

Current Trends in Efficient Production of 1,3-Propanediol

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Abstract

1,3-Propanediol, a highly needed multifunctional molecule, can be produced from different resources using microorganisms. According to its useful properties during the synthetic reactions, 1,3-Propanediol is a promising targeted product exceptionally in chemical industries such as polymer and cosmetic industries. By considering 1,3-PD as a natural product, it therefore be produced throughout fermentation processes by optimizing some related biochemical pathways. In this article, we reviewed and presented different strategies for the microbial production of 1, 3-propanediol with their advantages and disadvantages. Furthermore, metabolic and genetic engineering could significantly enhance product yields and therefore, overcome the challenges of fermentation engineering. This review describes the production of 1,3-propanediol by wild and engineered strains. It also tries to discuss about the different ways concerned in the use of crude glycerol for 1,3-PD production, with particular emphasis laid on biodiesel industries. Present review also summarizes the current state of technics applied for the downstream processing and purification of 1,3-propanediol produced biologically. The future prospect of 1,3-propanediol and its efficiency as a main bulk compound are also reviewed under the light of the present study.

Keywords: 1,3-Propanediol, glycerol, microorganisms, downstream processing, metabolic engineering.

Introduction

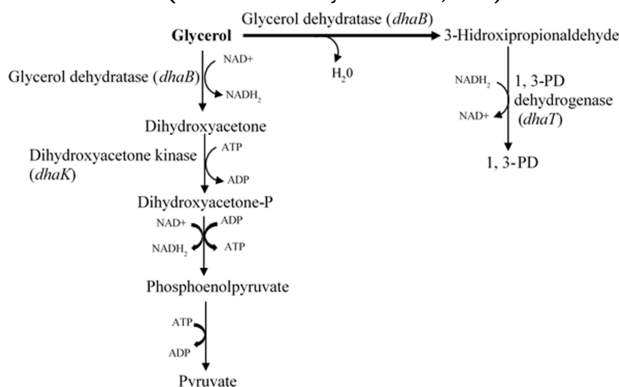
The current model of global industrialization, sustained by progressive decrease of petroleum stocks has raised the worldwide need for other sources of energy, such as biofuels, where biodiesel is considered as the most important. This compound is the main product of the transesterification of vegetable or animal fatty acids with short chain alcohols, producing a concentrated water-rich glycerol as by-product. The present global biodiesel high demand has caused an increase accumulation of raw glycerol, currently considered as waste. To valorize this compound into added value products, many glycerol conversion alternatives have been improved by using microbial biotechnology as a key role (Rodriguez *et al.*, 2016). Possibilities for new glycerol utilization have been reviewed; in chemical industry, glycerol is used as a raw material for the production of various chemicals such as: glyceryl ethers, glycerol carbonate, polyglycerol and hydroxypyruvic acid (Zheng *et al.*, 2008). Biotechnologically, microorganisms are used to convert glycerol into different types of valuable chemicals such as 1,3-propanediol, succinic, dihydroxyacetone, propionic, and citric acids, ethanol, pigments, polyhydroxyalkanoates, biosurfactants, 2,3-butanediol, amino acids, glyceric acids, d-xylulose, hydrogen, 3-hydroxypropionaldehyde, and fatty acids.

A greater selectivity on the final products, efficiency, and a shorter reaction time are some of the advantages of microbial bioconversion of glycerol comparing to its related chemical synthesis. In general, microbial bioconversion does not require several intermediate steps like chemical synthesis which needs several pathways, specific catalysts and extreme as well as controlled conditions like high temperatures and high pressures (DaSilva *et al.*, 2015) before reaching the targeted product and this leads to the use of biotechnological route of glycerol bioconversion (Maervoet *et al.*, 2011). As an interesting organic product, 1,3-PD has recently attracted many researchers due to its valuable importance on the market where it has many applications in different domains such as the production of polymers, medicines, cosmetics and lubricants (Przystałowska *et al.*, 2015). Chemical synthesis of 1,3-PD and microbial conversion of glycerol were done during the production of 1, 3 PD. Chemically, 1,3-PD is produced by using two different pathways. These methods were found to be of high cost, create pollutants and they depend on crude oil (Saxena *et al.*, 2009a) where one of them leads to 1,3-PD by using acrolein (2-propenal) as a raw material, it is hydrated to 3-hydroxypropionic acid, which is then hydrogenated in the presence of a catalyst.

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Another pathway requires the hydroformylation of ethylene oxide with CO and H under high pressure in the presence of a catalyst and a solvent. This reaction produces a dioxane as an intermediate product which is then hydrogenated to 1,3-PD. However, metabolic engineering was found to be the best procedure during the production of 1,3 PD through glycerol fermentations (Drożdżyńska et al., 2011) and this review is trying to describe the efficiency production of 1,3-PD by examining some of procedures and different factors which have been used (Fig. 1).

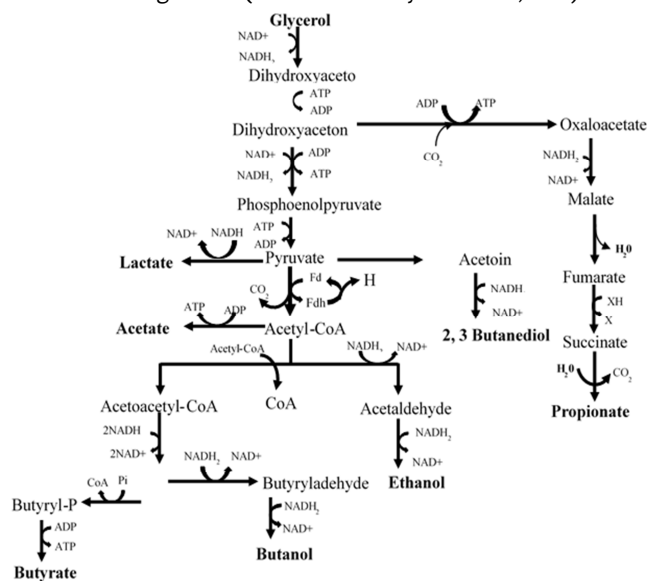
Fig. 1. Biochemical pathways of glycerol fermentation (Source: Drożdżyńska et al., 2011).



