CSCE Annual Conference Growing with youth – Croître avec les jeunes



Laval (Greater Montreal) June 12 - 15, 2019

PERFORMANCE ASSESSMENT OF LOW-TEMPERATURE ANAEROBIC DIGESTION BIOTECHNOLOGY FOR TREATING POULTRY MANURE

Adhikary, Suman^{1,2}; Khalil, Mario^{1,3}; Dorval, Daphneé^{1,4}; Goyette, Bernard¹; Dubreuil, Jérome.¹; Rahaman, Md. Saifur²; Rajagopal, Rajinikanth^{1,5} ¹Sherbrooke Research and Development Center, Agriculture and Agri-Food Canada, 2000 College Street, Sherbrooke, Quebec, Canada, J1M 0C8; ²Department of Building, Civil, and Environmental Engineering, Concordia University, 1515 Saint-Catherine St W, Montreal, Quebec, Canada, H3G 2W1; ³Département de Biologie, Université de Sherbrooke, Sherbrooke (QC), J1K 2R1, Canada; ⁴Centre Universitaire de formation en environnement et développement durable, Université de Sherbrooke, Sherbrooke, Sherbrooke (QC), J1K 2R1, Canada; ⁵ rajinikanth.rajagopal@canada.ca

Abstract: The treatment and disposal of livestock manure is always a major concern because of it's environmental, economic and legal issues. Anaerobic digestion (AD) is a widely used treatment method for the proper management of the high solid content wastes from livestock sectors. This is because AD is associated with energy recovery, solid by-products (digestate) as fertilizer and less generation of greenhouse gases. However, for the successful implementation of field scale AD process, lab scale operation is crucial to predict the performance. In this research, AD of chicken manure has been studied to determine the operational stability of the lab-scale AD process by performing the sequencing batch mode of operation in a 40 L (total volume) digester. Organic loading rate (OLR) during this phase of the study has been maintained in the range of 0.7-1.12 g COD_t/L/day, while digester temperature has been maintained at 20 ± 1 °C. For the initial 7 cycles of operation (cycle length = 14 days), COD_{total} has been reduced to 41-90%, with the highest specific methane yield (SMY) of 0.29 L CH₄/gCOD_{t fed}. The study also reveals that volatile fatty acids (VFA) can be reduced up to 90% whereas CH₄ quality can reach up to 80%, which ensures the process stability of maintaining favorable conditions for microbial communities. Total ammonia nitrogen (TAN) up to 5 gN/L did not affect the digestion process.

1 INTRODUCTION

The rapid increase of global poultry meat consumption (López-Andrés et al. 2018) has led to the production of a huge amount of chicken manure (CM). However, most of the farms across the world don't have adequate manure management strategies to handle this large amount of manure and prevent environmental pollution (Duan et al. 2019). To treat this manure, anaerobic digestion (AD) is proven biotechnology. This is not only because AD can effectively treat the manure by fully retrieving the inherent quality of the waste but also, it's a low-cost technology with the ability to produce clean energy and high-quality fertilizers. As a potential organic substrate for biogas production, CM has less utilized so far due to the high ammonia content which causes an adverse effect on the AD process. This inhibition owing to excess ammonia can be even more when the AD process is operated at high temperature (Rajagopal, Bellavance, and Rahaman 2017; Fuchs et al. 2018).

Many factors can cause a shift in the methanogenic community structure and affect the overall digestion process, thus making the AD process complex. These factors are: pH, temperature, substrate concentration, substrate composition, presence of toxic or inhibitory compounds (Venkiteshwaran et al. 2016), organic loading rate (OLR), and reactor configuration. Many studies have reported that temperature is one of the most crucial factors influencing the microbial communities, process kinetics, stability, substrate utilization rates, and methane yield (Riau, De la Rubia, and Pérez 2012; Habiba, Hassib, and Moktar 2009). The AD process is commonly operated in the mesophilic temperature, considering the process stability, energy expense and microorganisms sensitivity (El-Mashad, Van Loon, and Zeeman 2003; Ward et al. 2008).

