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Effect of Impeller Design on the Rate of Reaction of Hydrolysis in Batch Reactor

Nor Azyati Abd Muttalib[a,b], Dayang Norulfairuz Abang Zaidel*[,a,c], Muhd Nazrul Hisham Zainal Alam[a,b]

[a]Department of Bioprocess and Polymer Engineering, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bharu, Johor, Malaysia
[b]Process Systems Engineering (PROSPECT), Research Institute of Sustainable Environment, Universiti Teknologi Malaysia, 81310 UTM Johor Bharu, Johor, Malaysia
[c]Food and Biomaterial Engineering Research Group (FoBERG), Universiti Teknologi Malaysia, 81310 UTM Johor Bharu, Johor, Malaysia
dayang@cheme.utm.my

Lactose is a disaccharide found mostly in milk product. Lactose can cause digestive problem that commonly known as lactose intolerant. Manufacturer pre-treated the milk to produce lactose-free milk. This study aims to investigate the effect of impeller design on the rate of reaction of lactose hydrolysis in batch reactor by utilising immobilised enzyme. Lactose hydrolysis reactions were carried out by adding β-galactosidase (enzyme) into the milk. Alginate was utilised to immobilise the enzyme in order to reuse, enhanced stability and rapid separation of enzymes from the reaction mixture. In this reaction glucose was produced; hence increase sweetness of the milk. Replacing conventional enzymatic processes with immobilised (support) enzyme provides many advantages such as increase the enzyme activity, selectivity, stability and easy recovery from the reaction medium for their reuse. The rate of product formation with different variable such as agitation speed (150 rpm, 250 rpm and 300 rpm) and type of impeller (pitch blade turbine, Rushton turbine, marine propeller and pitch paddle) were investigated. It was found that the highest product formation was with marine propeller at speed 150 rpm with production rate 8.2 mg/L.min, followed by Rushton turbine at speed 250 rpm and pitch blade turbine at speed 300 rpm with production rate 6.2 mg/L.min and 5.4 mg/L.min. Pitch blade shown average of production rate around 3.4 - 4.6 mg/L.min.

1. Introduction

Milk hydrolysis has becoming a great interest in food industry due to its potential for economically feasible production of lactose. A lactose free product improves the texture consistency and sweetness of the product such as yogurt, ice cream and milk.

Based on present literature, many studies pertaining to enzymatic hydrolysis of milk were performed using a free-form enzymatic system. Whilst this particular process has several advantages such as easy to implement, did not produce any side reaction that might be harmful and reduce operational reaction cost (e.g. reduce reaction temperature), but recovery of enzyme is indeed an issue. Enzyme recovery is significant since enzyme is expensive and enzyme doesn't consume in reaction. It will be a waste to not reuse of enzyme with additional another unit operation for enzyme recovery process.

In this study, the enzymes were immobilised on beads and stirred as a free-form submerged enzyme-bead system in a stirred tank reactor. This is contrary to any classical immobilised enzyme system. The main reason underlying this concept is to resolve the mass transfer issues typically encountered in an immobilised enzyme system. By carrying the milk hydrolysis in this manner, it offers several advantages. The reactor equip with well-blended mixing system can minimise difference in temperature, pH, concentration and enhanced stability (Baysal and Karagöz 2005). In the midst of reaction, product can be separate from the catalyst before products itself inhibit the reaction (Petzelbauer et al., 2001). Many studies manipulate mixing to optimise the performance of bioreactor (Galaction et al., 2012).

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There are many factors affecting the milk hydrolysis process in this manner, however in the present study our focus is on impeller design (mixing and Reynolds number) effect on rate of reaction in hydrolysis milk in batch reactor. Four different types of impellers were investigated to assess the impeller suitability for carrying this sort of enzymatic hydrolysis system. All reactions were performed under various agitation rates and the enzymatic activity is calculated. Beads stability and mixing issues are discussed as well.