Glycerol metabolic pathway during 1,3-PD production

Glycerol can be metabolized during the production of 1,3-PD by fermentation through oxidative and reductive pathways by using different species of microorganisms such as *Klebsiella* spp., *Citrobacter* spp., *Clostridium* spp. and *Enterobacter* spp. (Drożdżyńska et al., 2011) (Fig. 2). 1,3-PD is formed in the reductive pathway. In the first stage of the reductive pathway, glycerol is dehydrated by glycerol dehydratase in the presence of coenzyme B12, with the formation of 3-hydroxypropionaldehyde (3-HPA) followed by NADH+H⁺ dependent 1,3 PD dehydrogenase reduction of 3-HPA to 1,3-PD and NAD⁺ (Przystałowska et al., 2015). In the oxidative pathway, the conversion of glycerol to dihydroxyacetone is catalyzed by the NAD⁺ dependent glycerol dehydrogenase. The next step is phosphorylation of the latter product, which is catalyzed by the glycolytic enzyme dihydroxyacetonekinase. Then, the phosphorylated product is subjected to glycolysis (Saxena et al., 2009a, Maervoet et al., 2011). In PD producing microorganisms, the first step of the oxidative pathway (i.e. glycerol consumption for growth and maintenance), provides NADH which is used in the last step of the reductive pathway forming PD. It was also reviewed that wild-type grown on glycerol and a co-substrate, glycerol partly enters the oxidative pathway yielding more NADH as when grown on the co-substrate as sole carbon source and therefore, more reducing equivalents such as NADH are formed in order to transform the all 3-HPA to PD (Maervoet et al., 2014).

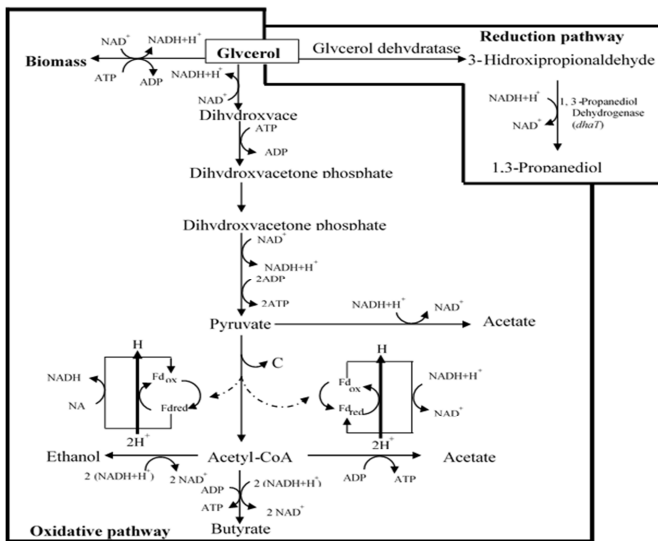
Fig. 2. The end-products of glycerol fermentation by different microorganisms (Source: Drożdżyńska et al., 2011).



Therefore, it was found that in all of the wild 1,3-PD producers, there is a low molar yield of 1,3-PD to glycerol conversion and that the pyruvate formed during glycerol conversion is used in several ways according to different types of the used microorganism. Also, depending on the type of used species and culture conditions, glycerol fermentation gives 1,3-PD as the main product but also other secondary products are generated (Leja et al., 2011). The production of 1,3 PD throughout glycerol fermentation by using “Natural producers” was challenged by the low molar yield of 1,3 PD (Chen et al., 2011), the presence of by products (Leja et al., 2011) which sometimes inhibits the formation of 1,3-PD (Galdeano et al., 2008), culture media conditions and the toxicity of some microorganisms (Wojtusik et al., 2015). However, except *Clostridium pasteurianum* whose synthesis of 1,3-PD is not a Vitamin B12-dependent process, a remarkable point of its economic advantage and industrial application (Drożdżyńska et al., 2011), metabolic engineering of microorganisms, the inhibition of the final by products and the analytic study of culture conditions were done in order to overcome all these challenges.

Metabolic engineering strategies and different production conditions of strains constructed for enhancing 1,3-PD yield: During this session of metabolic engineering on native strains, it was firstly shown that in most of 1,3-PD natural producers, both routes of glycerol metabolism pathways are important and the enzymes implicated in metabolic pathway for glycerol assimilation in native strain were studied as well as the genes involved in reductive pathway.

Fig. 3. Metabolic pathways for glycerol fermentation by *Clostridium butyricum*. Glycerol dehydratase is encoded by *dhaB1* and 1, 3-propanediol dehydrogenase by *dhaT* (Quilaguy-Ayure et al., 2010).



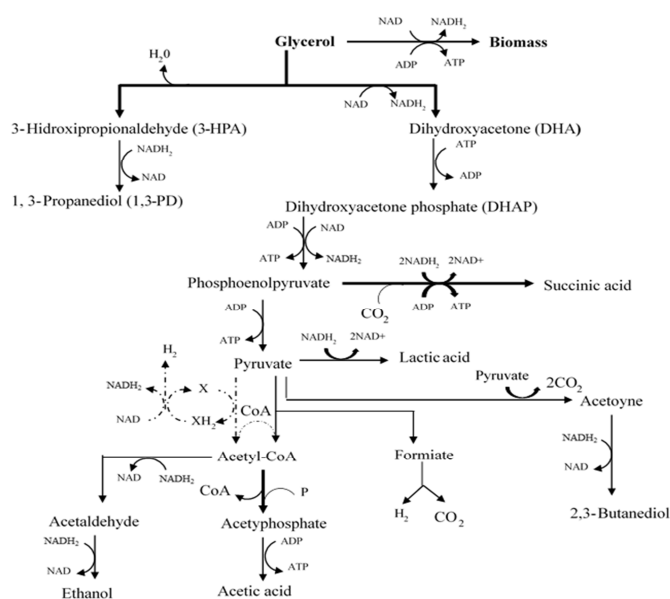
The gene *dhaT* found to be one of the genes responsible for the 1,3-propanediol production was analyzed in two native strains in order to know the physical structure of 1,3-PD operon in native strains, a knowledge which opened the way for developing genetic and metabolic engineering strategies for improving processes productivity (Fig. 3) (Quilaguy-Ayure et al., 2010).

