Recent innovations for reviving the ABE fermentation for production of butanol as a drop-in liquid biofuel

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HIGHLIGHTS

➢ Unique properties of butanol as a drop-in liquid biofuel are reviewed.
➢ Innovations in ABE production are scrutinized in three categories, upstream, mainstream, and downstream.
➢ Recent innovations in pretreatment for waste-based butanol production are reviewed and discussed.
➢ Process integrations with significant effects on butanol production are presented.
➢ Recent innovations for economically-viable butanol recovery are reviewed and discussed.

ABSTRACT

Butanol is a key microbial product that provides a route from renewable carbohydrate resources to a "drop-in" liquid biofuel, broadening its market in the near future. The acceptable performance of butanol as a neat or a blended fuel in different engines both from the technical and environmental points of view has attracted a wide range of research for reviving the old acetone-butanol-ethanol (ABE) fermentation. In this review, recent findings on fuel characteristics of butanol, different generations of substrate for large scale butanol production, and alternative process designs for upstream, mainstream, and downstream operations have been critically reviewed and discussed. In the upstream, studies devoted to designing and optimization of pretreatments based on prerequisites of butanol production, e.g., maximizing cellulose and hemicellulose recovery and minimizing lignin degradation, are presented. In the mainstream, different microbial systems and process integrations developed for facilitating ABE product ion (e.g., in-situ butanol removal) are scrutinized. Finally, innovations in ABE recovery and purification as “Achilles Heel” of butanol production processes which directly controls the energy return on investment (EROI), are reviewed and discussed.
Alcoholic fuels have been widely suggested by pioneering engineers like Henry Ford (Bernton et al., 2010), Charles Kettering (Kettering, 1919), and Sir Harry R. Ricardo (Ricardo, 1935), especially for internal combustion (IC) engines where anti-knock properties of alcoholic fuels are distinctive. Nevertheless, low-cost petroleum fuels have constantly precluded these eco-friendly energy carriers from reaching their full capacity for over a century. However, the growing environmental and public health challenges faced due to the widespread application of fuel-grade ethanol. These include blending issues, limited energy recovery, high volatility, high hygroscopicity, high corrosiveness, and pipe transportation difficulties (Amiri and Karimi, 2018). For instance, the current US regulations allow butanol blending with gasoline up to 16%, while this stands at 10% in the case of ethanol (National Research Council, 2012).

Despite its advantages, microbial production and purification of butanol are associated with some inherent challenges from the process point of view. Butanol is the most hydrophobic and toxic fermentation end product. Like ethanol, butanol is an amphiphatic molecule. It is partitioned in the hydrophobic regions of the membrane, increasing the polarity of the membrane's hydrophobic core, which significantly affects the cell metabolism due to (1) membrane leakage and (2) disruption of the membranes' phospholipids and proteins. Interestingly, solvent-producing *Clostridia* can produce butanol up to the threshold inhibitory concentrations, i.e., 1.2-1.6%. However, obtaining fuel-grade butanol from the relatively dilute beer is an energy-intensive process. In fact, the process' energy consumption may exceed the purified biofuel's energy content, questioning the process's main objective, which is a high energy return on investment (EROI). Tao et al. (2014) estimated the EROI of cellulotic butanol as 1.5:1 (about 6% lower than corn-based ethanol), which could be increased to 2.8:1 by counting in the co-product electricity credit. More importantly, to obtain an economically-viable biofuel, the yield of sugar bioconversion should be high enough to lose less than 50% of sugar combustion energy (assuming USD 0.18/kg sugar and USD 10.8/GJ) in both bioconversion and separation processes (Huang and Percival Zhang, 2011). Based on current technologies developed for butanol production, the energy-retaining efficiency is about 45%, which can be increased to as high as 78% through (1) increasing the ratio of butanol to total products from 0.7 to 0.95 (leading to an increase from 45 to 62%), (2) decreasing energy loss through separation from 23.2 to 12% (leading to increase from 62 to 68%), and (3) decreasing sugar to cell mass from 0.12 to 0.02 (leading to increase from 68 to 78%) (Huang and Percival Zhang, 2011).

