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REVIEW ARTICLE

# Application of heterogeneous catalysis to biodiesel synthesis using microalgae oil

**Violeta Makareviciene** (✉), **Egle Sendzikiene**, **Ieva Gaide**

Vytautas Magnus University, Agriculture Academy, K. Donelaicio str. 58, LT-44248 Kaunas, Lithuania

## HIGHLIGHTS

- Microalgae oil application for biodiesel synthesis is discussed.
- Catalytic effectiveness of ferment preparations and chemical catalyst is disputed.
- Application of heterogeneous catalysts for biodiesel synthesis is reviewed.
- Possibilities of catalyst regeneration is shown.

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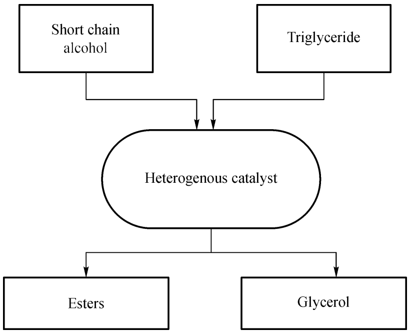
## GRAPHIC ABSTRACT



## ABSTRACT

Recently, there is a growing interest in the use of microalga in various fields. Microalgae have properties such as rapid reproduction and high biomass accumulation, and under certain conditions, some are able to accumulate a large amount of oil. However, microalgae oil often contains more free fatty acids than the vegetable oil and is therefore unsuitable for biodiesel synthesis using alkaline catalysts. For this reason, some authors suggest the application of heterogeneous catalysis. A particular interest in the use of immobilized enzymes has developed. Other solid substances can also be used as heterogeneous catalysts are usually metal oxides, carbonates or zeolites. The use of these catalysts results in simpler biodiesel synthesis, especially purification processes, a cleaner end product and a less polluted environment. The molar ratio of alcohol to oil is lower during enzymatic transesterification, and more than 90% ester yield is obtained using a molar ratio of alcohol to oil of 3:1 to 4.5:1. The alcohols do not have a negative effect on the effectiveness of chemical catalysts, so it is possible to use alcohols in molar ratio from 4:1 to 12:1. The optimal temperature of enzymatic process is 30°C–50°C. An ester yield of more than 95% was obtained in 12–48 h. Using chemical catalysts, greater than a 95% yield of esters was obtained at higher temperatures in a shorter time. Material costs of enzymatic catalysis can be reduced by reusing the catalysts directly or after regeneration.

## 1 Introduction

Recent decades have seen strongly increasing interest in the possibilities of using microalgae biomass for different purposes including biofuel production.

One of Europe's main raw materials for biodiesel production is rapeseed oil, which is also used for food. Increasing biofuel production increases competition between the food and fuel sectors. The advantage of microalgae over existing biodiesel feedstocks is that algae can be grown in infertile areas unsuitable for growing crops, as well as in industrial development areas and even on very limited land (Pokoo-Aikins et al., 2010), so algae does not compete for land with cultivated biomass for food production (Brennan and Owende, 2010). Compared to other biofuel feedstocks, algae have the ability to store high levels of lipids in their cells and at the same time have a high oil yield per unit area. In addition, these microorganisms can grow extremely rapidly under favorable conditions (Greenwell et al., 2010; Rawat et al., 2013). Microalgal growth requires nutrients such as nitrogen, phosphorus and microelements, what are found in wastewater and liquid waste; therefore, microalgae can be used efficiently for wastewater purification. Microalgae

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✉ Corresponding author
E-mail: violeta.makareviciene@vdu.lt

as other plants use carbon dioxide for photosynthesis, therefore application of microalgae for combustion gas or biogas purification is effective measure of reduction of greenhouse gases emissions (Lam and Lee, 2012; Moreno-Garcia et al., 2017; Enamala et al., 2018).

Currently options of microalgal biomass applications for liquid and gaseous fuel production are widely researched. The economic efficiency of these technologies is still low and methods to achieve higher economic efficiency are under investigation. One of such methods is production of biodiesel fuel from algae oil and biogas production from residual biomass.

Amount of oil in microalgae cells and fatty acid composition of microalgae oil differs depending on algae strain, cultivation media and conditions. Lipid content of microalgae biomass depending on algae strain is demonstrated in the Table 1.

The reported results show that under favorable conditions and when choosing certain types of microalgae for cultivation, 50%–70% lipids can be obtained. In microalgae cells, lipids are found in two forms, with polar lipids forming the cell's structural parts and nonpolar lipids, or triglycerides, constituting the cell's energy stocks. The oil content in microalgae biomass is influenced by cultivation conditions. The amounts of lipids and triglycerides are simultaneously increased by salt in the cultivation medium (sea water, in the case of *Dunaliella teraticola*) (Takagi et al., 2006). Adding $FeCl_3$ to the cultivation medium resulted in 3–7 times higher lipids content in *Chlorella vulgaris* microalgae biomass (Liu et al., 2008a). The most widely analyzed and applied method of increasing the amount of lipids in the microalgae biomass is nitrogen depletion (Mathimani et al., 2019). An analysis of the impact of nitrogen quantity during the cultivation of the microalgae and *Pavlova viridis* and *Nannochloropsis oculata* demonstrated that a larger amount of nitrogen in the cultivation medium results in faster increases in biomass, but a reduced amount of nitrogen in the medium

**Table 1** Lipid content of microalgae biomass and acid value of microalgae oil

| Algae | Microalgae biomass | | Acid value | |
|---|---|---|---|---|
| | Lipid content % | Ref. | Acid value mg KOH/g | Ref. |
| *B. braunii FACHB 357* | 51.6 | Cheng et al., 2013 | | |
| *B. braunii IPE 001* | 64.3 | Xu et al., 2012 | | |
| *B. braunii Showa* | 30–39 | Yoshimura et al., 2013 | | |
| *B. braunii UK 807-2* | 65–70 | Li and Qin, 2005 | | |
| *Botryococcus* sp. | 28.6 | Lee et al., 2010 | | |
| *C. vulgaris FACHB1068* | 42 | Feng et al., 2011a | | |
| *Chlorella vulgaris* | 10 | Lee et al., 2010 | | |
| *Chlorella vulgaris* | 16.41 | Converti et al., 2009 | | |
| *Chlorella vulgaris C7* | 56.6 | Liu et al., 2008a | | |
| *Chlorella vulgaris ESP-31* | 55.9 | Yeh and Chang, 2011 | | |
| *Chlorella vulgaris P12* | 11 | Anjos et al., 2013 | | |
| *Dunaliella tertiolecta ATCC 30929* | 70.6–71.4 | Takagi et al., 2006 | 167.25 | Krohn et al., 2011 |
| *Isochrysis zhangjiangensis* | 53 | Feng et al., 2011b | | |
| *Monoraphidium* sp. *FXY-10* | 51.72 | Zhao et al., 2014 | | |
| *N. oleoabundans* | 40 | Li et al., 2008 | | |
| *Nannochloris* sp. *UTEX LB1999* | 50.9 | Takagi et al., 2000 | | |
| *Nannochloropsis oculata* | 15.31 | Converti et al., 2009 | 83.4 | Krohn et al., 2011 |
| *Nannochloropsis* sp. | 59.9 | Jiang et al., 2011 | 6.5 | Chen et al., 2012 |
| *Neochloris oleabundans UTEX 1185* | 56 | Gouveia et al., 2009a | | |
| *Pond water algae* | | | 0.4 | Nautiyal et al., 2014 |
| *Porphyridium cruentum* | 8 | Biller and Ross, 2011 | | |
| *Scenedesmus obliquus CNW-N* | 22.4 | Ho et al., 2012 | | |
| *Scenedesmus* sp. | 10 | Lee et al., 2010 | 8.7 | Chen et al., 2012 |
| *Scenedesmus* sp. *LX1* | 53 | Xin et al., 2010 | | |
| *Spirulina* | | | 0.45 | Nautiyal et al., 2014 |
| *Tetraselmis subcordiformis* | 29.77 | Huang et al., 2013 | | |

results in a considerable increase in lipid quantity. Cultivating the microalgae *Nannochloropsis oculata* under nitrogen starvation conditions increased the amount of lipids almost 1.93 times, while in *Chlorella vulgaris*, the increase amounted to 2.8 times; these microalgae accumulated 15.31% and 16.41% lipids, respectively (Converti et al., 2009). The lipid percentage was still higher (64.3%) in *B. braunii IPE 001* microalgal biomass when using photobioreactors (Tasić et al., 2016), whereas *Nannochloropsis* sp. cultivated in sea water under nitrogen starvation conditions accumulated 59.9% lipids in its cells. Lipid accumulation in microalgae cells is also positively affected by reduced phosphorus and sulfur in the cultivation medium (Cakmak et al., 2012). Lipid accumulation is also determined by other microalgae cultivation conditions, such as illuminance, temperature and $CO_2$ concentration (Converti et al., 2009; Ho et al., 2012).

The lipid composition also plays a significant role in biodiesel production. For biodiesel synthesis, neutral lipids, namely, triglycerides, are generally used. These compounds are converted into biodiesel through the transesterification process, usually with the help of alkaline catalysts. The raw materials used for transesterification should contain less than 2% free fatty acids because they react with an alkaline catalyst, using it up for soap formation. Because they are good emulsifiers, soaps complicate the separation biodiesel from glycerol. Table 1 shows that the acid value in microalgae oil ranges from 0.4 to 167.25 mg KOH/g. This value indicates that in many cases, the direct transesterification of microalgae oil with alkaline catalysts is impossible, and the prior esterification of free fatty acids is required.

Fatty acid composition also has a major impact on biodiesel properties; it differs in different microalgae types and depends on their cultivation conditions. The data show (Table 2) that microalgae oil contains more saturated fatty acids, typical of fats of animal origin. It should be noted that microalgae oil is dominated by monounsaturated oleic acid (more than 40%), while rape oil is dominated by polyunsaturated linoleic acid. This suggests that microalgae biodiesel should be more stable for oxidation than biodiesel produced from rape oil, which contains more rapidly oxidising polyunsaturated fatty acids. Due to higher saturated fatty acid content, however, biodiesel produced from microalgae oil should have somewhat poorer low-temperature properties, that is, it should become turbid and crystallized at higher temperatures.

## 2 Application of heterogeneous catalysis during biodiesel synthesis

Biodiesel synthesis ordinarily uses homogeneous catalysis with reactants and the catalyst in the same state of aggregation; that is, it is performed in a liquid phase. This is attractive due to the closer contact between substances and a higher processing rate; on the other hand, it is difficult to clean and purify the reaction products at the end of the process. Principal technological scheme of biodiesel production from microalgae biomass is presented in the Fig. 1. Biodiesel synthesis can use heterogeneous solid catalysts (Goh et al., 2019).

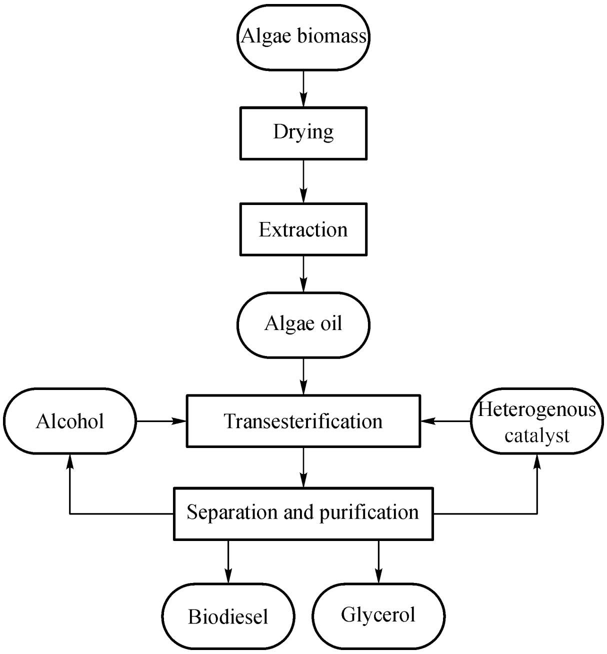

**Fig. 1** Principal technological scheme of biodiesel production from microalgae biomass.

### 2.1 Immobilised enzymes–heterogeneous catalysts for biodiesel synthesis

During biodiesel synthesis, triglycerides are transesterified with lower molecular mass alcohol, generally methanol. Fatty acid methyl esters (biodiesel) and glycerol are formed during transesterification process (see Fig. 2). If the raw materials are of high acidity, the free fatty acids reacts with the same alcohol using acid catalysts; this process results in increase of biodiesel yield.

It has been noted that enzymes have higher stability when used with substrates containing more free fatty acids because alcohols are more soluble in those acids. In addition, the free fatty acids contained in the substrate mitigate the enzyme inactivation caused by the alcohols (Watanabe et al. 2007; Véras et al., 2011). For these reasons, enzymatic processes are especially suitable for the transesterification of microalgae oil, which has a higher acidity.

