We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 9

Emerging Green Technologies for Biodiesel Production

Hanifa Taher and Sulaiman Al-Zuhair

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66187

Abstract

The current global energy demand is met by burning the non-renewable fossil fuels. As the demand is escalating, resources and reserves are diminishing. In addition, the environment is threatened by the continuous emission of greenhouse gases; mainly CO_2, which is worrying. Therefore, searching for alternatives is inevitable. Biodiesel received a considerable attention to potentially replace petroleum-based fuels. It can be produced from oil-rich feedstocks through several methods using different technologies, including transesterification. Although alkali catalyzed biodiesel process is commercially viable, several challenges were raised. In this chapter, an overview of the current status of biodiesel production approaches is discussed and the emerging technologies are highlighted. The chapter rewards the attention of using green processes, where the effectiveness of using; microalgae biomass as a green feedstock (compared to conventional crop-based seeds), lipases as green catalysts (compared to conventional chemical catalysts), and green and tunable solvents, such as neoteric solvents and supercritical fluids (compared to conventional volatile organic solvents) are addressed.

Keywords: biodiesel, microalgae, lipase, green solvents, microwave

1. Biodiesel

The continuous dwindling fossil fuels supply and increasing atmospheric carbon dioxide emission have put the pressure on finding and developing sustainable alternative fuels. Biodiesel, which is a mixture of fatty acids alkyl esters, is the proposed alternative that can replace to the conventional petroleum diesel. The physical properties of biodiesel are similar to petroleum diesel and can be used without any modifications in the engine [1, 2]. In addition, biodiesel is a renewable, non-toxic and biodegradable fuel that can decreases the

INTECH
open science | open minds

© 2017 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

reliance on fossil fuels and reduces harmful gasses emissions [3–8]. Furthermore, biodiesel has lower sulfur and aromatic contents. According to the U.S. Department of Energy statistics and analysis [9], in 2015, the production of biodiesel in the United States alone reached 1.2×10^9 gallons. The statistics also indicated that the current biodiesel production in first 6 months of 2016 is about 21% higher than that obtained in 2015, during same duration.

2. Feedstocks

2.1. Conventional feedstocks

Triglycerides from oil-rich feedstock's, such as soybean, rapeseed, canola, sunflower, and palm, have been commonly used due to their abundant availability [10–16]. The first initiation was by the diesel engine inventor, Rudolf Diesel, who tested the use of peanut oil. However, natural oils are viscose with inappropriate cetane number. Thus, the idea was not accepted and vegetable oils were replaced by petroleum oil [7, 17–19]. The recent concern about limited oil reservoirs and oil explosion increased activities recalled the attention to use oil-rich feedstocks. Oils dilution with solvent, thermal cracking, pyrolysis, micro emulsions and transesterification has been suggested to overcome this viscosity limitation [20, 21]. Among these, transesterification with short chain alcohols, such as methanol and ethanol, in the existence of proper catalyst is the preferred and commonly used approach.

Although vegetable oils are available in large quantities, biodiesel production from these vegetable oils competes with their use as food source, which results in increasing their prices and affect food market. In addition, cultivating oil-rich crops requires lands and freshwater. It was reported that vegetable oil accounts for more than 60% of biodiesel overall production cost [5, 22]. From that prospective, non-edible oils such as those from non-edible plants that are not used in nutrition and can grow in the unfertile lands were suggested. However, freshwater requirement still exist. The use of waste oils and fats have been recommended, where there use is a waste management process [23], but contains large amount of free fatty acids and water which increases the production cost. Furthermore, cannot satisfy the ever-increasing global demands of diesel [24–26].

2.2. Green feedstock

Choosing an inexpensive and more sustainable oil feedstock is the critical step to get cost-effective biodiesel. Currently, microalgae, which are micro-organisms, received a promising attention. Due to their high oil content and growth rate, they have been considered as potential feedstock that can replace the conventional diesel [27–31]. Furthermore, cultivation of micro-algae cells does not require land development neither freshwater. Several algae strains were found to grow in seawater and wastewater. The oil contents of such feedstocks are usually between 20% and 50% and reach be in some strains to 80% in dry basis. The strain may also change its composition by altering the growth conditions, such as light, nutrients, and temperature. The stressful environment usually results to higher oil productivity.

http://dx.doi.org/10.5772/66187

More interestingly, microalgae cells contain of protein, carbohydrates and lipids, which extend the application domains of produced biomass from food to biofuel. Microalgae cells are also used for CO_2 mitigation. However, to use microalgae biomass for biodiesel production, several steps have to be carried out, which are strain selection, biomass production and harvesting, and oil extraction and conversion.

3. Production technologies

Oils derivatization by transesterification is the most common approach used commercially. Typically, transesterification is a reaction between the oil and a short-chain alcohol that results to form an esters mixture and glycerol, as side product. The reaction is commonly take place in the presence of catalyst that can speed up the reaction, where three moles of alcohols are needed to react with 1 mol of the oil. Higher alcohol to oil molar ratios than the stoichiometry, however, is usually employed to produce more biodiesel.

3.1. Conventional catalysts

As mentioned earlier, transesterification reactions are chemically catalyzed, which can be either base or acid catalysts, depending on the oil quality free fatty acids (FFAs) and water contents). Alkali catalysts, such as sodium hydroxide (NaOH) and potassium hydroxide (KOH), are the commonly used, due to their low cost and high achievable yields of more than 98% within a hour at reasonable temperature of 60°C [7, 32]. The reaction starts by preparing the alkoxide solution and charging it to the reactor with oils. The reaction is then heated to the reaction temperature for few hours. Products are then separated by gravity and crude biodiesel is obtained, which needs further washing to recover unreacted oils and alcohols. In addition to neutralization to receive the catalyst used.

