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Dry fermentation of Manure with Straw in Continuous Plug flow Reactor: Reactor Development and Process Stability at Different Loading Rates

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Abstract

In this work, a plug flow reactor was developed for continuous dry digestion processes and its efficiency was investigated using untreated manure bedded with straw at 22 % total solids content. This newly developed reactor worked successfully for 230 days at increasing organic loading rates of 2.8, 4.2 and 6 gVS/L/d and retention times of 60, 40 and 28 days, respectively. Organic loading rates up to 4.2 gVS/L/d gave a better process stability, with methane yields up to 0.163 LCH4/gVS_{added}/d which is 56 % of the theoretical yield. Further increase of organic loading rate to 6 gVS/L/d caused process instability with lower volatile solid removal efficiency and cellulose degradation.

Keywords:

Dry fermentation; Plug flow reactor; Continuous process; Process stability; Reactor development

1. Introduction

Anaerobic digestion of organic wastes for biogas production has been successfully applied but there is a need for improved processes and design of less expensive reactors. In this vein the drive for dry anaerobic digestion processes is increasing both in research and the industry because of technical simplicity in design, together with low construction and operational costs (Karthikeyan & Visvanathan, 2013; Kothari *et al.,* 2014). Dry anaerobic digestion technology is designed to process more organic wastes per reactor volume with total solids (TS) content greater than 20 % (Demirer & Chen, 2008; Fernández *et al.,* 2008) treating food wastes, manure bedded with straw, garden wastes and other high solid waste fractions. In comparison with the wet anaerobic digestion process, this process allows higher organic loading rates (OLR), less pretreatment and gives better economic feasibility (Karthikeyan & Visvanathan, 2013) since the reactor volume is minimized and it is easier to handle the digestate residue.

Animal wastes and crop residues are one of the most abundant waste fractions generated worldwide, the amount of manure bedded with straw produced increases daily as the number of housed dairy herd increases. Cattle manure bedded with straw usually have a TS greater than 20 %, farmers can use low cost anaerobic digesters to convert these enormous waste streams to biogas, thereby improve water quality, reduce methane and nitrous oxide emissions and improve soil fertility. Dry anaerobic digestion is therefore a better option for processing these wastes. Since biogas production involves a complex biological process, monitoring of the process is essential to avoid process instability and failure of the digester (Drosg, 2013). For enhanced performance of dry anaerobic digestion processes, a suitable reactor is required; considering the substrate composition, amount of substrate to be treated, and process economy of the reactor.

Plug flow reactors have been reported to be efficient for dry anaerobic digestion processes. These reactors are inexpensive and easy to build which make them a suitable technology to improve the livelihoods of farmers (Lansing *et al.,* 2010; Lansing *et al.,* 2008). Plug flow reactors have also been reported to have the highest success rate in the United States, where 42% out of the 242 anaerobic digesters operating at livestock farms in 2015 were plug flow designs (USEPA, 2016). Nevertheless, some shortcomings, such as lower mass transfer due to lack of mixing, thermal stratification and solid sedimentation problems have been reported (Lansing *et al.,* 2010). These problems can be minimized by the use of impellers in plug flow reactors. The impellers allow minimal mixing for better performance in the reactors. In high solid digestion processes, however, continuous mixing have been reported to indicate unstable performance at high OLR and it was observed that the continuously mixed unstable reactor became stable when the mixing level was reduced (Stroot *et al.,* 2001). Researchers have also investigated the effectiveness of plug flow reactors on manure and other substrates with solid content in the range of 11 – 14 % TS (Adl *et al.,* 2012; Cantrell *et al.,* 2008). There have been studies on dry digestion of different substrates in batch reactors but little information is available on dry digestion in continuous plug flow reactors. The innovation of this paper in meeting this gap was the development of a novel type solid-state plug-flow laboratory reactor to treat substrates at higher TS levels, i.e. greater than 20 %.

The efficiency of this reactor was then investigated in a continuous dry anaerobic digestion process treating manure bedded with straw at 22 % TS content. The main objective was to identify the critical OLR, above which instability can occur in the reactor. The process was monitored by measuring VFA/Alkalinity ratio, pH, volatile fatty acid (VFA) and total ammonia nitrogen concentrations regularly. This is vital because the cost of starting the entire process over again far outweighs monitoring of the process.

2. Materials and Methods

2.1. Reactor Design

A horizontal plug flow reactor (Fig. 1) for continuous dry anaerobic digestion process was designed and made-up at the University of Borås, Sweden. This reactor has 9.2 L total volume, 1565 mm length and maximum inside pressure of 10 kPa. It was mounted on a base surface with which a clamp to the two edges of the reactor was associated for suspending the reactor. Mesophilic (37 ^{o}C) conditions were maintained by circulating water from a heater, a thermostatic water bath (GD 100, Grant instruments Ltd., Cambridgeshire, UK), through a water jacket (150 mm outer diameter with 4 mm wall thickness and capacity of 5 L) surrounding the reactor. The reactor was then shielded with a 10 mm thick-Styrofoam to avoid heat loss.

The reactor was sub-divided into 3 zones as follows. Inlet zone – this part was made of polyethylene material with sealed buffer system to avoid the release of gas and air entering the system. This part can be considered as an inactive zone in the inlet system. A shutoff valve was connected at the inlet with a piston rod (made gas tight by O-rings). The inlet storage volume is about 0.256 L with 0.0053 L of feedstock per rotation.

Main Zone – the main zone of the reactor was made of a Polymethylmethacrylate (PMMA) material with 110 mm outer diameter and 5 mm thickness for easy handling and to make the reactor transparent. An impeller, installed on a hexagonal shaft that runs through the reactor connected the inlet to the outlet. The hexagonal shaft had two oil seals that prevented leakage of materials and several O-rings making the different parts gas tight. The impeller allowed mixing of the feedstock at the bottom part of the inlet, and transported the materials very slowly towards the outlet taking several rotations: 100 cm^3 per rotation but it can also depend on the viscosity of feedstock and working volume of reactor.

Outlet zone – this part was also made of polyethylene material with an outlet pipe for the collection of the digestate residue. The material was transferred from inlet towards outlet at the end of the reactor by rotating of the impeller shaft; as a result the digested residue was discharged through the outlet pipe while new materials were added. The impeller handle was connected to the outlet for manual rotation. In this zone, the gas outlet was also connected where the daily volume of biogas produced was measured by the tipping device in the automated methane potential test system (AMPTs); meanwhile with an inserted thermocouple the temperature of the reactor was controlled.

Safety measures - a construction was made with a polyethylene material at the inlet and outlet with specific rubber connectors for sealing. If pressure inside the reactor would increase beyond 10 kPa due to blockage in the gas outlet or for any reason, this part of the reactor could open up avoiding an explosion. All interior parts were carefully selected in order to avoid any chemical reaction with the experimental materials as well as any corrosion during the test.

