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Effect of aeration and agitation rates and scale-up on oxygen transfer coefficient, $k_L a$ in exopolysaccharide production from *Enterobacter cloacae* WD7

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Abstract

In the exopolysaccharide production from *Enterobacter cloacae* WD7 under aerobic fermentation, biopolymer yield increased with aeration and agitation rates increasing at pilot plant scale, but decreased when agitation increased at bench scale because of shear thinning character (from 3.07 to 2.28 g/g over the range 200–800 rpm). However, it increased with increase of aeration rate (from 2.79 to 3.07 g/g over the range 0.5–1.25 vvm, respectively). By a dynamic gassing-out technique, the effects of aeration rate, agitation rate, character and size of fermentor and its accessories on the volumetric oxygen transfer coefficient $k_L a$ were studied. Values of $k_L a$ in the exponential and stationary phases increased with aeration and agitation rates to 9.97 and 9.72 h^{-1} in exponential and stationary phases at aeration rate 1.25 vvm of bench scale fermentation and 9.68 and 9.50 h^{-1} in exponential and stationary phases at aeration rate 1.75 vvm of pilot plant scale fermentation, respectively. Scale-up in the pilot plant performed by '$k_L a$ fixing', controlling the oxygen change in the fermentor broth by holding the $k_L a$ value to equal to the value obtained from small fermentor, resulted in a biopolymer yield of 3.20 g/g c.f. 3.07 g/g at bench scale.

Keywords: Biopolymer; *Enterobacter cloacae* WD7; Scale-up; Oxygen transfer coefficient; $k_L a$; Oxygen transfer rate, OTR; Oxygen uptake rate, OUR; Flow rheology

1. Introduction

Biopolymer synthesis generally occurs only when the microorganism is grown aerobically and usually under non-limited oxygen conditions, a polymer with higher molecular weight is produced (Sutherland, 1998). But the increased viscosity of broth formed a layer on cell surface and acts as a diffusion barrier, oxygen transfer to the cells becomes increasingly more difficult. The dissolved oxygen (DO) concentration becomes a limiting nutrient in processes of high oxygen demand (fast growing microorganisms, high biomass, and production of biopolymer) or when the rheological properties of broth offer a high resistance to the mass transfer, such as xanthan gum production (Casas, Santos, & Garćia-Ochoa, 2000; Lo, Hsu, Yang, & Min, 2001). The supply of oxygen (OTR) can be the controlling step in industrial bioprocesses, scale-up of aerobic biosynthesis systems (Al-Masry, 1999; Elibol & Ozer, 2000; Flores, Péres, & De La Torre, 1997; Gibbs & Seviour, 1996; Weuster-Botz, Hnnekes, & Hartbrich, 1998).

OTR is the most important parameter implied on the design and operation of aeration and agitation of bioreactors and in scale-up (Thiry & Cingolani, 2002; Wernersson & Trägårdh, 1998). Efficiency of aeration depends on oxygen solubilization, diffusion rate into broths, and bioreactor capacity to satisfy the oxygen demand of microbial population. However, the DO in the broths is limited by its consumption rate on cells or the oxygen uptake rate (OUR), as well as by its OTR. The OTR could be affected by several factors, such as geometry and characteristics of the vessels, liquid properties (viscosity, superficial tension,

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etc.), the dissipated energy in the fluid, biocatalyst properties, concentration, and morphology of microorganisms. The OTR value depends on the air flow rate, the stirrer speed, mixing, etc. On the other hand, the OUR is limited by increase in viscosity resulting from polymeric property (Çalik, Çalik, & Özdamar, 2000; Eickenbusch, Brunn, & Schumpe, 1995; Kobayashi, Okamoto, & Nishinari, 1994; Kwon et al., 1996).

Oxygen transfer can play an important role since it is often the limiting factor in order to obtain the appropriate volumetric oxygen transfer coefficient (k_La) that correlates with productivity in specific culture media (Montes, Catalan, & Galan, 1998; Tuffile & Pinho, 1970). There are many methods for k_La determination that have been reported by many authors and most k_La values are considerably affected by the geometry of the system. A dynamic biological method is widely used and involves physical oxygen absorption combined with oxygen consumption by a cell culture (Kouda, Yano, & Yoshinaga, 1997). The sulphite oxidation method is strongly discouraged and has come under severe criticism (Galaction, Cascaval, Oniscu, & Turnea, 2004). Because the reaction rate constant can vary in an unknown way, but be suitable in case of cell-free fermentation.

To reduce the complication of various variables and factors based on the theory of models and the principles of similarity, scaling-up for biopolymer production should be studied by consideration of the oxygen transfer parameters (Diaz & Acevedo, 1999; Nakayama, 1981; Winkler, 1983; Yuh-Lih & Wen-Teng, 2002). Fixing of k_La values has been commonly used criteria for scale-up of aerobic fermentations (Garćia-Ochoa, Gómez-Castro, & Santos, 2000; Gibbs & Seviour, 1996; Miura et al., 2003). The rationale of k_La values is to ensure a certain mass transfer capability that can cope with the oxygen demand of the culture and often serves to compare the efficiency of bioreactors and mixing devices as well as being an important scale-up factor.

Enterobacter cloacae WD7 was isolated from the sedimentation tank of a recycled sludge of a seafood processing plant in Songkhla region, Thailand (Dermlim, 1999). It produced the biopolymer, resulting in viscous broth, with a yield of 4.69 g/L after 3 days of batch cultivation in a 3 L fermentor under optimized conditions (30 °C, pH 7.0, at 2.0 vvm and 200 rpm) and medium (Wichienchot, 2000). This work focuses on scale-up with k_La fixing for biopolymer fermentation of *E. cloacae* WD7 from 5 to 72 L fermentors. The effect of various aeration and agitation rates on OTR and OUR values during fermentation and fluid flow behavior have been investigated.

The aims of this work were to study the mass production of biopolymer in pilot plant fermentor and overcome the problems of fermentation at different scales. In addition, the relation of aeration rate, agitation rate and k_La value are studied and then the EPS yields are compared under the similar k_La values of both scales. The elucidated conditions will be used for fermentation in pilot plant scale and large scale furthermore.

2. Materials and methods

2.1. Microorganism growth and medium

Enterobacter cloacae WD7 was maintained on nutrient agar slant at 4 °C. Inoculum was prepared by inoculating one loop of a 24 h culture into 200 mL of the medium (pH 7.0) in a 500 mL flask and cultivated on a rotary shaker (200 rpm) at 30 °C. Inoculum with the cell concentration of 10^5 CFU/mL was used as starter (5%) of fermentation.

The medium contained 3% sucrose, 0.05% yeast extract, 0.5% K_2HPO_4, 0.2% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$, and 0.01% NaCl, pH was adjusted to 7.0 and sterilized at 121 °C for 20 min (Wichienchot, 2000), the sucrose was sterilized separately.

2.2. Fermentors

Two sizes of fermentors used in this study were continuous stirrer tank reactors (CSTR) heights of tanks (0.25 and 1.00 m), heights of liquid medium within fermentors (0.23 and 0.70 m), diameters of tanks (0.15 and 0.40 m), diameter of impellers (0.08 and 0.10 m), and number of baffles within, (3 and 4) for bench and pilot plant scale fermentors, respectively. Bench scale fermentation was performed in a 5 L fermentor (Marubishi Co., Ltd, Japan) equipped with a DO electrode and a controller. The agitation system was a coupling of stirrer and control base that both were magnetic. The stirrer comprised two propellers on one axle and there were six flat-blades in each propeller. The agitation rate was controlled through electromagnetic impulse. The aeration system was an air inlet through a ring sparger with air-flow meter, filter, and dissolve oxygen measurement by dissolved oxygen tension, % DOT.

