Application of Simultaneous Oil Extraction and Transesterification in Biodiesel Fuel Synthesis: A Review

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Abstract: Increasing concentrations of greenhouse gases in the atmosphere are leading to increased production and use of biofuels. The industrial development of biodiesel production and the use of biodiesel in the EU transport sector have been ongoing for almost two decades. Compared to mineral diesel production, the process of producing biodiesel is quite complex and expensive, and the search for new raw materials and advanced technologies is needed to maintain production value and expand the industrial production of biodiesel. The purpose of this article is to review the application possibilities of one of the new technologies—simultaneous extraction of oil from oily feedstock and transesterification (in situ)—and to evaluate the effectiveness of the abovementioned process under various conditions.

Keywords: biodiesel; in situ; simultaneous oil extraction and transesterification

1. Introduction

With the increase in atmospheric pollution in the form of greenhouse gases, the development of the use of biofuels in the transport sector is receiving increasing attention. Biodiesel is a well-known type of biofuel that has been used for a relatively long time. It is produced from various oily raw materials. Recently, the possibilities of utilizing non-food raw materials and residual waste in the synthesis of biodiesel have been intensively studied. Despite substantial developments in the use of biodiesel, its production cost still exceeds the costs and market prices of comparable mineral fuels. There are various ways to solve this problem. These ways include the use of novel raw materials that are unsuitable for food, the use of more efficient oil extraction methods, and the use of innovative and efficient catalysts and transesterification technologies [1,2].

In conventional biodiesel production, oilseeds in the first stage are extracted from a feedstock by mechanical pressing or by solvent extraction and purified (by degumming, deacidification, dewaxing, dephosphorization, dehydration, etc.), and the second stage involves transesterification with alcohol, conventionally with methanol (Figure 1) [3–5]. The oil extraction and refining process is energy intensive, accounting for approximately 70% of the total process of biodiesel production [6]. The cost of biodiesel production could be reduced by eliminating the oil extraction process and applying the so-called in situ simultaneous oil extraction and transesterification process [1]. An extraction solvent may not be necessary in this process because the alcohol used as an acyl receptor for transesterification also acts as an oil extracting agent and the fatty acid esters formed during the process also have good oil solvent properties; therefore, the oil extraction efficiency is increased (Figure 1) [7].
The requirements for the quality of biodiesel are given in the European standard EN 14214. The parameters specified therein must also be met by the biodiesel produced using the in situ transesterification method. The main indicator of the efficiency of the transesterification process is the ester content, which according to the requirements of the standard must be at least 96.5%. This means that 96.5% of the oil is converted to esters during transesterification. The standard includes a number of indicators related to the nature of the oil. Indirectly related to the efficiency of the transesterification process are indicators, such as density at 15 °C (min 860 kg/m³; max 900 kg/m³), viscosity at 40 °C (min 3.50 mm²/s; max 5.00 mm²/s). The amount of unreacted triglycerides is determined by such indicators as monoglyceride content (max 0.6%), diglyceride content (max 0.2%), triglyceride content (max 0.2%). The currently used fuel is not pure biodiesel, but a blend of mineral diesel and biodiesel in a proportion of pure products. The simultaneous oil extraction and transesterification process using the addition of mineral diesel as an extraction solvent can eliminate not only the oil extraction process but also the stage of biofuel mixing with mineral diesel used for the production of fuel blends currently used in EU countries as fuels for diesel engines [8].

The paper summarizes the results of research investigating the in situ process for the synthesis of biodiesel from various types of feedstock and the influence of feedstock preparation methods, process conditions, and other means on the effectiveness of the process in terms of improving yield and product quality.

2. Raw Materials and Their Preparation Methods

Various fatty raw materials are suitable for the production of biodiesel [9]. The choice of raw materials is greatly influenced by the prevalence of reliably available raw materials in the region. Most of the biodiesel (approximately 84%) in the EU is produced from rapeseed oil, and the second most consumed source is sunflower oil, which accounts for approximately 13%. Soybean oil is widely used in the USA, and in warm countries, palm oil is used. As vegetable oil biodiesel production competes with the food industry, there is a search for fatty waste and raw materials that are unsuitable for food production but suitable for biodiesel production.

Food-grade rapeseed is also commonly used for in situ biofuel production research [8,10–12]. Substantial research has been performed with sunflower seed [13,14], soy flakes [15], cottonseed meal unfit for human consumption [16,17], *Jatropha curcas* L. seed [18–20], desert date kernels [21], caster/castor seed [22,23], rice bran [24], spent coffee grounds (waste) [25–27], rice bran [28], greasy sewage [29], municipal sludge [30,31], meat and bone meal, distillers dried grains with solubles [32], marine microalgae, microalgal biomass, and cyanobacteria [33–39].
The preparation of the raw material has a significant influence on the efficiency of biodiesel production applying an in situ process. To obtain a higher oil yield and higher ester content in the transesterification product, the biomass must be crushed to increase the access of the extraction solvent and transesterification agent to the oil in the cells. Biomass treatment is carried out by various methods and under various conditions. In addition to mechanical treatment, methods such as microwave and ultrasonic treatment and high-pressure homogenization are used to prepare biomass for the in situ process. These methods not only apply to the pretreatment of the raw material but are often applied during the simultaneous oil extraction and transesterification process. Ultrasonic and microwave processing has been extensively studied and used for in situ biodiesel synthesis using microalgae biomass.

Ultrasound treatment has been found to have a better effect than microwave treatment. When using yeast biomass for in situ transesterification, a 90.4% ester yield was achieved after 12 h, while under ultrasonication, 94.1% ester was obtained in 20 min using a 6-fold lower molar ratio of methanol to oil [40,41]. A similar but slightly lower effect of ultrasound on transesterification efficiency is reported in Einhem et al. (2012) in an in situ study on the use of microalgal (Chlorella sp.) biomass. The same ester yield (91%) was found upon applying ultrasound treatment and using a three-fold lower molar ratio of methanol to oil [42]. The high yield of fatty acid ethyl ester (94.6%) is reported by Sung and Han (2018) using 40% moisture microalgae Aurantiochytrium sp. KRS 101 biomass with sonication using K₂CO₃ catalyst at 70 °C temperature and 30 min [43]. However, the results are controversial—some authors point out that ultrasound has the same efficiency as mechanical mixing. Georgogianni et al. (2006) reported that ultrasound has the same efficiency as mechanical mixing in the in situ transesterification of sunflower seeds [44]. The degree of crushing of the feedstock also has a major influence on the efficiency of the in situ transesterification process. The finer the particles are, the greater the amount of oil in close proximity to their surface and the faster the process of oil extraction and subsequent transesterification. This effect reduces the amount of solvent needed for the extraction and diffusion processes and reduces the process duration required to obtain the same yield. The in situ treatment of crushed rapeseed particles with a size of 300–500 μm produced the highest ester yield in 1 h, whereas using 1000–1400 μm particles produced the same effect only in 3 h [10]. Similar results were obtained by chat et al., who investigated the in situ transesterification of Jatropha curcas L. seed [19]. Using milled feedstock with a particle size of less than 0.355 mm, the ester yield was 90% after a 24 h process duration. Using particles with a size of 1-0.355 mm, the yield of esters after the same time was only 60% [18].