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The yield of converting carbohydrates to alcohol is the other important inherent bottleneck in the economically-viable production of butanol. As listed in Table 1, the maximum theoretical yield of acetone-butanol-ethanol (ABE) from glucose, which is 0.2-0.4 g/g, is lower than that of ethanol, which is 0.51 g/g. Qureshi et al. (2007) reported a relatively high yield of 0.40 g/g ABE in the medium fermentation containing 50 g/L glucose by *C. beijerinckii* P260. They found that ABE yield was adversely proportional with glucose concentration, while a different observation was made for ABE titer. More specifically, ABE titer increased by increasing glucose concentration up to a certain limit (23.5 g/L for glucose concentration of 100 g/L) but declined upon further increments (Qureshi et al., 2007).

ABE fermentation is also associated with the inevitable production of a non-fuel product, i.e., acetone. Butanolate to acetone ratio varies depending on the solvent-producing *Clostridia* bio-catalyzing the ABE fermentation, and the substrate. In the fermentation of xylose-based medium, *C. butylicum* NRRL 597 showed butanol to acetone production ratio of as high as 8.8 (Ezeji and Blaschek, 2008). It seems that the ratio of butanol to acetone produced through fermentation of pentoses is generally higher than hexoses (Table 1).
2. Reviving the old ABE fermentation

Sugar fermentation, the genius microbial metabolism for survival in the absence of an electron acceptor, is a unique route for converting carbohydrate resources into alcoholic fuels, e.g., ethanol and butanol. ABE fermentation by *Clostridia* has a long history as one of the largest fermentation industries for acetone or butanol production. Despite the yeast fermentation, which won the competition with the petrochemical industry for ethanol production, ABE fermentation lost its economic attractiveness for butanol production in the 1950s has been reported as one of the main reasons for the closure of those plants (Sauer, 2016). The substrate selection is even more vital in the case of fuel-grade butanol production, given the enormous magnitude of the fuel market. From this viewpoint, lignocellulosic biomass is a “near-zero price” but mostly “land-based” waste known as second-generation substrate. The 1970-1990s developments in low-cost cellulase production facilitated the enzymatic downstream, recent studies on energy-efficient recovery and purification of ABE products to obtain promising EROIs are reviewed and discussed.

### 2.1. Substrate

The substrate choice has been historically a critical decision in the ABE fermentation plants. In the 1940s, along with increasing demands for maize, most commercial ABE fermentation plants decided to switch to blackstrap molasses, a waste-oriented substrate. However, the reduction of molasses quality through technological advancements in the cane sugar industry during the 1950s has been reported as one of the main reasons for the closure of those plants (Sauer, 2016). The substrate selection is even more vital in the case of fuel-grade butanol production, given the enormous magnitude of the fuel market. From this viewpoint, lignocellulosic biomass is the only non-edible source of carbon available on this scale. Lignocellulosic biomass is a “near-zero price” but mostly “land-based” waste known as second-generation substrate. The 1970-1990s developments in low-cost cellulase production facilitated the enzymatic profitability is one of the prerequisites of any commercial-scale production, the recently found application of butanol as a “drop-in” liquid fuel has been the main driver for reviving the old ABE fermentation.

