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Bioconversion technologies of crude glycerol to value added industrial products

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ABSTRACT

Crude glycerol that is produced as the by-product from biodiesel, has to be effectively utilized to contribute to the viability of biodiesel. Crude glycerol in large amounts can pose a threat to the environment. Therefore, there is a need to convert this crude glycerol into valued added products using biotechnological processes, which brings new revenue to biodiesel producers. Crude glycerol can serve as a feedstock for biopolymers, poly unsaturated fatty acids, ethanol, hydrogen and *n*-butanol production and as a raw material for different value added industrial products. Hence, in this review we have presented different bioconversion technologies of glycerol to value added industrial products.

Contents

1. Introduction

Nowadays, people are busy in inventing new machines and instruments which need a lot of energy for running their application, these energies that are supplied naturally, biologically, chemically, electrochemically or physically. One of these is petroleum and its products like petrol, diesel, gasoline, etc. These have certain drawbacks like they have created global ecological disturbance [1]. So this has resulted in the emergence of eco-friendly, alternative fuel biodiesel. There are many types of feedstock for its production of oils and fats [2]. Biodiesel can be defined as long chains of alkyl esters, which are formed by transesterification of triglycerides with alcohol that results in glycerol as a waste product [3]. It is estimated that the biodiesel market will reach to 37 billion gallons by 2016 with an annual

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growth of 42% which is indirectly producing 4 billion gallons of crude glycerol as a byproduct 10 kg of glycerol (crude) will be produced from for 100 kg of biodiesel [4]. The scheme of events for biodiesel production through transesetrification reaction is depicted in Fig. 1 [5].

The up gradation of biofuel byproduct facilitates the added value of the economy of the process; moreover it falls under 4th generation biofuel strategy of minimum waste production in the process. Crude glycerol up gradation to value added products impinges a substantial effect on the economy of the biodiesel sector. Purification of crude glycerol is a cumbersome and hence utilization of crude glycerol as intact as source for any industrial product is a value added approach. The abundant surplus of glycerol from biodiesel production, makes the utilization of glucose more expensive while compared with crude glycerol. Furthermore, glucose competes directly with food and feed production, which is not the case for glycerol [6]. Glycerol has a greater degree of reduction than does sugars, and it is also cheaper and more readily available. In comparison with glucose fermentation, the almost exclusive synthesis of reduced products during glycerol fermentation reflects the highly reducible state of glycerol. Conversion of glycerol to phosphoenolpyruvate, or pyruvate, generates twice the amount of reducing equivalents than does producing pyruvate from glucose or xylose. As an example, glycerol fermentation produced ethanol and formic acid (or ethanol and hydrogen) with overall a yield of twice that of glucose fermentation since half of the glucose lost as carbon dioxide during bioconversion of glucose. Moreover, utilization of crude glycerol also alleviates the carbon catabolic repression/glucose effect present in case of glucose utilization. In case of carbon catabolite repression, the presence of a rapidly metabolizable carbon source such as glucose inhibits the expression of genes encoding proteins required for the utilization of alternative carbon sources such as glycerol and lactose etc. [7]. Glycerol has many uses in the different type of industries like pharmaceutical, soaps, food, paint, cosmetics, toothpaste [8]. There are many microbes that can metabolize glycerol aerobically and few microbes are able to metabolise it anaerobically, so none of them is used at industrial scale. *Escherichia coli*,*Klebsiella*, *Enterobacter*, *Glucanobacter*, *Clostridium*, *Candida, Aspergillus* can convert crude glycerol into value added products [9]. About 10 kg of biodiesel produces glycerol equivalent to 1 kg. The production cost of biodiesel increases by $0.021/L for every $0.22/kg reduction in glycerol selling price [10].

2. Bioconversion technologies of glycerol to value added products

The left unattended crude glycerol from the biodiesel industry is also a threat to environment. Hence conversion to other value added products through biological routes with the aid of microbes is a viable resource and which subsequently enhances the economy of the process. Different value added products from crude glycerol have been summarized as follows.