The esterification of fatty acids and the transesterification of triglycerides are catalyzed by enzymes, namely, lipases. Pancreatic lipase is the most widely used lipase of animal origin. Plant lipases include lipases from papaya juice, oat seeds and castor bean seeds (Akoh et al., 2007).

**Table 2** Composition of fatty acids for the given types of microalgae oil compared to rape oil

| Fatty acid | Content % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rapeseed[1] | *Ankistrodesmus fusiformis*[2] | *Butyrococcus braunii*[2] | *Chlorella* sp.[1] | Chlorella vulgaris[2] | *Chlamidomonas* sp.[2] | *Haematococcus* sp.[1] | *Nanochloropsos* sp.[3] | *Nannochloris* sp.[1] | *Scenedesmus* sp.[1] | *Spirulina maxima*[3] | *Micractinium* sp. *IC-76*[4] |
| **Saturated**: | 5.40 | 37.33 | 9.85 | 36.91 | 49.46 | 78.61 | 18.78 | 30.96 | 14.27 | 22.81 | 41.68 | 32,5 |
| C14:0 Myristic | – | 2.02 | 0.73 | 0.31 | 0.63 | 1.61 | 0.23 | 7.16 | – | 0.73 | 0.34 | 2.2 |
| C16:0 Palmitic | 3.22 | 26.95 | 7.17 | 31.09 | 40.31 | 50.7 | 9.78 | 23.35 | 8.62 | 17.17 | 40.16 | 28.1 |
| C17:0 Margaric | | | | 0.47 | 0.51 | | 1.55 | | 0.93 | 0.51 | | |
| C18:0 Stearic | 2.18 | 2.01 | 1.59 | 4.11 | 8.01 | 11.54 | 3.99 | 0.45 | 2.81 | 2.87 | 1.18 | 2.2 |
| C20:0 Arachidic | | | | 0.57 | | | 1.37 | | 0.73 | 0.44 | 0.06 | |
| C22:0 Behenic | | | | 0.24 | | | 1.81 | | 1.09 | 0.92 | | |
| C24:0 Lignoceric | | | | 2.22 | | | 0.06 | | 0.1 | 3.17 | | |
| **Unsaturated**: | 94.60 | 52.63 | 90.15 | 63.09 | 45.02 | 21.39 | 81.24 | 59.20 | 85.74 | 77.19 | 51.9 | 67.5 |
| C16:1 Palmitoleic | | 0.25 | | 2.28 | 3.16 | 0.28 | 1.66 | 26.87 | 1.00 | 12.05 | 9.19 | 3.5 |
| C18:1 Oleic | 16.92 | 19.61 | 77.22 | 43.01 | 29.8 | 7.82 | 49.43 | 13.20 | 62.35 | 41.95 | 5.43 | 12.4 |
| C18:2 Linoleic | 66.32 | 12.23 | 5.16 | 10.95 | 8.54 | 3.93 | 13.67 | 1.21 | 12.72 | 13.02 | 17.89 | 29.2 |
| C18:3 Linolenic | 11.14 | 26.50 | 1.57 | 6.03 | 1.57 | 0.82 | 10.94 | | 5.44 | 17.97 | | 4.9 |
| C 20:1 Gadoleic | | | | 0.51 | 1.95 | | 4.59 | | 2.12 | 1.11 | | |
| C22:1 Erucic | 0.22 | | | 0.24 | | | 0.83 | | 0.62 | 0.20 | | |
| C24:1 Nervonic | | | | 0.02 | | | 0.12 | | 1.49 | 0.89 | | |

Note: [1]Andruleviciute et al., 2014; [2]Nascimento et al., 2013; [3]Gouveia et al., 2009b; [4]Piligaev et al., 2018.

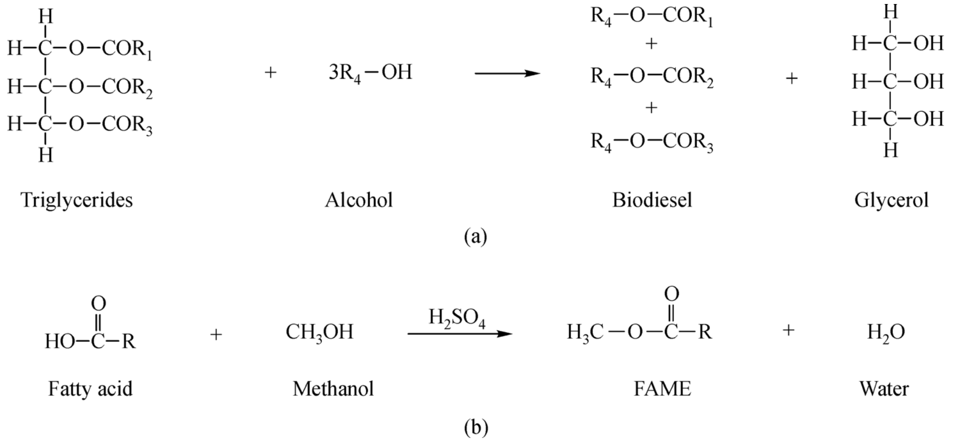

**Fig. 2** Transesterification reaction of triglycerides (a) and esterification reaction of free fatty acids (b).

Compared to animals and plants, microorganisms produce the largest range and amount of lipases, which are also more stable and less expensive; therefore, lipases from bacteria and fungi are produced industrially and are mostly used as biocatalysts in biotechnology (Christopher et al., 2014). Compared to lipases from animals or plants, microbial lipases are also more often used for biodiesel synthesis studies (Akoh et al., 2007; Antczak et al., 2009). Lipase production uses many microbial strains, but the lipases obtained from *Candida* sp., *Pseudomonas* sp. and *Rhizopus* sp. are used most frequently (Benjamin and Pandey, 1998).

Lipases have different properties, optimum operation temperatures, pH values and sensitivity to reactants. Lipases hydrolyse triglycerides naturally and can catalyze other reactions, namely, interesterification, esterification, alcoholysis, acidolysis and aminolysis (Jaeger and Reetz, 1998; Salis et al., 2005). Most lipases are specific and regioselective. The use of lipases in biodiesel synthesis involves regiospecificity and specificity regarding the length of the fatty acid chain (Marangoni, 2002). In terms of regioselectivity, i.e., selectivity with regards to the acyl group position, lipases are classified into three groups (Macrae, 1983; Chandler, 2001; Rahman et al., 2005), namely, *sn*-1.3-specific (they hydrolyse ester connections in positions R1 or R3 of a triglyceride molecule); *sn*-2-specific (they hydrolyse ester connections in position R2); and non-specific (there is no difference between the positions of the connections of a fissioning ester).

Narrowly regiospecific lipases are deemed unsuitable for biodiesel production; therefore, the majority of lipases used for this purpose are both specific and regiospecific with regard to the substrate. The following are the most popular sources of *sn*-1.3-specific lipases: *Rhizopus delemar, Rhizopus oryzae*, *Aspergillus niger, Rhizomucor miehei* and *Thermomyces lanuginosus* (Fernandez-Lafuente, 2010; Rodrigues et al., 2010). The only *sn*-2-specific lipase is that of *Geotrichum candidum*; however, it is not applied for transesterification (Macrae, 1983). The most popular non-specific lipase sources are *Pseudomonas cepacia, Pseudomonas fluorescens, Candida antarctica*, *Candida rugosa* and *Candida cylindracea*, (Aquieiras et al., 2015). The high yield of enzymatic transesterification is caused by the change of the acyl group position from *sn*-2 to the positions *sn*-1 and *sn*-3. *Geotrichum candidum* lipase splits the ester connections in position *sn*-2 of a triglyceride molecule.

Lipase, used in various processes, including biodiesel synthesis, can be applied in pure, liquid form or as a dried powder. However, these lipases have low activity and stability, and they are hard to regenerate and reuse (Yan et al., 2014). To increase their stability and the options for their reuse, lipases immobilised on various solid carriers are produced industrially. They have higher catalytic efficiency than pure lipases and a larger amount of active centers, facilitating synthesis reactions (Garcia-Galan et al., 2011). The porous natural or synthetic materials used as carriers for lipase immobilisation include zeolite, porous resins, silica gels, hydrotalcite, celite, acrylic resins, textile membranes, porous kaolinite, and diatomaceous earth. The lipase immobilisation methods differ in terms of the carrier and catalytic efficiency of the resulting products. Adsorption, covalent bonding, cross-linking and entrapment methods have long been known and used. To increase catalytic efficiency, the application of new carriers and immobilisation methods is under examination. Recent studies have focused on the use of magnetic particles, nanoparticles, nanofibers, protein-coated microcrystals and electrospun nanofibers for lipase immobilisation as well as the efficiency of these preparations in biodiesel synthesis (Xie and Ma, 2009; Xie and Ma, 2010; Tran et al., 2012; Cipolatti et al., 2014; Xie and Wang, 2014; Amini et al., 2017).

The carriers used for immobilisation and the nature of the lipases have great influence on the catalytic activity of enzyme preparations and the possibilities of their regeneration and reuse. The reuse of lipases is crucial in reducing the cost price of biodiesel production; the lipase price constitutes a large share of the cost. Biodiesel synthesis using enzymatic preparations is believed to be twice as expensive as synthesis with chemical catalysts (Jegannathan et al., 2011).

Bayramoglu et al. (2015) found that the efficiency of the lipase from *C. rugosa* when immobilised on bio-silicate for microalgae oil transesterification is 10% higher. Study findings show that this type of lipase can be employed for 6 cycles, after which the efficiency of biodiesel conversion stands at 83%. Additionally, this type of lipase has good thermal stability and resistance to solvents. Duraiarasan et al. (2016) describe the even better possibilities of lipase reuse in biodiesel synthesis from microalgae oil. According to them, the lipases that are immobilised on magnetic nanoparticles can be reused 10 times.

Considering that lipases are produced both plants and animals, recent studies have focused on the direct application of lipases contained in cell membranes during biotechnological processes. *Rhizopus oryzae* intracellular lipase showed quite strong activity (Andrade et al., 2014). Using *Rhizopus oryzae* lipase, Matsumoto et al. (2001) obtained a methyl ester yield of 71% over 165 h at 37°C, whereas Zeng et al. (2006) obtained a methyl ester yield of 86% using an intracellular lipase. The main advantage of intracellular lipase use is its simpler production and lower price. The attractiveness of the enzymatic process lies in the fact that fatty acid esters are obtained in one stage, while the chemical methods in this case require two stages, namely, esterification and subsequent transesterification.

### 2.1.1 Factors influencing the efficiency of enzymatic transesterification

The efficiency of biotechnological transesterification of oil is chiefly influenced by the following factors: the alcohol to oil molar ratio, the temperature, the amount of catalyst and the process duration. These variables determine the transesterification effectiveness.

#### 2.1.1.1 Process temperature

Chemical transesterification is usually carried out at higher temperatures. In biotechnological transesterification, significant increase of temperature is impossible because lipases lose their catalytic properties at high temperatures. For this reason, biotechnological processes are conducted in a temperature range of 30°C–55°C (Haas et al., 2002). Xu et al. (2004) noted that using lipase from *Thermomyces lanuginosus* and increasing the process temperature from 30° to 40° resulted in a gradual increase the process effectiveness, but transesterification with methanol at 50°C decreased the lipase stability and process rate. This adverse property of enzymes has also been noted by other authors (Shamel et al., 2005).

Using ethanol and the Lipozyme TL IM lipase obtained from *Thermomyces lanuginosus* for the transesterification of microalgae oil increased the transesterification efficiency at temperatures of 25°C to 35°C, whereas higher temperatures reduced the process efficiency (Gumbyte et al., 2018). Thus, the optimum process temperature must be chosen for different biocatalysts by accounting for different degrees of enzymatic stability.

#### 2.1.1.2 Nature and amount of the acyl receptor

During transesterification and esterification, a short-chain alcohol is used as the acyl receptor. For homogeneous catalysis with chemical catalysts, methanol is used most frequently. Methanol replaces glycerol bound to a triglyceride molecule, producing methyl esters with molecular masses that are almost three times lower than that of triglyceride. This lower molecular mass reduces the density and viscosity of the resulting biodiesel to values similar to those of mineral diesel fuel.

A stoichiometric reaction of one molecule of triglyceride requires three molecule of alcohol. Transesterification reaction is reversible; to increase the biodiesel amount, an alcohol surplus is usually used. In industrial production technologies, the molar ratio of methanol to oil stands at 6:1 (Marchetti et al., 2007).

