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Review

Biotechnological conversion of glycerol from biofuels to 1,3-propanediol using *Escherichia coli*

Hanna Przystałowska[1]✉, Daniel Lipiński[1,2] and Ryszard Słomski[1,2]

[1]Poznan University of Life Sciences, Department of Biochemistry and Biotechnology, Poznań, Poland; [2]Polish Academy of Sciences, Institute of Human Genetics, Poznań, Poland

In the face of shortage of fossil fuel supplies and climate warming triggered by excessive carbon dioxide emission, alternative resources for chemical industry have gained considerable attention. Renewable resources and their derivatives are of particular interest. Glycerol, which constitutes one of the by-products during biodiesel production, is such a substrate. Thus, generated excess glycerol may become an environmental problem, since it cannot be disposed of in the environment. The most promising products obtained from glycerol are polyols, including 1,3-propanediol, an important substrate in the production of synthetic materials, e.g. polyurethanes, unsaturated polyesters, and epoxy resins. Glycerol can be used as a carbon and energy source for microbial growth in industrial microbiology to produce 1,3-propanediol. This paper is a review of metabolic pathways of native producers and *E. coli* with the acquired ability to produce the diol via genetic manipulations. Culture conditions during 1,3-PDO production and genetic modifications of *E. coli* used in order to increase efficiency of glycerol bioconversion are also described in this paper.

Key words: bioconversion, biodiesel, carbon source, *Escherichia coli*, glycerol, 1,3-propanediol

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PRODUCTION OF BIODIESEL AND WASTE GLYCEROL

Currently, the primary trend in the fuel and chemical sector is to produce biofuels and chemical compounds using renewable energy sources. Biodiesel is a biofuel produced from vegetable oils or animal fats used to power compression-ignition (Diesel) engines as an admixture or complete fuel. Biofuels are renewable energy sources, in contrast to fossil fuels such as crude oil, natural gas or coal. The increasing oil prices and the fact that it is a non-renewable energy source have resulted in the need to use alternative energy sources. Biodiesel is a biodegradable fuel, which considerably reduces emissions of pollutants and toxins to the atmosphere and the natural environment, being its advantage over regular diesel oil, which combustion generates considerable emissions of these substances (Xu *et al.*, 2003; Chen *et al.*, 2008). It is formed as a result of transesterification of triglycerides (TG), mainly methanol (González-Pajuelo *et al.*, 2004; Mu *et al.*, 2006). At present, the greatest practical importance as a raw material in its production is attributed to rapeseed oil (Poland) and soybean oil (Brazil). For many years the European Union has been the primary producer of biodiesel worldwide (Demirbas & Balat, 2006). In several countries of Western Europe (Austria, France, Germany) and in Scandinavia methyl esters of fatty acids have been used for a long time (Fig. 1).

Data for the years 2015, 2020 — the estimate by OECD-FAO; Source: *Organisation for Economic Co-operation and Development – Food and Agriculture Organization, Agricultural Outlook 2011–2020, OECD-FAO 2011.*

Apart from methyl esters in the production of biodiesel the glycerol fraction is also produced, which depending on production parameters and the used substrate varies in terms of pure glycerol contents (Papanikolaou *et al.*, 2008). The components found in the glycerol fraction, i.e. free fatty acids, methanol, catalyst, soaps and salts, make it useless for the pharmaceutical or cosmetics industries. The glycerol fraction accounts for 10% produces ester, which means that from 1 ton produced biodiesel we obtain 100 kg waste glycerol. Excessive amounts of this waste have led to a decrease in glycerol prices (Yazdani *et al.*, 2007; Zhang, 2011; Yang *et al.*, 2012). Waste glycerol from the production of biofuels has rapidly become a competitive raw material in relation to sugars in the production of valuable chemicals, including 1,3-propanediol, in the biotechnological sector (Dharmadi *et al.*, 2006). The use of waste glycerol, being a derivative of a renewable energy source (e.g. longer-chain fatty acids of plant origin), as a source of carbon and energy for biotechnological processes would reduce production costs of biodiesel and would contribute to an increased share of renewable energy in the production of the desirable and extensive applications of chemical compounds (Ragauskas *et al.*, 2006). In the past, glucose was mainly used in fermentation processes due to its lower price in relation to glycerol, while at present excess waste glycerol has contributed to greater profitability of processes with its application (Nakamura & Whited, 2003).

✉e-mail: hanna.przystalowska@wp.pl

Abbreviations: ACK, acetate kinase; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AOR, alcohol dehydrogenase; BDH, 2,3-butanediol dehydrogenase; DHA, dihydroxyacetone; DHAK, dihydroxyacetone kinase; DHAP, dihydroxyacetone phosphate; GAL3P, glyceraldehyde-3-phosphate; GDH, glycerol dehydrogenase; GDHt, glycerol dehydratase; G3P, glycerol-3-phosphate; HA, hydroxyacetone; 3-HPA, 3-hydroxypropionaldehyde; IPTG, isopropyl β-D-thiogalactopyranoside; LAL, lactaldehyde; LALDH, lactaldehyde dehydrogenase; LDH, lactate dehydrogenase; MGS, methylglyoxal synthase; PEP, phosphoenolpyruvate; PTA, phosphate acetyltransferase; 1,2-PDO, 1,2-propanediol; 1,3-PDO, 1,3-propanediol; 1,3-PDDH, 1,3-propanediol dehydrogenase; TCA, cycle of tricarboxylic acids

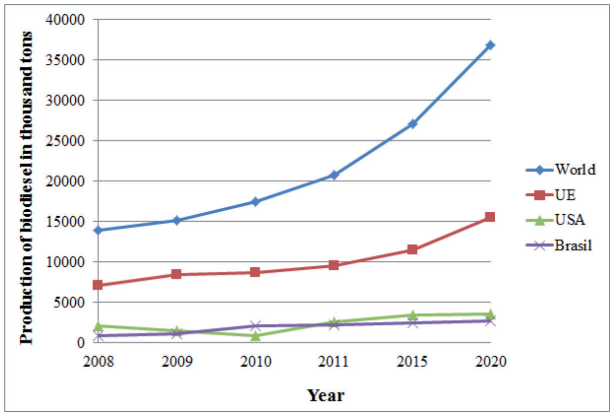

Figure 1. Production of biodiesel in selected countries in the years 2008–2020 (in thousand tons).

1,3-propanediol is produced mainly by chemical methods. However, it is possible to produce this compound using microorganisms in the fermentation processes of numerous carbon sources, e.g. glycerol. Pure glycerol does not pose a hazard and is extensively used in different branches of industry, while glycerol from the production of biofuels, containing numerous contaminants, is a noxious waste (Dharmadi *et al.*, 2006). Purification of waste glycerol is not profitable and its potential conversion to a valuable compound contributes to the solution of the problem of its bothersome management. Production of 1,3-PDO from waste glycerol is cheaper than the chemical method used to generate this compound (Rehman *et al.*, 2008). Results of studies concerning the effect of waste glycerol on bacterial growth and 1,3-PDO production show that there is no considerable difference in comparison to pure glycerol (Papanikolaou *et al.*, 2004). The potential to use non-purified waste glycerol from biofuel production to synthesize 1,3-PDO is a tremendous advantage of this process. This solution meets economic, ecological, social and ethical requirements imposed on contemporary biotechnology in the production of important molecules. There are "natural producers" of 1,3-PDO, as well as heterologous hosts of the 1,3-PDO synthesis pathway (Homann *et al.*, 1990; Gonzalez-Pajuelo *et al.*, 2006). Both the former and the latter are used in studies on the production of 1,3-PDO. Generation of increasingly efficient bacterial strains, optimization of glycerol conversion and 1,3-PDO purification will facilitate future advances in valuable technologies applying live organisms.

