Biofuel Research Journal 28 (2020) 1256-1266

Biofuel Research Journal

Journal homepage: www.biofueljournal.com

Review Paper

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

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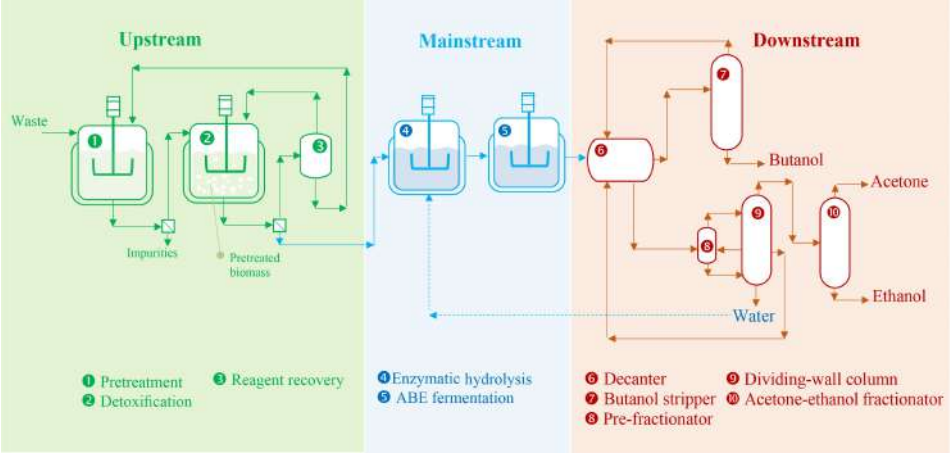
HIGHLIGHTS

- 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.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 September 2020
Received in revised form 20 October 2020
Accepted 18 November 2020
Available online 1 December 2020

Keywords:
Biobutanol
ABE fermentation
Clostridia
Lignocellulose
Pretreatment
Waste

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 production (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.

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Please cite this article as: Amiri H. Recent innovations for reviving the ABE fermentation for production of butanol as a drop-in liquid biofuel. Biofuel Research Journal 27 (2020) 1256-1266. DOI: 10.18331/BRJ2020.7.4.4

Contents

Abbreviations

ABE	Acetone-butanol-ethanol
A-DWC	Azeotropic dividing-wall column
AFEX	Ammonium fiber explosion
CI	Compression ignition
IC	Internal combustion
EROI	Energy return on investment
GAE	Gallic acid equivalent
HMF	Hydroxymethyl furfural
MSW	Municipal solid waste
PM	Particulate Matter
SCSF	Simultaneous co-saccharification and fermentation
SHF	Separate hydrolysis and fermentation
SI	Spark-ignition
SSCF	Simultaneous saccharification and co-fermentation
SSF	Simultaneous saccharification and fermentation
SSFR	Simultaneous saccharification, fermentation, and recovery

1. Introduction

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 utilization of fossil fuels have led to a renewed interest in alternative fuels, including alcoholic ones. The magnitude of these challenges and in particular global warming and climate change, and their adverse impacts on public health, demands an accelerated response (Watts et al., 2019). In other words, "gigaton problems need gigaton solutions" (Xu et al., 2010), and "sugar fermentation" for alcohol production from renewable carbohydrate resources is undoubtedly a major part of these solutions.

Among alcoholic fuels, the four-carbon butanol (also known as n-butanol) obtained through sugar fermentation has attracted a great deal of interest for its properties as a liquid fuel. Butanol as neat or blended fuel has been evaluated for use in both spark-ignition (SI) engines (Szwaja and Naber, 2010; Gu et al., 2012; Tornatore et al., 2012) and compression ignition (CI) engines (Zhang and Boehman, 2010; Doğan, 2011; Lujaji et al., 2011; Saisirirat et al., 2011). In 2005, McEnally and Pfefferle showed that the combustion of butanol isomers was accompanied by the lower formation of benzene, a precursor of soot, leading to lower particulate matter (PM) emissions from engines. Therefore, butanol could be regarded as a drop-in biofuel that can be used in the existing engines and infrastructure broadening its current market (USD 5.58 billion) to the potential market of liquid biofuel (USD 250 billion) (BP Energy Outlook, 2020). Besides, butanol is free from some of the major factors limiting the widespread application of fuel-grade ethanol. These include blending issues, 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 amphipathic 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's 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 cellulosic 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).

