18

Use of Glycerol in Biotechnological Applications

Volker F. Wendisch, Steffen N. Lindner and Tobias M. Meiswinkel
Chair of Genetics of Prokaryotes, Faculty of Biology & CeBiTec, Bielefeld University, Germany

1. Introduction

Since decades the limited access to petroleum oil is a major concern and substitutions for fossil fuels are needed. One promising substitute is biodiesel, which is widely produced from vegetable oils, e.g. from rape seeds, soybeans, sunflower seeds or animal fats. In the synthesis of biodiesel, oils and fats are transesterified to fatty acid methyl ester in the presence of sodium hydroxide or potassium hydroxide. In this process, glycerol is generated as stoichiometric byproduct with a ratio of 10% (w/w) with respect to biodiesel produced. In 2009, the biodiesel production of the world reached 16 million tons (Licht, 2010), with the lion's share produced by the European Union with 9 million tons (EBB, European Biodiesel Board 2010), followed by the United States with a production of 2.7 million tons (Licht, 2010). Hence, 1.6 million tons of glycerol were produced as obligatory by-product. Glycerol finds applications as an ingredient of various products, such as creams, food, feed, and pharmaceuticals, but the demand for glycerol in these processes is limited. Integrated conversion of raw glycerol from the biodiesel process to value-added products is a driver towards higher cost efficiency of biodiesel production.

Glycerol is a good source of carbon and energy for growth of several microorganisms and may be suitable for the biotechnological production of a number of chemicals in fermentative processes. To date several microbiological productions have been adjusted to glycerol as carbon and energy source or, if glycerol is close to the desired product, are based on glycerol as substrate anyway. For instance, the biotechnological production of 1,3-propanediol and dihydroxyacetone has predominantly been carried out from glycerol, since these processes are catalyzed in a two and one step reaction, respectively. Bacterial 1,3-propanediol production from glycerol is involving two enzymes. First glycerol is dehydrated by glycerol dehydratase to 3-hydroxypropionaldehyde, which is subsequently converted to 1,3-propanediol by 1,3-propanediol dehydrogenase. Predominantly, 1,3-propanediol production has been approved with *Clostridium* strains, *Klebsiella pneumoniae*, or *Escherichia coli* (Zhu et al. 2002, Biebl et al. 1998, Forsberg, 1987). 1,3-propanediol finds application in the production of the polyester polytrimethylene terephthalate. Strains of *Gluconobacter oxydans* are used for producing dihydroxyacetone (glycerone) from glycerol (Flickinger & Perlman, 1977). Dihydroxyacetone is used as a building block in organic chemistry and as a skin tanning agent in cosmetics.

Besides products that can be derived from glycerol in one or two reactions further products requiring more complex conversions have been investigated, e.g. succinic acid production

with *Anaerobiospirillum succiniciproducens* (Lee et al. 2001) or *Escherichia coli* (Blankschien et al., 2010, Zhang et al., 2010), citrate production with *Yarrowia lipolytica* (Rywinska & Rymowicz, 2010), ethanol production with *Escherichia coli*, *Saccharomyces cerevisiae*, or *Hansenula polymorpha* (Hong et al., 2010, Shams Yazdani & Gonzalez, 2008, Yu et al., 2010), production of amino acids with *Corynebacterium glutamicum* (Rittmann et al., 2008), or propionate production with *Propionibacteria* (Himmi et al., 2000).

Crude glycerol preparations from biodiesel factories differ considerably from the pure chemical glycerol, e.g. by their salt content. The applicability of crude glycerol from biodiesel production plants has already been demonstrated for production strains of *Clostridium* and *Klebsiella* in 1,3-propanediol production (Gonzalez-Pajuelo et al., 2004, Mu et al., 2006), *Yarrowia lipolytica* in citrate production (Papanikolaou et al., 2002), *Kluyvera cryocrescens* and *Klebsiella pneumoniae* in ethanol production (Choi et al., 2011, Oh et al., 2011), as well as *Basfia succiniciproducens* in succinic acid production (Scholten et al., 2009).

This chapter will summarize state-of-the-art of glycerol-based biotechnological processes and will discuss future developments.

2. Use of glycerol as a carbon source in biotechnological applications

Glycerol has many applications, it is used for the production of food, cosmetics, paints, pharmaceutics, paper, textiles, leather and for the production of various chemicals (Wang et al., 2001). It can be used as a stabilizing agent for storage of cells and proteins. Physiologically, glycerol is essential for the biosynthesis of membranes, since it is the backbone of glycerolipids. And for its function as a component of lipids and fats it is an abundant source of carbon and energy in nature.

Formerly, glycerol was a valuable product derived from glucose via dihydroxyacetone-phosphate and glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase (EC 1.1.1.94) and glycerol-3-phosphatase (EC 3.1.3.21). The yeast *Saccharomyces cerevisiae* which uses glycerol as an osmolyte under osmotic stress conditions was engineered for efficient glycerol production from glucose. *S. cerevisiae* possesses two isozymes of each, glycerol-3-phosphate forming glycerol-3-phosphate dehydrogenases and glycerol-3-phosphatases (Larsson et al., 1993, Pahlman et al., 2001). Sulphite treatment of yeasts enabled glycerol production (Petrovska et al., 1999), as did metabolic engineering on the glycerol production pathway, e.g. deletion of the triosephosphate isomerase gene (Overkamp et al., 2002), deletion of the alcohol dehydrogenase gene, overexpression of a glycerol-3-phosphate dehydrogenase gene (Navarro-Avino et al., 1999), or overexpression of a glycerol exporter gene (Cordier et al., 2007). Nowadays, glycerol arises from the biodiesel production. In 2010 glycerol formed as byproduct in the biodiesel process amounted to 1.6 million tons (Licht, 2010), which is extending the glycerol demand by far, thus, making microbial glycerol production unprofitable.

Glycerol as carbon source for growth

Glycerol can be used as a source of carbon and energy by many organisms. The initial step of glycerol utilization is its uptake into the cell. Albeit the small and uncharged molecule can diffuse through membranes without a transport system, many organisms possess glycerol transporters. In *Escherichia coli,* glycerol transport is mediated by the glycerol facilitator (Heller et al., 1980). In the wine bacterium *Pediococcus pentosaceus* active glycerol uptake has been reported (Pasteris & Strasser de Saad, 2008). Active glycerol transport has been

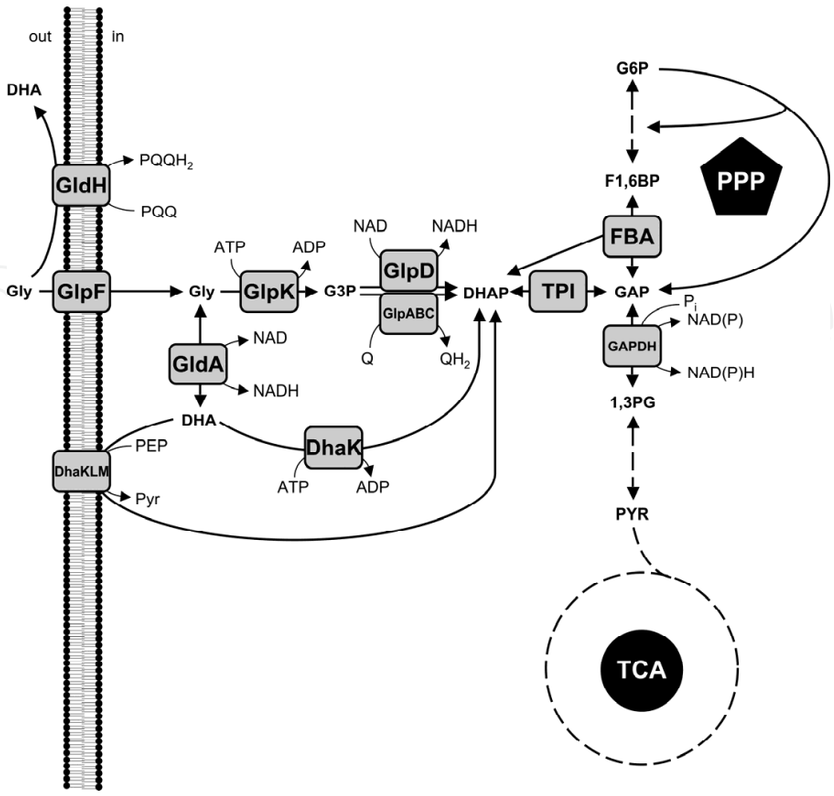


Fig. 1. Pathways of glycerol utilization. Abbreviations: 1,3-PG 1,3-phosphoglycerate, DHA dihydroxyacetone, DhaK ATP dependent dihydroxyacetone kinase, DhaKLM PEP dependent dihydroxyacetone kinase, DHAP dihydroxyacetone-phosphate, F1,6BP fructose-1,6-bisphosphate, G3P glycerol-3-phosphate, G6P glucose-6-phosphate, GAP glyceraldehyde-3-phosphate, GAPDH glyceraldehyde-3-phosphate dehydrogenase, GldA soluble glycerol dehydrogenase, GldH membrane bound glycerol dehydrogenase, GlpABC quinone dependent glycerol-3-phosphate dehydrogenase, GlpF glycerol facilitator, GlpK glycerol kinase, GlpD glycerol-3-phosphate dehydrogenase, Gly glycerol, PEP phosphoenolpyruvate, P_i inorganic phosphate, PPP pentose phosphate pathway, Pyr pyruvate, TCA tricarboxylic acid cycle, TPI triosephosphate isomerase.

described in yeasts, such as sodium dependent symport for *Debaryomyces hansenii* (Lucas et al., 1990) and proton dependent symport for *Pichia sorbitophila* (Lages & Lucas, 1995) and *Saccharomyces cerevisiae* (Lages & Lucas, 1997, Ferreira et al., 2005). In yeasts, active glycerol transport is mostly regarded of importance with respect to the use of glycerol as an osmolyte under osmotic stress conditions.

The imported glycerol can enter the metabolism at the level of the glycolytic intermediate dihydroxyacetone-phosphate. Two routes for the formation of dihydroxyacetone-phosphate from glycerol have been reported. In the first, ATP-dependent glycerol kinase (EC 2.7.1.30) phosphorylates glycerol to glycerol-3-phosphate, which is subsequently oxidized to dihydroxyacetone-phosphate by glycerol-3-phosphate dehydrogenase, which are either quinone or FAD-dependent (EC 1.1.5.3) or NAD-dependent (EC 1.1.1.8). In the second pathway, glycerol is oxidized to dihydroxyacetone by glycerol dehydrogenase (EC 1.1.1.6) before being phosphorylated to dihydroxyacetone-phosphate by ATP- or phosphoenolpyruvate-dependent dihydroxyacetone kinase (EC 2.7.1.29).

The glycerol-3-phosphate pathway is active in e.g. *Gluconobacter oxydans* and *Clostridium acetobutylicum* (Claret et al., 1994, Gonzalez-Pajuelo et al., 2006), whereas *Clostridium butyricum* uses the dihydroxyacetone pathway (Gonzalez-Pajuelo et al., 2006). In *E. coli*, *Klebsiella pneumoniae*, *Saccharomyces cerevisiae* and other yeasts, both pathways are present (Gonzalez et al., 2008, Wang et al., 2001, Ruch et al., 1974, Norbeck & Blomberg, 1997). In *S. cerevisiae,* glycerol is mainly utilized via glycerol-3-phosphate while the pathway via dihydroxyacetone is suggested to play a role during hyperosmotic stress for regulation of the intracellular glycerol concentration (Blomberg, 2000). In *E. coli,* the pathway via dihydroxyacetone operates under certain anaerobic conditions only (Gonzalez et al., 2008), but the major pathway is via glycerol-3-phosphate. In *K. pneumoniae,* the glycerol-3-phosphate pathway is active under aerobic and the dihydroxyaceton pathway is active under anaerobic conditions (Ruch et al., 1974).

2.1 Dihydroxyacetone

Dihydroxyacetone (DHA; 1,3-Dihydroxypropan-2-one, Glycerone) is produced biotechnologically with a global market of 2,000 tons per year (Pagliaro et al., 2007). DHA is produced biotechnologically, since its chemical synthesis is expensive and requires safety measures to cope with hazardous reactants (Hekmat et al., 2003). The most popular use of DHA is as coloring agent in sunless tanning products, such as creams and lotions (Levy, 1992). The tanning effect depends on Maillard-like reactions of DHA with the amino acids of the outer skin layer. Historically, also applications for medical treatments of glycogenesis, a glycogen storage disease, and diabetes mellitus have been described. Currently, the use of DHA as building block for chemical synthesis appears to have the highest potential for a production process based on biodiesel-derived glycerol (Enders et al., 2005, Zheng et al., 2008, Hekmat et al., 2003).

Gluconobacter oxydans is used for the production of DHA from glycerol. This bacterium belongs to the family of *Acetobacteraceae* (acetic acid bacteria), which are able to oxidize many carbohydrates and alcohols incompletely. To this end, *G. oxydans* possesses a variety of membrane-bound dehydrogenases. The membrane-bound pyrroloquinoline quinone (PQQ)-dependent glycerol dehydrogenase (EC 1.1.99.22) catalyzes oxidation of the secondary hydroxy group of glycerol to DHA (Matsushita et al., 1994). The enzyme is the protein product of *sldA* and *sldB* (Prust et al., 2005) and its localization allows extracellular oxidization of glycerol to DHA in the periplasma with a concurrent reduction of the membrane-localized PQQ. Besides membrane-bound glycerol dehydrogenase (Fig. 2), *G. oxydans* also possesses an intracellular catabolic pathway for the use of glycerol as a carbon source for growth (Fig. 1), in which glycerol is phosphorylated by ATP-dependent glycerol kinase to yield glycerol-3-phosphate which in turn is oxidized to dihydroxyacetone-phosphate by NAD-dependent glycerol-3-phosphate dehydrogenase (Claret et al., 1994). Since a functional glycolysis pathway is missing in *G. oxydans* and since its tricarboxylic acid cycle is incomplete, dihydroxyacetone-phosphate is metabolized via the pentose phosphate pathway (Greenfield & Claus, 1972).

Problems have occurred in the process of DHA production by *G. oxydans*, most importantly inhibition of the biotransformation process by the substrate glycerol and the product DHA as both inhibit growth and DHA production (Claret et al., 1992, Claret et al., 1993, Bauer et al., 2005). These problems have been met by optimizing production conditions including immobilization of *G. oxydans* cells to a polyvinyl alcohol matrix, which resulted in cells active for 14 days while maintaining glycerol dehydrogenase activity above 90 % (Wei et al., 2007).

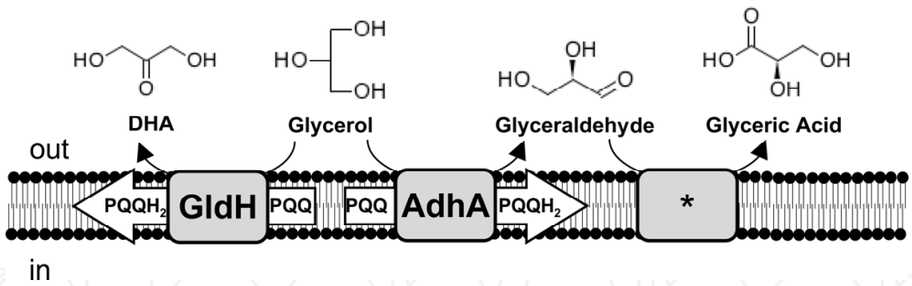


Fig. 2. Extracellular glycerol oxidation in *Gluconobacter oxydans*. Abbreviations: DHA dihydroxyacetone, PQQ oxidized pyrroloquinoline quinone, $PQQH_2$ reduced pyrroloquinoline quinone, AdhA alcohol dehydrogenase, * unidentified glyceric acid forming dehydrogenase.

Also fed-batch cultivations were shown to be supportive for DHA production compared to batch fermentations, as higher total glycerol amounts could be converted into DHA by avoiding inhibitory concentrations of glycerol, however, in this case production yields are limited with respect to DHA accumulation (Bories et al., 1991). Further enhancements of DHA production were achieved when using repeated fed-batch cultivations, in which DHA concentrations were kept below the inhibitory concentration. By this method immobilized cells were reused for up to 100 fed-batch cycles reducing costs and time for cleaning, sterilization, and inoculation (Hekmat et al., 2003). Other studies focused on optimizing culturing conditions for DHA production focused on cultivation media, aeration, or pH (Wethmar & Deckwer, 1999, Svitel & Sturdik, 1994, Tkac et al., 2001, Holst et al., 1985).

After the genome sequence of *G. oxydans* became public (Prust et al., 2005), metabolic engineering of *G. oxydans* has been reported for optimizing DHA production. Overexpression of the genes encoding the glycerol dehydrogenase, *sldAB*, led to increased biomass formation and higher DHA yields from glycerol (Herrmann et al., 2007). As the formation of glyceric acid as byproduct interferes with DHA production, disruption of *adhA*, the gene for the PQQ-dependent alcohol dehydrogenase, which is involved in the oxidation of glycerol to glyceric acid was shown to improve the product yield by abolishing glyceraldehyde and glyceric acid formation (Habe et al., 2010a). In addition, the mutant lacking *adhA* was less inhibited by high initial glycerol concentrations than the parent strain. In line with the notion that glyceric acid inhibits growth and DHA production by *G. oxydans*, it was found that addition of glyceric acid to the medium reduced growth and DHA production (Habe et al., 2010a) and that AdhA activity strongly increased when very high glycerol concentrations were used (Habe et al., 2009d). In the next step, the combination of *adhA* disruption and *sldAB* overexpression resulted in a strain with very high productivity and strongly increased tolerance towards glycerol (Li et al., 2010b). Since the production of DHA by the obligate aerobic *G. oxydans* is characterized by a very high demand for oxygen to oxidize reduced PQQ (Hekmat et al., 2003, Svitel & Sturdik, 1994), the gene encoding *Vitreoscilla* hemoglobin, an oxygen transporting protein, was shown to be beneficial (Li et al., 2010a).