### Table 1.

| Strain                        | Substrate          | Production titer (g/L) | Production yield (g/g) | Butanol to acetone ratio | Reference              |
|-------------------------------|--------------------|------------------------|------------------------|--------------------------|------------------------|
| *Clostridia acetobutylicum* ATCC 824 | Glucose *         | 18.70                  | 0.31                   | 2.91                     | Ezeji and Blaschek     |
|                               | Cellobose *       | 9.78                   | 0.16                   | 3.29                     | (2008)                 |
|                               | Galactose *       | 6.79                   | 0.11                   | 4.00                     | Ezeji and Blaschek     |
|                               | Mannose *         | 6.64                   | 0.11                   | 5.00                     | (2008)                 |
|                               | Arabinose *       | 14.18                  | 0.24                   | 5.50                     |                        |
|                               | Xylose *          | 12.04                  | 0.20                   | 4.65                     |                        |
| *C. acetobutylicum* ATCC 260   | Glucose *         | 20.99                  | 0.35                   | 1.92                     | Ezeji and Blaschek     |
|                               | Cellobose *       | 14.33                  | 0.24                   | 2.60                     | (2008)                 |
|                               | Galactose *       | 9.96                   | 0.17                   | 3.13                     | Ezeji and Blaschek     |
|                               | Mannose *         | 12.75                  | 0.21                   | 3.04                     | (2008)                 |
|                               | Arabinose *       | 12.07                  | 0.20                   | 2.53                     |                        |
|                               | Xylose *          | 9.53                   | 0.16                   | 4.10                     |                        |
| *C. beijerinckii* BA101       | Glucose *         | 17.82                  | 0.32                   | 3.25                     | Ezeji et al. (2007)    |
|                               | Cellobose *       | 19.10                  | 0.35                   | 3.21                     |                        |
|                               | Galactose *       | 10.01                  | 0.18                   | 4.22                     |                        |
|                               | Mannose *         | 14.29                  | 0.26                   | 6.00                     |                        |
|                               | Arabinose *       | 17.07                  | 0.31                   | 4.68                     |                        |
|                               | Xylose *          | 17.48                  | 0.32                   | 3.10                     |                        |
| *C. beijerinckii* P260        | Glucose (50 g/L)  | 20.10                  | 0.40                   | 1.89                     | Qureshi et al. (2007)  |
|                               | Glucose (60 g/L)  | 20.15                  | 0.33                   | 1.91                     |                        |
|                               | Glucose (100 g/L) | 23.49                  | 0.23                   | 1.65                     |                        |
|                               | Glucose (150 g/L) | 22.84                  | 0.15                   | 1.63                     |                        |
|                               | Glucose (200 g/L) | 14.65                  | 0.07                   | 2.23                     |                        |
|                               | Glucose (250 g/L) | <0.10                  | <0.01                  | NA                       |                        |
| *C. saccharobutylicum* 262     | Glucose *         | 16.72                  | 0.28                   | 1.93                     | Ezeji and Blaschek,    |
|                               | Cellobose *       | 10.72                  | 0.18                   | 3.44                     | (2008)                 |
|                               | Galactose *       | 11.02                  | 0.18                   | 3.45                     |                        |
|                               | Mannose *         | 5.31                   | 0.09                   | 4.57                     |                        |
|                               | Arabinose *       | 11.25                  | 0.19                   | 5.13                     |                        |
|                               | Xylose *          | 9.04                   | 0.15                   | 4.94                     |                        |
|                               | Glucose *         | 19.90                  | 0.33                   | 2.13                     |                        |
|                               | Cellobose *       | 18.28                  | 0.30                   | 1.80                     |                        |
|                               | Galactose *       | 13.13                  | 0.22                   | 2.85                     |                        |
|                               | Mannose *         | 16.41                  | 0.27                   | 2.56                     |                        |
|                               | Arabinose *       | 13.30                  | 0.22                   | 3.23                     |                        |
|                               | Xylose *          | 4.63                   | 0.08                   | 8.80                     |                        |

* a: 50 g/L
hydrolysis of cellulose and could be regarded as a turning point in converting lignocellulosic biomass into fermentable sugars (Wyman, 2001).

Different lignocellulosic biomass have been evaluated as substrates for biobutanol production, including rice straw (Amiri et al., 2014), corn stover (Qureshi et al., 2010b), corn cobs (Zhang et al., 2013), corn fiber (Qureshi et al., 2008a), wheat straw (Qureshi et al., 2007), barley straw (Qureshi et al., 2010a), and sorghum bagasse (Zafar et al., 2016). Besides, different types of wood such as pine (Amiri and Karimi, 2015a and b), elm (Amiri and Karimi, 2015a and b), and aspen poplar (Parekh et al., 1988) were also utilized for butanol production. Other lignocellulosic waste streams like wood pulping hydrolysate (Lu et al., 2013) and birch Kraft black liquor (Kudahetigie-Nilsen et al., 2015) as well as food wastes like potato peel waste (Abedini et al., 2020), banana peel waste (Mishra et al., 2020), orange peel waste (Joshi et al., 2015), and pineapple waste (Khedar et al., 2017) have also been suggested for biobutanol production.

