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Review

Microbial Conversion of Waste Glycerol from Biodiesel Production into Value-Added Products

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Abstract: Biodiesel has gained a significant amount of attention over the past decade as an environmentally friendly fuel that is capable of being utilized by a conventional diesel engine. However, the biodiesel production process generates glycerol-containing waste streams which have become a disposal issue for biodiesel plants and generated a surplus of glycerol. A value-added opportunity is needed in order to compensate for disposal-associated costs. Microbial conversions from glycerol to valuable chemicals performed by various bacteria, yeast, fungi, and microalgae are discussed in this review paper, as well as the possibility of extending these conversions to microbial electrochemical technologies.

Keywords: biodiesel waste glycerol; microbial conversion; 1,3-propanediol; ethanol; lactic acid; hydrogen; citric acid; microbial electrochemical technologies

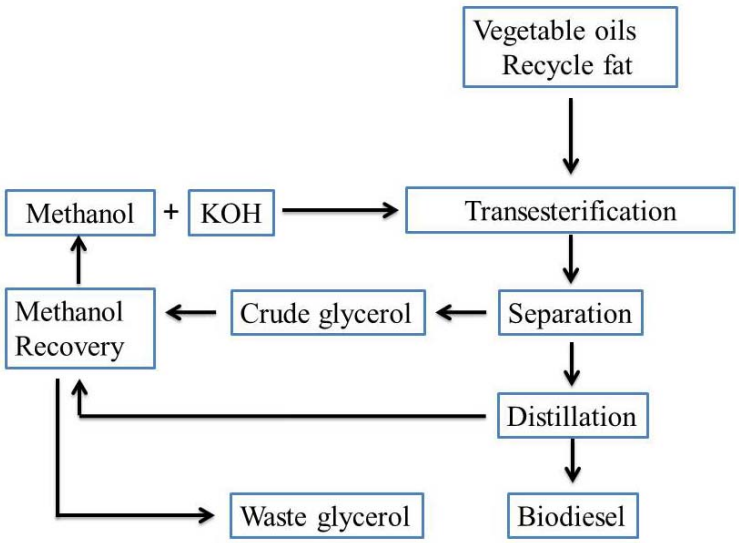
1. Introduction

Consumption of fossil energy is the foundation of modern society, from moving vehicles to lighting bulbs. However, many concerns have been raised about refining efficiency, effects on climate change, air pollution, and diminishing fossil fuel deposits [1]. Biodiesel is a fast growing alternative fuel that can be applied to a conventional diesel engine. The biodiesel demand is clearly increasing in the United States as annual production of biodiesel in 2012 (969 million gallons) was 2.83 times higher than in 2010 (343 million gallons) [2]. Furthermore, under a mandate by the renewable fuels standard

(RFS2) of the U.S. Energy Independence and Security Act of 2007, biomass-based diesel production is expected to increase to 36 billion by 2022 [3].

The flow chart of biodiesel production is shown in Figure 1. Biodiesel is an environmentally attractive alternative fuel, which compared to petro-diesel, holds technical advantages like inherent lubricity, low toxicity, biodegradability, renewability, *etc*. Biodiesel itself is composed of mono-alkyl esters of vegetable oils or animal fats and is produced by transesterification with a monohydric alcohol (methanol) [3]. During the transesterification reaction, the glycerol backbone of triacylglycerol is substituted by methanol in the presence of a catalyst at elevated temperature, leaving fatty acid alkyl esters and glycerol as the major liquid products. Waste glycerol, the liquid content after methanol recovery, has a pH around 10 and viscosity from 1213 to 1515 mPa·s [4]. The glycerol content in waste glycerol is from 27 wt% to 28 wt% with a methanol concentration that can vary from 6.2 wt% to 12.6 wt%. Trace amounts of soap formed in an undesirable saponification reaction can also be found in waste glycerol [4]. Previously, commercial glycerol synthesis was primarily performed by propylene chlorine hydrolyzation in caustic environments [5]. Nowadays, the chemical synthesis of glycerol only accounts for about 10% of the current market because of the increasing cost of petrochemical precursors and decreasing price of pure glycerol [6]. In the past few years, the price of refined glycerol had dropped from $1.15 per kilogram to $0.66 per kilogram while the price of waste glycerol has also dropped from $0.44 to $0.11 per kilogram [1,7]. The expansion of the biodiesel industry has thus created a surplus of glycerol, resulting in an inevitable abundance of waste glycerol now considered as a waste stream with associated disposal costs [6]. Therefore, the need to find efficient approaches to convert waste glycerol into more desirable products is urgent and necessary.

Figure 1. Scheme of biodiesel production and waste glycerol (adapted from [6]).



Value-added transformation processes of waste glycerol can be accomplished by direct application, chemical transformation, or microbial conversion [1,8–10]. Direct application refers to processes without any catalytic reaction, instead treating waste glycerol as a simple carbon source. One particular example of direct application is using biodiesel waste glycerol in animal feed [1,11]. However, some impurities within waste glycerol like methanol and potassium may result in harmful effects to animals, and thus concentrations need to be controlled [1]. Chemical transformation can be understood

through three major approaches: (1) oxidation/reduction of glycerol into other 3-carbon compounds; (2) synthesis of higher carbon compounds with glycerol and other substrates; and (3) industrial combustion [9]. These traditional chemical catalytic processes often include expensive metal catalysts, toxic intermediates, and low conversion rates [9]. Moreover, it is difficult to combust glycerol efficiently due to its low energy density, high viscosity, high auto-ignition temperature, and potential emission problems [8]. Compared to direct application and chemical transformation, microbial conversion is a viable alternative that avoids certain disadvantages such as low product specificity, high energy input (pressure/temperature) and intensive pretreatment requirements [1,6]. On the other hand, compared to conventional biorefinery substrates, such as glucose and sucrose, waste glycerol presents a class of substrates that are inexpensive, sustainable, and not considered a suitable human food source. In addition, glycerol has a higher degree of reductant and NADH generation rate than other common microbial feedstocks. Consuming one mole of glycerol generates two additional moles of NADH, while consuming a half mole of glucose (equally on a 3-carbon basis) only generates one additional mole of NADH [12,13].

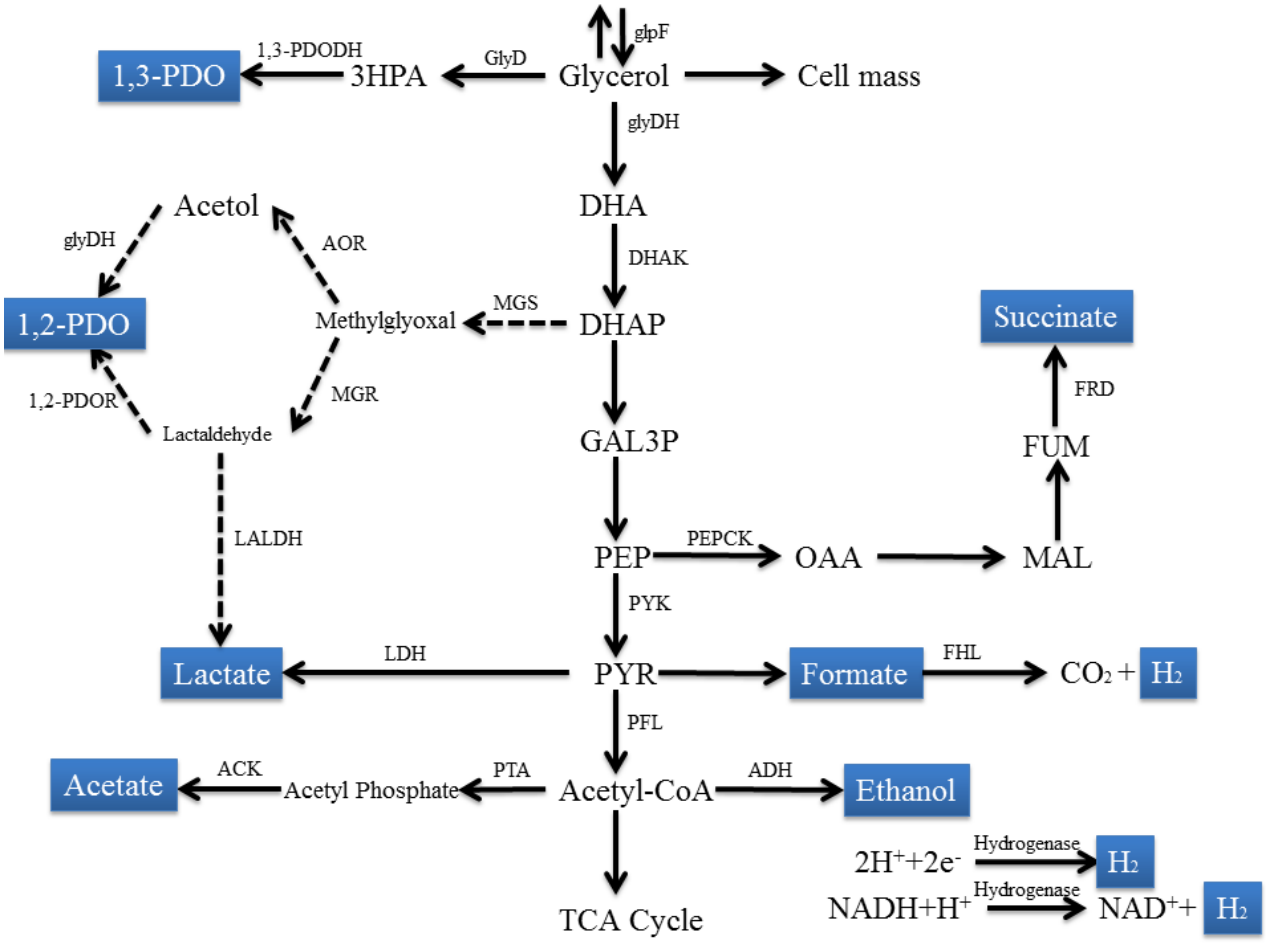
With the development of the biodiesel industry, new breakthroughs have been made each year using different microbial species and bioengineering techniques to convert waste glycerol to value-added products. There are several excellent reviews published in the past several years with a focus on specific products, microbial species, or chemical catalytic processes [1,6,9,14,15]. In this review, the glycerol metabolic pathways of representative bacterial and yeast species will be comprehensively discussed. This includes the capability of various microbial species to convert glycerol to value-added chemicals addressed in terms of yield, productivity and final concentration. We also introduced and discussed microbial electrochemical technologies that may be used as a strategy for generating value-added chemicals as well as electrical energy directly from glycerol.

2. Microbial Conversion of Glycerol

2.1. Fermentation of Glycerol by Enterobacteriaceae *Family*

Enterobacteriaceae is a large family of gram-negative bacteria that includes *Escherichia*, *Klebsiella*, and *Citrobacter* among others [16]. The glycerol utilization pathways in *Entrerobacteriaceae* have been intensively studied with the goal of industrial production of 1,3-propanediol (1,3-PDO) [6]. These pathways may also be applied to some other bacteria that contain similar metabolic grids. Figure 2 illustrates the general metabolic pathways for glycerol utilization in *Entrerobacteriaceae*.

Figure 2. Prokaryotic pathway of glycerol utilization in *Entrerobacteriaceae* species (adapted from [4,17]). Enzymes: ACK, acetate kinase; ADH, acetaldehyde/alcohol dehydrogenase; AOR, aldehyde oxidoreductase; DHAK, dihydroxyacetone kinase; FHL, formate hydrogen lyase complex; FRD, fumarate reductase; GlyD, glycerol dehydratase; glyDH, glycerol dehydrogenase; glpF, glycerol transporter, LALDH, lactaldehyde dehydrogenase; LDH, lactate dehydrogenase; MGR, methylglyoxal reductase; MGS, methylglyoxal synthase; PFL, pyruvate formate-lyase; PEPCK, phosphoenolpyruvate carboxykinase; PTA, phosphate acetyltransferase; PYK, pyruvate kinase; 1,2-PDOR, 1,2-propanediol reductase; 1,3-PDODH, 1,3-propanediol dehydrogenase. Chemical intermediates and products: DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; FUM, fumarate; GAL3P, glyceraldehyde-3-phosphate; 3HPA, 3-hydroxypropionaldehyde; MAL, malate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PYR, pyruvate; 1,2-PDO, 1,2-propanediol; 1,3-PDO, 1,3-propanediol. (Dash line indicates reactions for 1,2-PDO pathway; filled box indicates potential products).



Glycerol can be actively transported inside the cytoplasm by a transporter protein (glpF) [18], known as a glycerol facilitator [19]. Intracellular glycerol is converted to dihydroxyacetone (DHA) by a NAD^+-dependent glycerol dehydrogenase (glyDH), and then phosphorylated to dihydroxyacetone phosphate (DHAP) by a phosphoenolpyruvate (PEP) dependent DHA kinase (DHAK) [6]. DHAP is further oxidized through glycolysis to form building blocks like phosphoenolpyruvate (PEP), pyruvate

(PYR), and acetyl-CoA. The high-energy molecule PEP can be converted to oxaloacetate by phosphoenolpyruvate carboxykinase and then reduced stepwise to succinate in the presence of certain enzymes involved in the TCA cycle. Pyruvate can then be reduced to lactate by lactate dehydrogenase (LDH). Acetyl-CoA can be converted to acetate by both phosphate acetyltransferase (PTA) and acetate kinase (ACK), or be reduced to ethanol by acetaldehyde/alcohol dehydrogenase (ADH).

The reductive pathways act as an electron sink for oxidation, consuming reducing equivalents generated during the stepwise oxidation of glycerol to achieve redox balance. Differing from other substrates, glycerol has a high degree of reductant, therefore one particular challenge for glycerol fermenting species is the consumption of excess reducing equivalent [20]. A B_{12}-dependent glycerol dehydratase (GlyD) and a 1,3-propanediol dehydrogenase (1,3-PDODH) were identified in the reductive pathway for this purpose [21]. The 1,2-propanediol (1,2-PDO) pathway, which is present primarily in *Escherichia coli* strains, might act as an alternative mechanism for cells to regenerate NAD^+ [22]. During the reductive pathway, some of the electrons can be diverted to combine with protons and released as hydrogen gas under anaerobic conditions and low partial pressure of hydrogen [23]. It is also suggested that an acetaldehyde/alcohol dehydrogenase might carry out an important role in glycerol fermentation for regaining redox balance [18].

2.1.1. *Escherichia* Species

As the most well-studied member in *Enterobacteriaceae* family, both wild type and engineered strains of *Escherichia coli* can be considered a bacterial platform for producing value-added metabolites [24]. Glycerol fermentation performed by *Escherichia coli* can produce ethanol, lactic acid, 1,2-PDO, 1,3-PDO, and succinic acid, all of which represent value-added opportunities.

Ethanol is typically formed to fulfill the energy requirement of a bacterial cell. Ethanol can also serve as a direct fuel or as a gasoline additive for its octane booster effect [25], though ethanol conversion rates by wild type *E. coli* are often not high enough to satisfy the needs of industrial production [18,22]. To maximize ethanol production, strain SY03 was constructed by inactivating enzymes responsible for succinate and acetate synthesis (fumarate reductase and phosphate acetyltransferase). This strain was shown to be capable of producing 1 mole of ethanol and 1 mole of hydrogen gas per mole of glycerol consumed [26] (Table 1).

Table 1. Microbial conversion of glycerol to value-add products.

Bacterial species	Strain	Product	Yield (mol/mol glycerol)	Productivity (g/L/h)	Final concentration (g/L)	Ref.
Escherichia coli	Engineered *E. coli* SY03	Ethanol	1	0.051	5	[26]
	E. coli AC521	Lactic acid	0.9	0.97	85.8	[27]
	Engineered *E. coli*	D-lactic acid	0.82 (pure glycerol) 0.87 (waste glycerol)	1.5 (pure glycerol)	32 (pure glycerol) 34 (waste glycerol)	[28]
	Engineered *E. coli*	L-lactic acid	0.91	-	50	[29]
	Engineered *E. coli*	1,2-PDO	0.26	-	5.6	[30]
	Engineered *E. coli*	1,3-PDO	1.09	2.61	104.4	[31]
	Engineered *E. coli*	Succinate	0.8	-	12	[32]
Klebsiella	*K. pneumonia* (Encapsulated)	1,3-PDO	0.65 (batch) 0.43 (continuous)	4.46 (continuous)	-	[33]
	K. pneumonia (Pilot scale)	1,3-PDO	0.58	0.92	58.8	[34]
	K. oxytoca (Lactate deficient)	1,3-PDO	0.41–0.53	0.63–0.83	-	[35]
	K. pneumonia (Inactivated ADH)	1,3-PDO	0.70	1.07	-	[36]
	K. pneumonia	2,3-BD	0.36	0.18	49.2	[37]
	Engineered *K. pneumonia*	Ethanol	0.89	1.2	31.0	[38]
Citrobacter	*C. freundii* FMCC-B294	1,3-PDO	0.48	0.79	68.1	[39]
	C. werkmanii DSM 17579	1,3-PDO	0.62	2.84	-	[40]
	C. freundii H3	H_2	0.94	-	-	[41]
	Engineered *C. freundii*	Violacein	-	82.6 mg/L/h	4.13	[42]

Table 1. *Cont.*

Bacterial species	Strain	Product	Yield (mol/mol glycerol)	Productivity (g/L/h)	Final concentration (g/L)	Ref.
Clostridium	*C. butyricum* VPI 3266	1,3-PDO	0.65	10.3	-	[43]
	C. butyricum AKR102a	1,3-PDO	0.63 (pure glycerol)	3.3 (pure glycerol)	93.7 (pure glycerol)	[44]
	C. butyricum VPI 1718	1,3-PDO	0.665	-	67.9	[45]
	Engineered *C. acetobutylicum*	1,3-PDO	0.66	3	-	[46]
	C. pasteurianam (immobilized)	n-butanol	0.43	0.074	8.84	[47]
	C. pasteurianam ATCC 6013	1,3-PDO and butanol	0.17 (1,3-PDO) 0.28 (butanol)	0.42 (1,3-PDO) 2.49 (butanol)	-	[48]
Propionibacterium bacteria	Engineered *P. acidipropionici* strain	Propionic acid	0.66 (pure glycerol), 0.88 (waste glycerol)	0.10 (pure glycerol), 0.085 (waste glycerol)	106 (pure glycerol)	[49]
	P. freudenreichi subsp. Shermanii NCIM 5137	Trehalose	391 mg/g biomass	-	-	[50]
Other bacteria and mixed culture	*R. palustris* CGA009	H_2	6	-	-	[51]
	P. macerans	H_2	0.801	-	-	[52]
	Thermoanaerobacterium sp.	H_2	0.30	-	-	[53]
	Mixed culture	H_2	0.96	91 mL/L/h	-	[54]
	Mixed culture	H_2/formate	0.80	-	-	[55]
	Mixed culture	H_2	0.28 (pure glycerol) 0.31 (waste glycerol)	-	-	[23]
	L. acidophilus	Probiotic cell mass	0.37 g/g	-	2.11	
	L. diolivorans	1,3-PDO	-	-	73.7	
	Anaerobic co-digestion	Biogas	-	-	1210 mL/d	[56]
	C. necator DSM 545	PHAs	-	1.1	-	[57]
	Z. denitrificans MW1	PHAs	0.31 g/g glycerol	-	-	[58]
	P. putida GO16	PHAs	-	0.11	-	[59]

Table 1. *Cont.*

Bacterial species	Strain	Product	Yield (mol/mol glycerol)	Productivity (g/L/h)	Final concentration (g/L)	Ref.
Yeasts	*Y. lipolytica* NCIM 3589	Citric acid	-	-	77.4	[60]
	Y. lipolytica Wratislavia AWG7	Citric acid	0.33	1.16	139	[61]
	C. bombicola ATCC 22214	Sophorolipids	-	-	60	[62]
	P. antarctica JCM 10317	Mannosylerythritol lipid	-	-	16.3	[63]
	Cryptococcus curvatus	SCO	52% lipid content	-	17.1	[64]
	Rhodotorula glutinis	SCO	36.5% lipid content	-	5.4	[65]
	S. ruberrimus CSB 2636	Carotenoid	41.9 μg/g glycerol,	56.9μg/L/h	3425.9μg/L	[66]
	Engineered *S. cerevisiae*	Ethanol	-	-	2.4 g/L	[67]
	Engineered *S. cerevisiae*	1,2-PDO	0.258	-	2.19	[68]
Fungi	*L. edodes* strains	SCO	0.1 g/g biomass	-	0.52	[69]
	A. niger strains	SCO	0.41 to 0.57 g/g biomass	-	3.1 to 3.5	[69]
	Galactomyces geotrichum	SCO	0.44 g/g biomass	-	-	[70]
	Thamnidium elegans	SCO	-	-	11.6	[71]
	Pythium irregulare	EPA	-	14.9 mg/L/day	90 mg/L	[72]
	Blakeslea trispora	β-carotene	15 mg/g biomass	-	-	[73]
Microalgae	*S. limacinum* SR21	DHA	-	0.51	-	[74]
Microbial Electrochemical Technology	*B. subtilis* MFC	Electricity	Maximum power density 600 mW/m^2			[75]
	Single chamber MFC	Electricity	Maximum power density 2110 mW/m^2			[76]
	Single chamber MFC	Electricity	Maximum power density 4579 mW/m^3 with pure glycerol, 2324 mW/m^3 with waster glycerol			[77]
	E. aerogenes MEC	H_2	0.74	-	-	[78]
	Mixed culture MEC	H_2	3.9	-	-	[79]
	MEC with gas phase cathode	H_2	5.4	0.6 L/L/day	-	[80]

Lactic acid can be produced during glycerol fermentation by some *E. coli* strains as an alternative to NAD^+ regeneration in the absence of external electron acceptors [27]. Lactic acid has many applications as a food additive, acidulant, as well in the production of biodegradable polylactic acid [81,82]. Food-grade lactic acid has a price ranging between $1.38 per kilogram (50% purity) and $1.88 per kilogram (88% purity) [83,84]. Compared to traditional chemical synthesis methods, microbial conversion of lactic acid favors the formation of one specific configuration, either D- or L-, due to the high specificity of lactate dehydrogenase (LDH) [28,29], a property that can simplify downstream processes such as separation and purification. High chiral purity of D-lactate can be produced by fermenting glycerol using a recombinant strain that overexpresses enzymes that respond to glycolytic intermediates and inactivates fumarate reductase, phosphate acetyltransferase, alcohol/acetaldehyde dehydrogenase, and D-lactate dehydrogenase [28]. Thirty-two grams per liter of D-lactate (99.9% chiral purity) could be produced from 40 g/L of glycerol (0.82 mole lactate per mole of consumed glycerol, 1.5 g/L/h productivity). This strain was also tested for the ability to utilize waste glycerol as a substrate, and a higher yield was observed (0.87 mole lactate per mole of consumed glycerol) with the final concentration of D-lactic acid of 34 g/L [28]. Furthermore, an L-specific LDH from *Streptococcus bovis* was able to be introduced to replace the native *E. coli* D-specific LDH from the previous study. Fifty grams per liter of L-lactic acid (99.9% chiral purity) was produced from 56 g/L of waste glycerol with a yield of 0.91 mol/mol glycerol. Other than engineered strains, lactic acid production was also observed in glycerol fermentation by *E. coli* AC-521, a wild-type soil bacterium [27]. The yield reached 0.9 mole lactic acid per mole consumed glycerol with a final concentration of about 85.8 g/L (0.97 g/L/h productivity), however no data about chiral purity was presented in this study.

A 1,2-PDO-dependent glycerol fermentation capability has been discovered in some *E. coli* strains [18,22]. 1,2-PDO is a chemical that can serve as a building block for polyesters, anti-freeze agents, or solvents [85], and is currently priced around $1.08 to $1.59 per kg with an estimated global demand around 1.36 billion kilogram per year [86]. To enhance natural production of 1,2-PDO, an engineered *E. coli* was constructed by disrupting acetate and lactate synthesis and replacing the native PEP-dependent dihydroxyacetone kinase with an ATP-dependent dihydroxyacetone kinase from *Citrobacter freundii*. This causes the overexpressing enzymes responsible for the reductive 1,2-PDO pathway, and results in a strain that can produce 5.6 g/L of 1,2-PDO from the fermentation of glycerol with a yield of 0.26 mol/mol glycerol [30].

E. coli has also been genetically modified to generate desired products that are not naturally produced from glycerol by *E. coli* such as 1,3-PDO and succinate [6]. 1,3-PDO is a building block of biodegradable plastic polytrimethyleneterephthalate (PTT) and also a valuable product with various uses (resins, coolants, mortars and inks). A recombinant strain of *E. coli* was constructed by transferring the B_{12}-independent glycerol dehydratase *DhaB1* and its activating factor *DhaB2* from *Clostridium butyrium*. The final concentration, yield and overall productivity of 1,3-PDO was 104.4 g/L, 1.09 mol/mol and 2.61 g/L/h, respectively [31]. Another product capable of being produced by recombinant *E.coli.*is succinate. Succinate is one of the top twelve building block chemicals according to the U.S. Department of Energy and is typically used for creating biodegradable plastic polybutylene succinate [87]. Current glucose to succinic acid commercial production techniques are relatively sophisticated, and are capable of producing a succinic acid yield about 1.45 mol/mol glucose [88].

However, using glycerol as a substrate has advantages such as the low cost of the feedstock and higher yield of succinic acid (on an equal 3-carbon basis) [17]. A comparable yield (0.8 mol/mol glycerol) of succinic acid production was achieved in the recombinant *E. coli*, although the final concentration was low (12 g/L) [32]. To construct this strain, phosphoenolpyruvate carboxykinase (PEPCK) was upregulated, and genes responsible for ethanol and formate formation were deleted.

2.1.2. *Klebsiella* Species

As another member in the *Enterobacteriaceae* family, *Klebsiella* is considered one of the most promising candidates for producing 1,3-PDO from glycerol [34] and over the past few years a large amount of *Klebsiella* research has been focused on enhancing the production of 1,3-PDO production during the fermentation of waste glycerol. Pilot scale studies as large as 5000 L have been launched in order to test *Klebsiella's* potential for industrial application [34]. Syntheses of other products, like 2,3-butanediol (2,3-BD) and ethanol have also been reported in considerable amounts [37,38].

To increase the production of 1,3-PDO through the fermentation of glycerol by *Klebsiella* species, genetic enhancements were employed with different strategies such as minimizing undesired byproducts or increasing the utilization of glycerol. The formation of lactate and ethanol compete with the formation of 1,3-PDO through the consumption of reducing equivalents, and often impose difficulties on the downstream processes responsible for the purification and recovery of 1,3-PDO [35,36]. By creating a lactate deficient mutant (LDH3) from *K. oxytoca* M5a1, the productivity and yield of 1,3-PDO were increased from 0.63 to 0.83 g/L/h to 0.41 to 0.53 mol/mol glycerol, respectively [35]. By inactivating aldehyde dehydrogenase (ADH), the enzyme responsible for ethanol formation in *K. pneumoniae* YMU2, less ethanol was produced in the broth (from 9.26 g/L to 1.70 g/L) with a higher productivity (from 0.81 g/L/h to 1.07 g/L/h) and yield (from 0.36 mol/mol to 0.70 mol/mol) of 1,3-PDO observed [36]. To enhance both glycerol oxidative and reductive metabolisms, formate dehydrogenase (FDH) from *Candida boidinii* was expressed in *K. oxytoca* YMU1 [89]. The subsequent yield of 1,3-PDO increased about 17.3%, from 0.39 to 0.45 mol/mol glycerol.

Other value-added chemicals can also be produced through glycerol fermentation by *Klebsiella* species. For example, 2,3-BD was found to be the major product of fermentation by *K. pneumonia* G31 under microaerobic conditions with an initial alkaline pH [37]. 2,3-BD is a high-value chemical that can serve as a precursor for chemical products like methyl ethyl ketone, γ-butyrolactone, and 1,3-butadiene [90]. The final concentration of 2,3-BD reached 49.2 g/L after 280 h of fermentation (a productivity of 0.18 g/L/h) and the overall yield was 0.36 mol/mol glycerol. An engineered strain of *K. pneumonia* can produce ethanol when the lactate dehydrogenase was inactivated and pyruvate decarboxylase along with aldehyde dehydrogenase from *Zymomonas mobilis* were introduced. Compared to the wild type strain, final concentration, yield, and productivity were improved from 21.5 g/L to 31.0 g/L, 0.62 mol/mol glycerol to 0.89 mol/mol glycerol, and 0.93 g/L/h to 1.2 g/L/h, respectively [38].

2.1.3. *Citrobacter* Species

Citrobacter species are well-known for their ability to produce 1,3-PDO from fermenting glycerol. Studies using chemical grade glycerol in the mid-90s had revealed that the final concentration of

1,3-PDO produced by *C. freundii* to be comparable to those produced by *Klebsiella* and *Clostridium* species, although the reaction rate is much slower [91]. Recent studies have shown that both wild-type and engineered *Citrobacter* species are capable of using pretreated biodiesel waste glycerol as a substrate to produce various value-added products. For example, *C. freundii* strain (FMCC-B 294) can ferment pretreated waste glycerol to produce 1,3-PDO in fed-batch fermentation achieving final concentration of 68.1 g/L with yield of 0.48 mol/mol and volumetric productivity of 0.79 g/L/h [39]. This strain can also endure non-sterile feeding, which may reduce the energy requirement for pretreatment. Other than *C. freundii*, *C. werkmanii* DSM 17579 is another potential strain for 1,3-PDO production. The highest achieved yield per mole of glycerol consumed and productivity in fed-batch fermentation was 0.62 mol/mol and 2.84 g/L/h, respectively [40]. However, the highest yield, productivity, and final concentration of 1,3-PDO achieved by *C. freundii* was lower than those achieved by *Clostridium* species. Besides PDO, *C. freundii* H3 can ferment chemical grade glycerol to produce H_2 with a yield of 0.94 mol/mol [41]. A recombinant of *C. freundii* aimed at producing violacein was examined for its ability to ferment glycerol as substrate. Violacein is a blue-purple bacterial pigment that has antibacterial, antioxidant, antiviral, and anti-protozoal properties [92]. The maximum final concentration and productivity were 4.13 g/L and 82.6 mg/L/h, respectively [42].

2.2. Clostridium *Species*

Similar to *Klebsiella*, the glycerol fermenting ability of *Clostridium* species has been extensively studied with the goal of enhancing the production of 1,3-PDO [93]. The value-added product butanol was also discovered during fermentation.

The possible effects of dilution rate and substrate concentration on glycerol fermentation of *C. butyricum* VPI 3266 were examined [43]. The highest productivity of 1,3-PDO (10.3 g/L/h) was achieved under the dilution rate 0.30 h^{-1} and at substrate concentration of 60 g/L. The yield was around 0.65 mol/mol. Glycerol fermentation of *C. butyricum* AKR102a in an automatic fed-batch reactor can produce 93.7 g/L (3.3 g/L/h) 1,3-PDO from pure glycerol and 76.2 g/L from pretreated waste glycerol (2.3 g/L/h) [44]. *C. butyrium* VPI 1718 was tested for production of 1,3-PDO from non-sterile waste glycerol and was found to produce 1,3-PDO with a final concentration of 67.9 g/L, and yield of 0.67 mol/mol glycerol [45]. An engineered strain was constructed by introducing the reductive pathway of *C. butyricum* to *C. acetobutylicum* [46]. This recombinant, DG1(pSPD5), was capable of fermenting glycerol with a 1,3-PDO productivity of 3 g/L/h and a yield of 0.66 mol/mol glycerol.

Other than 1,3-PDO, butanol can also be produced through the fermentation of glycerol by *Clostridium* [47]. Butanol is a chemical that can be applied as a chemical synthesis building block, solvent, or as a potential biofuel. Microbial production of butanol lost its primary advantage in the 1960s due to the increasing cost of suitable substrates [94], but with the current low price of biodiesel waste glycerol combined with advances in genetic engineering techniques this alternative pathway may now become commercially practical. The simultaneous production of 1,3-PDO and butanol was reported in *C. pasteurianam* (ATCC 6013) by feeding pretreated waste glycerol with productivities of 0.42 g/L/h (0.17 mol/mol) and 2.49 g/L/h (0.28 mol/mol), respectively [48].

2.3. Propionibacterium *Species*

Fermentative glycerol dissimilation in *Propionibacteria* strains has been investigated for the production of propionic acid and trehalose [4,17]. As an important intermediate for cellulose fibers, herbicides and perfumes, large quantities of propionic acid are produced by chemical synthesis, e.g., oxidation of propionaldehyde with air and the catalytic dehydration of glycerol [49,95,96]. One of the best natural producers of propionate from glycerol is *Propionibacterium acidipropionici*, capable of achieving a final concentration of 42 g/L propionate with an overall yield of 0.84 mol/mol of glycerol consumed and a productivity of 0.36 g/L/h using technical grade glycerol [17]. A knockout mutant strain of *P. acidipropionici* lacking acetate kinase (ACK) was studied for its ability to use glycerol as sole carbon source during fermentation [49]. The final concentration of propionate (106 g/L) from the fermentation of glycerol was much higher than wild type (42 g/L). The yield and productivity, however, were lower than wild type (0.66 mol/mol consumed glycerol and 0.10 g/L/h). When pretreated waste glycerol was used as carbon source, this mutant strain achieved a higher yield of propionate (0.88 mol/mol glycerol) and a slightly lower productivity (0.09 g/L/h).

Trehalose can also accumulate in the biomass of some *Propionibacteria* strains during glycerol fermentation. Trehalose is a non-reducing sugar that can protect bacterial cells against osmotic stress and has value in the food and cosmetic industries as well as clinical applications [97]. Although enzymatic transformations have been reported as a viable method for trehalose production [98], accumulation of trehalose in microbial biomass is considered a less expensive method when a cheap carbon source is used [93]. Wild type and osmotic sensitive mutants of *P. freudenreichi subsp. Shermanii* were analyzed for the production of trehalose from waste glycerol [50]. The final concentration of trehalose was higher in the mutant strain than in wild type (678 mg/L to 158 mg/L). The use of waste glycerol further increased the final concentration of trehalose from 678 mg/L to 1303 mg/L in the mutant strain. It was possible that impurities in waste glycerol positively influenced trehalose accumulation by creating stressful conditions that signal the bacterial cells to accumulate trehalose [50].

2.4. Other Bacterial Species and Mixed Culture

Pure bacterial species and mixed culture communities outside of *Enterobacteriaceae*, *Clostridia*, and *Propionibacterium* species have also been investigated for their abilities to convert waste glycerol into value-added products like hydrogen, 1,3-PDO, probiotic biomass, and polyhydroxyalkanoates (PHAs). For example, *Rhodopseudomonas palustris* CGA009, a purple non-sulfur photosynthetic bacterium, has been demonstrated to have the ability to photo-ferment pretreated waste glycerol to hydrogen [51], a clean and efficient energy carrier that has received enormous attention. As previously described, glycerol fermentation theoretically produces more NADH than glucose fermentation on a 3-carbon basis, and thus has higher potential to generate hydrogen gas (one mole of H_2 per mole of excess NADH) [12,13]. Gene deletion experiments have indicated that hydrogen production in *R. palustris* is mainly due to nitrogenase, which catalyzes proton reduction in the absence of nitrogen gas. The yields of hydrogen were 6 mol/mol using pure glycerol and 4 mol/mol using waste glycerol. Some strains of *Paenibacillus macerans* were also reported to grow efficiently on glycerol as sole carbon

source, but hydrogen yield (0.80 mol/mol glycerol) was much lower than that by *R. palustris* CGA009 [52]. Ethanol was the dominant liquid product while 1,2-PDO and acetone were also detected [52]. Mixed cultures have also been investigated for potential hydrogen production from waste glycerol. The estimated maximum hydrogen yields ranged from 0.8 to 0.96 mol/mol, which is comparable to the hydrogen yield with glucose as substrate on a 3-carbon basis [54,55]. Thermophilic mixed cultures were also studied for their potential ultilization of waste glycerol to produce hydrogen [53]. Although the yield (0.30 mol/mol glycerol) was not as favorable, thermophilic mixed culture fermentation might provide several advantages over mesophilic mixed culture fermentation, such as more effective pathogen control and lower risk of methanogen contamination [53,99,100].

As one of the natural producers of 1,3-PDO, *Lactobacillus diolivorans* has the potential to produce 1,3-PDO from glycerol. The highest final concentration produced was 73.7 g/L under fed-batch cultivation [101]. A recent study suggests that biodiesel waste glycerol can support cell growth of *L. acidophilus*, *L. delbrueckii*, and *L. plantarum*, with resulting probiotic cell mass obtained as a value-added product.

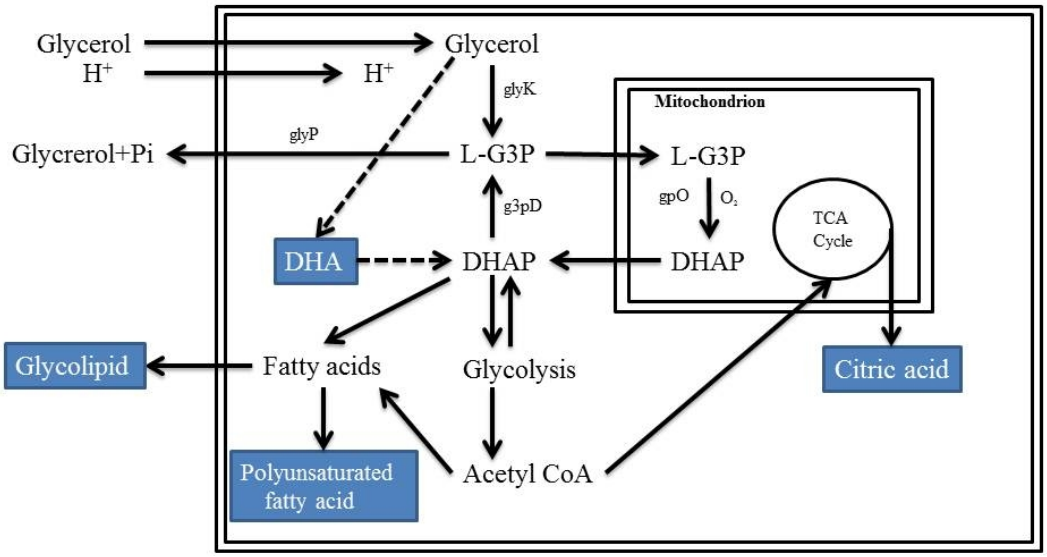
PHAs are typically stored by bacteria as a carbon source and energy reserve [102]. This chemical can also serve as an alternative to biodegradable polymers. Bacterial synthesis of PHAs is a cheaper way to produce this expensive compound compared to chemical synthesis techniques. *Cupriavidus necator* DSM 545 has demonstrated the capability to accumulate poly(3-hydroxybutyrate), a polyhydroxyalkanoate (PHA), in its cell mass by assimilating either pure glycerol or waste glycerol, with higher productivities able to be achieved using waste glycerol versus pure glycerol [57]. *Zobellella denitrificans* MW1 was also reported to use glycerol as sole substrate to accumulate poly(3-hydroxybutyrate) in its cell mass [58]. The highest yield was achieved using 10 g/L of glycerol (0.31 g/g glycerol, 80.4% of the cell dry weight). A waste glycerol supplement can increase the biomass production up to 1.7 fold and PHA accumulation up to 2.2 fold in *Pseudomonas putida* GO16 (0.11 g/L/h), compared to growth on sodium terephthalate (major plastic waste from pyrolysis of Polyethylene terephthalate) alone [59].

2.5. Microbial Conversion of Glycerol by Yeast

Glycerol is one of the byproducts of sugar fermentation in yeast, acting as an osmotic regulator and a way to balance excess reducing equivalent [103]. The glycerol transportation and dissimilation pathways of yeast have been intensively studied since the 1960s (Figure 3) [104–108]. A proton symport protein encoded by the *STL1* gene in both *Saccaromyces cerevisiae* and *Candida alba* is responsible for glycerol active transportation through the cell membrane [109,110]. However, simple diffusion was also observed in *S. cerevisiae* [107]. *GUP1*, a member of membrane-bound O-acyltransferases, has been indicated to involve extracellular glycerol transportation, and thus could also be one of the membrane-bound proteins that are responsible for the active uptake of glycerol via the proton symport system. After entering the cell membrane, glycerol is then phosphorlyated by glycerol kinase (glyK) to become glycerol-3-phosphate, a glycolytic intermediate that can be dehydrogenated in the mitochondria by glycerolphosphate oxidase (gpO) to form dyhydroxyacetone phosphate (DHAP) [107]. In another species of yeast, *Schizosaccharomyces pombe*, intracellular glycerol is directly oxidized by a NAD^+-dependent dehydrogenase to DHA. DHA is subsequently phosphorylated to DHAP after

which it may enter glycolysis. Glycolysis produces acetyl-CoA, one of the major building blocks to both TCA cycle and fatty acid synthesis, which is capable of subsequently generating valuable products like citric acid and glycolipids.

Figure 3. Eukaryotic pathway of glycerol utilization. Enzymes: glyK, glycerol kinase; glyP, glycerophosphatase; gpO, glycerophosphate oxidase; g3pD, glycerol-3-phosphate dehydrogenase. Chemical intermediates and products: DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; L-G3P, L-glycerophosphate. (Dash line indicates reactions in *Schizosaccharomyces pombe*; filled box indicates potential products).



Yeast species have been screened and investigated for their potential in converting waste glycerol to various products such as citric acid, biosurfactants, single cell oil (SCO), and carotenoids [111]. Additionally, products like ethanol and 1,2-PDO can also be produced by genetically engineered *S. cerevisiae* strains.

Citric acid is a flavoring additive and preservative agent in the food industry with a current estimated annual production of more than 800,000 tons which is expected to continue to increase by 5% per year [111–113]. Citric acid production from the submerged fermentation of *Aspergillus niger* (fungus) using sugar as substrate is a well-developed technique. However, feeding glycerol to *A. niger* does not favor citric acid production [114]. As an alternative to *A. niger*, *Yarrowia lipolytica* was investigated for citric acid production using glycerol as substrate [15]. Under optimal conditions, *Y. lipolytica* NCIM 3589 has shown the ability to yield a final citric acid concentration of 77.4 g/L, which is comparable to the final concentration obtained by *A. niger* [60]. Among all the strains tested, *Y. lipolytica* Wratislavia AWG7 produced an even higher citric acid concentration of 139 g/L after 120 h with an initial glycerol concentration of 200 g/L [61].

Glycolipids like sophorolipid and mannosylerythritol can be categorized as biosurfactants, which are a series of microbial produced molecules that have similar structures and chemical properties to surfactants [115]. Compared to other surfactants, biosurfactants contain advantages such as low toxicity and biodegradability, making them environmentally friendly for remediation processes in both liquid and on solid surfaces [116,117]. The production of biosurfactants was previously believed to

only be performed by bacterial species such as the rhamnolipid-producing *Pseudomonas aeruginosa*, but recent discoveries have shown similar levels of glycolipid production in *Candida* and *Pseudozyma* [115,118]. *Candida bombicola* ATCC 22214 has been proven to produce sophorolipids by fermenting biodiesel waste glycerol with a final concentration of about 60 g/L [62]. It has also been reported that *Pseudozyma antarctica* JCM 10317 was able to produce 16.3 g/L mannosylerythritol lipids from 100 g/L glycerol supplied with 2% mannose [63].

The oleaginous yeast *Cryptococcus curvatus* has the ability to grow on crude glycerol. Microbial lipophilic contents, often known as SCOs, contain multiple useful polyunsaturated fatty acids that have potential in both the medical and dietetic fields [119] in additional to potential use as a feedstock for second generation biodiesel production [69,111]. A fed-batch culture of *C. curvatus* was able to produce 32.9 g/L of biomass with a 52% lipid content within 12 days [64]. *Rhodotorula glutinis* is also oleaginous yeast that is able to grow on a mixture of crude glycerol and the thin stillage fraction from brewery waste to produce 14.8 g biomass per liter (36.50% lipid content) [65].

The value of carotenoids, a group of pigments, has increased along with the demand for use as a cosmetic additive and food colorant [14,120]. Yeast species *Sporobolomyces ruberrimus* CBS 2636 has recently been investigated for its potential to produce carotenoid using industrial glycerol from the soap manufacturing process as one of the co-substrates [14,66]. The maximum concentration that could be achieved was 3425.9 μg/L with a carotenoid productivity of 56.9 μg/L/h (41.9 μg/g glycerol).

S. cerevisiae can also be genetically engineered to produce some other products from glycerol fermentation, which are either not naturally produced or present in low concentrations, such as ethanol and 1,2-PDO. *S. cerevisiae* is an excellent ethanol producer when using sugar substrates. However, fermenting a more reduced substrate like glycerol to ethanol might require additional metabolic routes to handle the excess reducing equivalents. Simultaneous overexpression of glycerol dehydrogenase (*Gcy*) and dihydroxyacetone kinase (*Dak*) in *S. cerevisiae* was investigated for ethanol production by fermenting glycerol [67]. The final ethanol concentration was 1.66 g/L, 2.4 times greater than the wild type (0.69 g/L). The concentration was further increased to 2.4 g/L (3.4 fold improvement over the wild type) by overexpression of glycerol uptake protein GUP1. 1,2-PDO is not naturally produced by *S. cerevisiae,* yet recombinant strains of *S. cerevisiae* 499 were constructed to produce 1,2-PDO by fermenting glycerol [68]. A 1,2-PDO final concentration of 2.19 g/L with a yield of 0.258 mol/mol glycerol was achieved in strain *S. cerevisiae* 499 sJDPMG. This recombinant overexpressed glycerol dehydrogenase (*gdh*) and *GUP1* protein, along with the expressing methylglyoxal synthase (*mgs*) and glycerol dehydrogenase (*gldA*) from *E. coli*.

2.6. Microbial Conversion of Glycerol by Fungi

Microbial conversion of waste glycerol by fungi is another possible approach to generate value-added products, such as SCO, eicosapentanoic acid (EPA), and β-carotene, as studies have shown that fungi tend to accumulate lipids inside their mycelia [71]. *Lentinula edodes* strains AMRL 119 and AMRL 121 can produce a maximum of 5.2 g/L of biomass and a yield of 0.1 g/g biomass of lipid (mostly linoleic acid) under carbon limitation [69]. *A. niger* strains NRRL 364 and LFMB 1 were tested under nitrogen limiting conditions, resulting in 20.5–21.5g/L of oxalic acid production and 3.1–3.5 g/L of lipids with a yield of 0.41–0.57 g/g of biomass (composed by oleic acid and linoleic acid). *Galactomyces geotrichum*

is an ascomycetous fungus that has the ability to use glycerol and FFA within waste glycerol to produce 0.44 grams of lipid per gram biomass [70]. In addition to the fungi previously mentioned *Thamnidium elegans* has been shown to be able to produce up to 11.6 g/L of oil, corresponding to 71.1% wt/wt of oil in biomass [71].

As a specific member of microbial lipohilic content, EPA is an important member in omega-3 polyunsaturated fatty acid (PUFA) family, and has medical applications for treating cardiovascular disease, cancer and Alzheimer's disease [72,121,122]. PUFA is an important part of the human diet, with most dietary PUFA extracted from fish [123]. However, fish extracted PUFAs contain undesired odors and accumulated harmful heavy pollutants. Microalgae accumulation of PUFAs was evaluated as a commercial replacement for fish oil [123,124], but microalgae accumulation of EPA is often considered to be less efficient [72]. The fungus *Pythium irregulare* was capable of producing EPA from waste glycerol with a final concentration of 90 mg/L and a productivity of 14.9 mg/L/day [72]. Results from this study also suggest that impurities within waste glycerol like soap and methanol can inhibit cell growth, and thus should be removed by pretreatment.

A nutritional supplement, β-carotene, can be accumulated in the cell mass of *Blakeslea trispora* during grow on waste glycerol media. The highest concentration obtained was 15 mg β-carotene per gram of cell mass by using 60 g/L waste glycerol [73]. Impurities within waste glycerol did not inhibit the cell growth, but actually stimulated β-carotene synthesis.

2.7. Glycerol as Carbon Source for Microalgae Species

Being a potential biorefinery feedstock, microalgae species have raised considerable research interest [125] and accordingly waste glycerol has been studied as a possible cheap carbon source for growing microalgae biomass. Though glycerol can be metabolized by several microalgae species, even in the absence of carbon dioxide [126–128], the metabolic pathway of glycerol in microalgae has not been well developed. It is possible that in two of the *Mycobacterium* species, glycerol is first phosphorylated and then oxidized to triose phosphate [128]. Triose phosphate then goes through a stepwise oxidation and becomes pyruvate to enter TCA cycle. Therefore, triose phosphate might be one of the key intermediates for which oxygen is required. The alga species *Schizochytrium limacinum* SR21 can accumulate docosahexaenoic acid (DHA) in its cell mass, with DHA productivity of 0.51 g/L/day using waste glycerol as substrate [74]. DHA is an important omega-3 polyunsaturated fatty acid with research suggesting a role in preventing cardiovascular disease [74].

2.8. Converting Glycerol to Value-Added Products Using Fermentation, Co-Digestion, and Microbial Electrochemical Technologies

2.8.1. Fermentation

Generally speaking, fermentation processes can be operated in batch, fed-batch, and continuous modes [129]. In terms of glycerol microbial conversion, batch and fed-batch cultures were often employed because value-added metabolites and biomass can accumulate in relatively high final concentrations compared to continuous culture. For example, 67.9–104.4 g/L of 1,3-PDO can be produced by batch and fed-batch cultures [31,39,44,45], which are higher than concentrations achieved

in continuous culture (35.2–48.5 g/L) [130]. However, compounds other than the desired products also accumulate during the conversion process which might lead to inefficiencies due to inhibitory effects. On the other hand, continuous cultures can regulate accumulation by adjusting dilution rate, and thus have relatively high productivities. For example, 4.9–8.8 g/L/h of 1,3-PDO can be produced under low dilution rates [130]. Although low final concentrations often accompany continuous cultures, which might hinder downstream processes such as separation and purification [130].

Process-based enhancements like cell immobilization or co-digestion are often applied to these cultivation processes to improve glycerol utilization or metabolite production. Benefits conferred by cell immobilization might include increased stability in unfavorable environmental conditions during operation as well as avoiding washout [33,131,132]. For example, with microencapsulation, the biomass of *K. pneumoniae* was increased 2.6-fold compared to free cell cultures [33]. As the result, the final concentration of 1,3-PDO obtained in batch cultures was 63.1 g/L with a yield of 0.65 mol/mol glycerol. In continuous culture, the overall productivity of 1,3-PDO was 4.46 g/L/h with a yield of 0.43 mol/mol glycerol. By immobilizing *C. pasteurianam* cells on Amberlite, the maximum *n*-butanol yield of 0.43 mol/mol glycerol could be produced [47].

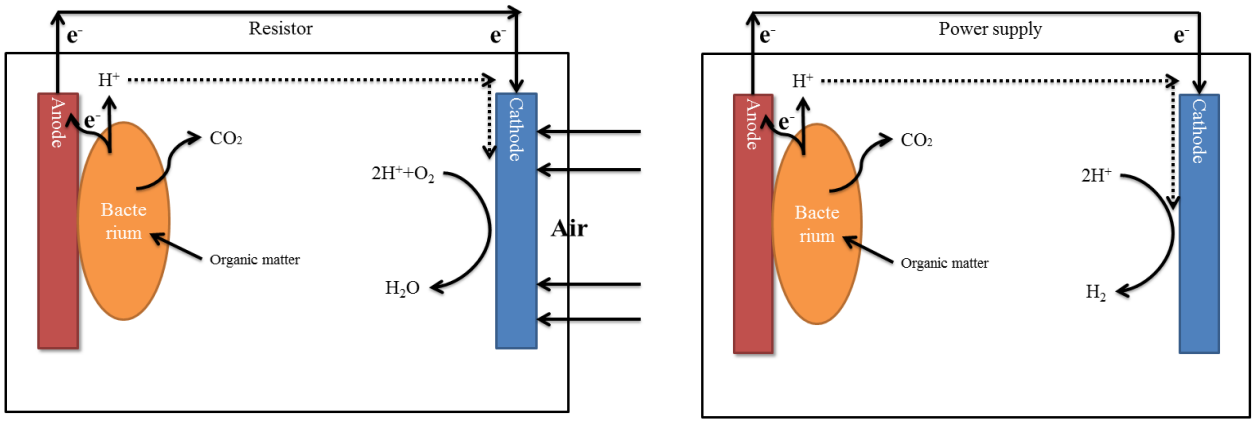
2.8.2. Anaerobic Co-Digestion

Anaerobic co-digestion of glycerol has been investigated to increase biogas productivity. Preceded by a mixed bacterial culture, this process consists of anaerobic digestion with waste glycerol as a complementary substrate to balance the C/N ratio [133]. As the result of co-digestion, useful products like biogas (consisting mainly CH_4 and CO_2) are produced. Direct utilization of waste glycerol without pretreatment could be problematic to microbes because of its alkalinity and high salt levels [134]. The application of pretreated waste glycerol has been reported as a positive supplement to increase the productivity of anaerobic digestion. For example, the methane production rate of a mixture of olive mill wastewater and slaughterhouse wastewater was increased from 479 mL/d to 1210 mL/d by volumetrically adding 1% waste glycerol as supplement [56]. Compared to mono-digestion, biogas production was also increased by about 400% under mesophilic conditions when pig manure was co-digested with 4% waste glycerol [135].

2.8.3. Microbial Electrochemical Conversion

Microbial electrochemical technologies (METs) can recover electrons from glycerol as electrical current using a microbial fuel cell (MFC) (Figure 4a) [136] or produce hydrogen using a microbial electrolysis cell (MEC) (Figure 4b) [137]. METs may contain either a mixed bacterial culture or pure culture that grows on the anode surface or in anodic chamber carrying out the oxidation of organic matter. During oxidation, electrons are released and then transferred to the anode through direct contact, electron shuttles, or conductive nanowires [136,138–141]. In an MFC, external electron acceptors (like oxygen or ferric cyanide) have to be fixed in order to create a potential difference large enough to allow current flow [142]. However, in an MEC, no external electron acceptor is added as the reduction of hydrogen ions on the cathode surface has a more negative redox potential than the anode, and thus an additional voltage must be applied to generate the current flow [143].

Figure 4. Single-Chamber microbial electrolysis cell (**a**) Single-Chamber microbial electrolysis cell (**b**) (adapted from [143]).



Both pure and mixed culture MFCs from which electrical power can be generated using glycerol are currently being investigated as an alternative method of biodiesel waste glycerol conversion. Enhancements have been made based on the selection of a favorable microbial consortia as well as improvements to MFC designs. Electrical power at a maximum density of 600 mW/m^2 can be generated from pure glycerol using a pure culture of *Bacillus subtilis* in a single chamber MFC with air cathode [75]. Although some pure bacterial strains, such as *Geobacter sulfurreducens*, have the ability to produce comparable power to mixed culture communities using defined substrates such as acetate [144], the use of mixed cultures in MFCs has the advantage of increased stability and a wider range of substrates capable of being utilized [145]. Electrical power can be directly generated from waste glycerol using a single chamber MFC with a mixed microbial consortium enriched from domestic wastewater [76]. The maximum power density generated from waste glycerol was 487 mW/m^2 with carbon cloth anode, but was further increased to 2110 mW/m^2 with a heat-treated carbon brush anode. An MFC with graphite fiber brush anodes can produce electrical current at power densities of 4.6 W/m^3 using pure glycerol and 2.3 W/m^3 using waste glycerol [77].

A recent study suggests that by externally applying current to a glycerol fermentation reactor inoculated with a mixed culture, metabolite formation can be significantly influenced with associated increases of highly reduced chemicals such as propanol and valerate [146]. Although the mechanism behind this metabolic shift was not clear, it is possibly related to the high hydrogen partial pressure caused by the current supplied in the vicinity of cathode. Besides producing more reduced, value-added products, attempts have also been made to achieve high levels of hydrogen production in pure and mixed culture MECs using glycerol as a substrate. Simultaneous production of hydrogen and ethanol from pretreated waste glycerol was reported in a two compartment MEC inoculated with *Enterobacter aerogenes* NBRC 12010 [69]. The yield of hydrogen and ethanol were 0.74 and 0.92 mol/mol glycerol respectively. The consumption of glycerol can be increased from 45.7% to 84.5% through the application of a potential of 0.2 V. Mixed culture MECs with a single chamber design can produce hydrogen yields of 3.9 mol/mol and 5.4 mol/mol glycerol, higher than pure culture MECs, with applied voltages of 0.9 and 1.0 V [79,80]. The application of pretreatments like heat shock should be considered to before inoculation as a method to prevent growth of methanogens.

3. Outlook

As previously discussed, the production of biodiesel waste will continue to increase with the growth of the biofuel market. The surplus of glycerol is not only creating difficulties for the glycerol production industry but also for biodiesel plants. Microbial conversion is an efficient and sustainable method for converting waste glycerol that avoids the disadvantages of direct application and chemical transformation, such as the inability to use waste glycerol directly and low product specificity [6]. Although there are still hurdles involved in constructing a suitable industrial scale reactor to more accurately predict real production costs, encouraging results have been demonstrated (Table 1).

1,3-Propanediol is one of the various value-added products that can be generated from glycerol fermentation, and is, a chemical that is already produced nationally on a level of about 31.6 million kg per year, and appears to be an encouraging target for future waste glycerol microbial conversion efforts [147,148]. Currently, commercial synthesis of 1,3-PDO is performed either by petroleum chemicals (acrolein and ethylene) [147,148] or by glucose fermentation of engineered bacterial strains [24,149]. As natural producers of 1,3-PDO, members of the *Klebsiella*, *Citrobacter*, and *Clostridium* families have been intensively studied for potential industrial applications. Compared to these bacteria, *E. coli* has an advantage being a highly tractable, and thus easier to be manipulated for various industrial needs [31]. In fact, one of the highest 1,3-PDO concentrations achieved by glycerol fermentation was the 104.4 g/L produced using an engineered *E. coli* strain, with a yield of 1.09 mol/mol (0.9 g/g) of glycerol [31].

On the other hand, citric acid generation from waste glycerol also represents a promising conversion route that can be achieved by yeast [60,61]. Strains of *Y. lipolytica* have been investigated as an alternative to citric acid production by *A. niger*. Although concentrations achieved are still lower than the concentrations from the commercialized process using glucose as substrate [150,151], economic analysis suggests that it is more profitable to generate value-added products like 1,3-PDO ($1.8 per kg [149]) and citric acid ($1.2 to 3.2 per kg) from waste glycerol ($0.11 per kg) [60,61,152].

One of the most challenging elements of the microbial conversion of waste glycerol to water-soluble products is downstream separation and purification, but microbial electrochemical technologies like MECs and MFCs have the potential to efficiently overcome this problem. Electrical power produced from waste glycerol requires no extra cost associated with separation or purification. It was estimated that the electrical power generated from waste glycerol by MFCs may be worth a total value of $98.4 million per year [77], a number that could be increased further by optimizing MFC designs for current generation. Similarly, as a gaseous product with low water solubility, hydrogen can be separated from reaction broth with no extensive separation cost regardless of hurdles in storage and transportation [153]. Current hydrogen production is dominated by steam reformation of natural gas, producing hydrogen at a cost ranging from $1.10 to $1.24 [154]. High hydrogen yields have been demonstrated using a photo-fermentative bacterium (6 mol/mol glycerol) [51]. However, the need for light may complicate the reactor design, increasing the difficulty in scaling-up for commercial applications. Under dark-fermentation, low hydrogen yields (0.28–0.96 mol/mol) are likely to be caused due to inefficient use of secondary metabolites such as acetate, lactate, and ethanol [23,52–55]. Through the use of an MEC design these secondary metabolites were able to be further utilized [155–157], and higher hydrogen yields (3.6–5.4 mol/mol glycerol) closer to the theoretical yields can be achieved

with a relatively low investment of energy compared to dark fermentation [23,79,80,158]. Further research is needed to demonstrate the ability to efficiently scale-up promising waste glycerol to hydrogen conversion technologies, as well as life cycle analyses comparing these technologies to current stream reformation techniques.

In summary, waste glycerol represents a carbon source that is widely available at relatively low-cost and potentially suitable for many applications. Although constraints are still largely present for practical utilization of waste glycerol from biodiesel plants, advancements have been made over the past decade that warrant further research in this fascinating area. Future advancements in this field could bring great social, economic and environmental benefits to society.

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Conflicts of Interest

The authors declare no conflict of interest.

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