Valorization of Biodiesel Byproduct Crude Glycerol for the Production of Bioenergy and Biochemicals

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Abstract: The rapid growth of global biodiesel production requires simultaneous effective utilization of glycerol obtained as a by-product of the transesterification process. Accumulation of the byproduct glycerol from biodiesel industries can lead to considerable environment issues. Hence, there is extensive research focus on the transformation of crude glycerol into value-added products. This paper makes an overview of the nature of crude glycerol and ongoing research on its conversion to value-added products. Both chemical and biological routes of glycerol valorization will be presented. Details of crude glycerol conversion into microbial lipid and subsequent products will also be highlighted.

Keywords: glycerol; valorization; purification; oxidative fuel additives; biological conversion; animal feed

1. Introduction

Global energy demand will increase due to population growth, industrialization and humankind's desire for a better quality of life. However, due to the limited nature of fossil fuel resources, and environmental and climate issues associated with its use, development of renewable energy has become imperative [1,2]. Biodiesel is a renewable biofuel that has the additional advantage of having lower levels of emission of Green House Gases (GHG) like carbon dioxide. It can thus help in mitigation of climate change and improve energy security. Chemically, biodiesel is a methyl or ethyl ester of fatty acid and is usually produced by transesterification of vegetable oils or animal fats with short chain alcohols [3]. However, increase in biodiesel production can lead to increase in the costs of edible vegetable oil. This leads to a debate of fuel versus food. In order to address such issues, use of alternative resources such as oils from non-edible plants [4], microbial sources [5] and use of waste-cooking oil [6] have been studied and even implemented. Even though the cost of production of biodiesel is currently higher than fossil-based diesel, its production across the world has increased due to the environmental benefits associated with it. One of the possible ways to improve the biodiesel production capacity would be to reduce the production of waste by-products and valorize the waste generated.

During the transesterification process, 1 mole of triglyceride produces 3 moles of biodiesel (ester) and 1 mole of glycerol. On this basis, every batch of biodiesel produces approximately 10 wt% of glycerol. The produced glycerol contains various impurities and is known as crude glycerol. Impurities in crude glycerol includes mainly methanol, soap, free fatty acids and salt (inorganic salts residues

from catalysts), unreacted mono-, di- and triglycerols and water [7]. Purification and refining of crude glycerol are carried out to different grades for pharmaceutical, food and cosmetic sectors. Purification techniques such as chemical pre-treatment, methanol removal, vacuum distillation, ion exchange, activated carbon and membrane separation technology are costly and hence are seldom economically feasible. The price of reformed glycerol is 40–50 cents/lb., which is 5- to 10-fold more than crude glycerol [8].

Many biodiesel producers have started treating crude glycerol as a waste rather than purifying it for commercial applications [9]. Crude oil is also directly used as an ingredient in animal feed [10]. While crude glycerol can seem like a liability for many biodiesel producers, from a biorefining point of view, it holds great potential for use as a starting material for value-added bio-chemicals. Such value-added chemicals can generate additional revenue for the existing biodiesel industries and make the process more sustainable. Valorization of crude glycerol will also help meet the circular bioeconomy guidelines by extending the value of every component in the chain as long as possible. Utilizing crude glycerol to produce commercially valuable compounds will simultaneously help to resolve the environmental issues associated with crude glycerol management [11,12].

Crude glycerol can serve as a low-cost and stable feedstock as the biodiesel market is estimated to have significant (42%) annual growth [13]. The cost of crude glycerol, which depends on the type of biodiesel feedstock, production process and location, varies between 3–20 cents/lb. [8,14]. Due to the low cost and stable nature of crude glycerol, researchers are focusing on its chemical and biological conversion into value-added products such as bioplastic, microbial oil, etc. Most of these conversion processes are constrained by the negative effects of impurities in crude glycerol on the chemical reaction or the microbial strains used for bioconversion. The use of robust microbes and processes can help overcome these problems and make the use of partially-treated crude glycerol possible.

Various methods have been developed to minimize the residual impurity present in crude glycerol [15]. These include development of heterogeneous catalysts used for the conversion of triglycerides to biodiesel. Interestingly, recent developments in bioconversion of crude glycerol to various value-added products show that certain microbial strains could take advantage of impurities present in crude glycerol [16]. Therefore, both pure, partially purified and non-purified glycerol could play a significant role in "waste to value" scenarios. Though conversion of glycerol to value-added biochemicals and biofuels has been widely investigated, these techniques are not extensively commercialized as yet. In this review, we will discuss the nature of crude glycerol and ongoing research of its conversion to value-added products. Both chemical and biological routes of glycerol valorization will be presented. Details of crude glycerol conversion into microbial lipid and subsequent products will also be highlighted. Figure 1. summarizes the routes for crude glycerol valorization.
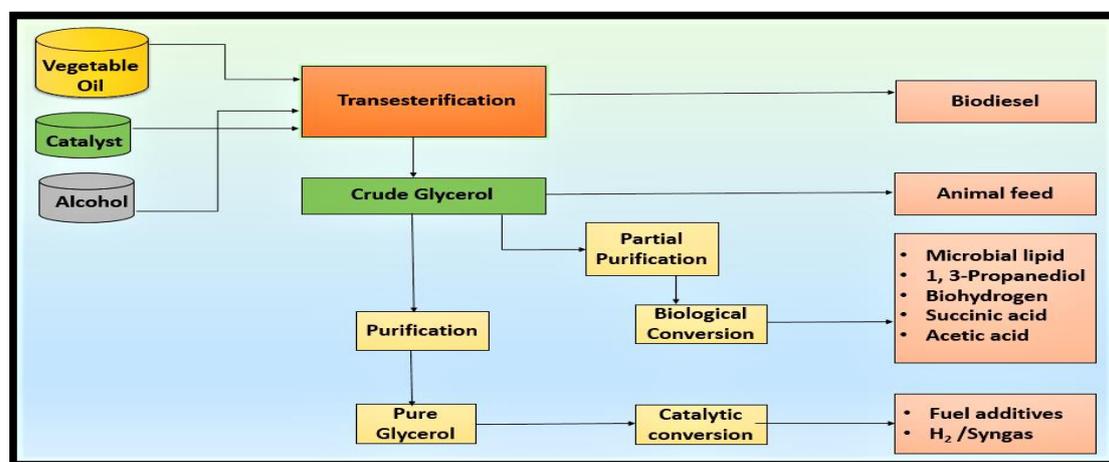

Figure 1. Crude glycerol valorization routes.

