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Boosting biogas yield of anaerobic digesters by utilizing concentrated molasses from 2nd generation bioethanol plant

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Abstract
Concentrated molasses (C_5 molasses) from 2nd generation bioethanol plant has been investigated for enhancing productivity of manure based digesters. A batch study at mesophilic condition (35±1°C) showed the maximum methane yield from molasses as 286 LCH_4/kgVS which was approximately 63% of the calculated theoretical yield. In addition to the batch study, co-digestion of molasses with cattle manure in a semi-continuously stirred reactor at thermophilic temperature (50±1°C) was also performed with a stepwise increase in molasses concentration. The results from this experiment revealed the maximum average biogas yield of 1.89 L/L/day when 23% $VS_{molasses}$ was co-digested with cattle manure. However, digesters fed with more than 32% $VS_{molasses}$ and with short adaptation period resulted in VFA accumulation and reduced methane productivity indicating that when using molasses as biogas booster this level should not be exceeded.

Keywords: Molasses; 2nd generation bio-ethanol plant; Anaerobic digesters; Biogas yield.

1. Introduction

The overwhelming dependence on fossil fuel and the escalating greenhouse gas emissions are the two concerns heavily contributing in rearranging most of the energy policies worldwide. In response to that, European energy council, has set a target of 20% renewable energy in proportion of total energy consumption and 10% bio-fuels in proportion of total fuel consumption by the year 2020 [1]. Coupled with policies and regulations, technologies encompassing renewable energy have also been diversified. Deploying lignocellulosic biomass for the production of bioethanol (2nd generation bioethanol) [2], is one example in that direction.

Conventionally, ethanol as a vehicle fuel is produced from different sources of biomass(sugar cane, corn, gain, rice etc), predominantly containing lower and higher carbohydrates [3]. Bioethanol plants dealing with biomass rich in sucrose and starch are termed as first generation plants [2]. Although majority of the World's ethanol is processed in first generation bio-ethanol plants, their negative impact to the environment has recently been brought into serious consideration. Competition with food or feed for fertile land and thereby increasing food prices is one of the long lasting dilemmas in regards of 1st generation ethanol industries [4]. Issues like eutrophication and acidification caused by high energy fossil fuel input for fertilization of ethanol feedstocks are also believed as the outcome of such ethanol

plants [5]. Avoiding these limitations yet maintaining continuously rising ethanol demand is a challenge to combat for which alternative solution is necessary. Second generation bio-ethanol plant (primarily based on agricultural and industrial residues), is potentially offering the solution of these burning issues of food or fuel while providing opportunities for the treatment of low value wastes and therefore is expected to play a vital role in the coming years.

Rooted in the notion of 2nd generation bio-ethanol plant, from the year 2003 and onward Inbicon A/S, Denmark developed the EU project idea of Integrated biomass utilization system (IBUS) [6] to convert lignocellulosic biomass into bio-ethanol. Since inception, extensive effort has been paid for the further improvement of its different aspects and now reached to the edge of a commercial reality [6]. Principally, the Inbicon A/S plant produces bio-ethanol from wheat straw by five processing steps i) pre-treatment ii) hydrolysis iii) fermentation iv) distillation and v) separation and uses solely steam and enzyme for the entire process. Pre-treatment, as an important part of this process itself is divided into two lines where one line is operating with lower capacity (100 kg biomass/hour) for the purpose of research, in contrast with the other with a higher capacity (1000 kg biomass/hour) for the purpose of mechanical development.

C_5 molasses as a by-product resulted from two of the above process streams. It is obtained as a residue either after pre-treatment or after separation. Characteristically, C_5 molasses is different depending on the point they are originated and on the qualities of the wheat straw it was derived from. Molasses originated after the pre-treatment unit was concentrated by the evaporation of water to enrich in dry matter content and used for this study. Previously, molasses was primarily used for animal feeding. But considering its storage potential and high degradability, it is recently exploited for anaerobic digestion also. Anaerobic digestion of molasses with a low dry-matter content of 4.4% that derived from the processing stream as described by Thomsen et al, 2008 was documented by Kaparaju et al, 2009 [7]. However, biogas production from molasses with a very high dry matter content (58%) has not been investigated before to the present knowledge of the authors.

Substrate with high dry-matter content is generally suitable for co-digestion which treats two or more materials with complementary attributes. Despite several advantages that include higher biogas production, lesser inhibition as well as higher buffering [8], the successful adoption of co-digestion strategy is challenged by the issue of scarcity of concentrated biomass that can be stored and utilized all around the year to meet the seasonal variation in energy demand. Biogas plant connected with CHP (combined heat and power plant) is typically designed for base load due inadequacy of the material characterized to boost the energy production when peak load is demanded. Generally, peak load is met from other source of energy often in fossil fuel nature. However, major effort has strongly been applied to substitute this concept and by displacing fossil fuel from the fuel renewable in nature. Considering this, the feasibility of utilizing concentrated C_5 molasses for biogas production and short term boosting of methane yield was examined in semi-continuously fed reactors. Together, the methane potential of C_5 molasses was measured in batch study.

