IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 12, Issue 1 Ver. I (January 2019), PP 24-31
www.iosrjournals.org

Biochemical methane potential of sheep manure: Focus in pathogen removal

Beily, M.E.[(1)*], Morici, G.[(2)], Crespo, D.[(1)], Schapiro, J.[(2)]
[1] *Laboratorio de transformación de residuos (LTR), Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria –(INTA.) Argentina*
[2]*Área de Parasitología, Instituto de Patobiología, Instituto Nacional de Tecnología Agropecuaria (INTA)*
Corresponding Author: Beily

Abstact: *Biochemical methane potential (BMP) test is a useful laboratory-scale tool to determine the biodegradability and methane potential from different manure. The aims of this study were to evaluate the BMP of sheep manure and the elimination of pathogens after going through anaerobic digestion. The BMP was carried out in mesophilic temperature (35ºC) with a substrate/inoculum (S/I) ratio of 0.5 gSV/grSV At the beginning and in the end of the experiment the following variables were determined, pH, total and soluble chemical oxygen demand (CODT and CODs respectively), total ammonia nitrogen (TAN), free ammonia nitrogen (FAN), volatile fatty acid (VFA), partial and total alkalinity (PA and TA, respectively), total coliforms, faecal coliforms, Escherichia coli, Salmonella spp. and gastrointestinal nematodes (GIN) eggs. Methane and carbon dioxide contents in the biogas were measured using gas chromatography. The results showed a specific methane production of 0.12 LCH_4 / gSVad and a CODS removal of 67%. No presence of faecal coliforms, E.coli and GIN eggs was detected. The BMP test demonstrated that mesophilic anaerobic digestion is a viable option to reduce organic and pathogenic load in sheep manure.*

Keyword: *Sheep manure; anaerobic digestion; pathogen removal; gastrointestinal nematodes eggs*

Date of Submission: 26-12-2018 Date of acceptance: 11-01-2019

I. Introduction

Sheep farming is an important component of the Argentine agricultural productive system [1]. The agribusiness generated by this activity has a high production potential with comparative and competitive advantages for its growth. Between 14 and 15 million sheep are owned by around 70-80,000 farmers [1]. About 85% of them own less than 100 sheeps in mixed production systems or small family farms. In the Patagonian region, two-thirds of the country's sheep are raised as the only productive activity, and over a third of the farmers own more than 1,000 sheeps, whereas some companies own more than 50,000. Consequently, there is a variety of production systems with different problems. Traditionally, Argentina has focused exclusively on wool production. More recently, dual-purpose sheep breeds were introduced to produce meat as well as wool. In the last decades, several breeds were adopted to obtain sheep's milk [1].

It is estimated that the total annual waste of an adult sheep (including manure, feed, hay, and other waste) can exceed 1,500 to 2,500 kg / year [2].The production of sheep in Argentina is not intensive. However, environmental problems are focused on intensive facilities, where optimal management and treatment of waste is not carried out. Inadequate waste management creates the following problems: 1 - risk due to the transmission of diseases for the human population to the presence of enterobacteria. The most common is Escherichia coli, which causes diarrhea and abdominal gas [3]. 2- high levels of nitrates that reduce the oxygen transport capacity in the blood, known as methemoglobinemia [4] 3- presence of hormones, mainly estrogen, related to a reduction in the amount of sperm in humans [5] 4- environmental impact such as the generation of greenhouse gases, the eutrophication of surface water bodies and the overload of nutrients in soils [6].

Biodegradation technologies, such as anaerobic digestion is a valid and rational strategy for excreta management. This technology reports some advantages like avoid volatile organic compound emissions, control odours, mineralize nutrients, elimination pathogens (zoonotic pests) and to recover energy through methane production [7 8, 9]. The potential biochemical methane test (BMP) is a useful tool at laboratory scale to determine the biodegradability, the potential of biomethane and the inhibitor compounds of an effluent [10, 11]. The aims of this study were to evaluate the biochemical potential of methane (BMP) from sheep manure and the elimination of pathogens after going through an anaerobic digestion process.