2.1. 1,3-Propanediol [$CH_2(CH_2OH)_2$]

This three carbon diol is a colorless viscous liquid used to produce polymers such as polytrimethylene terephthalate (PTT). It is used widely to produce aliphatic polyesters, co-polyesters, adhesives, composites, coatings, moldings, laminates, wood paints, and antifreeze [11]. 1,3-PDO have been reported to be produced by a recombinant strain of *E. coli* which was constructed by transferring DhaB1 (B12-independent glycerol dehydratase) and its activating factor DhaB2 from the species *Clostridium butyrium.* The overall yield, concentration and overall productivity of 1,3-PDO was 1.09 mol/mol, 104.4 g/L and 2.61 g/L/h respectively [12]. Bacteria *Citrobacter freundii* (DSM 15979) is another potential candidate for 1,3-PDO production through fed-batch fermentation [13]. *Klebsiella oxytoca* converts biodiesel derived crude-glycerol into 1,3-PDO by creating a lactate deficient mutant (LDH3) under batch and fed-batch fermentation conditions, the yield and productivity increased to 0.53 g/mol from 0.41 and 0.83 g/L/h from 0.63 respectively. In fed-batch fermentation, ethanol is formed along with 1,3-PDO with an initial glycerol concentration of 126 g/L [14].

2.2. Hydrogen [H_2]

Hydrogen, the only fuel to produce water as a by-product is seen as an ideal fuel for the future that can be produced in an eco-friendly manner. Crude glycerol serves as a raw material for

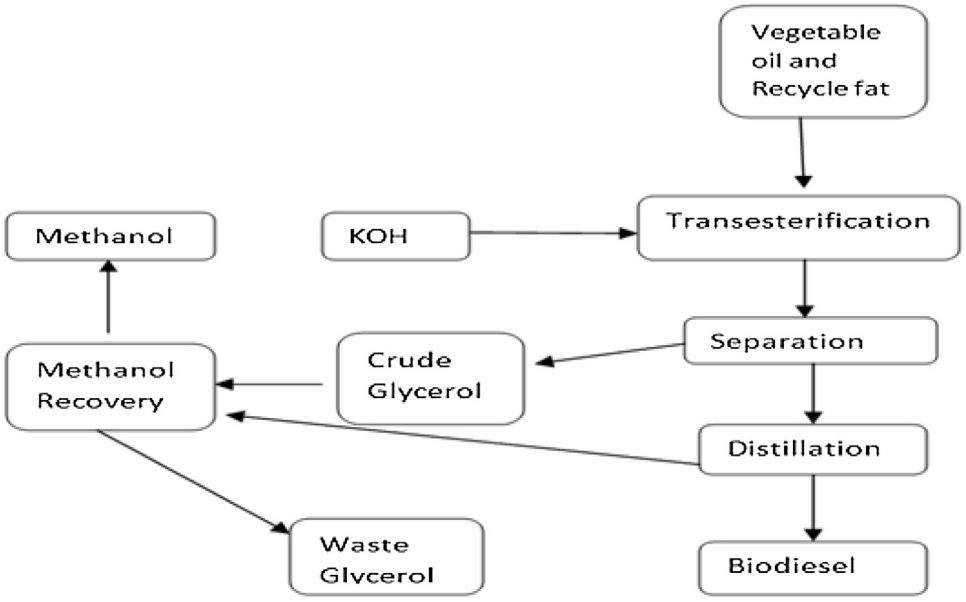

Fig. 1. Biodiesel production through transesterification reaction.

hydrogen production through microbial fermentation. The broad spectrum of applicable substrates in fermentative hydrogen production facilitates the possibility of combing the energetic utilization of biomass to hydrogen with the simultaneous treatment of waste materials. Hydrogen produced through microbial fermentation is a suitable alternative as combustion of hydrogen generates only water as byproduct which drastically reducing CO_2, NO_x, particulate and other emissions, usually accompanied with the fossil fuels usage [15,16].