Not only the degree of crushing but also the moisture content of the raw material influences the efficiency of in situ transesterification. The highest yield is obtained from dry feedstock because water in oilseeds impedes the transesterification process due to the reversibility of the transesterification reaction: water in the reaction medium slows the rate of the direct reaction and promotes a reversible reaction; at the same time, the hydrolysis process takes place of the resulting biodiesel to form fatty acids and alcohol. In addition, water, an ineffective oil solvent, acts as a barrier to oil-alcohol contact, thereby lowering the rate of the transesterification process. Reducing the water content of biomass from 8.7% to 1.9% has been found to increase the solubility of oil in methanol and to increase the yield of soy oil methyl esters from 18% to 98% [45]. When acid catalysts are used, water deactivates them [46].

To determine the influence of water on the in situ transesterification process, a number of studies have been carried out using various raw materials. Most authors cite the negative effects of water, while others show that high-moisture raw materials can yield high ester yields. In their study of the transesterification efficiency of cotton seed oil, Qian et al. (2013) observed that with a decrease in feedstock moisture from 8.7% to 1.9%, the yield of fatty acid methyl esters increased from 80% to 98% [45]. The authors point out that using dry raw materials not only improves the process efficiency but also reduces the alcohol and catalyst inputs. The negative influence of water on in situ process efficiency is also described by Haas et al. (2004), who found that 60% more methanol and 56% more sodium hydroxide were required to obtain the same ester yield when using wet soybeans than when
using absolute dry soybeans [15]. Drying soybeans to 2.6% moisture saves 40% methanol and 33% caustic soda. According to some authors, the use of biomass with a water content of 90% resulted in an ester yield of only 10.3%, while the use of fully dried biomass resulted in a 91.4% biodiesel yield [47]. When using 10% water containing biomass consisting of the microalgae Chlorella sp. in the in situ process with H₂SO₄ as the acid catalyst, the transesterification efficiency decreased by more than 20% compared to that obtained when utilizing dry biomass [48]. Nguyen et al. (2019) investigated the process of in situ transesterification of Chlorella vulgaris using H₂SO₄ as a catalyst and reported that high FAME yield (> 90 wt%) could still be attained at the moisture content lesser than 15 wt% [49]. A 19% lower yield of fatty acid esters was obtained using wet fatty sludge and acid catalysis compared to that obtained using dry fatty sludge [29].

From these results, it can be concluded that the moisture content of the raw material has a significant influence on the efficiency of biodiesel synthesis and the economic indicators of the in situ process since the removal of moisture requires additional energy costs. This effect is particularly true when microalgae biomass is used in biodiesel synthesis, with drying costs account for up to 70% of the biodiesel production cost [50].

However, in this case as well, the results of trials are controversial. While most authors describe a negative impact of water content on in situ transesterification efficiency, some authors have obtained completely different results in in situ studies of biodiesel synthesis from wet microalgae biomass. By studying the in situ transesterification process of several species of microalgae, Liu et al. (2015) found that transesterification efficiency obtained using wet biomass is only 6% lower than that obtained using dry algae biomass. The ester yield of 94% was obtained from wet microalgae biomass (water content of 90%) using sulfuric acid as a catalyst. Increase of water content to 99% resulted in a decrease of ester yield to 61% [51]. Tran et al. (2012) also investigated the efficiency of the direct enzymatic transesterification process using wet (water content of 86–91%) microalgae Chlorella vulgaris ESP-31 biomass and found that 90% biodiesel conversion can be obtained [52]. However, in this case, the biomass was pretreated by sonication to crush the cell walls. In addition, the use of solvents is necessary to achieve greater efficiency. Macías-Sánchez et al. (2015) obtained as much as 100% ester yield using the seaweed Nannochloropsis gaditana with 75% moisture content and found out that a high transesterification yield could be only obtained with the usage of additional solvent and high methanol content [33]. Slightly inferior results were obtained by Sangaletti-Gerhard et al. (2015), who noted that the difference in the transesterification efficiency (yield of esters) values obtained using dry and wet sludge was 19% [29]. Nguyen et al. (2020) investigated the acid catalyzed in situ transesterification of wet Chlorella vulgaris biomass and obtained that under wet microalgae conditions (75 wt% of moisture content), increasing of methanol content in the reaction media results in an improvement of the ester yield. Ester yield of 96.0 wt% was obtained using 650 mol/mol and 10,000 mol/mol of H₂SO₄ and methanol concentration, respectively. It was determined that the introduction of co-solvents (butanol, tetrahyfrofuran) increased the ester yield to 100% of esterifiable lipids [53].

It is difficult to reach an unequivocal conclusion from the summary of the obtained results, but on theoretical grounds, it is clear that water present in the feedstock still adversely affects the transesterification process, in both conventional biodiesel production and in situ transesterification processes applying chemical catalysis. Experimental results of wet microalgae transesterification in situ show that effectiveness is improved using a high amount of acid catalyst and alcohol. Introduction of co-solvent and application ultrasonication also positively influence the increase of ester yield [43,54].

3. Chemical Catalysis for Simultaneous Oil Extraction and Transesterification

In the in situ transesterification process, the ester yield is determined by four major factors: alcohol content (or molar ratio of alcohol to oil), amount of catalyst, temperature, and reaction time. Some of these factors have a greater influence on ester yield, while others have a smaller influence.
3.1. Influence of Alcohols/Solvents on Process Efficiency

Not only does the in situ chemical process eliminate the pressure or extraction step for the oil, but the solvent function could be performed by alcohols that both extract and transesterify the oil directly from the seeds. The most commonly used alcohol in the in situ process is methanol, which is also commonly used for conventional transesterification [31,37,55]; less commonly used alcohols include ethanol, propanol, and butanol [55].

Ozgul-Yucel and Turkay (2003) used various alcohols for rice bran transesterification using the same process conditions except temperature (the process temperature was close to the boiling point of the alcohol used). Longer-chain alcohols were found to have lower fatty ester yields: 85.8% for methanol, 78.3% for 99.1% ethanol, 76.4% for 96% ethanol, 74.8% for n-propanol, 69.7% for isopropanol, and 68% for n-butanol. The lowest transesterification efficiency was obtained when a branched chain alcohol, isobutanol, was used [55]. In addition, the purity and moisture content of the alcohol affect the efficiency of transesterification. For example, higher alcohol (absolute ethanol) produces higher ester yields. The lower yield obtained using 96% ethanol can be explained by the fact that the water it contains interferes with the transesterification reaction. Synthesis of fatty acid ester is a reversible reaction, and thus water presented in reaction medium induces hydrolysis resulting in methanol and free fatty acids formation. Water also serves as a shield that separates oil from alcohol and solvent, thus preventing transesterification reactions; acid catalysts may be deactivated by water, which competes for potential protons in the reaction.

Some scientists have used mixtures of alcohols (methanol-ethanol) to obtain mixtures of fatty acid esters in the process [56].

To increase the process rate, researchers have also studied the effect of the solvent additive in the reaction mixture. Most experiments were performed by using hexane [18]; less frequently, methylene dichloride [34] and petroleum ether [1] have been used. Solvents in the reaction mixture act as extraction solvents for the oil, increasing the solubility of the oil in the reaction media since methanol or ethanol used for transesterification does not exhibit good oil solvent properties. The solvent in the system is the initiator of the oil extraction process, and later on, the fatty acid esters formed during the transesterification reaction are also very effective oil solvents; the continuous increase in fatty acid esters in the reaction media further enhances oil extraction and thus increases the transesterification rate.

The alcohol content has a major influence on the yield of fatty esters. According to stoichiometry, three moles of alcohol are required to completely react with one mole of oil. According to the equilibrium reaction, excess alcohol is used to shift the equilibrium to the product side. In conventional biodiesel synthesis with homogeneous alkaline catalysis, the alcohol-to-oil molar ratio is 6:1. All researchers who have studied the in situ transesterification process also used a higher than stoichiometric alcohol content (Tables 1 and 2). It has been found that the in situ process requires a higher excess of alcohol than conventional biodiesel synthesis.

