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MINI-REVIEW

Present state and perspective of downstream processing of biologically produced 1,3-propanediol and 2,3-butanediol

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Abstract 1,3-Propanediol and 2,3-butanediol are two promising chemicals which have a wide range of applications and can be biologically produced. The separation of these diols from fermentation broth makes more than 50% of the total costs in their microbial production. This review summarizes the present state of methods studied for the recovery and purification of biologically produced diols, with particular emphasis on 1,3-propoanediol. Previous studies on the separation of 1,3-propanediol primarily include evaporation, distillation, membrane filtration, pervaporation, ion exchange chromatography, liquid–liquid extraction, and reactive extraction. Main methods for the recovery of 2,3-butanediol include steam stripping, pervaporation, and solvent extraction. No single method has proved to be simple and efficient, and improvements are especially needed with regard to yield, purity, and energy consumption. Perspectives for an improved downstream processing of biologically produced diols, especially 1,3-propanediol are discussed based on our own experience and recent work. It is argued that separation technologies such as aqueous two-phase extraction with short chain alcohols, pervaporation, reverse osmosis, and in situ extractive or pervaporative fermentations deserve more attention in the future.

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Introduction

During the last few years, considerable efforts and progresses have been made in the production of bio-based bulk chemicals from renewable resources as the price of petrochemical feedstocks continuously increases and their availability diminishes (Hermann and Patel 2007). Among the promising bulk chemicals, 1,3-propanediol (1,3-PD) and 2,3-butanediol (2,3-BD) are two bio-based diols which have a wide range of applications in cosmetics, foods, transport fuels (e.g., as antifreezes, lubricants, or fuel additives), and medicines as well as in the production of polymers. Because of their different structures and special properties, the application of 1,3-PD is mainly in the production of polymers, such as polyesters, polyethers, and polyurethanes, while 2,3-BD is especially in asymmetric syntheses and fuel additives. For example, a 1,3-PD-based new polyester, poly(trimethylene terephthalate) (PTT) has received much attention because of several unique properties for the production of fibers (Kurian 2005). 2,3-BD has been shown to have potential applications in the manufacture of printing inks, perfumes, fumigants, moistening and softening agents, explosives and plasticizers, and as a carrier for pharmaceuticals (Syu 2001). It can be readily dehydrated to methylethyl ketone (an excellent organic solvent for resins and lacquers), and to butadiene for the manufacture of synthetic rubber. It can also be easily dehydrogenated into acetoin and diacetyl which are flavoring agents used in dairy products, margarines, and cosmetics (Garg and Jain 1995).

So far 1,3-PD is mainly manufactured by chemical synthesis, which requires expensive catalyzers, high temperature, high pressure, and high level of safety measurement. Considering the yield and recovery of product, environmental protection, and sustainable development of 1,3-PD, much attention has been paid to its microbial production, either based on glycerol or on glucose (Deckwer 1995; Biebl et al. 1999; Hartlep et al. 2002; Zeng and Biebl 2002; Nakamura and Whitedy 2003; Cheng et al. 2007; Mu et al. 2006; Liu et al. 2007; Yang et al. 2007; Xiu et al. 2007a; Laffend et al. 2007;Yazdani and Gonzalez 2007). The chemical synthesis of 2,3-BD is unambiguously more costly than the microbial route and therefore commercial production of this compound would be limited to fermentation. The microbial production of 2,3-BD was developed to a commercial scale during the World War II (Othmer et al. 1945; Wheat et al. 1948). It is receiving renewed interest again in the new wave of white biotechnology because of its wide range of potential uses as a platform chemical and as biofuel or additive as briefly mentioned above (Zeng et al. 1994; Byun et al. 1994; Ghosh and Swaminathan 2003; Saha 2003). In this context, it is also interesting to note that 2,3-BD is a by-product in glycerol-based production of 1,3-PD by some organisms (Biebl et al. 1998). Under certain conditions, both 1,3-PD and 2,3-BD can be produced in quite high concentrations in the glycerol-based fermentation. For instance, the 1,3-PD and 2,3-BD concentrations are 83.56 and 60.11 g/l, respectively, in fed-batch glycerol-based fermentation with sucrose as cosubstrate by a lactate-deficient mutant of *Klebsiella oxytoca* under microaerobic conditions (Yang et al. 2007).

Glycerol is a renewable resource, especially formed as a by-product of alcoholic fermentation, fat saponification, and biodiesel production. Owing to the increased production of biodiesel and oleo-chemicals, there is an increasing surplus of glycerol on the world market. During the manufacture of biodiesel via transesterification of plant oils (such as rape, soya, and palm oils) and animal fats, glycerol is co-produced in a weight ratio of about 10% of the biodiesel. The production of glycerol in Europe has tripled within the last 10 years to ca. 600 thousand tons per year, and its production in the United States already averages more than 100 thousand tons per year (Yazdani and Gonzalez 2007). The surplus of glycerol will increase further as many nations are moving to substitute fossil fuels with more sustainable alternatives. The price of crude glycerol (80%) decreases from 55 cents/kg in 2004 to as low as 4.4 cents/kg in 2006 (Yazdani and Gonzalez 2007). Glycerol can be utilized by chemical and biological routes as shown in Fig. 1. Producing value-added products from glycerol would improve the economic viability of biodiesel manufacture and the biofuel supply chain. Bioconversion of glycerol to value-added products such as 1,3-PD has therefore attracted much attention.

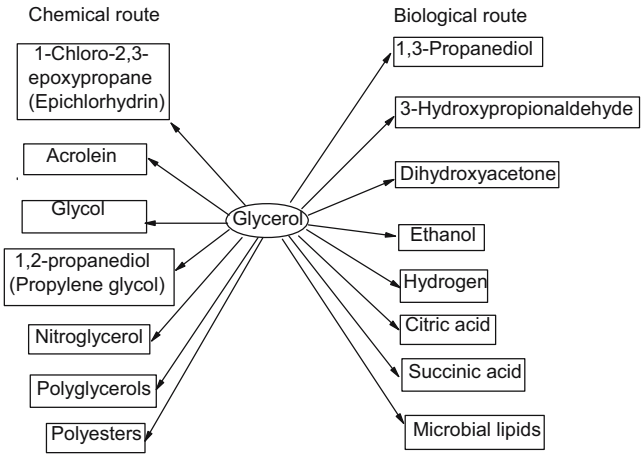

Fig. 1 Examples of potential products produced from glycerol by chemical and biological routes

