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Research paper

Simultaneous extraction–reaction process for biodiesel production from microalgae

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HIGHLIGHTS

- Supercritical CO_2 (SC-CO_2) was used as an extraction solvent and reaction medium.
- Immobilized lipase was used as the catalyst.
- Effects of temperature, reaction time, and methanol:oil molar ratio were studied.
- A biodiesel production yield of 19.3% was obtained.

GRAPHICAL ABSTRACT
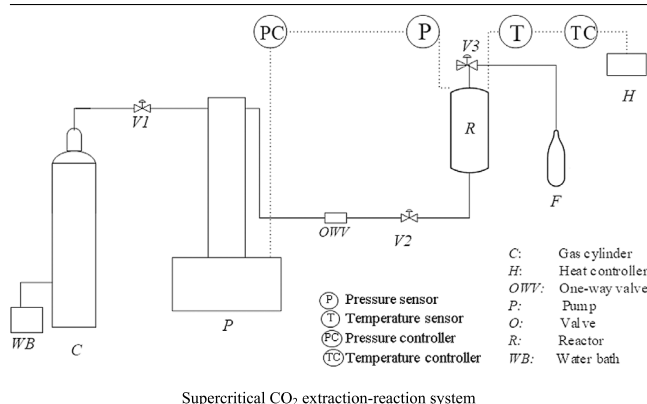
Supercritical CO_2 extraction-reaction system

ARTICLE INFO

ABSTRACT

Biodiesel is a promising sustainable alternative to non-renewable petrodiesel. In this work, oils extracted from microalgae are used for biodiesel production. To simplify the process, the extraction of the oils and their reaction are made to take place simultaneously in one step. Immobilized lipase was used as the catalyst, and supercritical CO_2 (SC-CO_2) was used as an extraction solvent and reaction medium. The use of SC-CO_2 allows easy separation of the products and leaves the leftover biomass uncontaminated, allowing it to be utilized in food and pharmaceutical applications. The effects of temperature (35–50 °C), reaction time (2-6 h), and methanol:oil (M:O) molar ratio (8:1–16:1) on biodiesel yield were investigated. Within 6 h, the maximum biodiesel production yield was found to be 19.3% at a temperature of 35 °C and at an M:O molar ratio of 8:1. The results hold promise in simplifying the microalgae-to-biodiesel production process.

1. Introduction

Finding alternatives to conventional fossil fuels is becoming increasingly important, because of their unsuitability and harmful environmental impact. Among the promising sustainable alternatives is biodiesel, the physical and chemical properties of which are similar to those of petrodiesel, allowing it to be used in conventional engines without requiring any modifications. In addition, it is renewable, non-toxic, biodegradable, and does not contribute to the net accumulation of greenhouse gases (Fjerbaek et al., 2009; Al-Zuhair et al., 2007; Mekhilef et al., 2011). Microalgae have received significant attention as a potential oil source, owing to their high oil content and growth rates (Lu et al., 2009). Cultivating microalgae cells does not require land development on freshwater, as several strains of algae have been found to grow in seawater and wastewater.

The conventional technique used for oil extraction from microalgae is the organic solvent extraction method (Mercer and Armenta, 2011). However, when an organic solvent is used, an

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additional downstream unit is required to separate the solvent from the products, resulting in an additional production cost. Moreover, most of these solvents are toxic and volatile, posing environmental problems and limiting further utilization of the left-over biomass. Once the oil is extracted, it is converted to biodiesel through a catalytic reaction with methanol. The use of lipase enzymes has been receiving increased attention, owing to their capacity to produce biodiesel from different sources of oils, with easy product separation and without any pretreatment or soap formation. However, the enzyme should be used in an immobilized form to overcome the high cost of the enzyme (Samukawa et al., 2000; Adamczak and Krishna, 2004). In this case, i.e., when an immobilized lipase is used, an organic solvent needs to be added to increase the solubility of the reaction substrates. This reduces the inhibition effect and lowers the viscosity and transport limitations of the reaction mixture (Yang et al., 2010; Rathore and Madras, 2007). Among the many organic solvents tested, n-hexane is by far the most commonly used. The transesterification yield of tallow fats was found to be 94.8% using *Mucor miehei* lipase, obtained after 5 h with n-hexane as the solvent, whereas in a solvent-free system, the yield did not exceed 20% (Nelson et al., 1996). As mentioned earlier, most organic solvents are toxic and require an additional separation unit.

