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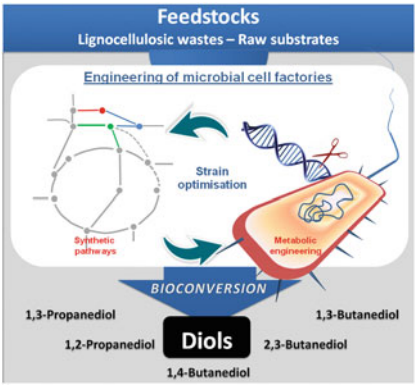
Microbial Cell Factories for Diol Production

W. Sabra, C. Groeger and An-Ping Zeng

Abstract Diols are compounds with two hydroxyl groups and have a wide range of appealing applications as chemicals and fuels. In particular, five low molecular diol compounds, namely 1,3-propanediol (1,3-PDO), 1,2-propanediol (1,2-PDO), 2,3-butanediol (2,3-BDO), 1,3-butanediol (1,3-BDO), and 1,4-butanediol (1,4-BDO), can be biotechnologically produced by direct microbial bioconversion of renewable materials. In this review, we summarize recent developments in the microbial production of diols, especially regarding the engineering of typical microbial strains as cell factory and the development of corresponding bioconversion processes.

Graphical Abstract



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Contents

1 Introduction

At the beginning of the twentieth century, before petroleum was introduced as raw material, the chemical industry had to rely on coal and renewable resources. Until 1930, the most important bulk products of that time, such as fuels (ethanol, butanol), organic acids (acetic acid, citric acid, lactic acid) and other basic chemicals, were biotechnologically produced from biomass. With the development of the petroleum industry, many of these biotechnological processes were replaced by chemical synthesis routes based on petroleum or natural gas. Nowadays, over 80 million tons of industrial chemicals are manufactured globally each year from fossil-based feedstocks [1]. These petrochemicals, which encompass building blocks, intermediate chemicals, and derived final products like polymers, are valued at over $2 trillion and provide the materials and products that impact and enable virtually every aspect of our daily existence [1]. However, these great benefits historically have come at great cost. While the chemicals themselves play a positive role in society, the petroleum-based processes used to manufacture chemicals engender challenges that can jeopardize the economy, the environment, and overall global security. Nowadays, the rapid advances in plant biotechnology, molecular biology, and new tools and concepts such as systems and synthetic biology, and biorefinery of renewable biomaterials have created new opportunities and markets for many biotechnologically produced (bio)chemicals. Many chemicals, which could only be produced by chemical processes in the past, could potentially be generated biologically from renewable resources. The microbial production of diols is a prominent example of success of the so-called white or industrial biotechnology

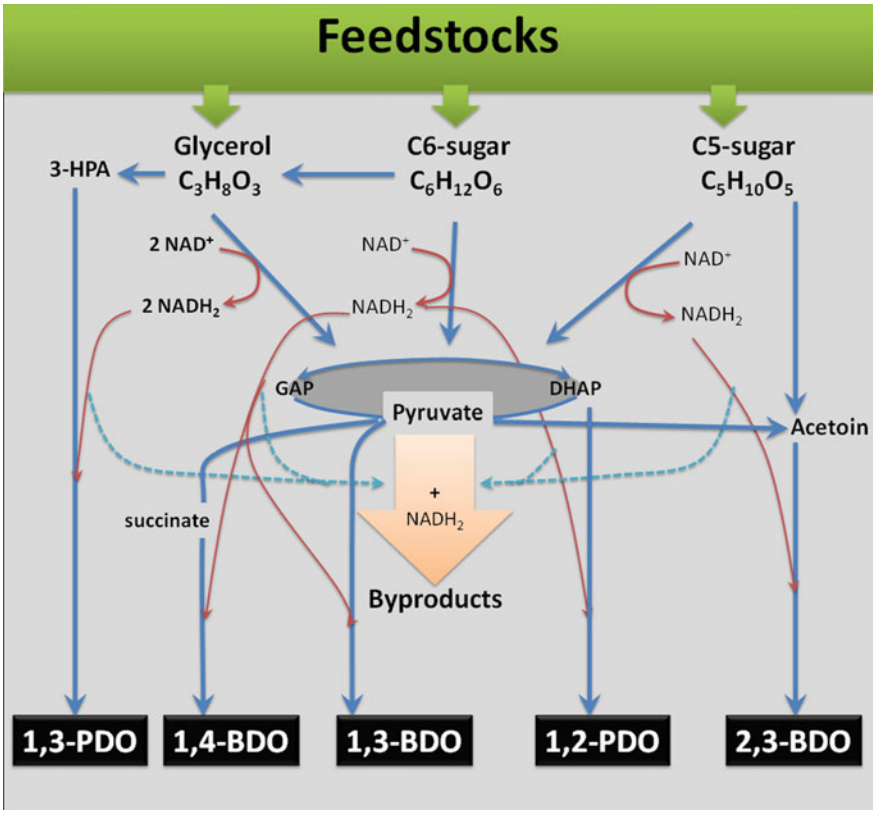

Fig. 1 Major routes for bioproduction of diols from different feedstocks (modified after [2]). *3-HPA* 3-hydroxypropionaldehyde, *GAP* glyceraldehyde 3-phosphate, *DHAP* dihydroxyacetone phosphate

in recent years. Diols are compounds with two hydroxyl groups which have a wide range of important applications as chemicals and fuels. They are considered as platform green chemicals for many industries. In particularly, the microbial production of 1,3-propanediol (1,3-PDO), 1,2-propanediol (1,2-PDO), 2,3-butanediol (2,3-BDO) and, more recently 1,4-butanediol (1,4-BDO) and 1,3-butanediol (1,3-BDO) has received much interest in industrial biotechnology. These diols can be produced from different renewable feedstocks and even waste materials from biofuel production (Fig. 1) [2].

The production of 1,3-PDO and 1,4-BDO has reached commercial scales. They are especially useful as biomonomers for the polyesters polypropylene terephthalate (PPT) and polybutylene terephthalate (PBT). Both PPT and PBT have the potential to steal market share from the classic polyester polyethylene terephthalate (PET) [3]. Pilot plant-scale production of 1,2-PDO and 2,3-BDO has also been reported. All these diols are of immense industrial interests because they are either

established chemicals presently produced from fossil resources in large production volumes (e.g. more than 1.5 Mio. t per year for 1,2-PDO and about 1.3 Mio. t per year for 1,4-BDO), or large market potentials [2]. Despite their large impact, relatively few publications are available for the biotechnological production of 1,2-PDO, 1,3-BDO, and 1,4-BDO. On the other hand, 1,3-PDO and 2,3-BDO have been most intensively studied in the last few years and several comprehensive reviews for the microbial production of these diols have been published [2, 4–7]. In this review article, microbial factories for the different diols and the pathways involved are illustrated. The current state of the art of strain improvement including synthetic pathways is also summarized.

2 Butanediol Production

Butanediol is a four-carbon diol having its hydroxy groups at various positions. 2,3-BDO is the only naturally occurring BDO, produced by several facultative and anaerobic bacteria. On the other hand, no natural metabolic pathways or micro-organisms are known which can produce 1,4-BDO or 1,3-BDO from sugar or other biomass. Although the market for synthetic 2,3-BDO is presently still very small, there is a shift towards the use of biobased 2,3-BDO. 1,4-BDO, the most widely used BDO compound, is currently produced from fossil fuel feedstocks. In the following, we summarize recent development in the microbial production of the different types of butanediol.

2.1 2,3-Butanediol

2,3-Butanediol (2,3-BDO) is one of the promising bulk chemicals which exhibits a wide range of potential applications [8–10]. It is used as the starting material for bulk chemicals such as methyl ethyl ketone, gamma-butyrolactone, and 1,3-butadiene [11]. Nowadays, the manufacture of 2,3-BDO is growing by an annual rate of 4–7 % due to the increased demand for many of its derivatives [12, 13]. 2,3-BDO is widely used in chemical, food, fuel, aeronautical, and other fields. Due to the presence of two chiral centres, 2,3-BDO has three isomers: *levo* (2*R,* 3*R*) and *dextro* (2*S,* 3*S*) forms with optical activity and the *meso*-form with no optical activity.

The optically active forms of 2,3-BDOs are very valuable chemicals in the directed asymmetric synthesis of chiral chemicals using boronic esters. Moreover, chiral compounds are especially important to provide chiral groups in drugs, in high-value pharmaceutical or for liquid crystals manufacture [14, 15]. The various applications of this polymer are summarized elsewhere [5, 16].

Although the first commercial production of 2,3-BDO was biotechnological one operated in Germany in the middle of the last century, currently the commercialized

process for its synthesis is based entirely on a chemical route. However, the synthetic (petroleum-based) 2,3-BDO does not have a very large market due to its unique structure and costly chemical synthesis. Also, there is no efficient method to convert the intermediate into downstream derivatives such as butadiene, methyl ethyl ketone, and butenes. Therefore, 2,3-BDO has not been produced on a large scale and is currently available as a laboratory chemical and is being sold as a small-volume intermediate for some niche applications like food flavouring. Moreover, its high price led also to inadequate development of its application [17]. Therefore, biobased 2,3-BDO is considered to be a highly attractive market and is expected to provide immense opportunities to the main players involved in the market.

