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Review

Bioprocesses for the Biodiesel Production from Waste Oils and Valorization of Glycerol

Cédric Decarpigny¹, Abdulhadi Aljawish², Cédric His², Bertrand Fertin¹, Muriel Bigan¹, Pascal Dhulster¹ , Michel Millares² and Rénato Froidevaux^{1,*} 

¹ UMR Transfrontalière 1158 BioEcoAgro, Univ. Lille, INRAE, Univ. Liège, UPJV, JUNIA, Univ. Artois, Univ. Littoral Côte d'Opale, ICV-Institut Charles Viollette, 59000 Lille, France; cedric.decarpigny@orange.fr (C.D.); bertrand.fertin@univ-lille.fr (B.F.); muriel.bigan@univ-lille.fr (M.B.); pascal.dhulster@univ-lille.fr (P.D.)

² GECCO—5011 rue des Marlières, ZA des Marlières, 59710 Avelin, France; abdulhadi.aljawish@gecco.fr (A.A.); cedric.his85@gmail.com (C.H.); michel.millares@gecco.fr (M.M.)

* Correspondence: renato.froidevaux@univ-lille.fr; Tel.: +33-320-417566

Abstract: The environmental context causes the use of renewable energy to increase, with the aim of finding alternatives to fossil-based products such as fuels. Biodiesel, an alternative to diesel, is now a well-developed solution, and its production from renewable resources makes it perfectly suitable in the environmental context. In addition, it is biodegradable, non-toxic and has low greenhouse gas emissions: reduced about 85% compared to diesel. However, the feedstock used to produce biodiesel competes with agriculture and the application of chemical reactions is not advantageous with a “green” process. Therefore, this review focuses only on bioprocesses currently taking an important place in the production of biodiesel and allow high yields, above 90%, and with very few produced impurities. In addition, the use of waste oils as feedstock, which now accounts for 10% of feedstocks used in the production of biodiesel, avoids competition with agriculture. To present a complete life-cycle of oils in this review, a second part will focus on the valorization of the biodiesel by-product, glycerol. About 10% of glycerol is generated during the production of biodiesel, so it should be recovered to high value-added products, always based on bioprocesses. This review will also present existing techniques to extract and purify glycerol. In the end, from the collection of feedstocks to the production of CO₂ during the combustion of biodiesel, this review presents the steps using the “greener” possible processes.

Keywords: biodiesel; bioprocesses; waste oils; crude glycerol; purification; valorization



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1. Introduction

The environmental context is resulting in the need to develop new technological processes using renewable resources due to the scarcity of fossil resources. Among the many alternatives based on the use of renewable resources, particularly biomass, the production of biodiesel from vegetable oils, or even animal fats, is nowadays widely accepted throughout the world due to its origin. It is biodegradable, non-toxic and has low greenhouse gas emissions [1]. However, the production of biodiesel creates competition with agriculture, resulting in high costs due to the cost of raw materials [2]. For this reason, it is important to focus on other feedstocks, constituting the five generations of biofuels, excluding petroleum-based diesel [3].

Currently, the first and second generations, including edible and non-edible oil crops, are the generations that compete with food and land treatment products (pesticides), resulting in higher costs and greenhouse gas emissions. On the other hand, the fourth and fifth generations, involving the use of algae and genetically modified crops, are a great step forward. The biodiesel produced from these feedstocks is very expensive, compared to petroleum diesel, and the production process requires considerable technological development. Therefore, third-generation biodiesel, using waste cooking oils as feedstock, is

currently the best option due to the low cost of this raw material and the lack of competition with foodstuffs. Moreover, this option represents a good solution to the problem of recovering waste oils.

The impact of biodiesel from waste oils on engines and their performances must also be considered [4]. The combustion characteristics of biodiesel from waste oils show that the cylinder peak pressure value increases and the heat release rate and ignition delay period decreases. For the engine performances, the brake-specific fuel consumption increases while brake-specific energy consumption, brake power and torque decrease. The use of biodiesel from waste oils reduces about 85% of gas emissions thanks to the decrease in hydrocarbon, SO₂, CO and smoke emissions in the exhaust. Nevertheless, CO₂ and NO_x increase compared to diesel, depending on its composition. This information can be confirmed with concrete results conducted by the GECCO company, which specializes in food waste valorization, as part of the BIOHEC LIFE program [5] with three other partners: Institut Charles Viollette, research laboratory in Lille University; Pour La Solidarité, think-tank based in Brussels; and RREUSE, all social companies that specialize in waste reuse. Table 1 shows results on exhaust gas measurements with two different biodiesels compared to diesel. The first biodiesel, B30, is composed of 30% of fatty acid ethyl esters and 70% of diesel and the second biodiesel, B100, is composed of 100% of fatty acid ethyl esters. The diesel is composed of 7% of fatty acid methyl esters and is called B7.

Table 1. Results on exhaust gas measurements on biodiesel compared to diesel.

Exhaust Gases Measurements	GECCO B30 vs. Gas Oil	GECCO B100 vs. Gas Oil
Fine particles emissions (PM10)	−46%	−63%
Unburned products (HC)	−37%	−50%
Carbon monoxide (CO)	−11%	−18%
Nitrogen oxide (NO _x)	Similar	+7%

This review will therefore focus on the most “green” and economical processes for recovering waste oils to produce biodiesel. The biotechnological processes of biodiesel production were reported in the first part. To complete this study, it is important to understand these processes, including the influence of waste oils used on the used processes, i.e., the impurities found in the waste oils and the impurities created during the reaction, as well as the present separation and purification techniques. The second part will be largely devoted to the impurities obtained, and more particularly to the by-product, glycerol. Indeed, glycerol has great potential for producing high value-added products [6]. However, the feasibility of converting biodiesel-derived glycerol must consider the impurities present and their impact on this conversion. Thus, the following review will consider the influence of impurities on glycerol quality and purification techniques. In the end, the biotechnological pathways of glycerol and the impact of impurities will be explained. Figure 1 exhibits the life cycle of oils through transformation into biodiesel and the valorization of glycerol into high value-added products. This life cycle is also called “Carbon Neutral Cycle” for biofuels due to converted plant source and emission with zero carbon dioxide. Indeed, biodiesel offers the advantage of sustainability since the CO₂ released on combustion will be used again by nature for feedstock preparation [7].

Some results on a life cycle analysis realized by the GECCO company are exhibited in Table 2. This life-cycle analysis starts with the waste oils collection following their pretreatment, the biodiesel production and distribution, then the use of biodiesel. These results are compared to gas oil and first-generation fatty acid methyl esters (FAME). To include this analysis in the supply chain management, more parameters must be considered. Supply chain management is the sequential arrangement or organizational functions, activities and facilities required to produce and deliver a product or service [8]. The functions and activities include different parameters such as purchasing, forecasting, inventory management, information management, scheduling, development of websites/mobile applications, delivery and customer service. Facilities, on the other hand, include production plants,

warehouses, factories, distribution centers, retail outlets, delivering products or services and offices. In the case of waste oils, especially waste cooking oils, the supply chain must include convenient waste collection and sorting systems along with transporting facilities for the waste feedstock to the biodiesel plants for producing waste-based biodiesel.

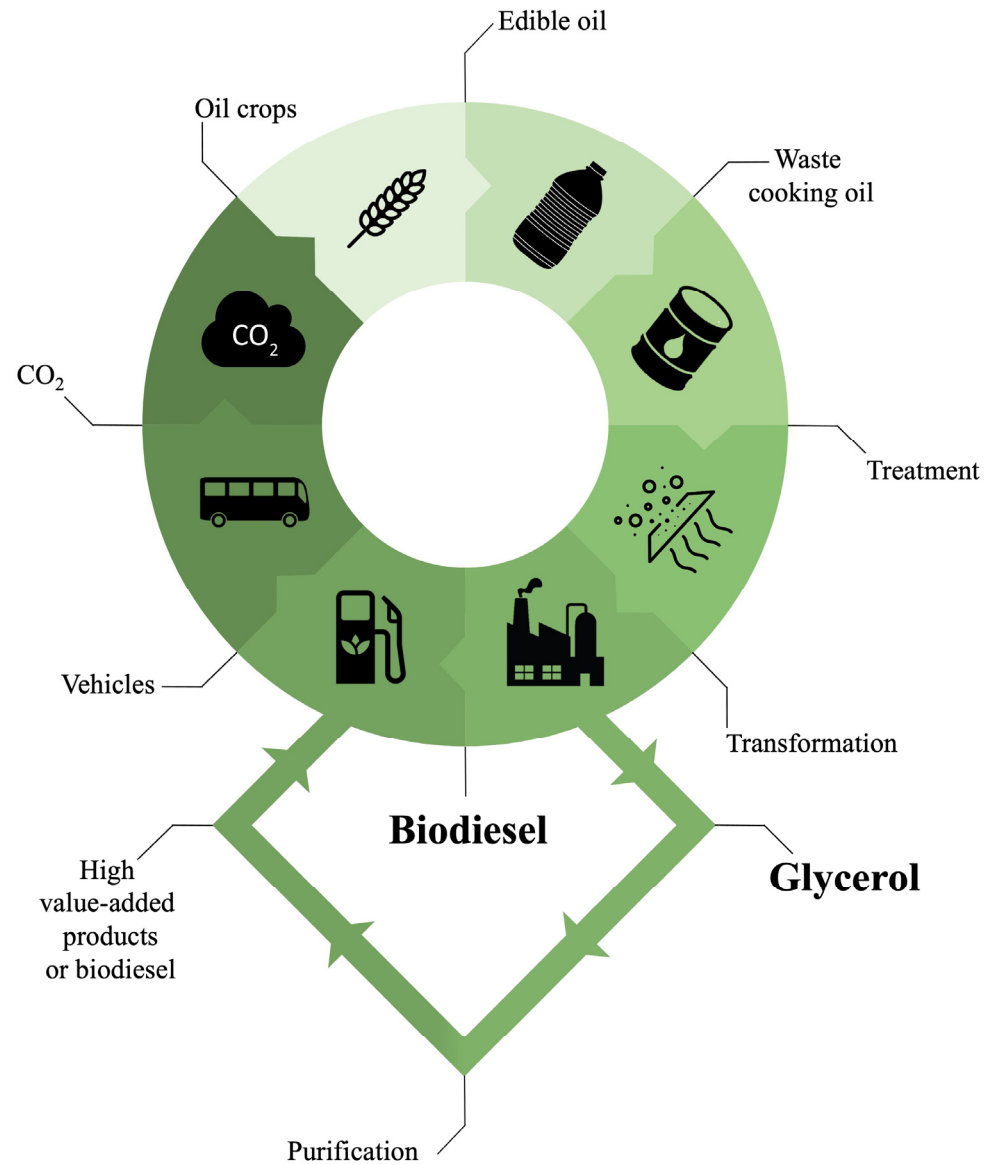


Figure 1. Life cycle of oils through transformation into biodiesel and valorization of its by-product (glycerol).

Table 2. Life-cycle analysis on waste oils compared to gas oil and first-generation FAME.