Supercritical carbon dioxide ($SC-CO_2$) has been suggested to replace conventional organic solvents for both oil extraction and reaction processes. CO_2 is a non-toxic and cheap fluid with moderate critical parameters (Celia et al., 2005; del Valle et al., 2004). Moreover, the products can be easily separated via a simple depressurization process. $SC-CO_2$ has been used for enhanced oil extraction from several sources such as oil crops (del Valle et al., 2004; Reverchon and Marrone, 2001) and microalgae cells (Andrich et al., 2005; Halim et al., 2011). The lipid extraction yield from *Scenedesmus sp.* microalgae increased by 20% using $SC-CO_2$ at a temperature of 50 °C and a pressure of 350 bar, as compared to that obtained using n-hexane (Taher et al., 2014a). This is mainly because of the higher diffusivity and lower viscosity of $SC-CO_2$, which allows it to penetrate easier within the biomass matrix. Higher extraction yields and rates have been reported when $SC-CO_2$ was used to extract lipids from *Nannochloropsis sp.* Andrich et al. (2005) and *Chlorococum sp.* Halim et al. (2011).

Because of the low critical temperature of CO_2 (31 °C), which is below the denaturation temperature of most proteins, lipases can maintain their activity in $SC-CO_2$. The moderate critical pressure of CO_2 (73 bar) has minimal effect on enzyme activity (Celia et al., 2005; Novak et al., 2003). Moreover, the diffusion of the reaction substrates and products to and from the enzyme active sites is enhanced with the use of $SC-CO_2$, which overcomes the main drawback of immobilized enzymes. In the transesterification of oil extracted from palm kernel in the presence of Novozym®435, an optimum conversion rate of 63.2% was obtained using $SC-CO_2$. However, even with double the amount of enzyme, the maximum conversion rate was only 58.3% using n-hexane (Oliveira and Oliveira, 2001).

Although employing a high-pressure $SC-CO_2$ process can be justified for oil extraction, this may not be the case for fuel production, despite the enhanced reaction yield and ease of product separation. Therefore, an integrated process was proposed, in which the extracted oils already dissolved in $SC-CO_2$ are directly fed into a bioreactor packed with immobilized lipase, to produce biodiesel without the need for further pumping. Thus, the advantages of performing the reaction in an $SC-CO_2$ medium are maintained while avoiding the additional cost of pumping. Such an integrated system was tested for the extraction-reaction of fats from lamb meat (Al-Zuhair et al., 2012) and from microalgae lipids (Taher et al., 2014b). In these studies, the extraction and reaction steps were carried out in separate units.

To reduce the overall cost associated with the microalgae-to-biodiesel conversion process and to avoid the complexity associated with the separate extraction of lipids, followed by the transesterification of the extracts, a simultaneous extraction–reaction process (SERP) has been proposed (Qian et al., 2008; Skorupskaite et al., 2016; Ehimen et al., 2010). The SERP was tested on *Chlorella sp.* microalgae using sulfuric acid as the catalyst (Wahlen et al., 2011), and an optimum conversion rate of 91% was achieved after 8 h of reaction at 60 °C. Sulfuric acid was also used as the catalyst when the SERP was tested on other microalgae strains such as *Synechocystis sp.* PCC 6803, *Synechococcus elongatus*, *Chlorella sorokiniana*, *Tetraselmis suecica*, and *Chaetoceros gracilis* (de Luna et al., 2017). However, the use of an acidic catalyst can be highly corrosive and is not recommended for fuel production. A base catalyst, i.e., LiOH-pumice, has been recently used in the SERP of *Chlorella sp.* microalgae (Shirazi et al., 2017). The highest production yield was 47%, obtained after a reaction time of 3 h at 80 °C. Near supercritical methanol has recently been tested for the SERP of *Spirulina* microalgae (Eggers and Schwudke, 2016). The optimum conversion rate was achieved at an extremely high temperature of 300 °C and a pressure of 120 bar. The use of such an energy intensive process may not be economical. Moreover, when the temperature was 200 °C, a significant drop in the yield was observed, with a conversion rate of only 1.78%. Owing to the advantages of enzymes over chemical catalysts, it was interesting to study the SERP for the enzymatic biodiesel production from microalgae using $SC-CO_2$, which is the focus of this work.