Besides *G. oxydans* strains other acetic acid bacteria have also been reported for DHA production from glycerol, e.g. *Gluconobacter melanogenus* (Flickinger & Perlman, 1977) and *Acetobacter xylinum* (Nabe et al., 1979). Until today, production of DHA from biodiesel-derived raw glycerol has not yet been reported.

2.2 1,2-Propanediol

1,2-propanediol (propylenglycol, 1,2-PDO) is a commodity chemical with a wide range of applications, including polyester resins, plastics, antifreeze agents, de-icing products, detergents, or paints. The global demand for 1,2-PDO is estimated to be up to 1.6 million tons per year (Shelley, 2007). Chemically, 1,2-PDO is produced from propylene.

A variety of microorganisms have been reported as natural producers of 1,2-PDO, such as the bacteria *E. coli* (Hacking & Lin, 1976, Gonzalez et al., 2008), *Thermoanaerobacterium thermosaccharolyticum* (Cameron & Cooney, 1986), *Bacteroides ruminicola* (Turner & Roberton, 1979), *Salmonella typhimurium*, and *Klebsiella pneumoniae* (Badia et al., 1985), but also yeasts have been shown to produce 1,2-PDO (Suzuki & Onishi, 1968).

Two main routes for the microbial production of 1,2-PDO exist. First, 1,2-PDO may be formed from lactaldehyde, an intermediate of dissimilatory desoxy sugar (e.g. fucose, rhamnose) utilization generated either by fuculose-1-phosphate aldolase (EC 4.1.2.17) or by rhamnulose-1-phosphate aldolase (EC 4.1.2.19). Lactaldehyde is reduced to 1,2-PDO by NADH-dependent lactaldehyde reductase (FucO, EC 1.1.1.77), as shown e.g. for *Salmonella typhimurium* (Suzuki & Onishi, 1968). Due to high prices of fucose and rhamnose this pathway is not applicable.

The second route to 1,2-PDO diverts from glycolysis and, thus, 1,2-PDO production from glucose is feasible. The glycolytic intermediate dihydroxyacetone-phosphate is funneled into the methylglyoxal pathway by methylglyoxal synthase (EC 4.2.3.3) forming methylglyoxal. Methylglyoxal synthases from e.g. *Escherichia coli*, *Clostridium acetobutylicum*, and *Saccharomyces cerevisiae* have been purified and characterized (Cooper & Anderson, 1970, Freedberg et al., 1971, Hopper & Cooper, 1972, Huang et al., 1999, Murata et al., 1985), all of which were inhibited by phosphate. Methylglyoxal in turn is reduced to 1,2-PDO in two subsequent NADH/NADPH-dependent reactions. Two variants of methylglyoxyal reduction to 1,2-PDO are known with either acetol or lactaldehyde as intermediate. Acetol a may arise from methylglyoxal e.g. by aldehyde dehydrogenase (EC 1.1.1.2) or alcohol dehydrogenases (EC 1.1.1.1) from *E. coli* (Misra et al., 1996), by methylglyoxal reductase from *S. cerevisiae* (EC 1.1.1.283) (Nakamura et al., 1997) or acetol oxidoreductase from *E. coli* (Boronat & Aguilar, 1981). In the second step acetol is converted to 1,2-PDO by e.g. *E. coli* glycerol dehydrogenase (EC 1.1.1.6). The variant with lactaldehyde as intermediate involves e.g. glycol dehydrogenase from *Enterobacter aerogenes* (EC 1.1.1.185) (Carballo et al., 1993) or glycerol dehydrogenase from *E. coli* (EC 1.1.1.6) (Altaras & Cameron, 1999) for the first reduction step and e.g. *E. coli* 1,2-PDO reductase for the second reduction. Both ways necessitate two reduction equivalents per 1,2-PDO formed.

1,2-PDO production by *E. coli*

First approaches towards metabolic engineering of *E. coli* strains for production of 1,2-PDO from glucose by Cameron et al. involved overexpression of genes for either aldose reductase or glycerol dehydrogenase (Cameron et al., 1998). Later on, the same group coexpressed glycerol dehydrogenase genes from *E. coli* or *K. pneumoniae* together with the *E. coli* methylglyoxal synthase gene in *E. coli* and reached up to 0.7 g l^{-1}, an almost three-fold increase when compared to overexpression of *E. coli* or *K. pneumoniae* glycerol dehydrogenase gene alone (0.25 g l^{-1}) (Altaras & Cameron, 1999). Additional overexpression of yeast alcohol dehydrogenase or *E. coli* 1,2-PDO reductase further improved production performance and 1,2-PDO concentrations of 4.5 g l^{-1} and a yield of 0.19 g (g glucose)$^{-1}$ were achieved in a fed-batch fermentation process (Altaras & Cameron, 2000). Elimination of

lactate dehydrogenase by gene deletion improved 1,2-PDO production by an *E. coli* strain overexpressing genes coding for methylglyoxal synthase from *Clostridium acetobutylicum* and glycerol dehydrogenase from *E. coli* (Berrios-Rivera et al., 2003).

Glycerol has a higher degree of reduction than glucose, thus, higher 1,2-PDO yields are theoretically possible using glycerol (0.72 g g^{-1}) as compared to glucose (0.63 g g^{-1}) (Clomburg & Gonzalez, 2011). Moreover, 1,2-PDO has been reported to be a natural product of anaerobic fermentation of glycerol in *E. coli* by Gonzalez et al. (Gonzalez et al., 2008).

Glycerol is converted to 1,2-PDO in a pathway consisting of glycerol dehydrogenase for oxidation of glycerol to dihydroxyacetone and phosphorylation of the latter to dihydroxyacetone-phosphate by phosphoenolypyruvate-dependent dihydroxyacetone kinase. Subsequently, dihydroxyacetone-phosphate is reduced to 1,2-PDO which requires two NADH. Thus, to generate NADH and ATP required in these reactions a portion of dihydroxyacetone-phosphate needs to be catabolized in glycolysis and onwards to ethanol (Gonzalez et al., 2008).

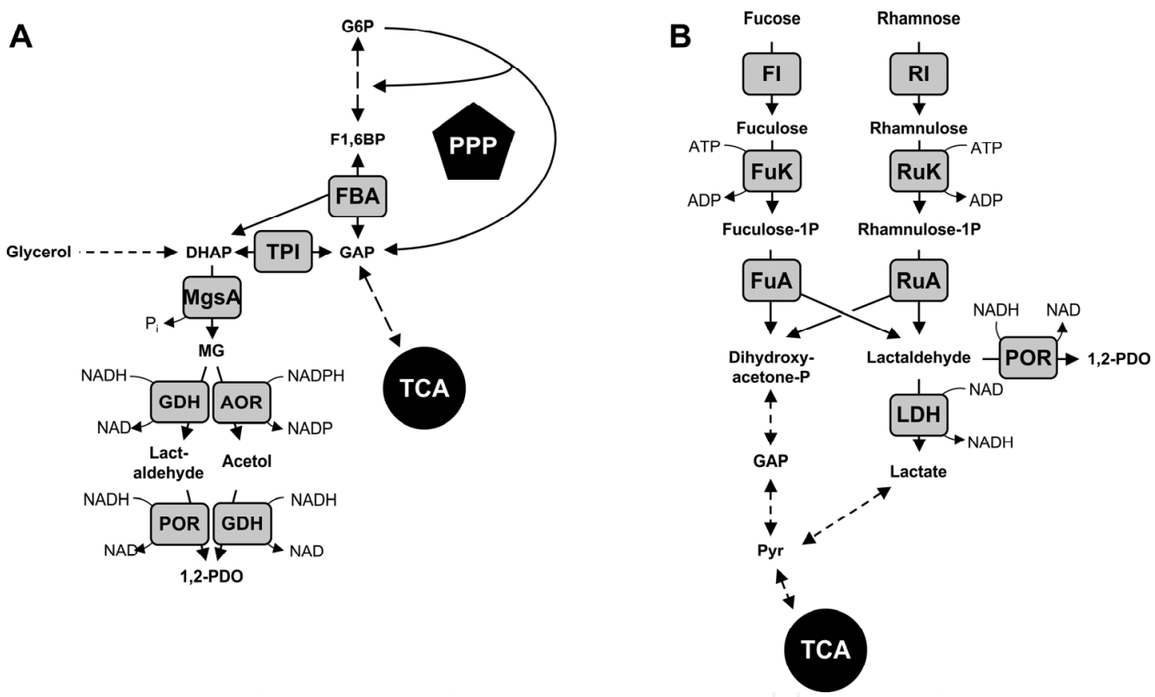


Fig. 3. 1,2-propanediol production pathways. A, 1,2-propanediol production from glycolytic intermediate dihydroxyacetone-phosphate; Abbreviations: 1,2-PDO 1,2-propanediol, AOR, acetol oxidoreductase, DHAP, dihydroxyacetone-phosphate, F1,6BP, fructose-1,6-bisphosphate, FBA, fructose-1,6-bisphosphate aldolase, G6P, glucose-6-phosphate, GAP, glyceraldehyde-3-phosphate, GDH, glycerol dehydrogenase, MG, methylglyoxal, MgsA, methylglyoxal synthase, P_i inorganic phosphate, POR 1,2-propanediol-oxidoreductase, TCA tricarboxylic acid cycle, TPI triosephosphate isomerase; B, 1,2-propanediol production from fucose and rhamnose; Abbreviations: FI fucose isomerase, FuA fuculose-1-phosphate aldolase, FuK fuculose kinase, LDH lactate dehydrogenase, RI rhamnose isomerase, RuK rhamnulose kinase, RuA rhamnulose-1-phosphate aldolase.

Recently, Clomburg et al. rationally engineered *E. coli* for effective 1,2-PDO production from glycerol. They introduced a 1,2-PDO production pathway via methylglyoxal synthase, glycerol dehydrogenase, and aldehyde oxidoreductase. Replacement of the phosphoenolpyruvate-dependent dihydroxyacetone kinase by ATP-dependent dihydroxyacetone kinase from *Citrobacter freudii* elevated dihydroxyacetone-phosphate availability and enhanced 1,2-PDO production from 0.02 to 0.15 g g^{-1}. Moreover, the formation of byproducts succinate, acetate, ethanol, and formate increased but lactate was reduced. To eliminate byproduct formation several gene deletions, e.g. of genes coding for lactate dehydrogenase, acetate kinase, phosphate acetyltransferase, formate hydrogen lyase, fumarate reductase, alcohol dehydrogenase, and pyruvate dehydrogenase, were tested alone or in combination. Ethanol formation could not be abolished as deletion of the alcohol dehydrogenase gene strongly decreased 1,2-PDO production (0.02 compared to 0.12 g g^{-1}) and glycerol consumption, but increased formation of acetate and succinate as byproducts. However, deletion of the genes for acetate kinase, phosphate acetyltransferase, and lactate dehydrogenase resulted in an increased product yield of 0.21 g g^{-1}, but entailed increased ethanol, formate, and pyruvate formation. The use of raw glycerol by this strain reduced formate formation and increased the 1,2-PDO yield (0.24 g g^{-1}) (Clomburg & Gonzalez, 2011). Taken together, engineered *E. coli* strains allow for 1,2-PDO yields from glycerol that are comparable to yields from glucose, making glycerol a feasible substrate for microbial 1,2-PDO production.

1,2-PDO production by other microorganisms

Recombinant strains of *S. cerevisiae* and of *C. glutamicum* have been developed for production of 1,2-PDO, as well. *S. cerevisiae* carrying multiple genome-integrated copies of *E. coli* methylglyoxal synthase and glycerol dehydrogenase genes was able to produce 1,2-PDO (Lee & da Silva, 2006). Plasmid-borne expression of the latter genes combined with the deletion of the endogenous triosephosphate isomerase gene resulted in the production of 1.1 g l^{-1} 1,2-PDO (Jung et al., 2008). Expression of *E. coli* methylglyoxal synthase gene and a *Corynebacterium glutamicum* aldo-keto reductase gene enabled *C. glutamicum* for the production of 1,2-PDO from glucose (1.8 g l^{-1}) (Niimi et al., 2011).

2.3 1,3-Propanediol

1,3-propanediol (1,3-PDO) can be used in several chemical applications. It is a substrate in the formulation reactions for polyesters, polyethers, polyurethanes, adhesives, composites, laminates, coatings, and moldings. In addition, 1,3-PDO itself is used e.g. as solvent or antifreeze agent. Most importantly, the production of polytrimethylene terephthalate (PTT) from 1,3-PDO and terephthalic acid is the driver of the growing market for 1,3-PDO. The polymer PTT is a promising polyester with numerous applications, e.g. as compound in textile, carpet, upholstery, or specialty resins. Due to its properties, PTT might be favored over polymers such as nylon, polyethylene terephthalate and polybutylene terephthalate. PTT has environmental benefits since it is biodegradable (for recent reviews see (da Silva et al., 2009, Liu et al., 2010, Carole et al., 2004). The chemical producers Shell and DuPont produce PTT from 1,3-PDO which is commercialized under the names Sorona, Hytrel (DuPont) and Corterra (Shell).

1,3-PDO is a success story of a glycerol-based biotechnological process. When 1,3-PDO was first discovered to be a fermentative product of glycerol in 1881 (Werkman & Gillen, 1932), it received little interest until the development of PTT in 1941 (Whinfield & Dickinson, 1946).

However, while terephthalic acid was readily available the expense of 1,3-PDO production limited efficient PTT production. Nowadays, chemical production of 1,3-PDO from ethylene oxide or acrolein as well as its biotechnological production enable supply of 1,3-PDO at low cost. It has to be noted that chemical synthesis of 1,3-PDO suffers from high energy consumption, toxic intermediates, and expensive catalysts as major drawbacks.
Currently, the market for 1,3-PDO is estimated to be about 50,000 tons per year (Liu et al., 2010), but due to a growing production of PTT a market volume of 230,000 tons is foreseen for 2020 (Carole et al., 2004). An indication of the growing interests and the potential of biotechnological 1,3-PDO production are the current decisions of the joint venture of DuPont and Tate & Lyle to extend their 1,3-PDO biotech plant in Louden, TN, USA, by 35 %. The actual capacity of the plant is 45,000 tons per year (Greenwood, 2010). Also the French company Metabolic Explorer (Clermont-Ferrand, France) decided to build a plant for biotechnological 1,3-PDO production in Malaysia in partnership with Malaysian Bio-XCell. The plant with a capacity to produce 50,000 tons 1,3-PDO annually is expected to start with a production of 8,000 tons per year (Degalard, 2011). While the process of DuPont and Tate & Lyle is based on sugars from corn hydrolysates, the Metabolic Explorer process will be based on crude glycerol.

Biotechnological production of 1,3-PDO

Several bacteria have been shown to naturally possess the ability of 1,3-PDO production, all of which belong either to enterobacteria or to firmicutes. Production of 1,3-PDO has been shown e.g. for strains of *Klebsiella pneumoniae*, *Clostridium butyricum*, *Clostridium acetobutylicum*, *Clostridium pasteurianum*, *Clostridium butylicum*, *Clostridium beijerinckii*, *Clostridium kainantoi*, *Lactobacillus brevis* and *Lactobacillus buchneri*, and *Enterobacter agglomerans* (Forage & Foster, 1982, Barbirato et al., 1996, Nakas et al., 1983, Schutz & Radler, 1984, Homann et al., 1990, Biebl, 1991, Biebl et al., 1992, Daniel et al., 1995).
Biosynthesis of 1,3-PDO from glycerol is catalyzed in a reducing pathway involving a cytosolic two-step process. First, glycerol dehydratase (EC 4.2.1.30) or diol dehydratase (EC 4.2.1.28) convert glycerol into 3-hydroxypropionaldehyde (3-HPA) (Schneider & Pawelkiewicz, 1966, Toraya et al., 1978). Glycerol dehydratase from *K. pneumoniae* has been reported to be inactivated by glycerol and necessitates a reactivating factor encoded by *gdrAB* (Tobimatsu et al., 1999). In the second step, 3-HPA is reduced to 1,3-PDO by NADPH- or NADH-dependent 1,3-PDO dehydrogenases (EC 1.1.1.202) e.g. by DhaT from *K. pneumoniae* or YqhD from *E. coli* (Johnson & Lin, 1987). Besides NADH-dependent DhaT, *K. pneumoniae* possesses a second NADPH dependent enzyme active as a 1,3-propanediol dehydrogenase. This enzyme was found, since a *dhaT* deletion mutant was still able to produce 1,3-PDO and 3-hydroxypropionic acid from glycerol albeit with reduced efficiency (Ashok et al., 2011). The sought enzyme was later identified as a homolog to *E. coli* YqhD and overexpression of the respective gene was shown to restore 1,3-PDO production by the *dhaT* deletion mutant (Seo et al., 2010).
The regeneration of reduction equivalents for 1,3-PDO production from glycerol is ensured in *K. pneumoniae* by simultaneous operation of the oxidative pathway of glycerol utilization. Here, NADH is generated by glycerol dehydrogenase during oxidation of glycerol to dihydroxyacetone and during catabolism of dihydroxyacetone phosphate, which is formed from dihydroxyacetone by dihydroxyacetone kinase (Seo et al., 2009). Thus, the demand of NADH limits the theoretical yield of 1,3-PDO production from glycerol. The requirement of some glycerol being oxidized and catabolized in glycolysis and the tricarboxylic acid cycle

entails the formation of unwanted byproducts such as acetic acid, lactic acid, formic acid, succinic acid, butyric acid, 2,3-butanediol, and ethanol. The main byproducts of *K. pneumoniae* strains are 2,3-butanediol, acetic acid, ethanol, and lactic acid (Menzel et al., 1997, Zhang et al., 2006, Kretschmann et al., 1993), whereas *C. butyricum* and metabolically engineered *C. acetobutylicum* strains mainly accumulate byproducts acetic acid and butyric acid during 1,3-PDO production (Papanikolaou et al., 2000, Papanikolaou et al., 2004, Saintamans et al., 1994, Gonzalez-Pajuelo et al., 2005, Soucaille, 2008, Sarcabal et al., 2007). With *C. pasteurianum,* butanol but not 1,3-PDO is the main fermentation product from glycerol, and ethanol, acetic acid, butyric acid and lactic acid are formed as further byproducts (Biebl, 2001). Metabolically engineered *E. coli* were reported to accumulate formic acid, acetic acid, lactic acid, and pyruvic acid as the major byproducts (Tong et al., 1991, Skraly et al., 1998) and accumulate growth inhibiting metabolites glycerol-3-phosphate and methylglyoxylate (Tkac et al., 2001, Zhu et al., 2001).

