Control of inhibition through anaerobic co-digestion of algae with sugarcane bagasse

Bilassé Zongo
Universite NAZI BONI
Sahand Iman Shayan
University of South Florida Tampa Campus: University of South Florida
Meng Wang
Penn State University: The Pennsylvania State University
Sarina J Ergas (sergas@usf.edu)
University of South Florida Tampa Campus: University of South Florida https://orcid.org/0000-0001-6820-7196

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Abstract

Anaerobic digestion (AD) of algal-bacterial biomass grown on wastewater has been used successfully for bio-methane production. However, challenges with AD of microalgae include inhibition due to accumulation of free ammonia (FA) and volatile fatty acids (VFAs), alkalinity depletion and/or pH outside optimal values. In this study, algal-bacterial biomass was co-digested with sugarcane bagasse, an agricultural waste product, to increase biogas production by controlling inhibition. Algal-bacterial biomass was cultured in bench-scale photo-sequencing batch reactors (PSBRs) used to treat high ammonia strength wastewater. Biochemical methane potential (BMP) assays were set up at approximately 2% solids content with varying ratios of algal-bacterial biomass and sugarcane bagasse to achieve carbon to nitrogen (C/N) ratios between 4.5 and 60. Addition of sugarcane bagasse helped balance the high nitrogen content of algae and control pH/Alkalinity, VFA and FA in BMPs. Methane content of the biogas was similar for all BMPs (~66 %). The highest total biogas production was observed for BMPs with substrates composed of algae (AL) and sugarcane bagasse (BG) at C/N ratios of 17 and 18. When the C/N ratio was maintained at about the optimal ratio, a significant correlation (r^2 = 0.88, p = 0.012) was observed between algae biomass content of the substrate and total gas production and thereby methane yield. The results show that sugarcane bagasse addition to algal-bacterial biomass reduced AD inhibition and led to greater methane yields.

Introduction

Microalgae have the potential to play an important role in resource recovery from wastewater treatment. During wastewater treatment in shallow facultative ponds, organic components and nutrients in wastewater are converted to microalgae-bacterial flocs with a continuous exchange of O_2 and CO_2 between algae and bacteria. Mixed species growth in open systems has been shown to improve biomass yield relative to algal monocultures [1]. This process is the dominant full-scale algal technology for treating wastewater [2].

Harvested microalgae and bacterial biomass can serve as an organic feedstock for energy production. Microalgae-based bioenergy production has received considerable interest because of the distinctive advantages of algae over other energy crops [3, 4]. Anaerobic digestion (AD) of algal biomass grown on wastewater has been used successfully for bio-methane production [5], which can contribute to the economic and environmental sustainability of wastewater treatment systems. The high nutrient availability in domestic wastewater and the high solar energy, particularly in urban areas of tropical and sub-tropical regions, is an advantage that can facilitate algae and bacteria biomass cultivation for biogas production.

It is important to ensure a stable AD process to achieve the goal of producing as much bio-methane as possible from a given substrate and to produce a stabilized residue of good quality [6]. The biodegradability index (BI), indicating the efficiency of the AD process, represents the methane yields measured relative to the theoretical methane yields based on the ultimate analysis [2]. In general, methane yields less than 50 % o that of common commercially exploited feedstocks (e.g., food wastes, sewage sludge, livestock manure) are obtained with raw algal biomass. Prior studies [7] reported that methane yields from AD of different algae species were between 19 and 81% of the theoretical value of about 575 mL CH_4 g^{-1} VS.

Problems with AD of microalgae include free ammonia (FA) and volatile fatty acid (VFA) inhibition, alkalinity depletion and/or pH outside the optimal range for methanogenesis [8]. This can be caused by several factors, such as cell wall structure, polysaccharides that are not readily hydrolyzed, polyphenols and low C/N ratio [9–11]. In the case of C/N, the organic loading rate must be controlled to maintain the release of total ammonia nitrogen (TAN) within the desired range in the digester. The optimal C/N ratio for biogas production is estimated to be between 20 and 30 [2, 12], while microalgae typically have a low C/N ratio that is estimated to be about 6 [2]. Therefore, co-digestion of algae with a high carbon content organic feedstock can increase the C/N ratio and reduce the inhibitory effects of free ammonia [13].

Sugarcane bagasse is a high carbon content biomass, with a C/N ratio of about 100 [14]. However, it is unsuitable for biogas production on its own due to poor buffering capacity and high C/N ratio. Sugarcane bagasse can therefore be used during anaerobic co-digestion with algal-bacterial biomass to balance the C/N ratio and avoid the risks of inhibition [15]. In prior studies, an increase in C/N to a ratio of 20 to 30 resulted in increased methane yields, which was attributed to the increment of volatile solids content and stability of pH within the range of 6.9 to 7.3, which is healthier for microbial activity [16].

Several prior studies have been conducted using sugarcane bagasse as a co-substrate with nitrogen rich biomass [15–20]. However, few prior studies have been conducted on enhancing methane production from algal biomass through a co-digestion with sugarcane bagasse [21]. In many sub-tropical and tropic regions around the world, the sugarcane industry occupies a major part in the economy. In general, sugarcane bagasse is used by the sugarcane industry as fuel to supply energy needs through combustion and cogeneration of electricity and heat [22]. Due to its potential feedstock properties, the technique of co-digestion of algal-bacterial biomass from wastewater treatment with sugarcane bagasse has the potential to enhance environmental sustainability in areas with large sugarcane industries.

Therefore, the goal of this study was to investigate co-digestion of mixed algal-bacterial biomass cultivated using wastewater and sugarcane bagasse to control AD inhibition. Specifically, the study aimed to: 1) characterize algal-bacterial biomass grown on wastewater and sugarcane bagasse in terms of its suitability as an AD substrate, 2) investigate the effect of different algal-bacterial/sugarcane bagasse ratios on bio-methane production, 3) measure parameters that could be inhibitory factors during the AD process, including ammonia, pH, alkalinity and VFAs.

Material And Methods

Biomass and pretreatment

Sugarcane bagasse was obtained from Florida Crystals Corporation. Dewatered sludge from mesophilic AD of sewage sludge was obtained from the Northeast Water Reclamation Facility (located in Clearwater, FL) and used as inoculum.

Algal-bacterial biomass was harvested from two 2-L bench-scale photo-sequencing bioreactors (PSBRs) that have been described elsewhere [23]. Briefly, PSBRs were set up with mixers, pumps and lights operated by timers, allowing them to run in the following stages: 1) feed, 2) light react (350 ± 3 μmol m^{-2} s^{-1} intensity),

3) dark react, 4) settle, 5) decant. The PSBRs were fed with screened raw wastewater collected from the Falkenburg Advanced Wastewater Treatment Plant in Hillsborough County, Florida, with added NH_4Cl, K_2HPO_4 and $MgCO_3$ to mimic N, P and alkalinity concentrations of AD sidestreams. Sodium acetate was added at the beginning of the dark period (Stage 3) to promote denitrification. The PSBRs were operated for > 1 year and consistently achieved > 90 % otal nitrogen (TN) removal without mechanical aeration due to the activity of the algal-bacterial consortium [23].

Genera and species of wild type algae in the harvested biomass from the PSBR were identified based on algal cell size, morphology and contents (Fig. 1). Microscopic analysis of harvested algal samples and estimation of species portions in samples showed that algal biomass consisted primarily of *Chlorella* spp. (30% of the total biomass), *Scotiellopsis* sp. (25%), and *Zynemopsis* sp. (25%) and *Actinastrum* sp. (15%). Some rare species of *Navicula* sp., *Pseudanabena* sp., *Dunalliena* sp. and *Euglena* sp. were also observed.

Thermal pretreatment was done on algal-bacterial biomass to disrupt the wall and structure of algae, increasing the availability of substrate and thereby increasing methane production [24]. The pretreatment consisted of incubating biomass samples at 90°C for 1h [24]. Sugarcane bagasse was not pretreated.

C:N ratios and BMP assays

Biochemical methane potential (BMP) assays were set up at approximately 2% solids content in 250 ml glass serum bottles. Six digestion sets were set up to obtain C/N ratios of 4.5, 17–18, 32 and 60 (Table 1) by varying the substrate composition of pretreated algae (AL), sugarcane bagasse (BG) or pretreated algae plus bagasse (AL + BG). Each digestion set was set up in three sets of duplicates to allow for chemical analysis at 1 week, 3 weeks and at the end of the BMP assay, when biogas production in test bottles was negligible. Bottles were filled with substrate and fresh inoculum and then flushed with nitrogen gas for 1 minute to ensure anaerobic conditions and sealed using rubber septa and metal crimp caps. All BMP assays included inoculum-only controls (IN), also done in duplicate, which had the same volume and source of inoculum as the test bottles.

Table 1
Mixing solids, C/N ratios and S/I in BMP assays with about 2 gTS

	Composition of substrates and inoculum in ~ 20g TS)	C/N ratios of substrates	S/I	Notation
1	IN (100%)	32	-	IN/32
2	IN (75%), AL (25%)	4.5	0.33	AL/4.5(0.33)
3	IN (93%), AL (7%)	4.5	0.08	AL/4.5(0.08)
4	IN (92%), BG (8%)	60	0.10	BG/60(0.10)
5	IN (25%), AL (50%), BG (25%)	17	3.00	AL + BG/17(3)
6	IN (36%), AL (36%), BG (28%)	18	1.8	AL + BG/18(1.8)

Note: IN = inoculum, AL = Algal-bacterial biomass, BG = Sugarcane Bagasse. In the notation, the first number corresponds to C/N ratio and the second number in parentheses corresponds to S/I

Chemical analysis and methane production

Standard Methods [25] were used to measure total solids (TS), volatile solids (VS), Chemical Oxygen Demand (COD), pH and alkalinity. TAN was measured using a Timberline TL-2800 Ammonia Analyzer (Timberline Instrument, USA). Total nitrogen (TN) and VFAs were measured using Hach (Loveland, CO, US) TNT 827 and TNT 872 test kits, respectively. The total carbon (TC) was estimated from the VS measurements by assuming biomass empirical formula of $C_5H_7O_2N$ [26, 27] for high carbon content biomass like sugarcane bagasse and the inoculum, and $C_5H_7O_3N$ [28] for algae biomass.

Biogas volume and methane content of the biogas was measured every three days until no methane production was observed. Biogas volume was measured using a glass frictionless syringe equipped with a 25-gauge BD PrecisionGlide needle. The methane content of the biogas was determined by injecting a biogas sample into a base solution (3M NaOH) and measuring the resulting liquid displacement (ASTM method D1827-92 2002). This method was verified by simultaneous measurement of selected samples using a GOW-MAC (Bethlehem, PA) gas chromatography system equipped with the thermal conductivity detector.

Statistical analysis

Nonparametric tests were used to compare data after determining an absence of normality. Univariate analysis was used for nonparametric (Kruskal–Wallis test) multiple comparison (post hoc) of methane yield between substrates. The different comparison tests were performed with a Type-1 error rate of $P < 0.05$, using Statistica software, version 7.1 (StatSoft, Tulsa, OK, USA). Using EXCEL software, Pearson correlation test was performed between the yield of produced methane and algae content in the substrates.

The Modified Gompertz model (MGM) vas used to fit the data to calculate the length of the lag phase and the rate of methane production [29, 30] (Eq. 1).

$$Y(t) = Aexp\left\{-\exp\left(\frac{\mu_m e}{A}(\acute{y} - t) + 1\right)\right\} \text{(Equation1)}$$

where,

Y(t) = Cumulative biogas yield at a digestion time t days (mL/g VS)

A = Biogas production potential (mL/g VS)

μ_m = Maximum biogas production rate (mL/g VS/day)

ý = Lag phase period or minimum time to produce biogas (days)

t = Cumulative time for biogas production (days)

e = Mathematical constant (2.718282)

Results And Discussion

Substrate mixtures

C/N ratios for inoculum, algal-bacterial biomass and sugarcane bagasse were found to be 32, 4.5 and 60 (Table 1), respectively, which was similar to other studies [21, 31, 32]. Substrate combinations were set up (Table 2) to achieve C/N ratios between 4.5 and 60 to help balance algal-bacterial biomass in some BMPs. Non-co-digestion sets were set up as controls. Ammonia concentrations below 200 mg/L as observed in the present study (45–56 mg/L) have been shown to be beneficial to AD processes, as nitrogen is an essential nutrient for anaerobic microorganisms [9, 33]. However, low C/N ratio as observed in substrates such as AL/4.5 leads to excessive TAN release and ammonia inhibition [34]. It is not the case in AL/4.5 substrates where TAN was low and estimated at around 50 mg/L (Table 2). This can be explained by the addition of inoculum (C/N = 32) that increased the final C/N ratios and limit the release of TAN in these BMPs.

TS contents in the BMPs were between 1.7 and 2.0% which is the recommended range for BMP assays [35]. The pH of around 7.8 in all digestion sets (Table 2) is close to the optimal pH for growth of methanogenic and acidogenic microorganisms [8, 9, 34].

Table 2
Initial characteristics of inoculum and substrates. Note: IN = inoculum, AL = Algal-bacterial biomass, BG = Sugarcane Bagasse, on the different notations, the first number corresponds to C/N ratio of the substrate and the second number in parentheses corresponds to S/I

Substrates	TS (g/L)	VS (g/L)	pH	Alkalinity (mg/L $CaCO_3$)	COD (mg/L)	TAN (mg/L)	VFA (mg/L)
IN/32	17.60	11.80	7.81	1,750	1,085	45.5	364
AL/4.5(0.33)	17.15	11.30	7.86	2,625	1,351	51.77	301
AL/4.5(0.08)	18.17	11.85	7.83	2,500	1,092	52.17	421
BG/60(0.10)	18.45	12.49	7.76	2,875	805	51.19	309
AL + BG/17(3)	18.78	13.49	7.87	2,250	1,897	54.54	740
AL + BG/18(1.8)	19.85	13.98	7.86	2,000	1,568	55.48	678

Biogas production and methane yield

Measurements of cumulative biogas (CH_4 and CO_2) production showed that the highest total gas production was observed for AL + BG at a C/N = 17 and S/I = 3 (AL + BG/17(3)), followed by AL + BG at a C/N = 18 and S/I = 1.8 (AL + BG/18(1.8)) as shown in Fig. 2A. The methane content of the biogas showed little variation among all BMPs: 66 ± 6% in IN/32, 66 ± 7% in AL/4.5(0.33), 67 ± 6% in AL/4.5(0.08), 66 ± 8% in BG/60(0.1), 61 ± 8% in AL + BG/17(3) and 62 ± 6% in AL + BG/18(1.8). Therefore, the volume of methane produced followed the same trend as the total biogas produced (Fig. 2B). A comparison of methane yield results is shown in Table 3. At $p < 0.05$, significantly higher cumulative methane yields were obtained from AL + BG/17(3) (145 ml methane/gVS) than AL + BG/18(1.8) (101 ml methane/gVS), compared with very low cumulative methane yields observed in IN/32, AL/4.5(0.33), AL/4.5(0.08), BG/60(0.1).

The high biomethane production at C/N ratios of 17–18 and S/I of 1.8 and 3 indicate that both C/N ratio and S/I positively affected biogas production. Note that variations in S/I ratios were allowed to achieve C/N ratios (17–18 in AL + GB) close to the optimal range for AD between 20 and 30 [2, 12]. S/I values applied were 1.8 in AL + BG/18 and 3 in AL + BG/17, which were within the range previously been reported to provide the best bio-methane production results of 1 to 3 [33, 35–37].

Even though substrates of AL + BG were found to produce higher biogas and methane yields (Table 4), methane yields of 145 mL/g VS as observed in AL + BG/17(3) remains low compared to the theoretical methane yield estimated at about 575 mL CH_4 g^{-1} VS [7]. Therefore, while maintaining S/I at an optimal range between 1 and 3, increasing the C/N ratios of 17–18 as set in AL + BG substrates to the optimal range (between 20 and 30) can significantly increase biogas production and methane yields in BMPs compared to what was obtained in this study during a 67 day experiment. By increasing C/N ratio from 8.5 to 24.7 in biomass of *Fucus serratus* (macroalgae), Tedesco and Daniels [38] found that the methane yield per gram in VS increased by up to 70%.

Table 3
Comparison of methane yields showing differences in p-values between BMPs. Note: *, significant difference at $p < 0.05$

BMPs	IN/32	AL/4.5(0.33)	AL/4.5(0.08)	BG/60(0.10)	AL + BG/17(3)	AL + BG/18 (1.8)
IN/32	1					
AL/4.5(0.33)	0.292	1				
AL/4.5(0.08)	0.936	0.847	1			
BG/60(0.10)	0.665	0.990	0.993	1		
AL + BG/17(3)	0.0001*	0.0001*	0.0001*	0.0001*	1	
AL + BG/18(1.8)	0.0001*	0.0154*	0.0003*	0.0022*	0.0265*	1

The presence of sugarcane bagasse in substrates containing algae helped balance C/N ratio and produce more biogas in the BMP bottles (Fig. 2). Indeed, AL/4.5(0.33) and BG/60(0.1) produced more biogas compared to AL/4.5(0.08). Moreover, even though substrates with C/N = 17 and S/I = 3 produced more biogas compared to those with C/N = 18 and S/I = 1.8, both AL + BG/17(3) and AL + BG/18(1.8) produced significantly higher biogas compared to BG/60(0.1) and the other BMP sets. In addition, the portion of algae biomass in substrates can also have positive effect on biogas production. Except for AL/4.5(0.33), algae biomass in the substrate positively affected the production of total gas and thereby methane yield. Therefore, a significant correlation ($r^2 = 0.88$, $p = 0.012$) between content of algae biomass in the substrate and total gas production was observed.

The MGM has been used to fit the cumulative biogas or methane production curves for AD of different substrates in batch studies [30]. Anaerobic co-digestion of microalgae and sugarcane bagasse showed a two-phase cumulative biogas production curve (Fig. 2A). There is initial biogas production, which ceases temporarily, and after a plateau phase, biogas production resumes. After setting up the BMPs, the first measurement on day 3 showed that only a small amount of biogas was produced in all BMPs, after which biogas production continuously increased. Therefore, we can assume that the lag phase for all BMP sets was about three (3) days. During this stage, also called acclimation phase, the microbes are adapting to their environment and the adapted cells begin to produce biogas [39]. The relatively very few days for the lag phase is due to inoculum that was already active and producing biogas during its incubation at 35°C. According to biogas production, AL + BG/17(3) and AL + BG/18(1.8) showed the highest cumulative gas yield during the process (Table 4).

Table 4
results from MGM showing the cumulative biogas yield (mL/g VS) during BMP process. Note: treatments represent the contents of the six different BMP sets, Y represents the cumulative biogas yields (mL/g VS) and the numbers in parenthesis represent the time of the BMP process in days.

Treatments (BMPs)	IN/32	AL/4.5(0.33)	AL/4.5(0.08)	BG/60(0.10)	AL + BG/17(3)	AL + BG/18 (1.8)
Y(7)	11.89	26.78	26.56	18.93	72.01	52.38
Y(21)	24.62	53	36	43.49	141.85	102.38
Y(67)	46.32	82.3	61.75	74.02	212.66	147.76

In the same way, considering the two BMP sets AL + BG/17(3) and AL + BG/18(1.8) which produce high biogas during BMP process, 67 and 69% of the total methane was produced during the first 21 days, respectively (Table 5), while over 40 days, ~ 90% of the total cumulative methane was obtained in the two BMPs. During biogas production, the objective is to determine the maximum volume of methane to be generated from a substrate, the longer the SRT the higher the overall methane production and reduction of biodegradable material [39]. Therefore, we assume that from this study, as more than 50% of the total methane was produced in 21 days, at least 21 days should be applied as an SRT for an acceptable biogas production and during anaerobic co-digestion of microalgae and sugarcane bagasse. Closer SRT (28 days) was used by Lee et al. [40]. In the same way, Xie et al. [41] noticed that the biogas production increased gradually after 35 days indicating that the SRT was also a key impact factor at high organic loading due to acclimation.

Table 5
Percentage of cumulative methane volume during time vs total produced methane volume in AL + BG/17(3) and AL + BG/18(1.8)

Time (Days)	0	7	10	16	21	34	40	46	52	58	67
% of cumulative methane in AL + BG/17(3)	0	31.52	43.82	56.47	67.1	83.32	88.79	92.73	95.66	98.08	100
% of cumulative methane in AL + BG/18(1.8)	0	32.63	43.82	56.96	69.33	85.29	89.66	93.68	96.48	98.3	100

Physio-chemical parameters

TS and VS during co-digestion

Reduction of TS and VS in all BMP bottles over the 67-day study is shown in Fig. 3. TS and VS reductions were significantly higher for BMPs containing both algae and bagasse (AL + BG/17(3) and AL + BG/18(1.8)) than those without AL and BG ($p < 0.05$). VS reduction is considered as an important parameter in

understanding the fate of organic matter in AD systems, indicating the degree of biodegradation and metabolic status [19]. During AD for biogas production, VS are degraded and converted into biogas and the degree of stabilization is often expressed as the percent VS reduction [36].

With about 45% VS reduction in AL + BG/17(3) and AL + BG/18(1.8) over 67 days, conditions during co-digestion of algae and bagasse could be improved for greater methane production. The inoculum was acclimated at 35°C and algae biomass was pretreated by incubation at 90°C for 1h. Pretreating algal biomass results in improved methane production [21]. Nevertheless, pretreatment of sugarcane bagasse has been suggested by several authors [15, 19, 21] because of its high cellulose and lignin content. Therefore, thermal pre-treatment of sugarcane bagasse could help facilitate digestion of VS leading to greater methane production during the process.

Chemical characteristics during co-digestion

Both pH and alkalinity values were in the optimal ranges for methane production, as shown in Fig. 4. pH plays an important role in partitioning between FA and ammonium. Therefore, maintaining a stable near-neutral pH reduces inhibition due to FA toxicity [35]. Alkalinity varied during the process but remained around the initial values in BMP substrates (Fig. 4B). Depletion of alkalinity to a level that can inhibit biogas production [8] was only observed in BMP containing only sugarcane bagasse (BG) at a C/N = 60, which is greater than the optimal range for biogas production.

Figure 5 shows an increase in COD in all BMP substrates from day 0 to 7, followed by significant decrease until the end of the process on day 67. The increase in COD at the start of the process resulted in relatively greater production of biogas in the BMPs. This is due to the lag phase, which represents the delayed response of the microbes and the combined action of extracellular enzymes and acetogenic bacteria converting the complex primary polymers (e.g., carbohydrates and proteins) into soluble organic compounds, and further into various intermediate products in the hydrolysis and acidogenesis steps [42, 43]. However, between day 7 and 21, COD was significantly removed by methanogenic activity, resulting in increased biogas production. After day 21 the considerable reduction in COD in BMPs consequently lowered biogas production.

Figure 6A shows an in initial increase in VFA at the beginning of the process in some BMP substrates, such as IN/32, AL/4.5(0.33), AL/4.5(0.08), BG/60(0.1), most likely due to high rates of hydrolysis and low initial methanogenic activity [40]. However, after the first week, the VFA concentrations leveled off or declined. VFA and FA both are potential inhibitors of the AD process.

Total ammonium nitrogen (TAN) concentrations in all BMPs followed similar trends (Fig. 6B). An increase was initially observed due to release of ammonia from nitrogen containing organic matter between days 0 to 21 followed by a decrease after day 21 until the end of the study on day 67 due to microbial assimilation. Chen et al. [8] reported that inhibitory TAN concentrations between 1.7 to 14 g/L caused a 50 %reduction in methane production. Therefore, TAN in all BMP bottles that ranged from 45 to 120 mg/L were suitable for biogas production.

Even though the concentrations of potential inhibitors were in the healthy range for biogas production, sugarcane bagasse could have had negative effect on algal biomass co-digestion due to the high content of cellulose and hemicellulose associated with lignin and the low cellulase activities present in digesters [15, 19]. Thermal, chemical, ultrasonic, thermo-chemical and biological pretreatment or solid-state fermentation (SSF) have been reported to enhance hydrolysis of lignocellulosic wastes [21]. As the sugarcane bagasse used in this study was not pre-treated, the low bioavailability of the sugarcane bagasse may have limited biogas production. Nevertheless, the combination of acclimated inoculum [40], co-digestion of algae biomass and sugarcane bagasse resulted in higher methane production compared to the other substrates such as only algae biomass (AL) or only sugarcane bagasse (BG).

Conclusions

Biomass generated from algal-bacterial treatment of wastewater has low C/N ratio (< 10) while anaerobic digestion for optimal biogas production requires an optimal C/N ratio ranging from 20 to 30. Co-digestion using sugarcane bagasse as a carbon rich substrate was able to balance the C/N ratio of algae biomass to achieve ratios close to the desired values for biogas production. After 67 days of AD, BMPs with AL + BG/17(3) and AL + BG/18(1.8) as substrates produced more biogas than digestion of either algal biomass or sugarcane bagasse alone. Although methane content of the biogas was similar for all BMPs, the combination of algal biomass and bagasse resulted in the highest methane yields (145 and 101 mL/g VS). However, pretreatment of sugarcane bagasse could help enhance VS reduction and biogas production and result in a shorter required SRT for co-digestion algae and sugarcane bagasse. Furthermore, S/I ratios between 1 and 3 and C/N ratios between 20 and 30 simultaneously should be considered to enhance methane production and yield. More research is needed on algae biomass and sugarcane bagasse as potential resources for biogas production in sub-tropical and tropical regions.

Declarations

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Competing interests

The authors have no relevant financing or non-financing interests to disclose.

Authors contributions

Bilassé Zongo and Sarina J. Ergas contributed to the study conception and design. Bilassé Zongo and Sahand Iman Shayan performed material preparation, data collection and analysis. The first draft of the manuscript was written by Bilassé Zongo and all authors reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

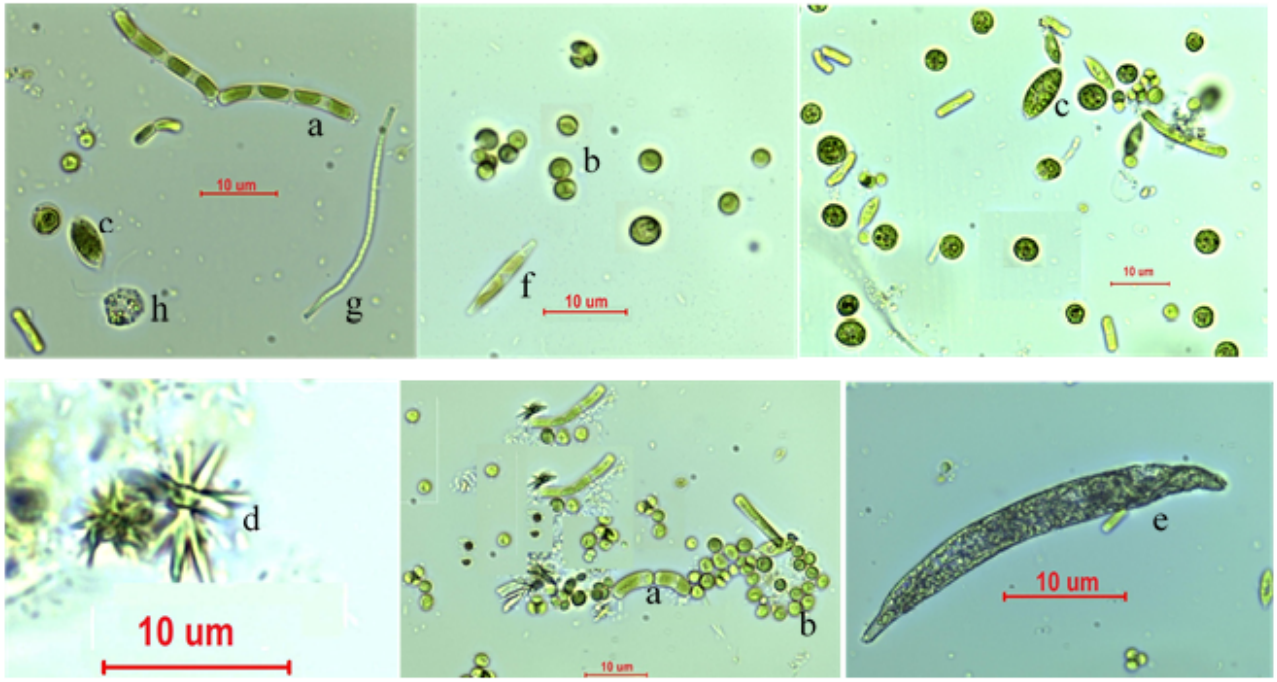


Figure 1

Algal strains in algal biomass: a. Zygnemopsis sp., b. Chlorella vulgaris, c. Scotiellopsis oocystiformis, d. Actinastrum aciculare, e. Euglena sp., f. Navicula sp., g. Pseudanabaena catenate, h. Dunaliella salina