The separation of 1,3-PD plays an important role in its microbial production. Previously, the production cost of 1,3-PD depends much on the cost of substrate (Deckwer 1995; Hermann and Patel 2007). However, with the availability of cheap and abundant substrates (glycerol or sugar), the cost of downstream processing can make a very high portion in the total production cost, mounting up to about 50–70%. Although the by-products of the fermentation, such as ethanol or acetic acid can be easily separated from 1,3-PD, the concentration of the target product (1,3-PD) is usually not very high in the fermentation broth, i.e., about 5–15% in glycerol-based and glucose-based fermentations. On the other hand, 1,3-PD is very hydrophilic and has a high boiling point. The boiling points of 2,3-BD, 1,3-PD, and glycerol are 184°C, 214°C, and 290°C under normal pressure, respectively. 2,3-BD is a main by-product in 1,3-PD microbial production from glycerol-based fermentation under certain conditions. In fact, it is a challenge to efficiently separate 1,3-PD from a mixture of multiple components, such as 1,3-PD, water, residual glycerol, or glucose, some by-products (e.g., 2,3-BD, ethanol, acetate, lactate, succinate, etc.), macromolecules (e.g., proteins, nucleic acids, polysaccharides), and salts. A similar situation is also encountered with the separation of 2,3-BD from fermentation broths.

This review first summarizes the present state of recovery and purification of biologically produced 1,3-PD and 2,3-BD. The separation methods studied for 1,3-PD mainly include evaporation, distillation, membrane filtration, pervaporation, ion exchange chromatography, liquid–liquid extraction, and reactive extraction. Major methods for the recovery of 2,3-BD are steam stripping, pervaporation, and solvent extraction. The methods for 1,3-PD recovery are then evaluated in terms of yield and energy consumption. Finally, perspectives for an improved downstream processing of biologically produced 1,3-propanediol are discussed based on our recent work.

Present state of recovery and purification of biologically produced 1,3-propoanediol

The downstream processing of biologically produced 1,3-PD usually includes three main steps as shown in Fig. 2. The first step is the removal of microbial cells, mostly by using membrane filtration or high-speed centrifugation, including pretreatment such as adjusting pH by base or adding a flocculant (e.g., chitose or synthetic cationic flocculants based on polyacryamide) into the broth. The second step is the removal of impurities and primary separation of 1,3-PD from the fermentative broth, e.g., using evaporation for removal of water, ethanol and acetic acid, electrodialysis for desalination, alcohol precipitation and dilution crystallization for removal of proteins and salts, solvent extraction and reactive extraction, ion exchange chromatography, adsorption with active charcoal or molecular sieve, and pervaporation with zeolite membrane. The last step is final purification of 1,3-PD by vacuum distillation and/or preparative liquid chromatography.

Pretreatment and solid–liquid separation

Solid–liquid separation techniques, e.g., microfiltration, centrifugation, and decanting, are usually used for the removal of cells from fermentation liquors. Flocculation precipitation attracts attention in industrial scale due to its simplicity, if cheap and effective flocculants are available. Chitosan and polyacrylamide have been tested for this purpose (Grothe 2000; Hao et al. 2006). Before centrifugation, the fermentation suspension was adjusted to pH 4 by phosphatic acid (Grothe 2000). The concentration of protein in the supernatant decreased from 0.6 g/l (pH=7) to 0.4 g/l (pH=4) (Grothe 2000). In another case, it is emphasized that adding base into the fermentation broth to raise the pH to a suitable level before distillation can reduce not only the reaction between acid and alcohol and thus the formation of ester, but also impurity formation during isolation of 1,3-PD, especially pigments in the broth (Kelsey 1996; Ames 2002).

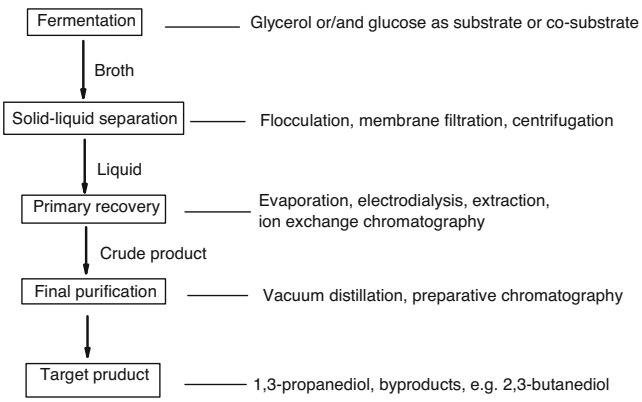

Fig. 2 General scheme and major methods studied for the recovery and purification of 1,3-propanediol from fermentation broth

Evaporation/distillation

The conventional evaporation and distillation techniques normally used in the removal of water and purification of 1,3-PD suffer from the problem of high energy consumption, leading to a high cost of the target product purified in this way. Compared with single-stage evaporation, multi-stage evaporation and down film vacuum evaporator can save much energy (Hermann and Patel 2007; Grothe 2000). After dewatering in a falling film evaporator, two vacuum rectification columns are used for the removal of water and acids and recovery of 1,3-PD, respectively (Grothe 2000). Isobaric vapor–liquid equilibrium data for the binary system (water 1,3-PD) and for the ternary system (water 1,3-PD glycerol) were determined (Grothe 2000; Sanz et al. 2001). The distillation point of 1,3-PD is in fact 214°C in the binary system under normal pressure. Vacuum distillation would save energy due to the decline of boiling points. For instance, the boiling points of 1,3-PD and glycerol were calculated to be 139.0°C and 202.5°C, respectively, in the ternary system at a vacuity of 0.095 MPa according to the Antoine equation (Sanz et al. 2001). Before distillation, desalination and deproteinization are required. Otherwise, the soluble macromolecules would be salting out after evaporation. The viscous slurry leads to low efficiency of evaporation/distillation and low yield of the target product. Electrodialysis can be used for desalination before evaporation (Gong et al. 2004; Hao and Liu 2005). The soluble proteins as well as salts have been precipitated by adding alcohol into the concentrated broth after evaporation due to alcohol precipitation and dilution crystallization (Gao et al. 2007). A flow sheet for this process is shown as Fig. 3.

Membrane separation

Membrane filtration, zeolite membrane pervaporation, and electrodialysis have been tested for the separation and purification of 1,3-PD (Adkesson et al. 2005; Li et al. 2001a, b, c, 2002; Gong et al. 2004; Hao and Liu 2005).

