Spent coffee grounds anaerobic digestion: Investigating substrate to inoculum ratio and dilute acid thermal pretreatment

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ABSTRACT

Spent coffee grounds have the potential of being used in further bioprocesses to produce materials and fuels. In Norway, the relative abundance and ease of collection of this waste substrate make it a candidate for investigation. For this study, the substrate-to-inoculum ratio as well as a combined dilute acid-thermal pretreatment were assessed by a series of biochemical methane potential assays using spent coffee grounds as a substrate. Reactors with substrate-to-inoculum ratio 2 demonstrated a relatively low hydrolysis rate constant (k_h) and comparatively high volatile fatty acids/alkalinity concentrations rendering them inapt to produce bio-CH_4. Pretreatment was conducted over varying contact times (15–45 min), dilute acid concentrations (1.5–2.5 %, v/v), and liquid-to-solid ratios (10–20 %, v/w) and evaluated using response surface methodology. To determine bio-CH_4 yield, pretreatment time and the interaction between acid concentration and liquid-to-solid ratio are considered significant variables, suggesting a shared importance. Chemical oxygen demand$_{removal}$ is primarily contingent upon changes in liquid-to-solid ratio. Finally, Fourier-transform infrared spectroscopy of the discarded solid phase showed that the major functional groups are still widely present in the coffee grounds even after pretreatment was applied. A better understanding of the biodegradability profile of spent coffee grounds as a function of substrate-to-inoculum ratio is achieved.

1. Introduction

Green energy production and organic waste processing technologies are intermediate milestones towards sustainable waste mitigating solutions. Anaerobic digestion (AD) is one such waste management technology. It can reliably and continuously process large amounts of organic waste while simultaneously producing bio-CH_4 and reducing waste adverse effects [17]. Coffee is an internationally traded commodity with global production reaching 9.92 million tons as of 2019 [4]. Coffee production is dominated by Brazil, Vietnam, and Colombia each producing an average of 3.18, 1.68, 0.84 million tons of raw coffee yearly, respectively [26]. On the other hand, consumption is dominated mainly by the Nordics. Throughout the period 2009 to 2019, Norway consumed a stable average of 45.36 kilotons coffee per year [13]. This coffee usually gets discarded with organic waste. However, its collection is relatively easy and so its viability as a raw material for industrial usage needs to be assessed.

The substrate-to-inoculum (SIR) mass ratio (VS basis) (g VS$_{substrate}$ g^{-1} VS$_{inoculum}$) plays a role in the degree of methanization of a substrate as well as sustained anaerobic digestor health. A high SIR results in substrate overloading and accumulation of volatile fatty acids (VFAs), oppositely a low SIR would result in washout, and increased reactor volumes [30]. Microbial consumption and production rates vary based on the selected SIR. Underloading or overloading (a too low or too high SIR) may result in unsustainable growth or a surge of VFA intermediates inhibiting bio-CH_4 yields [10]. To illustrate this, Sri Bala Kameswari et al. [30] co-digested fleshings and tannery wastewater and showed that decreasing the SIR from 1 to 0.43 nearly doubled the working volume of the reactor (from 18.7 to 34.8 m^3) while only marginally increasing the bio-CH_4 yield by 2.23 % (from 268 to 274 mL g^{-1} VS). Analogously, Li et al. [20] also demonstrated how the SIR plays a crucial role when digesting food waste. They showed that decreasing SIR allows

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for higher decrease in VS content as well as improved process kinetics and microbial activity, especially methanogenic archaea.

Studies have showed that the pretreatment of biomass has the ability of increasing bio-CH_4 yields by making the substrate more available for microbial organisms [4]. Using acid pretreatment, a near complete saccharification of hemicellulose can be induced if the conditions are optimized, though the crystalline cellulose fraction of lignocellulose is only partially solubilized [35]. Hydrothermal pretreatment, especially when used in conjunction with dilute acid, allows H_2O to pierce into the lignocellulosic structure and enables the hydrolysis of cellulose as well as the solubilization of parts of lignin and hemicellulose. Hydrothermal pretreatment is equivalent to "cooking" the biomass prior to AD. This enhances the digestion and biodegradation of the biomass feedstock. Studies on waste cassava pulp have shown that COD solubilization increases by 13 ± 2 g sCOD g^{-1} tCOD and hence bio-CH_4 yield increases by 244 ± 20 to 310 ± 0 mL bio-CH_4 g^{-1} VS when treated with 2 % HCl [21]. In the similar way, Rocha et al. [28] hydrolyzed de-oiled SCGs using 0.4 M H_2SO_4 and showed that any further increase in H_2SO_4 concentration led to a decrease in hydrolyzed sugars and an increase in organic acids. Hence, optimizing the acid concetration during pretreatment is a necessary step in maximizing fermentation production and subsequent bio-CH_4 yields.

Even though the SIR is known to be a critical factor in the AD of solid substrates, there is limited knowledge on how that affects the biodegradability and digestion of SCGs in particular. The large volumes of SCGs produced per capita in Norway encourages the research and exploitation of this waste biomass. The overall aim of this study to elucidate on two research problems. The first novelty is investigating how changes in the SIR mass ratio affect biomethanation during the AD of SCGs. At the same time, a parallel novel study assesses 3 distinct factors (pretreatment contact time, dilute HCl concentration, and the liquid–solid ratio (LS) ratio) on the digestibility of SCGs hydrolysate using a combined dilute acid-thermal pretreatment (DATP) step whilst holding the SIR constant. The simultaneous assessment of SIR and pretreatment conditions allow us to get a better understanding of the biodegradability profiles as well as possible biomethanation yields for SCGs. Post-experimental analyses were conducted using response surface methodology (RSM) using a Box-Behnken design (BBD). Finally, FTIR spectroscopy was used to determine functional group changes in SCG solids before and after DATP.

2. Materials and methods

2.1. Substrate and inoculum

SCGs were collected from an on-campus coffee shop at University of Stavanger, Norway. The beans are of the species *Coffea arabica*. The SCGs were not further milled as to their initial powdered nature. They were dried in an oven at 103 ºC for 24 h to expel moisture and stored at 4 ºC until further use. Table 1 shows the initial elemental and compositional analysis. For SIR optimization, the SCGs were used as received. When applying pretreatment, the solid residue was separated and only the liquid hydrolysate was used for AD. The solid residue was characterized by FTIR.

Two sets of experiments were run, and so different inocula were used. The inocula were standard anaerobic sludge (AS) and were obtained from the mesophilic digestor at the central wastewater treatment plant of North-Jaeren (SNJ), Randaberg, Norway. Characterization was conducted whilst the inoculum was still fresh and warm. Inocula characterization is given in Section 3.1.

2.2. Choosing the pretreatment

Initially, three pretreatments were considered. The one giving the highest dissolved COD (chemical oxygen demand) in the hydrolysate would qualify for further investigation and optimization. COD as a

Table 1
SCGs initial characterization.

