



Review Paper

Recent trends in acetone, butanol, and ethanol (ABE) production

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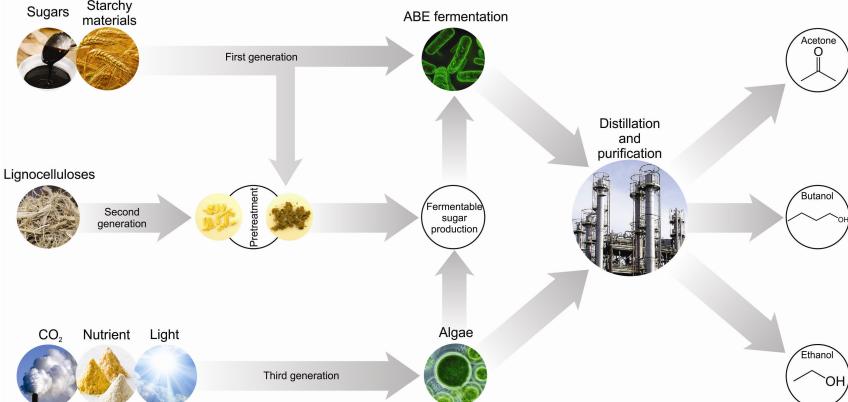
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HIGHLIGHTS

- Biobutanol is an advantageous competitor to most biofuels and petroleum-based fuels.
- Suitable substrates and low concentration of products are among the process challenges.
- In spite of different challenges, lignocelluloses are among the most suitable feedstocks.
- To revamp the old ABE or ethanol plants to butanol, more researches in different disciplines are necessary.
- This review paper discusses the basic and applied perspective of the process.

GRAPHICAL ABSTRACT



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ABSTRACT

Among the renewable fuels considered as a suitable substitute to petroleum-based gasoline, butanol has attracted a great deal of attention due to its unique properties. Acetone, butanol, and ethanol (ABE) can be produced biologically from different substrates, including sugars, starch, lignocelluloses, and algae. This process was among the very first biofuel production processes which was commercialized during the First World War. The present review paper discusses the different aspects of the ABE process and the recent progresses made. Moreover, the microorganisms and the biochemistry of the ABE fermentation as well as the feedstocks used are reviewed. Finally, the challenges faced such as low products concentration and products' inhibitory effects on the fermentation are explained and different possible solutions are presented and reviewed.

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1. Introduction

Among the renewable fuels, butanol is considered as a competitor to petroleum-based products. Butanol, compared to ethanol, has attracted more attention due to its unique properties (Durre, 2007; Ni and Sun, 2009; Patakova et al., 2013; Tiguanova et al., 2013; Karimi and Pandey, 2014; Li et al., 2014). A mixture of acetone, butanol, and ethanol (ABE) can be produced biologically from different sugars and starches. This process was commercialized in the Union of Soviet Socialist Republics, England, Canada, and the USA during the First World War. Several industrial units were also established in other countries including Japan, Australia, China, and South Africa (Linden et al., 1986; García et al., 2011; Köpke and Dürre, 2011; Dong et al., 2012). Initially, ABE fermentation was mainly being used for production of acetone as a solvent for military applications. However, nowadays there is more interest in butanol to be used as a liquid renewable fuel (Awang et al., 1988; Maddox, 1989; Durre, 2007).