2. Material Method

2.1 Materials

Milk was bought from a local supermarket from Good Day brand (highest lactose concentration). Sodium alginate Cica reagent, Kanto Chemical Co. Inc., Japan, calcium chloride anhydrous QReC (Asia) Sdn. Bhd., Buffer concentrate kit, YSI 2357, Artisan Technology group, Netherland, Standard D-Glucose, YSI 2776, USA. Lactase (β-galactosidase) from Escherichia coli lyophilized powder was purchased from, Sigma-Aldrich (Saint Louis, USA). All chemicals used were of analytical grade.

2.2 Preparation of immobilised enzyme

Immobilise enzyme was prepared according to Konsula and Liakopoulou-Kyriakides (2004) with modification. Sodium alginate powder (3 g) was mixed in 100 mL distilled water using homogeniser for 30 min. 80μL of enzyme was added to alginate mixture and mixed for the next 30 min. Sodium alginate solution was extruded drop wise through capillary tube to a stirred solution of 0.2 M calcium chloride (gelling bath) prepared in distilled water and continuously agitated for 2 h. The beads shape and size depend on the flow rate alginate drip into calcium chloride solution, agitation speed, viscosity of the mixture and diameter of the tube. The beads formed were washed thoroughly with distilled water and were stored in a refrigerator for storage.

2.3 Hydrolysis of lactose

The enzymatic reaction was carried out in a batch reactor by using 4 types of impeller (pitch blade turbine, Rushton turbine, marine propeller and pitch paddle) attached to a motor (IKA Labortechnik type RW20DZM). Figure 1 shows different types of impeller design; marine propeller, Rushton turbine, pitch blade turbine and pitch paddle.

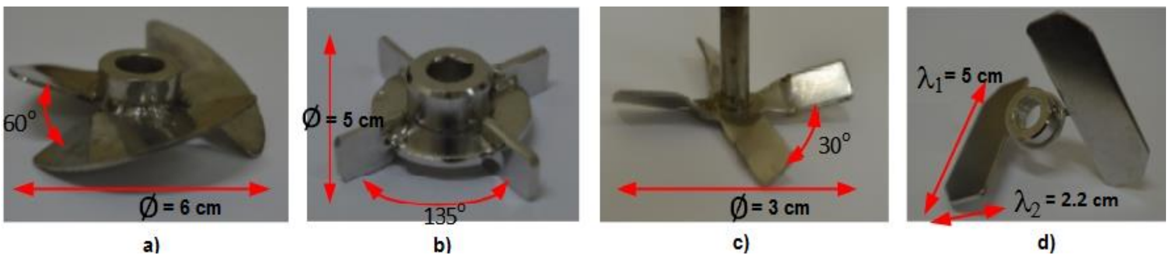

Figure 1: Various impeller designs ((a) marine propeller, diameter = 6 cm, height = 1.5 cm; (b) Rushton turbine, diameter = 5 cm, height = 1.5 cm; (c) pitch blade turbine, diameter = 3 cm, height = 0.6 cm; and (d) pitch paddle, diameter = 7 cm, height = 5 cm) on the milk hydrolysis rate of reaction

The lactose hydrolysis reaction was performed according to Obón et al. (2000) with modification. The experiments were carried out in 250 mL (pitch blade turbine, Rushton turbine and marine propeller) or 450 mL (pitch paddle) of milk added with immobilised enzyme bead in a batch system at 25 °C and 150 rpm, 250 rpm and 300 rpm (Caşcaval et al. 2010). The different in reactor volume specially used for pitch paddle in order to ensure the impeller immerse completely in the reactor. All impellers produced homogenous mixing and off-bottom suspension of beads.