Although the process is simple and commercially used, it is not practical with feedstocks containing high free fatty acids (FFAs) and water contents such as those from non-edible and waste oil due to soap formation that lowers the overall production yield and require large amount of catalysts [33, 34]. Pretreatment of oil prior transesterification by acid esterification have been suggested, where sulfuric acid (H_2SO_4) is commonly adopted. Although it could be beneficial in enhancing oil quality, the process very slow and requires large amount of alcohols. In addition, acids are corrosive [5, 17, 35].

3.2. Green catalysts

The use of enzymes, which are green catalysts, has been suggested. Among the several available enzymes, lipases (EC 3.1.1.3), which are of hydrolytic enzymes received an increasing attention in biodiesel production. Lipase-catalyzed biodiesel production due to their ability to act on ester bonds, at mild temperatures with less energy needs [35].

Lipases, namely the non-specific one, can convert oils from different sources, including from hose cooking, without any pre-treatment needs with easy product separation and no soap

formation. Among the several studied, lipases from *Candida antartica* [36–44], *Pseudomonas fluorescens* [45, 46], *Pseudomonas cepacia* [47], *Candida rugosa* [48–50] and *Rizhomucor miehei* [51, 52] are commonly used.

Although lipases are superior compared to chemical catalysts, accumulation of glycerol which is a by-product negatively affect the enzyme activity and reaction yield. Glycerol accumulation increases reaction mixture viscosity and forms hydrophilic layer around rhe enzyme, preventing the reaction substrate to reach enzyme active site [53, 54]. The highest glycerol inhibition effect was found when silica, which has the highest micro-pores structure, was used in the immobilization protocol of the lipase. Continuous removal of produced glycerol from reaction mixture and/or using *tert*-butanol as solvent was proposed [55, 56]. The used of silica gel that can absorb glycerol is also advantageous in such case [57].

The activity of the enzyme was also found to decrease when more than 1.5 molar equivalents of alcohol is used. This is because at certain concentration, alcohols which are hydrophilic becomes insoluble in oils and tends to strip-off the hydration layer of water from the lipase. Therefore, inhibition and lose in activity [2, 58–60]. Numerous solutions have been proposed to overcome short-chain alcohols inhibition limitation. These include step-wise alcohols addition [38, 61], use of acetates as acceptors [57, 58, 62], lipase pretreatment and activity enhancement [39], use of genetically modified methanol-tolerant lipase and improving the polarity of the reaction medium using organic solvents. The latter is commonly adopted method.

On the other hand, enzymatic biodiesel production is not yet commercialized due to enzymes high costs. Immobilization of the lipase is usually considered to re-use the enzyme in several cycles. Immobilization can also enhance the stability. For example, Novozym®435, which is an immobilized enzyme form of *Candida antartica,* was reused for 12 continuous cycles without any detectable loss in the activity [57] when the non-edible oil from Jatropha was transesterified with methanol. Whereas, when *tert*-butanol was used as reaction media Novozym®435 activity was maintained for 200 cycles [63].

4. Reaction medium

The solvent-free reaction systems are always the preferable one in enzyme catalyzed processes, however when the reaction is catalyzed by a lipase the use of solvents is essential to prevent the inhibition. By introducing hydrophobic solvents to the reaction, the solubility of reaction substrates increases resulting in reduced inhibition effect of hydrophilic substrates/products. In addition, the viscosity and transport limitations of reaction mixture to enzyme active sites decreases, which results in increased reaction yield [64, 65].

4.1. Conventional organic solvents

Numerous organic solvents have been used in biodiesel production, where the hydrophobicity was considered as main factor in selecting the proper solvent [66]. It was found that the

biodiesel production rate increases with the increase in the hydrophobicity of the solvent used, and hydrophilic solvents resulted tend to strip-off the bound water from the enzyme surface is used [39, 67–70]. Generally, the stripping was reported to take place when an organic solvent with Log P (hydrophobicity) <2 used. *n*-Hexane, which has log *P* = 3.5, has been commonly used, where its effect on enhancing the production yield, compared to solvent-free system, was observed in several studies. These includes the work of Nelson et al. [36], who tested the effect of using *n*-hexane in tallow fats transesterification with methanol at 3:1 methanol to oil molar ratio when catalyzed by *Mucor miehei* lipase. High yield reaching 95%, compared to 19% in solvent free, was obtained.

tert-Butanol is another solvent used in lipase catalyzed biodiesel processes. It has been selected as a capable alternative to *n*-hexane that cannot dissolve glycerol and minimize its inhibition effect [1, 13, 71–74]. A high methanol to oil molar ratio of 6:1 could be reached in soybean oils transesterification with Novozym®435, resulting in 60% yield, compared to only 10% in solvent-free system.

4.2. Green solvents

Although organic solvents enhance the production yield, an additional downstream unit is required to separate the solvent from the products, resulting in an additional production cost. Moreover, organic solvents are toxic and volatile and their use could pose several environmental issues that should be minimized. Efforts have been made to find alternative non-toxic and environmental benign solvents. In this regard, supercritical CO_2 and ionic liquids (ILs) have been suggested.

4.2.1. Supercritical carbon dioxide

Supercritical fluids are fluids at temperatures and pressures above their critical points. They have been used in several applications. Among the different fluids, supercritical carbon dioxide (SC—CO_2) and is been the most commonly used. Supercritical CO_2 is a non-toxic and cheap fluid that appear in abundant with moderate critical parameters [75]. Compared to organic solvents, SC—CO_2 has liquid solubilization capacity and gas diffusivity and viscosity, where small changes in the conditions can lead to a significant increase in the properties. These unique physiochemical properties allow it to be used in several applications, including separation and reaction [76–78]. Moreover, easy products separation can be achieved using SC—CO_2.

Although SC—CO_2 has been commonly used in esters transesterification in the presence of lipase, its employment in biodiesel production is still new [79]. The compatibility of SC—CO_2 with lipases is well recognized, and by using it in biodiesel production, the mass transfer of reaction substrates into enzyme active sites would be enhanced. In spite of the high pressure uses, it was clearly verified that it has minimal effect on enzyme inhibition at pressure less than 200 bars [80, 81]. Comparable yields to organic solvent were achieved when palm kernel and Jatropha oils were transesterified in the presence of Novozym®435 in SC—CO_2 [75, 82–84].