Butanol, C_4H_9OH , is a colorless liquid among the four-carbon alcohols. It can be produced by chemical and biological methods. The economy of butanol production by chemical methods highly depends on oil price while by biological methods, the cost of the raw material used is the determining factor (Linden et al., 1986). Nowadays, due to the increasing price of crude oil, biological processes of butanol production have attracted significant attentions (Amiri et al., 2015). Biobutanol, compared to ethanol, has several advantages. One of the most important advantages is that it could be blended with gasoline at any percentages. Furthermore, butanol has a lower vapor pressure and absorbs less moisture, and is less corrosive; thus, its transportation is more convenient. Butanol has higher energy content than ethanol and is more similar to diesel fuels, in terms of energy content (Awang et al., 1988; Maddox, 1989; Dong et al., 2012; Tiguanova et al., 2013). Different processes including batch, fed-batch, and continuous fermentation with and without in situ product removal with native and modified strains in the free and immobilized cells are currently applied (Bankar et al., 2012; Setlhaku et al., 2012; Survase et al., 2012; Xue et al., 2012; Chen et al., 2013; Ezeji et al., 2013; Jang et al., 2013; Millat et al., 2013; Chen et al., 2014; Rathore et al., 2015), and a number of review and book chapters have been published on this subject (Linden et al., 1986; Maddox, 1989; Durre, 2007; Kharkwal et al., 2009; Ni and Sun, 2009; Gu et al., 2010; García et al., 2011; Dong et al., 2012; Patakova et al., 2013; Tiguanova et al., 2013; Li et al., 2014). This review presents an introduction to the process and discusses the challenges and possible solutions for the ABE production.

2. Microorganisms for ABE fermentation

Certain species of microorganisms are used in biological ABE production process. The most important of these microorganisms is *Clostridium* genus that includes a variety of butanol-producing bacteria. Some of these bacteria are *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoacetobutylicum*, *C. aurantibutyricum*, and *C. sporogenes*. (Kharkwal et al., 2009; Ni and Sun, 2009; Patakova et al., 2013). Among these microorganisms, two species, *C. beijerinckii* and *C. acetobutylicum* are the most promising ones for commercial and laboratory applications with high efficiency (Mo et al., 2015). In fact, native and modified forms of these two strains are the most applied microorganisms in ABE production (Ni and Sun, 2009; Komonkhat and Cheirsilp, 2013; Patakova et al., 2013; Li et al., 2014). *C. acetobutylicum* is a rod-shaped, gram-positive, and obligate anaerobic bacterium that forms

spores. This is the first species of microorganisms that has been used in industrial ABE fermentation from starch and sugars (Maddox, 1989; Kharkwal et al., 2009; Ni and Sun, 2009). *Clostridium* spp. can utilize a wide range of simple sugars (i.e., glucose, galactose, and xylose) and disaccharides (i.e., maltose, sucrose, and lactose) (Loyarkat et al., 2013). Direct conversion of starch without necessity of hydrolysis is among the specific features of these strains (Madihah et al., 2001; Li et al., 2014; Thang and Kobayashi, 2014).

Selecting a category or group of microorganisms for biological production of ABE depends on many factors, including the type of the initial substrate, the desired production rate, required additional nutrients, and bacteriophages resistance (Kumar and Gayen, 2011). Overproduction of butanol by mutagenesis, evolutionary engineering, and recently genomic studies and transcriptional analysis is the subject of a high number of research activities in this area (Kumar and Gayen, 2011; Cooksley et al., 2012; Li et al., 2013). *C. acetobutylicum* capable of producing high concentration of butanol (as high as 20 g/L) was obtained by mutation, whereas the concentration of butanol in commercial fermentation is typically 12 g/L (Xue et al., 2012; Jiang et al., 2014). However, generally the success in screening by mutagenesis and evolutionary engineering highly depends on chance. One of the recent progresses in this field is based on the evolutionary dynamics and natural selection, referred to as artificial simulation of bio-evolution. Using this method, which is a repetitive evolutionary training, *C. acetobutylicum* that could tolerate 4% butanol was obtained (Liu et al., 2013).

3. Biochemistry of ABE fermentation

ABE production by clostridia species has a complex intracellular pathway. The most important products of the intracellular pathway of clostridia species fall into three main categories: (1) solvents (acetone, butanol, and ethanol), (2) organic acids (lactic acid, acetic acid, and butyric acid), and (3) gases (carbon dioxide and hydrogen) (Zheng et al., 2009; Xue et al., 2013). ABE fermentation process begins by the acidogenic phase within the exponential growth phase (Fig. 1). As indicated in Figure 1, each mole of glucose can be converted to either two moles of acetic acid or one mole of butyric acid via acidogenesis. The production of these acids reduces pH in batch cultivation; thus, without a proper pH control, an inhibition of the metabolic pathway occurs, referred to as acidic stress. The reason behind this acidic stress is the faster production of these acids compared to their consumption by the cells (Kumar and Gayen, 2011; Xue et al., 2013).

