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Published in:

Industrial Crops and Products

DOI:

[10.1016/j.indcrop.2020.112265](https://doi.org/10.1016/j.indcrop.2020.112265)

Published: 01/06/2020

Document Version

Peer reviewed version

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Please cite the original version:

Pratto, B., Chandgude, V., de Sousa, R., Cruz, A. J. G., & Bankar, S. (2020). Biobutanol production from sugarcane straw : Defining optimal biomass loading for improved ABE fermentation. *Industrial Crops and Products*, 148, [112265]. <https://doi.org/10.1016/j.indcrop.2020.112265>

Biobutanol production from sugarcane straw: Defining optimal biomass loading for improved ABE fermentation

Bruna Pratto^{a,b} ; Vijaya Chandgude^a; Ruy de Sousa Júnior^{b,c}; Antonio José Gonçalves Cruz^{b,c} and Sandip Bankar^{a}*

^aDepartment of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, P.O. Box 16100, FI-00076 Aalto, Finland

^bChemical Engineering Graduate Program, Federal University of São Carlos, Rod. Washington Luís-Km 235, CEP: 13565-905, São Carlos – SP, Brazil

^cChemical Engineering Department, Federal University of São Carlos, Rod. Washington Luís-Km 235, CEP: 13565-905, São Carlos – SP, Brazil

Authors' email addresses: brunapratto@ufscar.br (Pratto, B.); vijaya.chandgude@aalto.fi (Chandgude, V.); ruy@ufscar.br (Sousa Jr. R.); ajgcruz@ufscar.br (Cruz, A. J. G.) and sandip.bankar@aalto.fi (Bankar, S.)

*Corresponding author: sandip.bankar@aalto.fi

Highlights

- Biomass loads and hydrolysis-fermentation strategies for biobutanol fermentation
- 15% solid load resulted in poor fermentation due to weak acids and phenolics
- Detoxification was unnecessary when 10% solids were used for fermentation
- For 10% solids, simultaneous saccharification and fermentation enhanced production

Abstract

Key objective of this work was to evaluate the use of cellulosic fraction from sugarcane straw pretreated by liquid hot water (LHW) for butanol production via acetone-butanol-ethanol (ABE) fermentation. Separated hydrolysis and fermentation (SHF), and pre-saccharification and simultaneous saccharification and fermentation (PSSF) were investigated at 10 and 15 % w/v biomass loading. For 15 % w/v, the synergistic effect of weak acids and phenolic compounds made the sugarcane straw hydrolysate poorly fermentable. The 10 % w/v solid load was more favorable (~ 4-fold higher) in both SHF

and PSSF strategies with respect to the ABE production, without including a detoxification step. However, PSSF achieved higher ABE titer (10.5 g/L – SHF; 13.5 g/L – PSSF) and productivity (0.09 g/(L.h) – SHF; 0.14 g/(L.h) – PSSF) when compared with SHF. Using best condition (PSSF at 10 % w/v), it would be possible to estimate a yield of 169 L ABE per ton pretreated sugarcane straw (or 84.5 L ABE per ton of raw sugarcane straw), containing 65 L acetone, 95 L butanol, and 9 L ethanol. This result represents a process efficiency of 28 %, based on carbohydrates content in raw material.

Keywords: ABE fermentation; sugarcane straw; Liquid hot water pretreatment; separated hydrolysis and fermentation; Pre-saccharification and simultaneous saccharification and fermentation.

Graphical abstract

1. Introduction

The growing demand for liquid fuels has motivated the exploitation of renewable resources in substitution of fossil fuels. Pereira et al. (2015) have demonstrated that the introduction of *n*-butanol from lignocellulosic materials would result in higher revenues for biorefineries, compared to base scenarios, wherein ethanol is produced exclusively. Thus, the opportunities to develop an efficient biomass refining envisioning *n*-butanol market can contribute to the future implementation of biorefineries, bringing the progress for society and economy.

Besides the economic aspects, *n*-butanol stands out from other biofuels because of its similar fuel properties with gasoline. Biobutanol is superior to bioethanol in many ways, such as with higher calorific value, lower volatility and lower corrosiveness (Guan et al., 2016; Sasaki et al., 2014). Additionally, *n*-butanol can be blended with gasoline up to 40 % (v/v), without any adverse effects on the performance of spark-ignition engine (Merola et al., 2012), and can also be used as *biojet* fuel with further processing (Silva Braz and Pinto Mariano, 2018). Therefore, biobutanol is considered as an advanced combustible and excellent “green” substitute for fossil fuel (Bankar et al., 2013a).

n-butanol is naturally produced by *Clostridium* bacteria via conventional acetone-ethanol-butanol (ABE) fermentation process, with a ratio of 3:6:1, respectively. ABE fermentation occurs in two stages: acidogenic phase in the exponential growth; followed by the solventogenic phase at the end of the exponential growth. In acidogenic phase, the pathways for acid formation are activated to produce acetic and butyric acids, carbon dioxide and hydrogen as main products. At this stage, the pH of the medium drops down from 6.5-7.0 to 4.5-5.0. In response to lowered pH, the clostridial metabolic pathway is directed towards solvents production. Finally, in the solventogenic phase, acids are re-assimilated to produce ABE and gases (Bankar et al., 2013a).

Clostridium spp. is most favored microorganism for ABE production. However, it is highly sensitive to fermentation inhibitors produced during lignocellulosic biomass pretreatment. Hence, it is highly desirable to develop a pretreatment technique that does not (or at lower concentration) produce phenolics or other fermentation inhibitors. Incidentally, diluted-acid and steam explosion pretreatments are the most common techniques used to break down lignocellulosic materials. However, a considerable

inhibitors' concentration is generated, which is damaging to the clostridial fermentation. This happens in diluted-acid due to high severity, and in steam explosion because of low water content in the reaction medium (Amiri and Karimi, 2018; Sun et al., 2016). Hence, detoxification operation to remove these inhibitory compounds is imperative (Guan et al., 2018; Liu et al., 2015a, 2015b; Wang et al., 2019). Thereby, the number of steps operations (pretreatment-detoxification-hydrolysis, etc) substantially increases the bioprocess cost, making it noncompetitive compared to the conventional fossil fuel process.

Liquid hot water (LHW) pretreatment, on the other hand, produces lower toxic products. Thus, LHW as a promising pretreatment technique was explored in this study.

Additionally, better efficiency in enzymatic hydrolysis to release maximum fermentable sugars is also expected. Regarding the enzymatic hydrolysis stage, two main operation strategies can be used: the conventional separated hydrolysis and fermentation (SHF); and simultaneous hydrolysis and fermentation (SSF). The first one has as main advantage the fact that both hydrolysis and fermentation take place under their optimal conditions, whereas the SSF strategy is bit challenging, as it requires ideal conditions favoring both fermentation as well as hydrolysis operation simultaneously (Althuri et al., 2018; Paulova et al., 2015). SSF reduces the enzyme inhibition by sugars, since they are concurrently produced and utilized in single operation with lowered process cost (Cebreiros et al., 2019; Husin et al., 2019).