II. Materials And Methods

Collection and preparation of raw materials and inoculum

The sheep manure was collected in the experimental field of the Centro de Investigación en Ciencias Veterinarias y Agronómicas (CICVyA) from Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires (Argentina). The animals used were an early age stage. The sheep manure was characterized by the following parameters: total solid (TS), volatile solid (VS), total Kjeldahl nitrogen (TKN), total phosphorus (TP) and pH, measured by the U.S.D.A US Composting Council [12]. Total coliforms, faecal coliforms and *Escherichia coli* were determined by the method of the most probable number (MPN), with the multi-tube fermentation technique [13]. *Salmonella* spp. weas analyses by the method proposed by Caffer et al., 2008 [14] The gastrointestinal nematodes (GIN) eggs were determined according to the modified McMaster technique for cattle and sheep (Robert and O'Sullivan 1949) [15].

The inoculum used in biodegradability test was collected from an anaerobic digestion plant that treats sewage water. A specific methanogenic activity test (SMA) was carried out to ensure its activity. Its SMA was 0.11 gCOD/gVSS*day. The volatile suspended solid (VSS) of the inoculum was: 18.7 g VSS/ L.

Reactors

The experimental unit was each batch reactor. The reactor was 500 ml glass bottles, Schott -Duran model GL45 with threaded neck. In its top contained a plastic plug ended in a thread of ¼ NTP, which is coupled to a fast connection with retention.

BMP assays

The potential methane yield was determined as the total methane production after complete anaerobic degradation divided by the amount of VS in the sample. The experience consisted in one treatment, with three replicates, with a substratum/inoculum (S/I) ratio of 0.5 gSV/grSV. For the determination of endogenous methane production, blanks (control) contained the same quantity of inoculum without substrates were run. To develop the test, 20 % of headspace was set. The stirred was manually twice a day. The pH was adjusted to 7.0 in all cases. The reactor headspace was flushed with nitrogen in order to remove the oxygen. The pressurized reactor was immersed in water to verify the absence of gas leaks. Ensured air tightness is depressurized to atmospheric pressure. Then, the reactors were placed in an incubator at 35±1°.

For biodegradability assays, pH, total and soluble chemical oxygen demand (CODT and CODs respectively), total ammonia nitrogen (TAN), free ammonia nitrogen (FAN), volatile fatty acid (VFA), partial and total alkalinity (PA and TA, respectively) were determined at beginning and end of the trial. COD, NH_4^+, TS, and VS were determined according to Standard Methods for Examination of Water and Wastewater [13]. VFA, PA and TA were determined according to Jenkins et al., (1983) and DiLallo & Albertson (1961) [16, 17, 18]. FAN concentrations were calculated according to Hansen et al., (1998) [19] (Equation 1).

$$NH_3(free) = TANx\left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1} \quad \text{(Equation 1)}$$

Methane and carbon dioxide contents in the biogas were measured by gas chromatography (Hewlett Packard 5890 GC System) according to method ASTM D 1945-14 (2014) [20] using a molecular sieve 13X and HP PLOT Al2O3, a thermal conductivity detector (TCD and helium as a carrier gas at a flow rate of 2.1 mL/min). The column temperature was kept at 90º C whereas the injector and detector temperatures were set at 130º C and 250º C, respectively. Biogas and methane production were expressed at standard temperature and pressure conditions (0ºC; 1 atm). The methane yield (Specific methane Production) was calculated according to Bres et al., (2018)

$$SMP = \frac{Vmethane}{substrate\ mass}\left[\frac{NL\ methane}{Kg\ VSadded}\right] \quad \text{(Equation 2)}$$

where SMP is the Specific Methane Production, Vmethane is the accumulated volume of methane in standard conditions, and substrate mass is the weight of volatile solids in the substrate added to reactor.
Results were analysed with the T test using the statistical analysis software InfoStat®.

