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Review Article

BIOCONVERSION OF GLYCEROL

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ABSTRACT

The availability of petroleum sources in the near future is limited, so nowadays search for renewable energy sources are maximized. Biodiesel is one of the most important substituted for this problem. During biodiesel production, excessive glycerol is generated asbyproducts which contains impurities such as methanol, free fatty acid and salt. Thedisposal of glycerol leads to environmental problems. Alternatively glycerol can be utilized to obtain various valuable products viz.1,3-propanediol (1,3-PDO), 1,2-propanediol (1,2-PDO), ethanol, 2,3-butanediol, dihydroxyacetone, succinic acid, propionic acid and citric acid. Utilization of crude glycerol by means of chemical synthesis requires expensive catalysts like Ir, Cr, Ag etc. Comparatively biological method for utilizing crude glycerol is best to avoid environmental problems. Bioconversion of glycerol is carried out at 30-40^{0}C temperature and atmospheric pressure pressure which gives different products. Different microorganisms viz. *Escherichia coli, Pseudomonas, Enterobacter aerogen, Klebsiella pneumoniae, Clostridium butaricum* and *Clostridium pasterium* are reported to grow on glycerol to produce valuable chemicals. In this review article, bioconversion of glycerol to speciality chemicals such as ethanol, 1,3-PDO, 1,2-PDO, 2,3-butanediol, dihydroxyacetone, succinic acid, propionic acid and citric acid etc. Are discussed.

Keywords: Bioconversion, Glycerol, Biodiesel, Microorganisms, 1,3-propanediol.

INTRODUCTION

Glycerol was first discovered by Karl Wilhelm Scheele. He synthesized and characterized many other chemical compounds such as tartaric acid, citric acid and lactic acid. Glycerol is also known as 1,2,3-propanetriol as it contains three hydroxyl groups there so, it is also termed as polyol compound. It is the principal by-product obtained during transesterification of vegetable oils[1,2,3]. Glycerol is completely soluble in water and alcohol and slightly soluble in ether, ethyl acetate, and dioxane. It is insoluble in hydrocarbons. It has useful solvent properties which is similar water and simple aliphatic alcohols[4,5]. About 1.3 billion gallons glycerol was produced in the USA while 0.8 billion gallons glycerol was produced in India till 2013[6]. It is obtained from various sources viz. via acrolein route, via allyl chloride route, in fat splitting, saponification, ethanolic fermentation of glucose and major in biodiesel production.

Biodiesel is produced by transesterification of vegetable oils with methanol using sodium hydroxide as catalyst. Biodiesel is a mixture of methyl ester and fatty acids. Biodiesel can be used in the diesel engine motors. Germany is the largest producer and consumer of biodiesel in the world, which produces more than 2.5 billion liters annually[7]. Many countries use biodiesel as an admixture to diesel with different proportions. Brazil used 2% biodiesel till January 2008, which is now increasing to 5%. There are two reasons on the basis of which Brazil will become a major producer and consumer of biodiesel: Brazilian used alcohol in fuel cars since long and second, the conditions for cultivating oleaginous plants are extremely favorable in many areas of the Brazil [8]. The availability of petroleum is limited in the future, so biodiesel use will continually grow. In 2010, the gradual declining of petroleum production was started, and it assumes petroleum reserves may completely deplete by 2050. On the other hand, the demand of biofuel is rising worldwide[9].

Crude glycerol obtained from the biodiesel industry contains impurity such as methanol, fatty acid and salt. Purification of crude glycerol can be done by distillation method. But this method is quite costly if it is compared to the production cost required for traditional synthesis of glycerol. This technology produces high purity glycerol at high yields. But the distillation of glycerol is an energy intensive process because of its high heat capacity and required a high supply of energy for vaporization[10]. Ion exchange has also been used to purify raw glycerol, but this technique is not economically viable from an industrial view of point due to the high content of salts present in crude glycerol [11].

