Functional Diversity and biotechnological production of 1,3-Propanediol from Crude Glycerol

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Abstract: The conversion and utilization of bio renewable feedstock for the production of valuable materials has become an important research trend in recent years due to the decreasing supply of non-renewable resources, escalating global energy demand, and negative environmental impact. Emerging biofuel technology produces numerous by-products and waste, ranging from corn fiber and glycerol to animal manure, which serve as a basis for additional sources of bio energy (liquid biofuel and biogas). Once considered a valuable by-product, crude glycerol is rapidly becoming a 'waste product' with an associated disposal cost. Due to the rapid growth of biodiesel production, the price of pure glycerol drastically decreased.

I. Background

The global interest is widely increased towards research and commercialization of several microbial based fermentation technologies for the production of Industrial important chemical components. Several studies estimate that the biotechnological processes in the production of various industrial useful chemical components which is to be around 3.0% in 2004, however they estimated that this figure may increase by 15% in 2015 (Festel Capital, 2007). In response to this, there is a similar interest in producing industrially important bulk chemicals such as 1,3-propanediol, and many other components by fermentation using various microorganisms which mainly focuses on the environmental aspects, industrial safety and renewable nature and the mode of production. Among all the industrially important chemicals, the 1,3-propanediol is the challenging bulk chemical which has attracted worldwide attention due to its widespread applications in polymers, cosmetics, foods, adhesive, lubricants, laminates, solvents, antifreezing agents and medicine (Zhu et al., 2002, Cheng et al., 2007). 1,3-PDO has traditionally been considered as a 'specialty chemical' is now undergoing a 'commodity chemical' (Sheldon et al., 2007). In recent years the development of innovative biological processes and potent microbial strains, the biotechnologically produced 1,3-propanediol can now compete with the one which is synthesised by chemically. The conversion and utilization of biologically important chemical constituents has become an important research trend in recent years, which is mainly due to lack of non-renewable resources.

Newly developed biofuel technology produces numerous biofuel by-products and its associated raw components like fiber of corn, glycerol, animal manure, which mainly serves as a basis for additional sources of bio energy which consists of liquid biofuel and biogas. Crude glycerol is considered as valuable by-products which are derived from biofuel production unit. In recent years, with the development of new biological processes and novel strains, the biologically produced 1,3-PDO can now compete with the one produced from petrochemicals. The conversion and utilization of bio renewable feedstock for the production of valuable materials has become an important research trend in recent years due to the decreasing supply of non-renewable resources, escalating global energy demand, and negative environmental impact. Emerging biofuel technology produces numerous by-products and waste, ranging from corn fiber and glycerol to animal manure, which serve as a basis for additional sources of bio energy (liquid biofuel and biogas). Once considered a valuable by-product, crude glycerol is rapidly becoming a 'waste product' with an associated disposal cost. In 2007, due to the rapid growth of biodiesel production, the price of pure glycerol decreased. Manufacturers are forced to invest large amounts of money in removing the unwanted glycerol from their plants. It is, therefore, crucial to develop environmental-friendly solutions for glycerol waste. Interest in this new field of research, known as glycerol chemistry, has recently grown, raising possibilities for the use of unrefined glycerol, which, in turn, facilitates the sustainability of the biofuel market.

One of the promising strategies for glycerol utilization is the production of propanediol through selective glycerol hydrogenolysis. This process provides a clean and economically competitive route for the production of commercially valuable propanediol from renewable glycerol rather than from non-renewable petroleum. 1,3-Propanediol has received recent attention as a high-value specialty chemical used primarily in the preparation of polyester fibers, films, and coatings. It is a non-flammable, low toxicity liquid which is miscible with water, alcohols, and ethers, making it easy to transport. In 2012, global demand for 1,3-propanediol was 60.2 Kt (Kiloton) with a market value of $2.61 per Kg. Considering 1,3-propanediol is used in the textile industry, food packaging, lubricants, and medicine, demands will continue to rise. The biodegradable nature, higher light stability, and solubility of 1,3-propanediol based polyesters is most common solvents add to its already growing list of applications. By 2019, global

demand is expected to reach 150 Kt and the price of 1,3- propanediol is estimated to reach $3.73 per Kg. The high price of 1,3-propanediol indicates the economic sustainability of the glycerol conversion process.

Biotechnological methods have been widely used to obtain 1,3-propanediol from glycerol using bacteria of the genera *Clostridia, Klebsiella, Citrobacter, Lactobacilli,* and *Escherichia.* However, despite the fact that many microorganisms are able to metabolize glycerol in the presence of external electron acceptors (respiratory metabolism), few are able to do so fermentatively (i.e. in the absence of electron acceptors). Although the bioconversion of glycerol to 1,3- propanediol via fermentation has been extensively investigated, it appears that only several species of the *Enterobacteriaceae* family such as *Citrobacter freundii, Klebsiella pneumoniae, Enterobacter agglomerans, Lactobacilli brevis,* and *Lactobacilli buchneri,* as well as *Clostridium butyricum and Clostridium pasteurianum*, are able to form 1,3- propanediol. The dissimilation of glycerol in these organisms is strictly linked to their capacity to synthesize the highly reduced product 1,3-propanediol. The potential for using these organisms at the industrial level is limited due to their pathogenicity, the requirement for strict anaerobic conditions, the need for rich nutrient supplementation, and the lack of availability of the genetic tools and physiological knowledge necessary for their effective manipulation. In addition, because reducing power must be generated during the fermentation process, only a portion of the glycerol can be converted to 1,3-propanediol. Currently established fermentation processes that convert glycerol to 1,3-propanediol reach a maximum yield of 50–60% (mol/mol), with about 40-50% of the glycerol converted to undesirable by-products.

II. Chemical synthesis

Nowadays, there are two main chemical processes for 1,3-propanediol synthesis. The Degussa process, now owned by DuPont, Wilmington, DE, USA, is based on acrolein obtained by the catalytic oxidation of propylene. Acrolein is hydrated to 3-hydroxypropionaldehyde, followed by hydrogenation to produce 1, 3-propanediol. The yield of 1,3-propanediol obtained in this process is 40%. The other major process is owned by Shell Chemicals, USA. The process of Shell starts with the hydroformylation of ethylene oxide, followed by hydrogenation. In this process a yield up to 80% can be obtained. Both processes involve high production costs and formation of toxic intermediates (Saxena et al., 2009), that probably induced DuPont to develop a biological process.

III. Production through fermentation

1,3-Propanediol can be naturally produced by microorganisms. The carbon source used is glycerol or sugar co-fermented with glycerol, but no natural microorganism can convert only sugar into 1,3-propanediol (Cameron et al., 1998). The natural pathway for the production of 1,3-PDO is from glycerol and occurs in facultative aerobic or obligate anaerobic bacteria species. The facultative aerobic group includes microorganisms belonging to genera *Klebsiella, Citrobacter* and *Enterobacter (e.g., Citrobacter freundii, Klebsiella pneumoniae, Enterobacter agglomerans).* It was also shown that some *lactobacilli* species such as *Lactobacillus brevis, Lactobacillus buchneri and Bacillus welchii* are able to produce 1,3-propanediol from glycerol, when cultivated with a co-substrate like glucose or fructose, under anaerobic conditions. The obligate anaerobic bacteria able to produce 1,3-propanediol are some *Clostridia* species, like *Clostridium butyricum and Clostridium pasteurianum* (Schutz and Radler, 1984; Forsberg, 1987; Homann et al., 1990; Biebl et al., 1992; Dabrock et al., 1992; Barbirato et al., 1995; Daniel et al., 1995; Biebl et al., 1999). *C. acetobutylicum* was initially included among these species (Forsberg, 1987), but later on it was found that this microorganism does not carry the metabolic pathway to produce 1,3-propanediol and is not able to grow on glycerol as the sole carbon source (Vasconcelos et al., 1994). During the earlier years, various studies have been carried out with these microorganisms, aiming 1,3-PDO production (Boenigk et al., 1993; Daniel et al., 1995; Papanikolaou et al., 2000; Zeng and Biebl 2002; Gonzalez-Pajuelo et al., 2006). For all microorganisms tested for 1, 3-PDO production, *K. pneumoniae and C. butyricum* have been widely studied. *K. pneumoniae* is an opportunistic pathogenic microorganism and, consequently, *C. butyricum* is preferred for potential industrial applications.

IV. Historical overview

1,3-PDO is one of oldest known fermentation products. It was reliably identified as early as 1881 by August Freund, in a glycerol fermenting mixed culture containing *Clostridium pasteurianum* as the active organism (Freund, 1881). Later, in 1914, Voisenet described a wine spoiling *Bacillus* that produced the substance, but so far no comparable strain has been isolated. Quantitative analysis of 1,3-PDO produced by different *Enterobacteria* had started at the microbiology school of Delft and was successfully continued at Ames, Iowa (Mickelson and Werkman, 1940). In the 1960s, interest shifted to the glycerol attacking enzymes, in particular to the glycerol and diol dehydratase, as these enzymes were peculiar in the 1,3-PDO forming clostridia were first described in 1983 as part of a process to obtain a specialty product from glycerol excreting algae (Nakas et al., 1983).