2.4 Analysis of products

The enzyme activity was determined by the rate of glucose formation in the reaction medium. 25 μL sample was taken at specific time intervals and glucose concentration was measured by glucose analyser (YSI 2700 Select Biochemistry Analyzer, USA) for 1.5 h at 2 min to 10 min time interval (Zhou et al., 2012). After the experiment was completed, the beads were washed with distilled water for further used in reusability studies. The rate of reaction calculated based on Eq(1).

$$Rate\ of\ reaction = \frac{Glucose\ reading_{t2} - Glucose\ reading_{t1}}{t_2 - t_1} \quad (1)$$

2.5 Calculation of Reynolds number

Reynolds number was calculated using the following Eq(1) for different mixing time and types of impeller.

$$N_{Re} = \frac{ND^2\rho}{\mu} \quad (2)$$

where N = rev per second, D = diameter of impeller, ρ = density of the milk, μ = dynamic viscosity of milk at room temperature (The Engineering Toolbox n.d.).

2.6 Result and Discussion

2.6.1 Effect of impeller design on Reynolds number

Agitation speed showed significant effect on the Reynolds number as shown in Table 1. It can be seen that as Reynolds number was increased with the increasing in agitation speed. Reynolds number was also affected by impeller diameter (D) and speed of the agitation (N). Flow direction in the reactor is important to make sure beads mix homogenously in batch reactor. While pitch blade turbine and pitch paddle are, flat and set at ~45° angles, which produces a simultaneous axial and radial flow.

Marine propeller and Rushton turbine have similar diameter but enormous gap in Reynolds number. Rushton turbines have lowest range of Reynolds number compared to the other three types of impeller. Rushton turbine blades are flat and set vertically along an agitation shaft. This type of design produced a unidirectional radial flow. Marine propeller has a concave blade where back sides are convex. It produces axial flow to promote a better mixing. This design of marine propeller illustrates the higher Reynolds number. Previous study from Jirout and Rieger (2011) has reported that efficiency of mixing did not depend on dimension of impeller but vary on the force of agitation.

Pitch blade turbine has smallest dimension of impeller design with dual flow direction provides better overall mixing and low shear for gentle mixing (high Reynolds number). This impeller design was recommended for solid draw-down mechanism (Patel et al., 2014). At speed 250 rpm and 300 rpm pitch paddle has shown a significant increase in Reynolds number and almost reached full turbulent system (Re ~ 10,000) (Sinnott, 2005).

Table 1: Reynolds number for each impeller at difference speed (150 rpm, 250 rpm and 300 rpm)

Type Impeller	Speed (rpm)	Reynolds Number
Marine Propeller	150	3,000
	250	5,000
	300	6,000
Rushton Turbine	150	1,688
	250	2,813
	300	3,375
Pitch Blade Turbine	150	2,083
	250	3,472
	300	4,167
Pitched Paddle	150	4,083
	250	6,806
	300	8,167

2.6.2 Effect of impeller design on initial rate of reaction

The Michaelis–Menten kinetics for immobilised lactase was determined by quantifying concentrations of glucose following enzymatic hydrolysis of lactose. From Figure 2, the illustration showed that the change in product concentration over time according to Michaelis–Menten kinetic. The first 5 min, the reaction rate (slope) shows the highest rate of reaction in 5 min time interval. Even though the experiments run for 1.5 h the reaction almost reached equilibrium in 30 min. The result given in Figure 2 lead to following order of initial rate of reaction for different impeller design: Marine propeller > Rushton turbine > pitch paddle turbine > pitch paddle. Evaluation was performed at three different impeller rotational speeds ranging between 150 and 300 rpm over a period of 30 min at room temperature.

