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Valorization of sugarcane bagasse through biofuel and value-added soluble metabolites production: Optimization of alkaline hydrothermal pretreatment

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ABSTRACT

Sugarcane bagasse (SCB) is increasingly considered as a potential source for bioenergy or bulk chemicals production. However, efficient pretreatment techniques need to be developed to make full use of its potential. A central composite design was employed to evaluate the effect of temperature (146.4–213.6 °C), NaOH concentration (0.7–2.3 M) and treatment time (3.2–36.8 min) on the hydrothermal pretreatment of SCB. Glucose was the abundant fermentable sugar released in the hydrolysate (4.0 g/L) and its concentration was significantly (p -value < 0.05) affected by temperature and NaOH concentration. Sugars released in the hydrolysate and the remaining cellulose, hemicellulose, and lignin in the pretreated fiber were anaerobically co-digested in batch thermophilic assays (55 °C). Propionate, one of the most promising platform chemicals, was the main metabolite produced (0.5 g/L) and its concentration was significantly affected by temperature and NaOH concentration. Both NaOH concentration and pretreatment duration significantly affected methane composition in the biogas (p -value < 0.05 and 0.10 respectively). *Deftuviitoga*, and *Methanothermobacter* genera were favored in response to alkaline hydrothermal pretreatment at the central point conditions (180 °C, 1.5 M NaOH, 20 min).

1. Introduction

Lignocellulosic biomass mainly constitutes cellulose (40%), hemicellulose (25%) and lignin (25%) and is a potential substrate for the production of biofuels and commodity chemicals [1]. Sugarcane bagasse (SCB) is a lignocellulosic biomass widely produced in Brazil during the extraction of sugarcane juice for ethanol and sugar production, and is traditionally burned for heat and electricity generation [2]. However, potentially, lignocellulosic biomass, such as SCB, may play a key role as critical feedstock within the circular bioeconomy [2]. Due to the composition of lignocellulosic biomass, it could be converted to liquid fuels, commodity chemicals and biopolymers using clean catalytic processes, in a biobased economy [1].

Brazilian sugarcane bagasse has a high content of carbohydrates, i.e., 40.9 and 30.6% of cellulose and hemicellulose, respectively [3]. These carbohydrates are strongly interlinked with lignin, resulting in a very

compact, stable and resistant structure. Therefore, a pretreatment step is required in order to separate the main fibrous structural components. For instance, alkaline solutions may dissolve lignin, while reducing the biomass crystallization and enhancing the subsequent enzymatic hydrolysis of cellulose and hemicellulose [4]. Sodium hydroxide, potassium hydroxide, calcium hydroxide, and urea are the conventional alkaline reagents used in lignocellulosic biomass pretreatment [5]. In addition to alkaline, if the operational temperature of hydrothermal pretreatment exceeds 145 °C, it could result in the autohydrolysis of hemicellulose [6].

This subsequently, releases xylan oligomers and enriches the remainder of the fibers in terms of cellulose and lignin [3], which have been mainly used for biofuels and organic acids production [7]. Furthermore, addition of alkaline and/or hydrothermal pretreatment has been reported to reduce the recalcitrance of lignocellulosic biomass by facilitating additional delignification [8].

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Commonly, two main types of alkaline pretreatment are used, i.e., high-concentration (6–20% NaOH w/w) and low-concentration (0.4–4% NaOH w/w) pretreatment. In the low-concentration pretreatment, high temperatures and pressure are used with no recycling of NaOH. During this pretreatment, NaOH at high temperature disintegrates the lignin and hemicellulose and separates them from the solid fraction. In contrast, in the high-concentration pretreatment, ambient pressure and low temperatures are applied [9]. Mirahmadi et al. [9], reported that alkaline pretreatment resulted in a significant decrease in hemicellulose content and cellulose crystallinity, being responsible for an increase in enzymatic hydrolysis of birch from 6.9% to 82.3%, and of spruce from 14.1% to 35.7%, using 7% NaOH. Besides the reagent type and concentration used during the pretreatment, the efficiency of alkaline pretreatment was mainly affected by reaction temperature and pretreatment duration [8].

As far as authors are aware, there is limited information available in the literature on the quantification and interrelation of above mentioned parameters on the release of sugars to the hydrolysate and the amount of sugars that remain on the pretreated sugarcane bagasse fiber. In our present study, a central composite design was performed in order to quantify the effect of alkaline hydrothermal pretreatment on i) the release of fermentable sugars, ii) the amount of sugars that remain in the pretreated fibers, and iii) their bioconversion into value-added products.

2. Material and methods

2.1. Substrate and inoculum

SCB fibers were obtained from Laboratório Nacional de Bio-Renováveis (LNBR), Campinas, São Paulo, Brazil and maintained in vacuum sealed plastic bags under refrigeration until use. The cellulose, hemicellulose, lignin content, and non-identified compounds of the untreated SCB were $31 \pm 2.7\%$, $23 \pm 2.1\%$, $36 \pm 5.2\%$, and $10 \pm 0.4\%$, respectively.

The inoculum was supplied by an industrial biogas plant treating mixed organic waste from, amongst others, egg factories and slaughterhouses at 55 °C (Wabico B.V., Waalwijk, the Netherlands). Before the experimental tests, the inoculum was sieved through a 1-mm mesh aluminum screen and then incubated at 55 °C (Innova® 44 incubator) for one week.

Alkalinity, pH, conductivity, volatile fatty acids, ammonia, total and soluble chemical oxygen demand, total and volatile solids content of the inoculum were measured according to the protocols for accepting and validating biomethane potential (BMP) test results [10].

2.2. Central composite rotatable design (CCRD)

Sugarcane bagasse was subjected to an alkaline hydrothermal pretreatment to evaluate the effect of three independent variables: temperature (°C), NaOH concentration (M) and pretreatment duration (minutes) on sugars release, organic acids, and methane production according to CCRD using the Protimiza software (<https://experimental-design.protimiza.com.br/>). Fourteen different conditions were performed in triplicate in addition to six central points (Table 1).

The polynomial equations were derived from the experimental data to explain the impact of studied factors on the response propionate production and biogas composition.

The alkaline hydrothermal pretreatment was carried out in a stainless steel Parr pressure reactor (Moline, USA) (146.4–213.6 °C, 0.7–2.3 M, 3.2–36.8 min). The temperature was controlled by a 4838 Parr reactor controller (Moline, USA). The reactor chamber was filled with 300 ml of distilled water to account for evaporation losses. The solids: liquid ratio was fixed at 10% (w/w) with 10g of SCB fibers mixed with 100g of NaOH solution, according the CCRD conditions (Table 1).

After the predetermined time of CCRD, the heating was stopped and the pressure instantaneously released to obtain two fractions, fibers and

Table 1
Coded and decoded factor levels.