2. Impurities in Crude Glycerol and Hurdles to Use

The composition of glycerol and level of impurities present in it depends on the type of vegetable oil, catalysts and alcohol used and process conditions etc. [17,18]. However, various impurities that are generally present in crude glycerol include un-reacted triglycerides and alcohol (commonly methanol), residual fatty acid methyl esters (FAMEs), water, soap, free fatty acids (FFA), salts and catalyst [16,19,20]. The type of catalyst used for biodiesel production plays a significant role in determining the type of impurities in crude glycerol. Use of homogeneous catalysts (such as sodium hydroxide) for biodiesel production results in the formation of higher levels of salts which eventually deposit into a crude glycerol layer. Lesser salts are formed when heterogeneous catalysts (e.g., calcium oxide) are used instead [19]. Additionally, homogeneous catalysts also lead to the generation of soap and gel, which leads to phase separation problems at the end of the biodiesel production process and during the conversion of crude glycerol into value-added products. Thus, to make the crude glycerol valorization process efficient and economically feasible, heterogeneous catalysts should be used, as they can result in glycerol with higher purity [15].

The end product of the transesterification processes comprises of two phases which differ in density and polarity. The upper phase is made up of biodiesel, while the lower phase is rich in crude glycerol and other impurities. The obtained crude glycerol must be purified further into technical or food grade for its commercial application. This purification of glycerol comprises a combination of both chemical and physical treatment. Generally, crude glycerol refining involves a three-step process. This starts with neutralization for the removal of soaps and salts followed by vacuum evaporation to eliminate the excess methanol and water. Finally, other unit operations are used to get purer form of glycerol. Neutralization of crude glycerol can be carried out by adding strong acids (such as sulfuric acid, phosphoric or hydrochloric acid) into crude glycerol. The acid converts the soap present in crude glycerol into free fatty acids. Strong acids like sulfuric acid, phosphoric acid and hydrochloric acid have been used for neutralization. In a second stage, alcohols are removed by vacuum distillation due to their lower boiling point. At commercial level, advanced heat transfer technologies, i.e., a falling film evaporator and rising film evaporator, ensure the prevention of glycerol decomposition [21]. Physical deep separation technologies like vacuum distillation, activated carbon adsorption, membrane separation and ion exchange chromatography have been employed in the final stage to obtain glycerol with higher purity [3,22]. Ismail et al. [23] refined glycerol using neutralization followed by microfiltration and ion exchange resin adsorption. Glycerol purity to a level of 86 wt% has been reported without a distillation operation [24]. A higher purity of 95.7 wt% was obtained by Manosak et al. [24] using acidification and polar solvent extraction followed by activated carbon adsorption. Commonly, vacuum distillation at higher temperature (150–200 °C) must be employed to obtain technical grade glycerol (99.5 wt %). In terms of process economy, thermal separation processes, i.e., distillation, accounts for 50% of plant operation costs due to its higher energy consumption. However, new distillation technologies using cyclic distillation columns and dividing wall column distillation (DWC) systems help process economy criteria without compromising the quality of the final product [25–27]. However, due to the slump in the price of glycerol, purification of crude glycerol to higher grade glycerol has become uneconomical. Due to this, crude glycerol is being treated as a waste by-product by various biodiesel producers. This has necessitated the conversion of crude glycerol into value-added products rather than purifying it into a higher grade.

A lot of research has been carried out to valorize crude glycerol. However, the impurities in crude glycerol have always deterred the success of such conversion processes. For instance, Payle et al. [28] reported that the presence of methanol impurities negatively affects the algal production of docosahexaenoic acid (DHA) from crude glycerol. Similarly, during biogas generation from crude glycerol via anaerobic co-digestion, impurities such as salts (Na or K) have been shown to inhibit the growth of microbes [29]. Shengjun Hu et al. [30] carried out a characterization of crude glycerol samples generated from a biodiesel production unit and reported that the crude glycerol was made up of eight components, namely, methanol, free glycerol, water, soap, fatty acids methyl esters, free fatty

acids, glycerides and ash. The results show that 85% of the mass of crude glycerol is accounted for by glycerol, methanol, FAMEs, soap and water. On the other hand, glycerides, FFAs and ash make up less than 15%.

3. Technologies Studied for Value Addition of Glycerol

3.1. Chemo Catalytic Conversion

Due to its unique structure, properties, availability and renewability, a number of value-added products can be derived from glycerol via catalytic transformation. Different reaction pathways, namely, selective oxidation, selective dehydration, reforming, thermal reduction and selective etherification, have been attempted for value addition of glycerol. The major catalyst-assisted, glycerol-derived production processes are discussed below.

### 3.1.1. Oxidative Conversion of Glycerol to Fuel Additives

Gasoline, diesel and biodiesel blended with fuel additives shows better performance due to important changes in fuel properties. It can also reduce the emission of greenhouse gases (GHG). Improvement in the fuel viscosity, octane and cetane number ensures stability, ease of cleanliness and prevention of engine corrosion [31–33]. Petroleum-derived fuel additives like ethanol, methyl tert-butyl ether (MTBE) and ethyl tert-butyl ether (ETBE) can be replaced with glycerol-derived additives, more specifically by glycerol esters, glycerol ethers and glycerol formals [34]. Major reactions for the production of fuel additives are acetylation [35–39] and ketalisation [40], in which glycerol interacts with chemical groups like carbonyl compounds [26].

