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REVIEW

Fermentation of glycerol and production of valuable chemical and biofuel molecules

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Abstract Glycerol has attracted the attention of scientific and industrial communities due to its generation in bulk quantities as a byproduct of biofuel industries. With the rapid growth of these industries in recent years, glycerol is frequently treated as a very low-value byproduct or even a waste product with a disposal cost associated to it. Glycerol is not only abundant and inexpensive but also can generate more reducing equivalents than glucose or xylose. This unique characteristic of glycerol offers a tremendous opportunity for its biological conversion to valuable products at higher yield. This review focuses on research efforts to utilize glycerol as a carbon source for the production of a variety of fuels and chemicals by both native and metabolically engineered microorganisms.

Keywords Glycerol fermentation · Biofuels · Chemicals

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Introduction

It is now evident that the increasing demand of fuels will need to be compensated by their production from renewable sources. Currently, bioethanol and biodiesel represent the most common transportation fuels derived from renewable sources and their production results in the generation of glycerol as a by-product (Yazdani and Gonzalez 2007). It is estimated that for every 100 kg of biodiesel produced by the transesterification of triglycerides, 10 kg crude glycerol is generated as a reaction byproduct (Kolesarova et al. 2011). Due to increasing biofuel demand, the number of industries producing biodiesel has grown immensely in last two decades (Schubert 2006). This has created a surplus of crude glycerol and what once considered a desired co-product contributing to the economics of biofuel production has now become unwanted waste with the disposal cost attached it (Johnson and Taconi 2007).

This surplus of glycerol provides an opportunity to develop processes that can convert crude glycerol into high-value products and contribute to the viability of biofuel economy. Glycerol can be converted into higher value products by either biological or chemical transformations. Biological conversions are generally preferred to chemical means due to the higher specificity of the reaction, lower temperature and pressure involved in the reaction process, and lower levels of chemical contaminants (Johnson and Taconi 2007). Crude glycerol generated by biofuel industries

can be used as carbon source for microbial fermentations. The highly reduced nature of carbon in glycerol compared to sugar molecules provides a natural advantage when one considers the formation of various high value reduced products via biotransformation. The conversion of glycerol to glycolytic intermediates generates twice the amount of reducing equivalents generated by the metabolism of glucose or xylose (Yazdani and Gonzalez 2007). As a result, potential yields of fuels and chemicals are higher when synthesized from glycerol as opposed to monosaccharides (Dharmadi et al. 2006). When glycerol was used as the C source, wild type *E. coli* produced around 86 % ethanol and 7 % succinate and just traces of acetate and lactate (Dharmadi et al. 2006). However, when glucose was used, the yield of ethanol and succinate was only 29 and 6 %, respectively and an accumulation of lactate and acetate was observed (45 and 21 %) (Sawers and Clark 2004).

The availability and advantageous nature of glycerol has led to significant research efforts focused on the microbial conversion of glycerol into value added fuels and chemicals in recent years. This includes the use of native organisms capable of utilizing glycerol as well as certain organisms amenable to genetic manipulations (like *E. coli* and *Klebsiella pneumoniae*) enabling the application of rational metabolic engineering strategies to increase production of target compounds such as 1,3-propanediol, ethanol, butanol, succinic acid, and polyhydroxybutyrates. Here we review recent efforts exploiting glycerol as a carbon source for the production of a wide array of compounds by both native and metabolically engineered organisms. A schematic of pathways involved in the conversion of glycerol to these products is shown in Fig. 1, while Table 1 provides details on specific parameters of the conversion process.

Conversion of glycerol into value-added products by native organisms

A number of microbes have been reported to utilize glycerol as a carbon source for the production of

