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Bio-conversion of glycerol into commercial production of 1,3-propanediol - A review

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Abstract

Depletion of crude oil resources with consequent increase in price of petroleum fuel has led to significant increase in the production of fluid biofuels derived from renewable sources. Methyl esters of higher fatty acids, commonly called biodiesel, are made in the transesterification process. The dynamic growth of biodiesel production results in generation of huge amount of waste glycerol fraction, which constitutes a major obstacle in profitability of the process.Many microorganisms can utilize glycerol as a carbon and energy source for the production of high value products like bioplastics, ethanol, succinic acid, propanediol etc. Impurities present in waste glycerol may inhibit the growth of microorganism and hence, it requires purification process. Utilization of waste glycerol solves its disposal and environmental concerns. Products like polyesters and bioplastics obtained from 1, 3-propanediol are highly biodegradable with better specificity. This paper elucidates various microbes for production of emerging commodity 1, 3-propanediol by utilizing low grade waste glycerol.

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Introduction

Despite other energy sources such as coal, natural gas, hydroelectricity and nuclear power, fossil fuels are the foremost energy sources, which cover almost 62% of global energy demand (Yahaya *et al.,* 2014). However, continued use of fossil fuels is becoming unsustainable because of diminishing energy resource worldwide and additionally combustion of fossil fuels has encumbered negative impacts on environmental and climatic condition (Kumar *et al.,* 2010, Krishnan *et al.,* 2014). Such situation has triggered a rapid search of alternate energy sources. Biofuels constitute an alternative type of fuels, which is being produced from renewable source (Vasudevan PT and Briggs M 2008, Silva *et al*., 2009). Biodiesel is one of the most investigated biofuels, which has rapidly grown over the last two decades to meet our basic necessity (Chozhavendhan *et al*., 2015a). During the process of biodiesel production, one volume of glycerol is generated as a byproduct for every ten volume of biodiesel produced (Prisilla*et al.,* 2009, YazdhaniSS and Gonzalez R 2009). Glycerol is an important raw material for food, pharmaceutical and cosmetic manufacturing process (Perez *et al*., 2009). Waste glycerol produced during biodiesel production generally contain impurities, such as methanol, soap, salts, oils and solid organic materials (Ngo TA *et al.*, 2011; Selembo *et al*., 2009; Zhanyou *et al.*, 2007). However, high amount of impurities in the waste glycerol obstructs its direct use in food or pharmaceutical industries, and its purification is also expensive. Hence, surplus production of waste glycerol from biodiesel manufacturing industries have less commercial value (Escapa *et al.*, 2009). Moreover, if directly released without proper treatment it may be hazardous to the environment (Gervasio *et al.*, 2009). Glycerol can substitute traditional carbohydrates, such as sucrose, starch and glucose in some industrial fermentation process. (Saifuddin *et al*., 2015; Barbirato and Bories 1997;

Menzel *et al*., 1997). 1, 3-propanediol was produced during the fermentation of glycerol in 1881 (Wang *et al.,* 2001, Werkman and Gillen, 1932). Alternatively, waste glycerol from biodiesel manufacturing plant can be used as a cheaper substrate for the production of a wide range of chemicals and biofuels like 1,3-propanediol, ethanol, bioplastics, animal feed etc. (Samul *et al.,* 2014). 1, 3-propanediol ($C_3H_8O_2$) is colorless, water miscible liquid with boiling point $214^{o}C$ an emerging commodity chemical and renewed interest (Cameron *et al.,* 1993) for the development of new polyester, polypropylene terephthalate (Reynaud *et al*., 2003, Pajeulo *et al.,* 2006). Products obtained by 1, 3-propanediol polymerization are characterized by good biodegradability, better specificity and higher industrial safety, in addition to being cheaper than 1, 2-propanediol, ethylene glycol. In this review we focus on waste glycerol for the production of 1, 3-propanediol by various micro organisms. This offers a considerable scope for designing bio-refineries in a profitable way.