The fermentation broth of a recombinant *E. coli* culture that has been bioengineered to synthesize 1,3-PD from sugar was subjected successively to microfiltration, ultrafiltration, and nanofiltration, removing molecules or particles having a size greater than 0.2 μm, a molecule weight greater than about 5,000 Daltons, and about 200 to 400 Daltons, respectively (Adkesson et al. 2005). The final filtrate was then treated by ion exchange and distillation. The specific

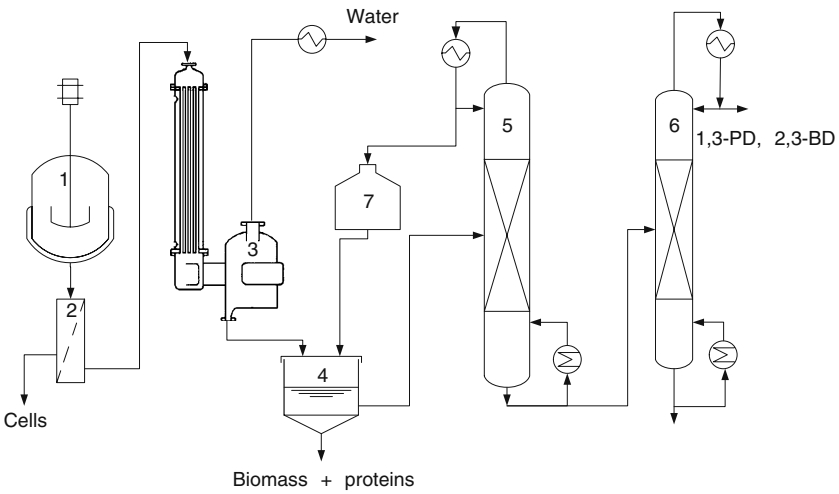

Fig. 3 Flow sheet of downstream processing of 1,3-propanediol from fermentation broth by alcohol precipitation and dilution crystallization (Gao et al. 2007). *1* Fermentator, *2* ultrafiltration module, *3* evaporator, *4* alcohol precipitation chamber, *5* ethanol recovery column, *6* rectifying column, *7* ethanol storage tank

energy consumptions of different membrane filtrations are very distinct, e.g., 2, 5, 7, and 9 kWh power per m^3 permeate for use of microfiltration, ultrafiltration, nanofiltration, and reverse osmosis, respectively (Hermann and Patel 2007).

A Na-ZSM-5 zeolite (Si/Al = 25) membrane was used in the separation of 1,3-PD from glycerol and glucose in water by pervaporation (Li et al. 2001a, b). Binary, ternary, and quaternary (1,3-PD/glycerol/glucose/water) solutions were used as feed mixtures. The separation of 1,3-PD is attributed to adsorption and diffusion. 1,3-PD/glycerol selectivity decreased from 54 to 21 over the temperature range 308–328 K, whereas for the same temperature range 1,3-PD/glucose selectivity increased from 330 to 2,100. The selectivity of 1,3-PD/glycerol was controlled by both preferential adsorption and differences in diffusion rates. The selectivity of 1,3-PD/glucose was considered to be mainly controlled by the differences in diffusion rates, with the larger glucose molecules diffusing through non-zeolite pores.

X-type zeolite membranes were prepared and used to separate 1,3-PD from glycerol in aqueous mixtures by pervaporation (Li et al. 2001c, 2002). The selectivity of 1,3-PD /glycerol was 41 at 300 K and increased with temperature. The high 1,3-PD/glycerol selectivity was due to preferential adsorption of 1,3-PD.

Electrodialysis membrane has been used for desalination before evaporation (Gong et al. 2004; Hao and Liu 2005). The salts could be effectively removed by electrodialysis. However, a low product yield was obtained due to loss of 1,3-PD during electrodialysis. Membrane pollution was observed during electrodialysis.

Chromatography

Ion exchange resin, molecular sieve adsorption, and preparative liquid chromatography have been recently reported in some patents (Roturier et al. 2002; Hilaly and Binder 2002; Corbin and Norton 2003; Wilkins and Lowe 2004; Adkesson et al. 2005) and a journal publication (Cho et al. 2006) for the purification of 1,3-PD.

In the patent applied by Roturier et al. (2002), a solution clarified by the removal of proteins and desalination was passed through a strongly acidic cation exchange resin of the polystyrenesulfonic acid type, on which cation is advantageously selected from the group consisting of lanthanum, lead, zinc, iron, and aluminum, and then a weakly and/or strongly basic anionic resin of the acrylic type. A fermentation medium containing 90.5 g/l of 1,3-PD and 28.7 g/l of glycerol was applied to the column. The chromatography was eluted by using water and gave a first fraction of 39 ml containing 2.0 g/l of 1,3-PD. The sample was diluted 45 times by water, leading to a high energy demand for the dewatering afterwards.

A strong cation exchange resin of polystyrene sulfonate in the Na form was employed to separate 1,3-PD from other impurities (Hilaly and Binder 2002). This process was conducted using a simulated moving bed apparatus. Water was added to elute the feed material. The effluent from 35 to 140 ml (a net volume of 105 ml) was obtained if 10 ml of the feed solution was applied. The original feed solution was thus diluted by ten times. The experiments resulted in a product with purity higher than 87%. The yield of 1,3-PD was more than 95% (Hilaly and Binder 2002).

Besides purification of 1,3-PD, ion exchange comprising a strong acidic cation exchange resin followed by exposing to a weak basic anion exchange resin was also used in the removal of anionic and cationic molecules (Adkesson et al. 2005). Ion exchange resin must be regenerated more frequently due to a large amount of anionic and cationic molecules in fermentative broths.

Adsorption techniques, especially adsorption on hydrophobic zeolites such as silicalite-1 or non-aluminous NaY

zeolites, or even active charcoal, were employed in the separation of 1,3-PD (Günzel et al. 1990; Günzel 1991; Schlieker et al. 1992; Schoellner et al. 1994; Corbin and Norton 2003; Wilkins and Lowe 2004). However, the capacity is quite low. Furthermore, a column of charcoal was also used to remove the proteins (Roturier et al. 2002) as well as pigments.

A preparative liquid chromatography column packed with silica resin was studied to separate 1,3-PD from a mixture containing 1,3-PD and 1,2-PD after phase separation using ethyl acetate (Cho et al. 2006). A mobile phase comprised of 98% ethyl acetate and 2% methanol was chosen to elute the two components. The overall purity and yield of 1,3-PD were 98% and 82% in the purification process, respectively.

Process chromatography was tested for removing the target molecule (e.g., 1,3-PD) in situ with the aim to prevent feedback inhibition of cell growth and product formation in the fermentation process (Wilkins and Lowe 2004). The chromatographic media or adsorbents include activated carbon, zeolites, polymeric neutral resins, chitosan beads, ion-exchange resins, and immobilized complexation materials. The eluent comprises a mixture of water and a non-aqueous eluent, e.g., a short chain alcohol or acetone.