Life Cycle Analysis Results	Gas Oil [9]	Rapeseed FAME [9]	Palm FAME [9]	GECCO FAEE	Variation vs. Gas Oil	Variation vs. Rapeseed FAME	Variation vs. Palm FAME
Global warming (kg CO ₂ eq./MJ)	9.14×10^{-2}	3.73×10^{-2}	2.18×10^{-2}	1.45×10^{-2}	−84%	−61%	−33%
Human toxicity (kg 1,4-DB eq./MJ)	4.12×10^{-1}	-6.48×10^{-1}	-6.46×10^{-1}	-6.72×10^{-1}	−263%	4%	4%
Eutrophication (kg PO ₄ eq./MJ)	3.71×10^{-5}	3.64×10^{-4}	1.84×10^{-4}	2.16×10^{-5}	−42%	−94%	−88%
Non-renewable energy (MJ primary/MJ)	1.25	4.31×10^{-1}	2.71×10^{-1}	3.45×10^{-1}	−72%	−20%	27%

DB: dichlorobenzene.

1.1. Biodiesel Production Routes

There are different processes to convert oils or fats into biodiesel, such as transesterification, esterification, blending, cracking, microemulsion and pyrolysis. However, transesterification is the most common method for biodiesel production [10]. This method consists of the reaction between oils or fats and a short-chain alcohol. The biodiesel production could be also realized by the esterification reaction when a high level of free fatty acids is present (Figure 2) [11,12]. More recently, the hydroesterification process is attracting attention because it allows the production of high-quality biodiesel. It combines the hydrolysis reaction with the esterification reaction; this reaction also occurs at the same time as transesterification, depending on the level of free fatty acids or water present [13]. Unlike transesterification, hydroesterification does not involve pretreatment of oils, as the reaction attacks the free fatty acids and the obtained glycerol is of high purity. The transesterification reaction can be carried out by acid, basic or enzymatic catalysis [14]. There are very few studies on acid catalysis due to high reaction times, the corrosion phenomenon, and the difficulty of separating catalyst from the main product. However, this method is preferred when the used oil has a low purity grade, which could be advantageous when using waste oils, as demonstrated by Zhang et al. [14,15]. Regarding the catalysis in an alkaline medium, reaction times can be thousands of times faster than acid catalysis and the reactions can be carried out under mild conditions and lower energy consumption. On the other hand, alkaline catalysts lead to excessive soap formation, which reduces the biodiesel yield. This type of reaction is currently the most widely used at the industrial level [16]. Finally, the use of enzymatic catalysis has increased for several years [17]. Indeed, like acid catalysis, it is very suitable for oils with low purity, especially oils with a high level of free fatty acids, since they can be directly converted into esters. Moreover, the reactions take place at mild temperature and under atmospheric pressure. Another interesting point is that enzymatic catalysis does not lead to the formation of many impurities, such as the formation of soaps. Thus, the by-product, glycerol, is obtained with high purity. Disadvantages of enzymatic catalysis include slow reaction times and sensitive enzymes for alcohols such as methanol, which leads to enzyme deactivation and high cost. The reaction times, the corrosion phenomenon using acids and the high cost of the enzyme make the alkaline reaction the most suitable method for industrial processes.

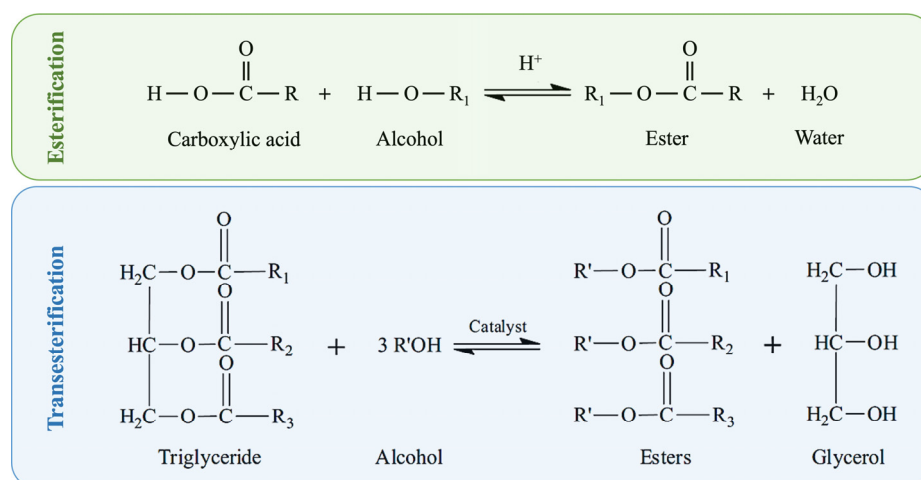


Figure 2. Esterification and transesterification reactions to produce esters [11,12].

1.2. Current Market for Biodiesel from Waste Oils

Over the last decade, biodiesel production has increased significantly from 20 billion liters in 2010 to 50 billion liters in 2020 (Figure 3) [18]. This represents an increase in demand, because of which the price per hectoliter falls from more than USD 120 to USD 80. On the other hand, this leads to a much higher consumption of vegetable oils, and more broadly, raw materials competing with food. Thus, waste oils and fats appear to be an

alternative to biodiesel produced by a source that is uncompetitive with food [19]. This also helps to reduce costs of biodiesel production when enzymatic catalysts are used [20].

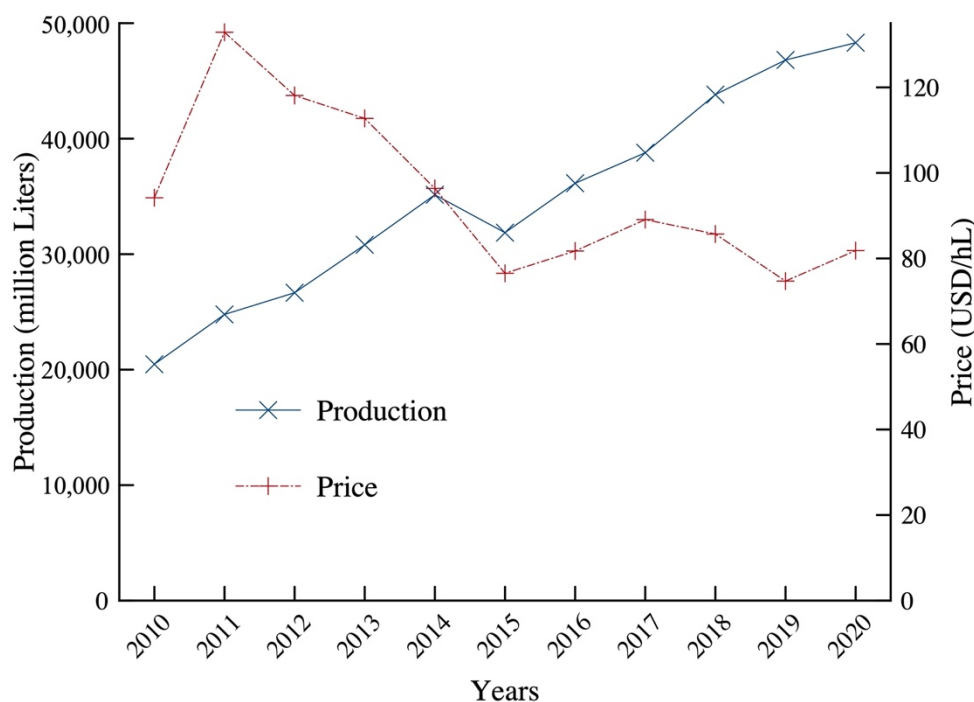


Figure 3. World biodiesel production and prices from 2010 to 2020 [18].

Currently, the European Union and the United States represent, together, the largest influencers of biodiesel demand [21]. In 2016, they produced nearly 44% of biodiesel. Some countries, such as Indonesia, Brazil, and Argentina, expanded their use of biodiesel; on the other side, biodiesel demand remained low in Central Asia and Eastern Europe as these regions are either oil and gas producers or lack biofuel incentive policies for producers or blending mandates for consumers. Policies such as tax exemptions, subsidies, fuel quality standards, import tariffs and investment backing are driven by a combination of factors, such as desires for increased energy security, environmental concerns and climate-related targets, lobby groups, feedstock availability and so on. Thus, the biodiesel development depends on each country policies, and different countries, such as India, China, Philippines, Malaysia, Thailand, and other countries mentioned above, have been encouraging biodiesel in their policies [22].

1.3. Current Glycerol Market and Applications

Glycerol, or propan-1,2,3-triol, also known as glycerin [23], is a colorless, viscous and odorless soluble liquid [24]. The number of industrial applications of glycerol would be more than 1500 [25], especially in cosmetics, pharmaceuticals and the food industry (Figure 4). These properties mean that glycerol is used as a softener in resins and plastics, but also as a lubricant, especially for the pharmaceutical industry. On the other hand, glycerol is non-toxic, and its sweet taste makes it suitable for use in the food industry. As glycerol is obtained from biodiesel, it contains various impurities which make it unusable in the above-mentioned fields of application. As a result, two solutions are possible: purification of glycerol to a high degree of purity to use in these application areas, or transformation of glycerol into other high value-added products, with or without pretreatment.

Currently, the percentages of used raw materials have remained stable in recent years, with waste oils accounting for around 10% of the basic resources for biodiesel production (Figure 5) [26]. These data, dating from 2017, have changed over the last three years, as the price for each compound varies over the years. The price of rapeseed oil has risen sharply, while palm and soybean oil are cheaper. The production of biodiesel from palm oil and

soybean oil is expected to increase. However, for the current environmental situation, the use of waste oils and fats is increasing. Moreover, the production of biodiesel from waste oils is environmentally friendly, as it allows the recycling of waste oils and distributes renewable energy by reducing pollution. It also configures a substitute for a quantity of imported petrochemical compounds and lowers the cost of waste management [27].

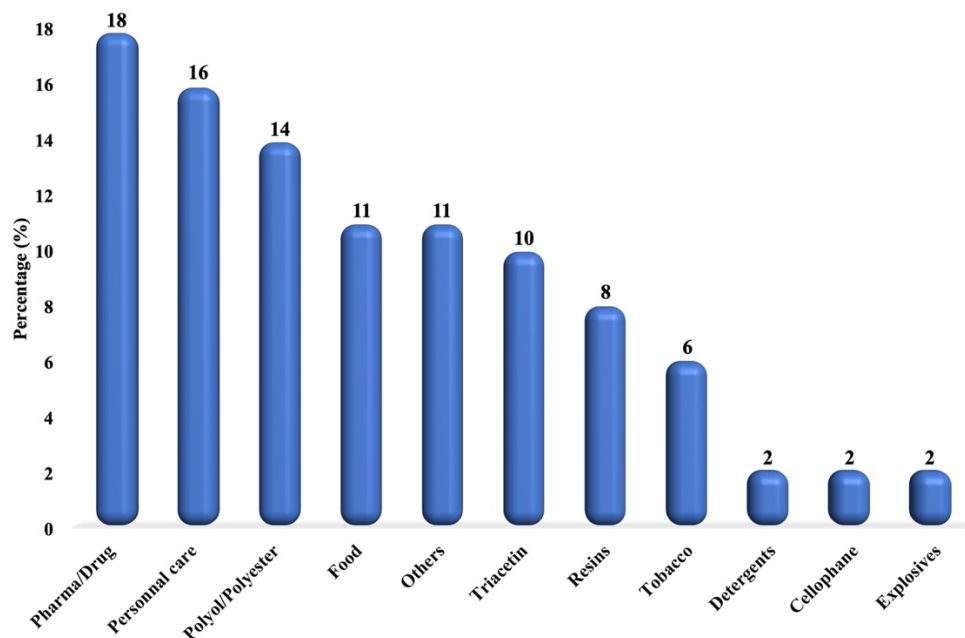


Figure 4. Distribution of glycerol consumption by sector/application [25].