2.1.1 Micro-organisms of Potential Significance for 2,3-BDO Production

Bacteria effectively producing 2,3-BDO belong mainly to the Enterobacteriaceae family. Their representative species are *Klebsiella pneumoniae*, *K. oxytoca*, *and E. aerogenes*. *Pseudomonas chlororaphis and Paenibacillus polymyxa* belonging to the families *Pseudomonadaceae* and *Paenibacillaceae*, respectively, have received attention due to the formation of a pure optically active stereoisomer (*L-form*) in plant rhizospheres. In general, the highest 2,3-BDO concentrations were obtained with pathogen (risk group 2) micro-organisms (Table 1) and thus not desirable for industrial-scale production. Interestingly, Jurchescu et al. [18] reported recently the production of 2,3-BDO by *Bacillus licheniformis* DSMZ 8785 grown on glucose in fed-batch cultivation. The maximum 2,3-BDO concentration obtained was 144.7 g/L, which was comparable to that achieved by the risk group 2 strains. Moreover, by using thermophilic *B. licheniformis* strains, high concentrations (103–115 g/L) of 2,3-BDO could be produced either from glucose [19, 20] or from plant polysaccharide inulin in a simultaneous saccharification and fermentation process [20]. Advantages of the thermophilic process include less contamination risk at high temperature and more efficient utilization of the plant substrate by simultaneous saccharification [19]. Indeed, species of *Bacillus* or *Paenibacillus* appear to be more suitable for commercial 2,3-BDO production. While a mixture of *levo* and *meso* (1:1 ratio) was formed by *B. licheniformis, P. polymyxa* has the ability to form almost exclusively the *levo*-isomer (over 98 %) when grown under anaerobic conditions [13, 21–25]. Recently, Fu et al. [26] showed that NADH played a vital role for chirally pure D-2,3-BDO production in *Bacillus subtilis* grown under limited oxygen conditions. Although the final concentrations in the 2,3-BDO fermentation are lower than those of *B. licheniformis,* the optical purity of the produced diol could be of interest for the fine chemical industry and specific synthesis. Under microaerobic conditions, the 2,3-BDO productivity of this bacterium is higher, but the optical purity decreases, since the *meso*-form is increasingly formed [23].

Table 1 Comparison of 2,3-BDO production by different species, substrates, and fermentation modes

Organism	2,3-BDO isomers	Substrate	Fermentation mode	Concentration (g/L)		Yield	Productivity (g/L h)	References
				2,3-BDO	Acetoin	(g/g)		
Risk group 2								
Klebsiella pneumoniae	*Meso*	Glucose	Fed batch	150	10	–	4.21	[8, 10]
		Glycerol	Fed batch	49.2		0.356	–	[9]
		Xylose + glucose	Fed batch	88	113	0.212	–	[21]
		Jerusalem artichoke powder	Fed-batch SSF	84	7.6	0.294	2.1	[21]
Enterobacter aerogenes	*Meso* *L*	Glucose	Fed batch with cell recycle	110		0.49	5.4	[27]
Enterobacter cloacae		Cassava powder	Fed batch	78		0.42	3.3	[28]
Klebsiella oxytoca	*Meso*	Glucose	Fed batch	95.5	1.9	0.478	1.71	[29]
	L	Xylose + glucose	Batch	23.2	2.5	0.387	0.52	[29]
Serratia marcescens	*Meso*	Sucrose	Batch	152	–	0.41	2.67	[30]
Risk group 1								
Bacillus polymyxa	*L*	Glucose	Fed batch	40.5	6	–	0.81	[21]
		Xylose	Shaking flask	7	–	0.248	–	
Bacillus licheniformis	*L, m*	Glucose Inulin	Fed-batch culture Fed batch	144 103	–	0.4	1.14 3.4	[18, 20]
		Corn stover	Fed batch	74			2.1	[31]
Paenibacillus polymyxa	*L*	Glucose	Fed-batch culture	50	–		0.8	[24]
	L	Glucose		20	–			[13]

(continued)

Table 1 (continued)

Organism	2,3-BDO isomers	Substrate	Fermentation mode	Concentration (g/L)		Yield	Productivity (g/L h)	References
				2,3-BDO	Acetoin	(g/g)		
Genetically modified micro-organisms								
E. coli *E. coli*	*D*	Glucose diacetyl	*Fed batch* *Fed batch*	74 31.7	–	0.4	1.2 2.3	[17, 32]
Saccharomyces cerevisiae		Glucose and galactose		100				[33]
Enterobacter cloacae	*L*	Glucose xylose	*Fed batch*	152			3.5	[11]
	L	Biomass hydrolysate	*Fed batch*	119.4			2.3	[11]

2.1.2 Metabolic Pathways of 2,3-BDO Biosynthesis

Several bacteria, yeasts, or even algae have the capability to produce 2,3-BDO, but the observed yields are often quite different [5, 34, 35]. The 2,3-BDO biosynthetic pathway has been intensively studied in bacteria (Fig. 2). 2,3-BDO synthesis is typically a part of a mixed-acid fermentation pathway observed under anaerobic or microaerobic growth conditions of different micro-organisms (Fig. 2). In addition to 2,3-BDO and depending on the micro-organism and cultivation conditions, other end products are formed, such as ethanol, acetate, lactate, formate, and succinate. In order to enhance the 2,3-BDO yield (theoretical maximum yield 0.5 g/g on glucose), most of the work done was concentrated on an efficient channelling of pyruvate to 2,3-BDO and not to the different by-products. The formation and selectivity of 2,3-BDO stereoisomers and in particular the control of their purity have not been completely understood. Consequently, various metabolic pathways have been proposed (Fig. 3).

Acetoin is the precursor of 2,3-BDO and is formed in bacteria from pyruvate through several enzymatic reactions. Under anaerobic conditions, α-acetolactate synthase catalyses the condensation of two pyruvate molecules with a single decarboxylation to form α-acetolactate that is converted to acetoin by α-acetolactate decarboxylase. Under low oxygen concentration, α-acetolactate can undergo a spontaneous decarboxylation, leading to the formation of diacetyl. Subsequently, a

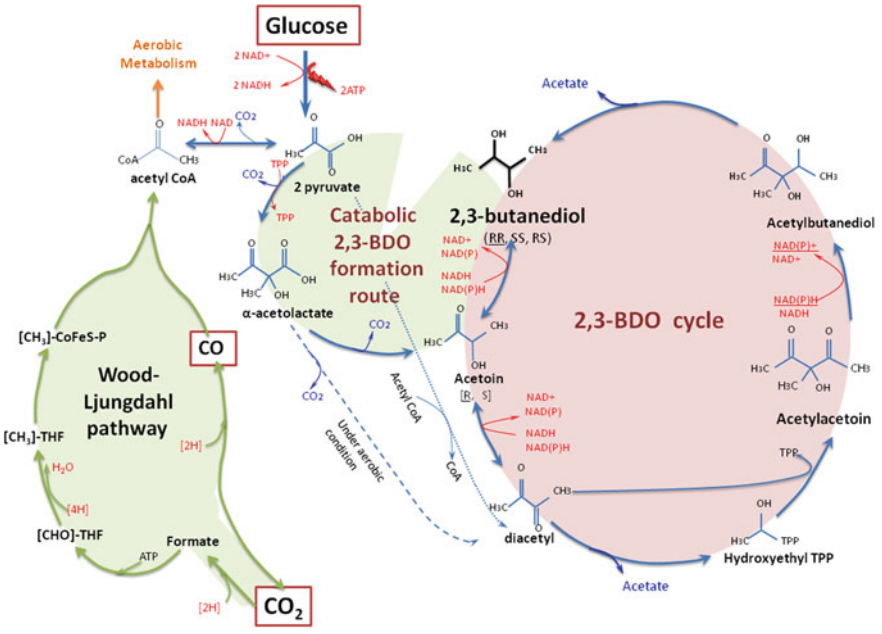

Fig. 2 Different pathways involved in the formation of 2,3-BDO in bacteria (after [5, 35]). *THF* tetrahydrofolate