Extraction

Compared with distillation, solvent or reactive extraction is considered to possess several advantages, such as large throughput and low energy consumption. Solvent extraction and reactive extraction have been paid much attention in the last 10 years. Liquid–liquid extraction with organic solvents can be directly applied to the recovery of the target product from dilute solutions, if a suitable solvent can be found. Many efforts have been made to separate 1,3-PD from fermentation broths by extraction. Malinowski (1999) evaluated the application of liquid–liquid extraction for the separation of 1,3-PD from dilute aqueous solutions. Solvent screening was performed by using an extraction screening program (ESP). According to the results of ESP, aliphatic alcohols and aldehydes were selected for experimental testing. Experimental results showed fairly large discrepancies between the predicted and experimental values. The distribution of 1,3-PD into extraction solvents appeared to be not good enough for developing a simple and efficient extraction process. An attempt to separate 1,3-PD from a dilute solution by normal physical or complex extraction was also not successful (Xiang et al. 2001). Although many solvent extractants were listed in a patent (Baniel et al. 2004), including pentanol, propanol, hexanol, oleyl alcohol, 4-methyl-2-pentanone, isopropyl acetate, tributyl phosphate, oleic acid, soya oil, and castor oil, the hydrophilic 1,3-PD in dilute broths is not apt to enter into hydrophobic solvents, except for adding a large amount of solvent into a concentrated broth. Similarly, a hydrophobic solvent, ethyl acetate, was also used in phase separation of 1,3-PD from a mixture containing 1,3-PD, 1,2-PD, glycerol, and glucose (Cho et al. 2006). Most of the glycerol and glucose moved down to the bottom aqueous phase. The top phase (ethyl acetate) contained 1,3-PD and 1,2-PD was used for subsequent chromatographic purification. The maximum solubility of 1,3-PD in ethyl acetate is only 40 g/l. Conventional liquid–liquid extraction process requires the handling of large quantities of solvents and, in particular, its 1,3-PD extraction and separation efficiency is too low. Therefore, other more promising downstream separation processing strategies should be applied to tackle the problem of separating 1,3-PD from a dilute aqueous system.

One such a possible way to tackle the problem is first to convert 1,3-PD into a substance without hydroxyl groups and then to recover it by means of liquid–liquid extraction. This is the so-called reactive extraction. Broekhuis et al. (1994) used chemicals of formaldehyde or acetaldehyde to form a dioxolane derivative of 1,3-PD. Likewise, recovery of propylene glycol (1,2-PD) from aqueous solution was studied in batch experiments using extractants consisting of ion pairs of Aliquat 336 and phenylboronate in 2-ethylhexanol, toluene, *o*-xylene, or diisobutyl ketone (Broekhuis et al. 1996). Up to 80% of the extracted 1,2-PD was back extracted into water after acidification with CO_2. The regeneration of extractant could cause its degradation at temperatures exceeding 110°C. Malinowski (2000) studied a reactive extraction process in which 1,3-PD was converted into 2-methyl-1,3-dioxane (2-MD) through a reversible reaction between 1,3-PD and acetaldehyde catalyzed by a Dowex or Amberlite ion-exchange resin, then 2-MD was extracted using an organic solvent such as *o*-xylene, toluene, or ethylbenzene. 1,3-PD was finally obtained by hydrolyzing 2-MD. This method seems to be very promising for a simulative artificial fermentation broth. It was reported that the yield of 2-MD was 91~92%, the overall conversion of 1,3-PD was 98%, and the recovery of dioxane into the organic extractant was 75%. However, the impurities in real fermentation broths are apt to cause inactivation of the catalyst for reaction between 1,3-PD and acetaldehyde, e.g., a strongly acidic cation-exchange resin. Furthermore, many substances in the broth can react with aldehyde, such as ethanol, 2,3-BD, glycerol as well as soluble proteins (Hao et al. 2005). Moreover, the utilization of extractant (*o*-xylene, toluene, or ethylbenzene) will be limited at a large scale due to their toxicity. Hao et al. (2005, 2006) found that butyraldehyde could act both as reactant and as extractant in reactive extraction. Proteins, cell debris must be removed and ethanol is best removed

before the reactive extraction. 1,3-PD, 2,3-BD, and glycerol react with butyraldehyde to form 1,3-PD acetal (2-propyl-1,3-dioxane), 2,3-BD acetal (2-propyl-4,5-dimethyl-1,3-dioxolane), and glycerol acetal. The acetals produced were hydrolyzed in a reactive distillation column using a strongly acidic cation-exchange resin as catalyst. The bottom product obtained was a mixture of 1,3-PD (407 g/l), 2,3-BD (252 g/l), glycerol (277 g/l), and glycerol acetals (146 g/l). The flow sheet of the reactive extraction process reported by Hao et al. (2006) is shown in Fig. 4. Because of the additional need to regenerate 1,3-PD from its dioxolane derivative, the complexity and the cost of the chemicals used make the extraction and purification process prohibitive (Fig. 4).

Recovery of biologically produced 2,3-butanediol

The biological production of 2,3-BD has been reviewed by Garg and Jain (1995) and Syu (2001), including recovery of 2,3-BD. Compared with the recovery and purification of 1,3-PD, few reports about separation of 2,3-BD have been published in the last decade. The reported separation techniques mainly include steam stripping (Wheat et al. 1948), solvent extraction (Othmer et al. 1945; Tsao 1978; Eiteman and Gainer 1989), reverse osmosis (Sridhar 1989), and pervaporation (Qureshi et al. 1994).

A countercurrent steam stripping was previously developed for recovery of 2,3-BD from whole fermentation broths at pilot plant (Wheat et al. 1948). Obviously, a large amount of energy is required for this process and prevents its application today.

Compared with single distillation, an integrated process of reverse osmosis and distillation can slightly decrease the processing cost (Sridhar 1989). The integrated process is much more economical than distillation combined with extraction using tributylphosphate as extractant. The costs for the recovery of 2,3-BD from a model medium on a production scale of 500 tons per year by using single distillation, reverse osmosis followed by distillation and combination of distillation and extraction were estimated to be 0.73, 0.69 and 1.09 DM/kg 2,3-BD respectively (Sridhar 1989).

