Optimization of anaerobic co-digestion of pasteurized slaughterhouse by-products incorporating residues from bioethanol industry to balance C/N ratios

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Abstract

Anaerobic digestion of pig slaughterhouse waste (SW) and corn sieving waste (CSW), and anaerobic co-digestion of CSW/SW were studied at lab scale employing several carbon to nitrogen ratio (C/N) and total solids (TS) content. Mixtures with highest biogas yield and suitable process stability values were scaled up to pilot scale. Results showed that SW and CSW co-digestion improved biogas yield over that obtained from mono-digestion of both substrates. Thus, CSW could be a proper substrate to balance C/N and improve biogas yield. Also, all studies reveal that the best biogas yield for each

C/N mixture was achieved for the lowest TS content. Moreover, SW/CSW mixture with C/N 15 and 5% TS achieved the highest biogas yield and the best process stability. Pilot scale assay demonstrates that biogas yield, methane yield and Organic Matter Removal (OMR) for C/N 15 mixture were 41%, 25%, and 24% higher than those using C/N 20, respectively. Methane content was similar for both C/N 15 and C/N 20 at pilot scale. However, other gasses composition (H_2, CO_2) presented variations.

Keywords: Anaerobic Digestion, Slaughterhouse Waste, Corn Sieving Waste, C/N ratio.

1. Introduction

Biogas energy is a renewable energy source obtained when organic matter decomposes producing a mixture of carbon dioxide and methane gases (biogas) [1]. Nowadays, there is an increasing interest in applying biomass energy supply, and it is predicted to include 15-50% of the world energy by 2050 [2]. Also, biofuels production and utilization has grown in the last years as a result of new renewable energy legislations. In this context, Argentina has promoted renewable energy production to diversify its national energy matrix by passing laws such as Act 26093/2006 (Regime of regulation and promotion for biofuels production and sustainable use) and Act 27191/2015 (National promotion regime for the use of renewable energy sources aimed at electric power production). For this reason, many bioethanol plants started to operate using mainly corn as a feedstock. In this context, bioethanol plants generate a solid waste from corn cleaning and sieving processes. This residue, here named Corn Sieving Waste (CSW), contains corn grain impurities that were set apart from the bioethanol process (earth, broken or damaged grains, small cobb pieces, etc.). However, CSW presents a high carbon content, mostly in the starch form.

Otherwise, pig slaughtering by-products are typical environmental liabilities of Argentina's center region. They are mostly converted into flour and then commercialized at marginal prices. These by-products, here called Slaughterhouse Waste (SW), can be used as a substrate for Anaerobic Digestion (AD) in order to produce biogas since they are an organic waste with high protein and lipid contents [3-7]. However, SW employed as raw material in biogas production presents several drawbacks, such as: slow hydrolysis rates, presence of foam, process inhibition on account of high ammonia and long chain fatty acid (LCFA) concentrations [8-10]. An interesting strategy to counteract the inhibition caused by ammonia is to optimize substrates carbon to nitrogen ratios (C/N) [11]. This can be done by anaerobically digesting diverse substrates with different C/N together at the same time, in a process known as anaerobic co-digestion (AcoD). The AcoD could be considered a potential process innovation in biogas production in order to increase biogas yield [12-14]. Several authors have probed that one of the most important factors in AcoD is to maintain the C/N ratio between 20 and 30 [15-17]. However, other studies state that the optimum C/N value is 15 [18]. Also, studies have shown that a disruption in this balance produces a negative effect on microbial activity, resulting in process depletion [19]. A well-organized microbial community that generates high quality biogas is related to a proper C/N balance [20]. Rodriguez-Abalde et al. [21] used a mixture of pig slurry, meat pasteurized byproducts and glycerin for AcoD, obtaining higher methane yields and higher process efficiency than with anaerobic digestion of pig slurry only.