2. Materials and methods

2.1 C_5 molasses

C_5 molasses, a by-product of a bio-ethanol industry [6], was obtained from a second generation bio-ethanol demonstration plant (Inbicon A/S, Kalundborg, Denmark) and used as a substrate for co-digestion in this study. It contains a high amount of oligosaccharides and sugars due to the breakdown of hemicelluloses during processing of input biomass (wheat straw). The physical and chemical properties of molasses (C_5 molasses) are given in Table 1.

2.2 Dairy cattle manure

Dairy cattle manure (DCM) was obtained from slurry reception tank at Research Center Foulum, Denmark, during February until March 2011. The average properties of slurry, collected several times during the experimental period, were: pH=7.7±0.5; Total Nitrogen =3.6±0.6%; Total Solid (TS) = 8.7±0.6, Volatile Solid (VS) =7.5±0.3 and Total ammonia nitrogen (TAN) =1.91±0.2 respectively.

2.3 Inoculum

Two types of inoculum was used for this study, thermophilic inoculum for the continuous reactors and mesophilic inoculum for the batch reactors. Effluent from main digester of biogas plant at research center Foulum (Denmark) was employed as thermophilic inoculum. The main digester operates at thermophilic

temperature (50±1.0°C) and treats various materials such as pig manure, cattle manure, maize silage and industrial wastes together. Average TS, VS, pH and TAN of thermophilic inoculum was measured as 3.6±0.5% , 2.2±0.5%, 8.3 and 0.5g/L respectively. Mesophilic inoculum, on the other hand, was collected from the same facility however from post digester tank where the digested slurry from the main reactor had been stored at a temperature of 35±0.5°C for further de-gasification. The properties of mesophilic inoculum when measured were TS=2.83±0.5%, VS=1.43±0.5%, pH= 8.1±0.4 and TAN=1.82±0.5 g/L respectively.

Table 1. Properties of C_5 molasses

Properties	Amount
Density (L/kg)	1.3
pH	4.2
TS (% w/w)	58.1
VS (% w/w)	43.0
Ash (% of TS)[a]	26.0
Ash (% of TS)[b]	19.0
Lipids (g/kg TS)	0.197
VFA (g/l)	25.4
Acetate (g/l)	24.8
Propionate (g/l)	0.412
Total Nitrogen (g/kgTS)	5.6
Protein (g/kgTS)[3]	35.0
Total P (g/kg TS)	1.3
Klason Lignin (g/kg TS)	4.3
Furfurals (g/l)	<0.5
5HMF (g/l)	<0.5
Phenols (g/kgTS)	0.88
Dietary fiber (g/kg TS)	54.0
Carbohydratres (mg/kg TS)	286.0
Fructose (g/kg TS)	1 (1^1, 0^2)
Arabinose (g/kg TS)	18 (18^1, 0^2)
Xylose (g/kgTS)	132 (82^1, 50^2)
Glucose (g/kgTS)	111 (56^1, 51^2)
Mannose (g/kgTS)	14 (7^1, 7^2)
Galactose (g/kgTS)	11 (10^1, 1^2)
Potassium (K^+) (g/kgTS)	32.0
Chloride (Cl^-) (g/kgTS)	6.3
Sodium (Na^+) (g/kgTS)	48.2
Magnesium (Mg^{2+}) (g/l)	1.5
Calcium (Ca^{2+}) (g/l)	4.5
Phosphorous (P^{2+}) (g/l)	1.3
Copper (Cu^{2+}) (g/l)	0
Manganese (Mn^{4+}) (g/l)	0.044
Zinc (Zn^{2+}) (g/l)	0.03
Iron (Fe^{3+}) (g/l)	0.92

[1] the monosaccharides present in sugar analysis

[2] the polysaccharides present in sugar analysis

[3] protein = 6.25 x total nitrogen

[a] incomplete evaporation of water from the sample analyse

[b] complete evaporation of water from the sample analysed

2.4 Methane potential of C_5 molasses

The biological methane potential of molasses (C_5 molasses) was estimated by batch assay as described by Møller et al. [9] and complied with the international standard ISO 11734 (1995) . The triplicate batch tests were conducted in 500 ml total volume infusion bottles for two different substrate concentrations. The inoculum to substrate ratio (VS/VS) for those two concentrations was 1:0.67 and 1:1.33 respectively which was prepared by adding 10 gram of molasses in 200 grams of inoculum for one set of bottles and 5 gram of molasses in 200 grams inoculum for other set of bottles. After inoculation, the glass bottles were flushed with pure N_2 for 5-10 minutes. The bottles were then closed with butyl rubber stoppers and sealed with aluminium screw tops and incubated for 90 days at 35±0.5°C. The assays with inoculum, typically defined as control, were also prepared to determine the biogas production from inoculum alone. Methane and biogas production from the batch tests were periodically measured, by using water displacement method, ten times in total during the whole incubation period. Water is acidified in the water displacement method (Figure 1) to reduce CO_2 solubility. To determine actual potential, produced methane from the samples was corrected from that produced by the inoculum alone. Theoretical methane yield (m^3/kgVS) was calculated on the basis of stoichiometric conversion of organic matter to methane and carbon dioxide as given below [7]:

$$B_u = \frac{0.415Carbohydrates + 0.496Proteins + 1.014Lipids + 0.373Acetate + 0.530Propionate}{(Carbohydrates + Proteins + Lipids + Acetate + Propionate)}$$