III. Results And Discussion

Sheep manure characteristic

The manure presented a neutral pH (7.1±0.1), this characteristic was evidenced by Medina et al., 2015 [21] when analyzing sheep manure to perform anaerobic digestion trials. On the other hand, it presented a TP concentration of 42.16 ± 1.2 mg/L; and 32.2 ± 0.4% ST; 90.8±0.13% SV and a TKN concentration of 17.5 g/L. Particularly important aspect for the anaerobic performance is the C/N, the suggested optimum C/N ratio is in the range of 20:1 to 30:1 [22]. In this study sheep manure had a C/N ratio of 26.7. The high nutrient and organic matter contents (VS and COD) in the raw materials indicated a favourable condition for a biologic process.

BMP assay

In this study the pH remained within the optimum range for the development of anaerobic bacteria throughout the experiments [23, 24]. Medina et al., (2015) [21] obtained similar results when performing BMP tests with sheep manure. The pH is an important control parameter during anaerobic digestion. The production of a large amount of VFA leads to the decrease of solution pH. Non-methanogenic microorganisms responsible for hydrolysis and fermentation can be adapted to low pH while methanogens will lose activity at low pH. Thus methanogenesis can be inhibited significantly at low pH [25]. VFA show reduction of 37% (Table 1) with a significant differences ($p<0.05$). At the beginning the concentration started increasing because the organic fraction of manure was hydrolyzed to intermediate organics and VFA, at the end of the assay a reduced of VFA was observed caused due to VFA was hydrolyzed and fermented to carbon dioxide and methane in subsequent steps [24]. VFA is an important parameter control because if the acidogenic and methanogenic phases are unbalanced in the final stage process the VFA is accumulated and inhibits the methanogenic bacteria, which in turn causes the reduction of the biogas production yield [26]. On the other hand, TA and PA show an increase at the end of the trial of 20.31% and 21.85%, respectively. For the digesters to operate efficiently, a large buffer capacity is required to maintain the pH between 6.7 and 7.4. In this pH range, the predominant buffers are carbonated. Under stable conditions, anaerobic digestion has a balance between the generation of volatile fatty acids (acetogenic phase) and the production of bicarbonates and carbonates (methanogenic phase) [27].

Table 1 shows an increase (50%) in the content of NH_4^+, showing significant differences ($p<0.001$) at the end of the assay. Organic nitrogen is mineralized by enzymatic hydrolysis of proteins, during anaerobic process. This is explained the increase of NH_4^+ take place along the assay. On the other hand, free ammonia (FAN) increase 60% ($p<0.001$) at the end of the trial (Table1). Free ammonia is the active component causing ammonia inhibition [28]. The free ammonia concentration depends mainly on three parameters: TAN concentration, temperature and pH [19]. A wide range of ammonia concentrations capable of inhibiting the process has been reported. The differences between inhibitory TAN and FAN levels can be attributed to different substrate type, dilution, acclimation period, pH and work temperature [29, 19, 30, 31].

Table 1: Physical and chemical properties of the manure and the digestate in BMP assay. Average (± SD) based on n=3. CODT: total chemical oxygen demand. CODs: soluble chemical oxygen demand. TAN: total ammonia nitrogen. FAN: Free ammonia nitrogen. VFA: Volatile fatty acid. TA: total alkalinity and PA: partial alkalinity. Different letters indicated significant differences between treatments * ($p<0.05$) ** ($p<0.001$).