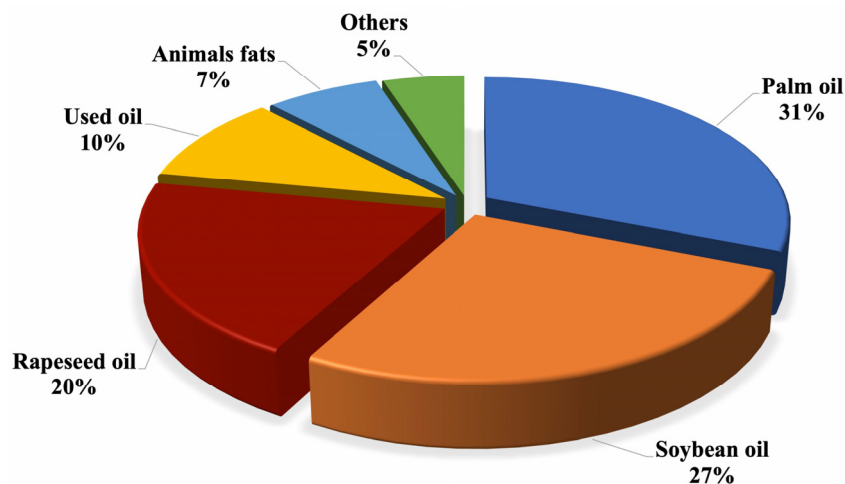


Figure 5. Feedstocks used for biodiesel production worldwide in 2017 [26].

The massive and increasing production of biodiesel has resulted in significant increases in glycerol levels. This dominance of glycerol production by the biodiesel industry, more than three-quarters of production, has had a significant impact on the glycerol surplus [28]. Since 2005, the biodiesel market has taken over the production of glycerol: the production of crude glycerol was more than 750 thousand tons, whereas in 2010, the production of crude glycerol increased to more than 1.2 million tons [29]. This year, crude glycerol was predicted to exceed 5 million tons [30,31]. This surplus production of glycerol led to a decrease in crude glycerol prices. In fact, between 2005 and 2010, the price fell from nearly USD 200 per ton to less than USD 70 per ton. Today, the price seems to vary around USD 100 per ton. At these low prices, the demand for glycerol is increasing considerably, so the glycerol market is expected to recover and grow in the coming years. Moreover, with

high-purity crude glycerol and the use of this many new fields, the future of glycerol is very promising [32].

1.4. Advantages and Disadvantages

The benefits of biodiesel production from waste oils are numerous [33]. From an environmental point of view, it is derived from renewable resources, especially waste oils. The use of biodiesel instead of diesel produces 78% less greenhouse gases and less particulate matter in the ambient air, thereby reducing the toxicity of air, which has a positive effect on health. These properties make it non-toxic, biodegradable and free of sulfur and aromatic content. Further, it is very safe for transport and does not require engine modifications to be used. Biodiesel is more advantageous compared to diesel since it does not involve drilling, transporting or refining steps, meaning that biodiesel can be locally produced. However, some of the drawbacks of biodiesel are significant enough to be exposed. It is less stable than diesel and may be more susceptible to oxidation, causing corrosion of the fuel tank and other vehicle components. Biodiesel has a high degree of oxygen, producing a higher amount of NO_x, and is not resistant to low temperatures. In addition, the use of biodiesel results in lower engine power, while its cost is higher.

2. Enzymatic Production of Biodiesel from Waste Oils

2.1. Enzymatic Transformation

In this section, the work performed on the biocatalytic transformation of waste oils will be highlighted (Table 3). Only some of the most recent works will briefly be described to compare results, but all works will be resumed in Table 3. Two categories will be exhibited: biocatalysts used in the free state and biocatalysts immobilized on a support. Among enzyme biocatalysts, lipases are known to be very effective to catalyze reactions such as esterification, hydrolysis, transesterification and aminolysis [34]. They have excellent catalytic activity and stability in non-aqueous media and their regioselectivity and enantioselectivity can be used for many applications in organic synthesis. The catalytic site of lipases is composed of three amino acid residues, Serine (Ser), Histidine (His) and Aspartate (Asp), which adopt a very precise spatial geometry to form the catalytic triad. Although lipases have very different specificities, they all act through a mechanism involving the catalytic triad. In recent years, there has been great interest in the use of lipases to produce biodiesel. During the transesterification reaction, the lipase mechanism can be described according to Figure 6 [35]. The first step of the reaction is to stabilize the serine alcohol through the formation of an oxyanion, which then attacks the carbonyl group of the substrate, creating intermediate 1. Then, the electrons making up the oxyanion are directed to the carbon of the carbonyl group and the proton of the histidine is transferred to the diglyceride, which is then released. The formed serine ester is reacted with alcohol to complete the transesterification. Histidine nitrogen removes hydrogen from the alcohol forming the alkyl oxide anion. The hydroxide group attacks the carbon of the carbonyl group, the intermediate oxyanion is stabilized by hydrogen bonds (intermediate 2), the electrons are repelled towards the carbon of the carbonyl group and the free fatty acid is formed. The oxygen atom of serine then takes over the hydrogen atom located on the histidine to re-establish the network of hydrogen bonds. Aspartic acid is used to draw a positive charge from histidine until it is fully positive.

A major advantage of using enzymes as catalyst is that other compounds in waste oils, such as water, free fatty acids or other solid impurities, do not influence the catalytic process compared to alkaline or acid catalysts [36]. Enzymes allow the conversion of free fatty acids into biodiesel, water in small amounts does not have an inhibition effect on the enzyme and other solid impurities can be easily removed, e.g., by filtration or centrifugation.

Among recent works on free enzyme, Chang et al. [37] reached an 97% yield in esters using Eversa Transform 2.0 lipase from a low-quality feedstock with high free fatty acids content. The reaction took place at 40 °C for 24 h with a methanol to oil ratio of 4:1, 2 wt% of water and 0.2 wt% of enzyme. Using the same lipase, Eversa Transform 2.0,

Wancura et al. [38] obtained 96.2% of esters yield in almost identical conditions: reaction temperature of 40 °C, 0.7 wt% of lipase, 4.0 wt% of water, molar ratio methanol to oil of 6.3:1 for 8 h of reaction time. Despite the increase in the lipase concentration, water and methanol, the difference in the reaction time could be explained by the quality of waste oil, which is better here. Najjar et al. [39] used lipases from *Kocuria flava* to obtain an ester yield of 83.08% under the following reaction conditions: 60 °C, 5 h, 1 mL of enzyme and molar ratio methanol to oil of 2:1. More work is presented in Table 3 [40–51].

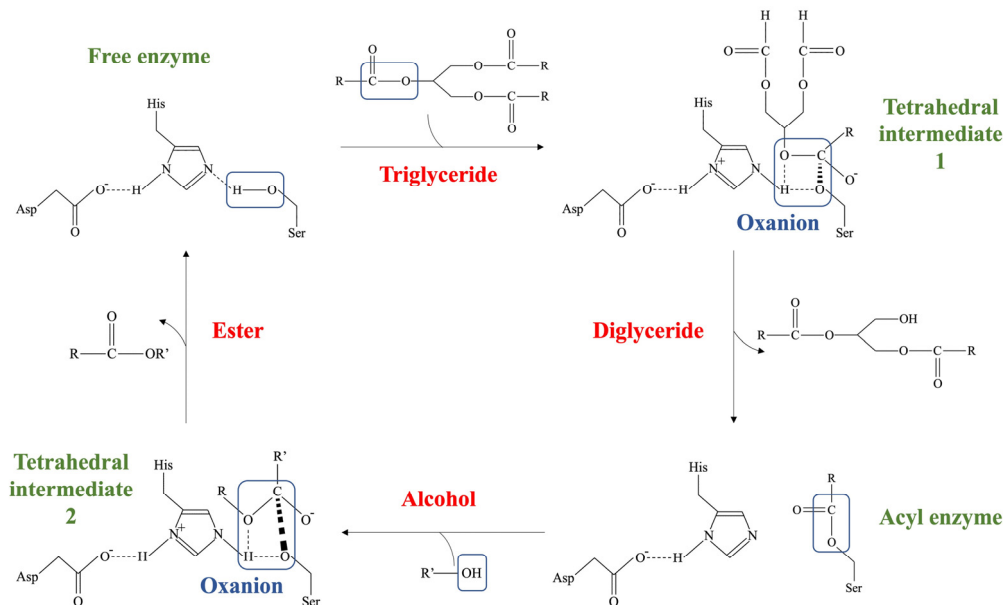


Figure 6. Mechanism of lipase in the transesterification reaction [35].

These few recent publications show the efficiency of biodiesel production from waste oils using free enzymes as a catalyst. The second part summarizes the works performed with immobilized enzymes. These have advantages over free enzymes, including repetitive reuse, simpler recycling and better stabilization of enzyme. However, immobilization may affect enzyme properties such as pH dependance and thermostability; all properties depend on the immobilization type and the carrier [52]. In addition, the use of carrier brings disadvantages such as a higher price compared to free enzymes, damage during stirring and inhibition caused by glycerol adsorption. These different parameters make the choice between free and immobilized enzymes complex. Enzymes can be immobilized in three different ways: binding to a support via covalent bonds, electrostatic interactions or physisorption; encapsulation; or cross-linking [53]. In terms of carriers, the most common examples are synthetic resins, biopolymers such as polysaccharides and inorganic solids such as mesoporous silicas and zeolites [54]. López-Fernández et al. [55] immobilized the lipase from *Rhizopus oryzae* on Purolite and obtained an ester yield of 100% within 16 h of reaction. The point, as in most of the work on immobilized enzymes, was to study the reuse of the biocatalyst. Here, there were up to five cycles. The reaction was performed at 30 °C with 0.2 wt% of catalyst and methanol to oil ratio of 2:1. Abdul Manab et al. [56] used commercial immobilized *Thermomyces lanuginose* lipase to reach an ester yield of 69.3% under the following conditions: 35 °C, 3:1 methanol:oil ratio, 0.5 wt% of enzyme and 3 h of reaction time. Guimarães et al. [57] immobilized Eversa Transform 2.0 lipase via the CLEAs method (cross-linked enzyme aggregates). A 90% ester yield was obtained over 72 h of reaction time at 40 °C, with a molar alcohol to oil ratio of 6:1 and 2.13 U/g of oil content. More work is presented in Table 3 [58–81].

