

Efecto de la carga orgánica de la gallinaza de jaula en el potencial de biometanización

Effect of chicken manure organic load on biomethane potential

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Resumen

El propósito de este estudio fue evaluar el efecto de la carga orgánica expresada en función de la relación inóculo/sustrato (RIS) sobre el potencial de biometanización de la gallinaza de jaula usando como inóculo lodo estiércol bovino. Se llevaron a cabo ensayos de biodegradación anaerobia a temperatura mesofílica de 39 °C. Para cada una de las cargas orgánicas evaluadas (16.6, 11.0, 8.3, 6.6 y 5.5 g SV/L) se cuantificaron las variables: ácidos grasos volátiles totales, alcalinidad, concentración amonio y volumen acumulado de metano. El mayor potencial de biometanización (0.58 m³ CH₄/kg SV) se alcanzó cuando la biodegradación anaerobia se llevó a cabo con una RIS de 1.0. Los resultados obtenidos demuestran que la gallinaza es un sustrato potencial para ser degradado por digestión anaerobia y el rendimiento del proceso es directamente proporcional a la concentración de sustrato. Este estudio también confirma que la RIS permite diluir la concentración de compuestos inhibitorios como el amonio en el caso de la gallinaza de jaula.

Palabras clave: digestión anaerobia, gallinaza de jaula, relación inóculo/sustrato, amonio.

Abstract

The aim of this study was to evaluate the effect of inoculum to substrate ratio (ISR) on biomethane potential of chicken manure using cattle slurry as inoculum. Biomethane potential assays were carried out at 39 °C mesophilic temperature. Total fatty acids, total alkalinity, ammonium concentration and accumulative methane volume were measured to evaluate organic load (16.6, 11.0, 8.3, 6.6 y 5.5 g VS/L). The highest biomethane potential (0.58 m³ CH₄/kg SV) was reached when anaerobic biodegradation was carried out to ISR of 1.0. The results demonstrated that chicken manure is a potential substrate to be degraded by anaerobic digestion and process performance is directly proportional to substrate concentration. This study also confirms that ISR allow dilution of inhibitory components as ammonium by the case of chicken manure.

Key words: anaerobic digestion, chicken manure, inoculum substrate ratio, ammonia.

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Introduction

The poultry sector is one of the foundations of the Colombian economy and diet. The majority of the country's poultry farms are found in the departments of Cundinamarca (28%), Santander (24%) and Valle del Cauca (13%) (Escalante *et al.* 2010). The different poultry farming processes generate waste such as laying-hen excreta, chicken excreta, dead birds, fatty waste and eggshells. This waste causes environmental contamination, public health and animal health

problems (Delgado, 2011). Caged laying hen excreta (CLHE), a mixture of liquid and solid animal excreta, is the most representative organic waste generated by poultry farming because of its volume, as well as its physiochemical properties (Rosales *et al.* 2007). During the industrial process of specialized rearing of farm birds, an average quantity of 107.98 tons of CLHE is generated per one million birds (Escalante *et al.* 2010). Through biological and thermal processes, it is possible to use the byproducts of poultry processes in

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the preparation of new products such as poultry manure and meat meal (Huang *et al.* 2011; Rosales *et al.* 2007). However, these technologies are insufficient to treat the volumes of waste generated by the sector. CLHE is mainly comprised of protein (20.5 %), cellulose (20 %), hemicellulose (23.2 %), lignin (1.6 %) and volatile fatty acids (VFAs) (0.3 %). This high content of organic matter makes CLHE an attractive substrate for anaerobic digestion (AD) (Li *et al.* 2013). Anaerobic bioconversion systems are biological processes where organic material is reduced to methane and carbon dioxide by the activity of different microbial groups that work in syntrophy. The microorganisms involved in AD are comprised of a) fermentative and hydrolytic bacteria b) acetogenic bacteria, and c) methanogens. The first group is represented by the *Peptostreptococcus*, *Propionibacterium*, *Bacteroides*, *Micrococcus* and *Clostridium* genera, which produce enzymes that divide the organic polymers into easily fermentable monomers. Then the acidogenic bacteria metabolize the monomers to generate acetate, propionate and butyrate, which are later reduced by the acetogenic bacteria to acetate, carbon dioxide and hydrogen. Finally, the methanogens act on the acetate and hydrogen to transform them into methane (Gerardi, 2003). Studies on AD in mesophilic conditions have reported yields of methane production from 0.29 to 0.50 m³ CH₄/kg VS, using CLHE as a substrate and anaerobic slurry from sewage treatment plants as an inoculum (Li *et al.* 2013; Nui *et al.* 2013).