Chemical properties

1,3-PDO is an compounds with a molecular formula $CH_2(CH_2OH)_2$, and molar mass at 76.09 g/mol. The synonym of 1, 3-PDO are trimethylene glycol, propylene glycol and 1,3-dihydroxypropane. The melting and boiling

point of 1,3-PDO are -28ºC and 210-212ºC respectively. Its density is 1.0597 g/l (25ºC) and is fully miscible in water. The structure of 1,3-PDO is depicted in Fig 1.

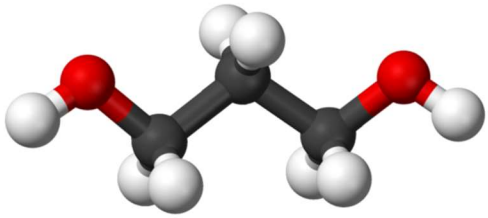


Fig 1: 1,3-Propanediol.

1,3-PDO is a linear aliphatic glycol with two hydroxyl groups at 1 and 3 positions. Due to presence of these two functional groups in 1,3-PDO, it finds application in the production of polymers, such as polyesters and polyurethanes and is a versatile intermediate compound used in the synthesis of heterocyclic.

V. Economic importance of 1,3-PDO

Earlier, 1,3-PDO had negligible value, when compared to other bulk chemicals due to the non-availability of sufficient quantity and quality. The price of 1, 3-PDO was very high in the past. According to Millet, (1993) the price of 1,3-PDO was about 30 U.S.$/Kg when chemical synthesis was used, meant that 1,3-PDO could not compete with the petro chemically available low priced (up to 2 U.S. $/Kg) diols like 1,2-ethanediol, 1,2-propanediol and 1,4-butanediol. Therefore, in the past 1,3-PDO has only found niche application of negligible market volume such as production of solvent, dioxanes and specialty polymers (Sullivan, 1993).

In 1995 the situation for 1,3-PDO changed significantly as Chemical Company announced the commercialization of a new polyester called Corterra^{TM,} based on terephthalic acid and 1,3-PDO. This poly trimethylene terephthalate (PTT) is appropriate particularly for fibre and textile applications and combines excellent properties (good resilience, inherent stain resistance, low static generation) with an environmentally benign manufacturing process (Chuah et al., 1995). Shell developed 1,3-PDO plant with a turnover of 80,000 t/year by a chemical route from ethylene oxide. Also Degussa had designed a new 1,3-PDO plant (around 10,000 t/year) from acrolein at Wesseling Geramany (McCoy, 1998). The Degussa process of manufacturing 1,3-PDO has been taken over recently by Dupont. However, DuPont's strategic interest is obviously to establish a monomer to produce the desired polyester, i.e. PTT, which was sold as Sorona R by DuPont. This Sorona R polymer can also be referred as 3GT, whereby 3G stands for 1,3-PDO and T for terephthalate). In a collaborative joint project by DuPont and Genencor International, metabolic engineering is being used to convert glucose to 1,3-PDO directly. By changing the feed stock basis for the fermentative 1,3-PDO production from glycerol to glucose, the raw material price could certainly be reduced considerably. In recent years technological breakthrough in the preparation of 1,3-PDO have resulted in lower market prices, currently in the $0.80 per pound range. The market for 1,3-PDO is over 100 million pounds per year and is growing rapidly (Kraus, 2008).

Biotechnological synthesis of 1,3-PDO

The need for a sustained resource supply, the rapid advances in biotechnology and microbial genetics and the strategic shift of major chemical companies into the area of life sciences are some of the driving forces for renewed interest in producing bulk chemicals from renewable resources by biological processes. The biotechnological synthesis of 1,3-PDO appear to be an alternative to chemical synthesis as it is carried out under milder operational conditions and it does not generate toxic by-products. Considering the yield, product recovery and environmental protection, much attention has been paid to microbial production of 1,3-PDO (Xiu et al., 2007). Moreover, the microbial process can use glycerol as substrate, which is a low-cost renewable resource appearing in increasing quantities as the principle by-product from fat saponification, alcoholic beverage manufacture and biodiesel production units. Glycerol is not only cheap and abundant, but its greater degree of reduction than sugars offer the opportunity to obtain reduced chemicals at higher yields than those obtained using sugars (Dharmadi et al., 2007).

Microorganisms and Industry

1,3-PDO is a typical product of glycerol fermentation and has been found in the fermentative conversion of other organic substrates. Only very few organisms, all of them bacteria, are able to form it (Bielb et al., 1999). A number of microorganisms can ferment sugars to glycerol but they cannot convert glycerol to 1,3-PDO. Others may have the ability to ferment glycerol 1,3-PDO and still others can ferment mixture of glycerol and sugars and sugars to 1,3-PDO, however, none can ferment sugars directly to 1,3-PDO (Cameron et al., 1998). These are reports where new

metabolic pathways have designed in cells by combining the ability to ferment sugar to glycerol and subsequently glycerol to 1,3-PDO (Forage and Lin, 1982; Tong and Cameron, 1920).

The biotechnological production of 1,3-PDO from glycerol has demonstrated for several bacteria, such as *Klebsiella, Clostridia, Citrobacter, Enterobacter* and *Lactobacilli* (Sauer et al., 2008). Several *Clostrodial* species such as non-pathogenic *Clostridium butyricum* (Papinikolaou et al., 2004), *C. pasteurianum* (Biebl, 2001) grow on glycerol and form 1,3-PDO. Facultative anaerobes such as *Klebsiella pneumoniae* (Yang et al., 2007; Vanajakshi and Annapurna, 2011) and *Citrobacter frendii* (Pfluamacher and Gottschalk, 1994; Danial et al., 1995; Barbirato et al., 1998; Boenigk et al., 1993) also appeared on to be suitable for 1,3-PDO production although it might be easier to handle facultative anaerobes, but since all these stains are classified as opportunistic pathogens, special safety precautions are required to grow them. Along with the *K. pneumonia,* other species same genera have also been exploited for 1,3-PDO production, such as *K. oxytoca* (Homan et al., 1990; Yang et al., 2007) and *K. planticola* (Homan et al., 1990). *Lactobaccillus brevis, L. buchneri and L. reuteri* (Pang et al., 2002) have been shown to use glycerol as an external hydrogen acceptor source during fermentation. Among these organisms, *C. butyricum* and *K. pnuemonia,* are considered the best "natural producers" and are paid more attention because of their appreciable substrate tolerance, yield and productivity (Gonzalelz-Pjuelo et al., 2006).

There are few thermophilic 1,3-PDO producers such as *Caloramator viterbensis* and *Closrtidium butyricum* are isolated from nature (Seyfried et al., 2002). The use of such organisms could be advantageous to make biotechnological process more cost efficient. In thermophilic fermentation, it is often possible to use hot effluents from preceding process steps, an energy consuming cooling of the fermenter is not necessary and downstream processing by distillation could save energy and money. Other 1,3-PDO forming species do not belong to the above mentioned groups are *Anaerovibrio burkinabensis, Iliobacter polytropus* and *Peliobacter venetianus.* They are forming 1,3-PDO from glycerol but only in small amounts (Willke and Vorlop, 2008).

The biotechnological conversion of glycerol to 1,3-PDO is mainly achieved by bacteria under anaerobic or micro aerobic conditions (Chen et al., 2003; Yang et al., 2007). A number of bacteria are able to grow on glycerol as the sole carbon and energy source, such as *Citrobacter freundii, Klebsiella pneumoniae, Clostridium pasteurianum, Clostridium butyricum, Enterobacter agglomerans, Enterobacter aerogenes* and *Lactobacillus reuteri.* The first publication describes the isolation and purification of 1,3-PDO from a bacterially (*Schizomycetes*) fermented glycerol solution (Freund, 1881). The best known 1,3-PDO producers investigated so far belong to the *Clostridia* and *Enetrobacteriaceae* (Willke and Vorlop, 2008). The two groups both use the pathway as a general part of NAD regeneration (Biebl et al., 1999), but have somewhat different metabolisms and differ in some features that are essential for economic production on an industrial scale.

Table 1: Comparison of potential industrial 1,3-PDO producers from glycerol (Willke and Vorlop, 2008)

Clostridia	***Enetrobacteriaceae***
Strictly anaerobic, therefore difficult to handle	Facultative aerobes, robust, easy handling
Spore forming	No sporulation
Main by-product: acetic acid, butyric acid	Main by-product: ethanol, acetic acid
Yield: about 0.5 Kg 1,3-PDO/Kg glycerol	Yield: about 0.5 Kg 1,3-PDO/Kg glycerol
Maximum theoretical yield: 0.72 mol/mol	Maximum theoretical yield: 0.64 mol/mol
Productivity (g/l/h): 2.8	Productivity (g/l/h): 2.4

VI. Bacteria Characterization

Systemic studies of 1,3-PDO production from glycerol with *Clostridia* are numerous (Biebl and Sproer, 2002). The best-known producer within this group is *Clostridium buityricum* followed by the acetone/butanol producers such as *C. acetobutyricum, C. pasteurianum* and *C. beijerinckii* (Dabrock et al., 1992). A novel strain of *Clostridium* i.e. *C. diolis* was found as potential producers of 1,3-PDO (Biebl and Sproer, 2002) and hydrogen (Matsumoto and Nishimura, 2007) from slurry. Attempts were made to modify *C. butyricum* by genetic engineering or to combine properties from different species to get a more efficient microorganism (Gonzalez-Pajuelo et al., 2006).