### Table 1. Summary of studies on using chemical acid catalysts for in situ transesterification.

| Raw Material          | Alcohol Solvent | Reaction Conditions                                                                 | Fatty Acid Esters Yield (%) | References |
|-----------------------|-----------------|--------------------------------------------------------------------------------------|----------------------------|------------|
| Jatropha. curcas L. seeds | Methanol/hexane  | Catalyst H$_2$SO$_4$—15 wt% of seed, 60 °C, 24 h, methanol to seed ratio—7.5 mL/g, hexane -10 vol% of solvent. | 99.8                      | [18]       |
| Chlorella sp.          | Methanol        | Catalyst H$_2$SO$_4$ lipid molar ratio 0.35:1, 60 °C, 19 h, methanol to seed molar ratio—600:1, 200 powdered microalgae, catalyst H$_2$SO$_4$ (at 80.8 mol/mol of H$^+$ to esterifiable lipid), 60 °C, 120 min. | 92 ± 2                    | [48]       |
| Chlorella vulgaris     | Methanol        | Catalyst H$_2$SO$_4$ lipid molar ratio 0.35:1, 60 °C, 19 h, methanol to seed molar ratio—600:1, 200 powdered microalgae, catalyst H$_2$SO$_4$ (at 80.8 mol/mol of H$^+$ to esterifiable lipid), 60 °C, 120 min. | 94.6                      | [53]       |
| Nannochloropsis       | Methanol        | Catalyst H$_2$SO$_4$ lipid molar ratio 0.35:1, 60 °C, 19 h, methanol to seed molar ratio—600:1, 200 powdered microalgae, catalyst H$_2$SO$_4$ (at 80.8 mol/mol of H$^+$ to esterifiable lipid), 60 °C, 120 min. | 73 ± 5                    | [48]       |
| Microalgae lipids     | Methanol        | Catalyst H$_2$SO$_4$ lipid molar ratio 0.35:1, 60 °C, 19 h, methanol to seed molar ratio—600:1, 200 powdered microalgae, catalyst H$_2$SO$_4$ (at 80.8 mol/mol of H$^+$ to esterifiable lipid), 60 °C, 120 min. | 91.3                      | [37]       |
| Dry water microalgae  | Methanol        | Catalyst H$_2$SO$_4$—3.361%, w/w, methanol/algae ratio—8:1, w/w, 50 °C, 60.4 min. | 89.58                     | [37]       |
In the performed studies, the molar ratio of alcohol to oil ranged from 12 to 655 [31,32] when using alcohol alone and between 6 and 60 [20,22] when using a mixture of alcohol and solvent, depending on the process conditions. It has been observed that at a relatively low molar ratio of methanol to oil (20:1), the transesterification reaction almost does not proceed [58]. In situ transesterification results for rapeseed oil methyl ester have shown that a 98% biodiesel yield can be achieved only at a methanol-to-oil molar ratio of 180:1 in 3 h at 40 °C [45]. When investigating the in situ transesterification of algae oil from *Nannochloropsis sp.*, Dianursanti and Wijanarko (2015) observed that by increasing the molar ratio of methanol to oil from 200 to 400, the ester yield could be increased from 56.1% to 79.87% [59]. Ehimen et al. (2010) stated that the optimum molar ratio of methanol to oil for the transesterification of the microalgae *Chlorella sp.* in situ is 315:1, while a higher alcohol content does not show a positive effect [37]. Velasquez-Orta and Harvey (2012) found that the highest ester yield (77.6%) in the transesterification of microalgae *Chlorella vulgaris* oil was obtained at a molar ratio of alcohol to oil of 600:1 [60].

Given that methanol is a poor solvent for oil and therefore limits the mass transfer of oil to alcohol, it is suggested to use additional solvents for the in situ transesterification process to increase the oil extraction efficiency from biomass. The research results related to solvent application are controversial.
Some scientists have noted that the solvent has a positive effect on process efficiency, while others have found that the solvent added does not have a significant effect on oil extraction and transesterification. Hincapié et al. (2011) [22] investigated the transesterification process of castor seeds using ethanol and ethanol and hexane mixtures under identical conditions and found that castor seeds (*Ricinus communis* L. red) produced a yield of fatty acid ethyl esters (FAEEs) of 95.3% when using ethanol and a yield of 95.6% when using an ethanol and hexane mixture. For castor seeds (BRS-149 nordestina), the FAEE yield was 98.0% for ethanol and 97.4% for ethanol and hexane (Table 3). Therefore, it can be argued that the FAEE yield differs only within the margin of error.

### Table 3. Summary of studies on using chemical acid/alkaline catalysts for in situ transesterification.

| Raw Material                  | Alcohol Solvent                  | Reaction Conditions                                                                 | Fatty Acid Esters Yield (%) | References |
|-------------------------------|----------------------------------|--------------------------------------------------------------------------------------|----------------------------|------------|
| Castor seeds (*Ricinus communis* L. red) | Ethanol                          | Seeds—20 g, catalyst H$_2$SO$_4$—1.0 wt%, 60 °C, 1 h, ethanol to oil molar ratio—40:1. After 1 h, catalyst KOH—1.0 wt%, 60 °C, 1 h, ethanol to oil molar ratio—20:1. | 95.3                     | [22]       |
| Castor seeds (BRS-149 nordestina) | Ethanol                          | Seeds—20 g, catalyst H$_2$SO$_4$—1.0 wt%, 60 °C, 1 h, ethanol to oil molar ratio—40:1. After 1 h, catalyst KOH—1.0 wt%, 60 °C, 1 h, ethanol to oil molar ratio—20:1. | 98.0                     | [22]       |
| Castor seeds (*Ricinus communis* L. red) | Ethanol/hexane                    | Seeds—20 g, catalyst H$_2$SO$_4$—1.0 wt%, 60 °C, 1 h, hexane 20% v/v, ethanol to oil molar ratio—40:1. | 95.6                     | [22]       |
| Castor seeds (BRS-149 nordestina) | Ethanol/hexane                    | Seeds—20 g, catalyst H$_2$SO$_4$—1.0 wt%, 60 °C, 1 h, hexane 20% v/v, ethanol to oil molar ratio—40:1. | 97.4                     | [22]       |
| Rice bran                     | Methanol/petroleum ether         | I STEP Rice bran 50 g (18.6% oil content). Catalyst H$_2$SO$_4$—0.75 g, 3 h, methanol—75 mL, petroleum ether—150 mL. II STEP Catalyst NaOH—0.71 g, 3 h, methanol—75 mL, petroleum ether—150 mL. | 95.16                    | [1]        |

Scientists analyzed the transesterification process of *Jatropha curcas* L. seeds using methanol, obtaining a uniform 87% FAEE yield when pure alcohol or its mixture with a solvent were used, although Kartika et al. (2013) used a methanol and hexane mixture (with KOH as the catalyst) [20], and Harvey et al. (2007) used a methanol and ethanol mixture (with NaOH as the catalyst) [56]. An ester yield of 99.8% was obtained for *Jatropha curcas* L. transesterification with a methanol and hexane mixture and H$_2$SO$_4$ as the catalyst. [18].

Li et al. (2011) analyzed the transesterification processes of the microalgae *Nannochloropsis* sp. using methanol and methylene dichloride mixtures. A 2:1 (v/v) mixture of methanol to solvent yielded an ester yield of 2.6%, and when a 3:1 (v/v) mixture of methanol to solvent was used, the ester yield increased to 28%. These results indicate that not only the molar ratio of alcohol to oil but also the ratio of alcohol to solvent significantly influences process efficiency [34].