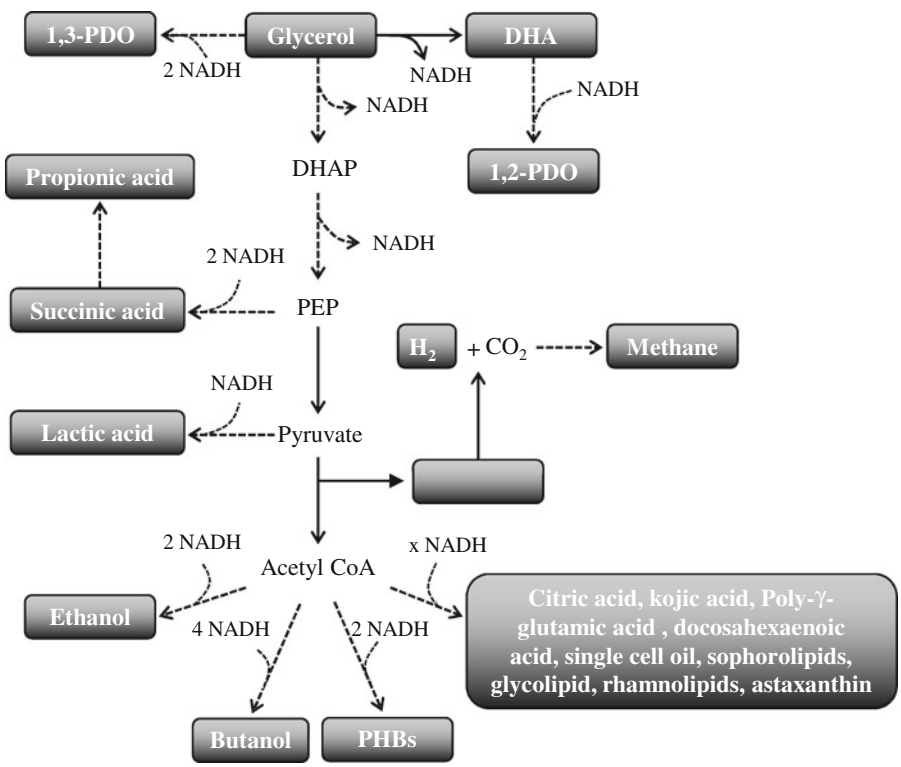

Fig. 1 Pathways for biological conversion of glycerol to various products. *Solid lines* represent single step reaction while *dotted lines* represent multiple step reactions. *1,3-PDO* 1,3-propanediol; *DHA* dihydroxyacetone; *PHB* polyhydroxybutyrate; *γ-PGA* poly-γ-glutamic acid; *H* reducing equivalents (NADH, NADPH, $FADH_2$, H_2)

Table 1 Native and engineered organisms producing various chemicals from crude glycerol

Nature	Organism	Product	Titer ($g\ l^{-1}$)	Yield ($g\ g^{-1}$)	Productivity ($g\ l^{-1}\ h^{-1}$)	References
Native	*Actinobacillus succinogenes*	Succinic acid	4.9	1.3	NA	Lee et al. (2001)
	Anaerobiospirillum succiniproducens	Succinic acid	19	NA	NA	Lee et al. (2004)
	Aspergillus flavuss NRRL 626	Kojic acid	29	NA	1.45[b]	Coelho et al. (2010)
	Bacillus subtilis NX-2	γ-PGA	31.7	NA	NA	Wu et al. (2010)
	Burkholderia cepacia	PHBs	7.4	NA	NA	Zhu et al. (2009)
	Candida bombicola	Glycolipids	60	NA	NA	Ashby et al. (2005)
	Chlorella protothecoides	Docosahexaenoic acid	NA	0.31	0.077	O'Grady and Morgan (2011)
	Clostridium butyricum	1,3-PDO	63.4	0.56	NA	Barbirato et al. (1998)
			46	NA	34	Papanikolaou et al. (2000)
			67.9	0.5	NA	Chatzifragkou et al. (2011)
	Clostridium pasteurianum	1,3-PDO	NA	NA	1.19	Moon et al. (2011)
		Butanol	NA	NA	0.98	
	Enterobacter aerogenes	Hydrogen	NA	NA	0.126	Nishio and Nakashimada (2007)
	Halanaerobium saccharolyticum sp. *senegalensis*	Hydrogen	0.013	0.035	NA	Kivisto et al. (2010)
	Klebsiella pneumoniae	1,3-PDO	35.2–48.5	NA	4.9–8.8	Menzel et al. (1997)
			3.27	0.69	1.29	Lin et al. (2005)
			71.58	0.78	1.93	Jin et al. (2011)
			66	NA	3.3	Xue et al. (2010)
		Ethanol	21.5	NA	0.93	Oh et al. (2011)
	Kluyvera cryocrescens S26	Ethanol	27	0.8	0.61	Choi et al. (2011)
	Labyrinthulomycota	Single cell oil	NA	0.32	NA	Scott et al. (2010)
	Methylobacterium rhodesianum MJ 126J and *Ralstonia eutropha* DSM 11348 (co-culture)	PHBs	50	0.17	NA	Bormann and Roth (1999)
	Pasteurellaceae strain DD1	Succinic acid	8.4	1.2	0.9	Scholten and Dagele (2008)
	Phaffia rhodozymo	Astaxanthin	0.034	NA	NA	Kusdiyantini et al. (1998)
	Propionibacterium acidipropioinici	Propionic acid	42	1.04	0.36	Barbirato et al. (1997)
			44.62	NA	0.2	Zhu et al. (2010)
	Pseudomonas aeruginosa	Sophorolipids	15.4	0.62	0.13	Zhang et al. (2005)
			45	NA	NA	Camilios-Neto et al. (2010)
	Schizochytrium limacinum	Single cell oil	4.91	NA	0.82	Chi et al. (2007)
	Serratia marcesens	Prodigiosin	0.583	NA	0.019	Tao et al. (2005)
	Ustilago maydis	Lipids	32.1	NA	0.16	Liu et al. (2010a)
	Vibrio strain BM1	PHBs	1.4	NA	NA	Wei et al. (2011)
	Yarrowia lipolytica	Citric acid	35	0.44	NA	Papanikolaou et al. (2002)
		Single cell oil	3.5	NA	1.2	Papanikolaou and Aggelis (2002)
	Zobellella denitrificans strain MW1	PHBs	4.27	0.3	NA	Ibrahim and Steinbuchel (2010)

