Agrociencia

# BIOGAS PRODUCTION BY ANAEROBIC CO-DIGESTION OF AGRO-INDUSTRIAL WASTE AND CRUDE GLYCEROL

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## ABSTRACT

The use of fossil fuels continues to represent a significant percentage of energy production in Mexico; this, together with the generation of considerable amounts and poor disposal of organic waste, has contributed greatly to the emission of greenhouse gases, exacerbating the problem of environmental pollution. In Mexico City's central supply center alone, more than 500 Mg of organic waste is produced daily, and its disposal is not the most appropriate. An alternative for the correct disposal of this waste is the anaerobic digestion process, which, in addition to being an environmentally friendly technology, offers benefits such as the possibility of obtaining value-added products such as biogas, which represents a viable alternative to the use of fossil fuels. In this study, waste generated in Mexico City's central supply center was collected for use in anaerobic co-digestion to produce biogas using crude glycerol obtained as a byproduct of the biodiesel plant located in the same center. Throughout the bioprocess lasting 12 weeks, with a hydraulic retention time of 33.3 ± 0.04 days and an organic matter removal efficiency of 77.6 %, it was possible to obtain perfectly flammable biogas with a methane composition between 60 and 70 %, with an average production of 0.576 ± 0.084 $m^3$ $d^{-1}$.

**Key words:** biodigestion, biomass, organic waste, gaseous biofuel.

**Citation:** Muñoz-Martínez S, Salmerón-Alcocer AM, Rodríguez-Casasola FN, Ahuatzi-Chacón D. 2025. Biogas production by anaerobic co-digestion of agro-industrial waste and crude glycerol.
**Agrociencia.** https://doi.org/ 10.47163/agrociencia.v59i4.3329

**Editor in Chief:**
Dr. Fernando C. Gómez Merino

Received: October 18, 2024.
Approved: May 07, 2025.
**Published in Agrociencia:**
May 12, 2025.

## INTRODUCTION

Agriculture is a very important economic sector in Mexico, with a share in gross domestic product (GDP) of 2.5 %; in addition, Mexico ranks first in vegetable production in Latin America and second in fruit cultivation (Statista Research Department, 2023). In 2023, the flow of foreign direct investment exceeded USD 109 million, and agri-food exports exceeded USD 50 billion for the first time. Avocado exports generated the highest income for the national economy, with a value of USD 3.153 million in 2023. As for vegetables, tomato exports generated the most revenue, with a value of USD 2.67 billion (Statista Research Department, 2023). This abundance of agricultural raw materials leads to a great potential for agroindustrial development (Martínez-Prats *et al*., 2022).

Agricultural activities (sowing and harvesting) represent 5 to 18 % of Latin America's GDP; however, when agro-industrial activities (transformation of farm products) are considered, the percentage increases to 21 %, which shows the importance of agro-industry (Sarandón, 2020). In Mexico, fresh products such as fruits, vegetables, leaves, and pods are processed, as well as other already transformed products such as flours, oils, juices, wines, jams, and concentrates, which generates a large amount of waste throughout the entire process, from harvesting, concentration, distribution, industrialization, marketing, and consumption (Mejías-Brizuela *et al*., 2016).

According to Mejías-Brizuela *et al*. (2016), who consider the definition given by the General Law for the Prevention and Integral Management of Waste in Mexico, "Agro-industrial waste are solid, semi-solid, and liquid organic products generated from the direct use of primary products or their industrialization, not useful for the process that generated them, but susceptible to a use or transformation that generates another product with economic value, of commercial and/or social interest."

In 2020, Mexico's Ministry of the Environment and Natural Resources published the Basic Diagnosis for Integrated Waste Management, which addresses the state of the country's waste generation and management. Composting or biodigestion can treat an estimated 56 427 Mg of organic waste produced per day; however, the number of waste treatment or utilization plants is very limited, as there are only 47 plants distributed in 15 states, which separate, grind, and compact. Of the 47 plants, 19 perform composting and only five perform biodigestion (SEMARNAT, 2020).