### 3.2. Chemical Catalysts Used for Simultaneous Oil Extraction and Transesterification

The in situ transesterification process uses both homogeneous and heterogeneous catalysts. Alkaline and earth alkaline hydroxides, sodium and potassium methylates, sulfur, phosphorus and some organic acids are the most commonly used homogeneous catalysts. H$_2$SO$_4$ is commonly used as an acid catalyst (Table 1) [18,19,31,55]; a commonly used alkaline homogeneous catalyst is NaOH [15,17,47,56], while less frequently used alkaline homogeneous catalysts include KOH [20] and CH$_3$ONa [47] (Table 2). Acid/alkaline catalysis is used to obtain higher yields and utilize higher-acidity raw materials. Most commonly, H$_2$SO$_4$ and KOH are used in this case [22] or H$_2$SO$_4$ and NaOH may be used [1] (Table 3).
Some authors point out that acid catalysts are more active in the in situ process than alkaline catalysts [54]. Velasquez-Orta et al. (2013) compared the efficacy of sodium methoxide, sodium hydroxide and sulfuric acid in the oil transesterification process of the microalgae *Chlorella* sp. and *Nannochloropsis oculata* and found that the highest yields were obtained using sulfuric acid [60]. The results of studies on the transesterification of *Jatropha curcas* L. seeds using different catalysts have shown that transesterification with a methanol and hexane mixture and 15 wt% (on a seed mass basis) H$_2$SO$_4$ as the catalyst resulted in the highest ester yield of 99.8% [18].

For transesterification with sulfuric acid, the fatty acid methyl ester (FAME) yield was 92% ± 2%; when the alkali catalysts sodium methoxide and sodium hydroxide were used, the FAME yield was 90% ± 10% and 79% ± 2%, respectively. The efficiency of the catalyst in the in situ transesterification process depends on the type of feedstock. Velasquez-Orta et al. (2013) used sulfuric acid as a catalyst for the transesterification of different algae species and found that for *Chlorella* sp. the FAME yield reached 92% ± 2%, and for *Nannochloropsis oculata*, it reached only 73% ± 5% [60] under the same reaction conditions. When using sulfuric acid as a catalyst, the FAEE yield varied from 2.5% for secondary municipal sludge to 99.8% for *Jatropha curcas* L. seeds [18,31]. Ehimen et al. (2010) compared the catalytic activity of sulfuric and hydrochloric acid under the same conditions and found that the usage of HCl resulted in a 10% higher ester yield [37].

Usual biodiesel production employs the alkaline transesterification process, which is, however, complicated when the content of FFA in the oil is higher than 2%. Acid catalysis process is mostly applied when raw material contains a high amount of free fatty acids. They are esterified with alcohol to produce the fatty acid esters. Esterification reaction is catalyzed by inorganic and organic acids. Microalgae oil is rich in free fatty acids; they are also formed due to the water presence in reaction media and occurrence of hydrolysis process, therefore, the higher efficiency the acid catalyst demonstrates for microalgae. However, compared with alkaline catalysis, acid catalysis has certain drawbacks: even though an acid catalyst is less expensive than an alkaline one, the reaction requires more acid catalyst than the alkaline reaction. In addition, in the case of acid catalysis, special requirements apply to reactors; therefore, biodiesel production costs also increase.

A comparison of alkaline catalysts showed that the highest efficiency of in situ transesterification of *Jatropha curcas* L. seed with ethanol was obtained with sodium methoxide as the catalyst with an optimal concentration of 2% [61]. Sodium hydroxide was found to have a higher catalytic efficiency than potassium hydroxide when used as a catalyst. In addition, NaOH makes the purification of fatty acid esters easier and is less expensive. However, according to other authors, the efficiency of NaOH and KOH is almost the same: Kartika et al. (2013) and Harvey et al. (2007) used alkaline homogeneous catalysts (0.075 mol/L KOH in methanol and 0.02 N NaOH) and observed the same 87% FAME yield [20,56].

The amount of catalyst has a major influence on the yield of fatty acid esters. Increasing the concentration of NaOH from 1% to 5% increased the yield of methyl esters from microalgae under the same conditions by 50%, but no positive effect was observed when using acid catalysts and increasing their amount from 1.2% to 2.5% [38,40,41].

Heterogeneous catalysts may be used for in situ transesterification (Table 4). Li et al. (2011) carried out in situ studies on the transesterification of the microalgae *Nannochloropsis* sp. by using an Mg-Zr catalyst. A methanol and methylene chloride mixture was used for transesterification under the following reaction conditions: Mg-Zr catalyst: 10%, reaction temperature: 65 °C, reaction duration: 4 h, and 45 mL of a 2:1 (v/v) methanol and methylene chloride mixture. Under these conditions, the ester yield was only 2.6%. Increasing the methanol and methylene chloride ratio to 3:1 (v/v) resulted in a 28% fatty acid methyl ester yield under the same conditions. When performing this process in two stages and increasing the methanol-to-oil molar ratio to 10:1 in the second stage, the FAME yield reached only 22.2%. Thus, the heterogeneous process is less efficient than the process using chemical catalysts [34].
### 3.3. Temperature of Simultaneous Oil Extraction and Transesterification

Most studies have shown that the temperature of the in situ transesterification process using chemical catalysts should be selected to be close to the evaporation/boiling point of the alcohol. Most commonly, researchers using methanol or mixtures of methanol with a solvent selected a process temperature of 60–65 °C [10,18,20,48,56]. The maximum obtained FAME yield under these conditions was 99.8% [18].

However, good results were also obtained at lower process temperatures. At process temperatures close to ambient (23 °C), the FAME yield from microalgae lipids reached 91.3% using an acid catalyst, whereas the usage of NaOH for soybean transesterification yielded 84% FAME [15,37]. Haas et al. (2007) carried out transesterification studies at 35 °C using residual raw materials from distillers dried grains with solubles and obtained a FAME yield of 91.1%; for meat and bone meal, a yield of 9.3% was observed. [32]. The transesterification of cottonseed meal at 40 °C resulted in a 98% FAME yield [17].

Ozgul-Yucel and Turkay (2003) produced rice bran esters using a variety of alcohols and process temperatures close to the boiling point of the alcohols. For in situ transesterification with methanol at 65 °C, a FAME yield of 85.8% was reported; at 78 °C for transesterification with ethanol, a yield of 76.4%–78.3% was obtained; at 82 °C for transesterification with isopropanol, an ester yield of 69.7% was reported; for transesterification with n-propanol at 97 °C, a yield of 74.8% was reached, and transesterification with butanol at 117 °C resulted in a yield of 68% [55]. Preparation of ethyl esters from castor seeds using ethanol or an ethanol and hexane mixture gave a 95.3%–97.4% ester yield at a process temperature of 60 °C [22].

### 3.4. Duration of Simultaneous Oil Extraction and Transesterification

As with conventional transesterification of oil, the efficiency of in situ transesterification depends on the duration of the process. Research has been carried out by scientists at a process duration of 1–24 h under different process conditions [18,55]. Ozgul-Yucel and Turkay (2003) produced rice bran esters using various alcohols and a reaction time of 1 h, obtaining fatty acid ester yields from 68% to 85.8% [55]. Distillers dried grains with solubles were transesterified with methanol for 1.2 h, resulting in a FAME yield of 91.1% [32]. The ester yield after transesterification of meat and bone meal was only 9.3% after 0.2 h [32]. A two-step castor seed ester production process yielded 95.3%–98.0% esters with 1 h for each process step [22]. It took some researchers 24 h to reach a 99.8% yield of *Jatropha curcas* L. seed fatty esters [18].

