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Two step process for volatile fatty acid production from brewery spent grain: Hydrolysis and direct acidogenic fermentation using anaerobic granular sludge

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ABSTRACT

Brewery spent grain (BSG) is an industrial waste stream with large potential for biorefining purposes. This work evaluated the production of volatile fatty acids (VFAs) by a two-step process using BSG as renewable feedstock by combining a single direct hydrolysis step (without removing the acid or potential inhibiting compounds) with an acidogenic fermentation step of the carbohydrate rich leachate. For the first step, a thermal diluted acid hydrolysis was carried (20 min at 121 °C), using eighteen different combinations in terms of total solid (TS) of BSG (4, 7 and 10 % w/w) and H_2SO_4 (0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 % v/v). The 7.0 % TS of BSG and 1.5 % of H_2SO_4 combination was the most efficient in terms of total carbohydrate recovery (0.44 g of total carbohydrates per gram of TS). For the second step, an acidogenic batch fermentation of the hydrolysate was performed using anaerobic granular sludge at five different pH conditions (uncontrolled pH from an initial pH 7.0, and constant pH controlled at 4.5, 5.0, 6.0 and 8.0). The highest VFAs concentration was obtained at pH 6.0 and reached 16.89 (± 1.33) g COD/L, composed of mainly (99.5–99.8 %) acetate and butyrate.

1. Introduction

The use of waste as feedstock for biorefineries is increasing with the aim to promote the valorisation of low-value residues and pursue the zero waste perspective [1]. In the last century, the treatment of organic waste streams has shifted from aerobic to anaerobic bioprocesses, which require a lower energy consumption and allow the simultaneous treatment of waste and production of energy. Anaerobic digestion is one of the sustainable processes driving the transition from fossil fuels to modern, renewable energy sources. This process mainly consists of four different steps: i) hydrolysis, ii) acidogenesis, iii) acetogenesis and iv) methanogenesis [2]. The partial or total inhibition of methanogenesis can shift biomethane production to some of the intermediate compounds as final products, such as lactate [3], hydrogen [4,5] or volatile fatty acids (VFAs) [6]. Some strategies to prevent methanogenesis and trigger VFA accumulation include the increase of the organic loading rate [7] or the ammonium concentration [8–11].

VFAs are short chain fatty acids (C_2 to C_6) with a broad range of industrial application and their current production relies on fossil sources [12]. VFAs can be produced from renewable biomass through anaerobic fermentation, but their recovery is still a bottleneck in this technological development. This is especially the case for low strength wastewaters, which yield only diluted VFA concentrations. Consequently, this process is till now mainly used as intermediate step to obtain compounds that are easier to recover, e.g. polyhydroxyalkanoates (PHA) or lipids for biodiesel production [13,14]. Nevertheless, recent improvements in the extraction systems may allow to efficiently extract the VFAs as the final products, especially if high VFA concentrations can be obtained [15,16]. Thus, VFA production depends less on fossil sources and provides a trade opportunity in countries that rely on importing gas and oil. However, some parameters of the acidogenesis phase, such as the VFA concentration and the operating pH must be further investigated.

One of the most important factors affecting the yield and stability of

Abbreviations: BSG, brewery spent grain; COD, chemical oxygen demand; TC, total carbohydrates; TS, total solids; TSS, total suspended solids; COD_{sol}, soluble chemical oxygen demand; VFAs, volatile fatty acids; VS, volatile solids; VSS, volatile suspended solids.

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a bioprocess is the consistent composition and availability of the feedstock throughout the year [17,18]. Lignocellulosic materials (LMs) represent the most abundant organic feedstocks for bio-industrial purposes, with low or no costs, in comparison to the more expensive energy crops. However, the main issue when using LMs in fermentation processes is associated with their complex structure, where cellulose and hemicellulose are interwoven within lignin [19], being recalcitrant to microbial degradation in the absence of a pretreatment [20]. Many studies have focused on different pretreatment methods to make LMs more accessible to microorganisms and thus release an elevated amount of high-value molecules, such as sugars, proteins or phenolic compounds. Some examples of these pretreatments are enzymatic hydrolysis [21], diluted acid treatment [22] and steam explosion [23]. With regard to chemical pretreatments, the hydrolysis efficiency of lignocellulosic wastes depends on the combination of different pretreatment parameters such as temperature, time and acid concentration. This combination of parameters can be captured in a single value, known as the severity factor [22,24,25].

Many agro-food industries could provide a constant supply of LMs to be used for either energy production [20,26] or the recovery of high value compounds [27]. One source of sustainable biomass for biorefinery platforms is the brewery sector. The basic ingredients of beer are water, barley, hops and yeast, and the most abundant waste stream deriving from the brewing industry is the malted barley obtained post boiling. This latter mentioned solid waste is known as brewery spent grain (BSG), and approximately 15–20 kg is produced per hectolitre of beer. Conventionally, BSG is used for animal feeding or burnt after drying. Rarely, BSG has been used for environmental applications, such as a co-substrate in anaerobic digesters aimed at methane production [28]. Recovery of valuable compounds, including lignin [21], polyphenols [29], prebiotics [30], proteins [31], and total carbohydrates (TC), mainly glucose, xylose and arabinose [32–34], from BSG has only recently gained interest.

The consistent composition and year-round availability make BSG a suitable renewable feedstock for VFA production. To enable this, a combination of different pretreatment methods and fermentation technologies is required, and the feasibility to combine them must also be evaluated. As pretreatment method for BSG, a thermal diluted sulfuric acid pretreatment was used in this study, as similar processes showed to be reliable methods used at the industrial scale, only requiring a single reaction step and a short time for the hydrolysis of the material. Therefore, we preliminarily focused on a first step on the hydrolysis of BSG using different sulfuric acid and BSG concentrations, in order to obtain a leachate rich in easily fermentable carbohydrates. The pretreatment conditions (i.e. low acid concentration, short reaction time of 20 min, and a temperature of 121 °C) allowed the solubilisation of complex carbohydrates without the production of toxic compounds (e.g. furfural, hydroxymethylfurfural, formic acid, levulinic acid or total phenolic compounds) that inhibit subsequent fermentation [22,35]. Subsequently, as a second step, two stirred tank reactors inoculated with anaerobic granular sludge were run in batch mode for the production of VFAs from the obtained carbohydrate-rich leachate. It should be noted that between step one and two, only pH neutralization was done after the thermal diluted sulfuric acid step and no further treatment was carried to remove possible inhibitory compounds co-released during the BSG hydrolysis. The VFA accumulation during acetogenic fermentation was promoted by the operating parameters initial organic concentration and pH. Different pH values were used to run the batch acetogenic fermenting reactors and evaluate the TC consumption, highest VFA production rate, total VFA concentration and VFA composition.

2. Material and methods

2.1. Feedstock and inoculum

BSG was supplied by a local brewery in Galway (Ireland) for the production of lager beer. Once collected, the BSG was mixed and frozen in individual bags of 3 kg each at −20 °C. Prior to the hydrolysis step, the BSG was defrosted overnight at 15 °C.

As inoculum, an anaerobic granular sludge collected from an UASB reactor treating dairy wastewater for methane production (Kilconnell, Ireland) at ambient temperature was used. The total (TSS) and volatile (VSS) suspended solid concentrations of the inoculum were 72.8 and 59.1 mg/g of wet inoculum, respectively. No pretreatment was applied to the inoculum.