Pure glycerol is required for utilizing in different application viz. in food, drugs, creams, tobacco processing, wrapping and packaging of materials, pharmaceutical industry, gaskets and cork products, as lubricants. As glycerol is obtained as a by-product in the production of biodiesel and it is assumed that by the year of 2020, production of glycerol will reach six times more [12]. The massive glycerol production forces a collapse in its market price and currently the market price of glycerol is reached to 60Rs per Kg.

A company like Dow Chemicals, Procter and Gamble closes their glycerol producing facilities. Therefore, alternative uses of glycerol are required. It can be utilized for combustion, animal feeding, thermo-chemical conversions, composting and biological conversion methods. The combustion of crude glycerol has been used for disposal. But, this method is not economical for large producers of biodiesel [13]. The process also generates the toxic greenhouse gases like CO and CO_2, which also have an adverse effect to the atmosphere and living organisms. It has also been suggested that glycerol can be composted or used to increase the biogas production of anaerobic digesters but it requires only 1% glycerol so this method is not solution for disposal[14]. Biodiesel-derived glycerol was fed to dairy cows in order to prevent ketosis, but found that it was not useful[15]. Glycerol can be thermochemically converted into propylene glycol. In which Raney nickel catalyst was used at 230^{0}C[16]. Glycerol is also used in the bioconversion process to obtain various products such as1,3-propanediol[17], ethanol[18], citric acid[19] and succinic acid[20]. These products also obtained by chemical synthesis too [21].

When the desired product is 1,3-PDO, it can be produced chemically by two methods: the hydration of acrolein and the hydroformylation of ethylene[22-24]. Chemical synthesis of 1,3-PDO requires high energy consumption, toxic intermediates like 3-hydroxypropyonaldehyde, expensive catalysts like Ir, Cr and Ag which leads to high costs of 1,3-PDO production[25-27]. More then 0.1 million tons of 1,3-PDO are produced yearly [28,29]. Currently, more than 2 million tons 1,3-PDO produced[30]. Consequently, chemical synthesis is expensive, thus, 1,3-PDO still has a low market volume[31]. Due to the environmental benefits and use of a renewable feed stock, the bioconversion of glycerol to 1,3-PDO is an attractive alternative to chemical synthesis[23]. Bioconversion of crude glycerol from the biodiesel process to value-added products is a driver towards higher cost efficiency of biodiesel production. Glycerol can be used by different microorganisms as an energy source. Microorganisms have the potential use in bioconversion of crude glycerol produced from biodiesel [32]. During industrial fermentation processes, glycerol can be used as a substitute for carbohydrates, such as sucrose, glucose and starch. Bioconversion of glycerol adds significant value to the productivity of the biodiesel industry. In this review, examples of possible bioconversion of glycerol are discussed and it demonstrates that inspite of simple chemical, glycerol is an important carbon sourcefor industrial microbiology.

Microorganisms

There are number of microorganisms can use glycerol as the sole carbon and energy source, viz. *Escherichia coli (E. coli)*[33], *Enterobacter aerogenes (E. aerogen)*[34,35]and *Lactobacillus reuteri*[36,37], *Pseudomonas*[38],*Clostridium acetobutylicum*, *Clostridium butylicum*[39,40], *Citrobacter freundli*[41-44], *Clostridium pasteurianum*[45-47], *Clostridium butyricum*[48-50],*Klebsiella pneumoniae*[51-53]and *Enterobacter agglomerans*[54] which are used to grow aerobically and anaerobically on glycerol and converts the glycerol into various products.

Glycerol is metabolized by oxidative and reducing pathwayin *Citrobacter, Clostridium, E. coli, Enterobacter and Klebsiella*[55]. In the oxidative pathway, glycerol converts to dihydroxyacetone [46]while in the reducing pathway, glycerol convertsinthe 3-hydroxypropionaldehyde [56]. The use ofthe $NADH^{+}H^{+}$-dependent enzyme reduce the 3-hydroxypropionaldehyde to 1,3-PDO[57]. The 1,3-PDOis highly specific for glycerol fermentation.