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. Butanol 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**).

In this review, besides presenting different generations of the substrate used for butanol production, recent innovations in process designs for upstream, mainstream, and downstream operations are critically reviewed and discussed. In the upstream, different pretreatments designed and optimized based on maximizing cellulose and hemicellulose recovery and minimizing lignin degradation are presented. In the mainstream, strain development by metabolic engineering and process integration strategies, including *in-situ* butanol removal, are scrutinized. Finally, in the

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Table 1.
ABE production from sugars by different wild strains of *Clostridia*.

Strain	Substrate	Production titer (g/L)	Production yield (g/g)	Butanol to acetone ratio	Reference
Clostridia acetobutylicum ATCC 824	Glucose [a]	18.70	0.31	2.91	Ezeji and Blaschek (2008)
	Cellobiose [a]	9.78	0.16	3.29	
	Galactose [a]	6.79	0.11	4.00	
	Mannose [a]	6.64	0.11	5.00	
	Arabinose [a]	14.18	0.24	5.50	
	Xylose [a]	12.04	0.20	4.65	
C. acetobutylicum ATCC 260	Glucose [a]	20.99	0.35	1.92	Ezeji and Blaschek (2008)
	Cellobiose [a]	14.33	0.24	2.60	
	Galactose [a]	9.96	0.17	3.13	
	Mannose [a]	12.75	0.21	3.04	
	Arabinose [a]	12.07	0.20	2.53	
	Xylose [a]	9.53	0.16	4.10	
C. beijerinckii BA101	Glucose [a]	17.82	0.32	3.25	Ezeji et al. (2007)
	Cellobiose [a]	19.10	0.35	3.21	
	Galactose [a]	10.01	0.18	4.22	
	Mannose [a]	14.29	0.26	6.00	
	Arabinose [a]	17.07	0.31	4.68	
	Xylose [a]	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 [a]	16.72	0.28	1.93	Ezeji and Blaschek, (2008)
	Cellobiose [a]	10.72	0.18	3.44	
	Galactose [a]	11.02	0.18	3.45	
	Mannose [a]	5.31	0.09	4.57	
	Arabinose [a]	11.25	0.19	5.13	
	Xylose [a]	9.04	0.15	4.94	
C. butylicum NRRL 592	Glucose [a]	19.90	0.33	2.13	Ezeji and Blaschek (2008)
	Cellobiose [a]	18.28	0.30	1.80	
	Galactose [a]	13.13	0.22	2.85	
	Mannose [a]	16.41	0.27	2.56	
	Arabinose [a]	13.30	0.22	3.23	
	Xylose [a]	4.63	0.08	8.80	

a: 50 g/L

downstream, recent studies on energy-efficient recovery and purification of ABE products to obtain promising EROIs are reviewed and discussed.

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. The propylene oxo synthesis, i.e., hydroformylation of propylene to aldehydes and their subsequent hydrogenation to butanol, was recognized as the main route for butanol production (Ndaba et al., 2015). In recent decades, however, several factories were built based on the fermentative process, especially in China, showing the feasibility of butanol production at least in some locations (Jiang et al., 2015). Besides, based on the techno-economic analysis performed, the utilization of lignocellulosic biomass for biobutanol production can turn the table by reducing the production cost beneath that of its petrochemical counterpart (Tao et al., 2014). Even though economic 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.

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

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

Different lignocellulosic biomasses have been evaluated as substrates for biobutanol production, including rice straw (Amiri et al., 2014), corn stover (Qureshi et al., 2010b), corncobs (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 (Jafari 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 (Kudahettige-Nilsson 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 (Khedkar et al., 2017) have also been suggested for biobutanol production.