Fig.1 shows a schematic diagram of the reactor and the experimental set up together with other accessories, such as heater and water bath for maintaining the temperature, sampling point for biogas composition analysis and the AMPTs system for measuring the biogas produced.

2.2. Substrates and Inoculum

The substrate, cattle manure bedded with straw, was collected from a cattle farm outside Borås (Sweden) and used as feedstock for the continuous anaerobic digestion process. During the experimental period two different batches, with similar content of total solids (TS) and volatile solids (VS) were obtained from the same farm. The manure was shredded manually to reduce the particle size of straw; then it was characterized, weighted and stored in plastic containers at -20 ^{o}C to prevent biodegradation until further use. During experiment, weighted frozen substrate was defrosted at room temperature and thoroughly mixed to gain a homogenized feed before use. Sludge used as inoculum was obtained from a digester treating waste water sludge and operating at mesophilic conditions (Vatten and Miljö i Väst AB, Varberg, Sweden). The inoculum was filtered through a 2 mm porosity sieve to remove sand, plastic and other unwanted particles after which it was acclimated for five days in an incubator at 37 ^{o}C prior to use. The inoculum was centrifuged at 10,000xg for 10 min to obtain a TS content of 7.8 ± 0.24 %. Table 1 shows the most important characteristics of the substrate and the inoculum used during the investigations.

2.3. Experimental Procedure

The substrate was inoculated with inoculum to start-up the reactor, keeping a volatile solids (VS) ratio ($VS_{substrate}$ to $VS_{inoculum}$) at 1:2 in the reactor. The pH of the mixture was adjusted to 7.47 by 2 M HCl solution. Furthermore, a nutrient solution with composition according to Angelidaki *et al.* (2009) was also added into the reactor. The experiment was started at batch mode until no further gas production was monitored (40 days of digestion period). After that the continuous feeding operation was started with an OLR of 2.8 gVS/L/d. The experiments continued with gradually increasing OLRs, thereby decreasing retention times with the aim of maintaining a fixed working volume. The OLR values investigated were 2.8 gVS/L/d (OLR 1), 4.2 gVS/L/d (OLR 2) and 6 gVS/L/d (OLR 3) with corresponding retention times of 60, 40 and 28 days. For the experimental period (start-up and OLR1) the first batch of manure was utilized, while another batch of the feedstock was used during the experimental period of OLR 2 and OLR 3. Each OLR condition was kept until a period of at least one corresponding retention time. The reactor was fed regularly in every second day and the digestate residue was withdrawn before feeding, and kept for analysis, while biogas composition was monitored daily. Process parameters, such as pH, VFA/Alkalinity ratio, VFA and total ammonia nitrogen concentration, TS, VS, as well as lignin, cellulose and hemicellulose contents in the digestate residue were also measured to monitor the digestion process. The reactor was mixed manually (once daily) with the impeller by moving its content to the inlet and back to the outlet to minimize stratification in the reactor. Overall, the experiment lasted for 230 days.

2.4. Theoretical BMP of Experimental Feedstock

The feedstock being solid was prepared according to Zupancˇicˇ and Rosˇ (2012) by blending 1g of the sample with water (dilution factor of 50) to reduce the particle size and allow homogenization (Raposo *et al.,* 2012). Thereafter, theoretical methane potential of the substrate used during digestion process was calculated from the amount and chemical oxygen demand (COD) concentration of the actual feeding using equation (1) (Nielfa *et al.,* 2015) assuming that the equation is valid for any substances or products (Tarvin & Buswell, 1934).

$$\mathrm{BMP}_{thCOD} = \frac{n_{CH4}\mathrm{RT}}{\mathrm{p}VS_{added}} \quad (1)$$

where:

BMP_{thCOD} = theoretical yield under laboratory condition

R = gas constant (R = 0.082 atm L/mol K)

T = working temperature (310 K)

P = atmospheric pressure (1 atm)

VS_{added} = volatile solids of the substrate added (g)

n_{CH4} = methane produced (mol) determined according to equation (2)

$$n_{CH4} = \frac{\mathrm{COD}}{64(\frac{\mathrm{g}}{\mathrm{mol}})} \quad (2)$$

2.5. Analytical Procedure

Moisture content, pH, total nitrogen, TS and VS were determined according to biomass analytical procedures (APHA-AWWA-WEF, 2005). Total nitrogen contents were measured using the Kjeldahl method, and the protein content was estimated by multiplying the Kjeldahl nitrogen content with a factor of 6.25 according to Gunaseelan (2009). The total carbon was obtained by correcting the total dry weight carbon value for the ash content (Haug, 1993; Zhou *et al.,* 2015). The fat content was determined using the Soxhlet extraction procedure (Carpenter, 2010). Alkalinity measured as the total inorganic carbonate and was determined by the Nordmann titration method according to Lossie and Pütz (2008). Samples were centrifuged at 4,000xg for 15 min and then 5 ml of the supernatant was titrated with 0.1 N sulfuric acid to pH 5, the titration was then continued until pH 4.4 was reached in order to determine the VFA concentration measured as acetic acid equivalent. Digestate samples for total ammonia nitrogen concentration were centrifuged (15 min at 4,000xg); supernatant diluted 50 times in deionized water to a final volume of 5 ml. Samples were then analyzed using Ammonium 100 test kit (Nanocolor, MACHEREY-NAGEL GmbH & Co. KG.

Germany) and concentration measured using Nanocolor 500D Photometer (MACHEREY-NAGEL GmbH & Co. KG. Germany). The free ammonia concentration was calculated from the total ammonia nitrogen concentration and pH values of samples according to Kayhanian (1999).

Total cellulose, hemicellulose and lignin content of the solid fraction of the digestates were determined according to NREL protocols (Sluiter *et al.,* 2011). The digestate was centrifuged at 4,000xg for 15 min and the solid fraction was washed with 100 ml distilled water and then air dried until moisture content was less than 10 %. The samples were then hydrolyzed using 72 % H_2SO_4 in a water bath at 30 °C for 60 min, samples were stirred every 5 min to ensure uniform hydrolysis, and then a second hydrolysis was performed using 4 % H_2SO_4 in an autoclave at 121 °C for 60 min. Monomeric sugars contained in the hydrolysis liquid were determined by HPLC. Mannose, glucose, galactose, xylose and arabinose were analyzed using Aminex HPX-87P column (Bio-Rad) at 85 °C and 0.6 mL/min ultrapure water as eluent. Acid soluble lignin (ASL) was determined using a UV spectrophotometer (Libra S60, Biochrom, England) at 320 nm. Acid insoluble lignin (AIL) was gravimetrically determined as residual solid after hydrolysis corrected with ash content. The ash content was determined as the remaining residue after keeping the samples in the muffle furnace at 575 °C for 24 h. The percentage of cellulose degraded was calculated according to Zhou *et al.* (2015).