Condition	Temperature (°C)	NaOH concentration (M)	Duration (minutes)
C1	160 (−1)	1 (−1)	10 (−1)
C2	200 (+1)	1 (−1)	10 (−1)
C3	160 (−1)	2 (+1)	10 (−1)
C4	200 (+1)	2 (+1)	10 (−1)
C5	160 (−1)	1 (−1)	30 (+1)
C6	200 (+1)	1 (−1)	30 (+1)
C7	160 (−1)	2 (+1)	30 (+1)
C8	200 (+1)	2 (+1)	30 (+1)
C9	146.4 (−1.68)	1.5 (0)	20 (0)
C10	213.6 (+1.68)	1.5 (0)	20 (0)
C11	180 (0)	0.7 (−1.68)	20 (0)
C12	180 (0)	2.3 (+1.68)	20 (0)
C13	180 (0)	1.5 (0)	3.2 (−1.68)
C14	180 (0)	1.5 (0)	36.8 (+1.68)
Central point (CP)	180 (0)	1.5 (0)	20 (0)

hydrolysate. Both fractions, fibers and hydrolysate obtained after being exposed to the conditions listed in Table 1, were then sampled to be used for sugar characterization and/or to be used as the substrate in BMP tests.

2.3. Biomethane potential test

Anaerobic incubations were performed at atmospheric pressure using 160 ml Wheaton glass serum bottles using SCB as the substrate under thermophilic conditions (55 °C) and stirring (55 rpm), using an Innova® 44 incubator (Eppendorf, USA). Positive (cellulose) and negative controls (only inoculum) were performed in triplicate [10]. The experiments were conducted at a gas to liquid ratio of 1.5:1 and inoculum to substrate ratio of 2:1 gVS/gVS. The liquid medium was complemented with macronutrient and micronutrient stock solution (15 mL/L and 7.5 mL/L) following the protocol of Lindeboom et al. (2011) plus 0.5 g VS of each substrate (fiber and hydrolysate). The initial pH was adjusted to 7.0 ± 0.2 using 1 M HCl or 1 M NaOH. The reactors were sealed using aluminum caps and butyl rubber stoppers and then flushed with N₂.

2.4. Fibers characterization

The composition of both untreated and alkaline hydrothermally treated fibers was evaluated according to NREL technical report [11]. Briefly, extractives from the untreated fiber were removed using a Soxhlet apparatus and a 1:1 mixture of cyclohexane: ethanol as solvent. After Soxhlet extraction, the fibers were digested using sulfuric acid (72%) for 1 h maintaining a constant temperature of 30 °C through a water bath. After this period, it was diluted to sulfuric acid 4% and autoclaved for 1 h at 121 °C. The acid treated fibers were then filtered in a glass crucible and dried at 105 °C for 24 h to determine Klason lignin. The liquid was injected in high performance chromatography (HPLC) for sugars and organic acids measurement, using a Bio-Rad Aminex HPX-87H column, operating at 60 °C, with 5 mM H₂SO₄ at a flow rate of 0.6 mL/min and refractive-index detector.

2.5. Analytical methods

The sugars concentration was analyzed by high-performance liquid chromatography (HPLC) equipped with a UV diode array detector (SPD-M10 AVP), a refraction index detector (RID-10A), a CTO-20A oven, an LC-10 ADVP Pump, an SCL 10 AVP control, and an Aminex HPX-87H column (300 mm × 7.8 mm) (BioRad). H₂SO₄ (0.01 N) was used at 0.5 mL/min flow rate as the mobile phase [12]. The CH₄ and CO₂ contents in the biogas were determined by gas chromatography using an

Agilent technology GC System [13].

The VFAs determination was analyzed using an Agilent technology GC automatic system according Ghasimi et al. [13]. Total solids, total suspended solids, total volatile solids and total volatile suspended solids were measured according to Standard methods 2540 B, D, and E [14], respectively, whereas pH was measured using a Multi 9620 IDS multi-parameter. Total and soluble COD was determined using HACH kits (Range 100–2000 mg/L) followed by digestion for 2 h at 148 °C after which the COD concentration was measured using a HACH spectrophotometer.

2.6. Sampling, DNA extraction, 16S rRNA sequencing and processing

Total DNA was extracted from samples of inoculum and experiments on the central point, at the end of operational condition (180 °C, 1.5 M, and 20 min), using a DNA extraction kit (DNeasy Ultraclean Microbial Kit QIAGEN, The Netherlands). The DNA quality and quantity were evaluated using Qubit. DNA sequencing was performed by Novogene (Hong Kong). The regions V3–V4 of the bacterial domain were analyzed using 515F-806R primers while the V4–V5 region of archaeal domain were evaluated using the 519F/915R primers.

The samples' libraries were generated with NEBNext® UltraTM DNA Library Prep Kit for Illumina, quantified via Qubit and Q-PCR, and analyzed through the Illumina platform. Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence.

Paired-end reads were merged using FLASH [15], and quality filtering on the raw tags was performed under specific filtering conditions to obtain the high-quality clean tags [16] with the Qiime quality-controlled process [17]. The effective tags were obtained after comparison with UCHIME algorithm [18], the reference database, to detect and remove chimera sequences.

Sequences analysis was performed using Uparse software [19], with all the effective tags. Sequences with $\geq 97\%$ similarity were assigned to the same operational taxonomic units (OTUs).

The representative sequence for each OTU was screened for further annotation. For each representative sequence, Mothur software was performed against the SSUrRNA database of SILVA Database [20] for species annotation at each taxonomic rank (Threshold:0.8–1) [21].

To get the phylogenetic relationship of all OTUs representative sequences, the MUSCLE algorithm [22] was applied to compare multiple sequences. OTUs abundance information was normalized using a standard of sequence number corresponding to the sample with the fewest sequences. The raw reads were uploaded in Sequence Read Archive (SRA) database (<http://www.ncbi.nlm.nih.gov/sra>) under the BioProject SUB 9608154 with the samples number SAMN 19130476 (SCB reactor) and SAMN 19130477 (thermophilic sludge).

3. Results and discussion

3.1. Raw sugarcane bagasse characterization

The untreated SCB composition was: $31 \pm 2.7\%$ cellulose, $23 \pm 2.1\%$ hemicellulose, $36 \pm 5.2\%$ Klason lignin, and $10 \pm 0.4\%$ not identified. Brienzo et al. [23] reported glucan, arabinoxylan, and lignin composition varying from 36.9 to 48.6%, 24.6–32.8%, and 13.3–21.5%, respectively, in African sugarcane bagasse. Da Cruz et al. [24] reported highest cellulose and hemicellulose (40.9 and 30.6%, respectively) in Brazilian SCB than observed in the present study.

