South African Journal of Chemical Engineering 41 (2022) 98–104

Contents lists available at ScienceDirect

South African Journal of Chemical Engineering

journal homepage: www.elsevier.com/locate/sajce

A rumen based anaerobic digestion approach for lignocellulosic biomass using barley straw as feedstock

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ARTICLE INFO

Keywords:
Biogas
Barley straw
Organic load
Rumen fluid
Bioenergy
RUBAD

ABSTRACT

The use of rumen-based anaerobic digestion (RUBAD) has been shown to have a high rate of biogas production and can lead to a lower retention time to digest lignocellulose biomass. Albeit the use of rumen fluid in anaerobic digestion is proven to increase the rate of digestion, there is a lack of literature focused on finding the optimal organic loading of barley straw biomass when using rumen fluid as an inoculum. Therefore, this study aimed to use rumen fluid as an inoculum to test different organic loadings (OLs) of barley straw on biogas production in batch mode and using a locally designed semi-continuous rumen digester. Batch digestions in 120 mL reactor volume were carried out in triplicates to test the effect of different concentrations of barley straw ranging between 2.03 gVS/L and 24.41 gVS/L. The locally designed digester functioned like the rumen simulation technique (RUSITEC) system with a total volume of 2 L inoculum. It was fed daily with 20 g (wet weight) barley straw contained in a nylon bag (20 μm pore size) to determine the daily biogas production. Results from the batch experiment showed the biogas yield increased with an increase in organic loading (OL). Most of the OLs have a biogas yield between 250 mL/gVSadded and 270 mL/gVSadded with a methane content of 22%. The OL of 16.24 gVS/L had the highest biogas yield of 269 ± 2 NmL/gVSadded while a further increase in OL to 24.41 gVS/L led to a 19% decrease in biogas yield (226 NmL/gVS added). The designed rumen reactor had a daily biogas production of 12.63 ml/gVS/day and had a stable pH between 6.8 and 7.4 for 15 days. However, only 37.74% and 48.40% of the total solids were digested after two days of solid retention time. More than half of the barley straw feedstock remained undigested, and therefore, would require a longer retention time to complete the digestion. This study demonstrates the importance of finding the optimal organic load for different feedstocks and led to a 43.78% increase in biogas yield under the same conditions. The use of rumen fluid as inoculum can significantly lower the volume of digesters required and lower the retention times for biogas production vital to the anaerobic digestion industry.

Abbreviations

RUBAD	Rumen-based anaerobic digestion
RUSITEC	Rumen simulation technique
TAN	Total ammonia nitrogen
TKN	Total Kjeldahl nitrogen
TS	Total solids
TSS	Total suspended solids
UASB	Up-flow anaerobic sludge blanket
VFAs	Volatile fatty acids
VS	Volatile solids

1. Introduction

Anaerobic digestion is a growing technology that can lower companies' carbon emissions worldwide and minimize organic waste being generated. The use of anaerobic digesters for electricity production has grown significantly throughout the world as they become aware of the impact of global warming and the benefits anaerobic digestion holds for the environment to achieve zero waste (Auer et al., 2016). However, for anaerobic digesters to continuously supply electricity, there is a growing need for constant waste feedstocks. The partial solution to this problem is using agricultural waste such as barley straw and other fast-growing

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https://doi.org/10.1016/j.sajce.2022.05.005
Received 15 December 2021; Received in revised form 14 April 2022; Accepted 18 May 2022
Available online 26 May 2022
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fibrous grasses as an alternative feedstock in anaerobic digestion due to its high carbon content and overall availability throughout the year (Clauser et al., 2021). The use of these biomasses will supply anaerobic digesters with continuous feedstock and can significantly impact the use of anaerobic digesters in various African countries.

The use of fibrous grasses in anaerobic digestion has seen significant growth in recent years and is usually co-digested with other waste streams to achieve a higher biogas yield (Nitsche et al., 2017; Sayara and Sánchez, 2019). However, these fibrous grasses consist of three polymers - hemicellulose, cellulose, and lignin, of which lignin limits the degradability during anaerobic digestion, biogas yield and leads to longer retention times (Chiumenti et al., 2018; Lizasoain et al., 2017). Different biological, chemical and physical pretreatments such as stream explosion, NaOH, and ultrasound have been used to increase the biogas produced from grasses, but it leads to increased cost and lowers the economic viability of the anaerobic digestion of the grasses (Lizasoain et al., 2017; Zhu et al., 2010; Yang and Wang, 2019). Another technique used by researchers is a biological pretreatment with the use of rumen fluid to increase the biogas produced and lower the retention time for biogas production from grass (Okeh et al., 2014; Jin et al., 2018; Nguyen et al., 2019). The rumen is known to be one of the most diverse microbial ecosystems shown to reduce the retention time for the digestion of lignocellulose biomass during anaerobic digestion (Pertiwiningrum et al., 2017; Zou et al., 2018; Jin et al., 2018). Rumen fluid is a readily available waste source from abattoirs for the mono-digestion of lignocellulose feedstocks during anaerobic digestion.