2.2. Diluted acid hydrolysis of brewery spent grain

After defrosting, the BSG was mixed with distilled water and blended using a laboratory Waring 2-Speed Laboratory blender (USA) for 1 min to break down the external structure of the grain and facilitate hydrolysis. Three different total solid (TS) concentrations of BSG (4, 7 and 10 % w/w) were used for the hydrolysis of BSG and the extraction of easily fermentable carbohydrates. The blended mixtures were placed in 500 mL Pyrex bottles with a working volume of 400 mL.

Sulfuric acid (95 % purity and 1.83 g/mL density), supplied from Fisher Chemical (UK), was used to perform diluted acid hydrolysis of BSG at 121 °C for 20 min in an autoclave (Sanyo Labo, Japan) as described by Carvalheiro et al. [22] and Djioleu et al. [35]. This cost-efficient method allows the hydrolysis of carbohydrates and proteins, while lignin remains as a precipitate [33]. Sulfuric acid was added at six different concentrations (0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 % v/v), resulting in a total of 18 different combinations in terms of sulfuric acid and TS concentration of BSG. Each combination was run in triplicate. The severity factor was calculated as proposed by Carvalheiro et al. [22] and Fockink et al. [25] for the selected combinations to be used as feedstock for the batch acetogenic fermentation.

Once hydrolysis was completed, the bottles were placed on ice to stop the reaction, and the hydrolysate was centrifuged at 5000 rpm for 30 min (Beckman Coulter, U.S.A.; JS-5.3 Rotor) to recover the supernatant.

The extraction efficiency was calculated taking into account the total concentration of carbohydrates extracted, the amount of liquid recovered, as well as the TS concentration and volume of sulfuric acid used.

2.3. Batch reactor operation and monitoring

As substrate for the acidogenic fermentation, the leachate obtained from the diluted acid hydrolysis performed at 7 % (w/v) of TS of BSG and 1.5 % of sulfuric acid (v/v) was used, as this pretreatment condition resulted in the highest extraction efficiency in terms of g TC/g TS (Table 1). The fermentation system consisted of two duplicate stirred tank reactors running as twin reactors operated at 37 °C. The reactors were made of a section of acrylic tube (inner diameter 15 cm) sealed at both sides with acrylic discs, thus creating a total volume of 2.1 L. A port in the side of the reactors allowed sampling, while a pH probe, gas outlet and tubing for NaOH addition were inserted through the top of the reactor (Fig. 1). The reactors were operated in batch mode with a working volume of 1.75 L each. In both reactors, the hydrolysate was mixed with distilled water to give a total initial carbohydrate concentration of 16 g COD/L. The reactors were seeded with 3.0 g/L VSS of granular sludge and sparged for 3 min with nitrogen gas (N_2) to remove oxygen from the medium. To maintain homogeneous conditions in each reactor, a magnetic stirrer (Stuart UC151, UK) was used and operated at 1500 rpm.

VFA production was analysed under both uncontrolled and controlled pH conditions over a period of 72 h. The experimental run at uncontrolled pH started from an adjusted pH of 7.0 (± 0.01), and no further correction was performed while the process was ongoing, with the aim determining the lowest tolerable pH by the system. In the experiments run at controlled pH, four experimental runs were performed at different pH, where the pH was maintained at either 4.5, 5.0, 6.0 or 8.0 (± 0.05 units) throughout. This was achieved using a pH controller

Table 1
Operating conditions used for the hydrolysis of brewery spent grain as well as the composition of carbohydrates extracted and efficiency of the hydrolysis process expressed as percentage of the total carbohydrates recovered per gram of total solid used.

H_2SO_4 %	TS (BSG) %	Cellobiose (g/L)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Efficiency (% gTC/gTS)
0	7	1.13 (± 0.13)	1.23 (± 0.83)	0.00 (± 0.00)	0.00 (± 0.00)	8.18 (± 1.3)
0	10	1.55 (± 0.25)	0.75 (± 0.75)	0.00 (± 0.00)	0.00 (± 0.00)	7.32(± 1.4)
1	7	0.70 (± 0.70)	10.05 (± 0.95)	10.40 (± 1.00)	11.10 (± 0.50)	40.35 (± 1.9)
1	10	2.20 (± 0.35)	12.50 (± 4.15)	14.10 (± 7.40)	14.50 (± 7.25)	34.7 (± 0.5)
1.5	7	0.00 (± 0.00)	12.80 (± 1.70)	12.05 (± 0.85)	11.80 (± 0.10)	43.8 (± 1.1)
1.5	10	2.00 (± 0.10)	16.70 (± 0.70)	15.65 (± 0.05)	12.75 (± 0.15)	36.6 (± 0.4)
3	7	0.00 (± 0.00)	11.32 (± 0.04)	6.58 (± 0.09)	5.96 (± 0.01)	44.6 (± 2.7)
3	10	0.60 (± 0.05)	20.74 (± 1.49)	19.08 (± 2.21)	8.68 (± 1.49)	42.5 (± 0.5)

(Alpha 100; Thermo Scientific, Singapore) connected to a peristaltic pump (Masterflex 323, UK) for the addition of a 3 M NaOH solution.

The reactor operation was monitored by analysing TC, monosaccharides, VFAs, lactic acid, ethanol, soluble chemical oxygen demand (COD_{sol}), TSS and VSS. Sulfate, proteins and ammonium were analysed at the beginning and the end of the batch incubations.

2.4. Physico-chemical analyses

The COD_{sol} was analysed using a commercial kit (Reagecon, Ireland) with a range from 0 to 1500 mg COD/L. 2 mL of sample was added to the vial and digested at 150 °C for 2 h, and the absorbance was measured with a DR2500 spectrophotometer (Hach, Germany). Protein analysis was performed using the Lowry method [36]. TC were analysed by the Dubois method [37]. Glucose, xylose, arabinose and cellobiose were measured using a 1260 Infinity II liquid chromatograph (Agilent, Germany) equipped with a Hi Plex H 7.7 × 300 mm and 8 µm (p/n PL1170−6830) column (Agilent, UK) kept at 60 °C and an RI detector at 55 °C. The mobile phase was sulfuric acid with a concentration of 0.005 M and a flow rate of 0.7 mL/min. Lactic acid and ethanol were analysed by liquid chromatography using a Prominence LC-20A Series HPLC (Shimadzu, Japan) with a Rezex ROA Organic Acid H + column (Phenomenex, USA) heated at 40 °C and an SPD-20A UV detector set at 220 nm [38]. As the mobile phase, a 0.0065 mM H_2SO_4 solution was used at a flow rate of 0.6 mL/min.

VFA analysis was performed with a 450-GC gas chromatograph (Varian, USA) equipped with a CombiPAL autosampler, a flame ionisation detector and a FFAP BP21 capillary column (SGE Analytical Science, Australia) of 30 m length, 0.25 mm internal diameter and a 0.25 µm film. Helium was used as carrier gas with a flow rate of 1 mL/min. The oven temperature was risen from 60 (10 s) to 110 °C (20 s) at a rate of 30 °C/min, and from 110 to 200 °C at a rate of 10 °C/min. The temperatures of the injector and detector were 250 and 300 °C, respectively.

Ammonium and sulfate analyses were performed using a Gallery Plus® discrete nutrient analyser (ThermoFisher, Finland) based on a colorimetric assay [39,40]. TSS and VSS concentrations were measured every 24 h, following the procedure reported in standard methods [41].

2.5. Calculations

To evaluate the effect of the operating pH, TC consumption and VFA production rates were calculated using the exponential phase after the initial acclimatization period from each of the replicate reactors. The rates were determined using 6–9 experimental points, always accepting an R-squared value above 0.89.