Micro- and macroalgal (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 polluted water 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. (2018b) 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 lignocellulose 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 hemicellulosic hydrolysates, where a 35% improvement in ABE production and 78% increase in xylose utilization were obtained (Mirfakhar et al., 2020). Therefore, it can be concluded that the combined utilization 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 size reduction, (2) possibility of preserving the hemicellulose 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 ethanolic 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 (Mirfakhar 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 than in ethanolic fermentation.

Different pretreatment methods, including steam explosion (Marchal et al., 1992), alkaline pretreatment (Cheng et al., 2012), ammonium fiber explosion (AFEX) (Ezeji and Blaschek, 2008), autohydrolysis (Amiri and Karimi, 2015a), dilute sulfuric acid pretreatment (Gottumukkala et al., 2013), ethanol organosolv pretreatment (Amiri et al., 2014), acetone organosolv pretreatment (Jafari et al., 2016), and phosphoric acid 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 hemicellulose content of lignocellulosic biomass is also a potential source of carbon for biobutanol production. Chemical hydrolysis of hemicellulose, detoxification of hydrolysate, followed by its fermentation are the stages of "hemicellulosic butanol" production. Dilute acid hydrolysis was extensively used for hemicellulose recovery and hemicellulosic butanol production from brewing bagasse (Juanbaró and Puigjaner, 1986), corn stover and switchgrass (Qureshi et al., 2010b), wheat bran (Liu et al., 2010), rice straw(Ranjan 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 hemicellulosic butanol production.

To obtain a hydrolysate with a higher total sugar concentration, also known as "overall hydrolysate", the cellulase-driven hydrolysis of lignocelluloses` cellulose fraction can be performed in a medium containing previously-obtained hemicellulosic hydrolysate (Amiri and Karimi, 2018). This approach has been implemented on wheat straw (Qureshi et al., 2008b), pinewood (Amiri and Karimi, 2015a), corncob (Zhang et al., 2013), and corn stover (Qureshi et al., 2010b) by the hemicellulosic 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 2**, the highest butanol yield and titer was obtained from corn stover through a process including (1) dilute sulfuric acid hydrolysis, (2)

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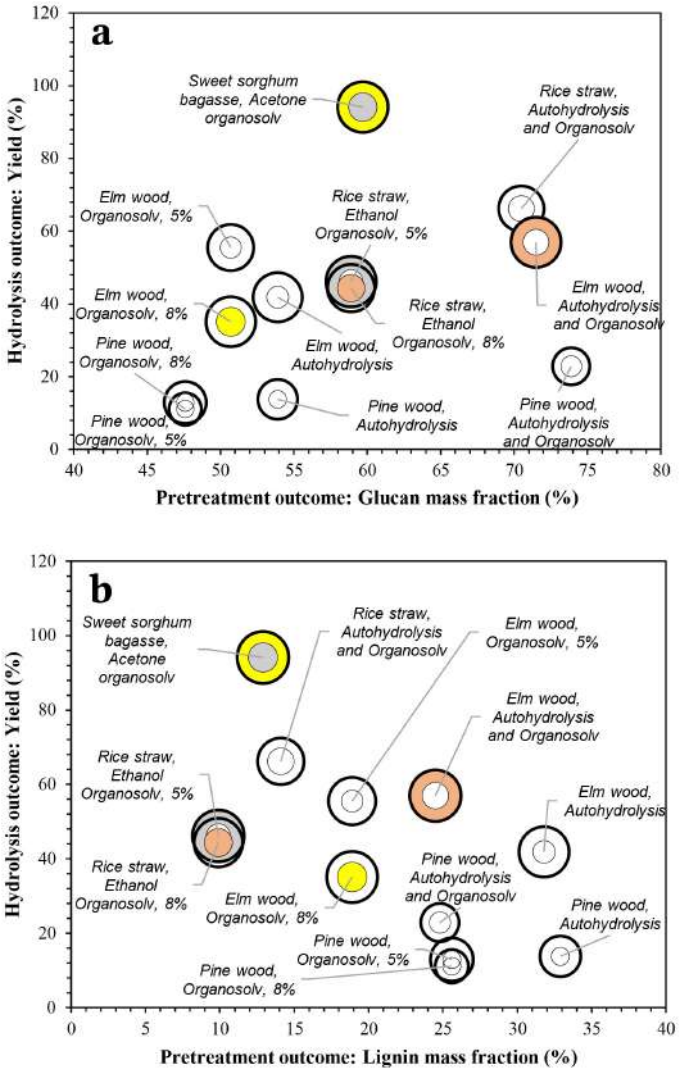

