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Preliminary analysis from slaughterhouse waste: energy potential of biohydrogen and biomethane in Tenerife

J. A. Perez-Remedios[1], K. E. Rodríguez[1], L.A. González[1] and D. J. Escalante[1]

[1] Chemical Engineering Department
University of La Laguna
Av. Astrofísico Fco. Sánchez s/n, La Laguna, Tenerife, Canary Island, 38200 (Spain)
Phone/Fax number:+0034 922318060, e-mail: jperezre@ull.edu.es, krodrige@ull.edu.es, lagonmen@ull.edu.es, descalan@ull.edu.es)

Abstract. Slaughterhouse waste generated in Tenerife is currently disposed of in landfills, highlighting the need for a sustainable alternative. This study explores anaerobic digestion as a viable solution, assessing hydrogen and methane production through one- and two-step processes. Four experimental conditions were tested, including co-digestion with glycerol and variations in acid hydrolysis time. Key performance indicators such as alkalinity, volatile fatty acids (VFA), and solid content were analyzed. The two-step process achieved the highest cumulative biogas yield, significantly enhancing substrate digestibility due to acid hydrolysis. Energy potential ranged from 1.87 to 27.82 J/g VS, with the best performance observed in the two-step system. The estimated annual energy production from slaughterhouse waste could reach 12,883 kWh annually. These findings underscore the potential of anaerobic digestion as a method for sustainable waste management, aligning with circular economy principles and contributing to energy self-sufficiency in the Canary Islands through a reliable renewable energy source.

Key words. Biohydrogen, anaerobic digestion, slaughterhouse waste, biomethane.

1. Introduction

The European Commission is striving to produce biofuels in pursuit of energy sovereignty, aiming to reduce its dependence on natural gas imports from countries like Russia. Biofuel production is possible from any type of waste. Moreover, an outermost region such as the Canary Islands is crucial for producing energy and biofuels and for avoiding fossil fuel imports.

In the Canary Islands, in accordance with European Regulations (EC) No 1069/2009 and No 142/2011, certain territories can benefit from specific exceptions regarding the treatment of animal by-products not intended for human consumption. Since 2012, the designation of the Canary Islands as a Remote Zone has been established and periodically extended, allowing the disposal of these animal by-products in landfills. These animal by-products are classified into three categories based on their risk level, as they can serve as vectors for transmitting diseases both from animals to human and among animals. For this reason, they must be handled safely to prevent such transmission. At present, the by-products undergo grinding and treatment with quicklime before being disposed of in landfills.

Given the limited land availability, landfill disposal is not a sustainable solution for this type of waste. Anaerobic digestion presents a well-established alternative, requiring low energy input due to its operation at moderate temperatures. Recent studies have explored system modifications to improve the performance of specific feedstocks. In particular, biohydrogen production through bacterial processes has gained attention. The two-step anaerobic digestion process consists of an initial acid hydrolysis phase (pH 5.5–6.5), followed by a methane production phase under neutral pH conditions. Hydrogen is a clean, renewable fuel whose combustion yields only water. Furthermore, it can serve as a reagent in the conversion of CO_2 into methanol and other biofuels, supporting both waste valorization and sustainable energy generation.

Previous studies have reported the co-digestion of chicken manure and food waste achieving hydrogen yields between 15.5–57.5 $mL \cdot g^{-1}$ VS and methane volumes of 247.3 $mL \cdot g^{-1}$ VS [1]. Additionally, municipal solid waste achieves 2.55 % H_2 and 47.03 % CH_4 [2].

This work focuses on the production of biohydrogen and biomethane from slaughterhouse waste using both one-step and two-step processes, comparing performance variables by using waste generated in slaughterhouses in Tenerife.

2. Materials and methods

A. Substrates

Animal by-products were collected from two of the main slaughterhouses in Tenerife. The mixture was prepared based on real proportions used in previous studies. It consisted of viscera, blood, and slaughterhouse sludges from rabbits, pigs, cows, goats, and sheep. As analyzed in earlier research, the composition included 92.7% viscera, 2.6% blood, and 4.7% slaughterhouse sludge. In the reactor, 360 g of slaughterhouse waste and 2.7 L of distilled water were introduced.

B. *Experimental system*

The system, as shown in Figure 1, consists of a two-stage continuously stirred tank reactor (CSTR). In the first stage, the pH was reduced to 5.55 using 1 M HCl to maximize hydrolysis, generating a gas composed of carbon dioxide and hydrogen. After this stage, the pH was raised to 7 by adding 4 M NaOH to promote methane production. The operating temperature was 31.5 °C. Four experiments were conducted: E2 and E5 were carried out using a one-step process, while E3 and E4 used a two-step process. The E5 samples involved the co-digestion of slaughterhouse waste (32.04 % wt.) and glycerol (67.96 % wt.).

