Dual Role of Grass Clippings As Buffering Agent and Biomass During Anaerobic Co-digestion With Food Waste

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Abstract

There is a dire need to replace the chemical buffers that regulate the redox environment in single-stage anaerobic digestion (AD) of food waste (FW). Hence, the applicability of grass clippings (GC) as an eco-friendly buffering agent and biomass during the anaerobic co-digestion of FW was explored. A focus was primarily given on the effects of GC on the redox environment and acidogenesis. Concomitantly the production of volatile fatty acids, hydrogen and methane in mesophilic conditions was monitored. Organic load and substrate to inoculum ratio were kept constant in all the experiments, and no chemical buffer was used. The results revealed that GC regulated the redox environment by inhibiting rapid pH drop in the digester with 10 % GC. The addition of 2, 4, and 6 % GC promoted acidogenesis with increased production of acetic and butyric acids; whereas, 8 and 10 % GC promoted solventogenesis with ethyl alcohol production. Hydrogen generation from the experiments with GC was in the range of 27-30 % of the total biogas, which was marginally higher than from the control (25 %). Methane concentration was negligible in the biogas generated from all experiments. The acidification rate, VFA production/consumption rate, specific hydrogen yield, hydrogen conversion efficiency, and volatile solid removal were maximum and minimum in the reactors with 6 and 10 % GC, respectively. From the above results, it can be concluded that the addition of GC to FW would regulate the sudden pH changes and enhance the production of value-added biochemicals, to make the process cost-effective.

1. Introduction

The United Nations Environment Program estimates that around 931 million tonnes of food waste (FW) was generated in 2019, indicating that 17 % of global food production may be wasted (UNEP 2021). According to the Food and Agriculture Organization (FAO 2011), 4.4 $GtCO_2eq$ were generated from global food loss and waste, which is about 8 % of total anthropogenic greenhouse gas emissions. In many countries, including India, FW gets disposed of along with municipal solid waste in landfills and open dump yards. This unscientific practice of FW disposal leads to a significant loss of the resource that has excellent potential to generate clean energy and value-added products via anaerobic digestion (AD) (Ferronato and Torretta 2019; Al-Wahaibi et al. 2020). However, single-stage AD of FW has limitations associated with a sudden drop of pH and concurrent inhibition of acidogenesis (Sarkar et al. 2019) and methanogenesis (Han et al. 2019). Drop of pH results mainly due to the accumulation of volatile fatty acids (VFA) produced during AD of carbohydrate-rich FW (Chakraborty et al. 2018b).

In conventional anaerobic systems, pH regulation is achieved using chemical buffering agents, such as sodium bicarbonate, sodium hydroxide, calcium oxide, and phosphate buffer (Selvam et al. 2010; Rabii et al. 2019; Sarkar et al. 2019). However, these chemicals are expensive and can adversely affect acidogenesis and methanogenesis processes (Yan 2016). Hence, there is a dire need for an organic co-substrate that could primarily act as a buffering agent to regulate the pH during single-stage AD of FW. Additionally, the co-substrate also gets digested and contributes to the production of desired products, such as VFA, ethanol and biogas. However, this buffering co-substrate should be readily available for free or at minimum cost to make the process cost-effective. One such promising co-substrate is grass clippings (GC), which are cut grasses collected in the mower when the lawn is mowed.

The grass is primarily made of cellulose, hemicellulose (xylans), lignin and phenolic acids (uronic acids), but the lignin content is significantly low compared to other plants. However, the lignin-carbohydrate association through covalent cross-linking between arabinoxylans and lignin tend to be stronger. The GC have free-monosaccharides (glucose and fructose ~ 1–3 %), disaccharides (sucrose ~ 2–8 %), and principal elements such as nitrogen (1.88–4 %), phosphorus (0.26–1 %), potassium (1.23–2 %), iron (0.18 %), manganese (0.0234 %, zinc (0.012 %) and copper (0.0007 %) which are essential for AD. Furthermore, the neutral pH of GC and its carbon to nitrogen (C/N) ratio of 23/1 are considered as the desired characteristics to be used as a natural buffering agent during AD of FW (Nitsche et al. 2017; Ramnarain et al. 2019).

The biodegradability of FW, which has a low C/N ratio, can be improved when it is co-digested with GC or other lignocellulosic wastes such as agricultural residues and weeds (Rabii et al. 2019; Vats et al. 2020). Anaerobic co-digestion is well-known for regulating acidogenesis and methanogenesis by diluting toxic compounds, maintaining the ideal C/N ratio, improving nutrient distribution, increasing organic loading rate, and favouring microbial syntrophy and growth (Chakraborty et al. 2018b). There are a few investigations done on anaerobic co-digestion of various substrates, including FW and GC (Table 1). Nevertheless, these investigations focused on controlling methanogenesis (Fitamo et al. 2016), producing liquid organic fertilizer (Mostafazadeh-Fard et al. 2019), increasing biogas yield and overall performance of the AD process (Solarte et al. 2017; Darimani and Pant 2020). However, other products of economic importance, such as VFA, ethanol and hydrogen, can also be produced from the anaerobic co-digestion of FW and GC.

Table 1
Selected studies on anaerobic co-digestion of various substrates and grass clippings

Sl. No.	Substrates	Reactor	Key operational parameters	Results/ Products	Reference
1	Food waste (FW) and Grass clippings (GC)	300 mL Glass bottles	FW (75 %):GC (25 %) HRT: 20 days Temperature: 35 ˚C ± 2 ˚C	Biogas: 840 mL	Darimani and Pant 2020
2	Molasses (M), water (W) and Grass (G)	Pilot scale	M (5.5 kg): W: (17 kg) G (9 kg) pH: 5.1–7.2 Temperature: 20–40°C Leachate recirculation	Liquid organic fertilizer	Mostafazadeh-Fard et al. 2019
3	Maize silage (MS), Cattle slurry (CS) and GC	250 mL Batch reactors	GC (1):MS (1):CS (1):Inoculum (1) HRT: 42 days Temperature: 39°C	Methane: 0.255 m^3/kgTS	Bedoic et al. 2019
4	Swine manure (SM) and Napier Grass (NG)	120 ml Serum bottles	NG (1):SM (1) C/N: 21.03 Microwave and acid pretreated, Inoculum concentration: 15 g-VSS/L. pH: 8	630.05 mL-CH_4/L	Kongjan et al. 2019
5	Poultry manure (PM) and GC	120 mL Reactor bottles	PM (81.24 %):GC (18.76 %)	Methane: 104.8 mL/g VS	Sukhesh and Rao 2019
6	FW and GC	3L Batch	FW (8):GC (5) Inoculum (25 %):Substrate (75 %) Temperature: 37°C	Biogas generation increased by 66 %	Toro et al. 2017
7	Potable water (PW) and Grass silage (GS)	20 L Polyethylene containers	GS (0.11 %): PW (99.89 %) Inoculum (2): Substrate (1) Temperature: 37°C	Methane: 291.86 kg/VS	Nitsche et al. 2017
8	Sludge (SL), FW, GC and garden waste (GW)	7.5 L Batch reactor	SL (10):FW (67.5):GC (15.75):GW (6.75) HRT: 15 days Temperature: 55°C	Methane:425 mL CH_4/g VS	Fitamo et al. 2016
9	Pig waste (PW) and GC	10 L Batch anaerobic digester	PW (1):GC (1) C/N ratio: 17.28 Temperature: 37°C pH: 6.9	Biogas: 7725 ml/g COD	Matheri et al. 2016

Sl. No.	Substrates	Reactor	Key operational parameters	Results/ Products	Reference
10	FW, cow dung (CD) and GC	250 mL Serum bottles	CD (11 %):FW (75 %):GC (14 %) Inoculum (2.7): Substrate (1)	Methane: 715 L/ kg VS	Poulsen and Adelard 2016
11	Dairy processing waste (DPW), and grass (G)	3800 L Fed batch system	DPW(98.4 %): G (1.61 %) Temperature: 39 to 40°C	Methane: 0.37 ± 0.01 ($Lg^{-1}COD$) with 64.2 ± 1.0 % of total biogas	Hansen et al. 2014
12	Pig manure (PM) and grass silage (GS)	1 L Glass bottles	PM (3):GS (1)	CH_4 yield: 304.2 ml CH_4/g VS	Xie et al. 2011
13	GC and FW	200 ml Serum bottles	FW with 0, 2, 4, 6, 8, 10 % GC HRT: 96 h Temperature: 37°C	*Mesophilic*: Ethanol: 0.613 g/gVS; Acetate: 0.468 g/gVS; Propionate: 0.21 g/gVS; Butyrate: 0.106 g/gVS; Hydrogen: 13.55 ml/gVS	Present investigation

The VFAs are essential building block chemicals; hence there is a tremendous global demand for them. At present, about 90 % of VFA available in the market is generated from petro-based production methods, causing adverse health and environmental effects (Dionisi and Silva 2016; Ramos-Suarez et al. 2021). Continuous depletion of fossil fuels has led to increased dependence on alternate energy sources, primarily alcohols. Ethanol, a second-generation fuel, has gained attention in the recent past, as it can be produced from a wide variety of lignocellulosic feedstocks, including FW (Ahorsu et al. 2018). Hydrogen formed during acidogenesis is a clean fuel, with a heat value of 142.354 kJg^{-1}; hence it has a huge market potential (Xia et al. 2019).

Till date, no investigation emphasized the specific roles played by GC as a co-substrate for enhancing the VFA and hydrogen production while acting as a buffer during AD of FW. Such experiments should focus on the first four to five days, which are critical for understanding the hydrolysis and acidogenesis processes (Sarkar et al. 2021). Regulating the redox environment during the initial hours of the experiment is imperative to stabilize the AD process, to enhance the bioresource recovery.

Hence an investigation was done to evaluate the specific role of GC both as a buffering agent and biomass during its anaerobic co-digestion with FW. Emphasize was given to optimizing the ideal GC to FW substrate ratio; assessing the effect of GC addition on the regulation of the redox environment and enhancement of VFA, ethanol, hydrogen and methane production; and evaluating VFA kinetics and biogas production.

2. Materials And Method

2.1 Substrate preparation

Microorganisms-laden slurry from an anaerobic digester treating FW was used as the inoculum. Before starting the experiments, the required inoculum volume was collected in 5L containers from the digester (Biourja, GPS, Bangalore, India). Fresh FW was collected from the student's hostel mess at BITS Pilani, Hyderabad Campus, India. Immediately after collecting the FW, it was grounded into a slurry using a mixer grinder and used as the primary substrate throughout the experiment. Clippings of grass (Family: *Poaceae*) were collected from the mower used to trim the lawns in the institute. The size of the GC typically ranged between 6 and 8 mm. The GC were directly mixed as the co-substrate with FW slurry in required quantities to obtain the desired substrate ratios.

2.2 Anaerobic co-digestion of food waste and grass clippings

Experiments were conducted in anaerobic reactors (200 ml reagent bottles, Borosil) in which FW slurry weighing 80 g (VS ~ 23.91 g) was mixed with 10 ml of inoculum to make a uniform substrate to inoculum ratio. GC were added to this mixture in five concentrations, i.e., 2, 4, 6, 8 and 10 % (w/v). Finally, 10 ml of Millipore water was added to each reactor to constant dilution. One set with all the contents except GC was used as the control. The digester bottles were sealed with a rubber septum and thoroughly mixed with a vortex mixer for about a minute. A 20 ml syringe with a needle was inserted into the rubber septum to measure the volume of biogas produced and sample the biogas to analyze its composition.

All the reactors were placed inside a temperature-controlled shaking incubator (RIS 24 Plus Orbital Shaking Incubator, Remi, Mumbai, India) maintained at 35˚C and set to 80 rpm, ensuring uniform mixing of the reactor contents. All the experiments were conducted for 96 h primarily to focus on the hydrolysis and acidogenesis phases, which determine the efficiency of biomass valorization and govern the production of desired value-added products. At 24 h intervals, 5 ml of the sample (mixed content) from the reactors was collected in 15 ml falcon tubes (Tarsons, India) and stored in a refrigerator for analyzing various parameters. All experiments were conducted in triplicates; two were used for sampling and one for measuring the volume of biogas.

2.3 Physicochemical characterization

Before starting the experiment, total chemical oxygen demand (tCOD), total solids (TS) and volatile solids (VS) analyses were performed for FW, inoculum, GC and the mixed reactor contents. These analyses were repeated at the end of the experiments for the reactor contents. pH, soluble COD (sCOD), total VFA, VFA distribution, ethanol and biogas analyses were carried out periodically for the samples collected from the reactors. Analyses of TS, VS, tCOD, total VFA and sCOD were done as per the procedure described in Standard Methods for the Examination of Water and Wastewater (APHA 2012). Freshly ground FW slurry was appropriately diluted for tCOD analysis. The supernatant was collected by centrifuging the FW slurry at 10,000 rpm for 10 minutes, filtered through 0.45 µm filter paper, diluted according to the organic load of the FW and used for sCOD analysis.

For VFA distribution analysis, samples were centrifuged at 10,000 rpm for 10 mins, and the clear supernatant was filtered through a 0.45 µm cellulose acetate membrane. To 100 µl of the filtrate, 850 µl of Millipore water and 50 µl of formic acid were added to get 1 ml of sample in gas chromatograph vials to analyze VFA distribution. Finally, soluble products were analyzed using gas chromatography (HP 6890 Series, Hewlett Packard) provided with injector and flame ionization detector (FID) and operated at a temperature of 250˚C. Nitrogen at 20 mL/min (25 psi) was used as the carrier gas. The oven temperature was programmed as follows: 120˚C for 5 min, increased to 180˚C at 5˚C/min, and then maintained at 180˚C for another 10 min. An Econo-Cap EC1000 (15 m × 0.53 mm × 1.20 µm) coated with 0.2 µm CP-Wax 57 CB column was used as the stationary phase in soluble VFA determination (Chakraborty et al. 2018b).

Total biogas volume was measured at 24 h intervals using a 20 ml syringe inserted into the rubber septum of the reactor. The composition of the biogas was analyzed using a gas chromatograph (HP 7890 Series, Hewlett Packard) equipped with a thermal conductivity detector (TCD) and PLOT-Q column (30 m × 0.53 mm × 15 µm). Initially, the standard biogas mixture (H_2:CH_4: CO2::1:60:30) was run at 100 ˚C for 5 min, where N_2 gas (40 ml/min) was used as carrier gas at an injection temperature of 200 ˚C to measure the retention time and peak area of standard gases. After this run, the biogas samples collected from the experiments were run to determine the distribution of various gases. Hydrogen conversion efficiency, degree of acidification, VFA kinetics were calculated to understand the treatment efficiency according to Sarkar and Venkata Mohan (2017). Two data points of each sample were used for statistical analysis where mean value and standard deviation were estimated using the analysis of variance (ANOVA) test. Regression and correlation analyses were carried out for the VFA metabolites to check possible connections between the final VFA production variables. The level of significance of the results was checked at $P \leq 0.05$.

3. Results And Discussion

3.1 pH flux and its influence on the redox environment

The physicochemical characterization of substrates and inoculum are presented in Table 2. After 12 h, in all digester bottles, the pH started falling from 6.6 to 4.6. Rapid solubilization and concomitant acid production made the pH decrease faster during the initial hours (Chakraborty and Venkata Mohan 2018; Sarkar et al. 2019). The addition of GC (2 to 8 %) did not significantly prevent pH decrease. After 96 h, pH was 3.2 in control and 3.8 in the other digester bottles with 2, 4, 6 % of GC. Nevertheless, in the experiment where 8 and 10 % GC were added, the pH was 4.2 and 4.6 after 96 h. The particulate nature of lignocellulosic materials in the GC helped to regulate the sharp decrease of pH as the difference was observed to be 1.4 units between control and the experiment with 10 % GC (Solarte et al. 2017).

Table 2
Summary of the reactor performance

Substrate	VS (g)	COD (g)	VS removal (%)	COD removal (%)	Hydrogen (ml/gVS)	Ethanol (g/gVS)	VFA yield (g/ gVS)					
							Acetate	Propionate	Butyrate	Valerate	Caproate	Cumulative
FW	23.91 ± 0.28	33.95 ± 0.4	30.05 ± 1.34	36.93 ± 0.72	8.63 ± 3.52	0.209 ± 0.05	0.228 ± 0.08	0.093 ± 0.01	0	0.194 ± 0.02	0.019 ± 0.001	1.26 ± 0.04
GC	0.43 ± 0.025	0.50 ± 0.03	ND	ND	ND	0	0.111 ± 0.03	0.061 ± 0.01	0	0.105 ± 0.05	0	0.33 ± 0.01
Inoculum	1.65 ± 0.017	3 ± 0.02	ND	ND	ND	0	0.017 ± 0.003	0.060 ± 0.002	0.021 ± 0.001	0	0	0
2 % GC + FW	24.76 ± 3.02	35.16 ± 4.2	41.11 ± 0.67	48.86 ± 0.25	10.35 ± 2.7	0.149 ± 0.02	0.468 ± 0.06	0.068 ± 0.005	0.095 ± 0.01	0.119 ± 0.007	0.058 ± 0.006	1.31 ± 0.07
4 % GC + FW	25.61 ± 1.03	36.37 ± 1.4	43.23 ± 1.42	49.95 ± 0.41	10.7 ± 2.43	0.293 ± 0.04	0.292 ± 0.07	0.063 ± 0.008	0.106 ± 0.008	0.190 ± 0.08	0.035 ± 0.003	1.44 ± 0.23
6 % GC + FW	26.47 ± 2.01	37.58 ± 2.8	47.37 ± 2.76	50.92 ± 0.82	13.55 ± 1.6	0.613 ± 0.01	0.355 ± 0.023	0.209 ± 0.004	0.085 ± 0.002	0.114 ± 0.004	0.064 ± 0.003	1.38 ± 0.35
8 % GC + FW	27.32 ± 1.27	38.79 ± 1.7	40.55 ± 0.64	47.39 ± 0.25	10.21 ± 0.9	0.572 ± 0.06	0.093 ± 0.082	0.050 ± 0.003	0.092 ± 0.005	0.138 ± 0.003	0.054 ± 0.001	1.31 ± 0.51
10 % GC + FW	28.17 ± 1.83	40.00 ± 2.6	40.20 ± 0.71	47.22 ± 0.71	10.27 ± 1.5	0.552 ± 0.07	0.101 ± 0.02	0.063 ± 0.001	0	0.119 ± 0.006	0.046 ± 0.001	1.26 ± 0.21

ND: Not determined

It is apparent that without GC addition (control), the pH drop continued after 12 h till the end of the experiment. Such sustained drop in pH was observed in AD of FW as the sole substrate (Moestedt et al. 2020). However, in the experiments with GC addition, after 12 h, no further drop in pH was observed; instead, after 24 h, the pH started increasing till the end of the experiment. Overall, results depicted that the addition of GC (10 %) has regulated the sudden drop of pH and controlled the redox environment. Hansen et al. (2014) reported that GC could regulate pH during anaerobic digestion of dairy processing waste. Co-digestion of FW and GC helped to balance the C/N ratio of the substrate, thereby leading to enhanced organic matter decomposition and increased microbial biomass. Ultimately, C and N got mineralized and soluble phenolic compounds are generated, which regulates the pH (Yao et al. 2009; Dias et al. 2014).

3.2 COD analysis

Hydrolysis and acidogenesis increase the solubilization of FW, which in turn increases the concentration of sCOD. Results depict that the sCOD production in mesophilic conditions increased until 24 h (20.65, 28.5, 30.87, 31.88, 32.2 g/L) after which it started decreasing till 48 h (11.3, 11.86, 12.25, 15.55, 16.38 g/L) for 2, 4, 6, 8 and 10 % GC, respectively (Fig. 1a). After 48 h, the sCOD concentrations started increasing until 96 h, in the experiments with 2, 4 and 6 % GC (15.2, 14.65, 13.78 g/L). Nevertheless, the sCOD concentrations continued to decrease for the experiments with 8% GC (13.5 g/L) and 10% GC (14 g/L) until 96 h. In the control experiment, sCOD production increased to 15 g/L after 24 h and kept increasing to a maximum of 18 g/L at 48 h, after which it decreased gradually to 7.65 g/L at 96 h. The addition of GC increased the sCOD production by 1.37 to 2.14 folds within 24 h, and the cumulative sCOD concentrations remained higher until the end of the experiment, compared to control. Macias-Corral et al. (2017) and Darimani and Pant (2020) reported that the addition of GC increased the substrate's solubilization, consequently increasing sCOD concentration. Compared to the experiments with 8 and 10 % GC, after 24 h, the sCOD concentration decreased rapidly and steadily in the digester bottles with 2, 4 and 6 % GC addition.

From Table 2, it is evident that the addition of 2, 4, 6, 8, and 10 % GC demonstrated increased COD removal efficiency of 48.86, 49.95, 50.92, 47.39 and 47.22 %, respectively, compared to the control (36.93 %). The results show that the hydrolysis and COD removal rates were minimal in the digester bottles with 8 and 10 % addition of GC because the lignin contents shield carbohydrates (cellulose and hemicelluloses) from hydrolytic enzyme activities (Ferdes et al. 2020). A tight extracellular matrix in GC and other lignocellulosic substrates prevents hydrolytic enzymes' penetration and must be pretreated thermally and chemically along with mechanical treatment to improve the rate of enzyme hydrolysis (Chakraborty and Venkata Mohan 2019; Venkata Mohan et al. 2020).

3.3 Fatty acid and alcohol profiles

After 24 h, the total VFA production was 14.09, 15.46, 16.21, 16.69, 11.50 and 8.67 g/L for 10, 8, 6, 4, 2 % of GC addition and control, respectively (Fig. 1b). However, at 48 h, the VFA concentrations decreased to 4.73, 4.80, 4.63, 4.21, 4.65 g/L in digester bottles fed with 10, 8, 6, 4, 2 % of GC, respectively. In control, the total VFA increased further to 10.14 g/L at 48 h and then reduced. Between 48 h and 96 h, the total VFA production increased minimally, except for 10 % GC. The addition of 4 and 6 % GC supported acidogenesis with good VFA production, whereas the acidogenesis process was slower with 8 and 10 % GC. Due to enhanced acidogenesis, the degree of acidification was maximum in 2, 4 and 6 % addition of GC (55.70, 58.56 and 57.83 %) than 8 % GC addition (48.49 %), 10 % GC addition (43.77 %) and in control (52.51 %).

On a similar line, it was observed that the total VFA concentration was considerably higher when sewage sludge was anaerobically co-digested with FW, wastewater and GC when compared to the sludge being used as a single substrate (Fitamo et al. 2016). Likewise, in the current investigation, the cumulative VFA concentrations were 30.18 and 32.45, 36.89, 36.43, 35.66, 35.39 g/L in control and with 2, 4, 6, 8 and 10 % of GC addition, respectively at 96 h. The addition of GC increased the solubilization of substrates, which increased the production of VFAs and other soluble metabolites such as ethanol (Eq. 1), acetic acid (Eq. 2 and Eq. 3), propionic acid (Eq. 4), butyric acid (Eq. 5). Further, the VFAs and alcohol produced during the acidogenic phase get oxidized to acetic acid, as shown in Eq. 6- Eq. 8. As could be noticed from the equations, hydrogen is generated as a byproduct from all the steps of acidogenesis.

$C_6H_{12}O_6 + 2H_2O + 2NADH \rightarrow 2CH_3CH_2OH + 2HCO_3^- + 2NAD^+ + 2H_2$ (ΔG=-234.8 kJ/mol)… Eq. 1

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (ΔG = -135.6 kJ/mol) … Eq. 2

$C_6H_{12}O_6 + 4H_2O + 2NAD^+ \rightarrow 2CH_3COO^- + 2HCO_3^- + 2NADH + 2H_2 + 6H^+$ (ΔG=-215.7 kJ/mol) … Eq. 3

$C_6H_{12}O_6 \rightarrow CH_3COO^- + CH_3CH_2COO^- + CO_2 + H_2 + 2H^+$ (ΔG = − 287.0 kJ/mol) … Eq. 4

$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2HCO_3^- + 2H_2 + 3H^+$ (ΔG = − 261.5kJ/mol) … Eq. 5

$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$ (ΔG = 76.1 kJ/mol) … Eq. 6

$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$ (ΔG = 48.1 kJ/mol) … Eq. 7

$CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$ (ΔG = 9.6 kJ/mol) … Eq. 8

Subsequently, profiling of VFA metabolites was carried out using gas chromatography with a flame ionization detector. While ethanol production increased with time in the control, its concentration was many folds higher in the digester bottles with GC. As depicted in Fig. 2, the maximum concentration of ethanol and its respective fraction out of the total soluble products were 1.71 g/L (35.80 %) at 72 h in control, and 8.51 g/L (50.12 %), 11.56 g/L (89.88 %) and 12.31 g/L (82.05 %), respectively, in digester bottles with 6, 8 and 10 % of GC at 24 h. With 2 and 4 % GC addition, the ethanol production was lower, 1.68 g/L (36.07 %) at 48 h and 4.03 g/L (41.68 %) at 72 h, respectively. The ethanol concentration decreased after 72 h in all the digester bottles and control, mainly because of the increase in pH. In the study done by Wainaina et al. (2019), lower pH (~ 3.2–5.0) led to alcohol biosynthesis and the generation of lactic and acetic acids. Similarly, in our experiment, the pH was 3.2–4.6, which was the main reason for ethanol production.

Acetic acid was dominant at various points of time in the digester bottles with and without addition of GC: 2 % (24 h), 4 % (24 h), 6 % (96 h), 8 % (72 h), 10 % (96 h) and control (96 h) contributing 8.8, 3.10, 7.57, 0.92, 1.15 and 2.39 g/L, respectively. As depicted in Fig. 2, it can be concluded that with the increased addition of GC, acetic acid production got delayed and decreased because lignocellulosic biomass, such as GC, affect hydrolysis, the rate-limiting step of the AD process (Chakraborty and Venkata Mohan 2018; Ferdes et al. 2020; Pilarski et al. 2020). Nonetheless, the addition of 2 to 6 % of GC was most favourable for the timely production of appropriate volumes of acetic acid. The concentration of propionic acid was 4.05 g/L in the digester bottles with 2 % GC. In the rest of the digester bottles and control, the propionic acid production was < 1 g/L (Fig. 2).

It is reported in the literature that the production of VFA, including acetic and propionic acids, is maximum at pH of 6 (Dahiya et al. 2015; Frohlich-Wyder et al. 2017; Wainaina et al. 2019). Additionally, during the redox phase of AD, different groups of acidogenic microorganisms would grow well only in their respective microenvironment with specific pH. To enhance the production of a particular VFA by the acidogens, it is necessary to maintain the pH more than the pKa value of that VFA. If the pH value goes below the pKa, then the VFA accumulates in the cytosol and hinders microbial growth, which will decrease the VFA production (Sarkar et al. 2021). As acetic acid is the main product of acidogenesis, its impact on the redox environment will be more significant than the other VFAs. Correspondingly, lower pH (~ 4.6) yielded lower proportions of acetic and propionic acids in the reactors in this investigation.

The maximum butyric acid production was at 96 h in all the digester bottles with GC, but it was at 24 h in control. The butyric acid concentrations were: 1.6, 2.36, 2.25, 2.51 and 2.36 g/L for digester bottles with 2, 4, 6, 8 % GC addition and control, respectively, and absent at 10 % GC (Fig. 2). Similar to acetic acid production, which happened during the initial hours of the experiment, butyric acid production also got delayed and could be measured only at 72 and 96 h in the digester bottles with GC. Results of this study are aligned with the reports of Sarkar et al. (2016) and Karthikeyan et al. (2016a), which explain the dominance of acetate-butyrate and mixed acid pathways that augment acidogenesis and enhances the production of acetate, butyrate, ethanol, H_2 and CO_2. Eventually, butyrate gets converted to acetate and hydrogen; hence the concentrations of acetate and butyrate are inversely proportional to each other, in all the digester bottles with GC addition, except 10 %. The main reason for no butyrate production at 10 % GC is higher ethanol production

(solventogenesis). Long-chain fatty acids (e.g., caproic acid) are generated through the β-oxidation pathway, where ethanol acts as electron donor and short-chain fatty acids (e.g., butyric acid) act as the electron acceptors (Wu et al. 2018).

Valeric (C5) and caproic (C6) acids were produced in all the digester bottles with GC, but isovaleric and isobutyric acids production was negligible. The maximum valeric acid concentrations were 1.83, 1.77, 2.02, 1.3, 2.25 and 2.34 g/L for control and the digester bottles with 2, 4, 6, 8 and 10 % addition of GC, respectively (Fig. 2). Caproic acid production increased with the addition of GC, but it was less than 1 g/L. The variation in the long-chain fatty acids was due to the acidic environment and the availability of ethanol, which promoted the β-oxidation pathway to produce long-chain fatty acids (Steinbusch et al. 2011).

3.4 Biogas production and volatile solids removal

A focus was given on the regulation of the redox environment by GC during the initial period (up to 96 h) of FW anaerobic co-digestion. Hence this investigation centred around acidogenesis, which primarily produces VFA and hydrogen (Eqs. 1 to 8) than methane. Gas chromatography analysis of biogas revealed that the maximum concentrations of hydrogen were ≈ 30 % and ≈ 25 % in experiments with GC and control, respectively, at 48 h. The specific hydrogen yield (13.56 ml/gVS, 29.68 %) was maximum in 6 % addition of GC, which was higher than 4% addition of GC (10.69 ml/gVS, 27 %), 2 % GC (10.36 ml/gVS, 27 %), 8 % GC (10.21 ml/gVS, 26.2 %), 10 % GC (10.27 ml/gVS, 26.1 %) and control (8.63 ml/gVS, 25 %) (Fig. 1c, Table 2). It has to be noted that the difference in hydrogen yields between the experiments with 2 and 6 % of GC addition is 0.82 ml/gVS. Hydrogen conversion efficiency was maximum in 6 % addition of GC (48.73 %) as compared to 2 % (47.51 %), 4 % (47.11 %), 8 % (46.26 %), 10 % addition of GC (45.51 %) and control (44.94 %).

Higher hydrogen production in the experiments with GC could be attributed to the higher production of acetate, butyrate and propionate in these digester bottles compared to control. Additionally, from the thermodynamics of anaerobic digestion, it was evident that the acetate formation pathway from Acetyl-CoA was favoured (Eqs. 6–8), which also led to hydrogen generation (Taheri et al. 2018). However, methane production was less till 96 h, mainly due to the inhibition of methanogens by the acidic environment (Han et al. 2019). During AD of FW, methane generation begins typically after 7 to 9 days from the start of the digestion due to the slow growth rate of methanogens (Chakraborty and Venkata Mohan 2018; Maus et al. 2020). For readily biodegradable substrates such as FW, accumulation of VFA and alcohols due to rapid hydrolysis and subsequent inhibition of acetogens and methanogens is a common phenomenon reported in several studies (Karthikeyan et al. 2016a; Karthikeyan et al. 2016b; Chakraborty et al. 2018b, Han et al. 2019). Additionally, due to its particulate nature, the addition of GC at higher concentrations will alter hydrolysis and VFA dynamics, which eventually hamper methanogenesis, as reported by Ferdes et al. (2020).

Compared to control, the VS removal and cumulative yields of ethanol and VFA were higher in all digester bottles with the addition of GC as co-substrate (Table 2). Digester bottles with 6 % of GC addition achieved the highest VS removal (47.37 %). The cumulative yields of ethanol (0.613 g/gVS), propionate (0.209 g/gVS) and caproate (0.064 g/gVS) were highest with 6 % of GC addition. Maximum cumulative yields of acetate (0.468 g/gVS) were with 2 % of GC, while at 4 % of GC, butyrate (0.106 g/gVS) and valerate (0.190 g/gVS) generation were maximum. Considering the VS removal, VFA and hydrogen production, the addition of 6 % of GC is recommended for anaerobic co-digestion of FW in mesophilic conditions.

3.5 VFA kinetics and statistical analyses

The acidogenic process is dynamic which gets influenced by the intermediate metabolites of anaerobic digestion, particularly the concentration and composition of the VFA (Yan 2016). Hence, VFA kinetics was evaluated as presented in Fig. 4. The maximum production of ethanol of 0.51 g/L.h (8 % GC), acetate of 0.37 g/L.h (2 % GC), propionate of 0.17 g/L.h (6 % GC), valerate of 0.08 g/L.h (10 % GC) and caproate of 0.035 g/L.h (8 % GC) were observed at 24 h, whereas for butyrate production of 0.098 g/L.h (6 % GC) was observed at 72 h. The maximum consumption of the metabolites happened at the same concentration of GC, however at different times, i.e., for ethanol − 0.215 g/L.h, acetate − 0.17 g/L.h, propionate − 0.073 g/L.h and valerate − 0.02 g/L.h at 48 h and caproate − 0.0083 g/L.h at 96 h. As seen in Fig. 4, the production and consumption rates of VFA and ethanol were higher in the digester bottles with GC addition than control. It has to be noted from Fig. 4 that the higher acetate, propionate and butyrate production in digester bottles with GC addition is also correlated with hydrogen production. A similar pattern of VFA kinetics was observed in the study by Sarkar and Venkata Mohan (2017).

To confirm the above-described relationship between ethanol and VFA in their production and consumption, a statistical correlation analysis was carried out for the digester bottles with maximum concentration (10 %) of GC and control. As explained in Sect. 3.3 for the digester bottles with higher GC (8 % and 10 %), a negative correlation was observed between the production of alcohol (increased) and acetic, butyric and propionic acids (decreased). The concentration of caproic acid was directly proportional to ethanol and inversely proportional to acetic acid. In control, acetic, butyric and caproic acids increased with reduced ethanol production, which showed a negative correlation. Propionic and butyric acids had a positive and negative correlation with acetic acid, respectively (Supplementary Table 1). It has to be noted that the dynamics of VFA production is directly dependent on the pH of the environment. In turn, the redox environment impacts the thermodynamic potential of biochemical reactions involved in acetogenesis (Eqs. 1 to 8) and the syntrophic relationship between hydrolytic, acidogenic and acetogenic microflora (Yan et al. 2016; Owusu-Agyeman et al. 2020). ANOVA single factor analysis of all experiments and control was expressed through the sum of square, mean square, F-value, and P-value, which revealed that the experiment is significant as it has a P value of ≤ 0.05 (Supplementary Table 2).

4. Prospects Of Producing Value-added Products

The VFA, ethanol and hydrogen are utilized to produce several environment-friendly products, such as biogas, biofuel, bioplastics and green electricity. Additionally, they serve as valuable raw materials for many industries, including pharmaceutical, food and chemical. The projected compound annual growth rate (CAGR) for hydrogen is around 6 % between 2017 and 2023 and is expected to generate around USD 183.34 billion by the end of 2023. The

CAGR for acetate is expected to grow at 4.27 % of 18,296.90 kilotons by 2023 (Mordor Intelligence 2018). The CAGR for butyric and propionic acids will grow at 5.4 % and over 3 %, and their market value was estimated to reach 827.6 and 708.57 Million USD during 2020–2026. The CAGR for ethanol is around 6.7 %, and it is expected to generate revenue of USD 115.65 billion by 2025 (Markets and markets 2021; Research Grand View 2021).

One of the main drawbacks of AD of FW is that the actual production efficiency of VFA, ethanol and hydrogen that could be achieved is only 80 % of the theoretical production efficiency, using the pure strain of microorganisms. The productivity decreases further if a mixed microbial consortium is used (Sarkar et al. 2016). One of the approaches for improving VFA, ethanol and hydrogen productivity is to provide the ideal microenvironment/ redox environment for the microorganisms. This can be achieved by maintaining the pH of the reactor higher than the respective pKa values of the desired short-chain fatty acids. By doing so, protonation of VFA can be avoided, which will check the accumulation of anions that are otherwise responsible for reducing the intracellular pH and causing metabolic disturbances.

Anaerobic co-digestion of FW with lignocellulosic substrates, such as GC, is considered as one of the best ways to control the pH in the reactor. In this investigation, the process pH was controlled by adding GC to FW and the desired value-added products (VFA, ethanol and hydrogen) were produced. Furthermore, any chemical buffering agent was not used to make the process eco-friendly and cost-effective. Certainly, this process could be easily scaled up for mass production. This investigation could salvage the industrial scale AD plants treating FW across the globe that are facing the major challenge of controlling the fluctuating pH and concomitant production of desired products. However, further research on adding GC to FW has to be done on a pilot scale to maintain process pH around 6, by focusing on various aspects, including the following, but not limited to optimizing: (i) mixing ratio of GC and FW, (ii) substrate to inoculum ratio, (iii) HRT, (iv) using enriched inoculum, and (v) pre-treatment of the substrates. Under such a favourable ambience in the digester, the desired syntrophic microbial diversity can be established, which will warrant the production of desired products.

5. Conclusions

The addition of GC to FW enhanced the rate of acidogenesis and removal of VS and COD. Consequently, the cumulative production of VFA, ethanol, and hydrogen was higher in the experiments with GC than in control. The role of GC as a buffering agent and biomass was best at 10 and 6 % addition, respectively. Furthermore, the addition of 4 and 6 % GC was best for acetate, butyrate, and hydrogen production, whereas 8 and 10 % of GC was best for ethanol production. Hence it could be concluded that the GC should be added in the appropriate ratio to FW to produce the desired value-added product(s). The procedure followed in this investigation to control the fluctuating pH during acidogenesis is simple yet efficient, cost-effective yet environment-friendly and could be easily replicated at the lab and industrial scales.

Declarations

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Availability of data and material: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Figures

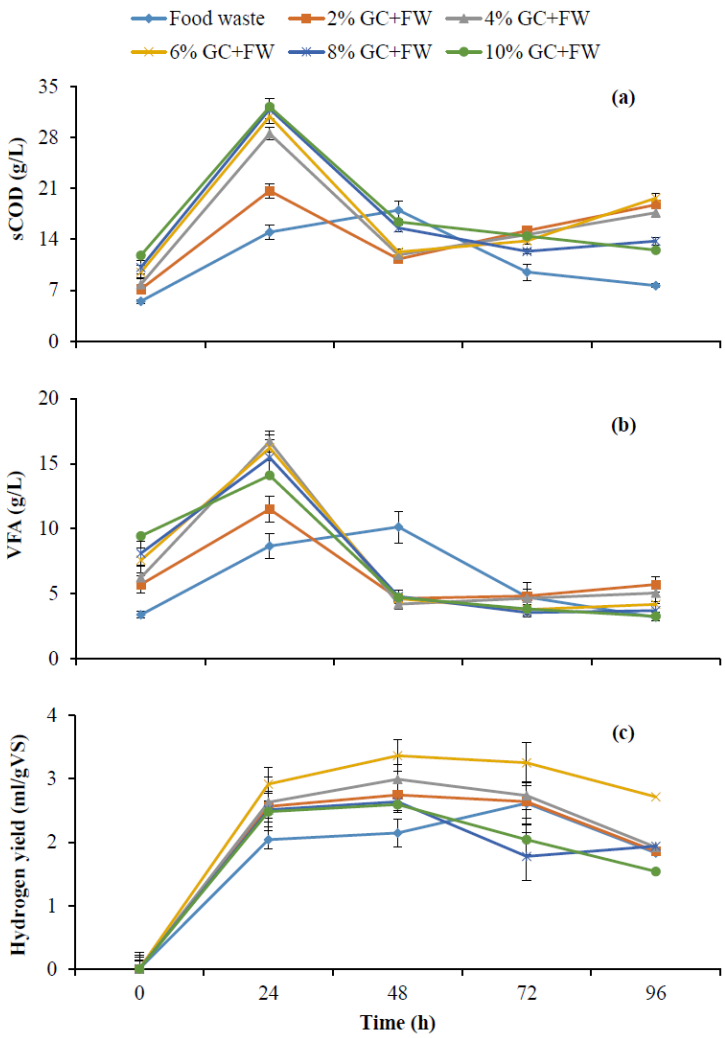

Figure 1

Effects of grass clippings on (a) sCOD leaching, (b) VFA production and (c) hydrogen yield

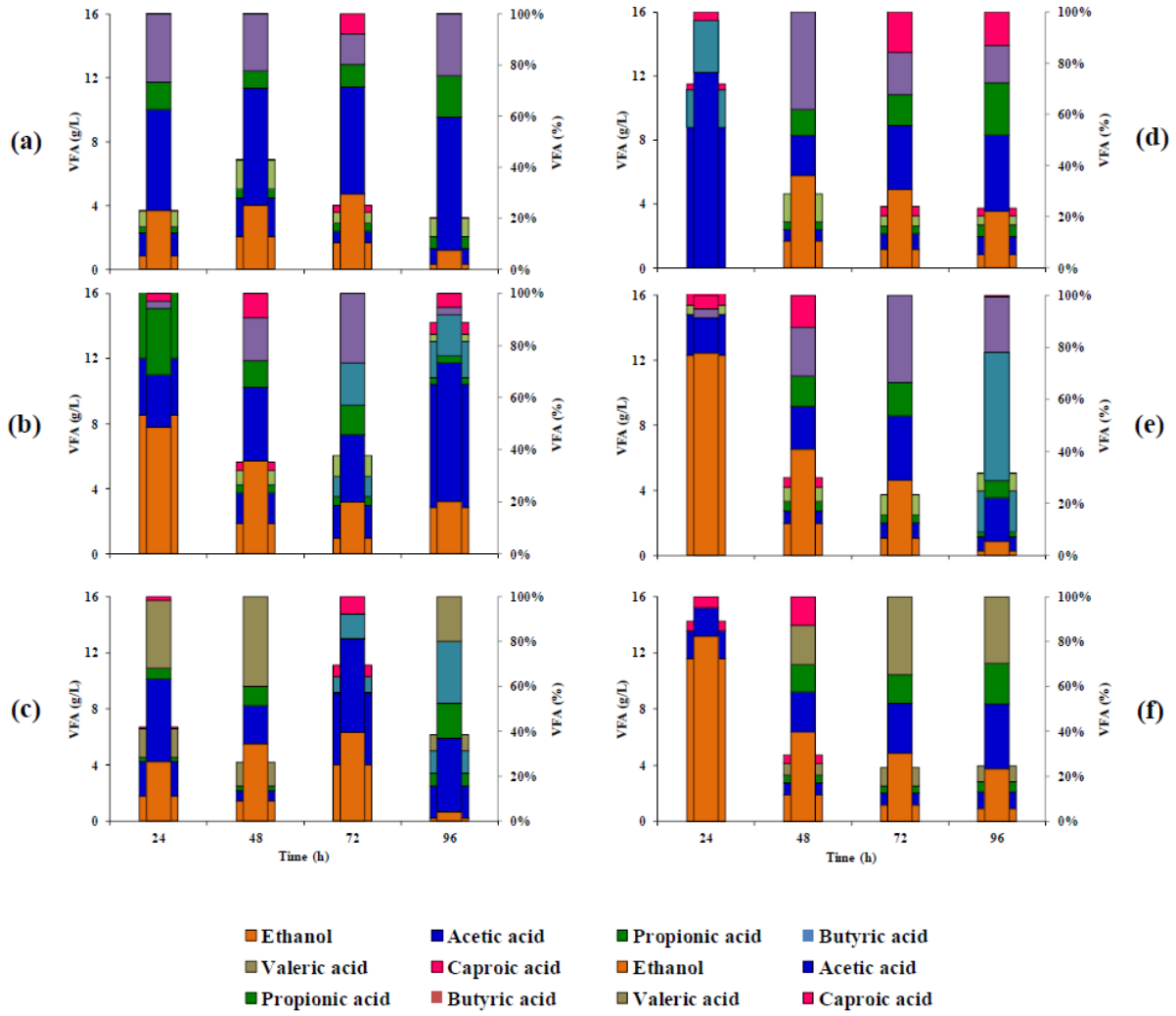

Figure 2

Ethanol and VFA distribution in (a) FW; (b) FW + 2 % GC; (c) FW + 4 % GC; (d) FW + 6 % GC; (e) FW + 8 % GC; (f) FW + 10 % GC

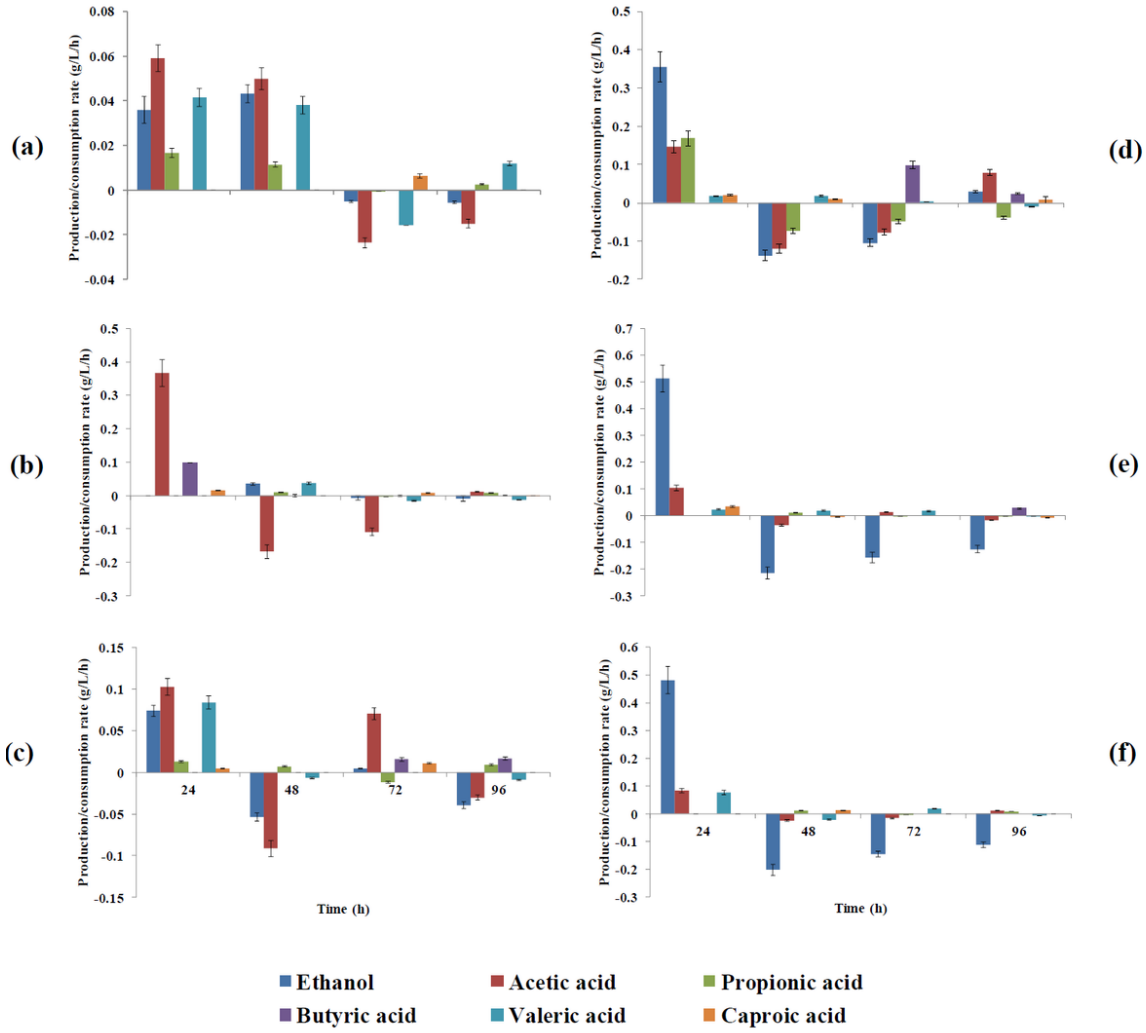

Figure 3

Ethanol and VFA kinetics in (a) FW; (b) FW + 2 % GC; (c) FW + 4 % GC; (d) FW + 6 % GC; (e) FW + 8 % GC; (f) FW + 10 % GC

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