Usage of methanol in biotechnological synthesis is complicated. The solubility of alcohol in oil is limited, and undissolved alcohol inactivates lipases; thus, it is impossible to increase the amount of alcohol without restriction (Akoh et al., 2007; Tan et al., 2010). However, the findings of some researchers are controversial. According to Tran et al. (2012), when using methanol and *Burkholderia* sp. *C20* lipase for the transesterification of *Chlorella vulgaris ESP-31* oil, the conversion efficiency of the microalgae oil increases when the molar ratio of methanol to oil is increased from 6.18 to 12.35 even without using a solvent. Further increases of alcohol amount reduce the process efficiency. Zheng et al. (2009) reported that the application of enzymatic catalysis requires a smaller surplus of methanol than chemical transesterification: when a methanol to oil ratio of 3:1 was used for transesterification, an ester yield of over 97% was obtained. Enzymes are more stable when used with substrates containing a higher count of free fatty acids because alcohols are more soluble in such substrates (Véras et al., 2011). With acid oil, a denaturation effect is achieved only at a molar ratio of 8:1 (Watanabe et al., 2007).

The gradual introduction of alcohol is the possible method for reducing the toxic impact of methanol and ethanol on enzymes. This approach keeps the alcohol concentration in the reaction medium low, and enzyme denaturation does not occur. The introduction of alcohol by few steps was investigated and gave the promising results. It was found that methanol addition in three portions results in a transesterification yield of 96% (Shimada et al.

(2002); Watanabe et al. (2000).

Other method for addressing the toxicity of methanol include the replacement of the acyl receptor with less harmful compounds. To solve the problems related to the toxic impact of methanol on lipases, other alcohols are recommended for use in transesterification. Studies show that an alcohol with a higher molecular mass inactivates lipases to a lesser degree, i.e., methanol has the most adverse effect on enzymes. Ethanol inactivates lipases less; therefore, methanol is often replaced by ethanol during enzymatic biodiesel synthesis (Zhang et al., 2014). With ethanol, *Thermomyces lanuginosus* lipase (Lipolase 100L) and immobilised *Candida antarctica* lipase (Lipozyme 435) are more promising (Makareviciene et al., 2017). Optimising the transesterification conditions with ethanol resulted in a transesterification degree of microalgae oil of over 96%. However, a larger amount of ethanol also has a negative impact on the effectiveness of transesterification (Watanabe et al. 2002); therefore higher molar ratio more than 3:1 is not recommended for the transesterification of microalgae oil by biotechnological methods (Makareviciene et al., 2017).

Alcohols in which the molecule contains 3 or more atoms of carbon (propanol, isopropanol, n-butanol, and isobutanol) are more soluble in vegetable oil, and simultaneously less harmful to enzymes (Shimada et al., 1999; Chen and Wu, 2003). The application of alternative acyl receptors has also been investigated. The most promising compounds are methyl or ethyl acetates. They do not inactivate enzymes, glycerol is not formed, and enzymatic preparations can be reused for transesterification without regeneration. However, the use of these acyl receptors requires a larger amount of the biocatalyst as well as a higher molar ratio of the reactants, and the rate of reaction and effectiveness of transesterification are lower (Xu et al., 2003; Du et al., 2004).

### 2.1.1.3 Solvent use in enzymatic transesterification

The glycerol produced during the transesterification process impedes the primary reaction because it adsorbs onto the lipase surface by covering the active centers of the enzyme, thus inactivating the biocatalyst. In addition, glycerol formation increases the viscosity of the reaction media, which makes mass transfer more complicated. The additional use of organic solvents increases the inter-solubility of alcohol, glycerol and oil and allows the introduction of the entire required alcohol quantity into the reaction medium at the same time, eliminating the need to add it gradually in small portions to avoid lipase inactivation (Li et al., 2006; Wang et al., 2006; Talukder et al., 2009; Azócar et al., 2011).

According to Wu et al. (2017), application of *Candida antarctica* for the transesterification of *Chlorella* sp. microalgae oil with a small amount of hexane, requires the molar ratio of methanol to oil of 12:1. Table 3 presents data on the conversion efficiencies of biodiesel production from *Chlorella* sp. oil using various solvents.

**Table 3** Efficiency of solvent use in enzymatic catalysis

| Solvent | Conversion efficiency (%) |
|---|---|
| Acetone | 53.05 |
| *1*-butanol | 11.36 |
| *t*-butanol | 79.69 |
| Dichlormethane | 19.78 |
| Ether | 71.25 |
| Ethyl acetate | 20.66 |
| Hexane | 51.81 |
| Isopropanol | 39.19 |
| MTBE | 77.66 |
| Pyridine | 6.98 |

The provided data show that during enzymatic transesterification, solvents such as *t*-butanol, ether, MTBE and hexane are more efficient. When solvents are used in biodiesel synthesis from highly acidic microalgae oil, it is necessary to evaluate the polarity of lipids and use polar or nonpolar solvents, respectively.

The process yield is influenced not only by the type but also the amount of solvent. Tests demonstrate that with the *C. antarctica* lipase, the highest ester yield is reached using *n*-hexane, while the lowest employs polar solvents such as acetone. The compound *tert*-butanol is among the most widely used solvents in biodiesel synthesis. The usage of this solvent considerably reduces the negative influence of glycerol and methanol on the enzyme efficiency because they are both highly soluble in *t*-butanol. The rapeseed oil methyl ester yield was increased by 65% when *t*-butanol was added to the transesterification reaction media (Li et al., 2006). The yield of 95% was achieved when using 3% of catalyst mixture of Lipozyme TL IM and Novozyme 435. Using *t*-butanol addresses the problems of catalyst reuse; the catalyst does not lose its activity even after 200 cycles. However, these results are subject to discussion because Lai et al. (2012a) report much poorer results regarding the efficiency of *t*-butanol use. Lee et al. (2013) obtained very good results using dimethyl carbonate (DMC) as a solvent for the enzymatic transesterification of *Chlorella* sp. oil, catalyzed by Novozyme 435 (Lee et al., 2013). DMC demonstrated good solvent properties; additionally it can replace methanol in transesterification process as effective acyl receptor and thus is effective both directly for microalgae oil transesterification and in the process of simultaneous oil extraction and transesterification from microalgae biomass (in situ). Using a mixture of DMC and methanol at a volume ratio of 7:3, Lee et al. (2013) extracted almost all the lipids from the biomass of the microalgae *Chlorella* sp. KR-1 (38.9%) and reached an oil conversion degree of higher than 90% with Novozyme 435 as a catalyst. It was reported that the catalyst remained active when used over 10 times. Some authors suggest to use supercritical carbon dioxide in enzymatic process of simultaneous algae oil extraction and transesterification as economically feasible. It was determined that pressure beyond 200 bar don't have negative effect on lipase effectiveness and stability (Taher et al., 2011).

Recently, scientists are interested in effectiveness of application of ionic liquids for transesterification of microalgae oil. Lai et al. (2012b) conducted transesterification tests on microalgae oil using Novozyme 435 and *Penicillium expansum* lipase as catalysts in two solvents, an ionic liquid [BMIm][PF6]) and *t*-butanol. It was determined that in the case of application of ionic liquid the lipase activity was considerably higher: under optimum conditions, the ester yield with the ionic liquid reached 90.7% and 86.2%, whereas with tert-butanol, 48.6% and 44.4% were observed, respectively.

However, solvent use in biodiesel synthesis also has an adverse effect, that is, the need for an additional solvent separation from the produced esters, which increases material and energy expenses (Antczak et al., 2009). To address the problem of solvent elimination, the application of mineral diesel as a solvent for enzymatic microalgae oil transesterification was investigated. Gumbyte et al. (2018) determined that the highest ester yield could be achieved in enzymatic transesterification of a mixture consisting of 93% mineral diesel and 7% microalgae using 13.2% immobilised Lipozyme RM IM, ethanol and oil molar ratio of 4.5:1, and at a duration of 13 h. Application of these conditions results in a product conforming to the quality requirements for mineral diesel indicated by standard EN 590.

#### 2.1.1.4 Other variables influencing the process efficiency

The effectiveness of enzymatic transesterification depends largely on the amount of catalyst. Although many authors reported an increase in transesterification effectiveness with the application of a larger amount of catalyst, using more than 10% biocatalyst is not recommended. This guideline applies to different lipases and transesterification agents (Selmi and Thomas, 1998; Lee et al., 2008; Makareviciene et al., 2017). Some investigators recommend using the smaller portion of the biocatalyst; the optimum amount of the Novozyme 435 catalyst in the reaction medium was reported to be 5% by Cervero et al. (2014). A smaller amount of biocatalyst makes the process more profitable. The regeneration and reuse of biocatalysts make the biotechnological process less expensive.

The ester yield is also substantially determined by the process duration. An increase in ester yield by increasing the process duration has been observed when using both ethanol and methanol for transesterification (Razack and Duraiarasan, 2016). The efficiency of enzymatic transesterification is also influenced by the presence of water. The small water content in reaction medium increase lipase catalytic activity. However, larger amount of water adversely affects the process of transesterification. The presence of water in the reaction medium causes oil hydrolysis and increases the free fatty acid content, the formation of enzyme particle aggregations, and a reduction in catalyst surface area and consequently the activity of the biocatalyst (Tan et al., 2006; Foresti et al., 2007).

Table 4 presents the conditions of enzymatic microalgae oil transesterification determined by different researchers. According to their findings, the optimal temperature for transesterification by ethanol and methanol is 30°C–50°C, a molar ratio of alcohol to oil from 3:1 to 6:1, duration more than 12 h, and a catalyst amount from 10% to 75%.

### 2.2 Biodiesel synthesis using chemical heterogeneous catalysts

Biodiesel synthesis can be performed using solid acids and solid bases as heterogeneous catalysts (Fig. 3).

Solid acid catalysts mostly consist of zeolite substances, heteropoly acids and pure or modified oxides of transitional metals. Using solid acids as catalysts causes fewer corrosion problems and is more environmentally friendly (Cheng et al., 2016). However, the activity of these acids depends greatly on the porosity of the given substances, which determines the speed of the adsorption-desorption and diffusion processes, which must be high to achieve a good catalytic effect.

When solid acid catalysts are used, the first stage involves a monohydroxy alcohol reaction with the catalyst's Lewis or Bronsted basic sites, resulting in the formation of alkoxides, which react with triglycerides during the next stage to form biodiesel and glycerol.

Acid zeolites have high porosity and thus are more suitable than metal oxides or carbonates. Their structure and properties can be modified easily to increase their biodiesel yield. Instead of the sulphuric acid used in homogeneous biodiesel synthesis, the recommendation is to use sulphated zirconia and organic sulpho acids as transesterification catalysts immobilised on porous silicon or aluminum carriers (Sani et al., 2013).

Heteropoly acids are also efficient catalysts. They are easily regenerated and reused and are also more active than homogeneous alkaline catalysts. The following heteropoly acids have been used for heterogeneous biodiesel synthesis: $H_3PW_{12}O_{40}$, $Cs_{2.5}H_{0.5}PW_{12}O_{40}$, $H_4SiW_{12}O_{40}$, $H_3PW_{12}O_{40}/Nb_2O_5$, $H_3PW_{12}O_{40}/ZrO_2$ and $H_3PW_{12}O_{40}/TiO_2$.

Basic zeolites, metal oxides and carbonates are effective basic heterogeneous catalysts for biodiesel synthesis (Galadima and Muraza, 2014).