Velasquez-Orta et al. (2013) achieved a 90%–92% yield of fatty acid methyl esters from *Chlorella* sp. after a 19 h process duration when CH$_3$ONa was used for transesterification by applying the acid catalyst H$_2$SO$_4$ [48], and Ehimem et al. (2010) obtained a 91.3% yield of microalgae lipid esters (with H$_2$SO$_4$ as the catalyst) after 8 h [37]. A longer duration of in situ transesterification than the
conventional process of biodiesel synthesis is believed to be necessary because the extraction and transesterification processes occur simultaneously.

4. Enzymatic Simultaneous Oil Extraction and Transesterification Method

The use of chemical catalysts in the transesterification process has certain disadvantages, such as high energy consumption, difficulties in regenerating the catalyst, and environmental pollution by the catalysts [64]. In addition, there are additional costs for the purification of the resulting product after chemical catalysis [52]. These problems can be eliminated by using enzymes (lipases) as catalysts instead of chemical catalysts. Lipases, such as those produced by Candida antarctica, Candida rugosa, Pseudomonas cepacia, Aspergillus niger, and Rhizopus oryzae, can be used in the biodiesel production process. Biocatalysts are less commonly used than chemical catalysts in the simultaneous oil extraction and transesterification process [65].

4.1. Catalysts for Enzymatic In Situ Transesterification

Immobilized lipases (Novozym 435, Lipozyme RM IM, and lipases from Candida cylindracea and Burkholderia) have been used for biotechnological in situ transesterification processes [8,29,52,66,67] (Table 5). Scientists studying the in situ process of rapeseed transesterification using butanol as the transesterification agent and mineral diesel as the solvent conducted studies on six industrially produced lipases: Lipex 100L, Lipolase 100L, Novozym 435, Resinase A 2X, Lipozyme RM IM, and Lipozyme TL IM. Lipozyme RM IM was found to be the most effective biocatalyst for this process. The in situ transesterification process was optimized using response surface methodology, and the optimal enzyme content was found to be 5.24%, resulting in a fatty acid ester yield of 97.6% [8].

Table 5. Summary of studies on using biocatalyst for in situ transesterification.

| Raw Material                  | Alcohol Solvent          | Reaction Conditions                                      | Fatty Acid Esters Yield (%) | References |
|-------------------------------|--------------------------|----------------------------------------------------------|-------------------------------|------------|
| Jatropha curcas L. seed       | Dimethyl carbonate       | Catalyst Novozym 435—10 wt%, 50 °C, 24 h, diethyl carbonate to oil ratio—10:1 | 95.5                          | [66]       |
| Jatropha curcas L. seed       | Diethyl carbonate        | Catalyst Novozym 435—10 wt%, 50 °C, 24 h, diethyl carbonate to oil ratio—10:1 | 94.5                          | [66]       |
| Pistacia chinensis Bunge seed | Dimethyl carbonate       | Catalyst Novozym 435—10 wt%, 50 °C, 24 h, diethyl carbonate to oil ratio—10:1 | 89.6                          | [66]       |
| Pistacia chinensis Bunge seed | Diethyl carbonate        | Catalyst Novozym 435—10 wt% of seed, reaction temperature—50 °C, reaction duration—24 h, diethyl carbonate to oil ratio—10:1 | 90.7                          | [66]       |
| Microalgae                    | Methanol/hexane          | Catalyst Burkholderia lipase—1203.11 U/g, lipase was immobilized on alkyl-grafted nanocomposites (Fe3O4–SiO2), 40 °C, water content—71.39 wt%, 48 h, methanol to oil molar ratio—67.93:1, hexane content—80.57 wt%. | 97.3                          | [52]       |
| Chlorella vulgaris             | Methanol                 | Catalyst Novozym 435—10% in mass | 52                            | [29]       |
| ESP-31                        |                         | fraction of sludge (wt weight base), 40 °C, 24 h, methanol to sludge ratio—4 mL/g | 55                            | [67]       |
| Greasy sewage sludge          | Methanol                 | Catalyst Candida cylindracea—10 wt%, 37 °C, 96 h, methanol to oil ratio—4:1 | 100                           | [67]       |
| Waste activated bleaching earth | Methanol                | Catalyst Candida cylindracea—10 wt%, 37 °C, 7 h, methanol to oil ratio—4:1, FAME content in sample 10% (w/w) | 100                           | [67]       |
| Waste activated bleaching earth | Methanol/hexane         | Catalyst Candida cylindracea—10 wt%, 37 °C, 2–3 h, methanol to oil ratio—4:1, FAME content in sample 10% (w/w) | 100                           | [67]       |
| Waste activated bleaching earth | Methanol/marine diesel   | Catalyst Lipozyme RMIM—5.2 wt%, mineral diesel to oil ratio (w/w)—9:1, 40 °C, 19.6 h, butanol to oil ratio—31:1 | 97.6                          | [8]        |

The results of using Novozym 435 in in situ transesterification showed that the optimal catalyst content was 10 wt% [29,67]. However, the obtained fatty ester yield from greasy sewage sludge was only 52 wt% [29], while the FAME yield from Jatropha curcas L. seed was 94.5%–95.5%, and that from Pistacia chinensis Bunge seed was 89.6%–90.7%, depending on the transesterification agent (dimethyl
carbonate or diethyl carbonate) [66]. Kojima et al. (2004) used the biocatalyst *Candida cylindracea* for the in situ transesterification with methanol of waste-activated bleaching earth. Studies have been carried out considering varying biocatalyst concentrations from 0.5 to 10 wt%, and the optimal concentration of catalyst was determined to be 10 wt% (on a seed mass basis) [67]. Tran et al. (2012) determined that the biocatalyst *Burkholderia sp. C20* immobilized on alkyl-grafted Fe₃O₄-SiO₂ is effective in the production of microalgae methyl esters by an in situ process; additionally, the biocatalyst could be used for six cycles (or 288 h) without loss of activity [52]. However, when aiming to achieve a high ester yield, additional solvents hexane or mineral diesel must be used in a mixture with methanol [67].

4.2. Alcohols and Solvents Used for Enzymatic In Situ Transesterification

As with chemical transesterification, methanol and ethanol are commonly used as transesterification agents in the case of enzymatic transesterification. The use of methanol is more problematic due to its negative effect on lipase activity [41]. This negative effect was reduced by using methanol for the transesterification of substrates with higher amounts of free fatty acids [68,69]. Considering lipase inactivation by methanol, the methanol content in the reaction medium should be less than that normally used in chemical catalysis. Typically, the molar ratio of methanol to oil used in conventional chemical catalysis is 6:1, while Kojima et al. (2004) studied the enzymatic process and suggested a lower molar ratio. However, in the absence of solvent, when using a catalyst from *Candida cylindracea* at an amount of 10 wt% (on a seed mass basis), a reaction temperature of 37 °C, a reaction time of 7 h, and a methanol to oil ratio of 4:1, only a 55% FAME yield was obtained [67].

Higher alcohols have fewer negative effects on the activity of lipases, and ethanol is often used during enzymatic transesterification [70,71]. To reduce the negative effects of alcohol, it has been proposed that other acyl receptors be used, in most cases dimethyl carbonate or diethyl carbonate. Su et al. (2009) used dimethyl carbonate and diethyl carbonate at a 10:1 molar ratio for in situ transesterification of *Jatropha curcas* L. seed and Pistacia chinensis Bunge seed. The yields of methyl esters from *Jatropha curcas* L. with both dimethyl carbonate (95.5%) and diethyl carbonate (94.5%) were very similar. The fatty acid ester yields of Pistacia chinensis Bunge seed were lower than those of *Jatropha curcas* L. seed, but the results were not significantly different when dimethyl carbonate (89.6%) or diethyl carbonate (90.7%) was used for transesterification [66].

