Kinetic modeling of biodiesel production by mixed immobilized and co-immobilized lipase systems under two pressure conditions

Jong Ho Lee*, Sung Bong Kim*, Hah Young Yoo*, Ja Hyun Lee*, Chulhwan Park**,
Sung Ok Han***, and Seung Wook Kim*,†

*Department of Chemical and Biological Engineering, Korea University, Seoul 136-701, Korea
**Department of Chemical Engineering, Kwangwoon University, Seoul 139-050, Korea
***College of Life Science and Biotechnology, Korea University, Seoul 136-701, Korea

Abstract−A kinetic model of mixed immobilized lipase (MIL) and co-immobilized lipase (CIL) systems was investigated by calculating the kinetic parameters based on the reaction mechanisms for lipase-catalyzed transesterification of soybean oil and methyl alcohol. The kinetic parameters were assessed under atmospheric and supercritical fluid conditions. Although the CIL system had a higher initial reaction rate, the effect of substrate inhibition by methanol was higher than that in the MIL system. The initial reaction rate of MIL and CIL decreased under atmospheric conditions as the methanol concentration increased. However, the initial reaction rate of MIL and CIL increased until methanol concentration increased to twice that of oil under the supercritical fluid condition. As a result, the inhibition effect by methanol was identified through a kinetic analysis. A simulated model can be used to predict the optimal conditions for biodiesel production under atmospheric and supercritical conditions.

Key words: Kinetic Model, Lipase, Enzyme Immobilization, Co-immobilization, Transesterification

INTRODUCTION

Lipase application for hydrolysis or esterification reactions has been studied for many years. Various esters are produced from a variety of alcohols and acids by lipases. In particular, non-aqueous media lipases catalyze a wide range of reactions such as alcoholysis, transesterification, ester synthesis, and asymmetric and regiospecificity acylation reactions [1-4]. Transesterification is a reaction in which triglycerides are reacted with short-chain alcohols such as methanol and ethanol. The reaction is composed of three stepwise reactions with intermediate formation of diglycerides and monoglycerides, resulting in the production of fatty acid methyl ester and glycerol (Fig. 1) [5,6].

The alkaline or acid catalysts form a well known method for bio-

diesel production. However, they are energy intensive, glycerol recovery is difficult, and the catalyst could be eliminated from the product. Additionally, transesterification using alkaline catalysts has low selectivity, leading to undesirable side reactions [7-10]. Therefore, many investigators have intensively studied enzymatic processes to solve these problems for biodiesel production. Actually, transesterification using lipases can overcome chemical processing problems [7-17]. However, transesterification using lipases has a fatal disadvantage of a slow reaction rate due to the acyl migration step and mass transfer limitation. Our previous studies investigated various systems to overcome the slow reaction such as mixed immobilized lipase (MIL) and co-immobilized lipase (CIL) systems under various conditions. However, despite these studies, no reports have considered the kinetics and mechanisms of MIL and CIL systems under various condi-
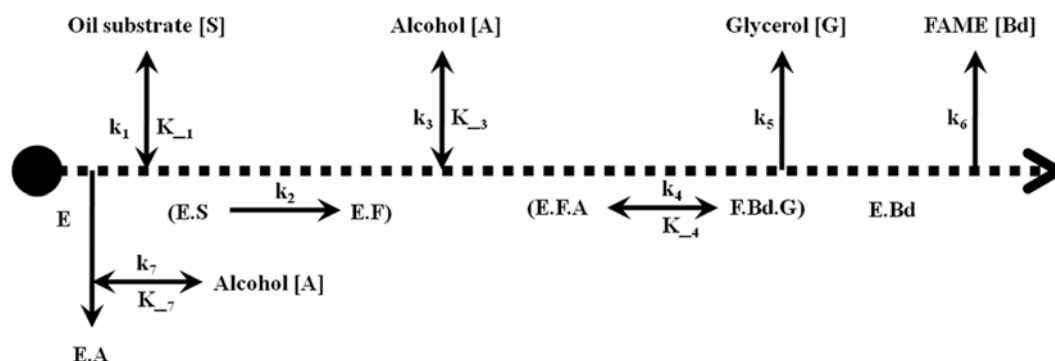

Fig. 1. Graphical representation of reaction mechanism steps of transesterification.

†To whom correspondence should be addressed.
E-mail: kimsw@korea.ac.kr

tions. Therefore, we investigated kinetic parameters and modeling of the MIL and CIL systems under atmospheric and supercritical conditions in this study. Moreover, the kinetics model was verified with experimental results of transesterification by the MIL and CIL systems under various conditions.