The basicity of the solid base catalyst has a major impact on the reaction rate. The higher the basicity is, the faster the reaction. Basic sites react with alcohol, removing hydrogen

**Table 4** Optimal conditions of enzymatic microalgae oil transesterification (Makareviciene and Skorupskaite, 2019)

| Catalyst | Solvent | Acyl receptor | Optimal conditions | Yield (%) | Ref. |
|---|---|---|---|---|---|
| Lipolase 100L | – | Ethanol | Ethanol to oil molar ratio 3:1, 30°C, 26 h, 10% of enzyme | 96.9 | Makareviciene et al., 2017 |
| Lipozyme TL IM | Diesel fuel | Ethanol | Ethanol to oil molar ratio 4.5:1, 30°C, 13 h, 13.3% of enzyme | 98 | Gumbyte et al., 2018 |
| *Candida antarctica* | *t*-butanol | Methanol | Methanol to oil molar ratio 12:1; oil and t-butanol ratio 1:1 (w/v), oil and lipase mass ratio 20:1; 40°C, 12 h, 250 r/min | >97 | Wu et al., 2017 |
| *Candida* sp. *99–125* and immobilized by physical absorption onto macroporous resins | Methanol | Methanol | 30% of lipase, 10% of water, methanol to oil molar ratio 3:1, 38°C, pH 7, 12 h | 98.15 | Xiong et al., 2008 |
| *Candida* sp. | Hexane | Methanol (stepwise) | Methanol to oil molar ratio 3:1, 38°C, 12 h., 75% of enzyme | 98.2 | Li et al., 2007 |
| *Penicillium expansum* lipase | [BMIm][PF6] | Methanol | Methanol to oil molar ratio 4:1, 50°C, 48 h., 20% of enzyme | 90.7 | Lai et al., 2012a |
| *Candida* sp.<br>Novozyme 435 | Dimethyl carbonate | Dimethyl carbonate | 50% of lipase, 60°C, 6 h | 75.5 | Lee et al., 2013 |

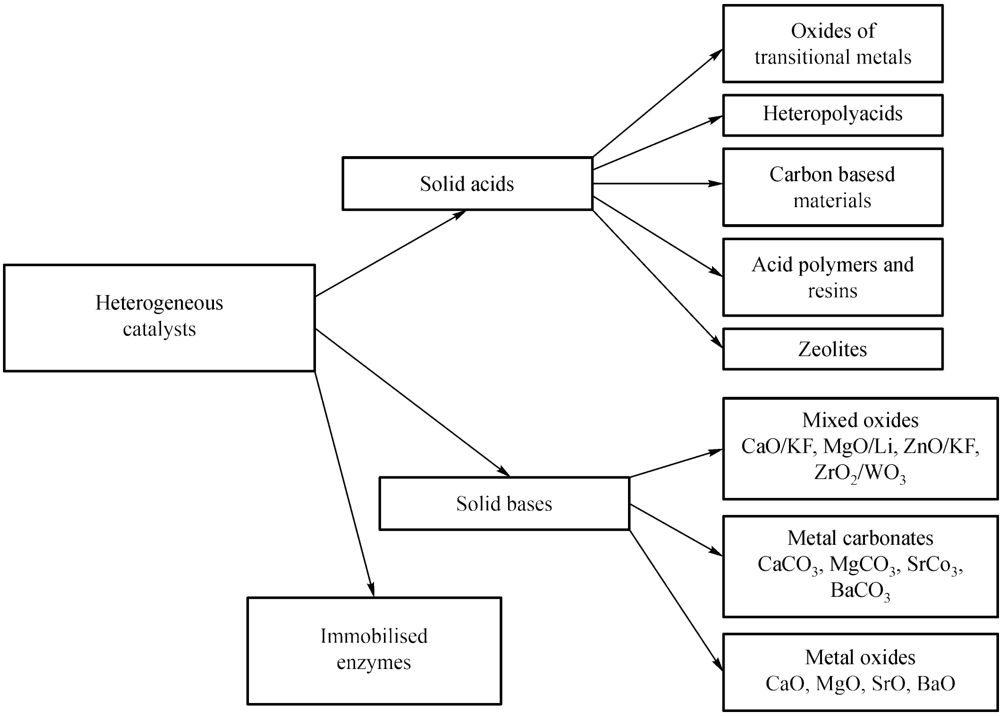

**Fig. 3** Possible chemical heterogeneous catalysts for biodiesel synthesis.

ions ($H^+$) and forming alkoxide groups (RO-), which actively react with triglycerides. The basicity of metal oxides has the following order: Ba>Sr>Ca>Mg.

Solid base catalysts are cheap and do not cause corrosion. Metal oxides can be extracted from natural rocks. Before they are used as catalysts, they undergo special preparation. One of the primary preparation methods is heating at a high temperature, i.e., calcination. This process substantially influences the catalytic activity the transesterification process. Mixtures of metal oxides are prepared by thermal treatment and impregnation. The optimum amount of metal oxides varies from 2% to 6%. The transesterification process is performed at approximately 65°C, and duration ranges between 100 and 180 min. Mixtures of metal oxide achieve higher efficiency of the transesterification process and possible reuse of catalysts (Naderi and Nayebzadeh, 2019). Most studies have been performed using mixtures of Mg-Al oxides. They have been found to be highly efficient when used for the transesterification in the fixed bed reactors (Frolich et al., 2019).

Mixed oxides of Ca and Fe, Ce, Zr and La are also quite promising; their use results in biodiesel yields greater than 95%. Recent studies have also examined the possibilities of using zeolites, hydrotalcites and cationites in heterogeneous biodiesel synthesis (Lee et al., 2014).

Less data have been published on microalgae oil transesterification with the use of heterogeneous catalysis than that of other types of oil. Carrero et al. (2011) explored the process of biodiesel synthesis from *Nannochloropsis gaditana* microalgae oil using hierarchical beta (h-Beta) zeolites and ZSM-5 (h-ZSM-5). The catalyst h-Beta was more effective than h-ZSM-5. Study findings show that the use of hierarchical h-Beta zeolites (Sani et al., 2013) results in high biodiesel yield (99.5%). Reyes et al. (2012) achieved a conversion of 94.2% during the in situ hydroesterification of *Monoraphidium contortum* (MORF-1) microalgae biomass with 10% niobium oxide powder (HY-340) at a temperature of 200°C, with a molar methanol to oil ratio of 30:1, a duration of 1 h and a mixing speed of 500 r/min. This process is attractive because humid microalgae biomass can be used, and the process duration is short. An ester yield of 99% was obtained when process was performed using mixture of 4% NiO and 18% $MoO_3$/alumina as heterogeneous catalyst. High ester yield (of 98%) was determined when 4% NiO and 18% $MoO_3$/h-ZSM-5 were used, and by application of (HY-340) niobium oxide and microporous titania a yield of over 94% was obtained. Amberlyst-15 also possesses very good properties (Dong et al., 2013), and its use as a catalyst for biodiesel synthesis from microalgae oil makes it possible to obtain a biodiesel yield of over 98%. The promising results show application of nano-catalysts for biodiesel production from microalgae. They have high surface area and catalytic efficiency. By using $Ca(OCH_3)_2$ nano-catalyst for *Nanochloropsis* sp. oil transesterification biodiesel yield of 99% was obtained, Using 8% of $SO_4^{2-}/Fe_3O_4–Al_2O_3$ nano-catalyst for transesterification of microalgae oil, 87.6% yield of esters was obtained (Safakish et al., 2020). CaO nanoparticles also have good catalytic properties: 96.3% ester yield was determined by using CaO nano-catalyst for microalgae oil transesterification (Akubude at al., 2019). Gupta and Agarwal (2016) reported the increase in the biodiesel yield form a 93% to 96% yield using CaO nano catalyst produced from snail shell. Even greater efficiencies were observed using CaO and MgO mixtures. Over 96% yield was reported using nano CaO catalyst obtained by calcination of egg and clam shells (Manikandan and Rajasekaran, 2013; Siva and Marimuthu, 2015).

Table 5 presents the results of selected studies on microalgae oil transesterification under heterogeneous conditions.

The conditions of heterogeneous catalysis (temperature, duration, mixing frequency, and amount of alcohol) depend on the type of catalyst, whether a solid acid or a solid base. The selected raw material and its pureness also have a substantial effect. The reaction temperature is usually close to the alcohol boiling point, and the reaction duration should not be longer than 1−3 h. During heterogeneous catalysis, one critical condition is the mixing intensity; an average frequency for the mixing intensity is recommended. Before the interaction with alcohol molecules, triglyceride molecules are adsorbed onto the solid catalyst surface. Alcohol access to the adsorbed triglycerides is affected by the mixing frequency, which greatly influences the process rate. At a low mixing speed, the transesterification reaction rate is low, but higher mixing intensity causes side reactions; therefore, an average mixing speed is applied during heterogeneous catalysis. As in homogeneous catalysis, an alcohol surplus is used during the heterogeneous process to increase the biodiesel yield.

The free fatty acids are unwanted impurities in the raw material, they must be esterified before the transesterification process; therefore, the recommended acidity of oil is less than 2%. This amount causes problems when applying heterogeneous catalysis for microalgae oil transesterification because the acidity of microalgae oil is often higher.

To increase the process efficiency and ester yield, supercritical conditions are applied, namely, transesterification occurs under alcohol supercritical conditions of high temperature and pressure. With acid catalysts, Helwani et al. (2009) obtained a high ester yield at temperatures higher than 350°C, even though Carrero et al. (2011) indicate that high transesterification effectiveness was also observed at a process temperature of 115°C. Table 6 provides the data on the heterocatalysts used for microalgae oil transesterification and the resulting product yields.

As observed from the given data, yields higher than 94% were obtained with niobium oxide, NiO, and $MoO_3/Al_2O_3$. Application of supercritical conditions for transesterification requires high temperature and pressure, and is thus is less promising than heterogeneous catalysis at atmospheric pressure and lower temperatures.

**Table 5** Heterogeneous catalysts used for microalgae oil transesterification and process conditions (Makareviciene and Skorupskaite, 2019)

| Catalyst | Amount (%) | Temperature (°C) | Yield (%) | Ref. |
|---|---|---|---|---|
| $CaOAl_2O_3$ | 1.56 | 50 | 88.9 | Narula et al., 2017 |
| Mg–Zr solid base catalyst | 10 | 65 | 28 | Li et al., 2011 |
| KF/CaO catalysts with different KF loadings | 12 | 60 | 93.07 | Ma et al., 2015 |
| $Cr_2O_3/Na_2Cr_2O_3/Al_2O_3$ | 20 | 80 | 98.28 | Guldhe et al., 2017a |
| Tungstated zirconium oxide ($WO_3/ZrO_2$) | 15 | 100 | 94.58 | Guldhe et al., 2017b |
| Amberlyst-15 | 30 | 90 | >98 | Dong et al., 2013 |
| Acid resins: | 0.8 (M) | 100 | >90 | Carrero et al., 2015 |
| CT-269 | | | 67 | |
| CT-275 | | | 18.2 | |
| Amberlite 15 | | | | |
| KSF klay | | | | |
| Silica-alumina | | | | |

**Table 6** Findings from studies on biodiesel synthesis from microalgae oil under supercritical conditions using heterocatalysts

| Catalyst | Amount (%) | Temperature (°C) | Pressure (kPa) | Yield (%) | Ref. |
|---|---|---|---|---|---|
| NiO, $MoO_3/Al_2O_3$ | 4<br>18 | 370 | 5000 | 99 | Vermaet al., 2011 |
| NiO, $MoO_3$/H-ZSM-5 | 5<br>18 | 375 | 1000 | 83 | Vermaet al., 2011 |
| Microporous titania | Continues | 340 | 15513.78 | 31 | Krohn et al., 2011 |
| (HY-340) niobium oxide | 10 | 200 | 20684.27 | 94.27 | Reyeset al., 2012 |

To increase the yield of biodiesel, some authors suggest application of ultrasound and microwave energy in oil transesterification process. These methods are often proposed for the simultaneous extraction and transesterification of microalgae oil. Ultrasonic and microwave energy breaks down the walls of microalgae cells, the oil is released faster and the transesterification efficiency increases. Jo et al. (2014) using ultrasound treatment for simultaneous microalgae *Chlorella* sp. KR-1 oil extraction and enzymatic transesterification obtained 91.8% ester yield. Sivaramakrishnan and Incharoensakdi (2017) under ultrasound treatment obtained 88% of *Botryococcus* sp. methyl ester yield, Sivaramakrishnan and Muthukumar (2014) achieved 86% yield of methyl esters of microalgae *Oedogonium* sp. However, the application of ultrasound has certain drawbacks. For some species of microalgae, this method shows low disruption effectiveness, and in addition, this method generates excess heat. The possibility of combining ultrasound with various solvent systems or other disruption methods is being investigated for reduction of energy consumption and increase of cell lysis efficiency.

Microwave treatment is other effective method of microalgae cell disruption. It is often used with solvent extraction, thus increasing the yield of oil, reducing the amount of solvent required and the process duration. Many researchers have investigated the possibilities of increasing the efficiency of oil extraction and transesterification using microwave assisted heating (Beneroso et al., 2017; Bundhoo, 2018). Some authors apply microwave treatment using solvents. However, obtained biodiesel yield is not high. Wahidin et al. (2016) investigated the direct biodiesel production process in combination with the microwave technique and several ionic liquids, and found that using 1-ethyl-3-methylimmidazolium methyl sulfate, a yield of 36.78% methyl esters of microalgae oil could be obtained. 42.22% biodiesel yield was determined by Wahidin et al. (2016) by using ionic liquid and application microwave technique during direct transesterification of wet microalgae biomass.

## 3 Multiple use and regeneration of heterogeneous catalysts

### 3.1 Biocatalysts

The industrial application of the enzymatic process for biodiesel synthesis is still restricted by the higher cost of the resulting product. This is determined by the high prices of the enzymatic preparations.

Jegannathan et al. (2011) specify that the price of biodiesel production with immobilised catalysts (per tonne) is 206.96% higher than that of biodiesel production by alkaline catalysis. The cost price with a production capacity of $10^3$/tonne and the batch-mode process is equal to 1166.67 USD/tonne using an alkaline catalyst. With a soluble lipase as the catalyst, the biodiesel cost reaches 7821.37 USD/tonne, and with an immobilised lipase, it is 2414.63 USD/tonne. For general biodiesel production expenses, the share of the lipase price constitutes 76.71% when using a soluble lipase and 49.69% when using an immobilised lipase; when using an alkaline catalyst, the catalyst price makes up only 1.56% of the general production expenses. The higher expenses of the enzymatic process are related to the higher price of the enzymatic preparation and the longer process duration. To reduce the production cost, even better possibilities of reuse are needed, and thus, recent studies have addressed efficient methods of lipase regeneration (Ramachandran et al., 2013).