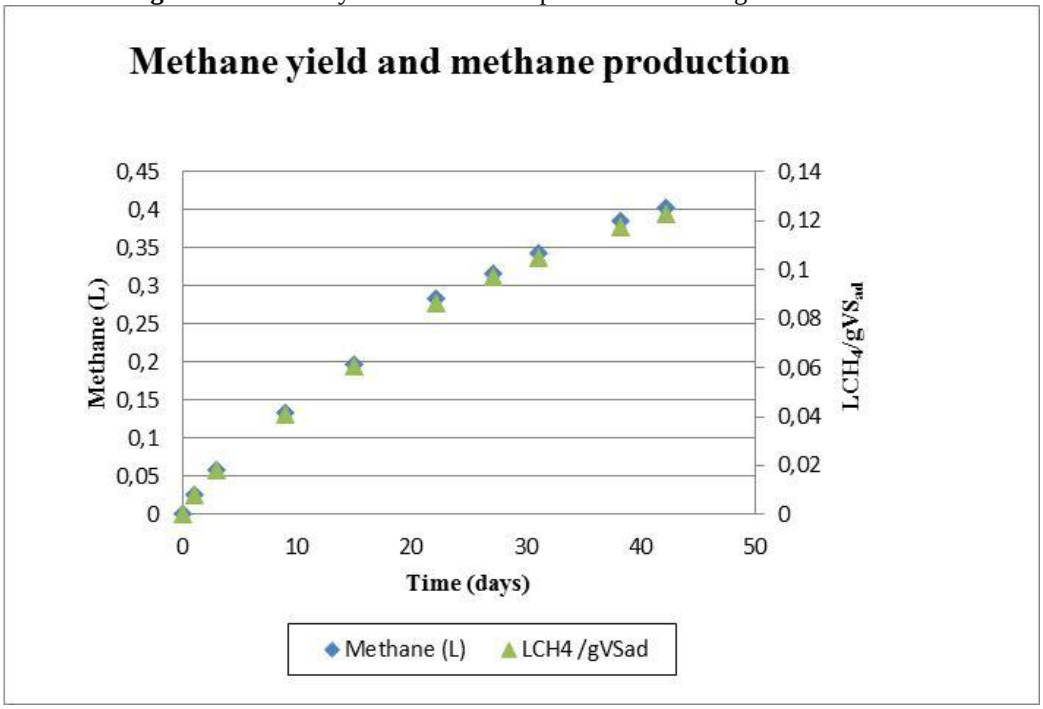
Parameter	Unit	Initial	Final
pH	upH	7.0 ± 0a	7.1 ± 0a
COD_T	gO_2/L	$15.45 \pm 0.55^{a**}$	$9.71 \pm 0.32^{b**}$
CODs	gO_2/L	$2.01 \pm 0.41^{a**}$	$0.66 \pm 0.18^{b**}$
TAN	gNH_4^+/L	$0.67 \pm 0.04^{a**}$	$1.34 \pm 0.01^{b**}$
FAN	$g\ NH_3/L$	$0.008 \pm 0.00^{a*}$	$0.02 \pm 0.00^{b*}$
VFA	mg Ac/L	$201.45 \pm 0.00^{a*}$	$126.40 \pm 13.64^{b*}$
TA	$gCaCO_3/L$	$1.53 \pm 41.89^{a**}$	$1.92 \pm 0.02^{b**}$
PA	$gCaCO_3/L$	$1.18 \pm 0.0^{a**}$	$1.51 \pm 0.04^{b**}$
Na^+	mg/L	$578.25 \pm 10.8^{a**}$	$368.55 \pm 10.82^{b**}$
K^+	mg/L	$354.6 \pm 6.3^{a**}$	$178.65 \pm 3.18^{b**}$
Cu^+	mg/L	$2.59 \pm 0.15^{a**}$	$1.66 \pm 0.24^{b**}$

On the other hand, the organic matter removal was evaluated through the soluble and total COD contents. The soluble COD shows a reduction of 67%, showing significant difference between the initial and final ($p < 0.001$). However, total COD showed a lower reduction (37%).The low removal in the total COD could be due to the interference of total solids in the sample.