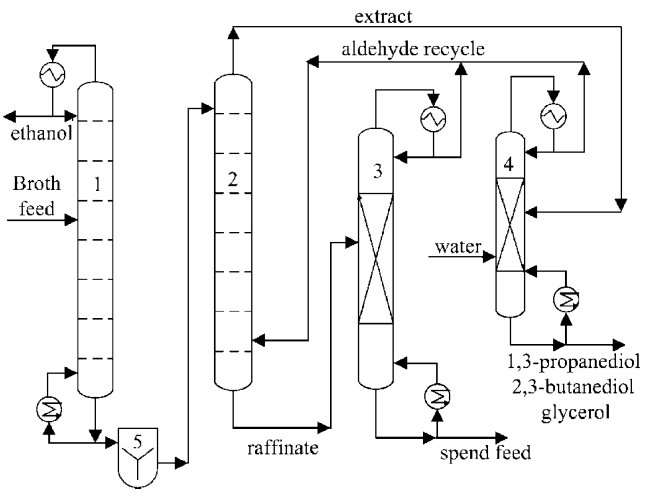

Fig. 4 Flow scheme of downstream processing of 1,3-propanediol fermentation broth by reactive extraction and distillation (Hao et al. 2006). *1* Distillation column, *2* reactive extraction column, *3* aldehyde recovery column, *4* reactive distillation column, *5* flocculation taker

Liquid–liquid extraction has been attracting much attention, including solvent extraction of 2,3-BD (Othmer et al. 1945; Tsao 1978; Eiteman and Gainer 1989) and aqueous two-phase extraction of 2,3-BD in PEG/dextran system (Ghosh and Swaminathan 2003). Alcohols or esters were chosen as solvent extractants, e.g., ethyl acetate, tributylphosphate, diethyl ether, *n*-butanol, dodecanol, and oleyl alcohol. A yield of 75% was obtained using diethyl ether as extractant to extract 2,3-BD from the fermentation slurry (Tsao 1978). Prior to exposure to solvent, the fermentation broth had to be dewatered by evaporation (Othmer et al. 1945) or both microfiltration and reverse osmosis (Sridhar 1989) because of the low partition coefficient and the low selectivity of 2,3-butanediol. Repulsive extraction or salting out using potassium chloride (KCl) or dehydrated K_2CO_3 was also investigated on the recovery of 2,3-BD (Syu 2001) like the salting-out effect of K_2CO_3 on extraction of butanol in acetone–butanol–ethanol fermentation (Xu 2001; Hu et al. 2003). The removal of water from the fermentation broth was also necessary before salting out because the concentration of 2,3-butanediol in the broth was too low to be salted out even if at a saturated KCl or K_2CO_3 solution.

As the reactive extraction of 1,3-propanediol, 2,3-butanediol can react with formaldehyde to form a formal under catalysis of acid (Senkus 1946). The 2,3-butanediol formal is collected in the top oil phase and allowed to react with acid methanol to form 2,3-butanediol and methylal. Methylal can be hydrolyzed to methanol and formaldehyde. Three-step reactions need acids as catalyst. Anticorrosion of devices due to acidity is a main problem in a large scale.

Pervaporation or vacuum membrane distillation used previously in ethanol and butanol fermentations was developed for the concentration of 2,3-BD (Qureshi et al. 1994). Using an integrated process for fed-batch fermentation and recovery of 2,3-BD by vacuum membrane distillation, 2,3-BD is concentrated to over 430 g/l from a fermentation broth. A microporous polytetrafluoroethylene (PTFE) membrane was used in the integrated process, while silicone membrane was usually used in pervaporative ethanol or butanol fermentations. No report about the concentration of 2,3-BD or 1,3-PD using pervaporation through the above organic membranes has been found up to date. However, inorganic zeolite membranes have been

developed to separate 1,3-PD from model solutions using pervaporation (Li et al. 2001a, b, c, 2002).

Challenges and perspectives

The difficulties in developing an efficient process to separate 1,3-PD from fermentation broths are associated with the hydrophilicity of the target product, its high boiling point, and the complexity of the fermentation broth. The abovementioned separation methods and techniques so far studied have some drawbacks or limitations as summarized in Table 1. The conventional evaporation and distillation require not only a high input of energy, but also desalination or deproteinization as pretreatment step(s). The desalination of broth by electrolysis gives a low product yield due to loss of 1,3-PD in the saline effluent. Additionally, the lifetime of electrolysis membrane can be relatively short because of membrane pollution of biomacromolecules, e.g., proteins, polysaccharides, and nucleic acids. A similar situation also occurs in ultrafiltration, nanofiltration, and zeolite membrane pervaporation. The performance (e.g., selectivity) of zeolite mem-

Table 1 Comparison of different separation techniques for 1,3-propanediol

Separation methods or unit operation	Application/investigation	Drawbacks or problems	References
Evaporation/ distillation	Evaporation was used in the removal of water from the fermentation liquors Distillation was used for the final purification of 1,3-PD	Evaporation and distillation suffer from a large amount of energy consumption. In addition, desalination and deproteinization are required before evaporation	Ames 2002; Sanz et al. 2001
Electrodialysis	Electrodialysis has been used for desalination before evaporation	Low product yield due to loss of 1,3-PD during electrodialysis. Membrane pollution can be very serious	Gong et al. 2004; Hao and Liu 2005
Pervaporation	Na-ZSM-5 and X-type zeolite membranes were used to separate 1,3-PD from an aqueous mixture by pervaporation. The high 1,3-PD/glycerol selectivity was due to preferential adsorption of 1,3-PD	The performance of pervaporation needs to be verified by using real fermentative broth in the presence of impurities, e.g., proteins and salts	Li et al. 2001a, b, c, 2002
Chromatography	Combined strongly acidic cationic and weakly basic anionic resins were used to desalinate in the fermentation broth A cationic exchange resin was used for recovery of 1,3-PD	Although high overall purity and yield of 1,3-PD could be obtained, the 1,3-PD solution was not concentrated but diluted because of the low selectivity and capacity of resin or adsorbent. This method consumed more energy than the simple evaporation and distillation	Roturier et al. 2002; Hilaly and Binder 2002; Corbin and Norton 2003; Wilkins and Lowe 2004; Adkesson et al. 2005; Cho et al. 2006
	Adsorption of 1,3-PD on hydrophobic zeolites or active charcoal was investigated for separation of 1,3-PD A preparative silica gel liquid chromatography was used to separate 1,3-PD after phase separation	In addition, the chromatographic matrix had to be regenerated frequently if the feed was not desalinated or deproteinized. This situation also occurred for ion-exchange resins used to desalinate due to high salt concentrations	
Solvent extraction/ liquid–liquid extraction	Many extractants have been investigated for the recovery of 1,3-PD from dilute broth. It is partly partitioned into the solvent phase only when adding a large amount of solvent into a concentrated broth	No effective extractant has been so far found for liquid–liquid extraction of 1,3-PD. Major problem is because 1,3-PD is hydrophilic	Malinowski 1999; Xiang et al. 2001; Baniel et al. 2004; Cho et al. 2006
Reactive extraction	Reactive extraction includes three key steps: reaction, extraction, and hydrolysis. A reversible reaction between 1,3-PD and aldehyde was used to form a dioxolane derivative (e.g., 2-MD). 2-MD is then extracted into an organic solvent and finally hydrolyzed into 1,3-PD	This process is quite complicated. The removal of proteins and ethanol as well as salts is necessary before reaction Additionally, the trace amount of aldehyde in 1,3-PD is prohibitive for polymerization of PTT	Broekhuis et al. 1994, 1996; Malinowski 2000; Hao et al. 2005, 2006