Several researchers have successfully implemented rumen fluid in co-digestion and mono-digestion of grass feedstocks to increase biogas yields (Gijzen et al., 1988; Budiyono et al., 2014; Nguyen et al., 2019). However, research remains unclear into what the optimum organic loading is for biogas production when making use of rumen fluid as an inoculum. For an anaerobic digester plant, it is essential to determine what the optimum organic loading is to maximize biogas production, increase the efficiency of the process, and limit inefficiencies. A too high organic loading in a digester will lead to acidification of the reactor, and this will cause the bacteria to succumb and lead to system failure and no biogas production. A too low organic loading in a digester will lead to low efficiency and lower the system's economic viability. Therefore, this study aimed to find the organic loading that led to the highest biogas yield for the mono-digestion of barley straw, which is a readily available waste feedstock while using rumen fluid as an inoculum. The study went further to test the optimal organic loading using a novel designed semi-continuous rumen based digester to determine the biogas and methane production.

2. Materials and methods

2.1. Sampling of feedstock and inoculum

The feedstock sample used in this study was barley straw was collected from a farm outside Malmesbury, South Africa. The feedstocks were cut with scissors and transported in air-tight containers to the laboratory. The feedstocks were milled through a hammer mill (Retsch ZM200, Germany) with a mesh size of 4 mm and stored at−4 °C until required.

Fresh rumen fluid was collected from two ruminal cannulated lactating Holstein cows located on the Welgevallen Experimental Farm at the University of Stellenbosch, South Africa. All rumen collections followed the rumen extraction protocol of the University of Stellenbosch, with the help of a research assistant. A thermos flask was prewarmed with boiling water before being transported to the farm to keep the flasks warm. Once on the farm, rumen fluid was collected from the rumen of the cows and filtered through two layers of cheesecloth to remove all solid materials before being transported to the laboratory in prewarmed thermos flasks (2 L). The rumen fluid was gassed with CO_2 before use to maintain an anaerobic environment and then measured for the pH and temperature.

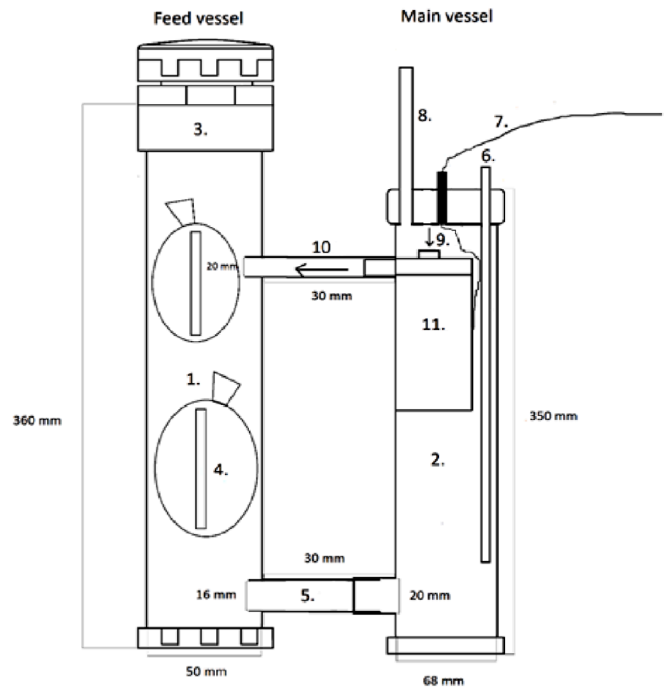

Fig. 1. . Design of rumen-anaerobic digester; 1 - Reactor one; 2 - Reactor two; 3 – Screw cap lid of reactor one; 4 - Nylon bag with rigid tube; 5 – Bottom flow through a pipe; 6 – Artificial saliva input port; 7 - Electrical cord for the magnetically coupled pump; 8 – Overflow pipe; 9 & 10 – Flow direction; 11 - Magnetically coupled pump (submersible).

2.2. Incubation medium and solutions

The preparation of all solutions followed descriptions by Goering and Van Soest (1970) and Van Soest, Robertson, and Lewis (1991). For reference, the chemical compositions of the buffer, macromineral, micromineral and reducing solutions are clearly listed in the above-cited literature. Resazurin solution was made by adding 0.1 g Resazurin in 100 mL distilled water to create a 0.1% solution. The macro- and micromineral solutions were stored at 4 °C in glass containers.