This problem is also present worldwide, where around 500 million Mg of agro-industrial waste is generated each year (Singh *et al*., 2021). Its poor disposal has resulted in a significant contribution to environmental pollution, causing negative impacts on the health of animals and humans (Herrera-Uchalin *et al*., 2023). Hence the importance of adding value to agro-industrial wastes through so-called clean technologies, which help reduce their environmental impact and follow the current trend of the circular economy (Matiacevich *et al*., 2023).

The valorization of agro-industrial wastes has two aspects: biotechnological and energetic utilization. Biotechnological valorization is done by direct extraction or by chemical or microbial transformation to higher value-added commercial products such as pigments, polymers, antioxidants, polyphenols, antibiotics, and enzymes (Singh *et al*., 2021). Energy valorization includes the chemical or microbiological transformation of agro-industrial by-products and residues containing significant amounts of biomass rich in cellulose, hemicellulose, lignin, glycosides, and fatty acids into bioenergy such as bioethanol, biodiesel, and biogas (Mejías-Brizuela *et al*., 2016). *Saccharomyces* yeasts ferment saccharides contained in sugarcane or corn and produce bioethanol (Pérez-Contreras *et al*., 2023). Biodiesel is produced through the transesterification reaction of fatty acids found in animal or plant oils (Singh *et al*., 2021).

In the case of biogas, specialized microorganisms degrade, under anaerobic conditions, the organic matter and produce the gaseous fuel, whose main component is methane, a high-quality energy source. Consequently, it can be used to generate electricity or steam in high-efficiency cogeneration systems (Jameel *et al*., 2024). The anaerobic digestion process can be improved by feeding more than one organic waste. Co-digestion is a process in which simultaneous anaerobic digestion of two or more wastes is carried out, improving the C/N ratio and increasing biogas production (Prasertsan *et al*., 2021). Several co-substrates have been used for biogas production, including sludge from wastewater treatment plants, municipal solid waste, cheese whey, grass (González *et al*., 2022), rice and banana husks (Olugbemide *et al*., 2023), and glycerol, among others (de Mello *et al*., 2024).

Biodiesel production generates crude glycerol as a byproduct; it is estimated that 10 kg of glycerol are obtained for every 100 kg of biodiesel produced (Chilakamarry *et al*., 2021). The purification process of this by-product is not economically viable, so its use in the food, pharmaceutical, and cosmetic industries is not recommended. Due to this, alternatives for its disposal have been sought, one of them being its use as a cosubstrate for biogas production, since its high carbon content increases the C/N ratio, avoiding inhibition by nitrogen and increasing biogas production from 50 to 200 % (Alves *et al*., 2020; Butkowska *et al*., 2022).

The objective of this work was to implement a bioprocess at the pilot plant level of anaerobic co-digestion, using agro-industrial waste and glycerol for biogas production, as a feasible solution to the serious pollution problem caused by the large amount of waste generated at Mexico City's Central de Abastos (CEDA) and, at the same time, the valorization of glycerol, a by-product of the biodiesel production plant of the same supply central.

## MATERIALS AND METHODS

### Biodigestor

The anaerobic digestion process was carried out in a 300 L pilot plant level biodigester with a programmable logic controller (PLC) (Figure 1).

The stainless steel biodigester with jacket-type exchanger was built at the Clean Technologies Laboratory, located in the Environmental Systems Engineering Department of the National School of Biological Sciences of the National Polytechnic Institute in Mexico City. The PLC controller receives information from connected sensors and input devices, processes the data, and issues outputs based on pre-programmed parameters (López *et al*., 2016). It has a home screen with a general menu where the user can monitor and record operating temperature information, as well as start and stop the heat exchanger operation automatically for temperature regulation. It also has programming for agitation control and dosing of the feed medium and acids or alkalis for pH control. The residues for anaerobic digestion were taken from fruit and vegetable waste from CEDA. Glycerol was provided by CEDA's biodiesel plant.