Micro- and macroalgae (seaweeds) biomass are considered the third-generation feedstock for biobutanol production and are associated with important advantages vs. plant-based biomass. Those advantages include higher photosynthetic efficiency, faster growth rate, and land-independency. Among three classes of seaweeds, i.e., Phaeophyceae (brown), Rhodophyceae (red) and Chlorophyceae (green), green seaweeds have higher protein, carbohydrate, and nutritional values (Potts et al., 2012b). Ulva lactuca, a green macroalgae with a high growth rate, has been evaluated for butanol production (Potts et al., 2012b; van der Wal et al., 2013). However, the high capital and operating costs of cultivation is the main drawback of this substrate. Even though pairing biofuel production with nitrogen and phosphorous remediation is recommended for cost-effective algal cultivation, seasonal variations of pollution and climate make the steady supply of the feedstock questionable (Wang et al., 2017). In addition, the processing of seaweeds with relatively low carbohydrate content leads to a relatively dilute hydrolysate and dilute fermentation beer. The presence of protein inhibitors in the algal structure and the formation of furan derivatives through chemical treatments are the other important drawbacks of using algae for butanol production.

Farmanbordar et al. (2013b) showed that complex and blended wastes in the organic fraction of municipal solid waste (MSW) are suitable substrates for biobutanol production. From the scale point of view, the MSW generated by each person daily can be converted to 220 g gasoline-equivalent butanol, providing 15-100% of that individual's energy requirement for daily transportation (Farmanbordar et al., 2020). MSW is a “negative-price” waste-oriented feedstock, and its utilization for liquid fuel production can simultaneously serve energy security and waste management purposes. Furthermore, it was found that co-processing of lignocellulosic wastes and organic fraction of MSW in an integrated process based on ABE fermentation led to 10-49% higher ABE production than what was obtained from the individual substrates (Farmanbordar et al., 2020). A similar synergistic effect was observed in the co-fermentation of starch and hemimcellulosic hydrolysates, where a 35% improvement in ABE production and 78% increase in xylose utilization were obtained (Mirifakhra et al., 2020). Therefore, it can be concluded that utilization of the co-fermentation of substrates is likely to result in yield improvements in addition to cost reductions.

2.2. Upstream process

Pretreatment, enzymatic hydrolysis (of the cellulose fraction of pretreated substrate), and ABE fermentation of the resulting hydrolysate are the main stages of “cellulosic butanol” production. The efficiency of cellulosic butanol production in terms of overall ABE yield and titer is affected by different parameters (Amiri and Karimi, 2018). In the earlier studies, such as those performed by Qureshi et al. (2008c and 2010a), dilute sulfuric acid pretreatment followed by enzymatic hydrolysis was mostly used without evaluating the effects of pretreatment conditions, i.e., temperature, acidity, and residence time on ABE production efficiency. However, further studies on lignocellulosic butanol production revealed the crucial roles of pretreatment type and conditions (Amiri and Karimi, 2018). After decades of research devoted to developing an effective pretreatment leading to enhanced enzymatic hydrolysis, the main criteria were first defined for lignocellulosic bioethanol production: (1) extent of required sized reduction, (2) possibility of preserving the hemimcellulosic fraction, (3) extent of inhibitory degradation products formation, (4) amount of energy consumption, (5) cost of pretreatment especially catalyst cost, and (6) generation of high-value lignin-derived products (Mosier et al., 2005). Even though these criteria are also valid for lignocellulosic butanol production, they may not cover all the biobutanol process features. First of all, unlike ethanol fermentation, the yield of ABE fermentation is highly controlled by the concentration of fermentable sugars. In a medium with sugar concentrations lower than a threshold, i.e., 7 g/L (Long et al., 1984), the shift from the acidogenesis phase to the solventogenesis phase (where butanol is produced) does not occur in C. acetobutylicum (Long et al., 1984) and C. beijerinckii (Ezeji et al., 2005). Secondly, furan aldehyde compounds, including furfural and hydroxymethyl furfural (HMF), which form respectively through dehydration of pentose and hexoses during pretreatment, are severe inhibitors of ethanolic fermentation, whereas these compounds have stimulatory effects on ABE fermentation. It was shown that through the ABE fermentation, C. acetobutylicum has the ability of biotransforming furfural and HMF to stimulatory compounds of furfuryl alcohol and 2,5-bis-hydroxymethylfuran, respectively (Zhang et al., 2012). On the other hand, several compounds, mainly phenolic compounds derived from lignin degradation (Ezeji et al., 2007), tannins present in organic wastes like sorghum grain (Mirifakhra et al., 2017) and acorn (Heidari et al., 2016), and glycoalkaloids present in potato peel waste (Abedini et al., 2020) exert significantly higher inhibitory effects in ABE fermentation then in ethanolic fermentation.