These many different studies show that it is very important to consider each parameter of the reaction, because each one may influence the lipase activity. Indeed, water, glycerol or other impurities may have a positive or negative effect depending on the lipase used, but

methanol always has an inhibitory effect on the enzyme. Most of the works involve the use of a carrier, although the use of free enzymes has many advantages, such as low cost, non-damage of carrier during stirring and non-inhibition by the adsorption of glycerol on the carrier. Improved stability and repetitive reuse brought by the carrier mean that research is focused on supported biocatalysts. In addition, the diversity of organic, inorganic or hybrid materials and the possibility of developing innovative carriers gives researchers a wide range of possibilities in terms of the design of supported biocatalysts. To conclude, free and immobilized lipases both have advantages and disadvantages. It is therefore necessary to determine the most important reaction parameters indicating the form of the used lipase.

Table 3. Work on the production of biodiesel from waste oils by enzymatic transesterification.

Pretreatment	Lipase	Acyl Acceptor	Support	Time (h)	Temp. (°C)	Enzyme Content	Water Content	Yield (%)	Ref.
<i>Free enzymes</i>									
None	Eversa Transform 2.0	MeOH (4:1 MeOH:oil)	-	24	40	0.2 wt%	2 wt%	97	[37]
Filtration	Eversa Transform 2.0	MeOH (6.3:1 MeOH:oil)	-	8	40	0.7 wt%	4 wt%	96.2	[38]
None	<i>Kocuria flava</i>	MeOH (2:1 MeOH:oil)	-	5	60	1 mL	None	83.08	[39]
None	<i>Thermomyces lanuginosus</i>	MeOH (2eq.; 6 shots)	-	12	37	0.2 wt%	3 wt%	96	[40]
None	<i>Thermomyces lanuginosus</i>	MeOH (3:1 MeOH:oil)	-	24	30	0.3 wt%	2.5:1 H ₂ O:oil	89.04	[41]
None	<i>Rhizopus stolonifer</i>	MeOH (3:1 MeOH:oil)	-	48	30	10 wt%	10 wt%	92.3	[42]
None	<i>Aspergillus tamarii</i>	MeOH (3:1 MeOH:oil)	-	48	30	10 wt%	10 wt%	92.3	[42]
None	Eversa Transform	MeOH (1.5 eq.)	-	24	45	0.3 wt%	1.5 wt%	94	[43]
None	<i>Candida antarctica</i>	EtOH (5:1 EtOH:oil)	-	3	30	15 wt%	None	89.95	[44]
None	<i>Rhizomucor miehi</i>	EtOH (5:1 EtOH:oil)	-	3	30	15 wt%	None	89.95	[44]
Degumming	<i>Callera Trans L</i>	MeOH (1.5 eq.)	-	24	35	1 wt%	3.5 wt%	>95	[45]
None	NS81006	MeOH (4.4:1 MeOH:oil)	-	8	55	1.5 mL	10 wt%	≈80	[46]
None	Eversa	MeOH (1.5 eq.)	-	16	35	1 wt%	2.5 wt%	94.89	[47]
None	<i>Thermomyces lanuginosus</i>	EtOH (2 eq.)	-	48	30	20 g	1.5 wt%	97.6	[48]
None	<i>Pseudozyma antarctica</i>	EtOH (2 eq.)	-	48	30	20 g	1.5 wt%	97.6	[48]
Heating	<i>Pseudomonas fluorescens</i>	MeOH (3:1 MeOH:oil)	-	24	45	5 wt%	None	55.6	[49]
None	<i>Streptomyces</i> sp.	MeOH (1:1 MeOH:oil)	-	48	40	0.2 mL	None	>80	[50]
None	<i>Oreochromis niloticus</i>	MeOH (4:1 MeOH:oil)	-	28	45	3 kU	3 wt%	96.5	[51]
<i>Immobilized enzymes</i>									
None	<i>Rhizopus oryzae</i>	MeOH (2:1 MeOH:oil)	Purolite D6308	16	30	0.2 wt%	None	100	[55]
None	<i>Thermomyces lanuginosus</i>	MeOH (3:1 MeOH:oil)	N.I.	3	35	0.5 wt%	None	69.3	[56]
None	Eversa Transform 2.0	Isoamyl alcohol (6:1 Alcohol:oil)	CLEA	72	40	2.13 U/g oil	None	90	[57]
None	<i>Thermomyces lanuginosus</i>	MeOH (4:1 MeOH:oil)	Hydrotalcite	105	45	4 wt%	None	92.8	[58]
None	<i>Candida antarctica</i>	MeOH (3:1 MeOH:oil)	Resin	12	40	4 wt%	None	88	[59]
None	<i>Candida antarctica</i>	MeOH (25:1 MeOH:oil)	Resin	4	50	10 wt%	None	89.1	[60]
None	<i>Thermomyces lanuginosus</i>	MeOH (6:1 MeOH:oil)	MPPM	24	65	1 g	15 wt%	90.2	[61]
Discoloration	<i>Penicillium expansum</i>	MeOH (1 eq.)	Resin	7	35	168 U	None	92.8	[62]
Filtration/	<i>Aspergillus niger</i>	MeOH (1 eq.)	Whole-cell	72	30	40 pcs	10 wt%	91.8	[63]
Acidification	<i>Rhizopus oryzae</i>	MeOH (3:1 MeOH:oil)	Whole-cell	24	30	10 wt%	None	94	[64]
Activated charcoal	<i>Rhizopus oryzae</i>	MeOH (3:1 MeOH:oil)	Whole-cell	24	30	10 wt%	None	94	[64]

Table 3. Cont.

Pretreatment	Lipase	Acyl Acceptor	Support	Time (h)	Temp. (°C)	Enzyme Content	Water Content	Yield (%)	Ref.
None	<i>Escherichia coli</i> (<i>Candida antarctica</i> / <i>Thermomyces lanuginosus</i>)	MeOH (1:1 MeOH:oil)	Whole-cell	48	30	4 wt%	None	95	[65]
None	<i>Pichia pastoris</i> (<i>Thermomyces lanuginosus</i>)	MeOH (4:1 MeOH:oil)	Whole-cell	84	40	12 wt%	None	82	[66]
None	<i>Candida antarctica</i>	MeOH (0.5 eq.)	Resin	10	30	N.I.	None	93.4	[67]
None	<i>Talaromyces thermophilus</i>	MeOH (6:1 MeOH:oil)	Chitosan	5	50	25 wt%	None	98	[68]
Heating	<i>Candida antarctica</i>	Dimethyl carbonate (6:1 DMC:oil)	Resin	4	60	10 wt%	None	86.61	[69]
None	<i>Candida antarctica</i>	MeOH (6.2:1 MeOH:oil)	Resin	8	50	1 wt%	None	90	[70]
Extraction	<i>Rhizomucor miehei</i>	MeOH (6:1 MeOH:oil)	Resin	N.I.	40	16 wt%	None	96.7	[71]
None	<i>Burkholderia cepacia</i>	MeOH (6:1 MeOH:oil)	SPION-silica	35	35	25 wt%	10 wt%	91	[72]
None	<i>Rhizomucor miehei</i>	MeOH (4:1 MeOH:oil)	MWCNTs-PAMAM	30	50	6 wt%	8 wt%	94	[73]
None	<i>Candida antarctica</i>	MeOH (14%)	Lewatit resin	6	40	N.I.	None	78	[74]
None	<i>Thermomyces lanuginosus</i>	MeOH (3:1 MeOH:oil)	styrene/methacrylate	6	30	10 wt%	1 wt%	79	[75]
None	<i>Candida antarctica</i>	MeOH (3:1 MeOH:oil)	Fe ₃ O ₄ @SiO ₂	96	50	4.5 wt%	None	≈100	[76]
None	<i>Bacillus licheniformis</i>	MeOH (3:1 MeOH:oil)	mCLEAs-lip	36	35	0.3 wt%	None	71	[77]
None	<i>Candida antarctica</i>	EtOH (36:1 EtOH:oil)	Resin	8	35	50 wt%	None	82.91	[78]
Filtration	<i>Aspergillus terreus</i>	MeOH (6:1 MeOH:oil)	Fe ₃ O ₄ _PDA	30	37	10 wt%	0.6 wt%	92	[79]
Filtration	<i>Pseudomonas fluorescens</i>	EtOH (4:1 MeOH:oil)	Na-SBA-15	48	37	N.I.	None	91.4	[80]
Extraction/ Filtration/ Drying	<i>Thermomyces lanuginosus</i>	MeOH (3:1 MeOH:oil)	octadecyl/methacrylate	24	35	10 wt%	1 wt%	75.3	[81]
None	<i>Pseudomonas fluorescens</i>	EtOH (1.5:1 EtOH:oil)	styrene-divinylbenzene	1.3	40	15 wt%	None	94.1	[13]

N.I.: Not Indicated.

2.2. Nature of Biodiesel by-Products

The nature and quantity of impurities are related to the catalysis used and the oils used. Typically, the impurities of biodiesel are free fatty acids, water, alcohol, very often methanol, glycerides, catalyst, soap in the case of basic catalyst, and the by-product, glycerol [82], which accounts for about 10% of production [83]. In the case of basic catalysis, the intensive production of soaps from the free fatty acids present in the oil (Figure 7) will lead to decrease the biodiesel yield and affect the quality of the end product [84]. This phenomenon is more noticeable as the waste oil will contain a higher amount of free fatty acids. Moreover, the recovery of biodiesel is more complicated. It has been concluded by several studies that the level of free fatty acids should be less than 1%; see 2%. Ma et al. [85] indicated that the content of free fatty acids should be below 0.5% to obtain the best conversion. Ramadhas et al. [86] indicated that the yield of esterification process decreases if the value of free fatty acids is greater than 2%. The importance of the free fatty acids level is due to generation of more water in the esterification reaction; then, water will hydrolyze the esters produced from transesterification [87].



Figure 7. Saponification of free fatty acids by soda [84].

2.3. Characterization of Biodiesel

A biodiesel must be characterized with certain physicochemical properties [88]. Biodiesel from waste oils is compared to diesel in Table 4. For viscosity measurements, the ASTM D445-19a [89] method is applied; the kinematic viscosity of biodiesel is around 4.06 mm²/s while diesel is about 2.42 mm²/s. The cloud and pour points are measured using ASTM D2500-17a [90] and ASTM D97-17b [91], respectively. The cloud point for biodiesel is 6 °C while the temperature for diesel is −5 °C, while the pour point is 5 °C for biodiesel and −6 °C for diesel. Higher temperatures of biodiesel for the cloud and pour point may be an issue, while diesel can lose its flow or crystal with low weather temperatures. To prevent those issues, the addition of additives or other fuels may be solutions [92]. The density of diesel is 0.857 g/cm³, while the density of biodiesel will 0.889 g/cm³ and the applied measurement method is ASTM D4052-18a [93]. The flash point is measured using the ASTM D7215-16 [94], 78 °C for diesel and it can rise to 153 °C for biodiesel. Finally, a very important characteristic is the cetane number, measured by the method ASTM D6890-18 [95]. Its value is around 53 for diesel and 59 for biodiesel. These values depend on the composition of the initial oil [96].