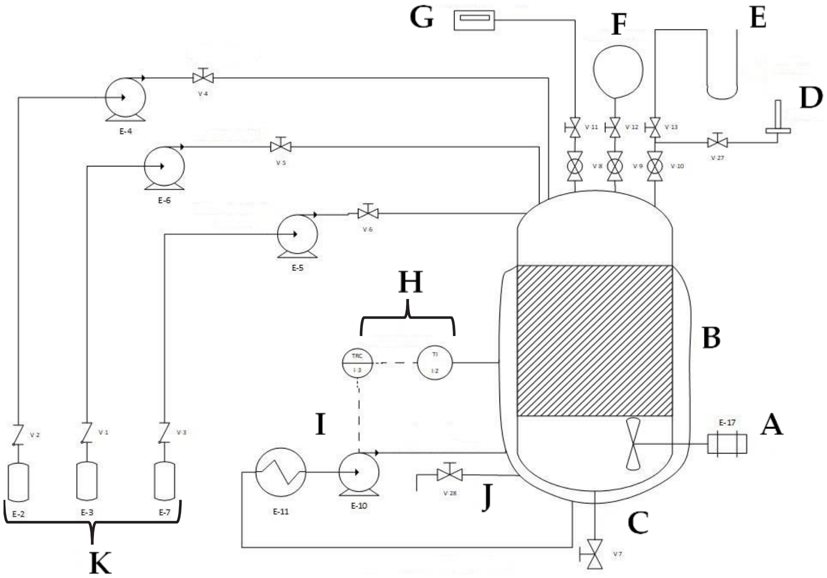

**Figure 1**. Diagram of the bioreactor used for biogas production. A: blade agitator; B: bioreactor body; C: drain; D: burner; E: differential pressure gauge; F: gasometer; G: gas detector; H: temperature control; I: heat exchanger with hot water recirculation; J: sampling; K: feed tanks.

## Analytical methods

The analytical methods were performed according to the official Mexican standard corresponding to each determination: moisture, NMX-F-083-1986 (DOF, 1986); ash, NMX-F-066-S-1978 (DOF, 1978a); fat, NMX-F-615-NORMEX-2018 (DOF, 2018); total carbohydrates, NMX-F-312-1978 (DOF, 1978b); proteins, NMX-F-068-S-1980 (DOF, 1980); pH, NMX-F-317-S-1978 (DOF, 1978c); and total solids and volatile solids, NMX-AA-034-SCFI-2015 (DOF, 2015). Determinations of total nitrogen, total phosphorus, total organic carbon, and chemical oxygen demand were performed according to Hach (2024).

## Volumetric consumption rate and chemical oxygen demand (COD) removal efficiency

The volumetric rate of COD consumption (VVC) was calculated through time ($t$) with the following relationship:

$$VVC = \frac{[COD]fed - [COD]soluble}{\Delta t}$$

The COD removal efficiency (η) was calculated with the following relationship:

$$\eta = \frac{[COD]fed - [COD]soluble}{[COD]fed} \times 100$$

### Hydraulic retention time

The hydraulic retention time (HRT) was calculated with the ratio:

$$HRT = \frac{Operation\ volume}{Feeding\ load}$$

### Biogas production measurement

Gas was purged from inside the biodigester through the tubing used for biogas measurement until a decrease in pressure was observed in both the gasometer and the U-shaped differential pressure gauge. After emptying the gasometer, the time it took to fill up to 2 L was registered. The pressure difference in the differential pressure gauge was calculated with the following expression (Tippens, 2011):

$$Differential\ pressure = \rho \times g \times h$$

where $g$ is the acceleration of gravity, $h$ is the height difference between the two levels of the "U" pipe, and $\rho$ is the density of the glycerin contained in the tubing.

With pressure, the number of moles was calculated using the ideal gas equation (Tippens, 2011):

$$PV = nRT$$

where $P$ is the pressure of the gas, $V$ is the volume, $n$ is the number of moles, $R$ is the ideal gas constant, and $T$ represents the temperature, with the values of $T = 311.15$ °K and $R = 8.31\ m^3\ Pa\ mol^{-1}\ °K^{-1}$.