Pre-treatment of waste glycerol : The presence of impurities like methanol, ash, salt, free fatty acids has obstructed direct usage of waste glycerol. Impurities in waste glycerol are partially purified to meet out industrial demand (Chozhavendhan *et al*., 2015b, Wen *et al*., 2009). Methanol, a major impurity in waste glycerol, exerts an inhibitory action on microbial growth and may be toxic to the environment. Hence, methanol can be removed by autoclaving, as boiling point of methanol is less as compared to water (Sneha *et al.,* 2009; Denver *et al.,* 2008). Soap, another major impurity in waste glycerol, can be removed by precipitation or altering the pH with various mineral acids (Shannon *et al*., 2011). In some pre-treatment methods dilute waste glycerol was mixed with hydrochloric acid to precipitate the soluble soap into residual fatty acids, and centrifuged for further purification (Moon *et al*., 2010, Venkatraman *et al*., 2012). In some cases, both distillation and precipitation were carried out to remove methanol and soap from waste glycerol. The purified glycerol has yellow to dull brown color, with purity ranging approximately from 80-88% that has a market price (Chiu *et al.,* 2005; Leung *et al*., 2010). In more complex operations, 99% of pure glycerol can be attained (Singhabhandhu and Tezuka, 2010).

Various microorganism used for the conversion of glycerol to 1, 3-propanediol

1, 3-propanediol has great prospect in commercial applications for synthetic reactions (Saxena *et al.* 2009), which predominates in the plastic industries as a monomer of polyesters, polyethers, polyurethanes and also used as an antifreezing agent. Plastics incorporating 1, 3-propanediol have unique properties and a high biodegradability factor. Over 10^5 tons of 1, 3-propanediol are produced every year, mostly through two different method: hydroformylation of ethylene oxide and hydration of acrolein (Gungormusler *et al.,* 2011). Production of 1, 3-propanediol by chemical methods require harmful reagents, expensive catalyst and also produces toxic intermediates. This has resulted in developing an environmental friendly and use of renewable feed stock such as waste glycerol for the production of 1, 3-propanediol by utilizing microorganisms. Several bacterial genera like *Clostridium*, *Citrobacter*, *Klebsiella* and *Escherichia* are capable of producing 1,3-propanediol (Rymowicz *et al.,*2009).

***Citrobacter* species:** *Citrobacter* species belong to Enterobacteriaceae family and is well known for their ability to produce 1, 3-propanediol from fermenting glycerol. Use of chemical grade glycerol has revealed that the final concentration of 1, 3-propanediol produced by *C. freundii*to be analogous to those produced by *Klebsiella* (Wolf-Dieter 1995). Recent studies have shown that both wild-type and engineered *Citrobacter* species are capable of using pre-treated waste glycerol as a substrate to produce high value chemicals (Cheng *et al.*, 2013).

Maria *et al.,* 2013 reported that newly isolated *C. freundii* FMCC-B 294when inoculated in sterile crude glycerol media gives a maximum yield of 68.1 gl^{-1} of propanediol during fed batch fermentation , whereas the unsterilized glycerol media yields 66.3 gl^{-1} of 1, 3-PDO. The difference in the yield was very meager and hence non sterile fermentation of crude glycerol by *C. freundii* FMCC-B 294 can create impact in the economics of production process. Similarly, on another study C. freundii was inoculated with the 4%V/V of inoculum in waste glycerol, pretreated glycerol and pure glycerol and incubated initially at $30^{0}C$ for pre-culture which enables the survival of microorganism and later the temperature raised to $37^{0}C$ for fermentation process at pH 7.0 with constant agitation of 150 rpm. From the study, it was concluded C. freundiiwas not resistant to the various impurities in the waste glycerol derived from biodiesel preparation. Furthermore, presence of Free Fatty Acids (FFAs) and Fatty Acid Methyl Esters (FAMEs) as impurities may inhibit the growth. Removing FFAs and FAMEs, not only increasesgrowth but also increased production of 1, 3-propanediol when compared to pure glycerol (Pinkiand Saxena, 2012).

E. coli: *E. coli* can be modified genetically to generate desired products that are not produced from glycerol by *E. coli* such as 1,3-propanediol and succinate (Zhang *et al.,* 2010). 1,3-propanediol is a building block of biodegradable plastic polytrimethylene terephthalate and also a valuable product with various uses (mortars coolants, resins, and inks). The novel recombinant strain *E. coli* BL21 used in production of 1, 3-propanediol (Rujananon *et al*., 2011) was the cultured on the medium consisting of 7g of glycerol, along with minimal supplementation of 100 µg ml^{-1} ampicillin to avoid contamination during cultivation. The optimum production was achieved at pH 8.0 for recombinant *E. coli* as same like *K. pneumoniae* (pH 8.0). This was due to the fact that low pH inhibits cell growth and product formation occurs at a higher pH value (Zhang *et al.,* 2006). Recombinant *E. coli* has an optimum incubation temperature for the production of 1, 3-propanediol at 37 ºC and growth ceases with increasing temperature and becomes zero at 45 ºC. The recombinant *E. coli* strains are able to tolerate glycerol concentration of 15 g l^{-1}, and the maximum yield of 1, 3-propanediol was achieved at glycerol concentration of 10 g l^{-1}. Other recombinant strains could tolerate only 5 g l^{-1} of glycerol concentration (Tang *et al.,* 2009).