The aim of this paper was to evaluate the AcoD of CSW, SW and pig slurry (PS) in order to balance C/N and, thus, to optimize biogas production. In the best of our knowledge, the use of CSW as a substrate or co-substrate for AD is not reported in the literature.

2. Materials and methods

2.1. Organic substrates and inoculum

Pig slurry was used as inoculum. It was obtained from a centralized pig facility located in Bell Ville, Córdoba, Argentina (Lat: S -32°40'12" Long: W 62°51'11"). Sampling was carried out according to American Public Health Association (APHA) [22] 1060 guidelines. PS was conserved, degasified, and characterized according to the methodology proposed by Angelidaki et al. [23] and Holliger et al. [24]. It was filtered to remove thick solids with a 5 mm pore-size mesh, and degasified in a 5L batch digester for 21 days at 37ºC±1 and 100 rpm.

SW is composed of a solid fraction (30% w/w) of previously minced pig stomach, viscera, kidneys, lungs and livers and a liquid fraction (70% w/w) of pig blood. Both fractions were collected from a pig meat process industry located in Justiniano Posse, Córdoba, Argentina (Lat: S -32°53'54" Long: W -62°40'37"W). SW was then pasteurized at 70ºC for 1 hour. CSW was collected form a bioethanol production plant located in Villa María, Córdoba, Argentina (Lat: S -32°41'54" Long: W 63°16'11"). Both SW and CSW were dried at 105ºC until TS exceeded 95%, ground and stored separately in zipper storage bags at room temperature.

2.2 Characterization of organic substrates and inoculum

Solid samples of SW were analyzed for humidity, Total Solids (TS) and Volatile Solids (VS) according to regulations 950.46, 950.46 and 923.153, respectively, issued by Association of Official Analytical Chemists (AOAC) [25]. Solid samples of CSW were analyzed for humidity, TS and VS according to AOAC 950.10, 950.10 and 923.03 respectively. Liquid samples were measured for TS, VS and Total Alkalinity (TA) according to APHA Standard Methods 2540 B, 2540 E and 2320 B, respectively. Volatile Fatty Acids (VFA) were measured according to Nordmann titration method. In addition, pH was measured by HANNA HI 8424 electronic pH meter. Chemical Oxygen Demand (COD), Total Ammonia Nitrogen (TAN) and Free Ammonia Nitrogen (FAN) were measured using HANNA spectrophotometer HI 83099 (Adaptation of USEPA 410.4 method for COD and Nessler method for TAN and FAN). Biological Oxygen Demand (BOD_5) was analyzed using VELP BOD EVO Sensor System 6. Lipids were measured according to AOAC 960.39, proteins were determined by multiplying the Total Kjeldahl Nitrogen (TKN) (APHA 4500 B) by a conversion factor of 6.25 [6]; and carbohydrates were calculated as the difference between organic matter (as VS), lipids and the estimated protein content [7]. Organic carbon was determined by considering an organic matter content to organic carbon ratio of 1.7241 [26].

2.3 Lab and pilot scale batch tests

Experimental set-up were done according to Deutsches Institut für Normung (DIN) International Organization for Standardization (ISO) 11734 method [27], Verein Deutscher Ingenieure (VDI) 4630 method [28], Angelidaki et al.[23] and Holliger et al. [24] in order to determine biogas and methane yields of co-digestions.

Several CSW/SW mixtures were made to carry out lab scale assays. Each mixture was diluted at three different TS concentrations: 5%, 10% and 15%. Table 2 shows characteristics of all mixtures. Each mixture was placed in 500 mL bottles with 100 mL of degasified PS which was used as active inoculum, and they were incubated at 37ºC into an orbital shaker at 100 rpm for 26 days. The assays were carried out by triplicate and with their corresponding blank (inoculum + deionized water). Biogas yield, organic matter removal (OMR) efficiency, and stability parameters (VFA, TA, TAN and FAN) were measured. Biogas was measured daily by water displacement, and then it was converted at Standard Temperature and Pressure (STP), considering the guidelines provided by Walker et al. [29] and Strömberg et al. [30]. Higher biogas yield mixtures with suitable stability parameter values were selected to be scaled up to pilot scale (5 L). Also, SW and CSW with 5% TS were digested separately (mono-digested) with the same criteria so that they can serve as references.