The products of acidogenesis are then transferred to solventogenic phase during the spore formation. Acetic acid can be converted to ethanol or acetone, while butyric acid is converted to butanol. Using *C. acetobutylicum*, aceton, butanol, and ethanol are produced in a ratio of 1:6:3, respectively, within the normal pathway. These solvents are toxic to the cells. About 50% of the cell growth is hindered by concentrations of 11, 51, and 84 g/l butanol, ethanol, and acetone, respectively. Thus, butanol is very toxic to the cells. The inhibitory effects of these solvents are known as solvent stress and a high number of studies have been conducted on this issue as well (Linden et al., 1986; Xue et al., 2013). Generally, clostridia are very sensitive to the medium composition and fermentation conditions. Small amounts of oxygen can completely stop

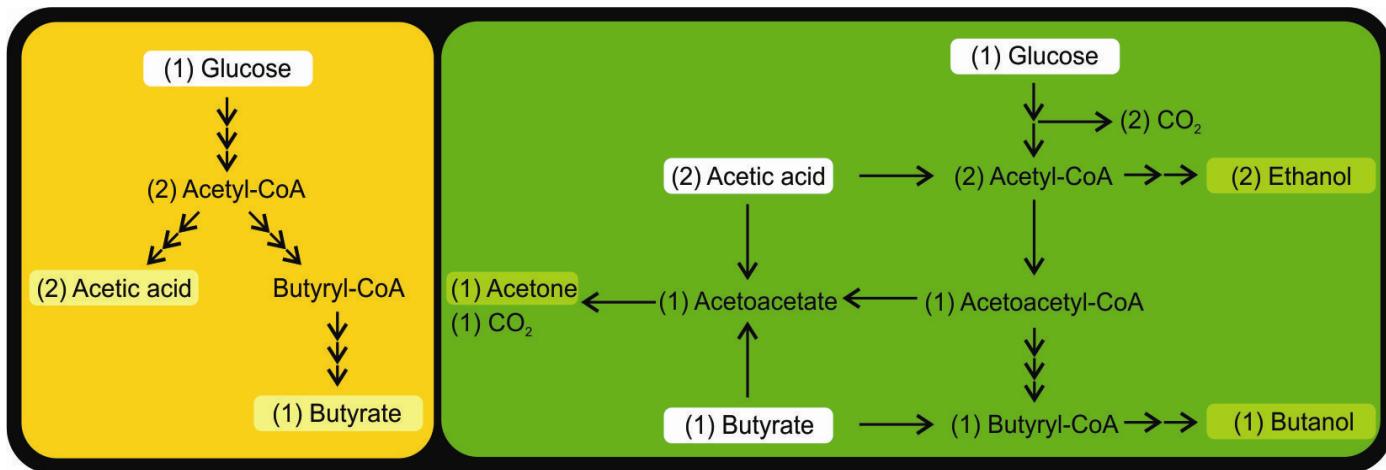


Fig.1. Primary metabolism of *C. acetobutylicum*. The numbers indicate the moles (Linden et al., 1986; Kumar and Gayen, 2011).

the activity of the cells, and some chemicals in minor amounts can affect the product distribution (Choi et al., 2012; Han et al., 2013). For instance, availability of small amount of zinc, e.g., 0.001 g/L ZnSO₄·7H₂O, can result in earlier shifting to solventogenesis (Wu et al., 2013).