A major concern in 1,3-PDO production is the fact that the substrate glycerol, the intermediate 3-HPA, the product 1,3-PDO, and several byproducts inhibit growth and production. In *K. pneumoniae,* 1,3-PDO yields decrease with increasing glycerol concentrations and metabolic flux analyses revealed a higher carbon flux via the oxidative glycerol utilization pathway to the loss of 1,3-PDO (Xiu et al., 2011). In *C. butyricum,* growth is completely inhibited at 1,3-PDO concentrations higher than 60 g l^{-1}. Also the byproducts acetic acid (27 g l^{-1}) and butyric acid (19 g l^{-1}) abolished growth of this bacterium as did glycerol concentrations of 80 g l^{-1} or more (Biebl, 1991, Colin et al., 2000). Growth of *K. pneumoniae* is inhibited at glycerol concentrations above 110 g l^{-1} under aerobic and above 133 g l^{-1} under anaerobic conditions. Also the byproducts acetic acid (15 g l^{-1}), lactic acid (19 g l^{-1}), and ethanol (26 g l^{-1}) (15, 19, 26 g l^{-1} under anaerobic and 24, 26, and 17 g l^{-1} under aerobic conditions) inhibit growth of *K. pneumoniae* (Cheng et al., 2005). The accumulation of 3-HPA, the intermediate product of 1,3-PDO production has a toxic effect on growth and 1,3-PDO fermentation in *K. pneumoniae*. Both, glycerol dehydratase and 1,3-propanediol dehydrogenase are sensitive to 3-HPA. 1,3-propanediol dehydrogenase activity decreased as 3-HPA accumulated, leading to a further increase in 3-HPA concentrations (Hao et al., 2008a). Purified 1,3-propanediol dehydrogenase from *E. agglomerans* CNCM 1210 was shown to be inhibited by NAD^+ (K_i 0.29 mM) and 1,3-PDO (K_i 13.7 mM) and therefore might be limiting production yields of 1,3-PDO (Barbirato et al., 1997). Also the glycerol dehydratases from *C. freundii* and metagenome samples were shown to be inhibited by 1,3-PDO (Knietsch et al., 2003), moreover glycerol dehydratases from *K. pneumoniae* and *C. freundii* are inhibited by deactivation by glycerol (Tobimatsu et al., 1999, Tobimatsu et al., 2000, Kajiura et al., 2001, Seifert et al., 2001).

To overcome production limitations by e.g. substrate and product inhibition or byproduct inhibition, several approaches to optimize cultivation conditions were followed. Because the oxidative glycerol utilization pathway is necessitated for NADH regeneration but also leads to the formation of unwanted byproducts the cultivations of *K. pneumoniae* is preferably carried out under micro-aerobic conditions. Chen et al. compared cultivation conditions during 1,3-PDO production with *K. pneumoniae*. They found that final 1,3-PDO concentrations and yields were increased in batch fermentations under micro-aerobic conditions. Productivity increased from 0.8 to 1.57 under anaerobic and micro-aerobic conditions, respectively, and ethanol was reduced as well (Chen et al., 2003). Hao et al. postulated that the use of *K. pneumoniae* in a fed batch fermentation process using initial

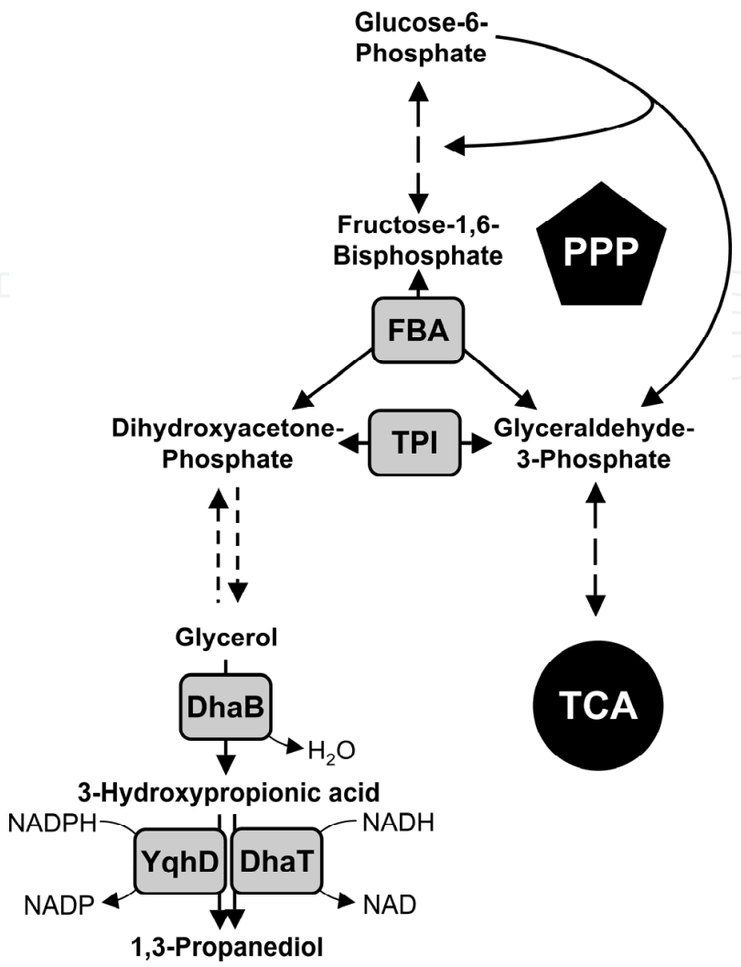


Fig. 4. 1,3-propanediol production pathway. Abbreviations: DhaB glycerol dehydratase, DhaT NADH dependent 1,3-propanediol dehydrogenase, FBA fructose-1,6-bisphosphate aldolase, PPP pentose phosphate pathway, TCA tricarboxylic acid cycle, TPI triosephosphate isomerase, YqhD NADPH dependent 1,3-propanediol dehydrogenase.

glycerol concentration of 30 g l^{-1} and subsequently keeping it to 7-8 g l^{-1} during exponential growth phase avoids toxic concentrations of 3-HPA (Hao et al., 2008a). For *Citrobacter freundii,* Pflugmacher et al. could show that cell immobilization to polyurethane carrier particles supports productivity to 8.2 g l^{-1} h^{-1} (Pflugmacher & Gottschalk, 1994). Gungormusler et al. reported 1,3-PDO production from raw glycerol with use *Clostridium beijerinckii* cells immobilized to ceramic rings and pumice stones in combination with a hydraulic retention time system. They could show an increase in productivity and yield for 1,3-PDO production and predicted a maximal 1,3-PDO production rate of 30 g l^{-1} h^{-1} (Gungormusler et al., 2011).

Metabolic engineering of *K. pneumoniae, C. acetobutylicum* and *E. coli* for 1,3-PDO production

Several metabolic engineering approaches were made for the optimization of 1,3-PDO production and reduction of byproduct formation with *K. pneumoniae* and *C. acetobutylicum*. *E. coli*, not a natural producer of 1,3-PDO, was engineered for 1,3-PDO production.

K. pneumoniae

K. pneumoniae was genetically manipulated to gain higher 1,3-PDO titers and production rates and to reduce process competing byproducts. When overexpressing the gene encoding the first enzyme of 1,3-PDO production glycerol dehydratase (*dhaB*) Zhao et al. (2009) found no effect on 1,3-PDO yields but reported decreased formation of the byproducts ethanol and 2,3-butanediol and an increase of acetic acid production (Ma et al., 2009). The overexpression of the 1,3-PDO dehydrogenase gene *dhaT* was shown to positively affect 1,3-PDO production in many studies and to reduce the formation of the toxic intermediate and substrate of 1,3-PDO dehydrogenase 3-HPA (Rao et al., 2010, Ma et al., 2009, Zhuge et al., 2010, Hao et al., 2008b). While aiming to reduce concentrations of the inhibitory intermediate 3-HPA Hao et al. (2008) overexpressed the 1,3-PDO dehydrogenase gene (*dhaT*) in *K. pneumoniae* TUAC01. During fermentation with 30 or 50 g l^{-1} glycerol 3-HPA accumulation was significantly reduced to 1.49 and 2.02 mM, respectively, compared to the parental strain which produced 7.55 and 12.57 mM, respectively (Hao et al., 2008b). Accordingly, Ma et al. (2010) overexpressed the 1,3-PDO dehydrogenase gene in *K. pneumoniae* and found a 3-fold decrease of 3-HPA production and an increase in 1,3-PDO production by 16.5% (Ma et al., 2010b). Similarly, Zhao et al. (2009) reported a strong decrease of the toxic intermediate 3-HPA by overexpression of the 1,3-PDO dehydrogenase gene leading to an increased molar yield of 0.64 mol mol^{-1} compared to 0.51 mol mol^{-1} of the parental strain and decreased lactic acid, ethanol and succinic acid concentrations in fed-batch fermentations (Ma et al., 2009). Zhuge et al. (2010) combined overexpression of *dhaT* and *yqhD* (encoding NADH- and NADPH-dependent 1,3-PDO dehydrogenases from *K. pneumoniae* and *E. coli*, respectively) in *K. pneumoniae*. The recombinant strain had a slightly elevated product titer (18.3 g l^{-1} compared to 17.1 g l^{-1}) and increased molar 1,3-PDO yield (0.51 to 0.57 mol mol^{-1}) in batch fermentations. Furthermore, the byproducts 3-HPA, succinic acid, lactic acid, acetic acid and ethanol were significantly reduced (Zhuge et al., 2010). When analyzing a *K. pneumoniae* mutant strain defective in the genes for NADH-dependent 1,3-PDO dehydrogenase and the oxydative glycerol utilization pathway Seo et al. (2009) reported that the mutant surprisingly retained the ability of 1,3-PDO production. 1,3-PDO yields were low but a strongly reduced byproduct formation was reported (Seo et al., 2009). Later, Seo et al. (2010) published the identification of a second 1,3-PDO dehydrogenase, which possesses high homology to *E. coli* YqhD and is, in contrast to the *dhaT* encoded enzyme, NADPH-dependent. Overproduction of the NADPH-dependent 1,3-PDO dehydrogenase from *K. pneumoniae* resulted in restoration of 1,3-PDO production in the mutant defective in the genes for NADH-dependent 1,3-PDO dehydrogenase and the oxydative glycerol utilization pathway and moreover byproduct formation remained low in the recombinant strain, as the oxidative pathway of glycerol utilization was absent. Although the 1,3-PDO concentrations were lower as compared to the parental strain (4.7 compared to 7.9 g l^{-1}), the molar yield of 0.54 mol mol^{-1} of the recombinant strain was higher compared to 0.48 mol mol^{-1} (Seo et al., 2010). To reduce byproduct formation Horng et al. (2010) constructed a *K. pneumoniae* mutant lacking the genes for glycerol dehydrogenase and dihydroxyacetone kinase. This mutant was reported to have ceased lactate, 2,3-butanediol, and ethanol byproduct formation and furthermore showed higher 1,3-PDO productivity when glycerol dehydratase and 1,3-PDO dehydrogenase were overexpressed (Horng et al., 2010). A lactate dehydrogenase mutant was constructed by

Xu et al. (2009) in *K. pneumoniae* HR526. The accumulation of lactate was reduced from 40 g l^{-1} in the parental strain to less than 3 g l^{-1} in the lactate dehydrogenase mutant, which showed very low lactate dehydrogenase activity. The mutant furthermore produced higher 1,3-PDO concentrations (95 g l^{-1} as compared to 102 g l^{-1}) with a higher yield (0.48 mol mol^{-1} to 0.52 mol mol^{-1}) and higher production rate (1.98 g l^{-1} h^{-1} to 2.13 g l^{-1} h^{-1}). Reduced lactate production increased NADH availability for 1,2-PDO production in *K. pneumoniae* (Xu et al., 2009) and *K. oxytoca* (Yang et al., 2007). Similarly, inactivation of the NADH-dependent aldehyde dehydrogenase gene in *K. pneumoniae* abolished ethanol formation, improved NADH availability and resulted in a 1,3-PDO production rate of 1.07 g l^{-1} h^{-1} and a yield of 0.70 mol mol^{-1} (Zhang et al., 2006).

E. coli

Wild-type *E. coli* is unable to convert glycerol to 1,3-PDO (Tong et al., 1991), but already in 1991 Tong et al. constructed a recombinant *E. coli* strain for the production of 1,3-PDO in an early application of metabolic engineering. A *K. pneumoniae* ATCC 25955 genomic library in *E. coli* was screened for anaerobic growth on glycerol and dihydroxyacetone and 1,3-PDO production. The selected recombinant possessed glycerol dehydratase, 1,3-PDO dehydrogenase, glycerol dehydrogenase, and dihydroxyacetone kinase from *K. pneumoniae* and produced 1 g l^{-1} 1,3-PDO with a yield of 0.46 mol mol^{-1} (Tong et al., 1991). Cofermentation of glycerol with glucose or xylose increased yields from 0.46 mol mol^{-1} to 0.63 mol mol^{-1} in the presence of glucose and to 0.55 mol mol^{-1} when cofermented with xylose (Tong & Cameron, 1992). Skraly et al. (1998) further optimized 1,3-PDO production with *E. coli* as they constructed an artificial operon containing the *K. pneumoniae* genes of 1,3-PDO production. They could show that the recombinant *E. coli* strain yielded 0.82 mol mol^{-1} (glycerol only) in a fed-batch process cofermenting glycerol and glucose, but only 9.3 g l^{-1} of glycerol where converted (Skraly et al., 1998). The additional expression of the glycerol dehydratase reactivating factor genes *gdrAB* together with the expression of *K. pneumoniae* genes encoding glycerol dehydratase and 1,3-PDO dehydrogenase yielded 8.6 g l^{-1} 1,3-PDO in a fed-batch fermentation (Wang et al., 2007). A higher final 1,3-PDO concentration of 13.2 g l^{-1} was obtained when they substituted *K. pneumoniae* 1,3-PDO dehydrogenase with the *E. coli* YqhD, which possesses NADPH-dependent 1,3-PDO dehydrogenase activity (Tan et al., 2007). *YqhD* from *E. coli* was also used in addition to the vitamin B12-independent glycerol dehydratase from *C. butyricum* in a two-stage fermentation process (aerobic biomass production from glucose followed by anaerobic 1,3-PDO production from glycerol). By this process 1,3-PDO concentrations of 104.4 g l^{-1} were reached, with a production rate of 2.62 g l^{-1} h^{-1} and a molar yield of 1.09 mol mol^{-1} (90.2% g g^{-1}) (referred to glycerol only) (Tang et al., 2009). Some efforts have also been made in the abolishment of toxic intermediate metabolites in *E. coli* during 1,3-PDO production. In *E. coli* high glycerol concentrations inhibit growth and 1,3-PDO production due to intracellular accumulation of glycerol-3-phosphate (Cozzarelli et al., 1965), the product of glycerol kinase, first enzyme in the oxidative pathway of glycerol utilization in *E. coli*. Zhu et al. (2002) could show that the glycerol-3-phosphate concentration increased when glycerol concentrations were elevated. The glycerol-3-phosphate accumulation was due to inefficient expression of the glycerol-3-phosphate dehydrogenase gene, and was overcome by usage of a glycerol kinase mutant, which showed 2.5-fold increased 1,3-PDO production (Zhu et al., 2002). The reduction of methylglyoxal formation by expression of the *Pseudomonas putida* glyoxalase I resulted in increased 1,3-PDO production by 50% (Zhu et al., 2001).

C. acetobutylicum

C. butyricum is a promising candidate for efficient 1,3-PDO production because it produces high concentrations of 1,3-PDO and possesses a vitamin B12-independent glycerol dehydratase circumventing the addition of expensive vitamin B12. Because tools for genetic manipulations of *C. butyricum* are unavailable, Gonzalez-Pajuelo et al. (2005) introduced the 1,3-PDO pathway from *C. butyricum* into *C. acetobutylicum*. The recombinant *C. acetobutylicum* strain (DG1(pSPD5)) accumulated 84 g l^{-1} 1,3-PDO in a fed-batch culture and reached a high production rate of 3 g l^{-1} h^{-1} (Gonzalez-Pajuelo et al., 2005).

Raw glycerol

Investigations of raw glycerol use for the production of 1,3-PDO have been made with *Clostridium* and *Klebsiella* strains. *K. pneumoniae* produced 1,3-PDO concentrations from raw glycerol from soybean oil biodiesel production close to that from pure glycerol (51.3 g l^{-1}) with a productivity of 1.7 g l^{-1} h^{-1} (compared to 2 g l^{-1} h^{-1}) (Mu et al., 2006). Similar concentrations (56 g l^{-1}) were reported by Hiremath et al. who used glycerol obtained from *Jatropha* seed biodiesel production (Hiremath et al., 2011). *C. beijerinckii* was also reported to produce 1,3-PDO from raw glycerol (Gungormusler et al., 2011). Notably, productivity was increased to 1.51 g l^{-1} h^{-1} when using raw glycerol as compared to 0.84 g l^{-1} h^{-1} when using pure glycerol (Um et al., 2010). Moon et al. compared 1,3-PDO production with *Klebsiella* and *Clostridium* strains from glycerol derived from biodiesel production from waste vegetable oil and soybean oil (Moon et al., 2010). Possibly due to inhibitory methanol concentrations, the use of soybean derived glycerol was better than use of raw glycerol derived from waste vegetable oil from different suppliers. In this study, a higher tolerance of *Klebsiella* strains to the different raw glycerols used compared to the *Clostridium* strains was observed (Moon et al., 2010). *C. butyricum* was also shown to utilize raw glycerol for 1,3-PDO production (Papanikolaou et al., 2000, Gonzalez-Pajuelo et al., 2004). Inhibitory effects of raw glycerol components on 1,3-PDO production with *C. butyricum* were found not to be due to NaCl or methanol, but rather due to oleic acid (Chatzifragkou et al., 2010).