Scaled up assays were carried out in 5-L bioreactors including water-displacement gasometers, as shown in Figure 1. Each bioreactor has temperature control device, rotating mixer with velocity control, and sensors that measure temperature and gas volume. Bioreactors were set-up at 37ºC±1 and 100 rpm. Biogas was measured daily and analyzed periodically to measure methane content. Methane, carbon dioxide, hydrogen and hydrogen sulfur content in the biogas were analyzed using Gas Chromatographer (Fuli Instrument) equipped with Thermal Conductivity Detector (TCD) and GDX-502 column (4m x 3mm). Biogas yield, OMR efficiency and stability parameters (VFA, TA, TAN, FAN) were determined.

3. Results and discussion

3.1. Inoculum and substrate characterization

Table 1 shows characteristics and chemical composition of inoculum and substrates used to carry out co-digestions. PS, TS and VS values here obtained were lower than those reported by Henjfelt and Angelidaki [6] and Rodriguez-Abalde et al. [21], and similar to those reported by Bonmati et al. [31] and Moukasis et al. [32]. Concentration differences could be due to different methods in farm work [33]. Most of regional farms surveyed do not have a proper waste management because they dilute PS. As it can be seen in Table 1, C/N was found between reported ranges (7.4-12.96) [34,35]. Also, it is possible to observe that inoculum presented proper characteristics in terms of nutrients, VFA and TA needed to face pH variations [24]. Moreover, SW composition was similar to those presented by Palatsi et al. [7] and lower than those reported by Hejnfelt and Angelidaki [6]. COD and BOD_5 values obtained were high, and BOD_5/COD ratio showed a low degradability. Finally, substrate characteristics used for co-digestion and inoculum presented a high protein and lipid content compared to other studies [36] and C/N value matched those of substrates used by Mouskasis et al. [32]. CSW presented a high carbon content, which is appropriate to balance C/N ratio. Also, CSW had high TS and VS content, which makes it suitable to combine with high humidity wastes for AcoD. These values cannot be compare due to it was not found in the literature ay about CSW characterization and its anaerobic digestion.

3.2. Analysis of lab scale assays

Table 2 shows co-digestions composition and biogas yields, organic matter removal values and stability parameters of all mixtures. It is possible to observe that TS of 5% achieved the highest biogas yields for each tested C/N mixtures. Biogas yields using 10% and 15% TS mixtures decreased 63.80% and 74.44%, respectively, compared with 5% TS mixture for C/N 10. In the case of C/N 15, biogas yields obtained employing 10% and 15% TS mixtures dropped 59.80% and 87.15%, respectively, compared to 5% TS mixture. C/N 20 mixtures with 10% and 15% TS presented a decrease in biogas yields of 72.86% and 86.65%, respectively, when it is compared to 5% TS mixture. Finally, biogas yields of C/N 30 mixtures with 10% and 15% TS decreased 22.57% and 60.15%, respectively, in comparison to 5% TS for the same C/N. Based on these results it is possible to conclude that biogas yield decrease as TS increase [6].

Also, Figure 2 shows biogas yield and OMR values for all experiences. When comparing the best biogas yields among all tested C/N, 15 C/N mixture reached the highest biogas yield. The best biogas yields for 10, 20 and 30 C/N presented a reduction of 41.87%, 30.01% and 82.39%, respectively, when compared to C/N 15 best biogas yield (Fig 2a). Similar studies differ in the optimum C/N ratio to produce biogas by means of AD or AcoD processes. Rodríguez-Abalde et al. [21] determined that 10.3

was the optimum C/N, while Riya et al. [19] fixed this value in 30. Zheng et al. [37] proposed a C/N between 26.41 and 27.5 as ideal values, but Sievers and Brune [18] stated an optimum C/N value around 15.