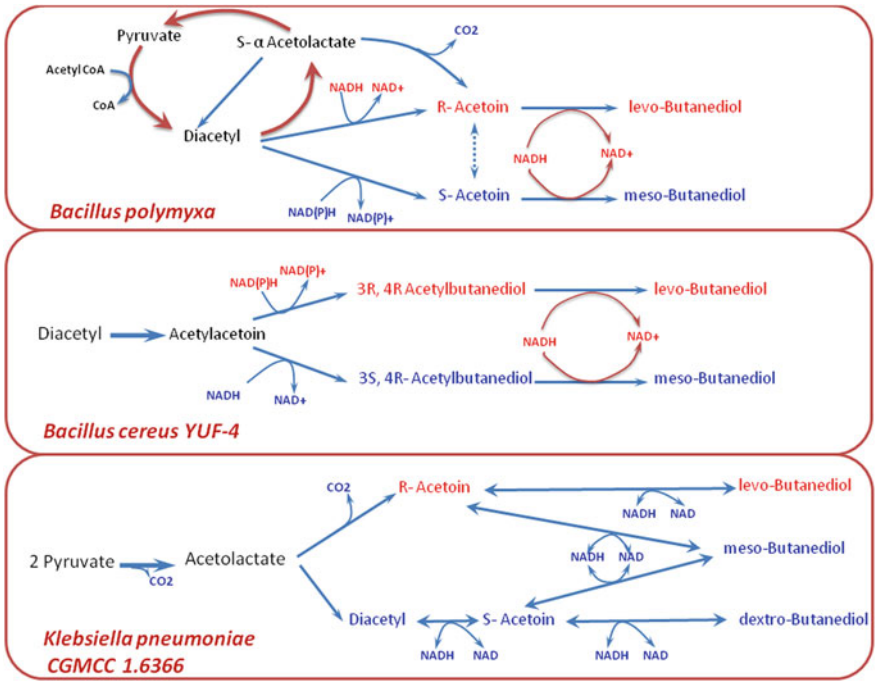

Fig. 3 Mechanisms of the formation of 2,3-BDO stereoisomers (modified after [5])

NADH-linked diacetyl reductase converts the latter to acetoin. Finally, 2,3-BDO of different isomeric forms is formed from acetoin by the action of different acetoin reductase enzymes with different stereospecificities, or by a cyclic pathway (the so-called butanediol cycle), the existence of which has been reported in different bacteria as shown in Fig. 3 [14, 36, 37]. Recently, autotrophic 2,3-BDO synthesis from CO_2 and/or CO plus H_2 was shown to exist in different acetogenic *Clostridium* species [35, 38]. Wood–Ljungdahl pathway was shown to be involved in which CO and/or CO_2 feeds the methyl and carbonyl branches of the pathway. In the methyl branch, CO or CO_2 is fixed in a sequence of tetrahydrofolate (THF)- and cobalamin-dependent reactions into a methyl group, which is then combined with CO (used either directly or after enzymatic reduction of CO_2) to form acetyl-CoA, in which the latter is catalysed by the CODH/ACS (carbon monoxide dehydrogenase/acetyl-CoA synthase) complex. Acetyl-CoA serves as a precursor for growth and 2,3-BDO production [35] (Fig. 2).

In the BDO cycle (Fig. 2), acetoin is oxidized to diacetyl by acetoin dehydrogenase, and then, 2 diacetyl molecules are converted to acetylacetoin and acetate by the enzyme acetylacetoin synthase. Acetylacetoin is further reduced to acetylbutanediol with different stereospecificities by either NAD(P)H- or NADH-linked acetylacetoin reductase. Different 2,3-BDO stereoisomers are then formed by the action of acetylbutanediol reductase. Through this butanediol cycle, 2 forms of

stereoisomers are formed in *B. cereus* as reported by Ui et al. [39]. Interestingly, in *P. polymyxa* grown under microaerobic conditions, diacetyl is converted to *S*-acetoin by a NAD(P)H-linked diacetyl reductase. Anaerobically, this bacterium produces 98 % of the *levo*-form through the catabolic 2,3-BDO formation route (Figs. 2, 3). Moreover, an acetoin racemase catalysing the conversion between the different forms of acetoin was proposed for the same bacterium [39]. Recently, Chen et al. [14] elaborated the mechanism of the different stereoisomer formation in *K. pneumonia.* They reported that glycerol dehydrogenase exhibited *2R,3R*-butanediol dehydrogenase activity and was responsible for *levo*-butanediol synthesis from *R*-acetoin. This enzyme also contributed to *meso*-2,3-butanediol synthesis from *S*-acetoin. Butanediol dehydrogenase was the only enzyme that catalyses the conversion of diacetyl to *S*-acetoin and further to *dextro*-butanediol (Fig. 3).

2.1.3 Pathway Engineering and Synthetic Pathway for 2,3-BDO Formation

Despite the intensive research done on enhancing 2,3-BDO production by its native risk group 2 bacteria (e.g. see [11, 40, 41]), the concerns associated with the utilization of potential pathogenic bacteria and/or the inefficient utilization of cellulosic sugars have led many scientists to engineer more safer strains. Oliver et al. [42] have developed a 2,3-BDO biosynthetic pathway in the photosynthetic cyanobacterium *Synechococcus elongatus*. The strain still has a limited productivity (2.38 g/L 2,3-BDO), and more research is needed to reach a desirable titre suitable for industrial application. Efforts were also done to enhance the production of optically active 2,3-BDO in native strains. A mutant of *P. polymyxa* with constitutive synthesis of the α-acetolactate synthase was constructed [21]. The mutant obtained grew more slowly than the wild type but produced fourfold more 2,3-BDO. By knocking out some by-product-producing genes in *Enterobacter cloacae*, Li et al. [11] were able to produce 119 g/L of enantiomerically pure 2,3-BDO using lignocellulosic hydrolysates.

Moreover, *E. coli* was extensively used as a host for many metabolic engineering studies for the production of 2,3-BDO, especially for the production of optically active one. Until recently, the synthetic pathways constructed in *E. coli* for enantiomerically pure 2,3-BDO using different stereospecific dehydrogenases from diverse species gave relatively low concentration of 2,3-BDO [5, 43]. Recently, applying a systematic metabolic engineering approach, Xu et al. [17] optimized the production of 2,3-BDO in recombinant *E. coli* strains. 2,3-BDO biosynthesis gene clusters were cloned from several native 2,3-BDO producers, including *B. subtilis*, *B. licheniformis, K. pneumoniae, Serratia marcescens*, and *E. cloacae*, inserted into the expression vector pET28a, and compared for 2,3-BDO synthesis. The best strain was then studied in fed-batch fermentation and was found to produce 74 g/L within 62 h [17].

Since no natural producers for the dextro-2,3-BDO (*2S,3S*) have been found, biosynthesis of this diol enantiomer has been achieved using engineered *E. coli* [32,

44]. Li et al. [32, 44] obtained 26.8 g/L of highly pure (>99 %) (*2S,3S*)-2,3-BDO in a fed-batch culture from diacetyl. Moreover, through introducing NADH regeneration enzymes into *E. coli*, a higher product titre (31.7 g/L) of (2*S,* 3*S*)-2,3-BDO was obtained [32].

Many industrial biotechnological processes are moving towards the use of yeast as a platform. Engineered yeast strains were also reported that are capable of producing 100 g/L of enantiomerically pure *levo*-2-3-BDO from a mixture of glucose and galactose with a yield over 70 % of the theoretical value [33, 45]. The high titre and yield of the optically active 2,3-BDO produced make the engineered yeast strain promising hosts for a cost-effective production of biobased 2,3-BDO.

2.2 1,4-Butanediol

1,4-Butanediol (1,4-BDO) is an important commodity chemical used to manufacture over 2.5 million tons of valuable products annually. The major use of 1,4-butanediol is in the production of tetrahydrofuran (THF) and PBT [46]. THF is used to produce spandex fibres and other performance polymers, resins, solvents, and printing inks for plastics. PBT is an engineering-grade thermoplastic that combines excellent mechanical and electrical properties with robust chemical resistance. The automotive and electronics industries heavily rely on PBT to produce connectors, insulators, wheel covers, gearshift knobs, and reinforcing beams. There is also growing demand in the apparel industry for renewable, biobased spandex. 1,4-BDO is also used as a plasticizer (e.g. in polyesters and cellulosics), as a carrier solvent in printing ink, a cleaning agent, an adhesive (in leather, plastics, polyester laminates, and polyurethane footwear), in agricultural and veterinary chemicals, and in coatings (in paints, varnishes, and films). 1,4-butanediol is also reportedly used as a solvent in cosmetic formulations and as a humectant in pharmaceuticals [47]. Recently, Diaz et al. [48] reviewed the various biodegradable polymers that can be synthesized from 1,4-BDO and dicarboxylic acids. Application of a series of polymers that cover a wide range of properties, namely materials from elastomeric to rigid characteristics that are suitable for applications such as hydrogels, soft tissue engineering, drug delivery systems, and liquid crystals, is reported.

