Green Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Green Chem.*, 2016, **18**, 4657

Production of 1,3-propanediol from glycerol *via* fermentation by *Saccharomyces cerevisiae*†

Betina Tabah,[a] Alexander Varvak,[b] Indra Neel Pulidindi,[a] Elizabeth Foran,[b] Ehud Banin[b] and Aharon Gedanken*[a]

Received 14th January 2016,
Accepted 17th May 2016

DOI: 10.1039/c6gc00125d

www.rsc.org/greenchem

The demand for 1,3-propanediol-based polymers is constantly increasing, necessitating an increase in 1,3-propanediol production. While the processes for the chemical and bacterial synthesis of 1,3-propanediol are well-known, we report for the first time the possibility of glycerol conversion to 1,3-propanediol by a fungal strain. The synthesis of 1,3-propanediol by biological means is extremely lucrative, and to the best of our knowledge, this is the first study focusing on the development of an optimized process for the production of the value-added chemical 1,3-propanediol from what can be considered as industrial waste, glycerol, *via* fermentation using instant baker's yeast (*Saccharomyces cerevisiae*). Various glycerol fermentation conditions (aerobic, semi-aerobic, and anaerobic) were tested at different reaction temperatures (25, 30, and 37 °C). Under optimal reaction conditions (anaerobic fermentation at 25 °C), 42.3 wt% 1,3-propanediol yield was achieved with 93.6 wt% glycerol conversion.

Introduction

The conversion and utilization of biorenewable feedstock for the production of valuable materials has become an important research trend in recent years due to the decreasing supply of non-renewable resources, escalating global energy demand, and negative environmental impact.[1–3] Emerging biofuel technology produces numerous by-products and waste, ranging from corn fiber and glycerol to animal manure, which serve as a basis for additional sources of bioenergy (liquid biofuels and biogas).[4] Once considered a valuable by-product, crude glycerol is rapidly becoming a 'waste product' with an associated disposal cost.[5] In 2007, due to the rapid growth of biodiesel production, the price of pure glycerol decreased from $1.50 per kg to $0.66 per kg and the price of crude glycerol dropped from $0.55 per kg to $0.11 per kg. Manufacturers are forced to invest large amounts of money in removing the unwanted glycerol from their plants.[6] It is, therefore, crucial to develop environmental-friendly solutions for glycerol waste. Interest in this new field of research, known as glycerol chemistry, has recently grown, raising possibilities for the use of unrefined glycerol, which, in turn, facilitates the sustainability of the biofuel market.[7]

One of the promising strategies for glycerol utilization is the production of propanediols through selective glycerol hydrogenolysis. This process provides a clean and economically competitive route for the production of commercially valuable propanediols from renewable glycerol rather than from non-renewable petroleum.[8] 1,3-Propanediol has received recent attention as a high-value specialty chemical used primarily in the preparation of polyester fibers, films, and coatings. It is a non-flammable, low toxicity liquid which is miscible with water, alcohols, and ethers, making it easy to transport.[2] In 2012, global demand for 1,3-propanediol was 60.2 kt with a market value of $2.61 per kg.[6] Considering 1,3-propanediol is used in the textile industry, food packaging, lubricants, and medicine, demands will continue to rise.[9] The biodegradable nature, higher light stability, and solubility of 1,3-propanediol-based polyesters in most common solvents add to its already growing list of applications.[10,11] By 2019, global demand is expected to reach 150 kt and the price of 1,3-propanediol is estimated to reach $3.73 per kg.[6] The high price of 1,3-propanediol indicates the economic sustainability of the glycerol conversion process.

[a]*Department of Chemistry and Institute for Nanotechnology and Advanced Materials (BINA), Bar-Ilan University, Ramat-Gan 5290002, Israel.*
E-mail: gedanken@mail.biu.ac.il; Fax: +972-3-7384053; Tel: +972-3-5318315
[b]*The Mina and Everard Goodman Faculty of Life Sciences and Institute for Nanotechnology and Advanced Materials (BINA), Bar-Ilan University, Ramat-Gan 5290002, Israel*

† Electronic supplementary information (ESI) available: ^{13}C NMR spectra of authentic glycerol and 1,3-propanediol (Fig. S1), ^{13}C NMR spectra of authentic ethanol, propionic acid, acetic acid, lactic acid, and formic acid (Fig. S2), HPLC chromatograms of metabolites from fermentation of glycerol (0.1 M) by *Saccharomyces cerevisiae* (3 g) at different temperatures (Fig. S3), HPLC chromatograms of organic acids from fermentation of glycerol (0.1 M) by *Saccharomyces cerevisiae* (3 g) at different temperatures (LA: lactic acid, FA: formic acid, AA: acetic acid, PA: propionic acid) (Fig. S4), ^{13}C NMR spectra of metabolites from fermentation of glycerol (0.1 M) by *Saccharomyces cerevisiae* (3 g) at different temperatures (Fig. S5). See DOI: 10.1039/c6gc00125d

The selective conversion of glycerol to 1,3-propanediol is still regarded as a challenging process.[7] Although several chemical conversions of glycerol have previously been explored and analyzed, they are less advantageous than biological conversion. Among the drawbacks of using chemical methods for the production of 1,3-propanediol are the requirements for high temperatures and pressures, use of expensive chemical catalysts, the addition of toxic organic solvents, production of unwanted by-products, release of toxic intermediates, dependence on non-renewable materials, and low product yields.[2,12,13] In contrast, the biological conversion of glycerol to 1,3-propanediol is comparatively more environmental friendly than chemical conversions, and, from an economic perspective, it is generally more advantageous since milder conditions are used, less energy is required, and greater yields are attainable for specific products. Furthermore, pursuing the biological route towards 1,3-propanediol production is particularly appealing, since it utilizes renewable feedstock and cultivation is performed at much lower temperatures and pressures, and generates no toxic by-products.[2,14,15]

Biotechnological methods have been widely used to obtain 1,3-propanediol from glycerol using bacteria of the genera *Clostridia*, *Klebsiella*, *Citrobacter*, *Lactobacilli*, and *Escherichia*.[16–20] However, despite the fact that many microorganisms are able to metabolize glycerol in the presence of external electron acceptors (respiratory metabolism), few are able to do so fermentatively (*i.e.* in the absence of electron acceptors).[21,22] Although the bioconversion of glycerol to 1,3-propanediol *via* fermentation has been extensively investigated, it appears that only several species of the Enterobacteriaceae family such as *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter agglomerans*, *Lactobacilli brevis*, and *Lactobacilli buchneri*, as well as *Clostridium butyricum* and *Clostridium pasteurianum*, are able to form 1,3-propanediol.[2,21,23,24–38] The dissimilation of glycerol in these organisms is strictly linked to their capacity to synthesize the highly reduced product 1,3-propanediol.[39] The potential for using these organisms at the industrial level is limited due to their pathogenicity, the requirement for strict anaerobic conditions, the need for rich nutrient supplementation, and the lack of availability of the genetic tools and physiological knowledge necessary for their effective manipulation.[40,41] In addition, because reducing power must be generated during the fermentation process, only a portion of the glycerol can be converted to 1,3-propanediol. Currently established fermentation processes that convert glycerol to 1,3-propanediol reach a maximum yield of 50–60% (mol/mol), with about 40–50% of the glycerol converted to undesirable by-products.[42,43] Finally, it should be noted that none of the abovementioned reports utilized yeast for the conversion of glycerol to 1,3-propanediol, which is the objective of the current study.