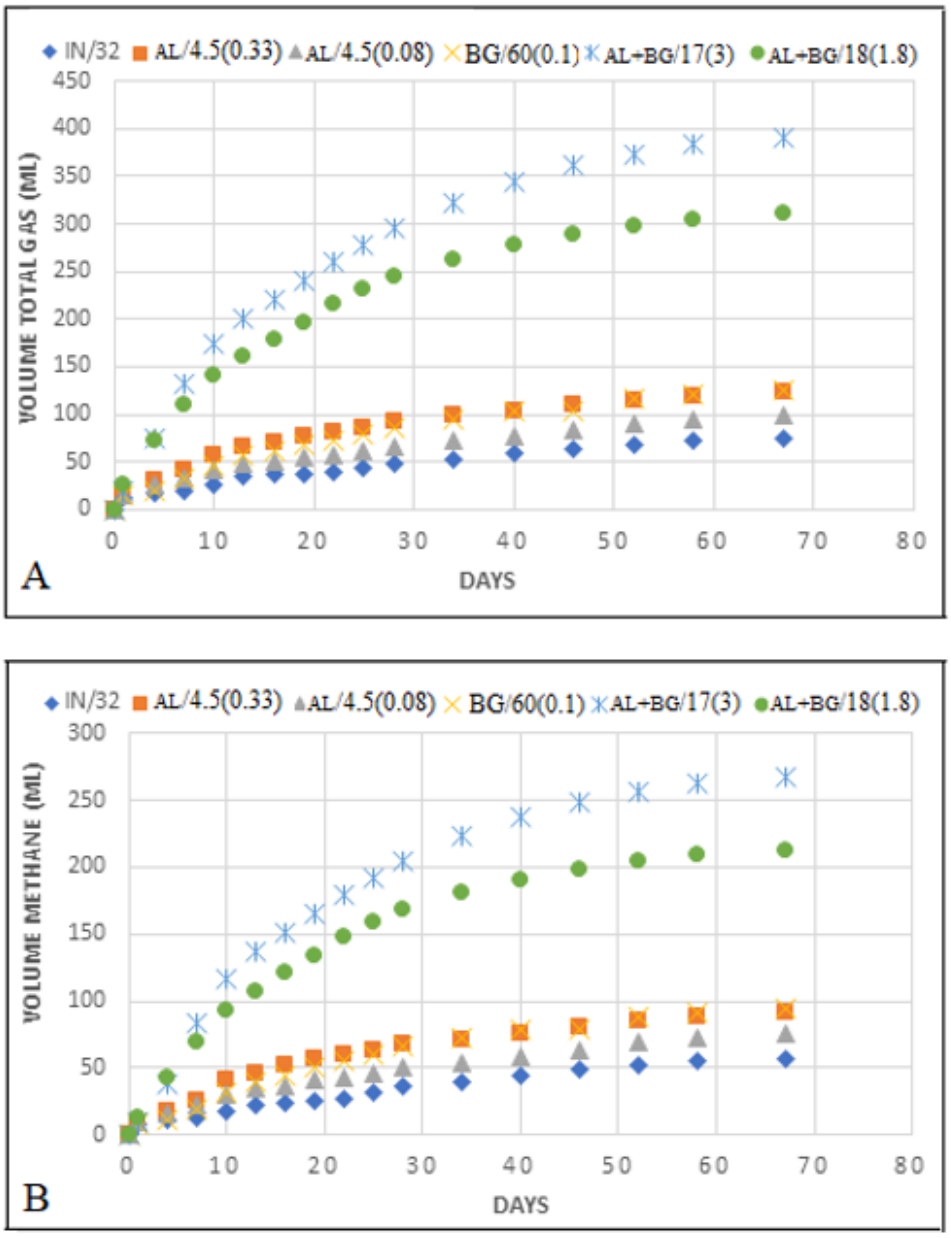


Figure 2

total gas (A) and methane production (B) during the process

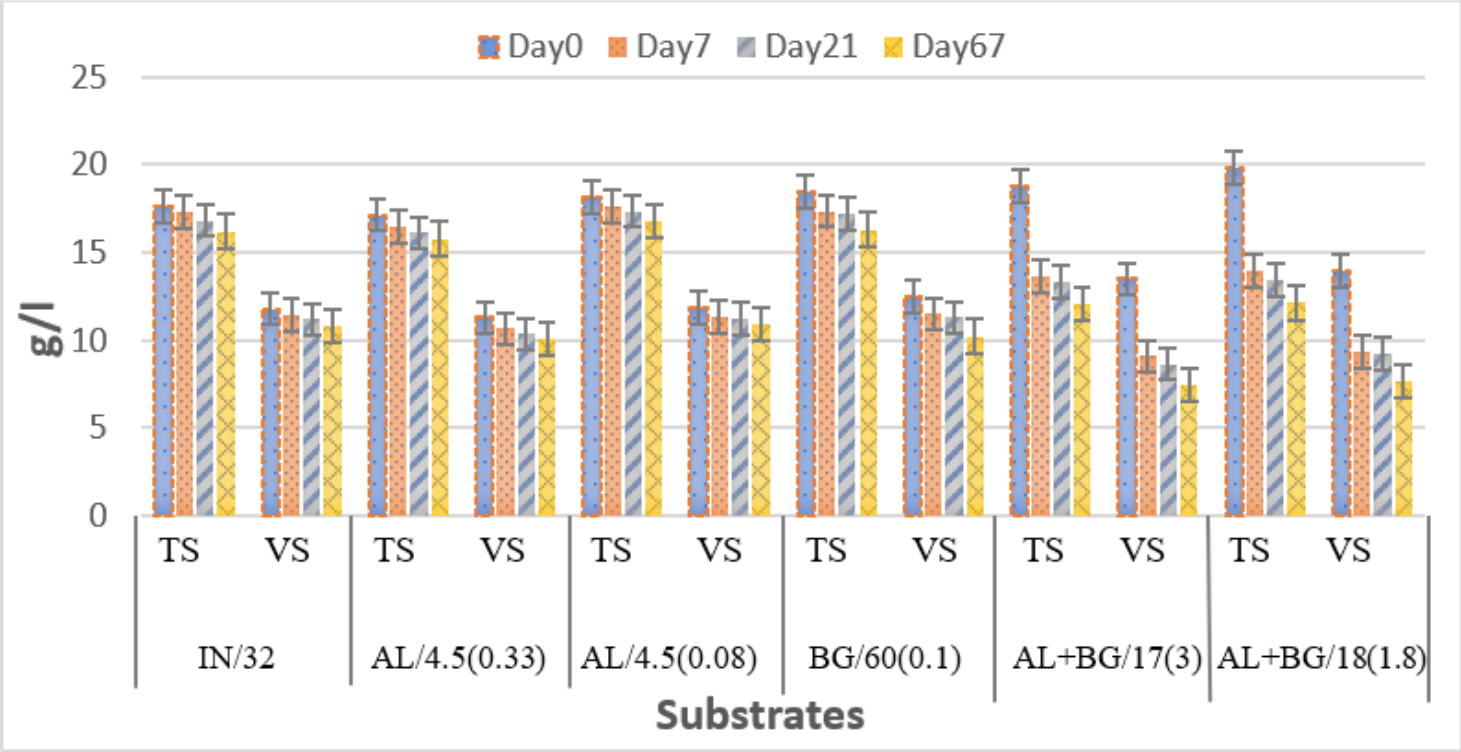
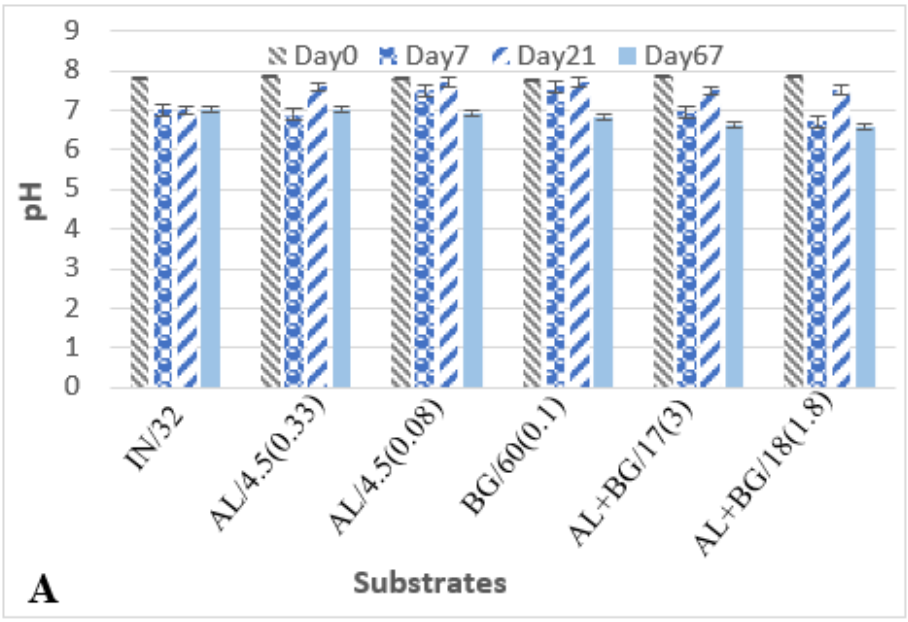


