Biotechnology Advances xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Prospective and development of butanol as an advanced biofuel

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ARTICLE INFO

Article history:
Received 1 February 2013
Received in revised form 31 July 2013
Accepted 5 August 2013
Available online xxxx

Keywords:
ABE fermentation
Sustainable feedstocks
Strain development
Process optimization
Butanol recovery

ABSTRACT

Butanol has been acknowledged as an advanced biofuel, but its production through acetone–butanol–ethanol (ABE) fermentation by Clostridia is still not economically competitive, due to low butanol yield and titer. In this article, updated progress in butanol production is reviewed. Low price and sustainable feedstocks such as lignocellulosic residues and dedicated energy crops are needed for butanol production at large scale to save feedstock cost, but processes are more complicated, compared to those established for ABE fermentation from sugar- and starch-based feedstocks. While rational designs targeting individual genes, enzymes or pathways are effective for improving butanol yield, global and systems strategies are more reasonable for engineering strains with stress tolerance controlled by multigenes. Compared to solvent-producing Clostridia, engineering heterologous species such as *Escherichia coli* and *Saccharomyces cerevisiae* with butanol pathways might be a solution for eliminating the formation of major byproducts acetone and ethanol so that butanol yield can be improved significantly. Although batch fermentation has been practiced for butanol production in industry, continuous operation is more productive for large scale production of butanol as a biofuel, but a single chemostat bioreactor cannot achieve this goal for the biphasic ABE fermentation, and tanks-in-series systems should be optimized for alternative feedstocks and new strains. Moreover, energy saving is limited for the distillation system, even total solvents in the fermentation broth are increased significantly, since solvents are distilled to ~40% by the beer stripper, and more than 95% water are removed with the stillage without phase change, even with conventional distillation systems, needless to say that advanced chemical engineering technologies can distil solvents up to ~90% with the beer stripper, and the multistage pressure columns can well balance energy consumption for solvent fraction. Indeed, an increase in butanol titer with ABE fermentation can significantly save energy consumption for medium sterilization and stillage treatment, since concentrated medium can be used, and consequently total mass flow with production systems can be reduced. As for various *in situ* butanol removal technologies, their energy efficiency, capital investment and contamination risk to the fermentation process need to be evaluated carefully.

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http://dx.doi.org/10.1016/j.biotechadv.2013.08.004

Please cite this article as: Xue C, et al, Prospective and development of butanol as an advanced biofuel, Biotechnol Adv (2013), http://dx.doi.org/10.1016/j.biotechadv.2013.08.004

1. Introduction

Concerns on shrinking reserve of crude oil and environmental impact caused by the over-consumption of petroleum-based products, particularly transportation fuels, have made it urgent to develop alternatives that are both renewable and environmentally friendly, and biofuels derived from biomass resources have thus been revitalized globally. At present, major biofuels are fuel ethanol and biodiesel, which are being produced from conventional sugar- and starch-based feedstocks such as sugarcane/molasses and corn for fuel ethanol production in Brazil and the US, respectively, and vegetable oil for biodiesel production in EU. Taking into account of global population and increased demand for food supply, such a production mode is not sustainable, and non-grain based feedstocks such as lignocellulosic biomass, particularly agricultural residues have been targeted for biofuels production (Ragauskas et al., 2006).

As four carbon primary alcohol, butanol is superior to ethanol as a fuel in many regards such as higher energy density, lower volatility and hydroscopicity, and less corrosion to existing infrastructure, which has thus been acknowledged as an advanced biofuel (Dürre, 2007), and attracted great attention across the world. Butanol is fermentatively produced by solvent-producing *Clostridium* spp., with acetone and ethanol as major byproducts (ABE fermentation). Unfortunately, such a sustainable pathway is not economically competitive compared to the petro-chemical route. However, rapid progress in biological sciences and applied technologies, not only inspire deep understanding of the intracellular metabolism of ABE fermentation by Clostridia, but also provide more efficient tools for their modifications, which were reviewed recently (Jang et al., 2012a; Lütke-Eversloh and Bahl, 2011; Papoutsakis, 2008). Meanwhile, non-solvent producing species such as *Escherichia coli* and *Saccharomyces cerevisiae* can also be engineered with butanol production pathway by synthetic biology strategy to overcome the intrinsic weakness of Clostridia in low butanol yield due to significant production of acetone and ethanol (Atsumi et al., 2008; Shen et al., 2011; Steen et al., 2008).