The use of microorganisms that are amenable to industrial applications, such as *Escherichia coli* and *Saccharomyces cerevisiae*, is highly desirable. The metabolism of glycerol in *E. coli* has been thought for many years to require the presence of external electron acceptors.[40,44–46] Yazdani *et al.* showed that *E. coli* can fermentatively metabolize glycerol to 1,2-propanediol, and established pathways, mechanisms, and conditions for this process.[4,40] Hong *et al.* also engineered *E. coli* to produce 1,3-propanediol from glycerol by introducing a synthetic pathway.[47] Glycerol transportation and dissimilation pathways of yeast have been intensively studied since the 1960s.[48] Yeast species have been screened and investigated for their potential in converting glycerol waste into various products such as citric acid, biosurfactants, single cell oil, and carotenoids.[49] Li *et al.* reported that *S. cerevisiae* can also be genetically engineered to produce alternative products from glycerol fermentation, which are either not produced naturally or present in low concentrations, such as ethanol and 1,2-propanediol.[48] Also, Jung *et al.* metabolically engineered *S. cerevisiae* strains in order to produce 1,2-propanediol using glycerol as the main carbon source.[50] Moreover, Rao *et al.* engineered *S. cerevisiae* strains to produce 1,3-propanediol at low cost by using D-glucose as feedstock.[51]

Our first report on the feasibility of glycerol conversion to 1,3-propanediol by a fungal strain has formed the basis for the current research.[52] Synthesizing 1,3-propanediol by biological means is extremely important and, to the best of our knowledge, this is the first study to develop an optimized process for the production of the value-added chemical 1,3-propanediol from industrial waste glycerol *via* fermentation using the biocatalyst instant baker's yeast *S. cerevisiae*.

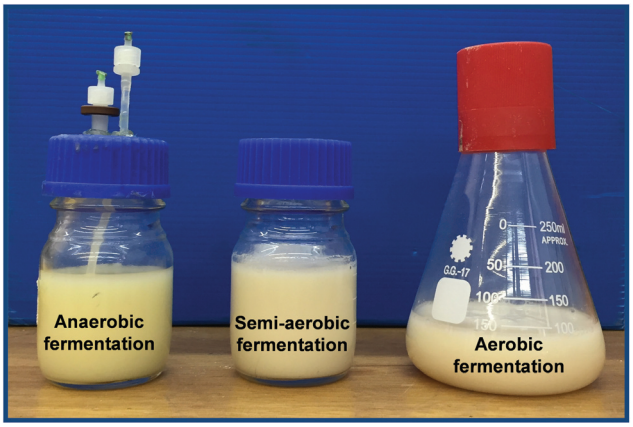
Experimental

Materials

1,3-Propanediol (98%, Product No. P50404) and DL-Lactic acid (90%, Product No. 69785) were purchased from Sigma-Aldrich, Israel. Glycerol (AR, Cat. No. 07120501) and absolute ethanol (AR, Cat. No. 05250502) were purchased from BioLab, Israel. Propionic acid (99%, Cat. No. A0217950001) and formic acid (99%, Cat. No. A0214654001) were purchased from Acros Organics, USA. Acetic acid (Glacial, Cat. No. P2I002102I) was purchased from Carlo Erba Reagents, France. The yeast *Saccharomyces cerevisiae* used for the fermentation of glycerol was purchased from various local supermarkets (instant baker's yeast). The materials were used as received without any pretreatment or further purification.

Fermentation of glycerol

The fermentation of glycerol was performed in an incubator (Heraeus® Functional Line Microbiological Incubator, Thermo Electron Corporation, Germany) at 25, 30, and 37 °C without shaking, under sunlight at 25–35 °C, and in the lab on a hot plate at ~35 °C, with stirring. Glycerol fermentation was performed under aerobic, semi-aerobic, and anaerobic conditions (see Scheme 1 for the experimental setup). The semi-aerobic reactions were performed in Schott Duran bottles containing 100 mL aqueous glycerol (0.1, 0.5, or 1.4 M) and a known amount of *S. cerevisiae* (0.5, 1, or 3 g, dry cell weight). The anaerobic reactions were performed in modified Schott Duran



Scheme 1 The experimental setup for anaerobic, semi-aerobic, and aerobic fermentation.

bottles containing 100 mL aqueous glycerol (0.1 M) and *S. cerevisiae* (3 g, dry cell weight). All the media underwent filter sterilization before the addition of the yeast. Aliquots were collected from the bottles at regular time intervals and, after each sampling, air was replaced by filtered nitrogen gas in order to maintain strict anaerobic conditions. The aerobic reactions were performed in Erlenmeyer flasks (with loose caps) containing 100 mL aqueous glycerol (0.1 M) and *S. cerevisiae* (3 g, dry cell weight). All reactions were performed in triplicate.

NMR analysis

The progress of glycerol metabolism was monitored using NMR spectroscopic analysis on a Bruker Avance DPX 300 instrument. Aliquots were collected from the reaction media at regular time intervals and analyzed. Qualitative analyses of the reaction products were performed by ^{13}C NMR spectroscopy, using D_2O as a solvent.

Yeast cell density measurements and HPLC analysis

The yeast cell optical density was measured using a UV/visible spectrophotometer (Ultraspec 2100 Pro, Amersham Biosciences) at a wavelength of 595 nm (OD_{595}). Cell culture was centrifuged at 16 000*g* for 10 minutes and the supernatant was filtered by HPLC filters (Nylon Syringe, 0.22 μm pore size, 25 mm diameter). Metabolite separation was achieved using the HPLC system (Merck-Hitachi LaChrom System L-7000 equipped with L-7455 Diode Array Detector (DAD) and Schambeck SFD RI 2000 Refractive Index (RI) Detector, Bad Honnef, Germany). Analyses were performed using a 300 × 7.8 mm REZEX-ROA ion-exclusion chromatography column equipped with a matching guard column (Phenomenex, Torrance, CA, USA). The filtered mobile phase of 0.005 N H_2SO_4 was used under isocratic conditions for 45 min at a constant flow rate of 0.5 mL min^{-1} with UV (210 nm) and RI detection at ambient temperature with a 10 μL injection volume. Organic acids (lactic, formic, acetic, and propionic acid) were analyzed at a wavelength of 210 nm (DAD), while ethanol, glycerol, and 1,3-propanediol were analyzed by the RI detector. To calibrate the system, standards of the metabolites were run at preset concentrations, and the areas under the peaks with particular retention times were used to generate a standard calibration curve. EZ Chrom Elite v. 3.1.7 software was used for data acquisition and processing, and the RI signal was acquired using an external analog input.

Results and discussion

Metabolism of glycerol by *Saccharomyces cerevisiae*

While many microorganisms can metabolize glycerol through the respiratory pathway, few are able to do so fermentatively.[21] Our findings have opened up new prospects by showing that *S. cerevisiae* can also fermentatively metabolize glycerol and convert it to an important value-added product, 1,3-propanediol.[52] Here we demonstrate *S. cerevisiae*'s ability to utilize glycerol as a sole source of carbon. Glycerol utilization in S. *cerevisiae* proceeds *via* a two-branch pathway, which results in the synthesis of a glycolytic intermediate, dihydroxyacetone phosphate, and the fermentation product ethanol. Respiratory and fermentative routes mediate the conversion of glycerol to glycolytic intermediates. The respiratory pathway involves glycerol kinase and mitochondrial glycerol 3-phosphate dehydrogenase.[53,54] In the fermentative pathway, glycerol is first converted into dihydroxyacetone by glycerol dehydrogenase and then to dihydroxyacetone phosphate by dihydroxyacetone kinase; however, this pathway is not yet fully described in *S. cerevisiae*.[55] In a number of bacterial species, there exists a separate reductive pathway which consumes NADH to produce 1,3-propanediol from glycerol by the sequential action of glycerol dehydratase (GDHt) and 1,3-propanediol dehydrogenase (PDO DH) *via* a 3-hydroxypropionaldehyde intermediate.[41,56] To the best of our knowledge, the production of 1,3-propanediol has never been reported using *S. cerevisiae*. Future efforts will aim to elucidate the biochemical pathway involved in 1,3-propanediol formation by *S. cerevisiae*.

