ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 07, pp.33982-33987, July, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

HORSE DUNG SLURRY AS AN OPTIONAL INOCULUM SELECTION FOR ANAEROBIC DIGESTION OF FOOD WASTE

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ARTICLE INFO

Article History:
Received 10th April, 2016
Received in revised form
05th May, 2016
Accepted 04th June, 2016
Published online 16th July, 2016

Key words:

Anaerobic digestion, Horse dung slurry, Inoculum preparation, Food waste digestion, Batch processing.

ABSTRACT

The mechanism of obtaining a reliable estimate of methane potential using an anaerobic bio-digester system is of high importance. The universality of the procedure undertaken to determine the methane production using an anaerobic system is essential to ensure inter-laboratory repeatability and accuracy of the results. Anaerobic sludge from UASB reactors are used as inoculum for seeding in anaerobic digesters. It is challenging to obtain anaerobic sludge with microbial community for startup of the bioreactor. This paper study the specific contribution of the Horse dung slurry inocula methanogenic community for startup reactor. The experimental results obtained discusses the operational parameters for preparation of inoculum from horse dung slurry and its low potential inhibiting effect.

Citation: Hatem Yazidi, Inshirah Al-Maskari, Joseph V. Thanikal and Abubacker, K. M. 2016. "Horse dung slurry as an optional inoculum selection for anaerobic digestion of food waste", *International Journal of Current Research*, 8, (07), 33982-33987.

INTRODUCTION

Anaerobic digestion has been applied to various biosolids waste streams, including agricultural waste, industrial waste, and municipal solid waste (MSW). This technology is an attractive treatment strategy for organic fraction of municipal solid waste (OFMSW), and has been considered the main commercially option for both treatment and recycling of biomass wastes being of great interest from an environmental point of view (Boualagui *et al*., 2004; De Baere, 2005). The anaerobic digestion is influenced by various factors, like demographic factors, composition of waste, temperature, microbial consortium and its growth (Thanikal *et al*., 2015a-b). There are several new methods and technology practiced for better decomposition of waste and significant biogas production (Pavan *et al*., 1994) and dry conditions (20–35% TS) (Bolzonella *et al*., 2003). Inoculum source together with its careful preparation is considered of high importance in the assessment of anaerobic biodegradability of solid waste and also the biogas produced. Inoculum source is also very important operational parameter. The inoculum source and the total solid percentage selected are responsible to accomplish rapid onset of a balanced microbial population.

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In case of the anaerobic biodegradability of solid waste, the use of a highly active anaerobic inoculum or animal inoculum waste will reduce significantly the experimental time, or reduced the amount of inoculum required in full scale batch digesters, and consequently, the corresponding digester volume (Obaja *et al*., 2003). The AD process involves biological conversions in which a consortium of interdependent microorganisms is responsible for the degradation of complex organic matter (Yazidiand Thanikal, 2016). Therefore, an estimation of methane production based only on the chemical composition of the substrate is not sufficient, despite the availability of well-developed complex inoculum/substrate systems, and an adequate biogas measurement is preferred (Ajeej *et al*., 2014; Yazidi and Thanikal, 2015). Batch assays have been suggested by Angelidaki *et al*. (2009), including the substrate characterization, the inoculum and its activity, the experimental procedure and the collection, interpretation and reporting of the data. The authors recommended for the inoculum to be 'fresh', homogenous, filtered and pre-incubated, to have a wide microbial diversity to ensure a sufficient level of hydrolytic and methanogenic activity, and to be tested towards model substrates, such as cellulose and acetic acid (Angelidaki *et al*., 2009). But, no recommendations about the methanogenic community composition or abundance were included. On 2011, Raposo *et al*., reviewed the factors affecting the performance of anaerobic batch assays, and

indicated that, although experimental conditions of batch assays are synchronized, a certain degree of variability in the results always remains due to the biological nature of the test systems. This biological difference can be assigned to the origin of the inoculum, as it comes with a different microbial population, leading to differences in initial activity and substrate adaptation (Wittebolle *et al*., 2009; Regueiro *et al*., 2012; Gough *et al*., 2013).Consequently, the type of inoculumhasa significant impact on the AD process ability to convert the substrate to methane. The influence of the inoculum source on methane production has recently been indicated by Chamy and Ramos (2011) for turkey manure and by Elbeshbishy *et al*. (2012) for food waste and primary sludge, and in both cases the methane yield depended on the inocula sample that was selected. However, this influence was not linked to the characteristics of the microbial community in the different inocula.