***Klebsiella* species :** *Klebsiella* is considered as one of the most auspicious organisms for producing 1, 3-propanediol from glycerol (Cheng *et al.,* 2007). Use of waste glycerol should simply reduce operational and substrate cost. Research on *Klebsiella* has been focused on enhancing the production of 1, 3-propanediol produced during fermentation of waste glycerol. *K. pneumoniae* are able to ferment waste glycerol consisting of 51 g - 53 g of glycerol and yield 1, 3-propanediol of 1.7g l^{-1} h^{-1} in fed-batch fermentation. With sucrose as co-substrate, the production was as 83.56 g of 1, 3-propanediol l^{-1}. (Yang *et al*., 2007). Another strain of *K. pneumoniae* M51 used 58.8 g1 of 3-propanediol l^{-1} and yielded 0.53 mol mol^{-1} glycerol with productivity of 0.92 g l^{-1} h^{-1}. On the other hand, *K. oxytoca* a mutant strain deficient in lactic acid biosynthesis produced 1.67 g l^{-1} h^{-1} of 1, 3-propanediol.

***Clostridium* species :** Similar to *Klebsiella*, *Clostridium* species has ability to ferment glycerol and has extensively been the studied for the production of 1, 3-propanediol. *C. butyricum* DSP1 strain was used for bio-conversion of waste glycerol to 1, 3-propanediol. Substrate was added repeatedly as glycerol concentration fell below 14%. The portion medium is removed and fresh sterile medium was added three times. Temperature was maintained at 37 ºC with pH 6.5 throughout fermentation process. Highest accumulation of biomass was observed for first 15 hr following inoculation and later the 1, 3-propanediol synthesis begins. A complete utilization of substrate (waste glycerol) occurred at 40 hr and corresponded to 1, 3-propanediol concentration of 31.8 g l^{-1}. Further by maintaining, fed batch condition productivity and concentration of 1, 3-PDO was increased. In another investigation, impact on waste glycerol on the growth of *C. butyricum* strains was carried. The results revealed that *C. butyricum* was unable to grow because of salt and catalyst in waste glycerol. Interestingly, *C. butyricum* ES strain could produce 1, 3-propanediol of 65 g l^{-1} in fed batch fermentation (Petitdemange *et al*., 1995). Recombinant *C. acteobutylicum* can also be used for continuous industrial production in 1, 3-propanediol which has a productivity of 1.70 g l^{-1} h^{-1} from waste glycerol. (Pajuelo *et al.*, 2005; Pajuelo *et al.,* 2006).

Market and applications scenario of 1, 3 propanediol : The global 1, 3-propanediol market is assessed to register a CAGR of 10.4% between 2014 and 2021, and the market value is estimated to reach $621.2 million by 2021.Emerging economies such as China, India, Brazil, and Argentina have taking ardent steps to reduce their fossil fuel dependence and encourage biodiesel production. Favorable regulations and directives regarding biodiesel in these economies are poised to contribute to higher glycerol production and in turn, assist the 1,3-propanediol market. 1,3-propanediol finds application in manufacturing of polytrimethylene terephthalate, polyurethane, cosmetics, personal care and cleaning products etc., 1, 3-Propanediol is an essential ingredient and approximately 90% used for the manufacture of PTT, followed by polyurethane. Growing polytrimethylene terephthalate demand across myriad industries is presumed to augment the polytrimethylene terephthalate market and in turn, assist the overall 1, 3 propanediol market.

Future prospects : As discussed in earlier sections, waste glycerol represents a versatile low cost carbon source and is easily utilized by numerous organism for the production of high value1, 3-propanediol. Higher value products represent a promising route to achieve the economic viability of biodiesel industry. The biotechnological method seems to be an attractive and effective for production of 1, 3-propanediol from glycerol. However, microorganisms which are used for production are pathogenic. Under this frame, researchers focus on to isolate and characterize non-pathogenic microorganism which should exhibit tolerance against impurities, present in waste glycerol. Further, advancement in this area would bring prodigious social, economic and environmental benefit to society.

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