Following our preliminary research,[52] we investigated the conversion of glycerol to 1,3-propanediol in detail. To maximize the yield of 1,3-propanediol, several reaction parameters such as the glycerol concentration, amount of catalyst, reaction temperature and fermentation type were varied. Initial studies were performed under semi-aerobic conditions using solar heating (25–35 °C) and various initial concentrations of glycerol (0.1, 0.5, and 1.4 M). ^{13}C NMR spectra of the reaction products (after 40 days) from the semi-aerobic fermentation of 0.1, 0.5, and 1.4 M glycerol using 3 g of *S. cerevisiae* are shown in Fig. 1. Irrespective of the initial glycerol concentration, the desired product 1,3-propanediol was observed in all three cases; however, complete conversion was observed only in the case of 0.1 M glycerol (Fig. 1c). In addition to the target product 1,3-propanediol, by-products such as ethanol and propionic acid were also observed (Fig. 1, see ESI Fig. S1 and S2† for ^{13}C NMR spectra of authentic glycerol, 1,3-propanediol, ethanol, and propionic acid). Subsequent studies were conducted using 0.1 M glycerol as a substrate. These results are

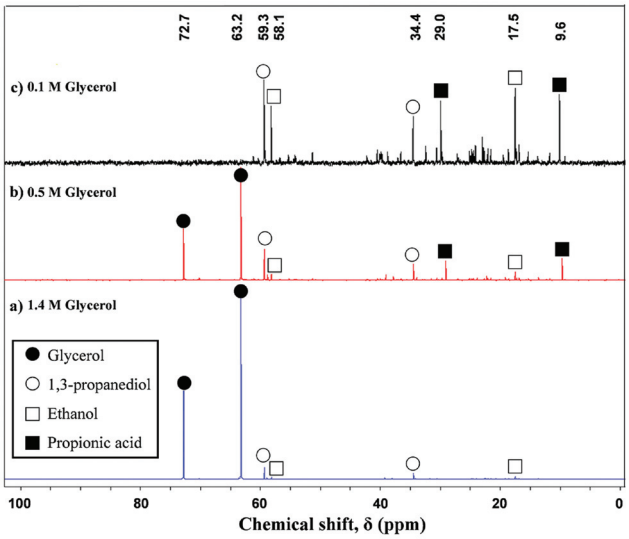


Fig. 1 ^{13}C NMR spectra of solar-heated semi-aerobic fermentation products (after 40 days) from (a) 1.4 M, (b) 0.5 M, and (c) 0.1 M glycerol and *Saccharomyces cerevisiae* (3 g).

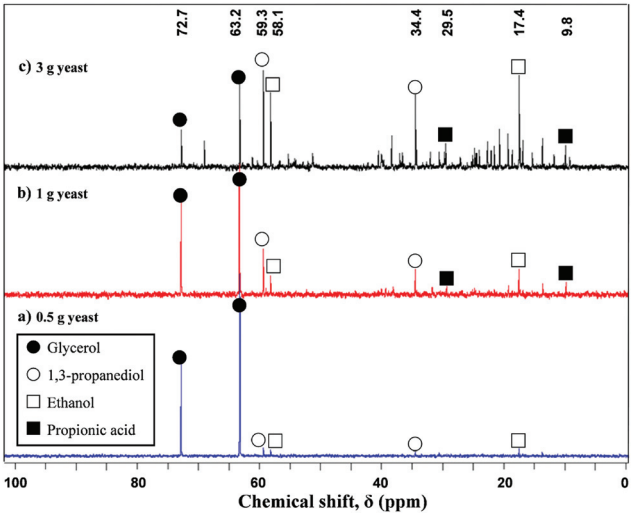


Fig. 2 ^{13}C NMR spectra of hot-plate-heated semi-aerobic fermentation products (after 27 days) from 0.1 M glycerol and (a) 0.5 g, (b) 1 g, and (c) 3 g of *Saccharomyces cerevisiae*.

consistent with our previous report where the secondary metabolite glycerol (maximal concentration of 0.11 M, formed in a solar-energy-driven simultaneous saccharification and fermentation of starch to bioethanol) was converted *in situ* to 1,3-propanediol (22 wt%) within 60 days at ~30 °C.[52]

After obtaining the desired product 1,3-propanediol in solar heated semi-aerobic experiments, the experiments were repeated in the lab on a hot plate (~35 °C) with stirring, and the amount of yeast used for glycerol (0.1 M) conversion was optimized. Different amounts of yeast (0.5, 1, and 3 g) were added to the reaction vessels and the fermentation reaction was monitored for 30 days. ^{13}C NMR spectra of the reaction products obtained on the 27th day using different amounts of *S. cerevisiae* are shown in Fig. 2. Even though the desired product 1,3-propanediol was observed in all three cases, the amount of glycerol converted to 1,3-propanediol was the highest with 3 g *S. cerevisiae*, as evident from the signal intensities of glycerol relative to 1,3-propanediol in the ^{13}C NMR (Fig. 2). Among the tested amounts, 3 g yeast was found to be the most effective, producing the highest amount of 1,3-propanediol from 0.1 M glycerol (Fig. 2c). It should be noted that the fermentation process of the 0.1 M glycerol with 3 g yeast lasted 40 days (Fig. 1c), and was therefore not yet complete by day 27 (Fig. 2c). This data again confirm the necessity of monitoring the reaction for 30–40 days.

In light of these preliminary findings, the fermentation of glycerol (aerobic, semi-aerobic, and anaerobic) was then performed under controlled temperature conditions (25, 30, and 37 °C in an incubator) without shaking for 40 days. Aliquots collected at regular time intervals were analyzed both qualitatively (^{13}C NMR) and quantitatively (HPLC). The yields (wt%) of the target product 1,3-propanediol and other metabolites in the samples collected on the 30th day from the reaction medium at different reaction temperatures (25, 30, and 37 °C) and different fermentation conditions (aerobic, semi-aerobic, and anaerobic) are summarized in the form of a histogram for brevity (Fig. 3). It is interesting to note from the HPLC analysis that the glycerol fermentation products consist of different metabolites such as ethanol, acetic acid, lactic acid, propionic acid, and formic acid, in addition to the desired product 1,3-propanediol (see ESI Fig. S3 and S4† for HPLC chromatograms). The products from glycerol fermentation were further confirmed by ^{13}C NMR analysis, which again showed the presence of organic acids (propionic, acetic, lactic, and formic acid) and ethanol in addition to the desired product, 1,3-propanediol (see ESI Fig. S5 for ^{13}C NMR spectra of metabolites from fermentation of glycerol and Fig. S1 and S2† for ^{13}C NMR spectra of the authentic samples). During the glycerol fermentation process in bacteria, pyruvate is obtained from the glycolysis pathway which competes with 3-hydroxypropionaldehyde for NADH-oxidoreductase (PDO DH) to form other by-products such as ethanol, citric acid, acetic acid, and butanol. Thus, by-product composition differs depending on the microorganisms involved in the process and the process conditions.[6]