An important operational parameter in AD is the organic load represented through the inoculum to substrate ratio (ISR) (Hashimoto *et al.* 1989). It has been demonstrated through experiments that the ISR affects the speed of the substrate's biodegradation. Therefore, this parameter allows the biomethane potential (BMP) to be specified and comparable AD studies to be conducted for any kind of substrate (Raposo *et al.* 2011). The first studies related to the influence of organic load on BMP in terms of ISR were carried out in discontinuous operation using corn silage as a substrate and bovine slurry as an inoculum in volume concentrations of 0% (control), 10%, 30%, 50%, 70% and 90%. The results show that for high organic loads (ISR values under 0.25), the speed of the substrate's biodegradation was slow compared to the biodegradation of the substrate for ISR values close to 2 (Hashimoto *et al.* 1989). Similar results were obtained by Raposo *et al.* (2006) for the AD of the same substrate using bovine slurry as an inoculum at an ISR from 1 to 3.

The influence of the ISR on BMP has also been studied for food and agribusiness waste. The existence of a declining linear relationship between biogas yields and ISR was proven (Liu *et al.* 2009). Neves *et al.* (2004) confirmed that for the AD of food waste using granular slurry and suspended slurry as an inoculum, the selection of the ISR becomes a greater determinant than the inoculum's own metabolic activity. Therefore, studies

on AD require that an organic load is established based on an ISR, which permits the initiation and stabilization of the process.

The criteria to establish an ISR value revolve around the complexity of the substrate's biodegradation. In the case of CLHE, concentrations of volatile fatty acids (VFAs) between 2000-4800 mg/L and alkalinity (TA) between 4,700-13,600 mg/L allow adequate initiation of the anaerobic process (Salminen and Rintala, 2002; Callaghana *et al.* 2002). However, CLHE also contains ammoniacal nitrogen, which in concentrations above 5,000 mg/L, has a toxic or inhibitive effect on microbial activity, affecting the stability of the process (Nue *et al.* 2013).

Owen *et al.* (1979), established that ISR values of more than one allow the dilution of the concentration of toxic compounds present in the substrate. Additionally, Chynoweth *et al.* (1993) observed that for substrates such as cellulose, an ISR of 2.0 is required to prevent inhibition from the elevated accumulation of volatile fatty acids (VFAs). The acidification produced by the low buffer capacity in the anaerobic biodegradation of kitchen waste and the inhibition caused by the release of orthophosphates in the AD of algae were controlled by establishing an ISR of 1.0, allowing the process to be operated in pH values within the appropriate range (6.0-8.5) for AD (Zeng *et al.* 2010; Neves *et al.* 2004).

The objective of this work was to assess the influence of the substrate/inoculum ratio on the biomethane potential of CLHE. The development of this objective allows the effect of the substrate's variability on the digestion of waste with a high ammonium content to be established.

Materials and Methods

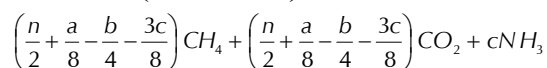
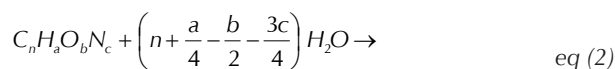
Substrate and Inoculum The substrate used in the experiment was CLHE collected from a poultry farm in the municipality of Lebrija-Santander, Colombia. The CLHE was kept refrigerated at a low temperature (4 °C) during the characterization and analysis stage. Bovine slurry from a slaughterhouse in Santander was used as the inoculum.

Experimental Design The biomethane potential (BMP) of the CLHE was established by experiment according to the protocol established by Angelidaki *et al.* (2009). The five organic loads were measured: 16.6 (ISR 1.0), 11.0 (ISR 1.5), 8.3 (ISR 2.0), 6.6 (ISR 2.5) and 5.5 (ISR 3.0) g VS/L, in biodigesters of 60 ml, with a fixed amount of inoculum of 0.58 g VS. To ensure anaerobic conditions, the biodigesters were purged with nitrogen for 2 minutes and sealed with butyl and aluminum stops. The biodegradation trials were carried out three times for 30 days in mesophilic conditions at a temperature of (39 °C±2) in an incubator (BD-53+ RS422 WTB BINDER).

Procedures and Methods of Analysis The volatile fatty acids (VFAs), total alkalinity (TA), pH, NH_4^+ and volume of accumulated methane were considered to be the response variables of the process. The VFAs were quantified by titration with NaOH (0.1 N) and the TA by titration with HCl (0.1 N) (Anderson and Yang, 1992). The pH was established using a pH meter (Metrohm 691) and the NH_4^+ according to the 4500-NH3D protocol from the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). The biogas produced during the experiment time bubbled in an alkaline solution (NaOH) with phenolphthalein as an indicator and pH 12, in which CO_2 is absorbed. The volume of displaced alkaline solution is equivalent to the volume of methane generated by the anaerobic system (Angelidaki et al. 2009). The methane was quantified in normal temperature and pressure conditions through the equation (1):

$$V_{NTP} = \frac{(P_0 - P)T_0}{P_{atm} \cdot T} \quad \text{eq (1)}$$

Where V_{NTP} is the volume of gas in normal conditions (ml), V is the volume of gas generated during the biomethanation test, P_0 is the pressure of methane in normal conditions (1013.25 mbar), T is the environment temperature in which the measurement was taken, P is the vapor pressure (mbar) at temperature T , and P_{atm} is the atmospheric pressure of the place in which the test was conducted. The GMP of the CLHE is expressed in terms of normalized accumulated methane volume over volatile solids added from the substrate (VS-Ad). The theoretical biomethane potential (BMP_{th}) of the substrate was calculated through stoichiometry represented in the Buswell equation (2 and 3), which assumes the full conversion of all the organic matter into biogas (Buswell and Neave, 1930).



$$PBM_{th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right) 22.4}{12n + a + 16b + 14c} \left(\frac{m^3 - CH_4}{kg - VS}\right) \quad \text{eq (3)}$$

Where 22.4 is the volume of one mole of gas at standard temperature and pressure conditions. The experiment's results were analyzed with the *StatGraphics plus 5.1*, *StatPoint®* Inc. (Virginia, USA) software. Fisher's exact test was used to verify the statistical differences between the results.

Results

Physiochemical Characterization of the Substrate and Inoculum Table 1 presents the physiochemical characterization of the substrate and inoculum, which

was carried out according to the protocols of the *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005).

The pH of the substrate and the inoculum is within the operating range for the development of AD reported in the literature (Raposo et al. 2006). The organic matter of the substrate represented by the concentration of total solids, volatile solids and volatile fatty acids of the caged laying hen excreta permit its identification as an attractive source of carbon for AD.