Production and productivity of 1,3-propanediol from glycerol by *Klebsiella* genera: *Klebsiella pneumoniae* is known as the most studied species and efficient microorganism for the production of 1,3-propanediol from glycerol. A new isolate of *K. pneumoniae* GLC29 was studied using response surface methodology by central composite design for the production of 1,3-propanediol using glycerol. The effects of temperature, pH, stirrer speed and glycerol concentration on the production and productivity of 1,3-PD were investigated. Considering both production and productivity, the best conditions for 1,3-PD production from glycerol are: a temperature between 33 and 38.5°C, a pH range of 6.9–7.1, a stirrer speed of 110–180 rpm, and a glycerol concentration of 39–49 g L⁻¹. About 20.4g L⁻¹ of Propanediol were obtained in a batch fermentation carried out at a temperature of 35°C, a pH of 7.0, a stirrer speed of 150 rpm, and a glycerol concentration of 40g L⁻¹, with a maximum volumetric productivity of 2.92 g L⁻¹h⁻¹ and a yield of 0.51 g g⁻¹. The main by products were acetic acid (approximately 7.0 g L⁻¹) and formate (approximately 3.7 g L⁻¹). Recently a new strain of *Klebsiella* genera (*K. pneumoniae* GLC29) was found to be a potential candidate in the conversion of glycerol into 1,3-PD, with high production and productivity (Silva et al., 2014).

However, further studies were conducted on *Klebsiella oxytoca* NRRL-B199 in order to optimize the medium composition by means of a statistical design based on Taguchi method and to evaluate the inhibition of the final products of both oxidative and reductive routes. During medium composition analysis, Strong influences of glycerol and phosphate concentrations have been detected on biomass and product yields. Other factors, such as magnesium concentration and K: Na ratio, have shown a small influence on responses, biomass and product concentrations. An optimized medium composition has been proposed, leading to a final 1,3-PD concentration of 12.4 g/L with a selectivity of 72% with respect to glycerol consumed at shaken bottle-scale. Once the medium composition had been optimized, the scale-up from shaken bottles to STBR was conducted. Several experiments in a 2 L STBR have been conducted in order to determine the best operating conditions concerning temperature and agitation. Wojtusik et al. (2015) obtained 13.5 g/L of 1,3-PD as the final concentration with a selectivity of 86% with respect to the glycerol consumed and under the best operating conditions such as a programmed variable stirring rate ranging from 50 to 100 rpm and a temperature of 37°C. On the other hand, the purpose was the evaluation of inhibition effects of 1,3-PD production and all its main by-products on *K. oxytoca* growth and on the production of 1,3-PD itself by using glycerol as the source of carbon. In the oxidative route; succinic acid (HSucc), Acetic acid (HAc), 2,3-butanediol (2,3-BD), lactic acid (HLac) and ethanol (EtOH) are the final products meanwhile 1,3-PD is the main end product which accumulates in the media in the reductive pathway. As the results, in *Enterobacter agglomerans*, the microbial production of 1,3-PD has been found to be inhibited by both oxidative and reductive routes when using glycerol as the carbon source (Galdeano et al., 2008). The growth inhibition of *K. oxytoca* was not only found during the production of 1,3-PD as the main product, but also all the by-products affected to the biomass yield and the most important growth inhibition was observed when EtOH was evaluated. The different compounds and concentrations affected to values of Yield in 1,3-PD (g/g). The highest decreases of Yield in 1,3-PD (g/g) have been observed when the inhibitions caused by EtOH and 1,3-PD were studied (Galdeano et al., 2008) (Fig. 4).

Metabolic engineering of *Escherichia coli* in 1,3-PD production using genes from *Citrobacter freundii* ATCC8090: The use of *E. coli* during metabolic engineering production of 1,3-PD was also found to be of the great support comparing to some pathogenic native producers of 1,3-PD because these bacteria are non- pathogenic, easy to culture and proliferate rapidly (Przystałowska et al., 2015; Saxena et al., 2009a).

Fig. 4. Routes of anaerobic glycerol metabolism in *Klebsiella* sp. (Galdeano et al., 2008).



For the better production of 1,3-PD, the strategy oriented *C. wekmannii* DSM17579 to metabolize glycerol strictly for 1,3-PD formation, while catabolizing a co-substrate for growth and maintenance. The separation of these two major biological pathways in *C. wekmannii* DSM17579, the deletion of *dhaD* gene encoding for glycerol dehydrogenase which is responsible of oxidative pathway (Przystałowska et al., 2015) was done and glucose was found as a promising co-substrate for growth and maintenance after a deep analysis between 13 co-substrates. Although, the competition between NADH- consuming enzymes and 1,3-PD dehydrogenase was emerged, the PD yield on glycerol was improved 1,5 times and this confirmed the idea of the industrial use of *Citrobacter wekmannii* DSM17579 as an interesting host for 1,3-PD production (Maervoet et al., 2014).

The use of a mixed culture for 1,3-PD production from low-cost feedstock: In order to make fermentation methods more competitive, more cost-effective technologies were developed (Kleerebezem and van Loosdrecht, 2007). One of them which uses the two-stage process for producing 1,3-PD from glucose has been studied, in this mechanism glucose was used first as the starting substrate to afford glycerol using recombinant yeast or *Escherichia coli*, and then the glycerol was finally converted to 1,3-propanediol by *Clostridium acetobutylicum* or *K. pneumoniae* (Mendes et al., 2011). However, the use of mixed culture for enhancing 1,3-PD production from low cost feed stock, had not been reported (Ma et al., 2012) till when Ma et al conducted a study on 1,3-PD production by using a mixed fermentation of *Zygosaccharomyces rouxii* JL2011 which is able to produce glycerol from glucose and *Klebsiella pneumoniae* S6 responsible of glycerol conversion to 1,3-PD in one bioreactor (Ma et al., 2012). Although 15.2 g/L of 1,3-PD were obtained from 200 g/L of glucose, such yield was found much lower compare to that obtained by using other microbial producing methods such as 1,3-PD production by using crude glycerol (Drożdżyńska et al., 2011, Posada et al., 2013) or two-stage from glucose (Homann et al., 1990; Mendes et al., 2011). Recovery process and purification of biologically produced 1,3-PD: After considering the yield and recovery of product, environmental protection and sustainable development of 1,3-PD, its microbial production was improved based on glycerol or on glucose (Homann et al., 1990; Deckwer, 1995; Pinto, 2014). However, the presence of cheap and available substrates (Xiu and Zeng, 2008), the process of downstream of biologically produced 1,3-PD can take 50 to 70% of the total production cost (Fig. 5). Although some of the potential by- products can be easily separated from 1,3-PD while on the other hand, some need specific process and high attention which leads to the cost top up of the entire process (Hao et al., 2006).