The metabolic pathway adopted by yeast (*S. cerevisiae*) in glycerol conversion is directed by both the reaction temperature and the availability of oxygen (fermentation type). For instance, 1,3-propanediol yield was the highest (42.3 wt%) under anaerobic conditions at the lowest reaction temperature studied (25 °C) and there was almost no formation of propionic acid (<1 wt%) (Fig. 3A). We observed similar performance of glycerol metabolism by yeast under anaerobic conditions at the highest reaction temperature (37 °C), where the yield of 1,3-propanediol was high (31.2 wt%). Again, there was almost no formation of propionic acid (<1 wt%) (Fig. 3C). On the contrary, in the semi-aerobic fermentation of glycerol (at 25 °C), where oxygen availability was greater, the propionic acid

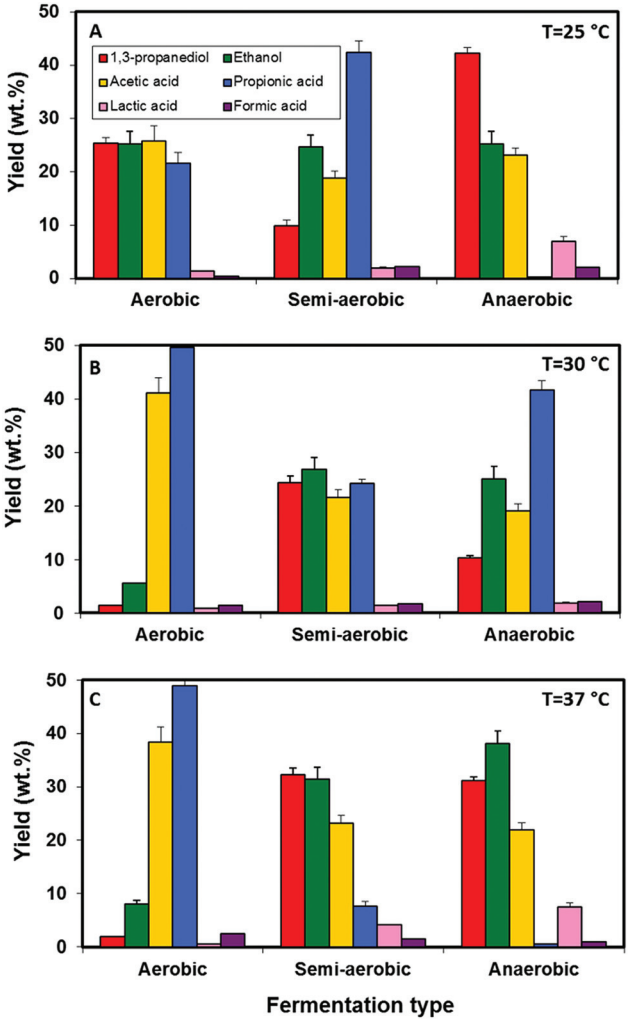


Fig. 3 Yield (wt%) of 1,3-propanediol and other metabolites (ethanol, acetic acid, lactic acid, propionic acid, and formic acid) through fermentation (aerobic, semi-aerobic and anaerobic) of glycerol (0.1 M) by *Saccharomyces cerevisiae* (3 g) at (A) 25, (B) 30, and (C) 37 °C on the 30th day.

pathway was preferred (42.4 wt%, the highest yield at this temperature) over the 1,3-propanediol pathway (9.9 wt%, the lowest yield at this temperature) (Fig. 3A). In the case of aerobic fermentation of glycerol at 25 °C, where there was no restriction to oxygen flow, the microorganism had no preferential pathway, as evident from the nearly equal yields of 1,3-propanediol (25.4 wt%) and propionic acid (21.7 wt%) (Fig. 3A).

When glycerol fermentation was performed under aerobic conditions at the lowest reaction temperature (25 °C), the presence of oxygen did not significantly affect the 1,3-propanediol yield (25.4 wt%) (Fig. 3A). However, aerobic fermentation of glycerol at higher reaction temperatures (either 30 or 37 °C) appeared to have a detrimental effect on the 1,3-propanediol yield (<2 wt%) (Fig. 3B and C). Under these conditions, the microorganisms were exposed to more oxygen, thus switching the pathway towards organic acid synthesis. In fact, the propionic acid yields in the aforementioned cases were *ca.* 50 wt%, which resulted in the suppression of 1,3-propanediol formation (<2 wt%) (Fig. 3B and C). The formation of organic acids such as propionic, acetic, formic, and lactic acid reduces the pH of the reaction medium and inhibits the metabolic pathway for 1,3-propenediol.[56] The optimal pH for the formation of 1,3-propanediol is 6.5–7.5.[6] Apart from the propionic acid formation, the formation of various pyruvate-derived by-products also results in a decrease in 1,3-propanediol yield.[6]

According to the results of Kivisto *et al.*, an acetate by-product that is formed in the glycerol fermentation process has an inhibitory effect on 1,3-propanediol yield.[57] Our observation that 1,3-propanediol yields are lowest (<2 wt%) following aerobic fermentation at 30 and 37 °C, where the yields of acetic acid are highest (*ca.* 40 wt%) (Fig. 3B and C), is consistent with the observations of Kivosto and his group.[57] The presence of excess oxygen, together with higher temperatures, is most likely attributed to the formation of the high amounts of propionic and acetic acids observed, leading to a subsequent reduction in 1,3-propanediol yield. In addition, through the formation of such acidic metabolites, particularly under aerobic conditions and at 37 °C, the yeast lysis was accelerated, as inferred from the decrease in OD_{595} values (Fig. 4C). Przystałowska *et al.* obtained similar results, in which large amounts of acetic acid and other metabolites produced in the culture inhibited the growth of microorganisms, subsequently decreasing the efficiency of 1,3-propanediol synthesis.[58,59] In contrast, in semi-aerobic fermentation at 37 °C, although the yeast lysis was accelerated (lowest OD_{595} values, Fig. 4C), the 1,3-propanediol pathway was preferred (32.3 wt% of 1,3-propanediol yield) due to the presence of lower amounts of organic acids. A similar correlation between 1,3-propanediol yield and yeast survival (lowest OD_{595} value corresponding to the highest 1,3-propanediol yield) was also observed when glycerol fermentation was performed at 25 and 30 °C (Fig. 4A and B).

Reaction kinetics of glycerol conversion using *Saccharomyces cerevisiae*

The effect of two vital reaction parameters, namely, the fermentation type and the reaction temperature, on the conversion of glycerol to 1,3-propanediol are summarized in Table 1. Anaerobic fermentation of glycerol at the lowest reaction temperature (25 °C) yielded the highest amount of 1,3-propanediol (42.3 wt%). This result is consistent with our observation of organic acid pathway suppression under these conditions, due to the absence of oxygen. The maximum theoretical yield of 1,3-propanediol production from glycerol in an ideal anaerobic fermentation process is 72.3 wt%.[6] Therefore, in the current study, under relatively modest fermentation reaction conditions, 58.5 wt% of the theoretical 1,3-propanediol yield was achieved with 93.6 wt% glycerol conversion.