Evolution of the Acidogenic Stage of Anaerobic Digestion of Caged Laying Hen Excreta The kinetics of VFA consumption during 30 days of biodegradation is presented for the ISRs of 1.0, 1.5, 2.0, 2.5 and 3.0 (Figure 2). It is observed that for the different inoculum to substrate ratios, the start of the process presents the same exponential trend, indicating the inoculum's affinity toward the substrate (Quintero et al. 2012). It has been demonstrated that VFA concentrations above 6,000 mg/L may cause inhibition of the anaerobic process (Castillo et al. 2007). In this study, the maximum concentration of VFAs was 5,400 mg/L (ISR 1); a value that is below the limit that would cause inhibition by acidification. From the sixth day, the VFA concentration decreased to below 450 mg/L, which demonstrates a high degree of reduction of organic matter and consequently, metabolic activity of the acetogenic microorganisms capable of reducing the VFAs, even acetate (Wang et al. 1999).

Stability of the Anaerobic Process The anaerobic processes achieve stability in VFA/TA ranges from 0.2-0.8 (Raposo et al. 2011). VFA/TA ranges above 0.8 indicate that there is no balance between the population of acidogenic and methanogenic microorganisms (Wan et al. 2011). Figure 2a shows the change in stability of the anaerobic digestion process of laying-hen excreta for different ISRs.

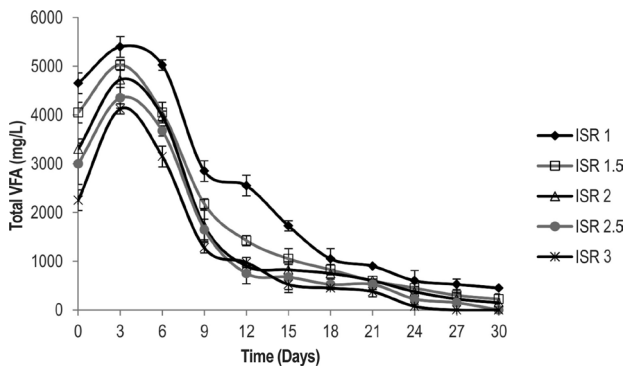
It was demonstrated that the anaerobic digestion process of laying hen excreta is promoted by organic loads below 16 g VS/l, equivalent to ISRs over 1.0. However, at the start of the process, instability was evident in the system for all the ratios studied with VFA/TA values over 0.8. This is due to the fact that the production speeds of acids are greater than the speeds of consumption of them. Therefore, the VFA/TA ratio is within the stability limits for the experiment with an ISR of 3. Due to the buffer capacity of the inoculum (0.23, see Table 1), the system achieves stability on the ninth day of biodegradation and the VFA/TA ratio maintains a range of 0.0 to 0.3, which is adequate to operate a process in stable conditions. These results coincide with the stability achieved (VFAs/TA = 0.4) for the digestion of bovine slurry and laying hen excreta diluted to 15% (Callaghan et al. 2002).

The pH evolution for the different ISRs is presented in Figure 2b. The pH for the measured ISRs oscillated

Table 1. Physiochemical Characterization of the Substrate and Inoculum

Parameter	Substrate	Inoculum
pH	6.3	8.5
Total solids (g/l)	226.2	29.2
Volatile solids (g/l)	107.7	20.5
Alkalinity (mg CaCO ₃)	13467	6023
Volatile fatty acids (mg/l)	15613	1370
Density (g/l)	1080	1003
C (%P/P)	28.0	ND
H (%P/P)	2.6	ND
O (%P/P)	18.4	ND
N (%P/P)	3.8	ND

ND: Not determined

**Figure 1.** Consumption kinetics of total fatty acids (VFAs)

within a range from 7.5 to 8.5, adequate values for the growth of methanogenic microbial consortia (Gerardi, 2003). During the first three days of the biodegradation process, the pH decreased as a result of increases in the concentration of VFAs. After the fourth day, the pH gradually increased until stabilizing. This behavior can be explained based on that reported by Lay *et al.* (1998), that the substrates with a high organic load, such as CLHE, tend to present rapid increases in the concentration of VFAs, causing strong drops in pH and destabilizing the reactor. However, progressive increases in ammonium concentration increase the pH, behaving like a base (Lay *et al.* 1998; Yenigun *et al.* 2013). Angelidaki *et al.* (2003) described similar

results for systems with different organic waste, specifying that for the case of CLHE, interactions may arise between the production of VFAs and pH, which lead to a state of balance.

Effect of Ammonium Concentration on the Anaerobic Digestion of Caged Laying Hen Excreta Figure 3 shows that the variation in the ammonium concentration for each one of the measured ratios has a similar trend. The ammonium concentrations are proportional to the added organic load. The maximum ammonium concentration (2,066 mg/L) is presented at the start of the process for the ISR of 1.0, which is equivalent to an organic load of laying hen excreta of 16.6 g VS/L. The process stabilizes at the ninth day of digestion, reaching ammonium concentrations that oscillate between 225 mg/L and 550 mg/L. Taking into account that ammonium concentrations above 2,000 mg/L inhibit the anaerobic system, it is observed that the anaerobic digestion of caged laying hen excreta does not present an inhibitory effect from ammonium ions, except for organic loads above 16.6 g VS/l (equivalent to an ISR of 1).

Anaerobic Biodegradability of Caged Laying Hen Excreta According to the element composition, the caged laying hen excreta used in this study (%p/p C:28; H:2.6; O:18.4; N:3.8) theoretically has a bi-methane potential (BMP) of 0.59 m³ CH₄/kg-VS. The specific methane production of the inoculum (bovine slurry) was 0.17 m³ CH₄/kg-VS. This value was subtracted from the respective BMP for each one of the trials (Figure 4). The maximum BMP was 0.58 m³ CH₄/

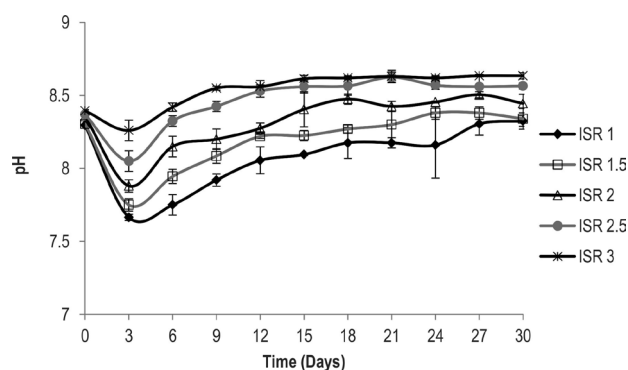
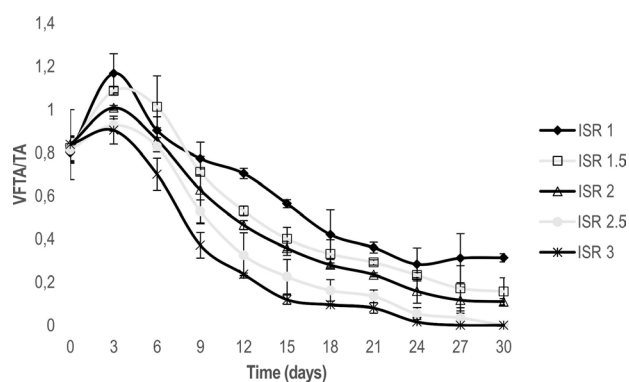


Figure 2. Stability of anaerobic digestion of caged laying hen excreta; a) VFA/TA ratio; and b) pH.

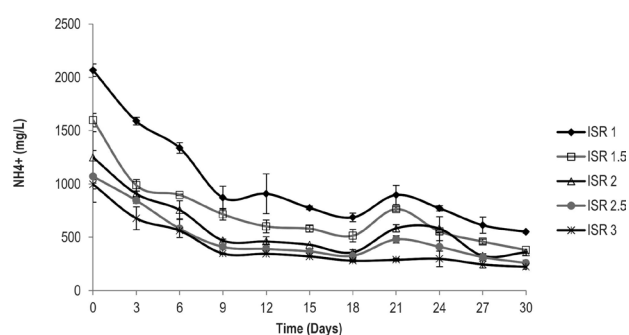


Figure 3. Variation of ammonium concentration in the anaerobic digestion of caged laying hen excreta.