Table 1 continued

Nature	Organism	Product	Titer ($g\ l^{-1}$)	Yield ($g\ g^{-1}$)	Productivity ($g\ l^{-1}\ h^{-1}$)	References
Engineered	*Escherichia coli*	Ethanol	10	0.51	0.21	Yazdani and Gonzalez (2008)
		1,3-PDO	104	90[a]	2.6	Tang et al. (2009)
			11.3	0.28	NA	Ma et al. (2009)
		1,2-PDO	5.6	21.3[a]	NA	Clomburg and Gonzalez (2011)
		PHBs	10.81	NA	0.18	Nikel et al. (2008)
		Succinic acid	14	0.69	0.26	Blankschien et al. (2010)
		D-lactic acid	32	0.80	1.5	Mazumdar et al. (2010)
	Gluconobacter oxydans		139.7	0.60	1.94	Habe et al. (2010)
			175.9	0.87	2.44	Hu et al. (2010)
			156.3	0.89	2.16	Hu et al. (2011)
			209.6	0.90	2.91	Hu et al. (2011)
	Klebsiella pneumoniae	Citric acid	78.13	0.64	1.95	Guo et al. (2010)
			16.1 and 16.0	0.51	0.70 and 0.66	Ashok et al. (2011)
	Wratislavia strain AWG7 strain A-101-1.22	Citric acid	154	0.78	1.05	Rywinska and Rymowicz (2010)
			124.2	0.77	0.85	Rymowicz et al. (2010)
	Streptomyces albulus strain M-Z18	DHA	30.11	NA	4.76[b]	Chen et al. (2010)

γ-PGA γ-polyglutamic acid; *PHB* polyhydroxybutyrates; *1,3-PDO* 1,3-propanediol; *3-HPA* 3-hydroxypropionaldehyde; *DHA* dihydroxyacetone; *NA* not available

[a] Data in % yield (g/g)

[b] Data in $g\ l^{-1}\ day^{-1}$

various value-added products, including 1,3-propanediol (1,3-PDO), ethanol, H_2, succinic acid, and propionic acid among others (Table 1; Fig. 1). Perhaps the best studied of these processes is the conversion of glycerol into 1,3-PDO by a variety of natural organisms. *Klebsiella pneumoniae* has been the most well studied as far as the production of 1,3-PDO is concerned. In a study, continuous fermentation with *K. pneumoniae* resulted in production of 35.2–48.5 g 1,3-PDO l^{-1}, which was close to the 1,3-PDO concentration obtained in batch and fed-batch fermentation (Menzel et al. 1997). The final volumetric productivity was 8.8 $g\ l^{-1}\ h^{-1}$ at a dilution rate of 0.25 h^{-1}. The addition of fumarate to the cultures of *K. pneumoniae* enhanced the utilization of glycerol and also led to increased 1,3-PDO formation. Although the net yield of 0.47 $g\ g^{-1}$ glycerol of 1,3-PDO was identical irrespective of the addition of fumarate, the volumetric productivity reached 1.29 $g\ l^{-1}\ h^{-1}$, an increase of almost 36 % when compared to the corresponding control (Lin et al. 2005). A study conducted by Jin et al. (2011) showed that when hemicellulosic hydrolysate was used as a co-substrate along with glycerol, a final 1,3-PDO concentration of 71.6 $g\ l^{-1}$ was observed with a net yield and productivity of 0.54 $g\ g^{-1}$ and 1.93 $g\ l^{-1}\ h^{-1}$. An increase of 18 % in production was observed as compared to single substrate (glycerol) fermentation and this can be attributed to the fact that the hydrolysate is rich in xylose and mannose which are known to exert a positive effect on both cell growth and solvent production. The authors have also shown for the first time that furfural and acetate (both components of hemicellulosic hydrolysate), which were earlier reported as inhibitory compounds actually had a mild stimulatory effect on 1,3-PDO production. When furfural and acetate were present in the hydrolysate at 0.28 and 1.46 $g\ l^{-1}$, respectively 1,3-PDO production increased from 8.77 to 11.7 $g\ l^{-1}$. A repeated fed-batch culture of *K. pneumoniae* supplied with an organic acid mixture (of succinate, citrate and fumarate) resulted in a significant increase in both cell

growth as well as PDO production with a maximum concentration of 66 g l^{-1} and the productivity reaching 3.3 g l^{-1} h^{-1} (Xue et al. 2010).