Figure 3 presents lab scale Final VFAs (a) and Final TAs (b) values. VFA values for all mixtures with 5% TS were found within the process stability range, except for C/N 30 which showed concentrations three times higher than those reported in the literature [24]. Moreover, it possible to observe as TS increased, VFA also increased (Fig 3a), and both yield and OMR decreased (Fig 2a and 2b). VFA increase and accumulation may indicate solids overloads.

TA value, was higher for mixtures with C/N 15 and 20 (Fig. 3b) and similar to those reported in the work by Rodríguez-Abalde et al. [21]. Moreover, FAN and TAN values for C/N 15 and 20 were within the stability range, while those for the other C/N mixtures were not. However, some authors established FAN and TAN limits higher than those obtained in this work [38,39].

When comparing the highest biogas yield obtained of all co-digested mixtures (C/N 15 and 5% TS) with biogas yields of SW and CSW monodigestion (5% TS), it is possible to conclude that co-digestion improved biogas production, as it can be seen in Figure 4. Biogas yield of SW/CSW co-digestion with 15 C/N and 5% TS was 3.47 and 1.85 times higher than SW (5% TS) and CSW (5% TS) mono-digestions, respectively.

Based on the results (best yields, stability parameter values and OMR efficiencies) both mixtures C/N 15 and 20 with 5% TS were chosen to scale up to 5 L.

3.3 Analysis of pilot scale assays

Figures 5a and 5b show daily biogas and methane production for C/N 15 and 20 mixtures at pilot scale, respectively. Figures 5c and 5d show cumulative biogas and methane production for C/N 15 and 20 mixtures at pilot scale, respectively. C/N 20 had a longer lag phase than C/N 15 for both biogas and methane production. However, lag phase value was not higher than that reported by Palatsi et al. [7]. Table 3 shows final values of stability parameters, biogas yields and OMRs for both pilot scale assays. Biogas and methane yields and OMR for C/N 15 were 41%, 21% and 24% higher than C/N 20, respectively. Final FAN and TAN values of C/N 20 were increased to 29% when compared to those for C/N 15. Final VFA of C/N 20 was 1.87 times higher than that of C/N 15, while Final TA of C/N 15 was 1.25 higher than that of C/N 20. This behavior could indicate that high carbon to nitrogen ratios may lead to an overproduction of VFA, which could cause AD inhibition due to a pH decrease if the inoculum does not present high TA values.

Figures 5e and 5f indicate daily biogas composition for each batch. C/N 15 presented two biogas production spikes on day 5 and 15, while C/N 20 showed one biogas spike on day 18. Both batches presented similar methane and carbon dioxide production kinetics, maintaining methane concentration in a range between 50-65% until the assay was finished. However in C/N 20 carbon dioxide composition was higher at the beginning than for C/N 15. This higher carbon dioxide composition could be probably due to a feeding overload which could result from initial VFA accumulation. Also, at the beginning of the experiment, H_2 composition was higher for C/N 15 than for C/N 20, while the opposite situation was presented at the end of the experiment. Ward et al. [40] establish that an increased H_2 concentration may indicate digester overload.

4. Conclusion

Anaerobic digestion of pig meat byproducts presents several drawbacks when they are used as a mono-substrate. Different AD and AcoD assays carried out in this work showed that CSW could be a proper substrate to co-digest with SW in order to balance C/N and improve biogas yield. Lab scale assays showed higher biogas yields when SW and CSW are digested together at low TS concentration due to a gradual C/N adjusting. Furthermore, pilot scale assays of the bests mixtures tested at lab scale revealed that C/N 15 mixture presented the highest biogas and methane yields. AcoD synergy needs to be further studied at pilot scale to provide new data, and thus, to improve biogas quality and AD stability.