The aim of the study was to evaluate horse dung as inoculum and to set batch operations under mesophilic conditions and to determine the start up of bioreactor. The paper also gives meticulous preparation of horse dung slurry which can be used for excellent inoculum preparation for anaerobic digestion.

MATERIALS AND METHODS

Inoculum preparation

The literature review concerning the effect of inoculums on the biogas production was conducted by several researchers and summarized in the following four points:

1. Inoculums are substantially relevant in the process kinetics of biogas production (Luengo and Alvarez, 1988);
2. The amount of methane produced seemed proportional to the initial inoculums (Castillo *et al*., 1995);
3. The higher percentage of inoculums gave the higher production of biogas (Forster-Carneiro *et al*., 2008), and;
4. The food to inoculums ration significantly affected the biogas production rate (F/I)-(Neves *et al*., 2004).

The context of the study was that, there are no anaerobic digestors operated in the region for recovery of anaerobic sludge as inoculm for degradation of solid waste. As shown in Fig.1, freshly collected horse dung was obtained from a local horse barn in Muscat (Sultanate of Oman). It was filtered through a strainer to remove all kinds of impurities before proceeding with slurry preparation, as it contains lots of undigested materials. The inoculum was mixed to ensure homogenous conditions prior to the beginning of the experiment phase. As mentioned at the beginning of this section, the inoculum plays a crucial role in obtaining an optimal biogas production. Therefore, it is very important to start the inoculum preparation process by using a minimum quantity and progressively increase the amount. For a one-stage batch process, the crucial point is to prevent volatile fatty acids (VFA) accumulation inside the “seed” particles beyond their assimilative methanogenic capacity. A slurry of 36g of solids per liter volume of tap water was prepared to fill the 6 liter lab reactor. The reactor was kept under continuous mixing, to keep the solids in suspension, at a temperature of 35°C. The reactor was kept under continuous mixing and controlled temperature for about 7 days. The solids concentration in the reactor was then increased to 72g per liter in the reactor. The aim was to succeed in preparing an inoculum portion with high methanogenic activity and low biodegradability. The chosen dilution rate was optimal to start the inoculum preparation process in order to obtain a highly active anaerobic inoculum. In other word the prepared inoculum should have enough volatile fatty acid accumulation inside the “seed” particles without compromising their assimilative methanogenic capacity.

Reactor design and operation

Biodegradation series of experiments were performed in anidentical double-walled bio-reactor of 6 liters effective volume (BR) maintained at 35°C by a regulated water bath (Fig.2 and Fig.3). Mixing in the BR was done by a system of magnetic stirring. The pH inside the reactor was continuously monitored online using Metler Toledo pH probe Inpro 4260i and maintained at 7.5±0.5. The BR was operated in batch mode without withdrawal. The flow rate was determined at each batch assay. The batch end was considered once the flow rate reached a threshold value of 1 ml/h.

Laboratory Experiment Configuration

We started our experiments on Feb. 16th, 2016at 5:10 PM. After almost half a day of lag phase a0.23ml of bio-gas was produced. It was only after almost 9 days that the first 1 liter of bio-gas was produced (fig. 4a). On the sixth day of the experiment, the pH observed to progressively drop down from 7.08 to 6.17. Subsequently, 8 g of bicarbonate was added each 24 hours to adjust the pH. Consequently, apH value progressively increased to stabilize at around 7.5 until the end of the experiment. Moreover, our results corroborated with Zhang *et al*. (2004) work. In fact, the authors found that pH adjustment could improve both hydrolysis and acid ogenesis rates. Furthermore, compared with pH at 5, 9 and 11, the authors demonstrated that pH 7 provided an optimum working condition for anaerobic digestion of food wastes (kitchen). At pH 7, they showed that about 86% of the total organic carbon (TOC) and 82% of chemical oxygen demand (COD) were solubilized and the maximum volatile fatty acid (VFA) concentration of 36 g l-1 was achieved on the fourth day. The latter results could explain the pH drops to its lower level (6.17),but in our case was on the sixth day.