Biogas and methane Production

Figure 1 shows the cumulative production of methane gas and SMP ($LCH_4/gVSa$).

Figure 1: Methane yield and methane production. Average based on n=3.



Gas production started immediately after loading the reactors and continued until day 25 of the test. Then, the volume of methane began to decrease progressively until day 42, where it was considered that the trial was finished. The accumulated biogas was 634 ml and the accumulated methane production was 402 ml. The maximum percentage of CH_4 was 55%. Medina et al., (2015) [21], found a lower percentage of methane in the biogas, when studying the anaerobic co-digestion of sheep and swine manure. The methane yield was 0.12 $LCH_4/gSVad$. This performance was lower than others described in the literature of different substrates. Chae et al., (2008) [32] found a porcine manure methane yield of 0.319 ± 0.014 LCH_4/gSV; Bres et al. (2018) [33] obtained a mesophilic anaerobic digestion of 0.21 ± 0.01 and 0.16 ± 0.03 $LCH_4/gVSad$ in poultry manure in co-digestion with fruit and vegetable residues and poultry manure respectively. The yield of SMP could be improved by co-digestion with substrates with a high content of organic matter, such as glycerol or different carbon-rich compounds [34]

Removal of pathogenic microorganisms and gastrointestinal nematodes (GIN) eggs

The results showed the presence of pathogens in the manure analyzed. Consequently, waste management must be carried out, especially in intensive production. One of the main concerns in the intensification of livestock is the increase in survival or resilience of these pathogens, many of which are dangerous zoonotic agents for the animals in production or their possibility of transmission in the human food chain [35]. In addition, they can contaminate soil, fresh product, surface and groundwater, and drinking water supplies [36]. The risk of transmission of infectious agents must be taken into account when recycling manure [37]. However, properly treated and disinfected manure can be assessed as an effective and safe biofertilizer for soil.

It is a well-established fact that bacterial pathogens can persist for long periods in animal fertilizers under typical farm conditions. This can be extended when temperatures are low, humidity is still optimal and aeration is not used. For instance, *Salmonella* and *E. Coli* O157:H7 survived for 4 – 6 months in animal manures and slurries kept at 1 – 9°C, which is up to 49 times longer than at 40 – 60°C [35]. Kudva et al., (1998) [38] study the survival of *E.coli* O157:H7 in ovine and bovine manure and manure slurry. In this study they found

that pathogen can survived for more than 1 year in non-aerated ovine manure pile that was exposed to environmental conditions while in similar aerated ovine manure piles, the organisms survived for 4 months.
Plachá et al., (2001) [39] evaluated the effect of summer and winter seasons on the survival of *S. typhimurium* and indicator micro-organisms during the storage of solid fraction of pig slurry. The results indicate that the survival of *S. typhimurium* and indicator bacteria was considerably affected by temperature, during summer time the survival of this pathogens was 26 days whilst in winter/spring was 85 days. On the other hand, when manures are applied to land, there will be some movement of the pathogens through the soil. The degree of mobility will affect the likelihood that pathogens will reach aquifers or surface waters. If these waters are subsequently used for the irrigation of products or for consumption by livestock, there are risks for food safety [35].

Table 2 shows the removed pathogens along the anaerobic process. At the end of the assay, the count of GIN, faecal coliforms and *E.Coli* were negative, showing a high removal of these pathogens. On the other hands, total coliforms showed a remove of 93%.

The pathogens removal found in this research could be mainly due to 3 important factors, the temperature, the retention time and the ammonia concentrations. Jenkins et al., (1998) [40] demonstrated that the high concentration of ammonium is directly proportional to the inactivation of *Cryptosporidium oocyst*, attributing to the ammonium the effect of biocide. They demonstrated that exposure to low concentrations of free ammonia in solution can have a deleterious effect on the survival of *C. parvum oocysts*. In the same research, the authors found that a 24-h exposure to 0.06 M ammonia inactivated between 64.5% - 83.7% of the *oocysts* [40].