Higher yield of 80% was obtained when SC—CO_2 was used in microalgae lipids transesterification in the presence of same lipase [42].

Supercritical CO_2 has been also used to extract oils for biodiesel production, such as those oils from vegetable crops [76, 77], microalgae cells [85–90] and fats from animal meat. The use of SC—CO_2 is adopted to minimize the use of toxic solvents and utilize the leftover, after extractions, in other applications such as in food and pharmaceutical industries, unlike *n*-hexane which is toxic and its use is an energy intensive process. Its effectiveness depends on the selected extraction conditions; namely the temperature, pressure and flow rate, where increasing the pressure increases SC—CO_2 density and the extraction yield whereas the temperature has two opposite effect that become equal at crossover pressure. By increasing the extraction temperature, SC—CO_2 density decreases and reduces its capability to solubilize the desired solute, while solute vapor pressure increases resulting in more solutes extraction. Several studies had considered the effectiveness of using SC—CO_2. For example, similar yields performance of *n*-hexane were reported for oils extraction from *Spirulina platensis* [86], *Spirulina maxima* [87] and *Pavlova* sp. [89] microalgae cells. A higher efficiency was reported in extracting oils from *Chlorococum* sp. and *Nannochloropsis* sp. [90, 91].

As mentioned earlier, SC—CO_2 has many advantages. However, high pressure is needed for pumping and reaching the supercritical state of CO_2, making the process costly. Depressurization to separate the biodiesel from enriched SC—CO_2 could negatively affect lipase structural confirmation, therefore its stability. To minimize the effect, continuous operation has been also considered. It has been successfully employed for soybean [92], corn oil [93, 94], microalgae and sunflower oils [95]. Taher et al. [55, 56] had stated that the feasibility of using SC—CO_2 for energy production is not evident, but combining oil extraction conversions to biodiesel in SC—CO_2 in one integrated system would be feasible and the additional pumping cost for energy production could be justified and make the overall process more feasible [42, 44, 96]. On the other hand, the presence of water could results to carbonic acid formation that change the reaction pH and denaturant the lipase. CO_2 may also react with the amine groups on the surface of lipase to form carbamates [97].

4.2.2. Ionic liquids

Ionic liquids (ILs) are liquids of low crystallization tendency. They are composed of cations and anions and distinguished from conventional solvents in their non-vapor pressure feature. Thus, known to as "designer solvents." They have developed as green alternative solvents to replace the conventional volatile solvents in several process, including biodiesel production. The first attempt to use them in lipase catalyzed reactions was with [bmim][PF_6] and [bmim][BF_4], which were used in several reactions, including transesterification [98]. The selection the proper IL depends on its effect to enhance the enzyme activity reaction substrates/products solubility [99–103].

Typically, by judicious selection of the alkali chain on the cation and anion group, the physiochemical properties of designed IL can be tuned. For example, symmetric and shorter alkyl chains cations in the IL result in a higher melting temperature than those with asymmetric cations [104, 105], and increasing chain branching results in an increased the melting point

http://dx.doi.org/10.5772/66187

[106, 107]. However, it decreases with the increase in anion size. On the other hand, ILs with symmetric and fluorinated anions, have high viscosity, which is not preferable in enzyme catalyzed reactions. Ionic liquids based on cations with aromatic phenyl ring also have high viscosity as well [108].

The miscibility of the reaction substrates with the IL and IL hydrophobicity are main factor affecting the overall reaction yield, where the high solubility of reaction substrates to enhance reaction rate and low solubility of the biodiesel in the IL are the desired features in biodiesel production. The hydrophobicity of the IL depends mainly on the anions used. For example, [PF_6^-] and [Tf_2N^-] anions, which are hydrophobic results in making hydrophobic ILs, where those with hydrophilic anions, such as [Cl^-], [Br^-], [I^-], [NO_3^-], [CH_3COO^-] and [CF_3COO^-] for hydrophilic ILs. The hydrophobicity of ILs can also be affected by the length of the alkyl chain on the cation, in which longer alkyl chain results in a more hydrophobic IL [109–111]. Similar to of organic solvents, hydrophobic ILs are preferable in enzyme catalyzed biodiesel process, wherein hydrophilic ILs may strip-off the essential hydration layer and deactivate the lipase. Moreover, the nucleophilicity of the anion used in the IL combination affect lipase activity and stability, where high nucleophilicity of an IL may affect lipase structure activity interacting with the positively charged sites in lipase [112].

Among the several tested ILs in biodiesel production, [PF_6^-] and [NTf_2^-] based ILs are commonly used. For example, [bmim][PF_6] and [emim][PF_6] where used in sunflower oil transesterification Novozyme®435. High yield, reaching 98%, was achieved in [emim][PF_6] due to its higher hydrophobicity, however, insignificant products were obtained when [BF_4^-]-based ILs were tested [113]. The high yield obtained in [emim][PF_6] compared to [bmim][PF_6] is due to the ability of long-chain cation-based ILs to dissolve reaction substrates, thus the reaction take place in a two-phase system resulting in moderate efficiency.

In addition to ILs uses in lipase catalyzed reaction, they have been employed as green catalyst to overcome the reaction complication and product purification issues in chemical catalyzed reactions. The brønsted acidic ILs [$PY(CH_2)_4SO_3H$][HSO_4] and [$CyN_{1,1}PrSO_3H$][Tos] was found to be effective in transesterifying cottonseed (92% yield) and coconut (98% yield) oils, respectively, where comparable to that using concentrated sulfuric acid were obtained [114, 115]. Similar yield was also obtained from esterification of long-chain free fatty acids in [NMP][CH_3SO_3] [116]. In addition, biodiesel yield of 87 and 97% were also achieved using the basic IL [bmim][OH] [117] and [hmm][OH], respectively [118].