Two gene constructs containing the 1,3-PD operon from *Citrobacter freundii* (pCF1 and pCF2) were used to transform the bacteria. It has been also reported that the pCF1 gene expression construct contained *dha* BCE genes encoding the three subunits of glycerol dehydratase, *dhaF* encoding the large subunit of the glycerol dehydratase reactivation factor and *dhaG* encoding the small subunit of the glycerol dehydratase reactivating factor while the pCF2 gene expression construct contained the *dhaT* gene encoding the 1,3-propanediol dehydrogenase. The above gene constructs expression was under T7lac promoter regulation and different technics such as SDS-PAGE analysis, RT-PCR and functional tests proved that the genes involved in the pathway synthesis of 1,3-PD were expressed at the RNA and protein levels, and worked flawlessly in the heterologous host. A batch flask culture has been used to study the 1,3-PD production in a preliminary research, with a short time applied, the recombinant *E. coli* bacteria afforded 1.53 g/L of 1,3-PD, using 21.2 g/L of glycerol in 72 h. In the Sartorius Biostat B Plus reactor, an efficiency of 0.58 mol 1,3-PD/mol glycerol was obtained when they used 24.2 g/L of glycerol to produce 11.7 g/L of 1,3-PD (Przystałowska et al., 2015).

1,3-Propanediol production with *Citrobacter wekmannii* DSM 17579: To improve the conversion of glycerol to 1,3-PD in producing strains, more methods have been used (Seo et al., 2009; Maervoet et al., 2011). However, in order to optimize 1,3-PD yield in *C. wekmannii* DSM17579, synthetic biology and protein engineering was used (Pirie et al., 2013) as well as strain improvement via metabolic engineering which used disconnection of the production and growth pathways (Dodge and Valle, 2003).

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Fig. 5. Examples of potential products produced from glycerol by chemical and biological routes (Xiu and Zeng, 2008).

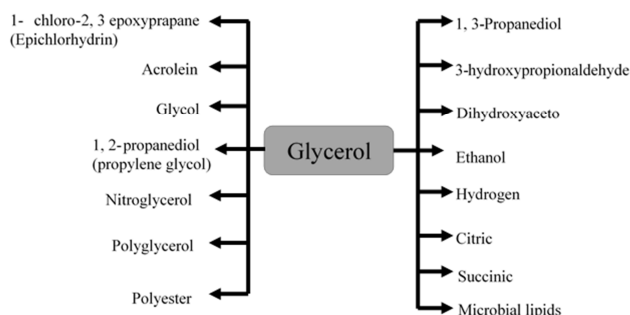
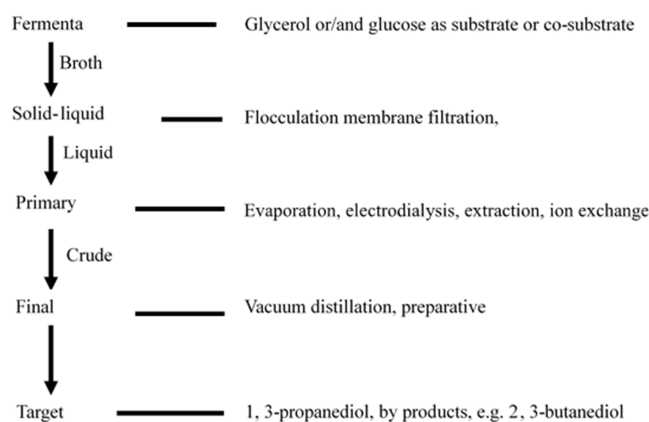


Figure 5 is a scheme depicting production route of some reviewed potential by-products of 1,3-PD production. Figure 6 shows the downstream processing of biologically produced 1,3-PD which normally has three major steps. The first step is the removal of microbial cells, which is most of the cases done by using high-speed centrifugation or membrane filtration, including pretreatment such as adjusting pH by base or adding the flocculants into the broth. After the cell debris removal, the second step comes over with the removal of impurities and primary separation of 1,3-PD from the fermentative broth. In this step, different methods have been documented and used for the proper and complete removal of impurities such as the use of evaporation for removal of water, ethanol and acetic acid, electrodialysis for desalination, alcohol precipitation and dilution crystallization for removal of proteins and salts, solvent extraction and reactive extraction, ion exchange chromatography, adsorption with active charcoal or molecular sieve, and pervaporation with zeolite membrane. Finally, vacuum distillation and/or preparative liquid chromatography are used as the last step to purify 1,3-PD (Hao *et al.*, 2006, Xiu and Zeng, 2008).

Pretreatment and solid-liquid separation: The techniques of Solid-liquid separation include microfiltration, centrifugation and decanting, and are most of the cases used to remove cell broth from different fermentation medium especially in fermentation liquors. Nowadays, when effective and cheap flocculants are available, flocculation precipitation is preferred in industrial scale due to its simplicity. For this purpose, polyacrylamide and Chitosan have been tested (Hao *et al.*, 2006). Before centrifugation, the PH of the fermentation suspension was adjusted at pH 4 by using phosphatic acid. The PH adjustment reduced gradually the concentration of protein in the supernatant from 0.6 g/L (pH=7) to 0.4 g/L (pH=4) (Grothe, 2000). In another case, it was reported that addition of base into the fermentation broth raised the pH to a remarkable level before distillation.