CHARACTERISTICS OF 1,3-PROPANEDIOL

1,3-propanediol (1,3-PDO), also referred to as trimethylene glycol, is an organic compound belonging to the group of diols, with the formula $C_3H_8O_2$ (Fig. 2), molar mass of 76.09 g/mol and density of 1.0597 g/cm^3. Under normal conditions it is a colorless liquid, readily soluble in water. 1,3-propanediol has many valuable properties, thanks to which for many years it has found many applications in different branches of industry (Igari *et al.*, 2000). 1,3-propanediol is mainly used as a monomer in the synthesis of biodegradable polyesters, polyurethanes and polyethers in the chemical industry, providing them with greater strength, while it is also contained in various products of the cosmetics, textile and medical industries. Countries of eastern and central

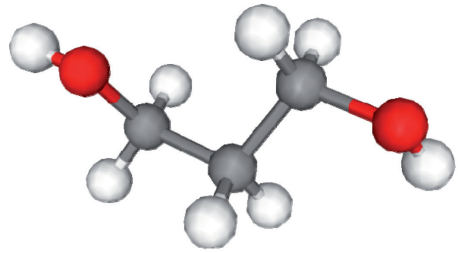

Figure 2. The structural model of a 1,3-propanediol molecule (Werle *et al.*, 2006).

Europe such as Poland, Turkey and Russia are those areas in this continent, which use and produce increasing amounts of polyurethanes (analysts in the team from IAL Consultants estimated that the present use in Europe, the Middle East and Africa is over 5 million ton annually). This compound provides specific properties to solvents, resins, laminates, detergents and cosmetics. 1,3-PDO is used to produce polymers applied in bullet-proof vests, and as a biocide (e.g. 2-nitro-1,3-PDO, a commercially available biocide protecting against contaminants) (Menzel *et al.*, 1997; Zeng & Biebl, 2002; Zhu *et al.*, 2002; Cheng *et al.*, 2007; Sauer *et al.*, 2008). It has been known for over 120 years that 1,3-propanediol is a product formed during microbial fermentation of glycerol. The molecule was identified for the first time in 1881 by a chemist, August Freund, as being a product of glycerol fermentation by *Clostridium pasteurianum* (Nakas *et al.*, 1983; Biebl *et al.*, 1999). In turn, in 1914 Voisenet described the production of 1,3-propanediol by *Bacillus ssp.* also in the fermentation process. In 1928 researchers from *Delft University of Technology* initiated studies on the production of 1,3-propanediol using *Enterobacteriaceae*, continued later by a team of Mickelson and Werkman, researchers from *Iowa State University of Science and Technology,* Ames, Iowa (USA). In the 1990's advances were made in biotechnological 1,3-PDO production from glucose and glycerol as well as chemical 1,3-PDO production methods (Katrlík *et al.*, 2007; Drożdżyńska *et al.*, 2011; Leja *et al.*, 2011). A breakthrough in the use of 1,3-PDO was connected with commercialization in 1995 by the British-Dutch petrochemical company Shell, of a new aromatic polyester Corterra® PTT poly(trimethylene terephthalate) based on 1,3-PDO (Shell Chemical Company Press release, 1995). This polymer is more durable, has greater tensile strength, is more elastic than polyester based on ethylene or butylene glycol, or 1,2-propanediol (1,2-PDO) (Chuah *et al.*, 1995; Kurian, 2005; Liu *et al.*, 2010). It is used as a fiber in the production of textiles and carpets (Chuah, 1996; Brown & Chuah, 1997). In 2000 one of the biggest multinational chemical companies, DuPont, the inventor of e.g. nylon and teflon, commercialized poly(trimethylene terephthalate) Sorona® based on 1,3-propanediol produced in the fermentation process (Chotani *et al.*, 2000; EMBO reports. White Biotechnology, 2003; Nakamura & Whited, 2003). It was one of the first advances on the world biotechnological market, applying a genetically modified *Escherichia coli* K-12 strain and a renewable energy source, i.e. corn glucose.

PRODUCTION OF 1,3-PDO BY CHEMICAL METHODS

In the 21st century the demand for 1,3-propanediol has increased drastically and annually 100 million kg

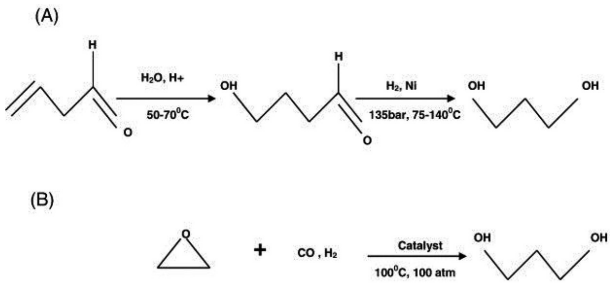

Figure 3. Chemical synthesis of 1,3-PDO : A) from acrolein, B) from ethylene oxide (Lawrence & Sullivan, 1972; Chuah *et al.*, 1995).

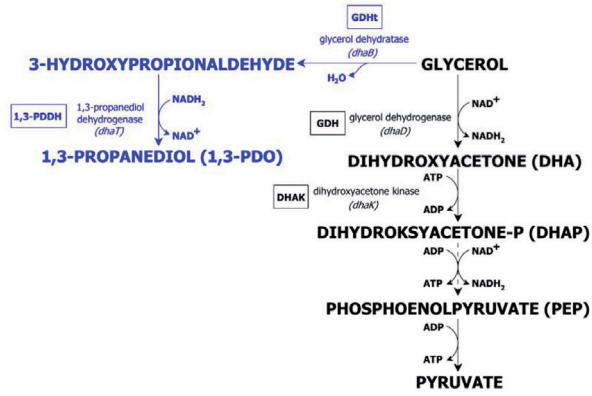

Figure 4. Catabolism of glycerol in natural 1,3-PDO producers. Enzymes of the *dha* regulon are marked in rectangles.
Pyruvate is reduced to different organic compounds depending on the microorganism and fermentation conditions. GDHt, glycerol dehydratase; 1,3-PDDH, 1,3-propanediol dehydrogenase; GDH, glycerol dehydrogenase; DHAK, dihydroxyacetone kinase. The blue colour marks the redox pathway, which incorporated in the *Escherichia coli* strain gives them capacity to produce 1,3-PDO. The figure was prepared based on (Biebl, 2001).

of this compound started to be produced worldwide (Németh *et al.*, 2003; Kraus, 2008; Tang *et al.*, 2009).

At present, there are two processes of chemical 1,3-PDO synthesis. DuPont uses acrylaldehyde to synthesize 1,3-PDO, while Shell uses ethylene oxide (Fig. 3). Acrylaldehyde is hydrated, then hydrogenated at high pressure in the presence of a catalyst, while in the case of ethylene oxide hydratation is preceded by hydroformylation (Cameron *et al.*, 1998; Hao *et al.*, 2006). Considering the fact that ethylene oxide is cheaper than acrylaldehyde, the chemical method used by Shell is economically more advantageous. However, obvious drawbacks of both these processes are related with the formed toxic intermediate products, the need to apply high pressure, high temperature, expensive catalysts, as well as moderate process efficiency and environmental noxiousness (Raynaud *et al.*, 2003; Lin *et al.*, 2005). For this reason starting from the 1990's attempts have been made to find biotechnological methods to produce 1,3-PDO from alternative energy sources (e.g. from waste glycerol generated by the production of biofuels) (Xiu *et al.*, 2007; Yang *et al.*, 2007). Already in the 20th century the primary method to obtain specialist chemical compounds, fuels, raw materials for heavy organic synthesis or plastics was a chemical method, in which crude oil or its derivatives were processed (rectified). Since it constitutes a non-renewable energy source and additionally its processing is connected with generation of environmental pollutants, chemical companies started to search for eco-friendly solutions applying e.g. glucose or glycerol as the energy sources. Obviously, it is promoted by the advances made in genetics, biotechnology and metabolic engineering of microorganisms (Ragauskas *et al.*, 2006). According to Festel Capital, the global share of biotechnological processes in the production of chemicals being substrates for the synthesis of polymers in 2015 will amount to 15% (www.festel.com), which in comparison to 2004 (3%) indicates the increasing importance of biotechnology of microorganisms and fermentation processes in the global industry, thus clearly indicating the direction for the world's development.