Parameter	Spent Coffee Grounds
TS [a] - %	92.1 ± 0.1
VS [a] - %	90.4 ± 0.05
VS$_{TS}$ [a] - %	98.2 ± 0.03
Moisture - %	58.4 ± 1.1
COD [a] – g COD/g SCGs	1.49 ± 0.07
COD [a] – g COD/g VS	1.65 ± 0.07
C — H — N — S — O [a] - %	52.35–7.04–2.27–0.09–38.25
Empirical Formula [a]	$C_{1553}H_{2488}O_{852}N_{58}S$
C:N Ratio [a] – mol C/mol N	26.9:1
Glucose [a] - %	17.8 ± 3.1
Xylose [a] - %	1.3 ± 0.4
Acid Soluble Lignin [a] – ASL – %	4.4 ± 0.6
Acid Insoluble Lignin [a] – AIL – %	21.1 ± 1.8
Proteins [a] - %	24.0 ± 2.7
Extractives [a] - %	28.3 ± 3.2

[a] – Dry basis. Measured after drying at 103 °C for 24 h.

marker was chosen due to its ease of measurement and its direct correlation to hydrolyzed chemical products i.e., '*pretreatment efficiency*' as well as the theoretical bio-CH_4 potential (397 mL CH_4/g COD at 37 °C, 1 atm).

First, hydrothermal pretreatment was conducted in an autoclave (Panasonic MLS-3751) using superheated water at 135ºC and 2.4 bar for 3 h at a H_2O/SCGs ratio of 10 (v/w). Second, a microwave assisted base pretreatment was employed. SCGs mixed with NaOH at 1 % (w/v) at 1 atm at an LS ratio of 10 (v/w) and were admitted into a standard microwave (LOGIK SJW20) at 300 W and 2450 Hz for 210 s. Finally, the third pretreatment was a combined dilute acid and thermal pretreatment (DATP). The dilute acid thermal pretreatment was done by autoclaving at 135ºC and 2.4 bar for 3 h with an LS ratio of 10 (v/w) and an HCl concentration of 0.5 % (v/v). A comparison (control) test was also conducted. The comparison was done using distilled water at room temperature with stirring for 6 h at 100 rpm at an LS ratio of 10 (v/w). The pretreatment mixtures were then centrifuged, and the COD of the supernatant was measured. DATP was chosen. The results from the pretreatment selection stage are given in Section 3.1.
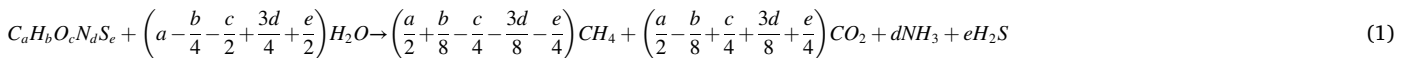
2.3. Biochemical methane potential assays

The BMP (biochemical methane potential) assay is a commonly used method for determining both the degree of biodegradability as well as bio-CH_4 production for the substrate at hand. Accurate and precise BMP tests are crucial for designing, managing, and assessing the technical and financial feasibility of AD plants [2]. All assays had residual CH_4 yields from their blanks removed according to the inoculum respectively used for that BMP.

The SIRs tested in this study were 2, 1, 0.5, 0.33, 0.25, 0.2 and 0.16 (g VS$_{SCGs}$ g^{-1} VS$_{AS}$). BMP assays were done using the AMPTS II (Automatic Methane Potential Test System 2) apparatus purchased from BPC Instruments AB (Lund, Sweden). Blanks (inoculum only) were used to standardize all other BMPs in order to re-adjust against residual biomethanation. Positive controls tests (glucose only) were conducted to assess the methanation potential of the inoculum. All other reactors were loaded in a specific ratio of SCGs and AS (g VS basis) according to its respective SIR. All reactors were purged with N_2 gas for at least 5 min to establish anaerobic environments. Prior to purging, pH was adjusted between 7 and 7.1 using 3 M HCl. Thereafter the glass bottled reactors were placed in a water bath fixed at 37 ± 1 °C. The reactors were mixed at 100 rpm for 10 min and rested for 1 min. The gas outlets were directly connected to the gas absorption units utilizing 80 mL of 3 M NaOH with thymolphthalein indicator dissolved in ethanol. The absorption units remove acid gasses, mainly CO_2 but H_2S too. The cumulative bio-CH_4 yield was obtained after removing the residual CH_4 generation of the blank tests. Thereafter the specific yield at each SIR was obtained by

dividing by the initial quantity of loaded SCGs (mL CH$_4$, STP g^{-1} VS$_{SCGs}$). The technical digestion times (t$_{80}$ and t$_{90}$) are arithmetically calculated as the times needed for the bio-CH$_4$ yield curves to reach 80 and 90 % production, respectively.

The anaerobic biodegradability and conversion of a substrate into CH$_4$ and CO$_2$ (biogas) may be stoichiometrically estimated from the elemental composition shown in Eq. (1). However, the biodegradability of substrate is usually incomplete. Complete biodegradability effectively implies complete conversion of COD to bio-CH$_4$. Eq. (2) shows the complete biodegradability (theoretical) of a substrate (BMP$_{Th}$) based on a substrate's elemental composition: where a–e are elemental co-efficients empirically determined from the molecular composition [27]. The actual biodegradability (BD) in Eq. (3) is simply the ratio of experimentally observed bio-CH$_4$ potential (BMP$_{Exp}$) to its theoretical estimate.

the samples were filtered through 1.5-µm GF/C microfiber glass filters (Whatman, VWR). The solids were discarded, and the liquid portion was taken for digestion. The liquid portions pH was then adjusted between 7 and 7.1, and 4 g VS$_{AS}$ was added and finally purged with N$_2$ for 5 min. The reactors were placed in a water bath fixed at 37 ± 1 °C. Stirring was set at 80 rpm for 10 min followed by no stirring for 20 min. The BMP assay ran for 22 days and were stopped when the yield curves plateaued.

2.4. Analytical techniques

Initial and final characterizations were performed to examine and monitor the results of the BMP assays. pH was measured for each pre-treatment as well as before and after digestion using a WTW Multi 340i probe. The VFA and ALK were determined by an automatic titrator (TitroLine® 5000) using 0.1 M HCl as the titrant. VFA (as g HAc m^{-3})

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \tag{1}$$

$$BMP_{Th} = \frac{\left[\left(\frac{a}{2}\right) + \left(\frac{b}{8}\right) - \left(\frac{c}{4}\right) - \left(\frac{3d}{8}\right) - \left(\frac{e}{4}\right)\right] \bullet 22,400}{12a + b + 16c + 14d + 32e} \tag{2}$$

$$BD(\%) = \frac{BMP_{Exp}}{BMP_{Th}} \bullet 100 \tag{3}$$

As for the DATP study, 4 g VS$_{SCGs}$ were weighed into a screw capped reagent bottle and dissolved in the appropriate HCl concentration and LS ratio as per the parameters provided in Table 2. SIR 1 (4 g VS$_{SCGs\ filtrate}$ 4 g^{-1} VS$_{AS}$) was chosen as it maintained a relatively high bio-CH$_4$ yield and was able to process higher volume of SCGs without compromising healthy reactor conditions. The samples were heated in an autoclave (Panasonic MLS-3751) for the time specified by the design. All test runs ran at 120ºC and 1.15 bar. A total of 15 data points were evaluated. A control trail (SCGs, H$_2$O only, 120ºC, and 1.15 bar, 30 mins, LS 15 v/w) corresponding to the middle levels of the BBD, was conducted for comparison. Blank tests were also used to determine the residual bio-CH$_4$ produced and COD conversion of the inoculum only. After pre-treatment, the sample bottles were cooled to room temperature and pH was adjusted to between 7 and 8 using 3 M NaOH. After neutralization,

and ALK (as g CaCO$_3$ m^{-3}) in the samples and the inoculum were determined according to the method proposed by Moosbrugger et al. [22]. This method utilizes a 5-point pH titration conjunction with the *TITRA5.exe* program. TS and VS of the SCGs and the anaerobic sludge were measured according the to the APHA 2450G standard method.

The SCGs COD was measured using a similar method to the one proposed by Andre et al. [1]. The COD of liquid samples (filtered hydrolysate and digestate) were diluted with a known dilution, before digestion for 2 h at 148 °C and spectrophotometrically measured after cooling down. All samples were analyzed using standard Merck COD cell test kits accompanied with a Spectroquant® Pharo 300 spectrophotometer. Response Y$_2$ of the BBD (COD$_{removal}$) was calculated as the difference between the initial total loading and the residual chemical oxygen demand at the end the BMP assay.

Compositional analysis was done according to the NREL published methods [29]. Simple sugar fractions (glucose and xylose) were determined by an HPLC (Waters 717 Plus, USA) equipped with an Aminex-HPX87P ion exclusion column (300 × 7.8 mm) and quantified by refractive index detection using 85 °C distilled water as the eluent. SCGs underwent elemental composition analysis using a FLASH 2000 CHNS elemental analyzer (ThermoFisher Scientific™). Elemental composition

Table 2
Design factors and levels with corresponding predicted and experimental response results.

Run	Factors (coded) – [uncoded]			Responses			
	A – time (min)	B – HCl conc (% v/v)	C – LS ratio (v/w)	CH$_4$ Yield (mL CH$_4$, STP/gVS) experimental	CH$_4$ Yield (mL CH$_4$, STP/gVS) predicted	COD$_{removal}$ (%) experimental	COD$_{removal}$ (%) predicted
1	(-1) – [15]	(-1) – [1.5]	(0) – [15]	95.8	98.7	65.1	65.6
2	(1) – [45]	(-1) – [1.5]	(0) – [15]	113.7	114.3	65.1	66.6
3	(-1) – [15]	(1) – [2.5]	(0) – [15]	98.5	97.9	67.7	66.2
4	(1) – [45]	(1) – [2.5]	(0) – [15]	119.7	116.7	66.0	65.5
5	(-1) – [15]	(0) – [2.0]	(-1) – [10]	93.1	90.6	71.6	72.6
6	(1) – [45]	(0) – [2.0]	(-1) – [10]	108.8	108.6	72.3	72.3
7	(-1) – [15]	(0) – [2.0]	(1) – [20]	95.1	95.2	64.0	64.0
8	(1) – [45]	(0) – [2.0]	(1) – [20]	109.2	111.6	65.8	64.7
9	(0) – [30]	(-1) – [1.5]	(-1) – [10]	95.8	95.4	72.4	70.9
10	(0) – [30]	(1) – [2.5]	(-1) – [10]	108.0	111.1	71.4	71.9
11	(0) – [30]	(-1) – [1.5]	(1) – [20]	117.2	114.1	64.6	64.1
12	(0) – [30]	(1) – [2.5]	(1) – [20]	99.6	100.0	60.9	62.4
13	(0) – [30]	(0) – [2.0]	(0) – [15]	102.0	104.3	67.2	66.9
14	(0) – [30]	(0) – [2.0]	(0) – [15]	105.7	104.3	68.2	66.9
15	(0) – [30]	(0) – [2.0]	(0) – [15]	105.3	104.3	65.6	66.9
Control	(0) – [30]	dH$_2$O only	(0) – [15]	30.1	–	44.1	–
Blank	–	Inoculum only	–	60.9	–	17.8	–

was calculated as mass % and the oxygen concentration is taken to be the difference between 100 % and %-CHNS.

Both untreated and pretreated SCGs were characterized by FTIR. Only the pretreated samples corresponding to BBD test run number 4 (45 mins – 2.5 % – LS 15) (highest bio-CH_4 yield) and test runs numbers 13/14/15 (30 mins – 2.0 % – LS 15) (mid-points) were analyzed. A Cary 630 FTIR (Agilent Technologies, USA) equipped with a diamond composite ATR (attenuated total reflectance) crystal was used. The spectra were measured in the range of 4000 to 650 cm^{-1} with a spectral resolution of 4 cm^{-1} and 32 scans per sample. Prior to FTIR-ATR analysis, the solids were dried at 65 °C overnight.

2.5. Modelling, data fitting and Parameter estimation

Two types of post-experimental kinetic modelling were applied. Further, one data fitting and parameter estimation model was performed. Programs used were either SigmaPlot V10.0 Windows by SyStat Software Inc or Excel Solver by Microsoft Corporation.

First, bio-CH_4 production curves were fit to a nonlinear modified Gompertz equation which describes the relationship between bio-CH_4 production and microbial growth and lag phases. The modified Gompertz equation used is given as Eq. (4). V(t) is given as the volume of bio-CH_4 (mL $CH_{4, STP}$ g^{-1} VS) with respect to time, V_{max} is given as the maximum bio-CH_4 volume achievable (mL $CH_{4, STP}$ g^{-1} VS), t is given as time (days), Vr_{max} is given as the maximum bio-CH_4 production rate achievable (mL $CH_{4, STP}$ g^{-1} VS day^{-1}), λ is given as the lag phase (days) and e is given as Euler's number (2.718).

Second, the first order hydrolysis kinetic model, show in Eq. (5) was fitted to the bio-CH_4 yield curve data and used to determine the hydrolysis rate constant (k_h) over the first 5 days of production [2]. This corresponds to the linear section in the beginning of the bio-CH_4 yield curve. k_h is given as the hydrolysis rate constant (day^{-1}).

Finally, batch mass balances for bio-CH_4 production were computed. Their output was plotted in comparison to the experimentally obtained specific bio-CH_4 yields (SMY_{SCGs}) shown in Fig. 2. Essentially, the total volumetric bio-CH_4 yield (VMY) comes from the loaded AS inoculum and SCG substrate as shown in Eq. (6). SIR at the start of the BMP is defined as shown in Eq. (7). Inserting Eq. (7) into Eq. (6) yields Eq. (8). It should be noted that $C_{tot}V_{tot} = C_{SCGs}V_{SCGs} + C_{AS}V_{AS}$. Eq. (8) is normalized for specific bio-CH_4 production from the sludge and so the term SMY_{AS} does not appear in the mass balance. Eq. (8) exhibits an inverse relationship between SMY_{SCGs} and SIR. $SMY_{SCGs, model}$ was calculated by minimizing the residual sum of square errors between $SMY_{SCGs, obs}$ and $SMY_{SCGs, experimental}$ using the Solver Add-in tool in Microsoft Excel.

$$V(t) = V_{max} \bullet \exp\left[-\exp\left(\frac{Vr_{max} \bullet e}{V_{max}}(\lambda - t) + 1\right)\right] \quad (4)$$

$$V(t) = V_{max} \bullet [1 - \exp(-k_h \bullet t)] \quad (5)$$

$$VMY_{tot} = SMY_{tot} \bullet C_{tot} \bullet V_{tot} = VMY_{SCGs} + VMY_{AS} \quad (6)$$

$$SIR = \frac{C_{SCGs} \bullet V_{SCGs}}{C_{AS} \bullet V_{AS}} \quad (7)$$

$$SMY_{SCGs\,obs} = \frac{SMY_{SCGs,model} \bullet SIR}{1 + SIR} = \frac{SMY_{SCGs,model}}{1 + \frac{1}{SIR}} \quad (8)$$

2.6. Box-Behnken design

A BBD with 3 factors and 3 levels was used, with a total of 15 experimental runs. The factors considered were pretreatment contact time (A), dilute HCl acid concentration (B), and LS ratio (C) at 3 equidistant levels (-1, 0, and 1). The factors and responses (coded and uncoded) are given in Table 2. The BBD consisted of 12 random leveled runs and 3 center pointed runs. The samples were tested for 2 responses:

bio-CH_4 yield (Y_1) and $COD_{removal}$ (Y_2). The experimental and predicted results are presented in Table 2. The aim is to measure the influence each factor (or a combination of factors) exhibits on a response, as well as the statistical deviation in the proposed model.

The software used was Design Expert 13® program (Stat-Ease, New York, USA). It computes and displays the statistical significance analysis as well as the graphical analysis based on the input variables A, B, and C, thereby fitting a polynomial (quadratic) regression across the design space. After, a model equation is generated with empirically derived coefficients for each of the variables as shown by Eq. (9). Coefficients α_0, α_i, α_{ij}, and α_{ii} are constant, linear, cross-product, and quadratic coefficients respectively and are determined by the models fit to the experimental data. Y_i denotes the responses. X_i and X_j denote the factors A, B, and C. The random error generated by the model is denoted by ε. After computing the model equations, validation runs were used to determine the validity of the model. The validation runs were conducted at random using two different runs. First at 45 mins – 1.5 % – LS 20 and second at 35 mins – 1.5 % – LS 10.

$$Y_i = \alpha_0 + \sum_{i=1}^{3} \alpha_i X_i + \sum_{i=1}^{3}\sum_{j=i+1}^{3} \alpha_{ij} X_i X_j + \sum_{i=1}^{3} \alpha_{ii} X_i^2 + \varepsilon \quad (9)$$

3. Results and discussion

3.1. Initial characterization

Physical and chemical characterization of the substrate was performed. The results are given in Table 1. Initial characterization is similar to results found in literature [3]. Using $C_{1553}H_{2488}O_{852}N_{58}S$ as the substrate's empirical formula, BMP_{Th} is calculated to be 652 NmL CH_4 g^{-1} VS_{SCGs}. This was then used to calculate the degree of biodegradability (BD) at the tested SIRs. The C:N ratio of the substrate (26.9 mol C mol^{-1} N) makes it suitable for AD processing. When the C:N ratio is low, the process produces NH_3 which is inhibitory, so a low nitrogen content of 2.27 wt% mitigates against this [5,25]. Evidence of this is shown by measuring pH_{final} of the SIR reactors (Table 3).

The inoculum used in the SIR study had TS (total solids) of 2.93 ± 0.10 %, VS (volatile solids) of 2.02 ± 0.04 %, VS_{TS} of 68.8 ± 2.5 %, ALK (alkalinity) of 3882 ± 121 mg L^{-1} as $CaCO_3$, total VFA of 426 ± 25 mg L^{-1} as CH_3COOH, VFA/ALK ratio of 0.11 ± 0.01, total COD of 36202 ± 1987 mg COD L^{-1}, pH of 7.53 ± 0.05 and finally NH_4^+ was found to be 1486 ± 23 mg NH_4^+–N L^{-1}. The inoculum used in the pretreatment study had TS (total solids) of 2.69 ± 0.10 %, VS (volatile solids) of 1.78 ± 0.10 %, VS_{TS} of 66.2 ± 0.44 %, ALK (alkalinity) of 5600 ± 330 mg L^{-1} as $CaCO_3$, total VFA of 660 ± 220 mg L^{-1} as CH_3COOH, VFA/ALK ratio of 0.12 ± 0.04, total COD of 36680 ± 2103 mg COD L^{-1}, pH of 7.45 ± 0.01 and finally NH_4^+ was found to be 1455 ± 4 mg NH_4^+–N L^{-1}. It is important to note that the VFA/ALK ratio of the anerobic sludge used in both experimental trails is relatively similar. Inocula quality is compliant according to the criteria outlined by Holliger et al. [12].

During pretreatment selection, the hydrolysate was measured for dissolved COD after phase separation. The results were as follows: control (9.4 g COD/L) > hydrothermal (32.1 g COD/L) > microwave base (37.9 g COD/L) > dilute acid thermal (77.1 g COD/L). Higher COD is linked to better pretreatment efficiency, and so based on the following COD results, we conclude that the most favored pretreatment for further study was chosen to be dilute acid thermal pretreatment (DATP).

3.2. CH4 production as a consequence of SIR variation

Fig. 1 shows the specific bio-CH_4 yield (top) and the daily average bio-CH_4 production rate (bottom) for each of the SIRs after subtracting residual bio-CH_4 produced by the inoculum. BMP profiles are characterized by their sigmoidal shape [23] with an initial lag phase followed by an exponential biomethanation stage and finally by a stable/non-

Table 3
BMP assay performance parameters.

Parameter	Units	Reactors						
		SIR 2.00	SIR 1.00	SIR 0.50	SIR 0.33	SIR 0.25	SIR 0.20	SIR 0.16
SMY_{SCGs}	mL $CH_{4,\ STP}$ g^{-1} VS	247 ± 0	307 ± 4	333 ± 50	412 ± 1	436 ± 44	464 ± 3	480 ± 44
VMY_{total}	mL $CH_{4,\ STP}$	1099 ± 1	1449 ± 18	1677 ± 100	1405 ± 2	1273 ± 94	1037 ± 4	990 ± 7
BD	%	40.6 ± 0	50.5 ± 0.7	54.8 ± 8.2	67.8 ± 0.2	71.7 ± 7.2	76.3 ± 0.5	79 ± 7.2
t_{80}	days	33.0 ± 1.0	9.2 ± 0.5	5.6 ± 0.6	6.8 ± 0.0	6.2 ± 0.8	5.5 ± 0.7	5.9 ± 1.6
t_{90}	days	38.0 ± 0.3	11.1 ± 0.7	7.5 ± 0.2	8.4 ± 0.0	8.8 ± 0.9	9.6 ± 2.1	8.9 ± 1.5
pH_{final}	–	6.83 ± 0.10	7.59 ± 0.02	7.64 ± 0.05	7.66 ± 0.01	7.64 ± 0.03	7.67 ± 0.04	7.68 ± 0.06
ALK	mg $CaCO_3$ L^{-1}	982 ± 56	2371 ± 246	4702 ± 638	N.A.	5203 ± 52	5624 ± 59	5415 ± 392
VFA	mg CH_3COOH L^{-1}	280 ± 22	284 ± 41	582 ± 117	N.A.	578 ± 64	624 ± 46	482 ± 40
VFA/ALK	mg CH_3COOH mg^{-1} $CaCO_3$	0.288 ± 0.038	0.121 ± 0.023	0.124 ± 0.020	N.A.	0.111 ± 0.011	0.111 ± 0.008	0.090 ± 0.014
V_{max}[a]	mL $CH_{4,\ STP}$ g^{-1} VS	237 ± 8	308 ± 4	332 ± 47	412 ± 1	434 ± 41	455 ± 1	474 ± 40
Vr_{max}[a]	mL $CH_{4,\ STP}$ g^{-1} VS day^{-1}	12.3 ± 0.5	36.2 ± 2.8	57.7 ± 12.0	51.9 ± 0.4	64.6 ± 11.8	74.5 ± 7.9	77.1 ± 19.8
λ[a]	days	11.48 ± 0.18	0.75 ± 0.15	0.26 ± 0.34	0.21 ± 0.04	0.09 ± 0.36	-0.36 ± 0.11	0.03 ± 0.43
$R^{2,a}$	–	0.988	0.998	0.995	0.992	0.991	0.981	0.989
k_h[b]	day^{-1}	0.01 ± 0.00	0.14 ± 0.01	0.27 ± 0.06	0.16 ± 0.00	0.24 ± 0.06	0.31 ± 0.03	0.27 ± 0.09

N.A. – Not available.
[a] – Modified Gompertz model.
[b] – First Order Hydrolysis model.
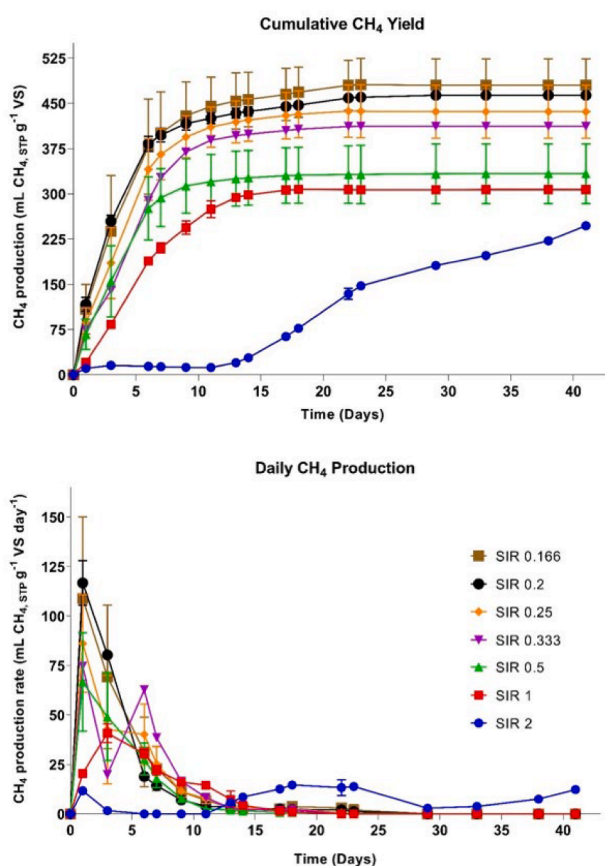
Fig. 1. Specific bio-CH_4 yield (top) and daily bio-CH_4 production (bottom).

Fig. 2. Regression model fitting: specific bio-CH_4 yield curves vs SIR.
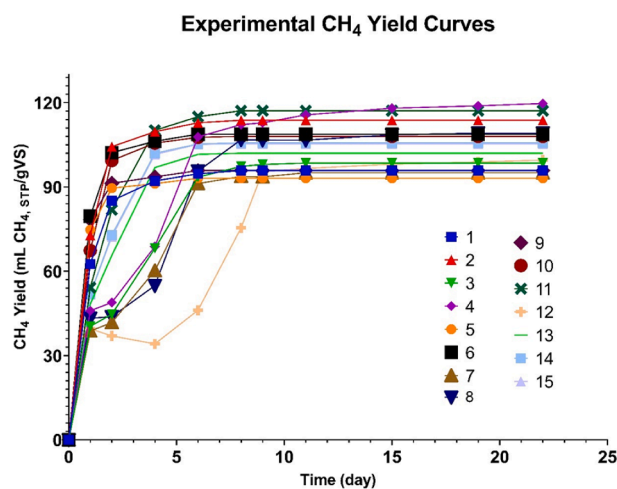
Fig. 3. Dilute acid thermal pretreatment bio-CH_4 yield curves.

producing plateau as seen in Figs. 1 and 3.

As for the relationship between SMY and SIR, a clear trend is visible in Fig. 2. As SIR decreases from 2 to 0.16 g VS g^{-1} VS the specific bio-CH_4 yield increases by more than twofold from 247 to 480 ± 44 mL $CH_{4,\ STP}$ g^{-1} VS respectively. As SIR decreases the anaerobic microbial ability to breakdown SCGs increases, this can be seen by an increasing k_h in Table 3. At the high SIR of 2, the concentrations of SCGs (as gVS) are 2 times higher than the concentration of inoculant. This is overwhelming and leads to substrate shock loading where the final bio-CH_4 yield drops to its lowest levels. Similar phenomena have been reported (Sri Bala
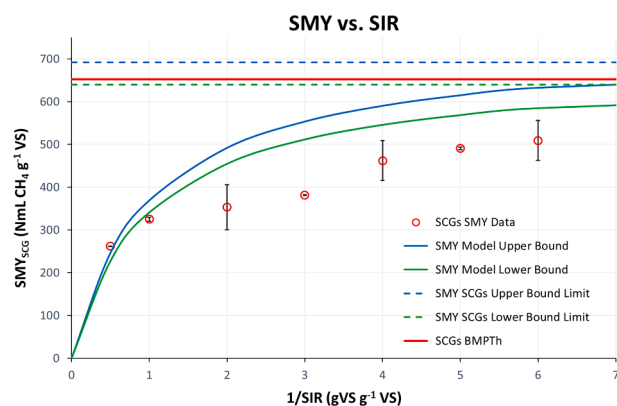
[30]). These results agree with similar precedents observed in the literature. For instance, Vítěz et al. [32] achieved similar ranged yields of $0.271 - 0.325$ m^3 $kg^{-1}_{dry\ organic\ matter}$ as obtained in this study. Li et al. [19] noted severe VFA accumulate when digesting SCGs, specifically propionate. This could possibly explain the extremely low bio-CH_4 yields and biodegradability at SIR 2 caused by methanogenesis inhibition [15]. The shape of the curve also indicated that it is a failed

"improper" digestion [15].

After obtaining the mass balance model equations in Section 2.5, experimentally obtained SMY data was fit using non-linear regression by minimizing the residual sum of squares error. The data and models are shown in Fig. 2. Open red circles represent SMY_{SCGs} from Table 3 with standard deviations. Residual CH_4 generation attributed to the inoculum (SMY_{AS}) is subtracted from the model and hence both the solid green and blue curves start at the origin. The difference between the upper and lower model bounds is attributed to this residual generation when using SMY_{AS} at 58.7 ± 27.8 NmL CH_4 g^{-1} VS_{AS}. The green and blue dashed lines represent the theoretical limits for SMY_{SCG} as SIR decreases. The upper limit tops out at 692.2 NmL CH_4 g^{-1} VS_{SCG}. The lower limit has a maximum of 639.5 NmL CH_4 g^{-1} VS_{SCG}. The actual BMP_{Th} from the empirical formula is calculated to be 651.9 NmL CH_4 g^{-1} VS_{SCG} and this value fits well within the range calculated (horizontal red line). The model curves had an R^2_{avg} of 0.928.

Biodegradability can be represented by the vertical distance between the model curve and its respective horizontal line. The curves suggest that the biodegradability of SCGs increases with decreasing SIR. However, the experimental data points (red circles) show that observed biodegradability is lower than the models expected values at lowers SIRs. This can be explained by SCGs lignocellulosic nature [26]. As SIR increases i.e., 1/SIR tends towards 0, then SMY_{SCGs} sharply falls as a consequence of substrate overloading. On the other hand, further decreasing SIR for a minimal increase in bio-CH_4 surely runs into increased reactor volume and digestate dilution issues [10]. Therefore, we conclude that SIRs between 1 and 0.25 g VS g^{-1} VS are within a reasonable and optimal range for SCGs mono-digestion.

3.3. BMP assay performance

Table 3 give presents all results regarding BMP assay. Reactors with SIR 2 resulted in poor performance. A combination of decreased ALK, increased VFAs, low yield and excessively long technical digestion times have rendered it substrate shocked and failed. SIR 2 reactors had a t_{80} and t_{90} of 33 ± 1 days and 38 ± 0.3 days, respectively. This is much longer than other SIRs tested. This occurs due to an increase in the substrate solids concentration in the reactor surpassing the healthy threshold. This overpowers the anaerobic sludge consortia and hence ends up producing less bio-CH_4 even though there is more substrate to be used for conversion. On the other hand, a minor decrease from SIR 2 to SIR 1 generates a significant increase of over 65 % in t_{80} and t_{90}.

Table 3 also shows the results of pH_{final}, ALK, VFA, and VFA/ALK ratios after AD had concluded. Increasing the SIR resulted in an overload of VFAs, in which there was rapid consumption of alkalinity and therefore less buffering capacity [10]. The initial pH of the reactors was adjusted to 7–7.1 before starting the process. After digestion, the pH for SIR 2 reactors was found to be is 6.83 ± 0.10. It is important to note that this is the only reactor with a lower pH at the end as opposed to the start. On top of that, upon removal of the caps from the bottles, these reactors omitted a strong foul smell whereas others did not. This is attributed to the build-up of VFAs and potentially H_2S. These observations as well as a high VFA/ALK ratio render these reactors failed. The digestion of SCGs has been associated with the accumulation of VFAs most notably propionic acid [19,25].

VFA/ALK ratios should be kept within operational limits to ensure a continual effective digestion. There is a pronounced decrease in the VFA/ALK ratio from 0.288 ± 0.038 to 0.121 ± 0.023 whilst moving down from SIR 2 to SIR 1. This implies that digestion of SCGs at high SIRs can lead to an overall decrease in reactor productivity as shown by a decrease in bio-CH_4 yields. VFA accumulation as a function of increasing SIRs has previously been observed [20]. At SIR 1 and lower, the VFA/ALK concentration ratios decrease and allow for improved reactor performance. The VFAs produced by acidogens and acetogens in low SIR reactors were equally consumed by methanogens without the risk of alkalinity depletion. Reactors with SIR 1 and lower had more

VFAs present in their digestate but were compensated by higher alkalinity which aided in equilibrating against substrate overloading. Therefore, we conclude that reactors with SIR 1 to 0.25 are best when it comes to the digestion of SCGs.

3.4. Kinetic model fitting

Table 3 displays the results of two kinds of kinetic analyses conducted. First, the modified Gompertz kinetic modelling and then the first order hydrolysis regression over the first 5 days of digestion. The modified Gompertz model is generally used to analyze microbial gas fermentations as it is based on the hypothesis that microbial growth and bio-CH_4 production are directly corelated. The modified Gompertz kinetic model also demonstrates how anaerobic microorganism cell growth rates and concentrations dynamically change over the course of digestion. As for the first order hydrolysis regression analysis, it sheds light on the kinetics of hydrolysis and hydrolysis rate constant (k_h). The interaction between the SCGs and sludge in the beginning/hydrolysis stages of the digestion is considered a crucial factor as it can dictate the overall process [10].

V_{max} and Vr_{max} follow an expected increasing positive trend as SIR decreases. The major changes occur between SIRs 2, 1, and 0.5. Initially λ is 11.48 ± 0.18 days at SIR 2 as the microorganisms are overwhelmed by the large influx of SCG solids and take >11 days to acclimate towards these conditions [10]. Analogously, the combination of a low k_h and a high λ is a statement of the inadequacy to biodegrade and produce bio-CH_4 efficiently at this SIR [24]. This is subsequently confirmed by the shape of its curve in Fig. 1. Now, a further decrease to SIR 1 and SIR 0.5 sees a substantial decrease in λ to 0.75 ± 0.15 and 0.26 ± 0.34 days, respectively. This suggests that microorganisms are adapting to the substrate. Similar observations between SIR and λ when digesting food waste to bio-H_2 [7]. A 2700 % increase in k_h is calculated between SIR 2 and 0.5. It is observed that λ in SIR 0.20 reactors is calculated to be negative. This is equivalent to assuming the λ to be 0 (negligible) due to firstly, the activity of the inoculum and secondly, the compatibility between the substrate and the inoculum which leads to elevated hydrolysis and increased yields. Therefore, based on the results from Table 3, we conclude that the optimal digestion SIR for SCGs is between 1 and 0.25.

3.5. Response surface methodology and Box Behnken design

For this part of the study, only the liquid hydrolysate was used for digestion. Therefore, it is immediately noticeable that the bio-CH_4 yield ranges in Table 2 are lower than those in Table 3. The solid fraction was either discarded after DATP or dried and used for FTIR characterization as seen in Fig. 6. This limits the concentration of substrate available for bioconversion, hence the lowered yields. The AD of whole SCGs solids generates more bio-CH_4, however, using it eliminates the opportunity for further value-added chemical recovery downstream.

Bio-CH_4 yields range between 93.1 and 119.7 mL CH_4, $_{STP}$ g^{-1} VS However, it is evident that production increases by a minimum of 200 % when using DATP as opposed to hydrothermal pretreatment (control test). This is due to there being more bioavailable substrate for conversion when pretreatment is applied [14]. When pretreating SCGs for digestion, Atelge et al. [4] and Girotto et al. [11] have been able to achieve yields up to 336 ± 7 mL CH_4 g^{-1} VS and 392 ± 3 mL CH_4 g^{-1} VS respectively. Therefore, the solid fraction helps boost bio-CH_4 production even after pretreatment. The bulk of the utilizable substrate lies within the lignocellulosic matrix and so it is believed that CDATP at 120ºC and 1.15 bar is not severe enough to break down lignin and completely release holocellulose fractions [34]. The less lignocellulosic penetration occurring, the lower the digestion yield, efficiency, and feasibility of biomethanation of the substrate.

DATP bio-CH_4 yield curves are shown in Fig. 3. The curves are numbered according to the scheme adopted in Table 2. Curves 3, 4, 7, 8, and 12 deviate in terms of their production profile from the rest. They

initially start off as all the others do, but approximately-one day into the fermentation they protrude outwards (shown by the positive concave bulge) before recovering upwards again. It is important to note that this phenomenon occurred in the validation tests too (curves not shown) and is considered to not be an anomaly.

From Table 2, it can be noted that the curves do not experience this bulge if the concentration of acid used is 1.5 % (v/v) and if the LS ratio is equal to 10 v/w. It is observed that a combination of high HCl concentration and high LS ratio may be why curves 3, 4, 7, 8, and 12 behave in this deaccelerated manner. The stepped/concaved shape may also be attributed to VFA inhibition issues caused by complex degradation kinetics as a consequence of the pretreatment conditions used [33].