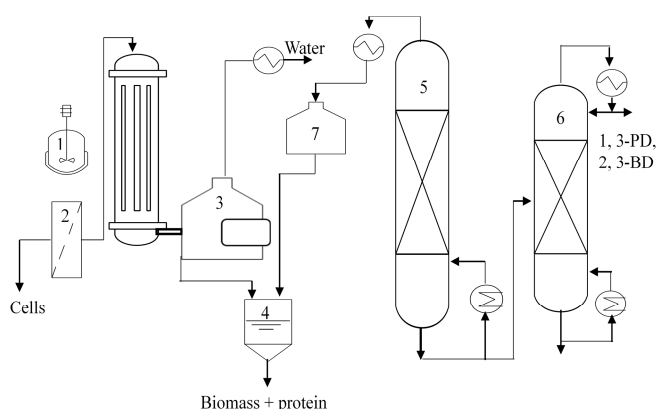
Fig. 6. General scheme and major methods reviewed for the recovery and purification of 1,3-PD from fermentation broth (Xiu and Zeng, 2008).



This decreased not only the reaction rate between acid and alcohol and thus the formation of ester, but also contributed to the formation of impurities during isolation of 1,3-PD, especially pigments in the broth (Kelsey, 1996).

Evaporation/distillation: Distillation and conventional evaporation techniques which are used for the removal of water and purification of 1,3-PD face the big challenge of a high cost of the final product due to the high energy consumption during these techniques. Grothe (2000) reported that the use of single-stage evaporation, multi-stage evaporation and down film vacuum evaporator can save more energy and thus lead to the affordable price of the final product purified by using such techniques. Dewatering in a falling film evaporator was performed and after water and acid were removed by using two vacuum rectification columns and recovery of 1,3-PD, respectively. Isobaric vapor-liquid equilibrium data for the binary system (water 1,3-PD) and for the ternary system (water 1,3-PD glycerol) were reported (Sanz *et al.*, 2001) and 1,3-PD has the distillation point of 214°C in the binary system under normal pressure. However, the use of vacuum distillation was recommended because of its remarkable decline of boiling points which at the end save the energy and promote the price affordability of the final product. For example, at a vacuity of 0.095 MPa in the ternary system, the boiling points of 1,3-PD and glycerol were found to be 139.0°C and 202.5°C respectively (Sanz *et al.*, 2001). Deproteinization and desalination are required before distillation. However, the soluble macromolecules would be salting out after evaporation. The factors which lead to low efficiency of evaporation/distillation such as viscous slurry should be avoided because they contribute to the low yield of the target product. Electrodialysis can be used for desalination before evaporation (Hao *et al.*, 2005).

Fig. 7. Description of the Flow sheet of downstream processing of 1,3- PD from fermentation broth by alcohol precipitation and dilution crystallization.



1-Fermentator, 2-ultrafiltration module, 3-evaporator, 4-alcohol precipitation chamber, 5-ethanol recovery column, 6-rectifying column, 7-ethanol storage tank (Source: Gao *et al.*, 2007).

The soluble proteins as well as salts have been precipitated by adding alcohol into the concentrated broth after evaporation due to alcohol precipitation and dilution crystallization (Gao *et al.*, 2007) (Fig. 7).

Membrane separation: During the separation and purification of 1,3-PD, several technics such as membrane filtration, zeolite membrane pervaporation and electro dialysis have been reported (Hao and Liu, 2005, Adkesson *et al.*, 2011b). To synthesize 1,3-PD from sugar, a recombinant *E. coli* was bioengineered and cultured and its fermentation broth was collected and subjected successively to microfiltration, ultrafiltration and nanofiltration in order to remove particles or molecules whose size is greater than 0.2 μm , whose molecule weight is greater than about 5,000 Daltons, and particles about 200-400 Daltons, respectively (Adkesson *et al.*, 2011a; Adkesson *et al.*, 2011a). Finally, ion exchange and distillation were performed to treat the final filtrate. However, it was reported that considering the energy consumptions for the use of these technics are different. The specific energy used were 2,5,7 and 9 kWh power per each m^3 permeate when using microfiltration, ultrafiltration, nanofiltration, and reverse osmosis, respectively (Hermann and Patel, 2007). A Na-ZSM-5 zeolite (Si/Al = 25) membrane was used in the separation of 1,3-PD from glycerol and glucose in water by pervaporation (Li *et al.*, 2001b). Feed mixtures of Binary, ternary and quaternary (1,3-PD/glycerol/glucose/water) solutions were used and the separation of 1,3-PD was attributed to adsorption and diffusion where 1,3-PD/glycerol selectivity decreased from 54 to 21 over the temperature range 308–328 K, while 1,3-PD/glucose selectivity increased from 330 to 2,100 for the same temperature range.

Although, the 1,3-PD/glycerol selectivity was monitored by both preferential adsorption and differences in diffusion rates, with the larger glucose molecules diffusing through non-zeolite pores, the selectivity of 1,3-PD/glucose was mainly controlled by the differences in diffusion rates. In aqueous mixtures, 1,3-PD was separated from glycerol with the X-type zeolite membranes by pervaporation (Li *et al.*, 2001a). During that process the selectivity of 1,3-PD/glycerol was 41 at 300 K and it was observed to be increased with temperature. Li *et al.* (2001b) reported that 1,3-PD preferential adsorption was the reason of this high 1,3-PD/glycerol selectivity. On the other hand, desalination before evaporation by electro dialysis membrane was also documented and used (Hao and Liu, 2005) where electro dialysis could effectively remove the salts. However, the use of electro dialysis presented a series of disadvantages such as the loss of 1,3-PD which lead to a low product yield and to a membrane pollution.