2.4 Ethanol

Ethanol as a bio-fuel is mainly gained from sugarcane in Brazil, from corn in the USA and from sugar beets in the EU (da Silva et al., 2009). Since ethanol production is already done in a tens of billions scale, production deriving from crude glycerol may contribute only a small fraction. Nevertheless there has been considerable research on this topic in order to use crude glycerol efficiently to produce ethanol (Licht, 2010).

Unfortunately, the well known ethanol producer *Saccharomyces cerevisiae* grows very slow on glycerol and, thus, the growth had to be considerably improved, e.g. by selecting *S. cerevisiae* strain CBS8066-FL20 which grows much faster (0.2 h^{-1} rather than at 0.01 h^{-1}) (Ochoa-Estopier et al., 2011). Ethanol accumulation of the yeast *Hansenula polymorpha* was improved from 0.83 g l^{-1} to 2.74 g l^{-1} by expression of genes encoding pyruvate decarboxylase (*pdc*) and aldehyde dehydrogenase II (*adhB*) from *Zymomonas mobilis*. Combined with the expression of glycerol dehydrogenase (*dhaD*) and dehydroxyacetone kinase (*dhaKLM*) genes from *Klebsiella pneumonia* even more ethanol (3.1 g l^{-1}) was produced (Hong et al., 2010). Elementary mode analysis and metabolic evolution of *E. coli* mutants led to conversion of 40 g l^{-1} glycerol to ethanol reaching 90% of the theoretical yield (Trinh & Srienc, 2009).

E. coli strains have been engineered to produce ethanol and H_2 or ethanol and formate from crude gycerol. Due to overexpression of genes for glycerol dehydrogenase (*gldA*) and

dihydroxyacetonekinase (*dhaKLM*) 95% of the theoretical maximum yield and specific production rates of 15-30 mmol (g cell)$^{-1}$ h^{-1} could be obtained (Shams Yazdani & Gonzalez, 2008). A newly isolated bacterium, *Kluyvera cryocrescens* S26, was able to produce 27 g l^{-1} ethanol with yield of 0.8 mol mol^{-1} and a productivity of 0.61 g l^{-1} h^{-1} (Choi et al., 2011).

2.5 Succinate

Succinic acid (succinate) is a so called platform chemical based on which a variety of other chemicals are produced, e.g. tetrahydrofuran, γ-butyrolactone, adipic acid, 1,4-butanediol and n-methyl-pyrrolidone. Based on this spectrum of products there are several markets succinic acid is involved in, such as pharmaceuticals, chemistry of biodegradable polymers, surfactants and detergents (Zeikus et al., 1999). Various microorganisms have been engineered for succinate production (Wendisch et al., 2006).

Succinic acid is an intermediate of the TCA cycle with four carbon atoms and two carboxylic groups. Today most of the produced succinic acid derives from the petrochemical industry and only a small part already comes from biotechnological processes. For the chemical synthesis the nonrenewable fossil fuel butane is the starting point leading through maleic anhydride to succinic acid (Zeikus et al., 1999).

Production of succinate from glycerol is interesting because both share the same level of reduction, thus, when produced from glycerol and CO_2 no further electron source is necessary. Various attempts have been made to use bacteria to efficiently produce succinate using natural succinic acid producers as well as metabolically engineered strains (Zeikus et al., 2007, Ingram et al., 2008, Lin et al., 2005, Samuelov et al., 1991, Okino et al., 2005, Singh et al., 2009, Van der Werf et al., 1997, Zhang et al., 2009). Among the natural producers, *Anaerobiospirillum succiniciproducens* was shown to use glycerol as sole or combined carbon source to efficiently produce succinic acid (Lee et al., 2004). They found a high succinic acid yield of 133% (or 160% when yeast extract was additionally fed) for glycerol concentrations of 6.5 g l^{-1}. Glycerol entailed less formation of acetic acid as byproduct and, thus, an easier downstream processing (Lee et al., 2001). Succinate production by the related bacterium *Basfia succiniciproducens* from crude glycerol in a continuous cultivation process allowed for product yields of about 1 g g^{-1} (Scholten et al., 2009).

Succinate production from glycerol by *E. coli* appeared to be favored under aerobic conditions as indicated by elementary mode analysis (Chen et al., 2010). Using microaerobic conditions, a recombinant producing pyruvate carboxylase from *Lactococcus lactis* and lacking pathways to byproducts showed succinic acid yields on glycerol comparable to those on glucose (Blankschien et al., 2010). About 80% of the maximum theoretical yield could be achieved by inserting three key mutations affecting phosphoenolpyruvate carboxykinase (*pck*), part of the phosphotransferase system (*ptsI*) and the pyruvate formate lyase (*pflB*) (Zhang et al., 2010).

2.6 Citrate

Citric acid is produced by fermentation at a scale of about 1,600,000 t/a (Papanikolaou et al., 2002, Berovic & Legisa, 2007), and it is sold for about 0.8 €/kg (Weusthuis et al., 2011). The main markets are the food and the pharmaceutical industries as well as applications in cosmetics, detergents and cleaning products. Citric acid is produced almost exclusively by *Aspergillus niger* in a submerged fermentation process using starch- or sucrose-based media like molasses (Soccol et al., 2006).

Different strains of the yeast *Yarrowia lipolytica* have been investigated for citric acid production using glycerol as a carbon source. For *Yarrowia lipolytica* Wratislavia AWG7 a maximal yield of 0.67 g g^{-1} was reported in continuous culture using glycerol as carbon source with only low contamination by the common byproduct isocitric acid (Rywinska et al., 2011, Rywinska & Rymowicz, 2010).
In 2002, Papanikolaou et al. reported about the *Y. lipolytica* strain LGAM S(7)1 being capable of growing on crude glycerol as carbon source and producing up to 35 g l^{-1} citric acid (Papanikolaou et al., 2002) . In 2010 and 2011, much higher citric acid concentrations of 112 g l^{-1} and a yield of 0.6 g g^{-1} using crude glycerol and the acetate-negative mutant strain *Y. lipolytica* A-101-1.22 (Rymowicz et al., 2010) or the acetate-negative strain *Y. lipolytica* N15 could be achieved (Kamzolova et al., 2011).

2.7 Amino acids

Amino acids are a multi-billion dollar business (Wendisch, 2007). They are used as flavor enhancers (L-glutamate), feed additives (L-lysine, L-methionine, L-threonine, L-tryptophane), to produce sweeteners such as aspartam (L-aspartate, L-phenylalanine), and in various pharmaceutical applications. The biggest products are L-glutamate (2,160,000 tons per year) and L-lysine (1,330,000 tons per year) (Ajinomoto, 2010a, Ajinomoto, 2010b). Pathways for amino acid synthesis start from intermediates of glycolysis (e.g. L-serine from 3-phosphoglycerate, L-valine from pyruvate), glycolysis and pentose phosphate pathway (aromatic amino acids L-tyrosine, L-phenylalanine, and L-tryptophane, from phosphoenolpyruvate (PEP) and erythrose-4-phosphate), and tricarboxylic acid cycle intermediates (L-glutamate, L-glutamine from 2-oxoglutarate, L-lysine, L-aspartate from oxaloacetate) (Schneider & Wendisch, 2001, Wendisch, 2007, Gopinath et al., 2011). In principle, production of amino acids from glycerol should be possible. In the following section the biosynthesis of L-glutamate, L-lysine, and L-phenylalanine are described, since strains for production of these from glycerol have been described.
Corynebacterium glutamicum is a natural L-glutamate producer (Eggeling & Bott, 2005), but the excretion of L-glutamate needs to be "triggered", e.g. by limitation of biotin (Shiio et al., 1962). Biotin is essential for the activity of acetyl-CoA carboxylase, necessary for fatty acid synthesis, and hence for membrane precursors, thus effect of biotin limitation on L-glutamate production is thought to be due to a higher permeability of the cell membrane (Shimizu & Hirasawa, 2007). Also addition of detergents like Tween 40 (Takinami et al., 1965), antibiotics like penicillin (Nara et al., 1964), and ethambutol (Radmacher et al., 2005, Stansen et al., 2005) trigger L-glutamate production. In *C. glutamicum* L-glutamate is mainly synthesized by NADPH dependent glutamate dehydrogenase from the tricarboxylic acid cycle intermediate 2-oxoglutarate (Bormann et al., 1992). This holds true for high ammonia concentrations, when ammonia is low L-glutamate is synthesized via L-glutamine by glutamine synthetase and glutamine-2-oxoglutarate aminotransferase. Crucial for L-glutamate production is the anaplerosis of the tricarboxylic acid cycle by either pyruvate carboxylase or PEP carboxylase. Pyruvate carboxylase has been shown to be indispensable under detergent triggered production conditions (Peters-Wendisch et al., 2001) and vice versa under biotin limiting conditions PEPcarboxylase is responsible for anaplerosis (Sato et al., 2008, Delaunay et al., 2004, Lapujade et al., 1999). *C. glutamicum* was engineered for glycerol utilization by expression of the genes for glycerol facilitator, glycerol kinase, and glycerol-3-phosphate dehydrogenase from *E. coli* (Rittmann et al., 2008). Under ethambutol

triggered L-glutamate production conditions recombinant *C. glutamicum* showed reduced L-glutamate yields from glycerol compared to glucose, 0.11 g g^{-1} compared to 0.20 g g^{-1}, respectively (Rittmann et al., 2008).

Production of L-lysine, which is used as a feed additive, is also carried out with *C. glutamicum* (Wendisch, 2007, Eggeling & Bott, 2005). The precursors of L-lysine production are the tricarboxylic acid cycle intermediate oxaloacetate and the glycolytic intermediate pyruvate. Deregulation of the L-lysine production pathway by introduction of feedback resistant variants of the key enzyme aspartate kinase, which usually is inhibited by L-lysine and L-threonine (Kalinowski et al., 1991) enables *C. glutamicum* for L-lysine production. Further increases were made by overexpression of the gene for pyruvate carboxylase (Peters-Wendisch et al., 2001), which provides L-lysine precursor oxaloacetate by the anaplerotic reaction from pyruvate (Peters-Wendisch et al., 1998). The anaplerotic reaction from PEP was shown to be dispendable for L-lysine production from glucose, however, it might play an important role if glucose is phosphorylated by use of ATP or polyphosphate and not PEP, which was shown to enhance L-lysine production and might elevate PEP availability (Lindner et al., 2011). Vice versa also an inactivation of the gene for PEP carboxykinase, catalyzing decarboxylation of oxaloacetate to PEP, entailed increased L-lysine production (Riedel et al., 2001). NADPH supply is very important for L-lysine production, as four molecules of NADPH are needed for one molecule L-lysine. The main path of NADPH generation is the oxidative branch of the pentose phosphate pathway (Marx et al., 1996), where NADP is reduced to NADPH by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, thus to enhance L-lysine production numerous attempts have been made towards increasing the pentose phosphate pathway flux, hence NADPH availability, hence L-lysine production. A deletion of the phosphoglucose isomerase gene drives the complete flux from glucose-6-phosphate into the pentose phosphate pathway and was shown to increase L-lysine production but to the cost of reduced growth (Marx et al., 2003). Redirection of the glycolytic flux towards the entry of the pentose phosphate pathway was furthermore achieved by overexpression of the fructose-bisphosphatase gene (Becker et al., 2005, Georgi et al., 2005) as well as use of feedback resistant variants of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Becker et al., 2007, Ohnishi et al., 2005). Also the increase of NADP availability by overexpression of a NAD kinase gene resulted in increased L-lysine production (Lindner et al., 2010). To establish L-lysine production from glycerol Rittmann et al. introduced the *Escherichia coli* glycerol utilization genes in a metabolic engineered *C. glutamicum* L-lysine producing strain (deregulated L-lysine pathway and higher anaplerotic from pyruvate to oxaloacetate). L-lysine yields were slightly lower from glycerol as glucose, 0.19 g g^{-1} compared to 0.26 g g^{-1}, respectively (Rittmann et al., 2008). Glycerol has also been used as a source of carbon for the production of the polymer of L-lysine ε-Poly-L-lysine with *Streptomyces sp.*(Chen et al., 2011b, Chen et al., 2011a). ε-Poly-L-lysine is an antimicrobial agent against bacteria, yeasts, and viruses (Shima et al., 1984) and therefore interesting for the pharmaceutical industry (Shih et al., 2004) and it is used as food preservative.

The main use of the aromatic amino acid phenylalanine is in production of the sweetener aspartam. Biosynthesis of aromatic amino acids from PEP and erythrose-4-phosphate involves the shikimic acid pathway and dedicated terminal biosynthesis pathways for tryptophan, tyrosine and phenylalanine (Sprenger, 2007). Biosynthesis of aromatic amino

acids e.g. in *Escherichia coli* and *C. glutamicum* was engineered e.g. by gene deregulation (Berry, 1996, Herry & Dunican, 1993), by gene copy number increase (Chan et al., 1993), and by the use of feedback-resistant enzyme variants, e.g. variants of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, the first enzyme of the shikimic acid pathway, variants of anthranilate synthase of the tryptophan pathway in *E. coli* (Tribe & Pittard, 1979) or anthranilate phosphoribosyltransferase of the tryptophan pathway in *C. glutamicum* (O'Gara & Dunican, 1995). In addition, strains were engineered for increased supply of the precursors PEP and erythrose-4-phosphate. In *C. glutamicum,* PEP availability was increased in PEP carboxylase mutants and erythrose-4-phosphate concentrations were elevated by overexpression of the transketolase gene (Ikeda & Katsumata, 1999, Ikeda et al., 1999, Katsumata & Kino, 1989). Similar approaches were made in *E. coli*, where PEP carboxylase or pyruvate kinase gene knock outs and overexpression of PEP carboxykinase increased PEP supply (Miller et al., 1987, Gosset et al., 1996, Chao & Liao, 1993, Backman, 1992). Furthermore, overexpression of genes encoding PEP synthase, PEP carboxykinase, and the use of an ATP-dependent glucose phosphorylation system instead of the PEP-dependent phosphotransferase system had positive effects on the availability of shikimic acid pathway precursor PEP (Patnaik et al., 1995, Liao, 1996, Gulevich et al., 2004). In *E. coli,* the availability of erythrose-4-phosphate could be increased by overproduction of transketolase and transaldolase or by phosphoglucose isomerase gene disruption (Draths & Frost, 1990, Draths et al., 1992, Lu & Liao, 1997, Mascarenhas et al., 1991, Frost, 1992). Up to now, only L-phenylalanine production from glycerol has been shown, but results might be transferable to the other aromatic amino acids. Similar final concentrations of L-phenylalanine were reported for an engineered *E. coli* strain regardless of the use of glycerol, glucose or sucrose as carbon source. Notably, a higher yield was reported when glycerol was used (0.58 g g^{-1}) as compared to the use of sucrose (0.25 g g^{-1}) (Khamduang et al., 2009).

Polyamines may be derived from amino acids (Schneider & Wendisch, 2010). While strain development for sugar-based production of polyamines such as the diamine 1,4-diaminobutane, which is used e.g. in the polyamide market, has been successful (Schneider & Wendisch, 2010), glycerol-based production of poylamines has not yet been reported.

2.8 2,3-Butanediol

2,3-Butanediol (2,3-BDO) is used as a solvent, fuel, and for the production of polymers and chemicals (Perego et al., 2003, Saha & Bothast, 1999). Bacterial 2,3-BDO production has been shown e.g. with strains of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Bacillus polymyxa*, and *Bacillus licheniformis* (Grover et al., 1990, Perego et al., 2000, De Mas et al., 1988, Nilegaonkar et al., 1996, Jansen et al., 1984). Biosynthesis of 2,3-BDO is funneled from pyruvate in three steps. First, acetolactate synthase (EC 2.2.1.6) catalyses the condensation of two pyruvate molecules to acetolactate with concomitant CO_2 liberation. Second, acetolactate is decarboxylated by acetolactate decarboxylase (EC 4.1.1.5) to acetoin. Third, acetoin is reduced to 2,3-butanediol by 2,3-BDO dehydrogenase (acetoin reductase; EC 1.1.1.4) (Juni, 1952). Thus for 2,3-BDO production all substrates first need to be converted to pyruvate, the intermediate of glycolysis.

2,3-BDO is a product of mixed acid fermentation and, thus, associated with byproduct formation. Byproduct reduction approaches were made with *K. oxytoca* mutants defective in genes encoding lactate dehydrogenase and phosphotransacetylase, reducing lactate and acetate byproduct formation by 88% and 92%, respectively, but increasing 2,3-BDO production only by 7.8% (Ji et al., 2008). Also formation of ethanol, a major byproduct of 2,3-

BDO production with *K. oxytoca*, could be eliminated by insertion mutagenesis of the aldehyde dehydrogenase gene and 2,3-BDO production from glucose in a fed-batch process was improved to yield 130 g l^{-1} 2,3-BDO with a productivity of 1.63 g l^{-1} h^{-1} and a yield of 0.48 g g^{-1} (Ji et al., 2010).
Many substrates have been used for the production of 2,3-BDO. Use of starch as a substrate for 2,3-BDO production has been shown with *K. pneumoniae* by overexpression of a secretory α-amylase (Wei et al., 2008). With *B. licheniformis* corn starch hydrolysates were applied to 2,3-BDO production (Perego et al., 2003). With *E. aerogenes*, food industry wastes such as starch hydrolysates, raw and decoloured molasses, and whey permeate were used for the fermentation of 2,3-BDO (Perego et al., 2000). The use of lignocellulosic compounds for 2,3-BDO has also been reported, e.g. corncob hydrolysates were used in processes with *K. oxytoca* (Cheng et al., 2010) and *K. pneumoniae* (Ma et al., 2010a).
Glycerol was used for the production of 2,3-BDO as well. Because 1,3-propanediol production is preferably carried out from glycerol e.g. by *K. pneumoniae* and because 2,3-BDO is a known byproduct of this process (Biebl et al., 1998), glycerol might be a good substrate for 2,3-BDO production. Production of 2,3-BDO from glycerol by *K. pneumoniae* G31 resulted in final concentrations of 49.2 g l^{-1}. The medium pH had a large influence on 2,3-BDO fermentation from glycerol with 2,3-BDO production being favored at alkaline pH (Petrov & Petrova, 2009). In addition, intense aeration increased 2,3-BDO synthesis and reduced byproducts (Petrov & Petrova, 2010).

