[\frac{-(e^{-N}-1)t}{\tau}] \quad (11)$$

Based on Eq. (11), the slope gradient term is obtained from the graph of ln (c_A/c_o) against time as shown in Eq. (12) and the value of N can therefore be determined subsequently.

$$\text{Slope} = \frac{-(e^{-N}-1)t}{\tau} \quad (12)$$

Since constant amount of immobilized enzyme particles are used throughout the reaction, the apparent reaction rate constant, k_p for each flow rate can be found from Eq. (6) when the value of N is known. The parameter k_p depicts the apparent rate constant which takes into account both the reaction and mass transfer phenomenon.

2.4.2 Apparent reaction rate as a function of external mass transfer limitation

The mass transfer rate of the substrate palm oil or TAG from the bulk reactant liquid to the outer surface of the immobilized enzyme particle is proportional to the external mass transfer coefficient, area of external mass transfer and the concentration gradient between the bulk liquid and the external surface of immobilized enzyme particle.

$$r_m = k_m a_m (C - C_S) \quad (13)$$

Where r_m shows the external mass transfer rate (mg g^{-1} h^{-1}); k_m is the external mass transfer coefficient (cm h^{-1}); a_m is the surface area per unit weight of immobilized particle for external mass transfer (cm^2 mg^{-1}); C is the substrate concentration in the bulk liquid (mg l^{-1}) and C_s represents the substrate concentration on the outer surface of the immobilized particle (mg l^{-1}).

Eq. (14) can be applied to evaluate the magnitude of a_m as shown below

$$a_m = \frac{6}{\rho_p d_p} \quad (14)$$

With d_p as the particle diameter (cm) and ρ_p being the particle density (mg cm^{-3})

Based on the assumption of first order reaction, the substrate utilization rate of each enzyme particle can be derived and written as follows

$$r = kC_S \quad (15)$$

The constant k is the intrinsic first-order reaction rate constant (l g^{-1} h^{-1}) which takes into account both the effective internal mass transfer and the intrinsic reaction. At steady state, both external mass transfer rate and substrate consumption rate by enzyme particle are identical. Equating and rearranging both Eq. (13) and (15) gives rise to Eq. (16).

$$C_S = \frac{k_m a_m C}{k + k_m a_m} \quad (16)$$

The mathematical expression describing the effects of external mass transfer on the apparent reaction rate constant, k_p is then formulated as given in the following equation by replacing Eq. (16) into Eq. (15) and equating with Eq. (2)

$$k_p = \frac{k k_m a_m}{k + k_m a_m} \quad (17)$$

Rearranging Eq. (17) gives

$$\frac{1}{k_p} = \frac{1}{k} + \frac{1}{k_m a_m} \quad (18)$$

The terms (1/k) and ($1/k_m a_m$) show the contributions of both substrate utilization reaction and external mass transfer resistance on the apparent reaction, k_p, respectively, at constant temperature.

2.4.3 External mass transfer correlation model

The reaction rate coefficient, k is always a constant value for a particular reaction, implying the independence of reaction rate on the operating conditions namely substrate flow rate and the scale of the system. On the contrary, the external mass transfer coefficient, k_m changes with respect to operating parameters such as flow rate, reactor dimension and fluid properties and thereby altering the apparent reaction rate. Consequently, the development of a mass transfer correlation model is of extreme importance to enable the estimation and quantification of mass transfer resistance at different operating parameters especially during up-scaling.

The mass transfer coefficients between the bulk fluid and particle surface in the fixed bed column system can be correlated in terms of dimensionless groups which characterize the flow conditions (Bailey and Ollis, 1986; Nath and Chand, 1996). The correlation of the external mass transfer coefficient, k_m, with variables such as flow rate, reactor diameter and fluid properties can be obtained by defining a dimensionless group as follows

$$J_D = \frac{k_m \rho}{G}(\frac{\mu}{\rho D_f})^{\frac{2}{3}} \quad (19)$$

Where J_D is the Colburn factor, defined in terms of Schmidt number (Sc) and Reynolds number (Re). The Schmidt number is given as

https://doi.org/10.26656/fr.2017.7(6).1005

$$Sc = \frac{\mu}{\rho D_f} \quad (20)$$

The symbols μ, ρ and D_f are the fluid viscosity (g cm^{-1} min^{-1}), density (g ml^{-1}) and diffusivity (cm min^{-1}), respectively. The Reynolds number can be defined as