However, in the context of a cold climate country like Canada, using low-temperature AD can offer several advantages; one of these advantages is that less energy is required to keep the temperature stable inside the digester when compared to mesophilic and thermophilic anaerobic digestion (Massé, Rajagopal, and Singh 2014). Another important advantage is that at a lower temperature, the hydrolysis of the complex organic materials is reduced which causes decreased acidogenesis and therefore the produced biogas contains a lower percentage of CO₂. One important consideration also is that the AD systems which are operated at low temperatures produce lower free ammonia nitrogen levels when compared with mesophilic and thermophilic processes (Rajagopal, Bellavance, and Rahaman 2017).

There are very few studies on treating CM under psychrophilic range so far. Our aim in this study is to adapt the inoculum for the high ammonia concentrations and then utilize them for the starting-up of high-solids anaerobic digester (closed loop percolation-recirculation system) treating dry CM. During this study, various physicochemical parameters have been studied to monitor the digester performance for better prediction of the adapted inoculum. Initial 7 cycle results have been included in this study because of it's availability.

2 MATERIAL AND METHODS

2.1 Feedstock and inoculum

The fresh CM used in this experiment for biogas production was obtained from "Groupe Robitaille" (located in Farnham, Quebec, Canada) and was transferred to the laboratory and stored at 4°C until the use. The CM had a very high proportion of carbohydrates (around 69% TS), followed by very high COD_t. After placing this fresh CM in the batch reactor, it was completely soaked with tap water (to be in line with real field scenario). A filter media at the bottom of the batch reactor was placed to pass the liquid leachate through it. Liquid leachate accumulated at the bottom of the tank was collected from the bottom and used as feed. Physicochemical parameters from the leachate and liquid inoculum are presented in Table 1.

Parameters	Liquid Inoculum	Leachate
	1.43–1.48	2.42-4.63
VS %	0.60-0.70	1.34–2.9
Alkalinity (mg/L as CaCO₃	9,195–10,453	11,423–16,297
Total COD (mg/L)	16,239–17,649	45,186–75,436
Soluble COD (mg/L)	2,061–2,561	24,781–69,479
TKN (mg/L)	2,128–2,236	4,452-6,929
NH ₃ (mg/L)	1,768–1,949	3,667–5,398
pH	7.71–7.73	5.84-6.63
VFA (mg/l)	549-839	21,510–36,759

2.2 Experimental setup

The experimental set up used in our study is presented in Fig 1(a) and (b). Three identical 40 L batch reactor with a working volume of 24 L was used for this study. Reactors were operated under psychrophilic condition at $20 \pm 1^{\circ}$ C. The bio-reactors (namely BR1, BR2, BR3) were manually fed with the leachate from the chicken manure at the start of the cycle (cycle length = 14 days). OLR was maintained at around 0.7-1.12 g CODt/L-day.



Fig 1: (a) Production of leachate; (b) Bioreactor operation

Tap water was sprinkled on top of the CM and leachate was collected from the bottom. To maintain the anaerobic condition, N_2 gas was sparged into the bioreactor at the beginning of the cycle & since the bioreactors were operated with perfectly airtight so anaerobic conditions prevails during the entire operation time. All the bio-reactors were mixed for 5 min/day using pumps. To analyze the process performance, samples were taken immediately after the feeding and after 3, 8 and 14 days. Various physicochemical parameters such as pH, alkalinity, TS, VS, COD_t, COD_s, TKN, NH₃, VFA, Biogas quality, and quantity were analyzed in the laboratory to observe the performance of the process.

2.3 Analytical methods

2.3.1 Physicochemical parameters

The TS, VS, total chemical oxygen demand (COD_t) and soluble chemical oxygen demand (COD_s) concentrations were determined following the guidelines given by the standard methods (APHA 2005). Analytical balances (Mettler Toledo, Mississauga, Ontario) were used for measuring the weight. Spectrophotometer (wavelength 600 nm) used for COD measurement was supplied by Thermo Scientific UV-Vis (Swedesboro, New Jersey, USA) whereas pH probe (Mettler Toledo, Mississauga, Ontario) was used for pH measurement. Total Kjeldahl Nitrogen (TKN) and NH₃ were measured following the standard procedure of the Kjeldahl method (Model 2400, KjeltecTM, Höganäs, Sweden).

2.3.2 Methane

Methane concentration in the biogas was analyzed using a gas chromatograph (Micro GC 490, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and Helium gas as the carrier gas at a flow rate of 20 mL/min. The injector and oven temperatures were 110°C and 180°C respectively.

2.3.3 Volatile fatty acids

The volatile fatty acids (VFAs) generated during methane production were detected using a PerkinElmer® Clarus® 500 Gas Chromatograph (Waltham, Massachusetts, United States). Samples collected from

digesters were first centrifuged at 41,000 *g* for 15 min and filtered through a 0.22 μ m membrane before injected. The injection volume was 0.1 μ L.

3 RESULTS AND DISCUSSION

3.1 Biogas Production

Methane production

A typical cycle of methane concentration and biogas production is presented in Fig. 2 (a) and (b). It is evident from the figure that, gas production increases rapidly (after 3 days approximately). Microbes present in an anaerobic digestion process took some time to get acclimatized, which was explained by the slight increase in gas production after 3 days. With time anaerobes becomes active and produces a high amount of biogas with the utilization of biodegradable organic matter. Then a decrease of biogas quality is observed because of the conversion of excess substrates into methane. The decreased phase of biogas production is evident from Fig 2(a) and (b). As the cycle proceeds towards the end of 14 days, methane production rate was decreased (evident from the decreased slope of Fig 2 (b)). Considering all the rate limiting factors - OLR, temperature, high ammonia concentration, and HRT, our system can produce biogas with approximately 80% methane concentration (with CO₂ approximately around 20%). Moreover, our system can produce up to 19.2 L CH₄/ L-feed which is comparable with the previous studies (Dalkilic and Ugurlu 2015).



Fig 2 (a) Methane concentration; and (b) biogas and methane concentration during a typical cycle of operation

H₂S production



Fig 3 Production of H₂S during a typical cycle

Figure 3 shows the H₂S quality for a typical cycle. H₂S production is found to be decreased significantly (~0.4%) after it reached a peak value of approximately 0.7%. H₂S usually has an inhibiting effect on methane production. In our process, we found a decrease of H₂S at the end of our cycle. It is unclear why H₂S production reduces over time. However, the probable reason could be the presence of sulfur content in the anaerobic process, which reduces over time, i.e. sulfur reducing bacteria (SRB) may not be able to produce more H₂S. Additionally, the pH range of our system varies between 7-8, which also signifies the hydrogen sulfide will be in the less toxic form in the system as HS⁻ (Paulo, Stams, and Sousa 2015). It can be also concluded that less amount of H₂S is desirable as a lesser amount of purification will be needed before the biogas will be used for energy production.

Specific methane yield (SMY)

In the anaerobic process, a certain amount of gas is produced per gram of COD or volatile solids are broken down or destroyed by the bacteria. This is referred as "specific methane yield" (SMY). The biogas substrate quality can be represented by SMY. SMY is also an indication of the efficiency of the system in terms of the utilization of organic loading. In our study, the SMY is presented in Fig 4 and the maximum SMY was found to be 0.29 L CH₄/ g COD_t. Even though we operate our process with low temperature, still the methane yield showed higher value than similar type of studies operated with mesophilic temperature range (Fuchs et al. 2018; Nie et al. 2015). This is another very good indication of the well-adaptation of the inoculum.



EN47-5

Fig 4 Specific methane yield during a typical cycle

Overview of the methane quality and quantity for all the cycles for different OLR

Despite variations of organic loading rate for different cycles, accumulation of VFA, high ammonia in the process as well as accumulation of TS and VS, anaerobic digestion process still has not shown any instability in terms of production of methane quality and quantity. Few factors that are contributing to the stable operation could be because of the high adaptability of inoculum, operational control in terms of retaining neutral pH range and high alkalinity. Another crucial factor is the operation of our system in the psychrophilic range. Most livestock manure (particularly swine and poultry) contain appreciable amounts of nitrogen, which will end up converting to ammonia resulting in toxicity in the digester (shown by fig. 5(c)). However, the effect of toxicity is expected to be reduced since we operate our digesters with psychrophilic range of temperature as methane quality and quantity shows steady results which is evident from fig 5 (a) and (b).



Fig 5 (a) Methane quality for 7 cycles; (b) Methane quantity for 7 cycles; (c) TKN and NH₃ concentration profile for a typical cycle

3.2 VFA reduction, pH and Alkalinity profile



Fig 5 (a) VFA reduction for a typical cycle; (b) pH profile; (c) Alkalinity profile for a typical cycle

Monitoring short-chain fatty acids levels can help to predict the performance of the anaerobic digestion process. Among the short chain fatty acids, approximately 70% of acetic acids directly contribute to the methane formation. This is because acetate is the major product of intermediate organic molecules and can be converted to methane directly (Dreher et al. 2012). However, excess accumulation of volatile fatty acids could lead the process towards fermentation by lowering pH value. From figure 5 (a), over 93% reduction of VFA has been obtained (26.323 g/L to 2.63 g/L). Overall from the 7 cycle results, VFA was reduced in the range of 37-93%. For the first 3 cycles, the reduction of VFA was very high, however, an accumulation of VFA occurs as we move towards further cycle. Usually, VFA accumulation occurs mostly due to the ammonia inhibition, which again reduces the pH. However, from a typical cycle, as presented in fig 5 (b), pH value in our system remains always in the range of 7-8. Stable pH range might be due to the high alkalinity value in our system (Fig 5 (c)). This good buffering capacity due to the alkalinity value of 9100 – 18000 mg/L as CaCO₃ maintains optimum pH value for the methanogens. It also indicates that there is no need to adjust pH from any external source. We can also conclude that psychrophilic anaerobic digestion of chicken manure can proceed successfully with our adapted inoculum at high ammonia concentration.

3.3 Chemical Oxygen Demand (COD) reduction

The chemical oxygen demand (COD) of the CM was considerably reduced by the anaerobic digestion process (Fig 6). The reduction of the COD implies the decrease of the organic loading from the substrate



Fig 6 CODt and CODs reduction during a typical cycle

during the treatment process. The COD_t reduction was found to be in the range of 41-90%. There is a similarity in the reduction pattern between COD_t and VFA. COD reduction is also a function of TS and VS. As the organic loading rate increased (from 0.7 g COD_t/L/day to approximately 1.12 g COD_t/L/day), the COD_t increased as well as the VS and TS content. Since there was an accumulation of VS (>100%) in the system over the operation time, this might contribute to the COD and hence COD was found decreasing with the increasing cycle number. Thus, the incomplete degradation and overload might be the reason for less reduction of COD_t. However, the production of methane in terms of quality and quantity always shows a similar pattern despite less reduction of organic loading in the process which will be described in the following section.

4. CHALLENGES DUE TO HIGH AMMONIA CONCENTRATION

Inoculum thus developed in this process which is already acclimatized with the leachate obtained from CM, will be directly utilized for the treatment of raw CM under psychrophilic temperature range. It is well-known that CM contains very high amount of ammonia (>6900 mg/L) (Nie et al. 2015). So, it is very important to use previously well-adapted inoculum for treating raw CM which can tolerate this high ammonia content as well as can maintain operational stability. This is because free ammonia present in the digester could pose potential toxic effect to the methanogens thus reducing methane quality resulting in digester failure. At low temperature and pH<8, conversion of free ammonia from total ammonia concentration has been hindered compared to mesophilic and thermophilic operating conditions, which further justifies the better performance of the digester operation without showing any inhibition in methane production. Additionally, it is important to ensure that liquid inoculum can maintain good buffering capacity as well as can tolerate any excess acidification that can occur while running the AD process.

5. CONCLUSIONS

This study gives some important insights about the adaption of liquid inoculum for treating dry CM. This study investigates the biogas production from leachate obtained from the dry CM at different organic loading rates (OLRs), under psychrophilic range anaerobic system. The system was operated on batch reactor mode under different OLRs (ranging from $0.7 \text{ g} - 1.12 \text{ g COD}_t/L/day$). It was observed that the anaerobic bacteria acclimatized to high total ammonia nitrogen concentration (>4000 mg/L) originated because of the degradation of CM. High volatile fatty acid concentrations were tolerated by the system due to the high pH in the reactors. The maximum average CH₄ production rate was found as 290 mL/g COD_{feed}. Average methane content of produced biogas was over 77% during the study.

ACKNOWLEDGMENTS

The authors thank Agriculture and Agri-Food Canada (Project No. 2335) for providing financial support for conducting this research.