4. Perspective of feedstocks for ABE production

The economy of ABE process highly depends on applied feedstocks (Lepiz-Aguilar et al., 2013). Like other first generation biofuels which are derived from sugars and starchy materials, butanol can create the conflict between fuel and food (Sims et al., 2010). Feedstock consumption is more challenging for ABE compared to ethanol, as production of each ton of butanol needs more than 6 tons of corn, while it is only 3 tons for ethanol production. Thus, recently most of the research activities have been shifted to the second generation butanol which is derived from lignocellulosic biomass such as bagasse, rice straw, wheat straw, grass, and waste woods (Naik et al., 2010). Lignocelluloses are nonfood feedstocks available in large quantity with low cost and seem to be the only promising raw materials for ABE production at large scale (Kumar et al., 2012). The old ABE processes should be revamped to use lignocelluloses substrates and the currently available ethanol plants can also be reorganized to produce ABE from lignocelluloses.

The overall process scheme of ABE production from different substrates including lignocelluloses is summarized in **Figure 2**. However, due to the complex and recalcitrant structure of lignocellulosic materials, they cannot be directly used by microorganisms. Therefore, a processing step, called pretreatment, is required to disrupt the lignocellulosic biomass matrix to make the carbohydrates accessible to enzymes and microorganisms. Then, cellulose and hemicelluloses polymers are hydrolyzed to obtain monomeric sugars (Taherzadeh and Karimi, 2007). Fermentation of sugars is then conducted for production of ABE by microorganisms. Produced ABE is then recovered and purified (Abdehagh et al., 2014). Separation and purification of biobutanol can be conducted during the fermentation (Huang et al., 2014).

5. Major challenges in the ABE processes and possible solutions

Although butanol, as an advanced liquid fuel, has several advantages compared to ethanol, the ABE process has several bottlenecks and challenges hindering its commercial production. Here is a brief introduction to the main challenges and some of their possible solutions.

5.1. Suitable substrates and their challenges

ABE is not a commercially profitable and competitive process without using an inexpensive and widely available substrate (García et al., 2011; Lepiz-Aguilar et al., 2013; Xue et al., 2013; Zhang et al., 2014; Becerra et al., 2015; Kheyandish et al., 2015). Lignocelluloses are suggested to be suitable

substrates because sources like municipal solid waste (MSW) and agricultural wastes (namely bagasse and rice straw) are available in huge amounts and mainly useless (Amiri et al., 2010; Hedayatkhan et al., 2013; Shafiei et al., 2013; Amiri et al., 2014; Amiri and Karimi, 2015). However, the main problem is the difficulty to produce fermentable sugar from such substrates, as they have recalcitrant structures and the hydrolytic enzymes are still expensive (Shafiei et al., 2011; Shafiei et al., 2013; Shafiei et al., 2014).

Therefore, the introduction of a pretreatment is needed, which is typically an expensive process step (Shafiei et al., 2010; Sims et al., 2010; Shafiei et al., 2014; Boonsombuti et al., 2015). Hence, investigating suitable pretreatment methods is the focus of a number of current studies and is considered as a key factor for efficient production of ABE from lignocelluloses. An ideal pretreatment process should efficiently improve the enzymatic hydrolysis, consume lower amounts of chemicals, and produce fewer by-products/inhibitors (Taherzadeh and Karimi, 2008; Karimi et al., 2013). Compared to ethanolic fermenting yeasts, ABE fermenting microorganisms are more sensitive to possible inhibitors present in lignocelluloses hydrolysates, e.g., hydroxymethylfurfural, furfural, and lignin derivatives (Kudahettige-Nilsson et al., 2015). These inhibitors have severe inhibitory effects both on clostridium growth and consequently the ABE production yield (Ezeji et al., 2007; Cai et al., 2013). Therefore, the applied pretreatment should not lead to the production of considerable amounts of inhibitors. For instance, dilute acid pretreatment which produces a high concentration of inhibitors should not be applied, otherwise an extra detoxification process is necessary that also consumes chemicals, and is accompanied with some sugar loss, and production of problematic wastes. Thus, special care should be taken in selection and optimization of the pretreatment processes. Liquid hot water, ammonia, ionic liquid, and organosolv treatments are among the most applied methods (Amiri et al., 2014; Ding et al., 2015), but all these methods have their own drawbacks (Taherzadeh and Karimi, 2008).