Table 7 presents the findings of these tests on the reuse and regeneration of enzymatic preparations. These findings show that the reuse possibilities depend on the catalyst and the alcohol used for transesterification. Some investigators have found that a well-known and highly efficient industrial preparation of Novozyme 435 can be reused in up to 50 cycles of transesterification with methanol and up to 100 cycles using methyl acetate as the acyl receptor (Shimada et al., 2002; Du et al., 2004; Modi et al., 2007; Suárez et al., 2019).

Methanol inactivates lipases/biocatalysts. Oil transesterification studies with methanol have shown that the activity of an enzyme decreases with each cycle and is influenced not only by the effect of methanol but also by glycerol adsorption on the biocatalyst surface (Shimada et al. 2002; Du et al., 2004). Tests by Jegannathan et al. (2011) have demonstrated that the enzymatic preparation can be reused five times. Nguyen et al. (2018) established that during the transesterification of insect fat with methanol, the enzymatic preparation Novozyme 435 did not lose its activity over 20 cycles. Better results were found when methanol was supplied to the process in stages. According to Watanabe et al. (2002), during the transesterification of soybean oil with methanol and the application of three-step methanolysis, the activity of Novozyme 435 does not decrease after 25 cycles. Shimada et al. (1999, 2002) obtained even better results; with a three-step methanolysis process, the activity of the enzymatic preparation was sufficient for 50–52 cycles. In a two-step methanolysis process, 95% conversion of vegetable oil was obtained when reusing the lipase for 70 cycles (Shimada et al., 2002).

Ethanol is less harmful to enzymes than methanol. During the transesterification of fish oil with ethanol, Novozyme 435 loses 16% of its activity after 10 cycles, whereas Lipozyme RM IM and Lipozyme TL IM are unsuitable for reuse because they lose 75% of their activity after the first cycle and 90% after the second cycle (Suárez et al., 2019). Supplying ethanol in stages increases the options for enzyme use. Novozyme 435 can be used for 37 cycles with a two-step ethanolysis process and for 54

**Table 7** Multiple reuse of biocatalysts in biodiesel synthesis

| Catalyst | Fatty raw material | Transesterification agent | Preparation of catalyst for reuse | No of cycles | Ref. |
|---|---|---|---|---|---|
| Novozym 435 | Soybean oil | Methanol | – | 1 | Du et al., 2004 |
| Novozym 435 | Insect fat | Methanol | Recovered Novozym 435 was immersed in tert-butanol for its regeneration, followed by washing with insect fat for the removal of tert-butanol on the catalyst | 20 | Nguyen et al., 2017 |
| Novozym 435 | Soybean oil | Three-step methanolysis | | 25 | Watanabe et al., 2002 |
| Novozym 435 | Vegetable oil | Two-step methanolysis | | 70 | Shimada et al., 2002 |
| Novozym 435 | Vegetable oil | Three-step methanolysis | | 50–52 | Shimada et al., 2002;<br>Shimada et al., 1999 |
| Novozym 435 | Fish oil | Ethanol | | 10 | Suárez et al. 2019 |
| Novozym 435 | Tuna oil | Two-step ethanolysis | | 37 | Shimada et al., 2002 |
| Novozym 435 | Tuna oil | Three-step ethanolysis | | 54 | Shimada et al., 2002 |
| Novozym 435 | Soybean oil | Methyl acetate | | 100 | Du et al., 2004 |
| *Rhodotorula mucilaginosa* MTCC8737 | *Chlorella salina* oil | Methyl acetate | | 10 | Surendhiran et al., 2014 |
| Novozym 435 | Insect fat | Methyl acetate | | 20 | Nguyen et al., 2018 |
| Novozym-435 | Crude jatropha oil | Ethyl acetate | | 12 | Modi et al., 2007 |
| Novozym 435 | Soybean oil | Methanol ultrasonic waves | | 5 | Yu et al., 2010 |

cycles with three-step ethanolysis (Shimada et al., 2002).

The use of low-mass carbonate esters in biodiesel synthesis does not result in the formation of glycerol, which can reduce the activity of the enzyme. Du et al. (2004) compared the activity of the biocatalyst Novozyme 435 when used with methanol and with methyl acetate. The enzyme lost its activity after one cycle of methanol use, whereas with methyl acetate, its activity remained unchanged after 100 cycles. Examining the transesterification of insect fat with methyl acetate, Nguyen et al. (2018) found that Novozyme 435 did not lose its activity after 20 cycles. For the transesterification of crude *Jatropha* oil with ethyl acetate, Novozyme 435 is suitable for use in 12 cycles (Modi et al., 2007).

During the transesterification of *Chlorella salina* microalgae oil with methyl acetate and the biocatalyst *Rhodotorula mucilaginosa* MTCC8737, the catalyst did not lose its activity after 10 cycles (Surendhiran et al., 2014). Yu et al. (2010) used Novozyme 435 soybean oil transesterification with methanol in an environment with ultrasonic waves and obtained that enzymes can be used in 5 cycles without losing their activity.

Aguieiras et al. (2016) explored the possibilities of reusing commercial immobilised lipases (Novozyme 435, Lipozyme RM IM, Lipozyme TL IM) for transesterification with ethanol. Before reuse, the lipases were washed with solvents (ethanol, butanol, and hexane). The highest glycerol removal efficiency (more than 85%) was observed when ethanol and butanol were used. Hexane inactivates Novozyme 435, whereas ethanol inactivates Lipozyme TL IM (Aguieiras et al., 2016). The fact that ethanol inactivates Lipozyme TL IM has also been confirmed by the research of Suárez et al. (2019).

## 3.2 Chemical catalysts

The multiple uses of heterocatalysts of mineral origin also enable reductions in the biodiesel production cost. It has been established that heterogeneous chemical catalysts are suitable for use in 2−50 cycles (Table 8).

When nanocrystal calcium oxide is used, the options for its reuse depend on the nature of oil/fat. This compound is inactivated after eight cycles for soybean oil and after three cycles in the transesterification of poultry fat (Reddy et al., 2006). Granados et al. (2007) confirmed that CaO can be used as a catalyst up to eight times in sunflower oil transesterification, but the biodiesel yield decreases from 94% to 81%. Yoosuk et al. (2010) found that both fresh and used CaO have the same morphological structure and can be used in palm oil transesterification five times without additional preparation. Some authors reported that calcium oxide can be used up to 20 times in soybean oil transesterification, with its activity decreasing only to a small degree (Liu et al.2008b).

Liu et al. (2008b) observed that when using calcium methoxide $Ca(OCH_3)_2$ for soybean oil transesterification with methanol, the catalyst don't lose the activity for 20 times. The possible multiple uses of KOH/NaX as a catalyst in soybean oil transesterification have also been analyzed. Before reuse, the catalyst was washed with cyclohexane and heated at 398 K for 2 h, but the conversion fell from 85.2% to 48.7%. Following the

**Table 8** Multiple uses of chemical/natural catalysts in biodiesel synthesis

| Catalyst | Fatty raw material | Preparation of catalyst for reuse | No of cycles | Ref. |
|---|---|---|---|---|
| Nanocrystalline CaO | Soyabean oil | – | 8 | Reddy et al., 2006 |
| Nanocrystalline CaO | Poultry fat | – | 3 | Reddy et al., 2006 |
| CaO | Sunflower oil | – | 8 | Granados et al., 2007 |
| CaO | Palm olein | – | 5 | Yoosuk et al., 2010 |
| CaO | Soyabean oil |  | 20 | Liu et al., 2008b |
| $Ca(OCH_3)_2$ | Soyabean oil | – | 20 | Liu et al., 2008b |
| KOH/NaX | Soyabean oil | Washing with cyclohexane and heating at 398 K for 2h | 1 | Xie et al., 2007 |
| KOH/NaX | Soyabean oil | Washing with cyclohexane and heating at 398 K for 2h, impregnation with 5% KOH | 1 | Xie et al., 2007 |
| $Mg_2CoAl$ | Canola oil | Filtration and drying at 100°C | 7 | Li, et al., 2009 |
| $Na/SiO_2$ | Jatropha curcus oil | Washing with methanol and drying at 100°C for 2 h | 5 | Kumar, et al., 2010 |
| Dolomite | Canola oil | – | 3 | Ilgen, 2011 |
| Dolomite | Sanflower oil | – | 2 | Sendzikiene et al., 2018 |
| Dolomite | Palm kernel oil | Centrifugation and washing with methanol | 7 | Ngamcharussrivichai et al., 2010 |
| waste eggshell | Soyabean oil | – | 13 | Wei et al., 2009 |
| Starch-derived solid acid catalyst | Waste cooking oils | – | 50 | Lou et al., 2008 |

impregnation of the catalyst with 5% KOH solution, the conversion reached 84.3% (Xie et al., 2007). $Mg_2CoAl$ is a stable catalyst and can be used for seven cycles without losing its activity; after each cycle, it must be filtered and dried at a temperature of 100°C/373.15 K (Li et al., 2009).

Kumar et al. (2010) used ultrasound and a $Na/SiO_2$ catalyst for *Jatropha* oil transesterification with methanol; the catalyst can be used for five cycles without a reduction in reaction efficiency. However, after each cycle, the catalyst must be regenerated by washing with methanol and drying at 100°C/373.15 K for 2 h. These catalyst regeneration methods require energy resources.

Ilgen (2011) also examined the options for multiple uses of dolomite. Dolomite can be used as a catalyst in only three cycles. After each cycle, the transesterification yield decreases by only 1.5%, but the maximum transesterification yield of the resulting biodiesel was equal to 91.78%. The same results were obtained by other researchers, who explored the possible multiple uses of dolomite in the transesterification process. Biodiesel that conformed to the standard requirements was obtained by using dolomite two times. After the third time, a high ester content was reached, at 95.72%, but the results did not conform to the requirements of the standard (Sendzikiene et al., 2018). Ngamcharussrivichai et al. (2010) successfully used dolomite in biodiesel synthesis seven times, but they regenerated it after each cycle by centrifuging and washing it with methanol.

Eggshells are waste products suitable for use as catalysts in biodiesel production; they can be used 13 times without losing their activity and are deactivated after more than 17 cycles (Wei et al., 2009). A starch-derived solid acid catalyst can be used successfully in 50 cycles of biodiesel synthesis; after each cycle, filtering the catalyst is sufficient (Lou et al., 2008).

## 4 Advantages and disadvantages of different types of heterogeneous catalysts in biodiesel synthesis

The advantages and disadvantages of heterogeneous catalysis compared to homogeneous catalysis are demonstrated in the Table 9. The advantages include the fact that purification of produced fatty acid esters is performed easily; separated catalyst after regeneration can be used repeatedly.

Results show that both heterogeneous and homogeneous catalysis use higher than stoichiometric alcohol content. However, comparing enzymatic and chemical catalysis reveals that the alcohol amount is lower during enzymatic transesterification, and more than 90% ester yield is obtained using alcohol to oil a molar ratio of 3:1 to 4.5:1. However, controversial results have also been reported. Wu et al. (2017) found that greater than 97% ester yield was obtained by using a molar ratio of methanol to oil of 12:1. The ability to use excess of alcohol in the enzymatic process is limited by the inactivation of lipases by alcohols. This problem can be solved by usage of additional solvents. However, application of additional solvents also poses certain problems: additional energy costs will be required to remove them from the esters. The alcohols do not have a negative effect on the effectiveness of chemical acid and alkali catalysts, so it is possible to use alcohols in larger quantities (molar ratio from 4:1 to 12:1) and not to use other solvents.

Process temperature cannot be higher than 50°C during the enzymatic process, because lipases are denatured and therefore inactivated at higher temperatures. Therefore, depending on the nature of the lipase, it is proposed to perform the enzymatic process at a temperature of 30°C–50°C. However, lower temperatures result in longer process times. During enzymatic transesterification, an ester yield of more than 95% was obtained only in 12–48 h. Meanwhile, using chemical catalysts, greater than a 95% yield of esters was obtained at higher temperatures in a shorter time. Dong et al. (2013) obtained >98% ester yield 10 min at 90°C, whereas Ma et al. (2015) yielded 93.07% esters at 60°C in only 4 h.

Given that enzymatic catalysts are still relatively expensive, the downside of enzymatic transesterification is that a relatively large amount of catalyst must be used for obtaining a higher yield of esters. Studies show that greater than 95% yield is obtained using at least 10% biocatalyst in 26 h (Makareviciene et al., 2017), while high ester yields in less time require 20% to 75% enzymatic preparation (Wu et al., 2017; Li, et al., 2007). Although higher yields of esters are obtained using a similar amount of catalyst (10%–20%) in chemical heterocatalysis, the price of chemical catalysts is lower than enzymatic catalysts.