Chromatography

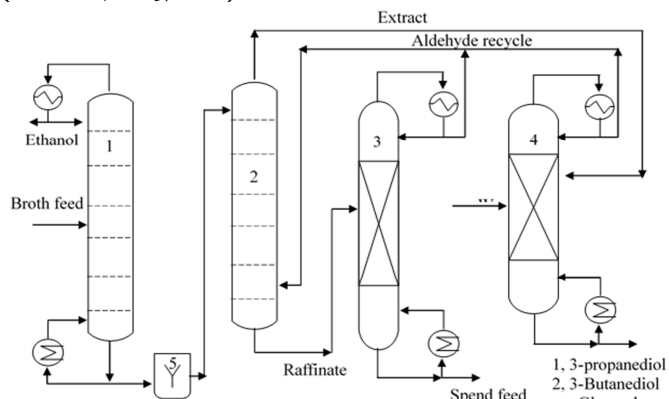
The purification of 1,3-PD by using molecular sieve adsorption, ion exchange resin and preparative liquid chromatography have been recently documented in some different patent and journal publication. Roturier *et al.* (2002) in their patent, they purified 1,3-PD by removing proteins and salts thereafter the obtained clear solution was passed through the type of resin of polystyrene sulfonic acid whose cation exchange is very strong and then through a weakly and/or a strong basic anionic resin of the acrylic type. In this process, cations from groups made of lanthanum, lead, zinc, iron and aluminium are advantageously selected. Using water as eluent, the flash column chromatography of a fermentation medium containing 90.5 g/L of 1,3-PD and 28.7 g/L of glycerol yielded 39 mL containing 2.0 g/L of 1,3-PD as the first fraction. Afterwards, the sample was diluted 45 times by water which lead to the high-energy consumption during the dewatering process and thereafter, other impurities were removed from 1,3-PD by using strong cation exchange resin of polystyrene sulfonate in the Na form (Hilaly and Binder, 2002). A simulated moving apparatus was used during this process. To elute the feed material, water was used and for each 10 ml of the applied feed solution, the effluent from 35 to 140 ml was obtained. The original feed solution was thus diluted by ten times. These essays lead to the pure product with 87% of purity and yielded more than 95% of 1,3-PD (Hilaly and Binder, 2002). A part of 1,3-PD purification, anionic and cationic molecules were removed by using the ion exchange comprising a strong acidic cation exchange resin followed by a weak basic anion exchange resin (Adkesson *et al.*, 2011b). The fermentative broths usually contain a large amount of anionic and cationic molecules which lead to the frequent ion exchange resin regeneration.

1,3-PD separation techniques such as adsorption were used, especially adsorption on hydrophobic zeolites like silicate-1 or non-aluminous NaY zeolites or sometimes the active charcoal (Wilkins and Lowe, 2004). Although all these separation techniques were developed, their quality was still below of the expected one; to overcome such challenge, they employed a column on charcoal to remove pigments and proteins (Roturier *et al.*, 2002). A mixture of 1,3-PD and 1,2-PD was used during the study conducted on the separation of 1,3-PD from such kind of mixtures and ethyl acetate was used as a phase separation reagent (Cho *et al.*, 2006). The elution of these two components was done by using 98% of ethyl acetate and 2% of methanol as a mobile phase, and this purification process yielded 82% of 1,3-PD pure at 98%. Furthermore, to prevent the feedback inhibition of cell growth and product formation in the fermentation process, chromatography was tested in order to remove the targeted molecule such as 1,3-PD *in situ* (Wilkins and Lowe, 2004). The chromatographic media can be of various types such as activated carbon, zeolites, polymeric neutral resins, chitosan beads, ion-exchange resins, and sometimes immobilized complexation materials can also be used. The elution reagent includes a mixture of water and a non-aqueous eluent such as acetone or any short chain alcohol (Xiu and Zeng, 2008).

Extraction: Extraction has been focused on in the last decade. It is subdivided into solvent and reactive, and both possess several advantages compared to classic distillation including large throughput and low energy consumption (Xiu and Zeng, 2008). In the presence of appropriate solvent, Liquid-liquid extraction using organic solvents can recover the target product from dilute solutions. For instance, extraction has been used to separate 1,3-PD from fermentation broths and diluted aqueous solutions (Malinowski, 1999). To screen the solvent, an extraction screening program (ESP) was used. ESP results provided aliphatic alcohols and aldehydes to be applied for experimental testing which resulted, however, to a fair large discrepancy between the predicted and experimental values. Consequently, the distribution of 1,3-PD into extraction solvents was not effective for developing a simple and efficient extraction process. Moreover, normal physical or complex extraction separation method of 1,3-PD from a dilute solution is not successful (Xiang *et al.*, 2001). Even though many solvent extractants including tributyl phosphate, isopropyl acetate, castor oil, pentanol, soya oil, oleyl alcohol, propanol, 4-methyl-2-pentanone, hexanol, and oleic acid are known; 1,3-PD is hydrophilic in dilute broths rendering it inappropriate to enter into hydrophobic solvents, but, this extraction can be enhanced by adding a large amount of solvent into a concentrated broth (Baniel *et al.*, 2009).

Additionally, the use of ethyl acetate, a hydrophobic solvent, in phase separation of 1,3-PD from a mixture containing 1,3-PD, 1,2-PD, glycerol, and glucose allowing the precipitation of glucose and glycerol (Hao *et al.*, 2006). The upper layer in the mixture containing ethyl acetate, 1,3-PD and 1,2-PD was subjected to successive chromatographic purification which resulted to the maximum solubility of 1,3-PD in ethyl acetate with only 40 g/L. Generally, the conventional liquid-liquid extraction process use high quantity of solvents. Thus, particularly this can be surmised that decreased the efficiency of 1,3-PD extraction and separation. Therefore, it is more critical to apply efficient downstream separation processes to enhance the 1,3-PD separation from a dilute aqueous system (Xiu and Zeng, 2008). One of the practical way is a 1,3-PD conversion into a hydroxyl-free substance, followed by reactive extraction, which is a liquid-liquid extraction recovery (Broekhuis *et al.*, 1994). For instance, it can be also achieved by use of formaldehyde or acetaldehyde to produce a 1,3-PD derivative called dioxolane (Broekhuis *et al.* 1994). In another way, the batch experiment can recover 80% of 1,2-PD from aqueous solution using extractant made of ion pairs of Aliquat 336 and phenylboronate in 2-ethylhexanol, di-isobutyl ketone, o-xylene, or toluene in combination with acidification-mediated reaction (Xiu and Zeng, 2008). For the regeneration of extractant, a temperature above 110°C can cause degradation of 1,2-PD. Moreover, the 1,2-PD can be produced by a reactive extraction process. Here, through a reversible reaction between 1,3-PD and acetaldehyde catalyzed by a Dowex or Amberlite ion-exchange resin, a 1,3-PD is converted into 2-methyl-1,3-dioxane (2-MD), then 2-MD is extracted using an organic solvent such as o-xylene, toluene, or ethylbenzene, and subsequently obtain 1,3-PD by hydrolyzing 2-MD (Malinowski, 2000). The above methodology can be applied in a simulative artificial fermentation broth, since its yield was 91~92% of 2-MD, 98% of the overall conversion of 1,3-PD, and 75% of the recovery of dioxane into the organic extractant. Though, one has to bear in mind that in real fermentation broths, impurities can cause catalyst inactivation between 1,3-PD and acetaldehyde (Xiu and Zeng, 2008). Also, unexpected reactions within the broth can occur, for example, with 2,3-BD, ethanol, aldehyde, glycerol or soluble proteins present in the mixture (Hao and Liu, 2005; Hao *et al.*, 2005). Moreover, high scale usage of extractant such as ethylbenzene, toluene, or o-xylene should be limited owing to their toxicity (Hao *et al.*, 2005; 2006). Finally, cell debris and proteins must be removed, preceded by ethanol removal before the reactive extraction. Furthermore, the 2,3-BD, 1,3-PD and glycerol react with butyraldehyde to produce 1,3-PD acetal (2-propyl-1,3-dioxane) and 2,3-BD acetal (2-propyl-4,5-dimethyl-1,3-dioxolane), and glycerol acetal.