Pilot plant scale was operated in a 72 L stainless steel reactor (Biostat-M of B. Braun, Germany) equipped with a temperature probe, pH electrode, antifoam probe, and dissolve oxygen electrode. The agitator consisted of three impellers (with six flat-blades each) on one axle and controlled through a controller. Air was given through spider sparger, air filter, and air flow meter and recorded as % DOT.

2.3. Analytical methods

Cell growth was determined as dry cell weight (DCW) by centrifugation of the broth at 7600*g* for 15 min at 4 °C and the washed cell being dried at 105 °C overnight and weighed (Yokoi et al., 1997). Biopolymer was precipitated from supernatant with 4 volumes of 95% cold ethanol kept in a freezer (−20 °C) and after centrifugation at 7600*g* for 15 min. The crude polymer was dried at 50 °C and weighed. DOT (% sat.) was measured by an oxygen electrode. Apparent viscosity (cp or mPa s) was determined by Brookfield viscometer (model LVDV-I+) with an SC4-18/13R spindle.

2.4. Fermentation studies

2.4.1. Effect of aeration and agitation rates on biopolymer production in bench scale

Batch fermentation of *E. cloacae* WD7 in a 5 L fermentor containing 4 L medium was carried out at 30 °C with controlled pH at 7.0 (using 0.1 N NaOH) for 3 days with varying aeration rates (0.5, 0.75, 1.00, and 1.25 vvm) and agitation rate (N) (200, 400, 600, and 800 rpm). Samples were taken every 6 h for determination of DCW, % DOT, biopolymer yield and viscosity.

2.4.2. Effect of aeration and agitation rates on OUR, OTR, and k_La values

In addition, during cultivation, the OUR, OTR, and k_La were determined by dynamic gassing-out technique according to the method of Garćia-Ochoa et al. (2000). From mass balance, equation for dissolve oxygen changes in batch fermentation could be established:

$$\mathrm{dCo}/\mathrm{d}t = \mathrm{OTR} - \mathrm{OUR} = k_\mathrm{L}\mathrm{a} \cdot (C^* - C) - (Q\mathrm{o}_2 \cdot Cx), \quad (1)$$

where k_La ($C^* - C$) and ($\mathrm{Qo}_2 \cdot Cx$) are the volumetric OTR and OUR, respectively. For OUR determination, the inlet of air flow was interrupted (OTR = 0) and DOT value decreased due to cellular respiration and OUR = slope of graph (DOT vs time). According to Eq. (2),

$$\mathrm{dCo}/\mathrm{d}t = \mathrm{OUR} = -(Q\mathrm{o}_2 \cdot Cx). \quad (2)$$

For OTR determination, the air inlet was restarted and caused the increase of % DOT and OTR value was obtained by calculation according to Eq. (3),

$$\mathrm{OTR} = \mathrm{dCo}/\mathrm{d}t + \mathrm{OUR}. \quad (3)$$

For k_La determination, Eq. (1) could be integrated at which the aeration begun ($t = t_1$, so $\mathrm{Co}_2 = C_1$) and another time ($t = t_2$, so $\mathrm{Co}_2 = C_2$), yielding the Eq. (4):

$$(Q\mathrm{o}_2 \cdot Cx)(t_2 - t_1) + (C_2 - C_1) = k_\mathrm{L}\mathrm{a} \cdot \int (C^* - C)\,\mathrm{d}t. \quad (4)$$

The k_La value was calculated by solving Eq. (4) of experimental values from the graph (DOT vs time) by means of the integration algorithm taken. The OUR, OTR, and k_La values in exponential and stationary phases (at 12 and 36 h cultivation, respectively) were investigated at various aeration and agitation rates at bench scale (0.5–1.25 vvm and 200–800 rpm, respectively) and at pilot plant scale (1.25–2.00 vvm and 200–600 rpm, respectively). The k_La values of both scales were compared to find out a control condition that gave the equal k_La for scale-up study.

2.4.3. Scale-up study

Batch cultivation in pilot plant scale was carried out in a 72 L fermentor with 50 L working volume at 30 °C, pH 7.0, for 3 days of cultivation using the aeration and agitation rates that gave the equal or very similar k_La values obtained from bench scale. Time course, the OTR, OUR, and k_La values and their effects from pilot plant fermentation were investigated and the EPS yield of both scales was compared.

2.5. Study on fluid properties of WD7 biopolymer

Flow data of crude WD7 biopolymer dispersed in distilled water (at 0–0.5% w/v) were investigated by using a Brookfield viscometer. The shear stress (τ, Pa) vs shear rate (γ, s^{-1}) were plotted in log forms as Y- and X-axis, respectively. The correlation of τ and γ is explained with the Ostwald–de Waele model of Eq. (5);

$$\tau = K(\gamma)^n, \quad (5)$$

where the K value is the consistency index (mPa s or cp) and the n value is flow index. The n value was used to identify the flow characteristic of shear thinning ($n < 1$) or shear thickening ($n > 1$) behavior of this biopolymer.

3. Results and discussion

Since the role of oxygen on microorganism growth and metabolism is important, the DO transfer due to cell consumption (OUR) and oxygen transfer (OTR) into a system during fermentation needs to be investigated. The fermentations of *E. cloacae* WD7 were carried out at bench scale by change of aeration (0.5, 0.75, 1.0, and 1.25 vvm) and agitation rate (200, 400, 600, and 800 rpm) for k_La values determination, especially in exponential phase and stationary phase.

3.1. Effect of aeration and agitation rates on DOT at bench scale

Effects of aeration rate and agitation rate on fermentation of *E. cloacae* WD7 at bench scale are illustrated in Figs. 1 and 2, respectively. From Figs. 1a and 2a, all DOT values of both tests decreased dramatically to a minimum level within 24 h of cultivation, and then kept constant afterwards at 10–30%. The remaining DOT levels in stationary phase (after fermentation 24 h) increased with increase of aeration rates tested, but did not depend on agitation rates. The viscosity was usually found to increase when the microorganism grew fast with high oxygen consumption. However, the DOT values in all runs never were less than 10%, indicating that oxygen support was enough for consumption of cells.

Aeration (as oxygen support) only affects retention time of oxygen flow with corresponding increase or decrease of gas velocity in the broth at both high or low rates of aeration, respectively. However, agitation, as is well known, affects both air bubble distribution and the mixing of the system. Mechanically agitated aerated vessels are widely used rather than aerated only vessels which can be in adequate to promote the liquid turbulence necessary for small air bubble generation. Although the agitation could maintain available dissolved oxygen in the fermentor, but the inappropriate speed of agitation results is poor oxygen