Clostridiaceae

Clostridia are rod-shaped, spore-forming Gram-positive bacteria and typically strict anaerobes. They can naturally produce butanol, acetone, ethanol, isopropanol and 1,3-propanediol. Moreover, clostridia can produce chiral products which are difficult to obtain by chemical synthesis and degrade a number of toxic chemicals. Solventogenic *Clostridia*, such as *C. acetobutylicum, C. beijerinckii, C. saccharobutylicum* and *C. saccharoperbutylacetonicum*, can utilize a wide range of substrates, from monosaccharide's including many pentose's and hexoses to polysaccharides (Lee et al., 2008). A variety of enzymes involved in degradation of hemicelluloses and starch have been identified in different strains. Cellulolytic *Clostridia,* classified by *Clostridium thermocellum*, produce a multi-enzyme cellulase

complex able to degrade cellulose, hemicelluloses and starch (Mitchell, 1997). A remarkable development of *Clostridial* toxins and spores has been their utility in the treatment of human diseases. *Botulinum neurotoxin* is used as a therapeutic agent for various neurological disorders, including dystonias, involuntary muscle disorders, pain and other maladies. Spores' systems of *clostridia* are being developed for the delivery of therapeutics to tumours (Johnson, 2009). The nutrient requirements for the growth of *clostridia* are simple. Complex nitrogen sources are generally required for good growth and solvent production. For all these reasons, clostridia are a valuable source of enzymes that are used in bio processing and in biotransformation. *Clostridia* are important in fermentation processes for the production of solvents and organic acids. When growing in glucose, three different metabolic states can be found in *C. acetobutylicum* depending on pH (Girbal and Soucaille, 1998). At neutral pH *C. acetobutylicum* behaviour is acidogenic and it produces acetic and butyric acids; but when the pH is neutral and, at the same time, the availability of NADPH is high, the behaviour is alcohologenic and it produces ethanol and butanol, but not acetone; at low pH the behaviour is solventogenic and it produces acetone, butanol and ethanol. Solventogenic behaviour is closely coupled to sporulation. The transcriptional factor responsible for initiation of sporulation is also responsible for the solvent production initiation in *C. acetobutylicum* (Lee et al., 2008) by activating transcription of acetoacetate decarboxylase, alcohol dehydrogenase and CoA transferase genes (Sullivan and Bennett, 2006). Although able to use several carbon sources, *C. acetobutylicum* cannot grow on glycerol as the sole carbon source, as it cannot re-oxidize the excess of NADH generated in glycerol catabolism (Vasconcelos et al., 1994). The best natural 1,3-PDO producer in terms of yield and titer is *C. butyricum.* Through a genetic engineering strategy, it was possible to introduce the NADH consuming 1,3-PDO pathway from *C. butyricum* into *C. acetobutylicum* and obtain the mutant strain *C. acetobutylicum* DG1 (pSPD5) able to grow on glycerol and produce 1,3-PDO as the main fermentation end-product. This strain was used in the present work.

Enterobacteriaceae

Only eight taxa of the *Enetrobacteriaceae* (out of 1123 strains from 128 taxa tested) are reported to grow fermentatively on glycerol and all produce 1,3-PDO and possess both glycerol dehydratase (GDHt) and 1,3-propanediol oxidoredutase (PDOR) enzymes (Bouvet et al., 1995). Among these eight taxa, numerous strains are able to convert glycerol into 1,3-PDO, with the most promising being *Klebsiella pneumonia* (Zhang et al., 2007; Vanajakshi and Annapurna, 2011b) and Citrobacter *freundii* (Boenigk et al., 1993), whereas *Klebsiella oxytoca* (Yang et al., 2007) and *K. Planticola* show somewhat lower yields and productiveness (Homann et al.,1990). 1,3-PDO yield strongly depends on the amount and type of by-products produced in the metabolic pathway of the organism. Little work exists on *Enterobacter agglomerans* (Barbiroto et al., 1995), which also produces 1,3-PDO but only to a lesser extent.

Glycerol fermentation by *Klebsiella* can result in the production of acetate, formate, lactate, succinate, ethanol, 2,3-butanediol, CO_2 and H_2, besides 1,3- propanediol (Streekstra et al., 1987; Homman et al., 1990; Barbirato et al., 1995). Acetate is the main acid formed and represents an energy source for cells (Zeng et al., 1993). When the substrate is in excess and the growth rate is high, small concentrations of Acetoin were also observed (Solomon et al., 1994).

Citrobacter, namely *C. freundii,* is able to use glycerol as the sole carbon source. The end-products of this fermentation are not only 1,3-propanediol, but also acetate, lactate, formate, pyruvate, ethanol, CO2 and H2 (Homann et al., 1990; Boenigk et al., 1993). *Citrobacter* produces lower amounts of ethanol than *Klebsiella.* 1,3-Propanediol molar yield can reach 65%, and in this case the main by-product is acetate. Barbirato et al., (1995) described an *Enterobacter, E. agglomerans,* that produces 1,3-propanediol as the major glycerol fermentation end-product. The by-products are acetate, ethanol, formate, lactate and succinate. When high glycerol concentrations were used (71 to 100 g/l), 1,3-propanediol yield reached 0.61 mol/mol of glycerol. During batch cultivations after the consumption of about 40 g/l of glycerol, cell growth, glycerol uptake and 1,3-propanediol production ceased. This phenomenon was related with the lethal accumulation of 3-HPA and was also observed in *K. pneumoniae and C. freundii* (Barbirato et al., 1996). For *K. pneumoniae*, the fermentation ceased when 3-HPA concentration was over 0.79 g/l (Zheng et al., 2008). A strategy using an engineered strain of *Escherichia coli,* was recently described by Tang et al., (2009). In this study, the first stage consisted of a significant increase of biomass using glucose and in the second stage glycerol added to the medium was converted to 1,3-PDO. The overall fermentation time was only 40 h. The 1,3-PDO obtained was 104.4 g/l, with a productivity of 2.61 g/l/h and a conversion of glycerol to 1,3-PDO of 90.2% (g/l).

Lactobacilli

Lactobacillus brevis and *L. buchneri* are well investigated strains (Ledesma et al., 2006), which can produce 1,3-PDO from glycerol while glucose is fermented to acetic acid or ethanol, and lactic acid. *Lactobacillus collinoides*, isolated from cider, degraded glycerol in the presence of sugar mainly to 1,3-PDO, acetic acid and ethanol (Claisse et al., 2000). *Lactobacillus reuteri* (Doleyres et al., 2005) and related strains have attracted attention since they are able to produce an antimicrobial agent called reuterin, containing 3-hydroxypropionaldehyde (3-HPA), which is further

metabolized to 1,3-PDO. There is also extensive research on the biotechnological production of 3-HPA, a promising intermediate for the chemical industry (Ruetti et al., 2007; Vanajakshi and Annapurna, 2010).

Thermophilic microorganisms

There are few works on isolating thermophilic 1,3-PDO producers from nature. The ability to proliferate at growth temperature optima well above 60ºC is associated with extremely thermally stable macromolecules of thermophilic microorganisms. As a consequence of growth at high temperature and unique macromolecular properties, thermophilic bacteria can possess high temperature and unique macromolecular properties, thermophilic bacteria can possess high metabolic rates, physically and chemically stable enzymes, and lower growth but higher end product yields than similar mesophilic species. Thermophilic processes appear more stable, rapid and less expensive. Among 60 screened strains, Wittlich et al., (2001) found 16, 1,3-PDO producers and of these, the best strain formed 6.4 g/l 1,3-PDO. Another thermophilic strain with an optimum temperature at 60ºC was described as *Caloramator viterbensis,* which produces 1,3-PDO from glycerol at high yields of 0.69 mol/mol with only acetic acid as organic by-product (Seyfried et al., 2002)**.**

Miscellaneous

Various other species of bacteria known for 1,3-PDO production are *Anaerovibrio burkinabensis, Iliobacter polytropus* and *Pelobacter venetianus* (Willke and Vorlop, 2008). They are all forming 1,3-PDO from glycerol in small amounts only. There is little work on biotechnological production of 1,3-PDO from glucose with natural strains. One project dealt with the synthesis via malonic acid. But only the step to malonic acid was done biotechnologically with a filamentous fungus such as *Phanaerochaete chrysosporium*; further reaction to 1,3-PDO should be performed with a specially developed chemical catalyst (Anonymos, 2008). Nakas et al., (1983) combined green algae *Dunaliella tertiolecta*, which converts glucose to glycerol (49 g/l). Dupont in cooperation with Genencor constructed a genetically engineered microorganism based on enzymes from *Saccharomyces* and *Klebsiella* combined in a single *E.coli K 12,* which converts glucose directly to 1,3-PDO. Product concentration reached up to 130 g/l, but only at low yields of 0.34 mol/mol (Cervin et al., 2004).