brane pervaporation needs to be verified using real fermentation broth instead of model solutions. Because of the low selectivity and capacity of resin, 1,3-PD solution is normally not concentrated but diluted using ion exchange chromatography or zeolite adsorption. Moreover, the chromatographic medium or matrix (resin, zeolite, charcoal) has to be regenerated frequently if the feed is not desalinated or deproteinized. The chromatography method seems to be economically less suitable for the recovery of 1,3-PD. Liquid–liquid extraction of 1,3-PD may represent a simple and efficient process. Unfortunately, no effective extractants has been so far found for this purpose, though many endeavors have been made. Reactive extraction needs complicated pretreatment (removal of proteins and ethanol as well as salts) and post-treatment (back extraction, hydrolysis, or reactive distillation). Additionally, the trace amount of aldehyde in 1,3-PD is prohibitive for polymerization in the production of PTT.

From the above comparison, it is therefore apparent that there is a need for further research to develop a process which should ideally be simple to carry out and allow the purification of 1,3-PD directly from the fermentation broth. A key challenge to successful separation of 1,3-PD from fermentation broths is how to apply separation technology to large-scale industrial processes in a cost- and time-effective manner that increases productivity and yield. It is worthy of mentioning that no reports about separation of 1,3-PD from its mixture with 2,3-BD has been found up to date. It will be a difficult task because 2,3-BD is similar to 1,3-PD in many aspects. Innovation in separation technology is needed to solve the problems or drawbacks. In this context, we would particularly mention a novel aqueous two-phase extraction based on our recent studies on the recovery of 1,3-PD from whole fermentation broth.

Repulsive extraction or salting out using potassium chloride or dehydrated K_2CO_3 has been used to separate 2,3-BD and butanol from the fermentation broths. The salting-out effect of inorganic salts (e.g., ammonium sulfate) is usually used to remove proteins from an aqueous mixture such as the concentrated fermentation broth (Xiu et al. 2007b). Organic solvent precipitation is also a usual means for removal of proteins, e.g., using alcohol precipitation (Gao et al. 2007). An aqueous two-phase system is formed if adding ethanol and ammonium sulfate together into the glycerol-based fermentation broth, leading to a novel and promising separation process for 1,3-PD (Xiu et al. 2007c). Based on our previous studies of separation of 1,3-PD from broths by using alcohol precipitation and ammonium sulfate salting out, we demonstrated that 1,3-PD can be recovered from fermentation broths by using aqueous two-phase extraction (Xiu et al. 2007c; Fig. 5).

Aqueous two-phase extraction (ATPE) has been widely applied in the separation of biomacromolecules, such as proteins and nucleic acids (Albertsson 1986; Kula et al. 1982) because of its mild conditions and high capacity. Up to now, most aqueous two-phase systems (ATPS) used for purification were based on either a polyethylene glycol (PEG)/salt system or a polymer/polymer (e.g., PEG/dextran) system. It should be mentioned that traditional ATPE has been rarely used in a large scale, especially for production of cheap and bulk chemicals, primarily due to the high cost of the polymers and the difficulty in isolating the extracted molecules from the polymer phase by back extraction. Although some efforts have been made, e.g., extractive fermentation of 2,3-BD in PEG/dextran aqueous two-phase system (Ghosh and Swaminathan 2003), the application of traditional ATPS on bio-based bulk chemicals is less promising.

In fact, short chain alcohols or hydrophilic organic solvents and salts are able to form aqueous two-phase systems (Greve and Kula 1990). This type of aqueous two-phase system has some advantages over the traditional one, such as low cost of extractant, easy recovery of hydrophilic organic solvent by evaporation and obviating the back extraction. Compared with the traditional solvent extrac-

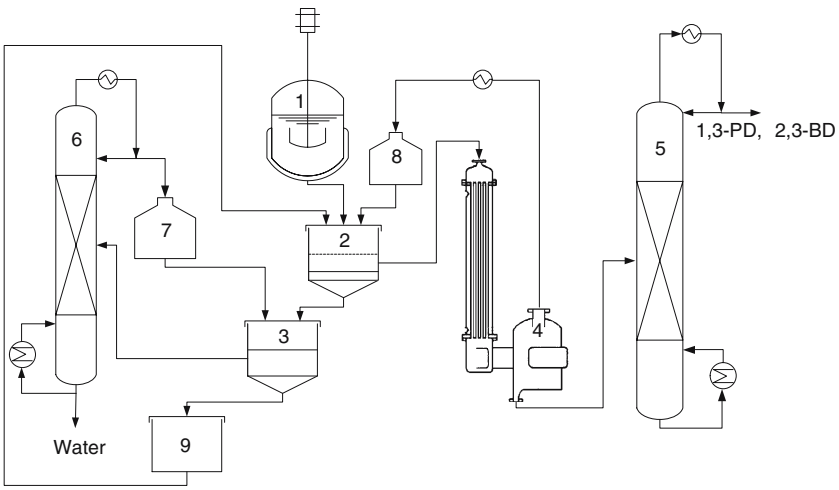

Fig. 5 Flow scheme of aqueous two-phase extraction of 1,3-propanediol from fermentation broth (Xiu et al. 2007c). *1* Fermentator, *2* aqueous two-phase extractor, *3* ammonium sulfate recovery chamber, *4* falling-film evaporator, *5* rectifying column, *6* methanol recovery column, *7* methanol storage tank, *8* ethanol storage tank, *9* ammonium sulfate storage tank

tions and reactive extractions, hydrophilic organic solvents and salts are green in terms of carcinogenic and toxic effects. Such a novel aqueous two-phase extraction was used for the separation of model proteins (Louwrier 1998). Recently, it has been applied to the recovery of natural products from crude extracts, e.g., glycyrrhizin from *Glycyrrhiza uralensis* Fisch (Tan et al. 2002) and salvianolic acid B from *Salvia miltiorrhiza* (Zhi and Deng 2006). However, there has been no reports on using this system to separate bulk chemicals from the fermentation broths. Our experiments showed that the novel ATPSs could be used to extract 1,3-PD from fermentative broth (unpublished results). The highest partition coefficient (4.77%) and recovery of 1,3-PD (93.7%) were obtained in single step extraction by an ATPS composed of 46% (*v*/*v*) ethanol and saturated ammonium sulfate. At the same time, the by-products, e.g., 2,3-BD and acetoin, were also extracted at high efficacy. The maximum selective coefficient of 1,3-PD to glycerol was 6.0 in the experimental range. Additionally, cells and proteins could be simultaneously removed from the fermentation broths. The removal ratio of cells and proteins reached 99.7% and 79.0%, respectively.