$$Re = \frac{d_p G}{\mu} \quad (21)$$

Where d_p is the particle diameter (cm). G is the mass flux (g cm^{-2} min^{-1}) and it can be calculated using Eq. (22) as follows

$$G = \frac{Q\rho}{a_c \varepsilon} \quad (22)$$

Where Q is the volumetric flow rate (mL min^{-1}), a_c is the cross sectional area of column (cm^2) and ε is the void fraction in a packed-bed. A few correlations for mass flow rates are available, varying in the dependence of the Colburn factor, J_D on Re. This correlation was suggested by Chilton and Colburn (1934) as follows.

$$J_D = KRe^{(n-1)} \quad (23)$$

Equating both Eq. (19) and (23) will lead to the following equation.

$$k_m = \left(\frac{K}{\rho}\right)\left(\frac{\mu}{\rho D_f}\right)^{-\frac{2}{3}}\left(\frac{d_p}{\mu}\right)^{n-1} G^n \quad (24)$$

Or

$$k_m = AG^n \quad (25)$$

Where

$$A = \left(\frac{K}{\rho}\right)\left(\frac{\mu}{\rho D_f}\right)^{\frac{2}{3}}\left(\frac{d_p}{\mu}\right)^{n-1}$$

Substituting Eq. (25) into Eq. (18) and rearranging it leads to the following equation:

$$\left(\frac{1}{k_p}\right) = \left(\frac{1}{Aa_m}\right)\left(\frac{1}{G^n}\right) + \left(\frac{1}{k}\right) \quad (26)$$

Eq. (26) can be analyzed for different values of n ranging from 0.1 to 1.0. A straight line of slope $1/(Aa_m)$ and intercept 1/k should be obtained if the experimentally measured values $1/k_p$ of versus $1/G^n$ for each value of n are plotted. The calculated values of A and k from the graph are then used to obtain the values of k_m by using Eq. (25) and the value k_p is then estimated by applying Eq. (18). A trial-and error procedure is repeated for all n values until the estimated k_p value is in good agreement with the experimental k_p value.

3. Results and discussion

Substrate flow rate is regarded as an important operating parameter in fixed bed reactor configuration as it affects the residence time of the reaction mixture with the immobilized enzyme particles. The effect of various volumetric flow rates on the DAG yield produced by lipase-catalyzed partial hydrolysis as a function of time is illustrated in Figure 1. The flow rates chosen for the current study range from 1 to 15 ml min^{-1}. Results demonstrated that the DAG synthesis rate increased in tandem with the increased flow rate from 1 to 5 ml min^{-1}, suggesting a diminution in external mass transfer resistance as the flow rate increases. Further increase in flow rate (> 5 ml min^{-1}), which corresponds to the insufficient residence time of the reactant mixture in the bed column, resulted in a gradual decline in reaction rate. In summary, approximately 30-wt% DAG yield could be achieved for each flow rate within the first 2 hrs. The reaction rate was found to decrease as the reaction continued until the equilibrium stage was reached in which the reaction rate was mainly controlled by the equilibrium thermodynamics rather than the intrinsic reaction or mass transfer. Therefore, only the data obtained for substrate flow rates of 1, 3 and 5 mL min^{-1} in the first 2 hrs was further used for the evaluation of the external mass transfer effect in PBR.

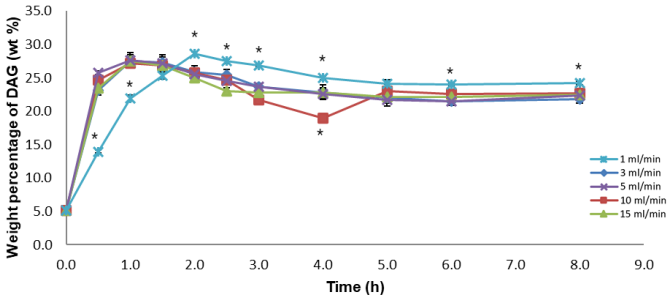

Figure 1. Diacylglycerol content as a function of time at different substrate flow rates.
*indicates a significant difference ($p < 0.05$) from other groups.