2.9 Hydrogen

Hydrogen production is highly desirable as a source of clean energy to be used, e.g. in fuel cells. Processes for the use of glycerol or crude glycerol respectively are under investigation. Besides microbial strategies to generate H_2 from crude glycerol there are also promising chemicals techniques such as steam reforming, partial oxidation, auto thermal reforming, aqueous-phase reforming, and supercritical water reforming (Xiaohu Fan, 2010). Currently, only low concentrations of glycerol can be used in microbial H_2 production process to avoid that other products like 1,3-propanediol or ethanol are produced along with H_2. *Enterobacter aerogenes* HU-101 showed hydrogen yields of 1.12 mol mol^{-1} using crude glycerol, but at relatively low glycerol concentrations of 1.7 g l^{-1} (Ito et al., 2005). Mixed cultures isolated from soil or wastewater converted crude glycerol to H_2 with a yield 0.31 mol mol^{-1} and to 1,3-Propanediol with a yield of 0.59 mol mol^{-1}. These values are lower than the ones on glucose but similar to the ones with pure glycerol, suggesting that inhibiting substances in crude glycerol may not be a problem in this process (Selembo et al., 2009). Production rates of 0.68 ± 0.16 mmol H_2 l^{-1} h^{-1} could be achieved by an evolved *E. coli* BW25113 *frdC* negative strain along with some ethanol production (Hu & Wood, 2010).

2.10 Glyceric acid

Glyceric acid is a known byproduct of dihydroxyacetone production from glycerol with *Gluconobacter oxydans*. The path from glycerol to glyceric aid, which might be suitable for chemical applications (Habe et al., 2009a), occurs via two dehydrogenases. First, alcohol dehydrogenase oxidizes glycerol to glyceraldehyde which is subsequently oxidized further to glyceric acid by a so far unidentified enzyme (Habe et al., 2009d). In a screen of various acetic acid bacteria *Acetobacter tropicalis* was the best glyceric acid producing strain (Habe et al., 2009b). *A. tropicalis* produced 101.8 g l^{-1} glyceric acid while *Gluconobacter frateurii*

accumulated 136.5 g l^{-1} (Habe et al., 2009d). The involvement of a membrane-bound alcohol dehydrogenase in glyceric acid production was investigated with *G. oxydans* IFO12528. Gene disruption of the alcohol dehydrogenase entailed severely reduced glyceric acid concentrations, indicating a role of the alcohol dehydrogenase in glyceric acid production (Habe et al., 2009d). *G. frateurii* was engineered for glyceric acid production by disruption of the glycerol dehydrogenase gene *sldA*, thus, eliminating dihydroxyacetone production. The growth retardation of this strain on glycerol alone was overcome by addition of sorbitol to the medium. A higher glyceric acid concentration of 89.1 g l^{-1} was reached with the *sldA* mutant compared to the parental strain (54.7 g l^{-1}) as production dihydroxyacetone as byproduct was avoided (Habe et al., 2010b). Glyceric acid production from raw glycerol pretreated with activated charcoal by *Gluconobacter sp.* NBRC3259 reached comparable concentration of glyceric acid (45.9 g l^{-1} and 54.7 g l^{-1}) and of dihydroxyacetone (28.2 g l^{-1} and 33.7 g l^{-1}) as production from pure glycerol (Habe et al., 2009c).

2.11 Biosurfactants

Surfactants are used in numerous applications such as cleaners, emulsifiers, in coatings, laundry detergents, or in paints. The global surfactant market is predicted to reach 17.9 billion dollars by 2015 (Global Industry Analysts, 2010). Biosurfactants consist of a hydrophilic part and a hydrophobic/lipophilic part, making them amphiphiles/tensides. The hydrophilic part can consist of a sugar, peptide or protein, while the hydrophobic part contains fatty acids or fatty alcohols. The great advantage of biosurfactants over chemically produced tensides is that they are biodegradable, hence environmentally friendly, less toxic, and they can be produced from renewable resources. Natural biosurfactant producers are bacteria, yeast, and fungi (Mulligan, 2005). Biosurfactants are already used in many applications, e.g. in the food, agriculture, chemical, pharmaceutical, and cosmetic industries (for recent review see (Pacwa-Plociniczak et al., 2011)). Glycerol is the backbone of the lipid component of biosurfactants and use of pure and raw glycerol in biosurfactant production has been investigated with a variety of organisms. The yeast *Pseudozyma antarctica* produced 16.3 g l^{-1} glycolipid biosurfactants from glycerol (Morita et al., 2007). Glycolipid surfactants production was also shown with *Candida bombicola* and product concentrations of 12.7 g l^{-1} of sophorolipids could be obtained from glycerol and oleic acid (Ashby & Solaiman, 2010). When a biodiesel co-product stream consisting of 40% glycerol, 34% hexane-solubles, and 26% water was used production of sophorolipids by *C. bombicola* was strongly increased (from 9 g l^{-1} to 60 g l^{-1}) (Ashby et al., 2005). Production of glucosylmannosyl-glycerolipid with *Microbacterium spec.* was reported to be 1.5-fold higher when glycerol instead of glucose is used in media containing the complex medium compounds peptone and yeast extract (Lang et al., 2004, Wicke et al., 2000). When several carbon sources were analyzed for rhamnolipid production by *Pseudomonas aeruginosa* EM1, glycerol was the best carbon source besides glucose, yielding 4.9 and 7.5 g l^{-1} respectively (Wu et al., 2008). Glycerol-based biosurfactant production with *Pseudomonas aeruginosa* UCP0992 yielded 8.0 g l^{-1} biosurfactants (Silva et al., 2010), processes with *Rhodococcus erythropolis* yielded 1.7 g l^{-1} biosurfactants (Ciapina et al., 2006) and with *Ustilago maydis* 32.1 g l^{-1} glycolipid biosurfactants from 50 g l^{-1} glycerol could be produced (Liu et al., 2011).

3. Conclusions

Glycerol availability has increased tremendously as it arises as byproduct of the biodiesel process. Besides using glycerol as a chemical in creams and other small-scale applications,

glycerol may be used as starting material for large-scale biotechnological processes. Several microbiological process are based on glycerol as substrate anyway, e.g. the biotechnological production of 1,3-propanediol and dihydroxyacetone. In addition, as glycerol is a good source of carbon and energy for growth of several microorganisms it may be suitable for the biotechnological production of a number of chemicals in fermentative processes. Microbial catalysts have been developed for the production of succinic acid, citric acid, glyceric acid, propionic acid, ethanol, 1,2-propanediol, 2,3-butanediol, biosurfactants and amino acids. Successful implementation of these processes critically depends on strain optimization, recalcitrance to inhibitors present in crude glycerol preparations from biodiesel factories and measures to reduce glycerol price volatility. If successful, coupling biodiesel production to the production of value-added products from the side-stream glycerol "on the spot" would represent an excellent example of applying the biorefinery concept to a large-scale process.

4. Acknowledgements

Work in the laboratory of the authors is supported in part by grants from the BMBF (0315589G, 0315598E, 316017A), ERA-IB (22009508B) and ESF (PAK529).

5. References

Ajinomoto, (2010a) Feed-Use Amino Acids Business. Available from World Wide Web: http://www.ajinomoto.com/ir/pdf/Feed-useAA-Oct2010.pdf. Cited 18 March 2011.

Ajinomoto, (2010b) Food Products Business. Available from World Wide Web: http://www.ajinomoto.com/ir/pdf/Food-Oct2010.pdf. Cited 18 March 2011.

Altaras, N. E. & D. C. Cameron, (1999) Metabolic engineering of a 1,2-propanediol pathway in *Escherichia coli*. *Appl Environ Microbiol* 65: 1180-1185.

Altaras, N. E. & D. C. Cameron, (2000) Enhanced production of (R)-1,2-propanediol by metabolically engineered *Escherichia coli*. *Biotechnol Prog* 16: 940-946.

Ashby, R. D., A. Nunez, D. K. Y. Solaiman & T. A. Foglia, (2005) Sophorolipid biosynthesis from a biodiesel co-product stream. *J Am Oil Chem Soc* 82: 625-630.

Ashby, R. D. & D. K. Solaiman, (2010) The influence of increasing media methanol concentration on sophorolipid biosynthesis from glycerol-based feedstocks. *Biotechnol Lett* 32: 1429-1437.

Ashok, S., S. M. Raj, C. Rathnasingh & S. Park, (2011) Development of recombinant *Klebsiella pneumoniae* Δ*dhaT* strain for the co-production of 3-hydroxypropionic acid and 1,3-propanediol from glycerol. *Appl Microbiol Biotechnol* 90: 1253-1265.

Backman, K., (1992) Method of biosynthesis of phenylalanine. U.S. Patent No. US 5169768.

Badia, J., J. Ros & J. Aguilar, (1985) Fermentation mechanism of fucose and rhamnose in *Salmonella typhimurium* and *Klebsiella pneumoniae*. *J Bacteriol* 161: 435-437.

Barbirato, F., J. P. Grivet, P. Soucaille & A. Bories, (1996) 3-Hydroxypropionaldehyde, an inhibitory metabolite of glycerol fermentation to 1,3-propanediol by enterobacterial species. *Appl Environ Microbiol* 62: 1448-1451.

Barbirato, F., A. Larguier, T. Conte, S. Astruc & A. Bories, (1997) Sensitivity to pH, product inhibition, and inhibition by NAD^+ of 1,3-propanediol dehydrogenase purified from *Enterobacter agglomerans* CNCM 1210. *Arch Microbiol* 168: 160-163.

Bauer, R., N. Katsikis, S. Varga & D. Hekmat, (2005) Study of the inhibitory effect of the product dihydroxyacetone on *Gluconobacter oxydans* in a semi-continuous two-stage repeated-fed-batch process. *Bioprocess Biosyst Eng* 28: 37-43.

Becker, J., C. Klopprogge, A. Herold, O. Zelder, C. J. Bolten & C. Wittmann, (2007) Metabolic flux engineering of L-lysine production in *Corynebacterium glutamicum*--over expression and modification of G6P dehydrogenase. *J Biotechnol* 132: 99-109.

Becker, J., C. Klopprogge, O. Zelder, E. Heinzle & C. Wittmann, (2005) Amplified expression of fructose 1,6-bisphosphatase in *Corynebacterium glutamicum* increases in vivo flux through the pentose phosphate pathway and lysine production on different carbon sources. *Appl Environ Microbiol* 71: 8587-8596.

Berovic, M. & M. Legisa, (2007) Citric acid production. *Biotechnol Annu Rev* 13: 303-343.

Berrios-Rivera, S. J., K. Y. San & G. N. Bennett, (2003) The effect of carbon sources and lactate dehydrogenase deletion on 1,2-propanediol production in *Escherichia coli*. *J Ind Microbiol Biotechnol* 30: 34-40.

Berry, A., (1996) Improving production of aromatic compounds in *Escherichia coli* by metabolic engineering. *Trends Biotechnol* 14: 250-256.

Biebl, H., (1991) Glycerol Fermentation of 1,3-Propanediol by *Clostridium butyricum* - Measurement of Product Inhibition by Use of a Ph-Auxostat. *Appl Microbiol Biot* 35: 701-705.

Biebl, H., (2001) Fermentation of glycerol by *Clostridium pasteurianum* - batch and continuous culture studies. *J Ind Microbiol Biot* 27: 18-26.

Biebl, H., S. Marten, H. Hippe & W. D. Deckwer, (1992) Glycerol Conversion to 1,3-Propanediol by Newly Isolated *Clostridia*. *Appl Microbiol Biot* 36: 592-597.

Biebl, H., A. P. Zeng, K. Menzel & W. D. Deckwer, (1998) Fermentation of glycerol to 1,3-propanediol and 2,3-butanediol by Klebsiella pneumoniae. *Appl Microbiol Biotechnol* 50: 24-29.

Blankschien, M. D., J. M. Clomburg & R. Gonzalez, (2010) Metabolic engineering of *Escherichia coli* for the production of succinate from glycerol. *Metab Eng* 12: 409-419.

Blomberg, A., (2000) Metabolic surprises in *Saccharomyces cerevisiae* during adaptation to saline conditions: questions, some answers and a model. *FEMS Microbiol Lett* 182: 1-8.

Bories, A., C. Claret & P. Soucaille, (1991) Kinetic-Study and Optimization of the Production of Dihydroxyacetone from Glycerol Using *Gluconobacter oxydans*. *Process Biochem* 26: 243-248.

Bormann, E. R., B. J. Eikmanns & H. Sahm, (1992) Molecular analysis of the *Corynebacterium glutamicum gdh* gene encoding glutamate dehydrogenase. *Mol Microbiol* 6: 317-326.

Boronat, A. & J. Aguilar, (1981) Experimental evolution of propanediol oxidoreductase in *Escherichia coli*. Comparative analysis of the wild-type and mutant enzymes. *Biochim Biophys Acta* 672: 98-107.

Cameron, D. C., N. E. Altaras, M. L. Hoffman & A. J. Shaw, (1998) Metabolic engineering of propanediol pathways. *Biotechnol Prog* 14: 116-125.

Cameron, D. C. & C. L. Cooney, (1986) A Novel Fermentation - the Production of R(-)-1,2-Propanediol and Acetol by *Clostridium thermosaccharolyticum*. *Bio-Technol* 4: 651-654.

Carballo, J., A. Bernardo, M. J. Prieto & R. M. Sarmiento, (1993) Kinetics of alpha-dicarbonyls reduction by L-glycol dehydrogenase (NAD+) from *Enterobacter aerogenes*. *Ital J Biochem* 42: 79-89.

Carole, T. M., J. Pellegrino & M. D. Paster, (2004) Opportunities in the industrial biobased products industry. *Appl Biochem Biotechnol* 113-116: 871-885.

Chan, E. C., H. L. Tsai, S. L. Chen & D. G. Mou, (1993) Amplification of the Tryptophan Operon Gene in *Escherichia coli* Chromosome to Increase L-Tryptophan Biosynthesis. *Appl Microbiol Biot* 40: 301-305.

Chao, Y. P. & J. C. Liao, (1993) Alteration of growth yield by overexpression of phosphoenolpyruvate carboxylase and phosphoenolpyruvate carboxykinase in *Escherichia coli*. *Appl Environ Microbiol* 59: 4261-4265.

Chatzifragkou, A., D. Dietz, M. Komaitis, A. P. Zeng & S. Papanikolaou, (2010) Effect of biodiesel-derived waste glycerol impurities on biomass and 1,3-propanediol production of *Clostridium butyricum* VPI 1718. *Biotechnol Bioeng* 107: 76-84.

Chen, X., L. Tang, S. Li, L. Liao, J. Zhang & Z. Mao, (2011a) Optimization of medium for enhancement of epsilon-poly-L-lysine production by *Streptomyces sp.* M-Z18 with glycerol as carbon source. *Bioresour Technol* 102: 1727-1732.

Chen, X., D. J. Zhang, W. T. Qi, S. J. Gao, Z. L. Xiu & P. Xu, (2003) Microbial fed-batch production of 1,3-propanediol by *Klebsiella pneumoniae* under micro-aerobic conditions. *Appl Microbiol Biot* 63: 143-146.

Chen, X. S., S. Li, L. J. Liao, X. D. Ren, F. Li, L. Tang, J. H. Zhang & Z. G. Mao, (2011b) Production of epsilon-poly-L: -lysine using a novel two-stage pH control strategy by *Streptomyces sp.* M-Z18 from glycerol. *Bioprocess Biosyst Eng* 34: 561-567.

Chen, Z., H. Liu, J. Zhang & D. Liu, (2010) Elementary mode analysis for the rational design of efficient succinate conversion from glycerol by *Escherichia coli*. *J Biomed Biotechnol* 2010: 518743.

Cheng, K. K., H. J. Liu & D. H. Liu, (2005) Multiple growth inhibition of *Klebsiella pneumoniae* in 1,3-propanediol fermentation. *Biotechnol Lett* 27: 19-22.

Cheng, K. K., Q. Liu, J. A. Zhang, J. P. Li, J. M. Xu & G. H. Wang, (2010) Improved 2,3-butanediol production from corncob acid hydrolysate by fed-batch fermentation using *Klebsiella oxytoca*. *Process Biochem* 45: 613-616.

Choi, W. J., M. R. Hartono, W. H. Chan & S. S. Yeo, (2011) Ethanol production from biodiesel-derived crude glycerol by newly isolated *Kluyvera cryocrescens*. *Appl Microbiol Biotechnol* 89: 1255-1264.

Ciapina, E. M., W. C. Melo, L. M. Santa Anna, A. S. Santos, D. M. Freire & N. Pereira, Jr., (2006) Biosurfactant production by *Rhodococcus erythropolis* grown on glycerol as sole carbon source. *Appl Biochem Biotechnol* 131: 880-886.

Claret, C., A. Bories & P. Soucaille, (1992) Glycerol Inhibition of Growth and Dihydroxyacetone Production by *Gluconobacter oxydans*. *Current Microbiology* 25: 149-155.

Claret, C., A. Bories & P. Soucaille, (1993) Inhibitory Effect of Dihydroxyacetone on *Gluconobacter oxydans* - Kinetic Aspects and Expression by Mathematical Equations. *J Ind Microbiol* 11: 105-112.

Claret, C., J. M. Salmon, C. Romieu & A. Bories, (1994) Physiology of *Gluconabacter oxydans* during Dihydroxyacetone Production from Glycerol. *Appl Microbiol Biot* 41: 359-365.

Clomburg, J. M. & R. Gonzalez, (2011) Metabolic engineering of *Escherichia coli* for the production of 1,2-propanediol from glycerol. *Biotechnol Bioeng* 108: 867-879.