In summary, enzymatic heterocatalysis is inferior to chemical heterogeneous catalysis due to the higher material costs associated with the higher cost of enzymatic catalysts, the need to evaporate the solvent, and the longer process duration.

The costs associated with the higher cost of enzymes can be reduced by reusing the catalysts directly or after regeneration. In this case, enzymatic preparations show good prospects. Although no studies have been performed with microalgae oil, the trends are sufficiently well demonstrated by transesterification studies of other types of oils with reuse of enzymatic preparations. It should be noted that the vast majority of reuse and recovery studies have been performed with the industrial preparation Novozyme 435. This preparation can be used for up to 52 cycles of transesterification using methanol (Shimada et al., 2002) and up to 100 cycles of transesterification with methyl acetate (Du et al., 2004) without regeneration. The use of tert-butanol to regenerate Novozyme 435 has been proposed (Nguyen et al., 2017).

Studies of chemical catalysts show that their reuse is more limited than that of enzymatic catalysts. Depending on the nature, chemical catalysts can be used for 2 to 50 cycles. It has been found that CaO can be used for up to 20 cycles without regeneration (Liu et al., 2008b), dolomite for up to 3 cycles (Ilgen, 2011), and starch-derived solid acid catalyst for up to 50 cycles (Wei et al., 2009). For the regeneration of these catalysts, it is proposed to wash them with cyclohexane or methanol, filter or triturate, and dry.

## 5 Other possible methods for fuel production from microalgae

Microalgae oil or biomass can be used for fuel production using thermochemical conversion technologies. One of the methods of thermochemical conversion is gasification, in which microalgae biomass is converted into a mixture of flammable gases at high temperature with limited oxygen content. Liquid fuels are produced using liquefaction and pyrolysis technologies. Liquefaction produces high viscosity oil, a process is performed at high temperatures using catalysts. Pyrolysis is considered a promising future technology for biofuel production. It can be applied to both oil and direct processing of biomass into fuel. It needs of high temperatures and airless environment or small amount of air.

During the pyrolysis process, gas, bio-oil and biochar are formed at 350°C–400°C in airless environment. For upgrading of bio-oil treatment of biomass and additional steps of pyrolysis are suggested. The pyrolysis process is divided into catalytic and non-catalytic. Metals (cobalt, platinum, nickel), metal oxides, natural rocks are used as catalysts. It has been determined that catalysts such as cement and clinker are effective catalysts for pyrolysis of spent oil. The use of catalysts increases the yield of hydrocarbons in the resulting oil compared to non-catalytic pyrolysis (Hussain et al., 2019). Clinker, which can also be used for direct biomass pyrolysis, is more efficient. Biochemical treatment of biomass and staged pyrolysis have been found to significantly increase bio-oil content (Hussain et al., 2017a; Hussain et al., 2017b). Due to high moisture content of microalgae biomass, the drying process is required before pyrolysis of algae biomass. The yield of biofuels from microalgal biomass by the pyrolysis process is relatively low. It is only 18%–24% (Miao and Wu, 2004). Due to the high energy and material costs, thermochemical treatment methods for microalgal biomass are so far rarely used in industry.

## 6 Conclusions

Recent decades have seen strongly increasing interest in the possibilities of microalgae for biodiesel production. Under favorable conditions, microalgae biomass can accumulate 50%–70% lipids. Microalgae cells contain a larger amount of polar lipids than vegetable oil; therefore, synthesizing biodiesel from microalgae oil using the heterogeneous catalysis method is advisable. Following the assessment of economic and environmental differences, heterogeneous catalysis has been found to have advantages over homogeneous catalysis.

Catalysts of both biological and mineral origin are used in heterogeneous catalysis. Among catalysts of biological

**Table 9** Comparison of homogeneous and heterogeneous catalysis

| Homogeneous catalysis | | Heterogeneous catalysis | | | |
|---|---|---|---|---|---|
| | | Chemical | | Enzymatic | |
| Advantages | Disadvantages | Advantages | Disadvantages | Advantages | Disadvantages |
| High process rate and yield | Sensitivity to moisture and a larger amount of free fatty acids in raw materials | Possibility to regenerate or reuse the catalyst | Average process speed | Water and free fatty acids contained in raw materials have no negative impact on process efficiency | Average process speed, long duration |
| Ability to use excess of alcohol | Corrosive environment | Easy separation of esters, clean glycerol non-contaminated with salts | | Possibility to regenerate or reuse the catalyst | Inactivation of ferment preparations by alcohols |
| Low cost of catalysts | Impossibility to regenerate or reuse the catalyst | Stability of catalysts effectiveness | | Easy separation of esters, clean glycerol non-contaminated with salts | Require high amount of catalysts |
| | Difficult cleaning and purification of the product or alcohol regeneration | Low cost of catalysts | | Low process temperature | High material costs associated with the high cost of enzymatic catalysts |
| | Difficulty of application of an continues process | Ability to use excess of alcohol | | | |
| | Quite high energy consumption and material expenses | Quite low energy consumption and material expenses | | | |

origin, lipases are able to catalyze the esterification and transesterification processes simultaneously. Industrially produced immobilised lipases are most frequently used in biodiesel synthesis. During enzymatic biodiesel synthesis, the recommended temperature is not higher than 40°C because at higher temperatures, enzymatic preparations lose their activity. The use of methanol as the acyl receptor is complicated due to the inactivation of lipases. Because of the toxic impact of methanol on enzymes the gradual addition of alcohol, the use of alcohols other than methanol, and application of alternative acyl receptors are supposed. Solvent use increases the transesterification efficiency. In enzymatic biodiesel synthesis from microalgae oil, the following solvents are more efficient: t-butanol, ether, MTBE, and hexane. The optimal conditions microalgae oil transesterification with methanol or ethanol are the following: 30°C−50°C temperature, molar ratio of alcohol to oil from 3:1 to 6:1, duration more than 12 h and 10%−75% of catalyst.

The implementation of industrial enzymatic processes for biodiesel synthesis is still restricted by the higher cost price of enzyme preparations. Multiple lipase use would solve this problem. Some researchers have found industrial preparation Novozyme 435 can be reused in up to 50 cycles of transesterification with methanol and up to 100 cycles with methyl acetate as the acyl receptor.

As heterogeneous catalysts of mineral origin, solid acids and solid bases can be used in biodiesel synthesis. Solid acid catalysts mostly consist of zeolite substances, heteropoly acids and pure or modified oxides of transitional metals. Basic zeolites, metal oxides and carbonates are effective basic heterogeneous chemical catalysts for biodiesel synthesis. The reaction temperature using these catalysts is usually close to the alcohol boiling point, and the reaction duration is 1−3 h, with a medium mixing intensity. An alcohol surplus is used in the heterogeneous process to increase the process effectiveness. A higher ester yield is obtained under supercritical conditions for alcohol, at high temperature and pressure, with or without usage of heterogeneous catalysts. The regeneration and reuse of chemical heterogeneous catalysts reduce the cost of biodiesel production. Study findings show that chemical heterogeneous catalysts can be used in 2−50 cycles depending on the type of catalyst. Regeneration is performed by using solvents to wash and dry the catalysts; however, this process also incurs energy and material expenses.

## References

Aguieiras E C, Ribeiro D S, Couteiro P P, Bastos C M, de Queiroz D S, Parreira J M, Langone M A (2016). Investigation of the reuse of immobilized lipases in biodiesel synthesis: Influence of different solvents in lipase activity. Applied Biochemistry and Biotechnology, 179(3): 485–496

Aguieiras E C G, Cavalcanti-Oliveira E D, Freire D M G (2015). Current status and new developments of biodiesel production using fungal lipazes. Fuel, 159: 52–67

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

Akubude V C, Nwaigwe K N, Dintwa E (2019). Production of biodiesel from microalgae via nanocatalyzed transesterification process: A review. Materials Science for Energy Technologies, 2(2): 216–225

Amini Z, Ong H C, Harrison M D, Kusumo F, Mazaheri H, Ilham Z (2017). Biodiesel production by lipase-catalyzed transesterification of *Ocimum basilicum* L. (sweet basil) seed oil. Energy Conversion and Management, 132: 82–90

Andrade G S S, Carvalho A K F, Romero C M, Oliveira P C, de Castro H F (2014). Mucor circinelloides whole-cells as a biocatalyst for the production of ethyl esters based on babassu oil. Bioprocess and Biosystems Engineering, 37(12): 2539–2548

Andruleviciute V, Makareviciene V, Skorupskaite V, Gumbyte M (2014). Biomass and oil content of *Chlorella* sp., *Haematococcus* sp., *Nannochloris* sp. and *Scenedesmus* sp. under mixotrophic growth conditions in the presence of technical glycerol. Journal of Applied Phycology, 26(1): 83–90

Anjos M, Fernandes B D, Vicente A A, Teixeira J A, Dragone G (2013). Optimization of $CO_2$ bio-mitigation by *Chlorella vulgaris.* Bioresource Technology, 139: 149–154

Szczęsna Antczak M, Kubiak A, Antczak T, Bielecki S (2009). Enzymatic biodiesel synthesis-Key factors affecting efficiency of the process. Renewable Energy, 34(5): 1185–1194

Azócar L, Ciudad G, Heipieper H J, Muńoz R, Navia R (2011). Lipase-catalyzed process in an anhydrous medium with enzyme reutilization to produce biodiesel with low acid value. Journal of Bioscience and Bioengineering, 112(6): 583–589

Bayramoglu G, Akbulut A, Ozalp V C, Arica M Y (2015). Imobilized lipase on micro-porous biosilica for enzymatic transesterification of algal oil. Chemical Engineering Research & Design, 95: 12–21

Beneroso D, Monti T, Kostas E T J, Robinson J (2017). Microwave pyrolysis of biomass for bio-oil production: scalable processing concepts. Chemical Engineering Journal, 316: 481–498

Benjamin S, Pandey A (1998). Candida rugosa lipases: Molecular biology and versatility in biotechnology. Yeast (Chichester, England), 14(12): 1069–1087

Biller P, Ross A B (2011). Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. Bioresource Technology, 102(1): 215–225

Brennan L, Owende P (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable & Sustanable Energy Reviews, 14(2): 557–577

Bundhoo Z M A (2018). Microwave-assisted conversion of biomass and waste materials to biofuels. Renewable & Sustainable Energy Reviews, 82: 1149–1177

Cakmak T, Angun P, Demiray Y E, Ozkan A D, Elibol Z, Tekinay T (2012). Differential effects of nitrogen and sulfur deprivation on growth and biodiesel feedstock production pf *Chlamydomonas reinhardtii.* Biotechnology and Bioengineering, 109(8): 1947–1957

Carrero A, Vicente G, Rodrı’guez R, Linares M, del Peso G L (2011). Hierarchical zeolites as catalysts for biodiesel production from *Nannochloropsis* microalga oil. Catalysis Today, 167(1): 148–153

Carrero A, Vicente G, Rodríguez R, Peso G L, Santos C(2015). Synthesis of fatty acids methyl esters (FAMEs) from *Nannochloropsis gaditana* microalga using heterogeneous acid catalysts. Biochemical Engineering Journal, 97: 119–124

Cerveró J M, Alvarez J R, Luque S (2014). Novozym 435-catalyzed synthesis of fatty acid ethyl esters from soybean oil for biodiesel production. Biomass and Bioenergy, 61: 131–137

Chandler I C (2001). Determining the regioselectivity of immobilized lipases in triacylglycerol acidolysis reactions. Journal of the American Oil Chemists’ Society, 78(7): 737–742

Chen J, Wu W (2003). Regeneration of Immobilized *Candida antarctica* lipase for transesterification. Journal of Bioscience and Bioengineering, 95(5): 466–469

Chen L, Liu T, Zhang W, Chen X, Wang J (2012). Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresource Technology, 111: 208–214

Cheng J, Qiu Y, Huang R, Yang W, Zhou J, Cen K (2016). Biodiesel production from wet microalgae by using graphene oxide as solid acid catalyst. Bioresource Technology, 221: 344–349

Cheng P, Ji B, Gao L, Zhang W, Wang J, Liu T (2013). The growth, lipid and hydrocarbon production of *Botryococcus braunii* with attached cultivation. Bioresource Technology, 138: 95–100

Christopher L P, Hemanathan Kumar, Zambare V P (2014). Enzymatic biodiesel: Challenges and opportunities. Applied Energy, 119: 497–520

Cipolatti E P, Silva M J A, Klein M, Feddern V, Feltes M M C, Oliveira J V, Ninow J L, de Oliveira D (2014). Current status and trends in enzymatic nanoimmobilization. Journal of Molecular Catalysis. B, Enzymatic, 99: 56–67

Converti A, Casazza A A, Ortiz E Y, Perego P, Del Borghi M.(2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chemical Engineering and Processing: Process Intensification, 48(6): 1146–1151