The concentration of substrate palm oil or TAG at each time interval (data not shown) was used to plot graphs of ln (C_1/C_o) as a function of time at different flow rates as presented in Figure 2. The gradient for each slope was determined and used to calculate the value of N using Eq. (12), followed by the evaluation of the observed reaction rate constant k_p using Eq. (6). The calculated k_p values at different flow rates are listed in Table 2. Results revealed that the observed rate constant, k_p increased from 0.32 ml g^{-1} h^{-1} to 0.52 ml g^{-1} h^{-1} in parallel with increasing flow rate from 1 ml min^{-1} to 5 ml min^{-1}, implying that a higher overall reaction rate could be achieved at a high flow rate. This may be attributed to the high turbulence created by the high flow rate which reduces the boundary layer effects surrounding the immobilized enzyme particles and therefore reduces the external mass transfer resistance. This observation is in accordance with previous literature (Chew *et al.*, 2008; Tepe and Dursun, 2008; Kathiravan *et al.*, 2010).

Using Eq. 26, various graphs of $1/k_p$ Versus $1/ G^n$

https://doi.org/10.26656/fr.2017.7(6).1005

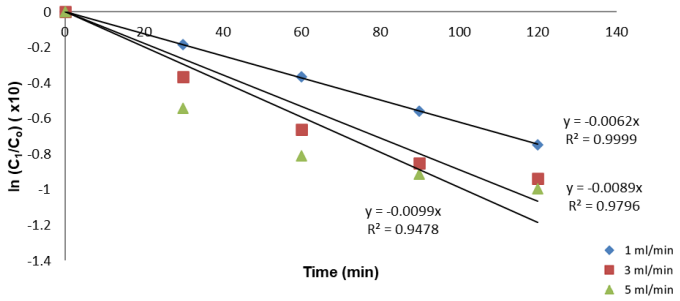

Figure 2. Overall rate of reaction at different flow rates.

were plotted for different n values (n = 0.1, 0.3, 0.5, 0.7, 0.8 and 0.9) and depicted in Figure 3. The range of n values proposed in this study, ranging from 0.1 to 1.0, comprised all the exponential values in the Colburn-Chilton factor that have been presented in the previous research studies (Rovito and Kittrell, 1973). Results indicated that negative intercepts were obtained for n < 0.5 and these n values were therefore not considered for further analysis. The magnitude of the intercept was found to increase with increasing n value from 0.1 to 0.9 whereas the gradient of a straight line demonstrated the reverse trend in which the slope value decreases with increasing values of n.

All n values between 0.5 and 0.9 were then further examined to determine the n value that provides the most satisfactory film diffusional model in predicting the external mass transfer resistance. Both gradient and intercept obtained for each straight line plotted which were related to the mathematical expression of $1/Aa_m$ and $1/k$, respectively, were subsequently used to determine the value of A and k. After that, the value of k_m at each flow rate was estimated using Eq. 25. In order to check the validity of the mass transfer models, the new k_p value which was determined by adding both calculated k and k_m, was compared with experimental k_p value. The n value which gives the closest k_p with respect to the experimental results indicates the most reliable model. The percentage deviation between the calculated k_p values and the experimental results for all flow rates are given in Table 3.

Table 2. Observed reaction rate constants (k_p) at different flow rates.

Flow rate (mL min^{-1})	Residence time (min)	Slope × 10^{-3} (min^{-1})	k_p × 10^{-4} (l g^{-1} h^{-1})
1	60.5	6.2	3.202
3	20.2	8.9	4.641
5	12.1	9.9	5.177