Colin, T., A. Bories & G. Moulin, (2000) Inhibition of *Clostridium butyricum* by 1,3-propanediol and diols during glycerol fermentation. *Appl Microbiol Biotechnol* 54: 201-205.

Cooper, R. A. & A. Anderson, (1970) Formation and Catabolism of Methylglyoxal during Glycolysis in *Escherichia coli*. *Febs Letters* 11: 273-&.

Cordier, H., F. Mendes, I. Vasconcelos & J. M. Francois, (2007) A metabolic and genomic study of engineered *Saccharomyces cerevisiae* strains for high glycerol production. *Metab Eng* 9: 364-378.

Cozzarelli, N. R., J. P. Koch, S. Hayashi & E. C. Lin, (1965) Growth stasis by accumulated L-alpha-glycerophosphate in *Escherichia coli*. *J Bacteriol* 90: 1325-1329.

da Silva, G. P., M. Mack & J. Contiero, (2009) Glycerol: a promising and abundant carbon source for industrial microbiology. *Biotechnol Adv* 27: 30-39.

Daniel, R., K. Stuertz & G. Gottschalk, (1995) Biochemical and Molecular Characterization of the Oxidative Branch of Glycerol Utilization by *Citrobacter freundii*. *Journal of Bacteriology* 177: 4392-4401.

De Mas, C., N. B. Jansen & G. T. Tsao, (1988) Production of optically active 2,3-butanediol by *Bacillus polymyxa*. *Biotechnol Bioeng* 31: 366-377.

Degalard, P., (2011) METabolic EXplorer develops its first plant in Malaysia. Available from World Wide Web: http://www.ubifrance.com/my/Posts-1483-METabolic-EXplorer-develops-its-first-plant-in-Malaysia. Cited 22 June 2011.

Delaunay, S., P. Daran-Lapujade, J. M. Engasser & J. L. Goergen, (2004) Glutamate as an inhibitor of phosphoenolpyruvate carboxylase activity in *Corynebacterium glutamicum*. *J Ind Microbiol Biotechnol* 31: 183-188.

Draths, K. M. & J. W. Frost, (1990) Synthesis Using Plasmid-Based Biocatalysis - Plasmid Assembly and 3-Deoxy-D-Arabino-Heptulosonate Production. *Journal of the American Chemical Society* 112: 1657-1659.

Draths, K. M., D. L. Pompliano, D. L. Conley, J. W. Frost, A. Berry, G. L. Disbrow, R. J. Staversky & J. C. Lievense, (1992) Biocatalytic Synthesis of Aromatics from D-Glucose - the Role of Transketolase. *Journal of the American Chemical Society* 114: 3956-3962.

EBB, (European Biodiesel Board 2010) 2009-2010: EU biodiesel industry restained growth in challenging times. Annual biodiesel production statistics. Available in: http://www.ebb-eu.org/EBBpressreleases/EBB press release 2009 prod 2010_capacity FINAL.pdf.

Eggeling, L. & M. Bott, (2005) Handbook of *Corynebacterium glutamicum*. *CRC Press, Boca Raton, USA*.

Enders, D., M. Voith & A. Lenzen, (2005) The dihydroxyacetone unit--a versatile C(3) building block in organic synthesis. *Angew Chem Int Ed Engl* 44: 1304-1325.

Ferreira, C., F. van Voorst, A. Martins, L. Neves, R. Oliveira, M. C. Kielland-Brandt, C. Lucas & A. Brandt, (2005) A member of the sugar transporter family, Stl1p is the glycerol/H^+ symporter in *Saccharomyces cerevisiae*. *Mol Biol Cell* 16: 2068-2076.

Flickinger, M. C. & D. Perlman, (1977) Application of Oxygen-Enriched Aeration in the Conversion of Glycerol to Dihydroxyacetone by *Gluconobacter melanogenus* IFO 3293. *Appl Environ Microbiol* 33: 706-712.

Forage, R. G. & M. A. Foster, (1982) Glycerol fermentation in *Klebsiella pneumoniae*: functions of the coenzyme B12-dependent glycerol and diol dehydratases. *J Bacteriol* 149: 413-419.

Forsberg, C. W., (1987) Production of 1,3-Propanediol from Glycerol by *Clostridium acetobutylicum* and Other *Clostridium* Species. *Appl Environ Microbiol* 53: 639-643.

Freedberg, W. B., W. S. Kistler & E. C. Lin, (1971) Lethal synthesis of methylglyoxal by *Escherichia coli* during unregulated glycerol metabolism. *J Bacteriol* 108: 137-144.

Frost, J., (1992) Enhanced production of common aromatic pathway compounds. U.S. Patent No. US 5168056.

Georgi, T., D. Rittmann & V. F. Wendisch, (2005) Lysine and glutamate production by *Corynebacterium glutamicum* on glucose, fructose and sucrose: Roles of malic enzyme and fructose-1,6-bisphosphatase. *Metab Eng* 7: 291-301.

Global Industry Analysts, I., (2010) MCP-2146: Surface active agents - a global strategic business report. Available from World Wide Web: http://www.strategyr.com/pressMCP-2146.asp. Cited 22 June 2011.

Gonzalez-Pajuelo, M., J. C. Andrade & I. Vasconcelos, (2004) Production of 1,3-propanediol by *Clostridium butyricum* VPI 3266 using a synthetic medium and raw glycerol. *J Ind Microbiol Biotechnol* 31: 442-446.

Gonzalez-Pajuelo, M., I. Meynial-Salles, F. Mendes, J. C. Andrade, I. Vasconcelos & P. Soucaille, (2005) Metabolic engineering of *Clostridium acetobutylicum* for the industrial production of 1,3-propanediol from glycerol. *Metab Eng* 7: 329-336.

Gonzalez-Pajuelo, M., I. Meynial-Salles, F. Mendes, P. Soucaille & I. Vasconcelos, (2006) Microbial conversion of glycerol to 1,3-propanediol: physiological comparison of a natural producer, *Clostridium butyricum* VPI 3266, and an engineered strain, *Clostridium acetobutylicum* DG1(pSPD5). *Appl Environ Microbiol* 72: 96-101.

Gonzalez, R., A. Murarka, Y. Dharmadi & S. S. Yazdani, (2008) A new model for the anaerobic fermentation of glycerol in enteric bacteria: trunk and auxiliary pathways in *Escherichia coli*. *Metab Eng* 10: 234-245.

Gopinath V, Meiswinkel TM, Wendisch VF, Nampoothiri KM (2011) Amino acid production from rice straw and wheat bran hydrolysates by recombinant pentose-utilizing Corynebacterium glutamicum. Appl Microbiol Biotechnol. doi:10.1007/s00253-011-3478-x

Gosset, G., J. Yong-Xiao & A. Berry, (1996) A direct comparison of approaches for increasing carbon flow to aromatic biosynthesis in *Escherichia coli*. *J Ind Microbiol* 17: 47-52.

Greenfield, S. & G. W. Claus, (1972) Nonfunctional tricarboxylic acid cycle and the mechanism of glutamate biosynthesis in *Acetobacter suboxydans*. *J Bacteriol* 112: 1295-1301.

Greenwood, A., (2010) DuPont, Tate & Lyle JV expand US propandiol plant. Available from World Wide Web: http://www.icis.com/Articles/2010/05/04/9356156/dupont-tate-lyle-jv-expand-us-propandiol-plant.html. Cited 22 June 2011.

Grover, B. P., S. K. Garg & J. Verma, (1990) Production of 2,3-Butanediol from Wood Hydrolysate by *Klebsiella-Pneumoniae*. *World J Microb Biot* 6: 328-332.

Gulevich, A., I. Biryukova, D. Zimenkov, A. Skorokhodova, A. Kivero, A. Belareva & S. Mashko, (2004) Method for producing l-amino acid using bacterium having enhanced expression of *pckA* gene. World Patent No.WO 090125.

Gungormusler, M., C. Gonen & N. Azbar, (2011) Continuous production of 1,3-propanediol using raw glycerol with immobilized *Clostridium beijerinckii* NRRL B-593 in comparison to suspended culture. *Bioprocess Biosyst Eng*.

Habe, H., T. Fukuoka, D. Kitamoto & K. Sakaki, (2009a) Biotechnological production of D-glyceric acid and its application. *Appl Microbiol Biotechnol* 84: 445-452.

Habe, H., T. Fukuoka, D. Kitamoto & K. Sakaki, (2009b) Biotransformation of glycerol to D-glyceric acid by Acetobacter tropicalis. *Appl Microbiol Biotechnol* 81: 1033-1039.

Habe, H., T. Fukuoka, T. Morita, D. Kitamoto, T. Yakushi, K. Matsushita & K. Sakaki, (2010a) Disruption of the membrane-bound alcohol dehydrogenase-encoding gene improved glycerol use and dihydroxyacetone productivity in *Gluconobacter oxydans*. *Biosci Biotechnol Biochem* 74: 1391-1395.

Habe, H., Y. Shimada, T. Fukuoka, D. Kitamoto, M. Itagaki, K. Watanabe, H. Yanagishita & K. Sakaki, (2009c) Production of glyceric acid by *Gluconobacter sp.* NBRC3259 using raw glycerol. *Biosci Biotechnol Biochem* 73: 1799-1805.

Habe, H., Y. Shimada, T. Fukuoka, D. Kitamoto, M. Itagaki, K. Watanabe, H. Yanagishita, T. Yakushi, K. Matsushita & K. Sakaki, (2010b) Use of a *Gluconobacter frateurii* mutant to prevent dihydroxyacetone accumulation during glyceric acid production from glycerol. *Biosci Biotechnol Biochem* 74: 2330-2332.

Habe, H., Y. Shimada, T. Yakushi, H. Hattori, Y. Ano, T. Fukuoka, D. Kitamoto, M. Itagaki, K. Watanabe, H. Yanagishita, and others, (2009d) Microbial production of glyceric acid, an organic acid that can be mass produced from glycerol. *Appl Environ Microbiol* 75: 7760-7766.

Hacking, A. J. & E. C. Lin, (1976) Disruption of the fucose pathway as a consequence of genetic adaptation to propanediol as a carbon source in *Escherichia coli*. *J Bacteriol* 126: 1166-1172.

Hao, J., R. Lin, Z. Zheng, Y. Sun & D. Liu, (2008a) 3-Hydroxypropionaldehyde guided glycerol feeding strategy in aerobic 1,3-propanediol production by *Klebsiella pneumoniae*. *J Ind Microbiol Biotechnol* 35: 1615-1624.

Hao, J., W. Wang, J. Tian, J. Li & D. Liu, (2008b) Decrease of 3-hydroxypropionaldehyde accumulation in 1,3-propanediol production by over-expressing *dhaT* gene in *Klebsiella pneumoniae* TUAC01. *J Ind Microbiol Biotechnol* 35: 735-741.

Hekmat, D., R. Bauer & J. Fricke, (2003) Optimization of the microbial synthesis of dihydroxyacetone from glycerol with *Gluconobacter oxydans*. *Bioprocess Biosyst Eng* 26: 109-116.

Heller, K. B., E. C. Lin & T. H. Wilson, (1980) Substrate specificity and transport properties of the glycerol facilitator of *Escherichia coli*. *J Bacteriol* 144: 274-278.

Herrmann, U., C. Gatgens, U. Degner & S. Bringer-Meyer, (2007) Biotransformation of glycerol to dihydroxyacetone by recombinant *Gluconobacter oxydans* DSM 2343. *Appl Microbiol Biot* 76: 553-559.

Herry, D. M. & L. K. Dunican, (1993) Cloning of the trp gene cluster from a tryptophan-hyperproducing strain of *Corynebacterium glutamicum*: identification of a mutation in the trp leader sequence. *Appl Environ Microbiol* 59: 791-799.

Himmi, E. H., A. Bories, A. Boussaid & L. Hassani, (2000) Propionic acid fermentation of glycerol and glucose by *Propionibacterium acidipropionici* and *Propionibacterium freudenreichii ssp. shermanii*. *Appl Microbiol Biotechnol* 53: 435-440.

Hiremath, A., M. Kannabiran & V. Rangaswamy, (2011) 1,3-Propanediol production from crude glycerol from *Jatropha* biodiesel process. *N Biotechnol* 28: 19-23.

Holst, O., H. Lundback & B. Mattiasson, (1985) Hydrogen-Peroxide as an Oxygen Source for Immobilized *Gluconobacter oxydans* Converting Glycerol to Dihydroxyacetone. *Appl Microbiol Biot* 22: 383-388.

Homann, T., C. Tag, H. Biebl, W. D. Deckwer & B. Schink, (1990) Fermentation of Glycerol to 1,3-Propanediol by *Klebsiella* and *Citrobacter* Strains. *Appl Microbiol Biot* 33: 121-126.

Hong, W. K., C. H. Kim, S. Y. Heo, L. H. Luo, B. R. Oh & J. W. Seo, (2010) Enhanced production of ethanol from glycerol by engineered *Hansenula polymorpha* expressing pyruvate decarboxylase and aldehyde dehydrogenase genes from *Zymomonas mobilis*. *Biotechnol Lett* 32: 1077-1082.

Hopper, D. J. & R. A. Cooper, (1972) The purification and properties of *Escherichia coli* methylglyoxal synthase. *Biochem J* 128: 321-329.

Horng, Y. T., K. C. Chang, T. C. Chou, C. J. Yu, C. C. Chien, Y. H. Wei & P. C. Soo, (2010) Inactivation of *dhaD* and *dhaK* abolishes by-product accumulation during 1,3-propanediol production in *Klebsiella pneumoniae*. *J Ind Microbiol Biotechnol* 37: 707-716.

Hu, H. & T. K. Wood, (2010) An evolved *Escherichia coli* strain for producing hydrogen and ethanol from glycerol. *Biochem Biophys Res Commun* 391: 1033-1038.

Huang, K., F. B. Rudolph & G. N. Bennett, (1999) Characterization of methylglyoxal synthase from *Clostridium acetobutylicum* ATCC 824 and its use in the formation of 1, 2-propanediol. *Appl Environ Microbiol* 65: 3244-3247.

Ikeda, M. & R. Katsumata, (1999) Hyperproduction of tryptophan by *Corynebacterium glutamicum* with the modified pentose phosphate pathway. *Appl Environ Microbiol* 65: 2497-2502.

Ikeda, M., K. Okamoto & R. Katsumata, (1999) Cloning of the transketolase gene and the effect of its dosage on aromatic amino acid production in *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol* 51: 201-206.

Ingram, L. O., K. Jantama, M. J. Haupt, S. A. Svoronos, X. L. Zhang, J. C. Moore & K. T. Shanmugam, (2008) Combining metabolic engineering and metabolic evolution to develop nonrecombinant strains of *Escherichia coli* C that produce succinate and malate. *Biotechnology and Bioengineering* 99: 1140-1153.

Ito, T., Y. Nakashimada, K. Senba, T. Matsui & N. Nishio, (2005) Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. *Journal of Bioscience and Bioengineering* 100: 260-265.

Jansen, N. B., M. C. Flickinger & G. T. Tsao, (1984) Production of 2,3-butanediol from D-xylose by *Klebsiella oxytoca* ATCC 8724. *Biotechnol Bioeng* 26: 362-369.

Ji, X. J., H. Huang, S. Li, J. Du & M. Lian, (2008) Enhanced 2,3-butanediol production by altering the mixed acid fermentation pathway in *Klebsiella oxytoca*. *Biotechnol Lett* 30: 731-734.

Ji, X. J., H. Huang, J. G. Zhu, L. J. Ren, Z. K. Nie, J. Du & S. Li, (2010) Engineering *Klebsiella oxytoca* for efficient 2, 3-butanediol production through insertional inactivation of acetaldehyde dehydrogenase gene. *Appl Microbiol Biotechnol* 85: 1751-1758.

Johnson, E. A. & E. C. Lin, (1987) *Klebsiella pneumoniae* 1,3-propanediol:NAD+ oxidoreductase. *J Bacteriol* 169: 2050-2054.

Jung, J. Y., E. S. Choi & M. K. Oh, (2008) Enhanced production of 1,2-propanediol by tpi1 deletion in *Saccharomyces cerevisiae*. *J Microbiol Biotechnol* 18: 1797-1802.

Juni, E., (1952) Mechanisms of Formation of Acetoin by Bacteria. *Journal of Biological Chemistry* 195: 715-726.

Kajiura, H., K. Mori, T. Tobimatsu & T. Toraya, (2001) Characterization and mechanism of action of a reactivating factor for adenosylcobalamin-dependent glycerol dehydratase. *Journal of Biological Chemistry* 276: 36514-36519.

Kalinowski, J., J. Cremer, B. Bachmann, L. Eggeling, H. Sahm & A. Puhler, (1991) Genetic and biochemical analysis of the aspartokinase from *Corynebacterium glutamicum*. *Mol Microbiol* 5: 1197-1204.

Kamzolova, S. V., A. R. Fatykhova, E. G. Dedyukhina, S. G. Anastassiadis, N. P. Golovchenko & I. G. Morgunov, (2011) Citric Acid Production by Yeast Grown on Glycerol-Containing Waste from Biodiesel Industry. *Food Technol Biotech* 49: 65-74.

Katsumata, R. & K. Kino, (1989) Process for producing amino acids by fermentation. Japan Patent 01317395 A (P2578488).

Khamduang, M., K. Packdibamrung, J. Chutmanop, Y. Chisti & P. Srinophakun, (2009) Production of L-phenylalanine from glycerol by a recombinant *Escherichia coli*. *J Ind Microbiol Biotechnol* 36: 1267-1274.

Knietsch, A., S. Bowien, G. Whited, G. Gottschalk & R. Daniel, (2003) Identification and characterization of coenzyme B12-dependent glycerol dehydratase- and diol dehydratase-encoding genes from metagenomic DNA libraries derived from enrichment cultures. *Appl Environ Microbiol* 69: 3048-3060.

Kretschmann, J., F.-J. Carduck, W.-D. Deckwer, C. Tag & B. H, (1993) Fermentive production of 1,3-propanediol. U.S. Patent No. US 5254467.