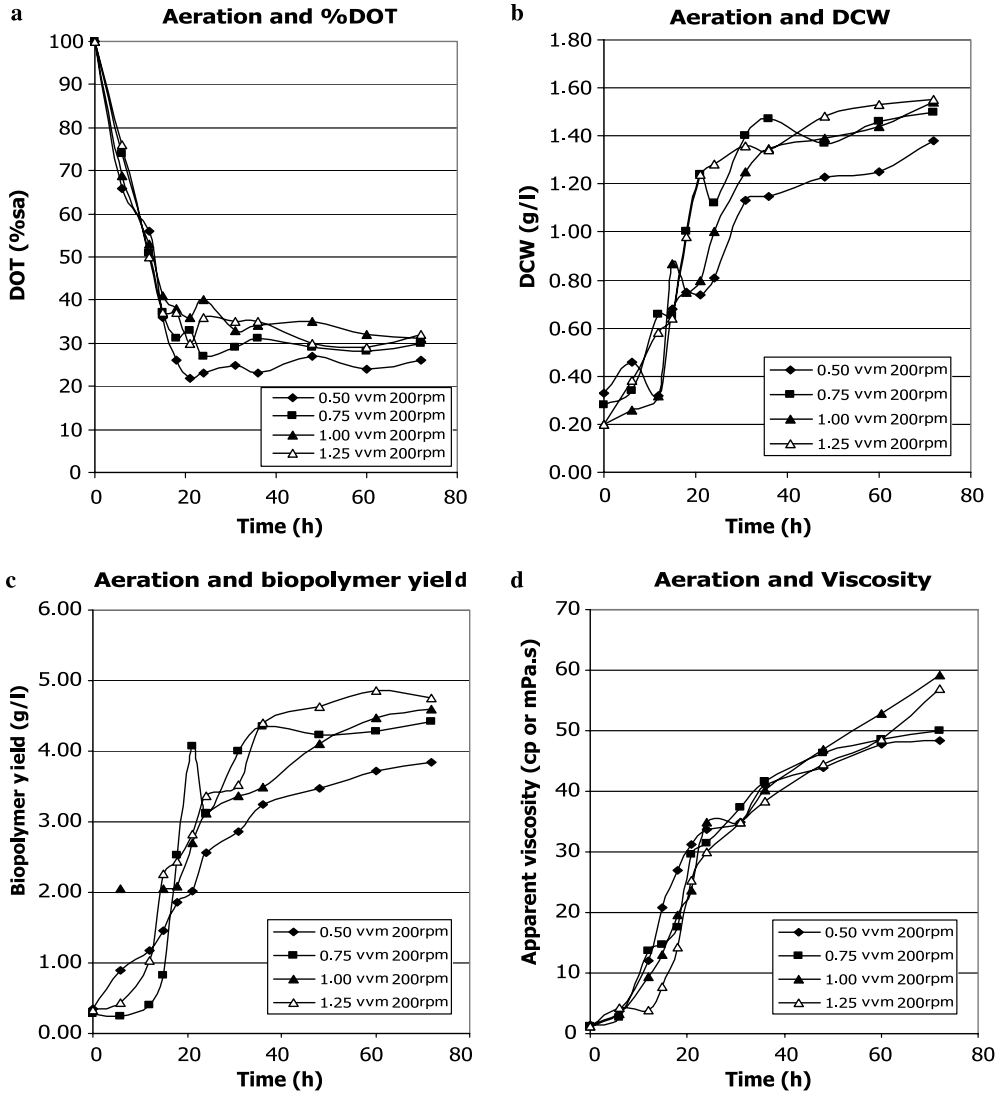

Fig. 1. Effect of aeration rate (0.5–1.25 vvm) on DOT (a), dry cell weight (b), biopolymer yield (c), and apparent viscosity (d) during cultivation of *E. cloacae* WD7 in a 5 L fermentor containing the optimal medium at 30 °C, 3 days and pH 7.0.

transfer especially in high viscous broths. At too high and too low speeds, the dissolved oxygen diffusion into the viscous broth was other than optimal. The fully mixed fermentor with the effective impeller is efficient on heat and mass transfer under fluid rheological behavior of EPS production. Therefore, the type of impeller may impact on various transport phenomena throughout the fermentor, for example, disc turbines having high velocity heads that give good small bubble formation but poor mixing in highly viscous systems.

3.2. Effect of aeration and agitation rates on DCW at bench scale

In the exponential phase, the DCW values did not vary in the tested ranges of aeration (0.75, 1.00, 0.75, and 0.98 g/L at 0.5, 0.75, 1.00, and 1.25 vvm, respectively) (Fig. 1b), but decreased when the agitation rate increased (0.98, 0.85, 0.76, and 0.67 g/L at 200, 400, 600, and 800 rpm, respectively) (Fig. 2b). In the stationary phase, the DCW increased with aeration rate increase (0.81, 1.12, 1.00, and 1.28 g/L at 0.5, 0.75, 1.00, and 1.25 vvm, respectively) (Fig. 1b), but became decreased at high agitation rate (1.28, 1.03, 0.91, and 0.89 g/L at 200, 400, 600, and 800 rpm, respectively) (Fig. 2b). Thus, the more the increase in aeration rates, the higher the DCW, but it decreased when agitation rates increased.

By consideration of Fig. 2b, the highest DCW value was found at 200 rpm stirring, but the DCW at higher speed (400–800 rpm) was reduced due to shear force and mixing effects. In the exponential phase, some growing cells became separated from medium and became stuck to the glass vessel walls over the broth at a too high stirring rate – this caused heterogeneous mixing (poor mixing). However, this did not happen in the stationary phase since cells were restricted from movement by the high viscosity of the broth. But DCW values decreased at high shear speed giving high shear stress that had influences on both the

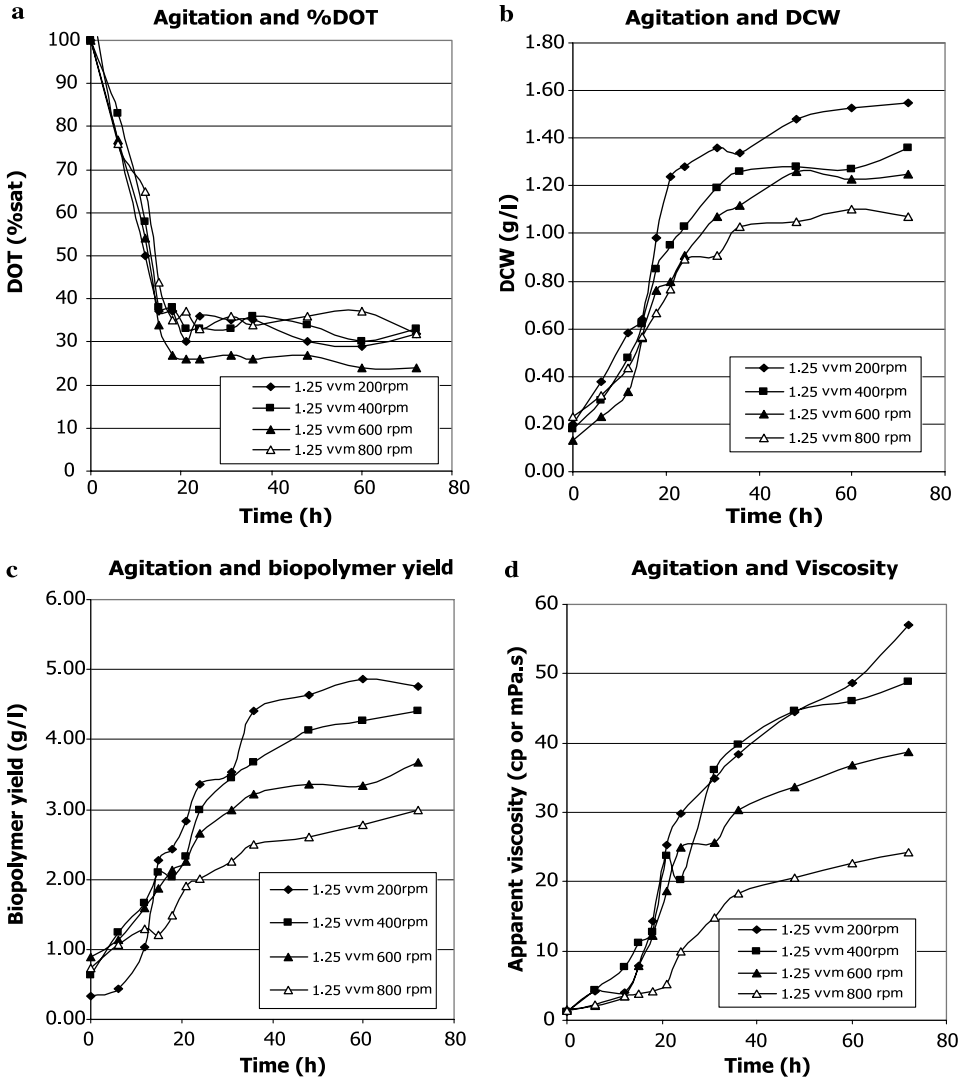

Fig. 2. Effect of agitation rate (200–800 rpm) on DOT (a), dry cell weight (b), biopolymer yield (c), and apparent viscosity (d) during cultivation of *E. cloacae* WD7 in a 5 L fermentor containing the optimal medium at 30 °C, 3 days and pH 7.0.

oxygen transfers and cell activities in the system (Gibbs & Seviour, 1996).