On the other hand, the hydrolysate produced through the pretreatment is a very complicated mixture of different components including sugars that cannot be simulated just by pure glucose (Karimi et al., 2005; Taherzadeh and Karimi, 2007; Taherzadeh and Karimi, 2007; Karimi et al., 2013). Lignocelluloses contain both cellulose and hemicellulose and to achieve an economically-feasible ABE production, the latter should not be ignored as it accounts for 14-37% of the lignocelluloses. If not hydrolyzed and fermented, the hemicellulose fraction ends up as a waste (Karimi et al., 2013). Besides glucose, the hydrolysate of hemicellulose and cellulose contains mainly xylose in the case of agricultural biomass and hardwoods and mannose in the case of softwoods. Other sugars and compounds, e.g., galactose, arabinose, rhamnose, glucuronic acid, methyl glucuronic acid, and galacturonic acid are also present in minor portions (Taherzadeh and Karimi, 2007; Taherzadeh and Karimi, 2008; Taherzadeh and Karimi, 2011; Karimi et al., 2013). Therefore, if all

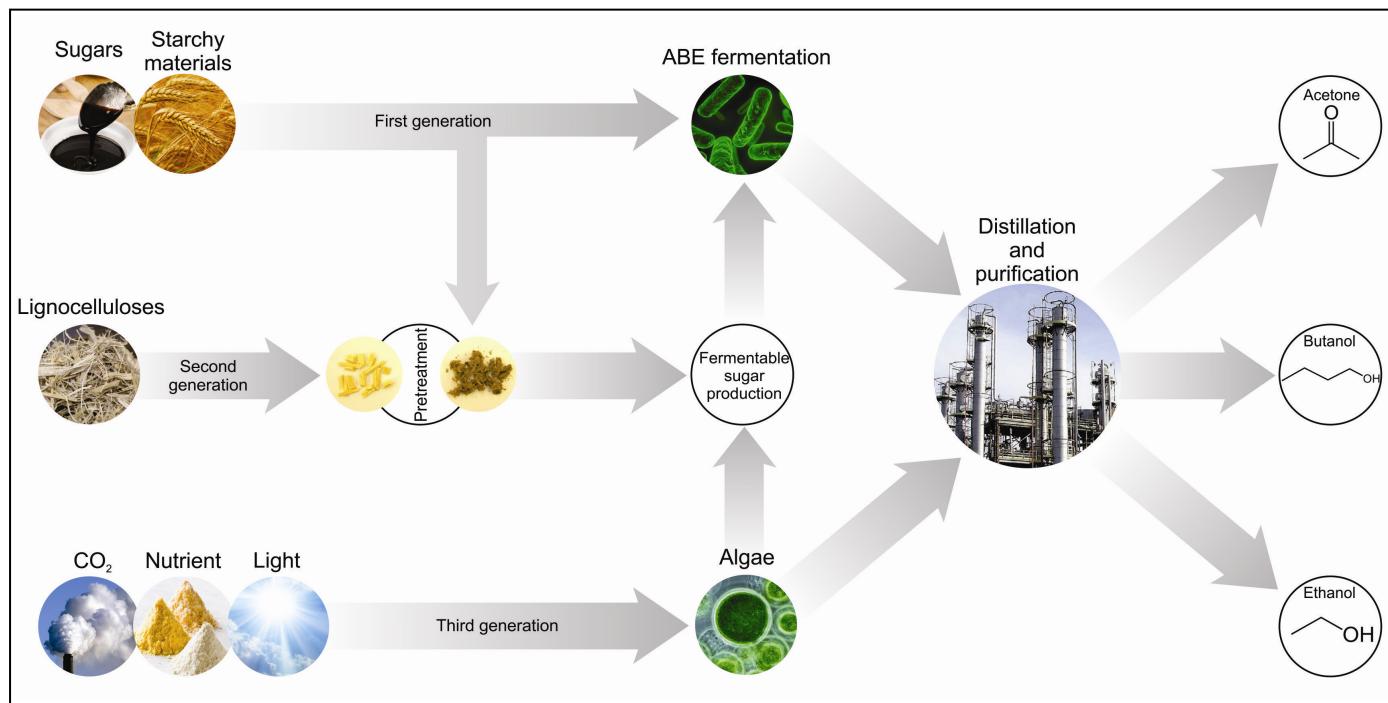


Fig.2. Overall process scheme for acetone, butanol, and ethanol production from lignocellulosic materials.