Although high levels of 1,3-PDO are produced by *Klebsiella* sp., their use on an industrial scale is limited because they are opportunistic pathogens capable of causing urinary tract and abdominal infections, and pneumonia. Additionally, these bacteria have a vitamin B_{12} dependent glycerol dehydratase enzyme. The continuous production of 1,3-PDO depends on the continuous utilization of glycerol which in turn, depends on the reactivation of the glycerol dehydratase enzyme. This reactivation of the enzyme is an ATP-dependent process in which the modified coenzyme is exchanged with an intact vitamin B_{12} molecule (Honda et al. 1980). However, as the *Klebsiella* sp. is not able to synthesize this vitamin, it needs to be added exogenously into the fermentation broth. Therefore, certain *Clostridia* strains such as *Clostridium butyricum*, *Clostridium pasteurianum*, are preferred because they are non- pathogenic and also have a vitamin B_{12} independent glycerol dehydratase enzyme. (O'Brien et al. 2004). This makes clostridial fermentations more cost-effective as they do not require the external addition of large amounts of this expensive molecule (Emptage et al. 2001).

Initial studies by Zeng (1996) suggested that the production of 1,3-PDO by *C. butyricum* was low because of the formation of side products, specifically butyric acid. This organic acid not only causes carbon loss from the solvent production pathway but is also extremely toxic (Zeng et al. 1994). Batch fermentations of *C. butyricum* using crude glycerol as the sole carbon source at 112 g l^{-1} yielded about 63 g 1,3-PDO l^{-1}, with a specific yield of 0.57 g g^{-1} glycerol (Barbirato et al. 1998). A field isolate of *C. butyricum* was found to produce 1,3-PDO at 46 g l^{-1} with a high volumetric productivity of 3.4 g l^{-1} h^{-1} (Papanikolaou et al. 2000). Fed-batch fermentations of *C. butyricum* VPI 1718 under non-sterile culture conditions using biodiesel-derived crude glycerol feedstocks resulted in a final 1,3-PDO titer of 67.9 g l^{-1} with a yield of 0.5 g g^{-1} (Chatzifragkou et al. 2011). 1,3-PDO was also produced by *C. pasteuranium* along with butanol with the productivity of 1.19 and 0.98 g l^{-1} h^{-1}, respectively (Moon et al. 2011).

In addition to these native organisms used for 1,3-PDO production, several other microbes have been exploited for the production of various other compounds from glycerol. The recent discovery that *E. coli* can utilize glycerol under fermentative conditions, a metabolic mode enabling increased yields of reduced compounds, has opened the possibly for the use of this well-studied organism for the production of value-added reduced products (Dharmadi et al. 2006). Under fermentative conditions, the authors reported ethanol as major product of glycerol fermentation in *E. coli*, which was produced at a yield of 0.43 g g^{-1} of glycerol. In addition to the high yields of ethanol produced by wild-type *E. coli*, the well-studied nature of this organism offers the opportunity to apply metabolic engineering strategies to diversify the portfolio of products that can be produced under fermentative or microaerobic conditions (See subsequent section).

Kluyvera cryocrescens S26, a strain obtained by screening of microbes that can utilize crude glycerol, produced ethanol with very high productivity (Choi et al. 2011). It was seen in this study that microaerobic fermentation resulted in higher cell growth as well as ethanol production and yield. This strain produced 27 g ethanol l^{-1} with a yield of 80 % of theoretical maximum and a productivity of 0.61 g l^{-1} h^{-1}. A mutant strain of *K. pneumoniae*, designated as GEM167, obtained by γ-irradiation produced 21.5 g ethanol l^{-1} with a productivity of 0.93 g l^{-1} h^{-1} (Oh et al. 2011). In addition, it was observed that products obtained from the oxidative pathway like 2,3-butanediol, lactate, succinate were significantly increased while reductive pathway metabolites like 1,3-propanediol and 3-hydroxypropionic acid were much lower compared to the wild type strain.

H_2 was produced by *Enterobacter aerogenes* from the crude glycerol at a rate of 0.126 g l^{-1} h^{-1} (Nishio and Nakashimada 2007; Ito et al. 2005). The glycerol obtained from biodiesel waste streams contains a very high concentration of metal ions and salts, which can interfere with the microbial utilization of such carbon sources. However, halophilic bacteria that normally survive under 0.5–2.5 M salt and, in some cases, even higher can comfortably utilize such glycerol-rich waste streams. Another advantage of using such specialized bacteria is that the chances of microbial contamination in such hypersaline conditions are minimal so costs related to sterilization are negligible. *Halanaerobium saccharolyticum* sp. *saccharolyticum* and *senegalensis* could grow on 2.5 g crude glycerol l^{-1} and 150 g l^{-1} salt to produce H_2 at a final yield of

0.01 g g^{-1} and 0.034 g g^{-1}, respectively. The other fermentation products include CO_2, acetate and interestingly, the former strain also produced 1,3-propanediol, butyric acid and ethanol (Kivisto et al. 2010).