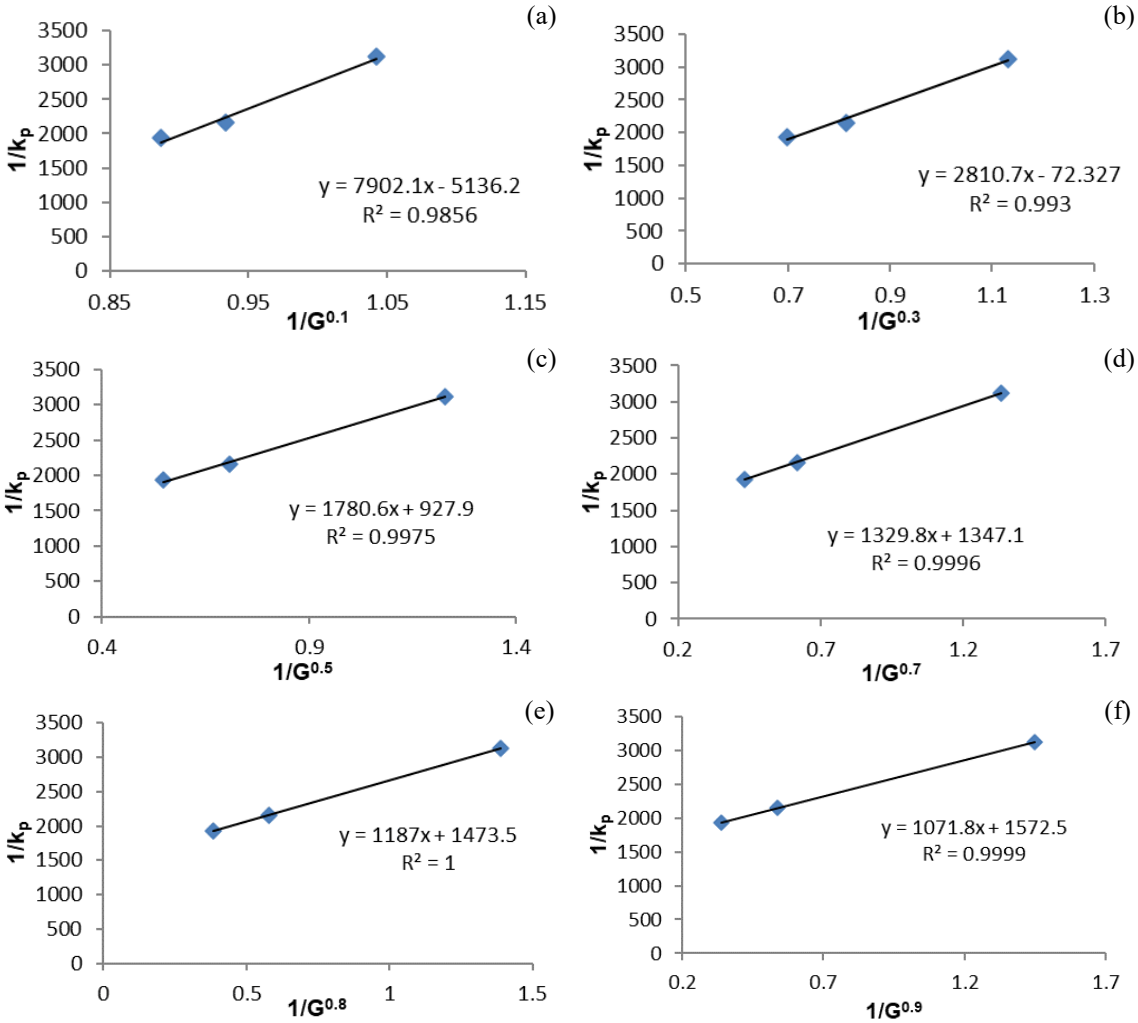

Figure 3. Plots of $1/k_p$ against $1/G^n$ for (a) n = 0.1; (b) n = 0.3; (c) n = 0.5; (d) n = 0.7; (e) n = 0.8 and (f) n = 0.9.

https://doi.org/10.26656/fr.2017.7(6).1005

In the view of these results, a model having n = 0.8 displayed the lowest normalized deviation of 0.12%, suggesting that the external mass transfer model with an exponent of 0.8 is capable of simulating and predicting the external mass transfer coefficient accurately for partial hydrolysis reaction using immobilized lipase PBR system. At n value equal to 0.8, the values of k and A were evaluated to be 6.79×10^{-4} l g^{-1} h^{-1} and 0.002247 $g^{-0.8}cm^{2.6}$ $min^{-0.2}$, respectively. The K value was then estimated to be 0.92. Thus, the external mass transfer correlation model for the present system can be written as

$$J_D = 0.92Re^{-0.2}$$

As can be observed from Table 3, the external mass transfer limitation which is a function of flow rate exhibited significant effects on the apparent reaction rate, k_p.