Fig. 1. The titer (inner bubbles) and yield (outer bubbles) of ABE production from rice straw pretreated by organosolv pretreatment (Amiri et al., 2014) or autohydrolysis-organosolv pretreatment (Amiri and Karimi, 2016), pinewood and elmwood pretreated by organosolv pretreatment (Amiri and Karimi, 2016), autohydrolysis (Amiri and Karimi, 2015a), or combined autohydrolysis-organosolv (Amiri and Karimi, 2016), and sweet sorghum bagasse pretreated by acetone organosolv pretreatment (Jafari et al., 2016). **(a)** Glucan mass fraction (%) and **(b)** Lignin mass fraction (%). The top three titer and yield values are shown by gold, silver, and bronze color, respectively.

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* P260.

Less severe upstream processes are needed for biobutanol production from microalgal and macroalgal biomass. In the absence of lignin and presence of amorph or less crystalline cellulose, a mild chemical hydrolysis (Potts et al., 2012a) or enzymatic hydrolysis after a mild pretreatment (van der Wal et al., 2013) can be utilized to obtain a fermentable hydrolysate. Potts et al. (2012a) utilized dilute acid hydrolysis at 125 °C for 30 min using 1% sulfuric acid for hydrolysis of Jamaica Bay macroalgae, *Ulva lactuca*, leading to 15.2 g/L total sugar, which was subsequently fermented to approx. 4 g/L butanol. Trying to produce ABE from rhamnose-rich *U. lactuca*, van der Wal et al. (2013) used dilute acid (1 M H_2SO_4 at 150 °C for 10 min) and dilute alkaline (6% NaOH at 85 °C for 4 h) treatments followed by enzymatic hydrolysis with commercial cellulase which led to 19.3 g sugars (7.5 g/L rhamnose, 8.4 g/L glucose, and 3.4 g/L other sugars) and 15.8 g sugars (6.2 g/L rhamnose, 6.9 g/L glucose, and 2.7 g/L other sugars), respectively. ABE fermentation of the resulting

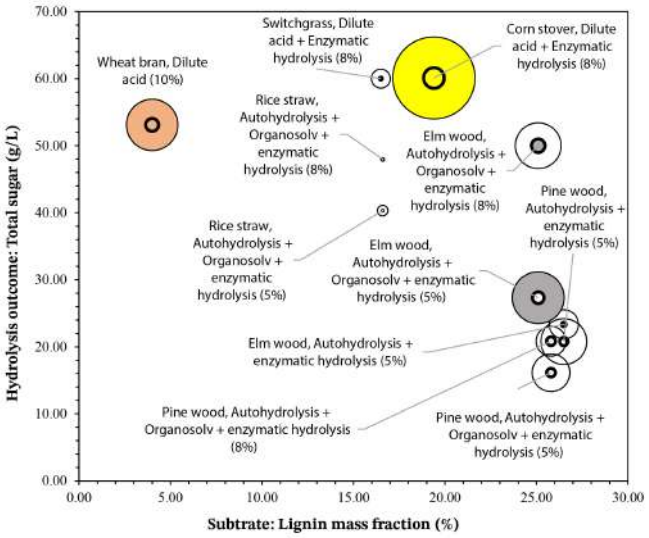

Fig. 2. The titer (inner bubbles) and yield (outer bubbles) of "hemicellulosic" and "overall" ABE production from rice straw, pinewood, elmwood, switchgrass, corn stover, and wheat bran using dilute acid hydrolysis, autohydrolysis, and integrated autohydrolysis-organosolv pretreatment.

hydrolysates by *C. beijerinckii* prodiced 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 extractives-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 starchy 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

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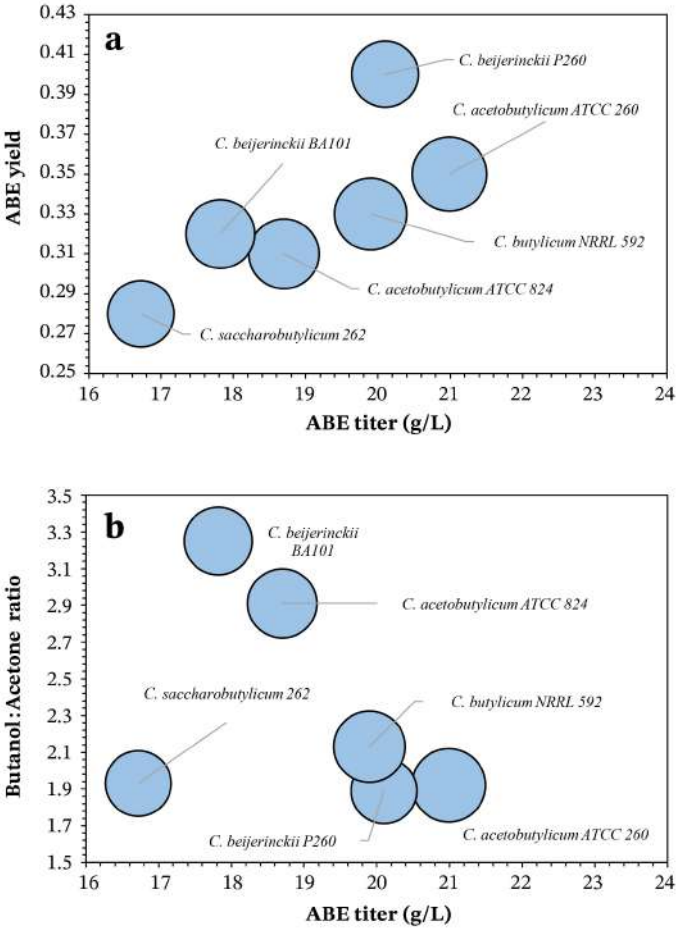

Fig. 3. Comparison of different studies in terms of **(a)** ABE titer *vs.* ABE yield and **(b)** ABE titer *vs.* butanol to acetone ratio. The size of the bubbles represents ABE productivities during the fermentation of 50 g/L glucose by *C. acetobutylicum* ATCC 260 (Ezeji and Blaschek, 2008), *C. acetobutylicum* ATCC 824 (Ezeji and Blaschek, 2008), *C. beijerinckii* BA101 (Ezeji et al., 2007), *beijerinckii* P260 (Qureshi et al., 2007), *C. saccharobutylicum* 262 (Ezeji and Blaschek, 2008), and *C. butylicum* NRRL 592 (Ezeji and Blaschek, 2008). The diameters of bubbles are scaled based on the productivity of 0.30 g/L.h obtained by *C. beijerinckii* BA101.

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 *Clostridia*, all sugars were utilized concurrently but with different rates. 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. acetobutylicum* ATCC 824 (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. acetobutylicum* ATCC 260 (Ezeji and Blaschek, 2008), *C. saccharobutylicum* 262 (Ezeji and Blaschek, 2008), and *C. butylicum* NRRL 592 (Ezeji and Blaschek, 2008). However, the butanol to acetone ratio is decreased when the ABE titer and yield from xylose are increased (**Fig. 4b**).

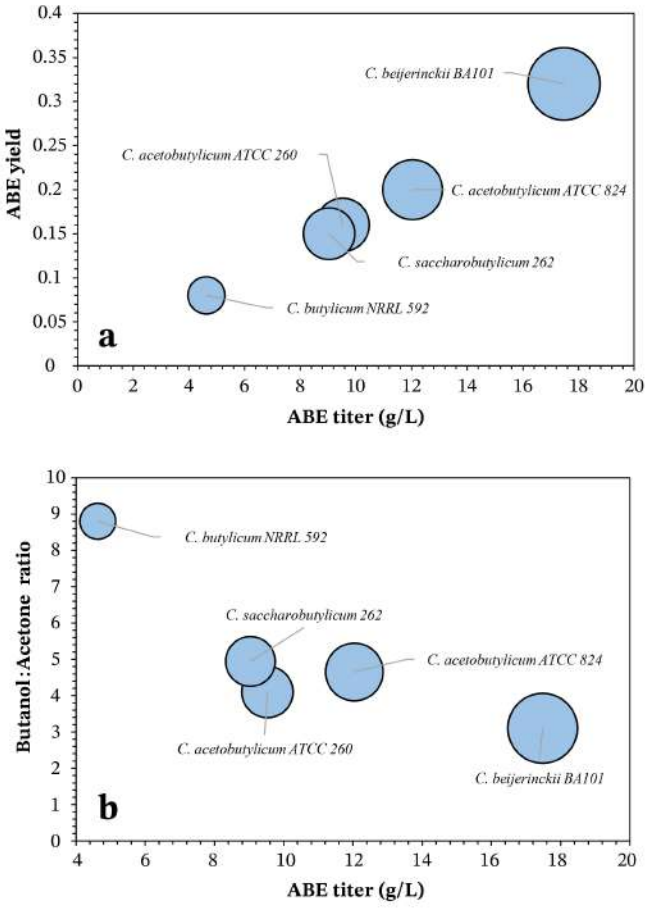

Fig. 4. Comparison of different studies in terms of **(a)** ABE titer *vs.* ABE yield and **(b)** ABE titer *vs.* butanol to acetone ratio. The size of the bubbles represents ABE productivities during the fermentation of 50 g/L xylose by *C. acetobutylicum* ATCC 260 (Ezeji and Blaschek, 2008), *C. acetobutylicum* ATCC 824 (Ezeji and Blaschek, 2008), *C. beijerinckii* BA101 (Ezeji et al., 2007), *C. saccharobutylicum* 262 (Ezeji and Blaschek, 2008), and *C. butylicum* NRRL 592 (Ezeji and Blaschek, 2008). The diameters of bubbles are scaled based on the productivity of 0.20 g/L.h obtained by *C. acetobutylicum* ATCC 824.

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; Jiang 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/CRISPR-associated protein 9 systems (CRISPR-Cas9) (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

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comprehensively reviewed previously by Lütke-Eversloh 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 Amiri, 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 Amiri, 2020), or a fermentable monomer (in SSCF), 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 (Oudshoorn et al., 2009; Sharma and Chung, 2011; Lin et al., 2012), gas stripping (Qureshi and Blaschek, 2001; Setlhaku 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 azeotrope 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 (Patraşcu et al., 2018).

In 2012, the utilization of pressure-swing azeotropic distillation was suggested, which reduced the process's energy consumption to some extent (Luyben, 2012). Later, Patraşcu 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 (Patraşcu 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 kt/yr 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 (Patraşcu et al., 2017). In continuation, the same researchers utilized a heat pump (vapor recompression)-assisted azeotropic dividing-wall column (A-DWC) and managed to further decrease the energy consumption from 4.46 to 2.70 MJ/kg butanol (Patraşcu et al., 2018). Based on the payback period of 10 months, investing in the new technology was found economically viable (Patraşcu 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 advances made in the domain 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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Please cite this article as: Amiri H. Recent innovations for reviving the ABE fermentation for production of butanol as a drop-in liquid biofuel. Biofuel Research Journal 27 (2020) 1256-1266. DOI: 10.18331/BRJ2020.7.4.4

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Please cite this article as: Amiri H. Recent innovations for reviving the ABE fermentation for production of butanol as a drop-in liquid biofuel. Biofuel Research Journal 27 (2020) 1256-1266. DOI: 10.18331/BRJ2020.7.4.4

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Please cite this article as: Amiri H. Recent innovations for reviving the ABE fermentation for production of butanol as a drop-in liquid biofuel. Biofuel Research Journal 27 (2020) 1256-1266. DOI: 10.18331/BRJ2020.7.4.4