Anaerobiospirillum succiniciproducens is one of the most efficient succinate producers. It produced 19 g succinate l^{-1} when glycerol was used as the sole carbon source in the culture medium (Lee et al. 2004). *Actinobacillus succinogenes* is another natural producer of succinate that produces 4.9 g succinate with a specific yield of 1.3 g g^{-1} glycerol (Lee et al. 2001). A novel succinic acid producing bacterial strain belonging to the family *Pasteurellaceae* and designated as DD1 was isolated from the rumen of a cannulated Holstein cow (Scholten and Dagele 2008). This organism could utilize crude glycerol as a carbon source and produced 8.4 g succinic acid l^{-1} in continuous fermentations, with a high yield and productivity of 1.2 g g^{-1} and 0.9 g l^{-1} h^{-1}, respectively.

Polyhydroxyalkanoates (PHA) are naturally-occurring polyesters that are synthesized by various microorganisms as a reserve material for carbon and energy. When *Methylobacterium rhodesianum* MJ 126-J and *Ralstonia eutropha* DSM11348 were cultured in a medium containing crude glycerol and casein hydrolysates, the amount of polyhydroxybutyrate (PHB) produced was 50 g l^{-1} and the net conversion of glycerol to PHB was 17 % (Bormann and Roth 1999). *Vibrio* BM1, isolated from a marine environment produced 1.4 g PHB l^{-1} after 12 h of cultivation. However when the fermentation time was increased to 40 h, PHB actually dropped to 0.25 g l^{-1} due to the secretion of the enzyme PHB depolymerase (Wei et al. 2011). *Burkholderia cepacia* was shown to utilize not only pure glycerol but also biodiesel-derived crude glycerol to form PHBs at 7.4 g l^{-1} (Zhu et al. 2009). *Zobellella denitrificans* strain MW1, a Gram-negative bacterium isolated from both Germany and Egypt, produced PHBs at 4.27 g l^{-1} when 15 g glycerol l^{-1} was used as the substrate (with a net yield of 0.3 g g^{-1}) (Ibrahim and Steinbuchel 2010). Interestingly, this organism could accumulate PHBs even in the presence of 20 g NaCl l^{-1} which is a routine contaminant in biodiesel derived crude glycerol feedstocks.

Gluconobacter oxydans is used industrially for the production of dihydroxyacetone (DHA). Media optimization as well as the use of an internal loop airlift bioreactor led to a maximal DHA concentration of 156.3 g l^{-1} with a net conversion rate of 89.8 % (Hu et al. 2011). A mutant strain designated as ZJB09112 produced 176 g l^{-1} (with a net yield of 0.87 g g^{-1}) in a DO-stat, fed-batch fermentation lasting 72 h (Hu et al. 2010). The air saturation rate was maintained at 30 % and the pH, initially maintained at 6 for 20 h, was then shifted to pH 5. The same group generated another mutant strain ZJB11001 by UV mutagenesis and by manipulating the DO rate i.e. increasing DO rate to 30 % after the initial 24 h and then to 40 % after 60 h, achieved a maximal DHA production of 209.6 g l^{-1} (Hu and Zheng 2011).

Propionibacterium acidipropionici can utilize glycerol to produce 42 g propionic acid l^{-1} while achieving a yield of 0.67 g g^{-1} glycerol and a productivity of 0.36 g l^{-1} h^{-1} (Barbirato et al. 1997). High glycerol concentrations, however, resulted in a lower cell growth rate. When the initial glycerol was kept at a low concentration of 30 g l^{-1} and then gradually fed at a rate of 0.01 l h^{-1} (from 72 to 120 h fermentation), propionic acid production increased to 44.6 g l^{-1} with a net productivity of 0.2 g l^{-1} h^{-1} (Zhu et al. 2010). A glycerol:glucose co-fermentation, in which the ratio between the two substrates is 4:1, can result in a better utilization of glycerol (Liu et al. 2010b). *Propionibacterium acnes* and *Clostridium propionicum* are other bacteria that are known to utilize crude glycerol to produce propionic acid (Barbirato et al. 1997).

Citric acid, a flavoring and preservative agent is commonly produced by *Yarrowia lipolytica*. This organism produces 35 g citrate l^{-1} with a net yield of 0.44 g g^{-1} glycerol (Papanikolaou et al. 2002). A mutant strain Wratislavia AWG7 that does not produce acetate, produced 154 g citric acid l^{-1} in long-term, repeated batch cultures that corresponded to a yield and net productivity of 0.78 g g^{-1} and 1.05 g l^{-1} h^{-1}, respectively (Rywinska and Rymowicz 2010). Another acetate-negative mutant generated by NTG mutagenesis, A-101-1.22, produced 112 g l^{-1} of the product, however the specific production rate gradually decreased in the late fermentation stages. Therefore, a repeated batch process was conducted in which the citric acid production went up to 124 g l^{-1} with a yield of 0.77 g g^{-1} and a productivity of 0.85 g l^{-1} h^{-1} (Rymowicz et al. 2010).