Biodiesel properties, like those of diesel, are important for engines. Indeed, in addition of the renewable energy of biodiesel, the behavior of biodiesel on engine and produced gas emissions must be improved. As mentioned in the introduction, biodiesel produces less greenhouse gas. For engines, different parameters must be studied—such as the tribological parameters, meaning the friction and wear characteristics. Jason et al. [97] made research to improve those properties and showed that the addition of additives such as graphene forms a protective film that prevents the interacting surfaces from rubbing, resulting in friction and wear reduction.

Table 4. Physical properties of biodiesel compared to diesel.

Physical Properties	Biodiesel	Diesel
Kinematic viscosity (40 °C, mm ² /s)	4.06	2.42
Cloud point (°C)	6	−5
Pour point (°C)	5	−6
Density (g/cm ^{−3})	0.889	0.857
Flash point (°C)	153	78
Cetane number	59	53

The European standards (Table 5) have defined properties that give biodiesel a guarantee of quality. One of these standardized qualities specifies the amount of esters to be achieved, the level of mono-, di- and triglycerides not to be exceeded and the total amount of glycerol [98].

Table 5. Properties of biodiesel according to the European standards.

Properties	Standard	Limits
Ester content	EN 14105	96.5% (min)
Monoglyceride levels	EN 14105	0.80% (max)
Diglyceride levels	EN 14105	0.20% (max)
Triglyceride levels	EN 14105	0.20% (max)
Total glycerol content	EN 14105	0.25% (max)

2.4. Separation and Purification of Biodiesel from Its By-Products

As indicated in Section 2.2, the composition of biodiesel will depend on the source used as well as the type of catalysis, so the separation and purification of biodiesel will also depend on the same. First of all, the by-product glycerol could be separated simply by decanting or centrifuging due to the difference in density between the biodiesel (≈ 0.850 g/cm³) and the obtained glycerol (>1.0 g/cm³). The ability of biodiesel to be more

easily separated or not will depend on the type of catalysis used. The use of heterogeneous catalysts (chemicals and immobilized enzymes) eliminates the neutralization and washing steps applied during the separation of biodiesel synthesized by homogeneous catalysis. The alcohol:oil ratio is an important parameter which should not exceed 6:1, as shown by Sharma et al. [99]. Increasing the ratio above 6:1 will not increase the ester yield. A high water presence will lead to the hydrolysis of triglycerides and produce free fatty acids. Finally, as previously mentioned, excessive soap production in the presence of alkaline catalysis complicates the separation of biodiesel.

The purification of biodiesel takes place after the glycerol separation. It can consist of four steps depending on the purity degree and the alcohol recycling [100]. In the first step, the alcohol excess can be recovered using simple distillation. The second stage involves washing the biodiesel with water either by depositing it on the biodiesel, or by bubbling or stirring to improve the biodiesel quality. Subsequently, the biodiesel is dried either by heating to 110 °C or by chemical compounds such as anhydrous sodium sulfate to remove all traces of water. The heating method also helps to remove the remaining alcohol residues. Finally, for a biodiesel of a quality respecting the previously mentioned European standards, distillation is applied.

3. Purification of Biodiesel Glycerol from Waste Oils

This part only considers the glycerol produced during the biodiesel synthesis and how it can be used as such or transformed in high value-added products. To facilitate the glycerol purification, it is important to know the composition of glycerol from the biodiesel obtained from waste oils and how these oils and the type of biodiesel synthesis influence the glycerol composition. First, different glycerol impurities were studied based on the oil origin and the used catalysts. Thus, the glycerol impurities are detailed to show their influence on the glycerol or on its transformation in other products. Different glycerol characterization techniques were used to determine the purity level and properties of glycerol. The main interest of this part is to describe different methods to purify glycerol and to understand its composition and its utilization.

3.1. Purity of Glycerol and Influence of Its Impurities

The composition of crude glycerol will depend on the feedstock used for biodiesel production, as well as the type of catalysis applied in the conversion reaction. Four different compositions will be compared (Figure 8), according to the glycerol obtained from the enzymatic or basic transformation of virgin oil or waste oil. The values of the graphs are indicative; the glycerol composition may vary according to the oil composition and the applied reaction conditions. Acid catalysis is not presented here because it is very rarely used; moreover, the glycerol composition is close to enzymatic catalysis. By comparing enzymatic catalysis to basic catalysis [101], a higher degree of glycerol purity will be obtained using enzymes, because the free fatty acids will be transformed into biodiesel, whereas in the case of basic catalysis, they will be transformed into soap, which is found with the glycerol.

In addition, the recovery of enzyme catalyst is much easier in comparison with basic catalyst because a certain amount of basic catalyst could be found with glycerol. For the oil type, the waste oil will generate a higher number of impurities compared to edible vegetable oil. In fact, among the impurities, ashes from food cooking are included in the glycerol composition; these can be up to 2%. The amount of water also increases in the waste oil because of cooking. Finally, the number of free fatty acids may be increased in the waste oil due to the presence of water and high-temperature cooking. This increase leads to an increase in the soaps found with the glycerol in the presence of basic catalyst. On balance, enzymatic catalysis is more advantageous due to higher degree of glycerol purity, which makes its use, or even its purification, simpler and cheaper.

Some impurities present with glycerol may be problematic during glycerol conversion, while others may be beneficial. Focusing on the glycerol bioconversion, many research

papers reported that the presence of methanol with glycerol has an inhibitory effect on some of the microorganisms used [102]. Depending on the aim of glycerol use, Sarma et al. [103] showed that methanol had a negative effect on hydrogen production or with the production of lipids, as exposed by Yang et al. [104]. Uprety et al. [105] observed that soap and fatty acid methyl esters had a positive effect by demonstrating the role of carbon source on the production of lipids from glycerol.

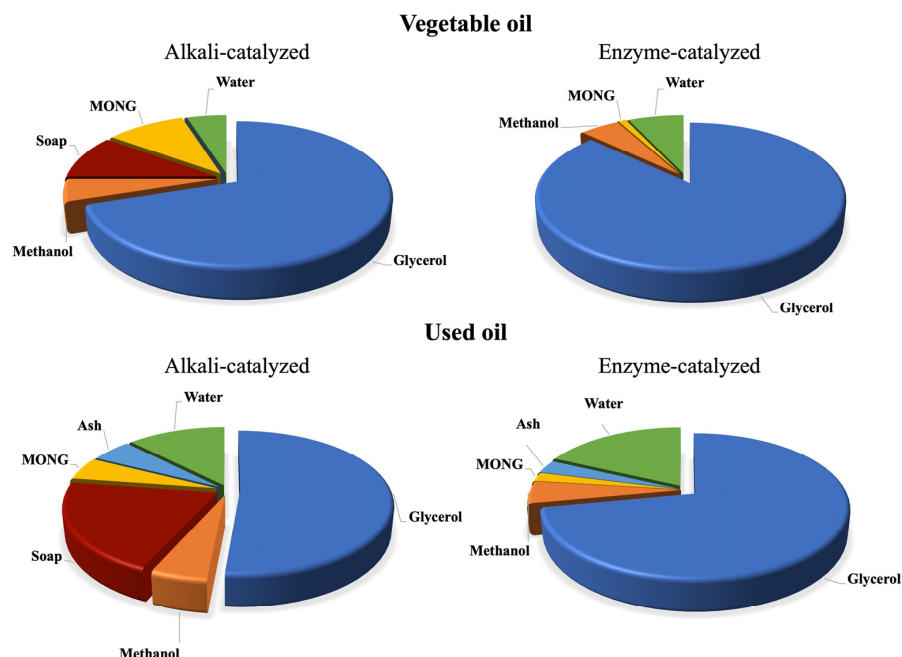


Figure 8. Composition of glycerol according to different feedstocks and different types of catalysis.

3.2. Glycerol Characterization Techniques

3.2.1. Properties of Glycerol

Most methods for characterizing glycerol are standardized [106], the physical properties of glycerol involve density measurement by ASTM D50002 [107] and viscosity measurement by ASTM D445-19a [89] (Figure 9). Crude glycerol derived from waste oil will have a density of about 1.0–1.1 g/cm³ [108] compared to 1.26 g/cm³ for commercial glycerol. The viscosity is about 270 cP for pure glycerol compared to about 50 cP for the glycerol obtained by Hunsom and Autthanit [109]. In fact, impurities such as fatty acids, solvent and water decrease density as well as viscosity. To measure the acidity, the ASTM D1093-98 [110] method is used, while the calorific value is measured by the ASTM D0240-19 [111]. The pH and color measurements are simply made using pH-metry and a UV/Vis spectrophotometer.

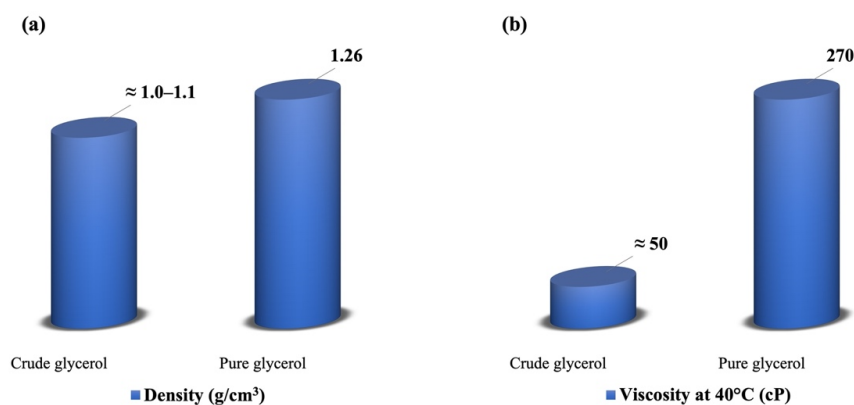


Figure 9. Density of crude glycerol compared to commercial glycerol (a). Viscosity of crude glycerol compared to commercial glycerol (b).

3.2.2. Purity of Glycerol

The measurement of glycerol purity is carried out by the Pharmacopeia standard method (USP 26), which also serves as a reference when glycerol is used in fields such as pharmacology or food. The glycerol purity must be greater than 99.7%. For technical grade, the glycerol purity must be higher than 98%. This is the most used glycerol for all applications except in pharmacology, personal care and food (Figure 10). Glycerol purity can also be measured using simple analytical techniques such as liquid or gas chromatography or NMR spectroscopy.

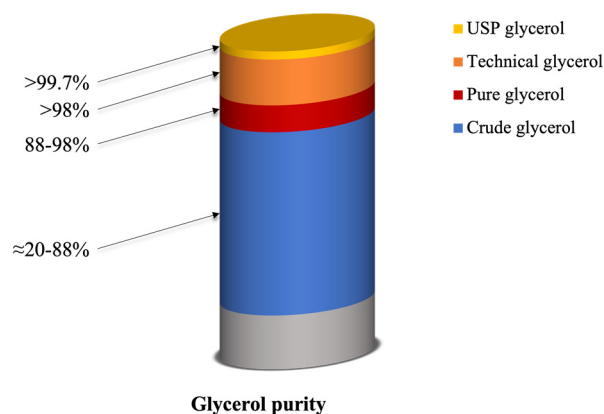


Figure 10. Qualifying label for glycerol according to its purity grade.