Different pretreatment methods, including steam explosion (Marchal et al., 1992), alkaline pretreatment (Cheng et al., 2012), ammonium fiber explosion (AFX) (Ezeji and Blaschek, 2008), autohydrolysis (Amiri and Karimi, 2015a), dilute sulfuric acid pretreatment (Gottumukkala et al., 2013), ethanol organosolv pretreatment (Amiri et al., 2014), acetic organosolv pretreatment (Schulz et al., 2016), and organosolv pretreatment (Moradi et al., 2013), were developed considering the butanol production features as mentioned above. In the process of lignocellulosic butanol production, the pretreatment type/conditions affect both enzymatic hydrolysis and ABE fermentation in direct and indirect manners. As shown in Figure 1, the pretreated sample might have different mass fractions of glucan and lignin as the most important components. The enzymatic hydrolysis yield of the pretreated sample might also vary considerably. Both of these could affect the ABE process parameters, including yield and titer. In the processes developed based on cellulose content of the lignocellulosic feedstock, between 30-130 g ABE/kg feedstock was produced. As shown in Figure 1, the highest yield of cellulosic ABE production, 124.3 g ABE/kg feedstock, was obtained using the organosolv pretreatment of sweet sorghum bagasse, through which a pretreated solid containing 60% glucan (Fig. 1a) and 13% lignin (Fig. 1b) was obtained. The pretreated biomass was subsequently hydrolyzed with a yield of 94% and fermented, resulting in 11.4 g/L ABE.

Since solvent-producing Clostridia can efficiently uptake pentoses, the hemimcellulosic content of lignocellulosic biomass is also a potential source of carbon for biobutanol production. Chemical hydrolysis of hemimcellulose, detoxification of hydrolysate, followed by its fermentation are the stages of “hemimcellulosic butanol” production. Hemimcellulose is extensively used for hemimcellulose recovery and hemimcellulosic butanol production from brewing bagasse (Jauanbaró and Puigjaner, 1986), corn stover and switchgrass (Qureshi et al., 2010b), wheat bran (Liu et al., 2010), rice straw (Kanjan et al., 2013), corn fiber (Ezeji et al., 2007), sweet sorghum bagasse (Cai et al., 2013), sugar maple (Sun and Liu, 2012), and brewer's spent grain (Plaza et al., 2017). Other chemical hydrolysis methods like autohydrolysis combined with dilute acid post-hydrolysis (Sun and Liu, 2012) and liquid hot water treatment (Qureshi et al., 2016) were also suggested for hemimcellulosic butanol production.

To obtain a hydrolysate with a higher total sugar concentration, also known as “overall hydrolysate”, the cellulose-driven hydrolysis of lignocelluloses’ cellulose fraction can be performed in a medium containing previously-obtained hemimcellulosic hydrolysate (Amiri and Karimi, 2018). This approach has been implemented on wheat straw (Qureshi et al., 2008b), pine wood (Amiri and Karimi, 2015a), corn cobs (Zhang et al., 2013), and corn stover (Qureshi et al., 2010b) by the hemimcellulosic hydrolysate obtained using alkaline peroxide pretreatment, autohydrolysis, wet disk milling, and dilute acid hydrolysis, leading to 29, 105, 282, and 305 g ABE/kg lignocellulose, respectively. As shown in Figure 1, the highest butanol yield and titer was obtained from corn stover through a process including (1) dilute sulfuric acid hydrolysis, (2)
enzymatic hydrolysis by addition of cellulase to the slurry leading to a hydrolysate with \( 60 \) g/L sugar, and (3) ABE fermentation of the resulting hydrolysate with \( C. \) beijerinckii produced less than \( 4.5 \) g/L ABE. However, due to inefficient rhamnose uptake, the hydrolysates were poorly fermented by \( C. \) acetobutylicum (van der Wal et al., 2013).