2. Materials and methods

2.1. Strain, Chemicals, and Enzyme

A dried biomass of *Scenedesmus sp.* microalgae was provided by AlgaOil Limited, Philippines. The biomass was cultivated in an organic fertilizer (NPK, grade 14-14-14) and then sun dried. An analytical grade methanol with a purity ≥ 99% was obtained from Fisher chemicals, USA. Novozym®435, with an activity of 11,900 PLU g^{-1}, was provided by Novozymes, Denmark. n-Hexane (purity of 96%) was obtained from DAEJUNG Co., Korea. A standard solution of high purity fatty acid methyl esters (FAME) containing 4% myristic acid (C14:0), 10% palmitic acid (C16:0), 6% stearic acid (C18:0), 35% oleic acid (C18:1), 36% linoleic acid (C18:2), 2% arachidonic acid (C20:0), and behenic acid (C22:0) was obtained from Sigma–Aldrich, USA. Ultra-pure air was supplied by Abu-Dhabi Oxygen Company, UAE, and high-purity helium was supplied by Air Product Company, UAE.

2.2. Experimental method

The harvested microalgae cells were lyophilized in a freeze drier operated at −80 °C and 0.01 mbar for 6 h. The lyophilized cells were then grinded for a short period of 15 s. Particles with sizes between 150 and 355 μm, determined using sieve analysis, were used. The total oil content was found to be 5.8 ± 0.16% using the Folch method (Eggers and Schwudke, 2016) with a chloroform:methanol solvent mixture of 2:1 (v:v).

The experimental setup comprises a CO_2 cylinder, a CO_2 high-pressure syringe pump with a maximum capacity of 500 bar (Model 260D, ISCO, USA), a pump controller (ISCO, SFX 200, USA), a high-pressure stainless steel reaction cell with a volume of 10 ml, and a temperature-controlled incubator with a maximum temperature of 150 °C (ISCO, SFX 220). The pressure within the chamber was measured and controlled by the system, whereas the temperature was measured and controlled in the incubator. The precision of the temperature measurements of the extraction
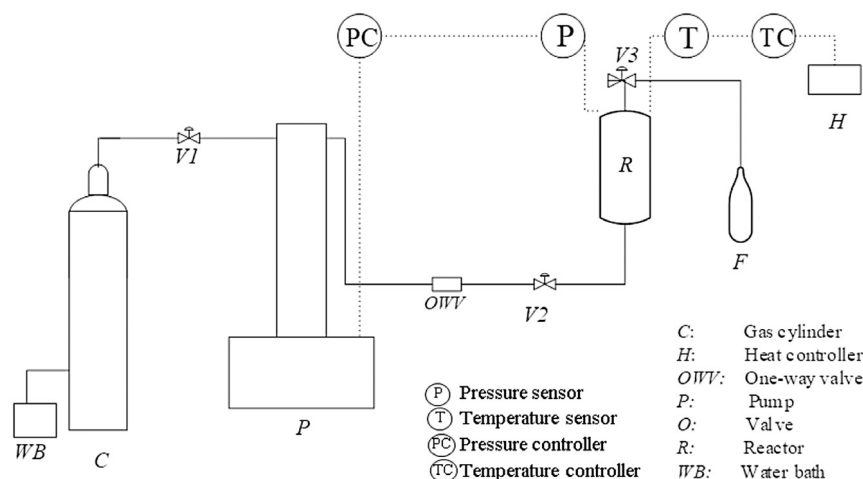
Fig. 1. Schematic of the supercritical CO_2 extraction–reaction system.

system was $\pm 0.1\,°C$. Fig. 1 shows the schematic of the supercritical fluid extraction (SFE) apparatus used.