According to ANOVA (analysis of variance), Y_1 is significantly affected ($p < 0.05$) by the interaction of factors BC, underpinning the fact that a suitable combination of LS ratio (w/v) and HCl concentration (%) are needed to optimize extraction and thereafter increase yield. Higher acid concentrations induce harsher pretreatment environments which does not necessarily translate into better bio-CH_4 yields. An increase in pretreatment contact time (factor A) is needed to improve bio-CH_4 yields.

As for Y_2, it ranged between 60.9 and 72.4 % after standardizing against the blank $COD_{removal}$. All of the responses having a $COD_{removal} > 70$ % occurred at the lowest LS ratio of 10 v/w, implying that pretreatment dilution factor possibly has the greatest effect in determining the degree of solubilization, and ultimately the pretreatment efficiency [16]. Also, as seen from runs 1 through 4, both factors A and B are less prominent in returning a higher $COD_{removal}$. Furthermore, $COD_{removal}$ decreases even further if a high LS ratio (C) is used as demonstrated in table 2 by runs 7, 8, 11, and 12. This can be attributed to the fact that pretreatment at conditions with low LS ratios promote the production acetic acid (HAc) from the substrate thus driving $COD_{removal}$ to be higher during digestion [31]. The ANOVA of Y_2 confirms that factor C holds the greatest significance ($p < 0.05$) in determining $COD_{removal}$. However, the LS ratio (C) alone is not as indicative of bio-CH_4 yield ($p > 0.05$). All second-degree polynomial model equations obtained were significant according to their p values and can be seen in Table 4 alongside R^2 values and significant factors.

Fig. 4 shows the three-dimensional 2nd degree polynomial response surface curves with their respective contour lines for Y_1 and Y_2, with varying A, B, and C. All the plots are taken as snapshots where one of A, B, and C is fixed in their middle levels (i.e., coded level = 0) while the other two variables span the coded levels between −1 and 1 on the horizontal axes. This gives 3 curves per response. Given the nature of the curve in Fig. 4a3), we can conclude that both factors B and C interact to determine Y_1. Analogously, Similarly, Fig. 4b2) and 4b3) in demonstrate the severe effect factor C has on Y_2. The relative flatness of Fig. 4b1) addresses the insignificance of A and B in determining the $COD_{removal}$.

The perturbation plots in Fig. 5 demonstrate and compare the deviation in each of the variable's A, B, and C caused by the variation of their respective parameters between −1 and 1. In essence, it shows the sensitivity in the response when A, B, and C reach out over the span of their coded values. Also, the steepness of a curve can be attributed to the degree of significance a factor holds in determining or inducing a change in a response [8].

It can be directly seen that from Fig. 5b) that factor C is the most pronounced spanning sharply between the extreme reference points.

However, in Fig. 5a), factor A holds more influence. Given the quadratic nature of the model equations we can see that factor A in Fig. 5a) acts in an increasing linear fashion. This suggests that further increase in pretreatment time may increase bio-CH_4 yield further. In Fig. 5a), the interaction between B and C is significant ($p < 0.05$) as they deviate from the center point in both directions implies that a negative correlation exists between the two factors on optimizing bio-CH_4 yield. This is reconfirmed by the pivotal shape of the curve in Fig. 4a3). Now, when comparing Fig. 5a) and 5b) it is observed that decreasing factor C to −1 may be beneficial in terms of increasing $COD_{removal}$ but is bad for the bio-CH_4 yield. For this research, it is important to optimize all three factors in order to maximize the response across all measured variables. However, factor C stands out as being a crucial factor to control in order to maximize responses Y_1 and Y_2.

Two different confirmation tests were chosen at random to assess the validity of the RSM model equations proposed in Table 4. Table 5 shows the results from running these confirmation tests. Errors in Y_2 have a smaller magnitude than those in Y_1. The largest error found was attributed to Y_1 in the run 45 mins – 1.5 % – 20 LS at 11.3 ± 0.7 %. The smallest error was attributed to Y_2 in the run 35 mins – 1.5 % – 10 LS at 3.2 ± 1.2 %. The predicted responses estimated by the models are within acceptable range when compared to the experimental responses.

3.6. FTIR characterization

ATR-FTIR was used to study changes in functional groups of SCGs before and after DATP was applied. With reference to Fig. 6, the broad band between 3550 and 3200 cm^{-1} is attributed to the stretching of intermolecular hydrogen (–OH) bonds in cellulose, and hemicellulose, and phenolic lignin [6]. It is obvious that pretreatment had an effect in lowering the severity of this peak indicating that acid induced hydrolysis took place. The dividing line differentiating between sp^2 C—H and sp^3 C—H is at around 3000 cm^{-1}. The sp^2 C—H stretch at 3008 cm^{-1} is indicative the presence unsaturated material such as lipids and carotenoids [9]. The band peak at 2921 cm^{-1} suggests the presence of aliphatic sp^3 C—H stretching in the methyl and methylene groups of carboxylic acids, cellulose, and hemicellulose [6]. Both pretreatments had relatively the same intensity here. Contrarily, the mild pretreatment conditions used do not result in major functional changes in the range of 2230 to 1990 cm^{-1}. However, it can be seen that increasing acid concentration and pretreatment time can result in slight functional changes in this region. The sharp band with a peak at 1742 cm^{-1} corresponds to C=O (carboxyl group) stretching in hemicellulose, lignin, caffeine, and other carboxylic compounds [6]. The peak bands found at 1650 and 1510 cm^{-1} attribute to C=O and C=C stretching in quinones and aromatic lignin rings [47]. The peak at 1156 cm^{-1} is due to the C—O—C stretching vibrations in cellulose and hemicellulose [6]. The band at 1040 cm^{-1} indicates C—O stretching in hemicellulose and cellulose [18]. The powerful drop in intensities between the untreated and pretreated curves is due to SCGs degradation using DATP. Qualitatively the FTIR curve show that major fractions of tannins, lignin, caffeine, and carotenoids are still present in the SCGs after pretreatment and can be used in further unit operations. Further study is needed to determine whether these value chemicals are present in large enough quantities to justify their recovery.

Table 4
Response model equations and statistical parameters.

Response	Proposed Model Equation	Significant parameters	Model p value	R^2	Adj. R^2
Y_1 Bio-CH_4 Yield	Y_1 [a] $= 104.35 + 8.61A + 0.40B + 1.90C + 0.82AB − 0.40AC − 7.43BC − 0.52A^2 + 3.11B^2 − 2.30C^2$	A – BC	0.014	0.940	0.833
Y_2 $COD_{removal}$	Y_2 [a] $= 66.97 + 0.09A − 0.15B − 4.06C − 0.42AB + 0.27AC − 0.68BC + 0.05A^2 − 1.05B^2 + 1.40C^2$	C	0.045	0.901	0.723