Biobutanol production from complex feedstocks, e.g., MSW, containing a broad spectrum of fermentable carbon sources (cellulose, starch, and hemicellulose) and different phenolic compounds acting as fermentation inhibitors, is challenging. Hence, upstream process design and optimization should be performed based on a trade-off between maximizing inhibitor removal and minimizing carbohydrate loss. It has been found that phenolic compounds, especially tannins, significantly inhibit ABE fermentation. In a study on butanol production from tannin-containing sorghum grain, Mirfakhar et al. (2017) showed that solvent-producing \( Clostridia \) could tolerate less than \( 0.20 \) mM gallic acid equivalent (GAE) tannin. Lower tannin concentrations led to a reduction in ABE production due to inhibiting the culture’s amylolytic activity (responsible for \( 62\% \) of the drop) and inhibiting the ABE fermentation itself (28% of the drop). Therefore, tannin-rich feedstocks such as MSW should be treated before the ABE fermentation process. Farmanbordar et al. (2018b) showed that most MSW phenolic compounds are extractable (up to \( 87\% \)) by different solvents, including acetone, butanol, or ethanol. A process including dilute acid hydrolysis of the extractsive-free MSW, enzymatic hydrolysis of the solid fraction, fermentation of the liquor obtained by dilute acid hydrolysis, and fermentation of the hydrolysate obtained by enzymatic hydrolysis led to the production of \( 142 \) g ABE from each kg MSW (Farmanbordar et al., 2018b).

On the contrary, when the same process was repeated using tannin-rich MSW (control), the ABE produced was undetectable. In another study, ethanol organosolv pretreatment was evaluated for the simultaneous extraction of phenolic compounds and pretreatment of the lignocellulosic and stalky fractions of organic matter of MSW. The hydrolysis of the pretreated solid followed by its fermentation resulted in the production of \( 160 \) g ABE from each kg MSW (Farmanbordar et al., 2018a).

2.3. Mainstream process

ABE fermentation by strictly anaerobic bacteria, e.g., \( C. \) acetobutylicum and \( C. \) beijerinckii, has been studied for butanol or acetone production for over a century. Different microbial aspects of this fermentation have been previously reviewed (Lan and Liao, 2013; Xue et al., 2017). Even though ABE fermentation was implemented at the commercial scale during World War I and II, mostly for acetone production, the inherent drawbacks of this natural metabolism stand in the way of developing a commercial-scale
process for the production of butanol as a biofuel (Amiri and Karimi, 2018). In recent years, many studies were performed to enhance the ABE fermentation using either strain modification (Jiang et al., 2018; Cho et al., 2019; Yu et al., 2019) or process engineering tools (Jafari et al., 2017; Seifollahi and Amiri, 2020).

As the most preferred sugar for most solvent-producing Clostridia to glucose fermentation ABE varies in yield, titer, and productivity depending on the strain (Fig. 3). In a study on fermentation of mixed sugars, Ezeji and Blaschek (2008) found that, although glucose was the most preferred monosaccharide of solventogenic acetobutylicum Blaschek (2008) found that, although glucose was the most preferred sugar for most solvent-producing Clostridia to glucose fermentation ABE varies in yield, titer, and productivity depending on the strain. As shown in Figure 3a, for ABE titer and yield during glucose fermentation, Clostridia species/strains are ranked in descending order as follows: C. beijerinckii P260 (Qureshi et al., 2007), C. acetobutylicum ATCC 260 (Ezeji and Blaschek, 2008), C. butylicum NRRL 592 (Ezeji and Blaschek, 2008), C. beijerinckii BA101 (Ezeji et al., 2007), and C. saccharobutylicum 262 (Ezeji and Blaschek, 2008). In the case of xylose fermentation, as shown in Figure 4a, the highest ABE yields and titers are associated with the following species/strains in descending order: C. beijerinckii BA101 (Ezeji et al., 2007), C. acetobutylicum ATCC 824 (Ezeji and Blaschek, 2008), C. butylicum NRRL 592 (Ezeji and Blaschek, 2008), and C. acetobutylicum ATCC 824. However, the butanol to acetone ratio is decreased when the ABE titer and yield from xylose are increased (Fig. 4b).