VII. Industrial applications

In the beginning of the twentieth century in response to a demand of the World War I, scientists developed the novel industrial-scale fermentation process, the production of acetone-butanol using the bacterium *Clostridium acetobutylicum.* After this, further innovations in fermentation technology were applied to industry, especially in the 1940s with the production of penicillin, and biotechnology began to emerge. For years biotechnology could not economically compete with the petrochemical industry in the synthesis of many products that could be produced from oil. However, growing concerns about petroleum depletion and environmental awareness are increasing the relevance of using industrial or white biotechnology. Industrial biotechnology uses living cells and enzymes, the so-called biocatalysts, to synthesise bio-based chemicals, materials and fuels. Besides the fact that most of the time these products are easily degradable, industrial biotechnology processes are considered more environmentally sustainable because generally they are low energy consuming and create less waste than the traditional chemical processes they replace. The use of biomass like starch, molasses, cellulose, vegetable oils and agricultural waste by industrial biotechnology is another advantage of those processes. Nevertheless, industrial biotechnology still is a relatively young technology and many of the processes have not yet proven that they are economically sustainable (Frazzetto, 2003). Some of the problems to be solved are the low product concentration, low productivity and also the high recovery cost. Although molasses and other renewable resources are cheap, the cost of downstream processing for these cases should not be neglected and can account for more than 50% of the total production cost. Research areas such as genetic and metabolic engineering have been developed and can contribute to the renewed interest in the production of chemicals from renewable resources.

1,3-Propanediol production from glycerol

Besides the microorganism, there are several factors influencing 1,3-PDO production, such as medium composition, temperature, pH, end-products and substrate concentrations. A fed-batch process can be a solution to avoid substrate inhibition. Chemostat cultures are also interesting if high productivity can be reached. The addition of sugars as co-substrate can also improve the production of 1,3-PDO from glycerol. Although sugar could not be converted into 1,3-PDO, it can be used to cells growth and regeneration of reducing power (Yang et al., 2007). All these strategies were evaluated and the results achieved depended on the microorganism used and on the process configuration and operating conditions. Gonzalez-Pajuelo et al., (2005) described a new *C. acetobutylicum* mutant able to produce 60 g.l-1 of 1,3-PDO from glycerol with a yield of 0.64 mol/mol and a productivity of 3 g/l/h in continuous cultures, what represented a twofold increase in 1,3-PDO titre and productivity when compared to the wild type *C. butyricum* that in the same conditions produced up to 35 g/l. Research on immobilized *K. pneumoniae* was carried out by Zhao et al., (2006) under batch, fed-batch and continuous cultures. Table 2.3.2, 1,3-Propanediol final concentration, yields and productivity of different glycerol (and co-substrate) fermentation processes.

Genetic, metabolic and evolutionary engineering

Fermentations used to be carried out spontaneously, and microorganisms were unknowingly transferred from fermentation to fermentation. The first pure yeast culture used for beverage production was obtained in 1883 by Emil Christian Hansen from the Carlsberg Brewery and in 1890 a pure culture of a wine yeast was obtained by Muller-Thurgau from Geisenheim, Germany (Dequin, 2001). Traditional techniques to improve the performance of microorganisms consisted of selecting the best producers for the desired phenotype. The evolution of microbial physiology knowledge, genetic manipulation, biochemistry advances and the development of fermentation technologies have contributed to obtain microorganisms or its derivatives capable of producing a final desired product with high efficiency, yields and titres. With genetic engineering, new tools were available for microorganism's genetic manipulation and transformation. It became possible to develop, improve, modify or eliminate metabolic pathways in order to increase the range of microbial native products as well as exogenous products commercially more viable. The new age of "omics", including genomics, transcriptomics, proteomics and metabolomics, has brought many advances and advantages to the area of study called metabolic engineering and introduced by Bailey in 1991 (Chotani, 2000; Ostergaard, 2000; Schuller and Casal, 2005; Nevoigt 2008). Since its expansion there are many examples of emerging applications of biotechnology using metabolic engineering in the design of systems wide experiments for the production of chemicals, materials and medicines. A relevant example is the genetically engineered human insulin production, in the yeast *Saccharomyces cerevisiae*, in 1987 (Kjeldsen, 2000).

Glycerol uptake

Like other small uncharged molecules, glycerol can cross the cytoplasmic membrane through passive diffusion. However, cells limited passive uptake has a growth disadvantage at low concentrations of substrate. Glycerol uptake frequently cited as only example of transport mediated by facilitated diffusion across *Escherichia coli* inner membrane. Facilitated diffusion is achieved by an integral membrane protein, the glycerol facilitator GlpF (Darbon et al., 1999). Intracellular glycerol is subsequently converted to glycerol-3-phosphate by glycerol kinase. Glycerol-3-phosphate remains trapped in the cell unit it is further metabolized because it is not a substrate for the glycerol facilitator (Darbon et al., 1999; Braun et al., 2000). GlpF acts as a highly selective channel, also conducting polyalcohol's and urea derivatives, for which it is stereo-selective enantio-selective. All these channels are strictly selective for non- ionic compounds, including hydroxide and hydronium ions, thus preventing the dissipation of membrane potential (Braun et al., 2000; Fu et al., 2000). The influx of glycerol mediated by GlpF is 100 to 1000-fold greater than expected for a transporter and is non saturable at a glycerol across the plasma membrane is controlled either by passive diffusion, a channel protein or an uptake mechanism (Wang et al., 2001).

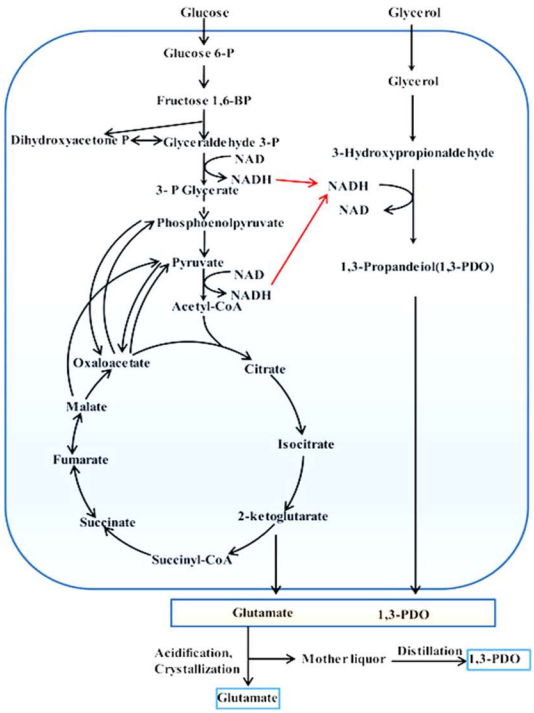


Fig. 2: Metabolic Pathway of Glycerol uptake and 1,3-Propanediol production. (Jinhai et al. 2017)**.**

Physiological formation of 1, 3-PDO

A variety of organisms such as *Klebsiella* and *Clostridia* produce 1,3-PDO from glycerol under very specific conditions. Glycerol metabolism involves two parallel pathways: the reductive and oxidative pathways. In the reductive pathway, glycerol is first converted to 3-hydroxypropanaldehyde (3-HPA) by glycerol dehydratase (GDHt) enzyme, which is then reduced to 1,3-PDO by $NADH_2$ dependent 1,3-propanediol oxidoreductase (PDOR) enzyme. Through the oxidative pathway glycerol is dehydrogenated by NAD^+ dependent glycerol dehydrogenase (GDH) enzyme to dihydroxyacetone (DHA), which is then phosphorylated by ATP dependent dihydroxyacetone kinase (DHAK) before entering glycolysis. This pathway produces by-products such as ethanol, succinic acid, 2,3-butanediol (2,3-BDO), also provides energy and reducing equivalents ($NADH^2$) for biomass and 1,3-PDO synthesis (Forage and Lin, 1982). The formation of 1,3-PDO in glycerol fermentation was considered for the regeneration of NAD^+ in which the necessary reducing equivalents, as well as the necessary energy, have to be produced by oxidation of glycerol to other products (Zeng et al., 1990). *Lactobacilli* have only this reductive conversion and need an additional fermentation substrate for growth and generation of reduction equivalents (Veiga da Cuhna and Foster, 1992).

The reactions down to the stage of pyruvate are common to all organisms involved. Glycerol is dehydrogenated to dihydroxyacetone which, after phosphorylation, can be converted to pyruvate in course of the known sequence glycolytic reactions involving another dehydrogenation and two ATP forming steps. The reductive glycerol conversion consists of vitamin B12 mediated dehydration of glycerol to 3-HPA and a reduction of the aldehyde to 1,3-PDO (Biebl et al., 1999). Glycerol fermentation by *Enterobacteriaceae* results in the accumulation of two main products, 1,3-PDO and acetate, whereas the secondary products, lactate, formate, succinate and ethanol, are produced in variable amounts depending on the culture conditions (Homann et al., 1990; Barbirato et al., 1998). With *C. butyricum,* 1,3-PDO is the main product, with butyric acid and acetic acid as by-products in addition to CO_2 and H_2 (Barbirato et al., 1998). The bacterium *C. pasteurianum* growing on glycerol produces a variety of metabolic end products, such as n-butanol, 1,3-PDO, ethanol, acetic acid, butyric acid and lactic acid (Biebl et al., 2001). 2,3-Butanediol (2,3-BDO) is another glycol that produced as a by-product from glycerol. According to Syu, (2001) 2,3-BDO is a compound that can be added as a flavouring agent in food products when converted to 1,3-butadiene, which is used in the production of synthetic rubber.