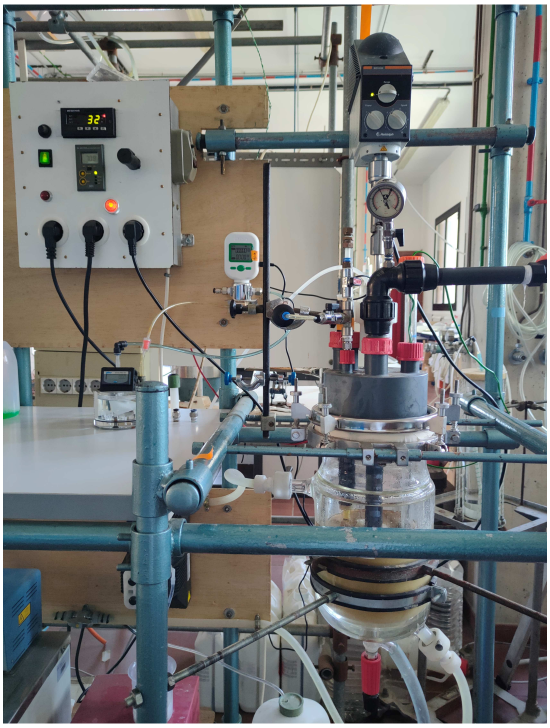

Fig. 1. Schematic representation of the anaerobic digestion system used in the experiments.

C. *Analysis*

The gas composition of each sample was analyzed using a gas chromatograph (Agilent 7820A GC) equipped with a thermal conductivity detector (TCD). The separation of compounds was performed using two parallel capillary columns: CP-Molsieve 5 Å (30 m, 0.53 mm ID, 0.53 µm film thickness) and CP-PoraBond Q (packed column), enabling the detection of CH_4, CO_2, CO, O_2, H_2 and N_2.
The analysis was conducted under the following operating conditions: the oven temperature was maintained isothermal at 40°C, the injector temperature was set to 175°C, and the TCD temperature was adjusted to 180°C. Helium was used as the carrier gas at a flow rate of 36 mL min^{-1} and a pressure of 9 psi. A gas sample volume of 1 mL was injected for each analysis. Besides, the solid analysis involved the determination of total solids (TS) and volatile solids (VS) content, using Standard Method 2540 G. The elemental composition of carbon, nitrogen, hydrogen, and sulfur (CNHS) was analyzed using the CNHS FLASH EA 1112 equipment. Another parameter to evaluate anaerobic digestion is the total alkalinity (TA), partial alkalinity (PA), and intermediate alkalinity (IA), which were analyzed following 2320 Standard Methods. However, the volatile fatty acid (VFA) analysis was used in the method of [3].

D. *Energy potential*

The energy yield (EY) of the gas mixture of gases was calculated according to Eq. (1) as is described by [4].

$$\mathrm{EY} = \frac{12.8 \cdot \mathrm{HY} + 35.8\mathrm{MY}}{1000} \qquad (1)$$

where HY is the hydrogen gas yield (mL H_2/g of VS) and MY is the methane yield (mL CH_4/g of VS). The factors 12.8 and 35.8 represent the energy values of hydrogen and methane, respectively, expressed in kJ/L.

3. Results and discussion

A. *Quantification of slaughterhouse waste*

According to previous works [5], the average annual generation of slaughterhouse waste was approximately 5,000 t per year during the period 2017–2021, showing a continuous growth trend. However, during the 2022–2023 period, the amount increased to 5,919 t and 6,153 t per year, respectively. This represents a 13 % increase over two years from 2021 and an average annual generation of approximately 5,500 t of slaughterhouse waste.

B. *Experimental samples analysis*

For the experimental samples, several performance variables were measured, such as solid content before and after the batch digestion process, as shown in Table I.

Table I. – Solid compositions before and after the batch anaerobic digestion.

Sample	STi (%)	SVi (%)	STf (%)	SVf (%)
E2	31.61	91.93	9.64	76.16
E3	35.30	94.44	7.21	76.39
E4	35.30	94.44	7.85	75.00
E5	55.57	97.89	23.85	72.25

In addition to other parameters, alkalinity and volatile fatty acid (VFA) contents were measured, as these factors influence the performance of methanogenic bacteria—the stage at which methane is produced. The alkalinity ratio as shown in Table II, defined as intermediate to partial alkalinity (IA/PA), was higher than the recommended range of 0.3 to 0.4, indicating potential imbalances in the system. The two-step experiments and the co-digestion setup demonstrated better performance compared to the digestion of slaughterhouse waste alone in the one-step process.

Table II. – Total alkalinity (AT), partial alkalinity (AP) and intermediate alkalinity (AI) (mg $CaCO_3 \cdot L^{-1}$), and alkalinity ratio (AI/AP).

Sample	AP	AI	AT	AI/AP
E2	10,425	6,900	17,325	0.66
E3	8,425	4,900	13,325	0.58
E4	9,025	5,025	14,050	0.56
E5	9,375	4,725	14,100	0.50

Regarding volatile fatty acids, as shown in the results in Table III. The VFA composition in the second stage showed a reduction, due to the transformation from VFA to hydrogen. The VFA/AT is a ratio, which shows an imbalance for the it should be lower than 0.30 [6].