1.   Glycerol Esters (Acetin)

Esters of glycerol or acetin are produced using esterification with carboxylic acid in the presence of catalysts [32,33]. Acetylation of glycerol with acetic anhydride is also a potential route for the acetin synthesis [41,42]. Mono, di and tri acetin glycerol (MAG, DAG and TAG) are derived via a three-step esterification process of glycerol with acetic acid (Scheme 1) or acetic anhydride (Scheme 2). Mixed catalysts of Zr phosphate–sulphate and Amberlyst-15 show the best catalytic performance in terms of complete conversion of glycerol and better selectivity of triacetin [35,43]. Information related to recent development of catalysts is presented in Table 1. Acetic anhydride is also a potential acetylating agent with higher selectivity of TAG due to the generation of an acetic acid molecule, which further reacts with the MAG and DAG produced in the first and second step, resulting in TAG [44]. Gonzalves et al. [35] reported the use of Amberlyst-15 as a catalyst for the esterification of glycerol with acetic acid. This resulted in excellent catalyst activity with 97% glycerol conversion and 90% selectivity of TAG obtained. Complete glycerol conversion is possible with 100% selectivity of TAG by performing the reaction in two steps: first, a reaction of glycerol with acetic acid and, second, a reaction with acetic anhydride [37]. Jinyan Sun et al. [45] prepared Fe-Sn-Ti (SO4 $^{2-}$)-400 catalyst and tested it at 80 °C for 30 min for glycerol esterification in the presence of acetic anhydride. It resulted in 99% selectivity of TAG with 100% conversion of glycerol.
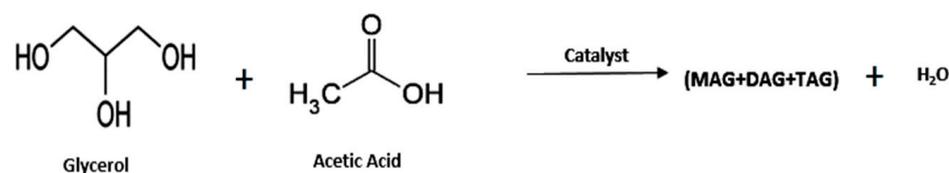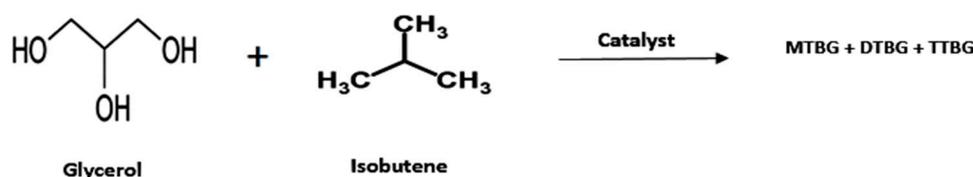

Scheme 1. Conversion of glycerol to acetin using carboxylic acids like acetic acid.
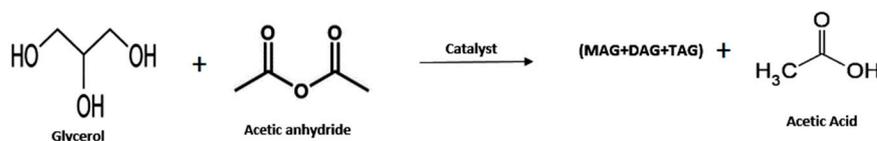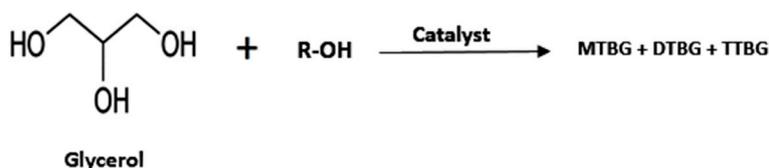
Scheme 2. Conversion of glycerol to acetin using acetic anhydride.

2. Glycerol Ethers

Highly branched materials can be derived via etherification of glycerol with alkenes (mainly isobutene) (Scheme 3) and alcohols like tert-butyl alcohol (TBA) (Scheme 4) in the presence of homogeneous or heterogeneous catalysts [35]. A blend of 1, 3-di, 1, 2-di and 1, 2, 3 tri-tert-butyl glycerol and aromatic diesel fuel drastically reduces the emission of particulate matter, hydrocarbons and carbon monoxide [46,47]. The low boiling point (−6.9 °C) of isobutene demands high pressure during the etherification reaction to keep it in a liquid stage for the reaction. Despite this, the reaction performance is governed by mass transfer between the two reactants [48]. However, the reaction conditions are mild with etherification of glycerol with TBA in the presence of an acid catalysts. In comparison with isobutene, TBA is more attractive for the production of glycerol tertiary butyl ethers (GTBEs) [49]. Behr and Obendorf [50] studied the reaction between isobutene and glycerol in the presence of p-toulene sulfonic acid and phosphorus tungstic acid. They observed low conversion of glycerol levels of 89% and 79%, respectively, with these homogeneous catalysts. Huang and Kim [51] reported that that Amberlyst-15 exhibits the best efficiency with 97% conversion of glycerol and 33% selectivity of TTBGs (tri-tert-butyl glycerol) and DTBGs with TBA. Amorphous carbon-based catalysts obtained using sulfonation of peanut shells shows very good catalytic performance, achieving complete glycerol conversion and 92% selectivity of GTBE at 70 °C [52]. The effectiveness of silica-based sulfonic catalyst that exhibits 78% conversion of glycerol in 30 min of reaction time has also been reported [52].
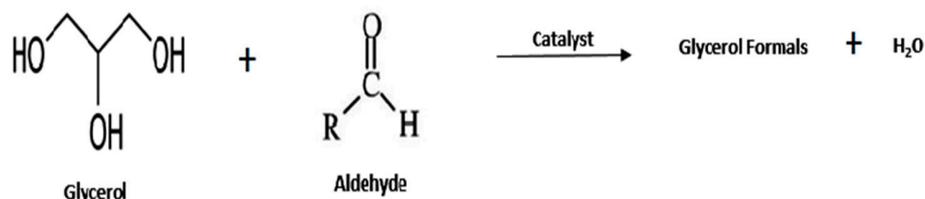

Scheme 3. Conversion of glycerol to glycerol ether using alkenes.

Scheme 4. Conversion of glycerol to glycerol ether using alcohols.

3. Glycerol Formal

Glycerol formal is a mixture of acetal and solketals and is derived via a condensation reaction of glycerol with aldehydes or ketones over homogeneous or heterogeneous catalysts (Scheme 5). The product composition of solketal and acetal is affected by reaction condition and type of catalyst [52,53]. Suriyaprapadilok and Kitiyanan [54] studied the reaction between glycerol and acetone in the presence of p-toluene sulfonic acid. The results revealed that excess of acetone leads to higher levels of solketal, rather than acetals at the end of the 12 h reaction time. Malaya et al. [55] reported that Amberlyst-36 catalyst show good efficiency with 96% selectivity of solketal. Silva et al. [56] reported that longer aldehyde chains prevent its interaction with glycerol, and the pore size of the catalyst does not impact the conversion of acetone. More details about catalysts are summarized in Table 1 below.