In contrast, using a Mexican SCB, Gonzales-Leos et al. [25], reported a similar composition to the values obtained in this work, i.e., 34.5, 29.7, and 35.4% of cellulose, hemicellulose and lignin, respectively.

Raw SCB characterization provides crucial information for assessing the potentials of anaerobic digestion for bio-energy and/or bio-chemical metabolites recovery. The SCB fiber composition affects its bioconversion potentials and, consequently, results in different intermediate and

final products, as previously demonstrate by Soares et al. [26].

3.2. Effects of alkaline hydrothermal pretreatment on lignin and residual sugars in the fibers

The remaining content of cellulose, hemicellulose and lignin at the SCB fibers changed according to the alkaline hydrothermal pretreatment conditions (Fig. 1). Temperature, NaOH concentration and its interaction had a positive and significant effect on the cellulose content in the pretreated fiber (p-value < 0.05). Highest cellulose contents (87.5 and 87.1%) were obtained at C10 (213.6 °C, 1.5 M NaOH, 20 min) and C4 (200 °C, 2.0 M NaOH, 10 min), respectively.

Batista et al. [27], evaluated the effect of temperature (170, 195, and 220 °C) and pretreatment duration (5, 10, and 15 min) on the hydrothermal pretreatment of sugarcane straw. According to these authors, application of intermediate temperatures results in distinct fiber modifications, high hemicellulose removal, but minor effects on cellulose solubilization, which is in agreement with the present study.

In the same way, McIntosh and Vancov [28], reported that increase in the NaOH concentration from 0.75% to 2.0% during alkali pretreatment of sorghum bicolor straw, resulted in high hemicellulose solubilization and, consequently, in a high cellulose content of the treated fiber.

High cellulose content after the alkaline hydrothermal pretreatment occurs due to the breakage of the ether and ester linkage between lignin and polysaccharides and removal of loose substances from the fiber surface during the pretreatment [29].

The increase in cellulose content can also be due to the increase in the pore size of the cellulose structure, due to hemicellulose removal, since both are physically linked [9].

In the present study, high cellulose concentrations, obtained at C4 and C10, were correlated with increasing levels of xylose and arabinoses in the hydrolysate at high temperature and NaOH concentration, corroborating the interactive effect of these variables on hemicellulose removal and consequent remaining cellulose composition in the pretreated fiber.

In addition, increasing NaOH concentration negatively affected the

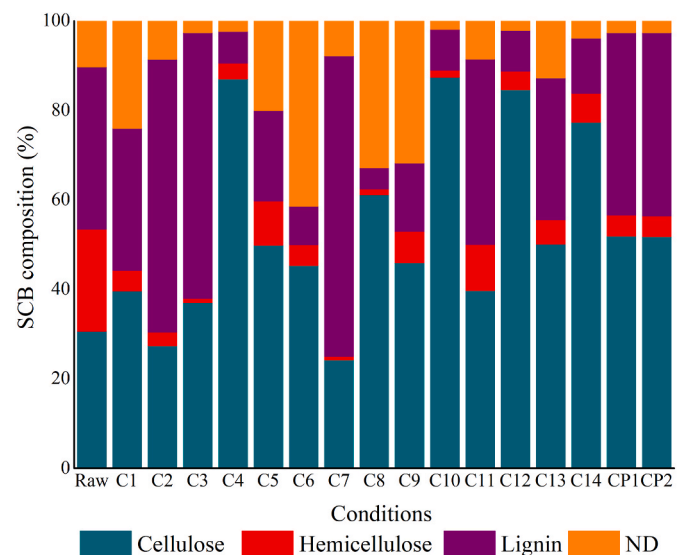


Fig. 1. Sugarcane bagasse (SCB) composition after alkaline hydrothermal pretreatment. Raw: non treated SCB, C1: 160 °C, 1M, 10 min, C2: 200 °C, 1M, 10 min, C3: 160 °C, 2M, 10 min, C4: 200 °C, 2M, 10 min, C5: 160 °C, 1M, 310 min, C6: 200 °C, 1M, 30 min, C7: 160 °C, 2M, 30 min, C8: 200 °C, 2M, 30 min, C9: 146.4 °C, 1.5M, 30 min, C10: 213 °C, 1.5M, 20 min, C11: 180 °C, 0.7M, 20 min, C12: 180 °C, 2.3M, 20 min, C13: 180 °C, 1.5M, 3.2 min, C14: 180 °C, 1.5M, 36.8 min, CP (Central point) 180 °C, 1.5M, 20 min.

remaining hemicellulose in the pretreated SCB (p-value < 0.05). At the lowest NaOH concentration (C11: 180 °C - 0.7 M NaOH - 20 min), the highest hemicellulose content was obtained (10.4%).

In general, in comparison with untreated SCB, the alkaline hydrothermal pretreated fibers had a lower percentage of hemicellulose and a higher percentage of cellulose. The same was reported by Deepa et al. [29], and Láinez et al. [30] after alkaline hydrothermal pretreatment of banana fiber and agave leaves.

Lastly, the lignin content in the pretreated SCB fibers was negatively affected (p-value < 0.05) by the interaction between the temperature and NaOH concentration (Supplementary Fig. S1).

Harsh process conditions will result in irreversible lignin degradation, resulting in an intractable fiber that is appropriate only for use as a low-cost energy source [1]. The lowest lignin content in the pretreated fibers (4.7%) was obtained at 200 °C, 2 M NaOH, and 30 min (C8). Low lignin content was also observed in C4 (7.1%), C6 (8.6%), C10 (9.1%) and C12 (9.1%), which are all conditions with high temperature or high NaOH concentrations (200 °C, 2 M NaOH, 10 min; 200 °C, 1 M NaOH, 30 min; 213.6 °C, 1.5 M NaOH, 20 min; and 180 °C, 2.3 M NaOH, 20 min, respectively).

In contrast, a lignin-rich fiber (67.2 and 59.4%) was achieved at C7 (160 °C, 2 M NaOH, 30 min) and C3 (160 °C, 2 M NaOH, 10 min), respectively. High lignin content in a pretreated fiber is advantageous for its use as a renewable aromatic building blocks source [1].

In summary, high temperature and high NaOH concentration are required to produce a pretreated fiber with high cellulose, low hemicellulose and low lignin contents. A high cellulose content fiber can be used for second generation (2G) cellulose based products [31], while hemicellulose can be converted into fermentable sugars, such as xylose, which can be transformed into valuable chemical feedstock like ethanol, hydrogen, organic acids, and methane [32]. Therefore, in order to convert all the content of 2G feedstock lignocellulose into biofuel or commodities chemicals, an integrated biorefinery strategy needs to be optimized in terms of pretreatment and biochemical pathways.

3.3. Effect of the alkaline hydrothermal pretreatment on sugars release on the hydrolysate

Pentoses (arabinose and xylose) and hexose (glucose) were released in the hydrolysate according to the alkaline hydrothermal pretreatment conditions (Supplementary Fig. S2). According to Medina et al. (2016) glucan corresponds to cellulose and hemicellulose fractions, while xylan and arabinan is theoretically released via hemicellulose degradation during the pretreatment. Only glucose was released in conditions C1 (160 °C, 1 M NaOH, 10 min), C2 (200 °C, 1 M NaOH, 10 min), C7 (160 °C, 2 M NaOH, 30 min), and C11 (180 °C, 0.66 M NaOH, 20 min), while arabinose and xylose were additionally released in the other pretreatment conditions. Using a central composite rotatable design it was possible to observe that temperature and NaOH concentration affected the percentage of glucose, xylose, and arabinose released in the hydrolyzed (p-value < 0.05) (Supplementary Fig. S3).

The glucose releases presented a convex behavior, with its minimal concentration at the central point (180 °C, 1.5 M NaOH, 20 min). This is an indication that the glucose released at low severities comes from hemicellulose degradation, while high severities pretreatment also affects the cellulose fraction. It is important to highlight that due to the lower thermal stability of hemicellulose, its degradation occurs before cellulose and lignin removal [33]. In contrast, higher arabinose plus xylose was released from hemicellulose at the central point of temperature and NaOH concentration (180 °C, 1.5 M, 20 min).

At high temperature and high NaOH concentration, sugars degradation can result in inhibitors formation. The pentoses decomposition during the fiber treatment could lead to production of furfural, while hexoses degradation can generate 5-hydroxymethylfurfural (5-HMF) [34]. C6/C5 sugars and 5-HMF can be used to produce bio-based polymers such as recyclable and/or biodegradable plastics [1]. In addition,

enzyme/microbial assisted processing can achieve biofuel and commodities chemicals production [1].

Microorganisms are able to synthesize added value chemicals from pentoses and hexoses, such as lactic and succinic acids, which are highly desired compounds for synthesis of environmentally benign materials in chemicals and food industries [35].

In the same way, fermentable sugars can be used for the production of hydrogen and bio-based building block chemicals, using the common methanogenic fermentative pathway. In this way, high-value products are produced instead of low-valued methane [36]. However, downstream processing of soluble chemicals from lignocellulosic biomass is, from an economic point of view, still not competitive, mainly due to the low products concentration and the high water-solubility of these products. Therefore, alternative strategies are required in order to minimize the costs for product recovery and successfully achieve its commercialization [37].

3.4. Effects of alkaline hydrothermal pretreatment on organic acids and methane production

An integrated bioprocess for the simultaneous production of organic acids and methane from fibers and hydrolysate of alkaline hydrothermal pretreated SCB was optimized. Volatile fatty acids (VFAs) were produced during anaerobic digestion of pretreated SCB (Fig. 2). The independent variables had no significant effect on the acetate concentration, although, accumulation of acetate was observed only in the conditions C4 (200 °C, 2 M, and 10 min), C8 (200 °C, 2 M, and 30 min), and C12 (180 °C, 2.34 M, and 20 min).

Under these conditions, high NaOH concentrations (2.0 and 2.3 M) and high temperature (180 and 200 °C) were applied during the hydrothermal pretreatment, resulting in a high release of sugars in the hydrolysate, i.e. 3.5, 2.7, and 3.1 g/L of total soluble sugars in C4, C8, and C12, respectively. Cellulose was the main constituent of the pretreated fibers in these conditions, i.e., 87.1, 61.2, and 84.6%, respectively, while hemicellulose and lignin contents were 3.5 and 7.1, 1.3 and 4.7, and 4.2 and 9.1%, respectively.

Acetate is mainly produced during acidogenesis, acetogenesis and homoacetogenesis and is converted via acetoclastic methanogenesis or acetate-oxidizing pathways for methane production [38].

Butyrate was observed in all experiments, but was not significantly affected by temperature, NaOH concentration or pretreatment duration. Butyrate and its derivatives have application in chemical, medicine,

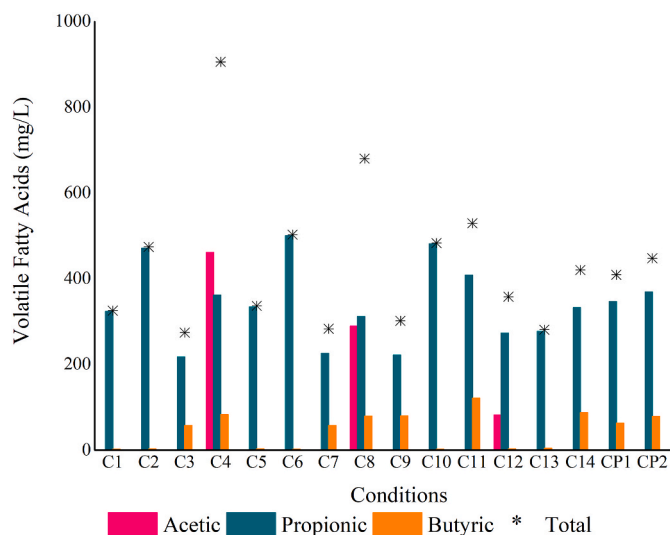


Fig. 2. Volatile fatty acids using SCB pretreated at different conditions with respect to temperature, NaOH, and duration of an alkaline hydrothermal pretreatment.

perfume, and animal feed production [39].

In contrast, propionate concentration was significantly affected ($p < 0.05$) by temperature and NaOH concentration (Supplementary Fig. S4). This acid and its salts can be used in agricultural, pharmaceutical, food, and biotechnological industries (Ali et al., 2021).

Under all the experimental conditions, propionate was the predominant intermediate produced. High temperature and low NaOH concentration resulted in increased propionate concentration.

High propionate concentrations, i.e. 500.3, 481.1, and 471.1 mg/L, accumulated at conditions C6, C10, and C2, respectively (200 °C, 1.0 M, 30 min; 213.6 °C, 1.5 M, 20 min; and 200 °C, 1.0 M, 10 min, respectively).

It must be noted that only a residual butyrate concentration was observed, i.e., 2.4, 2.2, and 3.1 mg/L at conditions C6, C10, and C2, respectively, indicating that all the sugars available in the pretreated fiber and hydrolysate were predominantly converted into propionate.

From the results an empirical equation was derived using Protimiza software to predict propionate production under the given conditions. ANOVA results are summarized in Supplementary Table S1.

$$\text{Propionic acid concentration} = 340.9 + 71.8X_1 - 54.2X_2 \quad \text{Equation 1}$$

Where: X_1 is Temperature (°C) and X_2 is NaOH concentration (M).

All hydrothermally pretreated SCB samples were subjected to anaerobic digestion for methane production during a period of 52 days in batch reactors (Fig. 3).

Notably, a higher methane content in the biogas (70, 69, and 69%) was obtained when high NaOH concentrations and long exposure times were used during pre-treatment, i.e., C8 (200 °C, 2.0 M NaOH, 30 min), C7 (160 °C, 2.0 M, NaOH, 30 min), C12 (180, 2.34 M NaOH, 20 min), respectively.

It is important to note that in condition C8, the lowest lignin content remained in the pretreated fiber (4.7%) and low lignin was also observed at C12 (9.1%). Although high lignin content was observed in C7 (67.2%), only glucose was released on the hydrolysate, which is easily converted by the microorganisms.

The applied NaOH concentration during pretreatment had linear, quadratic, and positive effects on the CH₄ content in the biogas at a significance level of 0.05, while time of exposure affected linearly and positively the response (p-value lower than 0.10).

ANOVA analysis of the model was performed to evaluate its statistical significance (Supplementary Table S2). equation (2) describes the

model for the methane content (%) on the biogas.

$$\text{CH}_4 \text{ content on the biogas} = 49.98 + 4.86X_2 + 6.06X_2^2 + 3.83X_3 \quad \text{Equation 2}$$

Equation 2

Where X_1 is Temperature (°C), X_2 is NaOH concentration (M), and X_3 is Time of exposition (minutes).

Fig. 4 shows the response surface graphs displaying the characteristic effects of the key process variables NaOH concentration during pre-treatment and pretreatment duration on the CH₄ content in the biogas, indicating a convex behavior (Fig. 4).

3.5. Microbial communities

The inoculum, as well as the biomass at the end of the incubation period from the batch reactor fed with SCB under central point conditions, were sampled and subjected to sequencing analysis on the Illumina platform to obtain their taxonomic classification.

The sequences were grouped into 404 and 316 OTUs for the inoculum and the batch reactor biomass fed with SCB under central point conditions, respectively (Supplementary Table S3).

Taxonomic analysis indicated that bacteria were dominant (~99% relative abundance), whereas a low abundance of archaea (<0.1%) were identified in both samples.

At the genus level (Fig. 5) ten bacterial genera showed relative abundance higher than 1% in the inoculum (*Gelria*, *Bacillus*, *Deftuviitoga*, *Advenella*, *Paenalcaligenes*, *Fastidiosipila*, *Tepidimicrobium*, *Anaerobaculum*, *Sporosarcina*, and *Tissirella*, 35.3%, 15.2%, 4.8%, 4.0%, 2.3%, 2.3%, 2.2%, 1.8%, 1.5%, and 1.0%, respectively) whereas *Deftuviitoga*, *Caldicoprobacter*, *Tepidimicrobium*, and *Gelria* were the most abundant genera in the reactor using SCB at central point (34.3, 29.9, 13.1, and 6.5%, respectively).

The genus *Gelria*, which showed a high relative abundance in the inoculum (35%), can establish syntrophic relationships with hydrogenotrophic methanogenic archaea and is inhibited by [Na+] in anaerobic digestion [40].

In contrast, the genus *Deftuviitoga* which showed a relative

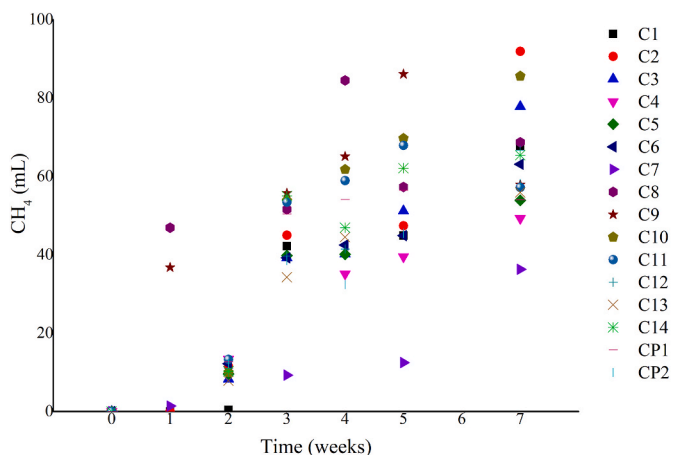


Fig. 3. Methane production versus time using SCB samples hydrothermally pretreated under all experimental conditions. C1: 160°C, 1M, 10 min; C2: 200°C, 1M, 10 min; C3: 160°C, 2M, 10min; C4: 200°C, 2M, 10 min; C5: 160°C, 1M, 310 min; C6: 200°C, 1M, 30 min; C7: 160°C, 2M, 30 min; C8: 200°C, 2M, 30 min; C9: 146.4°C, 1.5M, 30 min; C10: 213°C, 1.5M, 20 min; C11: 180°C, 0.7M, 20 min; C12: 1800°C, 2.3M, 20 min; C13: 180°C, 1.5M, 3.2 min; C14: 180°C, 1.5M, 36.8 min; CP (Central point) 180°C, 1.5M, 20 min.

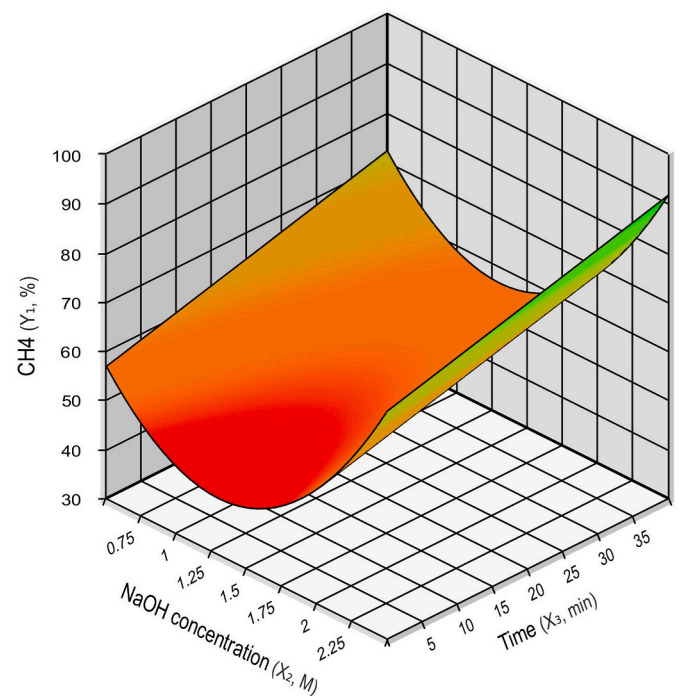


Fig. 4. 3D (A) and 2D (B) Response surface plot of Methane composition (CH₄%) as a function of temperature and pretreatment duration.

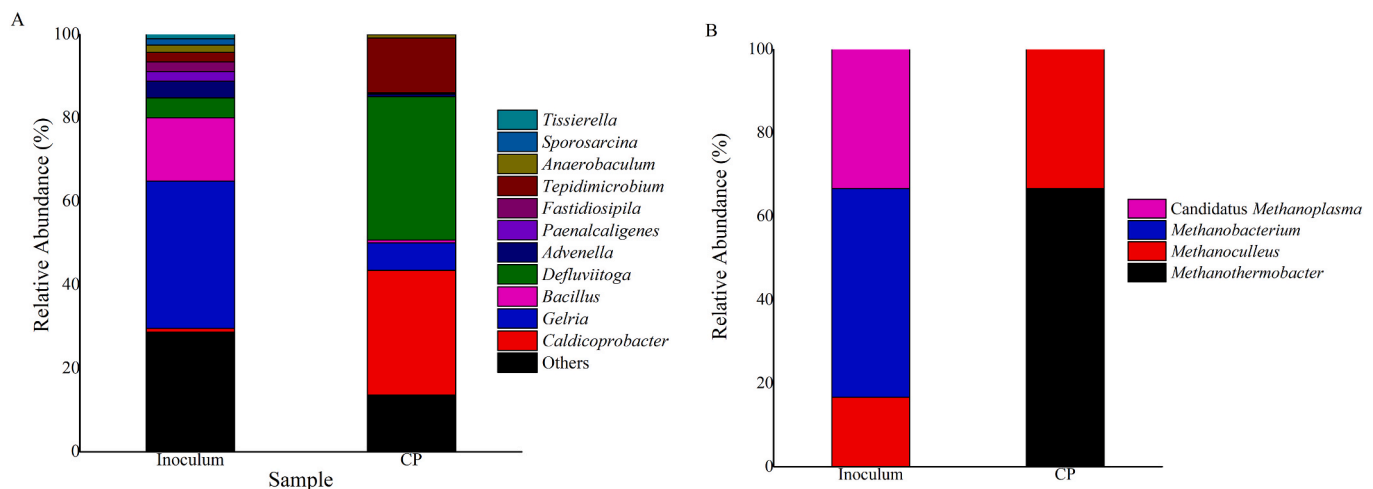


Fig. 5. Distribution of most abundant taxa at genera level in the inoculum and at the central point condition (CP) for Bacteria (A) and Archaea (B) domains.

abundance of 4.8% in the inoculum had a higher relative abundance (34.3%) at the end of the batch digestion. This genus participates in anaerobic digestion producing acetate, H₂, and CO₂, precursors of methanogenesis [41].

In the same way, the *Caldicoprobacter* and *Tepidimicrobium* genera, with low relative abundance in the inoculum (0.9, and 2.2% respectively) showed a high relative abundance, i.e., 29.9, and 13.1%, respectively, at the end of the incubation period.

The *Caldicoprobacter* genus includes bacteria able to produce extracellular thermostable xylanase [42] and cellulase activity [43], and could be related to lignocellulosic biomass bioconversion in this study.

Moreover, the hydrolytic and acidogenic *Tepidimicrobium* genus is able to convert and acidify many carbohydrates to acetate acid, ethanol, butyrate, H₂, and CO₂ [44]. At the end of the operational period its relative abundance was 13.1%, the total acids production reached 1.24 g/L, with 18.3% butyrate.

Considering the archaeal domain, *Methanobacterium*, *Candidatus Methanoplasma*, and *Methanoculleus* (50.0, 33.3, and 16.7%, respectively) were identified in the inoculum, whereas *Methanothermobacter* and *Methanoculleus* (66.7 and 33.3%, respectively) were found in the batch reactor incubated at central point conditions, using fiber and hydrolyzed SCB as the substrate.

It is important to note that *Methanothermobacter* was not identified in the inoculum, likely, due to its concentration below the detection limit. *Methanothermobacter* and *Methanoculleus* are common hydrogenotrophic methanogenic archaea, and were previously identified in anaerobic reactors treating SCB under thermophilic conditions [7].

Strikingly, no acetoclastic methanogenic archaea was identified in the reactor fed with SCB, nor acetate accumulated at central point condition incubations. Possibly, the acetate was used as substrate by syntrophic acetate-oxidizing bacteria (SAOB) producing H₂ and CO₂, which were subsequently converted into methane by hydrogenotrophic methanogenic archaea.

SAOB, such as *Caldicoprobacter* genus, also identified in the present study (29.9%), are associated to hydrogenotrophic methanogenic archaea that serve as electron sink during the anaerobic oxidation of fermentation products [45].

Many factors determine the ecological niche of acetate consumption, however, Dolfing (2014) demonstrated that under thermophilic conditions (55 °C) such as the one used in the present study, syntrophic acetate oxidation coupled to hydrogenotrophic methanogenesis becomes thermodynamically more favorable [46].

4. Conclusion

Cellulose-rich pretreated fiber (87.1%) from sugar cane bagasse was achieved applying hydrothermal pre-treatment under the following conditions: temperature 213.6 °C, 1.5 M NaOH, and 20 min duration. High hemicellulose content (10.4%) was obtained applying central point incubation conditions, i.e., 180 °C, 1.5 M NaOH, and 20 min. At temperatures of 146 and 160 °C, glucose was released in the hydrolysate coinciding with hemicellulose degradation, while at high temperatures (>200 °C), glucose release probably resulted from cellulose degradation. Arabinoses plus xylose were released as response to increased temperature and NaOH concentration. VFA and methane were also obtained from SCB alkaline hydrothermal pretreatment incubated at the central point (180 °C, 1.5 M, 20 min). *Defluviitoga* and *Methanothermobacter* were the most abundant bacterial and archaeal genera, respectively, identified at the end of the operational period at central point, and probably had an important role in sugarcane bagasse bioconversion.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biombioe.2022.106564>.

References

- [1] R.A. Sheldon, Biocatalysis and biomass conversion: enabling a circular economy: biocatalysis and biomass conversion, *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 378 (2020), <https://doi.org/10.1098/rsta.2019.0274>.
- [2] M. Meghana, Y. Shastri, Sustainable valorization of sugar industry waste: status, opportunities, and challenges, *Bioresour. Technol.* 303 (2020), 122929, <https://doi.org/10.1016/j.biortech.2020.122929>.
- [3] S.H. Da Cruz, N.N. Nichols, B.S. Dien, B.C. Saha, M.A. Cotta, Hydrothermal pretreatment of sugarcane bagasse using response surface methodology improves digestibility and ethanol production by SSF, *J. Ind. Microbiol. Biotechnol.* 39 (2012) 439–447, <https://doi.org/10.1007/s10295-011-1051-3>.