3.2.3. Impurities Measurement

Among the various impurities, characterization techniques are also standardized. Firstly, for the measurement of residual ashes that may be present during the cooking of the oil, the ASTM D0482-03 [112] method is used, mold is also part of the residue from waste oil, therefore the ASTM D4377-00e01 [113] method is used. The amount of solvent can be measured by chromatographic methods, while the amount of water is measured by Karl Fischer titration according to the ASTM D4017-02 [114] method. The amount of MONG (Non-Glycerol Organic Matter), which soaps often include, is measured by a simple Equation (1) [115].

$$\text{MONG} = 100 - (\% \text{ Glycerol content} + \% \text{ ash content} + \% \text{ water content}) \quad (1)$$

3.3. Glycerol Purification Methods

This section will present the glycerol purification techniques currently used. Figure 11 brings together these techniques and shows how these purification methods can be applied, and which paths are conductive. This diagram shows that these purification pathways usually involve a beginning, i.e., acidification, and an end, adsorption on activated charcoal. Although each of these techniques can be used independently, it can be seen that it is almost essential to begin and end with acidification and adsorption on activated carbon, respectively. The purification methods may also be applied or not and alone or in combination.

3.3.1. Acidification/Neutralization

This purification method is the most used for crude glycerol. It is applied mainly for the pretreatment of glycerol and is often followed by a second purification method. The process involves catalyst removal (basic catalyst) and soap [116]. It is important to differentiate between acidification and neutralization, although both processes involve the use of a strong acid and lead to a very similar result. During acidification, the medium is brought to a pH of 1–2, separating the crude glycerol into three phases: the fatty acids at the top, the glycerol-rich phase at the middle and the organic salts at the bottom [117]. Neutralization, as its name suggests, is used to remove the basic catalyst involved in the

transesterification of oils, bringing the pH close to neutrality, and obtaining two phases, the organic salts from the catalyst and the phase rich in glycerol and some free fatty acids when soaps are present, sometimes leading to the formation of three phases. Whatever the process, acidification or neutralization, the use of a strong acid is required. In a study by Hájek et al. [118], glycerol was derived from the biodiesel produced from virgin vegetable oil. This study aimed to compare three different acids: sulfuric acid, hydrochloric acid and phosphoric acid. The results showed that phosphoric acid had the better efficiency, leading to about 86% purity of glycerol, with an initial amount of glycerol between 50 and 60%. Thus, most studies have used phosphoric acid for this purification process. The low pH obtained after acidification requires neutralization with KOH or NaOH. This purification method is very effective but is still insufficient, so the further treatment of crude glycerol is necessary. It is possible to use this process alone; for this, the crude glycerol must have a high initial purity, as is the case in the research of Gil et al. [119]. In this study, the glycerol obtained via transesterification of rapeseed oil resulted in a high purity of 95.5% glycerol. For purification, after evaporation of methanol and water, leading to a purity of 96.4%, the neutralization step was performed by adding hydrochloric acid to remove the catalyst and the formed soap. Purification resulted in a final purity of 97.1%. The same process was applied in the study reported by Velázquez-Hernández et al. [120]; the virgin vegetable oils used for the synthesis of glycerol allowed high purity. Thus, the glycerol composition reached 95% after sulfuric acid treatment (pH = 2) followed by KOH neutralization of the glycerol-rich phase.

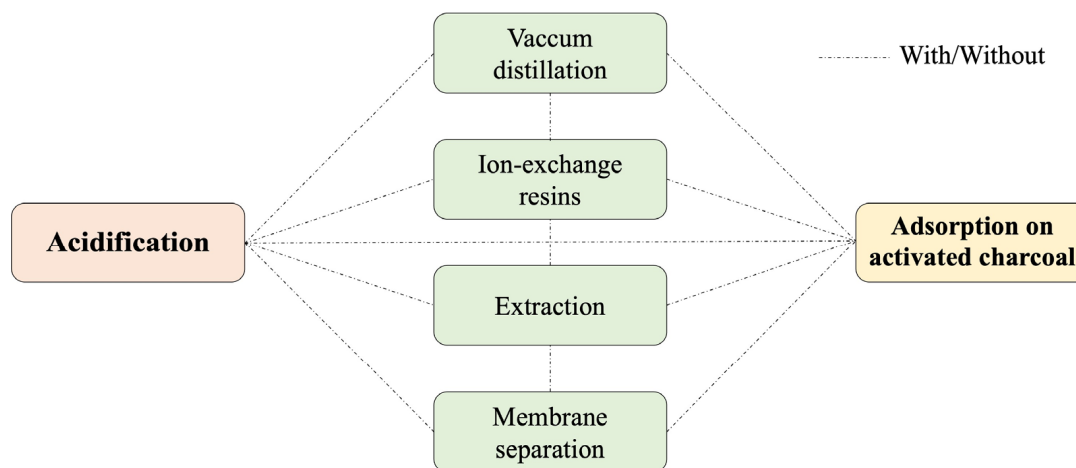


Figure 11. Glycerol purification methods and possible pathways between these methods.

3.3.2. Vacuum Distillation

Distillation is the most explored method. For many years, it was used alone to remove methanol, leaving glycerol with other impurities such as ashes, non-glycerol organic matter (MONG), water and soap; hence, there is a need for pretreatment [106]. The distillation efficiency depends on the temperature between 120 and 126 °C and the pressure between 4.0.10⁻¹ and 4.0.10⁻² mbar. This technique has largely proven itself but is still sensitive to unwanted reactions such as polymerization, dehydration, or oxidation. Posada et al. [121] pretreated crude glycerol by neutralization step with hydrochloric acid, hence the initial composition of glycerol was 60.05%. The combination with distillation resulted in a purity of 98%. Evaporation steps for methanol and water were applied during the purification process. The work of Skrzyńska et al. [122] is based on four-step purification process: catalyst neutralization, methanol distillation, water evaporation and glycerol distillation, allowing a purity of 98.2% compared to an initial purity of 40.3%. Each step attests to its effectiveness in terms of the quantities obtained for each step (neutralization: 47.4%, methanol distillation: 58.2%, evaporation: 92.8%, distillation: 98.2%). The obtained purity depends on the initial composition of crude glycerol. Remón et al. [123] have chosen

to neutralize glycerol with a purity of 63.17% with acetic acid for an optimum pH of 6. Once the second distillation stage was completed, a maximum purity of 85.25% was obtained. This study showed the influence of pH during the neutralization step for optimal purification, with pH values of 5, 6 and 7 being tested. Another work [124] reported glycerol synthesized through biodiesel production from waste frying oils. This study involved pretreatment during the purification process of glycerol, which has an initial purity of 15.4%. Thus, the crude glycerol was acidified by means of phosphoric acid to reach a pH of 2. In this case, two phases were obtained: the phase rich in free fatty acids above, and the glycerol-rich phase. The glycerol-rich phase with a pH of 2 was then neutralized with KOH. The second stage, distillation, resulted in a purity of 75.1%. Concretely, for glycerol with initial purity less than 50%, it becomes necessary to accumulate several purification processes.

3.3.3. Ion-Exchange Resins

More recently, the use of ion-exchange resins has shown great interest because, compared to the conventional distillation process, a better quality of purified glycerol up to a level of 99% is obtained thanks to this technique [125]. This process involves passing the crude glycerol through one or two chromatographic columns in which are placed either a cationic resin and anionic resin or a mixed resin (cationic and anionic). This process can be performed with or without pretreatment, as compared by Nasir et al. [126]. Firstly, a single column was loaded with a cationic resin, Amberlite IRN-78, with an anionic resin, Amberlite 200 C, silica beads were added to remove any mold that might be present. The pretreatment process includes the neutralization of glycerol by phosphoric acid followed by filtration. Subsequently, glycerol is placed in the chromatographic column, followed by methanol evaporation without pretreatment. The results showed a direct influence of glycerol composition depending on the type of reaction for biodiesel and the used source. Thus, both methods yielded a purity of up to 99.58%. Following a similar protocol, Isahak et al. [127] pretreated crude glycerol, with a purity of 77.4%, through a neutralization step involving phosphoric acid, followed by filtration, then the pretreated glycerol was purified by two resins, Amberlite IRN-78 and Amberlite 200C. A high purity of glycerol was obtained, reaching 99.4%. A disadvantage of using resins is that they need to be regenerated after use. In this study, Amberlite IRN-78 was regenerated by washing under a stream of dilute sodium hydroxide, while Amberlite 200C was regenerated from dilute sodium chloride. The work of Abdul Raman et al. [128] is based on the use of a highly cationic resin only, Amberlite 15, to purify glycerol preceded by phosphoric acidification pretreatment. The pretreatment purified glycerol from 35.60 to 77.42%, while the resin purified glycerol up to 98.20%. At present, the application of resins for glycerol purification is the most effective method, resulting in very high purity. Recently, Lopes et al. [129] developed a new glycerol pretreatment method based on the use of a cationic tannin with flocculation/coagulation capacity to reduce the amount of chloride and metals. This method therefore replaces the conventional pretreatment, acidification/neutralization. The authors used a glycerol synthesized from waste oils, the purity of which was determined to be 59.3%. This new pretreatment or pre-purification process significantly increased the purity to 77.3%. Subsequently, the application of cationic resin, Amberlite IRA120 Na, followed by anionic resin, Amberlite IRA410 Cl, increased the purity to 94.5%.

3.3.4. Adsorption on Activated Charcoal

Adsorption on activated charcoal is a simple method of purification. This process is mainly aimed at attenuating color and adsorbing small molecules such as lauric and myristic acids [130]. However, the use of activated charcoal alone remains insufficient to achieve high glycerol purity. Hunsom and Autthanit worked on the glycerol obtained from waste oils [109], initially pretreated with phosphoric acid to recover the glycerol-rich phase later neutralized by NaOH. This study was to purify glycerol with a sludge-derived activated charcoal prepared by activating different chemical agents such as H_3PO_4 ,

K_2CO_3 and KOH. KOH-impregnated activated charcoal carbonized at 800 °C (KOH-800AC) resulted in a glycerol purity of 93.0%.

3.3.5. Extraction

Extraction is a physical process combined with a chemical process, acidification. As has been shown previously, acidification will provide two or three phases. The phase of free fatty acids can thus be eliminated by decantation or extraction [131]. The work of Kongjao et al. [132] is based on the combination of chemical and physical treatment of glycerol of waste oils (27–30% (*w/w*)), whereas in this case the extraction step was performed using a solvent on the glycerol-rich phase. Firstly, acidification by sulfuric acid resulted in three phases: the upper phase rich in free fatty acids, which was decanted, and the middle phase rich in glycerol in the medium. The lower phase was rich in organic salts. After neutralization with NaOH, the glycerol-rich phase was extracted with ethanol to encourage salt precipitation, followed by further filtration leading to an acceptable purity of 93.34%. Demaman Oro et al. [133] reported a study on the extraction of glycerol using different solvents (methanol, ethanol, chloroform and dichloromethane). This study showed that methanol was the most effective solvent for the extraction of organic phase with excellent quality and yield of salts. Xiao et al. [134] used two solvents in series for glycerol extraction. Initially, the saponification phenomenon was amplified by adding NaOH to reach a pH of 11, which was then acidified with hydrochloric acid to bring the pH down to 1, followed by extraction with petroleum ether and then extraction with anhydrous ethanol. These different purification stages are separated by stages of filtration and evaporation of impurities. The purity of obtained glycerol increased from 74.5% to 95.6%.