The calculation of the total carbohydrate consumption rate is reported in Eq. 1:

$$-r_{TC} = \frac{[Y]_i - [Y]_f}{t_i - t_f} \left[gCOD/(L \cdot h) \right] \quad (1)$$

where $-r_{TC}$: total carbohydrate consumption rate, Y: total carbohydrate concentration, t: time of the log phase for TC consumption, i: at the beginning of log phase, f: at the end of the log phase.

The total VFA production rate was determined as reported in Eq. 2:

$$r_A = \frac{[A]_i - [A]_f}{t_i - t_f} \left[gCOD/(L \cdot h) \right] \quad (2)$$

where r_A: total VFA production rate, A: total VFA concentration, t: time

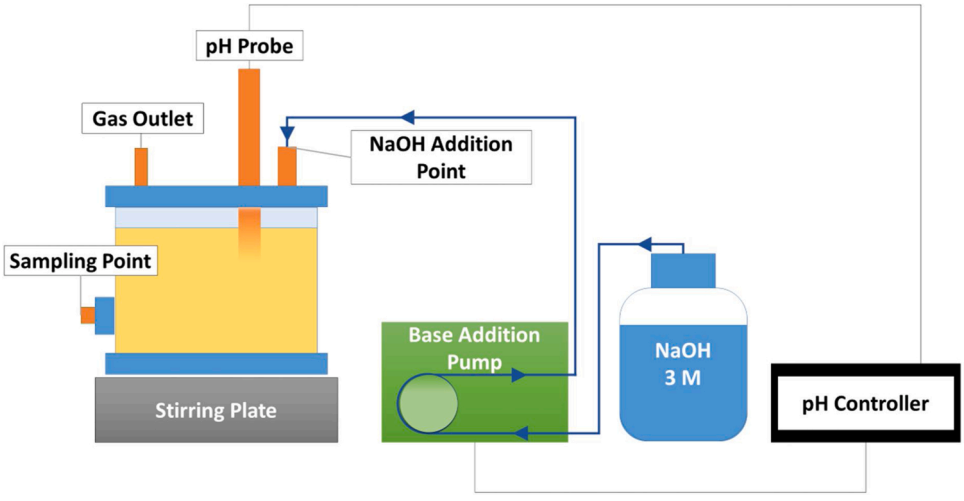

Fig. 1. Schematic experimental set up of the stirred tank reactors operated in batch mode for VFA production from the hydrolysate of brewery spent grain. The stirring plates, pH controller and probes as well as the dosing of the NaOH solution are shown.

of the log phase for VFA production, i: at the beginning of the log phase, f: at the end of the log phase.

At each pH, the acidification level was determined (Eq. 3) as previously reported by Bengtsson et al. [10] by calculating the percentage of the total amount of VFAs (in terms of g COD/L) above the total COD_{sol} (which includes the VFAs):

$$Acidification\ Level = \frac{Total\ VFA\ concentration}{Soluble\ COD}\left[\frac{gCOD}{gCOD_{soluble}}\right] \quad (3)$$

This percentage was used to evaluate the optimal pH at the end of each log phase as the highest amount of VFAs in terms of COD divided by the overall COD_{sol}. The TC depletion (Eq. 4) and the COD_{sol} consumption (Eq. 5) were calculated considering the initial and final points of the log phase for VFA production. The extent of sulfate reduction was calculated using the initial and final points of each experiment (Eq. 6).

$$Total\ carbohydrates\ depletion = \frac{[Y]_i - [Y]_f}{[Y]_i}\left[\frac{g\ consumed}{g\ beginning}\right] \quad (4)$$

$$sCOD_{consumption} = \frac{[sCOD]_i - [sCOD]_f}{[sCOD]_i}\left[g\ consumed/g\ beginning\right] \quad (5)$$

where Y: total carbohydrate concentration, t: time of log phase for VFAs production, i: at the beginning of the log phase, f: at the end of the log phase.

$$Sulphate\ reduction = \frac{[S]_0 - [S]_e}{t_0 - t_e}\left[g\ consumed/g\ beginning\right] \quad (6)$$

where S: sulfate concentration, t: time of log phase for VFAs production, 0: beginning of the trial, e: end of the trial.

3. Results

3.1. Efficiency of diluted acid hydrolysis of brewery spent grain

After acid hydrolysis, for the TC obtained from the 18 different pretreatment combinations; the lowest amount of carbohydrates recovered was in the absence of sulfuric acid, ranging between 2.5–8.5 g/L with increasing TS (Fig. 2a). The highest TC concentration was achieved using 10 % TS and 3 % H_2SO_4 (v/v). Standardising the extraction efficiencies by the TS concentration used, all the different conditions resulted in a similar ratio of 2.96 (± 0.20) g TC per gram of TS. The concentration of TC extracted increased linearly from 0.0–1.0 % v/v of sulfuric acid. At H_2SO_4 concentrations from 1.5 % upwards, the efficiency between the TC extracted and the volume of sulfuric acid used was lower. The composition of the carbohydrates recovered was mainly characterized in terms of glucose, xylose and arabinose (Table 1). The monosaccharides were analysed in the samples that were considered for the extraction at the fermentation step (7 and 10 % TS of BSG at 1.0 and 1.5 % of sulfuric acid), as well as for lowest and highest sulfuric acid concentration (Table 1). The presence of cellobiose was detected in all the samples for 10 % TS of BSG, and in the case of 7 % TS for 0.0 and 1.0 % of sulfuric acid.

For protein extraction, increasing the sulfuric acid concentration from 0.0 to 0.5 % (v/v) resulted in the highest release of proteins (Fig. 2c). After this point, the protein extraction efficiency decreased. Similarly, the extraction of ammonium was the most efficient at sulfuric acid concentrations between 0.0–1.0 % (v/v) (Fig. 2d).

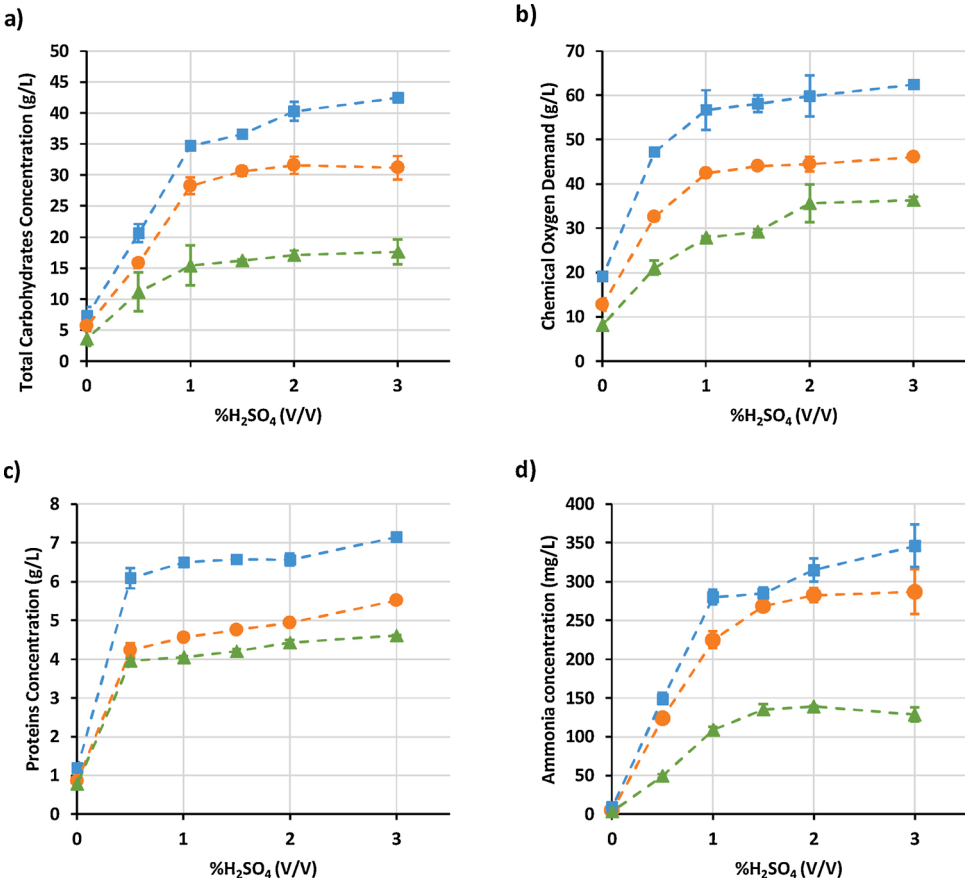

Fig. 2. Influence of the sulfuric acid concentration on the diluted acid hydrolysis of brewery spent grain (121 °C, 20 min) at three different total solid concentrations (▲ 4 % TS ● 7 % TS ■ 10 % TS): a) release of total carbohydrates, b) soluble chemical oxygen demand (COD), c) proteins extracted and d) ammonium concentration.

All combinations of sulfuric acid at 10 % TS of BSG and the combinations of 7 % TS of BSG at 1.0 % of sulfuric acid concentration or lower, resulted in an uncompleted hydrolysis, as indicated by the presence of cellobiose (Table 1). Taking into account the concentration of all the compounds extracted during the BSG diluted acid hydrolysis, the conditions operated at 1.5 % of sulfuric acid led to the highest overall efficiency, although this did not result in the highest total carbohydrate concentration. Furthermore, a 7 % TS concentration of BSG was considered the optimal as 0.44 (± 0.01) grams of TC were extracted per grams of TS. In the case of a TS concentration of 10 %, the amount of TC per TS was 0.37 (± 0.01) (Table 1).

3.2. Acidogenic fermentation of brewery spent grain with anaerobic granular sludge in batch reactors

3.2.1. Initial conditions

The pH of the liquid fraction obtained after BSG hydrolysis at 7 % TS and 1.5 % sulfuric acid was 0.93 (± 0.06). The pH was adjusted by the addition of NaOH before starting acidogenic fermentation in batch reactors. The average initial conditions for the acidogenic fermentation in all experiments were COD_{sol} 23.1 (± 2.1) g /L, TC 16.9 (± 2.2) g COD/L, proteins 3.35 (± 0.4) g/L, ammonium 194.9 (± 7.0) mg/L and sulfate 14.7 (± 1.8) g/L (Table 2).

3.2.2. Acidogenic fermentation at uncontrolled pH

In the experiment operated without pH control, the pH quickly decreased from 7.00 (± 0.01) to 6.66 (± 0.03) in 2.5 h and reached a final value of 4.08 (± 0.05) at the end of the experiment (72 h) (Fig. 3). The total carbohydrate concentration decreased at a constant rate of 0.24 (± 0.13) g COD/(L h) (Eq. 1) until reaching a concentration of 7.32 (± 1.93) g COD/L after 36 h, when a pH of 4.19 (± 0.18) was observed. Subsequently, carbohydrate consumption almost ceased with a final total carbohydrate consumption efficiency of 63.7 (± 4.4) % (Eq. 4). With regards to monosaccharides, glucose was completely consumed, while the arabinose and xylose concentration remained constant (Table 2).

In terms of VFAs produced, acetic acid production started after approximately 7 h, with a production rate of 0.06 (± 0.02) g COD/(L h) (Eq. 2). Butyric acid was produced after 11 h with a production rate of 0.15 (± 0.02) g COD/(L h) (Eq. 2), i.e. higher than that observed for acetic acid. The acidification level in terms of g COD of VFAs per 100 g of COD_{sol} was 44.0 (± 7.0) % (Eq. 3), with acetic and butyric acids as the major constituents and representing 95.5 % of the total COD_{sol} (33.3 and 62.2 %, respectively). Lactic acid production started after 11 h, but its concentration only reached a peak of 1.43 (± 0.43) g COD/L, i.e. 36.8 (± 12.1) and 24.6 (± 8.0) % lower than, respectively, the acetic and butyric acid concentration.

3.2.3. Acidogenic fermentation at controlled pH of 4.5, 5.0, 6.0 and 8.0

When keeping the pH constant and above 4.5 throughout the experiment, the percentage of TC consumption exceeded 90 % and was similar for each condition (Fig. 4a). The TC consumption rate increased at higher operating pH (Fig. 4b). Glucose was completely consumed after approximately 44 h when working at pH 4.5, and only 24 h were required to completely remove glucose when increasing the pH up to 8.0. After glucose was consumed, the degradation of xylose and arabinose started (data not shown).

The total amount of VFAs produced at each of the pH conditions tested (4.5, 5.0, 6.0 and 8.0) is shown in Fig. 5. The acetic and butyric acid content was the most significant fraction of the COD_{sol} (Fig. 5), ranging between 95.5 and 99.8 %. At pH 4.5 and 5.0, the VFA concentration remained stable from 29 to 35 h and from 11 to 23 h, prior to resume production until a final concentration of VFAs of 11.0 (± 0.04) and 12.2 (± 2.3) g COD/L, respectively (Supplementary material Fig. S1). In the case of pH 6.0 and 8.0, the intermediate phase with a stable VFA concentration was not observed. Furthermore, a delay of the VFA production compared to carbohydrate consumption was observed (Supplementary material Fig. S1 & S2). The acetic acid production rate increased when operating the process at a higher pH (Fig. 4b). The highest production rate for butyric acid was 0.52 (± 0.04) g COD/(L h achieved at pH 5.0 (Fig. 4b).

Lactic acid was produced under all conditions tested. Lactic acid production started earlier than VFA production at pH 4.5 and reached the highest concentration of 3.87 (± 0.76) g COD/L at 49 h and decreasing at the end of the trial to 2.07 (± 0.72) g COD/L. At pH 6.0, the production of lactic acid started simultaneously with VFAs, with lactic acid reaching the highest concentration of 1.08 (± 0.30) g COD/L at 17.5 (± 4.7) h, before being completely consumed after 49 h. At pH 8.0, lactic acid reached a peak of 0.62 (± 0.02) g COD/L after 18.5 h (Supplementary material Fig. S3).

3.2.4. Nitrogen compounds, sulfate and soluble chemical oxygen demand

The average initial proteins and ammonium concentrations for all the combinations corresponded, respectively, to 3.4 (± 0.4) g/L and 194.9 (± 7.0) mg/L. The final protein concentration was 1.7 (± 0.3) and 0.9 (± 0.2) g/L in the case of uncontrolled pH and pH 8.0, respectively. This led to a lower increase of the ammonium concentration in the case of uncontrolled pH with 273.1 (± 7.7) mg/L, and a higher increment at pH 8.0 up to 468.5 (± 6.5) mg/L.

The COD consumption and sulfate reduction percentage at the end of the acidogenic fermentation were investigated for each of the pH conditions tested (Fig. 4a). The lowest COD consumption of approximately 7.4 (± 2.3) % was obtained at uncontrolled pH, while the highest COD consumption of 18.9 (± 0.1) and 20.5 (± 3.1) % (Eq. 5) was observed at pH 6 and 8, respectively (Fig. 4a). The COD_{sol}/sulfate ratio in the reactors at the beginning of the experiments was 1.41 (± 0.10) and 1.28 (± 0.10) at the end of the experiments. The average initial and final sulfate concentrations in both reactors were 15.84 (±2.20) g/L and 14.99 (± 2.50) g/L, respectively, under all the conditions investigated. This implies an average sulfate reduction efficiency of 5.1 (± 1.8) % (Eq. 6) (Fig. 4a). The ethanol concentration constantly remained below the detection limit at all pH values investigated.

4. Discussion

4.1. Operational conditions during the acidogenic fermentation

This study shows for the first time the feasibility of producing high concentrations of VFA from BSG by combining a diluted acid hydrolysis

Table 2
Glucose, xylose and arabinose concentration in the reactor started at uncontrolled pH along the 72 h batch experiment.

Time (hour)	0	13	22	30	46	60	71
Glucose (g/L)	5.81 (± 0.19)	4.08 (± 0.63	2.63 (± 0.71)	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
Xylose (g/L)	5.53 (± 0.20)	5.46 (± 0.12)	4.60 (± 0.55)	5.22 (± 0.38)	4.95 (± 0.41	5.03 (± 0.28)	4.86 (± 0.41)
Arabinose (g/L)	2.75 (± 0.10)	2.35 (± 0.36)	2.67 (± 0.08)	2.55 (± 0.12)	2.52 (± 0.01)	2.55 (± 0.05)	2.45 (± 0.03)
pH	6.98 (± 0.01)	5.11 (± 0.23)	4.59 (± 0.18)	4.44 (± 0.17)	4.11 (± 0.07)	4.10 (± 0.06)	4.08 (± 0.05)

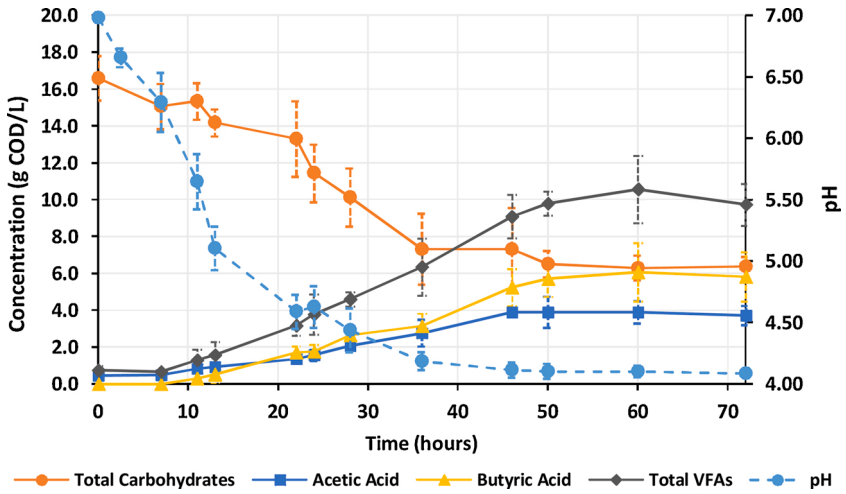

Fig. 3. Acidogenic fermentation of brewery spent grain hydrolysate at uncontrolled pH at 37 °C in two batch-operated stirred tank reactors. Profiles of total carbohydrates consumption, total volatile fatty acids (VFAs), acetic and butyric acid production, and pH.

of the BSG in step one with a direct acidogenic fermentation of the BSG hydrolysate using untreated anaerobic granular sludge. Despite the anaerobic granular sludge originated from an industrial reactor aimed at methane production, the operating conditions adopted in this study resulted in VFA accumulation rather than methanogenesis as no VFA consumption was observed after reaching the maximum VFA concentration (Supplementary material Fig. S1, S4 & S5). This supports previous research, which showed that a low pH and a high initial organic loading act as inhibitors of methanogenesis [8,11,42,43]. The highest acidification level was achieved at pH 6.0, with VFAs representing 93.0 % (± 2.1 %) of the soluble COD (17.6 (± 0.67) g/L) (Table 3). This is an

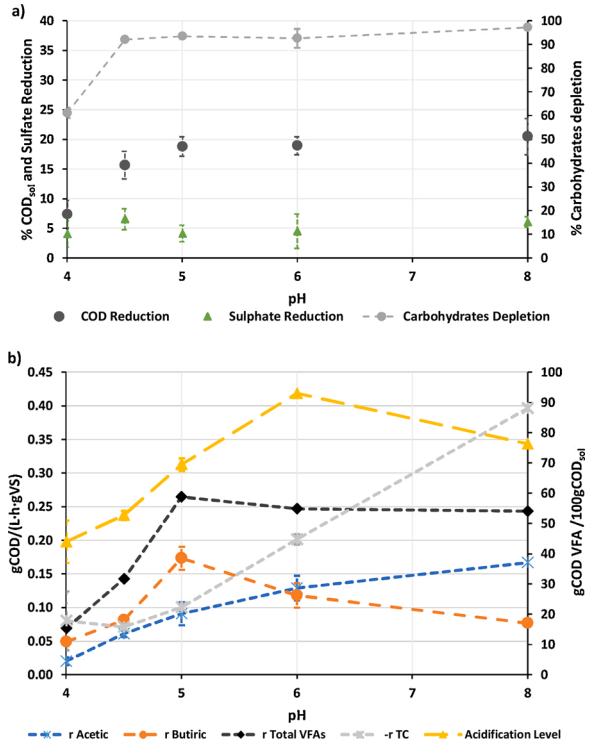

Fig. 4. Main parameters analysed during the acidogenic fermentation of hydrolysate from brewery spent grain in batch reactors at different pHs: a) Total carbohydrates depleted, reduction of soluble COD and reduction of sulphate; and b) production rate of the butyric acid, acetic acid and total VFA production, total carbohydrates consumption rate and acidification level.

improvement of the acidification yield when compared to systems using real wastewater as feedstock under similar optimised operational conditions (Table 3).

The VFA profile obtained here was similar to those observed in other studies. In the work of Domingos et al. [44] using lactose as source of carbohydrate at the same pH and temperature, butyric acid was the major VFA produced [44], although a lower TC consumption and VFAs production rate (0.14 and 0.07 g COD/(L·day), respectively) were observed compared to this study (Fig. 4b). Moreover, the sludge used by Domingos et al. [44] was an acclimated anaerobic acidogenic consortium from a continuous reactor for VFAs production. As in the present work no methane inhibitors or pretreatment of the granular sludge was applied, the observed high TC consumption rate (Fig. 4b) can be attributed to acidogenesis and acetogenesis of the monosaccharides (e.g. glucose, arabinose and xylose; Table 1) and protein released from the BSG during the acid hydrolysis step. Similarly, suppression of the methanogenesis when operating under similar conditions using sugarcane molasses as feedstock resulted in butyric acid as the major end product, but with a lower acidification level [45].

Interestingly, hydrolysates produced by alkaline fermentation (pH 8 in this study) give similar acidogenic fermentation end products (Fig. 5). Xie et al. [43] operated a (high-strength wastewater fed) system by correcting the pH up to 9.0 every 12 h obtaining butyric acid as major compound, which can be a result of pH fluctuating between 9.0 and 5.7. Nevertheless, when the system was run at constant pH 8.0 and above, butyric acid production was lower due to the increased pH used increased. Likewise, Shen et al. [46] used glycerol as feedstock at an initial pH of 10.0, resulting mostly in the production of propionic acid with an acidification level of 89.9 %. This results was similar to that obtained in our work, where operating the system at pH 8.0 decreased the butyric acid and increased the propionic acid concentration (Fig. 5). In contrast, Atasoy et al. [47] reported the production of solely butyric acid as the major compound at all alkaline pH values investigated, with cheese whey as feedstock and using granular sludge from an UASB reactor treating municipal wastewater.

4.1.1. Total carbohydrates and monosaccharides consumption during acidogenic fermentation of BSG

Under uncontrolled pH, when the pH achieved the final value of 4.08 (± 0.05), only glucose was completely consumed (Table 3). On the other hand, more than 90 % of the TC was consumed in all experiments with a controlled pH (Fig. 4a). The lowest pH achieved presumably led to process termination without arabinose and xylose consumption. Casey et al. [48] similarly investigated the effect of pH and acetic acid on the

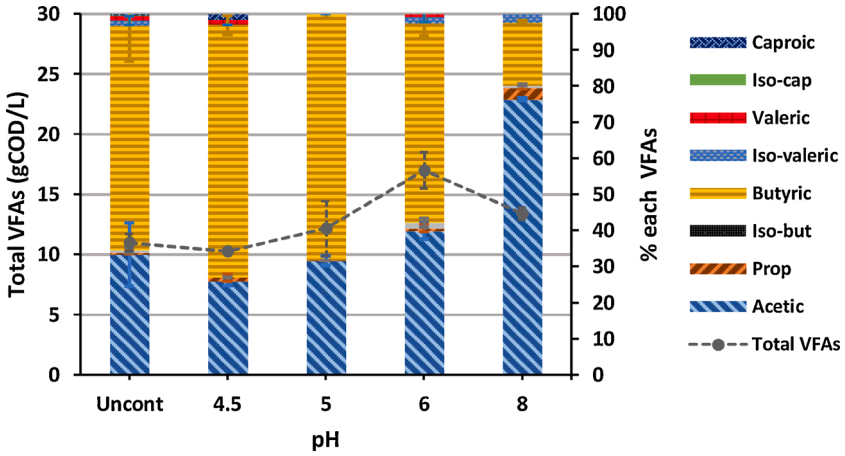

Fig. 5. Volatile fatty acids (VFAs) produced during the acidogenic fermentation of brewery spent grain hydrolysate at different pH conditions at 37 °C. The total VFA production and the percentage of each acid in the VFA mixture after the exponential phase are shown.

co-fermentation of glucose and xylose, where low pH and increasing acetic acid concentrations led to an inhibition of the xylose consumption, while glucose consumption was unaffected. These results are in agreement with those obtained in the present study, where at the lowest pH only glucose was consumed (see section 3.2.2). The accumulation of free acids likely led to product inhibition (e.g. acetate inhibition), which hindered the fermentation of xylose and arabinose.

The complete depletion of glucose under all pH values and the absent (at uncontrolled pH) (Table 2) or delayed (at pH 4.5 or higher) consumption of xylose and arabinose (data not shown) can also be explained by a mechanism of carbon catabolite repression [49] or the need for an extra ATP that microorganisms require for the uptake of xylose and arabinose [50,51]. Indeed, microorganisms start to consume pentoses (e.g. xylose and arabinose) only when the hexoses (e.g. glucose) are depleted [52].

4.1.2. Conversion of carbon compounds

The acidification level (i.e. the ratio between the total VFAs and COD_{sol}) increased from 44.0 (± 7.0) % at uncontrolled pH to 93.0 (± 2.1) % at pH 6.0, whereas it decreased to 76.4 (± 0.6) % at pH 8.0 (Fig. 4b). The highest VFA concentration was also obtained at pH 6.0. This increase in the acidification level from the lowest pH up to pH 6.0 can be explained by a minor concentration of the undissociated form of VFAs, which has a strong inhibitory effect on the microorganisms at low pH [53]. The relation of the acids between the dissociated and undissociated form is given by the pH and the dissociation constant [54], which is 4.76 and 4.82 for acetic and butyric acid, respectively [55]. In this case, the VFA production would result in a self-inhibitory effect at higher free acid concentrations, which can be obtained by either increasing the total VFA concentration or decreasing the pH [53]. Therefore, to achieve the same inhibitory effect a lower VFA concentration would be required when decreasing the pH. This would explain the lower acidification level at pH 4.5 and 5.0 (Fig. 4 ***b***), the incomplete sugar consumption and the lactic acid accumulation (Supplementary material Fig. S2 & S3). To overcome an excessive concentration of undissociated acids, the pH can be increased inducing a higher demand for alkali. Another strategy could be the use of continuous-flow systems, which withdraw the VFAs from the reactor mixed liquor [56,57]. For instance, bioreactor configurations with an immersed membrane system [15], or with a recirculation line coupled with a membrane contactor [15,57] can allow maintenance of a low free acid concentration and can thus work at a lower feed pH or a higher TC load, meanwhile keeping the same acidification level.

The trend for the VFA production was constant with a final stable concentration until the end of the trial, indicating a low extent of the methanogenesis or even its inhibition at the last stage of the batch experiments when a decrease in VFAs was not observed after reaching the maximum VFA concentration (Supplementary material Fig. S1, S4 & S5). This could explain also the higher VFA production rate at pH 5.0 compared to the uncontrolled pH or at pH 6.0 (Fig. 4b). The highest VFA production rate was at pH 5.0, where butyric acid peaked; meanwhile the acetic acid production rate increased along with the operating pH (Fig. 4b). This shows that a lower pH is more favourable for the production of butyric acid.

Under all the acidic pH conditions investigated, butyric acid was the most produced VFA in terms of COD, followed by acetic acid. pH 8.0 resulted in a lower butyric acid production (Fig. 5). The higher build-up of butyric acid and the absence of ethanol production has been related with the production of hydrogen gas [58]. Even though hydrogen was not measured in this study, a high hydrogen pressure likely led to the high butyric acid concentration [58,59].

Regarding the production of lactic acid, a similar trend was reported by Domingos et al. [44] using lactose as the feedstock, which was converted into lactic acid after five days without VFA production. In the present work, at low pH (4.5 and 5.0), lactic acid was produced before the VFAs (Supplementary material Fig. S1 & S3), while lactic acid and

Table 3
Average physical-chemical composition of different feedstocks for batch acidogenic fermentation aimed at volatile fatty acid production. The percentage of the main acids (i.e. Acetic, Propionic and Butyric) as well as the acidification level in each study is provided.

Feedstock	COD_{sol} (g/L)	TC (g COD/L)	pH	Temperature (°C)	VFAs (gCOD/L)	(Ac:Prop:But) (% of VFAs)	Acidification Level	Reference
Cassava alcohol wastewater	32.1	10.1	9.0 – 5.7	35	24.3	(37:20:40)	71.3	(43)
Cheese whey	–	16.5	6.0	37	19.2	(15:17:55)	N.G.	(44)
Sugarcanne molasses	8.1	N.G.	6.0	37	3.0	(53:09:26)	42.1	(45)
Glycerol	10.0	N.G.	10 (initial)	35	6.7	(08:91:00)	89.9	(46)
Cheese whey	3.0	N.G.	10.0 → 7.0	35.0	1.1	(07:34:49)	54.7	(47)
BSG hydrolysate	23.1	16.9	6.0	37.0	16.9	(40:01:55)	93.0	This study

*N.G.: data not given.

VFA production occurred simultaneously at higher pH (6.0 and 8.0), although a lower lactic acid concentration was observed (Supplementary material Fig. S3).