- [4] Y. Zhang, J. Yuan, L. Guo, Enhanced bio-hydrogen production from cornstarch hydrolysate pretreated by alkaline-enzymolysis with orthogonal design method, *Int. J. Hydrogen Energy* 45 (2020) 3750–3759, <https://doi.org/10.1016/j.ijhydene.2019.07.234>.
- [5] J. Du, Y. Qian, Y. Xi, X. Lü, Hydrothermal and alkaline thermal pretreatment at mild temperature in solid state for physicochemical properties and biogas production from anaerobic digestion of rice straw, *Renew. Energy* 139 (2019) 261–267, <https://doi.org/10.1016/j.renene.2019.01.097>.
- [6] M. Brienzo, A.F.A. Carvalho, F.C. de Figueiredo, P. de O. Neto, Sugarcane bagasse hemicellulose properties, extraction technologies and xylooligosaccharides production, https://www.novapublishers.com/catalog/product_info.php?products_id=60351, 2016.
- [7] L.A. Soares, C.A.B.S. Rabelo, T.P. Delforno, E.L. Silva, M.B.A. Varesche, Experimental design and syntrophic microbial pathways for biofuel production from sugarcane bagasse under thermophilic condition, *Renew. Energy* 140 (2019) 852–861, <https://doi.org/10.1016/j.renene.2019.03.103>.
- [8] F. Hu, A. Ragauskas, Pretreatment and lignocellulosic chemistry, *Bioenergy Res* 5 (2012) 1043–1066, <https://doi.org/10.1007/s12155-012-9208-0>.
- [9] K. Mirahmadi, M.M. Kabir, A. Jehanipour, K. Karimi, M.J. Taherzadeh, Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production, *Bioresources* 5 (2010) 928–938.
- [10] C. Holliger, M. Alves, D. Andrade, I. Angelidaki, S. Astals, U. Baier, C. Bougrier, P. Buffiere, M. Carballa, V. De Wilde, F. Ebertseder, B. Fernández, E. Ficara, I. Fotidis, J.C. Frigon, H.F. De Lacro, D.S.M. Ghasimi, G. Hack, M. Hartel, J. Heerenklage, I.S. Horvath, P. Jenicek, K. Koch, J. Krautwald, J. Lizasoain, J. Liu, L. Mosberger, M. Nistor, H. Oechsner, J.V. Oliveira, M. Paterson, A. Paus, S. Pommier, I. Porqueddu, F. Raposo, T. Ribeiro, F.R. Pfund, S. Strömberg, M. Torrijos, M. Van Eckert, J. Van Lier, H. Wedwitschka, I. Wierinck, Towards a standardization of biomethane potential tests, *Water Sci. Technol.* 74 (2016) 2515–2522, <https://doi.org/10.2166/wst.2016.336>.
- [11] A. Sluiter, B. Hames, R.O. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Energy, Determination of structural carbohydrates and lignin in Biomass, *Biomass Anal. Technol. Team Lab. Anal. Proced.* 1–18 (2012). NREL/TP-510-42618.
- [12] E.D. Penteado, C.Z. Lazaro, I.K. Sakamoto, M. Zaiat, Influence of seed sludge and pretreatment method on hydrogen production in packed-bed anaerobic reactors, *Int. J. Hydrogen Energy* 38 (2013) 6137–6145, <https://doi.org/10.1016/j.ijhydene.2013.01.067>.
- [13] D.S.M. Ghasimi, K. Aboudi, M. de Kreuk, M.H. Zandvoort, J.B. van Lier, Impact of lignocellulosic-waste intermediates on hydrolysis and methanogenesis under thermophilic and mesophilic conditions, *Chem. Eng. J.* 295 (2016) 181–191, <https://doi.org/10.1016/j.cej.2016.03.045>.
- [14] APHA, Standard Methods for Examination of Water and Wastewater, nineteenth ed., American Public Health Association/American Water Works Association/Water Environmental Federation, Washington, DC, USA, 2005.
- [15] T. Magoč, S.L. Salzberg, FLASH: fast length adjustment of short reads to improve genome assemblies, *Bioinformatics* (2011), <https://doi.org/10.1093/bioinformatics/btr507>.
- [16] N.A. Bokulich, S. Subramanian, J.J. Faith, D. Gevers, J.I. Gordon, R. Knight, D. A. Mills, J.G. Caporaso, Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing, *Nat. Methods* (2013), <https://doi.org/10.1038/nmeth.2276>.
- [17] J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E. K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S. T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data, *Nat. Methods* 7 (2010) 335–336, <https://doi.org/10.1038/nmeth.f.303>.
- [18] R.C. Edgar, B.J. Haas, J.C. Clemente, C. Quince, R. Knight, UCHIME improves sensitivity and speed of chimera detection, *Bioinformatics* (2011), <https://doi.org/10.1093/bioinformatics/btr381>.
- [19] R.C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads, *Nat. Methods* (2013), <https://doi.org/10.1038/nmeth.2604>.
- [20] C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, F. O. Glöckner, The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, *Nucleic Acids Res.* (2013), <https://doi.org/10.1093/nar/gks1219>.
- [21] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, *Appl. Environ. Microbiol.* (2007), <https://doi.org/10.1128/AEM.00062-07>.
- [22] R.C. Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Res.* (2004), <https://doi.org/10.1093/nar/gkh340>.
- [23] M. Brienzo, S. Fikizolo, Y. Benjamin, L. Tyhoda, J. Görgens, Influence of pretreatment severity on structural changes, lignin content and enzymatic hydrolysis of sugarcane bagasse samples, *Renew. Energy* 104 (2017) 271–280, <https://doi.org/10.1016/j.renene.2016.12.037>.
- [24] S.H. Da Cruz, N.N. Nichols, B.S. Dien, B.C. Saha, M.a. Cotta, Hydrothermal pretreatment of sugarcane bagasse using response surface methodology improves digestibility and ethanol production by SSF, *J. Ind. Microbiol. Biotechnol.* 39 (2012) 439–447, <https://doi.org/10.1007/s10295-011-1051-3>.
- [25] A. Gonzalez-Leos, M.G. Butos-Vásquez, L.V. Rodríguez-Castillejos, G.C. Rodríguez-Durán, A.D.Á.-D. Ángel, Kinetics of lactic acid fermentation from sugarcane bagasse by *Lactobacillus pentosus*, *Rev. Mex. Ing. Quim.* 19 (2020) 377–386, <http://www.redalyc.org/articulo.oa?id=62029966013>.