Scheme 5. Conversion of glycerol to glycerol formal.

Table 1. Summary of reactants and catalysts used for oxidative fuel additives production.

Entry	Reactant	Catalyst	Operating Condition	% Conv. (glycerol)	Yield (%)	Ref.
1	Acetic acid	Catalyst Free	MR acetic acid to glycerol 6:1, 378 K, 4 h	74	2 [1]	[37]
2	Acetic acid	$Zr_3(PO_4)_2(SO_4)_3$	Catalyst 5 wt%, MR acetic acid to glycerol 3:1, 105 °C, 3 h	100	53 [1]	[43]
3	Acetic acid	$Zr_4(PO_4)_2(SO_4)_5$	Catalyst 5 wt%, MR acetic acid to glycerol 3:1, 105 °C, 3 h	100	48 [1]	[43]
4	Acetic acid	$Zr(SO_4)_2$	Catalyst 5 wt%, MR acetic acid to Glycerol 3:1, 105 °C, 3 h	60	NA	[43]
5	Acetic acid	Amberlyst-15	Catalyst 0.2 mol%, MR acetic acid to glycerol 3:1, room temp, 30 min	97	90 [1]	[35]
6	Acetic acid	TAC-673 Catalyst	Catalyst 5 wt%, MR acetic acid to glycerol 9:1, 378 K, 4 h	>99	17 [1]	[57]
7	Acetic anhydride	H-Beta zeolite	Catalyst 2.0 mmol acid sites, MR acetic a hydride to glycerol 4:1, 393 K, 2 h	94	43 [1]	[56]
8	Acetic anhydride	Fe-Sn-Ti $(SO4^{2-})$-400	Catalyst 0.05 g, glycerol 1.5 g, acetic anhydride 8.39 g, 80 °C, 30 min	100	99 [1]	[45]
9	Acetic anhydride	Amberlyst-15	Catalyst 0.05 g, glycerol 1.5 g, acetic anhydride 8.39 g, 80 °C, 30 min	99	99 [1]	[45]
10	Acetic anhydride	HZSM-5	Catalyst 0.05 g, glycerol 1.5 g, acetic anhydride 8.39 g, 80 °C, 30 min	99	24 [1]	[45]
11	Tert Butyl alcohol (TBA)	Amberlyst-15	Catalyst 7.5 wt%, TBA/glycerol molar ratio 8, 70 °C, 5–8 h	97	30.3 [2]	[51]
12	Tert Butyl alcohol (TBA)	SiO_2-SO_3H	Catalyst 5 wt%, TBA/glycerol = 2 mol/mol, 30 min, 130 °C	78	24 [2]	[58]
13	Isobutene (IB)	p-toluene sulfonic acid	Catalyst 2.16 wt%, IB/glycerol = 4 mol/mol, 5 h, 90 °C, 1.4 bar	89	47 [2]	[50]
14	Isobutene (IB)	Amberlyst-15	Catalyst 1 g, IB/glycerol = 4 mol/mol, 7 h, 80 °C, 15 bar	>95	97 [2]	[59]
15	Isobutene (IB)	Sulfonated peanut shell	Catalyst 6 wt%, IB/glycerol = 4 mol/mol, 2 h, 70 °C, 15 bar	100	92 [2]	[60]
16	Acetone	Amberlyst-36	Acetone to glycerol ratio 4:1, 25 °C, 500 psi	100	96 [3]	[55]
17	Acetone	catalyst free	Reaction at super critical condition, 508 K, 8 MPa, 240 min	28	80 [3]	[61]
18	Acetone	$SnCl_2$	Catalyst: 1 wt%, acetone/glycerol = 8:1	78	76 [3]	[62]
19	Acetone	DT-851 sulfonic acid resin	Catalyst: 5%, acetone/glycerol: 20:1, 58 °C, 10 bar	95	99 [3]	[63]
20	Benzalde-hyde	K10 montmorillonite	Benzaldehyde/glycerol: 1.1:1, 40 °C, 6 h	83	99 [3]	[63]

[1] Tri acetin glycerol, NA not available, [2] glycerol tertiary butyl ethers, [3] solketal.

3.1.2. Hydrogen or Syngas Production from Glycerol

Hydrogen is a promising potential ecofriendly fuel as its combustion leads to the production of only water molecules as a by-product. Currently most hydrogen production processes (95%) use fossil fuel as the raw material. Similarly, syngas, a mixture of hydrogen and carbon monoxide, is also considered as a valuable intermediate for the production of methanol and hydrocarbons through Fischer–Tropsch synthesis [64,65]. The production of hydrogen and syngas is another promising use of glycerol (Scheme 6). Conversion techniques like pyrolysis, steam reforming, partial oxidation, auto thermal reforming and aqueous phase reforming can be used for the production of hydrogen and syngas from glycerol. Pyrolysis of glycerol is a thermal decomposition process in the absence of oxygen. Glycerol is converted to hydroxyacetone, 3-hydroxypropenal and glyceraldehyde by dehydration and dehydrogenation reactions and then transformed into syngas at higher temperature [66]. Higher heat inputs and unstable product distribution facilitates glycerol pyrolysis [34]. Steam reforming, a combination of pyrolysis and a water gas shift reaction, is a common technology applied for hydrogen

and syngas production from glycerol. Pre-vaporization of reactants demands higher heat input and process heat. However, the reaction is carried out at ambient condition of pressure [67,68]. Aqueous phase reforming, a process developed by Dumesic et al. [69], transforms glycerol in an aqueous phase without pre-vaporization under a moderate temperature (470–525 K) and pressure (16–40 bar). The automobile industry uses the high purity hydrogen synthesized by aqueous phase reforming for PEM fuel cells [70]. Partial oxidative reforming is an exothermic process in which glycerol conversion efficiency relies on controlling the amount of oxygen that enters into the reaction mixture. Partial oxidation reforming processes have high energy efficiency and also allow the production of syngas with control of the added amount of oxygen [71]. Supercritical water reforming is an innovative route for the production of hydrogen at higher pressure and lower temperature. Water takes part in the reaction at its critical point (374 °C, 218 atm) [72]. Table 2 includes a summary of the performance of some catalysts in the conversion of crude glycerol to hydrogen and syngas, as recently reported in the literature.

$$C_3H_8O_3 + H_2O + O_2 \xrightarrow{\text{Catalyst}} CO_2 + CO + H_2O + H_2 + CH_4 + \ldots$$

Scheme 6. Conversion of glycerol to hydrogen and syngas.