PRODUCTION OF 1,3-PDO FROM GLYCEROL USING BIOLOGICAL METHODS

In the last two decades progress has been made in biological methods of 1,3-PDO production from cheap renewable energy sources using newly developed strains in 1,3-PDO production (Deckwer, 1995; Zhu *et al.*, 2002). Glycerol, analogous to glucose, is the source of carbon (production of biomass) and energy for microorganisms. Because of the greater degree of glycerol reduction in relation to glucose, its bioconversion to valuable chemical compounds is more efficient than bioconversion of a glucose (Dharmadi *et al.*, 2006). 1,3-PDO is a product characteristic of glycerol fermentation only and is not obtained from conversion of any other organic compounds (Lin, 1976). The greater the availability of reduced coenzymes for reduction, the greater is the production of 1,3-PDO, which is not metabolized, but instead, it is accumulated in the culture medium (Johnson & Lin, 1987). The physiological role of 1,3-PDO synthesis in glycerol metabolism consists in the regeneration of a coenzyme NAD^+ from the reduced form produced during the oxidative pathway, in which glycerol is first oxidized to dihydroxyacetone (DHA), followed by the phosphorylation and the resulting production of dihydroxyacetone phosphate (DHAP), which is subsequently transformed to pyruvate (Zhu *et al.*, 2002; Forage & Lin, 1982) (Fig. 4). Commercialization of 1,3-PDO-based polymer produced by the biotechnological method by DuPont shows that there is a considerable demand for such product. This concept is realized by the research teams worldwide and subjected to competition in terms of scientific reports. Synthesis of 1,3-propanediol makes it possible for cells to reach the redox equilibrium using reduced coenzymes, formed during biomass production (Gonzalez *et al.*, 2008).

Natural 1,3-PDO producers

Natural 1,3-PDO production has been reported in some bacteria only (Biebl *et al.*, 1999). Numerous microorganisms may convert glucose to glycerol, while others are equipped with the glycerol conversion pathway to 1,3-PDO, but none are capable of converting glucose directly to 1,3-PDO (Cameron *et al.*, 1998). Natural producers of 1,3-PDO include bacteria from the genus *Klebsiella* (Huang *et al.*, 2002; Németh *et al.*, 2003), *Citrobacter* (Boenigk *et al.*, 1993; Seifert *et al.*, 2001), *Clostridia* (Colin *et al.*, 2001; Malaoui & Marczak, 2001), *Enterobacter* (Zhu *et al.*, 2002) and *Lactobacilli* (Schutz & Radler, 1984). Most frequently described species in the literature on the subject include e.g. *Citrobacter freundii* (Malinowski, 1999), *Klebsiella pneumoniae* (Biebl *et al.*, 1998), *Klebsiella oxytoca* (Homann *et al.*, 1990; Yang *et al.*, 2007), *Clostridium pasteurianum* (Nakas *et al.*, 1983; Biebl, 2001), *Clostridium butyricum* (Papanikolaou *et al.*, 2004), *Lactoba-*

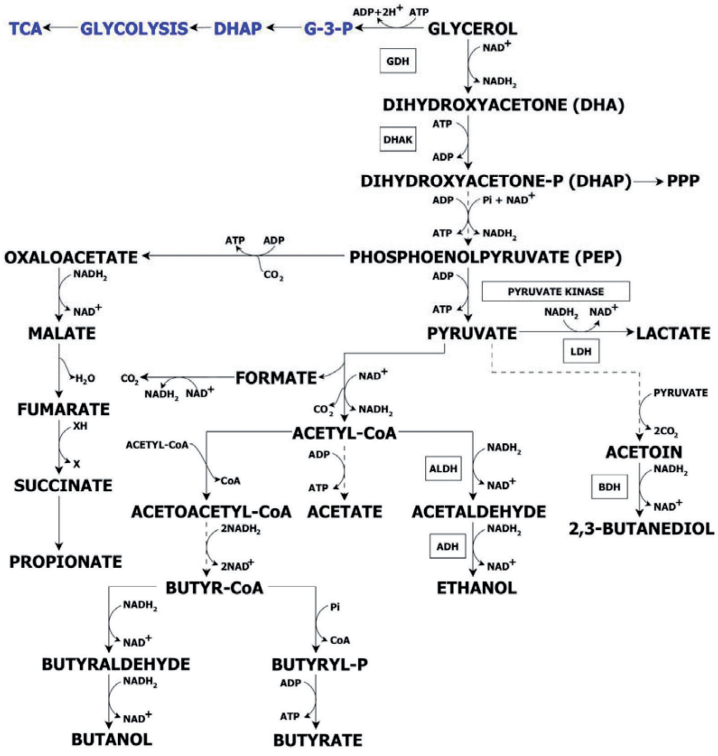

Figure 5. Catabolism of glycerol — the oxidative pathway and the situation of pyruvate reduced to different metabolites.
The type and concentrations of formed metabolites vary depending on the microorganism and fermentation conditions. The pathway occurring under aerobic conditions is marked in blue. GDH, glycerol dehydrogenase; DHAK, dihydroxyacetone kinase; LDH, lactate dehydrogenase; ALDH, aldehyde dehydrogenase; ADH, alcohol dehydrogenase; BDH, 2,3-butanediol dehydrogenase; PPP, pentosophosphate pathway; G-3-P, glycerol-3-phosphate; DHAP, dihydroxyacetone phosphate; TCA, cycle of tricarboxylic acids. The figure was prepared based on (Da Silva *et al.*, 2009).

cillus brevis and *Lactobacillus buchnerii* (Schutz & Radler, 1984), that have in their glycerol metabolizing pathway a branch, in which 1,3-PDO is formed in the process of fermentation. *Citrobacter freundii* and *Klebsiella pneumoniae* are good 1,3-PDO producers, but they are classified as the microbial risk group 2. For this reason protection of the production process is connected with the additional costs. In turn, *C. butyricum* is an extensively used organism in 1,3-PDO production, but the absolute anaerobic conditions required by that microorganism prevent its large-scale application (Willke & Vorlop, 2008). Despite being a potential pathogen. *K. pneumoniae* is the most frequently used microorganism in 1,3-PDO production. Efficiency of 1,3-PDO production is dependent on the applied *K. pneumoniae* strain (Huang *et al.*, 2002). Production of 1,3-PDO from glycerol is run most typically under the anaerobic conditions, although some strains from the genera *Klebsiella*, *Citrobacter* or *Enterobacter* synthesize 1,3-PDO also in the microaerobic and aerobic fermentation (Biebl *et al.*, 1998). Most natural 1,3-PDO producers metabolize glycerol in two pathways: redox and oxidative (Zhu *et al.*, 2002). 1,3-PDO is formed in the redox pathway. In the first stage of the redox pathway glycerol is dehydrated as a result of the action of the B_{12} coenzyme dependent glycerol dehydratase (GDHt), at the formation of 3-hydroxypropionaldehyde (3-HPA) (Figs. 4, 5) (Knietsch *et al.*, 2003). Next, the NADH+ H^+-dependent 1,3-PDO dehydrogenase (1,3-PD DH, 1,3-PDO PDOR-oxidoreductase) reduces 3-HPA to 1,3-PDO, regenerating NAD^+ (Ahrens *et al.*, 1998; Skraly *et al.*, 1998). In the oxidative pathway NAD^+-dependent glycerol dehydrogenase (GDH) catalyzes reactions of glycerol oxidation to dihydroxyacetone (DHA), which is then phosphorylated in the presence of the glycolytic enzyme ATP-dependent dihydroxyacetone kinase (DHAK) (Forage & Lin, 1982; Daniel *et al.*, 1995). Reduced co-enzymes formed in the oxidative pathway are required for the redox pathways, in which 1,3-PDO is synthesized together with other products of the glycerol metabolism. DHAP is further transformed in the process of glycolysis and the pentose phosphate pathway. During fermentation of glycerol from DHAP pyruvate is formed, which in *Enterobacteriaceae* is metabolized to acetyl coenzyme A or acetyl lactate (Biebl *et al.*, 1999). The primary end products formed during glycerol fermentation in *Enterobacteriaceae* include 1,3-PDO and acetate, although several other organic compounds such as formate, succinate, butanol, ethanol or 2,3-butanediol are also formed. Concentrations of end products to a considerable degree depend on the culture conditions and the applied bacterial strain (Homann *et al.*, 1990; Barbirato *et al.*, 1998). Catabolism of glycerol in natural 1,3-PDO producers is presented in Figs. 4 and 5. At the application of natural producers the efficiency of 1,3-PDO synthesis from glycerol ranges from approx. 0.51 to 0.64 ($mol_{1,3\text{-}PDO}/mol_{glycerol}$) depending on the concentration and quality of used glycerol (Zhang *et al.*, 2007).