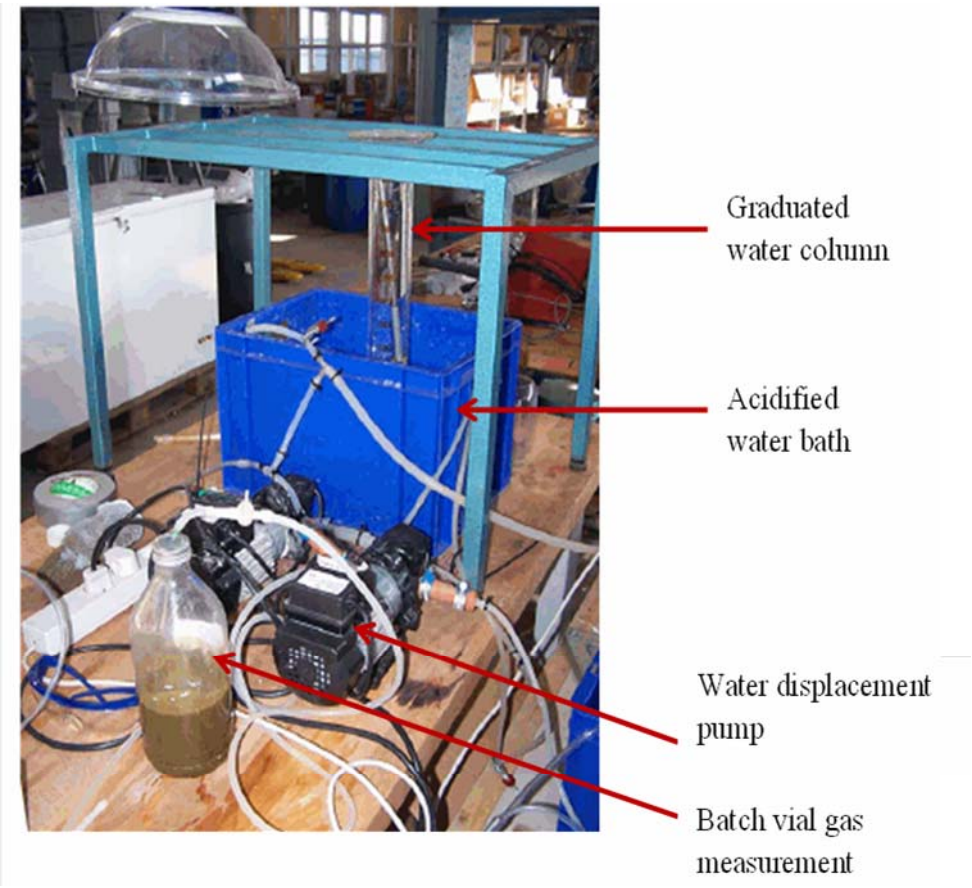


Figure 1. Water displacement method and measurement of biogas

2.5 Reactor experiment

The experiment was conducted by parallel running of two continuous reactors (CR), each with a capacity of 10 liters and 7 liters working volume, operated with 17 days hydraulic retention time (HRT). Reactors was stirred manually during feeding and collection of effluent and placed in an incubator where the temperature was maintained at 50±1°C.

Both the reactors were filled with 6.6 kg inoculum and 0.4 kg cattle manure during start-up. R(CM) was operated as a reference (control) reactor and was run with cattle manure throughout the experimental

period. R(CM+M), on the other hand, was the tested reactor undergone for mixed feeding of C_5 molasses and cattle manure. The experiment start-up (period 1, days 0-21) where R(CM) & R(CM+M) were fed with DCM alone so that stable performance between the reactors was achieved (average data is presented in Table 2). The stabilization period of this experiment is in accordance with other study, exemplified as maximum 5% variation of biogas production between the reactors [10].

After stabilization, molasses was introduced to R(CM+M) and between day 22 to day 38 (period 2), 10 grams of molasses was added with 390 grams of cattle manure. This, in terms of added VS, corresponded to the feeding ratio of 13:87 ($VS_{molasses}$: $VS_{cattle\ manure}$) representing 11% increase (4.3 to 4.8 gVS/L/d) in total OLR (Table 2). In the following period (day 39-51), the concentration of C_5 molasses was doubled where 20 grams of molasses was combined with 380 grams of cattle manure so that the feeding ratio in terms of added VS reached as 23:77 ($VS_{molasses}$: $VS_{cattle\ manure}$). This consequently raised the total OLR to 23% (5.3 gVS/L/d) from the start. C_5 concentration was further increased and each day in period 4 (day 52-71) 30 grams of molasses was mixed with 370 grams of cattle manure which simultaneously changed the corresponding feeding ratio to 32:68 ($VS_{molasses}$: $VS_{cattle\ manure}$) and resulted the increase of total OLR close to 35% (5.8 gVS/L/d) since the experiment was started. The entire feeding scheme from period 1 until period 4 was maintained for 17 days HRT (Table 2) by keeping total feeding and total extraction of materials from the digesters at the same volume.