In the *K. pneumoniae*, the genes functionally linked with activities of glycerol dehydratase, 1,3-PDO dehydrogenase, glycerol dehydrogenase[58]. Glycerol dehydratase is very oxygen sensitive and strongly associated with the cell membrane[59,60]. Glycerol is degraded via dihydroxyacetone using yeasts. Sometimes it is converted to glycerol-3-phosphate through glycerol kinase, which can be used as precursor for lipid biosynthesis and can serve as a substrate for the synthesis of other metabolites[61]. The aerobic degradation of glycerol was carried out using *K. pneumonia* [62]. Bioconversion of glycerol to ethanol or butanol does not depend on the by-products formed using *Clostridium pasteurianum*[52]. Another example is succinic acid obtained using the bioconversion of glycerol[63].

Bioconversion products

There are a number of products which are obtained through bioconversion of glycerol, which is shown in table 1.

Table 1: It shows the various products obtained from the bioconversion of glycerol

S. No.	Products	Microorganism	Application
1.	Ethanol	*Bacillus, Klebsiella planticola, Escherichia coli* and *Enterobacter aerogene*	Motor fuel, household heating, feedstock, antiseptic and as a fuel
2.	1,3-propanediol	*Escherichia coli, Lactobacillus, Citrobacter freundii, Klebsiella pneumoniae, Clostridium pasteurianum, Pseudomonas* and*Ennterobacter agglomerans*	Composites, adhesives, laminates, for coatings, moldings, manufacturing of polyesters and copolyesters, as a solvent, antifreeze
3.	1,2-propanediol	*Escherichia coli, Bacteroides ruminicola, Thermoanaerobacterium, Thermosaccharolyticum, Klebsiella pneumoniae*and *yeasts*	Manufacturing of polyester, as humectant, solvent, food preservatives and as antifreeze agent
4.	2,3-butanediol	*Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter aerogenes, Bacillus polymyxa* and *Bacillus*	Used in the resolution of carbonyl compounds in gas chromatography, used as precursor for various chemical manufacturing, flavoring agent, converted to 1,3-butadiene which is used for synthetic rubber, antifreeze agents, solvents, plastics, liquid fuel additives, in cosmetic industry
5.	Dihydroxyacetone	*Gluconobacter oxydans*	Used in wine making, as a colouring agent, in cosmetic industry
6.	Succinic acid	*Anaerobiospirillum succiniciproducens*	As precursor for manufacturing of specialized polyester, as a acidity regulator in food bevarages and as a food additive
7.	Propionic acid	*Propionibacterium acidipropionici, Propionibacterium acnes*and *Clostridium propionicum*	As apreservatives and food additives, as an intermediate in thermoplastic production
8.	Citric acid	*Aspergillus niger, Yarrowia lipolytica*	As a emulsifying agent in ice cream, as a chelating agent, as a cleaning agent, in pharmaceutical and cosmetic industry, in industrial construction, photography and in dyeing

Ethanol

Ethanol is generally produced from sugar cane, corn starch and sugar beets. Ethanol is produced from a glycerol-enriched algal mixture using Bacillus with final concentration 7.0-9.6 gm/L[64]. Major ethanol and formate are produce using fermetataion of glycerol by *Klebsiella planticola* which was isolated from the rumen[65]. *Escherichia coli* was used as biocatalyst for converting glycerol into ethanol. Glycerol was consumed within 84 hrs, with 86% of ethanol and 7% of succinic acid as the products. While acetate were produced in minor amount. Glycerol from biodiesel production was converted to ethanol using *E. aerogenes* using synthetic medium. Glycerol was consumed in 24 hrs and yields ethanol at 1.0 mol/mol glycerol. The yield of ethanol from glycerol fermentation is very low and future development is required[34].