References:

[1] Khoshgoftar Manesh M.H., Rezazadeh A., Kabiri S. 2020. A feasibility study on the potential, economic and environmental advantages of biogas production from poultry manure in Iran. Renew. Energy. 159, 87-106.

[2] Kazem H.A. 2011. Renewable energy in Oman: Status and future prospects. Renew. and Sustain Energy Rev. 15, 3465-3469.

[3] Pereira M. A., Pires O.C., Mota M., Alves M.M. 2002. Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitic acid as a key intermediate. Water Sci. Technol. 45, 139-144.

[4] Suzuki Y., Tsujimoto Y., Matsui H., Watanabe K. 2006. Decomposition of extremely hard-to-degrade animal protein by thermophilic bacteria. Biosci. and Bioeng. 102, 77-81.

[5] Vavilin V., Fernández B., Palatsi J., Flotats X., 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material. Waste Manag. 28, 939-951.

[6] Hejnfelt A., Angelidaki I. 2009. Anaerobic digestion of slaughterhouse by-products. Biomass & Bioenergy. 33, 1046-1054.

[7] Palatsi J., Vinas M., Fernández B., Flotats X. 2011. Anaerobic digestión of slaughterhouse waste: main process limitations and microbial community interactions. Bioresur. Technol. 102, 2219-2227.

[8] Chen Y., Cheng J.J., Creamer K.S. 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 99, 4044-4064.

[9] Rajagopal R., Massé D.I., Singh G. 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632-641.

[10] Rasit N., Idris A., Harun R., Ghani W. 2015. Effect of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: An overview. Renew. and Sustain. Energy Rev. 45, 351-358.

[11] Wang X., Lu X., Li F., Yang G. 2014. Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. PLOS ONE. 9, 5.

[12] Budzianowski W.M. 2016. A review of potential innovations for production, conditioning and utilization of biogas with multiple-criteria assessment. Renew. and Sustain. Energy Rev. 54, 1148-1171.

[13] Cuetos M.J., Fernández C., Gómez X., Morán A. 2011. Anaerobic co-digestion of swine manure with energy crop residues. Biotechnol. Bioprocess Eng. 16, 1044-1052.

[14] Labatut R.A., Angenent L.T., Scott N.R. 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol. 102, 2255-2264.

[15] Zhang C., Xiao G., Peng L., Su H., Tan T. 2013. The anaerobic co-digestion of food waste and cattle manure. Bioresour. Technol. 129, 170-176.

[16] Wang X., Yang G., Feng Y., Ren G., Han X. 2013. Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour. Technol. 120, 78-83.

[17] Zhu J., Zheng Y., Xu F., Li Y. 2014. Solid-state anaerobic co-digestion of hay and soybean processing waste for biogas production. Bioresour. Technol. 154, 240-247.

[18] Sievers D.M., Brune D.E. 1978. Carbon/nitrogen ratio and anaerobic digestion of swine waste. Transactions of the ASAE., 21, 537-541.

[19] Riya S., Suzuki K., Terada A., Hosomi M., Zhou S. 2016. Influence of C/N ratio on performance and microbial community structure of dry-thermophilic anaerobic codigestion of swine manure and rice straw. Med. Bioengin. 5, 11-14.

[20] Xu R., Zhang K., Liu P., Khan A., Xiong J., Tian F., Li X. 2018. A critical review on the interaction of substrate nutrient balance and function in anaerobic co-digestion. Bioresour. Technol., 247, 1119-1127.

[21] Rodríguez-Abalde A., Flotats X., Fernández B. 2017. Optimization of the anaerobic co-digestion of pasteurized slaughterhouse waste, pig slurry and glycerine. Waste Manag. 61, 521-528.

[22] American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA. 1998.

[23] Angelidaki I., Alves M., Bolzonella D., Bprzacconi L., Campos J. L., Guwy A. J., Kalyuzhnyi S., Jenicek P., Van Lier J. B. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. and Technol., 59, 927-934.