In addition to the acidity (pH), the biogas flow rate was also monitored during all the experimental steps. The produced biogas volume was measured using Ritter gas-counter. At the start of the experiment, the biogas components, mainly: CH4, CO2 and H2, were diluted in the headspace. At this stage and since we are only looking for an optimal environment for inoculum preparation and not for biogas production analysis, no measured biogas concentration was corrected. Furthermore, the instantaneously instruments recorded measure of the biogas that may be not the reflection of the real biogas production since water vapor could have an impact on the volume and composition measurements.

Fig.1. Step one in preparing the inoculum: Straws and impurities were removed from the freshly collected Horse Dung

Fig. 2. Step two in preparing the inoculum: Set the bio-reactor to be ready receiving the 6 l solution of Horse Dung Slurry

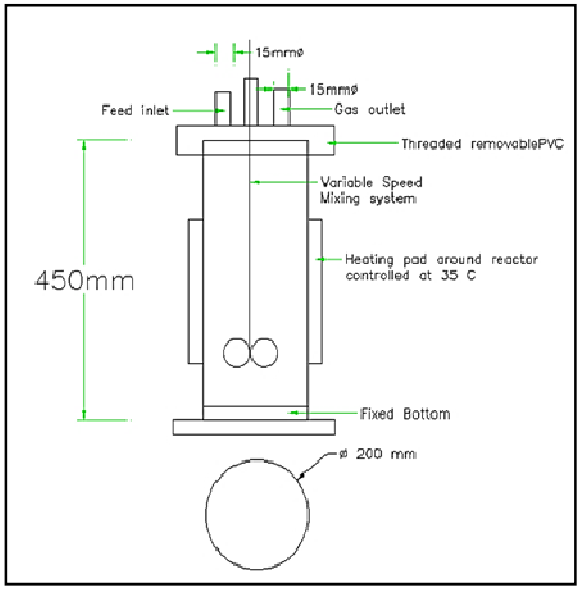

Fig.3. Schematic diagram of laboratory-scale digester

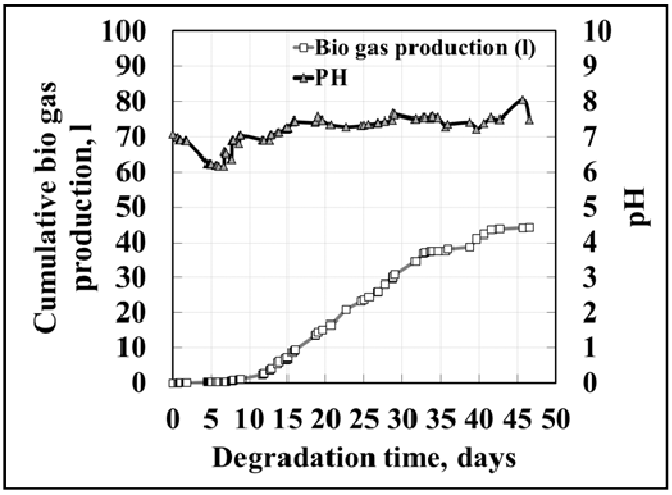

Fig.4a. Cumulative biogas curve of the HSD inoculum with pH time series

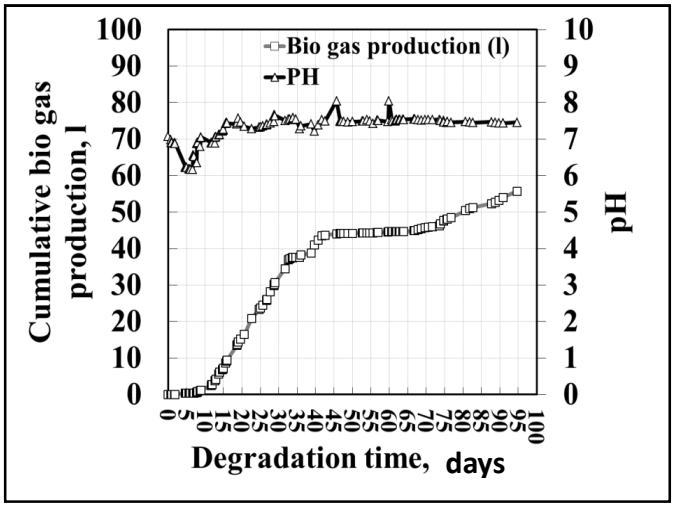

Fig.4b.Total cumulative biogas curve for all experiments (inoculum + substrates) of the HDS inoculum with pH time series

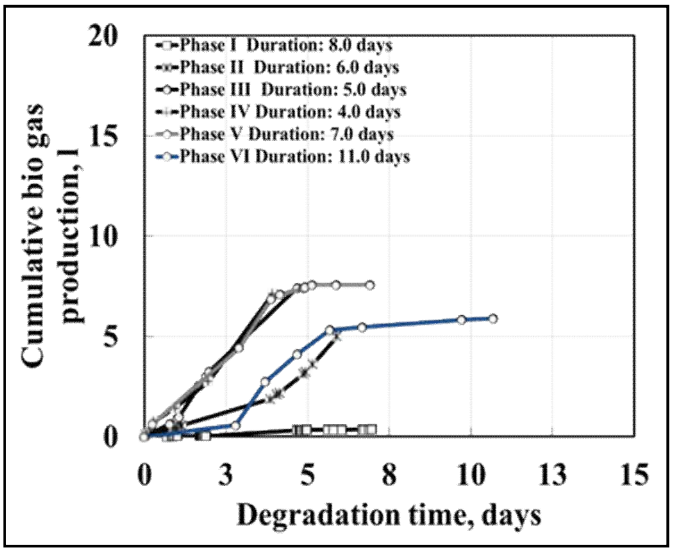

Fig.5. Total cumulative biogas curves of all observed phases (six) of the HDS inoculum preparation

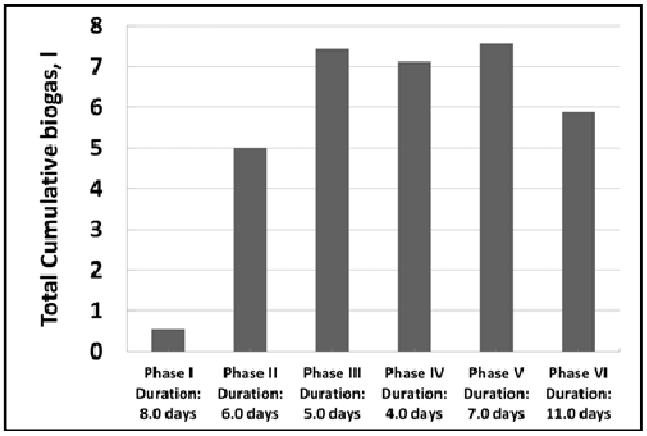

Fig.6. Total biogas production histograms obtained at the end of each HDS inoculum's preparatory phase

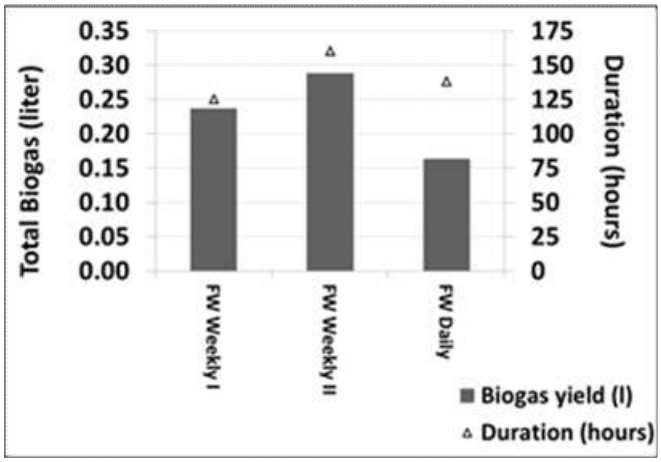

Fig.7. Biogas yield of each feeding cycle of: FW

Also the frequent addition of nitrogen could mislead the results. In fact, bicarbonate had significant effect on determining the VFA concentration when the titration method is used (Lutzhøft *et al*., 2014). One attempt to minimize the water vapor effect is to use of a long gas tubing (minimum 1.5 m) from the gas port of the reactor which contribute to cool the biogas to room temperature. The reactor was then added with 2 ml of ethanol, direct carbon source to test the methanogenic activity of the inoculum. Nitrogen gas was added to the reactor whenever the reactor was open for the addition of ethanol, to remove the residual oxygen. The biogas produced was monitored for methanogenic activity of the inoculum. The ethanol dosage was increased fewer times to 5 ml, to test the increase in activity.