2.3. Rumen digester design and functioning

The adapted, designed RUSITEC system consisted of two high-density polyvinylchlorides (HDPVC) reactors (Fig. 1). The two reactors are interlinked with two gas-tight HDPVC pipes at the top and the bottom, where rumen fluid can freely flow through without clogging the pipes. Reactor 1 has a screw cap lid where the nylon bags (100 µm) containing the feedstock and rumen solids can be added (Fig. 1). The cover of reactor 2 has three ports, each sealed with a PVC gland to ensure it is gas-tight and to maintain anaerobic conditions (Fig. 1). The reactor's functioning is similar to the functioning of other RUSITEC reactor systems (Czerkawski and Breckenridge, 1977; Martínez et al., 2010; Duarte et al., 2017). Both reactors are sealed using a screw cap, and the pipes are connected to the lid of reactor 2, where the gas and effluent will be able to move through the overflow pipe into the overflow vessel and then the gas bag (Fig. 2). The whole assembled RUSITEC system is placed in a thermostatically controlled water bath at 40 °C to function under mesophilic conditions (Fig. 2). On the first day of incubation, barley straw (20 g) and rumen solids (40 g) were added to separate nylon bags with a size of 8 cm x 15 cm (pore size of 100 µm), and the two nylon bags were inserted in reactor 1 (Fig. 1).

The reactor was filled with 1.5 L strained rumen fluid. The artificial

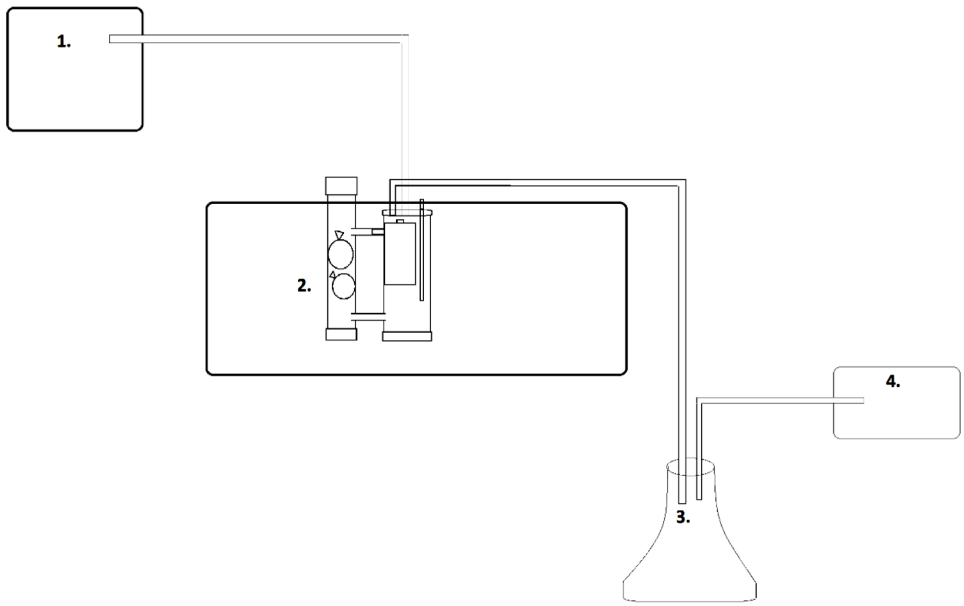

Fig. 2. . Layout of the system; 1 - Peristaltic pump for the input of artificial saliva; 2 - Rumen digesters in a thermostatically controlled water bath (37°C); 3 - Overflow vessel in an ice bath (2.5 L); 4 – 5 L Tedlar bag.

saliva solution with adjusted pH of 8.2 (van Soest, 1970) was infused with a peristaltic pump (Watson Marlow sci 323) at 1500 mL/day, where the inlet tube extended to the bottom of reactor 2 to mix the contents (**Fig. 1**). Each day the vessel was opened, and the nylon bags were removed and replaced with a new nylon bag filled with barley straw (20 wet weight). The original nylon bag with rumen solids was rejected. After every second day, the nylon bag that was incubated for two days was removed and gently squeezed and washed with 40 mL artificial saliva solution. After the wash, the fluid is added back into the reactor. The overflow pipe is connected to an overflow vessel, which is placed in an ice bath at 3–4 °C to store effluent (**Fig. 2**). The pressure from the peristaltic pump to add artificial saliva in the second reactor will cause the effluent to flow through the overflow pipe to the overflow vessel while the peristaltic pump adds artificial saliva in the system. The overflow vessel has a gas outlet pipe connected to a Tedlar bag (**Fig. 2**). For the testing of the reactor system, the reactor was run for 15 days for biogas production, similar to other RUSITEC systems (Duarte et al., 2017). The gas was measured using the water displacement method (Al Mamun et al., 2018). The quality of the biogas produced was measured with a Biogas Analyzer 5000 (Geotech, UK).