Shear rate is one of the indispensable parameters being used in the design of aerobic fermentor for viscous non-Newtonian systems. Vigorous mixing at high agitation speed always decreases the oxygen transfer that caused the cell growth and activities inhibition. High shear fields resulting from the fluid physical properties and hydrodynamics cause damage to fragile microorganism and reduced product formation (Al-Masry, 1999). Inappropriate agitation rates can inhibit cell growth because of both the heterogeneous mixing and shear stress effect. Hewitt, Caron, Axelsson, McFarlane, and Nienow (2000) reported that small-scale well-mixed fermentation gave the highest biomass yield, but surprisingly, the lowest cell viability for *Escherichia coli*.

Oxygen supply into the broths constitutes one of the decisive factors for cultivated microorganism growth and can play an important role in scale-up of aerobic biosynthesis systems. At constant agitation speed (200 rpm), as the aeration rate increased, higher DCW values were found as shown in Fig. 2b. The highest DCW yield (1.55 g/L) was obtained at the maximum rate of aeration (1.25 vvm). Oxygen contents in the cultivation should have been enough to supply demand by the microorganism tested but may be supplied at higher content if it has no effect on the microorganism and be non-sensitive on oxygen toxic due to too high oxygen concentration. Thus, not surprisingly, the growth and biopolymer production of *E. cloacae* WD7 were found at 1.25 vvm (maximum rate in this work), while they were found at 2 vvm (maximum rate in 3 L fermentor with 1.8 L working volume with the same conditions, medium and control) by Wichienchot (2000). The aeration efficiency depends on oxygen solubility and diffusion rate into the broths on the bioreactor capacity to satisfy the oxygen demand of microbial population (Galaction et al., 2004).

Cell growth and product formation should be related to both aeration rate and agitation at different rates,

especially under viscous conditions, to achieve higher yields. Galaction et al. (2004) reported that aeration with optimal turbulence agitation may counteract the increase of blocking effect and biomass coalescence in the high viscosity broth with non-Newtonian behavior. Too high biomass may influence growth and oxygen transfer, both by reducing the oxygen solubility in media – especially surface aeration, and by blocking the aeration and agitation system. The contribution of surface aeration to the total mass transfer of oxygen for some bacterial fermentation was about 15–20 times lower than for viscous broth without biomass.

3.3. Effect of aeration and agitation rates on biopolymer production at bench scale

In this work, the biopolymer formation at bench scale of all experiments increased when aeration rate increased (3.85, 4.43, 4.60 and 4.76 g/L at 0.5, 0.75, 1.00, and 1.25 vvm, respectively) (Fig. 1c). On the other hand, they decreased when agitation speed increased (4.76, 3.80, 3.67, and 3.00 g/L at 200, 400, 600, and 800 rpm, respectively) (Fig. 2c). Increase of aeration rate and decrease of agitation rate elevated the biopolymer yield, extrapolating the results of DCW values. This is a typical relationship showing the greater the increase in cell growth, the greater the increase in the product formation. Maximum biopolymer yield (4.76 g/L or 3.07 g/g DCW) was found at 1.25 vvm and 200 rpm (maximum aeration rate and minimum agitation rates in the test ranges, respectively). This is similar to previous report by Wichienchot (2000), the highest biopolymer yield (4.80 g/L) of *E. cloacae* WD7 cultivation was obtained at 2.0 vvm and 200 rpm (maximum aeration rate and minimum agitation rate). This shows the results obtained are different from those of Wichienchot (2000) who studied range of aeration rate in 3 L fermentor (c.f. 5 and 72 L fermentors in this work). This is due to the difference in sizes of vessel and working volumes of fermentation with different ranges of aeration rate. Thus, with the same aeration and agitation system, the maximum aeration rate was studied by control at maximum rate of 1.25 vvm for the 5 L fermentor used in this study, while it was 2 vvm for the 3 L fermentor used by Wichienchot (2000).

Oxygen transfer into microbial cells in aerobic fermentation processes strongly affects product formation by influencing metabolic pathways and changing metabolic fluxes (Çalik et al., 2000). Therefore, oxygen transfer in a fermentor depends on microorganism physiology and bioreactor efficiency. Most laboratory-scale stirred-tank fermentation systems are fitted with high speeds which impart a high shear stress on the medium, and this is known to reduce metabolite yields, such as in *Penicillium chrysogenum*, *Aspergillus* species, especially, pullulan EPS of *A. pullulans* (Gibbs & Seviour, 1996). High EPS yields were obtained under the combined conditions of low shear stress and optimal dissolved oxygen concentration. The biosynthesis of bacterial cellulose was mainly dependent on the energy compounds derived from respiration, i.e., processing (by oxidation in glycolysis or TCA cycle or other metabolisms) of macromolecular compounds (carbohydrate, protein, lipid) in the nutrient that can give the energy compounds such as AMP, ADP, ATP, etc., which are used in metabolism, activity or product formation of cells, and correlated with OUR (Kouda et al., 1997). Thus, at a too high speed of agitation, the biopolymer yields and cell growth decrease if OUR is unsuitable, although the efficiency and ability of oxygen transfer increases.

A reduction in metabolite production at high agitation rate has also been blamed on the cell deformation and damage suffered as a result of exposure to high shear rates. Therefore, the extreme flow and stress condition may inhibit the growth and activity of microorganisms by blocking the nutrient transfer and consumption of cells during growth. Therefore, the excessive oxygen supply caused the decrease in bacterial cellulose productivity because of a loss of substrate by direct oxidation. In addition, a too high agitation rate resulted in no mixing or slow movement of highly viscous broth outside of the stir zone, ultimately causing product decrease. However, the actual oxygen transfer to the cells would be reduced at low agitation rate that caused the large air-bubble size and poor bubble break-up, leading also to some viscous, non-stirred zones' formation (Gibbs & Seviour, 1996). Consequently, the OTR related to aeration rate and bioreactor performance and OUR related to agitation rate. Consequently, the OTR related to aeration rate and bioreactor performance but the OUR related to agitation rate, mixing and shear stress, while both rates had effects on growth and activities of cells.

3.4. Effect of aeration and agitation rates on viscosity at bench scale

Viscosity values increased if aeration rate increased (48.40, 50.70, 57.00, and 59.20 mPa s at 0.5, 0.75, 1.00, and 1.25 vvm, respectively) as presented in Fig. 1d, but decreased if agitation rate increased (59.20, 48.80, 43.80, and 24.30 mPa s at 200, 400, 600, and 800 rpm, respectively) as presented in Fig. 2d. Increase of broth viscosity from fast-growing microorganisms, high biomass and biopolymer lead to oxygen limitation or broth heterogeneity and act as a diffusion barrier. Therefore, if oxygen is a limiting nutrient in the processes and the transfer of oxygen from liquid phase to cells also is difficult, the product yield and the growth of microorganism will be affected directly. The rate of dissolved oxygen supply should be at least equal to the rate of oxygen demand. Parameters should be studied to obtain maximum biopolymer yield. Hence these parameters, OTR and OUR, were studied to in this work obtain maximum biopolymer yield.

Agitation rate has a direct effect on mixing which is an important factor that reduces the effects of high viscosity and increases the oxygen solubility and transfer, including enhancing the cells' assimilation of dissolved oxygen and

product formation at optimal stirring rate. In some CSTR, since the distance between some parts of the culture broth volume and the impellers was large, the homogeneous mixing was difficult to achieve in the high viscous culture broth furthest from the impeller (Kouda et al., 1997). Turbulent flow at high agitation rate with high viscosity in impeller zone was more than in the bulk zone (Wernersson & Trägårdh, 1998). In the present study, the highest shear rates were encountered in the bottom zone of the vessel; the shear rate was high in the impeller zone and low in the zone furthest from the impeller. The sluggish flow of highly viscous broth was found at agitation rates higher than 600 rpm. Stagnant zones have also been known to be developed in many biopolymer cultivations, such as in fermentation of bacterial cellulose (Kouda et al., 1997), xanthan, and hydroxypropyl guar (Eickenbusch et al., 1995) and *Monascus* sp. J101 biopolymer (Kim, Kim, Oh, & Shin, 2002).