Table 2. Review of literature on the conversion of glycerol to hydrogen and syngas.

Entry	Reforming Technology	System of Operation	Glycerol/Water	Catalyst	Temp (°C)	Pressure	H_2 Yield (%)	X_G (%)	Ref.
1	Steam reforming	Fixed bed reactor	1:16	Ni/Al_2O_3	600–700	atm	75–100	100	[57]
2	Steam reforming	Fixed bed reactor	1:9	Ni, Pt, Pt–Ni with γ- and La_2O_3	500–600	0.4 MPa	90	100	[73]
3	Steam reforming	Fixed bed reactor	1:3	Ni/Al_2O_3	400–700	atm	80	100	[74]
4	Partial oxidation reforming	Fixed bed reactor	1:3, 1:6, 1:9	$Ni/CeZrO_2/Al_2O_3$	550–650	atm	67–69	40–70	[75]
5	Aqueous phase reforming	Fixed bed reactor	1:3	AP Ni, Raney Ni	225	2.76 MPa	50–100	100	[72]
6	Aqueous phase reforming	Fixed bed reactor	1:3	Ni, Ni_5Cu, $Ni_{10}Cu$, $Ni_{20}Cu$	250–270	38–52 atm	80–90	60	[76]
7	Auto thermal Reforming	Fixed bed reactor	80 wt% glycerol and 20 wt% D.I. water	BASF Pt. and Rh/Pt. double-layer monolith	600–700	atm	75	100	[77]
8	Auto thermal Reforming	Fixed bed reactor	1:3	Rh Ce/γ-Al_2O_3	900–1200	atm	79	100	[78]
9	Super critical water reforming	Fixed bed reactor	1:3	Na_2CO_3	380–500	25 MPa	60	100	[79]

3.2. Direct Use or Minimal Treatment Products

Glycogenic amino acid and adipose triglycerides are valuable sources for glucose synthesis for animals that produce milk. Ketosis is a metabolic disorder that commonly takes place during the calving of an animal. Glycerol drenching is an effective treatment for the prevention of ketosis as the metabolic pathway of glycerol is pretty similar to glucose [80]. Cotrill et al. [81] reported high and rapid availability of net (2.27 Mcal/kg) and gross energy (4.32 Mcal/kg) from glycerol used as a lactation energy supplement. The possible use of glycerol as an animal food supplement depends on various parameters like the supply of glycerol, fuel demands and the price of the other feeds like oil seeds or fats which that are used for the production of biodiesel [82]. Bodarski et al. [83] showed that glycerol increases the blood insulin concentration in cows, which in turn improves the milk protein content and yield. In dairy cows, body weight (BW), body condition score (BCS), ruminal volatile fatty acids (VFA) and feed intake increases on glycerol administration in feed [10]. When added as feed to laying hens, egg quality, nutrient retention, metabolic energy and egg performance is unaffected

by incorporation of 6% glycerol in their diet [84]. Growth performance and nutrient digestibility is not affected by increasing the glycerol intake level in broiler diets [85]. Hampy et al. [86] indicates that 5% crude glycerol in a meat goat's diet is beneficial. Presence of impurities in crude glycerol is an important consideration while using crude glycerol as an animal feed supplement as it directly affects nutritive value and health. Drackley [82] reported the effect of methanol impurities in glycerol on pre-ruminant calves. Energy rich grain such as corn could be replaced by glycerol after removing the impurities of methanol [87]. While use of crude glycerol in this manner is possible, the conversion of glycerol to value-added products could lead to higher benefits.

3.3. Biological Conversion of Glycerol to Value-Added Chemicals

Most of the chemical routes for conversion of glycerol to value-added products discussed above make use of harsh and toxic chemicals. On the one hand, exposure to such chemicals can be detrimental to individual health. On the other, release of effluents containing such chemicals can seriously impact the existing eco-system. Furthermore, chemical conversions are quite energy intensive [88,89]. Thus, biological conversion of glycerol into different useful chemicals and compounds has been extensively explored. Like other biological conversions, glycerol conversion via the biological route is gaining popularity due to its use of enzymes and microbes, which have practically no negative impact upon the human population and environment. Some of the conversion processes (reactions) which are generally complex and infeasible via the chemical route can also be accomplished easily by using biological routes. However, the main drawbacks of the bioconversion process are its costs due to low productivity [90,91]. As both the conversion processes (chemical or biological) have certain drawbacks, a combination of these conversion processes has also been explored in order to increase the productivity of the overall process [92,93].

The carbon source which serves as the essential nutrient for microbes accounts for approximately 60% of the annual operation cost of fermentation systems. Thus, replacing the conventional carbon sources such as glucose with cheap substrates (e.g., crude glycerol, lignocellulose hydrolysate, effluents from pulp and paper industries, etc.) can reduce the overall production cost [90,94,95]. Use of such cheap substrates makes the biological conversion process economically feasible and sustainable. Additionally, from an environmental point of view, recycling of such waste by-products is desirable. Due to these reasons, crude glycerol has been extensively explored as an alternative carbon source for various biological conversion processes [96]. Glycerol also holds biochemical advantages over other sugars [97]. The amount of reducing equivalents produced from bioconversion of glycerol to pyruvate or phosphoenolpyruvate is twice more as compared with hexose and pentose [96,98].

Additionally, utilization of glycerol reduces concerns associated with the diauxic growth which often adversely affect the productivity of the conversion. For instance, when lignocellulosic hydrolysate/pre-hydrolysates are used as the carbon source for microbial growth, the microorganisms under study selectively consume preferred hexose sugars over the other types of sugar [97]. The problem of catabolic repression can be avoided by using glycerol. These characteristics of glycerol makes crude glycerol a potentially suitable substrate for its efficient biological conversion to commercially valuable products. Some of the important value-added biochemicals that have been studied for their production using crude glycerol via the biological route are discussed below.