The kinetics of glycerol conversion was systematically studied under various fermentation reaction conditions (aerobic, semi-anaerobic, and anaerobic) and temperatures (25, 30, and 37 °C). For a first-order reaction, a plot of ln[A] (where A is the chemical reactant) *versus* time is a straight line with a negative slope. The reaction rate constant, k, is the nega-

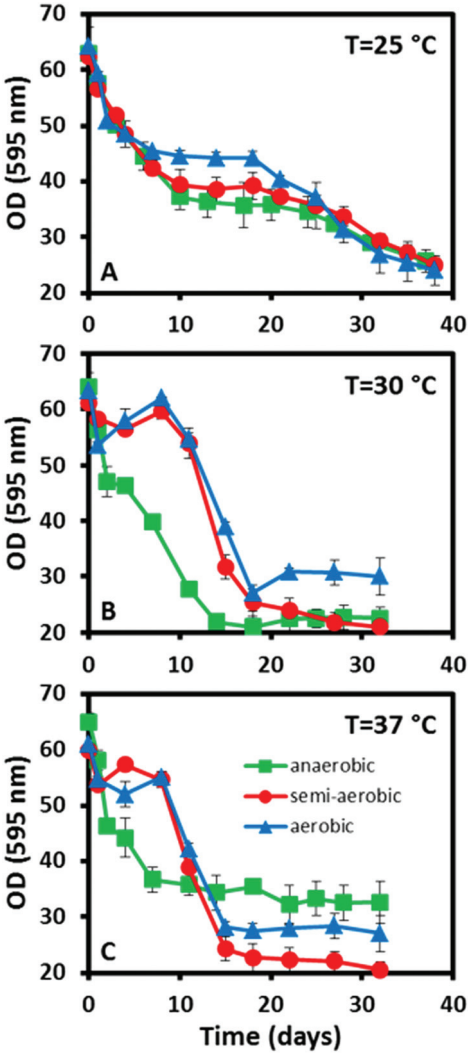


Fig. 4 Survival curves of *Saccharomyces cerevisiae* from different types of fermentation (anaerobic, semi-aerobic, and aerobic) at (A) 25, (B) 30, and (C) 37 °C.

Table 1 Kinetics of glycerol conversion and 1,3-propanediol production through different types of fermentation by *Saccharomyces cerevisiae* at different temperatures

Type of fermentation	Temperature (°C)	Conversion of glycerol (wt%)	Rate constant, k ($\times 10^{-2}$ per day)	Yield of 1,3-PDO (wt%)
Aerobic	25	98.8	25.2	25.4
	30	89.0	11.1	1.40
	37	100	2.14	1.90
Semi-aerobic	25	76.4	3.24	9.90
	30	99.6	2.84	24.4
	37	96.4	3.61	32.3
Anaerobic	25	93.6	3.28	42.3
	30	87.5	4.03	10.3
	37	70.4	8.44	31.2

tive of the slope. The linearity of ln[glycerol] *versus* reaction time indicates that, for all temperatures and fermentation types, glycerol fermentation by *S. cerevisiae* follows first-order kinetics ($R^2 > 0.97$ in all cases, Fig. 5). The values of the reaction rate constants of glycerol conversion by different types of fermentation at different reaction temperatures are shown in Table 1. Usually, for a chemical reaction, the rate is expected to double for every 10 °C increase in the reaction temperature. In fact, such a trend in kinetics was only observed in the case of glycerol fermentation performed under anaerobic conditions where the reaction rate increased from 3.28×10^{-2} to 8.44×10^{-2} per day by raising the temperature from 25 to 37 °C (Fig. 5 and Table 1). Although the rate was enhanced with an increase in temperature under anaerobic conditions, the glycerol conversion and the yield of 1,3-propanediol decreased due to the suppression of *S. cerevisiae* activity by the presence of excess ethanol and acetic acid (Fig. 3C). As Przystałowska *et al.* reported, large amounts of acetic acid and other metabolites produced in the culture inhibit the growth of fermenting microorganisms and subsequently decrease the efficiency of 1,3-propanediol synthesis. Therefore, in further studies

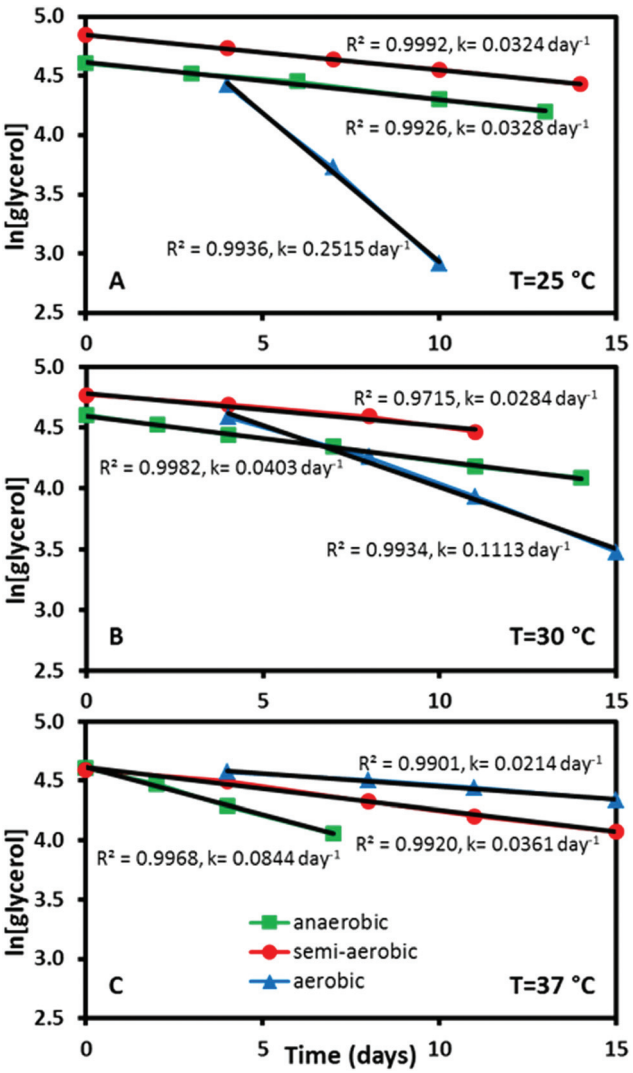


Fig. 5 Effect of fermentation type and temperature on the rate of glycerol conversion.

concerned with optimizing this value-added product, the removal of the growth medium should be considered in order to prevent negative impacts on the yield.[58,59]

The variation over time in the concentration of the target product 1,3-propanediol, at the expense of glycerol, is depicted in Fig. 6. One characteristic inference that can be deduced from the trend observed in 1,3-propanediol production from glycerol using *S. cerevisiae* is that a lower reaction temperature (25 °C) and the absence of oxygen create optimal conditions for 1,3-propanediol production (1.86 mM per day). Although we observed an initial lag period in 1,3-propanediol production under anaerobic reaction at 25 °C, its production increased linearly from day 13 to day 27 and then reached saturation (50.3 mM, 42.3 wt%). Conversely, the propanediol yield was suppressed (<4 mM, <2 wt%) when the reaction was performed in the presence of excess oxygen (aerobic) and at higher reaction temperatures (>25 °C) (0.04 mM per day at 30 °C; 0.12 mM per day at 37 °C). As stated earlier, under these reaction conditions, the yeast *S. cerevisiae* adopts an alternate metabolic pathway and converts glycerol to organic acids (mainly acetic and propionic acids).

Wojtusik *et al.* observed a remarkable impact of reaction temperature on 1,3-propanediol yield in anaerobic glycerol fermentation by the bacterium *Klebsiella oxytoca* when the reaction was performed at six different temperatures over the range of 30–39 °C. The 1,3-propanediol yield was in the range of 28–34 wt% over the temperature range of 30–38 °C, where a surprisingly drastic reduction in the yield from 32 to 8 wt% was observed solely by increasing the reaction temperature from 38 to 39 °C. This is attributed to the inhibition in the growth of the microorganism.[60] Again, in a study by Rodriguez *et al.*, the anaerobic glycerol fermentation by bacterium *Shimwellia blattae* was performed at four different temperatures over the range of 33–39 °C in order to find the optimum temperature conditions for this process. Increasing the temperature from 35 to 37 °C caused a 16 wt% increase in 1,3-propanediol yield (from 46 to 62 wt%), reaching maximum 1,3-propanediol concentration and productivity. Although the 1,3-propanediol yield at 39 °C was similar to that achieved at 37 °C, the productivity was lower at this temperature.[56] Thus, the reaction temperature as well as the fermentation environment seem to affect microorganism glycerol metabolism and, consequently, the yield of 1,3-propanediol.