At all agitation speeds, Marine propeller has shown a high rate of reaction compared to the other types of impeller. The rate of reaction obtained for marine propeller was in the range of 6.0 - 8.2 mg/L.min (Figure 2(a)). The highest rate of reaction (8.2 mg/L.min) was obtained with mixing speed at 150 rpm. Meneghel et al. (2014) have also found that the rate of reaction which results in optimum production rate can be achieved even though the agitation speed used was lower.
Initial rate of reaction for Rushton turbine and pitched-blade turbine were in between 5.0 - 6.2 mg/L.min (Figure 2(b)) and 5.0 - 6.2 mg/L.min (Figure 2(c)). Similar rate of reaction was achieved even though the dimension of pitch blade turbine was smaller compared to Rushton turbine and the blade design was different.
Initial rate of reaction for pitch paddle was the lowest compared to the three other impellers which were in between 3.4 - 4.6 mg/L.min (Figure 2(d)). This was most probably due to the highest range of Reynolds number and shorter blend time (shorter time for homogeneous mixing) and thus increased vibration and greater fluid forces that would affect immobilise enzyme beads (Caşcaval et al., 2010). The result was also supported by a study from Caşcaval et al. (2010) which reported the impact from collision between beads with the blade which can cause biocatalyst disruption and reduces enzyme activity compared to shear force generated during mixing. Physical properties of the beads such as pore diameter and surface area have effect on immobilise enzyme rate of reaction (Santagapita et al., 2011). Figure 2(b) 250 rpm and Figure 2(c) 300 rpm shows highest rate of reaction might be due to available active site on beads even though, the initial rate of reaction for the beads is a bit low compare to other.

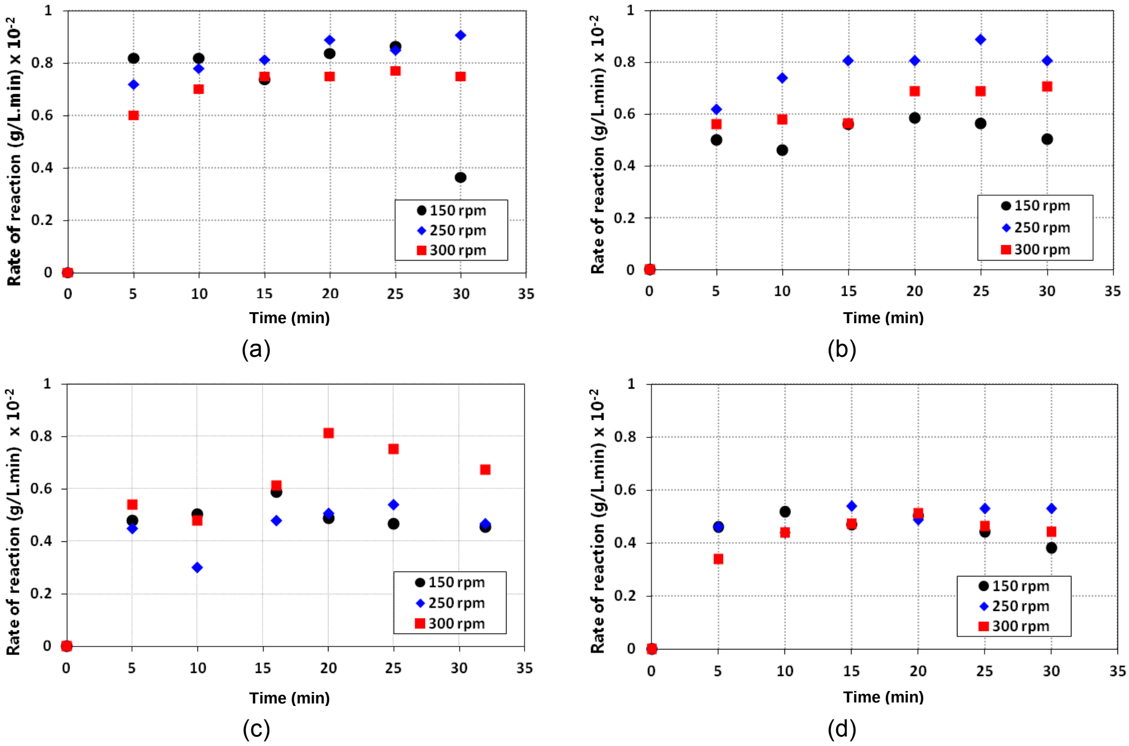

Figure 2: The effect of various impeller design (a) marine propeller, (b) Rushton turbine, (c) pitch blade turbine, and (d) pitch paddle on the milk hydrolysis rate of reaction