Zhang *et al.* (2008) investigated the effect of changing conditions on values of metabolic fluxes within the main nodes in glycerol metabolism based on *K. pneumoniae*. The distribution of metabolic fluxes within glycerol and dihydroxyacetone phosphate proved to be of low sensitivity to changes in external conditions, while the researchers showed that changes in the activity of enzymes involved in the metabolism of pyruvate and acetyl-CoA may contribute to the increase in 1,3-PDO production.

In *K. pneumoniae* and *C. freundii* genes encoding functionally interacting enzymes: glycerol dehydratase (*dhaB*), 1,3-propanediol dehydrogenase (*dhaT*), glycerol dehydrogenase (*dhaD*) and dihydroxyacetone kinase (*dhaK*) are contained in the *dha* regulon (Forage & Lin, 1982; Zhu *et al.*, 2002) (Fig. 6). The *dha* regulon is induced by the presence of dihydroxyacetone as well as glycerol (Tong *et al.*, 1991). Glycerol dehydratase (GDHt), which in *K. pneumoniae* and *C. freundii* require for its activity the presence of the B_{12} co-enzyme, facilitated dehydratation of glycerol with the formation of 3-HPA (Sun *et al.*, 2003). In the presence of glycerol the B_{12} co-enzyme is irreversibly degraded, thus it is necessary to exchange the inactive vitamin B_{12} with its active form in the presence of ATP and Mg^{2+}, which requires the action of the two-subunit factor reactivating glycerol dehydratase (Honda *et al.*, 1980; Kajiura *et al.*, 2007).

The structure of the *dha* regulon varies slightly between natural producers (Fig. 6). In this context many teams of researchers cloned genes of the 1,3-PDO operon, sequenced and characterized it (Daniel *et al.*,

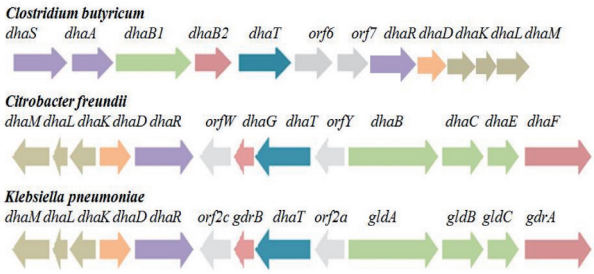

Figure 6. Genomic organisation of the *dha* regulon in *Clostridium butyricum, Citrobacter freundii* and *Klebsiella pneumoniae*. Arrows determine approximate length and direction of transcription for respective genes. Legend: *dhaK, dhaL, dhaM,* medium, small and large subunits of dihydroxyacetone kinase; *dhaD,*glycerol dehydrogenase; *dhaR,* transcription regulator; *orfW,* unknown product; *dhaG,* small subunit of the glycerol dehydratase reactivating factor; *dhaT*, 1,3-propanediol dehydrogenase (1,3-propanediol oxidoreductase); *orfY,* unknown product; *dhaB,* large subunit of glycerol dehydratase; *dhaC,* medium subunit of glycerol dehydratase; *dhaE,* small subunit of glycerol dehydratase; *dhaF,* large subunit of glycerol dehydratase reactivating factor; *orf2c,* unknown product; *orf2a,* unknown produkt; *gdrB,* small subunit of dehydratase glycerol reactivating factor; *gdrA,* large subunit of glycerol dehydratase reactivating factor; *gldA,* large subunit of glycerol dehydratase; *gldB,* medium subunit of glycerol dehydratase; *gldC,* small subunit of glycerol dehydratase; *dhaS* and *dhaA,* two-component signal transduction system — probably sensor protein and response regulator, respectively (role analogous to *dhaR*); *dhaB1,* B_{12}-independent glycerol dehydratase; *dhaB2,* protein activator; *orf6* and *orf7,* probably antioxidant proteins. The figure was prepared based on (Seifert *et al.,* 2001; Raynaud *et al.*, 2003; Kubiak *et al.*, 2012) and the NCBI database: GenBank U30903.1.

1995; Seyfried *et al.*, 1996; Skraly *et al.*, 1998; Seifert *et al.*, 2001). The gene encoding glycerol dehydratase (GDHt) was sequenced in certain strains from the family *Enterobacteriaceae*, i.e. *K. pneumoniae*, *C. freundii, K. oxytoca*, as well as strains from the family *Clostridiaceae* e.g. in *Clostridium butyricum*. In *Enterobacteriaceae* three subunits of this enzyme were identified. Nomenclature given in literature for ORFs includes *dhaB*, *dhaC*, *dhaE* in the case of *C. freundii*, occasionally *K. pneumoniae*; *gldA*, *gldB*, *gldC* or *dhaB1*, *dhaB2*, *dhaB3* for *K. pneumoniae*, as well as *pddA*, *pddB*, *pddC* for *K. oxytoca*, respectively. There is a significant homology between different strains of bacteria from the same genus as well as within a family (Daniel & Gottschalk, 1992; Tobimatsu *et al.*, 1995; Seyfried *et al.*, 1996; Seifert *et al.*, 2001; Xu *et al.*, 2009; GenBank U30903.1). In *C. butyricum* the *dhaB1* gene encodes glycerol dehydratase (Kubiak *et al.*, 2012). The *dhaT* gene was also characterized in different strains of *C. butyricum* (Malaoui & Marczak, 2000), *K. pneumoniae* (Zheng *et al.*, 2006), *C. freundii* (Daniel *et al.*, 1995), etc. It encodes an enzyme, 1,3-PDO oxidoreductase. The *dha* regulon comprises also genes encoding two subunits of the factor reactivating glycerol dehydratase. The available nomenclature include *dhaF* and *dhaG* for *C. freundii*, *gdrA* and *gdrB* or *dhaB4* and *orf2b* in the case of *K. pneumoniae*, *ddrA* and *ddrB* for *K. oxytoca* (Mori *et al.*, 1997; Seifert *et al.*, 2001; Wang *et al.*, 2007; GenBank U30903.1). In *C. butyricum* the reactivator of glycerol dehydratase is encoded by gene *dhaB2* (Kubiak *et al.*, 2012). The oxidative pathway of the *dha* regulon comprises the *dhaD* gene encoding glycerol dehydrogenase and genes *dhaK*, *dhaL*, *dhaM* encoding subunits of dihydroxyacetone kinase (Daniel *et al.*, 1995) (Fig. 6).