### Biogas composition measurement

Biogas is a mixture of different gases, mainly composed of methane and carbon dioxide, but it can also contain nitrogen, hydrogen sulfide, and traces of water vapor. Their total composition may depend on the type of material used as a substrate (Jameel *et al*., 2024). Biogas composition was determined using a NOVA Plus model MRU 947010US portable gas multidetector (MRU Instruments, TX, USA**).**

### Culture medium for biodigester start-up and conditioning stage

Before starting the biodigestion process with the organic waste, the aim was to adapt and strengthen the microbial consortia present in the inoculum and generate an adequate anaerobic environment inside the biodigester. For this purpose, a minimal mineral medium with glucose and glycerol as carbon sources (MMMC) was formulated using the following stoichiometric balances:

$$For\ glucose: C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

$$\textit{For glycerol}: C_3H_8O_3 + 3.5\ O_2 \rightarrow 3\ CO_2 + 4\ H_2O$$

The concentration of the components for the MMMC formulation was determined, considering a glucose concentration of 10 g $L^{-1}$ with 2.5 % glycerol. The percentage of glycerol was determined based on the maximum daily production of the CEDA biodiesel plant and considering that this production would work with 20 Mg of residue per day. Urea was used as a nitrogen source, considering a C/N ratio of 25:1, suitable for anaerobic digestion (Andlar *et al*., 2021), taking into account glucose and glycerol contained in the medium prepared for starting the bioreactor as a carbon source. Potassium phosphate was used as a phosphorus source, considering an N/P ratio of 6:1. Additionally, some nutrients were added at concentrations that stimulate methane production in anaerobic microorganisms (Jameel *et al*., 2024).

Taking all the above into account, the following composition of the culture medium was reached (in g $L^{-1}$): glucose 9.75, glycerol 0.22, urea 0.35, potassium phosphate 0.12, calcium 0.1, potassium 0.2, magnesium 0.075, iron 0.04, cobalt 0.004, molybdenum 0.0001, nickel 0.006, and one part of sodium bicarbonate was added for every 20 parts of glucose to regulate pH.

### Microorganisms

Inoculum for the process was collected from swine manure from a pig farm located in the State of Mexico. Swine manure contains a wide variety of microorganisms, including bacteria, fungi, yeasts, and actinomycetes that coexist in a complex symbiosis where they perform a specific and complementary function (Ruvalcaba-Gómez *et al*., 2019). When performing the physicochemical characterization of the inoculum, the following results were obtained: moisture 85 %, ash 0.9 %, total solids 0.14 g per gram of sample, volatile solids 0.11 g per gram of sample, COD 31.4 g of $O^2$ per liter, total phosphorus 2.97 g $L^{-1}$, and total nitrogen 3.2 g $L^{-1}$.

### Waste conditioning and characterization

Fruit and vegetable waste was collected from general containers at the CEDA. Three samples were taken in different months of the year and were physicochemically characterized; the percentages of moisture, ash, total solids, volatile solids, fats, proteins, and carbohydrates were determined for each sample. Likewise, COD, total phosphorus, total nitrogen, and total organic carbon were recorded (in g $L^{-1}$), as well as pH. Conditioning consisted of size reduction by liquefying waste using a professional Ninja blender (Mexico). The average solids content in each sample was 15 %, and 0.5 L of water was added to each kilogram of residue to obtain a fluid with a total solids content of 10 %.

### Inoculum conditioning

For the anaerobic digestion process, swine manure was used as inoculum. Manure has a large number of microorganisms that require acclimatization (Zhang *et al*., 2022). For this process, the biodigester was inoculated with 12 kg of pig manure (17 L in volume). Considering that solid excreta should enter the biodigester as a suspension in water with at least 3 % solids (Botero and Preston, 1987), 48 L of water and 15 L of MMMC were added, resulting in a proportion of 81 % inoculum and 19 % substrate in a total volume of 80 L.