Fig. 8. Flow scheme of downstream processing of 1,3-PD fermentation broth by reactive extraction and distillation (Hao *et al.*, 2005; 2006).



1-Distillation column, 2-reactive extraction column, 3-aldehyde recovery column, 4-reactive distillation column, 5-flocculation tank.

These acetals are hydrolyzed in a reactive distillation column using a strongly acidic cation-exchange resin as catalyst. The yield was a mixture of 2,3-BD (252 g/L), 1,3-PD (407 g/L), glycerol (277 g/L), and glycerol acetals (146 g/L).

Future prospects and challenges

The increasing of population leads to the high demand of World energy sources. In 2011, the overall biodiesel production in the EU, was reported to be increased twice during the last six preceding years (Drożdżyńska *et al.*, 2011). Moreover, such production increased up to 12 Mt and the global biodiesel market was expected to reach 37 billion gallons by 2016 (Saxena *et al.*, 2009a). Regarding this high demand in terms of biodiesel, and knowing that crude glycerol as a by-product during the production of biodiesel, we can assume that its bioconversion will be of great importance considering different reasons: First of all, it protects the environment against pollution by providing proper substrates for the production of biodegradable products. In addition, the use of biodegradable products would sustain the production of biodiesel as well as stopping petroleum dependency (Saxena *et al.*, 2009a,b). Polyethylene terephthalate (PET) which is one of the most known product of glycerol fermentation, belongs to the family of polymers based on fiber-grade 1,3-PD. It is a linear crystallizable polymer with a melting temperature of about 228°C and a glass transition temperature of about 50°C (Drożdżyńska *et al.*, 2011). The use of biological systems during the production of such polymers has shown several advantages comparing to its related classical chemical systems. For example, during the bio-production of 1,3-PD, the recorded greenhouse gas emission has proved to be about 40% less than for petrochemical 1,3-PD. Furthermore, the absence of heavy metals in the products such as PET has made its recycling process much easier comparing to PET and Nylon (Kurian, 2005).

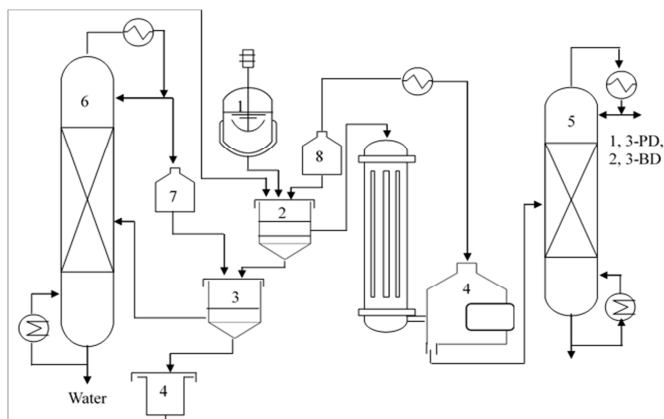
Complexity of the fermentation broth, Hydrophilicity and high boiling point of 1,3-PD are the most reported challenges during its separation process from fermentation broths. The above-discussed techniques and separation methods have shown some limitations which were summarized in Table 1 (Xiu and Zeng, 2008).

A part of high input of energy, deproteinization or desalination as pretreatment steps are also required during the conventional evaporation and distillation. The use of electrolysis to remove the salt in the broth will lead to the loss of 1,3-PD in the saline effluent and give a low product yield. Furthermore, the presence of bio macromolecules such as proteins, polysaccharides and nucleic acid; decreases the lifetime of electrolysis due to the membrane pollution of those compounds. The same situation also happens in other separation techniques such as nanofiltration, ultrafiltration and zeolite membrane pervaporation. Verification of the zeolite membrane pervaporation selectivity was recommended and should be done by using real fermentation broth instead of model solution. During chromatography method, 1,3-PD solution is normally diluted when zeolite adsorption or ion exchange chromatography is used due to the low selectivity and capacity of resins. Furthermore, if the feed is not deproteinized or desalinated, the chromatographic matrix needs to be regenerated frequently and this is why chromatography method was not economically recommended for the 1,3-PD recovery (Xiu and Zeng, 2008). Liquid-liquid extraction of 1,3-PD would be the most preferred and efficient method. However, the problem of the proper extractant remains up to now. Thus, many attempts have been performed and are promising. On the other hand, reactive extraction implies difficult methods on both sides of treatment. For example, the removal of salts, proteins and ethanol was found complicated during pretreatment as well as back extraction and hydrolysis or reactive distillation steps in post-treatment pathway. To all these mentioned complications, the additional challenge of the presence of aldehyde traces in 1,3-PD during the production of PET has not yet been found. Considering the above comparison, more efforts targeting the development of a simple and proper process for the purification of 1,3-PD directly from its fermentation broth, would be recommended. The main challenge in the separation of 1,3-PD from fermentation broths appears during the industrial large scale processes. Therefore, any separation technique which can increase the productivity and yield at this level in a cost-time dependent manner, would be highly appreciated. The separation of 1,3-PD from its mixture with 2,3-BD was still a challenge up to 2008 (Xiu and Zeng, 2008). However, separation problems and drawbacks need to be solved.