In nature, no metabolic pathway and no micro-organisms are found so far that can produce 1,4-BDO from sugar or other biomass. Therefore, fossil fuel-based feedstocks such as acetylene, butane, propylene, and butadiene are the current sources for its production. Recently, using genome-scale metabolic model of *E. coli* and biopathway prediction algorithms, the company Genomatica has established unnatural synthetic pathways and correspondingly engineered *E. coli* strains for 1,4-BDO bioproduction from sugars such as glucose, xylose, sucrose, and biomass-derived mixed-sugar streams [46, 49]. In one pathway, sugar is first

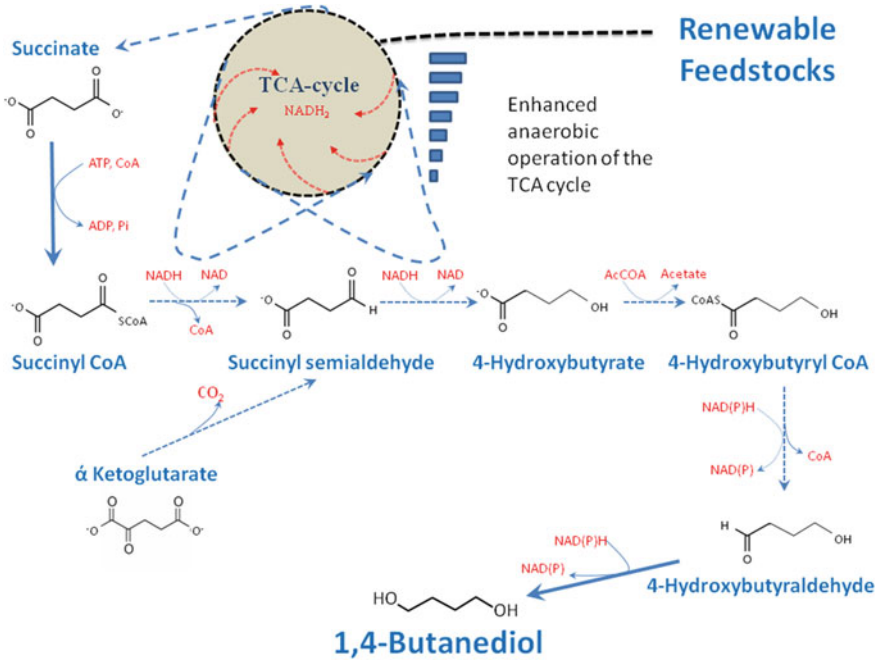

Fig. 4 1,4-BDO biosynthetic pathways introduced in *E. coli* (modified after Yim et al. [46]). *Solid lines* show reactions occurring naturally in *E. coli*, whereas *dotted lines* represent introduced synthetic reaction steps

converted into succinyl-CoA which is then further converted into 1,4-BDO over 4-hydroxybutyrate and other intermediates (Fig. 4), and a strain capable of producing 18 g/L 1,4-butanediol was engineered. The engineered *E. coli* has an enhanced anaerobic operation of the oxidative tricarboxylic acid cycle, thereby generating more reducing power to drive the synthetic 1,4-BDO pathway. According to Genomatica, they have done extensive work to optimize the yield and the rate of 1,4-BDO production, to minimize the by-products, and to enhance the 1,4-BDO tolerance of the engineered strain. Yim et al. [46] proposed that by rising the rates of key steps in the pathway, removing metabolic inefficiencies and substantially reducing by-products may increase the titre further. Burk [1] stated that the commercial production of 1,4-BDO from sugar will require much less energy and release significantly less carbon dioxide and is expected to have a substantial cost advantage relative to the current petrochemical process. Indeed, systems biology and fermentation process engineering approaches can identify and address bottlenecks that are obstacles to commercialization like achieving higher cell densities with improved specific productivity [46]. Recently, strains able to produce 30–40 g/L of 1,4-BDO in a continuous bioreactor were developed and patented by Genomatica [50].

2.3 1,3-Butanediol

1,3-Butanediol (1,3-BDO) is used as a chemical intermediate in the manufacture of polyester plasticizers, as a solvent for flavouring, and as a humectant in pet foods and tobacco. Its uses in cosmetics have been reviewed by the Cosmetic Ingredient Review which concluded that 1,3-butanediol is safe as normally used in cosmetics [51]. (*R*)-1,3-BDO, a non-natural alcohol, is a valuable building block for the synthesis of various optically active compounds such as pheromones, fragrances, and insecticides by direct incorporation into the target molecules, or is used as chiral template in the Lewis acid-mediated reactions of acetals with nucleophiles [52]. (*R*)-1,3-BDO is especially interesting as a starting material of chiral azetidinone derivatives and key intermediate of penems and carbapenems for industrial synthesis of ß-lactam antibiotics. Because these antibiotics are the mostly used antibacterial agents in clinical practice worldwide, the demand for *R*-1,3-BDO has been drastically increased, and as a consequence, the production method of *R*-1,3-BDO has been intensively studied [53–55]. So far, 1,3-BDO has been synthesized as a racemic mixture of *R*- and *S*-forms, mainly from petroleum-based chemicals such as a prochiral precursor, 4-hydroxy-2-butanone. Moreover, Eguchi and Mochida [56] attempted a kinetic resolution of 1,3-BDO by lipase-catalysed diacylations in organic solvent, resulting in (*R*)-1,3-diacetoxybutane with 23.4 % yield and 98.6 % enantiomeric purity. Using whole cells of recombinant *E. coli* expressing exogenous dehydrogenase from *Candida parapsilosis*, Daicel Chemical Industries Ltd. produce *R*-1,3-BDO with 48.4 % yield and 95 % enantiomeric purity [57]. Recently, Kataoka et al. [53] constructed an effective synthetic production route of 1,3-BDO from glucose in *E. coli* (Fig. 5). The high demand on reducing equivalents and cofactors for the production of 1,3-BDO (Fig. 5) reflects the importance of the aerobic catabolism of glucose for reducing equivalent regeneration. Hence, Kataoka et al. [54] optimized 1,3-BDO in an engineered *E. coli* by strict regulation of the overall oxygen transfer coefficient (k_La) during the cultivation. With optimized fermentation conditions, this recombinant *E. coli* strain was able to produce up to 9 g/L of 98.5 % enantiomeric purity of *R*-1,3-BDO. Although the titre reported by Kataoka et al. [53] was more than 8-fold higher than that reported in the patent published earlier in 2009 [58], still much work has to be done to reach an acceptable concentration suitable for commercialization.

3 Propanediol Production

Propanediol is a three-carbon diol having its hydroxy groups, at the first and the last carbon atom, in case of 1,3-PDO, or at the first and the second carbon atom in 1,2-PDO. 1,2-PDO is a chiral molecule and mostly available as a racematic mixture. Both 1,3-PDO and 1,2-PDO offer broad application spectra, either directly as solvents or as platform chemicals for a broad product spectrum. Even though a chemical synthesis is possible, the interest in biological production of propanediols

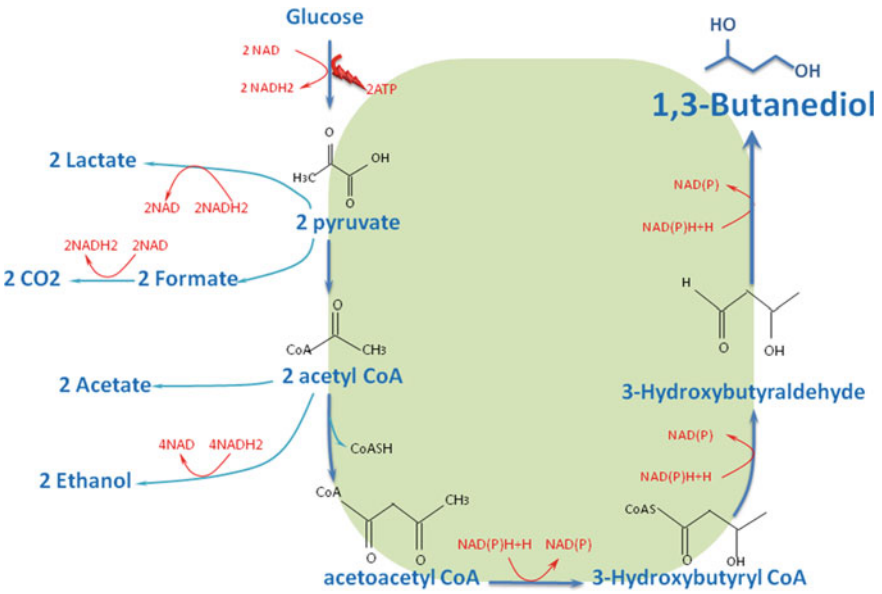

Fig. 5 Schematic diagram of 1,3-BDO biosynthetic pathways from glucose in an engineered *E. coli* (modified after [53, 54])

increases. Fermentation processes need less pressure, ambient temperature, and no expensive catalysts. Furthermore, they allow a sustainable process by transferring waste streams of biodiesel production or lignocellulosic residues into valuable side products.

3.1 1,3-Propanediol

Because of the attractive physical and chemical properties, and hence the various applications of 1,3-PDO, the interest in such polymer increased significantly in the last few years. On the one hand, it is used directly as solvent and antifreeze component in varnish, adhesives, or resins [6], as polyglycol-type lubricant, and in cosmetic products [59]. On the other hand, it is a very suitable monomer for synthetic reactions like polycondensation. 1,3-PDO is well known for the production of polytrimethylene terephthalate (PTT), biodegradable polyester which is utilized fibre not only in textiles and carpets but also in coatings. Furthermore, it can be used for the production of other biodegradable plastics polyesters, polyethers, and polyurethanes [60].