Table 4 shows the percentage contribution of both external mass transfer and overall utilization rate by enzyme particles on apparent reaction rate. Based on the calculations, it appears that the apparent reaction rate is limited by both film diffusion rate and substrate consumption rate which is the combination of both intrinsic reaction and internal mass transfer. At low mass flux of 0.66 g cm^{-2} min^{-1}, external mass transfer limitations contribute 52.8% of the apparent reaction rate, translating into dominance of external mass transfer effect on partial hydrolysis at low flow rate. As mass flux increases, the contribution of substrate utilization rate begins to surpass the contribution of external mass transfer resistance. Both overall utilization rate and external mass transfer effect were reported to contribute 76.4% and 23.6%, respectively, on the apparent reaction rate at high mass flux of 3.32 g cm^{-2} min^{-1}. Therefore, it was concluded that an increase in flow rate leads to the transition from mass transfer-limited regime to reaction kinetics-controlled regime. However, the external mass transfer effect could not be neglected completely as its percentage contribution remains significant, especially at large scales.

4. Conclusion

The overall results clearly demonstrated that the substrate flow rate plays an important role in the DAG formation rate *via* lipase-catalyzed partial hydrolysis reaction as well as affecting the external mass transfer coefficient in the PBR system. In the present study, high flow rate was found to reduce the external mass transfer resistance, thereby enhancing the apparent reaction rate to some extent. A further increase in flow rate would result in an insignificant improvement in the reaction rate. The external mass transfer correlation model in the form of $J_D = 0.92Re^{-0.2}$ predicts the experimental data in this study accurately. The model is valid for Reynolds number in the range of 0.002 – 0.01. The correlation model developed would be useful in predicting the external mass transfer coefficient for lipase-catalyzed partial hydrolysis reaction in PBR within this range.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This project is supported by a grant obtained by Sime Darby Research Sdn. Bhd. From Ministry of Science, Technology and Innovation Malaysia (MOSTI).

References

Arpi, N., Lubis, Y.M., Supardan, M.D. and Mustapha, W.A.W. (2016). Diacylglycerol-enriched oil production using chemical glycerolysis. *European Journal of Lipid Science and Technology*, 118(12), 1880-1890. https://doi.org/10.1002/ejlt.201500489

Bailey, J.E. and Ollis, D.F. (1986). Biochemical Engineering Fundamentals. New York, USA: McGraw-Hill.

Chew, Y.H., Lee, C.T., Sarmidi, M.R., Aziz, R.A. and Razali, F. (2008). External mass transfer model for the hydrolysis of palm olein using immobilized lipase. *Food and Bioproducts Processing*, 86(4), 276

Table 3. The percentage deviation of calculated values of k_p from the experimental values at different n values.

Flow rate (mL min^{-1})	Experimental $k_p \times10^{-4}$ (l g^{-1} h^{-1})	Percentage deviation (%)			
		n = 0.5	n = 0.7	n = 0.8	n = 0.9
1	3.202	-0.29	-0.10	0.00	0.03
3	4.641	1.62	0.65	0.14	-0.23
5	5.177	-1.40	-0.57	-0.21	0.26

Table 4. Effects of external mass transfer and overall substrate utilization rate in enzyme particles on the apparent reaction rate.

Mass flux (g cm^{-2} min^{-1})	$1/k_p$ (l^{-1} g h)	$1/k$ (l^{-1} g h)	$1/k_m a_m$ (l^{-1} g h)	% contribution	% contribution
0.66	3123	1474	1649	47.2	52.8
1.99	2158	1474	684	68.3	31.7
3.32	1928	1474	454	76.4	23.6

https://doi.org/10.26656/fr.2017.7(6).1005

-282. https://doi.org/10.1016/j.fbp.2008.02.001

Chilton, T.H. and Colburn, A.P. (1934). Mass transfer (absorption) coefficients prediction from data on heat transfer and fluid friction. *Industrial and Engineering Chemistry*, 26(11), 1183-1187. https://doi.org/10.1021/ie50299a012

Cozentino, I.D.S.C., Rodrigues, M.D.F., Mazziero, V.T., Cerri, M.O., Cavallini, D.C.U. and de Paula, A.V. (2020). Enzymatic synthesis of structured lipids from grape seed (Vitis vinifera L.) Oil in associated packed bed reactors. *Biotechnology and Applied Biochemistry*, 69(1), 101-109. https://doi.org/10.1002/bab.2085

Flickinger, B. And Matsuo, N. (2003). Nutritional characteristics of DAG oil. *Lipids*, 38(2), 129-132. https://doi.org/10.1007/s11745-003-1042-8

