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Microbial 2,3-butanediol production: A state-of-the-art review

Xiao-Jun Ji, He Huang *, Ping-Kai Ouyang

State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, No. 5 Xinmofan Road, Nanjing 210009, People's Republic of China

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ABSTRACT

2,3-Butanediol is a promising bulk chemical due to its extensive industry applications. The state-of-the-art nature of microbial 2,3-butanediol production is reviewed in this paper. Various strategies for efficient and economical microbial 2,3-butanediol production, including strain improvement, substrate alternation, and process development, are reviewed and compared with regard to their pros and cons. This review also summarizes value added derivatives of biologically produced 2,3-butanediol and different strategies for downstream processing. The future prospects of microbial 2,3-butanediol production are discussed in light of the current progress, challenges, and trends in this field. Guidelines for future studies are also proposed.

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* Corresponding author. Tel./fax: +86 25 83172094.
E-mail address: biotech@njut.edu.cn (H. Huang).

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1. Introduction

As crude oil reserves become increasingly scarce, bio-refinery systems that integrate biomass conversion processes and equipment to produce fuels, power, and chemicals from annually renewable resources are at the stage of worldwide development (Kamm and Kamm, 2004; Ragauskas et al., 2006). Many chemicals that could only be produced by chemical processes in the past can now have the potential to be generated biologically using renewable resources (Danner and Braun, 1999; Hatti-Kaul et al., 2007). Microbial production of 2,3-butanediol (2,3-BD) is one such example. Interest in this bioprocess has increased remarkably because 2,3-BD has a large number of industrial applications, and microbial production will alleviate the dependence on oil supply for the production of platform chemicals (Celińska and Grajek, 2009; Wu et al., 2008). Additionally, 2,3-BD has potential applications in the manufacture of printing inks, perfumes, fumigants, moistening and softening agents, explosives, plasticizers, foods, and pharmaceuticals (Garg and Jain, 1995; Syu, 2001).

Microbial 2,3-BD production has a history of more than 100 years. It was first investigated in 1906 by Harden and Walpole and in 1912 by Harden and Norris (Magee and Kosaric, 1987). The bacterium employed in these early studies was *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes* or *Klebsiella aerogenes*). In 1926, 2,3-BD accumulation in cultures of *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*; reclassified by Ash et al., 1993) was initially observed (Garg and Jain, 1995). Industrial-scale production of this compound is believed to have been first proposed by Fulmer et al. (1933). Shortages of the strategic compound 1,3-butadiene during World War II stimulated intense research efforts on 2,3-BD fermentation, culminating in the development of pilot-scale operations for both its manufacture and conversion to 1,3-butadiene. Development was discontinued because less expensive routes for chemically producing 1,3-butadiene from petroleum became available. In the 1970s, the long-term prospects of rising petroleum prices revived significant interest in producing 2,3-BD from biomass, especially in the United States (Voloch et al., 1985). Recently, microbial 2,3-BD production has attracted great attention worldwide, especially in China, as renewable biomass is a promising route for developing a low carbon economy and a gateway to a more sustainable future (Li et al., 2010c).

The objective of this paper is to review the state-of-the-art developments and technological achievements in microbial 2,3-BD production, including development of improved strains, alternative fermentation substrates, improved cultivation techniques, downstream processing, and derivative production. Furthermore, future perspectives on microbial 2,3-BD production are discussed.

2. Biochemistry and physiology

2.1. Microorganisms

A number of microorganism species are able to accumulate 2,3-BD but only a few do so in what might be considered significant quantities. Species which are noted for this ability include those belonging to the genera *Klebsiella*, *Enterobacter*, *Bacillus* and *Serratia*, which are considered of industrial importance in the production of 2,3-BD (Maddox, 1996). The wide distribution among bacteria of the ability to generate this compound is evident from the classification of key 2,3-BD producers.