Feeding was carried out once in a day by pouring substrate through the opening of a hollow tube which extends below the liquid level in order to prevent air trapping in the headspace. The opening was normally sealed by a rubber stopper before and after the feeding. Effluent was collected from the other opening at the lower end of the reactor wall which was also kept sealed except the instances when materials were removed. The Process performance was monitored by analyzing TS, VS, pH, VFA, gas production and gas composition of effluent and raw-materials on a regular interval.

Table 2. Governing parameters of Continuous reactor experiment (thermophilic, 50°C)

Parameters	Days of operation							
	0-21		22-38		39-52		53-70	
	R1	R2	R1	R2	R1	R2	R1	R2
Feed ratio (VS molasses :VS CM)	0:100	0:100	0:100	13:87	0:100	23:77	0:100	32:68
OLR of molasses (gVS/L/d)	0	0	0	0.61	0	1.23	0	1.84
Total influent OLR (gVS/L/d)	4.3	4.3	4.3	4.8	4.3	5.3	4.3	5.8
Biogas production (mL/day)	8230	8640	8954	10201	10754	13261	10164	12584
Biogas production (mL/gVS)	275	282	299	304	358	358	339	309
HRT (days)	17	17	17	17	17	17	17	17
Methane yield (mL/L/d)	694	728	755	845	906	1080	857	971
Methane yield (mL/gVS)	162	164	177	176	212	204	202	165
Methane composition, %	59	59	59	58	59	57	59	54
VFA (g/L)	-	-	0.42	0.5	0.5	1.22	0.94	3.47
TAN (g/L)	1.9	1.85	1.98	2	2	2.02	2.1	2
pH	8.03	8.05	8.16	8.24	8.02	8.06	8.08	8.05

2.6 Analytical methods

TS of cattle manure and C_5 molasses was measured after drying samples for 24 hours at 105°C. The dried samples were further heated at 550°C for 5±0.5 hours to determine ash content. VS was calculated by subtracting the amount of ash from the amount of TS [11]. pH was measured by using a glass pH probe (Knick Portamess, 911 pH, Germany) while total nitrogen was determined by using the standard Kjeldahl method [12] and a Kjell-Foss 16200 auto analyzer (Foss Electric, Hillerød, Denmark).

For volatile fatty acid (VFA) analysis, 1mL of sample was acidified with 4mL of pivalic acid and then centrifuged for 10 minutes at 12,000 rpm and afterwards filtered with 0.45µm filter before measuring on Gas Chromatograph (Hewlett Packard 6850A, USA) equipped with a flame ionization detector (FID) and HP-INNOWax column with a dimension of 30m x 0.25 mm x 0.25µm. The temperature of the column was gradually increased from 110°C to 220°C at the rate of 10°C/min. Helium (He) was used as carrier gas at a flow rate of 10 mL/min. Total ammonia nitrogen was analyzed colorometrically at 690®nm with Merck spectrophotometer (NOVA 60).

The produced biogas was accrued in aluminium coated plastic bags which were connected with the reactors through plastic tubes. Each reactor was joined with one aluminium coated plastic bag to facilitate gas collection and for subsequent volume and composition measurement. Collected gas was measured (for volume) on a daily basis by using acidified water displacement method. Gas samples were analysed (for composition) twice a week both for CO_2 and CH_4 content with a gas chromatograph (Perkin Elmer Clarus 500, USA) equipped with a Thermal Conductivity Detector and a Turbomatrix 16 Headspace auto sampler as described by Møller et al [9]. Methane and carbon dioxide was separated by using a 12' x 1/8" Haysep Q 80/100 column. The temperature of the injection port, oven and detector were 110, 40 and 150°C respectively. Helium (He) was used as a carrier gas with a flow rate of 30 mL/min.

Sugar (monosaccharides, polysaccharides, oligosaccharides etc), dietary fibres and klason lignin was analysed as described by Knudsen [13] whereas mineral analysis (primarily cations and anions) was conducted as according to Fang et al [14]. Fat was determined by using Danish standard infrared spectrometry method (DS/R 209:2006) whereas 5-Hyroxymethylfurfural (5-HMF), furfural and phenol were measured by adopting inductively coupled plasma (ICP) method.

3. Results and discussions

3.1 Characteristics of C_5 molasses

The physical and chemical characteristics of the studied molasses are presented in Table 1. The molasses was a by-product of the hydrothermal treatment (in 2nd generation bioethanol plant) of wheat straw and mainly composed with C_5- and C_6 sugars and alkali chlorides that was resulted from the lignocellulosic component of biomass. After analysis of molasses, average TS, VS, pH and total VFA content were recorded as 58%, 43%, 4.4±0.2 and 1.2 g/L respectively.

Sugar analysis revealed that the majority of sugars were xylose followed by glucose and arabinose (Table 1). Although not abundant, small amount of other sugars such as rhamnose (<1g/kgTS), mannose and galactose were also observed. Furthermore, analysis quantified approximately 0.5 g/L of 5-HMF (5-Hydroxymethylfurfural) (Table 1) that probably resulted from the degradation of hexose during hydrothermal pre-treatment [15]. The presence of 5-HMF in molasses was considered inhibitory to several microbial activities (enteric microorganisms) for fermentation [16] but favourable for anaerobic digestion when concentration is kept below 3 g/L [17].

Based on the chemical analysis, the concentration level of cations Mg^{2+},Zn^{2+},Fe^{2+} or Fe^{3+}, P^{2+},Cu^{2+},Mn^{4+} (Table 1) were found to be well within the acceptable ranges [14]. However, the concentration of other cations such as Na^{+}, K^{+}, Ca^{2+} (Table 1) was exhibited to be higher than the values previously published for normal molasses [14]. Na^{+}, Ca^{2+}, Mg^{2+} together with ammonia was reported [18] to present antagonistic behaviour for reducing ion inhibition. Fang et al. 2011 [14] however documented that sodium and potassium concentration of approximately 11 and 28 g/L jointly would be responsible for 50% methane inhibition whereas over 5g/L concentration of Ca^{2+} was found [19] as inhibitory for methanogenesis. These altogether are the potential reasons negatively influencing the choice of molasses with high dry-matter content as a substrate for mono-anaerobic digestion. However, co-digestion of molasses with cattle manure avoided some of these limitations, as demonstrated elsewhere in the present study.

3.2 Biological methane potential

The effect of molasses concentrations on biological methane potential is depicted by Figure 2. Methane yield was influenced by substrate concentration. For instance, where the maximum methane yield as an effect of low substrate concentration (24.4 gVS/L) was 286.3 L/kgVS, for the addition of higher concentration (34.1 gVS/L) it was 278.2 L/kgVS. Although the difference of these two yields was only about 8 L/kgVS (Figure 1), the pattern by which they developed was surprisingly different and characterized by the long lag phase due to the higher input of C_5. As illustrated in Figure 2, batch bottles fed with lower substrate concentration (24.4 gVS/L) gave approximately 86% of methane between day 0 to day 42 (246.42 L/kgVS) which gradually reached to maximum at day 93 (286.3 L/kgVS). On the contrary, experiment with higher feeding concentrations yielded merely 14% (39.9 L/kgVS) of methane between day 10 to 42 which thereafter swiftly rocketed to 278.2 L/kgVS until the end of the experiment at day 93.

Kaparaju et al [20] observed the similar phenomena, however, for a dissimilar experimental conditions where diluted wheat straw stillage (10.2% VS) was incubated (55°C) for about 65 days. High

concentration induced inhibition was also reported in several other research works such as by [21]. The low methane yield at high substrate concentration is possibly because of less dilution of inhibitory compounds that present in C_5 molasses. The long adaptation period of bacteria, on the other hand, was presumably responsible for the evolution of greater initial lag phase (Figure 2) when higher concentration feeding was adopted.

Calculation of experimental methane yield from batch assays revealed that approximately 65.3% of theoretical yield (438.39 L/kgVS as calculated) was realised, implying incomplete conversion partly due to the recalcitrant nature of organic contents or partly because of substrate and product inhibition as according to Mösche et al, 1999 [22].

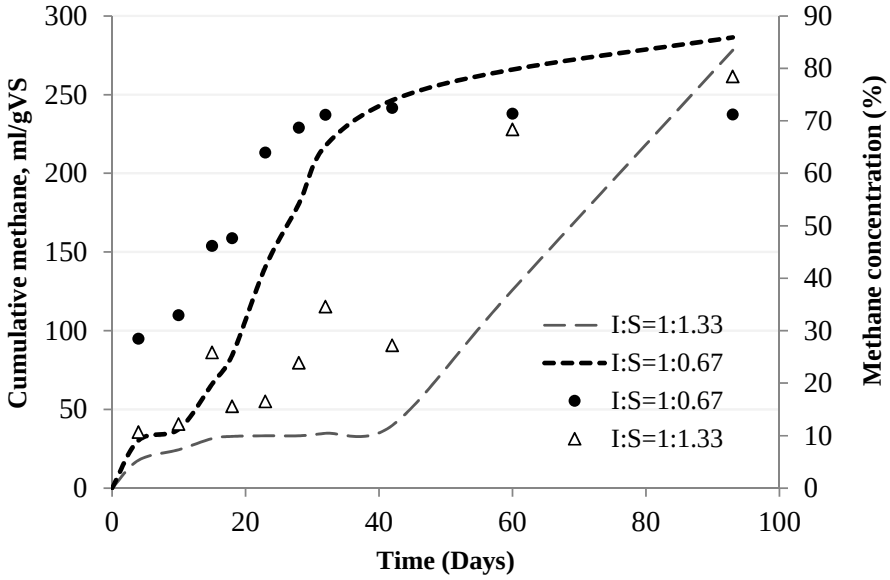


Figure 2. Cumulative methane yield vs methane concentration in batch experiment. Open triangles and closed circles are for methane concentration while black and gray lines are for cumulative methane yield

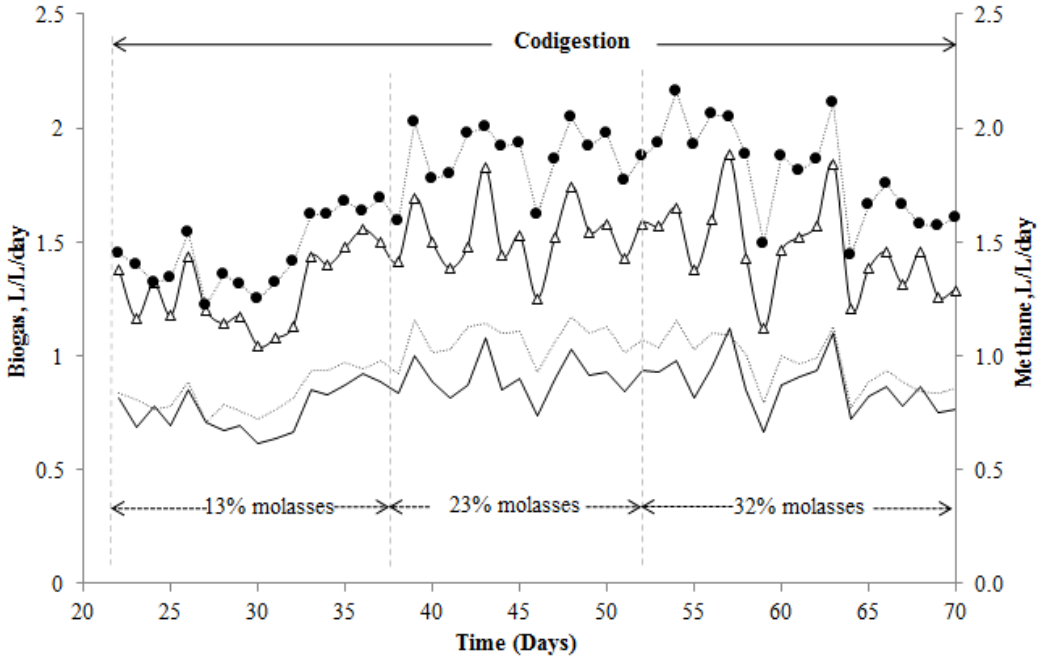
3.3 Continuous reactors (CR) experiment

The volumetric and weight based biogas and methane yields are demonstrated by Figure 3(a) & (b) while the effect of VFA and pH on feeding concentration is illustrated by Figure 4.

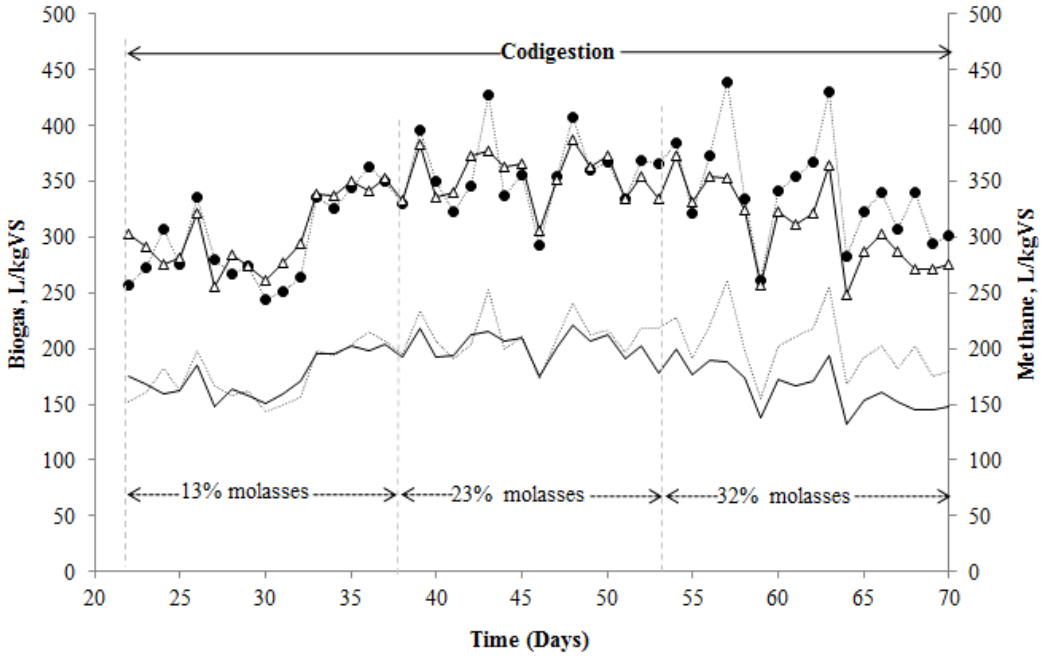
Co-digestion started at period 2 (day 22-38) when R (CM+M) was supplemented with molasses. There was an increase of about 14% daily volumetric yield of biogas (10.2 L/d) due to the addition of 0.61gVS/L/d molasses. The gas yield in terms of added volatile solid however was around 2% lower ($p>0.05$, $n=17$) than that from R(CM) (Figure 3(b)). Similar trend was observed for methane concentration which dropped approximately 1% ($p>0.05$, $n=7$) (from 59% to 58%; Table 2). Some accumulation of total VFA occurred in molasses reactor which rose about 19% than those from R(CM) (Figure 3(b)). pH was in the range of 8.16±0.2 (Figure 4) while average TAN was observed as 2±0.2g/L, indicating no serious inhibition at this stage.