2.4. Biogas production potential set-up

Glass vials with a total volume of (120 mL) were used as anaerobic digesters for the feedstocks. The vials were incubated in a controlled incubator and were maintained at a temperature of 38.8 °C. Each vial had a magnetic stirrer (0.2 mm) added which stirred the contents for two hours in three-hour intervals at 250 x rpm. The exact volume of each vial was predetermined as it was required to calculate the total gas produced. Each vial was filled with milled barley straw before the experimental set-up, and 40 mL of the reduced incubation medium was added to each vial. This step was followed by adding 10 mL of the rumen fluid to each vial, making up a total working volume of 50 mL. The digesters were incubated until there was little to no more biogas production.

2.5. Experimental set-up

Different organic loadings (2.03 gVS/L – 24.42 gVS/L) were used to determine the best organic loading for biogas production when using rumen fluid as inoculum and barley straw as a feedstock. The experiment was conducted under mesophilic conditions (38–39°C) to replicate the conditions in the rumen of a cow. Each treatment was run in triplicate with the reactors incubated as described above. At the end of the run, samples were taken from each vial to determine the volatile fatty acids (VFA) and digested total solids (TS).

2.6. Gasometer

The reading pressure technique (RPT) was used to determine the gas produced (Mauricio et al., 1999). The pressure in each vial was measured with a surgical needle and precision digital pressure gage model (WIKA, CPG1500, Germany) daily. The pressure was released at regular intervals with a surgical needle to prevent the pressure from becoming too high in the headspace of the vial ($\geq$ 9 psi).

The gas quality was measured at the end of the experimental run. The gas was sampled from the pressured vessels with a surgical needle and 50 mL syringe and analyzed with a portable Biogas Analyzer 5000 (Geotech, UK). The methane content was adjusted with **Eq. (1)** according to German standard methods (Kafle and Kim, 2012):

$$CH_{4\ Corr.} = \frac{CH_4.100}{CO_2 + CH_4} \tag{1}$$

Where: $CH_{4\ Corr.}$ = corrected methane content in the gas (%); CO_2 = Measured carbon dioxide content from Biogas Analyzer 5000 (%); CH_4 = Measured methane content from Biogas Analyzer 5000 (%).

The linear equation (**Eq. (2)**) was developed for the setup in the Department of Animal Sciences, University of Stellenbosch and was used to convert the pressure in the glass vial to the gas volume at standard temperature and pressure (STP):

$$Y = \frac{[(1000(0.0977X)C)]}{gVS} \tag{2}$$

Where: Y = Gas volume (mL/g VS), X = Gas pressure (psi) and C = Vial head space (mL).

2.7. Input and output analysis

The total solids (TS), total suspended solids (TSS), and ash were determined for both the inoculum and feedstocks according to standard procedures (APHA, 2005). According to the National Renewable Energy

Table 1
Characterization of feedstock and inoculum.

Characteristic	Inoculum (rumen fluid)	Barley straw
Total solids (%)	2.27 ± 0.22	86.83 ± 0.13
Volatile solids (%)	1.47 ± 0.16	81.37 ± 0.13
Ash (% of TS)	39.22 ± 2.97	7.26 ± 0.23
Water extractives (% of TS)	nd.	16.24 ± 1.11
Ethanol extractives (% of TS)	Nd	1.96 ± 1.29
Total Extractives (% TS)	Nd	17.61 ± 1.85
Hemicellulose (% of TS)	Nd	29.74 ± 2.88
Cellulose (% of TS)	Nd	33.78 ± 0.42
Lignin (% of TS)	Nd	20.70 ± 0.6
Carbon (% of TS)	Nd	45.89
Nitrogen (% of TS)	Nd	0.76
Kjeldahl-NH_4^+ (% WW)	0.148	nd
Oxygen (% of TS)	Nd	47.81
hydrogen (% of ts)	Nd	5.54
sulfur (% of ts)	Nd	bdl
pH	6.22 ± 0.07	nd

Bdl – below detection limit, nd, not determined.

Laboratory (NREL) analytical procedure, the ethanol and water extractives, lignin, cellulose, and hemicellulose were determined for the feedstock (Sluiter et al., 2008). The feedstock's carbon, nitrogen, hydrogen, sulfur, and oxygen were determined by central analytical facilities (CAF), Stellenbosch University, using an Elementar Vario EL Cube Analyzer. The pH was measured with a portable digital pH Pen Meter Tester (accuracy: +/− 0.1). The VFA of the inoculum and digestate were analyzed with high-performance-liquid chromatography (HPLC) using standard procedures (APHA, 2005). The total suspended solids (TSS) in the digestate were subtracted from the total solids added to the reactors to calculate the total percentage of digested.