Until now, *K. pneumoniae*, *Klebsiella oxytoca*, and *P. polymyxa* have been unbeatable in the efficient production of 2,3-BD. *Enterobacter aerogenes* and *Serratia marcescens* are also considered promising microorganisms for this application. Three stereoisomers of 2,3-BD exist: dextro- [L-(+)-] and levo- [D-(−)-] forms that are optically active, as well as an optically inactive meso- form (Fig. 1). In any given process, the isomer produced is dependent upon the particular

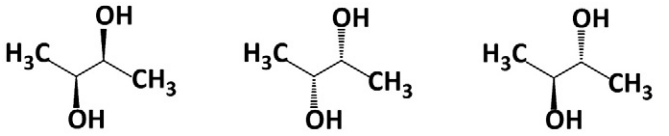

Fig. 1. Stereoisomers of 2,3-butanediol.

microorganism employed. Different microorganisms produce different stereoisomers, but a mixture of two stereoisomers is generally formed (Table 1) (Celińska and Grajek, 2009; Maddox, 1996). The product yields and conversion efficiencies of bacteria commonly used for 2,3-BD production are summarized in Table 1. Among these bacteria, *Klebsiella* sp. (*K. pneumoniae* or *K. oxytoca*) and *P. polymyxa* have been demonstrated to be potentially applicable in the industrial production of 2,3-BD. In light of this, it seems to be desirable to consider the advantages or disadvantages of each in somewhat greater detail. *Klebsiella* sp. typically produces at least twice the amount of 2,3-BD obtainable from *P. polymyxa*. Another advantage of 2,3-BD production using *Klebsiella* sp. is that the species are easy to cultivate. They grow rapidly in a simple medium and metabolize all of the major sugars present in hemicellulose and cellulose hydrolysates into 2,3-BD (Chandel et al., 2010; Jansen and Tsao, 1983; Kosaric et al., 1990, 1992). However, the pathogenicity of opportunistic infection caused by the encapsulated *Klebsiella* species is generally thought to be an obstacle hindering the large-scale 2,3-BD production using this method. On the other hand, *P. polymyxa* has the ability to produce the pure (R,R)-2,3-BD (D-(−)- isomer) that can be used as an antifreeze due to the special properties of this isomer (Marwoto et al., 2004; Nakashimada et al., 1998; Soltys et al., 2001). This would provide a justification for using this species in commercial operations. Besides, apart from monosugars present in hemicellulosic raw materials, this strain can ferment carbohydrate polymers such as xylan, inulin, and starch as it can secrete xylanase (EC 3.2.1.8), inulase (EC 3.2.1.7), and α-amylase (EC 3.2.1.1) which could simultaneously degrade those polymers to monosugars (Gao et al., 2010; Hespell, 1996; Kawazu et al., 1987). Therefore, if 2,3-BD is considered a simple industrial chemical, without concern for the isomer produced, *Klebsiella* sp. would be the culture of choice for commercial production. However, if chiral 2,3-BD is the desired product and some carbohydrate polymers are targeted to generate to 2,3-BD directly, *P. polymyxa* is the better choice.

2.2. Metabolic pathway

A variety of monosaccharides (hexoses or pentoses) can be fermented to produce 2,3-BD (Syu, 2001). In bacterial metabolism, monosaccharides must first be converted to pyruvate before generation of major products. From glucose, pyruvate is formed in a relatively simple manner via the Embden–Meyerhof pathway (glycolysis). In contrast, the production of pyruvate from pentoses must proceed via a combination of the pentose phosphate and Embden–Meyerhof pathways (Jansen and Tsao, 1983). In addition to 2,3-BD, the pyruvate produced from the monosaccharides is then channeled into a mixture of acetate, lactate, formate, succinate, acetoin, and ethanol, through the mixed acid-2,3-BD fermentation pathway illustrated in Fig. 2 (Maddox, 1996; Magee and Kosaric, 1987).

For 2,3-BD biosynthesis from pyruvate, three key enzymes are involved, i.e. α-acetolactate synthase (ALS, EC 4.1.3.18), α-acetolactate decarboxylase (ALDC, EC 4.1.1.5), and 2,3-BD dehydrogenase (BDH, EC 1.1.1.76; also called acetoin reductase, EC 1.1.1.4). The enzyme ALS has an optimum selectivity under slightly acidic conditions (pH 6 enzyme) and is thus distinct from the anabolic ALS, or 'pH 8 enzyme' (Gottshalk, 1986). The pH 6 enzyme conducts a