Strain development for butanol production can be achieved by improving (1) cellular performance and (2) cellular robustness. Several studies have been devoted to enhancing cellular performance in terms of solvent production (Harris et al., 2001; Jang et al., 2012b; Yu et al., 2019), butanol selectivity (Jiang et al., 2009; Lee et al., 2009; Cho et al., 2019), carbohydrate utilization (Perret et al., 2004; Xiao et al., 2011; Xiao et al., 2012; Yu et al., 2015; Jang et al., 2018), and stable and continuous production of butanol (Nguyen et al., 2018). These improvements/modifications are mainly accomplished by cell mutation (Hu et al., 2011; Li et al., 2016), metabolic engineering (Yoo et al., 2020), and enzyme engineering (Mann and Lütke-Eversloh, 2013). Metabolic engineering of Clostridia is mainly performed through gene inactivation (Jang et al., 2014), genome edition by CRISPR/Cas9 systems (Wang et al., 2015; Xu et al., 2015; Zhang et al., 2018), and control or knockdown of gene expression by a synthetic small regulatory RNAs (sRNAs) (Cho and Lee, 2017). The second class of cell modifications is performed by improving cell robustness through reducing acid stress (Borden et al., 2010), solvent stress (Borden and Papoutsakis, 2007), or synergistic stresses (Alsaker et al., 2010). The studies performed on strain modifications for improved ABE production have been
comprehensively reviewed previously by Little-Everslo and Bahl (2011), Yoo et al. (2020), Jang et al. (2012a), and Cheng et al. (2019).

Besides strain development, process engineering also has played an important role in developing mainstream processes. "Process integration" with several different strategies has shown promising impacts on ABE production. Integration of enzymatic hydrolysis and fermentation, i.e., simultaneous saccharification and fermentation (SSF), is one of the relatively oldest strategies firstly suggested by Gauss et al. (1976) for reduction of end-product inhibition in ethanolic fermentation and for saving on equipment and operation costs. This integration strategy was evaluated for butanol production from wheat straw (Qureshi et al., 2008c), rice straw (Valles et al., 2020), aspen wood (Shah et al., 1991), kraft paper mill sludge (Guan et al., 2016), and acorns and wood chips (Sasaki et al., 2014). SSF process led to improved butanol production in some cases, whereas it was unsuccessful in some other cases, primarily due to the relatively low hydrolysis rate, which was insufficient for Clostridial activity. For instance, in the study by Qureshi et al. (2008c) on wheat straw, a lower ABE production was recorded by SSF in comparison with separate hydrolysis and fermentation (SHF). In light of that, modifications of SSF process in the form of simultaneous co-saccharification and fermentation (SCSF) (Seifollahi and Amir, 2020) or simultaneous saccharification and co-fermentation (SSCF) (Jafari et al., 2017) were suggested. Providing additional source of carbon in the form of a readily digestible polymer (in SCSF), e.g., cellulose oligomers (Seifollahi and Amir, 2020), or a fermentable monomer (in SSSF), e.g., sweet sorghum juice (Jafari et al., 2017), for Clostridial activity led to improved ABE production.

In-situ butanol recovery during the fermentation process is a technically-successful but economically- and energetically-questionable option. Different butanol separation operations especially adsorption (Yang et al., 1994), liquid-liquid extraction (Teke and Pott, 2020), pervaporation (Azimi et al., 2019; Li et al., 2020; Zhu et al., 2020), and gas stripping (Xue et al., 2013a), were evaluated for butanol recovery. In a comparative study on in-situ butanol recovery technologies, Groot et al. (1992) found the pervaporation and liquid-liquid extraction more promising. Efforts have also been put into integrating in-situ butanol recovery into the SSF process. For instance, simultaneous saccharification, fermentation, and recovery (SSFR) was evaluated for biobutanol production from wheat straw (Qureshi et al., 2008c) and corn stover (Qureshi et al., 2014). Despite higher yields and productivities achieved by integrating in-situ butanol recovery, the cost and energy consumption associated with these processes may exceed their advantages (Xue et al., 2013b).

2.4. Downstream process

The fermentation beer obtained after ABE fermentation is an aqueous mixture of the main products, i.e., acetone, butanol, and ethanol along with several other chemicals ranging from unused nutrients to fermentation by-products, e.g., acetic and butyric acid. Several different separation technologies, including adsorption (Oudohoom et al., 2009; Sharma and Chander, 2009), gas stripping (Qureshi and Blaschek, 2001; Setthakut et al., 2013; Liao et al., 2014), liquid-liquid extraction (Kurkijärvi et al., 2014; Kurkijärvi and Lehtonen, 2014), membrane extraction (Qureshi and Maddox, 2005), membrane distillation (Banat and Al-Shannag, 2000), reverse osmosis (Garcia III et al., 1986), thermopervaporation (Borisov et al., 2011), sweeping gas pervaporation (Plaza et al., 2013), and vacuum pervaporation (Borisov et al., 2014; Liu et al., 2014; Rozicka et al., 2014) were evaluated for product recovery and purification in the downstream of ABE production processes.