Alternatively to these approaches, the pre-saccharification and simultaneous saccharification and fermentation (PSSF) process involves the pre-saccharification during short period of time earlier to SSF process (Althuri et al., 2018). This scheme has been proposed to overcome enzymatic hydrolysis constraints due to the different ideal temperatures between cellulolytic enzymes and *Clostridium* species. Thus, the PSSF configuration takes the advantages of both SHF and SSF strategies allowing an improvement in the process yield and productivity (Cebreiros et al., 2019). The pre-saccharification stage can also enhance the mixing properties due to the decrease in the viscosity of hydrolysate slurry before fermentation (Cebreiros et al., 2019; Paulová et al., 2014). As a consequence, the energy requirements would be reduced in PSSF operation (Corrêa et al., 2016).

Based on the considerations above, this study presents a proposal of using efficiently hydrothermally pretreated sugarcane straw for butanol production without detoxification requirements. Experiments were conducted to find the best strategy for ABE fermentation as well as the suitable biomass loading in the enzymatic hydrolysis that keeps fermentation inhibitors levels below the lethal concentration. Both SHF and PSSF were explored in this study with varied biomass loading which has a significant effect on ABE production.

2. Experimental section

2.1 Materials

The lignocellulosic material used in this work was sugarcane straw which was provided by Ipiranga Agroindustrial S.A. mill (Descalvado, SP, Brazil). The biomass was dried at room temperature until 10 % moisture content. Afterwards, it was milled in a Wiley type mill (model SP-30, SPLABOR, Presidente Prudente, SP, Brazil) to a particle size of 10 mesh (2 mm). The commercial enzymatic complex applied in the hydrolysis experiments was Cellic[®]CTec2 (Novozymes Bioag A/S, Denmark) with a filter paper activity of 240 FPU/mL (Ghose, 1987). All the chemicals and nutritional media components used in this study were of analytical grade and purchased from Sigma Aldrich, Finland Oy.

2.2. Pretreatment of sugarcane straw

The biomass was submitted to hydrothermal pretreatment in a 5.5 L stainless steel reactor (model 4584, Parr Instruments Company, Moline, IL, USA) equipped with propeller stirrer, heater, and temperature controller. 200 g dry and milled (2 mm particle size) sugarcane straw was mixed with distilled water (ratio 1:10 w/v) and set inside the reactor. The system was heated up (~70 min) to 195 °C, agitated at 200 rpm, and maintained for 10 min (previously optimized) (Batista et al., 2019; Santos-Rocha et al., 2017). After the operation, reactor was cooled down (~30 min) to 50 °C to separate solid and liquid fractions by using centrifugal filtration. Finally, the solid fraction was washed with water to remove solubilized contents, until neutral pH was reached. Only solid fraction was considered in present study for ABE fermentation. The pretreated

biomass was characterized to analyze its chemical composition, according to National Renewable Energy Laboratory's (NREL) procedure (Sluiter et al., 2008).

2.3. Microorganism and growth conditions

C. acetobutylicum NRRL B-527 was obtained from ARS (Agricultural Research Services) Culture Collection, USA. The pre-culture was anaerobically prepared by inoculating 2.5 % v/v of sporulated cells stock in 125-ml airtight glass bottles containing 100-mL sterile reinforced clostridial medium (RCM). RCM medium was composed by (g/L) meat extract, 10; peptone, 5.0; yeast extract, 3.0, glucose, 30; starch, 1.0; sodium chloride, 5.0; sodium acetate, 3.0; L-cysteine, 0.50 and pH adjusted to 6.8 ± 0.2 (Bankar et al., 2013b). The spore solution was activated by heat shock at 80 °C followed by sudden cooling in ice until room temperature which was then incubated at 37 °C for 20 h. 5 % inoculum after 20 h cultivation was re-suspended in hydrolyzed sugarcane straw supplemented with other necessary fermentation medium components as (in g/L): ammonium acetate (2.2), vitamins (para-amino-benzoic acid (0.1), thiamin (0.1), and biotin (0.01)), and mineral salts ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (0.01), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01), NaCl (0.01)).

2.4. Pre-saccharification, simultaneous saccharification and ABE fermentation (PSSF)

Pretreated sugarcane straw suspended in 50 mM acetate buffer (pH 5.0) was autoclaved (121 °C/20 min) prior to the PSSF experiment. Two solid concentrations (10 and 15 % w/v) were investigated in order to reach sugar levels similar to those commonly used in synthetic medium for optimal microbial growth and production. Biomass loadings lower than 10 % w/v does not meet minimal requirements for sugar levels (> 40 g/L) (Ibrahim et al., 2015) in clostridial ABE fermentation. On the other hand, biomass concentrations higher than 15 % w/v is unnecessary (> 60 g/L, from 24 h of enzymatic hydrolysis), which could result in substrate and/or product inhibition.

The PSSF assay was started with the pre-saccharification step where pretreated sugarcane straw (10 and 15 % w/v) was mixed with 20 FPU/g_{cellulose}. The pre-saccharification was carried out for 24 h, agitated in incubator shaker (Certomat® HK, Germany) at 250 rpm and 50 °C. The temperature, pH and stirring were adjusted to 37 °C, 6.5, and 100 rpm, respectively. The hydrolyzed slurry supplemented with

fermentation medium was purged with nitrogen to maintain anaerobic conditions. 5 % (v/v) actively growing cells were inoculated in fermentation medium for simultaneous saccharification and fermentation at 100 rpm for 96 h. The PSSF process was performed in 125-mL air-tight anaerobic glass bottles containing 75-mL reaction medium. Samples were taken after 96 h fermentation and analyzed for sugars, solvents and inhibitors concentration. All the experiments were performed at least in triplicate and the results presented are with average value $\pm$ standard deviation (SD).

2.5. Separated hydrolysis and ABE fermentation (SHF)

Pretreated sugarcane straw (10 and 15 % m/v) was submitted to enzymatic hydrolysis with 20 FPU/g_{cellulose} in 250-mL Erlenmeyer flasks containing 100 mL reaction medium at pH 5.0 (50 mM acetate buffer), 250 rpm and 50 °C for 24 h. The hydrolysate thus obtained was centrifuged (9,600 g, 20 min, 4 °C) to separate solids and liquids. The liquid fractions containing fermentable sugars were used for further ABE fermentation. Phenolics which are known fermentation inhibitors during ABE process were removed by adding 5 % (w/v) activated charcoal (Hydrotarco B, CABOT, Norit American, Inc., Marshall, USA), at 200 rpm and 28 °C for 1 h (adapted from Liu et al. 2015a). The detoxified hydrolysate was then recovered by centrifugation to obtain a solids-free liquid fraction. The non-detoxified and detoxified hydrolysates were supplemented with other essential fermentation nutrients and pH was adjusted to 6.5. Finally, the solution was autoclaved at 121 °C for 20 min for subsequent ABE fermentation. ABE fermentation was initiated as explained earlier (section 2.4). Control experiments used commercial glucose and xylose (Sigma-Aldrich, Finland OY) while keeping all other conditions same.

2.6. Analytical methods

Sugars (glucose and xylose) were analyzed by high-performance liquid chromatography (HPLC) (Waters Alliance e2695), equipped with a refractive index detector (Waters 2414) and ionic exclusion column (Rezex ROA-Organic acid H⁺ (8%)) using 5 mM H₂SO₄ at 0.6 mL/min and 65 °C. Solvents (acetone, n-butanol, and ethanol) and organic acids (acetic and butyric) were quantified by gas chromatography (GC) (Agilent Technologies 7890B) equipped with flame ionization detector (FID) and

AB-INNOWAX capillary column (30 m x 0.32 mm x 1 µm). Temperatures were maintained at 200 °C and 250 °C for the injector and detector, respectively, with an injection volume of 10 µL.

Total phenolics were estimated by the Folin-Ciocalteu method adapted from Xu and Chang (2009), with slight modification (Santos-Rocha et al., 2018). Samples (20 µL) were diluted in nano pure water (1580 µL), followed by the addition of 100 µL 2 M Folin-Ciocalteu reagent and incubated for 3 min in dark, at room temperature (25 °C). The reaction was stopped by adding 300 µL of Na₂CO₃ (200 g/L) and mixture was incubated for 25 min. Phenolic compounds were measured at 765 nm, using a UV-vis microplate spectrophotometer (PowerWave HT - BioTek). The total phenolic content of each sample was determined using a standard curve of gallic acid (5-500 µg/mL, R² = 0.998) and the concentrations were expressed as mg of gallic acid equivalent per g of dry biomass.

2.7. Calculations and statistical analysis

The polysaccharides-to-monomers conversion (X_{p-m}) during hydrolysis step was determined according to Eq. (1).

$$X_{p-m}(\%) = \frac{C_{glucose} + C_{xylose}}{C_{dry\ pretreated\ biomass}(f_C \times 1.11 + f_H \times 1.14)} \cdot 100 \quad (1)$$

where $C_{glucose}$ and C_{xylose} are the glucose and xylose concentrations (g/L) released at the end of the enzymatic hydrolysis, respectively. The term “ $C_{dry\ pretreated\ biomass}(f_C \times 1.11 + f_H \times 1.14)$ ” corresponds to theoretical sugars concentration available in the pretreated biomass, where $C_{dry\ pretreated\ biomass}$ is concentration (g/L) of dry pretreated sugarcane straw added at the beginning of hydrolysis process; f_C and f_H are the cellulose and hemicellulose fractions of pretreated sugarcane straw ($f_C = 0.55$ g/g and $f_H = 0.082$ g/g – please see Table 1 in section 3); 1.11 and 1.14 are the stoichiometric factors of cellulose to equivalent glucose and hemicellulose to equivalent xylose, respectively.

ABE yield and ABE productivity were calculated according to Eqs. 2 and 3, respectively.

$$ABE_{yield} (g_{ABE}/g_{sugars\ consumed}) = \frac{C_{ABE}}{C_{sugars\ consumed}} \quad (2)$$

where C_{ABE} is the concentration (g/L) of acetone, butanol and ethanol obtained; $C_{sugars\ consumed}$ is the concentration of sugars consumed (g/L).

$$ABE_{productivity} (g/(L \cdot h)) = \frac{C_{ABE}}{t_{total}} \quad (3)$$

where t_{total} (h) is the total processing time considering the pre-saccharification timing for PSSF experiment and hydrolysis timing for SHF experiment.

The overall ABE yield was expressed as gram ABE produced per available sugars at the beginning of fermentation, as indicated in Eq. 4.

$$Overall\ ABE_{yield} (g_{ABE}/g_{available\ sugars}) = \frac{C_{ABE}}{C_{available\ sugars}} \quad (4)$$

where $C_{available\ sugars}$ is the concentration of fermentable sugars at the beginning of ABE fermentation.

The process efficiency was evaluated by considering potential carbohydrates available in raw material, which consolidates the performance of pretreatment, enzymatic hydrolysis and ABE fermentation, as indicated in Eq. 5.

$$Process\ efficiency\ (\%) = \frac{C_{ABE}}{(0.362\ g_{ABE}/g_{sugars}) \cdot C_{dry\ raw\ biomass} (f_C \times 1.11 + f_H \times 1.14)} \cdot 100 \quad (5)$$

where 0.362 is the theoretical maximum amount of ABE produced by *Clostridia*, obtained from Sasaki et al. (2014) (more detailed studies by Yerushalmi et al. 1983). “ $C_{dry\ raw\ biomass} (f_C \times 1.11 + f_H \times 1.14)$ ” corresponds to theoretical sugar concentration available in the raw biomass (before pretreatment).

All the experiments were performed in triplicates. The results are reported as mean $\pm$ standard error. Tukey’s test was used to evaluate the statistical significance of the differences between groups, considering the confidence level of 95 % ($p < 0.05$).

Figure 1 illustrates the main steps performed during the experimental methodology.

Figure 1. Schematic diagram of experimental methodology. The dotted lines indicate the analyses carried out throughout the assays.

3. Results and discussion

3.1 Liquid hot water pretreatment (LHW)

The chemical compositions of sugarcane straw, before and after pretreatment, as well as the removal of cellulose, hemicellulose, lignin, ash and protein are shown in Table 1.

High amount of cellulose content (~80 %) was preserved in solid fraction, while most of the hemicellulose content (82 %) was solubilized after pretreatment, as usual in LHW pretreatment. The water ionization at elevated temperatures and pressures produces hydronium ions that behave as catalysts, cleaving acetyl groups from hemicellulose chains, generating organic acids that depolymerize the hemicellulosic fraction into xylooligomers as the major content (Batista et al., 2019). The values obtained after chemical characterization were consistent with literature data (Oliveira et al., 2014; Szczerbowski et al., 2014).

Table 1 – Chemical composition of sugarcane straw before and after LHW pretreatment (195 °C / 10 min) and the respective removal of the components.

Component (%)	Before pretreatment*	After pretreatment*	Removal after pretreatment*
Cellulose	34.2 ± 0.4	54.8 ± 0.3	19.16 ± 0.01
Hemicellulose	23.2 ± 0.3	8.2 ± 0.1	82.11 ± 0.02
Lignin	24.1 ± 0.5	26.7 ± 1.0	44.05 ± 0.02
Ash	7.1 ± 0.2	6.7 ± 0.1	52.67 ± 0.03
Protein	2.68 ± 0.03	2.27 ± 0.05	57.21 ± 0.04
Total (%)	99.6 ± 0.8	98.6 ± 1.0	-
Solid recovery (%)^a	-	50.5 ± 2.1	-

^aSolid recovery = $(m_{\text{final}}/m_{\text{initial}}) \times 100$, where m_{final} (g) is the amount of dry biomass after pretreatment and m_{initial} (g) is the amount of dry biomass before pretreatment.

* Values reported are average ± standard deviation of three replications

Different conditions (temperature and time) of LHW pretreatment were evaluated in our previous work (Batista et al., 2019) and found that 195 °C for 10 min maintained highest cellulose concentration in solid fraction. Additionally, fermentation inhibitor such as hydroxymethylfurfural (HMF) (0.2 g/L) and furfural (0.8 g/L) levels were also lowered liquid fraction. Here, the focus was to study the solid fraction in ABE fermentation.

3.2 Hydrolysis and fermentation strategies

3.2.1 PSSF strategy

10 and 15% biomass loading was considered for ABE production. Figure 2 (a) and (b) shows the solvents production, sugar consumption, and acids production profiles during ABE fermentation using PSSF strategy.

Figure 2. Effect of the biomass loading at 10 (a) and 15 (b) % w/v on ABE production via PSSF process. Glucose-xylose (squares); Acetone-Butanol-Ethanol (circles); Acetic-Butyric acids (turned-up triangles) Error bars correspond to standard deviation.

The total fermentable sugars produced after the pre-saccharification step was approximately 40 and 55 g/L for 10 and 15 % w/v, respectively. Both of these concentrations are sufficient for successful ABE fermentation.

The effect of biomass concentration is quite pronounced in ABE fermentation, with a total solvents production of 13.6 g/L in 10 % w/v and 3.2 g/L in 15 % w/v. For the lower biomass content, the available glucose was completely consumed after 96 h of

fermentation. On the other hand, by processing 15 % w/v of biomass, only 32 % of sugars were consumed, whose content remained constant after 24 h of fermentation.

As the biomass load increases, sugars concentration in fermentation media increases. However, it also releases higher concentrations of fermentation inhibitors such as phenolics and acids (Santos-Rocha et al., 2018), as shown in Table 2.

Table 2 – Effect of biomass loading on ABE production after 96 h of fermentation via PSSF strategy.

Biomass loading	10 % w/v	15 % w/v
Residual sugars (g/L)	0.44 ± 0.01	37.4 ± 1.2
Acetone (g/L)	5.38 ± 0.01	1.5 ± 0.2
Butanol (g/L)	7.6 ± 0.7	1.6 ± 0.2
Ethanol (g/L)	0.64 ± 0.01	0.09 ± 0.01
ABE yield (g_{ABE}/g_{sugars})	0.35 ± 0.02	0.18 ± 0.02
Acetic acid (g/L)	2.0 ± 0.7	5.4 ± 0.2
Butyric acid (g/L)	1.34 ± 0.04	3.2 ± 0.3
Acid production rate (g/(L.h))^a	0.10 ± 0.03	0.2 ± 0.1
Total phenolics (g/L)	0.65 ± 0.04	0.88 ± 0.02
pH^a	5.49 ± 0.01	4.72 ± 0.01

^a value measured at 24 h of fermentation.

The elevated rate of acids production at 15 % solid loading (about 2.2-fold higher than at 10 %) resulted in a rapid acid-formation, totaling 8.6 g/L (butyric plus acetic acids) at the end of fermentation. As a consequence, the commonly observed “acid crash” phenomenon occurs (Paniagua-garcía et al., 2018). Solvent production was started, but after 24 h of fermentation, the metabolic activities such as sugars and acids consumption and solvents production were unchanged. The acids produced in acidogenic phase were not adequately reassimilated during the late stages of the fermentation (Ibrahim et al., 2015). Thus, high acids concentration and low ABE production were observed at the end of the fermentation. Moreover, 15 % biomass loading observed the 1.5-fold higher phenolic compounds in the hydrolysate than 10 % biomass loading.

Maddox et al. (2000) verified that “acid crash” phenomenon occurs when acids concentration in their non-dissociated form exceed 60 mM. Based on results presented in Table 2, the total concentration of non-dissociated acids is 68.6 mM (48.1 mM from acetic acid, and 20.5 mM from butyric acid), indicating a possible cessation of glucose uptake and consequent limitation of the solventogenic phase.

According to Wang et al. (2013), the effect of “acid crash” is a result of the presence of inhibitors in the fermentation broth. Inhibitor compounds alter the cell permeability, leading to membrane disruption that causes release of proteins, RNAs, ATP, ADP and ions outside cells (Baral and Shah, 2014). *Clostridia* in attempt to regulate this redox stress directs carbon flow to produce more acetic and butyric acid, generating ATP (Ezeji et al., 2007; Ujor et al., 2014). As a result, an excess acid accumulation is observed, leading to “acid crash” effect.

Cho et al. (2009) showed that phenolic compounds interfere in the metabolic pathway, which affects the solvents production. They observed that even 1 g/L of phenolic compounds were sufficient to completely inhibit the butanol production. Results of Lee et al. (2015) corroborated the previous assertion by verifying that phenolic compounds concentration of 0.87 g/L in the rice straw hydrolysate inhibited the production of butyric acid by *C. tyrobutyricum* ATCC 25755, indicating high toxicity of these products to the microorganism. In current work 0.88 g/L phenolics were observed at 15 % w/v biomass load. Thus, it is suggested that the low butanol production at 15 % biomass load could be due to higher phenolics generation and subsequent “acid crash” phenomenon. Another potential cause for low ABE production at 15 % w/v is the mass transfer resistance during the mixing process, which tends to worsen with increase in biomass loading (Chen and Liu, 2017; Modenbach and Nokes, 2013).

Additionally, SHF strategy was studied to check the process performance and compare the overall ABE yields and productivities. For the SHF process, only the liquid fraction (hydrolysate) from the enzymatic hydrolysis step was fermented. Since in the SHF process hydrolysis and fermentation reactions take place separately, a detoxification step was considered before the ABE fermentation, in order to minimize the phenolic compounds.

3.2.2 SHF strategy

ABE fermentation was performed using non-detoxified and detoxified sugarcane straw hydrolysate (Table 3). After 24 h, the enzymatic hydrolysis released the following fractions: 45.3 g/L (average between assays 2 and 3) and 68.2 g/L (average between assays 5 and 6) glucose with 10 and 15 % w/v biomass loading, respectively. Assays 2 and 3 are replicates for hydrolysis process. The only difference between them is the detoxification procedure included in assay 3 after the hydrolysis reaction, which does not interfere in the glucose content available for fermentation. The similar approach is true for assays 5 and 6. The polysaccharides-to-monomers conversion was satisfactory (~ 71 %) during short hydrolysis time in both conditions.

Table 3 – Effect of biomass loading on ABE fermentation for 96 h using non-detoxified and detoxified hydrolyzed of sugarcane straw.

Assay	Assay 1 Control 10 %	Assay 2 Non- detoxified hydrolysed 10 %	Assay 3 Detoxified hydrolysed 10 %	Assay 4 Control 15 %	Assay 5 Non- detoxified hydrolysed 15 %	Assay 6 Detoxified hydrolysed 15 %
Initial glucose (g/L)	45	45.7 ± 1.9	45.0 ± 1.7	70	68.5 ± 1.4	68.0 ± 1.7
Initial xylose (g/L)	5	4.87 ± 0.03	4.7 ± 0.1	10	7.7 ± 0.3	7.0 ± 0.3
Sugars consumed (g/L)	47.8 ± 1.6	40.8 ± 2.6	47.2 ± 0.3	48.8 ± 2.1	22.0 ± 1.5	49.1 ± 1.2
Acetone (g/L)	4.3 ± 0.2	3.3 ± 0.4	3.51 ± 0.03	4.7 ± 0.3	0.9 ± 0.1	4.2 ± 0.2
Butanol (g/L)	10.3 ± 0.7 ^{a,b,c}	8.8 ± 0.3 ^a	9.1 ± 0.9 ^{a,b,c}	11.3 ± 0.4 ^c	2.1 ± 0.1 ^d	11.3 ± 0.7 ^{b,c}
Ethanol (g/L)	1.2 ± 0.1	0.9 ± 0.1	0.98 ± 0.02	1.3 ± 0.1	0.20 ± 0.02	1.4 ± 0.1
Total ABE (g/L)	15.9 ± 0.7 ^{a,b}	13.0 ± 0.5 ^a	13.6 ± 0.9 ^a	17.3 ± 0.5 ^b	3.2 ± 0.1 ^c	16.9 ± 0.7 ^b
ABE yield (g _{ABE} /g _{sugars})	0.33 ± 0.02 ^{e,f}	0.32 ± 0.02 ^{e,f}	0.29 ± 0.02 ^e	0.35 ± 0.02 ^f	0.15 ± 0.01 ^g	0.34 ± 0.02 ^{e,f}
Acetic acid (g/L)	0.8 ± 0.2	2.7 ± 0.4	2.2 ± 0.7	1.5 ± 0.4	5.5 ± 0.5	2.0 ± 0.4
Butyric acid (g/L)	0.6 ± 0.1	1.4 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	2.0 ± 0.7	1.4 ± 0.2
Total acids (g/L)	1.4 ± 0.2	4.2 ± 0.4	3.4 ± 0.7	2.4 ± 0.4	7.4 ± 0.9	3.4 ± 0.4
Total phenolics (g/L)	-	0.74 ± 0.04 ⁱ	0.04 ± 0.02	-	1.28 ± 0.01 ^j	0.06 ± 0.02

The overwritten lowercases represent the Tukey test for multiple comparisons between rows ($p < 0.05$) of each assay.

The butanol and total ABE production were similar ($p < 0.05$) in both assays 1 and 4, indicating that very high glucose concentrations (> 60 g/L) did not significantly increase the ABE production. However, sugar utilization in assay 1 was 95 %, against 61 % in assay 4. The incomplete sugar utilization (~ 30 g/L sugars left) in experiment 4 (also seen in assay 6) is probably due to end-products toxicity and other metabolic restrictions. Typically, in batch reactors, the ABE and butanol production is restricted to less than 20 g/L and 13 g/L, respectively (Ezeji et al., 2004; Mariano et al., 2012; Peralta-Yahya et al., 2012). These concentrations limit the sugars consumption to approximately 60 g/L (Ezeji et al., 2004, 2003; Ibrahim et al., 2017). Qureshi et al. (2014) employing barley straw hydrolysate for ABE fermentation by *C. beijerinckii* P260 also found that the highest sugar consumption was around 50-55 g/L, regardless of the initial sugar levels (63; 80; 100 and 125 g/L), producing 24 g/L and 19 g/L of ABE products for the lowest and highest sugar concentrations, respectively. Qureshi et al. (2008) verified that complete utilization of 128.3 g/L initial sugars (60 g/L of pure glucose plus 68.3 g/L of fermentable sugars from wheat straw hydrolysate) by *C. beijerinckii* P260 was just possible when simultaneous product-recovery system was adopted. Thus, it can be observed that the limitation of sugar consumption by *Clostridia* is more associated with butanol toxicity than substrate inhibition. Sugars inhibition could be experienced at high levels (> 150 g/L) of initial pure glucose.

Similar to the PSSF strategy, there is also an expressive difference in the solvents production for SHF experiments with 10 % (assay 2) and 15 % (assay 5) solid load, reaching 13.02 and 3.20 g/L, respectively. The ABE yield based on available sugars at the beginning of the fermentation was also drastically reduced from 0.26 g_{ABE}/g_{initial sugars} (assay 2) to 0.043 g_{ABE}/g_{initial sugars} (assay 5). With this observation, one could rule out the hypothesis of mixing and mass transfer problems associated with the high biomass loading, since only the liquid fraction is fermented in the SHF strategy. Thus, the most plausible reason for the low production is indeed the presence of inhibitors in the hydrolysate. The phenolic content present in the assay 5 possibly reached a threshold limit, causing the “acid crash” effect (high acid amount left at the end of the fermentation). Therefore, a detoxification step (activated charcoal treatment) was included in the process to improve the fermentability of *Clostridia*.

The detoxification step removed around 95 % phenolic compounds from the hydrolysate. Interestingly, the butanol and ABE productions were, respectively, increased from 2.1 to 11.3 g/L and from 3.2 to 16.9 g/L when assay 5 was submitted to detoxification (assay 6). These values were statistically equal to those of the control experiment (assay 4). In addition, sugar utilization was also improved from 29 % to 65.5 % when detoxification was employed. Clearly, this treatment had a positive effect on fermentation performance once the “acid crash” phenomenon was successfully bypassed.

Qureshi et al. (2016) observed a significant improvement in ABE production when sorghum bagasse hydrolysate was detoxified, increasing ABE production from 3.85 to 16.78 g/L; and productivity from 0.09 to 0.56 g/(L.h). Similarly, Guan et al. (2018) found that with inclusion of detoxification step, a satisfactory ABE production (13.2 g/L) was achieved from hemicellulosic hydrolysate.

In current study, there were no significant differences ($p < 0.05$) in butanol and ABE productions between non-detoxified and detoxified hydrolysates when 10 % biomass loading (assays 2 and 3), was studied and results were similar to the control (assay 1). Sugarcane straw hydrolysate with the lower biomass loading was successfully fermented by *C. acetobutylicum* ATCC 824, obtaining 13 g/L ABE solvents.

Although assay 6 produced higher ABE production when compared with assay 2, it required an additional detoxification step, which adds extra processing costs. Thus, the further experiments were conducted by employing 10 % w/v of initial biomass loading without adding detoxification step as a trade-off.

3.3. Comparison between SHF and PSSF strategies

The results obtained during the ABE fermentation via SHF and PSSF processes employing hydrolysate of sugarcane straw are presented in Figure 3 (a-d).

Figure 3. Comparison between SHF (a-b) and PSSF (c-d) processes on ABE fermentation of pretreated sugarcane straw (10% w/v) in the 2-L bioreactor. Acetic acid (close turned-down triangles); Acetone (open turned-up triangles); Butanol (open circles); Butyric acid (close turned-up triangles); Ethanol (open squares); Glucose (closed squares); Xylose (close circles); pH (open turned-down triangles). Error bars correspond to standard deviation.

The sugars consumption, acids production, solvent production and pH change profiles are similar for both SHF and PSSF processes. At the end of fermentation (96 h), the total ABE products obtained were 10.5 and 13.5 g/L for SHF and PSSF process, respectively. After 72 h of fermentation, the ABE solvents production remained steady in PSSF process (Fig. 3 (d)) thereby indicating higher productivity (0.14 g/(L.h)) compared to SHF approach (0.09 g/(L.h)). Zhang et al. (2013) observed 0.15 g/(L.h) of

ABE productivity for SSF process and 0.12 g/(L.h) for SHF process, using pretreated corncob as carbon source.

Residual sugar is also present at the end of the fermentation (Fig. 3 (c)), totaling 12.3 g/L of glucose and xylose. It is common that sugars are released during the process of simultaneous saccharification and fermentation. Probably the sugars accumulation was due to the enzymatic hydrolysis reaction is taking place while the microorganism is already in the stationary phase. Figure 4 shows comparative analysis of SHF and PSSF process in the light of ABE production.

Figure 4. Overall ABE yield from 100 g/L of pretreated sugarcane straw processed via SHF and PSSF strategies conducted in the 2-L bioreactor

For 100 g/L of pretreated sugarcane straw, the total ABE production was enhanced from 10.5 to 13 g/L when the sugarcane straw was fermented via PSSF process. The production estimation is 52.5 g and 65 g ABE per kg raw sugarcane straw using SHF and PSSF, respectively. For PSSF, the result represents 28% process efficiency (if all carbohydrates in the raw material were converted into ABE products). These results indicate that the simultaneous process of hydrolysis and fermentation is more favorable for upscaled operation.

The SSF strategy avoids the sugar (substrate) inhibition to enzymes because sugars are assimilated by the microorganism as they are formed. Besides, costs related to the equipment and operation are reduced when hydrolysis and fermentation are carried out in the same reactor. Wang et al. (2019) also proved that SSF process is a preferable strategy for butanol production compared to SHF by processing acid-pretreated switchgrass.

Table 4 summarizes recent literature studies regarding ABE fermentation via SSF employing various types of lignocellulosic biomasses without the inclusion of detoxification step.

Table 4 - ABE fermentation from different lignocellulosic materials without detoxification steps.

Pretreatment	Substrate	Strain	Process	Main results				Reference
				Butanol g/L	ABE g/L	ABE yield (g/g _{sugars consumed})	ABE productivity ^a (g/(L.h))	
Alkaline (2% NaOH)	50 g/L of pretreated oil palm empty fruit bunch	<i>C. acetobutylicum</i> ATCC 824	SSF	2.7	4.4	0.16	0.05	(Ibrahim et al., 2015)
Liquid hot water	160 g/L of pretreated sweet sorghum bagasse	<i>C. beijerinckii</i> P260	SHF	8.4	16.9	0.45	0.14	(Qureshi et al., 2016)
Alkaline (2.5% NaOH)	99 g/L of pretreated Napier grass	<i>C. acetobutylicum</i> ATCC 824	PSSF	9.4	15.0	0.35	0.12	(He et al., 2017)
Diluted acid (1% H ₂ SO ₄) with oxidate ammonolysis (5% NH ₃ H ₂ O and 6% H ₂ O)	60 g/L of pretreated sugarcane bagasse	<i>C. acetobutylicum</i> ATCC 824	SHF	7.7	12.1	0.39	0.06	(Li et al., 2017)
Starch gelatinization	70 g/L of sago hampas	<i>C. acetobutylicum</i> ATCC 824	PSSF	4.6	9.0	0.25	0.09	(Husin et al., 2019)
Liquid hot water	100 g/L of pretreated sugarcane straw	<i>C. acetobutylicum</i> ATCC 824	PSSF	7.4	13.0	0.46	0.14	This study (Figure 3 (c-d))

^aABE productivity was calculated with respect to the time required for enzymatic hydrolysis and ABE fermentation.

The present study and results of Qureshi et al. (2016) utilized the LHW technique, and reported the highest ABE yield and productivity. In this pretreatment, the amount of inhibitors released is generally at lower levels than those obtained in chemical pretreatment.

Hence, results obtained here demonstrate the potential of sugarcane straw to be utilized as a feedstock for biobutanol production (without pH control, detoxification step and product-recovery system). Further improvements to use fed-batch operation and continuous operation in modified continuous bioreactor with simultaneous solvent recovery are under investigation.

4. Conclusions

Biomass loading for pretreatment and hydrolysis experiment was investigated in this study. ABE fermentations are susceptible for inhibitor threshold levels in their operations. Hence, this study provides an insight on the relation among biomass loading, inhibitor production and enzymatic hydrolysis on ABE production. The results showed that lower (10%) biomass loading is sufficient for efficient ABE production (~13 g/L) without the detoxification step. Comparing the SHF and PSSF strategies in the 10 % w/v condition, both of them achieved similar butanol concentration. However, the PSSF was more efficient in terms of productivity (60 % higher). The prediction shows that 169 L ABE could be produced per ton pretreated sugarcane straw (or 84.5 L of ABE per ton raw sugarcane straw) processed, with 65 L of acetone, 95 L of butanol and 9 L of ethanol. This result represents a process efficiency of 28%, based on carbohydrates content in raw material. The ideal biomass loading chosen (10 % w/v) combined with the LHW pretreatment provided a sustainable route for a successful ABE fermentation from sugarcane straw. In addition, the integrated process of hydrolysis and fermentation eliminates problems of substrate inhibition.

Acknowledgements

The authors would like to thank EDUFI (Finnish National Agency for Education), CNPq (National Council for Scientific and Technological Development, Brazil, Process #312903/2018-9), and FAPESP (São Paulo State Research Funding Agency, Brazil, Process #2016/10636-8) for the financial support.

This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. Aalto University Bioeconomy infrastructure is acknowledged.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

- Althuri, A., Chintagunta, A.D., Sherpa, K.C., Banerjee, R., 2018. Simultaneous Saccharification and Fermentation of Lignocellulosic Biomass. pp. 265–285. doi:10.1007/978-3-319-67678-4_12
- Amiri, H., Karimi, K., 2018. Pretreatment and hydrolysis of lignocellulosic wastes for butanol production: Challenges and perspectives. *Bioresour. Technol.* 270, 702–721. doi:10.1016/j.biortech.2018.08.117
- Bankar, S., Survase, S.A., Ojamo, H., Granström, T., 2013a. Biobutanol: the outlook of an academic and industrialist. *RSC Adv.* 3, 24734. doi:10.1039/c3ra43011a
- Bankar, S., Survase, S.A., Ojamo, H., Granström, T., 2013b. The two stage immobilized column reactor with an integrated solvent recovery module for enhanced ABE production. *Bioresour. Technol.* 140, 269–276. doi:10.1016/j.biortech.2013.04.046
- Baral, N.R., Shah, A., 2014. Microbial inhibitors: formation and effects on acetone-butanol-ethanol fermentation of lignocellulosic biomass. *Appl. Microbiol. Biotechnol.* 98, 9151–9172. doi:10.1007/s00253-014-6106-8
- Batista, G., de Souza, R.B.A., Pratto, B., Santos-Rocha, M.S.R., da Cruz, A.J.G., 2019. Effect of severity factor on the hydrothermal pretreatment of sugarcane straw. *Bioresour. Technol.* 275, 321–327. doi:10.1016/j.biortech.2018.12.073
- Cebreiros, F., Ferrari, M.D., Lareo, C., 2019. Cellulose hydrolysis and IBE fermentation of eucalyptus sawdust for enhanced biobutanol production by *Clostridium beijerinckii* DSM 6423. *Ind. Crops Prod.* 134, 50–61. doi:10.1016/j.indcrop.2019.03.059
- Chen, H.-Z., Liu, Z.-H., 2017. Enzymatic hydrolysis of lignocellulosic biomass from low to high solids loading. *Eng. Life Sci.* 17, 489–499. doi:10.1002/elsc.201600102

- Cho, D.H., Lee, Y.J., Um, Y., 2009. Detoxification of model phenolic compounds in lignocellulosic hydrolysates with peroxidase for butanol production from *Clostridium beijerinckii* 1035–1043. doi:10.1007/s00253-009-1925-8
- Corrêa, L.J., Badino, A.C., Cruz, A.J.G., 2016. Power consumption evaluation of different fed-batch strategies for enzymatic hydrolysis of sugarcane bagasse. *Bioprocess Biosyst. Eng.* 39, 825–833. doi:10.1007/s00449-016-1562-4
- Ezeji, T.C., Qureshi, N., Blaschek, H.P., 2007. Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping. *J. Ind. Microbiol. Biotechnol.* 34, 771–777. doi:10.1007/s10295-007-0253-1
- Ezeji, T.C., Qureshi, N., Blaschek, H.P., 2004. Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. *Appl. Microbiol. Biotechnol.* 63, 653–658. doi:10.1007/s00253-003-1400-x
- Ezeji, T.C., Qureshi, N., Blaschek, H.P., 2003. Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and in situ recovery by gas stripping. *World J. Microbiol. Biotechnol.* 19, 595–603.
- Ghose, T., 1987. Measurement of cellulase activities. *Pure Appl. Chem.* 59, 257–268. doi:10.1351/pac198759020257
- Guan, W., Shi, S., Tu, M., Lee, Y.Y., 2016. Acetone-butanol-ethanol production from Kraft paper mill sludge by simultaneous saccharification and fermentation. *Bioresour. Technol.* 200, 713–721. doi:10.1016/j.biortech.2015.10.102
- Guan, W., Xu, G., Duan, J., Shi, S., 2018. Acetone–Butanol–Ethanol Production from Fermentation of Hot-Water-Extracted Hemicellulose Hydrolysate of Pulping Woods. *Ind. Eng. Chem. Res.* 57, 775–783. doi:10.1021/acs.iecr.7b03953
- He, C.R., Kuo, Y.Y., Li, S.Y., 2017. Lignocellulosic butanol production from Napier grass using semi-simultaneous saccharification fermentation. *Bioresour. Technol.* 231, 101–108. doi:10.1016/j.biortech.2017.01.039
- Husin, H., Faizal, M., Abd-aziz, S., Bahrin, E.K., 2019. Simultaneous saccharification and fermentation of sago hampas into biobutanol by *Clostridium acetobutylicum* ATCC 824 66–75. doi:10.1002/ese3.226
- Ibrahim, M.F., Abd-Aziz, S., Yusoff, M.E.M., Phang, L.Y., Hassan, M.A., 2015.

- Simultaneous enzymatic saccharification and ABE fermentation using pretreated oil palm empty fruit bunch as substrate to produce butanol and hydrogen as biofuel. *Renew. Energy* 77, 447–455. doi:10.1016/j.renene.2014.12.047
- Ibrahim, M.F., Ramli, N., Kamal Bahrin, E., Abd-Aziz, S., 2017. Cellulosic biobutanol by *Clostridia*: Challenges and improvements. *Renew. Sustain. Energy Rev.* 79, 1241–1254. doi:10.1016/j.rser.2017.05.184
- Lee, K.M., Kim, K.-Y., Choi, O., Woo, H.M., Kim, Y., Han, S.O., Sang, B.-I., Um, Y., 2015. In situ detoxification of lignocellulosic hydrolysate using a surfactant for butyric acid production by *Clostridium tyrobutyricum* ATCC 25755. *Process Biochem.* 50, 630–635. doi:10.1016/j.procbio.2015.01.020
- Li, H., Xiong, L., Chen, Xuefang, Wang, C., Qi, G., Huang, C., Luo, M., Chen, Xinde, 2017. Enhanced enzymatic hydrolysis and acetone-butanol-ethanol fermentation of sugarcane bagasse by combined diluted acid with oxidate ammonolysis pretreatment. *Bioresour. Technol.* 228, 257–263. doi:10.1016/j.biortech.2016.12.119
- Liu, K., Atiyeh, H.K., Pardo-Planas, O., Ezeji, T.C., Ujor, V., Overton, J.C., Berning, K., Wilkins, M.R., Tanner, R.S., 2015a. Butanol production from hydrothermolysis-pretreated switchgrass: Quantification of inhibitors and detoxification of hydrolyzate. *Bioresour. Technol.* 189, 292–301. doi:10.1016/j.biortech.2015.04.018
- Liu, K., Atiyeh, H.K., Pardo-planas, O., Ramachandriya, K.D., Wilkins, M.R., Ezeji, T.C., Ujor, V., Tanner, R.S., 2015b. Process development for biological production of butanol from Eastern redcedar. *Bioresour. Technol.* 176, 88–97. doi:10.1016/j.biortech.2014.10.148
- Maddox, I.S., Steiner, E., Hirsch, S., Wessner, S., Gutierrez, N.A., Gapes, J., Schuster, K., 2000. The cause of “acid-crash” and “acidogenic fermentations” during the batch acetone-butanol-ethanol (ABE) fermentation process. *J. Microbiol. Biotechnol.* 1, 95–100.
- Mariano, A.P., Filho, R.M., Ezeji, T.C., 2012. Energy requirements during butanol production and in situ recovery by cyclic vacuum. *Renew. Energy* 47, 183–187. doi:10.1016/j.renene.2012.04.041
- Merola, S.S., Tornatore, C., Marchitto, L., Valentino, G., Corcione, F.E., 2012.

