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Kinetic Study of Limonene and Glucose Adsorption on Immobilization and Co-immobilization Beads

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Abstract

Rotten oranges contain glucose and limonene, which is an inhibitor of microorganisms. Immobilization of mixed culture using entrapment method is the easiest method to protect the mixed culture from inhibitors. Entrapment method with extrusion drip is an efficient and effective technique to produce beads. This study aims to determine the adsorption rate of adsorbate (glucose and limonene) on the adsorbent surface (beads). Materials used in this study were glucose, DL-limonene, mixed culture, and beads. Three types of beads consisted of alginate-no mixed culture (A), alginate and activated carbon-no mixed culture (CA), alginate and activated carbon-free mixed culture (CB). Adsorption column consist of 30 ml nutrient, 15 mL substrate, and 5 mL beads. If the beads do not contain mixed culture, nutrients and substrate were replaced by distilled water, the reactor was done in a batch system at 37°C. The order of glucose adsorption capabilities starting from the lowest were AG, CAG, and CBG, while to limonene adsorption were AL, CBL, and CAL. Lagergren model was used to determined kinetic biosorption on limonene and glucose. The adsorption rate of the glucose in the pseudo-second order ($k_{2,ad}$) ranged between 0.025 to 0.087 min^{-1}, while the limonene ranged between 2.084 to 5.233 min^{-1}. Adsorption of glucose and limonene on three types of adsorbent surfaces reached the steady state at the 60th minute.

Keywords: *adsorption; immobilization; Lagergren model; limonene; orange*

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INTRODUCTION

Orange is the largest commodity in Indonesia with more than 1.8 million tons in 2014 (FAO, 2014). It is assumed that 40% of the fresh oranges become rotten and it can lead to the degradation of environmental quality. Orange waste still contains glucose (Pourbafrani *et al.*, 2010; Sanjaya *et al.*, 2015), so it is a potential substrate for hydrogen production through anaerobic fermentation. Unfortunately, orange peel contains antimicrobial compounds called limonene that can inhibit gas production (Mizuki *et al.*, 1990). One attempt to protect microbes/cells from limonene is by microbial immobilization (Kumar *et al.*, 1995). Physical application of microorganisms (immobilization) with polymers is one of the most widely used techniques to protect the mixed culture

from limonene. Entrapment in polymer matrix is the most common method as well as the easiest one to immobilize microorganism (Nawaz *et al.*, 2015). It can happen when a mixture containing immobilization materials and microorganism was dropped into the solution containing calcium cations (such as $CaCl_2$) during polymerization (Drichoutis *et al.*, 2007) to form beads (Lee *et al.*, 2013). Immobilization material/matrix derived from natural polymers, i.e alginate. Alginate beads are nontoxic (Hassan *et al.*, 2014) and hydrophilic due to the presence of carboxylic groups (Kumar *et al.*, 2013; Lin *et al.*, 2005). But, it also has weakness that it is easily. In this case, it is necessary to have activated carbon as supporting material (Dumitriu, 1998; *Mesran et al.*, 2014). Beads derived from two or more immobilized materials are called co-immobilization bead (Siahpush *et al.*, 1992). Cells immobilized in alginate gel have been applied to microbial degradations of toxic chemicals such as p-chlorophenol (Lin *et al.*, 2005). On the other hand, activated carbon has the high specific surface area, so it is able to efficiently adsorb many kinds of pollutants (Lin *et al.*, 2005).

A natural mixed culture is often chosen as hydrogen production of microorganism because it is easier to control, cheaper to operate, and has a broader choice of substrate (Li and Fang, 2007). Amekan *et al.* (2014) reported that the combination of three different digester sources, i.e cow dung, tofu waste, and fruit waste could produce the highest hydrogen concentration (231.02 mL/gVS) compared to mixed culture from one and two digester sources only. However, the diversity of hydrogen-producing bacteria (HPB) must be selected from mixed culture as well as acidification (Chen *et al.*, 2002; Cheong and Hansen, 2006) and with HPB enrichment (Marone *et al.*, 2012; Sivagurunathan *et al.*, 2014) repeatedly so the HPB life cycle is more stable (Sivagurunathan *et al.*, 2014).

Adsorption has been the most efficient and effective method for the removal of pollutant (Hassan *et al.*, 2014; Kumar *et al.*, 2013). Lagergren adsorption kinetics models have been widely used, for example the adsorption of pesticides with activated carbon from scrap tires (Hamadi *et al.*, 2004), dyestuff adsorption with activated carbon from coconut husk (Tan *et al.*, 2008), and adsorption of toxin Patulin using activated carbon-alginate bead (Yue *et al.*, 2013). However, adsorption of limonene and glucose on empty beads (without cells) and cells beads has not been widely studied.

In previous research biohydrogen was produced by rotten orange and egg shell using immobilized mixed culture (Damayanti *et al.*, 2017). Therefore, this research intends to determine the adsorption capacity of adsorbent which consisted of three types of beads (alginate without mixed culture, activated carbon-alginate without mixed culture, and activated carbon-alginate with mixed culture) to adsorbate i.e glucose and DL-limonene.

MATERIALS AND METHODS

Substrate and Medium Composition

The initial concentrations of glucose (Merck, 97.5%) and DL-limonene (Merck, >95%) was 10,000 ppm and 60 ppm, respectively. The same compositions of the fermentation enrichment medium and nutrients were used on the previous experiment (Damayanti *et al.*, 2015).

Mixed Culture

Mixed culture was obtained from biodigester of tofu waste, cow dung, and fruit waste in Indonesia. The characteristics and pretreatment of mixed culture were similar to the previous experiment (Damayanti *et al.*, 2015).

Preparation of Immobilization and Co-immobilization Beads

The bead making in this study was similar to that of the previous experiment (Damayanti *et al.*, 2015). Beads consisted of three types i.e no mixed culture of immobilization and co-immobilization beads, and at last, free mixed culture co-immobilization beads.

Batch Biosorption System

A batch reactor using 500 ml vial bottle which consisted of 60% (v/v) nutrient, 30% (v/v) substrate (glucose or DL-limonen), and 10% (v/v) beads. Medium composition was only used for beads containing mixed culture. If no mixed culture of beads, nutrient and substrate were substituted by distilled water. Five ml mixed culture was equivalent with 88 immobilization beads and 60 co-immobilization beads (Damayanti *et al.*, 2015).

The adsorption time calculation was started as the adsorption reactor containing the medium was flushed by N_2 for 3 minutes, then the reactor was placed on the waterbath at 37°C (Figure 1). Glucose and limonene samples were taken by a hypodermic syringe per 5 minutes and per 15 minutes, respectively. Each time 10 ml was taken for analysis. The glucose sample was filtered using a filter syringe with a pore size of 0.2 µm. The limonene sample was centrifuged at 4000 rpm for 15 min. This supernatant was taken for analysis. Limonene 0.2 ml was added by 2 ml n-hexane p.a and then it was shaken by vortex mixer Thermolyne Type 37600 for 5 min and then it was put into a bottle for GC analysis. The standard limonene solutions (ppm) were 10, 20, 30, 50, and 70. The sampling and analysis of each solution was duplicated.

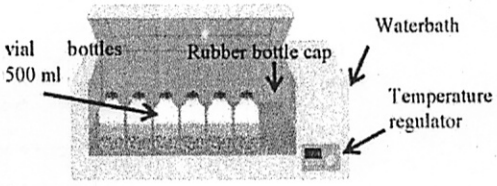

Figure 1. Biosorption reactor

The adsorption capacity of glucose and limonene at equilibrium was calculated by (1):

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (1)$$

With q_e is the amount of adsorbate adsorbed on the surface of the adsorbent at equilibrium (mg/g); C_0 is the concentration of adsorbate at 0 minute (mg/L); C_e is the concentration of adsorbate at equilibrium (mg/L).

KINETIC MODELING

To evaluate the biosorption kinetics of limonene and glucose, two kinetic models were used to fit experimental data on three types of beads.