3.3.6. Membrane Separation

Membranes are used for processes such as reverse osmosis, ultrafiltration, micro-filtration, gas separation, dialysis, pervaporation, electrolysis and electrodialysis [135]. Currently, they are widely used for water purification or gas or protein separation [136]. This separation process is already used for biodiesel purification. The use of membranes generates lower costs and lower energy consumption [137]. Nevertheless, the use of membranes has a big issue: their stability. This is mainly due to the addition of aggressive organic solvents. Thus, this is an interesting research topic to work on to improve the stability of membranes in particular on the chemical structure of membranes [138]. The general strategy to improve the structural stability of traditional polymer membranes is crosslinking. In the case of glycerol purification, membranes remove salts, fatty acids and triglycerides. A patent filed by EET Corporation [139] exposes glycerol purification technology combining electrodialysis and nanofiltration. The HEEPMTM (High Electro-Pressure Membrane) system can operate in batch, semi-batch or continuous flow. This technology designed for industry allows 99.9% salt removal. The obtained glycerol approaches USP-grade purity. Indok Nurul Hasyimah et al. [140] looked at the retention efficiency of polymeric membranes on a glycerol rich in triglycerides. The membranes used, polyether sulfone (PES) and polyvinylidene fluoride (PVDF), have a molecular weight cut-off (MWCO) of 25,000 and 30,000 Da, respectively. The effects of membrane surface chemistry, the solution-pH on the permeation flux and the ability to retain triglycerides were investigated. PVDF membranes were shown to provide higher permeation fluxes and lower triglyceride release rates (81%) than PES membranes (91%). In the same context, Mah et al. [141] studied the ability of ultrafiltration to remove palm oil and oleic acid present in glycerol solutions. A GE PVDF (GE Osmonics) membrane with a molecular-weight cut-off value of 30 kDa was used, and the effects of different concentrations of palm oil, oleic acid and blends of palm oil and oleic acid were investigated, as well as the effects of pH on membrane flow and discharge capacity. This work demonstrated that ultrafiltration was able to remove palm oil, oleic acid and blends of palm oil and oleic acid from glycerol solutions at 87.00, 98.59 and 95.60%, respectively. Another patent filed by Jeromin et al. [142] also offers membrane ultrafiltration to remove unreacted oils

or fats from the hydrolysis reaction. The purification process takes place continuously during the hydrolysis reaction. Fats with a larger diameter of 100 μm are separated from the aqueous phase by a plate allowing phase separation and redirected to the hydrolysis process. Thus, the pre-purified aqueous phase passes through an ultrafiltration membrane and the concentrate returns to the inlet of the phase separation plate. Vadthya et al. [143] found a method for the glycerol purification by electrodialysis to separate the salts from the crude glycerol. Two ion exchange membranes, cationic (CMI-7000) and anionic (AMI-7001), were used in batch and this process revealed that electrodialysis could remove salts to a percentage greater than 95%. This study also showed that this process is economical, with an estimated membrane and energy cost of USD 0.09/ m^3 . Kalafatakis et al. [144] used the direct osmosis process to recover glycerol water from the Aquaporin InsideTM direct osmosis system to achieve water flows of 10.5 L/ m^2 /h. This approach allows the recovery of water without further purification and an increase in the glycerol concentration in solution. A second strategy is the use of hybrid membranes, which have shown an improvement in membrane performance. Combining the properties of organic and inorganic materials increased mechanical and thermal properties [145]. Hybrid materials could be mixtures of polymethyl methacrylate (PMMA)/ SiO_2 or cellulose/ SiO_2 [146]. For example, Shaari and Rahman [147] used a composite thin-film membrane combined with a hybrid membrane. The membranes consisted of polyvinyl alcohol, sulfone resin pellet, polyethylene glycol and tetraorthosilicate. They showed that the addition of glycerol as a membrane preparation additive improved the purification of crude glycerol, resulting in a NaCl rejection rate of 48.02%. Finally, another emerging technology in terms of membrane separation is the use of membrane distillation. Membrane distillation is a combination of fluid stream and thermally conductive membrane, which allows the vapors to pass through a hydrophobic membrane [148]. Shirazi et al. [149] studied the effectiveness of membrane distillation in removing water from different glycerol solutions with different concentrations. They used hydrophobic microporous membrane based on polytetrafluoroethylene (PTFE). For each test carried out, a rejection rate higher than 99% was obtained. Pal et al. [150] used a hydrophobic polyvinylidene fluoride (PVDF) membrane and achieved a glycerol rejection rate of 99.9%. One of the major problems with membranes is membrane fouling [151], which can be defined as “a process resulting in loss of membrane performance due to the deposition of suspended or dissolved substances on its external surfaces, at the pore opening, or within the pores”. To solve this problem, membrane cleaning using chemicals such as nitric acid, hydrochloric acid or hydrogen peroxide can be applied; however, this comes at additional cost and with accelerated deterioration of the membranes. This creates complications for industrial application.

3.3.7. Multi-Step Methods

The use of several successive processes may be applied where a very high purity of glycerol is sought, or where the initial purity of glycerol is low and requires more extensive processing, in particular for glycerol obtained from the transesterification of waste oils. A list of different studies using multi-step purification is presented in Table 6. As shown in Figure 11, most methods apply acidification and adsorption on activated charcoal to begin and finish the purification of glycerol. Manosak et al. [152] purified the glycerol with an initial purity of 36.7%, obtained by the biodiesel production from waste oils. Initially, the acidification step was performed using different acids at different pHs, as reported by Hájek et al. [118]. They found that phosphoric acid was the most effective at pH 2.5. In the second step, solvent extraction, three types of polar solvents were tested: methanol, ethanol and propanol. They concluded that propanol was the most suitable for extraction. The extraction efficiency depends on the polarity and solubility of the solvent. Hunsom et al. [153] showed that n-butanol was a better solvent than hexane because it is a polar solvent, and it has better solubility in water. These first two steps allowed us to achieve a purity of 95.74%. Finally, to reduce the glycerol color, it was adsorbed on activated charcoal, which also showed that some fatty acids were removed during this

step, resulting in a final purity of 96.2%. The process added between acidification and adsorption on activated charcoal evolves with time. Distillation used to be one of the most applied methods, but the use of this process decreases over time because it is expensive and energy-consuming. Recently, extraction and membrane filtration were revealed to be better methods. It is also important to consider multi-step methods for industrial scale, such as the study by Pitt et al. [154] which compared the conventional process on waste oil at laboratory and industrial scales. The purification process includes acidification with phosphoric acid, followed by distillation and adsorption on activated charcoal. At the laboratory level, the obtained glycerol had a purity of 51.88% to 78.72% after purification, while at the industrial level, the purity obtained was 29.99% to 60.6%. Glycerol obtained from the industrial process was found to be denser, and therefore had more impurities; mass losses were also higher, resulting in lower purity. The conclusion is that it is essential to think about a purification process suitable for the industrial scale.

Table 6. Purification of glycerol by multi-step methods according to different authors.

Type of Oil	Purification Techniques	Glycerol (%)	Purified Glycerol (%)	Ref.
Waste oil	<ul style="list-style-type: none"> • Acidification • Extraction • Adsorption on activated charcoal 	36.7	96.2	[152]
Waste oil	<ul style="list-style-type: none"> • Acidification • Extraction • Adsorption on activated charcoal 	29.8	99.0	[153]
Waste oil (lab. scale)	<ul style="list-style-type: none"> • Acidification • Distillation • Adsorption on activated charcoal 	51.88	78.72	[154]
Waste oil (ind. scale)	<ul style="list-style-type: none"> • Acidification • Distillation • Adsorption on activated charcoal 	29.99	60.6	[154]
Waste oil	<ul style="list-style-type: none"> • Saponification • Acidification • Adsorption on activated charcoal 	40.6	96.08	[155]
N.I.	<ul style="list-style-type: none"> • Acidification • Extraction • Adsorption on activated charcoal 	12.0	96	[156]
Waste oil	<ul style="list-style-type: none"> • Sequential extraction • Adsorption on activated charcoal 	74.0	99.2	[157]
Waste oil	<ul style="list-style-type: none"> • Neutralization • Distillation • Extraction • Adsorption on activated charcoal • Distillation 	35.66	97.37	[158]
N.I.	<ul style="list-style-type: none"> • Saponification • Acidification • Extraction • Membrane filtration • Adsorption on activated charcoal 	40.0	97.5	[159]

Table 6. Cont.

Type of Oil	Purification Techniques	Glycerol (%)	Purified Glycerol (%)	Ref.
Virgin (hemp) oil	<ul style="list-style-type: none"> • Acidification • Extraction • Adsorption on activated charcoal 	51.38	93.89	[160]
N.I.	<ul style="list-style-type: none"> • Saponification • Acidification • Extraction • Membrane filtration • Adsorption on activated charcoal 	40.00	93.70	[161]
Virgin (canola) oil	<ul style="list-style-type: none"> • Acidification • Distillation • Adsorption on activated charcoal 	N.I.	98.1	[162]
N.I.	<ul style="list-style-type: none"> • Acidification • Distillation • Biosorption on nanofibers 	N.I.	N.I.	[163]

N.I.—not indicated.

3.4. Possible Applications of Glycerol Purification Methods from Biodiesel Produced by Enzymatic Processes on Waste Oils

At present, there are no reports of a protocol for glycerol purification from waste oil or virgin oil using enzymatic catalysts. There are two possible explanations for this. The first possibility is that, as shown in Section 3.1, glycerol obtained from enzymatic transesterification has a high degree of purity after separation from biodiesel. So, crude glycerol can be used in the state where the low impurity content does not influence the conversion of biodiesel. The second possibility is that the industries of biodiesel production apply basic catalysis, as reaction times are short, and the cost of biodiesel production is lower compared to enzyme catalysis. As a result, it is not common to obtain glycerol on an industrial scale from biodiesel produced by enzyme catalysis, and the purification protocols developed are based solely on glycerol from biodiesel produced by enzyme catalysis. However, it is acceptable to consider purification protocols and to understand the advantages of enzyme transesterification compared to basic catalysis. Firstly, as has been shown, the acidification—which is the purification method most used for the pretreatment of glycerol—involves the removal of the basic catalyst and the soaps present. However, in enzymatic way, the reaction does not result in the formation of soaps, and it is not necessary to remove the enzymatic catalyst. So, acidification is not an essential step to purify glycerol in the presence of enzyme catalysis. It is therefore highly possible to establish a single-step protocol with lower purification process cost, as outlined by R. Hobden [164]. In this case, glycerol purity can reach 80% by removing water and methanol using simple evaporation.