MATERIALS AND METHODS

1. Materials

3-Aminopropyltriethoxysilane (3-APTES) and 3-(*N*-morpholino) propane-1-sulfonic acid (MOPs) were purchased from Sigma (St. Louis, MO, USA). Glutaraldehyde was obtained from Fluka (Schweiz, Switzerland), and the silica gel was purchased from Grace Davison (Deerfield, IL, USA). *Candida rugosa* and *Rhizopus oryzae* lipases were purchased from Sigma and Fluka, respectively. All other chemicals were of reagent grade.

2. Lipase Preparation

C. rugosa and *R. oryzae* lipases (1 g) were suspended in 100 ml of a 0.25 M mixture of MOPs-sodium phosphate buffer (pH 6.5). The lipase solutions were centrifuged at 4,000 rpm for 15 min at 4 °C, and the supernatants were stored at 4 °C prior to immobilization [11-13].

3. Lipase Immobilization

One gram of dry silica gel was mixed with 10% 3-APTES in 20 ml acetone and incubated at 50 °C for 2 hr with constant mixing. The silica gel was then washed with water and dried at 60 °C for 2 hr. The dried silica gel was then suspended in 20 ml of 1 mM phosphate buffer solution (pH 7). A 25% (w/v) solution of glutaraldehyde (2 ml) was added to the mixture and incubated at 20 °C for 2 hr to activate the silica gel. The activated gel was then washed with water and dried at 60 °C for 2 hr. Prior to immobilization, *C. rugosa* and *R. oryzae* lipases were pretreated with 0.1% soybean oil at 40 °C with stirring at 150 rpm for 45 min. Activated silica gel (500 mg) was mixed with 10 ml of pretreated lipase solution and then incubated at 20 °C to immobilize the lipase. In the MIL system, 0.5 g of immobilized *C. rugosa* lipase on silica gel and 0.5 g of immobilized *R. oryzae* lipase were uniformly mixed. For co-immobilization, 1 g of activated silica gel was mixed with 10 ml of pretreated *C. rugosa* and 10 ml of pretreated *R. oryzae* lipase solutions simultaneously. The immobilized lipase was recovered by filtration, washed with water, and then dried overnight at room temperature [11-16].

4. Biodiesel Production by Mixed *C. rugosa* and *R. oryzae* Lipase and Co-immobilized Lipases under Two Conditions

Biodiesel was produced by using a mixture of immobilized *C. rugosa* and *R. oryzae* lipases and a co-immobilized system under various conditions. Under the atmospheric condition, 2-60 mmol of soybean oil and 20% immobilized and co-immobilized lipases were used for biodiesel production, and 2-180 mmol of methanol was initially added to the reaction medium. The reaction was carried out under optimal conditions (temperature, 45 °C; agitation speed, 300 rpm; and water content, 10%). Under supercritical fluid conditions, the reactants, which included 10-60 mmol of soybean oil with methanol, were loaded into the reactor and mixed with 20% immobilized lipases, and the reactor temperature was increased to 45 °C, which also increased the pressure to 130 bar. After the reaction was completed, the reactor was depressurized, and the products were

eluted in hexane. Next, the enzyme was removed by centrifugation and the samples were analyzed by gas chromatography (GC) [11-16].

5. Analytical Method

Biodiesel was analyzed by using a GC M600D (Younglin. Co. Ltd., Seoul, Korea) with an HP-Innowax 1909IN-133 column (30 m× 25 μm, Agilent Technologies, Santa Clara, CA, USA). Samples were collected from the reaction mixture and then centrifuged to obtain the upper layer. One micro liter of the treated sample was injected into the GC, and the column temperature was raised from 150-180 °C at a rate of 15 °C min^{-1}, and then from 180-240 °C by increasing the temperature at a rate of 5 °C min^{-1}, after which the temperature was maintained at 240 °C for 1 min. The injector and the detector temperature were both set at 260 °C [11-16].