The in situ transesterification process has been investigated not only by using alcohols as transesterification agents [29,66,67] but also by using mixtures of alcohols and solvents [8,67]. Kojima et al. (2004) found that the FAME yield after enzymatic transesterification with methanol at a 4:1 alcohol-to-oil molar ratio reached only 55% with a reaction time of 96 h. The addition of hexane or mineral diesel as solvent resulted in 100% FAME yield after 7 and 2–3 h, respectively [67]. A higher optimal molar ratio of methanol to oil was determined by Sangaletti-Gerhard et al. (2015). It was found that a FAME yield of 97.3% could be achieved by in situ enzymatic transesterification of the microalgae *Chlorella vulgaris* ESP-31 with a methanol-to-oil molar ratio of 67.93:1 and an 80.57 wt% hexane for a 48 h process duration [29].

The main results of in situ enzymatic transesterification prove that a higher molar ratio of alcohol to oil and the addition of solvent increase the reaction rate and maximize the product yield. Since pure biodiesel is rarely used in the transport sector, mineral diesel and biodiesel blends are the most common. During the in situ process, mineral diesel can be used as an extraction solvent. The advantage of the application of such a solvent is the simultaneous production of a biodiesel and mineral diesel mixture directly, avoiding the blending of pure mineral diesel with biodiesel. Scientists have produced mixtures containing 10% fatty acid esters in mineral diesel [8,67].

4.3. Temperature of Enzymatic In Situ Transesterification

Temperature is an important factor determining the rate of reaction. At higher temperatures, transesterification reactions occur faster, but biocatalysts are sensitive to higher temperatures. It is generally suggested that enzymatic reactions must be performed at 40–70 °C [72,73]. It has been found
that enzymatic activity decreases at higher temperatures due to the denaturation of enzymes [74]. A fatty acid ester yield of 52%–100% at 37–50 °C was reported by researchers investigating the enzymatic in situ transesterification process [29,66,67]. Su et al. (2009) reported *Jatropha curcas* L. and *Pistacia chinensis* Bunge fatty acid ester yields of 95.5% and 89.6%, respectively, when transesterification was performed at 50 °C under the same process conditions, which also demonstrates the influence of the origin of the oily feedstock on ester yield [66]. Transesterification of rapeseeds with butanol and mineral diesel as an extraction solvent resulted in 97.6% fatty acid butyl esters at a process temperature of 40 °C [8]. At the same transesterification temperature (40 °C), a 97.3% yield of FAME from the microalga *Chlorella vulgaris* was achieved [52]. Tran et al. (2012) found that biodiesel conversion and transesterification rates increased when the temperature was raised from 25 to 40 °C and decreased when the temperature was further increased to 50 °C. This result proved that the inactivation/denaturation process of the biocatalyst (*Burkholderia* lipase) had begun [52].

4.4. Duration of Enzymatic In Situ Transesterification

The duration of the enzymatic in situ process is longer than that of the conventional oil transesterification process, as oil extraction and transesterification are carried out simultaneously. Research shows that the optimum duration of the biotechnological process is 19–24 h. [8,59]. Tran et al. (2012) achieved a FAME yield of 97.3% by using *Chlorella vulgaris* (ESP-31) for in situ transesterification after a process duration of 48 h [52], whereas Kojima et al. (2004) reported that after a duration of 2–3 h, it was possible to obtain a 100% FAME yield by in situ transesterification of waste-activated bleaching earth using methanol and mineral diesel. When methanol and hexane were used, the same yield was obtained after 7 h. When methanol without the addition of solvent was used for transesterification, the FAME yield after 96 h was 55%. These results prove that the use of solvents significantly increases the rate of enzymatic transesterification in situ [67].

5. Conclusions

The oil extraction and refining process is energy intensive, accounting for approximately 70% of the total cost of biodiesel production. The cost of biodiesel production could be reduced by eliminating the oil extraction process and applying the so-called in situ simultaneous oil extraction and transesterification process.

Extraction solvents may be omitted in this process since alcohol is both an oil extractor and a transesterification agent. The in situ process can be performed by chemical and biochemical catalysis. Homogeneous acid and alkaline catalysts are more effective than heterogeneous catalysts in the in situ process. The results of studies on heterogeneous catalysis indicate that their efficiency in the in situ process is limited. Chemical catalysis is being explored more extensively, but with a view to sustainable development, opportunities are being sought to optimize enzymatic processes and implement them in industry. The effectiveness of the in situ transesterification process is essentially dependent on four independent variables: alcohol content, catalyst content, reaction duration, and temperature. The type of raw material, moisture content, degree of crushing, and method of preparation have a smaller influence on transesterification effectiveness.

Duration and temperature have a direct influence on ester yield, but increasing the process temperature above the boiling point of alcohol is not recommended due to energy and alcohol losses. Although more than a stoichiometric amount of alcohol is commonly used, the alcohol content is limited in the enzymatic process due to the negative influence of alcohol on the enzymatic preparation activity. Higher molar ratios may be used by replacing the methanol or ethanol used for in situ transesterification with dimethyl carbonate and diethyl carbonate or by using additional solvents that increase surface contact between lipids and alcohols and thus the rate of reaction and fatty acid ester yield. Further reductions in the cost of the product can be achieved by replacing the solvents with mineral diesel. Initial studies have shown that mineral diesel acts as an oil extracting agent. After determining the required mineral diesel-to-oil ratio in the reaction media, a mixture of mineral
diesel and biodiesel is produced; this reaction product is currently used in the transport sector. In this process, a mixture is obtained directly without additional mixing of pure mineral diesel and biodiesel.

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**References**

1. Lei, H.; Ding, X.; Zhang, H.; Chen, X.; Li, Y.; Zhang, H.; Wang, Z. In situ production of fatty acid methyl ester from low quality rice bran: An economical route for biodiesel production. *Fuel* 2010, 89, 1475–1479. [CrossRef]

2. Sendzikiene, E.; Sinkuniene, D.; Kazanceva, I.; Kazancev, K. Optimization of low quality rapeseed oil transesterification with butanol by applying the response surface methodology. *Renew. Energy* 2016, 87, 266–272. [CrossRef]

3. Basha, S.A.; Gopal, K.R.; Jebraj, S. A review on biodiesel production, combustion, emissions and performance. *Renew. Sustain. Energy Rev.* 2009, 13, 1628–1634. [CrossRef]

4. Wang, R.; Zhou, W.W.; Hanna, M.A.; Zhang, Y.P.; Bhadury, P.S.; Wanga, Y.; Song, B.A.; Yang, S. Biodiesel preparation, optimization, and fuel properties from non-edible feedstock, *Datura stramonium* L. *Fuel* 2012, 91, 182–186. [CrossRef]

5. Lin, C.Y.; Fan, C.L. Fuel properties of biodiesel produced from Camellia oleifera abel oil through supercritical-methanol transesterification. *Fuel* 2011, 90, 2240–2244. [CrossRef]

6. Zeng, J.; Wang, X.; Zhao, B.; Sun, J.; Wang, Y. Rapid in situ transesterification of sunflower oil. *Ind. Eng Chem Res.* 2009, 48, 850–856. [CrossRef]