Enzymes involved in 1,3-PDO production

Since the mid 1980 the pendulum has been swinging for the studies on metabolic pathways and enzyme kinetics of 1,3-PDO production. On the basis of these studies, the enzyme glycerol dehydratase (GDHt), 1,3-PDO oxidoreductase (PDOR), glycerol dehydrogenase (GDH) and dihydroxyacetone phosphate kinase (DHAK).

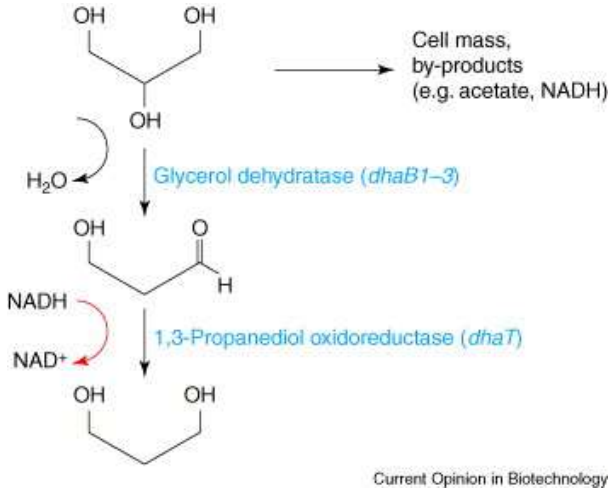


Fig 3: Enzymatic formation of 1,3-propanediol (*Charles E Nakamura.* 2013)

The first enzyme of glycerol fermentation, GDHt (E.C.4.2.1.3.0) requires adenosylcobalamine as a coenzyme, which removes a water molecule from glycerol to form 3-HPA (an intermediate in 1,3-PDO synthesis) by a radical mechanism (Sun et al., 2003). Similarly, the second enzyme of glycerol metabolism involved in the production of 1,3-PDO is PDOR (E.C.1.1.1.202). It catalyzes the transfer of reducing equivalent from NADH to 3-hydroxypropionaldehyde, yielding 1, 3-PDO (Skraly et al., 1998). PDOR had been purified from *L. breves* and *L. buchneri.* This enzyme is synthesized during the co-fermentation of glycerol with sugar. It provides an additional method of NADH disposal. The enzyme requires Mn^{2+} and is an octamer with a molecular mass of 350 KDa. Apart

from the enzymes discussed above, yet another enzyme glycerol phosphatase catalyzes the conversion of sugar to glycerol (Biebl et al., 1999). In nature, glycerol can be synthesised by microorganism like *Bacillus licheniformis*, *Saccharomyces cerevisiae, S.rouxii, Torulopsis magnolia* and *Pichia farinosa.* The glycerol phosphatase enzyme was found to have a unique activity, which is specific for D-glycerol-1-phosphate.

Table.2. Enzymes Involved in 1,3-propanediol synthesis.

Enzyme	Reference
glycerol dehydratase GDHt (E.C.4.2.1.3.0)	Sun et al., 2003
1,3-PDO oxidoreductase PDOR (E.C.1.1.1.202)	(Skraly et al., 1998
Glycerol dehydrogenase GDH (EC 1.1.1.6)	Mallinder et al.,1992
Dihydroxyacetone kinase DHAK (EC:2.7.1.29)	Regula Gutknecht et al 2001

VIII. Different strategies for the production of 1,3-PDO

Glycerol can be converted to 1,3-PDO, which is not only a useful final product, but also valuable starting compound for producing polymers (Papanikolaou et al., 2004). Both *Enterobacteria* and *Clostridia* appear to be suitable for a 1,3 PDO production process. Various strategies have been evaluated to produce 1,3-PDO from renewable feed stocks, which are important to the future competitiveness of the industry.

Process development for 1,3-PDO production

The biotechnological routes of 1,3-PDO production mainly includes processes such as batch fermentation, fed batch fermentation, continuous fermentation, two stage fermentation, micro aerobic fermentation and co-fermentation. Batch fermentation is simple and often used in primary investigation of 1,3-PDO production. 1,3-PDO production by *C. butyricum* DSM 5431 was studied in stirred tank and airlift reactors of various scales up to $2m^3$ and $1.2m^3$ respectively (Gunzel et al., 1991). The parameters studied in batch operation such as reactor type, N_2 gassing and changing the stirrer speed had no significant effect on 1,3-PDO production. As the airlift reactor is less expensive (lower investment and operational costs), its use appears more attractive. Inhibition by the initial substrate concentration in batch mode meant that fed-batch operation led to optimal results for 1,3-PDO production. Fed-batch fermentation is known to be an effective fermentation mode for producing a high concentration of 1,3-PDO without substrate inhibition (Jun et al., 2010). Some progress in fed-batch cultivation was made by controlling the nutrient supply. Saint-Amans et al., (1994) used CO_2 as the control parameter, Reimann and Biebl, (1996) combined pH correction by KOH with nutrient addition. By careful calculation of the ratio if the control parameter to nutrient addition, a slight but constant excess of glycerol was maintained, this is necessary to keep the amount of butyrate low. Continuous cultures have contributed considerably to the knowledge of product formation in 1,3-PDO fermentation as they are usually considered for their productivity.

In two stage fermentation approach, fermentation is carried out two consecutive steps. Two stage process has the advantages like better control of culture cultivation conditions for the individual stages and flexible of the use different substrates and to produce different products (glycerol or 1,3-PDO). A two stage 1,3-PDO fermentation has been carried out using *C. butyricum* (Papanikolaou et al., 2000). In co-fermentation strategy, mixture of glycerol and other sugars are used for the commercial production of 1,3-PDO in order to make the process less expensive. Abbad-Andaluossi et al., (1998) studied the effect of glucose on glycerol metabolism in case of *C. butyricum* DSM 5431. Vanajakshi and Annapurna, (2011a) have evaluated the sugars such as co-substrates in glycerol fermentation by *K. pneumoniae* 141B. Commonly, the biosynthesis of 1,3-PDO is done under anaerobic condition. Under anaerobic condition, glycerol can be converted to 3-HPA, which is toxic to cells. However, under micro aerobic condition or low aeration condition, it was found that 1,3-PDO can obtained by *K. pneumoniae* (Cheng et al., 2007.) Cheng et al., (2003) has reported that the micro aerobic production of 1,3-PDO by *K. pneumoniae* with improved 1,3-PDO productivity from 0.8 to 1.57 g/l/h by changing the fermentation from anaerobic to micro aerobic.

Strain improvement by mutagenesis for 1,3-PDO production

Strain improvement approaches by mutagenesis generated product tolerant mutants for 1,3-PDO production. Chemically generated mutants of strain *C. butyricum* DSM 5431 were selected in the presence of high 1,3-PDO concentrations and on bromide/bromate/glucose medium to obtain mutants that sustained substantially higher product concentrations and were strongly reduced in hydrogen evolution (Riemann et al., 1998a). If these stains are subjected to genetic improvement, further increases in the amount of the product can be expected.

Biotransformation

A novel enzymatic bioconversion utilizing a defined enzymatic process for the 1,3-PDO production was evaluated by Nemeth and Sevella (2008). Three key enzymes such as GDHt, PDOR and GDH were maintained in a batch stirred membrane reactor of 10 KDa ultra filter membrane, where the $NAD^+/NADH_2$ coenzyme were regenerated. It was observed that along with 1,3-PDO other unwanted by-products especially acetate was also formed. However, the results obtained by immobilization and enzymatic biotransformation studies are encouraging and merit further research and development.

Downstream processing of 1,3-PDO

For economical biological production of 1,3-PDO, an efficient and energy saving strategy for product separation and purification is essential. Since the product shall be used mainly in polymer chemistry, the grade of purification has to be from 95% up to over 99%, depending on the type of impurities and the demanded product properties. The development of an efficient purification strategy is posing as a technical barrier against the successful commercialization of 1,3-PDO from biological source. A fermentation broth containing mixture of multiple components, such as water residual glycerol, glucose, by-products (acetate, lactate, succinate, ethanol and 2,3-BDO), macromolecules (proteins, polysaccharides and nucleic acids), salts and residual medium makes the downstream processing of 1,3-PDO a potentially difficult separation challenge. In addition, 1,3-PDO is hydrophilic and has a high boiling point (Saxena et al., 2009). The boiling point of 1,3-PDO and glycerol are 214 and 290^{o}C at atmospheric pressure. This property makes the purification of 1,3-PDO from a complex fermentation broth a bottle neck for development of commercially viable process. Moreover, the purification of the product has to be considered as an integral part of the overall process as it is highly interconnected not only with biological process but also with economic factors, such as the choice of the carbon source. Purification costs can increase significantly with the use of less purified substrates.

Several methods for the purification of 1,3-PDO have been reported. The major methods for the recovery of 1,3-PDO studied are reactive extraction, liquid-liquid extraction, evaporation, distillation, membrane filtration, evaporation and ion exchange chromatography (Xiu and Zeng, 2008). In 1,3-PDO purification process, first the microorganisms are separated by filtration. Then ultra and nano filtration are employed to remove impurities with a molecular weight of more than 5000, or 200 Daltons, respectively. A series of ion exchange procedures removes ionic impurities. Subsequently, the amount of water in the product is reduced by evaporation. A series of two distillations removes first compounds having a boiling point exceeding that of 1,3-PDO and then compounds potentially giving rise to a discolouring of the products and a final series of two distillations concludes the process (Sauer et al., 2008).