2.8. Data and statistical analysis

All samples were run in triplicate, and the values are reported as the average of the triplicates. The results were statistically evaluated using STATISTICA software version 13 through the Tukey-test to test if the means of the samples are statistically different from one another. The results were statistically significant if the p-values were below 0.05.

3. Results and discussion

3.1. Feedstock and inoculum characterization

The characteristics of the inoculum and feedstock are summarized in Table 1. Barley straw showed a high number of total solids (TS) and volatile solids (VS), making 86.83% and 81.37% of the wet weight, respectively. Similar results have been reported in other studies that characterized barley straw (Serna-Díaz et al., 2020). In the present study, the cellulose, hemicellulose, and lignin of barley straw were 33.78%, 29.74%, and 20.7% of TS, respectively. Barley straw has 16.4% extractable in water and only 1.96% in ethanol. Based on barley straw's elemental analysis, the empirical formula can be extrapolated as follows: $C_{60}H_7O_{59}N$. The carbon to nitrogen ratio is 60:1 for barley straw, which is relatively lower than other studies, reporting a C/N ratio of 80:1 and 145:1 (Christensen, 1985; Serna-Diaz et al., 2020).

3.2. Biogas production and kinetics from different organic loadings

The biogas production potential curves when looking at different organic loadings (gVS/L) are shown in Fig. 3. The control, with no feedstock added, did not produce a significant amount of biogas with the organic loading of 2.03 gVS/L to 16.27 gVS/L leading to an increase in the biogas yield from 186 mL/gVS to 269 mL/gVS. However, a further increase of organic loading to 24.41 gVS/L lowered the biogas yield to 204 mL/gVS, which is a 25% decrease compared to the previous organic loading of 16.27 gVS/L (Fig. 3). The overall trend suggests that if more feedstock is available it will lead to a higher biogas production. However, due to the complexity and sensitivity of anaerobic digesters it can be seen that this is not the case. The results shown in this study is contrary to another study by Rajput & Sheikh (2019), which showed an increase in organic loading from 2 gVS/L to 14 gVS/L of wheat straw and sunflower seeds led to a decrease in the biogas production with various different feedstocks. However, rumen fluid was not an inoculum used in the study. Which might suggest that rumen fluid holds the key to increase the biogas production with an increased loading rate. The different feedstocks have different effects on the biogas production which highlights the importance for the mono-digestion of different feedstocks to fully understand what feedstocks is viable in anaerobic digestion. Another study done by Nguyen et al. (2019) tested the biogas production from lignocellulose feedstocks using anaerobic sludge and rumen fluid inoculums. The study found that rumen fluid had a lower biogas production compared to anaerobic sludge due to lower

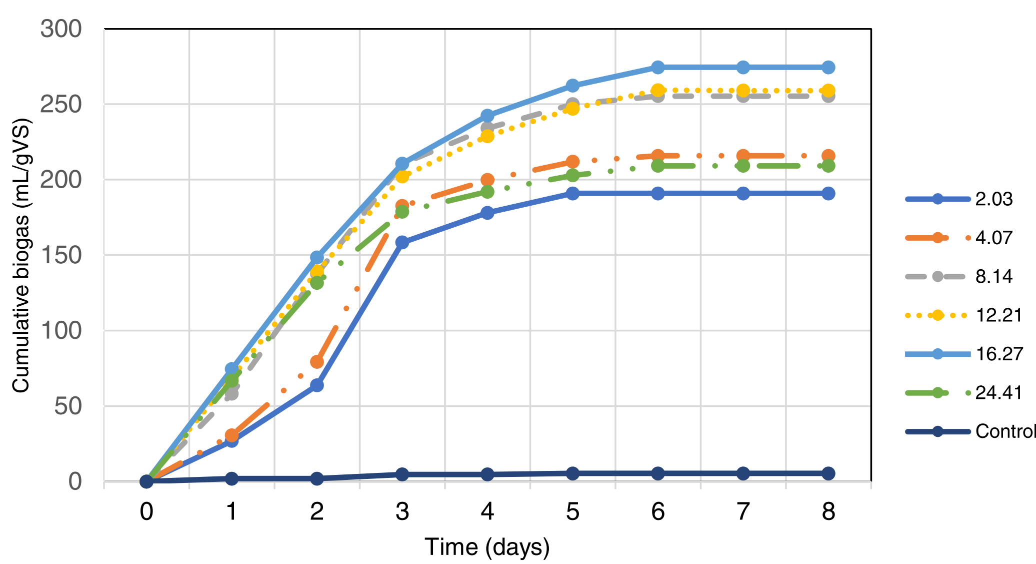

Fig. 3. . Cumulative biogas potential from different organic loadings (gVS/L). The values are reported as averages of samples run in triplicate. $n = 3$.