Shear rate is an important factor affecting the system viscosity, consequently vigorous mixing at high agitation speed is always used in viscous cultivation to increase the oxygen transfer and cause the inhibition of cell growth and activities with shear effect (Al-Masry, 1999). Therefore, a too high rate of aeration and agitation causes a flooding phenomenon (a decrease in viscosity with increasing shear) around the impellers and destroys the pseudoplastic product. High viscosity induces bubble coalescence and the mass-transfer characteristics deteriorate, while the oxygen mass transfer may become rate-controlling in aerobic cultivations (Eickenbusch et al., 1995; Kim et al., 2002). Consequently, the aeration and agitation had more effect on oxygen transfer in WD7 EPS fermentation due to having high viscosity and pseudoplastic characteristic.

3.5. Effect of aeration and agitation rates on OTR and OUR at bench scale

An example of an experimental graph obtained from dynamic gassing-out technique during the exponential phase of fermentation at 1.25 vvm, 200 rpm of *E. cloacae* WD7 cultivation in the 5 L fermentor, is shown in Fig. 3. The decline of initial curve showed the decrease of dissolved oxygen content in the fermentor (due to oxygen consumption of cells, OUR value) when air supply was stopped. The later part of the curve showed increase of dissolved oxygen content in the fermentor due to the air supply being restarted while the oxygen still was consumed by cells, indicating the change of oxygen content in the fermentor (dCo/dt value). Decrease of dissolved oxygen in the fermentor during cell oxygen consumption took a shorter time than the oxygen diffusion after restart of oxygen supply and both values at exponential phases took shorter times than those in stationary phase. These indicated that oxygen transfer was difficult in viscous broth. The effectiveness of oxygen transfer (OTR value) can be considered from OUR and dCo/dt values determined by Eq. (3). These parameters are important for determination of the

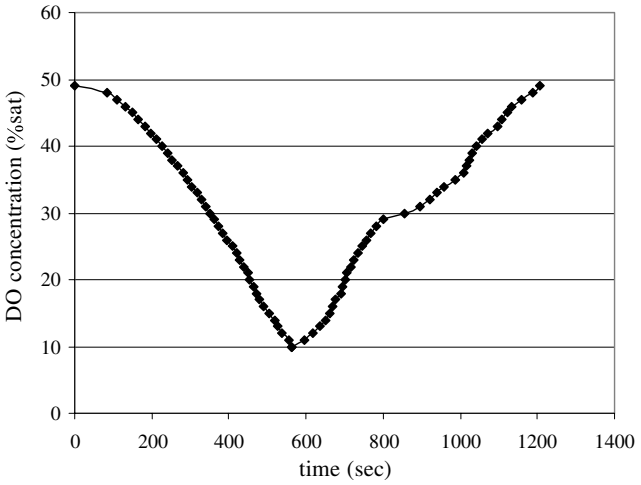

Fig. 3. Experimental graph obtained during dynamic gassing-out technique for the OUR and OTR determination during exponential phase of *E. cloacae* WD7 fermentation at 1.25 vvm, 200 rpm in 5 L fermentor.

efficiency of oxygen transfer and are widely used as the aeration and agitation controls in aerobic processes.

The OUR and OTR values in exponential and stationary phases at various aeration and agitation rates are summarized in Fig. 4, respectively. At various aeration rates tested (0.5–1.25 vvm), the OUR values were 6.47–6.90 and 4.67–5.07 ($\times 10^{-5}$ mol/L h), whereas the OTR values were 9.70–10.70 and 6.17–6.98 ($\times 10^{-5}$ mol/L h), in exponential and stationary phases, respectively. The OTR values of both phases were higher than OUR values indicating the oxygen supply was enough for cultivation. The OUR and OTR values during exponential phase were slightly higher than during stationary phase because the supplied oxygen was used for both cell growth and product formation, and it was not interrupted by viscosity as found in stationary phase. The OUR and OTR values increased with aeration rate increasing, the highest values were 6.90 and 5.07 ($\times 10^{-5}$ mol/L h) for OUR and 10.69 and 6.9 ($\times 10^{-5}$ mol/L h) for OTR in exponential and stationary phases, respectively, were found at the maximum aeration rate (1.25 vvm).

From Fig. 4, at various agitation rates tested (200–800 rpm), the OUR values in exponential and stationary phases were 6.79×10^{-5}–11.90×10^{-5} and 4.07×10^{-5}–7.14×10^{-5} (mol/L h), respectively, and the OTR values were 10.02–13.51 and 4.98–7.69 ($\times 10^{-5}$ mol/L h), respectively. The OUR and OTR values increased with agitation rate increase. The highest OUR and OTR values were found at the maximum speed (800 rpm) in both exponential and stationary phases. This showed that the increasing of agitation rate not only increased the OTR, but also increased the OUR value. It meant that if the oxygen was more transferred to the system it was more uptaken by cells. Consequently, the OTR also had an effect on OUR.

In this study, most OTR values were higher than OUR values in all runs resulting from enough of oxygen supply

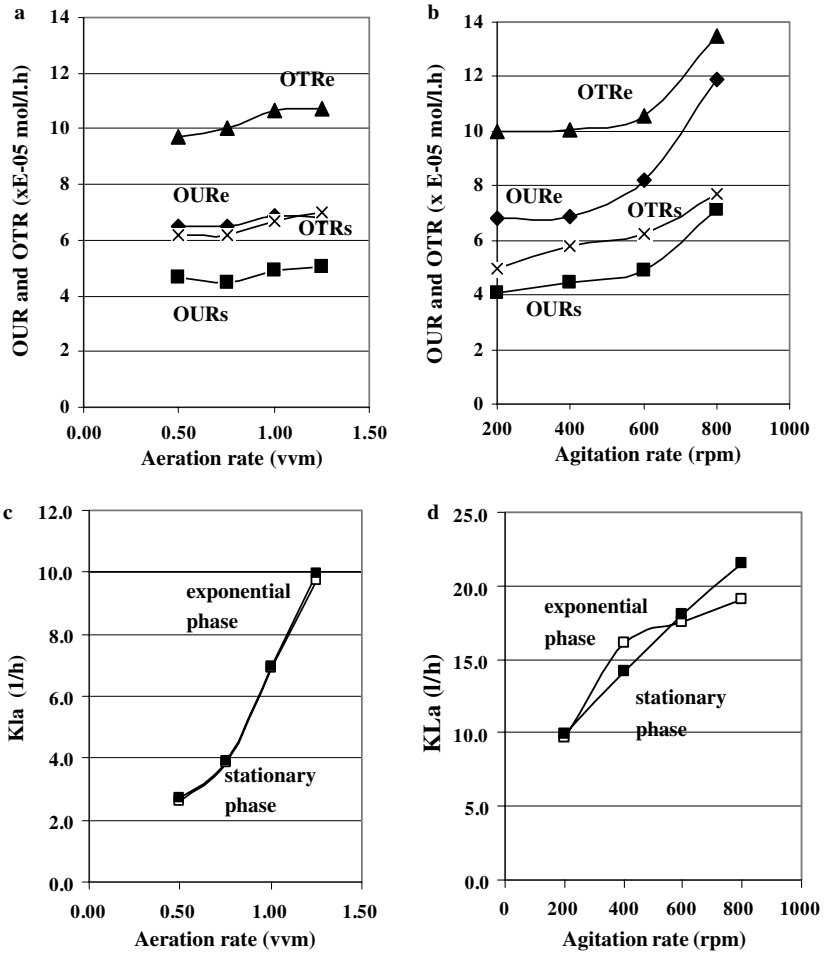

Fig. 4. Correlations of aeration and agitation rates on OUR and OTR (a and b) and k_La (c and d) during *E. cloacae* WD7 fermentation at bench scale (5 L fermentor) comparison between exponential, e (□) and stationary, s (■) phases.

for cultivation since the DOT did not drop lower than 10%. Rapid decrease of DOT in exponential phase was due to a high oxygen uptake rate by the cells. The OUR and OTR in all operations were slightly increased when the aeration and agitation rates increased, although the biopolymer yield in the stationary phase reduced with agitation rate increase. These indicated that *E. cloacae* WD7 can survive and has activity under oxygen-limiting conditions with high shear and viscosity. In contrast, the ESP produced by *A. tumefaciens* ID95-748 was strongly inhibited by oxygen limitation, with polymer yield decreasing from 9.40 to 2.06 g/L, and under non-limited oxygen conditions a polymer with higher molecular weight was produced.