3.3.1. Microbial Lipids

Microbial lipids, also known as single-cell oils (SCO), are obtained from a group of microbes known as oleaginous microorganisms. Oleaginous microbes are microorganisms that can accumulate lipids as more than 20% of their dried cell biomass. These microbes can fall into the categories of yeasts, fungus, bacteria or microalgae [99,100]. Most of the oleaginous yeasts and fungi (mainly molds) accumulate lipids in the range of 40%–70% [5,101,102]. Lipid accumulation in microalgae can vary between 1% and 70% [103] with some of them capable of accumulating up to 90% under certain conditions [104]. Lipids in yeast, fungus and microalgae are mostly neutral lipids, mainly triglycerides (TAG). The lipid also

contains free fatty acids, phospholipids, etc., but in much lower levels. Very few bacterial species can accumulate large quantities of lipids. Most of the lipids from bacteria are mostly complex lipids such as polyhydroxyalkanoic acids (PHA). However, few bacterial strains belonging to the actinomycetes group seems to accumulate a high amount of lipids (up to 70%). Additionally, unlike other bacteria, the lipids from actinomycetes genera are of the TAG form [5,100,102]. Lipid accumulation in oleaginous microorganisms takes places under stress environments, such as a shortage of major nutrients in the media. Under nutrient-limited conditions (usually nitrogen or phosphorus) and enough or excess amount of carbon sources, in oleaginous microbes (fungus and microalgae) the carbon flux is directed towards the accumulation of lipids [100,105]. Lipids obtained from oleaginous yeast, fungi and microalgae are in the form of neutral lipids and their fatty acids profiles are quite similar to the oils from plants and animals [100,102]. Thus, such lipids can potentially be used as an alternative to conventional oils in food, pharmaceutical and biofuel industries.

In recent decades, potential use of microbes to produce oils or oleo-chemical products is gaining popularity. This is because use of microbial lipids addresses some of the issues associated with the production of vegetable oils via a conventional route. Unlike conventional vegetable oil production, microbial lipid production does not make use of large amount of arable lands. In addition, the production process is less dependent on weather conditions, requires a lower amount of water and manpower, is easier to scale up and has a shorter life cycle compared to conventional vegetable oils [16,106]. Two types of microbial oil production pathways exist in oleaginous microbes: (i) the de novo pathway and (ii) ex novo pathway. Conventional sugar sources (such as glucose and glycerol) or organic acids present in the fermentation media are catabolized and converted into storage lipids via the de novo pathway.

Most of the hydrophobic compounds (e.g., fatty acids or oils) present in the media are taken up with the aid of active transport inside the microbial cell via the ex-novo pathway for growth or accumulation as lipids [100,107,108]. Compared to the ex- novo pathway, when microbial growth takes place on hydrophilic substrate, such as glycerol, lipids with higher quantities of triglycerides (TGA) are produced [109]. The biochemistry of lipid metabolism of oleaginous microbes differs from that of non-oleaginous microbes mainly in two ways. Firstly, in oleaginous microbes, citric acid is continuously converted to acetyl Coenzyme A (CoA) (a precursor for the fatty acid pathway) by adenosine triphosphate (ATP): citrase lyase (ACL).

Presence of such mechanism ensures the availability of enough precursor for the fatty acid biosynthesis. Secondly, nicotinamide adenine dinucleotide phosphate (NADPH; coenzyme), an essential reductant used in fatty acid biosynthesis, is generated sufficiently from malic acid or similar alternative NADPH-generating sources [100,109]. Generally, molds accumulate a large amount of lipids richer in polyunsaturated fatty acids (PUFA). Lipids with high levels of PUFAs such as gamma linoleic acid (GLA), eicosapentaenoic acid (EPA), arachidonic acid (ARA), and docosahexaenoic acid (DHA) have a number of health benefits and thus find a lot of food and medicine applications [108,110].

Microbial lipids obtained from yeasts have a high amount of monounsaturated fatty acids (MUFA) and thus can be used as suitable feedstock to produce biodiesel. We have demonstrated the use of oils rich in MUFA for the production of other compounds such as bioplastics [16,93,111,112]. The chemical composition of lipids obtained from oleaginous microbes also depends on factors such as the type of species or strain, media composition and growth conditions. We also grew *Rhodosporidium toruloides* ATCC 10788 in media with different concentrations of essential oil from orange. Microbial oils with different fatty acids profiles were obtained. Similarly, growth parameters such as temperature also have a direct effect on the chemical composition of the lipids obtained [111,112]. When oleaginous yeast *Lipomyces starkeyi* was grown under different temperature conditions (i.e., 15 °C to 28 °C), the lipids obtained were quite different in their fatty acids profiles. At a lower accumulation phase temperature (15 to 18 °C), the lipids obtained had a higher degree of unsaturation compared to that at 28 °C [113]. Though microbial lipids have a huge potential to replace conventional oils, they are currently still very expensive compared to vegetable oils. To make it cost competitive, cheap substrates, such as crude

glycerol from biodiesel industries, should be used for their production [110]. This will significantly reduce the overall production cost of microbial lipids. Due to this reason, recent studies have focused on utilization of crude glycerol to produce microbial lipids. However, like in other bioconversion processes, impurities present in the crude glycerol have posed a huge problem for such conversion. Methanol, salt and soap are three major impurities that have been found to hinder the production of microbial oils from crude glycerol [114–117]. Pyle et al. [28] found that the presence of methanol and soap in crude glycerol reduces the biomass and DHA production of *Schizochytrium limacinum* SR-21. Similarly, Gao et al. [118] reported that methanol can significantly reduce the lipid production of oleaginous yeast *Rhodosporidium toruloides* 32489. Compared to the control experiment, the presence of methanol in the media reduced the lipid production by 17.7%.

In another instance, salt (5.5% NaCl) present in crude glycerol was found to have a detrimental effect on the growth and poly(3-hydroxybutyrate) (PHB) content (reduced by 48%) of *P. denitrificans* [119]. It is due to these reasons that many researchers have focused on pre-treating crude glycerol before its utilization as a carbon substrate for the growth of these microbes. Purification of crude glycerol to a certain degree before its biological conversion requires an additional unit of operation in the overall production of microbial lipids. With the addition of such a unit of operation, the overall operational cost for the production of microbial lipids will increase. This will make conversion of crude glycerol to microbial oil production economically unattractive. Thus, from a commercial point of view, direct conversion of crude glycerol into microbial lipids is desirable. Thus, a robust strain that can withstand the harsh environment of crude glycerol has to be found. In this regard, we recently reported the robust oleaginous yeast, *Rhodosporidium toruloides* ATCC 10788, which grew well in crude glycerol with high levels of impurities [16].