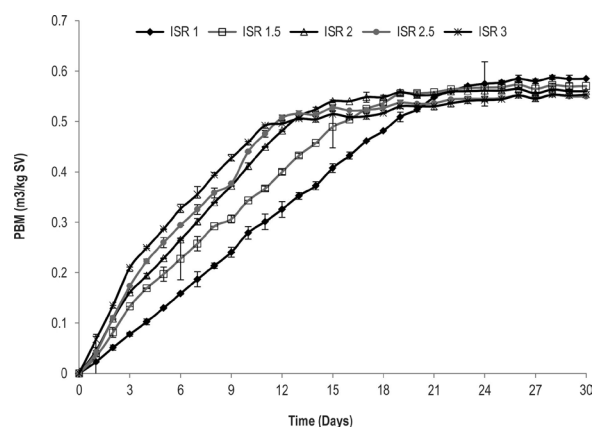


Figure 4. Specific methane production from caged laying hen excreta.

kg-VS for an ISR of 1.0, achieving 98% process efficiency. The lowest methane production was $0.55 \text{ m}^3 \text{ CH}_4/\text{kg VS}$ for the 2.5 and 3.0 ISR values, which correspond to added organic loads of 6.6 g VS/L and 5.5 g VS/L , respectively. The values obtained in this study are greater than those reported by Li *et al.* (2013), who achieved productions of $0.30 \text{ m}^3 \text{ CH}_4/\text{kg-VS}$ from an organic load of 3.0 g VS/L of caged laying hen excreta. The above indicates that the anaerobic digestion process of caged laying hen excreta presents high yields of methane production.

The comparison of means for the BMP of caged laying hen excreta at the measured ISR (statistical analysis) showed that there are significant differences between each of the experiments with a value of $p = 0.0001$ (Figure 5). Therefore, it may be confirmed that organic loads of less than 17 g VS/L do not inhibit the anaerobic process of caged laying hen excreta, demonstrating an inverse effect between the organic load of caged laying hen excreta (expressed according to the ISR) and the biomethane potential of this substrate.

Conclusions

The specific production of methane from caged laying hen excreta favors the operation of the process with organic loads below 17 g VS/L . Consequently, inoculum (bovine slurry) / substrate (caged laying hen excreta) ratios above 1.0 reduce the inhibition of anaerobic digestion caused by the accumulation of volatile fatty acids and ammonium ions, given that the maximum concentrations of these compounds are $5,400 \text{ mg/L}$ and $2,066 \text{ mg/L}$, respectively. As a result, the biomethane potential of caged laying hen excreta is $0.58 \text{ m}^3 \text{ CH}_4/\text{kg-VS}$, achieving a process efficiency of 98%.

Given that the anaerobic digestion process is inhibited at organic load concentrations above 17 g VS/L , it is recommendable to focus research with caged laying hen excreta on the co-digestion of this substrate with industrial waste rich in carbohydrates, lipids and proteins, for example sugar cane molasses, residual glycerol and whey.

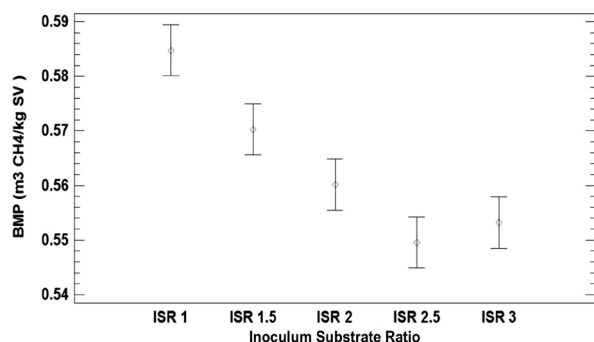


Figure 5. Analysis of biomethane potential means for caged laying hen excreta.

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