Ionic liquids have been used to extract oils, commonly from microalgae cells as they can be used with wet cells without the need cell walls disruption. In such processes, hydrophilic ILs are used where they have the capability to dissolve algal cell components leaving the oils insoluble and float. The extraction from wet cells of *Chlorella vulgaris* was tested using IL [emim][DEP], where 40% higher than *n*-hexane-methanol (7:3 v/v) mixture was obtained [119]. The effect of adding a polar solvent with the IL was also evaluated [120]. For example, a mixture of [emim][CH_3SO_4] and methanol was tested with *Chlorella sp.* cells containing 70% water, and an yield of 75% was achieved at 1:1.2 (w/w) solvents ratio.

The main challenge of employing ILs at industrial scale is in their high costs. Therefore, the recycling step is important. In addition, when long alkyl chain on cation-based ILs is used, the separation step is not easy and continuous recovery of biodiesel from reaction mixture as they produced is vital. Combination ILs with SC—CO_2 (IL-SC—CO_2) has been recently suggested, where biodiesel can be recovered using SC—CO_2 in an effective manner. Such system was tested for biodiesel production from triolein using Novozym®435 in different ILs and high yields reaching 98% was obtained after 6 h [121].

5. Conclusions

Biodiesel production using chemical catalysts and solvents from received the attention to replace conventional diesel fuel. However, the process is not commercialized due to many shortcomings raised. The employment of green catalysts and solvents, either by SC—CO_2 or by ILs has been suggested to several technical restrictions. The use of integrated processes that combine the use of different green catalysts and solvents in a one process to enhance product separation and solvent recover is discussed.

Author details

Hanifa Taher[1*] and Sulaiman Al-Zuhair[2]

*Address all correspondence to: healblooshi@masdar.ac.ae

1 Chemical and Environmental Engineering Department, Masdar Institute, Abu Dhabi, UAE

2 Chemical and Petroleum Engineering Department, UAE University, AlAin, UAE

References

[1] Demirbas, A., Progress and recent trends in biodiesel fuels. Energy Conversion and Management 2009, 50, 14–34.

[2] Fjerbaek, L.; Christensen, K. V.; Norddahl, B., A review of the current state of biodiesel production using enzymatic transesterification. Biotechnology and Bioengineering 2009, 102(5), 1298–1315.

[3] Bajpai, D.; Tyagi, V. K., Biodiesel: source, production, composition, properties and its benefits. Journal of Oleo Science 2006, 55(10), 487–502.

[4] Fellows, P., Food Processing Technology: Principles and Practice. Woodhead Publishing Limited and CRC Press LLC: USA, 2000.

[5] Al-Zuhair, S., Production of biodiesel:possibilities and challenges. Biofuels, Bioproducts and Biorefining 2007, 1(1), 57–66.

[6] Demirbas, A., Importance of biodiesel as transportation fuel. Energy Policy 2007, 35(9), 4661–4670

[7] Fukuda, H.; Kondo, A.; Noda, H., Biodiesel fuel production by transesterification of oils. Journal of Bioscience and Bioengineering 2001, 92(5), 405–416.

[8] Ranganathan, S. V.; Narasimhan, S. L.; Muthukumar, K., An overview of enzymatic production of biodiesel. Bioresource Technology 2008, 99(10), 3975–3981

[9] Monthly Biodiesel Production Report with Data for August 2016; U.S. Department of Energy, 2016; 1–10.

[10] Antunes, W. M.; Veloso, C. d. O.; Henriques, C. A., Transesterification of soybean oil with methanol catalyzed by basic solids. Catalysis Today 2008, 133–135, 548–554.

[11] Liu, X.; He, H.; Wang, Y.; Zhu, S.; Piao, X., Transesterification of soybean oil to biodiesel using CaO as a solid base catalyst. Fuel 2008, 87(2), 216–221.

[12] Dubé, M. A.; Tremblay, A. Y.; Liu, J., Biodiesel production using a membrane reactor. Bioresource Technology 2007, 98(3), 639–647

[13] Al-Zuhair, S.; Ling, F. W.; Jun, L. S., Proposed kinetic mechanism of the production of biodiesel from palm oil using lipase. Process Biochemistry 2007, 42(6), 951–960.

[14] Kalam, M. A.; Masjuki, H. H., Biodiesel from palmoil-an analysis of its properties and potential. Biomass Bioenergy 2002, 23(6), 471–479.

[15] Saka, S.; Kusdiana, D., Biodiesel fuel from rapeseed oil as prepared in supercritical methanol. Fuel 2001, 80(2), 225–231.

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

[17] Akoh, C. C.; Chang, S.-W.; Lee, G.-C.; Shaw, J.-F., Enzymatic approach to biodiesel production. Journal of Agricultural and Food Chemistry 2007, 55(22), 8995–9005.

[18] Basha, S. A.; Gopal, K. R.; Jebaraj, S., A review on biodiesel production, combustion, emissions and performance. Renewable Sustainable Energy Reviews. 2009, 13(6–7), 1628–1634.

[19] Sharma, Y. C.; Singh, B.; Upadhyay, S. N., Advancements in development and characterization of biodiesel: a review. Fuel 2008, 87(12), 2355–2373.

[20] Fan, X.; Burton, R., Recent development of biodiesel feedstocks and the applications of glycerol: a review. The Open Fuels and Energy Science Journal 2009, 1, 100–109.

[21] Helwani, Z.; Othman, M. R.; Aziz, N.; Fernando, W. J. N.; Kim, J., Technologies for production of biodiesel focusing on green catalytic techniques: a review. Fuel Process Technology. 2009, 90(12), 1502–1514.

[22] Lai, C.-C.; Zullaikah, S.; Vali, S. R.; Ju, Y.-H., Lipase-catalyzed production of biodiesel from rice bran oil. Journal of Chemical Technology and Biotechnology 2005, 80(3), 331–337.