Lages, F. & C. Lucas, (1995) Characterization of a Glycerol H^+ Symport in the Halotolerant Yeast *Pichia sorbitophila*. *Yeast* 11: 111-119.

Lages, F. & C. Lucas, (1997) Contribution to the physiological characterization of glycerol active uptake in *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 1322: 8-18.

Lang, S., W. Beil, H. Tokuda, C. Wicke & V. Lurtz, (2004) Improved production of bioactive glucosylmannosyl-glycerolipid by sponge-associated *Microbacterium* species. *Mar Biotechnol (NY)* 6: 152-156.

Lapujade, P., J. L. Goergen & J. M. Engasser, (1999) Glutamate excretion as a major kinetic bottleneck for the thermally triggered production of glutamic acid by *Corynebacterium glutamicum*. *Metab Eng* 1: 255-261.

Larsson, K., R. Ansell, P. Eriksson & L. Adler, (1993) A gene encoding sn-glycerol 3-phosphate dehydrogenase (NAD^+) complements an osmosensitive mutant of *Saccharomyces cerevisiae*. *Mol Microbiol* 10: 1101-1111.

Lee, P. C., W. G. Lee, S. Y. Lee & H. N. Chang, (2001) Succinic acid production with reduced by-product formation in the fermentation of *Anaerobiospirillum succiniciproducens* using glycerol as a carbon source. *Biotechnol Bioeng* 72: 41-48.

Lee, S. Y., S. H. Hong, S. H. Lee & S. J. Park, (2004) Fermentative production of chemicals that can be used for polymer synthesis. *Macromol Biosci* 4: 157-164.

Lee, W. & N. A. Dasilva, (2006) Application of sequential integration for metabolic engineering of 1,2-propanediol production in yeast. *Metab Eng* 8: 58-65.

Levy, S. B., (1992) Dihydroxyacetone-Containing Sunless or Self-Tanning Lotions. *J Am Acad Dermatol* 27: 989-993.

Li, M., J. Wu, J. Lin & D. Wei, (2010a) Expression of Vitreoscilla hemoglobin enhances cell growth and dihydroxyacetone production in *Gluconobacter oxydans*. *Curr Microbiol* 61: 370-375.

Li, M. H., J. Wu, X. Liu, J. P. Lin, D. Z. Wei & H. Chen, (2010b) Enhanced production of dihydroxyacetone from glycerol by overexpression of glycerol dehydrogenase in an alcohol dehydrogenase-deficient mutant of *Gluconobacter oxydans*. *Bioresour Technol* 101: 8294-8299.

Liao, J., (1996) Microorganisms and methods for overproduction of DAHP by cloned *pps* gene. World Patent No. WO 9608567.

Licht, F. O., (2010) World Ethanol and Biofuels Report. 8.

Lin, H., G. N. Bennett & K. Y. San, (2005) Fed-batch culture of a metabolically engineered *Escherichia coli* strain designed for high-level succinate production and yield under aerobic conditions. *Biotechnology and Bioengineering* 90: 775-779.

Lindner, S. N., H. Niederholtmeyer, K. Schmitz, S. M. Schoberth & V. F. Wendisch, (2010) Polyphosphate/ATP-dependent NAD kinase of *Corynebacterium glutamicum*: biochemical properties and impact of *ppnK* overexpression on lysine production. *Appl Microbiol Biotechnol* 87: 583-593.

Lindner, S. N., G. M. Seibold, A. Henrich, R. Kramer & V. F. Wendisch, (2011) Phosphotransferase System-Independent Glucose Utilization in *Corynebacterium glutamicum* by Inositol Permeases and Glucokinases. *Appl Environ Microbiol* 77: 3571-3581.

Lindner SN, Seibold GM, Kramer R, Wendisch VF (2011) Impact of a new glucose utilization pathway in amino acid-producing Corynebacterium glutamicum. Bio engineered Bugs 2 (5)

Liu, H., Y. Xu, Z. Zheng & D. Liu, (2010) 1,3-Propanediol and its copolymers: research, development and industrialization. *Biotechnol J* 5: 1137-1148.

Liu, Y., C. M. Koh & L. Ji, (2011) Bioconversion of crude glycerol to glycolipids in *Ustilago maydis*. *Bioresour Technol* 102: 3927-3933.

Lu, J. L. & J. C. Liao, (1997) Metabolic engineering and control analysis for production of aromatics: Role of transaldolase. *Biotechnol Bioeng* 53: 132-138.

Lucas, C., M. Dacosta & N. Vanuden, (1990) Osmoregulatory Active Sodium-Glycerol Cotransport in the Halotolerant Yeast *Debaryomyces-Hansenii*. *Yeast* 6: 187-191.

Ma, C. Q., A. L. Wang, Y. Wang, T. Y. Jiang, L. X. Li & P. Xu, (2010a) Production of 2,3-butanediol from corncob molasses, a waste by-product in xylitol production. *Appl Microbiol Biot* 87: 965-970.

Ma, X. Y., L. Zhao, Y. Zheng & D. Z. Wei, (2009) Effects of over-expression of glycerol dehydrogenase and 1,3-propanediol oxidoreductase on bioconversion of glycerol into 1,3-propandediol by *Klebsiella pneumoniae* under micro-aerobic conditions. *Bioproc Biosyst Eng* 32: 313-320.

Ma, Z., Z. Rao, B. Zhuge, H. Fang, X. Liao & J. Zhuge, (2010b) Construction of a novel expression system in *Klebsiella pneumoniae* and its application for 1,3-propanediol production. *Appl Biochem Biotechnol* 162: 399-407.

Marx, A., A. A. de Graaf, W. Wiechert, L. Eggeling & H. Sahm, (1996) Determination of the fluxes in the central metabolism of *Corynebacterium glutamicum* by nuclear magnetic resonance spetroscopy combined with metabolite balancing. *Biotechnol Bioeng* 49: 111-129.

Marx, A., S. Hans, B. Mockel, B. Bathe & A. A. de Graaf, (2003) Metabolic phenotype of phosphoglucose isomerase mutants of *Corynebacterium glutamicum*. *J Biotechnol* 104: 185-197.

Mascarenhas, D., D. J. Ashworth & C. S. Chen, (1991) Deletion of *pgi* alters tryptophan biosynthesis in a genetically engineered strain of *Escherichia coli*. *Appl Environ Microbiol* 57: 2995-2999.

Matsushita, K., H. Toyama & O. Adachi, (1994) Respiratory chains and bioenergetics of acetic acid bacteria. *Adv Microb Physiol* 36: 247-301.

Menzel, K., A. P. Zeng & W. D. Deckwer, (1997) High concentration and productivity of 1,3-propanediol from continuous fermentation of glycerol by *Klebsiella pneumoniae*. *Enzyme Microb Tech* 20: 82-86.

Miller, J. E., K. C. Backman, M. J. Oconnor & R. T. Hatch, (1987) Production of Phenylalanine and Organic-Acids by Phosphoenolpyruvate Carboxylase-Deficient Mutants of *Escherichia coli*. *J Ind Microbiol* 2: 143-149.

Misra, K., A. B. Banerjee, S. Ray & M. Ray, (1996) Reduction of methylglyoxal in *Escherichia coli* K12 by an aldehyde reductase and alcohol dehydrogenase. *Mol Cell Biochem* 156: 117-124.

Moon, C., J. H. Ahn, S. W. Kim, B. I. Sang & Y. Um, (2010) Effect of biodiesel-derived raw glycerol on 1,3-propanediol production by different microorganisms. *Appl Biochem Biotechnol* 161: 502-510.

Morita, T., M. Konishi, T. Fukuoka, T. Imura & D. Kitamoto, (2007) Microbial conversion of glycerol into glycolipid biosurfactants, mannosylerythritol lipids, by a basidiomycete yeast, *Pseudozyma antarctica* JCM 10317(T). *J Biosci Bioeng* 104: 78-81.

Mu, Y., H. Teng, D. J. Zhang, W. Wang & Z. L. Xiu, (2006) Microbial production of 1,3-propanediol by *Klebsiella pneumoniae* using crude glycerol from biodiesel preparations. *Biotechnol Lett* 28: 1755-1759.

Mulligan, C. N., (2005) Environmental applications for biosurfactants. *Environ Pollut* 133: 183-198.

Murata, K., Y. Fukuda, K. Watanabe, T. Saikusa, M. Shimosaka & A. Kimura, (1985) Characterization of methylglyoxal synthase in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 131: 190-198.

Nabe, K., N. Izuo, S. Yamada & I. Chibata, (1979) Conversion of Glycerol to Dihydroxyacetone by Immobilized Whole Cells of *Acetobacter xylinum*. *Appl Environ Microbiol* 38: 1056-1060.

Nakamura, K., S. Kondo, Y. Kawai, N. Nakajima & A. Ohno, (1997) Amino acid sequence and characterization of aldo-keto reductase from bakers' yeast. *Biosci Biotechnol Biochem* 61: 375-377.

Nakas, J. P., M. Schaedle, C. M. Parkinson, C. E. Coonley & S. W. Tanenbaum, (1983) System development for linked-fermentation production of solvents from algal biomass. *Appl Environ Microbiol* 46: 1017-1023.

Nara, T., S. Kinoshita & H. Samejima, (1964) Effect of Penicillin on Amino Acid Fermentation. *Agr Biol Chem Tokyo* 28: 120-124.

Navarro-Avino, J. P., R. Prasad, V. J. Miralles, R. M. Benito & R. Serrano, (1999) A proposal for nomenclature of aldehyde dehydrogenases in *Saccharomyces cerevisiae* and characterization of the stress-inducible *ALD2* and *ALD3* genes. *Yeast* 15: 829-842.

Niimi, S., N. Suzuki, M. Inui & H. Yukawa, (2011) Metabolic engineering of 1,2-propanediol pathways in *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol* 90: 1721-1729.

Nilegaonkar, S. S., S. B. Bhosale, C. N. Dandage & A. H. Kapadi, (1996) Potential of *Bacillus licheniformis* for the production of 2,3-butanediol. *J Ferment Bioeng* 82: 408-410.

Norbeck, J. & A. Blomberg, (1997) Metabolic and regulatory changes associated with growth of *Saccharomyces cerevisiae* in 1.4 M NaCl. Evidence for osmotic induction of glycerol dissimilation via the dihydroxyacetone pathway. *J Biol Chem* 272: 5544-5554.

O'Gara, J. P. & L. K. Dunican, (1995) Mutations in the *trpD* gene of *Corynebacterium glutamicum* confer 5-methyltryptophan resistance by encoding a feedback-resistant anthranilate phosphoribosyltransferase. *Appl Environ Microbiol* 61: 4477-4479.

Ochoa-Estopier, A., J. Lesage, N. Gorret & S. E. Guillouet, (2011) Kinetic analysis of a *Saccharomyces cerevisiae* strain adapted for improved growth on glycerol: Implications for the development of yeast bioprocesses on glycerol. *Bioresour Technol* 102: 1521-1527.

Oh, B. R., J. W. Seo, S. Y. Heo, W. K. Hong, L. H. Luo, M. H. Joe, D. H. Park & C. H. Kim, (2011) Efficient production of ethanol from crude glycerol by a *Klebsiella pneumoniae* mutant strain. *Bioresour Technol* 102: 3918-3922.

Ohnishi, J., R. Katahira, S. Mitsuhashi, S. Kakita & M. Ikeda, (2005) A novel *gnd* mutation leading to increased L-lysine production in *Corynebacterium glutamicum*. *FEMS Microbiol Lett* 242: 265-274.

Okino, S., M. Inui & H. Yukawa, (2005) Production of organic acids by *Corynebacterium glutamicum* under oxygen deprivation. *Appl Microbiol Biotechnol* 68: 475-480.

Overkamp, K. M., B. M. Bakker, P. Kotter, M. A. Luttik, J. P. Van Dijken & J. T. Pronk, (2002) Metabolic engineering of glycerol production in *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 68: 2814-2821.

Pacwa-Plociniczak, M., G. A. Plaza, Z. Piotrowska-Seget & S. S. Cameotra, (2011) Environmental applications of biosurfactants: recent advances. *Int J Mol Sci* 12: 633-654.

Pagliaro, M., R. Ciriminna, H. Kimura, M. Rossi & C. Della Pina, (2007) From glycerol to value-added products. *Angew Chem Int Ed Engl* 46: 4434-4440.

Pahlman, A. K., K. Granath, R. Ansell, S. Hohmann & L. Adler, (2001) The yeast glycerol 3-phosphatases Gpp1p and Gpp2p are required for glycerol biosynthesis and differentially involved in the cellular responses to osmotic, anaerobic, and oxidative stress. *J Biol Chem* 276: 3555-3563.

Papanikolaou, S., M. Fick & G. Aggelis, (2004) The effect of raw glycerol concentration on the production of 1,3-propanediol by *Clostridium butyricum*. *J Chem Technol Biot* 79: 1189-1196.

Papanikolaou, S., L. Muniglia, I. Chevalot, G. Aggelis & I. Marc, (2002) *Yarrowia lipolytica* as a potential producer of citric acid from raw glycerol. *J Appl Microbiol* 92: 737-744.

Papanikolaou, S., P. Ruiz-Sanchez, B. Pariset, F. Blanchard & M. Fick, (2000) High production of 1,3-propanediol from industrial glycerol by a newly isolated *Clostridium butyricum* strain. *J Biotechnol* 77: 191-208.

Pasteris, S. E. & A. M. Strasser de Saad, (2008) Transport of glycerol by *Pediococcus pentosaceus* isolated from wine. *Food Microbiol* 25: 545-549.

Patnaik, R., R. G. Spitzer & J. C. Liao, (1995) Pathway engineering for production of aromatics in *Escherichia coli*: Confirmation of stoichiometric analysis by independent modulation of AroG, TktA, and Pps activities. *Biotechnol Bioeng* 46: 361-370.

Perego, P., A. Converti, A. Del Borghi & P. Canepa, (2000) 2,3-butanediol production by *Enterobacter aerogenes*: selection of the optimal conditions and application to food industry residues. *Bioprocess Eng* 23: 613-620.

Perego, P., A. Converti & M. Del Borghi, (2003) Effects of temperature, inoculum size and starch hydrolyzate concentration on butanediol production by *Bacillus licheniformis*. *Bioresour Technol* 89: 125-131.

Peters-Wendisch, P. G., C. Kreutzer, J. Kalinowski, M. Patek, H. Sahm & B. J. Eikmanns, (1998) Pyruvate carboxylase from *Corynebacterium glutamicum*: characterization, expression and inactivation of the *pyc* gene. *Microbiology* 144: 915-927.

Peters-Wendisch, P. G., B. Schiel, V. F. Wendisch, E. Katsoulidis, B. Mockel, H. Sahm & B. J. Eikmanns, (2001) Pyruvate carboxylase is a major bottleneck for glutamate and lysine production by *Corynebacterium glutamicum*. *J Mol Microbiol Biotechnol* 3: 295-300.

Petrov, K. & P. Petrova, (2009) High production of 2,3-butanediol from glycerol by *Klebsiella pneumoniae* G31. *Appl Microbiol Biotechnol* 84: 659-665.

Petrov, K. & P. Petrova, (2010) Enhanced production of 2,3-butanediol from glycerol by forced pH fluctuations. *Appl Microbiol Biotechnol* 87: 943-949.

Petrovska, B., E. Winkelhausen & S. Kuzmanova, (1999) Glycerol production by yeasts under osmotic and sulfite stress. *Can J Microbiol* 45: 695-699.

Pflugmacher, U. & G. Gottschalk, (1994) Development of an Immobilized Cell Reactor for the Production of 1,3-Propanediol by *Citrobacter-Freundii*. *Appl Microbiol Biot* 41: 313-316.

Prust, C., M. Hoffmeister, H. Liesegang, A. Wiezer, W. F. Fricke, A. Ehrenreich, G. Gottschalk & U. Deppenmeier, (2005) Complete genome sequence of the acetic acid bacterium *Gluconobacter oxydans*. *Nat Biotechnol* 23: 195-200.

Radmacher, E., K. C. Stansen, G. S. Besra, L. J. Alderwick, W. N. Maughan, G. Hollweg, H. Sahm, V. F. Wendisch & L. Eggeling, (2005) Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits L-glutamate efflux of *Corynebacterium glutamicum*. *Microbiology* 151: 1359-1368.

Rao, Z. M., Z. Ma, B. Zhuge, H. Y. Fang, X. R. Liao & J. Zhuge, (2010) Construction of a Novel Expression System in *Klebsiella pneumoniae* and its Application for 1,3-Propanediol Production. *Appl Biochem Biotech* 162: 399-407.

Riedel, C., D. Rittmann, P. Dangel, B. Mockel, S. Petersen, H. Sahm & B. J. Eikmanns, (2001) Characterization of the phosphoenolpyruvate carboxykinase gene from *Corynebacterium glutamicum* and significance of the enzyme for growth and amino acid production. *J Mol Microbiol Biotechnol* 3: 573-583.

Rittmann, D., S. N. Lindner & V. F. Wendisch, (2008) Engineering of a glycerol utilization pathway for amino acid production by Corynebacterium glutamicum. *Appl Environ Microbiol* 74: 6216-6222.

Ruch, F. E., J. Lengeler & E. C. Lin, (1974) Regulation of glycerol catabolism in *Klebsiella aerogenes*. *J Bacteriol* 119: 50-56.

Rymowicz, W., A. R. Fatykhova, S. V. Kamzolova, A. Rywinska & I. G. Morgunov, (2010) Citric acid production from glycerol-containing waste of biodiesel industry by *Yarrowia lipolytica* in batch, repeated batch, and cell recycle regimes. *Appl Microbiol Biotechnol* 87: 971-979.

Rywinska, A., P. Juszczyk, M. Wojtatowicz & W. Rymowicz, (2011) Chemostat study of citric acid production from glycerol by *Yarrowia lipolytica*. *J Biotechnol* 152: 54-57.

Rywinska, A. & W. Rymowicz, (2010) High-yield production of citric acid by *Yarrowia lipolytica* on glycerol in repeated-batch bioreactors. *J Ind Microbiol Biotechnol* 37: 431-435.