Based on the differences in the boiling point of the main products, distillation is a reasonable, robust and proven but at the same time energy-intensive separation technology for ABE recovery and purification (Green, 2011). Also, the formation of a heterogeneous water-butanol azetropote facilitates the distillation process without requiring the addition of any compounds. However, purification of butanol from the fermentation beer with a low butanol concentration requires relatively high energy consumption, in the range of 14.7-79.05 MJ/kg butanol (Kujawska et al., 2015).

The classic distillation process consists of four columns, i.e., acetone column, ethanol column, water stripper, and butanol stripper, where acetone (99.5 wt%), ethanol (95 wt%), and butanol (99.7 wt%) are obtained from the top of the first column, top of the second column, and bottom of the fourth column, respectively. Having separated acetone and ethanol in the first two columns, the stream remaining in the bottom of the ethanol column contains two immiscible phases of water and butanol. After splitting these phases in a decanter, the water stripper and butanol stripper columns are used to recover butanol from the water-phase and the butanol-phase, respectively. However, the energy balance of this classic process is not favorable and could be even negative in some cases. More specifically, the energy consumption to recover butanol through distillation stands at 14.5-79.5 MJ/kg butanol, which might exceed the final product's energy density of 36 MJ/kg (Patrašcu et al., 2018).

In 2012, the utilization of pressure-swing azetotropic distillation was suggested, which reduced the process's energy consumption to some extent (Løyben, 2012). Later, Patrašcu et al. (2017) proposed a new distillation sequence to perform the separation process with less energy and less equipment. In this sequence, a decanter was used as the first unit to (1) eliminate the necessity of beer stripper for initial water removal and (2) to prevent phase splitting throughout the columns (Patrašcu et al., 2017). The butanol phase was processed in a butanol stripper, while the water phase was sent to a pre-fractionator interconnected with a dividing-wall column to separate water (bottom product), recycle butanol (middle product), and provide an acetone-ethanol stream as feed for the final column. This distillation sequence reduced downstream energy consumption of a 40 ktpy butanol plant from 11,428 to 8,635 kW (from 5.90 to 4.46 MJ/kg butanol), corresponding to 1.44 MJ/kg butanol (Patrašcu et al., 2017). In continuation, the same researchers utilized a heat pump (vapor recompression)-assisted azetropic dividing-wall column (A-DWC) and managed to further decrease the energy consumption from 4.46 to 2.70 MJ/kg butanol (Patrašcu et al., 2018). Based on the payback period of 10 months, investing in the new technology was found economically viable (Patrašcu et al., 2018).

3. Concluding remarks and future directions

Butanol can be used as a drop-in fuel in the existing engines, supplying the transportation sector with a sustainable and renewable energy source. Compared with ethanol, butanol is advantageous in terms of ease of blending, volatility, hygroscopicity, corrosiveness, and pipe transportation. Accordingly, the butanol market could be extended by two orders of magnitude. However, there are critical inherent challenges to be addressed before commercial-scale butanol production could be realized. Butanol is a toxic chemical even for Clostridia, and its purification from the resulting dilute beer requires relatively high energy input. Furthermore, the yield of biobutanol production is relatively low, primarily due to the co-production of acetone, ethanol and acids.

To address these bottlenecks, process development for utilization of negative price substrates and strain modifications for obtaining higher cellular performance or robustness, process integrations for improving ABE yield and productivity, and developing more efficient separation technologies to reduce energy consumption have been suggested. Despite advancements made in the field mentioned above, there are still significant challenges to overcome for cost-effective and energy-efficient biobutanol production. Hence, future research should focus on developing (1) a cost- and energy-efficient pretreatment stage satisfying the requirements of ABE fermentation, in particular, maximizing hemicellulose and cellulose recovery with least degradation of lignin, (2) a microbial system either single or co-culture with enhanced butanol yield and titer, and (3) processes for maximizing mass and heat integration.

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