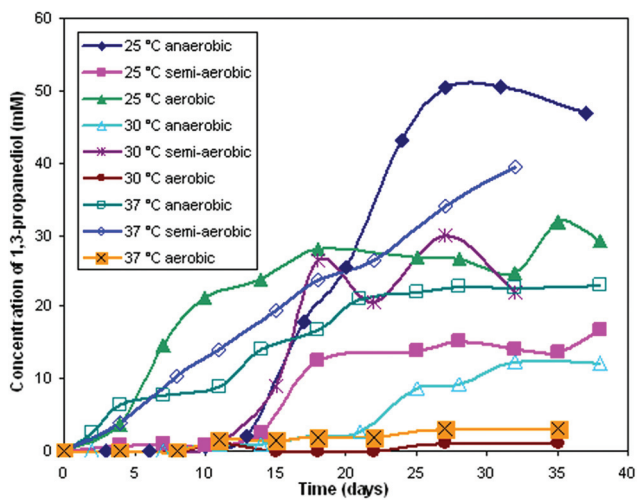


Fig. 6 Concentrations of 1,3-propanediol produced from different types of glycerol (0.1 M) fermentation (anaerobic, semi-aerobic, aerobic) by *Saccharomyces cerevisiae* (3 g) at different temperatures (25, 30, and 37 °C).

Comparison of biological and chemical catalysts for 1,3-propanediol production from glycerol

Biocatalytic 1,3-propanediol production (fermentation) from glycerol using different bacterial strains is summarized in Table 2. Despite the promising reported yields of 1,3-propanediol (23–58 wt%), they are still far from the theoretical yield (72.3 wt%). Although the greatest 1,3-propanediol production was obtained by *K. pneumoniae*, the use of this bacterium in an industrial process is not desirable due to its pathogenicity.[56,61] Our results from this study using the fungal strain *S. cerevisiae* (42.3 wt%) are in line with the data published for bacterial fermentation. It is important to note that the yeast cells in this study are not pathogenic and were not supplemented with any other nutrients, and glycerol was used as the sole carbon source.

In the biological production of 1,3-propanediol from glycerol, the formation of various by-products (acetic acid,

Table 2 1,3-propanediol production from glycerol through biological method (fermentation)

Type of fermentation	Biocatalyst	Yield of 1,3-PDO (wt%)	Ref.
Batch	*S. cerevisiae*	42.3	This work
	C. butyricum DSM5431	46.3	29
	C. butyricum VPI3266	47.9	62
	C. butyricum	34.0	63
	K. pneumoniae	23.0	64
	K. pneumoniae	33.0	65
	K. pneumoniae	34.0	66
	K. pneumoniae DSM2026	43.8	67
	H. saccharolyticum	51.2	57
	C. viterbensis	57.0	68
	C. diolis	32.0	2
	K. oxytoca NRRL-B199	31.0	60
	H. alvei	45.0	69
	S. blattae ATCC33430	49.0	56
Fed-batch	*C. butyricum* VPI3266	57.0	36
	C. butyricum DSM5431	56.2	70
	C. butyricum mutant 2/2	54.5	70
	C. butyricum IK124	54.5	71
	C. butyricum AKR102a	51.2	68
	C. butyricum VPI1718	55.4	72
	C. butyricum VPI1718	54.5	73
	K. pneumoniae ME-308	57.8	74
	K. pneumoniae ME-303	53.7	75
	Engineered *E. coli*	49.0	46
Continuous	*K. pneumoniae* DSM2026	50.4	76
	C. butyricum VPI3266	49.6	62
	C. butyricum VPI3266	53.7	77
	C. butyricum F2b	55.4	78,79
	C. butyricum VPI3266	53.7	72

lactic acid, ethanol, 2,3-butanediol, succinic acid, formic acid, *etc.*) contributes to the reduction of propanediol yield. Taking into account that microbial growth, production rates, and product distribution are affected by operational conditions and media composition, these variables must be optimized in order to develop a cost-effective bioprocess on the industrial scale.[60] Although the research in this field is getting closer to finding a cheap, safe, and efficient method of biotechnological 1,3-propanediol production, much still remains to be accomplished. Within the cultures used in this study, production stopped when all glycerol had been consumed. Therefore, as Przystałowska *et al.* suggested, supply of glycerol at a predetermined rate in fed-batch or continuous fermentation can increase the productivity.[58] Additionally, both fed-batch and continuous-flow fermentations may help overcome substrate inhibition.[80] Further improvement in productivity may be achieved by removing the target product 1,3-propanediol from the fermentation broth.[81] Even though the biotechnological route holds great promise for glycerol conversion to value-added products, the reaction rates are often slow. Thus, in order to have a demand-based supply of useful chemicals and also minimize the risk of large quantities of glycerol being produced as an industrial by-product, parallel green pathways for the catalytic conversion of glycerol should be developed.

Recent developments in catalytic glycerol hydrogenolysis (a chemical method) are summarized in Table 3. Among various chemical catalysts reported, Pt-sulfated zirconia was the most effective catalyst for the conversion of glycerol to 1,3-propanediol (55.6 wt% yield).[82] However, the disadvantages of using a chemical method include the requirements for high temperature and pressure, the use of expensive chemical catalysts and toxic organic solvents, the production of unwanted by-products, the release of toxic intermediates, and dependence on non-renewable materials. The use of *S. cerevisiae* as a biocatalyst, suggested in this report, has the advantage of higher glycerol conversion (93.6 wt%) and higher yield of 1,3-propanediol (42.3 wt%) compared to reported chemical studies (Table 3). This biological route is particularly appealing since it utilizes a renewable feedstock, and fermentation reactions occur at a much lower temperature and atmospheric pressure, with no generation of toxic by-products.

Table 3 1,3-propanediol production from glycerol through the chemical method (catalytic hydrogenolysis)

Catalyst	Conversion of glycerol (wt%)	Yield of 1,3-PDO (wt%)	Ref.
Rh/C + H_2WO_4	32.0	4.00	76
Rh-ReOx/SiO_2	78.0	10.1	83
Pt/WO_3/ZrO_2	86.0	24.2	84
Pt/WO_3/ZrO_2	70.2	32.0	85
Rh-complex catalyst	—	21.0	86
ZrO_2	46.1	16.6	82
Sulfated ZrO_2	53.6	27.3	82
Pt–sulfated ZrO_2	62.9	12.3	82
Pt–sulfated ZrO_2	66.5	55.6	82
Ru–sulfated ZrO_2	83.0	25.7	82
Ni–sulfated ZrO_2	51.7	3.00	82
Cu–sulfated ZrO_2	50.8	3.20	82
Fe–sulfated ZrO_2	51.4	13.8	82
Mn–sulfated ZrO_2	56.4	14.5	82
Al–sulfated ZrO_2	58.2	15.6	82
Pt/STA/ZrO_2	50.2	17.2	82
Pt/STA/ZrO_2	48.4	15.4	82

The superior features of the current approach to 1,3-propanediol production from glycerol are (i) the biotechnological conversion of glycerol using a fungal strain, thereby providing a potentially viable alternative to the existing methods, (ii) the capability of synthesizing 1,3-propanediol from a renewable substrate (industrial waste glycerol) through fermentation, (iii) the simplicity of the methodology and reproducibility of the results (which were similar for various supermarket yeasts), (iv) the availability and low cost of instant baker's yeast *S. cerevisiae*, making the 1,3-propanediol a cheap product, (v) the lack of need for any nutrients to support the *S. cerevisiae* fermentation, (vi) *S. cerevisiae*'s non-restricted applicability in industrial processes due to the fact that unlike previously utilized bacterial strains, *S. cerevisiae* is edible and not pathogenic, and, finally, (vii) the lack of toxic by-products or requirements for high temperatures, expensive catalysts, or a high-hydrogen-pressure reduction step that is usually needed in chemical catalytic processes. Thus, a novel methodology for optimal and green conversion of glycerol to 1,3-propanediol has been developed under modest reaction conditions.