A sample of lyophilized cells (1 g) was placed along with 2.7% w/w enzyme loading and a pre-specified amount of methanol in the reaction cell, which was covered by two 5/8 filters. Glass wools were placed at the top and bottom of the sample to prevent particle carryover. The cell was tightly sealed and heated up to the desired temperature. SC-CO_2 was then passed from the CO_2 cylinder into the high-pressure syringe pump and was pressurized to the desired pressure. Once the desired pressure was reached, the reaction cell was filled with SC-CO_2. At this point, the reaction starts. After a specified reaction time, the products dissolved in SC-CO_2 were eluted by depressurizing the cell. The reaction products were diluted in 10 ml of *n*-hexane and taken for analysis to determine the FAME content using gas chromatography (GC). The experiment was carried out at a pressure of 400 bar, at different temperatures (35, 40, and 50 °C), and at different methanol:oil (M:O) ratios (8:1, 12:1, and 16:1). As the rate-determining step in this work was found to be the lipid extraction process, a pressure of 400 bar was used. This is because it was shown that the extraction yield from the same strain of microalgae used in another study increased with the increase in the pressure of SC-CO_2 (Taher et al., 2014a). This is mainly because of the increase in the density, which has a positive effect on the solubility. At the same time, the pressure was not excessively high, thus avoiding any possible negative effect on the enzyme. The quantity of FAME produced was represented as a percentage of the total oil content, determined using the Folch method as expressed in Eq. (1).

$$\text{FAME yield} = \frac{m_{FAME}}{m_{oil\ content}} \times 100\% \qquad (1)$$

where m_{FAME} and $m_{oil\ content}$ are the weights of the FAME produced and the oil in the biomass used, respectively. The experiments were carried out in duplicate, and the presented results are the average values (with the standard deviation shown in the form of error bars in the figures).

3. Results and discussions

The FAME production yield was determined after 2, 4, and 6 h of reaction at 400 bar, for a 2.7% w/w enzyme loading, at a temperature of 35 °C, and at an M:O molar ratio of 8:1. As shown in Fig. 2, the highest production yield is 19.3% obtained after 6 h. Comparing the results of this work with previous results obtained
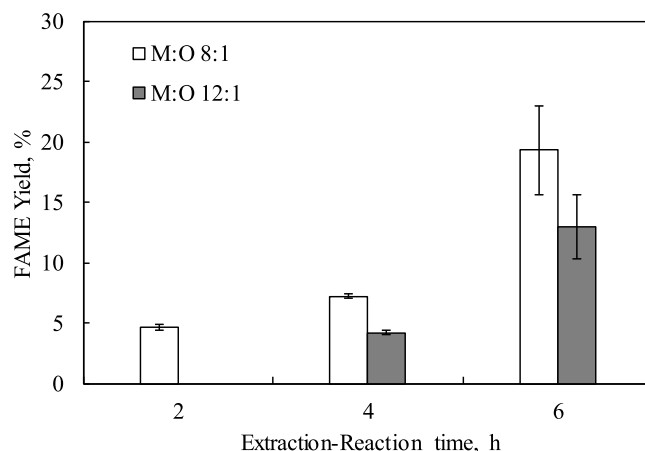

Fig. 2. Effects of reaction time on the FAME yield at 400 bar, 35 °C, 2.7% w/w enzyme loading, and different methanol:oil (M:O) molar ratios.

using the same enzyme, oil extracted from the same microalgae biomass, at the same temperature but at a lower pressure of 200 bar, with a similar enzyme loading, and for the same M:O molar ratio, a much better yield of 62% was obtained after 4 h (Taher et al., 2014b). This is expected, as the oil in this case was readily available for the reaction, whereas in the current study, the two processes take place simultaneously. This also suggests that the lipid extraction is the rate-determining step.

The experiment was then repeated using a higher M:O molar ratio of 12:1 and tested at reaction times of 4 and 6 h and at the same temperature and pressure. As shown in Fig. 2, the yield decreases with the increase in the M:O molar ratio at both the tested times, mainly because of the inhibition of methanol. This result is in good agreement with that of the previous study conducted on the extracted oil, wherein the optimum yield was obtained at an M:O molar ratio of 9:1 (Taher et al., 2014b). It noteworthy that the error bars increase at 6 h. Nevertheless, the standard deviations are relatively low. The most important takeaway is the successful use of the SC-CO_2 system for a simultaneous extraction–reaction.