6. Kinetic Modeling

Most previous studies on lipase-catalyzed transesterification of triglycerides have been described by the Ping Pong Bi Bi and Michaelis-Menten kinetic models with competitive inhibition by the alcohol [9,17-20]. Moreover, as mentioned by Al-Zuhair, all steps except deacylation of the lipase were assumed to be in a quasi-steady state [1]. Based on these reaction mechanisms, the transesterification reaction rate can be written as Eq. (1):

$$V = k_6[E \cdot Bd] = \frac{V_{max}[S]}{1 + K_{iS}[S] + \dfrac{K_S}{[S]}\left(1 + \dfrac{[A]}{K_{iA}}\right) + \dfrac{K_A}{[A]}} \quad (1)$$

where V is the initial reaction, V_{max} is the maximum initial reaction rate, K_S and K_A are the binding constants for the oil and alcohol, respectively, and K_{iS} and K_{iA} are the dissociation constants for i from the specific enzyme form-inhibitor complex. K_S, K_A, K_{iS}, K_{iA}, and V_{max} values were calculated by measuring the initial reaction rate of the MIL and CIL systems under atmospheric and supercritical conditions.

RESULTS AND DISCUSSION

1. Determination of Kinetic Parameters

One of the most important characteristics of an enzyme is substrate affinity, usually assessed as apparent K_S, K_A, K_{iS}, and K_{iA} with respect to specific substrates. When an enzyme is immobilized or co-immobilized, variations in kinetic parameters such as K_S, K_A, K_{iS}, K_{iA}, and V_{max} are observed because of the short mass transfer distance. Moreover, the kinetic parameters can fluctuate markedly due to high steric hindrance and diffusional effects under the supercritical fluid condition [21-24]. Therefore, we studied the kinetic parameters to confirm the optimum conditions. When the values of these kinetic parameters were substituted in Eq. (1), the maximum reaction rate was estimated under the two conditions. Table 1 shows the numerical values of the kinetic parameters in the MIL and CIL systems under the two conditions.

V_{max} of the CIL system was generally higher than that of the MIL system under atmospheric and supercritical conditions, indicating that the CIL system had higher transesterification activity than that of MIL system under the same conditions. Moreover, the K_{iA} value of the MIL system was higher than that of the CIL system, whereas the K_A value of the MIL system was lower than that of the CIL system. These results show that the inhibitory effect of the alcohol in the MIL system was lower than that of the CIL system. However,

Table 1. Numerical values of the kinetic parameters on MIL and CIL systems under atmospheric and supercritical conditions

Parameter	Atmospheric condition		Supercritical condition	
	MIL system	CIL system	MIL system	CIL system
V_{max} (mmol/s)	0.00299	0.00317	0.06900	0.12630
K_S (mmol/L)	0.35000	0.10000	0.09800	0.06930
K_{iS} (mmol/L)	1.00002	0.99998	1.00180	0.99967
K_A (mmol/L)	0.05000	0.08000	0.27500	0.70559
K_{iA} (mmol/L)	0.03144	0.00927	0.00453	0.00211

MIL: mixed immobilized lipases
CIL: co-immobilized lipases

because co-immobilized lipases are proximally located, high activity and maintenance occurred. As expected, the CIL system activity was about five-times higher and maintenance of the CIL system increased 1.6 times compared to those in the MIL system. The K_A value under the supercritical condition was much higher than that under the atmospheric condition. Moreover, the K_{iA} value under the supercritical condition also decreased markedly. These results suggest that lipase activity and the inhibitory effect depend on mass transfer. Despite the increased inhibition effect by the improved mass transfer, activity can be increased much higher than the inhibitory effect [13,20-24].

2. Comparison of Simulation Results and Experimental Data on Biodiesel Production under Atmospheric and Supercritical Conditions