Examples of 1,3-PDO production by natural producers and modified natural producers

Mu and co-workers used a strain of *K. pneumoniae* DSM 2026 in the process of glycerol fermentation yielding 51.3–53 g/L of 1,3-PDO (Mu *et al.*, 2006). In turn, a research team headed by Yang *et al.* (2007) obtained over 80 g/L diol with an efficiency of 0.62 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ using a modified strain of *K. oxytoca* (the knockout gene of lactate dehydrogenase). A wild *K. pneumoniae* strain M5a1 used by a team headed by Cheng produced 58.8 g 1,3-PDO with a yield of 0.53 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ (Cheng *et al.*, 2007). Efficiency of 1,3-PDO production depends on the type of fermentation, used glycerol, applied strain, culture conditions and accumulation of metabolites. Example yields and productivity of bacterial strains depending on the type of fermentation towards 1,3-PDO are given in Table 1.

Synthesis of 1,3-PDO under microaerobic and aerobic conditions

It was commonly assumed that the synthesis of 1,3-PDO in the process of glycerol fermentation may only occur under anaerobic conditions. However, many research teams showed that it may be run under microaerobic as well as aerobic conditions (Huang *et al.*, 2002; Ma *et al.*, 2009). Researchers from China in their study presented 1,3-PDO production by *K. pneumoniae* under

Table 1. Several examples of 1,3-PDO production.

Fermentation method	Strain	1,3-PDO [g/L]	Yield [$mol_{1,3\text{-}PDO}/mol_{glycerol}$]	References
Batch	*C. butyricum* CNCM 1211	67.0	0.63	(Himmi *et al.*, 1999)
Fed-batch	*K. pneumoniae* AC 15	71.0	0.64	(Zheng *et al.*, 2008)
Continuous, two-step strategy	*C. freundii* DSM 30040	41.5	0.62	(Boenigk *et al.*, 1993)
Continuous, two-step strategy	*C. butyricum* F 2b	43.5	0.49	(Papanikolaou *et al.*, 2008)
Fed-batch, aerobic	*K. pneumoniae* XJPD-Li	65.2	0.56	(Ma *et al.*, 2009)

varying aerobic conditions, obtaining e.g. in fed-batch culture the yield of 0.56 $mol_{1,3\text{-PDO}}/mol_{glycerol}$ (Ma *et al.*, 2009). The team headed by Cheng presented results of anaerobic-aerobic strategy of 1,3-PDO production, receiving 70 g/L diol (Cheng *et al.*, 2004). A report by Hao *et al.* (2008) also showed potential production of 1,3-PDO in *Klebsiella* under aerobic conditions. Thanks to the above, it is possible to reduce production costs as well as minimize accumulation of a toxic metabolite, 3-hydroxypropionaldehyde (3-HPA). In another study Hao *et al.* (2008a) reported an efficient 1,3-PDO production by strains of *Citrobacter* and *Klebsiella* under aerobic conditions, with one strain of *K. pneumoniae* producing as much as 60.1 g/L 1,3-PDO from glycerol. Reports may also be found in literature on 1,3-PDO production by modified strains of *E. coli* in the presence of oxygen. Under aerobic conditions *E. coli* modified with genes from *Clostridium butyricum* produced 3.7 g/L 1,3-PDO with a yield of 0.3 $g_{1,3\text{-PDO}}/1\ g_{glycerol}$ (Dąbrowski *et al.*, 2012). A study by Wang *et al.* (2007) described production of 1,3-PDO (approx. 8.6 g/L) from glycerol by genetically modified *E. coli* under microaerobic conditions.

E. coli as a host of heterologous 1,3-PDO synthesis pathway

Organisms, which in their glycerol metabolism do not have the 1,3-PDO synthesis pathway, include e.g. *Escherichia coli*, *Saccharomyces cerevisiae* and *Clostridium acetobutylicum*. *Escherichia coli* is a Gram-negative, facultative anaerobic bacterium belonging to the family *Enterobacteriaceae*, owing their name to their discoverer, an Austrian pediatrician and bacteriologist Theodor Escherich. At present, among bacterial systems it is one of the most extensively applied systems for the production of heterologous proteins. *Escherichia coli* is a model organism in today's experiments in genetic and metabolic engineering. It constitutes the best known and the cheapest system to test new gene constructs and in attempts at production of proteins and enzymes in bench scale and commercial scale systems. Its application in commercial projects is undisputable. Obviously, the most significant advantages of bacteria *E. coli* include a high degree of genome characterization (defined transcription and translation systems), availability of a broad spectrum of vectors and strains, as well as capacity of rapid growth on cheap culture media (successive generations are produced very fast) using at the same time simple selection factors, mainly antibiotics. *Escherichia coli* is a non-pathogenic species, for which culture methods are already well-established. Human albumin, growth hormones as well as enzymes extensively used in molecular biology are successfully produced in *E. coli* cells. Potential applicability of the above mentioned properties of this host facilitates modification, use of genetic engineering methods, glycerol metabolism in *E. coli* cells leading to the acquisition of capacity to produce 1,3-PDO.

Glycerol metabolism in *E. coli*

In *E. coli* glycerol is transported to cells by facilitated diffusion, where the critical role is played by integral membrane protein of GlpF (Voegele *et al.*, 1993; Lu *et al.*, 2003). Only eight bacterial genera from the family *Enterobacteriaceae* (e.g. *Citrobacter* and *Klebsiella*) may ferment glycerol and at the same time produce 1,3-PDO, as they are equipped with glycerol dehydrogenase I and 1,3-PDO dehydrogenase (Bouvet *et al.*, 1995). The capacity to synthesize 1,3-PDO has not been identified in *E. coli* and glycerol metabolism itself was, as it had been assumed before, dependent on the availability of external electron acceptors (Bouvet *et al.*, 1994; Booth, 2005). Glycerol metabolism under aerobic conditions (or the presence of other electron acceptors, e.g. fumarate) occurs in the presence of glycerol transporter encoded by gene *glpF*, glycerol kinase (GK) and two glycerol-3-phosphate G-3-P dehydrogenases (G3PDHs) (Schryvers & Weiner, 1982; Borgnia & Agre, 2001; Walz *et al.*, 2002; Booth, 2005) (Fig. 7). In the presence of oxygen active dehydrogenase, a-G3PDH (a-aerobic), encoded by the *glpD* gene, is required for glycerol metabolism, while in the presence of fumarate an-G3PDH dehydrogenase (an-anaerobic), encoded by gene *glpABC*, catalyses conversion of glycerol-3-phosphate (G-3-P) to DHAP (Fig. 7). At a lack of oxygen or another electron acceptor (e.g. fumarate) the above mentioned dehydrogenases were not active, which contributed to the accumulation of G-3-P in amounts inhibiting bacterial growth, thanks to which it was stated that *E. coli* are not capable of glycerol fermentation (Booth, 2005). However, further studies concerning glycerol metabolism in *E. coli* conducted by Dharmadi *et al.* (2006) showed that *E. coli* may ferment glycerol when external electron acceptors are not available, and that it is capable of synthesizing 1,2-propanediol (1,2-PDO) (Gonzalez *et al.*, 2008). Earlier attempts at observing glycerol fermentation in *E. coli* under anaerobic conditions failed due to culture conditions and culture medium composition, which had a negative effect on glycerol fermentation (e.g. pH, concentrations of glycerol, phosphate, potassium, accumulation of H_2) at pH 7.5 (Gonzalez *et al.*, 2008). Strains devoid of genes encoding aerobic and anaerobic glycerol-3-phosphate dehydrogenase, respectively, were capable of fermenting glycerol, which indicated the existence of an alternative pathway metabolizing glycerol at the lack of electron acceptors (Murarka *et al.*, 2008).