Under batch fermentation, *Clostridium* species forms hydrogen. *C. freundii* H3 produces H_2 to give a yield of 0.94 mol/mol by fermentation from chemical grade glycerol [17]. Under thermotolerant fermentation, *Klebsiella pneumoniae* TR17 forms 20 g/L of hydrogen by batch as well as continuous fermentation [18]. Pre-treated waste glycerol can be photo-fermented to hydrogen through *Rhodopseudomonas palustris* CGA009, a purple non-sulphur photosynthetic bacterium. It produces 6.1 mol/h/mol of glycerol [19]. A hydrogen yield of 0.80 mol/mol glycerol has been reported from *Paenibacillus macerans* strains through utilization of glycerol as a sole carbon source. The reported was very low as compared to the later research studies, where H_2 has been produced by *R. palustris* and *Enterobacter aerogenes* [20].

Microbial electrolysis cells also used as a source for hydrogen production uses crude glycerol as a carbon source. The basic mechanism of microbial electrolysis cell for hydrogen production is—an electron is donated to the anode with the help of an external power supply during bioconversion of glycerol, then the free protons will be reduced on the cathode that subsequently produce H_2 [21]. Different researchers reported the efficiency of glycerol as a substrate for hydrogen production through microbial electrolysis cells (MEC's). Conversion of crude glycerol into H_2 and electricity has been reported by Chookaew et al. [22] through a two-stage process of linking dark fermentation with a microbial fuel cell (MFC) or microbial electrolysis cell (MEC) and achieved a maximum H_2 rate of 332 ml/L and a yield of 0.55 mol H_2/mol glycerol. A hydrogen yield of 3.9 mol–H_2/mol has been reported using glycerol as carbon source through MEC, which is higher than that possible by fermentation, at relatively high rates of $2.0 \pm 0.4\ m^3/m^3\ d$ (Eap = 0.9 V) [23]. Sakai and Yagishita have reported the hydrogen production by *E. aerogenes* NBRC 12010 using crude glycerol as a feedstock in a bio-electrochemical two-compartment reactor with a cation-exchange membrane separating the compartments [24].

2.3. Propanoic acid and trehalose

Propanoic acid [CH_3CH_2COOH] is a universal preservative derived directly from a metabolic pathway similar to that of succinic acid. The numerous industrial applications of Propanoic acid account for increasing interest in the development of a biotechnological production process as it is used to produce solvents, pesticides, artificial flavours, thermoplastics and pharmaceutical products [25]. *Propionibacterium acidipropionici*, *Propionibacterium acnes* and *Clostridium propionicum* are the three major bacterial strains used to produce propionate from glycerol. Both in terms of yield and productivity, authors have concluded that for Propanoic acid production, glycerol is a promising substrate. Trehalose, a disaccharide of sugar is a reducing sugar with nutraceutical properties and sed as a stabilizer in therapeutic products (Herceptin®, Avastin®, Lucentis®, and Advate®). *Propionibacterium freudenreichii* subspecies *shermanii* produces Propanoic acid, lactic acid and trehalose from crude glycerol [26].

2.4. Single cell oil

Single cell oil or simply SCO has drawn interest in industrial applications as microbial lipids has the potential to replace the plant triacylglycerols. Fed-batch systems appear to enable an increase in lipid content and cell density. Batch experimentations that though have been predominantly used to investigate lipid production from glycerol were negatively impacted for species such as *Cryptococcus curvatus* by the impurities found in crude glycerol. Comparable cell densities were observed even in crude glycerol by switching to the fed-batch culture system. High lipid content above 70% was reported using the fungus *Thamnidium elegans* in batch cultures [27] while 60.7% lipid was accumulated in fed-batch system using the yeast *Rhodotorula glutinis* [28]. Novel fungi strains have to be fetched out that produce high cell densities that contain a copious amount of lipid in the form of SCO from crude glycerol.