7. Kaul, S.; Porwal, J.; Garg, M.O. Parametric study of jatropha seeds for biodiesel production by reactive extraction. *J. Am. Oil Chem. Soc.* 2010, 87, 903–908. [CrossRef]

8. Sendzikiene, E.; Makarevičienė, V.; Gumbyte, M. Reactive extraction and fermentable transesterification of rapeseed oil with butanol in diesel fuel media. *Fuel Process. Technol.* 2015, 138, 758–764. [CrossRef]

9. Ambat, I.; Srivastava, V.; Sillapāp, M. Recent advancement in biodiesel production methodologies using various feedstock: A review. *Renew. Sustain. Energy Rev.* 2018, 90, 356–369. [CrossRef]

10. Zakaria, R.; Harvey, A.P. Direct production of biodiesel from rapeseed by reactive extraction/insitu transesterification. *Fuel Process. Technol.* 2012, 102, 53–60. [CrossRef]

11. Abo El-Enin, S.A.; Attia, N.K.; El-Ibiari, N.N.; El-Diwani, G.I.; El-Khatib, K.M. In-situ transesterification of rapeseed and cost indicators for biodiesel production. *Renew. Sustain. Energy Rev.* 2013, 18, 471–477. [CrossRef]

12. Haagenson, D.M.; Brudvik, R.L.; Lin, H.; Wiesenborn, D.P. Implementing an in Situ alkaline transesterification method for canola biodiesel quality screening. *J. Am. Oil Chem. Soc.* 2010, 87, 1351–1358. [CrossRef]

13. Siler-Marinkovic, S.; Tomasevic, A. Transesterification of sunflower oil in situ. *Fuel* 1998, 77, 1389–1391. [CrossRef]

14. Harrington, K.J.; d’Arcy-Evans, C. Transesterification in situ of sunflower seed oil. *Ind. Eng. Chem. Prod. Res. Dev.* 1985, 24, 314–318. [CrossRef]

15. Haas, M.J.; Scott, K.M.; Marmer, W.N.; Foglia, T.A. In situ alkali transesterification: An effective method for the production of fatty acid esters from vegetable oils. *J. Am. Oil Chem. Soc.* 2004, 81, 83–89. [CrossRef]

16. Wu, H.; Liu, Y.; Zhang, J.; Li, G. In situ reactive extraction of cottonseeds with methyl acetate for biodiesel production using magnetic solid acid catalysts. *Bioresour. Technol.* 2014, 174, 182–189. [CrossRef] [PubMed]

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

18. Shuit, S.H.; Lee, K.T.; Kamaruddin, A.H.; Yusup, S. Reactive extraction and in situ esterification of *Jatropha curcas* L. seeds for the production of biodiesel. *Fuel* 2010, 89, 527–530. [CrossRef]

19. Shuit, S.H.; Lee, K.T.; Kamaruddin, A.H.; Yusup, S. Reactive extraction of *Jatropha curcas* L. seed for production of biodiesel: Process optimization study. *Environ. Sci. Technol.* 2010, 44, 4361–4367. [CrossRef]

20. Kartika, I.A.; Yani, M.; Ariono, D.; Evon, P.; Rigal, L. Biodiesel production from jatropha seeds: Solvent extraction and in situ transesterification in a single step. *Fuel* 2013, 106, 111–117. [CrossRef]
21. Chapagain, B.P.; Yehoshua, Y.; Wiesman, Z. Desert date (Balanites aegyptiaca) as an arid lands sustainable bioresource for biodiesel. *Bioresour. Technol.* 2009, 100, 1221–1226. [CrossRef][PubMed]

22. Hincapié, G.; Mondragón, F.; López, D. Conventional and in situ transesterification of castor seed oil for biodiesel production. *Fuel* 2011, 90, 1618–1623. [CrossRef]

23. Dasari, S.R.; Borugadda, V.B.; Goud, V.V. Reactive extraction of castor seeds and storage stability characteristics of produced biodiesel. *Process. Saf. Environ. 2016, 100, 252–263. [CrossRef]*

24. Özgül, S.; Türkay, S. Variables affecting the yields of methyl esters derived from in situ esterification of rice bran oil. *J. Am. Oil Chem. Soc.* 2002, 79, 611–614. [CrossRef]

25. Liu, Y.; Tu, Q.; Knothe, G.; Lu, M. Direct transesterification of spent coffee grounds for biodiesel production. *Fuel* 2017, 199, 157–161. [CrossRef]

26. Tuntiwattananup, N.; Monono, E.; Wiesenborn, D.; Tongcumpou, C. In-situ transesterification process for biodiesel production using spent coffee grounds from the instant coffee industry. *Ind. Crops Prod.* 2017, 102, 23–31. [CrossRef]

27. Park, J.; Kim, B.; Lee, J.W. In-situ transesterification of wet spent coffee grounds for sustainable biodiesel production. *Bioresour. Technol.* 2016, 221, 55–60. [CrossRef]

28. Shiu, P.J.; Gunawan, S.; Hsieh, W.H.; Kasim, N.S.; Ju, Y.H. Biodiesel production from rice bran by a two-step in-situ process. *Bioresour. Technol.* 2010, 101, 3984–3989. [CrossRef]

29. Sangaletti-Gerhard, N.; Cea, M.; Risco, V.; Navia, R. In situ biodiesel production from greasy sewage sludge using acid and enzymatic catalysts. *Bioresour. Technol.* 2015, 179, 63–70. [CrossRef]

30. Choi, O.K.; Song, J.S.; Cha, D.K.; Lee, J.W. Biodiesel production from wet municipal sludge: Evaluation of in situ transesterification using xylene as a cosolvent. *Bioresour. Technol. Vol.* 2014, 166, 51–56. [CrossRef]

31. Mondala, A.; Liang, K.; Toghiani, H.; Hernandez, R.; French, T. Biodiesel production by in situ transesterification of municipal primary and secondary sludges. *Bioresour. Technol.* 2009, 100, 1203–1210. [CrossRef][PubMed]

32. Haas, M.; Scott, K.; Foglia, T.; Mariner, W. The general applicability of in situ transesterification for the production of fatty acid esters from a variety of feedstocks. *J. Am. Chem. Soc.* 2007, 84, 963–970. [CrossRef]

33. Macías-Sánchez, M.D.; Robles-Medina, A.; Hita-Peña, E.; Jiménez-Callejón, M.J.; Esteban-Cerdán, L.; González- Moreno, P.A.; Molina-Grima, E. Biodiesel production from wet microalgal biomass by direct transesterification. *Fuel* 2015, 150, 14–20. [CrossRef]

34. Li, Y.S.; Lian, S.A.; Tong, D.M.; Song, R.L.; Yang, W.Y.; Fan, Y.; Qing, R.W.; Hu, C.W. One-step production of biodiesel from Nannochloropsis sp. on solid base Mg-Zr catalyst. *Appl. Energy 2011, 88, 3313–3317. [CrossRef]*

35. Xu, R.; Mi, Y. Simplifying the process of microalgal biodiesel production through in situ transesterification technology. *J. Am. Oil Chem. Soc.* 2011, 88, 91–99. [CrossRef]

36. Johnson, M.B.; Wen, Z. Production of biodiesel fuel from microalga Schizochytrium limacinum by direct transesterification of algal biomass. *Energy Fuel 2009, 23, 5179–5183. [CrossRef]*

37. Ehimen, E.A.; Sun, Z.F.; Carrington, C.G. Variables affecting the in situ transesterification of microalgae lipids. *Fuel* 2010, 89, 677–684. [CrossRef]

38. Wahlen, B.D.; Willis, R.M.; Seefeldt, L.C. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresour. Technol.* 2011, 102, 2724–2730.