[23] Phan, A. N.; Phan, T. M., Biodiesel production from waste cooking oils. Fuel 2008, 87(17–18), 3490–3496.

[24] Predojevi?, Z. J., The production of biodiesel from waste frying oils: a comparison of different purification steps. Fuel 2008, 87(17–18), 3522–3528.

[25] Taufiqurrahmi, N.; Mohamed, A. R.; Bhatia, S., Production of biofuel from waste cooking palm oil using nanocrystalline zeolite as catalyst: process optimization studies. Bioresource Technology 2011, 102(22), 10686–10694.

[26] Zhang, Y.; Dube, M. A.; McLean, D. D.; Kates, M., Biodiesel production from waste cooking oil: 1. process design and technological assessment. Bioresource Technology 2003, 89(1), 1–16.

[27] Adamczak, M.; Bornscheuer, U. T.; Bednarski, W., The application of biotechnological methods for the synthesis of biodiesel. European Journal of Lipid Science and Technology 2009, 111, 800–813.

[28] Chisti, Y., Biodiesel from microalgae. Biotechnology Advances 2007, 25(3), 294–306.

[29] Spolaore, P.; Joannis-Cassan, C.; Duran, E.; Isambert, A., Commercial applications of microalgae. Journal of Bioscience and Bioengineering 2006, 101(2), 87–96.

[30] Vijayaraghavan, K.; Hemanathan, K., Biodiesel production from freshwater algae. Energy and Fuels 2009, 23(11), 5448–5453.

[31] Sheehan, J.; Dunahay, T.; Benemann, R.; Roessler, G.; Weissman, C., A look back at the U.S. department of energy- aquatic species program: biodiesel from algae; National Renewable Energy Laboratory, USA, 1998.

[32] Atadashi, I. M.; Aroua, M. K.; Abdul Aziz, A. R.; Sulaiman, N. M. N., The effects of water on biodiesel production and refining technologies: a review. Renewable and Sustainable Energy Reviews 2012, 16(5), 3456–3470.

[33] Ma, F.; Clements, L. D.; Ha, M. A., Biodiesel fuel from animal fat. Industrial and Engineering Chemistry Research 1998, 37(9), 3768–3771.

[34] Sivasamy, A.; Cheah, K. Y.; Fornasiero, P.; Kemausuor, F.; Zinoviev, S.; Miertus, S., Catalytic applications in the production of biodiesel from vegetable oils. ChemSusChem 2009, 2(4), 278–300.

[35] Marchetti, J. M.; Miguel, V. U.; Errazu, A. F., Possible methods for biodiesel production. Renewable & Sustainable Energy Reviews. 2007, 11, 1300–1311.

[36] Nelson, L. A.; Foglia, T. A.; Marmer, W. N., Lipase-catalyzed production of biodiesel. Journal of American Oil Chemists' Society 1996, 73(9), 1191–1195.

[37] Shimada, Y.; Watanabe, Y.; Samukawa, T.; Sugihara, A.; Noda, H.; Fukuda, H.; Tominaga, Y., Conversion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase. Journal of the American Oil Chemists' Society 1999, 76 (7), 789–793.

[38] Watanabe, Y.; Shimada, Y.; Sugihara, A.; Noda, H.; Fukuda, H.; Tominaga, Y., Continuous production of biodiesel fuel from vegetable oil using immobilized *Candida antarctica* lipase. Journal of the American Oil Chemists' Society 2000, 77(4), 355–360.

[39] Samukawa, T.; Kaieda, M.; Matsumoto, T.; Ban, K.; Kondo, A.; Shimada, Y.; Noda, H.; Fukuda, H., Pretreatment of immobilized *Candida antarctica* lipase for biodiesel fuel production from plant oil. Journal of Bioscience and Bioengineering 2000, 90(2), 180–183.

[40] Fedosov, S. N.; Brask, J.; Pedersen, A. K.; Nordblad, M.; Woodley, J. M.; Xu, X., Kinetic model of biodiesel production using immobilized lipase *Candida antarctica* lipase b. Journal of Molecular Catalysis B: Enzymatic 2013, 85–86, 156–168.

[41] Watanabe, Y.; Shimada, Y.; Sugihara, A.; Tominaga, Y., Conversion of degummed soybean oil to biodiesel fuel with immobilized *Candida antarctica* lipase. Journal of Molecular Catalysis B: Enzymatic 2002, 17(3–5), 151–155.

[42] Taher, H.; Al-Zuhair, S.; Al-Marzouqi, A. H.; Haik, Y.; Farid, M., Enzymatic biodiesel production of microalgae lipids under supercritical carbon dioxide: process optimization and integration. Biochemical Engineering Journal 2014, 90, 103–113.

[43] Taher, H.; Al-Zuhair, S.; AlMarzouqui, A.; Hashim, I., Extracted fat from lamb meat by supercritical CO_2 as feedstock for biodiesel production. Biochemical Engineering Journal 2011, 55(1), 23–31.

[44] Al-Zuhair, S.; Hussein, A.; Al-Marzouqi, A. H.; Hashim, I., Continuous production of biodiesel from fat extracted from lamb meat in supercritical CO_2 media. Biochemical Engineering Journal 2012, 60, 106–110.

[45] Guldhe, A.; Singh, B.; Rawat, I.; Permaul, K.; Bux, F., Biocatalytic conversion of lipids from microalgae *Scenedesmus obliquus* to biodiesel using *Pseudomonas fluorescens* lipase. Fuel 2015, 147, 117–124.

[46] Devanesan, M. G.; Viruthagiri, T.; Sugumar, N., Transesterification of jatropha oil using immobilized *Pseudomonas fluorescens*. African Journal of Biotechnology 2007, 6(21), 2497–2501.

[47] Noureddini, H.; Gao, X.; Philkana, R. S., Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil. Bioresource Technology 2005, 96(7), 769–777.