In the following period (period 3) between day 39 to day 52, the concentration of molasses for R(CM+M) was further increased (0.61 to 1.23 gVS/L/d) to 23% of total added VS (Table 2) which yielded approximately 23% higher biogas (Figure 3(a)) than that from R(CM), in terms of unit reactor volume. In terms of volatile solid, however, identical ($p>0.05$, $n=14$) mean biogas yields (358 L/ $kgVS_{added}$) was observed. Generally, in this period, the average gas production from both the reactors increased, compared to the previous period. Although acceptable for R(CM+M), this implied the unexpected yielding pattern of R(CM), possibly resulted due to the variation in cattle manure properties that varied every time fresh manure was collected (three instances for this entire experiment) and stored. Stored cattle manure was reported to impact other parameters of anaerobic digestion also, such as for VFA [23]. In respect of methane composition, R(CM+M) was still showing the decreasing trend and resulted approximately 2% lower concentration ($p>0.01$, $n=5$) (Table 2) as compared to R(CM). There was a dramatic increase in total VFA which jumped to approximately 144% (1.22g/L) in contrast with control. VFA accumulation is tightly linked to OLR and expected to play a critical role in this period, as it (OLR) was increased close to 24% (Table 2). Noticeably, the major part of this total VFA in

R(CM+M) was acetic acid (0.64 g/L) that followed by propionic acid (0.51g/L) and trace amount of higher molecular weight VFAs. Total VFA from control, as increased from the previous period, also dominated by the concentration of acetic acid (0.40 g/L) and propionic acid (0.08 g/L) (Figure 4). This is not surprising as total VFA for digestion of cattle manure alone can reach to a range of 0.2- 3.8 g/L for a fair adaptation period of over 100 days [24]. Since VFA rose, pH for the experimental reactor dropped (8.02±0.6) from the previous period. Average TAN remained nearly unchanged (2.02±1.0 g/L) although the variation among the observed data (for TAN) was quite high.



(a) Specific volumetric yield. Closed circles: Biogas yield from R (CM+M); Open triangles: Biogas yield from R (CM); Dotted line: Methane yield from R (CM+M); Solid line: Methane yield from R(CM)



(b) Specific yield in terms of VS. Closed circles: Biogas yield from R (CM+M); Open triangles: Biogas yield from R (CM); Dotted line: Methane yield from R (CM+M); Solid line: Methane yield from R(CM)

Figure 3. Specific biogas and methane yield from continuous reactors in terms of volume and volatile solid addition