The simulation results and experimental data were compared to investigate the suitability of the kinetic parameters. Fig. 2 shows the derivation of the simulation results and experimental values for establishing the MIL and CIL systems under the two conditions. The
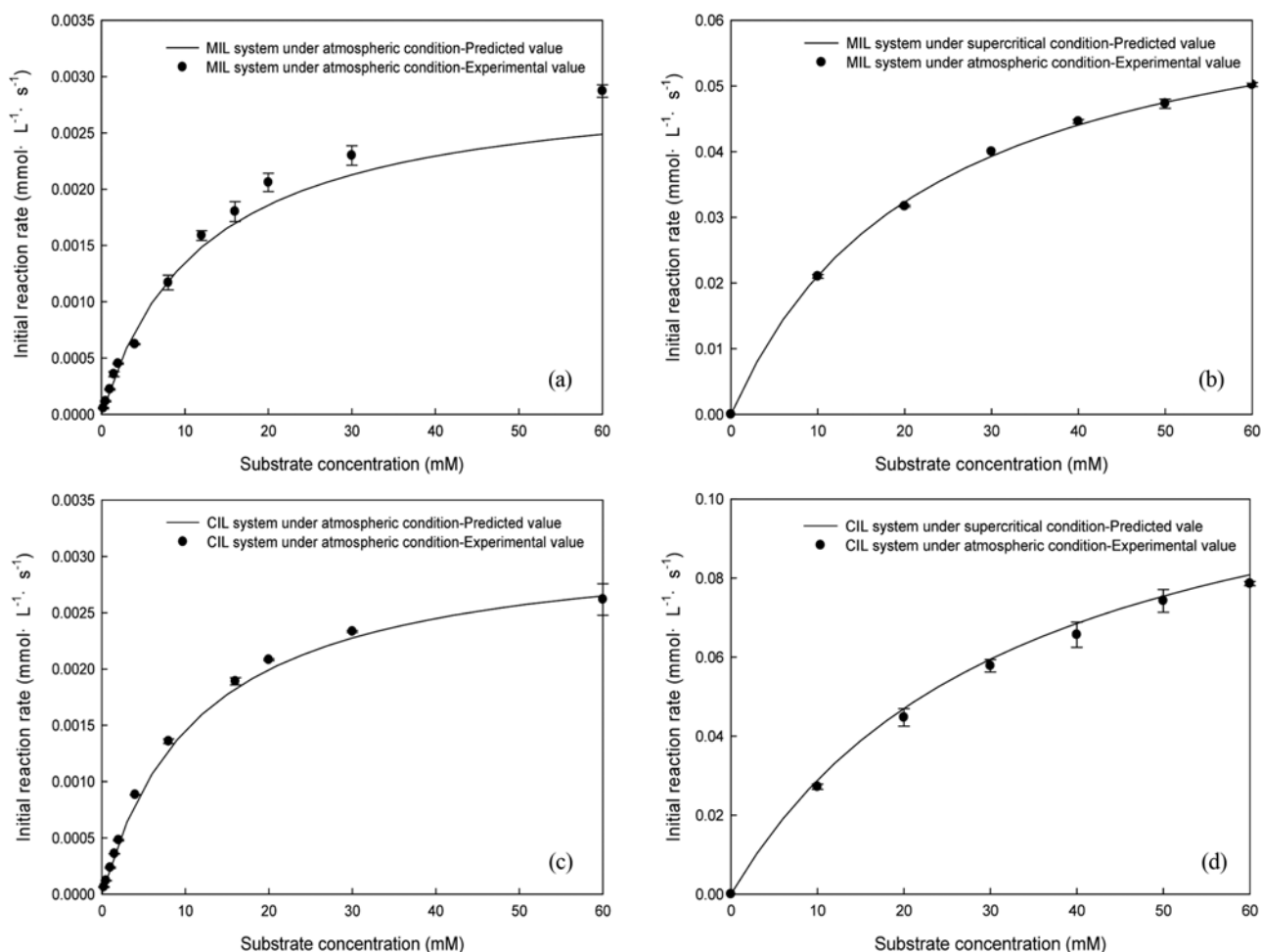