Till recently, biotechnology could not economically compete with the chemical synthesis of 1,3-PDO. In 2004, however, DuPont constructed a biochemical plant in Loudon for manufacturing 1,3-propanediol using *E. coli* with a synthetic pathway

from glucose. The plant was commissioned in November 2006. Very recently, the two companies METabolic Explorer (France) and SK Chemicals (South Korea) recently announced a joint agreement to manufacture 1,3-propanediol from crude glycerol. Together, they will market it in Europe and Asia to fulfil the expanding global demand for 1,3-PDO (www.metabolic-explorer.com, www.skchemicals.com, 2014). Biotechnological plants for 1,3-PDO from glycerol were also built in China. It is not clear if any of these plants are in operation.

3.1.1 Micro-organism of Potential Significance for 1,3-PDO Production

1,3-PDO is one of the natural products of the anaerobic degradation of glycerol in many bacteria. Therefore, and as by-products of biodiesel industry, crude glycerol was intensively used for the production of 1,3-PDO. However, the productive strains should be used that can tolerate impurities normally found in crude glycerol (salts, free fatty acids, and methanol [61]). The production of 1,3-PDO from glycerol is mainly performed by micro-organisms of the families *Clostridiaceae* and *Enterobacteriaceae, and* several species of *Klebsiella, Clostridia, Citrobacte*r, and *Enterobacter* are known to convert glycerol to 1,3-PDO under anaerobic conditions. The most-studied and well-known species are *K. pneumoniae* and *Clostridium butyricum*, because of their high substrate tolerance as well as high yield and productivity. Although *C. butyricum* is strictly anaerobic and *K. pneumonia* is facultative anaerobic (easier to handle), species of *Clostridia* are more interesting for industrial application. *K. pneumoniae* is classified as an opportunistic pathogen, and hence, special safety precautions are needed to use *K. pneumonia* for fermentation. Recently, in a cocultivation of cyanobacteria with *K. pneumoniae,* Wang et al. reported the production of 1,3-PDO from CO_2 [62]. Moreover, it was shown that 1,3-PDO can be produced in an unsterile process from raw glycerol using either mixed culture [63] or pure culture of *C. butyricum* [64] and *C. pasteurianum* [65]. This new development makes it economically very competitive. Moreover, the incorporation of the 1,3-PDO production into a biorefinery concept can further increase the ecological advantage and the commercial chance of the glycerol-based process. Friedmann and Zeng [66] proposed to use a mixed culture to produce 1,3-PDO and methane from glycerol. This concept was successfully demonstrated within a European 7th Framework research project (www.propnergy.eu) in laboratory and pilot scale. The basic idea was to use acidogenic and methanogenic bacteria for converting the by-products simultaneously into methane. Alternatively, the by-products can be degraded in a following biogas bioreactor. Formerly, a theoretical and metabolic flux study of syntrophic-like growth of *C. butyricum* and *Methanosarcina mazei*, a methanogenic archeon, under anaerobic conditions was carried out to analyse the several possible scenarios, especially to examine the preference of *M. mazei* in scavenging acetate and formate under conditions of different substrate availability, including methanol as a cosubstrate in biodiesel-derived raw glycerol [67]. Zhou et al. [68] studied the bioconversion of

glycerol to 1,3-PDO with a mixed population in a microbial bioelectrochemical system (BES). Though the mixed population used in this study was less effective, the use of BES system for delivering the necessary reducing power for 1,3-PDO production represents an interesting development. More recently, Choi et al. [69] showed that *C. pasteurianum*, a promising 1,3-PDO producer as mentioned above, can directly use electrons from cathode for the regeneration of reducing power in glycerol fermentation. However, the electron flow from the cathode was relatively low and the effect on the glycerol fermentation was not significant. In fact, microbial electrochemical processes for biosynthesis are still poorly understood [70, 71]. The use of a mixed culture in BSE is even more complicated. In general, it is essential to better understand the regulation and metabolic interactions and to control the dynamics of microbial consortia suitable for such processes and to inhibit the 1,3-PDO degradation.

Mixtures of glucose and glycerol have also been used for the production of 1,3-PDO by using members of *Lactobacillaceae*. *Lactobacilli* have only the reductive conversion and need an additional substrate for the growth and generation of the reducing equivalents. *L. reuteri*, *L. brevis*, *L. buchneri*, *L. collonoides,* and *L. panis* were reported to produce 1,3-PDO in mixed substrate fermentation [72]. Pflügl et al. [73] reported the production of 42 g/L 1,3-PDO from glycerol by *L. diolivorans.* However, after the addition of glucose, the 1,3-PDO production increased up to 74 g/L [73]. Recently, Sabra et al. [74] reported the simultaneous production of 1,3-PDO and *n*-butanol in mixtures of glucose and glycerol in different ratios using *C. pasteurianum*. On the other hand, with glucose as mono-substrate, several approaches with genetically modified organisms have been reported (see Sect. 4.1.2). An overview of the potential 1,3-PDO productive strains is given in Table 2.

3.1.2 Biosynthetic Pathways and Pathway Engineering of 1,3-PDO

The natural pathway for the production of 1,3-PDO in different micro-organisms is shown in Fig. 6. Generally, the pathway is divided into two parallel routes, a reductive route for the production of 1,3-PDO (A) and an oxidative route (B) where glycerol is metabolized via glycolysis into pyruvate and energy is produced. Only about 5 % of the glycerol is used for biomass production, when it is the sole carbon source [59].

In the reductive route, glycerol is dehydrated into 3-hydroxypropionaldehyde (3-HPA) by glycerol dehydratase. 3-HPA is subsequently reduced to 1,3-PDO by 1,3-propanediol oxidoreductase (PDOR) under consumption of nicotinamide adenine dinucleotide ($NADH_2$). This reducing equivalent is generated in the oxidative route through the synthesis of pyruvate and the transformation of pyruvate into acetyl-CoA. Different micro-organisms convert pyruvate into different by-products (Fig. 6). Indeed, the yield of 1,3-PDO per glycerol depends on the availability of $NADH_2$. The availability is not only determined by the micro-organism itself but also dependent on the process conditions of the fermentation [89]. Hence, the yield

Table 2 Comparison of 1,3-PDO production by different species, substrates, and fermentation modes

Organism	Substrate	Fermentation mode	Media Supplements	1,3-PDO (g/L)	Yield (g/g)	Productivity (g/L h)	References
C. butyricum DSM 5431	Glycerol	Fed batch	1 g/L yeast extract	58	0.56	2.7	[75]
C. butyricum VPI 3266			0.04 mg/L biotin 8 mg/L *p*-aminobenzoic acid	65	0.57	1.2	[76]
C. butyricum DSM 5431 mutant 2/2			1 g/L yeast extract	70.6	0.54	0.8	[77]
C. butyricum E5			2 g/L yeast extract	65.6	0.54	1.4	[78]
C. butyricum DSM 5431			1 g/L yeast extract	47.5	0.51	2.4	[79]
C. butyricum mutant 2/2			1 g/L yeast extract	70.4	0.56	1.4	[79]
C. butyricum			–	86.6	–	–	[80]
C. butyricum VPI 1718			1 g/L yeast extract crude glycerol	68	0.5	–	[64]
Clostridia mixed culture			0.024 mg/L biotin 0.015 mg/L pantothenate	70	0.46	2.6	[63]
C. butyricum DSP1		Repeated batch	Crude glycerol	62	0.53	0.76	[81]
C. butyricum CNCM 1211		batch	0.004 mg/L biotin	67	0.52	–	[82]
C. acetobutylicum DG1 (pSPD5)		Fed batch	0.04 mg/L biotin 8 mg/L *p*-aminobenzoic acid Crude glycerol	84	0.54	1.8	[83]
		Continuous		60	0.53	3	[83]

(continued)

Table 2 (continued)

Organism	Substrate	Fermentation mode	Media Supplements	1,3-PDO (g/L)	Yield (g/g)	Productivity (g/L h)	References
Clostridium IK124		Fed batch	5 g/L yeast extract	87.7	0.54	1.9	[84]
C. pasteurianum mutant			1 g/L yeast extract in situ *gas stripping*	27	0.21	1.2	[85]
Citrobacter freundii [FMCC-B 294 (VK-19)			5 g/L peptone 5 g/L meat extract 2.5 g/L yeast extract	68	0.4	0.79	[86]
K. pneumoniae				83.5	–	–	[80]
Klebsiella oxytoca M5alΔ ldhA	Sucrose + glycerol	Fed batch	1 g/L yeast extract	84	0.62 mol/mol glycerol	1.6	[87]
Lactobacillus reuteri	Glucose + glycerol		(Repeated fed batch) 10 g/L bact. peptone 10 g/L meat extract 5 g/L yeast extract	65	0.97 mol/mol glycerol	1.2	[88]
L. diolivorans	Glucose + glycerol		5 mg/L vitamin B_{12} 10 g/L casein peptone 10 g/L meat extract 5 g/L yeast extract	84.5	0.55 mol/mol substrate	0.45	[73]

