Biotechnology Advances 34 (2016) 984–996

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Microbial production of propanol

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ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form 8 April 2016
Accepted 31 May 2016
Available online 1 June 2016

Keywords:
n-propanol
Isopropanol
Metabolic engineering
Synthetic biology
Biofuel

ABSTRACT

Both, *n*-propanol and isopropanol are industrially attractive value-added molecules that can be produced by microbes from renewable resources. The development of cost-effective fermentation processes may allow using these alcohols as a biofuel component, or as a precursor for the chemical synthesis of propylene. This review reports and discusses the recent progress which has been made in the biochemical production of propanol. Several synthetic propanol-producing pathways were developed that vary with respect to stoichiometry and metabolic entry point. These pathways were expressed in different host organisms and enabled propanol production from various renewable feedstocks. Furthermore, it was shown that the optimization of fermentation conditions greatly improved process performance, in particular, when continuous product removal prevented accumulation of toxic propanol levels. Although these advanced metabolic engineering and fermentation strategies have facilitated significant progress in the biochemical production of propanol, the currently achieved propanol yields and productivities appear to be insufficient to compete with chemical propanol synthesis. The development of biosynthetic pathways with improved propanol yields, the breeding or identification of microorganisms with higher propanol tolerance, and the engineering of propanol producer strains that efficiently utilize low-cost feedstocks are the major challenges on the way to industrially relevant microbial propanol production processes.

Contents

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http://dx.doi.org/10.1016/j.biotechadv.2016.05.011
0734-9750/

1. Introduction

Propanol exists in the form of two isomers, 1-propanol (also called *n*-propanol), and 2-propanol (also called isopropanol or isopropyl alcohol). Both propanols are mainly used as solvents, but they also serve as chemical intermediates in the production of various esters and amines. Furthermore, isopropanol is often applied as a disinfectant in pharmaceutical products, or as an antifreezing agent. *n*-propanol is industrially produced via hydroformylation of ethylene. Isopropanol can be chemically produced by reduction of acetone in the presence of excess hydrogen, or from propylene by hydration over an acid catalyst (Papa, 2011).

Growing shortage of fossil resources has increased the interest in microbial synthesis of propanol from renewable raw materials. The development of cost-effective propanol fermentation processes may not only satisfy the demand of 2 Mt/year of isopropanol and 0.2 Mt/year of *n*-propanol. In fact much larger markets could be addressed if the production costs of biosourced propanol would allow using this alcohol as a biofuel (Choi et al., 2014; Dusséaux et al., 2013; Huo et al., 2011; Lee et al., 2012; Shen and Liao, 2008), or as a precursor for propylene which can be chemically produced from either propanol by dehydration (Kibby and Hall, 1972).

Whether propanol is a good target molecule to develop biofuels is still matter of debate. This alcohol provides high octane numbers and is less corrosive than ethanol (Fernando et al., 2007). On the other hand, its energy density is not much higher than that of ethanol, and significantly smaller than of alternative biofuels such as butanol or biodiesel (Biofuels - Types of Biofuels - Bioalcohols, 2010). Hence, pure propanol may not be the ideal biofuel, but it could account for a significant fraction of biofuel alcohol mixtures that are obtained in mixed fermentation processes such as the isopropanol-butanol-ethanol (IBE) fermentation (Dusséaux et al., 2013; George et al., 1983; Lee et al., 2012), or from the fermentation of innovative feedstocks such as algae proteins (Choi et al., 2014; Huo et al., 2011).

The development of fermentation processes for the production of pure propanol is mainly motivated by its potential use as a precursor for propylene, which is one of the most important building blocks in the chemical industry. Propylene is used for the production of plastics and is a precursor for the chemical synthesis of propylene oxide, acrylonitrile, cumene, butyraldehyde, and acrylic acid. Currently, it is exclusively produced from petrol, and its annual production volume amounted to 85 Mt in 2013 (Propylene – Study: Market, Analysis, Trends, 2016). Thus, the development of competitive microbial propanol production processes which enable an economically viable propylene production from renewable resources represents a significant market opportunity.