[48] Moreno-Pirajàn, J. C.; Giraldo, L., Study of immobilized *Candida rugosa* lipase for biodiesel fuel production from palm oil by flow Microcalorimetry. Arabian Journal of Chemistry 2011, 4(1), 55–62.

[49] Tan, Z.; Fang, M.; Du, H.; Song, L.; Ren, D.; Tang, X.; Han, X.; Liang, X., Production of biodiesel catalyzed by *Candida rugosa* lipase at interface of W/O microemulsion system. Journal of the Brazilian Chemical Society 2014, 25, 1704–1711.

[50] Lee, J. H.; Kim, S. B.; Kang, S. W.; Song, Y. S.; Park, C.; Han, S. O.; Kim, S. W., Biodiesel production by a mixture of *Candida rugosa* and *Rhizopus oryzae* lipases using a supercritical carbon dioxide process. Bioresource Technology 2011, 102(2), 2105–2108.

[51] Huang, D.; Han, S.; Han, Z.; Lin, Y., Biodiesel production catalyzed by *Rhizomucor miehei* lipase-displaying *Pichia pastoris* whole cells in an isooctane system. Biochemical Engineering Journal 2012, 63, 10–14.

[52] Huang, J.; Xia, J.; Yang, Z.; Guan, F.; Cui, D.; Guan, G.; Jiang, W.; Li, Y., Improved production of a recombinant *Rhizomucor miehei* lipase expressed in *Pichia pastoris* and its application for conversion of microalgae oil to biodiesel. Biotechnology for Biofuels 2014, 7(1), 1–11.

[53] Dossat, V.; Combes, D.; Marty, A., Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: influence of the glycerol production. Enzyme and Microbial Technology 1999, 25(3–5), 194–200.

[54] Xu, Y.; Nordblad, M.; Nielsen, P. M.; Brask, J.; Woodley, J. M., In Situ visualization and effect of glycerol in lipase-catalyzed ethanolysis of rapeseed oil. Journal of Molecular Catalysis B: Enzymatic 2011, 72 (3–4), 213–219.

[55] Chen, H.-C.; Ju, H.-Y.; Wu, T.-T.; Liu, Y.-C.; Lee, C.-C.; Chang, C.; Chung, Y.-L.; Shieh, C.-J., Continuous production of lipase-catalyzed biodiesel in a packed-bed reactor: optimization and enzyme reuse study. Journal of Biomedicine and Biotechnology, 2011, 1-6.

[56] Azócar, L.; Navia, R.; Beroiz, L.; Jeison, D.; Ciudad, G., Enzymatic biodiesel production kinetics using co-solvent and an anhydrous medium: a strategy to improve lipase performance in a semi-continuous reactor. New Biotechnology 2014, 31(5), 422–429.

[57] Modi, M. K.; Reddy, J. R. C.; Rao, B. V. S. K.; Prasad, R. B. N., Lipase-mediated conversion of vegetable oils into biodiesel using ethyl acetate as acyl acceptor. Bioresource Technology 2007, 98(6), 1260–1264

[58] Du, W.; Xu, Y.; Liu, D.; Zeng, J., Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors. Journal of Molecular Catalysis B - Enzymatic 2004, 30(3–4), 125–129.

[59] Li, L.; Du, W.; Liu, D.; Wang, L.; Li, Z., Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium. Journal of Molecular Catalysis B: Enzymatic. 2006, 43(1–4), 58–62

[60] Zheng, Y.; Quan, J.; Ning, X.; Zhu, L.-M.; Jiang, B.; He, Z.-Y., Lipase-catalyzed transesterification of soybean oil for biodiesel production in tert-amyl alcohol. World Journal of Microbiology and Biotechnology 2009, 25(1), 41–46.

[61] Shimada, Y.; Watanabe, Y.; Sugihara, A.; Tominaga, Y., Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. Journal of Molecular Catalysis B: Enzymatic 2002, 17(3–5), 133–142.

[62] Modi, M. K.; Reddy, J. R. C.; Rao, B. V. S. K.; Prasad, R. B. N., Lipase-mediated transformation of vegetable oils into biodiesel using propan-2-ol as acyl acceptor. Biotechnology Letters 2006, 28(9), 637–640.

[63] Lu, J.; Chen, Y.; Wang, F.; Tan, T., Effect of water on methanolysis of glycerol trioleate catalyzed by immobilized lipase *Candida* sp. 99–125 in organic solvent system. Journal of Molecular Catalysis B: Enzymatic 2009, 56(2–3), 122–125.

[64] Klibanov, A. M., Improving enzymes by using them in organic solvents. Nature 2001, 209, 241–246.

[65] Zaks, A.; Klibanov, A., Enzymatic catalysis in organic media at 100 degrees C. Science 1984, 224, 1249–1251.

[66] Adamczak, M.; Krishna, S. H., Strategies for improving enzymes for efficient biocatalysis. Food Technology and Biotechnology 2004, 42(4), 251–264.

[67] Klibanov, A. M., Why are enzymes less active in organic solvents than in water? Trends in Biotechnology 1997, 15(3), 97–101.

[68] Doukyu, N.; Ogino, H., Organic solvent-tolerant enzymes. Biochemical Engineering Journal 2010, 48(3), 270–282.

[69] Yang, L.; Dordick, J. S.; Garde, S., Hydration of enzyme in nonaqueous media is consistent with solvent dependence of its activity. Biophysical Journal 2004, 87(2), 812–821.

[70] Gorman, L. A. S.; Dordick, J. S., Organic solvents strip water off enzymes. Biotechnology and Bioengineering 1992, 39(4), 392–397.

[71] Yang, Z.; Zhang, K.-P.; Huang, Y.; Wang, Z., Both hydrolytic and transesterification activities of *Penicillium expansum* lipase are significantly enhanced in ionic liquid [Bmim][Pf6]. Journal of Molecular Catalysis B: Enzymatic 2010, 63(1–2), 23–30.