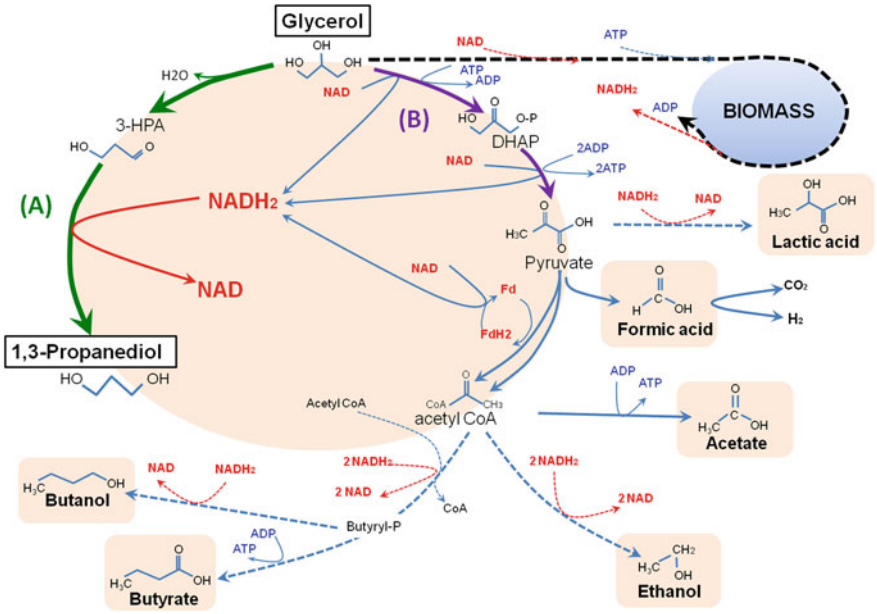

Fig. 6 Metabolic pathway of glycerol fermentation

of 1,3-PDO depends on the combination and stoichiometry of the reductive and oxidative pathways. Consequently, the maximum yield of 1,3-PDO formation from glycerol in clostridia represents 0.67 mol/mol and is achieved under conditions where acetic acid is the main by-product and not butyric acid, ethanol, or butanol [89–91]. If no hydrogen and butyric acid are produced at all during the fermentation, the theoretical yield can be further increased to 0.72 mol/mol [89, 90, 92]. The 1,3-PDO yield from glycerol can be additionally enhanced with an in vitro approach using crude enzymes from different organisms [93]. These systems feature several biomanufacturing advantages, such as fast reaction rate, easy product separation, broad reaction condition and tolerance to toxic substrates or products [94]. Nevertheless, the cost and stability of enzyme and coenzymes restrict the use of such systems in industrial scale.

Intensive work has been done to genetically modify micro-organisms to convert glucose to 1,3-PDO in one micro-organism. In the DuPont PDO process, a synthetic pathway was successfully developed to produce PDO from glucose, in which the glycerol synthesis pathway from *S. cerevisiae* (catalysed by glycerol 3-phosphate dehydrogenase (DAR1) and glycerol 3-phosphate phosphatase (GPP1/2) and the metabolic pathway of converting glycerol to PDO from *K. pneumonia* (glycerol dehydratase, encoded by the genes *dhaB1*, *dhaB2*, and *dhaB3*) were integrated into *E. coli* (Fig. 7, [95]). The last step, the formation of 1,3-PDO is realized by a 1,3-propanediol oxidoreductase isoenzyme from *E. coli* (YqhD). Continuous strain development was made by DuPont/Genencor, and the most fundamental changes

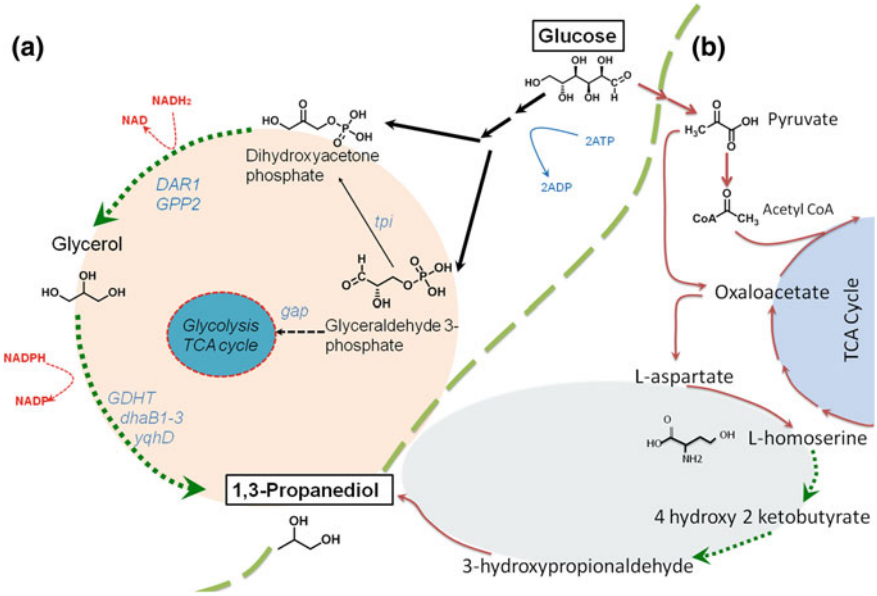

Fig. 7 Engineered *E. coli* strains for the production of 1,3-PDO from glucose. **a** Glycerol-dependent synthetic pathway [95] and **b** non-glycerol-dependent pathway [96]. *Dotted arrows* indicate introduced synthetic pathway steps

done were probably the elimination of D-glucose transport by the phosphotransferase system (PTS) and the downregulation of glyceraldehyde 3-phosphate dehydrogenase (gap) together with reactivation of tpi. Finally, the yield could be increased to 135 g/L with a productivity of 3.5 g/L h [95].

Still, in such a production system, the substrate suicide of glycerol dehydratase (GDHt) that could limit the productivity has to be overcome [97]. Recently, Chen et al. [96] constructed a new non-glycerol-derived synthetic 1,3-PDO synthesis in *E. coli* (Fig. 7). With protein engineering of glutamate dehydrogenase, they extended the pathway of homoserine, a natural intermediate of cellular amino acid metabolism. At first, homoserine is converted by deamination into 4-hydroxy-2-ketobutyrate, followed by decarboxylation into 3-hydroxypropionaldehyde (3HPA). Like in the conventional pathway, 3HPA is subsequently transformed by alcohol dehydrogenase into 1,3-PDO. The theoretical maximum yield (1.5 mol 1,3-PDO/mol glucose) of the new 1,3-PDO pathway is the same as that of the DuPont route. Since homoserine synthesis is a common pathway in most of the bacteria, the proposed route can be engineered into selected hosts with the more favourable ability to utilize different and cheap sugars. Moreover, the proposed pathway does not utilize GDHt and thus can avoid the serious problems associated with vitamin B12 and substrate suicide. This non-natural pathway is thus very appealing for 1,3-PDO production.

3.2 1,2-Propanediol

1,2-propanediol (1,2-PDO), generally called propylene glycol, is a major commodity chemical with a global demand estimated to be around 1.36 Mio. t/a for several industries [98]. It appears as a colourless hygroscopic liquid with low volatility and an oily consistency. This industrial important compound is mainly utilized as solvent, antifreeze, de-icer, and heat transfer fluids [99]. Furthermore, it could be applied as colour compound and flavour and fragrance carrier in foods, beverages, cosmetics, and pharmaceuticals, or even as tobacco humectants [100]. The interest in 1,2-PDO increases since it is less toxic than products based on ethylene glycol for humans and animals. The US Food and Drug Administration (FDA) has determined 1,2-PDO to be "generally recognized as safe" for use in food, cosmetics, and medicines [98].

3.2.1 Microbial Cell Factories for the Production of 1,2-PDO

The biological route for producing 1,2-PDO from sugars is known since many years. Early studies on *Thermoanaerobacterium thermosaccharolyticum* [101, 102], *Bacteroides ruminicola* [103], *C. sphenoides* [104], *L. Buchneri* [105]), and *E. coli* [99] have demonstrated 1,2-PDO formation. In comparison with other diols, the 1,2-PDO yields are much lower, either from sugars or from glycerol [92]. The biosynthesis of 1,2-PDO requires the conversion of the main carbon source into DHAP with the glycolytic pathways (Fig. 8). Therefore, due to higher reduction degree of glycerol, the yield of 1,2-PDO is higher than that from glucose (theoretical maximum yield of 0.63 and 0.72 g/g from glucose and glycerol, respectively). In either way, the biosynthesis consumes redox equivalent and ATP [99].