When crude glycerol with 44.56 wt% glycerol, 13.86 wt% methanol, 10.74 wt% of ash and 32.97 wt% of soap was used as a carbon source for *Rhodosporidium toruloides* ATCC 10788, double the biomass (21.16 g/L) and triple the lipid content (11.27 g/L) were obtained. Similarly, oleaginous microorganisms such as *Mortierella isabelline*, *Yarrowia lipolytica* [120], *Trichosporon fermentans* CICC 1368 and *Trichosporon cutaneum* [121] have also been shown to grow well and accumulate lipids in the presence of impurities present in crude glycerol. In our lab, besides crude glycerol, we have also explored the potential use of hemicellulose hydrolysate, a waste by-product from a lignocellulose processing plant, as a cheap carbon substrate for the production of microbial lipid. The hemicellulose prehydrolysates from lignocellulosic biomass have similar issues with the presence of toxic compounds present in them. We reported that under optimum growth conditions, *Cryptococcus curvatus* ATCC 20509 can successfully grow in hemicellulose hydrolysate and produce 16.54 g/L of cell biomass and 6.97 g/L of lipid concentration at the end of 164 h without detoxification in a batch bioreactor [122].

3.3.2. Constraints for the Commercial Production of Microbial Lipid from Crude Glycerol

Even though various new approaches (screening robust screen, genetic modification, novel feeding strategies, pretreatment, etc.) have been explored for the valorization of waste by-products such as crude glycerol and lignocellulose hydrolysate, most of them have been limited to the research levels [100]. This is mainly because of variations in composition of these waste by-products, which have a direct impact on the bioconversion process. While this statement holds true in most of the biorefining processes, here we will limit our discussion to crude glycerol from biodiesel industries. The impurities present in crude glycerol are directly dependent on the type of substrate used for biodiesel production, production processes and the degree of downstream separation involved. All three factors vary considerably between industries and even between batches from the same production facility. A systematic study is required to determine the exact reason for such variations. There are also some additional reasons for these processes not to be easily scaled up to commercial levels. Currently, most of the biodiesel production processes are mainly designed focusing on the quantity and quality of biodiesel produced but overlook the quality of glycerol produced. To make the biodiesel more cost competitive and

enhance the company's revenue, biodiesel producers often use cheaper feedstock, catalysts (such as sodium hydroxide) and downstream processes.

Cheaper feedstock used for biodiesel industries varies from plant-based oil to waste cooking oil. Most commonly, sodium hydroxide is used as a catalyst. While use of such feedstock and catalyst might help in the production of biodiesel that meets the specifications for commercial applications, the glycerol obtained is quite low in quality and even inconsistent from batch to batch. Such low-quality glycerol needs to be purified to a certain degree before use. However, due to the glut of glycerol, even purified glycerol prices have slumped [100]. Due to this, crude glycerol is currently treated as waste. Such waste glycerol has very few applications, and for its bioconversion, we need its composition to be consistent throughout. This leaves biodiesel producers in a dilemma of using improved biodiesel processes which would be costly or sticking to the conventional process and treat glycerol as waste. In this regard, we recently reported a comparative study between different types of biodiesel production catalysts.

We also provided a recommendation on what type of catalyst to use if a company wants to reduce the generation of crude glycerol waste without compromising on their current biodiesel production cost [15]. We found that even though the use of homogeneous catalyst (such as sodium hydroxide) can generate biodiesel that meets the commercial specification, it can generate low grade glycerol. To generate high quality biodiesel and glycerol, we recommended the use of a heterogeneous catalyst in an anchored or unanchored form. Use of such heterogeneous catalyst can produce commercial grade biodiesel and simultaneously a purer form of glycerol for its direct bioconversion into the value-added products. Integration of such a production process into existing biodiesel production plants can reduce the generation of glycerol waste and improve a company's revenue. To address the cost constraints involved with the use of heterogeneous catalyst, we have also demonstrated the potential use of ash as heterogeneous catalyst for biodiesel production. Even though biodiesel a production process simultaneously focusing on the quality of glycerol for its valorization and subsequently the revenue generation of a company is recommended, it will take a while before this is implemented by biodiesel producers. A major constraint to research in the use of crude glycerol valorization is the use of samples from a single batch. Such samples cannot be good enough to represent crude glycerol whose composition varies from batch to batch in real case scenarios. With so much variation in the composition of crude glycerol, it is even hard to prepare a representative synthetic crude glycerol in the lab to conduct any research. Hence, a comprehensive investigation of the effect of impurities and their interactions has to be carried out to overcome the stated constraints.

3.3.3. Production of 1, 3-Propanediol

1, 3-Propanediol (1, 3-PD) is perhaps the most investigated biochemical via bioconversion of glycerol. 1, 3-PD is a colorless viscous liquid belonging to the group of diols. It is one of the main precursors in the synthesis of polytrimethylene terephthalate (PTT) and other polymers. Furthermore, it has a variety of applications in cosmetics, foods, lubricants, and medicines [123,124]. Bacteria of the genera *clostridium*, *klebsiella*, *citrobacter*, *hafnia*, and *lactobacillus* are mainly reported for production of 1, 3-PD from glycerol [123,125,126]. Among these, *Clostridium butyricum* and *Klebsiella pneumoniae* are well-studied bacteria. Even though *Klebsiella pneumoniae* produces a high level of 1, 3-PD, *Clostridium* strains such as *C. butyricum* and *C. pasteurianum* have attracted more attention as they are non-pathogenic and also have a vitamin B12 independent glycerol dehydratase enzyme, as opposed to *Klebsiella pneumoniae*, which is a pathogen and requires medium with exogenously-provided B12 [127,128].