[72] Peng, W.; Wu, Q.; Tu, P., Pyrolytic Characteristics of heterotrophic chlorella protothecoides for renewable bio-fuel production. Journal of Applied Phycology 2001, 13(1), 5–12.

[73] Lai, J.-Q.; Hu, Z.-L.; Wang, P.-W.; Yang, Z., Enzymatic production of microalgal biodiesel in ionic liquid [Bmim][Pf6]. Fuel 2012, 95, 329–333.

[74] Royon, D.; Daz, M.; Ellenrieder, G.; Locatelli, S., Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent. Bioresource Technology 2007, 98(3), 648–653.

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

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

[77] Reverchon, E.; Marrone, C., Modeling and simulation of the supercritical CO_2 extraction of vegetable oils. Journal of Supercritical Fluids 2001, 19(2), 161–175.

[78] Sovova, H.; Zarevucka, M.; Vacek, M.; Stransky, K., Solubility of two vegetable oils in supercritical CO_2. Journal of Supercritical Fluids 2001, 20(1), 15–28.

[79] Romero, M. D.; Calvo, L.; Alba, C.; Habulin, M.; Primoži?, M.; Knez, Ž., Enzymatic synthesis of isoamyl acetate with immobilized *Candida antarctica* lipase in supercritical carbon dioxide. The Journal of Supercritical Fluids 2005, 33(1), 77–84.

[80] Celia, E.; Cernia, E.; Palocci, C.; Soro, S.; Turchet, T., Tuning *Pseudomonas cepacea* lipase (PCL) activity in supercritical fluids. The Journal of Supercritical Fluids 2005, 33(2), 193–199.

[81] Novak, Z.; Habulin, M.; Krmelj, V.; Knez, Å. E., Silica aerogels as supports for lipase catalyzed esterifications at sub- and supercritical conditions. The Journal of Supercritical Fluids 2003, 27(2), 169–178.

[82] Oliveira, D.; Oliveira, J. V., Enzymatic alcoholysis of palm kernel oil in n-hexane and $SCCO_2$. The Journal of Supercritical Fluids 2001, 19(2), 141–148.

[83] Madras, G.; Kolluru, C.; Kumar, R., Synthesis of biodiesel in supercritical fluids. Fuel 2004, 83(14–15), 2029–2033.

[84] Varma, M. N.; Madras, G., Synthesis of biodiesel from castor oil and linseed oil in supercritical fluids. Industrial and Engineering Chemistry Research 2007, 46(1), 1–6.

[85] Taher, H.; Al-Zuhair, S.; Al-Marzouqi, A. H.; Haik, Y.; Farid, M.; Tariq, S., Supercritical carbon dioxide extraction of microalgae lipid: process optimization and laboratory scale-up. The Journal of Supercritical Fluids 2014, 86, 57–66.

[86] Andrich, G.; Zinnai, A.; Nesti, U.; Venturi, F., Supercritical fluid extraction of oil from Microalga Spirulina (Arthrospira) platensis. Acta Alimentaria 2006, 35(2), 195–203.

[87] Mendes, R. L.; Nobre, B. P.; Cardoso, M. T.; Pereira, A. P.; Palavra, A. N. F., Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorganica Chimica Acta 2003, 356, 328–334.

[88] Mendes, R. L.; Fernandes, H. L.; Coelho, J. P.; Reis, E. C.; Cabral, J. M. S.; Novais, J. L. M.; Palavra, A. N. F., Supercritical CO_2 extraction of carotenoids and other lipids from Chlorella vulgaris. Food Chemistry 1995, 53(1), 99–103.

[89] Cheng, C.-H.; Du, T.-B.; Pi, H.-C.; Jang, S.-M.; Lin, Y.-H.; Lee, H.-T., Comparative study of lipid extraction from microalgae by organic solvent and supercritical CO_2. Bioresource Technology 2011, 102(21), 10151–10153.

[90] Halim, R.; Gladman, B.; Danquah, M. K.; Webley, P. A., Oil extraction from microalgae for biodiesel production. Bioresource Technology 2011, 102, 178–185.

[91] Andrich, G.; Nesti, U.; Venturi, F.; Zinnai, A.; Fiorentini, R., Supercritical fluid extraction of bioactive lipids from the microalga *Nannochloropsis* sp. European Journal of Lipid Science and Technology 2005, 107(6), 381–386.

[92] Dalla Rosa, C.; Morandim, M. B.; Ninow, J. L.; Oliveira, D.; Treichel, H.; Oliveira, J. V., Continuous lipase-catalyzed production of fatty acid ethyl esters from soybean oil in compressed fluids. Bioresource Technology 2009, 100(23), 5818–5826.

[93] Ciftci, O. N.; Temelli, F., Continuous production of fatty acid methyl esters from corn oil in a supercritical carbon dioxide bioreactor. The Journal of Supercritical Fluids 2011, 58(1), 79–87.

[94] Ciftci, O. N.; Temelli, F., Continuous biocatalytic conversion of the oil of corn distiller's dried grains with solubles to fatty acid methyl esters in supercritical carbon dioxide. Biomass and Bioenergy 2013, 54, 140–146.

[95] Rodrigues, A. R.; Paiva, A.; da Silva, M. G.; Sim??es, P.; Barreiros, S., Continuous enzymatic production of biodiesel from virgin and waste sunflower oil in supercritical carbon dioxide. The Journal of Supercritical Fluids 2011, 56(3), 259–264.

[96] Al-Zuhair, S.; Taher, H., Supercritical Fluids Technology in Lipase Catalyzed Processes. CRC Press, USA, 2016.

[97] Wright, H. B.; Moore, M. B., Reactions of aralkyl amines with carbon dioxide. Journal of the American Chemical Society 1948, 70(11), 3865–3866.

[98] Madeira Lau, R.; Van Rantwijk, F.; Seddon, K. R.; Sheldon, R. A., Lipase-catalyzed reactions in ionic liquids. Organic Letters 2000, 2(26), 4189–4191.