optimizations are conducted on pure glucose, it does not necessarily give the same results on the hydrolysates. In fact, co-consumption of different sugars takes place in the case of fermentation of hydrolysates to ABE, which is much more complicated than the fermentation of pure glucose. This may also influence the inhibitory effects of the hydrolysate on the bacteria (Su et al., 2015).

By applying a suitable pretreatment, the consumption of hydrolytic enzymes can be significantly reduced (Boonsombuti et al., 2015). The price of the hydrolytic enzymes, cellulases, has recently significantly reduced via the global attempts within this area (Karimi et al., 2013). However, cellulases are still much more expensive than the enzymes used for the hydrolysis of starch. Thus, the process for ABE production should use a minimal amounts of these enzymes to achieve an economically feasible production cycle (Karimi et al., 2013). Plant genetic engineering is also an option to develop plants with less recalcitrant structures. In spite of a number of attempts, it is not possible yet to develop plants with less recalcitrant biomass achieving same yields. Development of plants in which cell wall-degrading enzymes are expressed (Saathoff et al., 2011) as well as changing enzymes involved in lignin biosynthesis (Saathoff et al., 2011), both aiming at the production of easily convertible biomass have also been investigated.

Glycerol, an important byproduct of biodiesel industry, has been suggested as a suitable substrate for ABE production (Khanna et al., 2013; Li et al., 2014; Yadav et al., 2014). Algae are also among the suggested alternative substrates. The algae biomass has several advantages compared to the other substrates (Ellis et al., 2012; van der Wal et al., 2013; Yazdani et al., 2015); however, the production of algae fuels is generally in the early stages of development and a number of serious challenges are still needed to be addressed first. For instance, the biomass of algae is produced in a very dilute solution and its separation and downstream processing are costly. Another option is the direct conversion of solar energy and CO₂ to isobutanol by certain algae species (Atsumi et al., 2009; Jang et al., 2012). This strategy is also in its early stage of development and it is not possible to consider yet whether this process would be economically feasible for large scale production.

5.2. Low concentration of products and possible solutions

The concentration of ethanol in the commercial scale processes is typically between 5-9%, while it is possible to reach a concentration as high as 16% (Taherzadeh and Karimi, 2008; Breisha, 2010; Taherzadeh and Karimi, 2011), whereas the concentration of total produced ABE is typically between 2-4% (Xue et al., 2013; Huang et al., 2014; Ye et al., 2015). Therefore, the cost of separation and purification of ABE is much higher than that of ethanol. This is principally related to toxicity of the produced solvents on the ABE producing bacteria (Awang et al., 1988). The suggested solutions are using more tolerant strains, recovery of the solvents during the fermentation, and using less energy demanding and inexpensive purification processes (García et al., 2011; Xue et al., 2013; Huang et al., 2014; Dhamole et al., 2015). The solvents, especially butanol, are severe inhibitors of the solvent-producing bacteria (Xue et al., 2013). Thus, product removal technologies, also referred to as *in situ* butanol removal, are suggested and applied in laboratory and pilot scales (Abdehagh et al., 2014; Huang et al., 2014). Pervaporation (Friedl et al., 1991; Jitesh et al., 2000; Cai et al., 2013), liquid-liquid extraction (Yen and Wang, 2013), gas stripping (Ezeji et al., 2004; Xue et al., 2012), vacuum fermentation (Mariano et al., 2012; Qureshi et al., 2014), perstraction (Qureshi and Maddox, 2005), and adsorption (Liu et al., 2014; Thompson et al., 2014) of the solvents are among the most applied techniques. Pervaporation and perstraction are highly selective and efficient; however, the high cost and the possibility for fouling limit their potentials for large scale applications. Among the membrane separation processes, polydimethylsiloxane (PDMS)/ceramic composite membranes have been shown to lead to less fouling problems (Chen et al., 2014). Liquid-liquid extraction is highly selective (Bankar et al., 2012) but the applied solvents are typically toxic to the bacteria; thus, special nontoxic extractants such as biodiesel should be applied (Yen and Wang, 2013). Moreover, after butanol extraction, a distillation/evaporation step is necessary to recover butanol which is an energy demanding process. On the other hand, another method, gas stripping, can be simply applied in