The spectrum of fermentation products obtained can be explained by the use of the anaerobic granular sludge as inoculum. These granules harbour a highly diverse microbial community that results in a complex different combinations of pathways [49,60]. These different pathways are influenced by the operational conditions (e.g. different pH and higher organic loading concentration), with pyruvate as the intermediate compound between the monosaccharides and the different spectrum of products obtained during the fermentation process, e.g. lactic acid, acetic acid, butyric acid and propionic acid [47,48,50]. The adaptation period of the microorganisms resulted in a delay for the VFA production, which was longer as lowering the pH (Supplementary material Fig. S2, S3, S4 & S5). However, the TC concentration was partially consumed before the lactic acid or VFA production. This could have resulted in the accumulation of pyruvate, which can increase the intracellular NADH. Thus, in combination with a possible decreased intracellular pH, it would have promoted the activation of lactate dehydrogenase resulting in the conversion of pyruvate into lactic acid [61,62]. In addition, the longer lag phase for VFA production resulted in a higher accumulation of lactic acid at pH 4.5 and 5.0 (Supplementary material Fig. S1). Upon prolonged batch incubation, however, the microorganisms eventually consume the lactic acid for VFA production [63], as also observed in this study (Supplementary material Fig. S1 & S3)

4.1.3. Conversion of nitrogen and sulfur compounds

The consumption of the proteins during the acidogenic fermentation led to an increment of the ammonium concentration in the fermented liquor. Although the ammonium concentration has a low influence on the anaerobic digestion process, the strongest inhibition effect is due to the free ammonium (FA), which is a function of the total ammonium concentration and the pH through the Anthonisen equation [64]. FA has been reported to hinder the different steps of the anaerobic digestion when its concentration is increased, the methanogenesis being the most sensitive step. An FA concentration above 30 mg/L starts to inhibit methanogenesis, being almost completely inhibited at a concentration close to 60 mg/L [8]. In contrast, the acidogenesis is negatively affected only when above 300 mg/L of FA [9]. During this work, when operating the system under acidic conditions the FA was much lower (between 0.0 and 0.7 mg/L at pH 4.5 and 6.0, and 65.2 mg/L at pH 8.0), which was below the inhibitory threshold for VFA production.

Sulfate removal was only observed to a limited extent (i.e. 5 %) and was similar at all pH values studied (Fig. 4a). This probably occurred through a partial sulfate reduction towards sulfide production. However, as the hydrogen sulfide concentration was below the detection limit (data not shown), this suggests that the sulfate reducing bacteria (SRB) were unlikely to be active or were outcompeted by the acidogens populations. Sulfate reducers can use simple organic electron donors such as ethanol and lactate [65]. However, during the degradation of carbohydrates to VFAs and lactic acid by acidogens, SRB were likely not favoured kinetically to use these simple compounds at the low pH in the 72 h of the batch experiment.

4.2. Efficiency of the hydrolytic pretreatment

The thermal diluted acid hydrolysis at 121 °C for 20 min allowed the release of glucose, xylose and arabinose from BSG (Table 1). The use of 1.5 % and 2.0 % H_2SO_4 (v/v) with 7 % of TS (w/w) resulted in the most efficient combination in terms of TC per g of TS, 0.44 g and 0.45 TC/g BSG, respectively (Supplementary material Table S1). As their efficiencies were similar, the lower sulfuric acid concentration was used to progress forward, with a severity factor corresponding to 1.09 under these conditions. In the case of 10 % TS, the presence of cellobiose indicated (Table 1) the occurrence of an incomplete hydrolysis, while conversely in the case of 7 % no cellobiose was detected.

The extraction efficiency was similar for both TC and COD_{sol} and increased linearly when the sulfuric acid concentration was increased from 0.0 to 1.0 % (v/v) (Fig. 2). After this point, the efficiency in terms of carbohydrates release and sulfuric acid used decreased, resulting in a lower improvement of the carbohydrates extracted from 1 to 3 %. The highest recovery of monosaccharides in the present work was achieved at 1.5 % of sulfuric acid and decreased using 3 % sulfuric acid at 7 % BSG (w/w). For the combinations with 10 % of BSG (w/w), glucose and xylose increased for all the sulfuric acid increments used, meanwhile the arabinose concentration decrease in the case of 3 % sulfuric acid (Table 1). Even if this combination resulted in the highest TC concentration, the loss of arabinose and the incomplete hydrolysis of the BSG, as indicated by the presence of cellobiose, decreased the efficiency of the TC extracted per amount of BSG used (Supplementary material Table S1). As a consequence, not only should the severity factor be used to calculate the efficiency of the hydrolysis, but also the TS concentration of the lignocellulosic biomass.

In the last years, studies focusing on the use of BSG as feedstock have proposed different strategies for the storage and initial treatment of the BSG. Few researchers used the BSG as received [30,66] and others dried or milled the BSG into different sizes [14,17,67,68]. These physical changes are not considered as a pretreatment. However, Niemi et al. [21] determined their effect during the hydrolysis. In the case of wet milled samples, a more homogeneous slurry was produced. Dried samples have been reported to form aggregations and more quickly sediment after submersion in water, being this related with the hornification of the BSG [21].

The hydrolysis of BSG for recovery of carbohydrates has been studied using a single or two step-process (Table 4). Carvalheiro et al. [22] studied the hydrolysis of BSG using a single step, a thermal diluted acid hydrolysis, achieving a high xylose and arabinose recovery at a low severity factor and maximum glucose extraction under a higher severity factor (1.94). These observations are slightly different than those of the present work as a higher glucose extraction was obtained (Table 4) using a lower severity factor (1.09). This could be explained by how Carvalheiro et al. [22] stored the BSG: washing and drying remove some of the remaining sugars from the brewery process and hornify some of the carbohydrates. Therefore, the use of the raw BSG not only reduces the energy consumption, but also increases the TC recovery.

In studies using a two step hydrolytic pretreatment, some have mainly focused on glucose recovery, using the first step for the enrichment of cellulose in the solid fraction for the next extraction, without quantifying the sugars extracted (mainly xylose and arabinose). Ravindram et al. [17] carried out different pretreatments followed by enzymatic hydrolysis, with the microwave assisted alkali hydrolysis being the most effective, followed by diluted sulfuric acid. However, the overall efficiency was much lower as only the TC from the second extraction were quantified (Table 4). Similarly, Patel et al. [14] followed a method for cellulose concentration using an organosolvent pretreatment achieving a TC concentration of 71.6 g/L, mainly made up of glucose and xylose.

In the pretreatments where the overall carbohydrates extracted are considered, the obtained efficiency values are seen to be higher. Rojas-Chamorro et al. [67] and Plaza et al. [68] used a two step process, with the first step being a thermal diluted acid hydrolysis (similar to the first step applied in this study). Both studies used a lower sulfuric concentration and a higher concentration of BSG than those used in this study, resulting in a lower efficiency recovery and a higher TC concentration compared to this study. However, the final overall efficiency of the two step hydrolysis process recovered almost all the initial carbohydrates from the BSG [67,68].

Besides carbohydrates, the use of sulfuric acid as a pretreatment agent allowed the release of high-value compounds such as lignin, proteins and soluble fibres from BSG (Fig. 2). Rommi et al. [32] compared different pretreatment combinations (i.e. raw BSG, steam explosion, alkaline and acid extraction) for lignin and protein recovery,

Table 4
Different studies for the hydrolysis of the brewery spent grain using different process in one or two combined steps for the recovery of monosaccharides.