ε-Poly-L-lysine (ε-PL) is an unusual polyamide of lysine in which amide linkages are formed between the ε-amino and the α-carboxyl group. This naturally

occurring polymer is heat stable and has a strong antimicrobial activity underlying its use in some countries as a natural food preservative. This compound can also act as an emulsifier, drug delivery agent, and disinfectant. *Streptomyces albulus* M-Z18 (generated by UV and NTG mutagenesis) produced 2.7 g ε-PL l^{-1} when 59.5 g glycerol l^{-1} was used (Chen et al. 2010). A two stage pH control strategy in which the pH was initially maintained at 3.5 for the first 36 h and subsequently at 3.8 led to a final ε-PL concentration of 9.13 g l^{-1} and a productivity of 4.76 g l^{-1} day^{-1}. A fed-batch fermentation with the same pH control strategy led to the highest titer of 30.1 g ε-PL l^{-1}.

Poly-γ-glutamic acid (γ-PGA) is a polymer of D- and L-glutamic acid that is used in the food, pharmaceutical and plastic industries. The molecular weight of γ-PGA produced by *Bacillus* sp. is usually very high resulting in high viscosity and difficulty in chemical reagent modification. *B. subtilis* NX-2, isolated from a soil sample, grown in a medium containing 20 g glycerol l^{-1} produced 31.7 g γ-PGA l^{-1} as opposed to 26.7 g l^{-1} in the absence of glycerol. Interestingly, the average molecular weight of γ-PGA also decreased from 2.43 to 1.86; which not only decreased the culture broth viscosity but also improved cell growth and increased γ-PGA production (Wu et al. 2010).

Serratia marcescens used crude glycerol derived from biodiesel wastes to produce the pigment prodigiosin at 583 mg l^{-1} (Tao et al. 2005). Prodigiosin is a red pigment that can induce apoptosis specifically in cancer cell lines and hence, is a potential anti-cancer agent. This pigment is naturally produced by *Pseudomonas magneslorubra* and *Vibrio psychoerythrous*. *Phaffia rhodozyma* ferments crude glycerol to produce 34 mg astaxanthin l^{-1}, a pigment that is usually incorporated in marine feed to enhance the visual appeal of fish (Kusdiyantini et al. 1998). *Aspergillus flavus* NRRL 626 has been used to produce kojic acid, a tyrosinase inhibitor that is used in the cosmetic, pharmaceutical and food industries, from glycerol. A routine fermentation normally yields 18.8 g l^{-1} of this compound in 22 days but media optimization in which 220 g glycerol l^{-1} was used as a C source and 7 g corn steep liquor l^{-1} was used as a N source resulted in a final kojic acid production of 29 g l^{-1} (Coelho et al. 2010).

Pseudomonas aeruginosa utilizes crude glycerol to produce 15.4 g l^{-1} of rhamnolipids, a biosurfactant that has wide environmental applications (Zhang et al. 2005). However, submerged liquid cultures are associated with foaming so an attempt at solid state cultivation (SSC) was made. In this report, a solid medium made of 50:50 (w/w) mixture of sugarcane bagasse and corn meal, impregnated with a 6 % (v/v) solution of glycerol and soybean oil, led to a final rhamnolipid production of 45 g l^{-1} (Camilios-Neto et al. 2010). The same study showed that glycerol derived from biodiesel waste streams actually inhibited the rhamnolipid production.

Candida bombicola produces an extracellular glycolipid known as sophorolipid (SL). This is composed of a disaccharide sophorose attached to a hydroxy-acyl fatty acid group that may be saturated or unsaturated in nature. *Candida bombicola* produces 9 g l^{-1} of the lipid in pure glycerol medium but when grown in the waste stream obtained from a biodiesel plant (containing crude glycerol), up to 60 g sophorolipids l^{-1} was obtained. This dramatic increase in SL production was attributed to low osmotic stress and presence of fatty acids in the crude glycerol (Ashby et al. 2005). *Ustilago maydis* can secrete large amounts of glycolipid type biosurfactants, especially mannosylerythritol lipids and ustilagic acid under certain growth conditions. The supply of trace amino acids, group B vitamins, mannose, erythritol along with 50 g crude glycerol l^{-1} and 20.3 mg ammonium citrate l^{-1} as the nitrogen source resulted in a final glycolipid production of 32.1 g l^{-1} after 8.2 days of a fed batch process in which the pH and temperature were maintained at 4.0 and 30 °C, respectively (Liu et al. 2010a). *Chlorella protothecoides* is a unique member of the algae family that can grow on minimal media and utilize either glycerol or glucose equally well, to form lipids. This alga, when grown on only glycerol, produced lipid with a net productivity of 0.077 g l^{-1} h^{-1}, but when grown in a 9:1 mixture of glycerol and glucose, a lipid productivity of 0.098 g l^{-1} h^{-1} was observed. The use of crude glycerol had absolutely no impact on either specific growth rate or productivity (O'Grady and Morgan 2011).