The glycerol metabolism pathway, including biosynthesis of 1,2-propanediol from glycerol in *E. coli*, was described by Gonzalez *et al.* (2008). The glycerol metabolism pathway in *E. coli* was presented in Fig. 7. In order to identify key enzymes of glycerol metabolism in *E. coli*, the 1,2-propanediol synthesis pathway and external conditions significantly affecting this process, Gonzalez *et al.* (2008) and Murarka *et al.* (2008), applied several genetic and biochemical approaches. Gonzalez *et al.* (2008) has demonstrated the effect of the gene encoding glycerol dehydrogenase type II (GlyDH-II; GldA) in *E. coli* on glycerol metabolism under anaerobic conditions. Glycerol dehydrogenase type II transforms glycerol to dihydroxyacetone (DHA), as well as hydroxyacetone (HA) to 1,2-propanediol (1,2-PDO) (Gonzalez *et al.*, 2008). Next, DHA in *E. coli* undergoes phosphorylation catalyzed by PEP-dependent dihydroxyacetone kinase DHAK (Paulsen *et al.*, 2000) and it is not released to the culture medium. A study by Gonzalez describes a model of glycerol fermentation in *E. coli*, which includes synthesis of 1,2-PDO (utilization of reduced coenzymes) and synthesis of ethanol (generation of ATP in substrate phosphorylation). The presented model is composed of the oxidative pathway comprising genes encoding glycerol dehydrogenase type II and dihydroxyacetone kinase (DHAK) leading to dihydroxyacetone phosphate (DHAP) (an intermediate product of glycolysis) and the redox pathway using first of all the activity of methylglyoxal synthase (MGS), aldehyde reductase (AOR) and glycerol dehydrogenase type II, with DHAP as the substrate, leading to the synthesis of 1,2-propanediol (Fig. 7). The pathways leading to 1,2-PDO and ethanol, and other minor products obtained from the reduction of pyruvate and acetyl-CoA,

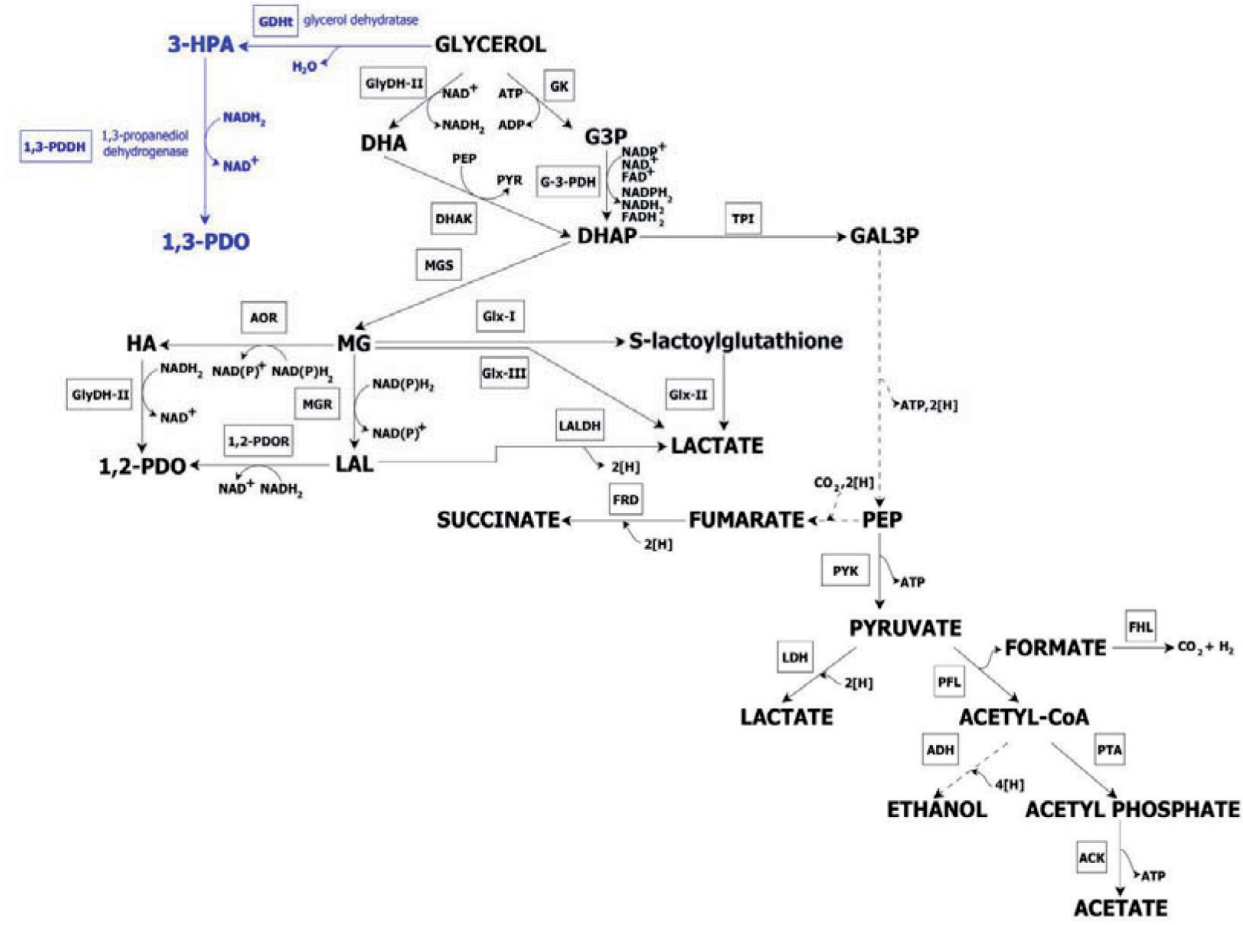

Figure 7. Glycerol metabolic pathway in *Escherichia coli*.
Pathway from glycerol to DHAP *via* G-3-P occurs in the presence of external electron acceptors. The heterologous pathway not found in *E. coli* is marked in blue. 3-HPA, hydroxypropionaldehyde; 1,3-PDO, 1,3-propanediol; DHA, dihydroxyacetone; G3P, glycerol-3-phosphate; DHAP, dihydroxyacetone phosphate; GAL3P, glyceraldehyde-3-phosphate; HA, hydroxyacetone; MG, methylglyoxal; 1,2-PDO, 1,2-propanediol; LAL, lactaldehyde; PEP, phosphoenolpyruvate; GlyDH-II, glycerol dehydrogenase; DHAK, dihydroxyacetone kinase; MGS, methylglyoxal synthase; AOR, aldehyde oxidoreductase; 1,2-PDOR, 1,2-propanediol reductase; MGR, methylglyoxal reductase; GK, glycerol kinase; G-3-PDH, glycerol-3-phosphate dehydrogenase; TPI, triosephosphate isomerase; Glx-I, glyoxalase type I; Glx-II, glyoxalase type II; Glx-III, glyoxylase type III; LALDH, lactaldehyde dehydrogenase; FRD, fumarate reductase; PYK, pyruvate kinase; LDH, lactate dehydrogenase; PFL, pyruvate formate lyase; FHL, formate hydrogen lyase; ADH, alcohol dehydrogenase; PTA, phosphate acetyltransferase; ACK, acetate kinase. The figure was prepared based on (Gonzalez *et al*., 2008; Clomburg & Gonzalez, 2010).

ensure the maintenance of redox equilibrium and ATP synthesis. The synthesis of 1,2-PDO and glycerol metabolism are significantly affected by pH of fermentation (Dharmadi *et al*., 2006; Murarka *et al*., 2008). Researchers also indicated the crucial role of formate:H_2 (FHL) lyase and F_oF_1-ATP synthase. In the process of glycerol fermentation in *E. coli* FHL catalyzes oxidation of formate to carbon dioxide and hydrogen, thanks to which it ensures CO_2 supply required for the cell growth, while it also participates in the establishment of proton motive force (PMF) (Hakobyan *et al*., 2005; Dharmadi *et al*., 2006; Gonzalez *et al*., 2008). ATP F_oF_1-synthase uses PMF produced by FHL in an acid medium, while in an alkaline environment PMF produced independently from the activity of FHL. The use of PMF by ATP F_oF_1-synthase is necessary for the generation of energy and metabolite transport, and thus — also key processes for glycerol fermentation in *E. coli* (Gonzalez *et al*., 2008). Apart from ethanol, other metabolites are also formed, such as succinate, lactate, formate and acetate (in greater amounts, when external electron acceptors are present) (Dharmadi *et al*., 2006).