Both OUR and OTR in the exponential phase were higher than in the stationary phase, with increasing of aeration rate and agitation rate (Figs. 4a and b). They were still high at 800 rpm, even so, the cells growth, biopolymer yield and broth viscosity were all less than for a lower agitation rate. Increasing of shear rate enhanced the OTR value, but it caused the diminution of cell growth and biopolymer yield by mechanical damage. However, the OUR values became increase for cell survival although the cells concentration decreased as found in aerobic cultivation of *Aureobasidium pullulans* (Gibbs & Seviour, 1996). However, the OUR in the viscous broth of *Monascus sp.* fermentation was decreased at high agitation rate (>500 rpm) while the DOT level was maintained as highly as 40%, despite the fact that the results for OTR were opposite to the OUR of cells. This suggests the presence of mechanical cell damage resulting from strong shear stress at high speed of agitation (Kim et al., 2002).

3.6. Effect of aeration and agitation rates on k_La at bench scale

The k_La values increased with aeration and agitation rate increase (Figs. 4c and d). At various aeration rates (0.5–2.0 vvm giving gas velocity of 0.0018–0.0047 m/s respectively), the k_La values were about 2.63–9.72 h^{-1} and 2.71–9.97 h^{-1} in the exponential and stationary phases, respectively. At various agitation rates, the k_La values were 9.72–19.10 h^{-1} and 9.97–21.54 h^{-1} in the exponential and stationary phases, respectively. The k_La values increased with agitation rate more than with aeration rate increase. Increase of agitation rate (500–1200 rpm) gave an increase of k_La value (approximately from 10 to

90 h^{-1}), investigated by using a static gassing-out technique, during fermentation for cellulose production by *Acetobacter xylinum* in aerated and agitated vessel (3 L) with 1.8 L working volume (Kouda et al., 1997). The k_La values of *Monascus sp.* fermentation increased gradually from 0.003 to 0.029 s^{-1} at rotational speed increase from 200 to 700 rpm (Kim et al., 2002).

The k_La values at optimal condition (1.25 vvm and 200 rpm) were 9.72 and 9.97 h^{-1} in the exponential and stationary phases, respectively. The k_La values increased with aeration rate increase in both phases (Fig. 4c). The k_La values in the exponential phase at high agitation rate seem to be depressed when compared with the results at stationary phase; this was due to OUR increase (Fig. 4d). These results indicated that agitation had more effect on k_La value than aeration especially during the exponential phase. In addition to aeration and agitation rates, many factors influenced the k_La values during fermentation, such as the mixing, broth viscosity, product formation, biomass content, etc. There are many factors influencing the OTR, OUR, and k_La values that were not considered in this study (i.e., distribution and size of air bubble, mixing delay time, shear force, and stress) as reported by others (Galaction et al., 2004) and they should be further studied subsequently.

3.7. Correlation of k_La values on biopolymer productivity at bench scale

In this work, the k_La values at bench scale increased with increasing of aeration and agitation rate, while cell growth and biopolymer yield decreased with agitation rate increase. Thus, the positive correlation of k_La values on biopolymer productivity is found with aeration effect, but negative correlation of k_La values on biopolymer productivity is found with agitation effect. These correlations were found to be typical in many studies of fermentation for production of biopolymers. Garćia-Ochoa et al. (2000) reported that the xanthan gum concentration increased when k_La value decreased at high agitation rate, but low productivity was found although the oxygen transferred into viscous broth increased. High shear stress (high agitation rate) affects the structure and property of biopolymer (i.e., viscosity) and depresses cell growth and activity. Consequently, the DCW and biopolymer yield were low due to a too high agitation rate and heterogeneous mixing, resulting in low viscosity. However, the EPS produced by *Aureobasidium pullulans* was reduced with increasing agitation rate but no shear forces occurred (Gibbs & Seviour, 1996).

Basically, the biopolymer productivity of most aerobic processes increased with the aeration rate and agitation speed increasing and the negative effect of agitation speed was stronger than aeration rate. This was anticipated in a mechanically agitated vessel since the agitation affected the rate of oxygen transfer more strongly than aeration (Elibol & Ozer, 2000). DO concentrations directly correlate with the OTR and k_La values. The data for k_La under various aeration rates and agitation speeds indicated a stronger dependence of OTR on agitation than on aeration; therefore, the productivity with increasing of k_La was a satisfactory correlation.

Mixing was another important factor for oxygen transfer and cell activity in the bioprocess and its scale-up. Therefore, mixing had a significant impact on the rate of biomass production and on product polysaccharide physical properties (Audet, Gagnon, Lounes, & Thibault, 1998). The mixing intensification may lead to a reducing of k_La and this effect is more pronounced at lower aeration rate and lower biomass concentrations, being the results of finest dispersion of air and, consequently, of easier adsorption of cells on to the bubble surfaces (Galaction et al., 2004). The increasing of k_La value is the result of bubble surfaces being blocked by cells adsorption, phenomena that can be described by means of the ratio between k_La value for viscous suspensions with and without biomass. The magnitude of blocking effect on k_La values also depends on microorganism type. Therefore, the k_La around the impellers is high since the mixing is appropriate while k_La low where mixing is heterogeneous (Kouda et al., 1997). Heterogeneous mixing was also found during WD7 biopolymer production at too high on agitation rate (>600 rpm) which affected the k_La value.

3.8. Effect of aeration and agitation rate on k_La at pilot plant scale

When the scale of operation is increased, the broth will become more and more heterogeneous and oxygen can be depleted in some areas of the reactor (Thiry & Cingolani, 2002). The supply of oxygen to the growing cells is usually the limiting operation in scale-up. In this study, the DOT values in all runs at pilot plant scale decreased in the exponential phase, then increased and kept constant in the stationary phase; i.e., the same as those found at bench scale. The residue DOT levels were high at high rates of aeration and agitation, with higher than 10% of DOT in all runs where there was sufficient oxygen support in the system.

The effects of aeration (0.5, 1.0, 1.5, and 2.0 vvm) and agitation rates (200, 400, and 600 rpm) on OTR, OUR, and k_La values were also studied at pilot plant scale. By the dynamic gassing-out technique, the OTR and OUR values increased with increasing aeration and agitation rates. Agitation rate had still more effect on these parameters than the aeration rate, and the k_La values at various rates of aeration and agitation are shown in Fig. 5.