IX. Application of 1,3-PDO

As a bifunctional organic molecule 1,3-PDO has several promising properties for many synthetic reactions, particular as a monomer for polycondensation to produce polyesters, polyether and polyurethanes. These applications have led to a strong market need for the production of bulk chemical preferable by biological route. The main advantage of this biodegradable polyester compared to polyhydroxy alkanote or polylacticides (two biodegradable polyesters from renewable sources) is the possibility that the physical properties of the polyester can be easily modified to meet the manufacturing specifications for the final article made from the plastic (BioMat Net Item, 2000).

Polypropylene terephthalate (PTT) or polytrimethylene terephthalate (PTT), is an aromatic polyester made by the polycondensation of 1,3-PDO and terephthalic acid. PTT has wide range of applications for fibre industry and it was first synthesized by Whinfield and Dickson of Caligo Printing Ink. Shell Company has released the commercial PTT polymer as Corterra^{R} polymer. PTT combines excellent properties like good resilience, inherent stain resistance, and low static generation with an eco- friendly manufacturing process. PTT promise its potential to be used in the carpet, textile, film and packaging and it has properties comparable to nylon (Hao et al., 2006). The additional applications of PTT include, it acts as co-monomer in polyesters, lacquers, casting resins, binders, adhesives as well as sealants inn industry building. The unsaturated polyesters derived from the condensation of 1,3-PDO with itaconic acid are versatile non-toxic and odourless polymers from with numerous applications (Willke and Vorlop, 2008) .

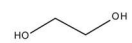
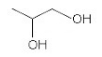
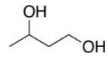
Plans to manufacture a T-shirt from corn have reached the same impasse. DuPont, the company that inverted nylon, has for many years been developing a polymer based on 1,3-PDO, with new levels of performance, resilience and softness. Adding an environmentally responsible dimension to the production, DuPont's polymerization plant in Decatur, illinoise (USA) has successfully manufactured 1,3-PDO from corn sugar, a renewable resource. Their corn-based polymer, called Sorona^{TM}, is more eco-friendly and has improved characteristics (EMBO reports, 2003). In addition to benefits of softness, stretch recovery, vibrant colour and strain resistance, the textiles made with Sorona^{TM} also have dimension of a naturally sourced material.

1,3-PDO also finds application in making transparent ballistic polymer which is a lifesaving technology, and the war fighter is the primary beneficiary. Ballistic polymer is based family transparent materials whose composition can tailor to enhance properties such as transparency, impact resistance and UV stability. The unique properties of these coployesters stem from the combination of 2,2,4,4-tetramethyl-1,3-cyclobutanediol with 1,3-PDO and dimethyl terephthalate. This polymer can be utilized in impact resistant eyeglass lenses, hurricane glass, and architectural ballistic glass (Saxena et al., 2009).

1,3-PDO also has a plethora of other interesting applications in addition to that of a polymer constituent. It may also be used as minor tranquilizer. Merprobamate, which was synthesized in 1951, is a prototype of propanediol. It was the first known propanediol tranquilizer. 1,3-PDO may also be used as biocides for example-PCT 3015; this is a low-cost high-performance biocide. Besides this, 2-bromo-2nitro-1,3-PDO is used as an industrial biocide for the

prevention of bio fouling in area such as recirculating water in cooling tower and evaporation condenser, air conditioners and humidifier systems. It can give improved properties for solvents, adhesives, laminates, resins, detergents and cosmetics (Zeng and Biebl, 2002). It is also used in the preservation of consumer, household and institutional products such as an industrial disinfectant and freshness keeping agents of cut flowers. It may also serve as a solvent and as a polyglycol-type lubricant (Papanikolaou et al., 2000). In engine coolant formulations, 1,3-PDO demonstrate improved heat stability, less corrosion and lower toxicity than conventionally coolant compounds. It was also used as component of animal feed.

Table.3. 1,3-propanediol can be used across a variety of consumer and industrial applications to replace or enhance the following ingredients:

Components	Ingredient	Formula	Structure	MP 0°C	BP 0 °C	MW	Density
Ethylene glycol	1,2-Ethanediol	$C_2H_6O_2$		-12.7	197.6	62.1	1.116
Propylene glycol	1,2-Propanediol	$C3H_8O_2$		-60	187.3	76.1	1.038
Butylene glycol	1,3-Butanediol	$C_4H_{10}O_2$		-50	204	90.1	1.005
Propanediol	1,3-Propanediol	$C_3H_8O_2$		-28	212	76.1	1.059

Biochemistry of 1,3-propanediol formation

Microbial anaerobic glycerol fermentation involves an oxidative and a reductive pathway. 1,3-Propanediol is produced through the reductive pathway in two enzymatic steps. First, glycerol is dehydrated to 3-hydroxypropionaldehyde (3-HPA) by a glycerol dehydratase, followed by the reduction of the aldehyde to 1,3-propanediol by the enzyme 1,3-propanediol dehydrogenase under the oxidation of NADH. In the species *K. pneumoniae* (Forage and Lin, 1982) and *C. freundii,* glycerol dehydratase is encoded by dhaB gene and is B12-dependent, and 1,3-propanediol dehydrogenase is encoded by dhaT. For *C. butyricum*, the conversion of glycerol to 1,3-propanediol involves three genes arranged in an operon. Glycerol dehydratase is encoded by dhaB1 and dhaB2; dhaT codifies for 1,3-propanediol dehydrogenase (Raynaud et al., 2003). The enzyme glycerol dehydatase is very sensitive to oxygen and it was shown that for *C. butyricum VPI 3266* the enzyme is B12- independent (Saint-Amans et al., 2001). In the second step of 1, 3-propanediol biosynthesis, NADH is consumed. NADH regeneration occurs in the oxidative pathway. In the oxidative branch, glycerol is converted to dihydroxyacetone by a NAD+ dependent enzyme glycerol dehydrogenase, which is phosphorylated by the enzyme dihydroxyacetone kinase and then converted into pyruvate through the glycolytic pathway. Biomass is produced and pyruvate can be converted into several end-products, depending on the microorganism (Figure 1.5.). Butyrate and n-butanol are produced by clostridia, while 2,3-butanediol is only formed by *Enterobacteria*. Acetate and ethanol are produced by both bacterial groups (Biebl et al., 1999). The intermediate product 3-hydroxypropionaldehyde (3-HPA) is a very toxic compound produced in first enzymatic step of the reductive pathway for 1,3-PDO formation and when accumulated can cause growth inhibition.

Fermentation End Product

Fermentation of glycerol by *Clostridium* results in production of 1,3-PDO but also in several secondary products such as lactic, acetic and butyric acid, ethanol, CO_2 and H_2. The theoretical maximum yield that can be obtained from anaerobic fermentation of glycerol occurs when acetate is the only by-product produced (Saxena et al., 2009; Chotani et al., 2000). The theoretical maximum 1,3-propanediol yield is 0.72 mol/mol glycerol, which was calculated for a culture without H_2 and butyric acid productions (Zeng, 1996). The production of by-products not only reduces the amount of carbon available, but can also inhibit the growth of microorganisms.

C. pasteurianum also synthesises butanol, sometimes the predominant product (Bieble et al., 1999). Other Clostridium able to produce butanol is *C. acetobutylicum*. Besides this product it can also produce acetone and ethanol however, it cannot produce 1,3-PDO (Fig 1.6.). A characteristic reaction of *C. acetobutylicum* and others is the phosphoroclastic reaction. In this reaction pyruvate is cleaved by pyruvate ferredoxin oxireductase, in the presence of coenzyme A (CoA), to yield CO_2 and acetyl-CoA. The reduced ferredoxin (Fd red) also formed is reoxidized in different reactions, and the most important one is the cleavage into hydrogen and oxidized ferredoxin (Fdox). Ferredoxin is an iron-sulphur containing protein that accepts or gives electrons at a very low potential and together with NADH-ferredoxin oxidoreductase, plays a crucial role as an electron carrier in electron distribution system in cells (Jungermann et al., 1973; Petitdemange et al., 1976). In the conversion of glycerol into 1,3-PDO, the reducing equivalents ($NADH_2$) generated by the oxidative branch during the production of organic acids determines the amount of 1,3-propanediol that can be formed by the reductive branch. The genetic engineered *C. acetobutylicum DG1 (pSPD5)* contain the genes for the production of 1,3-propanediol from *C. butyricum VPI 3266.* A comparison between these two strains showed a similar physiological “global behaviour” when grown on glycerol (Gonzalez-Pajuelo et

al., 2006). In both strains the main fermentation end-product resulting from glycerol metabolism was 1,3-PDO, and the hydrogen specific production rate was very slow. Most of the reduced ferredoxin produced by the decarboxylation of pyruvate was used to generate NADH, which caused the low hydrogen production.