Bioconversion of glycerol to 1, 3-PD takes place in a two-step enzymatic reaction sequence. Firstly, glycerol is converted to 3 hydroxypropionaldehyde (3-HPA) and water by glycerol dehydratase, and then, NADH dependent 1, 3-PD dehydrogenase reduces 3 hydroxypropionaldehyde to 1, 3-PD [129]. Both pure and crude glycerol were compared for the production of 1, 3-PD. In general, higher yields for production of not only 1, 3-PD but also other metabolites were reported with pure glycerol [129,130].

However, reports are available that show approximately the same yield from 1, 3-PD production for both pure as well as non-purified glycerol. For instance, 11.3 g/L of 1, 3-PD was obtained using *C. butyricum* grown on both pure glycerol (99%) and crude glycerol (81.0%) during batch cultures in a 3-L bioreactor [131]. A positive influence of impurities has even been reported. Productivity of 1.51 g/L h using crude glycerol as compared with 0.84 g/L h with pure glycerol was reported by Jun et al. [132] after 47 h of fermentation using *Klebsiella pneumoniae*.

3.3.4. Microbial Hydrogen Production

Hydrogen (H_2) is a clean and renewable fuel that produces only water on combustion. This can significantly reduce CO2, NOx, particulate and other emissions. Hydrogen not only serves as a clean fuel, but it is a valuable feedstock for production of food, pharmaceuticals, specialty chemicals and petrochemical products [133]. Four groups of microorganisms including anaerobic, facultative anaerobic, aerobic and phototrophic are involved in the production of hydrogen based on the biosynthesis pathways. The main fermentative bacteria known to produce hydrogen include *Enterobacter* sp., *Bacillus* sp., *Clostridium* sp., *Klebsiella* sp. and *Citrobacter* sp. [134]. Dark fermentation, photo fermentation, microbial electrolysis cells (MECs) and microbial fuel cells (MFCs) and combined methods were applied using glycerol as substrate for production of biohydrogen [8,13]. Dark fermentation showed better performance in terms of production rate, yield and cost as compared with H_2 production usingt photosynthetic techniques. The cost of H_2 production via the photosynthetic pathway is 300-fold higher than fermentative H_2 production [135]. Microorganisms such as *Clostridium* sp. and *Klebsiella pneumoniae* [136,137] in dark fermentation and *Rhodopseudomonas palustris* [138] in photosynthetic fermentation were used. A combination of dark fermentation with a MEC was studied using glycerol, and a maximum H_2 rate of 332 mL/L and a yield of 0.55 mol H_2/mol glycerol was achieved [139].

3.3.5. Succinic Acid

Succinic acid (SA) is a C4 linear saturated dicarboxylic acid and as a platform chemical plays a significant role in the synthesis of polymers such as polyesters and polyurethane. Furthermore, it has several applications in food, cosmetics and pharmaceuticals [140]. In 2018, the global production of (SA) was 131.71 million tones, and it is predicted that its market will increase further [141]. Production of SA on an industrial scale is based on the oxidation of maleic anhydride [142]. The high cost of this chemical conversion has motivated scientists to seek alternative economical processes. SA can be produced using microbial fermentation and a low-cost substrate such as glycerol, which will help to reduce the cost of production substantially. Moreover, biological conversion of SA requires the addition of CO2 to the culture broth, which contributes to the reduction of CO2 emissions [143].

Anaerobic facultative bacteria like *Mannheimia succiniciproducens* [144], *Anaerobiospirillum succiniciproducens* [145], *Actinobacillus succinogenes* [146] or *Basfia succiniciproducens* DD1 [147] have been reported to produce SA as a fermentation end-product. Engineered strains such as *Yarrowia lipolytica* [148] and *Escherichia coli* [149] have also been reported. *Anaerobiospirillum succinici* is one of the best SA producers, with production of 19 g/L of succinate using glycerol as the only carbon source in the medium [150]. One of the limiting factors to achieving a higher yield is the redox imbalance during cell growth. Utilization of an external electron acceptor such as dimethylsulfoxide can improve the SA production in this strain [151].

3.3.6. Citric Acid

Citric acid is an organic acid with various applications, mainly in the food industry and pharmaceutical industry. Several microorganisms including bacteria, fungi and yeasts have been studied for the production of citric acid. *Aspergillus niger* has been used on an industrial scale [152] for microbial production of citric acid for a long time. Among citric acid producers, yeasts showed better resistance against metal ions which facilitate the use of non-pure substrate such as crude glycerol.

This in turn improves the overall economy of the process [152]. In addition, the unicellular nature of yeast allows a better process control [153,154]. *Candida (Yarrowia) lipolytica*, *Candida guilliermondii*, *Candida oleophila*, *Candida intermedia*, *Candida paratropicalis*, *Candida zeylanoides*, *Candida catenulata*, *Candida parapsilosis* and *Pichia anomala* are the yeast species that were reported to produce citric acid [155,156]. Mutant strains of *Yarrowia lipolytica* were also reported to increase the production on glycerol [157]. It is a potential citric acid producer using this cheap substrate. Submerged fermentation in batch mode is the most common technique for this bioconversion [156].

4. Conclusions

The biodiesel industry is increasing globally, however, the economics aspect of this production remains a challenge. The fluctuation of the crude oil industry also affects the competitive price of biodiesel and other biofuels. Utilizing the desired by-product of biodiesel, glycerol, can contribute to the sustainable growth and economics of this high demand biofuel. Finding markets for crude glycerol will also address the environmental issues associated with the surplus generation of glycerol with expected growth of biodiesel production. We have attempted to highlight and evaluate various facets of glycerol valorization, ranging from crude glycerol composition, its purification and multiple routes of and the importance of taking into account glycerol purity when designing biodiesel plants. Glycerol esters, glycerol ether, glycerol formal, fuel additives and syngas can be obtained by chemical modification of glycerol. In addition, the reduced nature of carbon atoms in glycerol makes this low-cost substrate more attractive as compared with more oxidized carbon sources for biological conversion of glycerol to value-added biochemicals such as microbial lipid, 1, 3-propanediol, microbial hydrogen, succinic acid and citric acid. Both native and engineered microorganisms were able to consume pure as well as crude glycerol efficiently. In general, the high potential of using glycerol generated by the biodiesel industry needs to be harnessed as it will bring about economic and environmental benefits locally and globally. This in turn will help to move towards a circular bioeconomy.

Conflicts of Interest: The authors declare no conflict of interest.

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