Dong T, Wang J, Miao C, Zheng Y, Chen S (2013). Two-step *in situ* biodiesel production from microalgae with high free fatty acid content. Bioresource Technology, 136: 8–15

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

Duraiarasan S, Razack S A, Manickam A, Munusamy A, Syed M B, Ali M Y, Ahmed G M, Mohiuddin M S (2016). Direct conversion of lipids from marine microalga *C. salina* to biodiesel with immobilised enzymes using magnetic nanoparticle. Journal of Environmental Chemical Engineering, 4(1): 1393–1398

Enamala M K, Enamala S, Chavali M, Donepudi J, Yadavalli R, Kolapalli B, Aradhyula T V, Velpuri J, Kuppam C (2018). Production of biofuels from microalgae: A review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renewable & Sustainable Energy Reviews, 94: 49–68

Feng D, Chen Z, Xue S, Zhang W (2011b). Increased lipid production of the marine oleaginous microalgae *Isochrysis zhangjiangensis* (Chrysophyta) by nitrogen supplement. Bioresource Technology, 102(12): 6710–6716

Feng Y, Li C, Zhang D (2011a). Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium. Bioresource Technology, 102(1): 101–105

Fernandez-Lafuente R (2010). Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. Journal of Molecular

Catalysis. B, Enzymatic, 62(3–4): 197–212

Foresti M L, Pedernera M, Bucalá V, Ferreira M L (2007). Multiple effects of water on solvent-free enzymatic esterifications. Enzyme and Microbial Technology, 41(1–2): 62–70

Frolich K, Vavra A, Kocik J, Hajek M, Jílková A (2019). The long-term catalytic performance of mixed oxides in fixed-bed reactors in transesterification. Renewable Energy, 143: 1259–1267

Galadima A, Muraza O (2014). Biodiesel production from algae by using heterogeneous catalysts: A critical review. Energy, 78: 72–83

Garcia-Galan C, Berenguer-Murcia A, Fernandez-Lafuente R, Rodrigues R C (2011). Potential of different enzyme immobilization strategies to improve enzyme performance. Advanced Synthesis & Catalysis, 353(16): 2885–2904

Goh B H H, Ong H C, Cheah M Y, Chen W H, Yu K L, Mahlia T M I (2019). Sustainability of direct biodiesel synthesis from microalgae biomass: A critical review. Renewable & Sustainable Energy Reviews, 107: 59–74

Gouveia L, Marques A E, da Silva T L, Reis A (2009a). Neochloris oleabundans UTEX #1185: A suitable renewable lipid source for biofuel production. Journal of Industrial Microbiology & Biotechnology, 36(6): 821–826

Gouveia L, Oliveira A C (2009b). Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology & Biotechnology, 36(2): 269–274

Granados M L, Poves M D Z, Alonso D M, Mariscal R, Galisteo F C, Moreno-Tost R, Santamaría J, Fierro J L G (2007). Biodiesel from sunflower oil by using activated calcium oxide. Applied Catalysis B: Environmental, 73(3–4): 317–326

Greenwell H C, Laurens L M L, Shields R J, Lovitt R W, Flyn K J (2010). Placing microalgae on the biofuels priority list: A review of the technological challenges. Journal of the Royal Society, Interface, 7(46): 703–726

Guldhe A, Moura C V R, Singh P, Rawat I, Moura E M, Sharma Y, Bux F (2017a). Conversion of microalgal lipids to biodiesel using chromium-aluminum mixed oxide as a heterogeneous solid acid catalyst. Renewable Energy, 105: 175–182

Guldhe A, Singh P, Ansari F A, Singh B, Bux F (2017b). Biodiesel synthesis from microalgal lipids using tungstated zirconia as a heterogeneous acid catalyst and its comparison with homogeneous acid and enzyme catalysts. Fuel, 187: 180–188

Gumbytė M, Makareviciene V, Skorupskaite V, Sendzikiene E, Kondratavicius M (2018). Enzymatic microalgae oil transesterification with ethanol in mineral diesel fuel media. Journal of Renewable and Sustainable Energy, 10(1): 013105

Gupta J, Agarwal M (2016). Preparation and characterization of CaO nanoparticle for biodiesel production. AIP Conference Proceedings, 1724

Haas M J, Piazza G J, Foglia T A(2002). Enzymatic approaches to the production of biodiesel fuels. In: Kuo T M, Gardner H W, eds., Lipid Biotechnology. New York: Marcel Dekker, 587–598

Helwani Z, Othman M R, Aziz N, Kim J, Fernando W J N (2009). Solid heterogeneous catalysis for transesterification of triglycerides with methanol: A review. Applied Catalysis A, General, 363(1–2): 1–10

Ho S H, Chen C Y, Chang J S (2012). Effect of light intensity and nitrogen starvation on $CO_2$ fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. Bioresource Technology, 113: 244–252

Huang X, Huang Z, Wen W, Yan J (2013). Effects of nitrogen supplementation of the culture medium on the growth, total lipid content and fatty acid profiles of three microalgae (*Tetraselmis subcordiformis, Nannochloropsis oculata* and *Pavlova viridis*). Journal of Applied Phycology, 25(1): 129–137

Hussain Z, Bashir N, Khan M I, Hussain K, Sulaiman S A, Naz M Y, Ibrahim K A, AbdEl-Salam N M (2017a). Production of highly upgraded bio-oils through two-step catalytic pyrolysis of water hyacinth. Energy & Fuels, 31(11): 12100–12107

Hussain Z, Naz H, Rafique M, Gulab H, Naz M Y, Sulaiman S A, Khan K M (2019). Conversion of spent fat oil into liquid and gaseous fuels through clinker catalyse pyrolysis. Brazilian Journal of Chemical Engineering, 36(2): 949–957

Hussain Z, Hussain K, Sulaiman S A, Naz M Y, Ibrahim K A, Abdel-Salam N M (2017b). Preparation of upgraded bio-oil using two-step catalytic pyrolysis of fresh, putrefied and microbe treated biomass. Environmental Progress & Sustainable Energy, 37: 1836–1844

Ilgen O (2011). Dolomite as a heterogeneous catalyst for transesterification of canola oil. Fuel Processing Technology, 92(3): 452–455

Jaeger K E, Reetz M T (1998). Microbial lipazes form versatile tools for biotechnology. Trends in Biotechnology, 16(9): 396–403

Jegannathan K R, Eng-Seng C, Ravindra P (2011). Economic assessment of biodiesel production: Comparison of alkali and biocatalyst processes. Renewable & Sustainable Energy Reviews, 15(1): 745–751

Jiang L, Luo S, Fan X, Yang Z, Guo R (2011). Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of $CO_2$. Applied Energy, 88(10): 3336–3341

Jo Y J, Lee O K, Lee E Y (2014). Dimethyl carbonate mediated lipid extraction and lipase-catalyzed in situ transesterification for simultaneous preparation of fatty acid methyl esters and glycerol carbonate from *Chlorella* sp. KR-1 biomass. Bioresource Technology, 158: 105–110

Krohn B J, McNeff C V, Yan B, Nowlan D (2011). Production of algae-based biodiesel using the continuous catalytic Mcgyan® process. Bioresource Technology, 102(1): 94–100

Kumar D, Poonam G K, Singh C P (2010). Ultrasonic-assisted transesterification of *Jatropha curcus* oil using solid catalyst, Na/ $SiO_2$. Ultrasonics Sonochemistry, 17(5): 839–844

Lai J Q, Hu Z L, Sheldon R A, Yang Z (2012a). Catalytic performance of crosslinked enzyme aggregates of *Penicillium expansum* lipase and their use as catalyst for biodiesel production. Process Biochemistry, 47(12): 2058–2063

Lai J Q, Hu Z L, Wang P W, Yang Z (2012b). Enzymatic production of microalgal biodiesel in ionic liquid. Fuel, 95: 329–333 (BMIm) (PF6)

Lam M K, Lee K T (2012). Microalgae biofuels: A critical review of issues, problems and the way forward. Biotechnology Advances, 30 (3): 673–690

Lee A F, Bennett J A, Manayil J C, Wilson K (2014). Heterogeneous catalysis for sustainable biodiesel production via esterification and transesterification. Chemical Society Reviews, 43(22): 7887–7916

Lee J H, Lee D H, Lim J S, Um B H, Park C, Kang S, Kim S W (2008). Optimization of the process for biodiesel production using a mixture of immobilized *Rhizopus oryzae* and *Candida rugosa* lipases. Journal of Microbiology and Biotechnology, 18(12): 1927–1931

Lee J Y, Yoo C, Jun S Y, Ahn C Y, Oh H M (2010). Comparison of several methods for effective lipid extraction from microalgae. Bioresource Technology, 101(1): S75–S77

Lee O K, Kim Y H, Na J G, Oh Y K, Lee E Y (2013). Highly efficient extraction and lipase-catalyzed transesterification of triglycerides from *Chlorella* sp. KR-1 for production of biodiesel. Bioresource Technology, 147: 240–245

Li E, Xu Z P, Rudolph V (2009). MgCoAl–LDH derived heterogeneous catalysts for the ethanol transesterification of canola oil to biodiesel. Applied Catalysis B: Environmental, 88(1–2): 42–49

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

Li X, Xu H, Wu Q (2007). Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. Biotechnology and Bioengineering, 98(4): 764–771

Li Y, Horsman M, Wang B, Wu N, Lan C Q (2008). Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans.* Applied Microbiology and Biotechnology, 81(4): 629–636

Li Y, Qin J G (2005). Comparison of growth and lipid content in three *Botryococcus braunii* strains. Journal of Applied Phycology, 17(6): 551–556

Li Y S, Lian S, Tong D M, Song R L, Yang W Y, Fan Y, Qing R, Hu Ch (2011). One-step production of biodiesel from *Nannochloropsis* sp. on solid base Mg–Zr catalyst. Applied Energy, 88(10): 3313–3317

Li Y, Qin J G (2005). Comparison of growth and lipid content in three *Botyrococcuc braunii* strains. Journal of Applied Phycology, 17(6): 551–556

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

Liu Z Y, Wang G C, Zhou B C (2008a). Effect of iron on growth and lipid accumulation in *Chlorella vulgaris.* Bioresource Technology, 99 (11): 4717–4722

Lou W Y, Zong M H, Duan Z Q (2008). Efficient production of biodiesel from high free fatty acid-containing waste oils using various carbohydrate-derived solid acid catalysts. Bioresource Technology, 99(18): 8752–8758

Ma G, Hu W, Pei H, Jiang L, Song M, Mu R (2015). In situ heterogeneous transesterification of microalgae using combined ultrasound and microwave irradiation. Energy Conversion and Management, 90: 41–46

Macrae A R (1983). Lipase-catalyzed interesterification of oils and fats. Journal of the American Oil Chemists' Society, 60(2Part1): 291–294

Makareviciene V, Gumbyte M, Skorupskaite V, Sendzikiene E (2017). Biodiesel fuel production by enzymatic microalgae oil transesterification with ethanol. Journal of Renewable and Sustainable Energy, 9(2): 023101

Makareviciene V, Skorupskaite V(2019). Transesterification of microalgae for biodiesel production. In: Basile A and Dalena F, eds. Second and Thirds Generation Feedstocks. The Evaluation of Biofuels. Amsterdam: Elsevier, 469–509

Manikandan G, Rajasekaran R (2013). Transesterification of algal oil using nano CaO catalyst. International Journal of Chemical Science, 11(1): 591–597

Marangoni A G(2002). Lipases: Structure function and properties. In: Kuo T M, Gardner H W, eds. Lipid Biotechnology. New York: Marcel Dekker, 357–384

Marchetti J M, Miguel V U, Errazu A F (2007). Possible methods for biodiesel production. Renewable & Sustainable Energy Reviews, 11 (6): 1300–1311

Mathimani T, Baldinelli A, Rajendran K, Prabakar D, Matheswaran M, Pieter van Leeuwen R,Pugazhendhi A (2019). Review on cultivation and thermochemical conversion of microalgae to fuels and chemicals: Process evaluation and knowledge gaps. Journal of Cleaner Production, 208: 1053–1064

Matsumoto T, Takahashi S, Kaieda M, Ueda M, Tanaka A, Fukuda H, Kondo A (2001). Yeast whole-cell biocatalyst constructed by intracellular overproduction of *Rhizopus oryzae* lipase is applicable to biodiesel fuel production. Applied Microbiology and Biotechnology, 57(4): 515–520

Miao X, Wu Q (2004). High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella* protothecoides. Journal of Biotechnology, 110(1): 85–93

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

Moreno-Garcia L, Adjallé K, Barnabé S, Raghavan G S V (2017). Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. Renewable & Sustainable Energy Reviews, 76: 493–506