2.7 Correlation between Reynolds number and rate of reaction

In immobilise enzyme reaction mass transfer limitation can be overcome with convective mixing. It can be achieved by maximise ratio of beads surface area to bioreactor volume or minimise the working volume of reactor. Figure 3 shows the correlation between Reynolds number and rate of reaction. Evaluation was performed at three different impeller rotational speeds ranging between 150 to 300 rpm over a period of 30 minutes at room temperature. The Reynolds number increase with increase in rate of reaction. The Reynolds number show flow regime in the reactor. The higher the Reynolds numbers the higher ratio of inertial force to viscosity force in the fluid. Chemically, enzymatic reaction in immobilise beads depend on diffusion barrier between enzyme and substrate. Accessibility of enzyme with substrate may affect rate of reaction by optimise fluid rotational region to increase immobilise enzyme reaction. The trend goes well until Reynolds number reaches almost 3,000 (Figure 3).

Increasing velocity causes an increase in the mixing rate relative to the reaction rate (Pompano et al., 2008). The optimum condition for enzymatic reaction can be seen from Figure 2(a) and 2(b). The reaction rate drops when Reynolds numbers reach 4,000 and above as illustrated at Figure 3. Abd Rahim et al. (2015) have found similar trend in enzymatic reaction where the reaction increases until reach maximum point then the reaction rate dropped after optimum agitation speed. Theoretically at low agitation speed external mass transfer rate between beads and mixture phase is low. At speed (300 rpm) reaction rate is low because mechanical force was too high and would also disturb the complex molecule of enzyme which will denature the protein (Elibol and Özer, 2000).

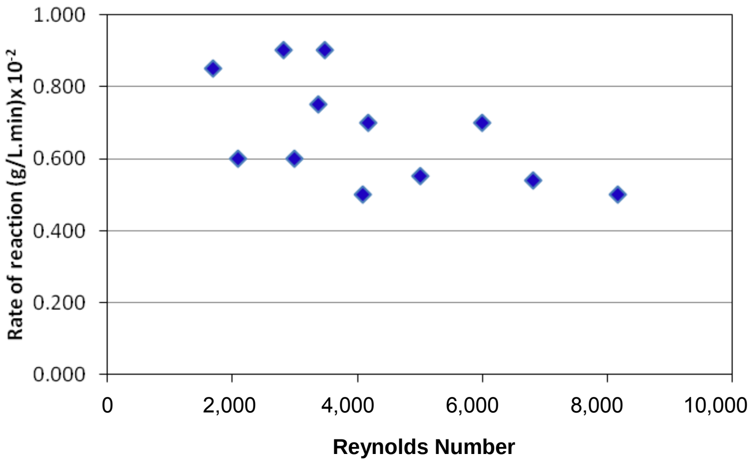

Figure 3: The correlation of Reynolds number on the initial rate of reaction of milk hydrolysis

As a result, suitable speed and impeller type for mixing homogenously (off-bottom suspensions of beads) and homogenous mixing must be considered in order to achieve complete enzymatic reaction. It becomes necessary to consider dual axial flow impellers or radial flow to perform the reaction effectively. It is because this element of impeller design can increase rate of enzymatic reaction.

3. Conclusion

Reynolds numbers were varied in between 3,000 - 4,000 at optimum mixing to achieve high rate of reaction. The highest rates of reaction reach 8.2 mg/L.min with agitation speed 150 rpm. Marine propeller was the best impeller design compared to other three because it produces axial flow to promote a better mixing and illustrates the higher Reynolds number. As the Reynolds number increase, it will increase the rate of reaction until it reach optimum mixing (up to N_{Re} 4,000). Out of four impellers design, marine propeller produced highest rate of enzymatic reaction which made it as the most suitable propeller to be used for hydrolysis reaction in batch reactor.