Figure 3

Variation of TS and VS during the AD process: IN, inoculum, AL, Algae, BG, Sugarcane Bagasse, values after substrate composition indicate initial C/N ratios and S/I



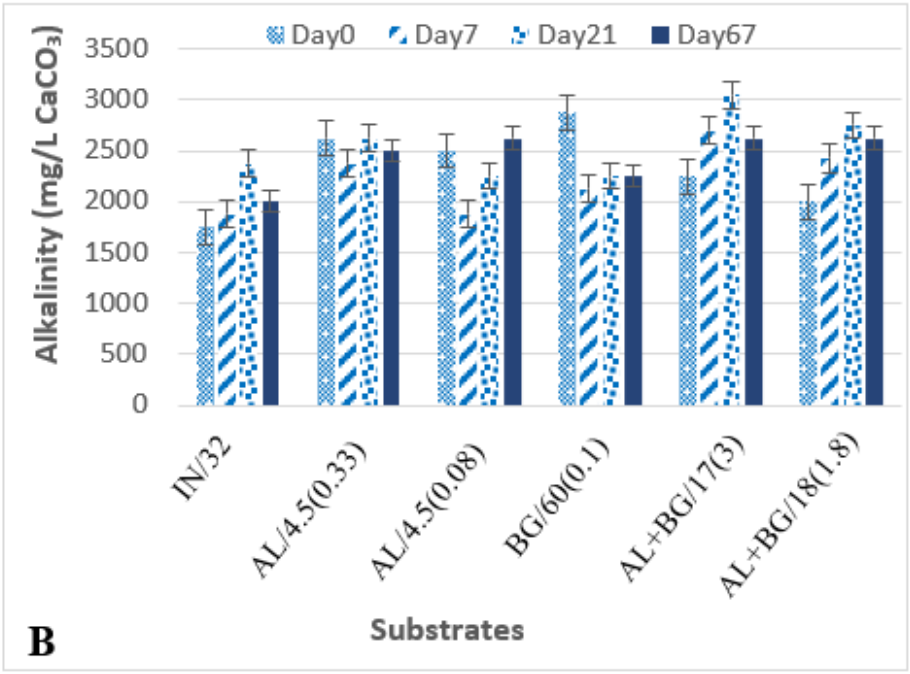


Figure 4

Variation of pH and alkalinity during the process: a, pH, b, Alkalinity