Fig. 2. Comparison of simulation results and experimental data on initial reaction rate by MIL and CIL systems under atmospheric and supercritical condition: (a) MIL system under atmospheric condition, (b) MIL system under supercritical condition, (c) CIL system under atmospheric condition, (d) CIL system under supercritical condition.
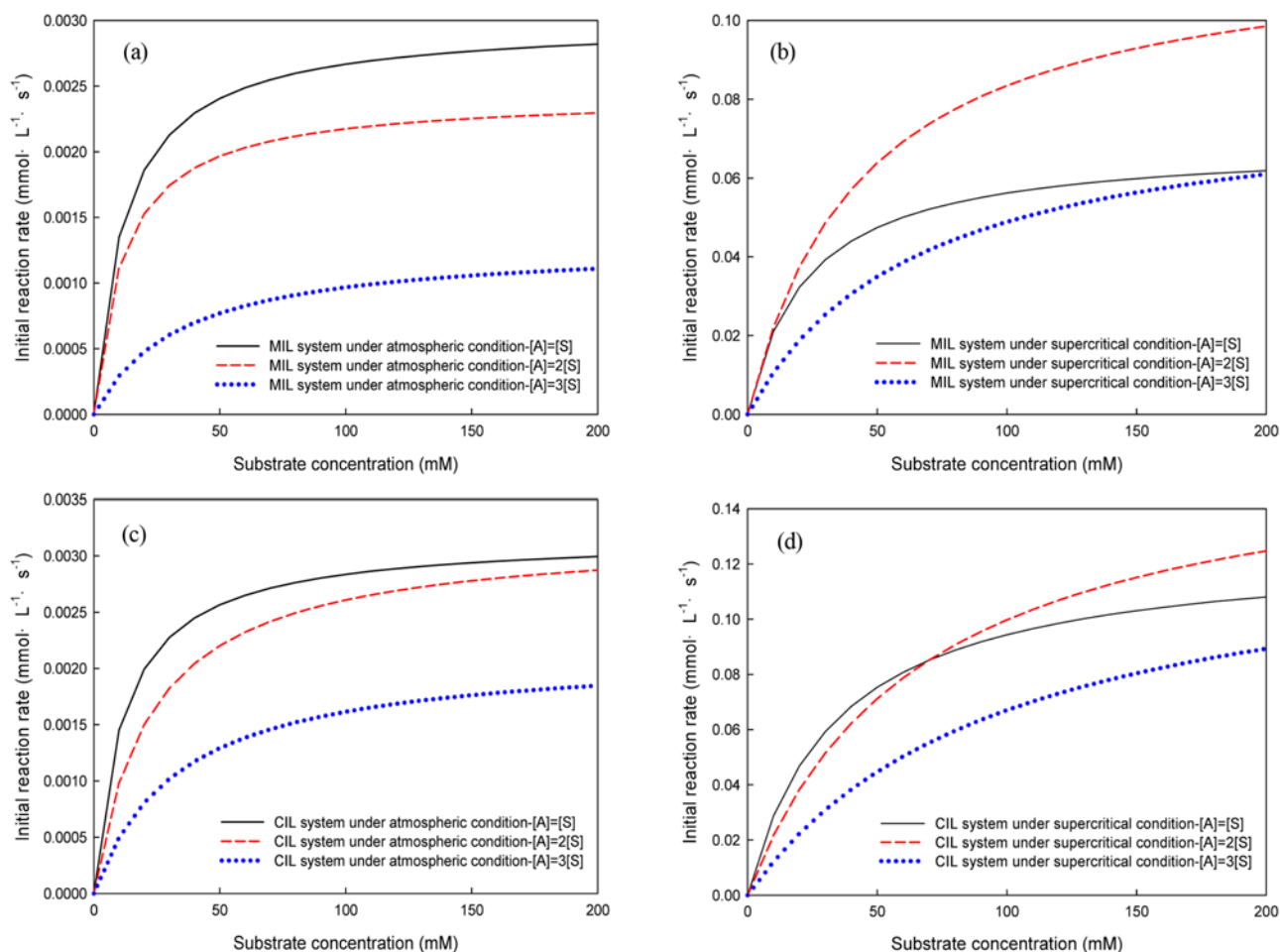
Fig. 3. Effect of methanol concentration on initial reaction rate by MIL and CIL systems under atmospheric and supercritical condition: (a) MIL system under atmospheric condition, (b) MIL system under supercritical condition, (c) CIL system under atmospheric condition, (d) CIL system under supercritical condition.

results showed that the CIL system had a higher initial reaction rate than that of the MIL system under the same conditions, and the supercritical condition had a higher initial reaction rate. The experimental values were applied to a computer curve-fitting simulation model. The relative error of this model was 3.83%, which was acceptable for a kinetic study in these systems. Therefore, these models will be applicable to experimental substrate concentration for this kind of transesterification catalyzed by the MIL and CIL systems [20-24].

Fig. 3 shows the effect of methanol concentration. Under atmospheric conditions, the initial reaction rate of the MIL and CIL systems decreased as the methanol concentration increased. However, under the supercritical condition, the initial reaction rate increased as the methanol concentration increased to two times that of oil. When methanol concentration was increased more than two times, initial reaction rate decreased markedly. The diffusional effect was more effective than that of the inhibitory effect as mass transfer increased. However, the inhibitory effect was increased at high methanol concentrations. These results suggest that the lipases can be deactivated markedly during biodiesel production at high methanol concentrations. In particular, a stepwise reaction was applied in our previous studies to prevent deactivation of the MIL and CIL sys-

tems under the two conditions, because of their deactivation by methanol [11-16]. Finally, the inhibitory effect caused by alcohol was identified through the kinetic analysis and the simulated model and could be used to predict the optimal condition for biodiesel production under various conditions.