1,3-propanediol

Bacterial fermentation has been known for almost 120 years. in which glycerol is converted to 1,3-PDO[66]. The 1,3-PDO is the main product obtained through bioconversion of glycerol. 1,3-PDO is the oldest fermentation product and was first observed as a product in 1881 in fermentation of glycerol[67]. Then in 1914, production of 1,3-PDO by *Bacillus sp.* Was described. Microbiology School of Delft was analyzed 1,3-PDO using different *Enterobacteriaceae* in 1928[68]. The 1,3-PDO is an emerging speciality chemical. 1,3-PDO can be used to produce polyesters, polyethers and polyurethanes[69]. It is also used as asolvent and lubricant[70].

The bioconversion of glycerol to 1,3-PDO has been demonstrated for several bacteria, such as *E. coli*[33], *Pseudomonas*[38], *Lactobacillus*[71], *Citrobacter freundii*[41], *Klebsiella pneumonia* [72], *Clostridium pasteurianum(C. pasteurianum)*[73],*Ennterobacter agglomerans (E. agglomerans)*[74] and *E. aerogen*[35]. As an additional reducing equivalent required so complete conversion of glycerol to 1,3-PDO is notpossible[75]. Glycerol is converted to 1,3-PDO by two steps, using any of the microorganisms. The first one is the conversion of glycerol to 3-hydroxypropionaldehyde and water and then 3-hydroxypropionaldehyde is reduced to 1,3-PDO by

NAD+-linked oxidoreductase [76]. The production of 1,3-PDO from glycerol is carried out under aerobic as well as anaerobic conditions where glycerol is used as a carbon source, In *Citrobacter, Klebsiella* and *Clostridium strains*, a parallel pathway for glycerol conversion is used. In which glycerol is oxidized to dihydroxyacetone (DHA) by NAD+ followed by phosphorylation of the dihydroxyacetone gives dihydroxyacetone phosphate. This is an oxidative pathway[55].

Bioconversion of glycerol by *Enterobacter strain* results in the 1,3-PDO while the secondary productslike formate, succinate and ethanol are produced in variable amounts[77]. Bacterium *C. pasteurianum*converts glycerol into different products, such as n-butanol, 1,3-propanediol, ethanol,acetic acid, butyric acid and lactic acid[47].

The maximum concentrations that inhibit glycerolfermentation by *C. butyricum* are 60 gm/L for 1,3-PDO[48]. During batch fermentationsusing*C. butyricum*,112 gm/L glycerol was used to produce 63.4 gm/L 1,3-PDO[4]. The conversion yield was0.69 mol/mol and maximal 1,3-PDO productivity was 1.85 gm/L/h.

The highest 1,3-PDOconcentration obtained in continuous culture was 31-48 gm/L, with aconversion yield of 0.55 gm 1,3-PDO/gm glycerol. K. pneumoniae was used to ferment crude glycerol and obtained a concentration of51.3-53 gm/L of 1,3-PDO[78]. Waste glycerol was utilized to produce 1,3-PDO using Pseudomonas strain. 0.514 mol 1,3-PDO was obtained per mole of waste glycerol[38]. Bioconversion of glycerol was carried out using *E. aerogen* where 0.615 mole 1,3-PDO was obtained at 30^0C[35]. *E. coli* was grow on glycerol to yield 1,3-PDO which was further utilized for polyester manufacturing[33].

A recombinant *E. coli strain* produces glycerol fromD-glucose. Then glycerol is converted to 1,3-PDO by *K. pneumoniae*. This two-stage process renders up to 60-70 gm/L 1,3-PDO/L[51]. In the DuPont and Genencor patent, a process using a recombinant *E. coli* strain containing the genes from *K. pneumoniae* for 1,3-PDO production is described. This recombinant microorganism reached a final 1,3-PDO concentration of 135 gm/L using glucose as substrate. The efficiency of substrate conversion was 51%[79].

E. coli was used to convert glycerol to 1,3-PDO have been constructed by over expressing genes of the dihydroxy acetone from *K. pneumoniae*. But, the glycerol conversion is low due to toxic by-products, such as glycerol-3-phosphate[55]. A recombinant *E. coli JM109 strain* containing the genes (coding for 1,3-propanediol oxidoreductase from wild type *E. coli*)[29]. This recombinant strain produced up to 41.1 gm/L of 1,3-PDO in an optimized culture medium containing 61.8 gm glycerol/L. Several metabolic engineering approaches for 1,2 and 1,3-propanediol production[80].