Genetic modification in *E. coli*

Production of 1,3-PDO using a wild strain of *E. coli* both from glucose and glycerol is based on a completely heterologous pathway (Cameron *et al*., 1998). It needs to be mentioned here that numerous reports describe 1,3-PDO production from glucose using genetically modified bacteria *E. coli*. Chotani *et al.* (2000) modified *E. coli* with genes from *S. cerevisiae* and *K. pneumoniae* in order to produce glycerol and next 1,3-PDO. A team headed by Hartlep reported a two-stage 1,3-PDO production, in which a recombinant strain of *E. coli* produced glycerol from glucose and next, glycerol was transformed by *K. pneumoniae* to 1,3-PDO (Hartlep *et al*., 2002). Yeasts are the best producers of glycerol from glucose (Zhuge *et al*., 2001), thus frequently if the modified organism was capable of 1,3-PDO synthesis from glycerol and the aim was to optimize production starting with two sources of carbon (co-fermentation with the use of glycerol and glucose), genes of *S. cerevisiae* were incorporated. DuPont and Genencor patented the most efficient to date biotechnological 1,3-PDO production from glucose, modifying *E. coli* with genes coming from *S. cerevisiae* and *K.*

pneumoniae (Nakamura & Whited, 2003). They obtained 135 g/L 1,3-PDO with an efficiency of 0.6 $mol_{1,3\text{-}PDO}/mol_{glucose}$.

The literature on the subject contains results of several more or less efficient attempts at the production of 1,3-propanediol from glycerol by genetically modified *E. coli*. In a report by Tong and Cameron (1992) a strain *E. coli* AG1 was transformed with the *dha* regulon from *K. pneumoniae* and the yield of 1,3-PDO production in co-fermentation (glycerol + glucose) was 0.63 $mol_{1,3\text{-}PDO}/mol_{glycerol}$. The authors suggested that an addition of glucose which use constitutes the additional source of reducing co-factors, improves 1,3-PDO production. According to those authors, elimination of pathways leading to ethanol, succinate and lactate could also improve efficiency of 1,3-PDO synthesis. A research team headed by Skraly published results concerning 1,3-PDO production by *E. coli* containing in vector pSL301 controlled by promoter *trc* the *dhaB* and *dhaT* genes from *K. pneumoniae*. This strain during fed-batch fermentation, following induction of IPTG expression, produced 6.3 g/L 1,3-PDO from 9.3 g/L glycerol with a yield of 0.82 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ (Skraly *et al.*, 1998). A team headed by Zhu obtained a strain of *E. coli* capable of glycerol conversion to 1,3-propanediol through overexpression of the *dha* regulon from *K. pneumoniae* and *C. freundii*. However, efficiency was not spectacular because of the accumulation of toxic metabolites, e.g. glycerol-3 phosphate and methylglyoxal (MG) (Da Silva *et al.*, 2009). For this reason, it was attempted to upgrade 1,3-PDO production by limiting the accumulation of these compounds. In order to reduce MG concentration they transformed *E. coli* with a gene coding glyoxylase I coming from a strain of *Pseudomonas putida* and the 1,3-PDO regulon from *K. pneumoniae* induced with IPTG, which caused a considerable reduction of the inhibitory metabolite and 1,3-PDO production increased by 50% under anaerobic conditions (efficiency of 1,3-PDO synthesis increased from 0.33 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ to 0.52 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ (Zhu *et al.*, 2001). In another experiment recombinant bacteria *E. coli* containing the *dha* regulon from *K. pneumoniae* were used in 1,3-PDO production, adding fumarate as an exogenous electron acceptor, which increases anaerobic activity of G-3-P dehydrogenase, thus reducing the concentration of G-3-P (inhibitor of 1,3-PDO production) and improving cell growth and efficiency of 1,3-PDO synthesis (Zhu *et al.*, 2002). An addition of fumarate was also investigated in the case of *K. pneumoniae*, in which it had an advantageous effect on GDHt activity by an enhanced activation of the reactivating factor, which resulted from the increased ATP production in the presence of fumarate, and in turn improved 1,3-PDO production (Lin *et al.*, 2005). In *E. coli* an isoenzyme of 1,3-PD DH was identified, encoded by the *yqhD* gene (Emptage *et al.*, 2003). An important characteristic of *yqhD* is connected with lesser sensitivity to oxygen and capacity to use coenzymes NADH and NADPH, thanks to which it catalyzes more efficiently the transformation of 3-HPA to 1,3-PDO (Sulzenbacher *et al.*, 2004; Zhuge *et al.*, 2010). Zhang and co-workers (2005) (a team from China) modified *E. coli* JM109 with IPTG-induced plasmids pUCtac-dhaB and pEtac-yqhD, containing under the *tac* promoter glycerol dehydratase from *C. freundii* and alcohol dehydrogenase from *E. coli*. Under aerobic conditions the recombinant strain produced 38 g/L 1,3-PDO from 50 g/L glycerol. In turn, a strain *E. coli* JM109 containing in the pHsh vector (thermal induction) the *yqhD* gene (being an isoenzyme, 1,3-propanediol oxidoreductase, naturally found in *E. coli*) and the *dhaB* gene from *C. freundii* produced 41.1 g/L 1,3-PDO from 62 g/L glycerol with an efficiency of 0.81 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ (Zhang *et al.*, 2006a). In this report the authors showed that production may be considerably increased using a mathematical model defining optimal concentrations of nutrients in the culture medium. Wang *et al.* (2007) in the modification of *E. coli* used two plasmids, i.e. pET28a carrying glycerol dehydratase and 1,3-PDO dehydrogenase, and pET22 carrying a gene encoding factor reactivating glycerol dehydratase. A strain of *K. pneumoniae* was the donor of genes under the T7 promoter. The recombinant strain *E. coli* in fed-batch fermentation on glycerol with an addition of glucose produced 8.6 g/L 1,3-PDO from 14.3 g/L glycerol at the efficiency of 0.68 $mol_{1,3\text{-}PDO}/mol_{glycerol}$. Ma and co-workers described the generation of a recombinant strain *E. coli* JM 109 incorporating genes *dhaB* and *dhaT* from *K. pneumoniae* in pACYCDuet-1, each controlled by a separate T7 promoter. The microorganism under aerobic conditions produced 11.3 g/L 1,3-PDO from 40 g/L glycerol with an efficiency of 0.35 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ (Ma *et al.*, 2009a). A study by Tang *et al.* (2009) describes the highest to date 1,3-PDO production from glycerol. The authors transformed *E. coli* with the thermally induced pDY220 plasmid, carrying genes of glycerol dehydratase (independent from the B_{12} coenzyme) together with the reactivating cofactor from *C. butyricum* and 1,3-PDO oxidoreductase *yqhD* from *E. coli*. In the two-stage (aerobic/30ºC-anaerobic 42ºC) fed-batch fermentation they finally obtained 104.4 g/L 1,3-PDO at a yield of 90.2% g/g glycerol conversion to 1,3-PDO. In another publication genes of the *dha* regulon, i.e. *dhaB*, *dhaT*, *gdrA* and *gdrB* from *K. pneumoniae,* were incorporated in *E. coli* and *S. cerevisiae* W303-1A. Modified *E. coli* produced 8–9 g/L 1,3-PDO from 40 g/L glycerol (plus 30 g/L glucose) at the efficiency of 0.26 $mol_{1,3\text{-}PDO}/mol_{glycerol}$, while no such production was observed in W303-1A (Ma *et al.*, 2010). A report by Qi *et al.* (2011) presented another attempt at 1,3-PDO production by a strain *E. coli* JM109 modified with plasmid pSE380 carrying a gene encoding GDHt from *K. pneumoniae* and the *yqhD* gene from *E. coli* BL21, localized under the *trc* promoter. They showed that glycerol dehydratase exhibits higher activity at 45ºC and pH 9 than at 37ºC and pH 7. The obtained production amounted to 28 g/L 1,3-PDO. A team headed by Dąbrowski constructed a recombinant strain of *E. coli* transforming it with genes from the *dha* regulon (*dhaB1*, *dhaB2* i *dhaT*) coming from the *C. butyricum* strain 2CR371.5, which under aerobic conditions produced 3.7 g/L 1,3-PDO with an efficiency of 0.36 $mol_{1,3\text{-}PDO}/mol_{glycerol}$ (Dąbrowski *et al.*, 2012).