2.5. n-Butanol

The production of biobutanol is of particular interest as it offers better physical properties in comparison to ethanol as an alternative fuel. Glycerol as a byproduct from biodiesel production has also been served as a potential substrate for *n*-butanol production. A maximum yield of 0.28 g/L × h *n*-butanol was obtained by the use of *Clostridium pasteurianum*, a gram positive anaerobe with the initial substrate concentration of 25 g/L at 37 °C [29]. Through in situ *n*-butanol removal by gas striping, Jensen et al. achieved a *n*-butanol productivity of as high as 1.3 g/L × h [30,31]. *C. pasteurianum* DSMZ 525 have been reported as a promising strain for *n*-butanol production from mixed substrates of biomass hydrolysate and glycerol. The produced *n*-butanol has less downstream processing steps as the process is not producing acetone as a by-product. In this case, the formation of butanol was checked at different temperatures and also at different initial substrate's concentration. Out of all the cases, the best temperature at which there was a maximum production of butanol was 37 °C [32]. *Clostridium acetobutylicum* KF158795 have been reported as a promising *n*-butanol producer using glycerol as a substrate and produced 13.57 g/L of butanol in 96 h under optimized conditions [33].

2.6. Glyceric acid

Glyceric acid is produced as a small by product of dihydroxyacetone production from glycerol by *Glucanobacter* sp. The microbe used in this was isolated from rotten apple they also optimized the amounts of glyceric acid by using various volumes of $CaCl_2$, characterized trace elements present in the waste glycerol. Hence, they proposed that glyceric acid be potentially mass producible from waste glycerol. During fermentation it was observed that the initial pH of 6.0 favored the growth of microbes and as the conversion proceeded the pH dropped to 2.5 which tended to increase the production of glyceric acid. It was also observed that there was a maximum precipitation of glyceric acid calcium salt solution at various concentration ranges from 30 to 70%, 50% [34].

2.7. Citric acid

Citric acid is used as an emulsifying agent in ice creams, as a cleansing agent, in pharmaceutical industry, cosmetics, etc. It is usually produced by submerged microbial fermentation of molasses using *Aspergillus niger*, *Yarrowia lipolytica* [35]. The final concentration of citric acid that obtained was 77.4 g/L in the fermentation broth when raw glycerol as a substrate was used. However, by using *Y. lipolytica* N15, higher citric acid concentrations of 112 mg/g were obtained [36].

2.8. Ethanol

It is the highest produced alcohol during the fermentation. It is used in thermometers, as a solvent, as a fuel, etc. *Pachysolen tannophilus* (CBS4044) is used to produce ethanol by submerged batch fermentation from crude glycerol. The efficiency of this bacterium to use glycerol was found to be 50% v/v [37]. *K. pneumoniae* (GEM167) is used to produce ethanol in batch and fed batch fermentation conditions with a production rate of 0.93 g/L/h [38].

E. coli is also used to produce ethanol. To maximize the ethanol production, strain SY03 was constructed by inactivating fumarate reductase and phosphate acetyltransferase, which resulted in the production of 1 mol of ethanol and 1 mol of hydrogen gas per mole of glycerol consumed [39].

2.9. Polyunsaturated fatty acids

It was observed that *T. elegans* was able to produce 371 mg at an initial crude glycerol concentration of 90 g. In another study, *Cunninghamella echinulata* and *Mortierella isabellina* also produced GLA at concentrations of 190 and 116 mg, respectively from crude glycerol [40]. DHA (Docosahexanoic acid) and EPA (Eicosahexanoic acid) are both important omega-3 PUFA's due to their essential role in the treatment of cancer, Alzheimer's disease and cardiovascular disease. Most of the PUFA's are fish extracted which are less preferred due to unpleasant odor and harmful pollutants that they might accumulate. EPA was produced from waste glycerol from the fungus *Pythium irregular* at a final concentration of 90 mg/L and a productivity of 14.9 mg/L/day [41]. DHA was produced using waste glycerol as substrate by the algal species *Schizochytrium limacinum* SR21 with DHA productivity of 0.51 g/L/day [42].