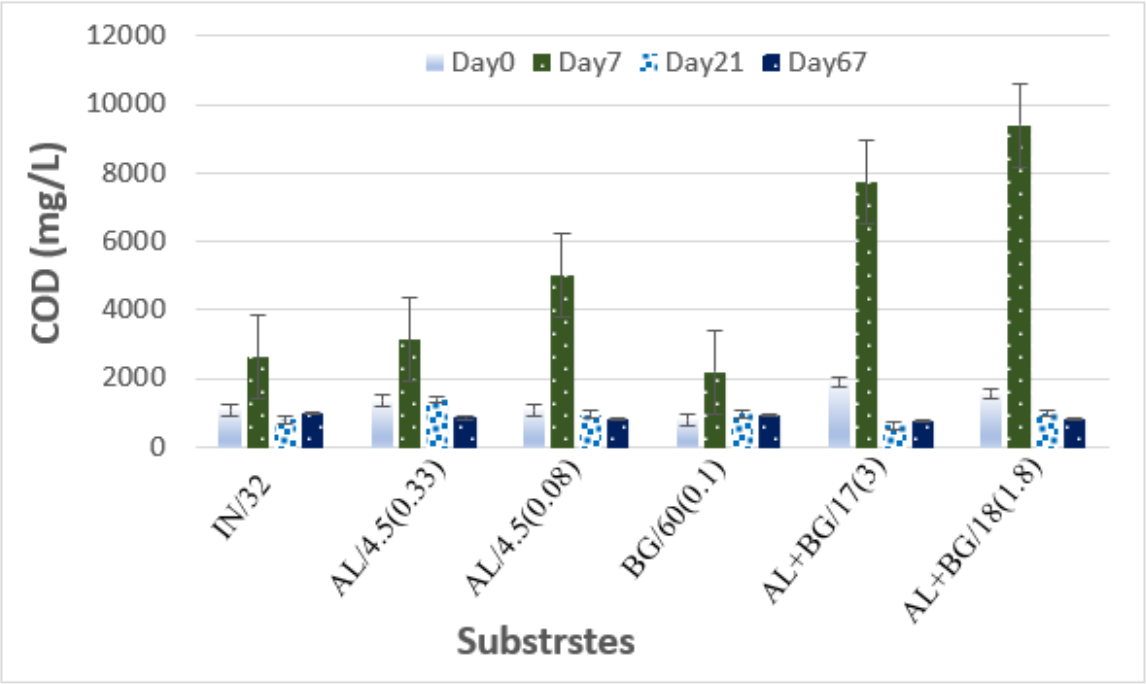
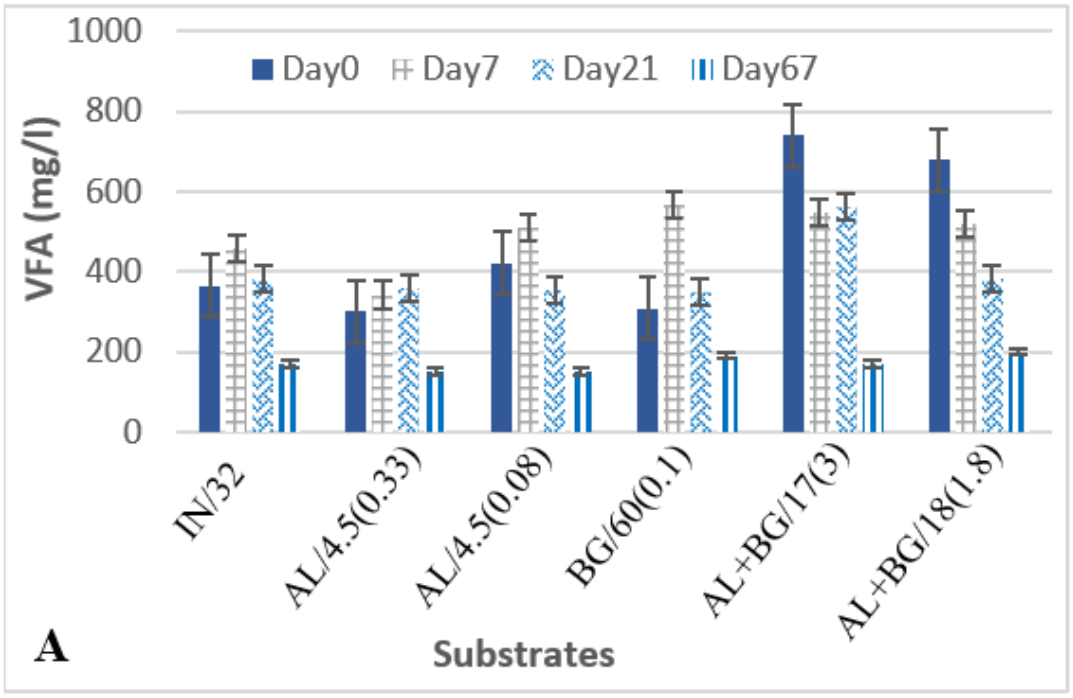


Figure 5

Variations in COD during the process



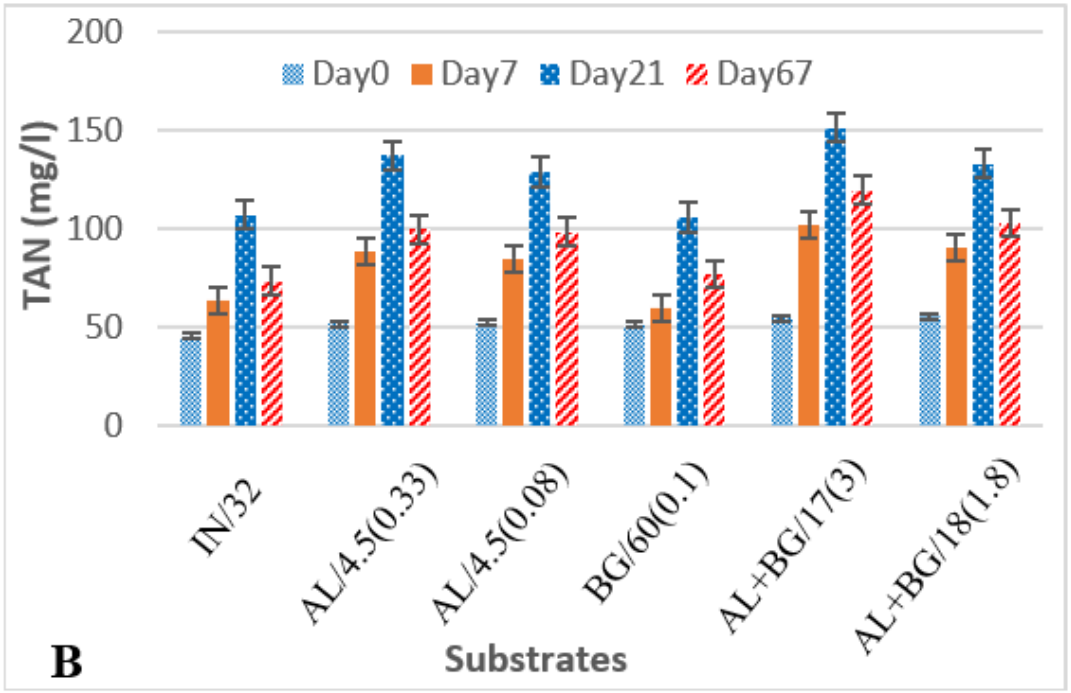


Figure 6

Variations in VFA (A) and TAN (B) during the process