It is therefore not surprising that much research efforts have been invested in recent years to improve microbial propanol production. Production organisms and process conditions have been optimized to increase the propanol yield and the final product titers; and the engineering of different metabolic pathways and production organisms has enabled the use of new feedstocks, such as protein waste, cellobiose, lignocellulose, or carbon dioxide. We herein review all of these aspects but pay particular attention to the description of the nine natural and synthetic propanol-producing pathways (and pathway variants) that have been recently identified and constructed. This growing number of pathways reflects our ever increasing capacity to conceive and implement new metabolic routes by making use of synthetic biology principles, and to use renewable carbon feedstocks more and more efficiently. However, given the current oil market prices, the replacement of petrol-based propanol and propylene production by sugar-based biochemical processes appears to be a long way ahead.

2. Natural propanol-producing organisms

2.1. Biosynthesis of isopropanol in the mixed isopropanol-butanol-ethanol (IBE) fermentation

The mixed acetone-butanol-ethanol (ABE) fermentation, which occurs during anaerobic growth of solvent-producing *Clostridia* species, was one of the most important industrial fermentation processes in the early 20th century. During World War One, the ABE fermentation was the major source for acetone which was required in the production of ammunition. In the period between the two world wars butanol became a highly used solvent in the car industry, and its supply was mainly guaranteed by ABE fermentation plants (see Jones and Woods, 1986 for an excellent review). With the rise of the petrochemical industry, the contribution of ABE fermentation processes to the production of solvents became gradually smaller and eventually marginal. Due to the expected shortage of oil supply, and because butanol is considered as one of the most promising drop-in biofuels, this fermentation process recently regained considerable interest. Most anaerobically growing *Clostridia* species naturally produce mixtures of acetone, butanol, and ethanol (ABE) at an approximate ratio of 3:6:1, respectively (Chen and Hiu, 1986; George et al., 1983). The production of solvents is considered a defense mechanism against medium acidification that is caused by the secretion of acetic and butyric acid, which are alternative fermentation end-products (Hüsemann and Papoutsakis, 1988). At decreasing pH, these organic acids are re-assimilated and converted into alcohols. Upon adjustment of a slightly acidic pH of the cultivation medium, organic acid production can be almost completely prevented and solvents become the major fermentation products.

The acetone-butanol pathway of *Clostridia* is shown in Fig. 1. Two molecules of acetyl-CoA are condensed to one molecule of acetoacetyl-CoA by acetyl-CoA:acetyl-CoA C-acetyltransferase (also named acetoacetyl-CoA thiolase, or acetoacetyl-CoA synthase). In the next step, acetoacetyl-CoA:acetate CoA-transferase (also named coenzyme A transferase, or acetoacetyl-CoA transferase) catalyzes the transfer of CoASH to acetate, yielding one molecule acetoacetate and regenerating one molecule acetyl-CoA. Acetoacetate is either decarboxylated by acetoacetate decarboxylase yielding acetone, or it is reduced and dehydrated via a sequence of 5 reactions to yield butanol (Fig. 1). For some *Clostridia* species, reduced acetone production and concomitant accumulation of isopropanol was observed (George et al., 1983). Formation of isopropanol in these strains was shown to be a consequence of the increased specificity of the strictly NADPH-dependent primary/secondary alcohol dehydrogenase for acetone (Hiu et al., 1987). However, isopropanol production in natural *Clostridia* strains remained small compared to the production of butanol, and did not exceed 25 mol% of the total alcohol fraction (George et al., 1983) (Table 1). The metabolic pathway which produces isopropanol via the characteristic intermediate acetone is in the following sections termed the acetone-dependent isopropanol pathway (Fig. 1). It has been optimized in *Clostridia* strains for the simultaneous production of isopropanol, butanol, and ethanol; and it was heterologously expressed in different bacteria and yeast to facilitate the exclusive production of isopropanol (see below).