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Reference

Baysal S.H., Karagöz R., 2005. Preparation and characterization of κ-carrageenan immobilized urease, Preparative Biochemistry and Biotechnology 35 (2), 135–143.

Caşcaval D., Galaction A.-I., Lupăşteanu A.-M., 2010, Comparative evaluation of radial impellers efficiency for bioreactors with stirred bed of immobilized cells, 4. Studies on mechanical effect on biocatalysts integrity. Romanian Biotechnological Letters 15 (1), 4931 - 4939.

Elibol M., Özer D., 2000. Lipase production by immobilised Rhizopus arrhizus, Process Biochemistry 36 (3), 219–223.

Jirout T., Rieger F., 2011, Impeller design for mixing of suspensions, Chemical Engineering Research and Design 89 (7), 1144–1151.

Konsula Z., Liakopoulou-Kyriakides M., 2004, Hydrolysis of starches by the action of an α-amylase from Bacillus subtilis, Process Biochemistry 39 (11), 1745–1749.
Meneghel L., Reis G.P., Reginatto C., Malvessi E., da Silveira M.M., 2014. Assessment of pectinase production by Aspergillus oryzae in growth-limiting liquid medium under limited and non-limited oxygen supply, Process Biochemistry 49 (11), 1800–1807.
Noraida Abd Rahim, S. Sulaiman A., Ku Hamid K.H., Edama N.A., Baharuddin A.S., 2015, Effect of agitation speed for enzymatic hydrolysis of tapioca slurry using encapsulated enzymes in an enzyme bioreactor. International Journal of Chemical Engineering and Applications 6 (1), 38–41.
Obón J.M., Castellar M.R., Iborra J.L., Manjón A., 2000, β-Galactosidase immobilization for milk lactose hydrolysis: A simple experimental and modelling study of batch and continuous reactors, Biochemical Education 28 (3), 164–168.
Patel D., Ein-Mozaffari F., Mehrvar M., 2014, Using tomography to visualize the continuous-flow mixing of biopolymer solutions inside a stirred tank reactor, Chemical Engineering Journal 239, 257–273.
Petzelbauer I., Splechtna B., Nidetzky B., 2001, Galactosyl transfer catalyzed by thermostable beta-glycosidases from Sulfolobus solfataricus and Pyrococcus furiosus: kinetic studies of the reactions of galactosylated enzyme intermediates with a range of nucleophiles, Journal of biochemistry 130 (3), 341–349.
Pompano R.R., Li H.-W., Ismagilov R.F., 2008, Rate of mixing controls rate and outcome of autocatalytic processes: theory and microfluidic experiments with chemical reactions and blood coagulation, Biophysical journal 95 (3), 1531–1543.
Santagapita P.R., Mazzobre M.F., Buera M.P., 2011, Formulation and drying of alginate beads for controlled release and stabilization of invertase, Biomacromolecules 12 (9), 3147–3155.
Sinnott R.K., 2005, Coulson & Richardsons Chemical Engineering Design, Elsevier Butterworth-Heinemann, Oxford, United Kingdom.
The Engineering Toolbox, 2016, Dynamic Viscosities for some common Liquids, The Engineering Toolbox <www.engineeringtoolbox.com/absolute-viscosity-liquids-d_1259.html> acessed 20.04.2016.
Zhou J., Lv X., Mu Y., Wang X., Li J., Zhang X., Wu J., Bao Y., Jia W., 2012, The accuracy and efficacy of real-time continuous glucose monitoring sensor in Chinese diabetes patients: a multicenter study, Diabetes Technology & Therapeutics 14 (8), 710–718.