The objective of this article is to provide update and critical review, with a focus on feedstock selection, strain development, and process engineering aspects, and in the meantime challenges for butanol to be economically competitive as an advanced biofuel and strategies to address these challenges are addressed.

2. Feedstock selection and process development

At present, sugar- and starch-based feedstocks are used for butanol production. While sugar-based feedstocks such as sugarcane molasses are geographically available at bulk amount for biofuels production, starch-based feedstocks such as potato, corn and wheat are food supplies or main ingredients of animal feed to produce protein products for human being, which are not sustainable for butanol production at large scales for fuel use. Therefore, alternative feedstocks that are abundantly available and not food-related need to be developed.

Different feedstocks require different strategies for process development, as illustrated in Fig. 1. The simplest process for butanol production is with molasses. Although the process for butanol production from starch-based feedstocks is more complicated with millhouse to comminute the feedstocks which is energy-intensive, even a complicate process to separate starch from other components like fiber, gluten and germ from corn and wheat, it is still more economically competitive compared to processes developed for butanol production from lignocellulosic biomass, since clostridial strains are saccharolytic and enzymatic hydrolysis of starch as that required with ethanol fermentation by *S. cerevisiae* is not necessary. No doubt, ABE fermentation technologies previously established with sugar- and starch-based feedstocks lay solid foundation for the revitalization of this old industry, even for butanol production from alternative feedstocks.

Lignocellulosic biomass, particularly residues from agriculture and forest industry that are abundantly available at low cost, and dedicated energy crops that can grow well in marginal land not suitable for grain production are sustainable feedstocks (Heaton et al., 2008). However, like ethanol production from lignocellulosic biomass, these feedstocks also present challenges for butanol production due to their recalcitrance to degradation and release sugars for fermentations (Himmel et al., 2007).

Plant genetic engineering cannot be employed to grain crops to render the recalcitrant issue of their residues for biofuel production due to the risk in compromising grain output. In contrast, when grain crops are modified for high yield, their residues become more recalcitrant to

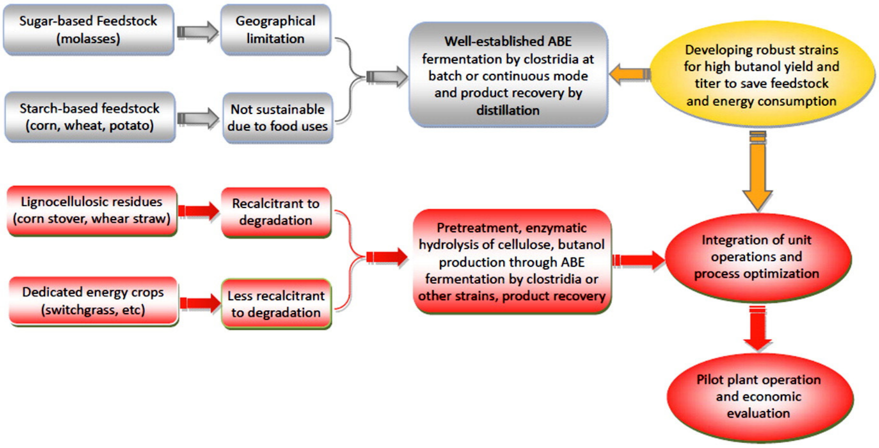

Fig. 1. Feedstocks for butanol production and corresponding processes.

Please cite this article as: Xue C, et al, Prospective and development of butanol as an advanced biofuel, Biotechnol Adv (2013), http://dx.doi.org/10.1016/j.biotechadv.2013.08.004