REFERENCES

- APHA. 2005. Standard Methods for the Examination of Water and Wastewater, 21st Ed.; APHA:Washington, DC, USA, 2005. American Water Works Association/American Public Works Association/Water Environment Federation. https://doi.org/10.2105/AJPH.51.6.940-a.
- Dalkilic, Kenan, and Aysenur Ugurlu. 2015. "Biogas Production from Chicken Manure at Different Organic Loading Rates in a Mesophilic-Thermopilic Two Stage Anaerobic System." *Journal of Bioscience and Bioengineering*. https://doi.org/10.1016/j.jbiosc.2015.01.021.
- Dreher, Teal M., Henry V. Mott, Christopher D. Lupo, Aaron S. Oswald, Sharon A. Clay, and James J. Stone. 2012. "Effects of Chlortetracycline Amended Feed on Anaerobic Sequencing Batch Reactor Performance of Swine Manure Digestion." *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2012.08.077.
- Duan, Na, Duojiao Zhang, Cong Lin, Yifeng Zhang, Lingying Zhao, Hongbin Liu, and Zhidan Liu. 2019. "Effect of Organic Loading Rate on Anaerobic Digestion of Pig Manure: Methane Production, Mass Flow, Reactor Scale and Heating Scenarios." *Journal of Environmental Management*. https://doi.org/10.1016/j.jenvman.2018.10.062.
- El-Mashad, Hamed M., Wilko K.P. Van Loon, and Grietje Zeeman. 2003. "A Model of Solar Energy Utilisation in the Anaerobic Digestion of Cattle Manure." *Biosystems Engineering*. https://doi.org/10.1016/S1537-5110(02)00245-3.
- Fuchs, Werner, Xuemei Wang, Wolfgang Gabauer, Markus Ortner, and Zifu Li. 2018. "Tackling Ammonia Inhibition for Efficient Biogas Production from Chicken Manure: Status and Technical Trends in Europe and China." *Renewable and Sustainable Energy Reviews*. https://doi.org/10.1016/j.rser.2018.08.038.
- Habiba, Lahdheb, Bouallagui Hassib, and Hamdi Moktar. 2009. "Improvement of Activated Sludge Stabilisation and Filterability during Anaerobic Digestion by Fruit and Vegetable Waste Addition." *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2008.09.019.
- López-Andrés, Jhony Josué, Alberto Alfonso Aguilar-Lasserre, Luis Fernando Morales-Mendoza, Catherine Azzaro-Pantel, Jorge Raúl Pérez-Gallardo, and José Octavio Rico-Contreras. 2018. "Environmental Impact Assessment of Chicken Meat Production via an Integrated Methodology Based on LCA, Simulation and Genetic Algorithms." *Journal of Cleaner Production* 174: 477–91. https://doi.org/10.1016/j.jclepro.2017.10.307.
- Massé, Daniel I., Rajinikanth Rajagopal, and Gursharan Singh. 2014. "Technical and Operational Feasibility of Psychrophilic Anaerobic Digestion Biotechnology for Processing Ammonia-Rich Waste." *Applied Energy*. https://doi.org/10.1016/j.apenergy.2014.01.034.
- Nie, Hong, H. Fabian Jacobi, Katrin Strach, Chunming Xu, Hongjun Zhou, and Jan Liebetrau. 2015. "Mono-Fermentation of Chicken Manure: Ammonia Inhibition and Recirculation of the Digestate." *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2014.09.029.
- Paulo, Lara M., Alfons J.M. Stams, and Diana Z. Sousa. 2015. "Methanogens, Sulphate and Heavy Metals: A Complex System." *Reviews in Environmental Science and Biotechnology*. https://doi.org/10.1007/s11157-015-9387-1.
- Rajagopal, Rajinikanth, David Bellavance, and Md Saifur Rahaman. 2017. "Psychrophilic Anaerobic Digestion of Semi-Dry Mixed Municipal Food Waste: For North American Context." *Process Safety and Environmental Protection*. https://doi.org/10.1016/j.psep.2016.10.014.
- Riau, V., M. A. De la Rubia, and M. Pérez. 2012. "Assessment of Solid Retention Time of a Temperature Phased Anaerobic Digestion System on Performance and Final Sludge Characteristics." *Journal of Chemical Technology and Biotechnology*. https://doi.org/10.1002/jctb.3709.
- Venkiteshwaran, Kaushik, Benjamin Bocher, James Maki, and Daniel Zitomer. 2016. "Relating Anaerobic Digestion Microbial Community and Process Function." *Microbiology Insights*. https://doi.org/10.4137/MBI.S33593.
- Ward, Alastair J., Phil J. Hobbs, Peter J. Holliman, and David L. Jones. 2008. "Optimisation of the

Anaerobic Digestion of Agricultural Resources." *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2008.02.044.