The k_La values increased when agitation and aeration rates increased in both phases. With various aeration rates (1.25–2.00 vvm), the k_La ranges at 200 rpm were 9.25–10.67 and 9.09–10.18 h^{-1}, 11.51–16.75 and 10.21–14.57 h^{-1} at 400 rpm, while the maximum k_La values (21.16 and 18.11 h^{-1}) were found at 2.00 vvm and 600 rpm in the exponential and stationary phase, respectively. The maximum k_La value was still found at maximum rates of aeration and agitation and still higher in the exponential

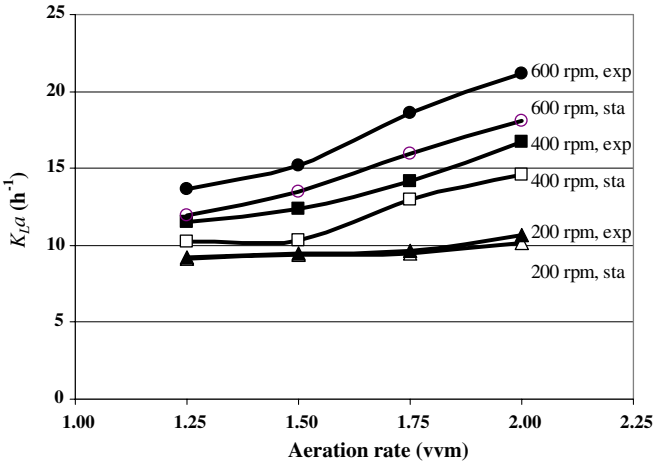

Fig. 5. Correlations of aeration and agitation rates on $k_L a$ (h^{-1}) during fermentation of *E. cloacae* WD7 on pilot plant scale (72 L fermentor) at exponential (exp) and stationary (sta) phases.

phase rather than stationary phase because of high OUR under high viscosity. By comparison, under the same conditions (1.25 vvm and 200 rpm) as the optimal condition at bench scale, the $k_L a$ values at pilot plant sale (9.25 and 9.09 h^{-1}) were lower than those at bench scale (9.97 and 9.72 h^{-1}) in exponential and stationary phases, respectively. Therefore, the $k_L a$ values at pilot plant scale (9.68 and 9.50 h^{-1}) found at 1.75 vvm, 200 rpm, were very close to the $k_L a$ value at optimal condition at bench scale (9.97 and 9.72 h^{-1}), therefore these were chosen for scale-up of WD7 biopolymer production.

3.9. Biopolymer fermentation at pilot plant scale

Fermentations at pilot plant scale were carried out for the massive production of WD7 EPS. Three batch cultivations at various conditions in the pilot plant fermentor were chosen for examples as follows: (a) 1.25 vvm and 200 rpm, an optimal condition from bench scale, (b) 2.00 vvm and 200 rpm, the aeration rate was increased while the agitation rate was maintained constant, and (c) 2.00 vvm and 600 rpm, the maximum rates of aeration and agitation at pilot plant scale. Time courses and some data of fermentations at pilot scale under these difference conditions are shown in Figs. 6 (a, b, and c). All time courses were similar characteristics to those for the bench scale, the DCW, biopolymer yield and viscosity concentration still increasing corresponding to cultivation time. Therefore, when aeration and agitation rates increased, in which the DCW values were 1.58, 1.65, and 1.67 g/L, the biopolymer yields were 3.07, 3.18, and 3.20 g/g, respectively, with corresponding viscosities of 66.8, 76.0, and 77.2 cp (mPa s), at operating conditions of (a), (b), and (c), respectively (Table 1). The best condition for highest biopolymer production at pilot plant scale was 2.00 vvm and 600 rpm, which differed from that at bench scale (at 1.25 vvm and 200 rpm). The maximum biopolymer yield was found at maximum aeration rate at both scales. The effect of mechanical shear rate on biopolymer production (as found at bench scale) was not found at pilot plant scale because of differences in geometric characteristics, impeller size and number and diameter ratio of impeller to fermentor. Thus, no effect of shear stress on cell growth and biopolymer production occurred at pilot plant scale in the aeration and agitation ranges tested.

3.10. Scale-up by $k_L a$ fixing

Since the $k_L a$ values at bench scale had differences from those at pilot plant scale and both fermentors had different geometric characteristics and agitation systems, the scale-up in this study was operated by fixing of $k_L a$ at bench and pilot scales to be equal. By comparison, the $k_L a$ values at pilot plant scale (ps) were 9.50 and 9.68 h^{-1} at 1.75 vvm and 200 rpm, which were much closer to those of bench scale (9.97 and 9.72 h^{-1}) at 1.25 vvm and 200 rpm in both exponential and stationary phases, respectively. These gave $k_L a$ ratios of 1.03 ($k_L a_{(ps)}/k_L a_{(bs)} = 9.97/9.68$) in exponential phase and 0.98 ($k_L a_{(ps)}/k_L a_{(bs)} = 9.50/9.72$) in stationary phase. In the successful scale-up for WD7 biopolymer production, biopolymer yield (3.20 g/g) was slightly higher than at bench scale (3.07 g/g) (Table 1). This is normally found in other scale-ups of biopolymer production due to different geometric characteristics with aeration and agitation systems (Flores et al., 1997; Galaction et al., 2004; Garćia-Ochoa et al., 2000).

In this work, although heterogeneity is usually found in viscous culture, no such problem was found in the tested ranges of variables that could not be solved easily by using appropriate agitation rate control. Using the appropriate geometry, stirrer and impeller types one can increase the oxygen supplement and reduce the extreme flow. At bench scales with only two impellers, the extreme flow caused serious stress conditions for microorganisms which were higher than for the pilot plant reactor with three impellers with six flat-blade type. Oxygen was highly transferred into the pilot plant system by the control of agitation with suitable mixing and without shear stress. Cell concentration and biopolymer yield at pilot scale were high, even so high values of OTR, OUR, and $k_L a$ were found.

From this study, in addition to the effects of agitation and aeration, there are also many factors affecting aerobic process and scale-up such as shear stress, mixing, growth of microorganism, product concentration and viscosity, which are important directly on oxygen transfer rate in different scales and on $k_L a$ determination (Hsu & Wu, 2002). These effects of environmental state variables need to be studied more on cultivation. If the fermentation system tested in a small-scale fermentor were insensitive to a wide range of these environment state variables, scale-up is not a problem. Therefore, the knowledge of how a cell reacts is needed so one can accurately predict the, process parameters, influence of environmental factors and product yield in scale-up (Hewitt et al., 2000).