- [26] L.A. Soares, E.L. Silva, M.B.A. Varesche, Dissecting the role of heterogeneity and hydrothermal pretreatment of sugarcane bagasse in metabolic pathways for biofuels production, *Ind. Crop. Prod.* 160 (2021), <https://doi.org/10.1016/j.indcrop.2020.113120>.
- [27] G. Batista, R.B.A. Souza, B. Pratto, M.S.R. dos Santos-Rocha, A.J.G. Cruz, Effect of severity factor on the hydrothermal pretreatment of sugarcane straw, *Bioresour. Technol.* 275 (2019) 321–327, <https://doi.org/10.1016/j.biortech.2018.12.073>.
- [28] S. McIntosh, T. Vancov, Enhanced enzyme saccharification of Sorghum bicolor straw using dilute alkali pretreatment, *Bioresour. Technol.* 101 (2010) 6718–6727, <https://doi.org/10.1016/j.biortech.2010.03.116>.
- [29] B. Deepa, E. Abraham, B.M. Cherian, A. Bismarck, J.J. Blaker, L.A. Pothan, A. L. Leao, S.F. de Souza, M. Kottaisamy, Structure, morphology and thermal characteristics of banana nano fibers obtained by steam explosion, *Bioresour. Technol.* 102 (2011) 1988–1997, <https://doi.org/10.1016/j.biortech.2010.09.030>.
- [30] M. Láinez, H.A. Ruiz, A.A. Castro-Luna, S. Martínez-Hernández, Release of simple sugars from lignocellulosic biomass of Agave salmiana leaves subject to sequential pretreatment and enzymatic saccharification, *Biomass Bioenergy* 118 (2018) 133–140, <https://doi.org/10.1016/j.biombioe.2018.08.012>.
- [31] M.S. Rahman, M.I.H. Mondal, M.S. Yasmin, M. Abu Sayeed, M.A. Hossain, M. B. Ahmed, Conversion of lignocellulosic corn agro-waste into cellulose derivative and its potential application as pharmaceutical excipient, *Processes* 8 (2020), <https://doi.org/10.3390/PR8060711>.
- [32] V. Silva, R.P. Ratti, I.K. Sakamoto, M.V.F. Andrade, M.B.A. Varesche, Biotechnological products in batch reactors obtained from cellulose, glucose and xylose using thermophilic anaerobic consortium, *Renew. Energy* 125 (2018) 537–545, <https://doi.org/10.1016/j.renene.2018.02.124>.
- [33] D.K. Shen, S. Gu, The mechanism for thermal decomposition of cellulose and its main products, *Bioresour. Technol.* 100 (2009) 6496–6504, <https://doi.org/10.1016/j.biortech.2009.06.095>.
- [34] P.K. Rai, S.P. Singh, R.K. Asthana, S. Singh, Biohydrogen production from sugarcane bagasse by integrating dark- and photo-fermentation, *Bioresour. Technol.* 152 (2014) 140–146, <https://doi.org/10.1016/j.biortech.2013.10.117>.
- [35] D. Pleissner, Q. Qi, C. Gao, C.P. Rivero, C. Webb, C.S.K. Lin, J. Venus, Valorization of organic residues for the production of added value chemicals: a contribution to the bio-based economy, *Biochem. Eng. J.* 116 (2016) 3–16, <https://doi.org/10.1016/j.bej.2015.12.016>.
- [36] J. De Vrieze, K. Verbeeck, I. Pikaar, J. Boere, A. Van Wijk, K. Rabaey, W. Verstraete, The hydrogen gas bio-based economy and the production of renewable building block chemicals, food and energy, *N. Biotech.* 55 (2020) 12–18, <https://doi.org/10.1016/j.nbt.2019.09.004>.
- [37] K.K. Cheng, X.B. Zhao, J. Zeng, R.C. Wu, Y.Z. Xu, D.H. Liu, J.A. Zhang, Downstream processing of biotechnological produced succinic acid, *Appl. Microbiol. Biotechnol.* 95 (2012) 841–850, <https://doi.org/10.1007/s00253-012-4214-x>.
- [38] A. Ghimire, L. Frunzo, F. Pirozzi, E. Trably, R. Escudie, P.N.L. Lens, G. Esposito, A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products, *Appl. Energy* 144 (2015) 73–95, <https://doi.org/10.1016/j.apenergy.2015.01.045>.
- [39] L. Jiang, H. Fu, H.K. Yang, W. Xu, J. Wang, S.T. Yang, Butyric acid: applications and recent advances in its bioproduction, *Biotechnol. Adv.* 36 (2018) 2101–2117, <https://doi.org/10.1016/j.biotechadv.2018.09.005>.
- [40] C.M. Plugge, M. Balk, E.G. Zoetendal, A.J.M. Stams, *Gelria glutamica* gen. nov., sp. nov., a thermophilic, obligately syntrophic, glutamate-degrading anaerobe, *Int. J. Syst. Evol. Microbiol.* 52 (2002) 401–407, <https://doi.org/10.1099/00207713-52-2-401>.
- [41] I. Maus, K.G. Cibis, D. Wibberg, A. Winkler, Y. Stolze, H. König, A. Pühler, A. Schlüter, Complete genome sequence of the strain Defluviitoga tunisiensis L3, isolated from a thermophilic, production-scale biogas plant, *J. Biotechnol.* 203 (2015) 17–18, <https://doi.org/10.1016/j.jbiotec.2015.03.006>.
- [42] B.D. Amel, B. Nawel, B. Khelifa, G. Mohammed, J. Manon, K.G. Salima, N. Farida, H. Hocine, O. Bernard, C. Jean-Luc, F. Marie-Laure, Characterization of a purified thermostable xylanase from *Caldicoprobacter algeriensis* sp. nov. strain TH7C1T, *Carbohydr. Res.* 419 (2016) 60–68, <https://doi.org/10.1016/j.carres.2015.10.013>.
- [43] E. Widyastika, A. Shikata, R. Hashim, O. Sulaiman, K. Sudesh, E. Wahjono, A. Kosugi, Biodegradation of fibrillated oil palm trunk fiber by a novel thermophilic, anaerobic, xylanolytic bacterium *Caldicoprobacter* sp. CL-2 isolated from compost, *Enzym. Microb. Technol.* 111 (2018) 21–28, <https://doi.org/10.1016/j.enzmictec.2017.12.009>.
- [44] Y. Huang, Y. Wang, S. Liu, W. Huang, L. He, J. Zhou, Enhanced hydrolysis-acidification of high-solids and low-organic-content sludge by biological thermal-alkaline synergism, *Bioresour. Technol.* 294 (2019), 122234, <https://doi.org/10.1016/j.biortech.2019.122234>.
- [45] S. Dykstra, L. Jansen, C. Gallert, Syntrophic acetate oxidation replaces acetoclastic methanogenesis during thermophilic digestion of biowaste, *Microbiome* 8 (2020) 1–14, <https://doi.org/10.1186/s40168-020-00862-5>.
- [46] J.B. Van Lier, Limitations of thermophilic anaerobic wastewater treatment and the consequences for process design, Antonie van Leeuwenhoek, *Int. J. Gen. Mol. Microbiol.* 69 (1996) 1–14, <https://doi.org/10.1007/BF00641606>.