39. Koberg, M.; Cohen, M.; Ben-Amotz, A.; Gedanke, A. Bio-diesel production directly from the microalgal biomass of Nannochloropsis by microwave and ultrasound radiation. *Bioresour. Technol.* 2011, 102, 4265–4269.

40. Zhang, J.; Cui, C.; Chen, H.; Liu, J. The completion of esterification of free fatty acids in Zanthoxylum bungeanum seed oil with ethanol. *Int. J. Green Energy 2014, 11, 822–832. [CrossRef]*

41. Zhang, X.; Yan, S.; Tyagi, R.D.; Surampalli, R.Y.; Valero, J.R. Ultrasonication aided in-situ transesterification of microbial lipids to biodiesel. *Bioresour. Technol. 2014, 169, 175–180. [CrossRef][PubMed]*

42. Ehimen, E.A.; Sun, Z.; Carrington, G.C. Use of ultrasound and co-solvents to improve the in situ transesterification of microalgal biomass. *Procedia Environ. Sci.* 2012, 15, 47–55.

43. Sung, M.; Han, J.I. Ultrasound-assisted in-situ transesterification of wet Aurantiocystis sp. KRS 101 using potassium carbonate. *Bioresour. Technol. 2018, 261, 117–121. [CrossRef]*

44. Georgogianni, K.G.; Kontominas, M.G.; Avlonitis, D.; Gergis, V. Transesterification of sunflower seed oil for the production of biodiesel: Effect of catalyst concentration and ultrasonication. In Proceedings of the 2006 IASME/WSEAS International Conference on Energy & Environmental Systems, Chalkida, Greece, 8–10 May 2006; pp. 425–429.
45. Qian, J.; Yang, Q.; Sun, F.; He, M.; Chen, Q.; Yun, Z.; Qin, L. Cogeneration of biodiesel and nontoxic rapeseed meal from rapeseed through in-situ alkaline transesterification. Bioresour. Technol. 2013, 128, 8–13. [CrossRef]
46. Liu, B.; Zhao, Z.K. Biodiesel production by direct methanolysis of oleaginous microbial biomass. Chem. Technol. Biotechnol. 2007, 82, 775–780. [CrossRef]
47. Cao, H.; Zhang, Z.; Wu, X.; Miao, X. Direct biodiesel production from wet microalgae biomass of Chlorella pyrenoidosa through in situ transesterification. Biomed. Res. Int. 2013, 2013, 930686. [CrossRef]
48. Velasquez-Orta, S.B.; Lee, J.G.M.; Harvey, A.P. Evaluation of FAME production from wet marine and freshwater microalgae by in situ transesterification. Biochem. Eng. 2013, 76, 83–89. [CrossRef]
49. Nguyen, T.T.; Uemura, Y.; Lam, M.K.; Mansor, N.; Lim, J.W. Revealing the effect of reaction parameters towards alkyl group distribution in in-situ transesterification of Chlorella vulgaris. Energy Convers. Manag. 2019, 185, 223–231. [CrossRef]
50. Bruton, T.; Lyons, H.; Lerat, Y.; Stanley, M.; Rasmussen, M.B. A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland, Report, Sustainable Energy Ireland. 2009. Available online: http://www.fao.org/uploads/media/9025_9025_524015593045.pdf (accessed on 30 March 2020).
51. Liu, J.; Liu, Y.; Wang, H.; Xue, S. Direct transesterification of fresh microalgal cells. Bioresour. Technol. 2015, 176, 284–287. [CrossRef]
52. Tran, D.T.; Yeh, K.L.; Chen, C.L.; Chang, J.S. Enzymatic transesterification of microalgal oil from marine microalgae. Energy Convers. Manag. 2013, 61, 83–89. [CrossRef]
53. Ginting, M.S.A.; Azizan, M.T.; Yusup, S. Alkaline in situ ethanolysis of Jatropha curcas L. seeds using homogeneous and heterogeneous basic catalysts. Fuel 2019, 235, 277–287. [CrossRef]
54. Kazemifard, S.; Nayebzadeh, H.; Saghatoleslami, N.; Safakish, E. Application of magnetic alumina-ferric oxide nanocatalyst supported by KOH for in-situ transesterification of microalgae cultivated in wastewater medium. Biomass Bioenergy 2019, 129, 105338. [CrossRef]
55. Sivaramakrishnan, R.; Muthukumar, K. Direct transesterification of Oedogonium sp. oil be using immobilized isolated novel Bacillus sp. Lipase. J. Biosci. Bioeng. 2014, 117, 86–91. [CrossRef]
56. Ghaly, A.E.; Dave, D.; Brooks, M.S.; Budge, S. Production of Biodiesel by Enzymatic Transesterification: Review. J. Biochem. Biotechnol. 2010, 6, 54–76. [CrossRef]
57. Su, E.; You, P.; Wei, D. In situ lipase-catalyzed reactive extraction of oilseeds with short-chained dialkyl carbonates for biodiesel production. Bioresour. Technol. 2009, 100, 5813–5817. [CrossRef]
67. Kojima, S.; Du, D.; Sato, M.; Park, E.Y. Efficient production of fatty acid methyl ester from waste activated bleaching earth using diesel oil as organic solvent. *J. Biosci. Bioeng.* 2004, 98, 420–424. [CrossRef]

68. Ve’ras, I.C.; Silva, F.A.L.; Ferr’co-Gonzales, A.D.; Moreau, V.H. One-step enzymatic production of fatty acid ethyl ester from high-acidity waste feedstocks in solvent-free media. *Bioresour. Technol.* 2011, 102, 9653–9658. [CrossRef] [PubMed]

69. Watanabe, Y.; Pinsirodom, P.; Nagao, T.; Yamauchi, A.; Kobayashi, T.; Nishida, Y.; Takagi, Y.; Shimada, Y. Conversion of acid oil by-produced in vegetable oil refining to biodiesel fuel by immobilized *Candida antarctica* lipase. *J. Mol. Catal. B Enzym.* 2007, 44, 99–105. [CrossRef]

70. Chen, J.; Wu, W. Regeneration of immobilized *Candida antarctica* lipase for transesterification. *J. Biosci. Bioeng.* 2003, 95, 466–469. [CrossRef]

71. Shimada, Y.; Watanabe, Y.; Samukawa, T.; Sugiara, A.; Noda, H.; Fukuda, H.; Tominaga, Y. Conversion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase. *J. Am. Oil Chem. Soc.* 1999, 76, 789–793. [CrossRef]

72. Ochoa-Gómez, J.R.; Gómez-Jiménez-Aberasturib, O.; Maestro-Madurgab, B.; Pesquera-Rodriguezb, A.; Ramírez-Lópezb, C.; Lorenzo-Ibarretab, L.; Torrecilla-Soria, J.; Villarán-Velasco, M.C. Synthesis of glycerol carbonate from glycerol and dimethyl carbonate by transesterification: Catalyst screening and reaction optimization. *Appl. Catal. A Gen.* 2009, 366, 315–324. [CrossRef]

73. Jo, Y.J.; Lee, O.K.; Lee, E.Y. 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. *Bioresour. Technol.* 2014, 158, 105–110. [CrossRef]

74. Ceni, G.; Lerin, L.A.; Conto, J.F.; Brancher, V.; Silva, P.C.; Toniazzo, G.; Treichel, H.; de Oliveira, D.; Oliveira, J.V.; Oestreicher, E.G.; et al. Optimization of 1-glycerol benzoate production by enzymatic transesterification in organic solvents. *Enzyme Microb. Technol.* 2010, 46, 107–112. [CrossRef]

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