The conversion of glycerol to single cell oil (SCO) by microbes is another commercially important process due to its use as nutraceutical, pharmaceutical and as a feed ingredient for aquaculture. *Yarrowia lipolytica*, when grown in a medium containing crude glycerol, produced 3.5 g SCO/l at the dilution rate of

0.03/h with maximum productivity of 1.2 g l^{-1} h^{-1} (Papanikolaou and Aggelis 2002). Thraustochytrids—a class of eukaryotic organisms belonging to the phylum *Labyrinthulomycota*, can convert crude glycerol to produce SCO that is especially rich in polyunsaturated fatty acids (PUFA). The strain was able to utilize both neat glycerol as well as crude glycerol obtained from a fish oil processing plant to produce microbial oil with a yield of 320 mg g^{-1} and a PUFA yield of 145 mg g^{-1}, after 6 days of fermentation (Scott et al. 2010). The study indicated that the biomass as well as oil production was higher when crude glycerol was used as a substrate as this is a very rich source of Na^+, K^+ and PO_4^{3-} that are essential for growth. The marine alga *Schizochytrium limacinum* can grow on crude glycerol to produce 4.91 g docosahexaenoic acid/l, an omega-3 PUFA that has wide applications as food additive, aquaculture feed ingredient and pharmaceutical agent (Chi et al. 2007).

Metabolic engineering approaches to improve the synthesis of fuels and chemicals from glycerol

Metabolic engineering of *E. coli*

Until a few years ago, *E. coli* was not considered a suitable organism for fermentative utilization of glycerol since the metabolism of this carbon source required the presence of an external electron acceptor (Tong et al. 1991). However, identification of glycerol fermentative pathways in *E. coli* has opened up a new horizon to engineer this industrial microorganism for production of various reduced molecules (Dharmadi et al. 2006; Murarka et al. 2008; Yazdani and Gonzalez 2007).

Ethanol is the major product of glycerol fermentation in *E. coli*. By deleting the genes responsible for competing by-products, such as succinate and acetate, a near theoretical maximum yield of 0.51 g ethanol per gram of glycerol utilized was achieved (Yazdani and Gonzalez, 2008). In addition, strains were engineered to achieve co-production of either ethanol and H_2 or ethanol and formate (Yazdani and Gonzalez 2008), something not attainable from glucose due to its higher oxidation state. Further studies have established the complementary roles of the respiratory and fermentative pathways for glycerol utilization in *E. coli* under micro-aerobic conditions (Durnin et al. 2009). In a separate study using elementary mode analysis, 15,000 possible pathways of central metabolism were reduced to a total of 28 glycerol-utilizing pathways that support cell function by introducing nine gene-knockout mutations (Trinh and Srienc 2009). These pathways included both aerobic and anaerobic pathways that together supported cell growth and glycerol utilization. The engineered strain following metabolic evolution converted 40 g glycerol l^{-1} to ethanol in 48 h achieving 90 % of the theoretical ethanol yield.

In addition to ethanol, *E. coli* has also been engineered to produce various other useful products, such as 1,3-PDO, 1,2-PDO, PHB, succinate and lactate. Tang et al. (2009) reported production of 1,3-PDO in engineered *E. coli* using glycerol as carbon source. A synthetic operon was made comprising three genes, *dhaB1* and *dhaB2* from *C. butyricum* that encode vitamin B_{12}-independent glycerol dehydratase DhaB1 and its activating factor DhaB2, respectively, and *yqhD* from wild-type *E. coli* that encodes 1,3-propanediol oxidoreductase isoenzyme. With the help of a two-stage fermentation process, the first stage under aerobic conditions to grow the biomass and the second stage under anaerobic conditions to make the product, the authors reported the production of 104 g 1,3-PDO l^{-1} at a rate of 2.6 g l^{-1} h^{-1} with 90 % of the theoretical yield. Genes responsible for 1,3-PDO production in *K. pneumoniae* were also expressed in *E. coli* to increase the productivity (Ma et al. 2009). The recombinant *E. coli* produced 11.3 g 1,3-PDO l^{-1} with the consumption of 40 g glycerol l^{-1}. *Escherichia coli* has also been engineered to produce 1,2-PDO from glycerol by overexpression of native enzyme methylglyoxal synthase (MgsA), glycerol dehydrogenase (GldA) and aldehyde oxidoreductase (YqhD) and an ATP-dependent dihydroxyacetone kinase (DHAK) from *Citrobacter freundii* (Clomburg and Gonzalez 2011). The engineered *E. coli* strain produced 5.6 g 1,2-PDO l^{-1} at a yield of 21.3 % (w/w).

Glycerol was also used as substrate to produce poly(3-hydroxybutyrate) (PHB) in an *arcA* mutant of *E. coli* transformed with *phb* biosynthetic genes of *Azotobacter* sp. (Nikel et al. 2008). After optimization of various bioprocess parameters using statistical design, 10.81 g PHB l^{-1} was achieved in 60 h in fed-batch cultivation under microaerobic condition. A related compound, poly(3-hydroxypropionate) [poly (3HP)], was synthesized in *E. coli* by heterologous

expression of (i) glycerol dehydratase DhaB1 from *C. butyricum*, (ii) propionaldehyde dehydrogenase PduP from *Salmonella enterica* and (iii) PHA synthase PhaC1 from *R. eutropha.* Poly(3HP) accumulated up to 12 % (w/w) in an engineered *E. coli* strain.