[a] – The model equations are given as a function of their coded values. The values for A, B, and C can be −1, 0, and 1, or a combination of values in this range.
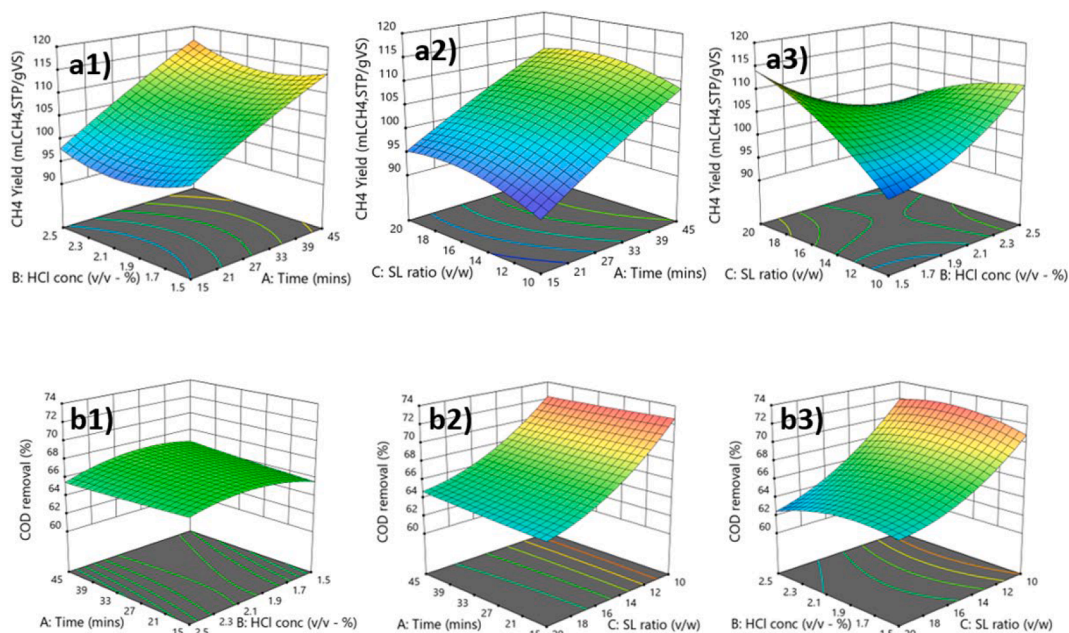
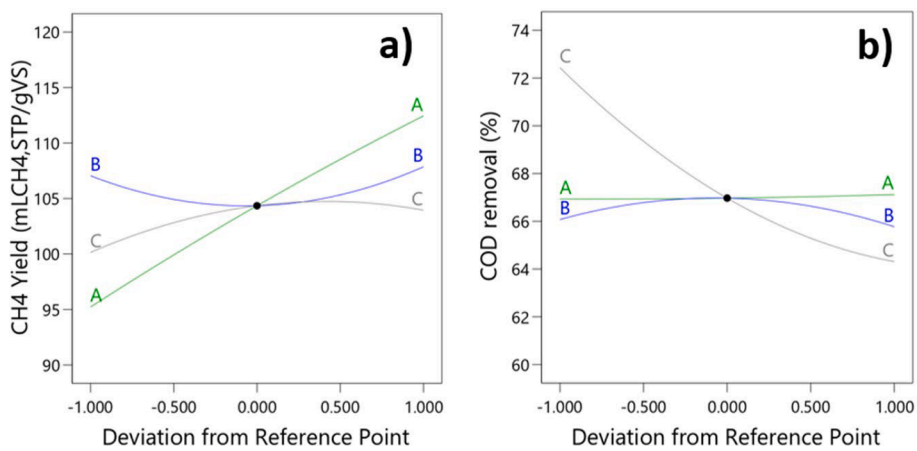**Fig. 4.** Response surface plots for a) bio-CH$_4$ yield and b) COD$_{removal}$.

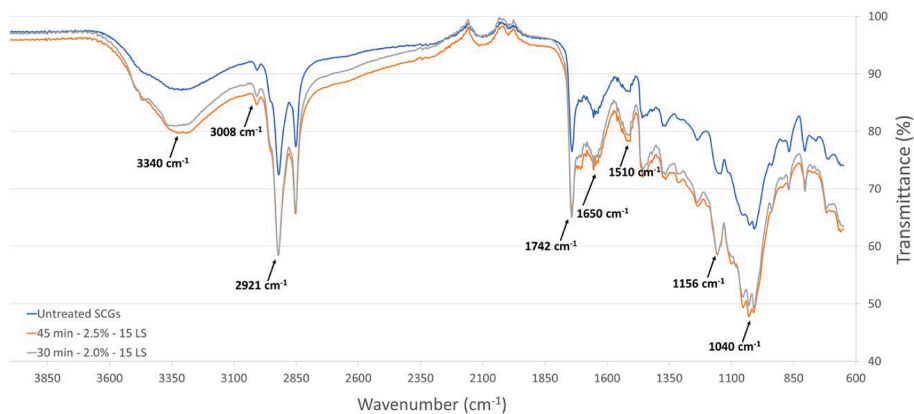**Fig. 5.** Perturbation plots for a) bio-CH$_4$ yield and b) COD$_{removal}$.

Fig. 6. FTIR spectra comparing residual SCGs before and after pretreatment.

Table 5
Experimental validation tests.

Run (Uncoded)	Y$_1$ Experimental (mL CH$_4$ g^{-1} VS)	Y$_1$ Predicted (mL CH$_4$ g^{-1} VS)	Y$_1$ Error (%)	Y$_2$ Experimental (%)	Y$_2$ Predicted (%)	Y$_2$ Error (%)
45 mins – 1.5 % – 20 LS	107.3 ± 0.8	121.0	11.3 ± 0.7	60.6 ± 1.5	64.9	6.8 ± 2.3
35 mins – 1.5 % – 10 LS	93.5 ± 2.4	97.6	4.2 ± 2.5	68.6 ± 0.8	70.9	3.2 ± 1.2

4. Conclusion

Anaerobic digestion is a powerful tool which can be employed as a sustainable waste mitigating solution. Two complementary novel studies on the AD of SCGs are conducted. First the digestion of SCGs at different SIRs and second the digestion of SCGs hydrolysate post thermal acid pretreatment. The main conclusions are as follows:

- Reactors with SIR 2 exhibited delayed growth, high VFA/ALK ratios and low specific bio-CH$_4$ yields. The highest bio-CH$_4$ yield was 480 ± 44 mL CH$_{4,\ STP}$ g^{-1}VS at SIR 0.16.
- Pretreated SCGs bio-CH$_4$ yields depend on the interaction between acid concentration and LS ratio. LS ratios are the main factor to determine COD$_{removal}$. There exists a trade-off between bio-CH$_4$ yield and COD$_{removal}$. Increasing the LS ratio decreases the COD$_{removal}$ but increases the bio-CH$_4$ yield. A maximum bio-CH$_4$ yield of 119.7 mL CH$_{4,\ STP}$ g^{-1} VS and 72.4 % COD$_{removal}$ was achieved.
- FTIR spectroscopy shows decreased hemicellulose and cellulose level in pretreated SCGs. However, a large fraction still remains in the solids.

Nonetheless, future focus on fractionation studies on the residual biomass after pretreatment should be conducted to determine their viability for further bioprocessing. Also, developing an understanding of how SCGs degradation changes microbial communities through all stages of AD (hydrolysis through methanogenesis) is important to work around the bottlenecks of high SIRs.

CRediT authorship contribution statement

Georgeio Semaan: Writing – original draft, Conceptualization, Methodology, Investigation. **M.R. Atelge:** Writing – review & editing, Validation. **Roent Dune Cayetano:** Investigation, Validation. **Gopalakrishnan Kumar:** Project administration, Supervision. **Roald Kommedal:** 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.

Data availability

Data will be made available on request.

Acknowledgements

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