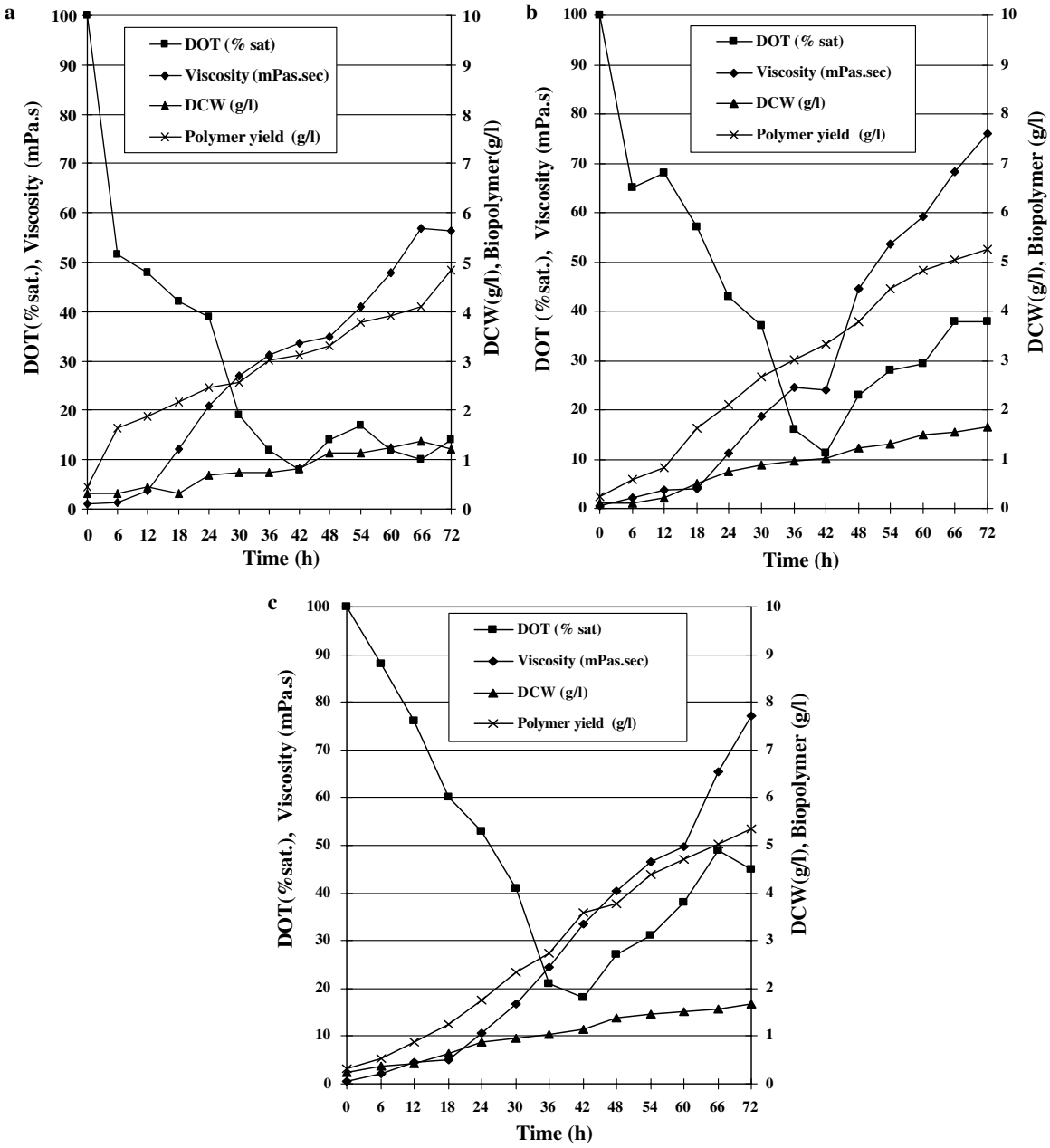

Fig. 6. Time courses of biopolymer production of *E. cloacae* WD7 in 72 L fermentor containing optimal medium at 30 °C, pH 7.0 and (a) 1.25 vvm and 200 rpm, the optimal condition from bench scale (b) 2.00 vvm and 200 rpm and (c) 2.0 vvm and 600 rpm (▲ = cell concentration, × = biopolymer concentration, ■ = dissolve oxygen tension, and ◆ = viscosity).

3.11. Study on flow properties of WD7 biopolymer

Rheological properties of the broth offer a high resistance to the mass transfer, the supply of oxygen to the growing cells usually being the limiting operation in scale-up. When scale is increased, the broth will become more and more heterogeneous and oxygen can be depleted in some areas of the reactor (Thiry & Cingolani, 2002). Characteristics and flow behavior of the viscous broth were studied in order to describe some of the phenomena found in EPS fermentation. The relationship of apparent viscosities and shear rate at various tested concentrations (0.1–0.5% w/v) of WD7 biopolymer is shown in Fig. 7, which indicates the viscosity decreased when shear rates increased. Table 2 presents the values of viscosity (3.18, 4.90, 5.34, 7.35 and 12.80 mPa s) at shear stress (419.76, 646.80, 704.88, 970.20 and 1,689.60 mPa), the K values of about 6.92, 20.41, 26.30, 38.02, and 83.18×10^{-3} Pa s and n values of about 0.85, 0.74, 0.68, 0.66, and 0.63, respectively. Thus, this WD7 biopolymer solution is a non-Newtonian fluid with pseudoplastic and shear thinning characteristics due to $n < 1$.

Normally, the substances in the pseudoplastic class are long molecules, randomly oriented and with no connected structure. These fluids need an efficient rate of agitation for overall mixing to homogeneity. The application of a

Table 1
Effect of various conditions on biopolymer fermentation of *E. cloacae* WD7 at 30 °C, pH 7 after 3 day cultivation in 72 L fermentor when comparison with results of cultivation at 5 L fermentor for scale-up consideration

Parameters	Fermentors			
	72 L			5 L
	1.25 vvm, 200 rpm	2.00 vvm, 200 rpm	2.00 vvm, 600 rpm	1.25 vvm, 200 rpm
Final cell concentration (g/L)	1.58	1.65	1.67	1.55
EPS concentration (g/L)	4.86	5.25	5.35	4.76
EPS yield based on biomass, Yp/x (g/g)	3.07	3.18	3.20	3.07
Apparent viscosity (mPa s)	68.8	76.0	77.2	59.20
K_La at exponential phase (h^{-1})	9.09	9.50	18.11	9.72
K_La at stationary phase (h^{-1})	9.25	9.68	21.16	9.97

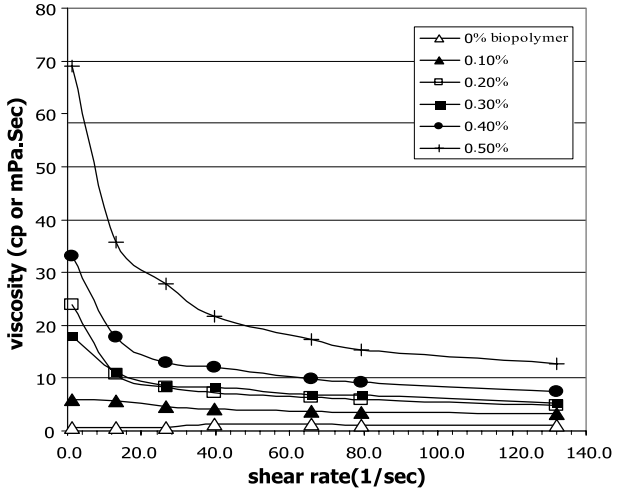

Fig. 7. Relationship between shear rate (s^{-1}) and viscosity (mPa s) of WD7 biopolymer samples at various concentrations (0–0.5% w/v) by Brookfield viscometer.

Table 2
The *K* values (consistency index) and *n* values (flow index) at various concentrations of WD7 biopolymer obtained from curves of log shear rate vs log shear stress

EPS concentration (%)	Viscosity ($\times 10^{-3}$ Pa s)	*K* values ($\times 10^{-3}$ Pa s)	Flow index (*n*)
0	1.14	4.9	1.19
0.1	3.18	6.92	0.85
0.2	4.90	20.41	0.74
0.3	5.34	26.30	0.68
0.4	7.35	38.02	0.66
0.5	12.8	83.18	0.63

shearing stress tends to align the molecules so that the viscous resistance between the adjacent layers diminishes, resulting in a progressive fall in viscosity as the shear rate increases. Therefore, the shear rate causes the stress that inhibits cell growth and biopolymer production (Audet et al., 1998) as was also found in this study.

4. Conclusions

Cell growth and biopolymer yield of *E. cloacae* WD7 under aerobic fermentation increased with increasing aeration and agitation rates at pilot plant scale (72 L), but decreased when agitation increased at bench scale (5 L), because of shear thinning. The highest biopolymer yield at bench scale (3.07 g/g) was found at optimum condition (1.25 vvm and 200 rpm), but the increase of biopolymer yield (3.20 g/g) was found at 2 vvm and 600 rpm in pilot plant cultivation. The OTR was higher than OUR in both phases indicating adequate oxygen supply in both systems. The OUR and OTR values in exponential phase were lower than in the stationary phase due to having high concentrations of cell and biopolymer with high viscosity. The k_La values at both scales increased with increasing of aeration and agitation rates. At 1.75 vvm and 200 rpm, the k_La values in exponential and stationary phases at bench scale were 9.97 and 9.72 h^{-1}, which were very close to those of pilot plant scales (9.68 and 9.50 h^{-1}), respectively. Under these conditions, scale-up differences in geometry and stirrer types were successfully overcome by k_La fixing (with ratio of k_La fixing 1.03 and 0.98 in exponential and stationary phases, respectively) giving biopolymer yields of 3.07 and 3.20 g/g per gram of DCW (dry cell weight) at bench and pilot plant scales, respectively, without system failure.

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