1,2-propanediol

1,2-propanediol (1,2-PDO) is also known as propylenglycol, which is a commodity chemical with a wide range of applications, including polyester resins, plastics, antifreeze agents, detergents, paints[81].

There are number of microorganisms reported as natural producers of 1,2-PDOviz. *E. coli*[82,83], *Bacteroides ruminicola*[84], *Thermoanaerobacterium thermosaccharolyticum*[85], *Klebsiella pneumonia* [86] and *yeasts*. The 1,2-PDO may formed from lactaldehyde in which lactaldehyde is reduced to 1,2-PDO byNADH-dependent lactaldehyde reductase[87].

First approaches towards the metabolic engineering of *E. coli* strains for production of 1,2-PDOfrom glucose involved overexpression of genes for glycerol dehydrogenase and produce 0.7 gm/L using *E. coli* or *K. pneumoniae* together with the *E. coli* methylglyoxal synthase gene[80]. Additional overexpressionof *yeast* alcohol dehydrogenase further improved productionperformance of 1,2-PDO giving 4.5 gm/L which was achieved in a fed batch fermentation process[88]. Elimination of lactate dehydrogenase by gene deletion improved 1,2-PDO production by an *E. coli strain*[89]. As glycerol has a higher degree of reduction than glucose, 0.72 gm 1,2-PDO produce per gm of glycerol while 0.63 gm 1,2-PDO produces per gm of glucose. Also, 1,2-PDO has been reported to be a natural productof anaerobic fermentation of glycerol in *E. coli*[90].

Glycerol is converted to 1,2-PDO in a pathway consisting of glycerol dehydrogenase forthe oxidation of glycerol leads to dihydroxyacetone and phosphorylation of the dihydroxyacetone givesdihydroxyacetone-phosphate by dihydroxyacetonekinase. Subsequently, dihydroxyacetone-phosphate is reduced to 1,2-PDO[83].

During production of 1,2-PDO different by-products viz. succinate, acetate, ethanol, and formate were formed. To eliminate by-product formation genes was tested. However, removal of the genes for acetate kinase, phosphate acetyltransferase, and lactatedehydrogenase resulted in an increased product yield of 0.21 gm/gm, but increasedethanol, formate, and pyruvate formation. The use of raw glycerol by *E. coli*reducedformate formation and increased the 1,2-PDO yield (0.24 gm/gm)[90]. Recombinant strains of *S. cerevisiae* and *C. glutamicum* have been developed forproduction of 1,2-PDOwhich produces 1.1 gm/L 1,2-PDO[91,92].

2,3-butanediol

2,3-butanediol is another glycol that can be produced from the bioconversion of glycerol[93,94]. 2,3-butanediol can be added as a flavoring agent in food products when converted to diacetyl through oxidation. 2,3-Butanediol can be converted to 1,3-butadiene, which is used in the production of synthetic rubber, antifreeze agents, solvents, plastics, liquid fuel additives, polyurethanes for drugs and cosmetic products [95]. The 2,3-butanediol production carried out at low pH and an excess of glycerol. It is used as a solvent, fuel and for the production of polymers and chemicals [96].

2,3-BDO production has been possible by using various strain viz. *Klebsiella pneumoniae* [97], *Klebsiella oxytoca* [98], *Enterobacter aerogenes* [99], *Bacillus polymyxa* [100]and *Bacillus* [101]. Glycerol is a good substrate for 2,3-BDO production. Bioproduction of 2,3-BDO is carried out from pyruvate in three steps. In the first step, acetolactate synthase catalyses the condensation of two pyruvate molecules to acetolactate with CO_2 liberation. In thesecond step, acetolactate is decarboxylated by acetolactate decarboxylase to acetoin while third step consists reduction of acetoin to 2,3-butanediol[102]. In 2,3-BDO production, ethanol was produced as a by-product which could be eliminated by insertion mutagenesis of the aldehyde dehydrogenase gene and gives 0.48 gm 2,3-BDO per gm of glycerol[103].