Succinate production from glycerol has also recently been reported by metabolically engineered *E. coli* strains (Blankschien et al. 2010). The pathways leading to synthesis of competing by-products lactate, ethanol and acetate were blocked and pyruvate carboxylase of *Lactococcus lactis* was expressed to convert pyruvate (instead of phosphoenolpyruvate) into 4-C intermediates required for succinate production. The engineered bacterium had a maximum specific productivity of ~400 mg succinate g cell^{-1} h^{-1} and a yield of 0.69 g succinate g^{-1} glycerol. In an effort to re-engineer *E. coli* to produce succinate from glycerol without the use of any foreign gene, another study deleted native *ptsI* and *pflB* genes and mutated the *pck* promoter to upregulate the expression of native phosphoenolpyruvate carboxykinase (Zhang et al. 2010). The *ptsI* deletion was introduced to block the glycerol dehydrogenase and dihydroxyacetone kinase pathway of glycerol utilization and increase the yield of succinate. All three mutations together were responsible for fermentative metabolism of glycerol to succinate at 80 % of the maximum theoretical yield.

D-Lactic acid was also manufactured in *E. coli* using glycerol as carbon source by overexpressing GlpK-GlpD/GldA-DHAK pathways and D-lactate dehydrogenase enzymes and blocking the synthesis of succinate, acetate and ethanol. The engineered strain produced 32 g D-lactate l^{-1} from 40 g glycerol l^{-1} at a yield of 85 % of the theoretical maximum (Mazumdar et al. 2010).

Metabolic engineering of other microbes—*Corynebacterium glutamicum*, *Klebsiella pneumoniae* and *Gluconobacter oxydans*

Corynebacterium glutamicum is known for producing high amounts of amino acids for various biotechnological applications. However, this organism cannot utilize glycerol. Rittmann et al. (2008) expressed *E. coli* genes for glycerol kinase (*glpK*), glycerol-3-phosphate dehydrogenase (*glpD*) and aquaglyceroporin (*glpF*) in a glutamate- and lysine-producing *C. glutamicum* and showed that glutamate and lysine could be produced from glycerol as sole carbon substrate. As previously discussed, *K. pneumoniae* has been used extensively to produce 1,3-PDO from glycerol. Further improvements to 1,3-PDO production have been investigated through a mutation in capsular polysaccharides gene (CPS) of *K. pneumoniae* that resulted in 78 g 1,3-PDO/l with a yield of 0.43 g g^{-1} and productivity of 1.95 g l^{-1} h^{-1} (Guo et al. 2010). In another study, a gene, *puuC*, involved in production of 3-hydroxypropionic acid was overexpressed in *K. pneumoniae*, which led to co-production of 3-HP and 1,3-PDO at the titer of 16 g and 16.8 g l^{-1}, respectively with cumulative yield of 51 % on glycerol carbon (Ashok et al. 2011).

Gluconobacter oxydans is commonly used for production of dihydroxyacetone (DHA) (Habe et al. 2010). However, the high glycerol content in the medium inhibits DHA production. Deletion of membrane-bound alcohol dehydrogenase gene (*adhA*), however, markedly improved the capability of *G. oxydans* to convert high concentration of glycerol at 230 g l^{-1} into 140 g DHA/l. In addition, overexpressing the glycerol dehydrogenase gene (*sldAB*) in this organism has been shown to increase the concentration of DHA from 18–25 to 30 g l^{-1} and reduce the inhibitory effect of DHA on cell viability (Gatgens et al. 2007).

Conclusions

The abundance and low price of glycerol has made it an ideal feedstock to exploit for the production of fuels and chemicals. In addition, the reduced nature of carbon atoms in glycerol makes the biological production of reduced compounds from glycerol advantageous compared to more oxidized substrates. Capitalizing on these characteristics, recent research efforts have focused on the use of both native and engineered organisms to convert glycerol into value-added products. Currently, the use of native organisms has provided a route to numerous compounds with 1,3-PDO production drawing the most industrial attention. In addition to 1,3-PDO, native organisms have been utilized for the production of high titers and yields of ethanol, propionic acid, polyhydroxyalcanoates, citric acid, and dihydroxyacetone among others. Furthermore, advancements in the field of metabolic

engineering has provided an additional avenue to utilize glycerol as a carbon source for fuel and chemical production in organisms such as *E. coli*. Through various metabolic engineering strategies, *E. coli* has been engineered for the production of compounds such as ethanol. 1,2-PDO, succinic acid, D-lactic acid, and poly(3-hydroxybutyrate) (Yazdani and Gonzalez 2008; Clomburg and Gonzalez 2011; Blankschien et al. 2010; Mazumdar et al. 2010; Nikel et al. 2008). Further advancements in the fields of metabolic engineering and synthetic biology should increase the number of products that can be synthesized from glycerol and help to determine how many of these processes can be commercially successful.

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