2.10. Biopolymers (PHA, PHB and acrylates)

It was observed that the biopolymers were produced by using the crude glycerol—a byproduct of biodiesel under the limited amount of nitrogen and phosphate. Several researchers reported the production of biopolymers through microbial fermentation using crude glycerol as a carbon source. Ammonium molybdate and ammonium sulphate were used in the medium that was nitrogen deficient instead of ammonium chloride. Hence, maximum biopolymer productions were observed in *Bacillus* sp. and *Pseudomonas* sp. That was equal to 13.3 g/100 ml and 11.2 g/100 ml respectively [43]. It has been seen that the *Burkholderia cepacia* ATCC 17759 produced poly-3-hydroxybutyrate (PHB) from different concentrations of glycerol ranging from 3% to 9% (v/v). It was observed that with increasing the concentration of glycerol, there was a gradual reduction of biomass, the yield of PHA, and molecular mass of PHB. In the end, the fermentation was successfully scaled up to 200 L for its production which resulted in 23.6 g/L yield of dry biomass and 7.4 g/L yield of PHB [44]. Polyhydroxyalkanoates (PHAs) represent naturally occurring biopolymers produced intracellularly through fermentation by several bacterial strains using glycerol as a carbon source. Halomonas sp. SA8, a soil bacteria from Finnish soils and sediments have been reported for the intracellular accumulation of PHA (56%) through fermentation by using crude glycerol as a carbon source using mineral medium. The produced PHA has been identified as a PHB homopolymer, which shows a great hindrance in the commercial production of PHA's [45]. mcl-PHA produced through microbial fermentation by *Pseudomonas* spp. *P. mediterranea* 9.1 (CFBP 5447) using crude glycerol as a carbon source is a potential to use as a softener for biopolymeric blends of food packaging and medical devices material [46]. *Bacillus thuringiensis* EGU45 also reported for its efficiency in production of different PHA's under

Table 1
Microbial conversion of crude glycerol to value-added products.

Sl. no.	Species	Strain	Product/yield (mol/mol glycerol)	References
1	*Clostridium*	*C.pasteurianam* (immobilized)	*n*-butanol/0.43	Wilkens et al. [51]
		C. butyricum AKR102a	1,3-PDO/0.63	Gonzales et al. [52]
		C. butyricum VPI	1,3-PDO/0.65	Gonzales et al. [53]
		3266	1,3-PDO/0.66	Jensen et al. [54]
		Engineered *C. acetobutylicum*		
2	*E. coli*	Engineered *E. coli* SY03	Ethanol/1	Hong et al. [55]
		E. coli AC521	Lactic acid/0.9	Zhang et al. [56]
		Engineered *E. coli*	Succinate/0.8	Metsoviti et al. [57]
3	*Citrobacter*	*C. freundii* FMCC-B294	1,3-PDO/0.48	Maervoet et al. [58]
		C. werkmanii DSM 17579	1,3-PDO/0.62	Maru et al. [59]
		C. freundii H3	H_2/0.94	Yang et al. [60]
4	*Klebsiella*	Engineered *K. pneumonia*	Ethanol/0.89	Zhao et al. [61]
		K. pneumonia (Encapsulated)	1,3-PDO/0.65	Yang et al. [62]
		K. oxytoca (Lactate deficient)	1,3-PDO/0.41	Zhang et al. [63]
		K. pneumonia (Inactivated ADH)	1,3-PDO/0.70	Zhang et al. [64]
5	*Propionibacterium*	Engineered *P. acidipropionici*	Propionic acid/0.66	Ruhal et al. [65]
	Bacteria	Strain	Trehalose/391 mg/g biomass	Sabourin et al. [66]
		P. freudenreichi subsp. *Shermanii* NCIM 5137		
6	Other bacteria and mixed culture	*R. palustris* CGA009	H_2/6	Gupta et al. [67]
		P. macerans	H_2/0.801	Andre et al. [68]
7	Fungi	*L. edodes* strains	SCO/0.1 g/g biomass	Chatzifragku et al. [69]
		A. niger strains	SCO/0.41 1 g/g biomass	Imandi et al. [70]
		Thamnidium elegans	SCO and PUFAs	Rywinska et al. [71]
8	Yeast	*Y. lipolytica* Wratislavia AWG7	Citric acid/0.33	Liang et al. [72]
		Cryptococcus curvatus	SCO/52% lipid content	Jung et al. [73]
		Rhodotorula glutinis	SCO/36.5% lipid content	Chi et al. [74]
		Engineered *S. cerevisiae*	Ethanol	Sánchez et al. [75]
9	Microalgae	*S. limacinum* SR21	DHA	Pyle et al. [76]