Table 2
Experimental data fitted to the modified Gompertz model.

Organic loading (gVS/L)	Biogas production (mL/gVS) Predicted	Measured	Error %	μm (mL/gVS/day)	λ(Day)
2.03	188	186	1.34	84.950	1.129
4.07	213	210	1.00	91.295	0.990
8.14	252	250	0.65	90.433	0.430
12.210	255	254	0.61	79.494	0.234
16.27	271	269	0.72	82.675	0.203
24.41	203	204	−0.40	75.740	0.197

μm - maximum biogas production; λ – lag time.

methanogens present in rumen fluid. The lower biogas production of barley straw in a two-staged system was also noted at an organic loading of 24.41 gVS/L which led to acidification of the methanogenic digester (Gijzen et al., 1988).

The organic loading of 2.04 gVS/L and 16.27 gVS/L reported in this study suggests that rumen fluid can digest an extremely high organic loading resulting in the possibility to lower digester volumes and increase the economic viability of anaerobic digestion. The standard time for biogas production is between 15 and 30 days, whereas the biogas production potential in this study started to plateau after six days and fully plateaued at eight days independent of the organic loading used (Raposo et al., 2011; Rajput and Sheikh, 2019).

The kinetics of the different organic loadings were determined by fitting the experimental results to the Modified Gompertz. The lag phase between the different organic loadings decreased with a concomitant increase in organic loading (**Table 2**). The lower lag phase during start-up indicated that there are more than enough living microorganisms present in rumen fluid to digest the biomass feedstock to initiate the digestion of barley straw. The lag phase at an organic load of 2.03 gVS/L was 1.129 days and decreased to 0.197 days at an organic loading of 24.41 gVS/L (**Table 2**). The results show that low organic loadings led to a higher lag phase, and this might be attributed to the vast number of microorganisms present in rumen fluid as the microorganisms do not survive in the reactor due to inadequate amount of nutrients in the reactor. The kinetics result also suggests that an adequate number of microorganisms is present in the specific organic loadings 8.14 gVS/L and 16.24 gVS/L is sufficient for biogas production when using rumen fluid. The maximum biogas production rate per day (μ_m) was the highest between an organic loading of 4.07 gVS/L and 8.14 gVS/L (91.295 mL/gVS/day and 90.433 mL/gVS/day); with the lowest maximum biogas production rate per day (μ_m) was achieved for the organic load of 24.41 gVS/L which was 75.74 mL/gVS/day. The high maximum production rate may also be attributed to the lower lag times in lower organic loadings. However, the general maximum biogas production was above 79 mL/gVS/day for the organic loadings between 2.03 gVS/L and 16.27 gVS/L. The maximum rate of biogas production in this study is 23 times higher compared to a study digesting cow manure with rumen fluid (Budiyono et al., 2014; Seno and Nyooman, 2010).

3.3. Production of volatile fatty acids and total solids digested

The total volatile fatty acids (VFAs) in the digestate indicate the efficiency of hydrolysis and acidification of the microorganisms in the system. It also serves as an indicator of the methanogenic activity in the reactor, as this is the intermediate step for methane production (Nguyen et al., 2019). Biogas production is desired to have a low VFA concentration, as too high a VFA concentration will lead to acidification in the reactor and system failure. An organic loading of 2.03 gVS/L, 4.07 gVS/L, and 8.14 gVS/L yielded a total of 2.07 g/L, 2.53 g/L, 2.57 g/L VFAs, respectively (Fig. 4), while the organic loading of 12.21 gVS/L, 16.27 gVS/L, and 24.41 gVS/L yielded a total of 3.47 g/L, 5.38 g/L, and 6.64 g/L VFAs, respectively (Fig. 4). Acetic acid and propionic acid are the highest producing volatile fatty acids in the digesters, making up 68% and 19% of the VFAs, respectively. The amount of VFAs produced in the digestate correlates well with the drop in the pH of the digestate (Table 3). The total VFAs increased as the organic load increased from 2.03 gVS/L to 24.41 gVS/L ($r = 0.97$). Similar results have been reported in other studies done with rumen fluid, with a rapid increase in the VFAs during anaerobic digestion of biomass (Gijzen et al., 1988; Kivaisi et al., 1992; Nguyen et al., 2019; Rajput and Sheikh, 2019). However, it has been stated that high organic loadings of barley straw will not lead to

Table 3
Summary of results from different organic loadings (OL) of Barley straw. The standard deviation is shown from the triplicates.