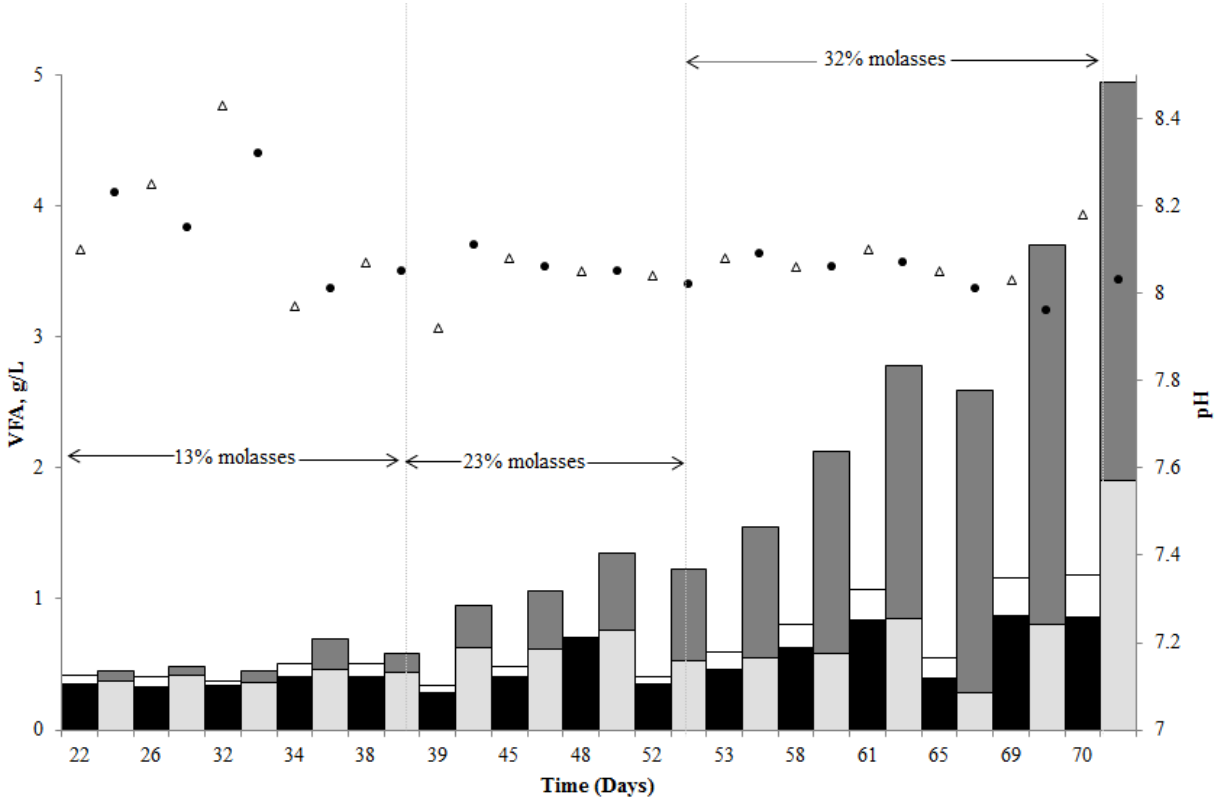


Figure 4. Effect of VFA and pH on experimental reactor. Columns are for VFA and the symbols are for pH. Column with black shades: acetic acid from R (CM); Columns with white shades: propionic acid for R(CM); Column with light grey: acetic acid from R (CM+M); Columns with dark grey: propionic acid for R(CM+M). Open triangles: pH of R (CM); Closed circles: pH of R (CM+M)

In period 4, between day 53 to 71 the concentration of molasses for reactor R (CM+M) was finally augmented (1.23 to 1.84 gVS/L/d) to 32% of total added VS which stimulated 24% growth of biogas (Figure 3(a)) in terms of volume. In terms of added volatile solid, however, the yield from R(CM+M) (309 L/kg VS_{added}) approximately reduced to 10 % compared to R(CM) (339 L/kg VS_{added}). While volumetric methane yield was still increasing, methane composition declined and reached to a level (54%), lowest for the whole experiment. Furthermore, there was approximately fourfold increase in total VFA (table 2) that unlike the previous period was alternated by the concentration of propionic acid (2.28 g/L) followed by acetic acid (0.85 g/L). The rapid climb in propionic acid along with acetic acid was the serious indication of process stress with a possibility to complete failure. In fact, propionic acid alone is a very potential candidate to severely trigger process imbalance [25]. There were several other potential factors played a significant role to characterize such VFA pattern of R(CM+M). One, for instance, the lignin decomposition, as a consequence of which lower molecular weight VFA forms during hydrothermal treatment of upstream biomass (wheat straw in this case) [20]. This was expected for C_5 molasses as the type of process (section 1) it was involved to originate. Moreover, there was an issue of present feeding strategy where instead of slow increase in molasses OLR (will be published later), R(CM+M) was tested for sudden OLR rise to achieve optimum boosting of biogas which presumably had a strong influence on VFA rise too. Based on these VFA facts, the feeding of R(CM+M) beyond this period was decisively stopped. Meanwhile, the average pH and TAN for molasses reactor showed very little variation from the earlier periods as their corresponding values in this level was 8.08±0.05 and 2.0±0.2 respectively. For R(CM), on the other hand, the total VFA along with acetic acid and other compounds exhibited no serious implications as they were tended to stabilize in this period (Figure 4).

As discussed above, throughout the experiment, rise in VFA compounds was serious concern while pH was fairly safe with apparently stable values (Figure 4). This was probably attributed to the fact that co-digestion of C_5 molasses with cattle manure facilitated buffering by neutralizing pH at varying substrate concentrations and thus sustained the process for perceivably higher OLR input. Similar phenomena was observed by Fang et al. [14] who noticed higher VFA but stable pH for high concentration feeding.

Despite the fact, straining the process with even higher loading beyond the present level of maximum OLR might lead to a process imbalance causing buffering capacity to deplete and eventually deteriorating system stability.

All in all, C_5 molasses played a significant role in the yielding pattern of biogas from R(CM+M) which generally decreased in terms of added VS as molasses concentration was increased. Essentially, the subsequent increase in substrate concentration was purposefully adopted in order to optimize the daily boosting of biogas which was well achieved (23% extra biogas yield) in the middle part of the experiment, with a tolerable VFA and other parameters. However, between the last two periods due to the sudden rise in C_5 concentration from 23% to 32% of added VS, the system was stressed and imbalanced as evidenced by the higher VFA accumulation, although volumetric yield of biogas continued to increase. Boosting biogas in conditions of later part of the experiment as a result of high concentration feeding hence should not be replicated in commercial scale biogas digesters, as indicated by this work.

4. Conclusions

Improvement in productivity of anaerobic digesters together with sustainable utilization of 2nd generation bioethanol plant product are the two potential benefits the present study revealed. The maximum biogas yield of 358 L/kgVS (1.3 L/L/d) was obtained for the continuous reactor experiment with a total organic loading rate of 5.3 gVS/L/day beyond which the process was rather unstable. Utilizing C_5 molasses above 5.3 gVS/L/day of total OLR, or, in other words above 23% concentration of molasses VS, therefore, is not recommended when the adaption period is shorter.

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