Table 2: Pathogenic content of the raw manure and the digestate in BMP assay. Average based on n=12.

	Units	Initial	Final
Total Coliforms	MPN/ml	460	28
Fecal Coliforms	MPN/ml	150	< 3
E. coli	MPN/ml	93	< 3
Salmonella spp.	-------	absence	absence
Gastrointestinal nematodes (GIN) eggs	Eggs/L	6600	0

Olsen & Nansen (1987) [41] studied the viability of an important cattle nematodes (*Cooperia oncophora*) after mesophilic (35°C) and thermophilic (53°C) anaerobic digestion of bovine slurry. In this study they found that the eggs apparently lost viability very rapidly at 35°C in that they failed to develop after the second day of digestion. At 20°C they failed to develop after day 22, but at 4°C viable eggs were recorded throughout the experimental period. At 53°C, the eggs of *Cooperia oncophora* had an atypical, slightly amorphous, appearance after only 1 h, and after 24h no eggs could be recovered. On the other hand, Olsen & Larsen (1987) [37] studied the interaction of temperature with the bacterial decimation times in anaerobic digestions of animal slurries. In this research they conclude that the thermophilic and mesophilic anaerobic digestion resulted in faster inactivation of vegetative pathogenic bacteria in animal liquid manures than that normally described for conventional storage procedures. Furthermore in this research they found that the thermophilic temperature during digestion resulted in a considerably faster reduction of the vegetative pathogenic bacteria than the mesophilic range; conferring to the temperature a decisive factor for bacterial survival during anaerobic digestion.

IV. Conclusions

The BMP test demonstrated that mesophilic anaerobic digestion is a viable option to reduce the organic and pathogenic load of sheep manure. Therefore, the use of anaerobic digestion as a method of treatment of manure could be a good option to add value to the waste, to reduce the negative impact on the environment and to avoid the proliferation of pathogens in intensive production.

Acknowledgement

This research was funded by National Program of Natural Resources and Environmental Management - National Institute of Agricultural Technology (INTA), Argentina. A special acknowledgement to the ALARALAB Laboratory, for its contribution and support in the chromatographic analyzes.

References

[1]. SENASA http://www.senasa.gob.ar/cadena-animal/ovinos
[2]. Lobato Fuertes, A. 2012. Estudio de la co-digestión anaerobia de residuos ganaderos con otros substratos agroindustriales. Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad (Área de Ingenieria Química). Universidad de León, España.
[3]. LeJeune, J. T., and A. N. Wetzel. 2007. Preharvest control of Escherichia coli O157 in cattle. J. Anim. Sci. 85:73-80.,
[4]. Miner, J. R., F. J. Humenik, and M. R. Overchash. 2000. Managing Livestock Wastes to Preserve Environmental Quality. Environmental Quality. Iowa State Univertisy Press. Ames, IA, USA. pp: 318.
[5]. Sharpe, R., and N Skakkebæk. 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? The Lancet 341: 1392. https://doi.org/10.1016/0140-6736(93)90953-E
[6]. Nelson, C. J. 1999. Managing nutrients across regions of the United States. J. Anim. Sci. 77: 90-100
[7]. Bonmatí, A., Flotats X., Mateu, L. and Campos, E. 2001. Study of thermal hydrolysis as a pretreatment to mesophilic anaerobic digestion of pig slurry. Water Science and technology. 44, 109-116
[8]. Nelson, C. & Lamb, J. 2002. Final Report: Haubenschild Farms Anaerobic Digester. Minnesota Project. www.mnproject.org.
[9]. Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928–7940.https://doi.org/10.1016/j.biortech.2008.02.044.
[10]. Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L.Y., McCarty, P. L. 1978. Biossay for monitoring biochemical methane potential and anaerobic toxicity. Water research 13, 485-492
[11]. Angelidaki, I., Sanders, W. 2004. Assessment of the anaerobic biodegradability of macropollutants. Reviews in Environmental Science and Bio/Technology3: 117–129.
[12]. USDA., USCC, 2001. Test Methods for the Examination of Composting and Compost (TMECC). Edaphos International, Houston, USA
[13]. APHA, 1992. Standard methods for examination of water and wastewater.18th ed.,American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington DC, USA
[14]. Caffer, M.I., Terragno, R., Binsztein, N. 2008. Manual de Procedimientos: Diagnostico y caracterización de Salmonella spp. Departamento Bacteriología Instituto Nacional de Enfermedades Infecciosas A.N.L.I.S. "Dr. Carlos G. Malbrán" Centro Regional de Referencia del WHO Global Salm Surv para América del Sur.
[15]. Roberts, F. & O'Sullivan, P. 1949. Methods for egg count and larval cultures for strongyles infesting gastrointestinal tract of cattle. Australian Journal of Agricultural Research 1: 99-102.
[16]. Jenkins, S.R., Morgan, J.M., Sawyer, C.L., 1983. Measuring digestion simple and alkalimetric sludge by titration a growth. Water Pollut. Control Fed. 55, 448–453.
[17]. Jenkins, S.R., Morgan, J.M., Zhang, X., 1991. Measuring the usable carbonate alkalinity of operating anaerobic digesters. Res. J. Water Pollut. Control Fed. 63, 28–34.https://doi.org/10.2307/25043948.
[18]. DiLallo, R. & Albertson, O. 1961. Volatile acids by direct titration. Journal WPCF, 33(4): 356-365.
[19]. Hansen, K.H., Angelidaki, I., Ahring, B.K., 1998. Anaerobic digestion of swine manure: Inhibition by ammonia. Water Res. 32, 5–12.https://doi.org/10.1016/S0043-1354 (97) 00201-7.
[20]. ASTM D1945-14. 2014. Standard test methods for analisis of gas natural by gas chromatography. ASTM International, West Conshohocken, PA, USA. DOI: 10.1520/D1945-14
[21]. Medina, A., Quipuzco, L., Juscamaita, J. 2015. Anales Cientificos, 76 (1) 116-124. DOI: http://dx.doi.org/10.21704/ac.v76i1.772
[22]. Zaher U. P., Grau P. L., Benedetti E. A. y Vanrolleghem P. A. (2007). Transformers for interfacing anaerobic digestion models to pre-and post-treatment processes in a plant-wide modeling context. Environmental Modeling & Software22, 1, 40-58.
[23]. Sakar, S., Yetilmezsoy, K., Kocak, E., 2009. Anaerobic digestion technology in poultry and livestock waste treatment: a literature review. Waste Manag. Res. 27, 3–18.
[24]. https://doi.org/10.1177/0734242X07079060.
[25]. Don, J.; Zhao, Y.; Hong, M.; Zhang, W. 2009. Influence of alkalinity on the stabilization of municipal solid waste in anaerobic simulated bioreactor. Journal of hazardous materials, 163(2-3): 717-722.
[26]. Zhang P., Zeng G., Zhang G, Li Y., Zhang B. y Fan M. (2008). Anaerobic co-digestion of biosolids and organic fraction of municipal solid waste by sequencing batch process. Fuel processing technology 89(4):485-489.
[27]. Elango, D., Pulikesi, M., Baskaralingam, P., Ramamurthi, V., Sivanesan, S. 2007. Production of biogas from municipal solid waste with domestic sewage. Journal of Hazardous Materials 141 (2007) 301–304. https://doi:10.1016/j.jhazmat.2006.07.003
[28]. Bres, P.; Beily, M.; Rizzo, P.; Giampaoli, O.; Crespo, D. 2010. Monitoreo de reactor anaerobico semi-continuo para el tratamiento de residuos de cereales. Parte I. Avances en Energías renovables y medio ambiente, 14: 29-34.
[29]. Angelidaki I; Ahring, B. (1993) Thermophilic anaerobic digestión of livestock waste: the effect of ammonia. App. Microbol Biotecnol., 38, 560-564
[30]. Gallert, C.; Bauer, S.; Winter, J. 1998. Effect of ammonia on the anaerobic degradation of protein by mesophilic and thermophilic biowaste population. Applied microbiology and biotechnology, 50: 495-501. https://doi.org/10.1007/s002530051326
[31]. Hashimoto, A. 1986. Ammonia inhibition of methanogenesis from cattle wastes. Agricultural Wastes, 17: 241-261. https://doi.org/10.1016/0141-4607(86)90133-2
[32]. Krylova, N.I., Khabiboulline, R.E., Naumova, R.P., Nagel, M.A., 1997. The influence of ammonium and methods for removal during the anaerobic treatment of poultry manure. J. Chem. Technol. Biotechnol. 70, 99–105. https://doi.org/10.1002/(SICI)1097-4660(199709)70:1<99::AID-JCTB684>3.0.CO;2-C
[33]. Chae, K.; Jang, A.; Yim, S.; Kim, I. 2008. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. Bioresource Technology, 99: 1-6. https://doi.org/10.1016/j.biortech.2006.11.063
[34]. Bres, P., Beily, M.E., Young, B.Y., Gasulla, J., Butti, M., Crespo, D., Candal, R., Komilis, D. 2018. Performance of semi-continuous anaerobic co-digestion of poultry manure with fruit and vegetable waste and analysis of digestate quality: A bench scale study. Waste Management 82 (2018) 276–284.
[35]. Astals, S., Nolla-Ardèvol, V., Mata-Alvarez, J. 2012. Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate. Bioresource Technology 110 (2012) 63–70. https://doi.org/10.1016/j.biortech.2012.01.080
[36]. Venglovsky, J., Sasakova, N., & Placha, I. (2009). Bioresource Technology Pathogens and antibiotic residues in animal manures and hygienic and ecological risks related to subsequent land application. Bioresource Technology, 100(22), 5386-5391. https://doi.org/10.1016/j.biortech.2009.03.068
[37]. Vanotti, M., Szogi, A., Hunt, P., Millner, P., Humenik, F. 2007. Development of environmentally superior treatment system to replace anaerobic swine lagoons in the USA. Bioresource Technology 98 (2007) 3184–3194. https://doi.org/10.1016/Bj.biortech.2006.07.009