There are two possible pathways for the biosynthesis of 1,2-PDO. The first one metabolizes deoxy sugars (methyl pentoses (Fig. 8a)), whereas the second one converts DHAP into methylglyoxal (Fig. 8b) and further to 1,2-PDO. The deoxy pathway is well studied in *E. coli* and is reviewed by Bennett and San [106]. At first, *L*-rhamnose is converted into *L*-rhamnulose-1-phosphate, which is subsequently split into dihydroxyacetone phosphate and S-lactaldehyde by the enzyme RhaD (*L*-rhamnose dehydrogenase). Fucose, on the other hand, is first isomerized into *L*-fuculose and transformed into *L*-fuculose-1-phosphate by the enzyme *L*-fuculose kinase. Another enzyme, fucA (*L*-fuculose-1-phosphate aldolase), cleaves it into dihydroxyacetone phosphate and *L*-lactaldehyde. Depending on the redox conditions, the lactaldehyde can be either reduced to 1,2-PDO or oxidized to lactic acid. Anaerobic conditions lead to conversion into *S*-1,2-PDO, catalysed by a NAD-oxidoreductase fucO (*S*)-1,2-propanediol oxidoreductase [106]. Since deoxy sugars are quite expensive as substrate, the deoxy pathway is considered to be uneconomical as an industrial process.

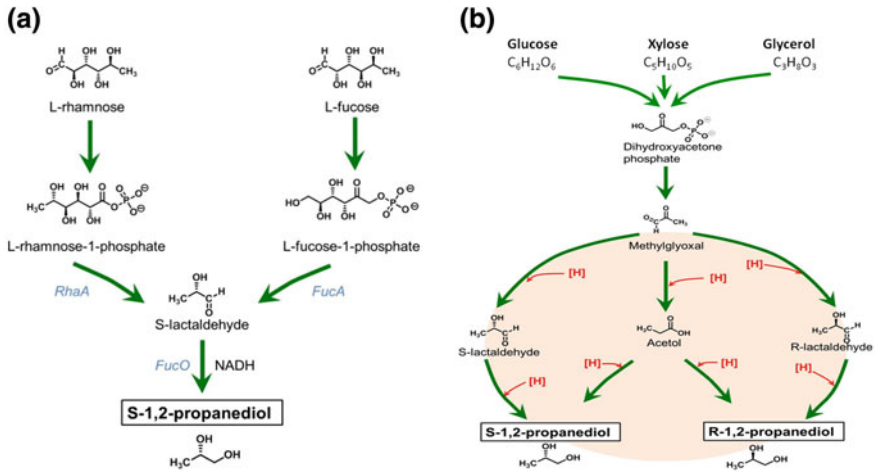

Fig. 8 **a** Metabolic pathways of deoxy sugar fermentation into 1,2-propanediol by *E. coli* and **b** the various routes of the conversion of methylglyoxal to 1,2-PDO in different micro-organisms (modified after [106])

In *T. thermosaccharolyticum*, the second pathway is found. At first, DHAP is produced from glucose, xylose, mannose, or cellobiose. DHAP is then converted with methylglyoxal synthase into methylglyoxal (MG). Subsequently, MG is reduced to *R*-1,2-PDO with aldose reductase or glycerol dehydrogenase. If glucose is fermented only into *R*-1,2-PDO, acetate, and CO_2, a theoretical yield of 0.42 g *R*-1,2-PDO/g glucose is possible. In *E. coli*, MG is converted into acetol with the NADPH- or NADH-dependent lactaldehyde oxidoreductase, and alcohol or aldehyde dehydrogenases. *E. coli* also converts MG into *R*-lactaldehyde with NADH-dependent glycerol dehydrogenase. On the contrary, the yeast *S. cerevisiae* produces S-lactaldehyde from MG, which is subsequently converted into *S*-1,2-PDO by a NADPH-dependent aldose reductase. However, the MG production in *S. cerevisiae* is non-enzymatic and spontaneous, and the final 1,2-PDO titre is quite low [101].

Recently, Clomburg and Gonzales [99] developed a new strain of *E. coli* with increased production of 1,2-PDO. The functional pathway was engineered by combining different strategies (Fig. 9a): (I) to ensure DHAP availability, they changed the PEP-dependent DHAK (dihydroxyacetone kinase) with the ATP-dependent DHAK from *Citrobacter freundii*; (II) they overexpressed the genes for 1,2-PDO synthesis from DHAP; and (III) competitive pathways for acetate and lactate were deleted. Other side products were maintained to ensure the necessary redox balance and ATP generation. The recombinant *E. coli* strain produced 5.6 g/L 1,2-PDO with a yield of 0.21 g/g glycerol [99]. More recently, Koch et al. [107] established a recombinant *E. coli* to enhance 1,2-PDO production from several carbon sources with three newly integrated and highly expressed enzymes.

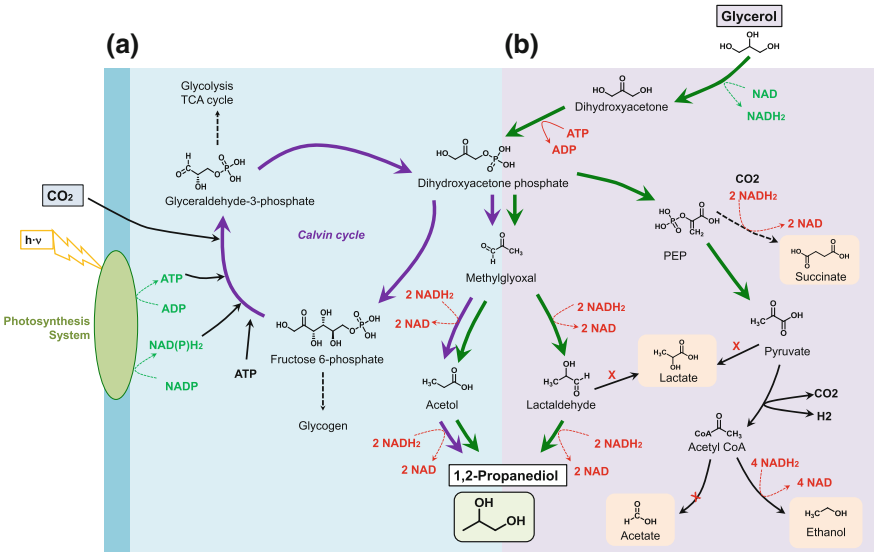

Fig. 9 Synthetic pathways for the production of 1,2-PDO: **a** from CO_2 by *Synechococcus elongates* (according to [108]) and **b** from glycerol by *E. coli*. *Red crosses* indicate deleted pathways, and *dashed lines* represent intermediate steps. (modified after [99])

This new pathway avoids the toxic intermediate methylglyoxal and use the natural formation of lactate. The latter one is transferred into lactyl-CoA by lactate-CoA transferase and then into lactaldehyde by lactyl-CoA reductase. In the last step 1,2-PDO is formed with the help of lactaldehyde reductase (also 1,2-propanediol oxidoreductase). Due to this, a maximum yield of 0.55 g PDO/glycerol and additional ATP can be achieved.

Furthermore, in a more sustainable approach, Li and Liao [108] described a photosynthetic conversion of carbon dioxide with a newly engineered cyanobacterium *S. elongatus* PCC 7942 (Fig. 9b). For the production of 1,2-PDO, genes for methylglyoxal synthase (mgsA), glycerol dehydrogenase (gldA), and aldehyde reductase (yqhD) from *E. coli* have been inserted. Additionally, the alcohol dehydrogenase (sADHs) from *C. beijerinkii* is induced into the cyanobacterium. The NADPH pool of *S. elongates* itself was taken into account for the 1,2-PDO production that requires many reducing equivalents. Therefore, the NADPH-specific secondary alcohol dehydrogenase was newly implemented in the pathway, resulting in the production of 150 mg/L 1,2-PDO from environmental CO_2 and light. Despite the progresses made in the implementation of metabolic engineering strategies and developing different new strains, the low reaction rate and product concentration are the most important barriers in its industrial production.

4 Recovery of Diols

The production of diols suitable for chemical or pharmaceutical applications is only achievable by using suitable separation and purification steps (downstream processing) after the fermentation. The downstream processing is one of the most influencing factors, contributing up to 50–70 % of the total product costs [109]. Thus, to have a suitable downstream process is of major interest for an economic and sustainable production of biobased diols. Xiu and Zeng [109] reviewed extensively the downstream processing of 2,3-butanediol and 1,3-propanediol fermentation broths. In the following, the main steps and challenges of recovering diols are briefly mentioned and some of the recent studies are then highlighted.