However, a major difference was found between the two strains relatively to the oxidative glycerol pathway. While in *C. butyricum VPI 3266* glycerol oxidation is carried out by glycerol dehydrogenase and dihydroxyacetone kinase (Saint Amans et al., 2001), Gonzalez-Pajuelo and Co-authors (2006) demonstrated that the mutant strain *C. acetobutylicum DG1 (pSPD5)* uses only glycerol kinase and glycerol-3-phosphate dehydrogenase to oxidize glycerol. No glycerol dehydrogenase and dihydroxyacetone kinase activities were found in *C. acetobutylicum DG1 (pSPD5).* Another difference reported by the same authors, was the fact that lactate dehydrogenase activity was only detected in *C. acetobutylicum DG1 (pSPD5).* In glycerol fermentation by *C. butyricum VPI 3266* no lactate was produced and no lactate dehydrogenase activity was found.

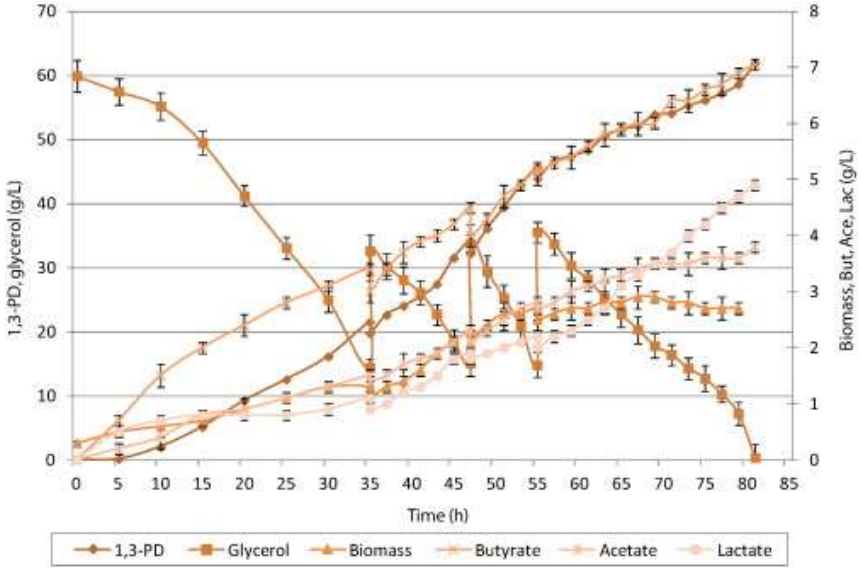


Fig.4. Glycerol consumption and metabolite (1,3-PD and organic acids) production during batch fermentation with crude glycerol as a carbon source

(Daria *Szymanowska-Powalowska- Electron. J. Biotechnol. 2014).*

Process strategies for 1,3-pronanediol fermentation

In an attempt to enhance the production and reduce the final cost of 1,3- PDO, many efforts have been focused on the development of new production processes. Fermentative production seems to be a feasible solution due to the present world situation. The use of renewable feedstock and agricultural and industrial wastes in biological processes appears to be the desired solution to cheaper substrates and a lowered demand for diminishing crude oil supplies. Glucose, starch and glycerol appear as possible cheap carbon sources for 1,3-PDO production; however, as no native microorganism can convert glucose or starch into 1,3-PDO, metabolic engineering has been used to generate novel 1,3-PDO producers' biological systems. Whether using sugar or glycerol different approaches have been implemented involving two-step fermentation, co-fermentation, mixed cultures and cells immobilization in batch, fed-batch or continuous cultures.

Table 4: 1,3-propanediol producing useful microorganisms

Fermentation process	Microorganisms	1,3-PD (g/l)	YPD (mol/mol)	q 1,3-PD (g/l/h)	Reference
Batch	*K.pneumoniaeATCC15380* *K.pneumoniae M 5al* *C.butyricum DSM5431* *C.butyricum VPI3266*	57.7 58.8 56.0 35.0	0.56 0.53 0.62 0.65	2.4 0.92 2.2 0.6	Tag (1990) Cheng et al (2007) Biebl et al (1992) Saint-Amans et al. (1994)
Fed-batch	*K.pneumoniaeATCC25955* *K.pneumoniaeATCC25955*	58.1 73.3	0.44	1.0	Held, (1996).

X. Conclusion

Besides the microorganism, there are several factors influencing 1,3-PDO production, such as medium composition, temperature, pH, end-products and substrate concentrations. A fed-batch process can be a solution to

avoid substrate inhibition. Chemostat cultures are also interesting if high productivity can be reached. The addition of sugars as co-substrate can also improve the production of 1,3-PDO from glycerol. Although sugar could not be converted into 1,3-PDO, it can be used to cells growth and regeneration of reducing power (Yang et al., 2007). All these strategies were evaluated and the results achieved depended on the microorganism used and on the process configuration and operating conditions. Gonzalez-Pajuelo et al., (2005) described a new *C. acetobutylicum* mutant able to produce 60 g.l-1 of 1,3-PDO from glycerol with a yield of 0.64 mol/mol and a productivity of 3 g/l/h in continuous cultures, what represented a twofold increase in 1,3-PDO titre and productivity when compared to the wild type *C. butyricum* that in the same conditions produced up to 35 g/l. Research on immobilized *K. pneumoniae* was carried out by Zhao et al., (2006) under batch, fed-batch and continuous cultures. Table 2.3.2, 1,3-Propanediol final concentration, yields and productivity of different glycerol (and co-substrate) fermentation processes.

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References

1. Barbirato F, Camarasaclaret C, Grivet J P and Bories A. Glycerol fermentation by a new 1,3-propanediol-producing microorganism – *Enterobacter agglomerans*. *Appl. Microbiol. Biotechnol.* (1995) 43: 786-93.
2. Barbirato F, Himmi E H, Conte T and Bories A. 1,3-propandiol production bt fermentation: an interesting way to valorize glycerin from the ester and ethanol industries. *Ind. Crop. Prod.* (1998) 7: 281-9.
3. Biebl H and Sproer C. Taxonomy of the glycerol fermenting *Clostridia* and description of *Clostridium diolis* sp nov. *Syst. Appl. Microbiol.* (2002) 25: 491-97.
4. Biebl H, Marten S, Hippe H and Deckwer W D. Glycerol conversion to 1,3-propandiol by newly isolated *Clostridia*. *Appl. Microbiol. Biotechnol.* (1992) 36: 542-7.
5. Biebl H, Marten S, Hippe H and Deckwer W D. Glycerol conversion to 1,3-propandiol by newly isolated *Clostridia*. *Appl. Microbiol. Biotechnol.* (1992) 36: 542-7.
6. Biebl H, Menzel K, Zeng A P and Deckwer W D. Microbial production of 1,3-propandiol. *Appl. Microbiol. Biotechnol.* (1999) 52: 289-97.
7. Boenigk R, Bowien S and Gottschalk G. Fermentation of glycerol to 1,3-propandiol in continuous cultures of *Citrobacter freundii*. *Appl. Microbiol. Biotechnol.* (1993) 38: 453-57.
8. Bouvet O M, Lenormand P, Ageron E and Grimont P A. Taxonomic diversity of anaerobic glycerol dissimilation in the *Enterobacteriaceae*. *Res. Microbiol.* (1995) 146: 279-90.
9. Braun T, Philippsen A, Wirtz S, Borgnia M J, Agre P, Kuhlbrant W, et al. The 3.7 A projection map of the glycerol facilitator GlpF: a variant of the aquaporin tetramer. *EMBO Rep.* (2000) 11: 183-9.
10. Cameron D C, Altaras N E, Hoffman M L and Straw A J. Metabolic engineering of propanediol pathways. *Biotechnol. Prog.* (1998) 14: 116-25.
11. Cameron D C, Altaras N E, Hoffman M L and Straw A J. Metabolic engineering of propanediol pathways. *Biotechnol. Prog.* (1998) 14: 116-25.
12. Cervin M A, Soucaile P and Valle F. Process for the biological production of 1,3-propandiol with high yield (2004), US patent 2004/0152174A1.
13. Charles E Nakamura. (2013) “Metabolic engineering for the microbial production of 1,3-propanediol”. Current opinion in Biotechnology Volume 14, Issue 5, October 2003, 454-459.
14. Chen X, Xiu Z, Wang J, Zhang D and Xu P. Stoichiometric analysis and experimental investigation of glycerol bioconversion to 1,3-propandiol by *Klebsiella pneumoniae* under micro aerobic conditions. *Enzymes. Microb. Technol.* (2003) 33(4): 386-94.
15. Cheng K K, Liu D H, Sun Y, Liu H J and Yang M D. Pilot-scale production of 1,3-Propanediol using *Klebsiella pneumonia.*. *Process. Biochem.* (2007) 42:19-22.
16. Cheng K K, Liu D H, Sun Y, Liu H J and Yang M D. Pilot-scale production of 1,3-Propanediol using *Klebsiella pneumonia.*. *Process. Biochem.* (2007) 42:19-22.
17. Chuah H H, Brown H S and Dalton P A. Corterra poly (trimethylene tetraphthalate)- a new performance carpet fiber. *Int. Fiber. J.* (1995) October.
18. Claisse O and Norton T. Process to separate 1,3-propandiol or glycerol, or a mixture thereof from a biological mixture (2003), US Patent 6,603,048.
19. Dabrock B, Bahl H and Gottschalk G. Parametrs affecting solvents production by *Colstridium pasteurianum*. *Appl. Environ. Microbiol.* (1992) 58: 1233-39.
20. Daria Szymanowska-Powalowska (2014 “1,3-Propanediol production from crude glycerol by Clostridium butyricum DSP1 in repeated batch” Electron. J. Biotechnol. vol.17 no.6 Valparaíso nov. 2014
21. EMBO reports. *White Biotechnology. Eur. Mol. Bio. Org.* (2003) 4(9): 835-7.