FACTORS LIMITING 1,3-PDO PRODUCTION

The primary causes for the low efficiency and productivity of glycerol fermentation to 1,3-PDO are connected with the conditions, under which fermentation is run, i.e. physiological temperature, atmospheric pressure as well as the type of fermentation, typically being batch fermentation (Zeng & Biebl, 2002). Increasing the density of bacterial cells as well as application of fed-batch fermentation or continuous fermentation with immobilized cells instead of batch fermentation constitute an important step in the optimization of the process (Saxena *et al.*, 2009). In *Enterobacteriaceae* we may observe accumulation of 3-hydroxypropionaldehyde, which is a toxic metabolite having an adverse effect on bacterial growth and 1,3-PDO production (Zeng *et al.*, 1993; Biebl *et al.*, 1998). Minor products formed during glycerol fermenta-

tion, such as ethanol, 2,3-butanediol (in *K. pneumoniae*), lactate or acetate, may also negatively influence the efficiency of 1,3-PDO biosynthesis (Zeng *et al*., 1994; Menzel *et al*., 1997). Targeted mutagenesis and different genetic engineering strategies offer a chance to overcome this problem (Zeng & Biebl, 2002). It would be an interesting strategy to shut the synthesis pathway for acetate, which is the most toxic metabolite for bacterial cells; however, it has not been attempted to date. Inactivation of the *ldhA* gene encoding lactate dehydrogenase (LDH) in *K. pneumoniae* improved efficiency of 1,3-PDO synthesis (Xu *et al*., 2009a), alike the inactivation of the *aldA* gene in *K. pneumoniae* YMU2 encoding aldehyde dehydrogenase (ALDH), catalyzing conversion of acetyl-CoA to ethanol, which resulted from greater availability of $NADH_2$ cofactors for 1,3-PDO oxidoreductase in *K. pneumoniae* (Zhang *et al*., 2006). Many research teams analyzed the effect of the substrate and products of glycerol metabolism on 1,3-PDO production. Xiu *et al.* (2004) showed that an advantageous effect on the efficiency of 1,3-PDO synthesis, productivity and final concentration may be provided by the two-stage structure of the bioprocess, using two bioreactors, with a greater dilution rate applied in the second bioreactor. Numerous bacteria developed the mechanism of tolerance to different high concentrations of the substrate or products, and a method to produce such strains is their culture at high concentrations of metabolites, where the most resistant remain and the others are eliminated by a natural selection (Saxena *et al*., 2009). Efficiency of 1,3-PDO synthesis is significantly affected by the availability of $NADH_2$ cofactors, formed in the oxidative pathway leading to minor products. 3-hydroxypropionaldehyde (3-HPA) formed in the redox pathway is a toxic metabolite, which limits 1,3-PDO production (Hao *et al*., 2008). Accumulation of this compound in the medium reduces GDHt activity, which results in a deteriorated catabolism of glycerol (Barbirato *et al*., 1996). Accumulation of 3-HPA is caused by an inappropriate ratio of GDHt activity to 1,3-PD DH, and it is suggested that increasing the activity of 1,3-PD DH would have a positive effect on 1,3-PDO production (Chen *et al*., 2009). High concentration of 1,3-PDO also has an inhibitory effect on bacterial cells, which results from the generally harmful effect of alcohols on the cell growth. The use of 1,3-PDO dehydrogenases, which also catalyze the reaction from 1,3-PDO to 3-HPA may lead to additional accumulation of 3-HPA, thus the potential application of oxidoreductases with lower activity from 1,3-PDO to 3-HPA, would reduce accumulation of the toxic metabolite (an example in this respect may be HOR oxidoreductase — *hypothetical oxidoreductase* from *K. pneumoniae*) (Wang *et al*., 2003).

PURIFICATION OF 1,3-PDO FROM SPENT MEDIUM

Spent medium contains numerous different components such as water, glycerol residue, acetate, succinate, lactate, proteins, polysaccharides, nucleic acids, etc., which hinders further utilization of 1,3-PDO, and at the same time is a challenge for further commercialization of the process. There are several methods of 1,3-PDO purification described to date in literature and applied in practice. These include reactive extraction, evaporation, distillation, membrane filtration or ion-exchange chromatography. However, each is burdened with certain drawbacks (Xiu & Zeng, 2008). An efficient purification process most frequently combines several of the above mentioned techniques. It is also possible to utilize organic products applying other strains (from methanogenesis) with no negative effect on the production of 1,3-propanediol (Szymanowska-Powałowska *et al*., 2013). It seems essential to develop a technology with a greater efficiency, with lower energy consumption and ensuring greater purity of the desired product (Xiu & Zeng, 2008).

CONCLUSIONS

Nowadays, there is a lager industrial need to exchange the chemical way of various chemicals synthesis for biotechnological — more ecological and sustainable manner. The 1,3-PDO production from glycerol is an desirable alternative to the traditional production from non-renewable resources. Despite few technical barriers and some economical challenges, *E. coli* are promising as a biomaterial used for 1,3-PDO synthesis from glycerol, which is widely exploited in this direction (imitating 1,3-PDO synthesis from glucose by *E. coli*, which has already had many patents). The selection of *E. coli* bacteria for the production of 1,3-PDO seems fully justified, since they are thoroughly studied model organisms, easy to culture, rapidly proliferating in cheap media, and non-pathogenic. For these reasons *E. coli* bacteria comprise one among the best heterologous system entirely sufficient for such use i.e. the production of valuable chemical compounds.

Currently used variants include also microorganisms naturally producing 1,3-PDO (despite their invasiveness), while the emphasis is placed on the use of recombinant microorganisms. In already optimized processes, in fed-batch or continuous cultures using natural producers, typically production level is 40–50 g/L at a yield of 0.6 $mol_{1,3\text{-}PDO}/mol_{glycerol}$, max. 60–70 g/L 1,3-PDO with a comparable yield (Saxena *et al*., 2009). In the case of recombinant *E. coli* maximum production of slightly over 100 g/L 1,3-PDO from glycerol was reported at a glycerol conversion rate of 90.2% (g/g) in a two-stage optimized culture (Tang *et al*., 2009). Thus, obtaining higher efficiency with the use of recombinant *E. coli* rather than natural producers is feasible and it is possible to use even antibiotic selection, as evidenced by the above mentioned report.

For efficient biosynthesis of 1,3-PDO by *E. coli* not only genetic manipulations, optimization of culture conditions and media composition need to be conducted, but also emphasis should be put on means of selection for gaining particular strains which will be more resistant for majority stress factors.

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