[99] Lozano, P.; de Diego, T.; Carrié, D.; Vaultier, M.; Iborra, J. L., Lipase catalysis in ionic liquids and supercritical carbon dioxide at 150°C. Biotechnology Progress 2003, 19(2), 380–382.

[100] Lozano, P.; De Diego, T.; Carrie, D.; Vaultier, M.; Iborra, J. L., Over-stabilization of *Candida antarctica* lipase B by ionic liquids in ester synthesis. Biotechnology Letters 2001, 23(18), 1529–1533.

[101] Dang, D. T.; Ha, S. H.; Lee, S.-M.; Chang, W.-J.; Koo, Y.-M., Enhanced activity and stability of ionic liquid-pretreated lipase. Journal of Molecular Catalysis B: Enzymatic 2007, 45 (3–4), 118–121.

[102] Kaar, J. L.; Jesionowski, A. M.; Berberich, J. A.; Moulton, R.; Russell, A. J., Impact of ionic liquid physical properties on lipase activity and stability. Journal of the American Chemical Society 2003, 125(14), 4125–4131.

[103] Klahn, M.; Lim, G. S.; Wu, P., How ion properties determine the stability of a lipase enzyme in ionic liquids: a molecular dynamics study. Physical Chemistry Chemical Physics 2011, 13(41), 18647–18660.

[104] Ohno, H.; Yoshizawa, M., Ion conductive characteristics of ionic liquids prepared by neutralization of alkylimidazoles. Solid State Ionics 2002, 154, 303–309.

[105] Endres, F.; Zein El Abedin, S., Air and water stable ionic liquids in physical chemistry. Physical Chemistry Chemical Physics 2006, 8(18), 2101–2116.

[106] Xue, L.; Gurung, E.; Tamas, G.; Koh, Y. P.; Shadeck, M.; Simon, S. L.; Maroncelli, M.; Quitevis, E. L., Effect of alkyl chain branching on physicochemical properties of imidazolium-based ionic liquids. Journal of Chemical and Engineering Data 2016, 61(3), 1078–1091.

[107] Zhang, S.; Lu, X.; Zhou, Q.; Li, X.; Zhang, X.; Li, S., Ionic Liquids: Physicochemical Properties. Elsevier Science, Netherlands, 2009.

[108] Ochędzan-Siodłak, W.; Dziubek, K.; Siod?ak, D., Densities and viscosities of imidazolium and pyridinium chloroaluminate ionic liquids. Journal of Molecular Liquids 2013, 177, 85–93.

[109] Hernández-Fernández, F. J.; Ríos, A. P. D. L.; Tomás-Alonso, F.; Gómez, D.; Víllora, G., Stability of hydrolase enzymes in ionic liquids. The Canadian Journal of Chemical Engineering 2009, 87(6), 910–914.

[110] Aki, S. N. V. K.; Brennecke, J. F.; Samanta, A., How polar are room-temperature ionic liquids? Chemical Communications 2001, 5, 413–414.

[111] Huddleston, J. G.; Visser, A. E.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Rogers, R. D., Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. Green Chemistry 2001, 3(4), 156–164.

[112] Lozano, P.; de Diego, T.; Guegan, J.-P.; Vaultier, M.; Iborra, J. L., Stabilization of a-chymotrypsin by ionic liquids in transesterification reactions. Biotechnology and Bioengineering 2001, 75(5), 563–569.

[113] Sunitha, S.; Kanjilal, S.; Reddy, P. S.; Prasad, R. B. N., Ionic liquids as a reaction medium for lipase-catalyzed methanolysis of sunflower oil. Biotechnology Letters 2007, 29(12), 1881–1885.

[114] Han, M.; Yi, W.; Wu, Q.; Liu, Y.; Hong, Y.; Wang, D., Preparation of biodiesel from waste oils catalyzed by a brønsted acidic ionic liquid. Bioresource Technology 2009, 100(7), 2308–2310.

[115] Wu, Q.; Chen, H.; Han, M.; Wang, D.; Wang, J., Transesterification of cottonseed oil catalyzed by brønsted acidic ionic liquids. Industrial and Engineering Chemistry Research 2007, 46(24), 7955–7960.

[116] Zhang, L.; Xian, M.; He, Y.; Li, L.; Yang, J.; Yu, S.; Xu, X., A brønsted acidic ionic liquid as an efficient and environmentally benign catalyst for biodiesel synthesis from free fatty acids and alcohols. Bioresource Technology 2009, 100(19), 4368–4373.

[117] Zhou, S.; Liu, L.; Wang, B.; Xu, F.; Sun, R. C., Biodiesel preparation from transesterification of glycerol trioleate catalyzed by basic ionic liquids. Chinese Chemical Letters 2012, 23(4), 379–382.

[118] Ren, Q.; Zuo, T.; Pan, J.; Chen, C.; Li, W., Preparation of biodiesel from soybean catalyzed by basic ionic liquids [Hnmm]oh. Materials 2014, 7(12), 8012.

[119] Choi, S.-A.; Oh, Y.-K.; Jeong, M.-J.; Kim, S. W.; Lee, J.-S.; Park, J.-Y., Effects of ionic liquid mixtures on lipid extraction from *Chlorella vulgaris*. Renewable Energy 2014, 65, 169–174.

[120] Young, G.; Nippgen, F.; Titterbrandt, S.; Cooney, M. J., Lipid extraction from biomass using co-solvent mixtures of ionic liquids and polar covalent molecules. Separation and Purification Technology 2010, 72(1), 118–121.

[121] Lozano, P.; Bernal, J. M.; Vaultier, M., Towards continuous sustainable processes for enzymatic synthesis of biodiesel in hydrophobic ionic liquids/supercritical carbon dioxide biphasic systems. Fuel 2011, 90(11), 3461–3467.

IntechOpen

IntechOpen

IntechOpen