Model 1. Pseudo first order-Lagergren model

$$\frac{dq}{dt} = k_{1,ad}(q_e - q) \quad (2)$$

With $k_{1,ad}$ is the first order adsorption rate constant (min^{-1}); q is the amount of adsorbate adsorbed (mg/g); q_e is the adsorption capacity at equilibrium (mg/g); t is the time in minute. After rearrangement, equation (1) can be written as follow:

$$q = \frac{(C_0 - C_t)V}{m} \quad (3)$$

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (4)$$

With C_0 is the concentration of adsorbate at minute 0 (mg/L); C_t is the concentration of adsorbate at minute (mg/L); C_e is the concentration of adsorbate at equilibrium (mg/L). The mass of immobilization beads (adsorbate) without mixed culture and co-immobilized mixed culture were 3.3088, 3.717, and 3.7453 g, respectively.

Equation (2) was integrated into

$$\ln\left[\frac{(C_t - C_e)}{(C_0 - C_e)}\right] = -k_{1,ad}t \quad (5)$$

Equation (2) was a linear regression equation, Y = mX.

Model 2. Pseudo second order-Lagergren model

$$dq = k_{2,ad}(q_e - q)^2 dt \quad (6)$$

Equation (6) was integrated into

$$\frac{(C_0 - C_t)}{(C_0 - C_e)\frac{V}{m}[(C_t - C_e)]} = k_{2,ad}t \quad (7)$$

Equation (7) was a linear regression equation, Y = mX.

Sample Analysis

The limonene samples were analyzed by Shimadzu 14B gas chromatography with temperature of column, detector, and injector were 80, 250, and 220°C, respectively. It used flame ionization detector (FID) and 2 m column packed with OV-17/AP. The carrier gas was ultra high purity nitrogen. Reducing sugar was analyzed by Nelson-Somogy method (Nelson, 1944; Somogy, 1951).

RESULTS AND DISCUSSION

The results of the adsorption test on three types of beads on glucose and limonene were presented in Figure 2 and Figure 3.

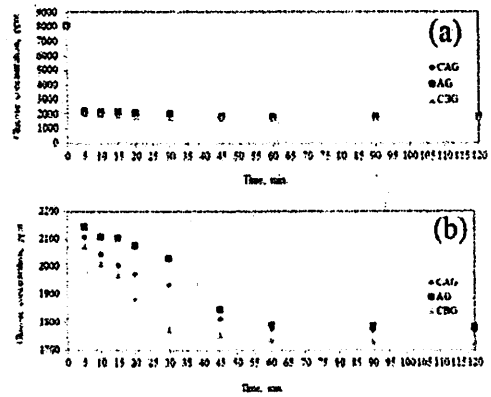

Figure 2. Adsorption of glucose in 3 types of beads with (a) initial glucose and (b) without initial glucose. CBG = alginate-activated carbon-mixed culture, CAG = alginate-activated carbon, AG = alginate.

Figure 2(a) shows that glucose concentrations degrade drastically to 6000 ppm. Figure 2(b) shows that steady state conditions for glucose adsorption started to occur at the 60th minutes. The lowest order of bead's ability to adsorb glucose solution was AG followed by CAG and finally CBG.

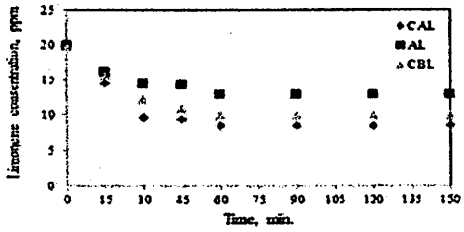

Figure 3. Adsorption of limonene in 3 types of beads, CBL = alginate-activated carbon-mixed culture, CAL = alginate-activated carbon, AL = alginate

Figure 3 shows that the limonene degradation was only about 5 ppm and steady conditions began to occur after 60-minute. The lowest order of beads' ability to adsorb limonene concentrations was AL followed by CBL and CAL. Based on these results, AL was lower than CBL and CAL, it can be assumed that the alginate gel was hydrophilic while the limonene was hydrophobic, so alginates gel have no capability to adsorb limonene. Meanwhile, co-immobilization beads can remove limonene due to hydrophobic interaction with activated carbon in alginate bead. Hence, limonene were adsorbed more strongly to the activated carbon in alginate gel than alginate gel (Lin *et al.*, 2005). The adsorption of limonene by CBL is lower than by CAL and this phenomenon was apparently caused by microorganism that entirely filled activated carbon pores and made limonene difficult to penetrate. Other factor was due to the polarity difference between limonene and alginate.