CONCLUSIONS

We found good agreement between the experimental results of the initial reaction rate and the expected simulated model results. Consequently, the powerful simulated models were identified and are applicable to experimental substrate concentrations for this kind of transesterification catalyzed by MIL and CIL systems. Compared with the V_{max} and kinetic parameters (K_S, K_A, K_{iS} and K_{iA}) of the MIL and CIL systems, the diffusional effect was more effective than that of inhibitory effect. Moreover, the initial reaction rate was associated with alcohol concentration to produce biodiesel under each condition. As the methanol concentration increased, the initial reaction rate of the MIL and CIL systems decreased under the atmospheric condition. However, the initial reaction rate of the MIL and CIL systems increased as methanol concentration increased to two times that of oil under the supercritical fluid condition. As a result,

the inhibitory effect by alcohol was identified through the kinetic analysis, and the simulated model can be used to predict the optimal condition for biodiesel production under the two conditions.

ACKNOWLEDGEMENTS

REFERENCES

1. W. Chulalaksananukul, J. S. Condoret, P. Delorme and R. M. Willemot, *FEBS. Lett.*, **276**, 181 (1990).
2. A. T. Bull, *Korean J. Chem. Eng.*, **18**, 137 (2001).
3. C. H. Kwon, D. Y. Shin, J. H. Lee, S. W. Kim and J. W. Kang, *J. Microbiol. Biotechnol.*, **17**, 1098 (2007).
4. G. W. Zhou, G. Z. Li, J. Xu and Q. Sheng, *Collids Surf.*, **194**, 41 (2001).
5. B. Freedman, R. O. Butterfield and E. H. Pryde, *J. Am. Oil Chem. Soc.*, **63**, 1375 (1986).
6. Joelianingsih, H. Maeda, S. Hagiwara, H. Nabetani, Y. Sagara, T. H. Soerawidjaya, A. H. Tambunan and K. Abdullah, *Renew. Energy*, **33**, 1629 (2008).
7. H. Fukuda, A. Kondo and H. Noda, *J. Biosci. Bioeng.*, **92**, 405 (2001).
8. M. M. Soumanou and U. T. Bronscheuer, *Enzyme Microbiol. Technol.*, **33**, 97 (2003).
9. A. Z. Sulaiman, *Biotechnol. Prog.*, **21**, 1442 (2005).
10. K. Park, S. Lee, S. Maken, W. Koh, B. Min and J. Park, *Korean J. Chem. Eng.*, **23**, 601 (2006).
11. D. H. Lee, J. M. Kim, H. Y. Shin, S. W. Kang and S. W. Kim, *Biotechnol. Bioprocess Eng.*, **11**, 522 (2006).
12. J. H. Lee, S. B. Kim, S. W. Kang, Y. S. Song, C. Park, S. O. Han and S. W. Kim, *Bioresour. Technol.*, **102**, 2105 (2011).
13. J. H. Lee, S. B. Kim, C. Park and S. W. Kim, *Bioresour. Technol.*, **101**, S66 (2010).
14. J. H Lee, C. H Kwong, J. W. Kang, C. Park, B. Tae and S. W. Kim, *Appl. Biochem. Biotechnol.*, **156**, 24 (2009).
15. J. H. Lee, D. H. Lee, J. S. Lim, B. H. Um, C. Park and S. W. Kim, *J. Microbiol. Biotechnol.*, **18**, 1927 (2008).
16. W. Malilas, S. W. Kang, S. B. Kim, H. Y. Yoo, W. Chulalaksananukul and S. W. Kim, *Korean J. Chem. Eng.*, **30**, 405 (2013).
17. A. Zaidi, J. L. Gainer, G. Carta, A. Mrani, T. Kadiri, Y. Belarbi and A. Mir, *J. Biotechnol.*, **93**, 209 (2002).
18. D. Mukesh, S. Jadhav, A. A. Banerji, K. Thakkar and H. S. Bevinakatti, *J. Chem. Technol. Biotechnol.*, **69**, 179 (1997).
19. A. Z. Sulaiman, W. L. Fan and S. J. Lim, *Proc. Biochem.*, **42**, 951 (2007).
20. C. D. Rosa, M. B. Morandim, J. L. Minow, D. Oliveira, H. Treichel and J. V. Oliveira, *J. Supercrit. Fluids*, **47**, 49 (2008).
21. M. Giridhar, K. Chandana and K. Rajnish, *Fuel*, **83**, 2029 (2004).
22. Z. Guo and X. Xu, *Green Chem.*, **8**, 54 (2006).
23. M. Habulin and Z. Knez, *J. Chem. Technol. Biotechnol.*, **76**, 1260 (2001).
24. D. Oliveira and J. V. Oliveira, *J. Supercrit. Fluids*, **19**, 141 (2001).