To test the effect of temperature, the experiment was repeated at different temperatures but at the same pressure, i.e., 400 bar, and for an enzyme loading of 2.7%. The results, shown in Fig. 3, indicate that the production yield decreases with the increase
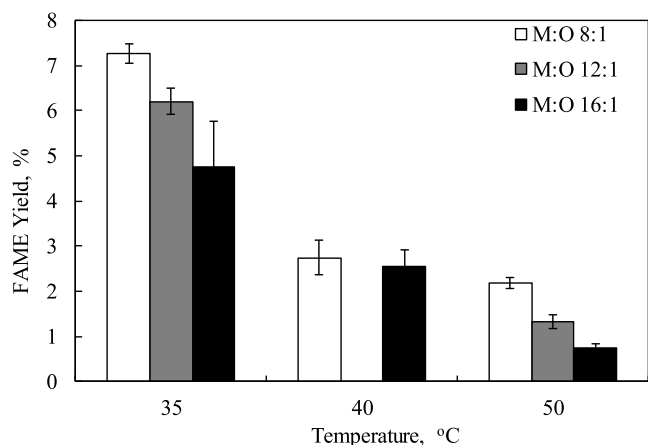
Fig. 3. Effect of temperature on FAME yield after 4 h in SC-CO_2 at 400 bar for 2.7% w/w enzyme loading and different methanol: oil (M:O) molar ratios.

in the temperature. However, when the extracted oil was used instead of the biomass, the optimum temperature is 50 °C (Taher et al., 2014b). This difference is due to the pressure used in the current simultaneous extraction–reaction system, i.e., 400 bar, whereas that used in the previous separate units was 200 bar. In addition, the temperature in the current study affected not only the reaction, as in the previous study, but also the solubility in SC-CO_2. As mentioned earlier, the rate-determining step in the SERP is the lipid extraction process. As the temperature increases, the solubility of the oils in SC-CO_2 decreases because of the reduction in the SC-CO_2 density (Al-Zuhair et al., 2012). This in turn reduces the extraction rate.

The main challenge in this work was the low extraction rate, which affected the overall biodiesel production. This was mainly because of the low lipid content in the biomass used. Therefore, it is recommended to extend this work by testing other microalgae biomasses having a higher lipid content for a more efficient extraction.

4. Conclusions

Biodiesel was directly produced from microalgae via a simultaneous extraction–reaction process (SERP) using SC-CO_2. The effects of temperature, reaction time, and methanol:oil (M:O) molar ratio on the biodiesel yield were investigated. A biodiesel production yield of 19.3% was obtained after a reaction time of 6 h, at a temperature of 35 °C, and at an M:O molar ratio of 8:1. The production yield was found to decrease with the increase in the temperature and M:O molar ratio. Despite having a lower yield compared to that obtained via separate extraction and reaction processes, the successful production of biodiesel via the simultaneous process is expected to simplify the overall biodiesel production process and make it more economical.

Acknowledgment

References

Adamczak, M., Krishna, S.H., 2004. Strategies for improving enzymes for efficient biocatalysis. Food Technol. Biotechnol. 42 (4), 251–264.

Al-Zuhair, S., Hussein, A., Al-Marzouqi, A.H., Hashim, I., 2012. Continuous production of biodiesel from fat extracted from lamb meat in supercritical CO_2 media. Biochem. Eng. J. 60, 106–110.

Al-Zuhair, S., Ling, F.W., Jun, L.S., 2007. Proposed kinetic mechanism of the production of biodiesel from palm oil using lipase. Proc. Biochem. 42 (6), 951–960.

Andrich, G., Nesti, U., Venturi, F., Zinnai, A., Fiorentini, R., 2005. Supercritical fluid extraction of bioactive lipids from the microalga Nannochloropsis sp. Eur. J. Lipid Sci. Technol. 107 (6), 381–386.

Celia, E., Cernia, E., Palocci, C., Soro, S., Turchet, T., 2005. Tuning Pseudomonas cepacea lipase (PCL) activity in supercritical fluids. J. Supercrit. Fluids 33 (2), 193–199.

Eggers, L.F., Schwudke, D., 2016. Liquid extraction: Folch. In: Wenk, M. (Ed.), Encyclopedia of Lipidomics. Springer, Dordrecht.

Ehimen, E.A., Sun, Z.F., Carrington, C.G., 2010. Variables affecting the in situ transesterification of microalgae lipids. Fuel 89 (3), 677–684.

Fjerbaek, L., Christensen, K.V., Norddahl, B., 2009. A review of the current state of biodiesel production using enzymatic transesterification. Biotechnol. Bioeng. 102 (5), 1298–1315.