4.1 Recovery of Butanediol

The principle process of product recovery is almost the same for all diols. After cultivation, the final fermentation broth is a multicomponent mixture not only consisting of water, residual substrate and salts, and side products (e.g. alcohols, organic acids), but also consisting of cells and cell debris in addition to the target product. The initial step is the separation of biomass, which can be performed via centrifugation, filtration, or flocculation [110]. Residual salts may cause fouling on heating devices or inactivation of catalysts. Therefore, they may need to be removed, e.g., by electrodialysis, salting out, or ion exchange chromatography. The excess amount of water in the broth can be reduced by evaporation. The last step to obtain high purification grades is mainly conducted via distillation.

Difficulties in the recovery of butanediol-like 2,3-BDO are mainly caused by the high boiling point (180 °C for 2,3-BDO) and high hydrophilicity. Extractive separation is hampered by its low selectivity and a relatively low distribution coefficient towards extracting solvents. Promising solvents studied include ethyl acetate, tributyl phosphate, diethyl ether, *n*-butanol, dodecanol, and oleyl alcohol. For example, Anvari and Kayati used the non-toxic oleyl alcohol for an in situ extraction, but separated only 68 % of the total 2,3-BDO produced by *K. pneumonia* [111]. Improvement of extraction methods represents the combination of solvent extraction and salting-out techniques. The salting-out technique is based on a system of two aqueous phases: one with a hydrophilic solvent and one with highly concentrated salts. The increased ionic strength in the salt phase forces more diols to dissolve in the solvent phase, which is in this case an extractant. Li et al. [112] use a mixture of 32 % (w/w) ethanol and 16 % (w/w) ammonium sulphate to recover 91.7 % of 2,3-BDO next to 99.7 % of cells and 91.2 % of proteins. With 34 % (w/w) 2-propanol and 20 % (w/w) ammonium sulphate, Sun et al. [113] separated 93.7 % of 2,3-BDO. Also here, 99 % of the cells could be removed and reused for a new inoculation, which has a positive effect on the process economics. With butanol and potassium, phosphate salts up to 99 % of the 2,3-BDO can be

separated, as revealed by an Aspen Plus simulation performed by Birajdar et al. [114]. However, the 2,3-BDO has to be separated again from the extractants by evaporation, which means additional downstream units with additional costs. The purification of 2,3-BDO directly by distillation is hampered due to the high boiling, and it might be only used to enhance the concentration. Qureshi et al. [115] described a vacuum membrane distillation process, where the membrane retains the 2,3-BDO and let the more volatile compounds (water, ethanol) pass through. The concentration could be increased from 40 to 430 g/L. However, medium components caused membrane fouling, and the water flux decreased at higher 2,3-BDO concentrations [115]. A newly developed process combines reactive extraction and reactive distillation. Li et al. [116] used *n*-butyraldehyde (BA) as reactant and extractant at the same time. It reacts with 2,3-BDO to 2-propyl-4,5-dimethyl-1,3-dioxolane (PDD), which is extracted by BA itself. Both BA and PDD are transferred into a reactive distillation column, where the catalysts sulphuric acid and hydrochloric acid cleave PDD again into BA and 2,3-BDO. Li et al. were able to recover 90 % of the 2,3-BDO with purity higher than 99 %.

4.2 Recovery of 1,3-Propanediol and 1,2-Propanediol

The downstream processing of 1,2-PDO and 1,3-PDO from fermentation broth is similar, but could be even more challenging than the recovery of butanediol because of their higher boiling points (188 and 233 °C, respectively).

Liquid–liquid extraction could be more advantageous for PDO because it is selective and more energy efficient than distillation. Malinowsky tested different solvents, such as the series of pentanol until nonanol and hexanal until decanal, and other organic solvent. The best results were achieved with aliphatic alcohols and aldehydes, but the distribution of PDO in the solvents has been very low. Thus, large amounts of solvents would be required [117]. Li et al. [118] described the extraction and salting-out method using ethanol and sodium carbonate. They could separate 97.9 % of the 1,3-PDO and were able to separate 99.1 % of cells and other fermentation products, such as organic acids, in one step with this method. A combination of methanol and dipotassium hydrogen phosphate leads to a slightly higher 1,3-PDO recovery of 98.1 % [119]. In addition, the main side product 2,3-BDO, as well as organic acids, could also be recovered. Müller et al. [120] used ionic liquids as extractants in combination with phosphate salts. Despite the fact that high distribution coefficients for 1,3-PDO could be achieved, extraction with ionic liquids is too expensive and not available for in situ processes due to their high toxicity for the bacteria. Another possibility is the reactive extraction of 1,3-PDO with formaldehyde or acetaldehyde into 2-methyl-1,3-dioxane. The extraction of the product is enabled by the organic solvent extractants *o*-xylene, toluene, or ethylbenzene [121]. In a recent approach, Matsumoto et al. [122] use 1-butanal as reactant and toluene as diluent together with a hydrophobic acidic ionic liquid as a catalyst for the acetalization of 1,3-PDO into a dioxan. With this method, 96 % of the

1,3-PDO could be converted and extracted [122]. Possible drawbacks of this method are undesired reactions of reactant and fermentation by-products, forming further undesired components and causing loss of reactant. The reactive extraction of 1,2-PDO from aqueous environment was described by Broekhuis et al. [123]. 1,2-PDO reacts with acetaldehyde to form 2,4-dimethyl-1,3-dioxolane. In the next step, dioxalan is cleaved via hydrolysis into 1,2-PDO and acetaldehyde. Again, the last step in purification comprises a distillation column. Separation and purification combined in one operation unit can be realized in adsorption processes. With a sulphonate exchange resin, Hilaly and Binder were able to separate 95 % of the 1,3-PDO with a purity of 87 %. This process, however, had a high water demand, resulting in high energy cost [124]. For further cost reduction, Wang et al. [125] used a low-cost cation exchange resin based on polystyrene with high adsorption capacity to recover 1,3-PDO from fermentation broth. Other possibilities are adsorption on silica resin [126] or on beta zeolites [127]. In general, they are very selective, exhibit a simple design, are easy to operate, and are environmentally friendly because the absorbance material can be recovered [125]. However, they are difficult to be implemented in large-scale processes, due to high exchange surfaces and subsequent large pressure loss, together with high tendencies for fouling [109]. In addition, every adsorption process also requires a desorption step with additional costs.

5 Concluding Remarks

As summarized above, significant progresses have been made in the biosynthesis of different diols from various substrates. Quite clearly, for some of the diols, the microbial inherent weaknesses, such as the low product yield, slow reaction rate, high separation cost, and intolerance to toxic products, are the largest obstacles to the cost-competitive biotechnological production (e.g. 1,2-PDO and 1,3-BDO). A more profound comprehension of cell factories' physiology and stress responses would necessarily offer improved tools (at either genetic, metabolic, or system levels) to favour high diol yield and high-quality production. In the past few years, steps taken towards these goals enhanced the bioproduction process economics of some diols significantly. Biobased 1,3-PDO, 1,4-BDO, and 2,3-BDO are successful examples. Still, providing low-cost production process limits the competitiveness of some processes, and hence, much R&D efforts are further needed which may include:

1. Production of high-quality diols suitable for high-value products. This requires system-level understanding of the synthetic pathways to target the formation of desired isomers of diols within cell factories. Pure compounds of optically active 2,3-BDO, 1,3-BDO, 1,4-BDO, or 1,2-PDO are considered as high-value products. It is worth mentioning that chiral synthesis or separation remains a costly step in chemical synthesis, and hence, using enzymes or cells to synthesize compounds with high enantiomeric purity represents an alternative and effective approach.

2. Formation of multiproduct in a biorefinery approach will reduce the process costs significantly. Hence, several conversion technologies (thermochemical, biochemical, etc.) are combined together to reduce the overall cost, as well as to have a better flexibility in product generation and to provide its own power. Examples are the simultaneous production of 1,3-PDO and biogas in unsterile process or the coproduction of 1,3-PDO and butanol.
3. Development of robust microbial cell factories with wide substrate utilization specificities that can dominate in wide number of niches. Lignocellulosic residues that are plentiful and cheap have been widely investigated but their recalcitrance to degradation challenge the production of diols biotechnologically. Hence, adapted cell factories to inhibitors and environmental stresses in such raw substrates are then crucial for forthcoming diol production.
4. Exploring new derivatives or uses of diols that will open new markets. Whereas bioprocesses for 2,3-BDO are well established in terms of productivity, yield, and titre, the market size for 2,3-BDO itself is still relatively small.
5. Downstream processing of diols is technically feasible, and a relatively high purification grade can be achieved, though the costs could be rather high. As a cost-effective method, in situ product recovery integrated with the fermentation process should gain more attention in the future.

Designing new-generation bioprocesses increasingly depend on engineering process-compatible cell factories. The latter, whether through genetic or physiological manipulations, can be greatly assisted by metabolic engineering. To achieve these goals, more fundamental knowledge is needed about metabolic pathways, control mechanisms, and process dynamics to optimally design integrated systems. Chemical engineers, metabolic engineers, and microbial physiologist will have to work for such integrated process. We argue that only by developing cost-efficient processes through integration of fermentation and downstream processing, the microbial production of diols can fulfil their potentials as platform chemicals.

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