[38]. Olsen, J. E.& Larsen, H.E. 1987. Bacterial decimation times in anaerobic digestions of animal slurries. Biological Wastes 21, 153-168. https://doi.org/10.1016/0269-7483(87)90121-2
[39]. Kudva, I., Blanch, K., Hovde, C.1998 Analysis of Escherichia coli O157:H7 Survival in Ovine or Bovine Manure and Manure Slurry. Applied and Enviromental Microbiology, 64 (9) 3166-3174
[40]. Plachá, I., Venglovský, J., Sasáková, N., Svoboda, I.F. 2002. The effect of summer and winter seasons on the survival of Salmonella typhimurium and indicator microorganisms during the storage of solid fraction of pig slurry. Journal of Applied Microbiology, 91, 1036-1043. https://doi.org/10.1046/j.1365-2672.2001.01471.x.
[41]. Jenkins, M., Bowman, D., Ghiorse, W. 1998. Inactivation of Cryptosporidium parvum Oocysts by Ammonia. Applied and environmental microbiology, 64(2) 784-788
[42]. Olsen, J. E. & Nansen, P. 1987. Inactivation of Some Parasites by Anaerobic Digestion of Cattle Slurry. Biological Wastes 22, 107-114. https://doi.org/10.1016/0269-7483(87)90043-7

Beily" Biochemical methane potential of sheep manure: Focus in pathogen removal" IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 12.1 (2019): PP-24-31.