[24] Holliger C., Alves M., Andrade D., Angelidaki I., Astals S., Baier U., Bougrier C., Buffiere P., Carballa M., Wilde V., Ebertseder F., Fernández B., Ficara E., Fotidis I., Frigon J., Fruteau de Laclos H., Ghasimi D. S. M., Hack G., Hartel M., Heerenklage J., Horvarth I. S., Jenicek P., Koch K., Krautwald J., Lizasoain J., Liu J., Mosberger L., Nistor M., Oechsner H., Oliveira J. V., Peterson M., Pauss A., Pommier S., Porqueddu I., Raposo F., Ribeiro T., Pfund F. R., Stromberg S., Torrijos M., van Eekert M., van Lier J., Wedwitschka H., Wierinck I. 2016. Towards a standarization of biomethane potential tests. Water Sci. and Technol. 74, 2515-2522.

[25] Association of Official Analytical Chemists. Official Methods of Analysis of AOAC INTERNATIONAL, Three-volume set, 21st Edition 2019.

[26] Cuetos M. J., Gómez X., Otero M., Morán A. 2008. Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). Bioch. Eng. 40, 99-106.

[27] DIN EN ISO 11734. 1998. Water quality – Evaluation of the 'ultimate' anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production. Deutsches Istitut für Normung.

[28] VDI 4630. Fermentation of organic materials – Characterization of the substrate, sampling, collection of material data, fermentation tests. 2016. VDI-Handbuch Energietechnik.

[29] Walker M., Zhang Y.; Heaven S., Banks C. 2009. Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. Bioresour. Technol. 100, 6339-6346.

[30] Strömberg S., Nistor M., Liu J. 2014. Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests. Waste Manag. 34, 1939-1948.

[31] Bonmatí A., Campos E., Flotats X. 2003. Concentration of pig slurry by evaporation: anaerobic digestion as the key process. Water Sci. and Technol. 48(4): 189-194.

[32] Moukasis F., Pellera Gidarakos E. 2018. Slaughterhouse by products treatment using anaerobic digestion. Waste Manag. 71, 652-662.

[33] Flotats X., Bonmatí A., Fernández B. Magrí, A. 2009. Manure treatment technologies: on-farm versus centralized strategies. NE Spain as case study. Bioresour. Technol. 100, 5519-5526.

[34] Ren J., Yuan X., Li J., Ma X., Zhao Y., Zhu W., Wang X., Cui, Z. 2014. Performance and microbial community dynamics in a two-phase anaerobic co-digestion system using cassava dregs and pig manure. Bioresour. Technol. 155, 342-351.

[35] Zhou S., Nikolausz M., Zhang J., Riya S., Terada A., Hosomi M. 2016. Variation of the microbial community in thermophilic anaerobic digestion of pig manure mixed with different ratios of rice straw. J. Biosci. Bioeng. 122, 334-340.

[36] Del Pozo R., Okutman Tas D., Dulkadirog lu H., Orchon D., Diez V. 2003. Biodegradability of slaughterhouse wastewater with high blood content under anaerobic and aerobic conditions. Chem. Technol. Biotechnol. 78, 384-391.

[37] Zheng Z., Liu J., Yuan X., Wang X., Zhu W., Yang F., Cui Z. 2015. Effect of dairy manure to switchgrass co-digestion ratio on methane production and the bacterial community in batch anaerobic digestion. Appl. Energy. 151, 249-257.

[38] Siegrist H., Hunziker W., Hofer H. 2005. Anaerobic digestion of slaughterhouse waste with UF-membrane separation and recycling of permeate after free ammonia stripping. Water Sci. Technol. 52, 531-536.

[39] Ortner M., Leitzinger K., Skupien S., Bochmann G., Fuchs W. 2014. Efficient anaerobic mono-digestion of N-rich slaughterhouse waste: Influence of ammonia, temperature and trace elements. Bioresour. Technol. 174, 222–232.

[40] Ward A., Hobbs P., Holliman P., Jones D. 2008. Optimization of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928-7940.