Table 1. Comparison of different separation techniques for 1,3-PD (Xiu and Zeng, 2008).

Separation, methods or unit operation	Drawbacks	Application/investigation
Evaporation/distillation	Evaporation and distillation suffer from a large amount of energy consumption. In addition, desalination and deproteinization are required before.	Evaporation was used in the removal of water from the fermentation liquors Distillation was used for the final purification of 1,3-PD.
Electrodialysis	Low product yield due to loss of 1, 3-PD during electrodialysis. Membrane pollution can be very serious.	Electrodialysis has been used for desalination before evaporation.
Pervaporation	The performance of pervaporation needs to be verified by using real fermentative broth in the presence of impurities, e.g., proteins and salts.	Na-ZSM-5 and X-type zeolite Membranes were used to separate 1,3-PD from an aqueous mixture by pervaporation. The high 1,3 PD/glycerol selectivity was due to preferential adsorption of 1,3-PD
Chromatography	Although high overall purity and yield of 1, 3-PD could be obtained, the 1,3-PD solution was not concentrated but diluted because of the low selectivity and capacity of resin or adsorbent. This method consumed more energy than the simple evaporation and distillation. In addition, the chromatographic matrix had to be regenerated frequently if the feed was not desalinated or deproteinized. This situation also occurred for ionexchange resins used to desalinate due to high salt concentrations	Combined strongly acidic cationic and weakly basic anionic resins were used to desalinate in the fermentation broth A cationic exchange resin was used for recovery of 1,3-PD. Adsorption of 1,3-PD on hydrophobic zeolites or active charcoal is done for separation of 1,3-PD A preparative silica gel liquid chromatography was used to separate 1,3- PD after phase separation
Solvent extraction/ liquid-liquid extraction	No effective extractant has been so far found for liquid-liquid extraction of 1,3-PD. Major problem is because 1,3-PD is hydrophilic.	Many extractants have been investigated for the recovery of 1,3-PD from dilute broth. It is partly partitioned into the solvent phase only when adding a large amount of solvent into a concentrated broth.
Reactive extraction	This process is quite complicated. The removal of proteins and ethanol as well as salts is necessary before reaction Additionally, the trace amount of aldehyde in 1,3-PD is prohibitive for polymerization of PTT.	Reactive extraction includes three key steps: reaction, extraction, and hydrolysis. A reversible reaction between 1,3-PD and aldehyde was used to form a dioxolane derivative (e.g., 2-MD). 2-MD is then extracted into an organic solvent and finally hydrolyzed into 1,3-PD.

Fig. 9. Flow scheme of aqueous two-phase extraction of 1,3-propanediol from fermentation broth (Xiu et al., 2007a).



1-Fermentator, 2-Aqueous two-phase extractor, 3-ammonium sulfate recovery chamber, 4-falling-film evaporator, 5-Rectifying column, 6-Methanol recovery column, 7-Methanol storage tank, 8-Ethanol storage tank, 9-Ammonium sulfate storage tank.

It is in this context that a novel separation technology based on aqueous two-phase extraction can be used during 1,3-PD recovery from whole fermentation broth. The use of inorganic salt (e.g., ammonium sulfate) can be used to remove proteins mixture in the concentrated fermentation broth (Xiu et al., 2007b; Xiu and Zeng, 2008). On the other hand, organic solvent precipitation (e.g., the use of alcohol precipitation) has been used to remove proteins (Gao et al., 2007). The combination of these two procedures leads to an aqueous two-phase system. This novel separation process of 1,3-PD has been tried by adding together ammonium sulfate and ethanol into the glycerol-based fermentation broth and the results were promising (Xiu et al., 2007a) and showed that the use of alcohol and ammonium sulfate salting out as the aqueous two phase extraction system can recover 1,3-PD from fermentation broths (Xiu et al., 2007a; Xiu and Zeng, 2008) (Fig. 9).

Furthermore, another new method based on the synthesis and the application of allylcyclohexylamine functionalized siloxane and its phase separated blend with styrenebutyl acrylate copolymer which allows the recovery of mechanical strength lost due to functionalization has been recently reported. This system was used during pervaporative enrichment of 1,3-PD from dilute aqueous solution without loss in separation performance and it reached the separation factors of 9–15 with functionalization levels of 50–90%, while 1,3-propanediol flux was 1.5×10^{-3} – $1.6 \times 10^{-3} \text{ g/m}^2 \cdot \text{s}$ (5.5 – $5.8 \text{ g/m}^2 \cdot \text{h}$). Increase of feed concentration and temperature was found as the main property which decreased the separation efficiency while this one was increased with functionalization.

Computation of concentration polarization, overall mass transfer coefficients and intrinsic material mass transport properties was done by using a model of solution diffusion and the dominance of the membrane on the transport resistance was reported due to the data analyzed which showed that when the boundary layer mass transfer coefficient ranged from $5 \times 10^{-7} \text{ m/s}$ to $18 \times 10^{-7} \text{ m/s}$, the overall mass transfer coefficient for 1,3-propanediol was between 1.0×10^{-7} – $1.4 \times 10^{-7} \text{ m/s}$. To underscore the results, computation of Hansens solubility parameters by a group contribution method was done. The membrane, with excellent mechanical integrity and its good cost/performance tradeoff offered the possibility of fabrication into modules and scale up (Kanjilal et al., 2015).

Conclusion

The current industrial biotechnology concern targets the substitution of traditional petrochemical routes to commodity chemicals in order to respond to the environmental challenges and the general concepts of sustainability. More efforts need to be invested in the promotion of research centered on the elevation of production by using bio-based products, enhanced biological processes, engineered more robust strains and targeted affordable downstream processing. As these challenges are arranged, different factories will not only provide valuable products but also protect the environment by using various safe and sustainable mechanisms such as the use of cells and enzymes as the chemical factories. In this context, the realization of this vision needs collaboration of different aspects such as big policies and commercialization which must work hands in hands by supporting developmental research in the field of bioresources as well as creating new opportunities for the current industrial status.

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