Kathiravan, M.N., Karthiga Rani, R., Karthick, R. And Muthukumar, K. (2010). Mass transfer studies on the reduction of Cr(VI) using calcium alginate immobilized *Bacillus* sp. In packed bed reactor. *Bioresource Technology*, 101(3), 853-858. https://doi.org/10.1016/j.biortech.2009.08.088

Lai, O.M., Yusoff, M.S.A., Lo, S.K., Kamariah, L., Tan, C.P., Tahiruddin, S. and Hashim, K. (2007). Production of acylglycerol esters. US8076497B2. Washington, DC: U.S. Patent and Trademark Office.

Lee, Y.Y., Tang, T.K., Phuah, E.T., Tan, C.P., Wang, Y., Li, Y., Cheong, L.Z. and Lai, O.M. (2020). Production, safety, health effects and applications of diacylglycerol functional oil in food systems: a review. *Critical Reviews in Food Science and Nutrition*, 60(15), 2509-2525. https://doi.org/10.1080/10408398.2019.1650001

Louie, S.M., Roberts, L.S. and Nomura, D.K. (2013). Mechanisms linking obesity and cancer. *Biochimica et Biophysica Acta*, 1831(10), 1499-1508. https://doi.org/10.1016/j.bbalip.2013.02.008

Macabe, W.L., Smith, J.C. and Harriott, P. (2005). Unit operations of chemical engineering. 7th ed. New York, USA: McGraw-Hill.

Nath, S. and Chand, S. (1996). Mass transfer and biochemical reaction in immobilized cell packed bed reactors: Correlation of experiment with theory. *Journal of Chemical Technology and Biotechnology*, 66(3), 286-292. https://doi.org/10.1002/(SICI)1097-4660(199607)66:3<286::AID-JCTB492>3.0.CO;2-Q

O'Sullivan, M.G. (2016). Low-fat foods: Types and manufacture. *Reference Module in Food Science: Encyclopedia of Food and Health*, 2016, 571-575. https://doi.org/10.1016/B978-0-12-384947-2.00430-X

Phuah, E.T., Lai, O.M., Choong, T.S.Y., Tan, C.P. and Lo, S.K. (2012). Kinetic study on partial hydrolysis of palm oil catalyzed by *Rhizomucor miehei* lipase. *Journal of Molecular Catalysis B: Enzymatic*, 78, 91-97. https://doi.org/10.1016/j.molcatb.2012.03.009

Phuah, E.T., Lee, Y.Y., Tang, T.K., Lai, O.M., Choong, T.S.Y., Tan, C.P., Ng, W.N. and Lo, S.K. (2016). Modeling and Optimization of Lipase-Catalyzed Partial Hydrolysis for Diacylglycerol Production in Packed Bed Reactor. *International Journal of Food Engineering*, 12(7), 681-689. https://doi.org/10.1515/ijfe-2015-0381

Phuah, E.T., Tang, T.K., Lee, Y.Y., Choong, T.S.Y., Tan, C.P. and Lai, O.M. (2015). Review on the current state of diacylglycerol production using enzymatic approach. *Food and Bioprocess Technology*, 8(6), 1169-1186. https://doi.org/10.1007/s11947-015-1505-0

Rovito, B.J. and Kittrell, J.R. (1973). Film and pore diffusion studies with immobilized glucose oxidase. *Biotechnology and Bioengineering*, 15(1), 143-161. https://doi.org/10.1002/bit.260150111

Schetz, M., De Jong, A., Deane, A.M., Druml, W., Hemelaar, P., Pelosi, P., Pickkers, P., Reintam-Blaser, A., Roberts, J., Sakr, Y. And Jaber, S. (2019). Obesity in the critically ill: a narrative review. *Intensive Care Medicine*, 45, 757–769. https://doi.org/10.1007/s00134-019-05594-1

Tepe, O. And Dursun, A.Y. (2008). Combined effects of external mass transfer and biodegradation rates on removal of phenol by immobilized ralstonia eutropha in a packed bed reactor. *Journal of Hazardous Materials*, 151(1), 9-16. https://doi.org/10.1016/j.jhazmat.2007.05.049

World Health Organization (WHO). (2021). Obesity and overweight. Retrieved on December 02, 2021 from WHO Website: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