Conclusions

Recovery and purification of 1,3-PD and 2,3-BD represent a technological challenge and an economical obstacle for an efficient microbial production of these two promising bulk chemicals in a large scale. Methods and technologies studied so far have their limitations or drawbacks in terms of yield and energy consumption. For further development, classic separation techniques need to be improved or combined with other new technologies. For instance, evaporation may be improved by adopting multi-stage evaporation instead of single-stage evaporation. In situ extractive and pervaporative fermentations could be promising. Furthermore, the novel aqueous two-phase extraction method with short chain alcohols or hydrophilic organic solvents deserves attention in the future.

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References

Adkesson DM, Alsop AW, Ames TT, Chu LA, Disney JM, Dravis BC, Fitzgibbon P, Gaddy JM, Gallagher FG, Lehnhardt WF, Lievense JC, Luyben ML, Seapan M, Trotter RE, Wenndt GM, Yu EK (2005) Purification of biologically-produced 1,3-propanediol, United States Patent 20050069997

Albertsson PA (1986) Partition of cell particles and macromolecules, 3rd edn. Wiley, New York

Ames TT (2002) Process for the isolation of 1,3-propanediol from fermentation broth. US Patent 6. 361. 983 B1

Baniel AM, Jansen RP, Vitner A, Baiada A (2004) Process for producing 1, 3-propanediol. United States Patent 20040222153

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

Biebl H, Menzel K, Zeng AP, Deckwer WD (1999) Microbial production of 1,3-propanediol. Appl Microbiol Biotechnol 52:289–297

Broekhuis RR, Lynn S, King CJ (1994) Recovery of propylene glycol from dilute aqueous solutions via reversible reaction with aldehydes. Ind Eng Chem Res 33:3230–3237

Broekhuis RR, Lynn S, King CJ (1996) Recovery of propylene glycol from dilute aqueous solutions by complexation with organoboronates in ion-pair extractants. Ind Eng Chem Res 35:1206–1214

Byun T-G, Zeng A-P, Deckwer W-D (1994) Reactor comparison and scale-up for the microaerobic production of 2,3-butanediol by *Enterobacter aerogenes* at constant oxygen transfer rate. Bioprocess Eng 11:167–175

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

Cheng K-K, Zhang J-A, Liu D-H, Sun Y, Liu H-J, Yang M-D, Xu J-M (2007) Pilot-scale production of 1,3-propanediol using *Klebsiella pneumoniae*. Process Biochem 42:740–744

Cho M-H, Joen SI, Pyo S-H, Mun S, Kim J-H (2006) A novel separation and purification process for 1,3-propanediol. Process Biochem 41(3):739–744

Corbin DR, Norton T (2003) Process to separate 1,3-propanediol or glycerol, or a mixture thereof from a biological mixture. United States Patent 6 603 048

Deckwer WD (1995) Microbial conversion of glycerol production to 1,3-propanediol. FEMS Microbiol Rev 16:143–149

Eiteman MA, Gainer JL (1989) In situ extraction versus the use of an external column in fermentation. Appl Microbiol Biotechnol 30:614–618

Gao S, Zhang D, Sun Y, Xiu Z (2007) Separation of 1,3-propanediol from glycerol-based fermentations of *Klebsiella pneumoniae* by alcohol dilution crystallization. Front Chem Eng China 1(2):202–207

Garg SK, Jain A (1995) Fermentative production of 2,3-butanediol: a review. Bioresour Technol 51:103–109

Ghosh S, Swaminathan T (2003) Optimization of process variables for the extractive fermentation of 2,3-butanediol by *Klebsiella oxytoca* in aqueous two-phase system using response surface methodology. Chem Biochem Eng Q 17(4):319–325

Gong Y, Tong Y, Wang XL, Liu DH (2004) The possibility of the desalination of actual 1,3-propanediol fermentation broth by electrodialysis. Desalination 161:169–178

Greve A, Kula MR (1990) Cost structure and estimation for the recycling of salt in a protein extraction process system. Bioprocess Eng 6:173–177

Grothe E (2000) Konzeption und Wirtschaftlichkeit der industriellen Glycerinvergärung zu 1,3-Propandiol. Dissertation, VDI Verlag GmbH, Düsseldorf 2000

Günzel B (1991) Mikrobielle Herstellung von 1,3-Propandiol durch Clostridium butyricum und adsorptive Aufarbeitung von diolen. Dissertation, Technischen Universität Braunschweig

Günzel B, Berke CH, Ernst S (1990) Adsorption von Diolen aus Fermentationsmedien an hydrophobe Zeolithe. Chem Ing Tech 62(9):7482750

Hao J, Liu DH (2005) Desalination of fermented broth containing 1,3-propanediol by electrodialysis. Chinese J Proc Eng 5:36–39

Hao J, Liu HJ, Liu DH (2005) Novel route of reactive extraction to recover 1,3-propanediol from a dilute aqueous solution. Ind Eng Chem Res 44:4380–4385

Hao J, Xu F, Liu H, Liu D (2006) Downstream processing of 1,3-propanediol fermentation broth. J Chem Technol Biotechnol 81:102–108

Hartlep M, Hussmann W, Prayitno N, Meynial-Salles I, Zeng AP (2002) Study of two-stage processes for the microbial production of 1,3-propanediol from glucose. Appl Microbiol Biotechnol 60:60–66

Hermann BG, Patel M (2007) Today's and tomorrow's bio-based bulk chemicals from white biotechnology: a techno-economic analysis. Appl Biochem Biotechnol 136:361–388

Hilaly AK, Binder TP (2002) Method of recovering 1,3-propanediol from fermentation broth. United States Patent 6 479 716

Hu B-L, Qiu X-Q, Yang D-J (2003) Separation of butanol–acetone–water system using repulsive extraction. Journal of South China University of Technology (Natural Science Edition) 31(12):58–62

Kelsey DR (1996) Purification of 1,3-propanediol. US Patent 5. 527. 973

Kula MR, Krone KH, Hustedt H (1982) Purification of enzymes by liquid–liquid extraction. In: Fiechter A (ed) Advances in biochemical engineering, vol 24. Springer, Berlin, pp 73–118