Once the biodigester was inoculated, it was sealed, and anaerobic biodigestion began. To achieve a system as homogeneous as possible, the stirrer was activated, keeping it at a speed of 48 rpm to avoid the incorporation of air. The temperature was maintained in the mesophilic regime at 38 °C by means of the jacket-type heat exchanger, recirculating hot water. Samples of the digestate mixture were taken every 48 hours and analyzed for COD, total nitrogen, total phosphorus, total solids, and volatile solids. In addition, measurements of the biogas produced with the gas multidetector were performed.

Once COD levels decreased by 25 %, the first feeding was performed, adding 12 L of MMMC. Feedings were performed every 48 h until a greater than 80% decrease in COD was observed; at that time, the feeding load was increased to 15 L of MMMC every 48 h until the difference between soluble COD and fed COD remained constant.

### Biodigestion with agro-industrial waste

Biodigestion of agro-industrial wastes started when the soluble COD remained approximately constant during the inoculum conditioning process. At that time, the substrate was changed with a 10 L load of a mixture of 50 % MMMC and 50 % of previously conditioned organic residues. This feeding was maintained until a decrease in soluble COD and an increase in methane production were observed again, at which time a new substrate change was made to only organic waste with 2.5 % glycerol.

## RESULTS AND DISCUSSION

### Waste conditioning and characterization

The values obtained in the characterization did not have a large variation among the three samples collected at different times of the year, so the values obtained were averaged to take into account the variation of the parameters according to the type of fruits and vegetables available in different seasons (Table 1).

Conditioning organic waste is recommended to break down the compact structures of cellulose, hemicellulose, and lignin found in fruits and vegetables. These structures form a complex network that is difficult for microorganisms to degrade directly, increasing fermentation time and decreasing the efficiency and speed of biogas production (Zhang *et al*., 2022). Cellulose, hemicellulose, and lignin must be hydrolyzed to simpler subunits by extracellular enzymes. Hydrolysis of cellulose to glucose units is carried out by enzymes called cellulases. Lignin is degraded by a complex of enzymes, including laccases, lignin peroxidase, Mn peroxidases, and tyrosinases, which act synergistically (González-Rentería *et al*., 2011).

**Table 1.** Characterization of agro-industrial organic wastes generated in the central supply center (Central de Abasto) of Mexico City, Mexico, used in biogas production.

| Parameter | Value |
|---|---|
| Moisture (%) | 85.00 ± 5.91 |
| Ash (%) | 0.54 ± 0.23 |
| Total solids (%) | 15.00 ± 2.23 |
| Volatile solids (%) | 13.00 ± 1.98 |
| COD (g of $O_2$ $L^{-1}$) | 99.00 ± 8.77 |
| Total phosphorus (g $L^{-1}$) | 2.50 ± 3.76 |
| Total nitrogen (g $L^{-1}$) | 2.20 ± 1.15 |
| Total organic carbon (g $L^{-1}$) | 21.00 ± 1.56 |
| Fat (%) | 0.38 ± 0.09 |
| Proteins (%) | 1.00 ± 0.11 |
| Total carbohydrates (%) | 7.60 ± 3.40 |
| pH | 4.80 ± 0.20 |

Organic waste can be treated in a variety of ways, including physical, chemical, biological, and combined. By using physical methods, the cell wall structure of the raw materials can be destroyed, increasing the accessibility of enzymes and anaerobic bacteria to the organic matter, which facilitates its digestion. In the case of chemical methods, the use of acids or alkalis that break cellulose, hemicellulose, and lignin structures increases enzyme accessibility; however, they can be toxic to microorganisms, affecting anaerobic digestion (Espinosa-Negrín *et al*., 2021). Biological methods use microorganisms that can degrade cellulose, hemicellulose, and lignin, but, in this case, the bioprocess can be prolonged for much longer, increasing the substrate consumption to produce the required conditions of the degrading microorganisms, affecting biogas production (Zhang *et al*., 2022).

In this work, the physical method used for the conditioning of organic waste allowed reducing the particle size and increasing the rate of the anaerobic digestion process and biogas production by increasing the contact surface and the substrate/inoculum mass transfer (Theuretzbacher *et al*., 2015).

## Inoculum conditioning

Adaptation of microorganisms to a new substrate is essential for good results in biogas production (Zhang *et al*., 2022; Atelge *et al*., 2020). Once the COD level decreased by 25 % after one week of culture (Figure 2), the first feeding was performed, adding 12 L of MMMC. Immediately afterwards, a sample was taken and analyzed, obtaining the following values for the mixture in the biodigester: COD 13.4 g $L^{-1}$, total nitrogen 1.1 g $L^{-1}$, total phosphorus 0.21 g $L^{-1}$, total solids (TS) 7.7 g $L^{-1}$, and volatile solids (VS) 5.45 g $L^{-1}$, at a pH of 7.

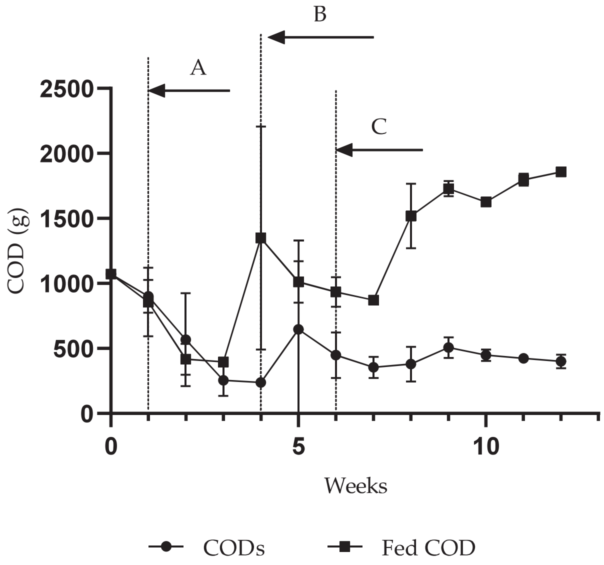

**Figure 2.** Chemical oxygen demand (COD) and its average consumption during anaerobic digestion. A: start of feeding with minimal mineral medium with glucose and glycerol as carbon sources (MMMC); B: change to MMMC mixture with residue; C: total substrate change to only organic residue in mixture with 2.5 % glycerol. CODs: soluble COD.

When COD decreased by about 80 %, the feed load was increased to 15 L of MMMC every 48 h until the difference between soluble COD and fed COD remained in a constant range. During that time, the composition of the biogas produced was measured (Figure 3), which initially could be increased by the addition of more organic matter

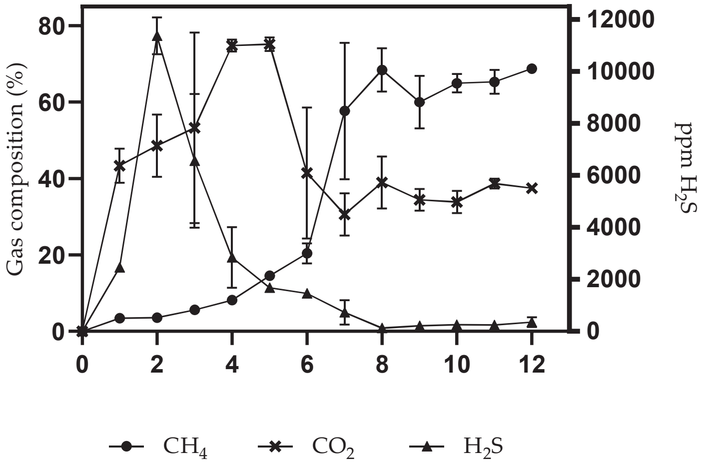

**Figure 3.** Average composition of biogas produced in the anaerobic digestion of agro-industrial organic waste generated in the central supply center (Central de Abasto) of Mexico City, Mexico.

(Jameel *et al*., 2024). After 30 days of processing, with 15 % of methane produced, the substrate was changed (Figure 2), initiating the anaerobic digestion stage of the agro-industrial waste.

### Anaerobic digestion of agro-industrial waste

Anaerobic digestion of organic waste was initiated with a 10 L load of a mixture of 50 % MMMC and 50 % waste. After the substrate change, a volume of 217 L was reached in the biodigester. This volume was considered the final operating volume and was kept constant by draining 5 L of sludge before each 5 L waste feed with MMMC. The feeding with MMMC/waste mixture was maintained for two weeks, during which time the COD decreased by 30 % and the methane percentage increased from 10 to 25 % (Figure 3), which is why the total change of substrate to only waste in mixture with 2.5 % glycerol (R/G) was carried out (Figure 2).

A total of 5 L of the latter substrate (R/G) were added every 48 hours due to the rapid consumption of COD and the high percentage of methane produced. The feeding load was increased until a volume of 13 L of substrate (R/G) was reached every 48 hours. Methane production continued to increase until it reached a biogas composition with a methane percentage of 72 % (Figure 3). The $CO_2$ percentage peaked at week five and subsequently decreased to 30 %. Biogas production was maintained for more than 2 weeks with a methane percentage that ranged between 60 and 70 % and a $CO_2$ percentage between 40 and 30 % (Figure 3). At this point, the biogas produced was already fully flammable.

These results confirm that in order to increase the yield, speed, and efficiency of biogas production, simultaneous digestion of two or more substrates is necessary, since a better nutritional balance, adequate C/N ratio, buffering capacity, and stability in the biodigestion system are achieved (Andlar *et al*., 2021). Co-digestion of agro-industrial waste with glycerol increased biogas and methane production from 15 to 70 %, i.e., 4.6 times more than when only glycerol was fed. This increase is greater than that obtained in other glycerol co-digestion processes (Alves *et al*., 2020; Butkowska *et al*., 2022). In addition, stability was achieved in the bioprocess, maintaining biogas production with a high percentage of methane for more than 2 weeks (Figure 3).

### Phosphorus, nitrogen, and solids concentrations during bioprocessing

At start-up, nitrogen and phosphorus concentrations were balanced at a 6:1 ratio. However, throughout the process, both residual concentrations had variations, equalizing at some times and at others increasing the phosphorus concentration. These variations did not affect methane production, since neither nutrient accumulated in excess nor was limited during the bioprocess.

During biodigestion, ammonia may be produced as a result of the anaerobic degradation of proteins or amino acids present in the feedstock. Although ammonia nitrogen is an important nutrient for bacterial growth, if found in excess, it can limit bacterial growth (Jameel *et al*., 2024). In this case, the total nitrogen concentration remained in the range of 0.3 to 1.5 g $L^{-1}$, which was still lower than those reported as toxic. Concentrations higher than 3 g $L^{-1}$ may be inhibitory (Jameel *et al*., 2024).

Phosphorus concentration also did not show large variations (between 0.21 and 1.7 g $L^{-1}$), which reflects that the microorganisms had an adequate proportion of these nutrients during the bioprocess. As for the concentration of total solids, it was maintained in a range of 8 to 12 g $L^{-1}$; this concentration was sufficient for the microorganisms to utilize the organic material and transform it to biogas (Jameel *et al*., 2024). Volatile solids represented more than 50 % of total solids.

### Temperature and pH

A temperature of 38 °C was maintained from the beginning of the process. Temperature affects the reaction rate, and although anaerobic digestion can be performed at thermophilic temperatures (50 to 60 °C), many biodigesters are operated at mesophilic conditions (30 to 40 °C) because of the ease in system control and lower energy demand (Atelge *et al*., 2020). During the first three weeks, the pH was decreasing, indicating acidification of the medium to a value close to six due to the accumulation of volatile fatty acids. At this point it was necessary to add 5 L of a 5% sodium bicarbonate solution to avoid further acidification that could inhibit the activity of methanogenic bacteria (Jameel *et al*., 2024). After 4 weeks, when a new decrease in pH was observed, sodium bicarbonate was added again. Once the methane composition increased, a parallel increase in pH was observed, remaining between 7.1 and 7.5 during methane production. The high methane production implies a high consumption of organic acids produced, which explains the increase and stabilization of pH.