Conclusions

The biological conversion of glycerol to 1,3-propanediol has received attention as it is performed at a low temperature and atmospheric pressure, avoiding the generation of toxic by-products. To the best of our knowledge, we are the first group to report that *S. cerevisiae* can fermentatively metabolize glycerol to 1,3-propanediol. This report focuses on the development of an optimized process for the production of 1,3-propanediol from industrial waste glycerol *via* different types of fermentation (aerobic, anaerobic, and semi-aerobic) using instant baker's yeast, *S. cerevisiae*. The highest 1,3-propanediol yield (42.3 wt%) was achieved under anaerobic fermentation at 25 °C with 93.6 wt% glycerol conversion. Although slower than bacterial fermentation, the ability of *S. cerevisiae* to directly utilize glycerol (in the absence of other carbon sources) is itself a significant finding. 1,3-propanediol production from glycerol is a promising alternative to traditional methods that use non-renewable fossil-based resources. Moreover, as finding alternatives to chemical synthesis remains an important goal, biological synthesis involving non-pathogenic microorganisms as biocatalysts is a significantly promising and innovative green method.

Acknowledgements

This work was supported by the Israel Science Foundation (ISF, Grant No. 598/12) and the Israel Ministry of Science, Technology and Space (Grant Nos. 3-9802 and 3-99763).

References

1 Y. Q. Peng, S. Z. Wang, L. Lan, W. Chen and B. S. Fang, *Eng. Life Sci.*, 2013, **13**, 479–486.
2 G. Kaur, A. K. Srivastava and S. Chand, *Biochem. Eng. J.*, 2012, **64**, 106–118.
3 A. Szabolcs, M. Molnar, G. Dibo and L. T. Mika, *Green Chem.*, 2013, **15**, 439–445.
4 S. S. Yazdani, A. J. Mattam and R. Gonzalez, in *Biofuels from Agricultural Wastes and Byproducts*, ed. H. P. Blaschek, T. C. Ezeji and J. Scheffran, Wiley-Blackwell, Oxford, UK, 2010, DOI: 10.1002/9780813822716.ch6.
5 J. M. Cavalheiro, M. C. M. de Almeida, C. Grandfils and M. M. R. Da Fonseca, *Process Biochem.*, 2009, **44**, 509–515.
6 C. S. Lee, M. K. Aroua, W. M. A. W. Daud, P. Cognet, Y. Pérès-Lucchese, P.-L. Fabre, O. Reynes and L. Latapie, *Renewable Sustainable Energy Rev.*, 2015, **42**, 963–972.
7 A. B. Leoneti, V. Aragao-Leoneti and S. V. W. B. De Oliveira, *Renew. Energy*, 2012, **45**, 138–145.
8 S. S. Priya, V. P. Kumar, M. L. Kantam, S. K. Bhargava and K. V. Chary, *RSC Adv.*, 2014, **4**, 51893–51903.
9 D. A. S. Grahame, T. S. Kang, N. H. Khan and T. Tanaka, *World J. Microbiol. Biotechnol.*, 2013, **29**, 1207–1215.
10 S. S. Umare, A. S. Chandure and R. A. Pandey, *Polym. Degrad. Stab.*, 2007, **92**, 467–497.
11 U. Witt, R. J. Müller, J. Augusta, H. Widdecke and W. D. Deckwer, *Macromol. Chem. Phys.*, 1994, **195**, 793–802.
12 T. Willke and K. Vorlop, *Appl. Microbiol. Biotechnol.*, 2004, **66**, 131–142.
13 W. D. Deckwer, *FEMS Microbiol. Rev.*, 1995, **16**, 143–149.
14 S. Jun, C. Moon, C. Kang, S. Kong, B. Snag and Y. Um, *Appl. Biochem. Biotechnol.*, 2010, **161**, 491–501.
15 R. Dobson, V. Gray and K. Rumbold, *J. Ind. Microbiol. Biotechnol.*, 2012, **39**, 217–226.
16 S. Papanikolaou, S. Fakas, M. Fick, I. Chevalot, M. Galiotou-Panayotou, M. Komaitis, I. Marc and G. Aggelis, *Biomass Bioenergy*, 2008, **32**, 60–71.
17 M. Metsoviti, S. Paramithiotis, E. H. Drosinos, M. Galiotou-Panayotou, G. J. E. Nychas, A. P. Zeng and S. Papa-Nikolaou, *Eng. Life Sci.*, 2012, **12**, 57–68.
18 D. Szymanowska-Powalowska, A. Drozdzynska and N. Remszel, *Adv. Microbiol.*, 2013, **3**, 171–180.
19 W. Rymowicz, A. Rywijska, B. Zlarowska and M. Wojtatowicz, *Food Technol. Biotechnol.*, 2009, **47**, 1–6.
20 R. K. Saxena, P. Anand, S. Saran and J. Isar, *Biotechnol. Adv.*, 2009, **27**, 895–913.
21 S. S. Yazdani and R. Gonzalez, *Curr. Opin. Biotechnol.*, 2007, **18**, 213–219.
22 H. J. Schuller, *Curr. Genet.*, 2003, **43**, 139–160.
23 H. Biebl, K. Menzel, A. P. Zeng and W. D. Deckwer, *Appl. Microbiol. Biotechnol.*, 1999, **52**, 289–297.
24 S. Abbad-Andaloussi, E. Guedon, E. Spiesser and H. Petitdemange, *Lett. Appl. Microbiol.*, 1996, **22**, 311–314.
25 F. Barbirato, C. Camarasa, J. P. Claret Grivet and A. Bories, *Appl. Microbiol. Biotechnol.*, 1995, **43**, 786–793.
26 F. Barbirato, J. P. Grivet, P. Soucaille and A. Bories, *Appl. Environ. Microbiol.*, 1996, **62**, 1448–1451.
27 F. Barbirato, E. H. Himmi, T. Conte and A. Bories, *Ind. Crops Prod.*, 1998, **7**, 281–289.
28 H. Biebl, *Appl. Microbiol. Biotechnol.*, 1991, **35**, 701–705.
29 R. Boenigk, S. Bowien and G. Gottschalk, *Appl. Microbiol. Biotechnol.*, 1993, **38**, 453–457.
30 T. Colin, A. Bories and G. Moulin, *Appl. Microbiol. Biotechnol.*, 2000, **54**, 201–205.
31 R. Daniel, R. Boenigk and G. Gottschalk, *J. Bacteriol.*, 1995, **177**, 2151–2156.
32 E. H. Himmi, A. Bories and F. Barbirato, *Bioresour. Technol.*, 1999, **67**, 128–231.
33 H. Huang, C. S. Gong and G. T. Tsao, *Appl. Biochem. Biotechnol.*, 2002, **98**, 687–690.
34 B. Otte, E. Grunwaldt, O. Mahmound and S. Jennewein, *Appl. Environ. Microbiol.*, 2009, **75**, 7610–7616.
35 U. Plugmacher and G. Gottschalk, *Appl. Microbiol. Biotechnol.*, 1994, **41**, 313–316.
36 H. Schutz and F. Radler, *Syst. Appl. Microbiol.*, 1984, **5**, 169–178.
37 G. Yang, J. Tian and J. Li, *Appl. Microbiol. Biotechnol.*, 2007, **73**, 1017–1024.
38 G. Kaur, R. Sharma, A. K. Srivastava and S. Chand, *Appl. Biochem. Biotechnol.*, 2012, **166**, 138–145.
39 O. M. Bouvet, P. Lenormand, E. Ageron and P. A. Grimont, *Res. Microbiol.*, 1995, **146**, 279–290.
40 A. Murarka, Y. Dharmadi, S. S. Yazdani and R. Gonzalez, *Appl. Environ. Microbiol.*, 2008, **74**, 1124–1135.
41 J. M. Clomburg and R. Gonzalez, *Trends Biotechnol.*, 2013, **31**, 20–28.
42 K. Ahrens, K. Menzel, A. P. Zeng and W. D. Deckwer, *Biotechnol. Bioeng.*, 1998, **59**, 544–552.
43 F. Rieckenberg, I. Ardao, R. Rujananon and A. P. Zeng, *Eng. Life Sci.*, 2014, **14**, 380–386.
44 E. C. C. Lin, *Annu. Rev. Microbiol.*, 1976, **30**, 535–578.
45 J. H. Quastel and M. Stephenson, *Biochem. J.*, 1925, **19**, 660–666.
46 J. H. Quastel, M. Stephenson and M. D. Whetham, *Biochem. J.*, 1925, **19**, 304–317.
47 E. Hong, J. Kim, S. J. Ha and Y. Ryu, *Biotechnol. Bioprocess Eng.*, 2015, **20**, 849–855.
48 C. Li, L. K. Lesnik and H. Liu, *Energies*, 2013, **6**, 4739–4768.
49 R. W. Nicol, K. Marchand and W. D. Lubitz, *Appl. Microbiol. Biotechnol.*, 2012, **93**, 1865–1875.
50 J. Y. Jung and J. W. Lee, *J. Microb. Biotechnol.*, 2011, **21**, 846–853.
51 Z. Rao, Z. Ma, W. Shen, H. Fang, J. Zhuge and X. Wang, *J. Appl. Microbiol.*, 2008, **105**, 1768–1776.
52 B. Tabah, I. N. Pulidindi, V. R. Chitturi, L. M. R. Arava and A. Gedanken, *ChemSusChem*, 2015, **8**, 3497–3503.
53 P. Pavlik, M. Simon, T. Schuster and H. Ruis, *Curr. Genet.*, 1993, **24**, 21–25.
54 M. Krantz, D. Ahmadpour, L. G. Ottosson, J. Warringer, C. Waltermann, B. Nordlander, E. Klipp, A. Blomberg, S. Hohmann and H. Kitano, *Mol. Syst. Biol.*, 2009, **5**, 281.