Bioconversion of glycerol to 2,3-BDO was carried out using *K. pneumoniae* G31 which resulted in final concentrations of 49.2 gm/L. The medium pH had a large influence on 2,3-BDO production as 2,3-BDO production being favored at alkaline pH. By applying strong aeration, increased the 2,3-BDO production and reduced by-products[104].

Dihydroxyacetone

Dihydroxyacetone (DHA) is serves as a versatile building block for the organic synthesis of a fine chemicals and generally used in the cosmetic industry[105,106]. It can be produced by oxidation of glycerol by acetic acid bacterium *Gluconobacter oxydans*in a process that requires good oxygenation and a medium containing *yeast* extract[107-109]. The chemical production of DHA is so expensive as it requires higher safety. Thus, the production of DHA is performed more economically using a microbial process[110,111].

For glycerol catabolism, there are two pathways used in *Gluconobacter oxydans*[112]. Glycerol is phosphorylated to glycerol-3-phosphate and then dehydrogenated to DHA-phosphate, which is ATP- and NAD-dependent. DHA production occurs via a membrane bound glycerol dehydrogenase, which appears to be the only process responsible for DHA synthesis. Both the substrate and product have an inhibitory effect on bacterial growth is observed in aDHA microbial synthesis[113]. The culture was able to grow in up to 80 gm/L DHA concentration while the formation of the product was observed maximum up to 220 gm/L DHA concentration. The effect of the over expression of glycerol dehydrogenase on glycerol oxidation, demonstrating that growth on glycerol was significantly improved in the over expression strains which having optical density 2.8-2.9 compared to the control strains having optical density 1.8-2.0. The velocity of total glycerol dehydrogenase inactivation can be reduced by the higher concentration of enzyme and slowed down the inactivation of glycerol oxidation[114].

DHA was produced from glycerol using *Gluconobacter oxydans* which is belongs to the family of *Acetobacteraceae*. They are able to oxidize many carbohydrates and alcohols incompletely. Problems have occurred in the process of DHA production by *G. oxydans* was inhibition of the biotransformation process by the substrate glycerol and the product DHA. Both inhibit the growth and DHA production[115].

Succinic acid

Succinate has a great importance as speciality chemical in industries which produces food and pharmaceutical products, green solvents, biodegradable plastics, surfactants, detergents, and ingredients to stimulate plant growth. Due to its structure as a linear saturated dicarboxylic acid, succinate can be used as an intermediate chemical and can be converted to 1,4-butanediol[116], tetrahydrofuran, γ-butyrolactone and linear aliphatic esters[117]. An increasing demand for succinic acid is expected as its use is extended to the synthesis of biodegradable polymers such as polybutyrate succinate (PBS) and polyamides[118]. The synthesis of a new biodegradable polymer, poly(1,3-propylene succinate), obtained through the thermal polycondensation of succinic acid with 1,3-PDO[119]. Succinate is currently produced petrochemically from butane through maleic anhydride[120]; only natural succinic acid sold in the food market is produced by fermentation. Using several different metabolic pathways like pyruvate carboxylation, succinate is produced under anaerobic conditions. By utilizing *anaerobiospirillum succiniciproducens (A. succiniciproducens)*, succinate can be produced using the carboxylation pathway, catalyzed by malate dehydrogenase, fumarase and fumarate dehydrogenase[121].