Kurian JV (2005) A new polymer platform for the future—Sorona from corn derived 1,3-propanediol. J Polym Environ 13(2):159–167

Laffend LA, Nagarajan V, Nakamura CE (2007) Bioconversion of a fermentable carbon source to *1,3-propanediol* by a single microorganism, United States Patent Application, 20070048849A1

Li S, Tuan VA, Falconer JL, Noble RD (2001a) Separation of 1,3-propanediol from glycerol and glucose using a ZSM-5 zeolite membrane. J Membr Sci 191:53–59

Li S, Tuan VA, Falconer JL, Noble RD (2001b) Separation of 1,3-propanediol from aqueous solutions using pervaporation through an X-type zeolite membrane. Ind Eng Chem Res 40(8):1952–1959

Li S, Tuan VA, Falconer JL, Noble RD (2001c) Effects of zeolite membrane structure on the separation of 1,3-propanediol from glycerol and glucose by pervaporation. Chem Mater 13(5):1865–1873

Li S, Tuan VA, Falconer JL, Noble RD (2002) X-type zeolite membranes: preparation, characterization, and pervaporation performance. Microporous Mesoporous Mater 53(1–3):59–70

Liu H-J, Zhang D-J, Xu Y-H, Mu Y, Sun Y-Q, Xiu Z-L (2007) Microbial production of 1,3-propanediol from glycerol by *Klebsiella pneumoniae* under micro-aerobic conditions up to a pilot scale. Biotechnol Lett 29(8):1281–1285

Louwrier A (1998) Model phase separations of proteins using aqueous/ethanol components. Biotechnol Tech 12(5):363–365

Malinowski JJ (1999) Evaluation of liquid extraction potentials for downstream separation of 1,3-propanediol. Biotechnol Tech 13:127–130

Malinowski JJ (2000) Reactive extraction for downstream separation of 1,3-propanediol. Biotechnol Prog 16:76–79

Menzel K, Zeng AP, Deckwer WD (1997) High concentration and productivity of 1,3-propanediol from continuous fermentation of glycerol by *Klebsiella pneumoniae*. Enzyme Microb Technol 20:82–86

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

Nakamura CE, Whitedy GM (2003) Metabolic engineering for the microbial production of 1,3-propanediol. Curr Opin Biotechnol 14:454–459

Othmer DF, Bergen WS, Shlechter N, Bruins PF (1945) Liquid–liquid extraction data: systems used in butadiene manufacture from butylenes glycol. Ind Eng Chem 37(9):890–894

Qureshi N, Meagher MM, Hutjins RW (1994) Recovery of 2,3-butanediol by vacuum membrane distillation. Sep Sci Technol 29 (13):1733–1748

Roturier JM, Fouache C, Berghmans E (2002) Process for the purification of 1,3-propanediol from a fermentation medium. United States Patent 6 428 992

Saha BC (2003) Hemocellulose bioconversion. J Ind Microbiol Biotech 30:279–291

Sanz MT, Blanco B, Beltran S, Cabezas JI (2001) Vapor liquid equilibria of binary and ternary systems with water, 1,3-propanediol, and glycerol. J Chem Eng Data 46:635–639

Schlieker H, Günzel B, Deckwer WD (1992) Einsatz der Adsorption zur Produtsabtrennung bei der Glycerinvergarung zu 1, 3-Propanediol. Chem Ing Tech 64(8):7272728

Schoellner R, EInicke WD, Unverricht S (1994) Investigations of adsorptive separation of glycerol/propane-1,3-diol in aqueous solution on zeolites by liquid phase adsorption. J Prakt Chem 336(5):404–407

Senkus M (1946) Recovery of 2,3-butanediol produced by fermentation. Ind Eng Chem 38:913–916

Sridhar S (1989) Zur Abtrennung von Butandiol-2,3 aus Fermenter-Brühen mit Hife der Umkehrosmose. Chem Ing Tech 61(3):252–253

Syu M-J (2001) Biological production of 2,3-butanediol. Appl Microbiol Biotechnol 55:10–18

Tan T, Huo Q, Ling Q (2002) Purification of glycyrrhizin from *Glycyrrhiza uralensis* Fisch with ethanol/phosphate aqueous two phase system. Biotechnol Lett 24:1417–1420

Tsao GT (1978) Conversion of biomass from agriculture into useful products. Final Report, USDDE Contact No. EG-77-S-02-4298

Wheat JA, Leslie JD, Tomkins RV, Mitton HE, Scott DS, Ledingham GA (1948) Production and properties of 2,3-butanediol. XXVIII. Pilot plant recovery of levo-2,3-butanediol from whole wheat mashes fermented by *Aerobacillus polymyxa*. Can J Res 26F:469–496

Wilkins AE, Lowe DJ (2004) Product removal process for use in a biofermentation system, United States Patent 6,812,000

Xiang BT, Chen SF, Liu DH (2001) Extraction of 1,3-propanediol from in dilute fermentation broth. Journal of Tsinghua University (Science and Technology) 41(12):53–55

Xiu ZL, Chen X, Sun YQ, Zhang DJ (2007a) Stoichiometric analysis and experimental investigation of glycerol–glucose cofermentation in *Klebsiella pneumoniae* under microaerobic conditions. Biochem Eng J 33:42–52

Xiu ZL, Zhang D, Li X (2007b) Recovery of 1,3-propanediol from fermentation broths by using ammonium sulfate salting out, Chinese Patent, 2006101346958

Xiu ZL, Li Z, Jianng B, Sun Y, Zhang D (2007c) Aqueous two-phase extraction of 1,3-propanediol from fermentation broth, Chinese Patent, 200710010201.X

Xu W-Y (2001) Separation of ethanol–acetone–butanol–water system using potassium carbonate. Chinese J Proc Eng 1(3):318–320

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

Yazdani SS, Gonzalez R (2007) Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr Opin Biotechnol 18:213–219

Zeng AP, Biebl H (2002) Bulk chemicals from biotechnology: the case of 1,3-propanediol production and the new trends. Adv Biochem Eng 74:239–59

Zeng A-P, Byun T-G, Posten C, Deckwer W-D (1994) Use of the respiratory quotient as a control parameter for optimum oxygen supply and scale-up of 2,3-butanediol production under microaerobic conditions. Biotechnol Bioeng 44:1107–1114

Zhi W, Deng Q (2006) Purification of salvianolic acid B from the crude extract of *Salvia miltiorrhiza* with hydrophilic organic/salt-containing aqueous two-phase system by counter-current chromatography. J Chromatogr A 1116:149–152