optimized nitrogen conditions using crude glycerol as a carbon source. In presence of high nitrogen contents as media components, *B. thuringiensis* EGU45 produces a PHA in a concentrations around 1.5–3.5 g. *B. thuringiensis* EGU45 has been also reported for the production of co-polymer of P(3HB-co-3HV) under non-limiting N conditions (N containing media supplemented with propionic acid) using crude glycerol as a carbon source [47]. Several recombinant strains also have been reported for the production of biopolymers using crude glycerol as a carbon source. In a batch fermentation, P(3HB) has been reported to be produced by recombinant *E. coli* JM109 as glycerol as a carbon source [48]. An attempt of producing poly(3-hydroxypropionate), P(3HP) has been reported in the literature in a two stage process. The first stage involves conversion of glycerol to 3-hydroxypropionaldehyde (3HPA) by *Lactobacillus reuteri* followed by second stage of transformation of 3HPA to P(3HP) using recombinant *E. coli* strain with the co-expression of A-acylating propionaldehyde dehydrogenase (PduP) from *L. reuteri* and polyhydroxyalkanoate synthase (PhaCcs) from *Chromobacterium* sp. [49].

The several research attempts for production of value added products (with the yield information) and microbes used for the bioconversion of crude glycerol have been tabulated under Table 1 [50].

3. Future prospects

New technologies which involve glycerin-to-methanol production pathway and glycerin-to-ethanol production pathway can rapidly change the market scenario biofuels. Instead of seeing glycerin as a waste by biofuel producers, they have come to know that this by-product is a valuable resource for their own production processes. This could change the market situation for other industries which now fully rely on the glycerin production and supply from the biofuels industry. Compared to conventional catalysis, the biotransformation/bioconversion of crude glycerol are a less studied domain. As a sustainable approach, more focus has to be given for bioconversion of crude glycerol to poly urethane foams, biopolyols, polyglycerols and pigments. A more research has to done to enhance the yields of lactic acid, *n*-butanol and PUFA's through crude glycerol based bioconversions. Scarce literature reports are available on crude glycerol pretreatment for better utilization in MEC for *r* hydrogen production. Hence, further investigation is still required like collective removal method for different types of impurities (like acetic acid and butyric acid) will fetch the process to industrial scale that may be helpful for crude glycerol to hydrogen production in near future. Further investigation of microbial hydrogen production on continuous mode and application of co-culture system is recommended. Further, genetically engineered microbial strains may enhance the hydrogen production potential of crude glycerol. However, many of the existing technologies offered still need further development to make them cost-effective and operationally feasible for incorporation into biorefineries.

4. Conclusion

Biodiesel, designated to be a future alternative fuel produces crude glycerol as a waste byproduct. The bioconversion of this crude glycerol to value added products is a sustainable approach compared to chemical conversion. With the advent of directed evolution and systems biology approaches, the constructed novel strains can convert to some more valuable products from the crude glycerol reservoir. There are many products which can be made from crude glycerol which can be used in cosmetics, pharmaceuticals, feed products etc. Bioconversion approaches seems to a viable source for economic development and also towards maintenance of nature.

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