The daily volume of biogas produced was measured by the tipping device in the Automatic Methane Potential Testing System (AMPTS, Bioprocess control AB, Lund, Sweden), which is based on the principle of water displacement buoyancy. The composition (methane and carbon dioxide) of the produced gas was determined using a

GC (Perkin-Elmer, USA) equipped with a packed column (6'×1.8" OD, 80/100, Mesh, Perkin Elmer, USA), and a thermal conductivity detector (Perkin-Elmer, USA), with an inject temperature of 150 °C. The carrier gas was nitrogen operated with a flow rate of 20 ml/min at 60 °C. A 250-µl pressure-lock gas syringe (VICI, precious sampling Inc., USA) was used for taking samples for the gas composition analysis.

VFAs in the digestate filtrates were measured using a high-performance liquid chromatograph (HPLC, water 2695, Waters Corporation, Milford, MA, USA) equipped with an RI detector (Waters 2414, Waters Corporation Milford, MA, USA) and a biohydrogen-ion exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) operating at 60 ^{o}C. A UV absorbance detector (Walters 2487), operating at 210 nm wavelength was used in series with a refractive index (RI) detector (Walters 2414) operating at 60 ^{o}C. A UV absorbance detector (Walters 2487), operating at 210 nm wavelength was used in series with a refractive index (RI) detector (Walters 2414).

3. Results and Discussion

3.1. Reactor Development

During preliminary studies a syphon mechanism was applied initially at the inlet of the reactor to make it gas tight and avoid entering of air during feeding. This mechanism worked well at lower TS rates up to 11 %. However, it was very troublesome when working with higher TS concentrations; i.e. when feedstock with 13 % TS was applied, this completely blocked the syphon system for the feed. After that, the inlet part of the reactor was modified to a sealed buffer system described in subsection 2.1 and Fig. 1. The shutoff valve connected at the inlet was always closed at the start of feeding to avoid gas leakage. When materials were fed in, the piston rod was inserted and tightened, to avoid the release of any gas. After this, the valve was opened, and the material was pressed down into the reactor via the piston rod and then the valve was closed again. The impeller in this reactor allows minimal mixing by moving the reactor content manually to the inlet and back to the outlet for better performance of the process. This new modification worked well even with an inlet feed of 22 % TS without any blockage at the inlet. The developed plug flow reactor worked successfully treating cattle manure bedded with straw at 22 % TS for 230 days using organic loading rates of 2.8, 4.2, and 6 gVS/L/d at retention times of 60, 40 and 28 days respectively.

3.2. Substrate Characterization

The most important characteristics of the substrate as collected from the cattle farm are shown in Table 1. The substrate contains 22.29 % TS of which 70.44 % are organic matter. The carbon nitrogen (C/N) ratio was 16.8:1 which is within the required range for stable anaerobic digestion process. Previously, optimal C/N ratios between 20:1 and 35:1 have been stated (Habiba et al., 2009; Kayhanian, 1999), also, other researchers, i.e. Friehe *et al.* (2010); Pagés Díaz *et al.* (2011) reported a wider range of between 10:1 and 30:1 as optimal C/N ratios. However, the availability of carbon and nitrogen for the microorganisms is more significant than the actual C:N ratio calculated on the basis of elementary composition of the feedstock. Hence the C:N ratio of straw might be overestimated due to the limited availability of carbon fraction in the lignocellulosic structure of straw (Herrmann et al., 2016). The theoretical methane potential of the feedstock used in this experiment was calculated to be 0.290 LCH_4/gVS_{added}.

3.3. Biogas Production

The startup batch digestion period ran for 40 days, the daily gas production was high at the beginning; hence the digestion process started up properly and the feedstock were degraded until the end of this batch digestion period. The methane content in the produced gas reached 63 % ± 3.86 at the end of the startup (batch) period as shown in Fig. 2b. This start-up period was allowed until gas production nearly stopped (40 days) after which the continuous digestion mode with feeding and withdrawing in every second day was started.

Fig. 2a illustrated the daily biogas production at different OLR. Experiments were carried out using progressive organic loading rates; the loading rate of 2.8 gVS/L/d (OLR 1) produced only about 1 L/d in the initial days. Then it gradually increased to around 3 L/d after 36 days and remained at the same level until the end of the OLR1 (2.8 gVS/L/d) period. Similar pattern was observed for the period when OLR 2, (4.2 gVS/L/d) was applied, it started from 3.5 L/d daily methane production initially which increased gradually to around 5 L/d after 18 days, and thereafter it remained stable. The process performed differently when OLR 3, i.e. 6 gVS/L/d was introduced. There were larger fluctuations observed in the daily gas production and only a slightly stable period could be achieved after 20 days maintaining a daily biogas production of around 7 L/d. The reactor was then fed keeping the OLR 3 (6 gVS/L/d) for an additional corresponding retention period of 28 days to check the stability of the process under these conditions. However, the daily gas production could not be kept stable with decreasing values towards the end of this period. In parallel, the VFA concentration continued to increase (Fig. 4a) leading to increased VFA/alkalinity ratios with a maximum of around 0.9 at the end of the period (Fig. 3a) showing instability in the process. To examine if VFA could be reduced and restore stability in the process, the OLR was then reduced to 4.2 gVS/L/d (OLR 2) again. As a consequence of the reduced load the biogas production reduced gradually and remained stable after 16 days maintaining a daily biogas production around 4.2 L/d which is slightly lower than that obtained when the same loading rate (OLR 2) was used previously.

The average methane content in the biogas was 64.9 %, 65.1 % and 63.3 % for OLR of 2.8, 4.2 and 6 gVS/L/d respectively. Overall, OLR up to 4.2 gVS/L/d gave a better process stability with methane yield of 0.163 LCH_4/gVS_{added} accounting around 56 % of the theoretical methane yield of 0.290 LCH_4/gVS_{added} as shown in Table 2. The methane yield at OLR 2.8 gVS/L/d was slightly lower than the yield at OLR 4.2 gVS/L/d probably due to slight differences in the feedstock composition, since these two experimental periods were performed with two different batches of the substrate. This lower methane yield could be associated with the presence of untreated straw in the cattle manure. Hydrolysis of the cellulose in untreated straw has been reported to be the rate limiting step (Noike *et al.,* 1985) especially when it is mixed with cattle manure (Myint & Nirmalakhandan, 2006). However, our result is similar to the methane yield of 0.170 LCH_4/gVS_{added} obtained by Kusch *et al.* (2008) when the digestion of horse dung with straw was investigated in batch-operated solid phase digestion.

3.4. Process Performance

Fig. 3a shows the variation of pH and VFA/Alkalinity ratio at different OLR while Fig. 4a illustrates the variation of total and individual VFA for all the experiments carried out. At 2.8 gVS/L/d (OLR 1), the pH was increased slightly to around 8 initially but dropped to around 7.5 after 4 days of digestion and it remains steady afterwards which is within the favourable pH range of 7 – 8 for anaerobic digestion (Drosg, 2013). The VFA/Alkalinity ratio was lower (Fig. 3a) than the reported failure limit value of 0.3 which shows that the process was stable during these conditions. Anaerobic digestion process has been reported to be working favourably without acidification possibility when this ratio is less than 0.3 (Drosg, 2013). When the OLR was increased to 4.2 gVS/L/d, the pH remained still within the favourable range around 7.6 throughout this period and the VFA/Alkalinity ratio was below 0.3. So, an appropriate buffering capacity and high stability of the process was observed for OLR of 2.8 and 4.2 gVS/L/d and at corresponding retention times of 60 and 40 days, respectively.

No VFA accumulation was observed during the process applying 2.8 gVS/L/d (OLR 1) and 4.2 gVS/L/d (OLR 2) as shown in Fig. 4a. This signifies the complete degradation of the intermediary produced volatile fatty acids. Fast consumption of VFAs was also reported by Liu et al. (2007) as a proof of stable and properly functioning process without danger of failure. However, when the OLR was increased to 6 gVS/L/d with corresponding decreased retention time of 28 days the stability of the system deteriorated. At this 6 gVS/L/d (OLR 3), the process started to show signs for instability already during the first retention time of 28 days, though the pH was still within the favourable range, i.e. an average of 7.7. However, the VFA/Alkalinity ratio increased to 0.4 and the acetic acid concentration increased to 2100 mg/l which signifies a slightly unstable process (Drosg, 2013). There was fluctuations in the daily biogas production (Fig.2a) as well at this loading rate (OLR3). Continuing the loading at the same conditions, i.e. 6 gVS/L/d, into the second retention time period caused considerably disturbances in the system. As shown in Fig. 3a, the VFA/Alkalinity value increased upto 0.9 and the total VFA content also increased gradually up to around 7,000 mg/L

(Fig. 4a) which is a typical sign for overloading. The acetic acids increased up to around 3,800 mg/L and the proprionic acid concentration increased slightly to around 1000 mg/L during the process. Proprionic acid accumulation within the range of 250 - 1000 mg/L has been reported as an indication of instability in the process (Drosg, 2013) and concentrations greater than 1000 mg/L has been reported as a first sign for overloading in the system (Björnsson *et al.,* 1997). Running the process at a higher OLR (6 gVS/L/d) led to a shorter retention time of 28 days, since the reactor was kept at a fixed working volume during the whole experimental period. This shorter retention time could cause the removal of the slow-growing methanogenic community, which led to process instability as well as overloading of the reactor.

Despite the increase in VFA the pH still remained within the range of 7.6 and 8 during the second retention period of 6 gVS/L/d (OLR3). Weiland (2010) has also reported that manure can have a surplus of akalinity which stabilizes the pH value even at higher VFA accumulation and as such will not always result in pH drop. To examine if the VFA could be reduced and restore the process back to stability, the loading rate was reduced again to 4.2 gVS/L/d (OLR 2). It was observed that the VFA/Alkalinity ratio reduced gradually to 0.2 (Fig. 3a) and the VFA accumulation reduced drastically (Fig. 4a) and so the process was restored back to stability.

During digestion, at OLR of 2.8 and 4.2 gVS/L/d the total ammonia nitrogen concentration was between 1350 and 2000 mg/l (Fig. 4b) and the VFA/Alkalinity ratio was below 0.3 which shows good buffering capacity. However, at OLR of 6 gVS/L/d, the total ammonia nitrogen concentration was slightly reduced to around 1000 mg/l probably due to reduced time for feedstock degradation and as such the buffering capacity was slightly lower. This together with the VFA accumulation resulted to higher VFA/Alkalinty ratio showing instability in the process. The free ammonia concentration throughout the process at different OLR was between 47 and 145 mg/l which shows that the reported tolerable free ammonia concentration of 150 mg/l (Kayhanian, 1999) was not exceeded, hence the process was not inhibited by free ammonia.

3.5. Volatile Solids Removal Efficiency

An important factor for assessing the efficiency of an anaerobic digestion process is following up the reduction in VS. As shown in Table 2, 2.8 gVS/L/d (OLR 1) has the highest VS removal efficiency, an average of 74.87 ± 3.75 %, while 6 gVS/L/d (OLR 3) has the lowest with an average of 41.17 ± 4.80 %. It was observed that as VFAs getting accumulated the removal efficiency reduces. The removal effiency decreases as the OLR increases, since with increasing OLR the retention time in the digester decreases, so that the organic matter loaded into the digester has not enough time for the degradation, especially not if difficult to digest fractions, as untreated straw is present in the feedstock.

3.6. Degradation of Lignocelluloses

As shown in Fig. 3b, the amount of cellulose and hemicellulose decreases gradually compared to those of the initial composition in the feed during the digestion process, while the lignin content increases. This shows that the carbohydrates were consumed during the process at all loading rates examined. However, the highest cellulose degradation was obtained at the lowest loading rate 2.8 gVS/L/d (OLR 1); an average of 60.9% ± 6.37, while at 4.2 gVS/L/d (OLR 2) an average of 48.8% ± 6.73 cellulose degradation was observed, and finally 6 gVS/L/d (OLR 3) having the lowest celluose degradation of an average of 30.1 % ± 7.05. For the same reason as explained above percentage of cellulose degraded decreases as the loading rate increases and with reducing retention time.

4. Conclusions

The new plug flow reactor developed can operate sucessfully for continuous dry digestion of manure bedded with straw at 22% TS when operated at OLR of 2.8 and 4.2 gVS/L/d with retention time of 60 and 40 days respectively. OLR of 6 gVS/L/d and retention of 28 days favoured process instability decreasing the VS removal efficiency and cellulose degraded. Digestion of manure bedded with straw without pretreatment at 22% TS was successful, methane yield 0.163 LCH4/gVS_{added} counting around 56 % of the theoretical yield with 57 % VS removal efficiency was obtained at organic loading rate of 4.2 gVS/L/d.

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References

1. Adl, M., Sheng, K.C., Xia, Y.H., Gharibi, A., Chen, X. 2012. Examining a hybrid plug-flow pilot reactor for anaerobic digestion of farm-based biodegradable solids. *Int. J. Environ. Res.*, **6**(1), 335-344.
2. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Sci. Technol.*, **59**(5), 927 - 934.
3. APHA-AWWA-WEF. 2005. *Standard Methods for the Examination of Water and Wastewater 21st ed*, American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), Washington DC.
4. Björnsson, L., Mattiasson, B., Henrysson, T. 1997. Effects of support material on the pattern of volatile fatty acid accumulation at overload in anaerobic digestion of semi-solid waste. *Appl. Microbiol. Biotechnol.*, **47**(6), 640-644.
5. Cantrell, K.B., Ducey, T., Ro, K.S., Hunt, P.G. 2008. Livestock waste-to-bioenergy generation opportunities. *Bioresour. Technol.*, **99**(17), 7941-7953.
6. Carpenter, C. 2010. Determination of Fat Content. in: *Food Analysis Laboratory Manual*, (Ed.) S.S. Nielsen, Springer US, pp. 29-37.
7. Demirer, G.N., Chen, S. 2008. Anaerobic biogasification of undiluted dairy manure in leaching bed reactors. *Waste Manage.*, **28**(1), 112-119.
8. Drosg, B. 2013. Process Monitoring in Biogas Plants. IEA Bioenergy 978-1-910154-03-8.

9. Fernández, J., Pérez, M., Romero, L.I. 2008. Effect of substrate concentration on dry mesophilic anaerobic digestion of organic fraction of municipal solid waste (OFMSW). *Bioresour. Technol.*, **99**(14), 6075-6080.
10. Friehe, J., Weiland, P., Schattauer, A. 2010. Fundamentals of anaerobic digestion. in: Guide to Biogas From Production to Use. Fachagentur Nachwachsende Rohstoffe e. V. (FNR). 5th revised edition. .
11. Gunaseelan, N.V. 2009. Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcus on dry lands. *Biomass and Bioenergy*, **33**(4), 589-596.
12. Habiba, L., Hassib, B., Moktar, H. 2009. Improvement of activated sludge stabilisation and filterability during anaerobic digestion by fruit and vegetable waste addition. *Bioresour. Technol.*, **100**(4), 1555-1560.
13. Haug, R.T. 1993. Feed Conditioning - Physical and Chemical. in: *The Practical Handbook of Compost Engineering.*, Taylor & Francis, pp. 247-257
14. Herrmann, C., Kalita, N., Wall, D., Xia, A., Murphy, J.D. 2016. Optimised biogas production from microalgae through co-digestion with carbon-rich co-substrates. *Bioresour. Technol.*, **214**, 328-337.
15. Karthikeyan, O.P., Visvanathan, C. 2013. Bio-energy recovery from high-solid organic substrates by dry anaerobic bio-conversion processes: a review. *Rev. Environ. Sci. Bio/Technol.*, **12**(3), 257-284.
16. Kayhanian, M. 1999. Ammonia inhibition in high-solids biogasification: an overview and practical solutions. *Environ. Technol.*, **20**(4), 355-365.

17. Kothari, R., Pandey, A.K., Kumar, S., Tyagi, V.V., Tyagi, S.K. 2014. Different aspects of dry anaerobic digestion for bio-energy: An overview. *Renew. Sust. Energ. Rev.*, **39**, 174-195.
18. Kusch, S., Oechsner, H., Jungbluth, T. 2008. Biogas production with horse dung in solid-phase digestion systems. *Bioresour. Technol.*, **99**(5), 1280-1292.
19. Lansing, S., Martin, J.F., Botero, R.B., da Silva, T.N., da Silva, E.D. 2010. Methane production in low-cost, unheated, plug-flow digesters treating swine manure and used cooking grease. *Bioresour. Technol.*, **101**(12), 4362-4370.
20. Lansing, S., Víquez, J., Martínez, H., Botero, R., Martin, J. 2008. Quantifying electricity generation and waste transformations in a low-cost, plug-flow anaerobic digestion system. *Ecol. Eng.*, **34**(4), 332-348.
21. Liu, G., Zhang, R., Sun, Z., Li, X., Dong, R. 2007. Research Progress in Anaerobic Digestion of High Moisture. *Agric. Eng. Int. : CIGR Journal*, **9**(13), 1 - 10.
22. Lossie, U., Pütz, P. 2008. Targeted control of biogas plants with the help of FOS/TAC. Practice Report, Laboratory Analysis Titration FOS/TAC. HACH LANGE
23. Myint, M., Nirmalakhandan, N. 2006. Evaluation of first-order, second-order, and surface-limiting reactions in anaerobic hydrolysis of cattle manure. *Environ. Eng. Sci.*, **23**(6), 970-980.
24. Nielfa, A., Cano, R., Fdz-Polanco, M. 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnol. Rep.*, **5**, 14-21.

25. Noike, T., Endo, G., Chang, J.E. 1985. Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion. *Biotechnol. Bioeng.*, **27**(10), 1482-1489.

26. Pagés Díaz, J., Pereda Reyes, I., Lundin, M., Sárvári Horváth, I. 2011. Co-digestion of different waste mixtures from agro-industrial activities: Kinetic evaluation and synergetic effects. *Bioresour. Technol.*, **102**(23), 10834-10840.

27. Raposo, F., De la Rubia, M.A., Fernández-Cegrí, V., Borja, R. 2012. Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. *Renew. Sust. Energ. Rev.*, **16**(1), 861-877.

28. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2011. Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure. NREL/TP-510-42618.

29. Stroot, P.G., McMahon, K.D., Mackie, R.I., Raskin, L. 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions—I. digester performance. *Water Res.*, **35**(7), 1804-1816.

30. Tarvin, D., Buswell, A.M. 1934. The Methane Fermentation of Organic Acids and Carbohydrates1,2. *J. Am. Chem. Soc.*, **56**(8), 1751-1755.

31. USEPA. 2016. US Environmental Protention Agency. AgSTAR Data and Trends - Anaerobic digester facts and trends https://www.epa.gov/agstar/agstar-data-and-trends#adfacts Accessed 16/10/18.

32. Weiland, P. 2010. Biogas production: current state and perspectives. *Appl. Microbiol. Biotechnol*, **85**(4), 849-860.

33. Zhou, C., Liu, Z., Huang, Z.-L., Dong, M., Yu, X.-L., Ning, P. 2015. A new strategy for co-composting dairy manure with rice straw: Addition of different inocula at three stages of composting. *Waste Manage.*, **40**, 38-43.

34. Zupancˇicˇ, G.D., Rosˇ, M. 2012. Determination of Chemical Oxygen Demand in Substrates from Anaerobic Treatment of Solid Organic Waste. *Waste Biomass Valor.*, **3**, 89–98.

Figure Captions

Figure 1. Schematic diagram of developed reactor and experimental set-up

Figure 2. Biogas production (a) and biogas composition (b) at different organic loading rate (OLR) during experiments. The symbols represent daily biogas production (◊), cumulative biogas production (□), methane composition (♦) and carbon dioxide composition (■)

Figure 3. pH and VFA/Alkalinity ratio variation (a) and composition of cellulose, hemicellulose and lignin in digestate (b) at different organic loading rate (OLR) during experiments. The symbols represent pH (◊), VFA/Alkalinity ratio (□), total lignin (♦), cellulose (■) and hemicellulose (▲). Presented values are mean values of duplicate measurements with error bars as standard deviation between the two values.

Figure 4. Total and individual volatile fatty acids (VFA) variation (a) and Total ammonia nitrogen with free ammonia concentration (b) at different organic loading rate (OLR) during experiments. The symbols represent Total VFA (◊), Acetic (□), Butyric (Δ), isobutyric (×), isovaleric (*), propionic (○), valeric (|), Total ammonia nitrogen (♦) and free ammonia (■)

Tables

Table 1: Characteristics of Substrate and Inoculum used during experiment (standard deviation based on at least duplicate measurements)

Parameters	Manure with Straw	Anaerobic sludge
Total solids (%)	22.29 ± 2.78	7.80 ± 0.24
Volatile solids (%)[a]	70.44 ± 2.68	40.46 ± 1.12
Moisture (%)	77.72 ± 2.78	92.20 ± 0.30
Ash (%)[a]	29.56 ± 2.68	59.54 ± 1.12
Fat content (%)[a]	1.37 ± 0.24	ND
Total carbon (%)[a]	39.14 ± 1.49	22.48 ± 0.62
Kjeldahl Nitrogen (%)[a]	2.33 ± 0.30	4.16 ± 0.15
C/N	16.80 ± 0.64	5.4 ± 0.15
pH	8.81 ± 0.45	8.19 ± 0.30
Bulk density (kg/m3)	967.20 ± 14.18	1001.33 ± 17.38
Protein Content (%TS)	14.56 ± 0.30	26.00 ± 0.15
Total COD ($gCOD/gVS_{added}$)	0.73 ± 0.02	ND
$BMP_{theoretical}$ (LCH_4/gVS_{added})	0.290 ± 0.01	ND

[a] Dry basis
ND = Not determined; COD = chemical oxygen demand; C/N = carbon nitrogen ratio

Table 2: Experimental Results at different loading rate (values are averages with standard deviation)

Parameter	Loading 1	Loading 2	Loading 3
Loading rate (gVS/d)	13.80	20.70	29.90
Organic Loading rate (OLR) (gVS/L/d)	2.80	4.20	6.00
Retention time	60	40	28
Biogas produced (L/gVS_{added})	0.23 ± 0.01	0.25 ± 0.01	0.23 ± 0.01
Average methane content (%)	64.90 ± 1.75	65.10 ± 2.81	63.30 ± 2.06
Methane yield (LCH_4/gVS_{added})	0.15 ± 0.01	0.163 ± 0.01	0.146 ± 0.01
Initial VS concentration (%)	17.17	17.17	17.17
Final VS concentration (%)	4.31 ± 0.64	7.31 ± 1.19	10.10 ± 0.82
VS removal efficiency (%)	74.87 ± 3.75	57.42 ± 6.96	41.17 ± 4.80

Figures

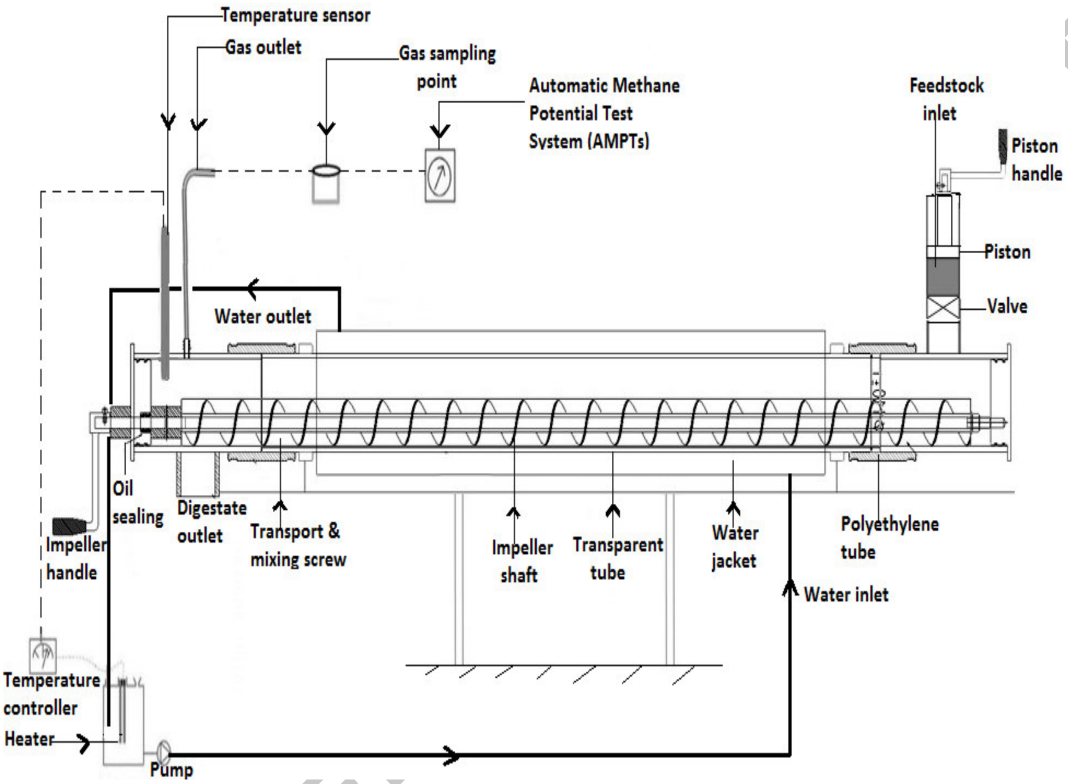


Figure 1.

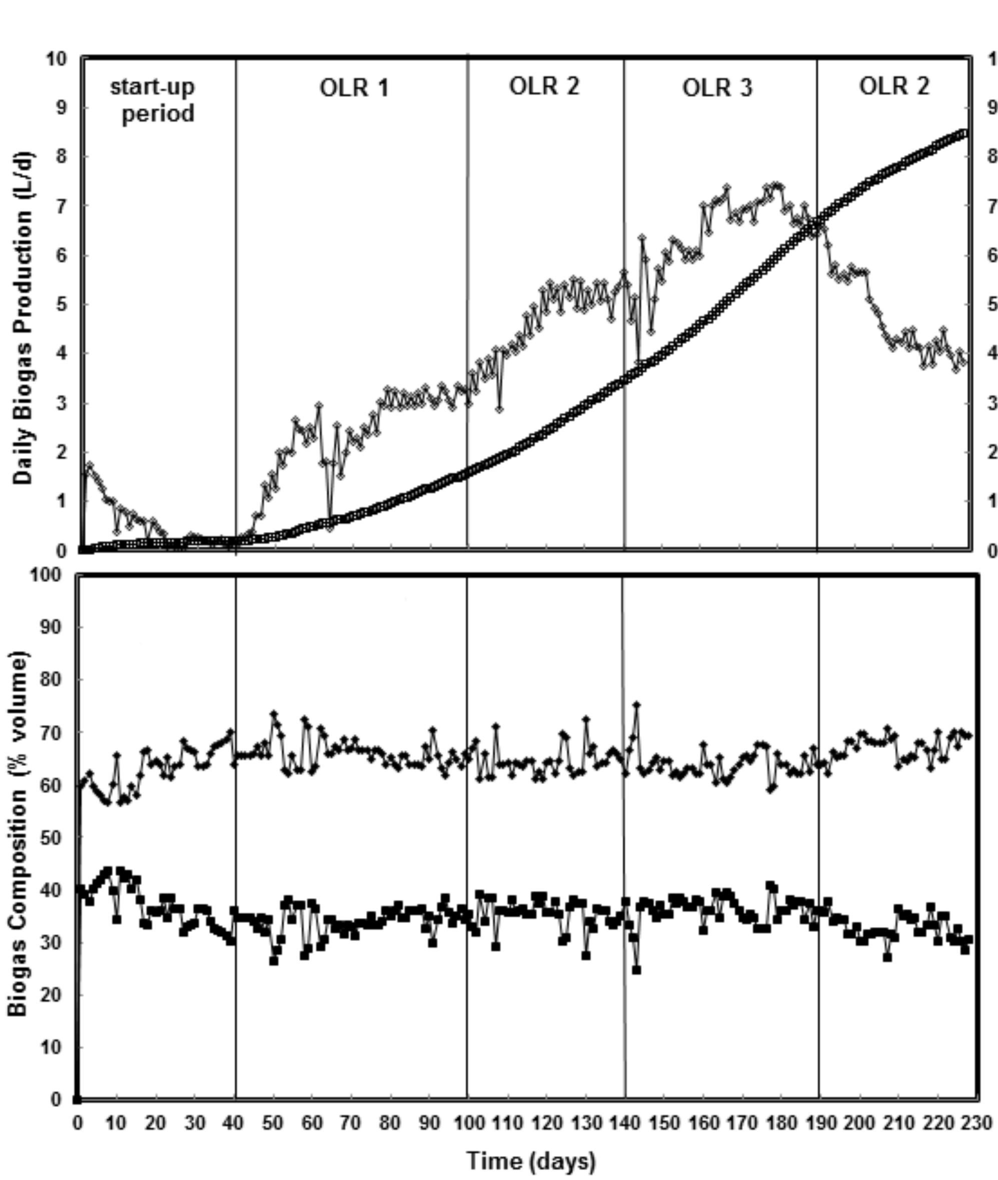

(a)
start-up period
OLR 1
OLR 2
OLR 3
OLR 2
Daily Biogas Production (L/d)
Cumulative Biogas Production (L)
(b)
Biogas Composition (% volume)
Time (days)

Figure 2.

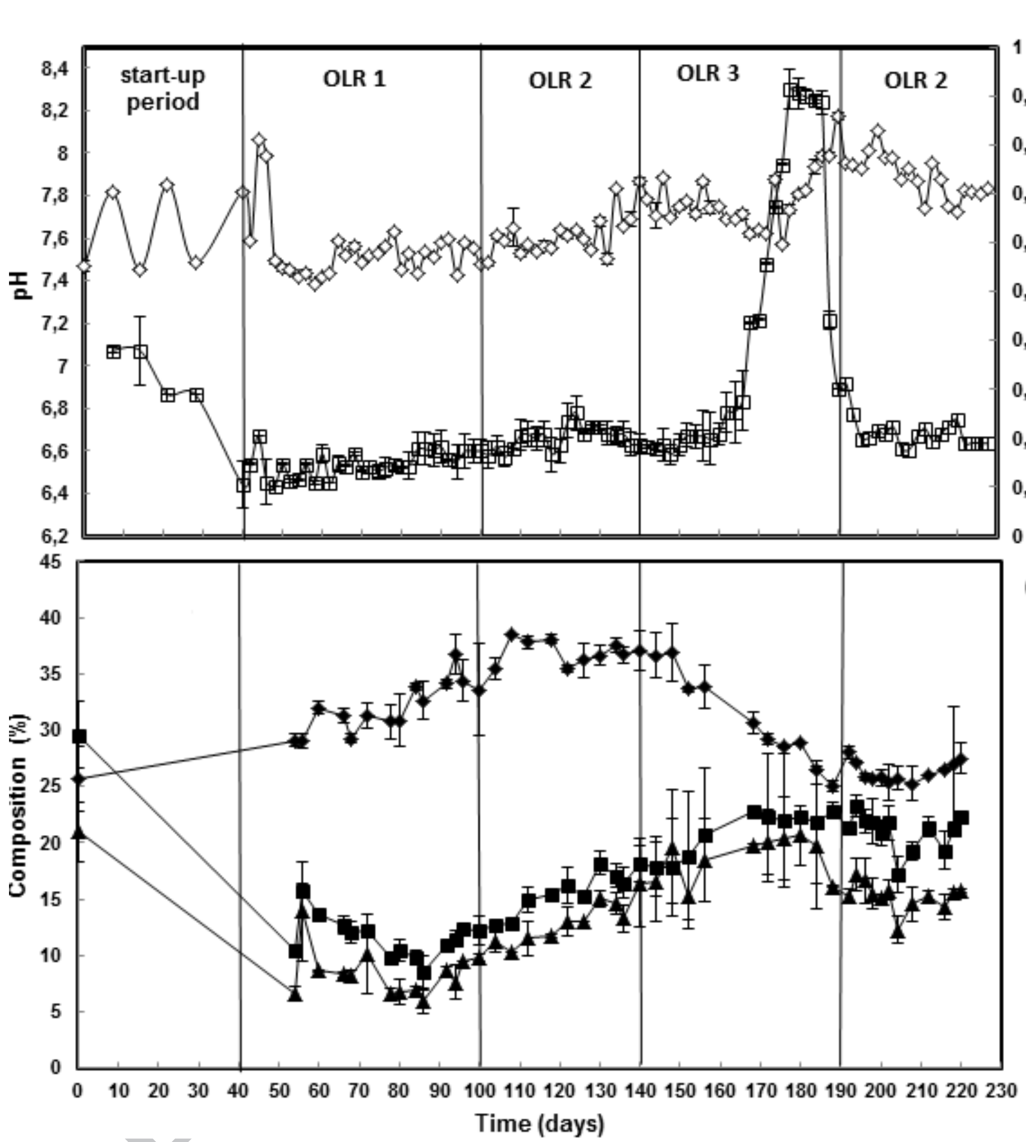

(a)
start-up period
OLR 1
OLR 2
OLR 3
OLR 2
pH
VFA/Alkalinity
(b)
Composition (%)
Time (days)

Figure 3.