Narula V, Khan M F, Negi A, Kalra S, Thakur A, Jain S (2017). Low temperature optimization of biodiesel production from algal oil using CaO and CaO/$Al_2O_3$ as catalyst by the application of response surface methodology. Energy, 140(1): 879–884

Nascimento I A, Marques S S I, Cabanelas I T D, Pereira S A, Druzian J I, de Souza C, Vich D V, de Carvalho G C, Nascimento M A (2013). Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. BioEnergy Research, 6(1): 1–13

Nautiyal P, Subramanian K A, Dastidar M G (2014). Production and characterization of biodiesel from algae. Fuel Processing Technology, 120: 79–88

Ngamcharussrivichai C, Nunthasanti P, Tanachai S, Bunyakiat K (2010). Biodiesel production through transesterification over natural calciums. Fuel Processing Technology, 91(11): 1409–1415

Nguyen H C, Liang S H, Chen S S, Su C H, Lin J, Chien C C (2018). Enzymatic production of biodiesel from insect fat using methyl acetate as an acyl acceptor: Optimization by using response surface methodology. Energy Conversion and Management, 158: 168–175

Nguyen H C, Liang S H, Doan T T, Su C H, Yang P C (2017). Lipase-catalyzed synthesis of biodiesel from black soldier fly (*Hermetica illucens*): Optimization by using response surface methodology. Energy Conversion and Management, 145: 335–342

Piligaev A V, Sorokina K N, Samoylova Y V, Parmon V N (2018). Lipid production by microalga *Micractinium* sp. IC-76 in a flat panel photobioreactor and its transesterification with cross-linked enzyme aggregates of Burkholderia cepacia lipase. Energy Conversion and Management, 156: 1–9

Pokoo-Aikins G, Nadim A, El-Halwagi M M, Mahalec V (2010). Design and analysis of biodiesel production from algae grown through carbon sequestration. Clean Technologies and Environmental Policy, 12: 239–254

Rahman R N Z R A, Baharum S N, Basri M, Salleh A B (2005). High-yield purification of an organic solvent-tolerant lipase from *Pseudomonas* sp. strain S5. Analytical Biochemistry, 341(2): 267–274

Ramachandran K, Suganya T, Nagendra Gandhi N, Renganathan S (2013). Recent developments for biodiesel production by ultrasonic assist transesterification using different heterogeneous catalyst: A review. Renewable & Sustainable Energy Reviews, 22: 410–418

Rawat I, Ranjith Kumar R, Mutanda T, Bux F (2013). Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Applied Energy, 103: 444–467

Razack A, Duraiarasan S (2016). Response surface methodology assisted biodiesel production from waste cooking oil using encapsulated mixed enzyme. Waste Management, 47(A): 98–104

Venkat Reddy C R, Oshel R, Verkade J G (2006). Room-temperature conversion of soybean oil and poultry fat to biodiesel catalyzed by nanocrystalline calcium oxides. Energy & Fuels, 20(3): 1310–1314

Reyes Y, Chenard G, Aranda D, Mesquita C, Fortes M, Joao R, Bacellar L (2012). Biodiesel production by hydroesterification of microalgal biomass using heterogeneous catalyst. Nature and Science, 4(10): 778–783

Rodrigues R C, Pessela B C C, Volpato G, Fernandez-Lafuente R, Guisan J M, Ayub M A Z (2010). Two step ethanolysis: A simple and efficient way to improve the enzymatic biodiesel synthesis catalyzed by an immobilized–stabilized lipase from *Thermomyces lanuginosus.* Process Biochemistry, 45(8): 1268–1273

Safakish E, Nayebzadeh H, Saghatoleslami N, Kazemifard S (2020). Comprehensive assessment of the preparation conditions of a separable magnetic nanocatalyst for biodiesel production frim algae. Algal Research, 49: 101949

Salis A, Pinna M, Monduzzi M, Solinas V (2005). Biodiesel production from triolein and short chain alcohols through biocatalysis. Journal of Biotechnology, 119(3): 291–299

Sani Y M, Daud W M A W, Abdul Aziz A (2013). Solid acid-catalyzed biodiesel production from microalgal oil: The dual advantage. Journal of Environmental Chemical Engineering, 1(3): 113–121

Selmi B, Thomas D (1998). Immobilized lipase-catalyzed ethanolysis of sunflower oil in a solvent-free medium. Journal of the American Oil Chemists' Society, 75(6): 691–695

Sendžikiene E, Makareviciene V, Kazancev K (2018). Application of dolomite as a heterogeneous catalyst of biodiesel synthesis. Transport, 33(5): 1155–1161

Shamel M M, Hasan M, Ramachandran B (2005). Operational stability of lipase enzyme: Effect of temperature and shear. Asia-Pacific Journal of Chemical Engineering, 13(5–6): 599–604

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

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

Siva S, Marimuthu C (2015). Production of biodiesel by transesterification of algae oil with an assistance of nano-CaO catalyst derived from egg shell. International Journal of ChemTech Research, 7(4): 2112–2116

Sivaramakrishnan R, Incharoensakdi A (2017). Direct transesterification of *Botryococcus* sp. catalysed by immobilized lipase: Ultrasound treatment can reduce reaction time with high yield of methyl ester. Fuel, 191: 363–370

Sivaramakrishnan R, Muthukumar K (2014). Direct transesterification of *Oedogonium* sp. oil be using immobilized isolated novel *Bacillus* sp. lipase. Journal of Bioscience and Bioengineering, 117(1): 86–91

Marín-Suárez M, Méndez-Mateos D, Guadix A, Guadix E M (2019). Reuse of immobilized lipases in the transesterification of waste fish oil for the production of biodiesel. Renewable Energy, 140: 1–8

Surendhiran D, Vijay M, Sirajunnisa A R (2014). Biodiesel production from marine microalga *Chlorella salina* using whole cell yeast immobilized on sugarcane bagasse. Journal of Environmental Chemical Engineering, 2(3): 1294–1300

Taher H, Al-Zuhair S, Al-Marzouqi A H, Haik Y, Farid M M (2011). A review of enzymatic transesterification of microalgal oil-based biodiesel using supercritical technology. Enzyme Research, Article ID 468292

Takagi M, Watanabe K, Yamaberi K, Yoshida T (2000). Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of *Nannochloris* sp. UTEX LB1999. Applied Microbiology and Biotechnology, 54(1): 112–117

Takagi M, Karseno K, Yoshida T K (2006). Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. Journal of Bioscience and Bioengineering, 101(3): 223–226

Talukder M M R, Wu J C, Van Nguyen T B, Fen N M, Melissa Y L S (2009). Novozym 435 for production of biodiesel from unrefined palm oil: Comparison of methanolysis methods. Journal of Molecular Catalysis. B, Enzymatic, 60(3–4): 106–112

Tan T, Lu J, Nie K, Deng L, Wang F (2010). Biodiesel production with immobilized lipase: A review. Biotechnology Advances, 28(5): 628–634

Tan T, Nie K, Wang F (2006). Production of biodiesel by immobilized *Candida* sp. lipase at high water content. Applied Biochemistry and Biotechnology, 128(2): 109–116

Tasić M B, Pinto L F R, Klein B K, Veljković V B, Filho R M (2016). Botryococcus braunii for biodieselproduction. Renewable & Sustainable Energy Reviews, 64: 260–270

Tran D T, Chen C L, Chang J S (2012). Immobilization of *Burkholderia* sp. lipase on a ferric silica nanocomposite for biodiesel production. Journal of Biotechnology, 158(3): 112–119

Véras I C, Silva F A L, Ferrão-Gonzales A D, Moreau V H (2011). One-step enzymatic production of fatty acid ethyl ester from high-acidity waste feedstocks in solvent-free media. Bioresource Technology, 102 (20): 9653–9658

Verma D, Kumar R, Rana B S, Sinha A K (2011). Aviation fuel production from lipids by a single-step route using hierarchical mesoporous zeolites. Energy & Environmental Science, 4(5): 1667–1671

Wahidin S, Idris A, Shaleh S R M (2016). Ionic liquid as a promising biobased green solvent in combination with microwave irradiation for direct biodiesel production. Bioresource Technology, 206: 150–154

Wang L, Du W, Liu D, Li L, Dai N (2006). Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in *tert*-butanol system. Journal of Molecular Catalysis. B, Enzymatic, 43(1–4): 29–32

Wang Y, Liu J, Gerken H, Zhang C, Hu Q, Li Y (2014). Highly-efficient enzymatic conversion of crude algal oils into biodiesel. Bioresource Technology, 172: 143–149

Watanabe Y, Pinsirodom P, Nagao T, Yamauchi A, Kobayashi T, Nishida Y, Takagi Y, Shimada Y (2007). Conversion of acid oil by-produced in vegetable oil refining to biodiesel fuel by immobilized *Candida antarctica* lipase. Journal of Molecular Catalysis. B, Enzymatic, 44(3–4): 99–105

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

Wei Z, Xu C, Li B (2009). Application of waste eggshell as low-cost solid catalyst for biodiesel production. Bioresource Technology, 100 (11): 2883–2885

Wu S, Song L, Sommerfeld M, Hu Q, Chen W (2017). Optimization of an effective method for the conversion of crude algal lipids into biodiesel. Fuel, 197: 467–473

Xie W, Huang X, Li H (2007). Soybean oil methyl esters preparation using NaX zeolites loaded with KOH as a heterogeneous catalyst. Bioresource Technology, 98(4): 936–939

Xie W, Ma N (2009). Immobilized lipase on $Fe_3O_4$ nanoparticles as biocatalyst for biodiesel production. Energy & Fuels, 23(3): 1347–1353

Xie W, Ma N (2010). Enzymatic transesterification of soybean oil by using immobilized lipase on magnetic nano-particles. Biomass and Bioenergy, 34(6): 890–896

Xie W, Wang J (2014). Enzymatic production of biodiesel from soybean oil by using immobilized lipase on $Fe_3O_4$/poly (styrene-methacrylic acid) magnetic microsphere as a biocatalyst. Energy & Fuels, 28(4): 2624–2631

Xin L, Hong-ying H, Ke G, Ying-xue S (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. Bioresource Technology, 101(14): 5494–5500

Xiong W, Li X, Xiang J, Wu Q (2008). High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. Applied Microbiology and Biotechnology, 78(1): 29–36

Xu L, Wang F, Guo C, Liu C Z (2012). Improved algal oil production from *Botryococcus braunii* by feeding nitrate and phosphate in an airlift bioreactor. Engineering in Life Sciences, 12(2): 171–177

Xu Y, Du W, Liu D, Zeng J (2003). A novel enzymatic route for biodiesel production from renewable oils in a solvent-free medium. Biotechnology Letters, 25(15): 1239–1241

Xu Y, Du W, Zeng J, Liu D (2004). Conversion of soybean oil to biodiesel fuel using Lipozyme TL IM in a solvent-free medium. Biocatalysis and Biotransformation, 22(1): 45–48

Yan J, Zheng X, Li S (2014). A novel and robust recombinant *Pichia pastoris* yeast whole cell biocatalyst with intracellular overexpression of a *Thermomyces lanuginosus* lipase: Preparation, characterization and application in biodiesel production. Bioresource Technology, 151: 43–48

Yeh K L, Chang J S (2011). Nitrogen starvation strategies and photobioreactor design for enhancing lipid content and lipid production of a newly isolated microalga *Chlorella vulgaris* ESP-31: Implications for biofuels. Biotechnology Journal, 6(11): 1358–1366

Yoosuk B, Udomsap P, Puttasawat B, Krasae P (2010). Modification of calcite by hydration–dehydration method for heterogeneous biodiesel production process: The effects of water on properties and activity. Chemical Engineering Journal, 162(1): 135–141

Yoshimura Y, Okada S, Honda M (2013). Culture of the hydrocarbon producing microalga *Botryococcus braunii* strain Showa: Optimal $CO_2$, salinity, temperature, and irradiance conditions. Bioresource Technology, 133: 232–239

Yu D, Tian L, Wu H, Wang S, Wang Y, Ma D, Fang X (2010). Ultrasonic irradiation with vibration for biodiesel production from soybean oil by Novozym 435. Process Biochemistry, 45(4): 519–525

Zeng J, Du W, Liu X, Liu D, Dai L (2006). Study on the effect of cultivation parameters and pretreatment on *Rhizopus oryzae* cell-catalyzed transesterification of vegetable oils for biodiesel production. Journal of Molecular Catalysis. B, Enzymatic, 43(1–4): 15–18

Zhang X, Yan S, Tyagi R D, Surampalli R Y, Valero J R (2014). Ultrasonication aided *in-situ* transesterification of microbial lipids to biodiesel. Bioresource Technology, 169: 175–180

Zhao P, Yu X, Li J, Tang X, Huang Z (2014). Enhancing lipid productivity by co-cultivation of *Chlorella* sp. U4341 and *Monoraphidium* sp. FXY-10. Journal of Bioscience and Bioengineering, 118(1): 72–77

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