- Experimental investigations of butanol-gasoline blends effects on the combustion process in a SI engine. *Int. J. Energy Environ. Eng.* 3, 1–14. doi:10.1186/2251-6832-3-6
- Modenbach, A.A., Nokes, S.E., 2013. Enzymatic hydrolysis of biomass at high-solids loadings – A review. *Biomass and Bioenergy* 56, 526–544. doi:10.1016/j.biombioe.2013.05.031
- Oliveira, L.R.M., Nascimento, V.M., Gonçalves, A.R., Rocha, G.J.M., 2014. Combined process system for the production of bioethanol from sugarcane straw. *Ind. Crops Prod.* 58, 1–7. doi:10.1016/j.indcrop.2014.03.037
- Paniagua-garcía, A.I., Hijosa-valsero, M., Díez-antolínez, R., Sánchez, M.E., 2018. Enzymatic hydrolysis and detoxification of lignocellulosic biomass are not always necessary for ABE fermentation : The case of *Panicum virgatum*. *Biomass and Bioenergy* 116, 131–139. doi:10.1016/j.biombioe.2018.06.006
- Paulova, L., Patakova, P., Branska, B., Rychtera, M., Melzoch, K., 2015. Lignocellulosic ethanol: Technology design and its impact on process efficiency. *Biotechnol. Adv.* 33, 1091–1107. doi:10.1016/j.biotechadv.2014.12.002
- Paulová, L., Patáková, P., Rychtera, M., Melzoch, K., 2014. High solid fed-batch SSF with delayed inoculation for improved production of bioethanol from wheat straw. *Fuel* 122, 294–300. doi:10.1016/j.fuel.2014.01.020
- Peralta-Yahya, P.P., Zhang, F., del Cardayre, S.B., Keasling, J.D., 2012. Microbial engineering for the production of advanced biofuels. *Nature* 488, 320–328. doi:10.1038/nature11478
- Pereira, L.G., Chagas, M.F., Dias, M.O.S., Cavalett, O., Bonomi, A., 2015. Life cycle assessment of butanol production in sugarcane biorefineries in Brazil. *J. Clean. Prod.* 96, 557–568. doi:10.1016/j.jclepro.2014.01.059
- Qureshi, N., Cotta, M.A., Saha, B.C., 2014. Bioconversion of barley straw and corn stover to butanol (a biofuel) in integrated fermentation and simultaneous product recovery bioreactors. *Food Bioprod. Process.* 92, 298–308. doi:10.1016/j.fbp.2013.11.005
- Qureshi, N., Liu, S., Hughes, S., Palmquist, D., Dien, B., Saha, B., 2016. Cellulosic Butanol (ABE) Biofuel Production from Sweet Sorghum Bagasse (SSB): Impact of Hot Water Pretreatment and Solid Loadings on Fermentation Employing

- Clostridium beijerinckii* P260. *BioEnergy Res.* doi:10.1007/s12155-016-9761-z
- Qureshi, N., Saha, B.C., Cotta, M.A., 2008. Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: Part II-Fed-batch fermentation. *Biomass and Bioenergy* 32, 176–183. doi:10.1016/j.biombioe.2007.07.005
- Santos-Rocha, M.S.R., Pratto, B., de Sousa, R., Almeida, R.M.R.G., Cruz, A.J.G., 2017. A kinetic model for hydrothermal pretreatment of sugarcane straw. *Bioresour. Technol.* 228, 176–185. doi:10.1016/j.biortech.2016.12.087
- Santos-Rocha, M.S.R., Pratto, B., Jacob, L., Colli, A., Maria, R., Garcia, R., José, A., Cruz, G., 2018. Assessment of different biomass feeding strategies for improving the enzymatic hydrolysis of sugarcane straw. *Ind. Crop. Prod.* 125, 293–302. doi:10.1016/j.indcrop.2018.09.005
- Sasaki, C., Kushiki, Y., Asada, C., Nakamura, Y., 2014. Acetone-butanol-ethanol production by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) methods using acorns and wood chips of *Quercus acutissima* as a carbon source. *Ind. Crops Prod.* 62, 286–292. doi:10.1016/j.indcrop.2014.08.049
- Silva Braz, D., Pinto Mariano, A., 2018. Jet fuel production in eucalyptus pulp mills: Economics and carbon footprint of ethanol vs. butanol pathway. *Bioresour. Technol.* 268, 9–19. doi:10.1016/j.biortech.2018.07.102
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determination of structural carbohydrates and lignin in biomass. Technical Report NREL/TP-510-42618.
- Sun, Shaoni, Sun, Shaolong, Cao, X., Sun, R., 2016. The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresour. Technol.* 199, 49–58. doi:10.1016/j.biortech.2015.08.061
- Szczerbowski, D., Pitarelo, A.P., Zandon?? Filho, A., Ramos, L.P., 2014. Sugarcane biomass for biorefineries: Comparative composition of carbohydrate and non-carbohydrate components of bagasse and straw. *Carbohydr. Polym.* 114, 95–101. doi:10.1016/j.carbpol.2014.07.052
- Ujor, V., Agu, C.V., Gopalan, V., Ezeji, T.C., 2014. Glycerol supplementation of the growth medium enhances in situ detoxification of furfural by *Clostridium*

- beijerinckii during butanol fermentation. Appl. Microbiol. Biotechnol. 101. doi:10.1007/s00253-014-5802-8
- Wang, P., Chen, Y.M., Wang, Yifen, Lee, Y.Y., Zong, W., Taylor, S., McDonald, T., Wang, Yi, 2019. Towards comprehensive lignocellulosic biomass utilization for bioenergy production: Efficient biobutanol production from acetic acid pretreated switchgrass with *Clostridium saccharoperbutylacetonicum* N1-4. Appl. Energy 236, 551–559. doi:10.1016/j.apenergy.2018.12.011
- Wang, Y., Li, X., Milne, C.B., Janssen, H., Lin, W., Phan, G., Hu, H., Jin, Y.S., Price, N.D., Blascheka, H.P., 2013. Development of a gene knockout system using mobile group II introns (Targetron) and genetic disruption of acid production pathways in *Clostridium beijerinckii*. Appl. Environ. Microbiol. 79, 5853–5863. doi:10.1128/AEM.00971-13
- Xu, B., Chang, S.K., 2009. Total Phenolic , Phenolic Acid , Anthocyanin , Flavan-3-ol , and Flavonol Profiles and Antioxidant Properties of Pinto and Black Beans (*Phaseolus vulgaris* L.) as Affected by Thermal Processing 57, 4754–4764. doi:10.1021/jf900695s
- Yerushalmi, L., Volesky, B., Leung, W.K., Neufeld, R.J., 1983. Variations of solvent yield in acetone-butanol fermentation. Eur. J. Appl. Microbiol. Biotechnol. 18, 279–286. doi:10.1007/BF00500492
- Zhang, J., Wang, M., Gao, M., Fang, X., Yano, S., Qin, S., Xia, R., 2013. Efficient Acetone-Butanol-Ethanol Production from Corn cob with a New Pretreatment Technology-Wet Disk Milling. Bioenergy Res. 6, 35–43. doi:10.1007/s12155-012-9226-y