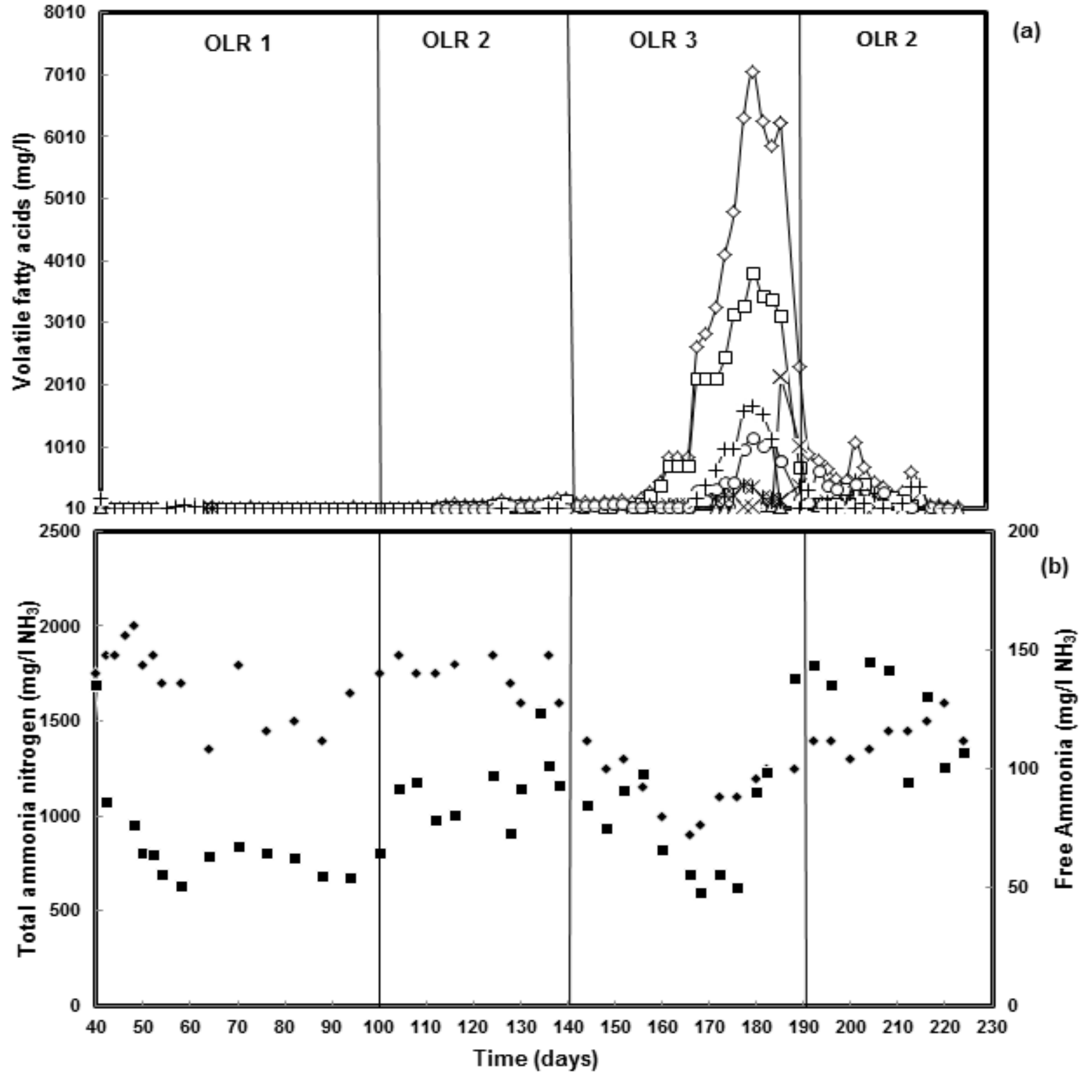
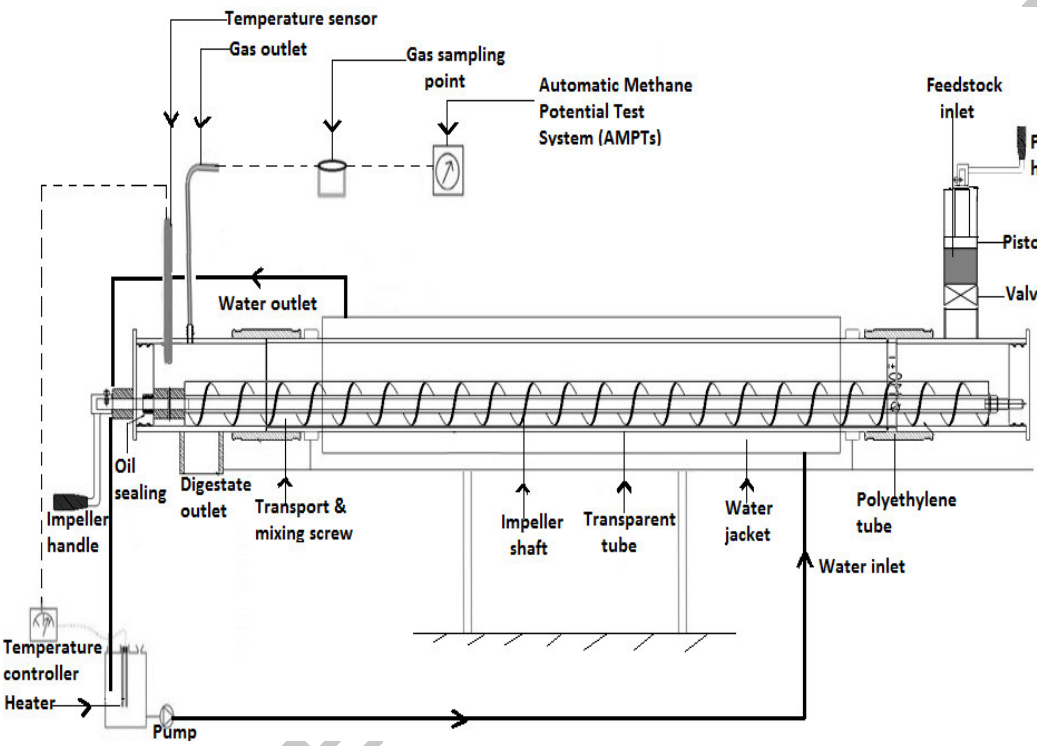

(a)
OLR 1
OLR 2
OLR 3
OLR 2
Volatile fatty acids (mg/l)
8010
7010
6010
5010
4010
3010
2010
1010
10
(b)
Total ammonia nitrogen (mg/l NH3)
2500
2000
1500
1000
500
0
Free Ammonia (mg/l NH3)
200
150
100
50
0
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230
Time (days)

Figure 4.



Temperature sensor
Gas outlet
Gas sampling point
Automatic Methane Potential Test System (AMPTs)
Feedstock inlet
Piston handle
Piston
Valve
Water outlet
Oil sealing
Digestate outlet
Transport & mixing screw
Impeller shaft
Transparent tube
Water jacket
Polyethylene tube
Water inlet
Impeller handle
Temperature controller
Heater
Pump

- New plug flow reactor developed for continuous dry digestion processes
- Reactor worked successfully for 230 days using untreated manure with straw at 22 %TS
- Methane yield of 56 % of the theoretical value was obtained at OLR of 4.2 gVS/L/d
- OLR of 6 gVS/L/d caused process instability with 41 %VS removal efficiency