22. Festel Capital. (2007) (available on http://www.Festel.com).
23. Forage R and Lin ECC. Dha system mediating aerobic and anaerobic dissimilation of glycerol in *Klebsiella pneumonia* NCIB418. *J. Bacteriol.* (1982) 15: 591-9.
24. Forage R and Lin ECC. Dha system mediating aerobic and anaerobic dissimilation of glycerol in *Klebsiella pneumonia* NCIB418. *J. Bacteriol.* (1982) 15: 591-9.
25. Gunzel B, Yonsel S and Deckwer W D. Fermentative production of 1,3-propandiol from glycerol by *Clostridium butyricum* up to a scale of 2 m^3. *Appl. Microbiol. Biotechnol.* (1991) 36: 289-94.
26. Hao J , Xu F and Liu D. Downstream processing of 1,3-propandiol fermentation broth. *J. Chem. Technol. Biotechnol.* (2006) 81: 102-8.
27. Held A M. The fermentation of glycerol to 1,3-Propanediol by *Klebsiella pneumoniae.* Master's thesis, *University of Wisconsin Madison, USA*, (1996).
28. Homann T, Tag C, Biebl H, Deckwer W D and Schink B. Fermentation of glycerol to 1,3-propandiolby *Klebsiella* and *Citrobacter* strains. *Appl. Microbiol. Biotechnol.* (1990) 33: 121-6.
29. Jinhai Huang et al (2017) "Cofactor recycling for co-production of 1,3-propanediol and glutamate by metabolically engineered Corynebacterium glutamicum" doi:10.1038/srep42246. Scientific Reports 7, Article number: 42246 (2017)
30. Johnson D T and Taconi K A. The glycerin glut: option for the value-added conversion of crude glycerol resulting from biodiesel production. *Environ. Prog.* (2007) 26: 338-48.
31. Jun S A, Moon C, Kang C H, Kong S W, Sang B I and Um Y. Microbial fed-batch production of 1,3-propandiol using raw glycerol with suspended and immobilized *Klebsiella pneumonia. Appl. Biochem. Biotechnol.* (2010) 161: 491-501.
32. Kraus G A. Synthetic methods for the preparation of 1,3-propandiol. *Clean* (2008) 36: 648-51.
33. Mallinder, Phillip R.; Pritchard, Andrew; Moir, Anne (1992). "Cloning and characterization of a gene from Bacillus stearothermophilus var. non-diastaticus encoding a glycerol dehydrogenase". Gene. 110 (1): 9–16. doi:10.1016/0378-1119(92)90438-U. ISSN 0378-1119. PMID 1339360.
34. McCoy M. Chemical makers try biotech paths. *Chem. Eng. News* (1998) 22: 13-9.
35. Mickelson M N and Werkman C H. Formation of trimethylene glycol from glycerol by Aerobacter. *Enzymologia* (1940) 8: 252-6.
36. Millet P. Retournement de la situation de la glyceA rine. *Information Chimie* (1993) 345: 102-4.
37. Nakas J P, Schaedle M, Parkinson C M, Coonley C E and Tanenbaum S W. System development for linked-fermentation products of solvents from algal biomass. *Appl. Environ. Microbiol.* (1983) 46: 1017-23.
38. Nemeth A and Sevella B. Development of a new Bioprocess for production of 1,3-propanediol I; modeling of glycerol bioconversion to 1,3-propanediol with *Klebsiella pneumoniae* enzymes. *Appl. Biochem. Biotechnol.* (2008) 144: 47-58.
39. Papanikolaou S, Ruiz-Sanchez P, Parisat B, Blanchard F and Fick M. High production of 1,3-propanediol from industrial glycerol by a newly isolated *Clostridium butyricum* strain. *J. Biotechnol.* (2000) 77: 191-208.
40. Pflugmacher U and Gottschalk G. Development of an immobilized cell reactor for the production of 1,3-propnediol by *Citrobacter freundii*. *Appl. Microbiol. Biotechno.l* (1994) 41: 313-16.
41. Regula Gutknecht, Rudolf Beutler, Luis F. Garcia-Alles, Ulrich Baumann, and Bernhard Erni1 (2001) "The dihydroxyacetone kinase of Escherichia coli utilizes a phosphoprotein instead of ATP as phosphoryl donor" doi: 10.1093/emboj/20.10.2480, EMBO J.; 2480–2486.
42. Ruetti D P, Vollenweider S, Maular X, Jeramic T, Mathias M, Die A and Lacroix C. Biotechnological production of 3-hydroxypropionaldehyde combined with in situ product removal process to purify this compound. *J. Biotechnol.* (2007) 131: S192-2.
43. Saint-Amanas S, Perlot P, Goma G and Soucaille P. High production of 1,3-propanediol from glycerol by *Clostridium butyricum* VPI 3266 in a simply controlled fed-batch system. *Biotechnol. Let.t* (1994) 16: 832-36.
44. Saint-Amanas S, Perlot P, Goma G and Soucaille P. High production of 1,3-propanediol from glycerol by *Clostridium butyricum* VPI 3266 in a simply controlled fed-batch system. *Biotechnol. Let.t* (1994) 16: 832-36.
45. Sauer M, Marx H and Mattanovich D. Microbial production of 1,3-propanediol. *Recent patents on Biotechnol.* (2008) 2: 191-7.
46. Saxena R K, Anand P, Saran S and Isar J. Microbial production of 1,3-propanediol: Recent developments and emerging opportunities. *Biotechnol. Adv.* (2009) 27: 895-913.
47. Seyfried M, Lyon D, Rainley F A and Weigel J. *Caloramator viterbensis* sp nov., a novel thermophilic, glycerol-fermenting bacterium isolated from a hot spring in Italy. *Int. J. Syst. Evol. Microbiol.* (2002) 52: 1177-84.
48. Sheldon R A, Arends I and Hanefeld U. Chemicals from renewable raw materials. In: Tundo P, Perosa A, Zecchini F, editors. *Green chemistry and catalysis. New York: Wiley-VCH*; (2007) 329-88.

49. Skraly F A, Lytle B L and Cameron D C. Construction and characterization of a 1,3-propanediol operon. *Appl. Environ. Microbiol.* (1998) 64: 98-105.
50. Sullivan C J. Propanediols, in *Ullmann's Encyclopedia of Industrial Chemistry,* (1993) Vol A 22. VCH, Weinheim.
51. Syu M J. Biological production of 2,3-butanediol. *Appl. Microbiol. Biotechnol.* (2001) 55: 10-8.
52. Tag C G and Mikrobielle Herstellung Von. 1,3-Propanediol. 1990. Dissertation, *University of Oldenburg, Germany.*
53. Vanajakshi J and Annapurna J. Isolation and identification of 1,3-propanediol producing strain of *K. pneumoniae* 141B from soil and optimization of process Parameters. *Res. J. Biotechnol.* (2011) 6:29-36.
54. Vanajakshi J and Annapurna J. Isolation and identification of 1,3-propanediol producing strain of *K. pneumoniae* 141B from soil and optimization of process Parameters. *Res. J. Biotechnol.* (2011) 6:29-36.
55. Vanajakshi J and Annapurna J. Optimization of 1,3-propanediol production by *Klebsiella pneumoniae* 141B using Taguchi methodology: improvement in production by cofermentation studies. *Res. Biotechnol.* (2011a) 2: 90-104.
56. Vanajakshi J and Annapurna J. Studies on production of 3-hydroxypropionaldehyde from glucose by a novel Enterococcus dispar: optimization of medium components by statistical experimental design. *Int. J. Biotechnol. Biochem.* (2010) 6: 743-55.
57. Veiga da Cunha M and Foster M A. 1,3-propanediol: NAD+ oxidoreductase of *Lactobacillus brevis* and *Lactobacillus buchneri*. *J. Bacteriol.* (1992) 174: 1013-9.
58. Wang Z X, Zhuge J, Fang H and Prior B A. Glycerol production by microbial fermentation: a review. *Biotechnol. Adv.* (2001) 19: 201-23.
59. Wilke T and Vorlop K. Biotransformation of glycerol into 1,3-propanediol. *Eur. J. Lipid Sci. Technol.* (2008) 110: 831-40.
60. Yang G, Tian J and Li J. Fermentation of 1,3-propanediol by *Klebsiella oxytoca* under microaerobic conditions. *Appl. Microbio.l Biotechnol.* (2007) 73: 1017-24.
61. Zhu M M, Lawman P D and Cameron D C. Improving 1,3-propanediol production from glycerol in a metabolically engineered Escherichia coli by reducing accumulation of sn-glycerol-3-phosphate. *Biotechnol. Prog.* (2002) 18: 694-9.
62. Zhu M M, Lawman P D and Cameron D C. Improving 1,3-propanediol production from glycerol in a metabolically engineered Escherichia coli by reducing accumulation of sn-glycerol-3-phosphate. *Biotechnol. Prog.* (2002) 18: 694-9.