Saha, B. C. & R. J. Bothast, (1999) Production of 2,3-butanediol by newly isolated *Enterobacter cloacae*. *Appl Microbiol Biotechnol* 52: 321-326.

Saintamans, S., P. Perlot, G. Goma & P. Soucaille, (1994) High Production of 1,3-Propanediol from Glycerol by *Clostridium butyricum* Vpi-3266 in a Simply Controlled Fed-Batch System. *Biotechnol Lett* 16: 831-836.

Samuelov, N. S., R. Lamed, S. Lowe & J. G. Zeikus, (1991) Influence of CO_2-HCO_3- Levels and Ph on Growth, Succinate Production, and Enzyme-Activities of *Anaerobiospirillum succiniciproducens*. *Appl Environ Microb* 57: 3013-3019.

Sarcabal, P., C. Croux & P. Soucaille, (2007) Method for preparing 1,3-propanediol by a recombinant micro-organism in the absence of coenzyme b12 or one of its precursors. U.S. Patent No. US 7267972

Sato, H., K. Orishimo, T. Shirai, T. Hirasawa, K. Nagahisa, H. Shimizu & M. Wachi, (2008) Distinct roles of two anaplerotic pathways in glutamate production induced by biotin limitation in *Corynebacterium glutamicum*. *J Biosci Bioeng* 106: 51-58.

Schneider, J. & V. F. Wendisch, (2010) Putrescine production by engineered *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol*. 88(4):859–868

Schneider J, Niermann K, Wendisch VF (2011) Production of the amino acids l-glutamate, l-lysine, l-ornithine and l-arginine from arabinose by recombinant Corynebacterium glutamicum. J Biotechnol 154 (2-3):191-198.

Schneider, Z. & J. Pawelkiewicz, (1966) The properties of glycerol dehydratase isolated from *Aerobacter aerogenes*, and the properties of the apoenzyme subunits. *Acta Biochim Pol* 13: 311-328.

Scholten, E., T. Renz & J. Thomas, (2009) Continuous cultivation approach for fermentative succinic acid production from crude glycerol by *Basfia succiniciproducens* DD1. *Biotechnol Lett* 31: 1947-1951.

Schutz, H. & F. Radler, (1984) Anaerobic Reduction of Glycerol to Propanediol-1.3 by *Lactobacillus brevis* and *Lactobacillus buchneri*. *Syst Appl Microbiol* 5: 169-178.

Seifert, C., S. Bowien, G. Gottschalk & R. Daniel, (2001) Identification and expression of the genes and purification and characterization of the gene products involved in reactivation of coenzyme B-12-dependent glycerol dehydratase of *Citrobacter freundii*. *European Journal of Biochemistry* 268: 2369-2378.

Selembo, P. A., J. M. Perez, W. A. Lloyd & B. E. Logan, (2009) Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures. *Biotechnol Bioeng* 104: 1098-1106.

Seo, J. W., M. Y. Seo, B. R. Oh, S. Y. Heo, J. O. Baek, D. Rairakhwada, L. H. Luo, W. K. Hong & C. H. Kim, (2010) Identification and utilization of a 1,3-propanediol oxidoreductase isoenzyme for production of 1,3-propanediol from glycerol in *Klebsiella pneumoniae*. *Appl Microbiol Biotechnol* 85: 659-666.

Seo, M. Y., J. W. Seo, S. Y. Heo, J. O. Baek, D. Rairakhwada, B. R. Oh, P. S. Seo, M. H. Choi & C. H. Kim, (2009) Elimination of by-product formation during production of 1,3-propanediol in *Klebsiella pneumoniae* by inactivation of glycerol oxidative pathway. *Appl Microbiol Biotechnol* 84: 527-534.

Shams Yazdani, S. & R. Gonzalez, (2008) Engineering *Escherichia coli* for the efficient conversion of glycerol to ethanol and co-products. *Metab Eng* 10: 340-351.

Shelley, S., (2007) A renewable route to propylene glycol. *Chem Eng Prog* 103: 6-9.

Shih, I. L., Y. T. Van & M. H. Shen, (2004) Biomedical applications of chemically and microbiologically synthesized poly(glutamic acid) and poly(lysine). *Mini Rev Med Chem* 4: 179-188.

Shiio, I., S. I. Otsuka & M. Takahashi, (1962) Effect of biotin on the bacterial formation of glutamic acid. I. Glutamate formation and cellular premeability of amino acids. *J Biochem* 51: 56-62.

Shima, S., H. Matsuoka, T. Iwamoto & H. Sakai, (1984) Antimicrobial Action of Epsilon-Poly-L-Lysine. *J Antibiot* 37: 1449-1455.

Shimizu, H. & T. Hirasawa, (2007) Production of Glutamate and Glutamate-Related Amino Acids: MolecularMechanism Analysis and Metabolic Engineering. *In Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering (Wendisch V.F., ed), Springer, Heidelberg, Germany*: DOI 10.1007/7171_2006_1064.

Silva, S. N., C. B. Farias, R. D. Rufino, J. M. Luna & L. A. Sarubbo, (2010) Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP0992. *Colloids Surf B Biointerfaces* 79: 174-183.

Singh, A., M. D. Lynch & R. T. Gill, (2009) Genes restoring redox balance in fermentation-deficient *E. coli* NZN111. *Metab Eng* 11: 347-354.

Skraly, F. A., B. L. Lytle & D. C. Cameron, (1998) Construction and characterization of a 1,3-propanediol operon. *Appl Environ Microbiol* 64: 98-105.

Soccol, C. R., L. P. S. Vandenberghe, C. Rodrigues & A. Pandey, (2006) New perspectives for citric acid production and application. *Food Technol Biotech* 44: 141-149.

Soucaille, P., (2008) Process for the biological production of 1, 3-propanediol from glycerol with high yield. World Patent No. WO08052595

Sprenger, G., (2007) Aromatic Amino Acids *In* Amino Acid Biosynthesis - Pathways, Regulation and Metabolic Engineering (Wendisch, VF., ed), Springer, Berlin, Germany, pp. 93-128.

Stansen, C., D. Uy, S. Delaunay, L. Eggeling, J. L. Goergen & V. F. Wendisch, (2005) Characterization of a *Corynebacterium glutamicum* lactate utilization operon induced during temperature-triggered glutamate production. *Appl Environ Microbiol* 71: 5920-5928.

Suzuki, T. & H. Onishi, (1968) Aerobic Dissimilation of L-Rhamnose and Production of L-Rhamnonic Acid and 1,2-Propanediol by Yeasts. *Agr Biol Chem Tokyo* 32: 888-&.

Svitel, J. & E. Sturdik, (1994) Product Yield and by-Product Formation in Glycerol Conversion to Dihydroxyacetone by *Gluconobacter oxydans*. *J Ferment Bioeng* 78: 351-355.

Takinami, K., H. Yoshii, H. Tsuri & H. Okada, (1965) Biochemical Effects of Fatty Acid and Its Derivatives on L-Glutamic Acid Fermentation .3. Biotin-Tween 60 Relationship in Accumulation of L-Glutamic Acid and Growth of *Brevibacterium lactofermentum*. *Agr Biol Chem Tokyo* 29: 351-&.

Tan, T. W., F. H. Wang, H. J. Qu, D. W. Zhang & P. F. Tian, (2007) Production of 1,3-propanediol from glycerol by recombinant *E. coli* using incompatible plasmids system. *Mol Biotechnol* 37: 112-119.

Tang, X., Y. Tan, H. Zhu, K. Zhao & W. Shen, (2009) Microbial conversion of glycerol to 1,3-propanediol by an engineered strain of *Escherichia coli*. *Appl Environ Microbiol* 75: 1628-1634.

Tkac, J., M. Navratil, E. Sturdik & P. Gemeiner, (2001) Monitoring of dihydroxyacetone production during oxidation of glycerol by immobilized *Gluconobacter oxydans* cells with an enzyme biosensor. *Enzyme Microb Tech* 28: 383-388.

Tobimatsu, T., H. Kajiura & T. Toraya, (2000) Specificities of reactivating factors for adenosylcobalamin-dependent diol dehydratase and glycerol dehydratase. *Archives of Microbiology* 174: 81-88.

Tobimatsu, T., H. Kajiura, M. Yunoki, M. Azuma & T. Toraya, (1999) Identification and expression of the genes encoding a reactivating factor for adenosylcobalamin-dependent glycerol dehydratase. *J Bacteriol* 181: 4110-4113.

Tong, I. T. & D. C. Cameron, (1992) Enhancement of 1,3-propanediol production by cofermentation in *Escherichia coli* expressing *Klebsiella pneumoniae dha* regulon genes. *Appl Biochem Biotechnol* 34-35: 149-159.

Tong, I. T., H. H. Liao & D. C. Cameron, (1991) 1,3-Propanediol production by *Escherichia coli* expressing genes from the *Klebsiella pneumoniae dha* regulon. *Appl Environ Microbiol* 57: 3541-3546.

Toraya, T., S. Honda, S. Kuno & S. Fukui, (1978) Coenzyme B12-dependent diol dehydratase: regulation of apoenzyme synthesis in *Klebsiella pneumoniae* (*Aerobacter aerogenes*) ATCC 8724. *J Bacteriol* 135: 726-729.

Tribe, D. E. & J. Pittard, (1979) Hyperproduction of tryptophan by *Escherichia coli*: genetic manipulation of the pathways leading to tryptophan formation. *Appl Environ Microbiol* 38: 181-190.

Trinh, C. T. & F. Srienc, (2009) Metabolic engineering of *Escherichia coli* for efficient conversion of glycerol to ethanol. *Appl Environ Microbiol* 75: 6696-6705.

Turner, K. W. & A. M. Roberton, (1979) Xylose, arabinose, and rhamnose fermentation by *Bacteroides ruminicola*. *Appl Environ Microbiol* 38: 7-12.

Um, Y., S. A. Jun, C. Moon, C. H. Kang, S. W. Kong & B. I. Sang, (2010) Microbial Fed-batch Production of 1,3-Propanediol Using Raw Glycerol with Suspended and Immobilized *Klebsiella pneumoniae*. *Appl Biochem Biotech* 161: 491-501.

Van der Werf, M. J., M. V. Guettler, M. K. Jain & J. G. Zeikus, (1997) Environmental and physiological factors affecting the succinate product ratio during carbohydrate fermentation by *Actinobacillus sp.* 130Z. *Arch Microbiol* 167: 332-342.

Wang, Z. X., J. Zhuge, H. Fang & B. A. Prior, (2001) Glycerol production by microbial fermentation: a review. *Biotechnol Adv* 19: 201-223.

Wang F, Qu H, Zhang D, Tian P, Tan T (2007) Production of 1,3-propanediol from glycerol by recombinant E. coli using incompatible plasmids system. Mol Biotechnol 37 (2):112-119.

Wei, D. Z., Y. Zheng, H. Y. Zhang, L. Zhao, L. J. Wei & X. Y. Ma, (2008) One-step production of 2,3-butanediol from starch by secretory over-expression of amylase in *Klebsiella pneumoniae*. *J Chem Technol Biot* 83: 1409-1412.

Wei, S., Q. Song & D. Wei, (2007) Repeated use of immobilized *Gluconobacter oxydans* cells for conversion of glycerol to dihydroxyacetone. *Prep Biochem Biotechnol* 37: 67-76.

Wendisch, V. F., (2007) Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering. In: Microbiology Monographs. A. Steinbüchel (ed). Berlin: Springer, pp.

Wendisch, V. F., M. Bott & B. J. Eikmanns, (2006) Metabolic engineering of *Escherichia coli* and *Corynebacterium glutamicum* for biotechnological production of organic acids and amino acids. *Curr Opin Microbiol* 9: 268-274.

Werkman, C. H. & G. F. Gillen, (1932) Bacteria Producing Trimethylene Glycol. *J Bacteriol* 23: 167-182.

Wethmar, M. & W. D. Deckwer, (1999) Semisynthetic culture medium for growth and dihydroxyacetone production by *Gluconobacter oxydans*. *Biotechnol Tech* 13: 283-287.

Weusthuis, R. A., I. Lamot, J. van der Oost & J. P. Sanders, (2011) Microbial production of bulk chemicals: development of anaerobic processes. *Trends Biotechnol* 29: 153-158.

Whinfield, J. & J. Dickinson, (1946) Improvements relating to the manufacture of highly polymeric substances. UK Patent No. GB 578079

Wicke, C., M. Huners, V. Wray, M. Nimtz, U. Bilitewski & S. Lang, (2000) Production and structure elucidation of glycoglycerolipids from a marine sponge-associated *Microbacterium* species. *J Nat Prod* 63: 621-626.

Wu, J. Y., K. L. Yeh, W. B. Lu, C. L. Lin & J. S. Chang, (2008) Rhamnolipid production with indigenous *Pseudomonas aeruginosa* EM1 isolated from oil-contaminated site. *Bioresour Technol* 99: 1157-1164.

Xiaohu Fan, R. B. a. Y. Z., (2010) Glycerol (Byproduct of Biodiesel Production) as a Source for Fuels and Chemicals – Mini Review. *The Open Fuels & Energy Science Journal* 3: 17-22.

Xiu, Z. L., Y. H. Wang & H. Teng, (2011) Effect of aeration strategy on the metabolic flux of *Klebsiella pneumoniae* producing 1,3-propanediol in continuous cultures at different glycerol concentrations. *J Ind Microbiol Biot* 38: 705-715.

Xu, Y. Z., N. N. Guo, Z. M. Zheng, X. J. Ou, H. J. Liu & D. H. Liu, (2009) Metabolism in 1,3-propanediol fed-batch fermentation by a D-lactate deficient mutant of *Klebsiella pneumoniae*. *Biotechnol Bioeng* 104: 965-972.

Yang, G., J. Tian & J. Li, (2007) Fermentation of 1,3-propanediol by a lactate deficient mutant of *Klebsiella oxytoca* under microaerobic conditions. *Appl Microbiol Biotechnol* 73: 1017-1024.

Yu, K. O., S. W. Kim & S. O. Han, (2010) Engineering of glycerol utilization pathway for ethanol production by *Saccharomyces cerevisiae*. *Bioresour Technol* 101: 4157-4161.

Zeikus, J. G., M. K. Jain & P. Elankovan, (1999) Biotechnology of succinic acid production and markets for derived industrial products. *Appl Microbiol Biot* 51: 545-552.

Zeikus, J. G., J. B. McKinlay & C. Vieille, (2007) Prospects for a bio-based succinate industry. *Appl Microbiol Biot* 76: 727-740.

Zhang, X., K. Jantama, J. C. Moore, L. R. Jarboe, K. T. Shanmugam & L. O. Ingram, (2009) Metabolic evolution of energy-conserving pathways for succinate production in *Escherichia coli*. *Proc Natl Acad Sci U S A* 106: 20180-20185.

Zhang, X., K. T. Shanmugam & L. O. Ingram, (2010) Fermentation of glycerol to succinate by metabolically engineered strains of *Escherichia coli*. *Appl Environ Microbiol* 76: 2397-2401.

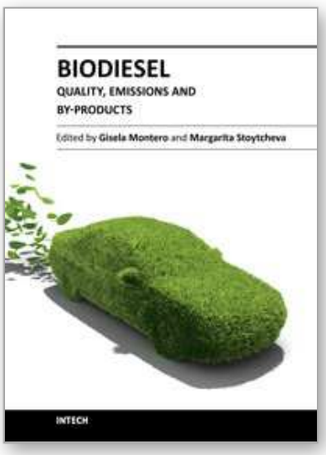
Zhang, Y., Y. Li, C. Du, M. Liu & Z. Cao, (2006) Inactivation of aldehyde dehydrogenase: a key factor for engineering 1,3-propanediol production by *Klebsiella pneumoniae*. *Metab Eng* 8: 578-586.

Zheng, Y., X. Chen & Y. Shen, (2008) Commodity chemicals derived from glycerol, an important biorefinery feedstock. *Chem Rev* 108: 5253-5277.

Zhu, M. M., P. D. Lawman & D. C. Cameron, (2002) Improving 1,3-propanediol production from glycerol in a metabolically engineered *Escherichia coli* by reducing accumulation of sn-glycerol-3-phosphate. *Biotechnol Prog* 18: 694-699.

Zhu, M. M., F. A. Skraly & D. C. Cameron, (2001) Accumulation of methylglyoxal in anaerobically grown *Escherichia coli* and its detoxification by expression of the *Pseudomonas putida* glyoxalase I gene. *Metab Eng* 3: 218-225.

Zhuge, B., C. Zhang, H. Y. Fang, J. A. Zhuge & K. Permaul, (2010) Expression of 1,3-propanediol oxidoreductase and its isoenzyme in *Klebsiella pneumoniae* for bioconversion of glycerol into 1,3-propanediol. *Appl Microbiol Biot* 87: 2177-2184.



Biodiesel- Quality, Emissions and By-Products

Edited by Dr. Gisela Montero

ISBN 978-953-307-784-0
Hard cover, 380 pages
Publisher InTech
Published online 16, November, 2011
Published in print edition November, 2011

This book entitled "Biodiesel: Quality, Emissions and By-products" covers topics related to biodiesel quality, performance of combustion engines that use biodiesel and the emissions they generate. New routes to determinate biodiesel properties are proposed and the process how the raw material source, impurities and production practices can affect the quality of the biodiesel is analyzed. In relation to the utilization of biofuel, the performance of combustion engines fuelled by biodiesel and biodiesels blends are evaluated. The applications of glycerol, a byproduct of the biodiesel production process as a feedstock for biotechnological processes, and a key compound of the biorefinery of the future is also emphasized.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Volker F. Wendisch, Steffen N. Lindner and Tobias M. Meiswinkel (2011). Use of Glycerol in Biotechnological Applications, Biodiesel- Quality, Emissions and By-Products, Dr. Gisela Montero (Ed.), ISBN: 978-953-307-784-0, InTech, Available from: http://www.intechopen.com/books/biodiesel-quality-emissions-and-by-products/use-of-glycerol-in-biotechnological-applications

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen