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Prospect on agro-industrial residues usage for biobutanol production

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Abstract. Climate changes, environmental pollution and resource depletion are one of the numerous major problems humanity faces. United Nations sustainable development goals are aimed at solving these problems. The requirement for affordable, renewable, sustainable, biodegradable and environmentally friendly fossil fuel alternative sources is prompted by the development and advancement of biofuel production technologies. Of the various biofuel alternatives, biobutanol has increased the interests of researchers due to its desirable characteristics such as hydrophobicity, relatively high heating value and energy density, relatively low vapour pressure, etc. Nowadays, sustainable production of the biobutanol depends on the used feedstock source and its pre-treatment method, selected enhancing microorganism strain, acetone–butanol–ethanol fermentation effectiveness and titer of biobutanol. The main research challenges in biobutanol production are an improvement of production efficiency and increasing the financial viability of the technology. This review summarizes the latest results of lignocellulosic components content and fermentable sugars composition in different agro-industrial residues; biobutanol production depending on the *Clostridium* enhancing strategy, process optimization and selection of substrate. Such analysis provides a better perception of the capability of using agro-industrial residues for biobutanol production efficiency.

Key words: ABE fermentation, agricultural residues, biobutanol, *Clostridium*, lignocellulosic.

INTRODUCTION

The awareness of harmful impacts over global warming, environmental pollution and limitation of fossil fuels creates a need to find an alternate source for renewable energy resources (Anandharaj et al., 2020) Consequently, a variety of sustainable potent biofuels have been explored such as biodiesel, bioethanol, biobutanol, biomethanol, biogas etc. (Rathour et al., 2018). Butanol is a four-carbon straight-chained alcohol with a formula of C_4H_9OH (Lv et al., 2021). It is a promising alternative biofuel, owing to its appropriate physical properties. Compared to bioethanol it has a greater heating value and 25% higher energy content, higher viscosity, lower heat of vaporization and lower corrosivity (Jiang et al., 2018; Anandharaj et al., 2020). Butanol has better intersolubility than that of ethanol fuel, it is hydrophobic, can be blended in any concentration with gasoline without any modification of current vehicle engines, and can be transported in existing

infrastructure (Rathour et al., 2018; Lv et al., 2021). To assess the profitability of biofuels, including biobutanol, the important criteria is energy return on investment (EROI) defined as the ratio of the total energy output to the energy input. Because of relatively high energy density of butanol, biobutanol production process has the potential to have higher EROI than it is for corn-based ethanol (EROI ranges from 0.8 to 1.6) (Tao et al., 2013; Rezaei et al., 2021). Research by Tao et al. (2013) shows that cellulosic n-butanol lower than that of ethanol, by-product appearance during the butanol production process eventually compensate and increase the EROI of butanol (Tao et al., 2013).

Selection of the feedstock is other important factor affecting EROI of the butanol production. Shift from first to the second generation biofuels by utilizing lignocellulose, instead of edible resources - is accompanied by the increase of EROI (Rezaei et al., 2021). Compared with the first generation of biofuels production using starch-based feedstock, the second generation of biofuels is suitable for acetone-butanol-ethanol (ABE) fermentation, because does not compete with food market (Li et al., 2019; Jiang et al., 2019; Tsai et al., 2020). Utilizing lignocellulosic biomass seems to be the best alternative for biobutanol production since it is a renewable and widely available low cost resource (Huzir et al., 2018). Additionally, utilizing lignocellulosic biomass helps to properly manage the waste generation (Huzir et al., 2018).

The selection of biomass of feedstock for butanol production should be considered is the variety of cultivated agricultural crops available in each country, its growth time, the request for each crop for other purposes, and harvesting, transportation and pre-treatment costs (Procentese et al., 2017). Lignocellulosic biomass from agricultural residues like rice straw, sugarcane bagasse, wheat straw, corn cob and corn stover are potential sources for bioethanol and biobutanol production. These crops have a short-harvest rotation, thus allowing greater availability of these residues throughout the year (Sindhu et al., 2016; Araújo et al., 2018). Very interesting lignocellulosic by-product of the mushroom industry is the spent mushroom substrate. Food and Agriculture Organization have estimated, that in 2019 year world production of spent mushroom substrate was about 12 million tons. For every ton of mushroom produced, about 5 tons of spent mushroom substrate is generated (FAO, 2019) As a kind of lignocellulosic materials, it could be a source of reducing sugars for producing biofuels (Rajavat et al., 2019).

Many lignocellulosic biomasses with high cellulose and hemicellulose content and low lignin content are the ideal substrate for biobutanol production (Galbe & Wallberg, 2019). However, lignocellulosic biomass cannot be converted into biofuels directly and has to be pre–treated to release the fermentable sugars for solventogenic *Clostridium sp*., which produce butanol via ABE fermentation (Jiang et al., 2018; Kolesinska et al., 2019). Lignocellulosic biomass feedstock selection, ABE fermentation time and biobutanol yield were some of the major factors which predominantly affect the cost and sustainability of the butanol production process. The production cost of biobutanol can be reduced by various *Clostridium* strain metabolic engineering and fermentation process optimization strategies (Tian et al., 2019b; Gao et al., 2020).

In recent years, a numerous researchers has reviewed the potential of agricultural feedstock, lignocellulose pre-treatment methods and process optimization for sustainable production of biobutanol, for example, Ravindran & Jaiswal (2016), Araújo et al. (2018), Kolesinska et al. (2019), Vivek et al. (2019). In this study the latest results for lignocellulosic components content and fermentable sugars composition in different

agro-industrial residues were summarized. Based on this, biobutanol production depending on the *Clostridium* enhancing strategy, process optimization and selection of substrate were analyzed. Such analysis provides a better perception of the capability of using agro-industrial residues for biobutanol production efficiency.

AGRO-INDUSTRIAL RESIDUES

Agricultural crops capable of generating residues still in the harvesting phase and obtained residues at different stages of industrial processing must be considered for a potential feedstock for ABE fermentation (Araújo et al., 2018). Most studies emphasize that agricultural residues and waste with high cellulose and hemicellulose and low lignin content is appropriate substrate for biobutanol production (Huzir et al., 2018). According to Araújo et al. (2018), among the main agro-industrial residues, the most promising for use as raw materials, based on cellulose content in its composition, are soy straw, sugarcane leaves, corn husks and straw, as well as sugarcane pulp. The least potential was attributed to apple pomace, potato skin and tomato pomace. Most suitable residues originate from the most productive crops which have high cellulose content. Some residues such as sugarcane bagasse, mango seed, coffee husk and pineapple peel are potential feedstock due to low lignin content (Araújo et al., 2018).

Lignocellulosic biomass consists mainly of three essential polymers in plant cell walls, which are cellulose, hemicellulose and lignin (Huzir et al., 2018). The interactions of these components create a highly resistant and recalcitrant biomass. Cellulose is the major component of lignocellulose and the most abundant polysaccharide present on earth (Madeira et al., 2017). It is made up of D–glucose units attached via β–1,4 glycosidic bonds. Due to crystallinity and hydrogen bonding cellulose possesses high resistance to enzymatic hydrolysis (Jiang et al., 2019). Hemicellulose is a heterogenous polymer made of short chains of polusaccharide molecules. They constitute 15–35% of the plant biomass and are composed of penthoses (xylose, arabinose), hexoses (glucose, mannose, galactose, fucose, rhamnose) and sugar acids (Ravindran & Jaiswal, 2016); (Chong et al., 2020) The component sugars in hemicellulose may vary depending on the source of the plant biomass (Ravindran et al., 2018). It helps strengthen the cell wall by interactions with cellulose or lignin via hydrogen bonds (Jiang et al., 2019). Lignin is a phenolic heteropolymer, which is formed by oxidative polymerization of plant p-hydroxycinnamyl alcohols (Chaudhary & Verma, 2020). Lignin is providing structural support, resistance against microbial attack and water impermeability to the secondary cell walls of plants. However, lignin also serves as both a physical and biochemical barrier that impedes most biomass-to-bioproducts conversion processes (Madeira et al., 2017).

Regardless of lignocellulosic biomass source it is difficult to use it as a substrate in fermentation and usually lignocellulose has to be pre-treated to release the fermentable sugars, which are then convert into biofuels by microorganisms. Pre-treatment methods for lignocellulosic biomass have been extensively studied using physical, chemical and biological means, with the aim of improving the efficiency of hydrolysis. The pre-treatment processes disrupt the highly crystalized cellulose structure and the lignin–carbohydrate complex, remove lignin, and ultimately hydrolyse cellulose and hemicellulose to simple sugars (Wang et al., 2014; Ibrahim et al., 2015; Narayanasamy et al., 2019). These pre-treatment methods are usually combined because no pretreatment technique alone can meet the objectives cited above (Houfani et al., 2020).

Depending on the morphological structure and pre-treatment method the ratio of cellulose, hemicellulose and lignin varies in different lignocellulosic materials (Shirkavand et al., 2016; Araújo et al., 2018). However, the main constituents are basically the same, although the contents of individual carbohydrates, aromatics and other compounds vary: about 50–60% are carbohydrates, i.e. cellulose and hemicelluloses, 20–30% lignin, while the rest consist of extractives, fatty acids, ash, etc. (Galbe & Wallberg, 2019). In general, lignocellulosic biomass consists of 39–50% of cellulose, 24–31% of hemicellulose and 15–25% of lignin (Jiang et al., 2019; Houfani et al., 2020).

Table 1. Chemical composition of agro-industrial residues

Agro-industrial residues	Pre-treatment	Chemical composition (% dry mass)		
		Cellulose	Hemicellulose	Lignin
Rice straw[1]	untreated	36.8	25.8	15.8
Rice straw[1]	ammonia	57.4	22.1	8.6
Rice straw[2]	alkaline	71.20	22	1.6
Brewers spent grain[3]	untreated	23.1	22.9	14.1
Soybean straw[4]	untreated	44.2	5.9	19.2
Soybean straw[4]	alkaline	74	10.3	10.1
Palm empty fruit bunches[5]	untreated	41.32		10.8
Palm empty fruit bunches[5]	acid	63		16.0
Palm empty fruit bunches[5]	alkaline	63		13.2
Palm empty fruit bunches[5]	alkaline and acid	68.4		15.1
Bamboo[6]	milling	42.5	20.1	17.1
Wheat straw[7]	acid	38.7	19	17.3
Sugarcane top[8]	acid	39.8	28.6	22.5
Sugarcane bagasse[9]	untreated	43.1	22.8	24.1
Sugarcane bagasse[9]	alkaline	68.4	6.9	17
Hazelnut shell[10]	alkaline	42.1	28.2	25.2
Barley straw[11]	milling	31–45	27-38	14-19
Barley hull[12]	acid	30.6	46.8	9.5
Coconut husks[13]	milling	18.2–21.3	11.3–17.3	46.4–53.1
Coconut husks[13]	alkaline	33.7–36.9	22.6–24.2	36.8–37.6
Coconut husks[13]	acid	17–25.6	13.2-22.4	48.7–51.5
Sorghum straw[14]	untreated	37.7	28.1	21.5
Sorghum straw[14]	alkaline	71.4	16.2	6.3
Sorghum straw[14]	acid	57.8	11.8	17.8
Sorghum straw[14]	oxidising agents	54.6	24.5	11.6
Sweet sorghum bagasse[15]	alkaline	36.9	17.8	19.5
Corn stover[16]	milling	36.3	31.4	17.2
Corn strover[17]	alkaline	64	16	13
Corn stalk[18]	acid	34.5	27.6	21.8
Deshelled corn cobs[18]	alkaline	69.8	27.4	1.5
Oat straw[19]	milling	34.8	26.7	8.7
Spent mushroom substrate[20]	organosolv	52.7	14.6	10.5
Spent mushroom substrate[21]	thermal drying	37.5	18.6	20.5

[1](Nguyen et al., 2010); [2](Cheng et al., 2012); [3](Plaza et al., 2017); [4](Kim, 2018); [5](Noomtim & Cheirsilp, 2011); [6](Kumar et al., 2017); [7](Pérez–Rangel et al., 2015); [8](Szczerbowski et al., 2014); [9](Yue et al., 2015); [10](Demirbaş, 2005); [11](Saini et al., 2015); [12](Guerfali et al., 2018); [13](Ding et al., 2012); [14](Dong et al., 2019); [15](Umagiliyage et al., 2015); [16](Saha et al., 2016); [17](Yoav et al., 2017); [18](Ma et al., 2011); [19](Arreola–Vargas et al., 2014); [20](Zhu et al., 2016); [21](Rajavat et al., 2019).

Table 1 present cellulose, hemicellulose and lignin values for a range of chemical pre-treated and untreated or physical treated lignocellulosic biomass wastes derived from food and agricultural industries. Depending on the converting process of lignocellulose (Table 1), agro-industrial residues consist of 18–74% of cellulose, 4–47% of hemicellulose and 4–53% of lignin. For example, untreated and pre–treated sorghum straw contains 38% and 55–71% of cellulose, 28% and 16–25% of hemicellulose, 21% and 6–18% of lignin, respectively (Dong et al., 2019). Residues particle size is important factor to. For example, coconut husks particle size diminishing from 850–1500 μm^2 to 300–600 μm^2 increases content of cellulose to 3%, hemicellulose to 6% and reduce lignin to 7% (Ding et al., 2012).

In studies an alkaline and acid pre-treatment of residues often was used, which effectiveness is different between crop and waste types. Alkaline was an effective chemical pre-treatment method used for agro-industrial residues, such as coconut husks (Ding et al., 2012) sorghum straw (Dong et al., 2019), sorghum bagasse (Umagiliyage et al., 2015), sugarcane bagasse (Yue et al., 2015), rice straw (Cheng et al., 2012), soybean straw (Kim, 2018) and deshelled corn cobs (Wen et al., 2014). A study by Dong et al. (2019) demonstrates how the selection of treatment method influences the final ratio of components in sorghum straw. Alkaline pre-treatment increases cellulose content in it by almost 34%, while the lignin content was reduced by 15%, what makes sorghum straw more available for further use in ABE fermentation (Dong et al., 2019). The result of Noomtim & Cheirslip (2011) study showed, that the treatment efficiency of palm empty fruit bunches with acid and alkaline was equal for cellulose and lignin content (Noomtim & Cheirsilp, 2011), compared to Dong et al. (2019). The usage of H_2O_2 as oxidising agents is able to lower the lignin content by almost 10% in comparison with untreated sample, and appears to be more effective than acid treatment, having higher total cellulose and hemicellulose content by 10% (Dong et al., 2019). One of potential by-product types is the spent mushroom substrate, which is high in a cellulose content and moderate in lignin. After organosolv treatment, it has increases by 15% of cellulose and reduced by 10% of lignin (Zhu et al., 2016; Rajavat et al., 2019).

These chemical composition values describe the possibility of dividing lignocellulosic material into different effectiveness. The ratios should not be interpreted directly, as each study used different acid or alkaline concentrations, different liquid to solid ratio, and other parameters as particle size. Although chemical pretreatment can be effective at deconstruction, but the production of toxic materials, carbohydrate loss and the high cost of the process - are common disadvantages (Shirkavand et al., 2016). Therefore, parameters and method selection of lignocellulosic biomass pretreatment is an important step for efficient use of wastes. Abundantly available agricultural wastes from rice, sugarcane and wheat, with high level of cellulose and hemicelluloses is the main advantage for their usage for the production of biobutanol.

LIGNOCELLULOSE CONVERSION TO SUGARS

The degradation of cellulose into glucose molecules requires a combined hydrolysis by three key enzymes: endoglucanase, exoglucanase and β-glucosidase. They are categorized in the glycoside hydrolase family and catalyse the cleavage of glycosidic bonds (Ravindran et al., 2018). Hemicellulose degradation needs depolymerase and debranching hemicellulases enzyms, such as a xylanase, mannanse, β-glucanase,

xyloglucanase, α–glucuronidase, α-arabinfuranosidease, α-d-galactosidase, acetyl xylan esterase and ferulic acid esterase (Chen & Wang, 2017; Ravindran et al., 2018). Commercial enzymes with cellulose and hemicellulose degradation activity are mainly produced by aerobic fungi and anaerobic bacteria (Chen & Wang, 2017). Fungi, mainly *Aspergillus* and *Trichoderma* species are potentially useful for because, generally, their secreted enzyme levels are much higher than those of yeasts and bacteria (Godoy et al., 2018). However, for the more cost–effective production of biobutanol, the use of cellulolytic *Clostridium* (Ou et al., 2017; Wen et al., 2020b) and *Thermoanaerobacterium* sp. with xylanases and β–xylosidases activity is preferred (Jiang et al., 2018).

Glucose is the major monosaccharide present in lignocellulosic biomass hydrolysates, xylose is the next, followed by arabionos and mannose, galactose etc. (Tsai et al., 2020). Considering the amount of reducing sugars recovered in the pre-treated enzymatic hydrolysates from agro-industrial residues, the sugar yields are 3.1–21.7 g L^{-1} for glucose, 0.3–18.8 g $L^{-1}$for xylose, 0.9–12.2 g L^{-1} for arabinose, 0.5–3.2 g L^{-1} for mannose (Table 2). The rice straw and sugarcane bagasse hydrolysates in Cheng et al. (2012) study shows higher sugar yields, 52.3 and 50.7 g L^{-1} for glucose, 7.7 and 15.2 for xylose, respectively (Cheng et al., 2012). High glucose values of 34.8 g L^{-1} were observed in spent mushroom substrate pre–treated with organosolv and enzymatic hydrolysis (Zhu et al., 2016). Corn fiber (Ezeji et al., 2007), brewers spent grain (Plaza et al., 2017), wheat straw (Quershi et al., 2008) and barley straw (Qureshi et al., 2010) hydrolysates shows high xylose content - 18.8 g L^{-1}, 18.4 g L^{-1}, 17.3 g L^{-1} and 15.9 g L^{-1}, respectively. High arabinose and galactose content was founded in corn fiber hydrolysate (Ezeji et al., 2007). These agro-industrial residues are promising for use as substrate for ABE fermentation.

Table 2. Sugars composition in agro-industrial residues

Agro-industrial residues	Pre-treatment	Hydrolysate sugar contain, g L^{-1}		
		Glucose	Xylose	Arabinose
Spent mushroom substrate[1]	organosolv and enzymatic	34.8	1.03	0.055
Rice straw[2]	alkaline and enzymatic	52.3	7.7	
Sugarcane bagasse[2]	alkaline and enzymatic	50.7	15.2	
Sugarcane bagasse[3]	acid	11	14	
Brewers spent grain[4]	acid	20	18.4	
Corn fyber[5]	acid and enzymatic	20.9	18.8	12.2
Wheat straw[6]	acid and enzymatic	3.1	17.3	3.1
Rice bran[7]	acid and enzymatic	21.74	0.37	1.44
Rice bran[7]	enzymatic	6.2	0.26	0.88
Rice bran[7]	acid	12.66	0.29	1.14
Barley straw[8]	acid and enzymatic	20.2	15.9	6.1
Oat straw[9]	acid	1.53	3.69	1.3
Apple peel 1/10 to water ratio[10]	hydrothermal	25		

[1](Zhu et al., 2016); [2](Tsai et al., 2020); [3](Narayanasamy et al., 2019); [4](Plaza et al., 2017); [5](Ezeji et al., 2007); [6](Qureshi et al., 2008); [7](Lee et al., 2009); [8](Qureshi et al., 2010); [9](Arreola–Vargas et al., 2014); [10](Raganati et al., 2016).

POTENTIAL CLOSTRIDIUM SP. FOR PRODUCTION OF BIOBUTANOL

Biobutanol production is usually done by solventogenic bacteria from *Clostridium* genus, such as *C. acetobutylicum* (Ibrahim et al., 2015), *C. beijerinckii* (Plaza et al., 2017), *C. saccharoperbutylacetonicum* (Zetty–Arenas et al., 2019) and *C. pasteurianum* (Lipovsky et al., 2016). These bacteria produce biobutanol by fermenting sugars through acetone, butanol and ethanol fermentation (Zetty–Arenas et al., 2019; Ashani et al., 2020). ABE fermentation can be divided into two phases: acidogenic phase, where cell growth occurs and acids (butyric acid, acetic acid) are the main metabolites; and solventogenic phase, where acids are reassimilated and solvents are produced. Later, fermentation ceases and cells form endospores (Birgen et al., 2019; Xue & Cheng, 2019);

Butanol producing *Clostridium* sp. are able to use a wide variety of carbohydrates such as starch, cellobiose, sucrose, glucose, fructose, mannose, dextrin, galactose, xylose and arabinose (Plaza et al., 2017). Glucose is the most preferred carbon source for *Clostridium* sp., and all the central carbon metabolic pathways are expressed constitutively enabling efficient and rapid glucose utilization. (Jang et al., 2013; Ibrahim et al., 2015; Tsai et al., 2020). Other simple sugars, such as xylose, could not be consumed by bacteria in the presence of higher concentrations of glucose, due to a phenomenon called carbon catabolite repression, which might reduce butanol yield from the lignocellulosic biomass (Wen et al., 2020b). Bacteria have developed sophisticated mechanisms to adapt to environmental changes. For example, carbon catabolite repression allows bacteria the assimilation of a preferred (i.e. rapidly metabolisable) carbon source when they are exposed to more than one carbohydrate (Deutscher, 2008). Improvement of either native or genetically engineered strains for simultaneous utilization of hexoses and pentoses without carbon catabolite repression, could improve the biobutanol production efficiency, resulting in a more economicaly feasible process (Vivek et al., 2019).

Acidogenic *Clostridium* such as *C. tyrobutyricum*, *C. thermocellum*, *C. cellulolyticum* and *C. cellulovorans* produces butyric and acetic acids as the main metabolic product, but not butanol, because of lacking some key enzymes, including CoA transferase (ctfAB), acetoacetate decarboxylase (adc), and aldehyde dehydrogenase (ald), in the pathways leading to ABE production (Yu et al., 2015; Xue & Cheng, 2019); (Bao et al., 2019). These strains have had evolving interest of researchers, due to the possibility of engineering their metabolic pathways in benefit for butanol production. Especially, *C. thermocellum*, *C. cellulolyticum* and *C. cellulovorans* with cellulolytic activity, because they natively secrete cellulases and consume cellulose, xylan and mannan (Yang et al., 2015; Ou et al., 2017; Wen et al., 2020b).

ENHANCING PRODUCTIVITY OF BIOBUTANOL

Solvent producing *Clostridium* strains rarely tolerate more than 10–20 g L^{-1} butanol in fermentation broth (Yang et al., 2015; Amiri & Karimi 2018; LV et al., 2020). Depending on the species, 10–20 g L^{-1} butanol concentration induces an adverse change in phospholipid and fatty acid composition in cell membrane (LV et al., 2020). Which induces bacteria sporulation, that results in, viable cell metabolism and end of solvent biosynthesis (Kumar & Gayen, 2011; Cheng et al., 2019). Most solventogenic *Clostridium* sp. share similar central carbon metabolic pathway and may encounter

similar problems, including low butanol yield (g of butanol from g of consumed sugar) and final titer (g butanol in L of fermentation broth), in the fermentation (Cheng et al., 2019).

To improve *Clostridium* sp. biobutanol yield and titer various strategies have been explored, such as genetic modification, metabolic engineering, randomly induced mutation by UV light, irradiation and chemical mutagenesis (Jang et al., 2013; Schwarz et al., 2017; Tian et al., 2019b; Gao et al., 2020). These strategies have been used to engineer (1) solventogenic *Clostridium* with improved biobutanol tolerance and productivity, (2) acidogenic *Clostridium* to produce butanol, (3) cellulolytic *Clostridium* to produce butanol and improve cellulases activity (Wen et al., 2017; Xin et al., 2018; Cheng et al., 2019).

Metabolic engineered *Clostridium* sp.

Metabolic engineering (ME) has been widely applied for *Clostridium* strain improvement for enhanced production of butanol (Raganati, 2016; Zhang et al., 2018b; Bao et al., 2019; Wen, 2020b). In study by Jang et al. (2012) butanol production in engineered *C. acetobutylicum* BKW increased by 60% from 11.8 g L^{-1} to 18.9 g L^{-1}, compared with a wild strain (Jang et al., 2012). By using ME to enhance solvent tolerance of *C. acetobutylicum* strain an increase of 61% butanol titer from 12.6 to 20.3 g L^{-1} with increase in yield from 0.20 to 0.23 g/g was observed (Xu et al., 2015). In another study, Tian et al. (2019b) have reported significant increase in butanol tolerance by *C. thermocellum* of 15 g L^{-1}, which is up to 300% higher, compared to wild strain (Tian et al., 2019b). In Wen et al. (2020b) study, metabolic engineered *C. cellulovorans* was metabolic engineered, resulting in 4.96 g L^{-1} butanol titer from alkali extracted corn cob xylose (Wen et al., 2020b). Some *Clostridium* wild type strains and engineered strains are able to convert acetone to isopropanol, producing a mixture of isopropanol, butanol and ethanol, all of which can be used as biofuels without purification (Youn et al., 2016). Youn et al. (2016) reported on an effective isopropanol and butanol (IB) fermentation using a newly isolated *Clostridium* sp. A1424 capable 13.92 g L^{-1} of IB and 9.43 g L^{-1} of butanol producing from glucose with a small amounts of residual acetone (Youn et al., 2016). Zhang et al. (2018a) engineered *C. tyrobutyricum* and these recombinant bacteria produced 26.2 g L^{-1} butanol and 38.2 g L^{-1} butanol and ethanol mixture without acetone, which is the highest value when cultivated on glucose based batch fermentations using natural butanol–ethanol producers (Zhang et al., 2018a).

Table 3 summarizes the highest butanol titer results from 24 studies, depending on the choice of *Clostridium* specie, substrate, fermentation process and enhancing strategy. The highest titer results showed the ME *C. tyrobutyricum* 26.2 g L^{-1} (Zhang et al., 2018a), ME *C. acetobutylicum* 20.3 g L^{-1} (Xu, 2014), chemical mutant *C. acetobutylicum* 17.6 g L^{-1}, which have used glucose as substrate (Jang et al., 2013) and ME *C. cellulovarans* 15.8 g L^{-1} from fructose (Wen et al., 2020b). It should be noted that such high titer values are observed in experiments, where sugar content in batch was more than 60 g L^{-1}. Rather well butanol titer rates of 7.6–11.8 g L^{-1} were obtained from other carbon sources as mannitol, xylose, mannose, arabinose and crude glycerol (Raganati 2016; Xin 2017; Wen et al., 2020a). Sugarcane bagasse showed good results as substrate, resulting in 14.5 g L^{-1} of butanol, fermented by *C. saccharoperbutylacetonicum* (Zetty-Arenas 2019). Although not large, but optimistic results have ME *C. cellulovarans* that consumes cellulose, with butanol titer of

1.11–2.31 g L^{-1} (Bao, 2019; Ou, 2017) and 3.37–4.96 g L^{-1}, fermented from pre-treated corn cob hydrolysate without enzymatic treatment (Ou 2017; Wen et al., 2020b). That is notably higher than it was obtained by ME *C. thermocellum*, when biobutanol titer was 0.04–02 g L^{-1} from cellulose (Tian 2019a). *C. cellulovarans* seems to be suitable microorganism for cellulose degradation and butanol production with solventogenic *Clostridium* consortium.

Table 3. Production of biobutanol with *Clostridium* sp. from different substrate

Process	Microorganism	Carbon source	Biobutanol titer, g L^{-1}
Mono–culture, wild type			
batch	*C. acetobutylicum* ATCC 824[1]	sago pith residues	5.41
simultaneous saccharification and fermentation	*C. acetobutylicum* ATCC 824[2]	pre–treated oil palm empty fruit bunch	2.75
batch	*C. beijerinckii* BGS1[3]	glucose	10.21
consolidated bioprocessing	*Clostridium* sp. NJ4[4]	Jerusalem artichoke	13.25
batch	*Clostridium* sp. A1424[5]	glucose	9.43
fed–batch	*C. pasteurianum* NRRL B–598[6]	glucose	8.3
batch	*C. beijerinckii* DSM 6422[7]	brewer's spent grains	6.6
batch	*C. beijerinckii* DSM 6422[7]	sucrose	9.61
batch	*C. saccharoperbutylacetonicum* DSM 14923[8]	sugarcane bagasse	14.5
Mono–culture, mutagenesis			
batch	*C. acetobutylicum* BKM19[9]	glucose	17.6
batch	*Clostridium* sp. CT7[10]	crude glycerol	11.8
batch	*C. acetobutylicum* ATTC 824[11]	rice straw	9.1
Mono–culture, metabolic engineered			
batch	*C. tyrobutyricum* ATCC 2575 (Δcat1, adhE2)[12]	glucose	26.2
batch	*C. cellulovarans*[13]	cellulose	1.11–2
consolidated bioprocessing	*C. cellulovarans* (adhE2)[14]	corn cobs	3.37
consolidated bioprocessing	*C. cellulovarans* (adhE2)[14]	glucose	3.08
consolidated bioprocessing	*C. cellulovarans* (adhE2)[14]	cellulose	2.31
batch	*C. acetobutylicum*[15]	glucose	13.2
batch	*C. acetobutylicum*[15]	mannose	8.91
batch	*C. acetobutylicum*[15]	arabinose	9.62
batch	*C. acetobutylicum*[15]	xylose	8.45
batch	*C. acetobutylicum* BEKW (buk)[16]	glucose	18.9
batch	*C. tyrobutyricum* (adhE2), (ctfAB)[17]	glucose	10–13.4
batch	*C. acetobutylicum* JB200[18]	glucose	20.3
batch	*C. cellulovorans* DSM 743B[19]	corn cobs	4.96
consolidated bioprocessing	*C. cellulovorans* DSM 743B[19]	fructose	15.81
batch	*C. tyrobutyricum* ATCC 25755 CTpM2[20]	glucose	3.47
batch	*C. tyrobutyricum* ATCC 25755 CTpM2[20]	mannitol	7.55

Table 3 continued

Co–culture consolidated bioprocessing	Phlebia sp. & *C. saccharoperbutylacetonicum*[21]	cellulose	3.2
consolidated bioprocessing	*C. cellulovorans* DSM 743B & *C. beijerinckii* 8052[22]	extracted corn cob	11.5
consolidated bioprocessing	*C. thermocellum* NBRC 103400 & *C. saccharoperbutylacetonium* N1–4[23]	rice straw	5.5
consolidated bioprocessing	*Thermoanaerobacterium* sp. M5 & *C. acetobutylicum* NJ4[24]	xylan	8.34
consolidated bioprocessing	*Thermoanaerobacterium* sp. M5 & *C. acetobutylicum* NJ4[25]	xylan	13.25

[1](Linggang et al., 2013) [2](Ibrahim et al., 2015); [3](Zhang et al., 2018b); [4](Jiang et al., 2020a); [5](Youn et al., 2016); [6](Lipovsky et al., 2016); [7](Plaza et al., 2017); [8](Zetty–Arenas et al., 2019); [9](Jang et al., 2013); [10](Xin et al., 2017); [11](Tsai et al., 2020); [12](Zhang et al., 2018a); [13](Bao et al., 2019); [14](Ou et al., 2017);[15](Raganati et al., 2016); [16](Jang et al., 2012); [17](Yu et al., 2015); [18](Xu et al., 2015); [19](Wen et al., 2020b); [20](Yu et al., 2012) [21](Tri & Kamei, 2020); [22](Wen et al., 2017); [23](Kiyoshi et al., 2015); [24](Jiang et al., 2018); [25](Jiang et al., 2020b).

Process optimization

It should also be noted that with optimization in the medium composition, fermentation conditions and process integrations, higher butanol productivity and titer can also be achieved (Yang et al., 2015). Supplementation of micronutrients and chemical compounds has been proven to be an effective (Xin et al., 2017). For example, supplementation of zinc and iron could enhance butanol titer production to 12.8 g L^{-1} compared to 4.5 g L^{-1} of butanol (Wu et al., 2016). Calcium carbonate had ability to enhance *Clostridium* tolerance to butanol by stabilizing cell membrane (Zhang et al., 2018b). In Raganati et al. (2016) study, the highest butanol titer was obtained from glucose, mannose, arabinose and xylose, when added 5 g L^{-1} and 10 g L^{-1} calcium carbonate in fermentation broth, respectively (Raganati et al., 2016). Yang et al., 2015 reported on synergistic effect of surfant PEG 4000 with xylanase on saccharification process efficiency, when used glucose and xylose yield increased from 53.2% to 86.0% and from 36.2% to 70.2%, respectively (Yang et al., 2015.) The influence of pH has been recognized as an important factor in determining the outcome of ABE fermentation, that the initiation of solvent production occurred only after the medium pH had decreased between 4.5 and 5.5 (Xin et al., 2017; Wen et al., 2020b). Generally, cultures at high pH values mainly produce acids, whereas in cultures maintained at a low pH, solvent production usually predominates. However, the pH range, over which solvent formation may occur, appears to vary quite widely depending on the particular strain and applied culture conditions (Xin et al., 2017).

In the last decade, to reduce the butanol toxicity during the fermentation process, various *in situ* solvent recovery techniques were successfully employed. These recovery techniques are integrated into the fermentation process for simultaneous production of solvents and its removal, which leads to increase in consumption of fermentable sugars by solventogenic Clostridium, prolongs the time of solventogenic phase and improves fermentation productivity and biobutanol yield (Jiménez-Bonilla & Wang, 2018; Azimi et al., 2019). Solvents removal methods, that have been studied and optimized all the time, are liquid–liquid extraction (Díaz & Tost, 2018), pervaporation (Zhu et al., 2020),

perstraction (Merlet et al., 2017), adsorption (Raganati et al., 2020), gas stripping (Naidoo et al., 2018), vacuum fermentation (Mariano et al., 2012) and other. These methods are admitted to have numerous advantages and disadvantages, that are reviewed in detail by Jiménez-Bonilla & Wang, (2018), Outram et al. (2017), Xue et al. (2017), Cai et al. (2018), Azimi et al. (2019).