BSG Characterisation			1st extraction						2nd extraction		Carbohydrates Recovery	Efficiency recovery	Reference
TC (%)	Proteins (%)	Lignin (%)		TC g/L	Glu g/L	Xyl g/L	Gal g/L	Ara g/L		TC g/L	g TC/ g BSG	g TC recovered/ 100 g TC raw BSG	
55.1	12.54	13.7	Organosolv pretreatment (10 % BSG w/w; 60 % ethanol; 1 % H_2SO_4 wt dry BSG; 175 °C, 60 min)	NG	NG	NG	NG	NG	Enzymatic hydrolysis (10 % TS; 50 °C; 27 h)	71.6	NG	NG	Patel et al., 2018 (14)
43.5	23.3	19.4	Wet milling (95 % particle size < 40 μm) + Enzymatic extraction (3 % TS; 50 °C; 5 h)	6.7	2.8	2.1	0.28	1.12	None	–	0.15	0.35	Niemi et al., 2012 (21)
47.8	23.5	22.8	TDAH (8 % BSG w/w; 3 % H_2SO_4 V/V; 130 °C; 15 min)	43.5	3.99	26.74	–	12.77	None	–	NG	NG	Carvalheiro et al., 2004 (22)
46.2	–	–	Microwave assisted alkali (1 % BSG w/v, 0.5 % NaOH (w/v); 400 W; 60 s)	NG	NG	NG	NG	NG	Enzymatic hydrolysis (10 % TS; 50 °C; 120 h)	18.6	0.23	0.50	Ravindram et al., 2018 (33)
43.5	22.6	4.1[#]	Steam explosion	–	5.69	8.23	–	3.84	Enzymatic hydrolysis (20 % TS)	NG	0.26	0.62	Kemppainin et al., 2016 (66)
45.6	23.1	12.5	TDAH (11.11 % BSG; 1 % H_2SO_4 wt TS; 130 °C; 26 min)	43.4	9.44	21.68	2.31	11.08	Enzymatic hydrolysis (5 % TS ; 50 °C; 24 h)	14.4 (72.04*)	0.48	0.94	Rojas-Chamorro et al., 2020 (67)
46.0	NG	NG	TDAH (15 % BSG w/w; 1 % H_2SO_4 wt dry BSG; 121 °C; 30 min)	47.0	20.0	18.4		8.6	Enzymatic hydrolysis (10 % TS; 50 °C; 48 h)	32.9	0.43	0.95	Plaza et al., 2017 (68)
ND	ND	ND	TDAH (7 % BSG w/w; 1.5 % H2SO4 v/v; 121 °C; 20 min)	32.0	12.8	12.05	–	11.8	None	–	0.44	ND	This study

*TC: Total carbohydrates.
*ND: not determined.
*NG: data not given.
[#] :acid insoluble lignin.

based on a previous work focused on carbohydrates extraction from BSG [67]. They observed that the solubilisation of different organic compounds like lignin, proteins and polysaccharides strongly depended on the pretreatment combination. For example, steam explosion and hydrolysis increased lignin solubilisation, while protease was required to improve protein extraction [32]. Similarly, the diluted acid hydrolysis and neutralization used in the present study could be developed to recover some of the proteins extracted using the lowest solubility point of proteins (i.e. pH 3–5) [69,70]. This was, however, out of the scope of the present study, although the recovery of a broader range of end compounds beyond VFAs would be beneficial for the overall process.

4.3. Future development of the process

BSG is a renewable feedstock with great potential for future biorefinery platforms as it retains a considerable pool of high value compounds, which can be recovered and reused in industrial applications, e.g. cosmetic or food production. However, the whole treatment chain including a pretreatment step and the subsequent acidogenic fermentation needs to be improved in future applications prior to a potential scale up of the technology.

In the case of the hydrolysis, application of a single thermal diluted acid hydrolysis step showed similar recovery efficiencies for the total amount of carbohydrates compared to two step hydrolysis processes used elsewhere (Table 4), thus decreasing the complexity of the process. In addition, the fermented hydrolytic liquid fraction achieved a high acidification level (pH 6.0, Fig. 4b), implying that any of the possible inhibiting compounds co-released during the hydrolysis step did not affect the carbohydrates consumption and the VFA production. On the other hand, the production of a more concentrated fermented liquor was limited by the initial TC concentration. However, increasing the BSG concentration during the hydrolysis resulted in a lower efficiency (Table 1, Supplementary material Table S1), thus in a loss of carbohydrates.

Different hydrolytic techniques can also be investigated, for instance combining a chemical diluted acid with a biological enzymatic hydrolysis to obtain a higher TC concentration as feedstock [67,68]. The efficiency, complexity and cost of the hydrolysis step need to be evaluated, as more steps would be required, however this could be compensated by the simultaneous recovery of proteins and lignin during the pretreatment step [29,32]. On the other hand, decreasing the amount of sulfuric acid used would make the pretreatment solution less corrosive and less hazardous, thus avoiding the use of specific non-metallic constructions or expensive alloys [71] and high alkali inputs for neutralization. Similar processes for the hydrolysis of other lignocellulosic materials (e.g. forestry waste, sugarcane bagasse or corn cob) have been successfully

scaled up for bioethanol production at pilot and industrial scales [23,26,72]. The development of a similar approach for BSG with the subsequent recovery of valuable compounds in a cascade process would make the entire process more sustainable, mitigating the pretreatment costs and valorising BSG that is currently considered as a waste.

The obtained carbohydrate-rich BSG hydrolysate can be fermented at low retention times using anaerobic granular sludge to produce VFAs under acidogenic conditions, as demonstrated in this study (Fig. 5). This can be further improved using a continuous-flow high-rate unit, such as an expanded granular sludge bed (EGSB) reactor, keeping a high VFA concentration that even contributes to inhibit methanogenesis. Moreover, this configuration allows the uncoupling of the liquid and solid retention times, thus requiring smaller bioreactor volumes [73]. These systems have been previously used for hydrogen and VFA production both with [7] and without [74] an inoculum pretreatment. Other configurations, such as a continuously stirred tank reactor treating the solid lignocellulosic material without pretreatment, would require a longer residence time and give a lower conversion of the feedstock into VFAs [75]. Another configuration is the leach-bed reactor, which requires lower maintenance and consumption of chemicals, but demands a high residence time [76]. A deeper understanding of the process in terms of stability of the VFA production, evolution of the microbial communities and the granular sludge integrity in the EGSB reactor over long-term operation is required for the further development of the technology for future scaling up of the process.

5. Conclusions

The feasibility of VFA production from BSG was demonstrated in this study, reinforcing the change in the point of view of BSG from a solid waste into a valuable resource. First, a mixed liquor rich in carbohydrates with 0.44 g of carbohydrates per gram of total solid of BSG was obtained using a single step of a thermal diluted sulfuric acid hydrolysis (1.5 % H_2SO_4 v/v). Then, the BSG hydrolysate was used for VFA production during carbohydrate fermentation by an anaerobic granular sludge. At the different pH values tested, the contribution of VFAs to the soluble COD at pH 6.0 was approximately 93.0 (± 2.1) %. Therefore BSG has a great potential as a raw feedstock for VFA production. Further research is required for the development of the process at a larger scale and in a continuous-flow mode.

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CRediT authorship contribution statement

Juan Castilla-Archilla: Conceptualization, Methodology, Investigation, Writing - original draft. **Stefano Papirio:** Methodology, Validation, Supervision, Writing - review & editing. **Piet N.L. Lens:** Funding acquisition, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.procbio.2020.10.011.

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