Table III. – Volatile fatty acid (VFA) concentrations and volatile fatty acid and total alkalinity (VFA/AT) ratio.

Sample	VFA	VFA/AT
E2	14,065	0.81
E3	11,527	0.87
E4	11,139	0.79
E5	9,729	0.69

C. Gas composition and production

The highest methane potential was achieved in the one-step process using slaughterhouse waste as the sole substrate. However, the two-step process or the one-step process with glycerol as a co-substrate also showed promising results. Glycerol is a substrate with high carbon content, which improves the C/N ratio, and its high biodegradability makes it easily assimilable by bacteria and yeasts under anaerobic conditions [7].

Table IV. – Specific gas production and maximum hydrogen and methane concentrations

Sample	Gas prod. (mL/g of VS)	H_2 (%)	CH_4 (%)
E2	17.15	0.00	1.58
E3	56.55	0.18	1.31
E4	63.51	0.23	0.00
E5	53.03	2.23	0.00

Figure 2 shows the experimental data, highlighting the variation in biogas generation over time for different experimental samples, with the specific cumulative gas potential presented in Table IV. The figure effectively illustrates the dynamic changes in gas production rates throughout the experiment, with E4 achieving the highest cumulative production and E2 the lowest. The figure also reveals that E4 and E3 exhibited the fastest kinetics, reaching higher cumulative biogas production in a shorter period compared to the other treatments. These findings are characteristic of the two-step process, due to the enhanced hydrolysis step, which makes the substrate more digested and more easily accessible to the bacteria [8].

The E5 experiment involved the co-digestion of slaughterhouse waste with glycerol in one-stage. Glycerol, a by-product of biodiesel production, is rich in carbon and highly biodegradable, which enhances microbial activity under anaerobic conditions. In this setup, E5 yielded the highest hydrogen concentration among all experiments, reaching 2.23 %, while methane was not detected. The addition of glycerol significantly altered the fermentation pathway, favoring methanogenic processes. This suggests that co-digestion with glycerol not only boosts overall gas generation but can also shift the microbial metabolism toward specific bioenergy outputs. Although the cumulative gas volume in E5 was slightly lower than in E4, the quality of the gas produced, enriched in hydrogen, highlights the potential of glycerol as a strategic co-substrate for optimizing hydrogen yields in anaerobic digestion systems. These results underline the importance of substrate selection in steering the process toward desired energy vectors, offering a flexible approach to waste valorization depending on energy needs and end-use applications.

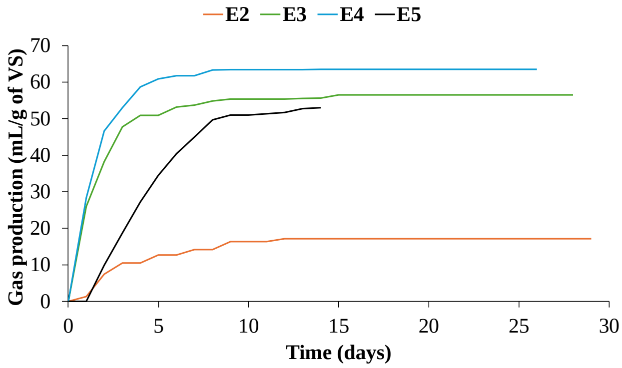

Fig. 2. Cumulative gas production over time.

D. Energy production

For each sample, the energy potential ranged from 1.87 to 27.82 J/g of VS, with the highest energy yield achieved in the two-step anaerobic digestion process, where the acid hydrolysis time was the shortest (27.82 J/g of VS). Using this maximum potential to estimate the annual energy production and considering an average slaughterhouse waste generation rate of 5,500 t per year, the process could produce an energy output of approximately 12,883 kWh annually.

4. Conclusion

In two-step anaerobic digestion, the hydrolysis step plays a fundamental role in enhancing the energy potential of the resulting gases, particularly by increasing the production of hydrogen. This stage breaks down complex organic matter, making it more accessible to microbial activity and leading to greater conversion efficiency. The increased hydrogen yield is especially significant, as hydrogen is not only a clean and renewable energy carrier—whose combustion produces only water—but also a versatile compound that can be used as a reagent in the transformation of CO_2 into value-added products such as methanol and synthetic fuels. This aligns strongly with the principles of the circular economy and supports the Canary Islands' strategic objective of achieving energy sovereignty through renewable and locally sourced energy alternatives. However, it is important to note that the improvement in hydrogen production was accompanied by a slight reduction in methane concentration, decreasing from 1.58 % to 1.31 %. This trade-off highlights the need to carefully balance the operational conditions of the hydrolysis step. Future research should focus on optimizing variables such as pH, retention time, and temperature to maximize hydrogen generation without compromising methane output, thereby improving the overall energy efficiency and scalability of the process for real-world applications.

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