Co-cultivation

In recent years, co-cultivation of cellulolytic and solventogenic *Clostridium,* by means of consolidated bioprocessing, has been actively studied (Jiang et al., 2018; Xin et al., 2019; Wen et al., 2020a). Consolidated bioprocessing (CBP), which combines hydrolytic enzymes production, cellulose and hemicellulose hydrolysis and sugars fermentation through ABE production, has a promising potential (Ou et al., 2017). The combination of cellulose saccharification and fermentation in CBP shows numerous advantages, including reduction of contamination risk, increased overall production yield and ability to reduce the reactor and enzyme costs (Vivek et al., 2019; Olguin–Maciel et al., 2020; Tri & Kamei, 2020). A co–culture system will also increase the substrate utilization rate when both bacteria can use the substrate to produce the target product (Du et al., 2020).

However, such consolidated bioprocessing implementation strongly depends on pH regulation (Wen et al., 2020b). Solventogenic *Clostridium* usually produce solvents after acidogenesis, when the culture pH decreases to 4.5–5.5 (Wen et al., 2020b), which, however, would inhibit growth and hamper cellulose degradation by cellulolytic *Clostridium* (Cheng et al., 2019). The change of pH during ABE fermentation by *C. acetobutylicum* could initiate the switch from acidogenesis to solventogenesis (Ou et al., 2017). Ou et al. (2017) study showed, that cell growth of the *C. cellulovorans* stopped when the culture pH dropped below 5.5, but high fermentation pH could reduce the production and selectivity of butanol. Therefore, the pH of 6.5 was identified as the optimal cellulosic fermentation pH in CBP using *C. cellulovorans*, resulting in 3.07 g L^{-1} butanol production. Higher pH value reduces titer 1.82 g L^{-1} and 1.33 g L^{-1} at pH of 7.0 and 7.5, respectively (Ou et al., 2017).

Recently, metabolic engineered strains with tolerance to butanol and low pH, or with higher butanol production capability, was studied towards Clostridium species, Clostridium sp. & bacteria, Clostridium sp. & fungi co-cultivation (Table 3) (Kiyoshi et al., 2015; Jiang et al., 2018; Wen et al., 2017; Jiang et al., 2020b; Tri & Kamei, 2020). In Jiang et al. (2018) study, thermophilic Thermoanaerobacterium sp. M5 was used, which possesses the indigenous capability of butanol production (1.17 g L^{-1}). Optimization of co-cultivation process of mesofilic *C. acetobutylicum* NJ4 has increased butanol titer to 8.34 g L^{-1} from xylan (Jiang et al., 2018). In the other study, thermophilic *C. thermocellum* and mesophilic *C. saccharoperbutylacetonicum* was co–cultivated, resulting in 5.5 g L^{-1} of butanol synthesis from 40 g L^{-1} of cellulose, indicating the highest butanol biosynthesis from cellulose (Kiyoshi et al., 2015). High result was obtained from engineered *C. cellulovorans* with *C. beijerinckii* in consortium, where butanol titer was 11.5 g L^{-1} from alkali pre-treated corn cobs (Wen et al., 2017). Interesting results were obtained in the Tri & Kamei (2020) study, where the synergistic effect of the white–rot fungus Phlebia sp. KO77 and *C. saccharoperbutylacetonicum* co-culture was successful in terms of both butanol production and enhancement of

saccharification, resulting in 3.2 g L^{-1} production of butanol from cellulose (Tri & Kamei, 2020).

CONCLUSIONS

Although intense research and development have been made in the field of production of biobutanol using a lignocellulosic substrate, it is necessary to select the best pre-treatment method for each type of lignocellulosic feedstock for efficient biomass conversion to sugars. The most promising feedstock from agro-industrial residues are rice straw, rice bagasse, soybean straw, sorgum straw, corn strover and spent mushroom substrate. Due to the high results of metabolic engineering strains, it is possible to use lignocellulosic biomass more efficiently. Cellulolytic *C. cellulovorans* shows sufficient enzyme activity for cellulose degradation to fermentable sugars, which allows cost–effective lignocellulose conversion for ABE fermentation with *C. acetobutylicum*, *C. beijerinckii* or *C. saccharoperbutylacetonicum* co–cultivation. Further research is needed on co-cultivation optimization of solventogenic with cellulolytic engineered *Clostridium* or with other potential microorganisms capable of degrading lignocellulosic biomass for technically feasible and simplified strategy to produce butanol from agro-industrial residues. A consolidated bioprocessing is well suited for these purpose, because it increases substrate utilization rate from lignocellulosic wastes through co-cultivation process. At the moment, there are not many studies on the production of butanol by co-cultivation of bacteria using CBP, and further research is needed.

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