Organic loading (gVS/L)	Digested total solids (%)	Total VFA in digestate (g/L)	VFA/gVS added	pH after digestion	Biogas yield (NmL/gVS)
2.03	74	2.07 ± 0.1	1.02	6.83 ± 0.03	185 ± 14
4.07	65	2.53 ± 0.24	0.62	6.83 ± 0.03	210 ± 17
8.14	69	2.57 ± 0.30	0.32	6.7	250 ± 20
12.21	59	3.47 ± 0.62	0.28	6.56 ± 0.03	257 ± 2
16.28	60	5.38 ± 0.62	0.33	6.3	269 ± 2
24.41	54	6.64 ± 0.44	0.27	5.97 ± 0.03	226 ± 17

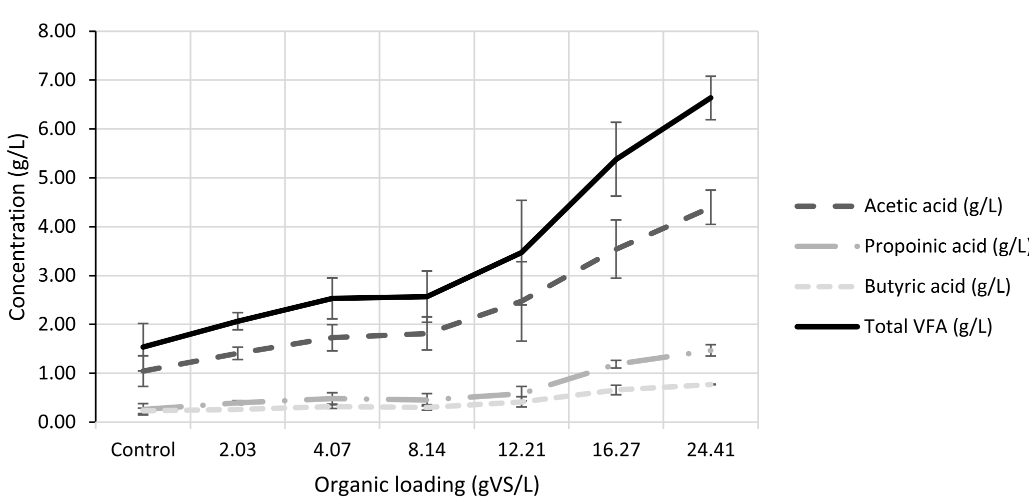

Fig. 4. . Total volatile fatty acids produced from different organic loads in the reactors (gVS/L). The error bars indicate the standard deviation of the samples, $n = 3$.

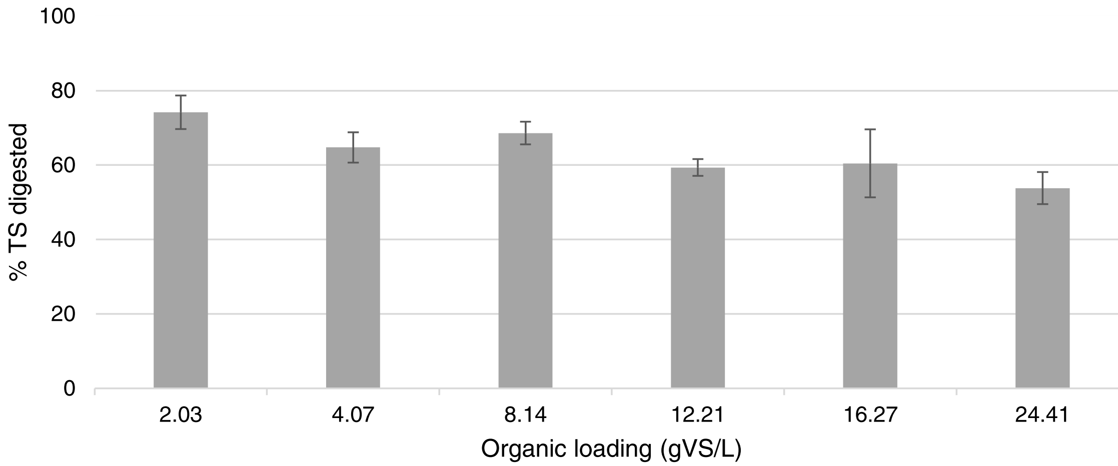

Fig. 5. . The percentage of total solids digested from different organic loadings (gVS/L). The error bars indicate the standard error of replicates ($n = 3$).

acidification in the reactor (Dubrovskis et al., 2012). This observation is contrary to the results reported in this study, as the higher organic loadings of barley straw led to acidification of the digester (Table 3). The cellulolytic microorganisms in rumen fluid produce a high amount of VFAs from the barley straw in a short amount of time and led to acidification in the reactor at high organic loadings. It has been desired to have a pH value between 6 and 6.5 for anaerobic digestion for the methanogenic bacteria to produce methane gas (Noxolo et al., 2014). This study supports the findings by Nguyen et al. (2019) where rumen fluid leads to the fast production of VFA from grasses, leading to higher organic loadings and smaller digesters, which will reduce the capital input to produce biogas.

The organic load of 2.03 gVS/L had the highest percentage of degraded total solids, 74%. The high degradation indicates that the microorganisms had limited substrate and are trying to digest the undegradable parts of the biomass, therefore leading to a higher percentage of total solids being digested. The organic load of 4.07 gVS/L; 8.14 gVS/L; 12.21 gVS/L; 16.27 gVS/L, and 24.41 gVS/L had a degradability of total solids of 65%, 69%, 59%, 60% and 54% respectively (**Fig. 5**). In this study, the percentage of total solids degraded is similar to other studies testing the degradation of lignocellulose biomass in rumen fluid, with values ranging 55%−60% (Czerkawski and Breckenridge, 1977; Gijzen et al., 1988; Kivaisi et al., 1992). The low degradation of barley at a high organic load (24.41 gVS/L) may be attributed to the low pH value of 5.97 and high VFAs, which caused the microorganisms to succumb in the digester inhibiting the further degradation the barley straw.

3.4. Biogas and methane production from the designed rumen digester

The average biogas produced each day was 12.63 mL/gVS/day (**Fig. 6B**). The cumulative biogas and methane produced from the designed digester followed a linear line for the duration of the experiment, **Fig. 6A**. The overall biogas produced in the semi-continuous system is lower compared to other studies digesting barley straw as a substrate which may be due to the low solid and hydraulic retention time (HRT) in the digester, Day1 and Day2, respectively (Kivaisi et al., 1992; Nguyen et al., 2019). The sudden drop of biogas production on Day4 and Day6 is due to a malfunction in the peristaltic pump, which could not add artificial saliva to the reactor, leading to a pH drops from 6.9 to 6.6 in the reactor and decrease the buffer capacity. However, on Day 7, the reactor recovered to a pH value of 7. The average methane percentage of the biogas produced each day was 41.5%. This methane yield is slightly higher than other reactors using rumen fluid as an inoculum (Gijzen et al., 1988b; Kivaisi et al., 1992). The daily methane production was relatively low compared to other anaerobic digesters due to the methanogenic bacteria washing out during the short HRT. Only half of the added substrate was digested during the reactor's running, which resulted in low biogas production. Comparing the batch mode of digestion with the semi-continuous system it can be seen that the batch mode has a very high maximum biogas yield of 79 mL/gVS/day when using rumen fluid to digest the biomass. However, the residual solid digestate is considered to be a highly valuable protein source and is to be further explored in future research.

4. Conclusion

Rumen fluid has been shown to digest barley straw during the monodigestion to produce biogas effectively. The organic loading between 8.14 gVS/L and 16.27 gVS/L had no statistically significant biogas production potentials. However, the organic loading of 16.27 gVS/L had between 5.65% and 43.79% more biogas yield than other organic loadings. The increase of the organic loading to 24.41 gVS/L had detrimental effects on the biogas production potential, leading to an increase in VFA in the reactor and a biogas production decreased by 25%. A suggestion is to work with an organic loading between 8.14 gVS/L and 16.27 gVS/L when using rumen fluid as an inoculum. The designed rumen reactor had a daily biogas production of 12.63 ml/gVS/day and had a stable pH between 6.8 and 7.4 for 15 days. The use of rumen fluid as inoculum can significantly lower the volume of digesters required and lower the retention times for biogas production. It is worth investigating the co-inoculation of rumen fluid and anaerobic sludge for a better biogas yield and lower the VFA acclimation.

CRediT authorship contribution statement

Gustav Meyer: Methodology, Formal analysis, Investigation, Writing – original draft. **Vincent Okudoh:** Conceptualization, Formal analysis, Resources, Methodology, Writing – review & editing, Supervision. **Eugene van Rensburg:** Conceptualization, Resources, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

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

Acknowledgments

This work was funded by the Department of Process Engineering, Stellenbosch University. Our sincere thanks go to the staff of the

Chemical Engineering Department at the Cape Peninsula University of Technology and the Department of Process Engineering at Stellenbosch University, especially Prof Johann Gorgens, for their contribution and help in completing this study.

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