Glycerol can be converted to succinic acid using *A. succiniciproducens*[121]. Fermentation of glycerol as the sole carbon source using *A. succiniciproducens* gives 19 gm/L of succinic acid where medium is supplemented with *yeast* extract while glycerol was fed with glucose 29.6 gm/L of succinic acid wasobtained. Succinic acid production from glycerol have some advantages over glucose, like high succinic acid produces with low acetic acid as by-product. Acetic acid imposes difficulties during downstream process for the recovery of succinic acid. As looking to the separation cost of succinate from fermentation broth, the formation of by-products like acetic acid is a problem to be solved through fermentation process optimization[118]. Fumaric acid has several industrial applications, that it is used as an acidulant in the food industry and promising candidate for manufacturing the various polymers. It is a direct precursor of succinic acid, it can be obtained from glycerol fermentation[122].

Propionic acid

Propionic acid is derived directly from ametabolic pathway and it is synthesized in a similar pathway to that of succinic acid[2]. Propionate is used as an antifungal agent in food and feed and asa basic chemical to produce cellulose-based plastics, herbicides, solvents, perfumes, arthritis drugs, flavors andthermoplastics[123]. The numerous industrial applications of propionic acid account for an increasing interest in thedevelopment of a biotechnological production process based on therenewable resource glycerol[124]. The production of propionate fromglycerol can be carried out using three bacterial strains: *Propionibacterium acidipropionici, Propionibacterium acnes* and *Clostridium propionicum*[2]. Consideringfermentation time and conversion yield, the best strain for glycerolconversion to propionate was *P. acidipropionici*. The fermentationprofile of this bacterium revealed five end products, consisting of propionic acid as the major product (0.84 mol/mol), with the following minimal by-products: succinate,acetate and n-propanol. The maximal propionic acid concentrationwas 42 gm/L using 80 gm/L glycerol in the medium[125]. As the efficiency of propionic acid extractionthrough distillation is strongly limited by acetic acid, the extremely low acetic acid concentrationobtained by using glycerol as substrate should greatly increase the yield of propionic acid recovered through distillation and simplifythe distillation procedure. The authorsconclude that glycerol is a promising substrate for propionic acid production, both in terms of conversion yield and productivity (0.35 gm/L/h)[126,127].

Citric acid

Citric acid can be produced in large quantities by fermentation method. Citric acid is widely used to impart a pleasant, fruity flavor to foods and beverages and used as an additive in detergents, pharmaceuticals, cosmetics and toiletries[128]. Citric acid is produced by submerged microbial fermentation of molasses using *Aspergillus Niger*[129].

In recent years, considerable interest has arisen in finding less expensive carbon sources for citric acid production[130]. *Yarrowia lipolytica (Y. lipolytica)* grows on glycerol to produce citric acid, so concluding that raw glycerol may be a suitable substrate for citric acid production. This *yeast* produced up to 35 gm/L of citric acid when a high initial concentration of glycerol was used in the culture medium. Growth and citric acid production parameters on glycerol were similar to those obtained using glucose. The final concentration of citric acid was 77.4 gm/L in the fermentation broth when using raw glycerol as a substrate[130]. Using *Y. lipolytica N15*, higher citric acid concentrations of 112 gm/gm was obtained[131,132].

CONCLUSION

Glycerol availability increased extremely as it arises as by-product of the biodiesel process. As glycerol is a renewable resource, utilizing this renewable waste substrate is the choice of an environmentally friendly process. As we utilized waste glycerol from biodiesel industry for thebioconversionprocess to obtain value added products, it will help to reduce the cost of waste treatment and disposal problem. Bioconversion of crude glycerol gives speciality chemicals which are used for the manufacturing of biodegradable polymer. As well utilization of crude glycerol promotes the use of biodiesel and reduce the petroleumdependency. If the biodiesel production is more economical and it will help to establish more bio refineries which also helps to develop the bioconversion process for converting glycerol into higher value products. Different microorganisms were developed for the production of ethanol, 1,3-propanediol, 1,2-propanediol, 2,3-butanediol, succinic acid, citric acid, propionic acid. Glycerol is a versatile compound and important carbon source which produces different chemicals in industrial microbiology. Future research shall also concentrate their efforts for increasing the % conversion of glycerol to useful products by bioconversion route as well as develop the most efficient and economic techniques.

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