Glucose and limonene degradation in Figure 2 and Figure 3 show that they have a stark difference of as much as 1200 times. The small adsorption of limonene in this study is similar to the research done by Fabra *et al.* (2012) who stated that limonene released from the iota-carrageenan matrix into water was lowest at 37°C although rising temperatures lead to increased limonene diffusion through the matrix. In addition, the properties of iota-carrageenan was used by Fabra *et al.* (2012) was similar to alginates beads because they were derived from natural polysaccharides, hydrophilic, and at the same temperature, it can be indicated that the amount of limonene attached to the three types of beads were low.

The values of the adsorption constant (k_{ad}) and adsorption capacity at equilibrium (q_e) for both glucose and limonene were presented in Table 1 and Table 2.

Table 1 and Table 2 show that negative values of k_{1ad} indicate that pseudo-first order cannot represent the kinetic data. Both of them that the correlation coefficient (R^2) of the pseudo- second order was above 0.7 means. It mean that the pseudo-second order of Lagregren models can be used to study the kinetics of sugar adsorption and limonene on the three beads. The value of R^2 above 0.5 to 0.99 means that the correlation coefficient is strong (Sarwono, 2007).

The suitable kinetic model was the pseudo-second order because the adsorption rate constant (k_{1ad}) of the pseudo-second order is positive, whereas the pseudo-first order was negative. Although the pseudo-first order k values for CBG and CBL are positive, their R^2 value was less than R^2 at the pseudo-second order.

Table 1 and Table 2 explain that the adsorption velocity contants (k_{2ad}) at pseudo-second order for glucose solution was ranged between 0.025-0.087 min^{-1}, while the adsorption velocity constants (k_{2ad}) of limonene solution was ranged between 2.084-5.233 min^{-1}. It suggests that the adsorption rate of glucose solution was much faster 60-83 times than the adsorption rate of limonene solution due to the molecular size of glucose, limonene, and sodium alginate gel (nm) was 2,845 (ChemSketch); 3.2570 (ChemSketch); and ~5 (Lee and Mooney, 2012), respectively.

Table 1 and Table 2 show that the value of the adsorption capacity at equilibrium at 60 minute are 75.468-84.769 mg/g for glucose solution and 0.086-0.137 mg/g for limonene solution. This suggests that the ability of three types of beads at equilibrium to adsorb glucose solution ranged between 619-877 times higher than limonene solution due to the smaller size of the glucose molecule.

CONCLUSION

It is concluded that AG beads had the highest adsorption capacity for glucose followed by CAG and CBG, while for highest limonene adsorption was AL followed by CBL and CAL. The suitable kinetic model was pseudo second order-Lagergren model because it resulted the best value of coefficient of correlation (R^2). i.e above 0.7. The mean of the reaction constants (min^{-1}) for glucose and limonene on the three beads (A, CA, and CB) were 0.051 and 3.15, respectively.

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Table 1. Glucose adsorption constant in 3 types of beads

Beads type	Pseudo-first order			Pseudo-second order			
	Linear equations	R^2	k_{1ad} ($minute^{-1}$)	Linear equations	R^2	k_{2ad} ($minute^{-1}$)	q_e (mg/g)
AG	Y= -0.043X	0.8157	-0.043	Y= 0.025X	0.725	0.025	84.769
CAG	Y= -0.029X	0.9849	-0.029	Y= 0.054X	0.752	0.041	75.574
CBG	Y= 0.019X	0.0434	0.019	Y= 0.087X	0.781	0.087	75.468

Tabel 2. Limonene adsorption constant in 3 types of beads

Beads type	Pseudo-first order			Pseudo-second order			
	Linear equations	R^2	k_{1ad} ($minute^{-1}$)	Linear equations	R^2	k_{2ad} ($minute^{-1}$)	q_e (mg/g)
AL	Y= -0.051X	0.975	-0.051	Y= 2.084X	0.9662	2.084	0.136
CBL	Y= 0.011X	0.028	0.011	Y= 5.233X	0.779	5.233	0.086
CAL	Y= -0.030X	0.868	-0.030	Y= 2.143X	0.776	2.143	0.137

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