Halim, R., Gladman, B., Danquah, M.K., Webley, P.A., 2011. Oil extraction from microalgae for biodiesel production. Bioresour. Technol. 102 (1), 178–185.

Lu, J., Chen, Y., Wang, F., Tan, T., 2009. Effect of water on methanolysis of glycerol trioleate catalyzed by immobilized lipase Candida sp. 99–125 in organic solvent system. J. Mol. Catal. B 56 (2–3), 122–125.

de Luna, M.D.G., Doliente, L.M.T., Ido, A.L., Chung, T.W., 2017. In situ transesterification of Chlorella sp. microalgae using LiOH-pumice catalyst. J. Environ. Chem. Eng. 5 (3), 2830–2835.

Mekhilef, S., Siga, S., Saidur, R., 2011. A review on palm oil biodiesel as a source of renewable fuel. Renewable Sustainable Energy Rev. 15 (4), 1937–1949.

Mercer, P., Armenta, R.E., 2011. Developments in oil extraction from microalgae. Eur. J. Lipid Sci. Technol. 113, 539–547.

Nelson, L.A., Foglia, T.A., Marmer, W.N., 1996. Lipase-catalyzed production of biodiesel. J. Am. Oil Chem. Soc. 73 (9), 1191–1195.

Novak, J., Habulin, M., Krmelj, V., Knez, Å.E., 2003. Silica aerogels as supports for lipase catalyzed esterifications at sub- and supercritical conditions. J. Supercrit. Fluids 27 (2), 169–178.

Oliveira, D., Oliveira, J.V., 2001. Enzymatic alcoholysis of palm kernel oil in n-hexane and $SCCO_2$. J. Supercrit. Fluids 19 (2), 141–148.

Qian, J., Wang, F., Liu, S., Yun, Z., 2008. In situ alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal. Bioresour. Technol. 99, 9009–9012.

Rathore, V., Madras, G., 2007. Synthesis of biodiesel from edible and non-edible oils in supercritical alcohols and enzymatic synthesis in supercritical carbon dioxide. Fuel 86 (17–18), 2650–2659.

Reverchon, E., Marrone, C., 2001. Modeling and simulation of the supercritical CO_2 extraction of vegetable oils. J. Supercrit. Fluids 19 (2), 161–175.

Samukawa, T., Kaieda, M., Matsumoto, T., Ban, K., Shimada, Y., Noda, H., et al., 2000. Pretreatment of immobilized Candida antarcticalipase for biodiesel fuel production from plant oil. J. Biosci. Bioeng. 90 (2), 180–183.

Shirazi, H.M., Karimi-Sabet, J., Ghotbi, C., 2017. Biodiesel production from Spirulina microalgae feedstock using direct transesterification near supercritical methanol condition. Bioresour. Technol. 239, 378–386.

Skorupskaite, V., Makareviciene, V., Gumbyte, M., 2016. Opportunities for simultaneous oil extraction and transesterification during biodiesel fuel production from microalgae: A review. Fuel Proc. Technol. 150, 78–87.

Taher, H., Al-Zuhair, S., Al-Marzouqi, A.H., Haik, Y., Farid, M., 2014a. Supercritical carbon dioxide extraction of microalgae lipid: Process optimization and laboratory scale-up. J. Supercrit. Fluids 86, 57–66.

Taher, H., Al-Zuhair, S., Al-Marzouqi, A.H., Haik, Y., Farid, M., 2014b. Enzymatic biodiesel production of microalgae lipids under supercritical carbon dioxide: process optimization and integration. Biochem. Eng. J. 90, 103–113.

del Valle, J.M., Rivera, O., Mattea, M., Ruetsch, L., Daghero, J., Flores, A.S., 2004. Supercritical CO_2 processing of pretreated rosehip seeds: effect of process scale on oil extraction kinetics. J. Supercrit. Fluids 31 (2), 159–174.

Wahlen, B.D., Willis, R.M., Seefeldt, L.C., 2011. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixedcultures. Bioresour. Technol. 102 (3), 2724–2730.

Yang, Z., Zhang, K.P., Huang, Y., Wang, Z., 2010. Both hydrolytic and transesterification activities of Penicillium expansum lipase are significantly enhanced in ionic liquid [BMIm][PF$_6$]. J. Mol. Catal. B 63 (1–2), 23–30.