Analytical

Total solids (TS) and Volatile Suspended Solids (VSS) were measured according to the standard methods (APHA, 2005). The biogas production was measured on-line every 2 mins by Milligas counter MGC-1 flow meters manufactured by Ritter gas meters fitted with a 4-20 mA output for acquiring the data (APHA, 2005). The software supplied by Ritter Company was used to log the gas output. The samples were centrifuged at 15,000 RPM for 15 min at 20°C; 2 ml of the clear supernatant is then added to HACH COD vial and digested in a COD digester for 2h. The $COD_{soluble}$ was determined by spectrophotometry at 620 nm according to the APHA Standard Methods. The VFA was determined by titration method (Lutzhøft *et al*., 2014). Methane content in the biogas was measured using the online methane analyser supplied by BlueSens (Germany). The substrates homogenized mixture was then characterized before fed into the bio-reactor and the results are shown in table 1.

RESULTS AND DISCUSSION

After careful preparation of inoculum, the reactor was fed with low food wastes' (FW) loading rate of 0.5g of the VSS / liter volume of reactor to test the biodegradability.

Inoculum with low organic load of substratetests

Fig. 4a shows the reactor performance data: temporal evolution of the cumulative biogas of Inoculum (cycle one) and Fig.4b shows the same, but with the addition of FW (cycle two), starting on day 47. As can be seen, in the first 8 days of operation (start-up stage), the reactor showed low biogas production (low organic matter degradation) due to the acclimation period of biomass substrate. During the same period a drop in pH values was observed. The latter can be explained by the hydrolytic and acidogenic activity among 0–8 days charcterised by a high fatty acid generation. The total observed pH variation can be attributed to the different biodegradability of organic matter fractions contents in the digester. From day 8 to day 43, the hydrolyzed organic matter was transformed to volatile fatty acids (mainly butyric and acetic acids) suggesting the stabilization phase (linear increasing of the biogas rate). The concentration of these volatile acids in the digester was conductedby their production rate and their removal rate. The outputs of the latter statements could be interpreted by approximately 70% of the digester biogas produced could be originated from acetate (the most important precursor on which to focus) and the remainder of the digester methane (approximately 30%) could be originated mainly from the reduction of dioxide of carbon by hydrogen (Chynoweth *et al*., 1993).Six phases were observed during the inoculum acclimation period (Fig. 5). Numerical values of biogas yield in several days' observation time are presented in Fig. 6. The first phase has the lowest total cumulative biogas. Fig. 6 shows that, in general the inoculum exhibit progressively increasing cumulative biogas production with an observed plateau at the end. In other terms, the total bacteria abundance was significantly lower at the start of the experiment and started to grow during the experiment.

Biogas generation and methane efficiency

Fig. 7 shows the total cumulative methane obtained from testing low rate OLR FW substrate. To start the feeding two cycles of weekly feeding was performed. The first week feeding cumulative biogas (FW Weekly I) amounted to 0.24 liters, for week two (FW Weekly II) the total cumulative biogas was amounted to 0.29 liters, which is not significantly different from week one feeding. The second feeding cycle was performed on a daily basis (FW Daily). The total cumulative biogas production of daily feeding was observed

lower than those obtained from weekly feeding. The amounts of biogas produced despite the low substrate load indicate that digestion quality in the lab-scale reactor with HDS as inoculum was good. As the quality of horse dung may differ with regard to its contents, age, and storage prior to being digested, a future work on a higher proportion of inoculum may be useful in order to determine the rate-limiting steps in the degradation of each substrate, and to assess the effects of the inoculum fractions on the methanogenic activity of the AD process.

Conclusion

In today's energy saving challenging, AD is demonstrated to be a promising renewable way of producing energy in the form of biogas. In this work a filtered HDS was shown to be a potential inoculum for mono substrate and co-digested substrate processing in batch-operated solid-phase digestion. During the inoculum preparation phase, we showed that only half a day of lag phase was enough to produce the first 0.23 ml of bio-gas. But it took almost 9 days to obtain the first 1 liter of bio-gas. It was observed that all inoculum acclimation phases (6 phases) were performed without any major bioreactor functionality problems. The experimental results showed a progressively biogas loading of the HDS. These collected data during the experiment pointed out the fact that HDS is suitable to be used as inoculum in the anaerobic digester process.

Acknowledgments

This research work is a part of the funded research from the "The Research Council of Oman" (TRC), Al Mawelah central vegetable market Muscat, The sultanate of Oman.

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