3.5. Bioconversion of Glycerol from Waste Oils to High Value-Added Products

Glycerol can be converted into high value-added products either chemically or biologically. Some products resulting from chemical reactions are shown in Figure 12 [165]. Other products such as succinic acid, citric acid, propionic acid, lactic acid or lipids can be produced from glycerol by biological tools as described by Vivek et al. [166]. In this case, the bioconversion of glycerol does not necessarily involve a purification step. However, the source used to produce biodiesel may influence the glycerol composition and how the conversion reaction will occur. For example, this section will focus on the bioconversion of glycerol from waste oils and the importance of impurities requiring purification and non-purification (Table 7).

A study by Nuchdang et Phalakornkule [167] used glycerol to produce methane through anaerobic co-digestion with pig manure. Since glycerol was highly contaminated with oils and fats and the acidification with sulfuric acid was necessary. This pretreatment resulted in a higher production of hydrogen sulfide produced during glucose digestion, so it is recommended that alternative techniques can be used to treat glycerol. Following the same principle, Siles López et al. [168] treated glycerol to neutralize the catalyst (KOH) with phosphoric acid followed by distillation to remove water and methanol from biodiesel production. The anaerobic reclamation of glycerol with granular sludge successfully demonstrated 100% biodegradability. For Liu et al. [169], treatment of glycerol was not necessary due to its initial high purity of 83.41%, despite the use of waste cooking oil for biodiesel production. They therefore produced glycolipids from *Ustilago maydis* and showed an adverse effect of methanol above a level of 2%. Suzuki et al. [170] showed the effect of crude glycerol impurities on ethanol production by the bacterium *Klebsiella variicola*. They showed that the use of glycerol from biodiesel produced from waste cooking oil with a higher level of impurities improved the production of ethanol. They also showed that the optimum pH was 8–9, which is the pH of glycerol after transesterification of oil in alkaline medium. Thus, no purification process was necessary. Maru et al. [171] used microorganisms to produce hydrogen from glycerol that is unaffected by the impurities present in glycerol and thus avoids glycerol treatment. They then showed that the mixed culture of *Escherichia coli* and *Enterobacter spH1* increased the fermentation effect on crude hydrogen glycerol. If a different microorganism, here from granular sludge, is used for hydrogen production, Rodrigues et al. [172,173] showed that the impurities in glycerol have an inhibitory effect on the biological process of hydrogen production. For example, the pretreatment with hydrochloric acid was performed to purify glycerol to be bio-converted to hydrogen. Yuwa-amornpitak et Chookietwatana [174] also concluded that the impurities present in glycerol, such as free fatty acids, had a positive effect on lactic-acid production, since free fatty acids play the role of nutrient in microbial growth. However, since glycerol is derived from waste cooking oils, a treatment to neutralize and remove some of the impurities was carried out. Since pH played an important role in the reaction, it was necessary to raise the pH of glycerol to seven by neutralizing it with hydrochloric acid. Thus, better production of lactic acid by fermentation via *Rhizopus microsporus* was achieved with crude glycerol compared to pure glycerol. Like Maru et al. [171], Wang et al. [175] applied *Escherichia coli* and *Enterobacter spH1* mixed culture, which was not affected by glycerol impurities, to produce 1,3-propanediol and lactate. The fermentations were performed in batch system at 37 °C with continuous stirring at 200 rpm for 72h. Ma et al. [176] also produced 1,3-propanediol from glycerol with a purity of 69%, again from waste cooking oils. The microorganism used to produce 1,3-propanediol is *Klebsiella pneumoniae*. A very interesting point of this work is that the main impurities present in crude glycerol were studied to determine the impact on the production of 1,3-propanediol. In general, the impurities had no influence except for one fatty acid, linoleic acid, with a tendency to reduce cell growth. Chen et al. [177] found a method to maximize the productivity of glycerol obtained from biodiesel production. In addition, the simple purification of glycerol played a considerable role due to the large number of soaps contained in the glycerol from alkaline transesterification. Indeed, the acidification of glycerol, here by phosphoric acid, leads to the transformation of the soap into free fatty acids, which are then converted into biodiesel via the esterification process. The purified glycerol is bio-converted into lipids by the *Trichosporon oleaginosus* bacterium, which can then be converted into biodiesel by a conventional transesterification process. With an initial glycerol purity of 31.8% and soap concentration of 21.1%, 99.2% of these soaps were converted into free fatty acids and the glycerol purity after treatment was 54.96%. For other impurities in glycerol, such as esters, organic salts or methanol, the study by Xu et al. [178] showed that methanol could have an inhibitory effect, while other impurities showed a positive effect in lipid production. Additionally, for biodiesel production, Guerfali et al. [179] searched for a microorganism capable of converting glycerol without inhibition effect by impurities. For example, the

yeast *Candida viwanathii* Y-E4 showed its potential for bioconversion of the glycerol into lipids and then into biodiesel. Contrary to Chen et al. [177], who treated crude glycerol with phosphoric acid, no treatment of glycerol has been carried out to transform soaps into free fatty acids. This depends on the glycerol composition. Here the percentage of soaps is 4.1%, so it is not profitable to add a step by acidifying the glycerol. More recently, Brage et al. [180] used crude glycerol as a carbon source to transform L-phenylamine to 2-phenylethanol using the strain *Yarrowia lipolytica* CH1/5. Crude glycerol had an initial purity of 82%, so no treatment was needed, and the remained impurities had no effect on this reaction because glycerol is not the substrate. Ripoll et al. [181] converted crude glycerol into serinol by immobilized *Gluconobacter oxydans* and a transaminase coupling together. The impurities in crude glycerol were not considered; the work was only based on the biocatalyst and how the immobilization allows reuse.

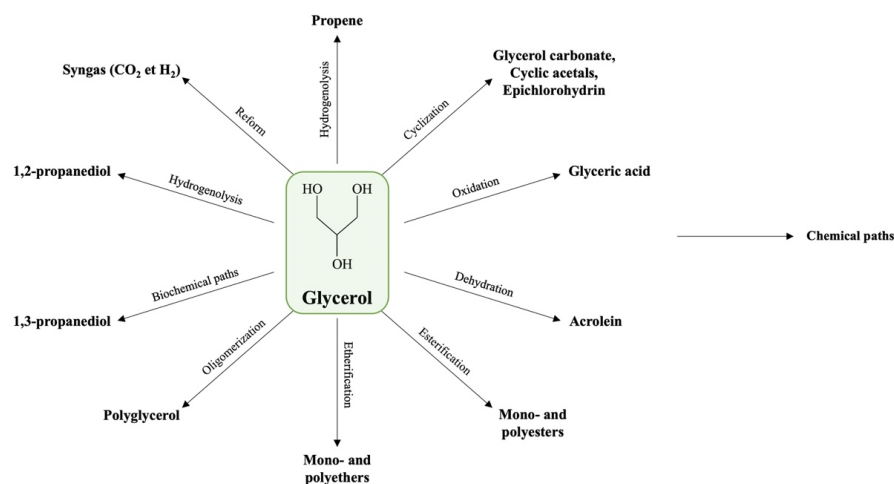


Figure 12. Reaction pathways for possible glycerol derivatives. According to Monteiro et al. [165].

Table 7. Summary of studies on the conversion of glycerol according to its purity level.

Glycerol (%)	Glycerol Treatment	Final Product	Process or Microorganism	Temperature (°C)	Time (h)	Yield	Ref.
N.I.	• Acidification	Methane	Anerobic digestion	25	2	54%	[167]
N.I.	• Neutralization • Distillation	Methane	Anerobic digestion	2	0.8	0.306 m ³ CH ₄ /kg	[168]
83.41	• None	Glycolipids	<i>Ustilago maydis</i>	30	196.8	32.1 g/L	[169]
N.I.	• None	Ethanol	<i>Klebsiella variicola</i>	25	24	9.8 g/L	[170]
47.5	• None	Hydrogen	<i>E. coli/Enterobacter spH1</i>	37	120	69.1 mM	[171]
10.41	• Acidification	Hydrogen	Anerobic digestion	37	19.1	2.2 mol H ₂ L ⁻¹	[172,173]
N.I.	• Neutralization	Lactic acid	<i>Rhizopus microsporus</i>	37	1.3	1.33 g/L	[174]
49.30	• None	1,3-propanediol / lactate	<i>E. coli/Enterobacter spH1</i>	37	1.3	27.77 g/L 1,3-PDO 14.68 g/L LA	[175]
69	• None	1,3-propanediol	<i>Klebsiella pneumoniae</i>	37	12	0.64 mol1,3-PDO/mol glycerol	[176]
31.8	• Acidification	Biodiesel	<i>Trichosporon oleaginosus</i>	28	72	5.24 g/L	[177]
32.97	• None	Triacylglycerols	<i>Rhodospiridium toruloides</i>	30	160	13.4 g/L	[178]
64.5	• None	Biodiesel	<i>Candida viwanathii</i> Y-E4	30	166	13.6 g/L	[179]
82	• None	2-phenylethanol	<i>Yarrowia lipolytica</i> CH1/5	27	200	2.2 g/L	[180]
N.I.	• None	Serinol	<i>Gluconobacter oxydans</i> transaminase	30	44	36 mM	[181]

N.I.—not indicated.

In recent years, the discovery that bioconversion allows crude glycerol to be used as a feedstock without any pretreatment has been a breakthrough for the biodiesel industry [182]. However, the use of low-purity crude glycerol, often due to the use of waste oils for biodiesel production, means that it may still be necessary to pretreat the glycerol. In some cases, pretreatment can even be used to make good use of impurities in the production of lipids.

4. Conclusions

The content of this review followed the life cycle of oils, or the “Carbon Neutral Cycle”, and how it can be improved by working on the best approaches to produce biodiesel and valorize its by-product through a greener path. To initiate this system, the use of waste oils is the best alternative to produce biodiesel, since the third-generation fuels use inexpensive feedstocks. It is derived from recycling, and it does not compete with the food market. Subsequently, to catalyze the reaction of biodiesel, enzymes have become the best option. Even though alkali catalysis is currently the most suitable method due to its shorter reaction times and low costs, studies on biocatalysts increased through the years to improve the stability and recyclability of enzymes to lower their cost, while allowing better reaction specificity than chemical catalysis. Additionally, using biocatalysts permits high yields of biodiesel, above 90%, and produces very few impurities, which reduces the treatment of biodiesel, and thus the cost. Once biodiesel is produced, about 10% of glycerol is generated. The life cycle of cooked oils for biodiesel production does not include the treatment of crude glycerol, yet it can be transformed into high value-added products. Therefore, glycerol must be purified, and a large part of this review presented the techniques to purify crude glycerol and the potential products obtained after biotransformation, using only bioprocesses. As mentioned above, thanks to the biocatalysts applied in the production of biodiesel, very few impurities are produced, which is another advantage, reducing the steps of treatment for the glycerol and making the cost lower. To end the life cycle of cooked oils, biodiesel allows reductions on gases emissions of about 85%. CO₂ and NO_x increase, but CO₂ released from combustion will be used again by nature for feedstock preparation. However, there is still work to do to improve the system, in particular the use of better robust enzymes, better treatment of products and their properties for use in engines.

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