laboratory, pilot, and commercial scales without causing toxicity. Promising results were obtained by the combination of batch, fed-batch, and continuous fermentation with gas stripping (Xue et al., 2012). However, gas stripping is accompanied with very low selectivity. Despite advantages such as no fouling and no toxicity, vacuum fermentation and adsorption are also accompanied with low selectivity (Lin et al., 2012). Besides an excess cost of *in situ* butanol removal, the problems associated with addition of air to the system, energy consumption, and contamination should be considered (Xue et al., 2013; Huang et al., 2014).

In addition to process-based solutions, using solvent tolerant strains of clostridia and other metabolically-engineered bacteria are also among the possible solutions (Abdehagh et al., 2014; Huang et al., 2014). However, using strains of clostridia with a higher tolerance to butanol can improve the ABE concentration to a minor extent. On the other hand, butanol productivity and yields as well as efficient consumption of a wide variety of sugars present in the substrates by the genetically-engineered microorganisms, including *Escherichia coli* (Zheng et al., 2009; Huffer et al., 2012), *Bacillus subtilis* (Kataoka et al., 2011), *Saccharomyces cerevisiae* (Krivoruchko et al., 2013), and *Pseudomonas putida* (Ruhl et al., 2009), are still insufficient compared to native strains of ABE fermenting clostridia.

Increasing butanol concentration and yield, e.g., by elimination of by-products, increasing the substrate utilization, development of aerotolerant strains have also been investigated by metabolic engineering approaches (Kumar and Gayen, 2011). Inactivation of ethanol, acetone, acetate, and butyrate production can also help to improve the yield of butanol production. However, development of such homo-butanol producers in which more than one by-product is eliminated is still challenging (Papoutsakis, 2008; Kumar and Gayen, 2011; Lütke-Eversloh and Bahl, 2011). Multi-pressure distillation systems and process heat integration as well as membrane separation are suggested to reduce the cost of purification. However, the price for ABE purification is still much higher than that of ethanol, although the gap is now narrower (Xue et al., 2013).

6. Concluding remarks

Butanol, a competitor to most of the other biofuels and petroleum-based products, can be produced biologically *via* ABE processes. It can be mixed with gasoline at any concentrations and easily transported without the risk of absorbing moisture. Moreover, butanol contains higher energy and has a lower corrosion rate than ethanol. In fact, the ABE process is among the biological processes industrialized long time ago but was stopped for economical reasons 30 years ago. Nevertheless, this process has gained renewed interest recently due to its unique advantages. On the other hand, the ABE production process is still accompanied with several challenges such as low concentration and difficulty in separation of products, higher feedstock consumption rate (i.e., lower production yield), and sensitivity to substrate composition, inhibitors, and to the presence of oxygen. Different techniques, e.g., pervaporation, vacuum fermentation, gas stripping, perstraction, and adsorption liquid-liquid extraction can help to overcome some of the above-mentioned challenges but each of these techniques is accompanied with some drawbacks as well. Strain enhancement to achieve butanol overproduction with higher tolerance is also among the alternative solutions. It is worth mentioning that lignocellulosic wastes seem to be the most promising feedstocks for ABE production in the future. However, the high price of hydrolytic enzymes and the necessity to implement an expensive pretreatment step are still the challenges that need to be overcome. In summary, more research is necessary to revamp the old ABE production processes or the current ethanol production processes to better reflect the requirements of the second generation butanol production in the future.

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