55 K. O. Yu, S. W. Kim and O. S. Han, *Bioresour. Technol.*, 2010, **101**, 4157–4161.
56 A. Rodriguez, M. Wojtusik, V. Ripoll, V. E. Santos and F. Garcia-Ochoa, *Bioresour. Technol.*, 2016, **200**, 830–837.
57 A. Kivisto, V. Santala and M. Karp, *J. Biotechnol.*, 2012, **158**, 242–247.
58 H. Przystałowska, J. Zeyland, D. Szymanowska-Powałowska, M. Szalata, R. Słomski and D. Lipiński, *Microbiol. Res.*, 2015, **171**, 1–7.
59 X. Tang, Y. Tan, H. Zhu, K. Zhao and W. Shen, *Appl. Environ. Microbiol.*, 2009, **75**, 1628–1634.
60 M. Wojtusik, A. Rodríguez, V. Ripoll, V. E. Santos, J. L. García and F. García-Ochoa, *Biotechnol. Rep.*, 2015, **6**, 100–107.
61 S. Pflugl, H. Marx, D. Mattanovich and M. Sauer, *Bioresour. Technol.*, 2012, **119**, 133–140.
62 V. Jalasutram, A. Jetty and G. R. Anupoju, *Afr. J. Biotechnol.*, 2011, **10**, 493–502.
63 A. Chatzifragkou, G. Aggelis, C. Gardeli, M. Galiotou-Panayotou, M. Komaitis and S. Papanikolaou, *Appl. Microbiol. Biotechnol.*, 2012, **95**, 1541–1552.
64 Z. M. Zheng, K. K. Cheng, Q. L. Hu, H. J. Liu, N. N. Guo and D. H. Liu, *Biochem. Eng. J.*, 2008, **39**, 305–310.
65 K. Petrov and A. Stoyanov, *Bioprocess Biosyst. Eng.*, 2012, **35**, 317–321.
66 M. Rossi, E. A. de Souza, S. H. Flores and M. A. Zachia Ayub, *Renew. Energy*, 2013, **55**, 404–409.
67 P. Ledesma, C. F. Gonzalez, J. D. Breccia and F. Sineriz, *Sci. Aliments*, 2006, **26**, 283–287.
68 M. Seyfried, D. Lyon, F. A. Rainey and J. Wiegel, *Int. J. Syst. Evol. Microbiol.*, 2002, **52**, 1177–1184.
69 A. Drozdzynska, J. Pawlicka, P. Kubiak, A. Kosmider, D. Pranke, A. Olejnik-Schmidt and K. Czaczyk, *New Biotechnol.*, 2014, **31**, 402–410.
70 M. V. Dacunha and M. A. Foster, *J. Bacteriol.*, 1992, **174**, 1013–1019.
71 P. Wittlich, A. Themann and K. D. Vorlop, *Biotechnol. Lett.*, 2001, **23**, 463–466.
72 A. S. Ouattara, A. S. Traore and J. L. Garcia, *Int. J. Syst. Bacteriol.*, 1992, **42**, 390–397.
73 M. Stieb and B. Schink, *Arch. Microbiol.*, 1984, **140**, 139–146.
74 B. Schink and M. Stieb, *Appl. Environ. Microbiol.*, 1983, **45**, 1905–1913.
75 A. Kivisto, V. Santala and M. Karp, *Bioresour. Technol.*, 2010, **101**, 8671–8677.
76 J. Chaminand, L. Djakovitch, P. Gallezot, P. Marion, C. Pinel and C. Rosier, *Green Chem.*, 2004, **6**, 359–361.
77 M. Matsumoto and Y. J. Nishimura, *Biosci. Bioeng.*, 2007, **103**, 236–241.
78 L. Huang, Y. Zhu, H. Zheng, G. Ding and Y. Li, *Catal. Lett.*, 2009, **131**, 312–320.
79 T. M. Che, *US Patent* 4642394, In: Corporation C, editor, 1985.
80 T. C. Ezeji, N. Qureshi and H. P. Blaschek, *Appl. Microbiol. Biotechnol.*, 2004, **63**, 653–658.
81 N. Qureshi and H. P. Blaschek, *Renew. Energy*, 2001, **22**, 557–564.
82 J. Oh, S. Dash and H. Lee, *Green Chem.*, 2011, **13**, 2004–2007.
83 Y. Shinmi, S. Koso, T. Kubota, Y. Nakagawa and K. Tomishige, *Appl. Catal., B*, 2010, **94**, 318–326.
84 T. Kurosaka, H. Maruyama, I. Naribayashi and Y. Sasaki, *Catal. Commun.*, 2008, **9**, 1360–1363.
85 L.-Z. Qin, M.-J. Song and C.-L. Chen, *Green Chem.*, 2010, **12**, 1466–1472.
86 Y. Zheng, X. Chen and Y. Shen, *Chem. Rev.*, 2008, 108.