### Volumetric rate of consumption and COD removal efficiency

During the first weeks of the process, the volumetric rate of COD consumption (VVC) increased. This can be explained by the fact that the first substrate is easily assimilated. Over the next 4 weeks, the VVC decreased and then increased as the feeding of the MMMC/residue mixture was initiated. At week 12, VVC took an average value of approximately 2.84 ± 0.2 g $L^{-1}$ $d^{-1}$.

The COD removal efficiency ($\eta$) showed a similar behavior, reaching 60 % in the first weeks of the process. Subsequently, it decreased in the same week as VVC did, and at the same time, it increased to an average value of 77.6 ± 0.3 %.

### Hydraulic retention time and biogas production

The hydraulic retention time (HRT) was 28 days at week four. When the substrate was changed to only glycerol residue with a constant volume of 217 L, the feed was reduced to 5 L due to the high concentration of COD in the residue. At week 6, the HRT was 87 days; to reduce it, the feeding volume was doubled, and at 8 weeks, an HRT of 43 days was reached. Feeding was again increased to 13 L, and finally an HRT of 33 days was reached. The average HRT for anaerobic digestion processes was between 20 and 30 days (Atelge *et al*., 2020), so the HRT obtained in this study is in line with the average.

On the other hand, biogas production was increasing, obtaining in the last week a volume of 0.576 ± 0.084 $m^3$ $d^{-1}$ (Table 2). This value, which corresponds to 0.681 ± 0.098 $m^3$ of $CH_4$ $kg^{-1}$ VS, is higher than that reported for fruit residues as substrate (Andlar *et al*., 2021), demonstrating that co-digestion of organic residues with glycerol increases the production and quality of biogas obtained during biodigestion.

**Table 2.** Biogas production at different bioprocessing stages.

| Time (weeks) | Volume ($m^3$ $d^{-1}$) | Pressure (Pa) | Feeding (L $d^{-1}$) | Feeding (kg of waste $d^{-1}$) | HRT (days) |
|---|---|---|---|---|---|
| 4 | 0.048 ± 0.023 | 469.22 ± 2.51 | 7.5 ± 0.3 | - | 28.9 ± 0.5 |
| 6 | 0.144 ± 0.035 | 617.40 ± 1.92 | 2.5 ± 0.4 | 1.6 ± 0.1 | 86.8 ± 0.2 |
| 8 | 0.360 ± 0.103 | 1173.06 ± 3.28 | 5.0 ± 0.5 | 3.0 ± 0.3 | 43.4 ± 0.3 |
| 11 | 0.576 ± 0.084 | 1111.32 ± 2.84 | 6.5 ± 0.2 | 4.5 ± 0.2 | 33.3 ± 0.4 |

HRT: Hydraulic retention time.

Alves *et al*. (2020) demonstrated that the process of co-digestion of sludge from a wastewater treatment plant and glycerol for biogas production increases methane production by 5.7 % compared to digestion of only sewage treatment plant sludge, achieving 368.8 L of $CH_4$ $kg^{-1}$ VS. Butkowska *et al*. (2022) were able to increase methane production 3.1 times by adding glycerol as a cosubstrate to the manure digestion process. The results obtained in this work confirm that biogas production using agro-industrial wastes improves methane production more than four times when glycerol is added as a cosubstrate, obtaining up to 681 ± 0.098 L of $CH_4$ $kg^{-1}$ VS.

The co-digestion process stabilized in this research represents a good alternative for the disposal of organic waste generated at the CEDA, providing a solution to the social problem, with the option of regularizing scavengers and recyclers and, above all, mitigating the problem of environmental pollution caused by the disposal of waste in open-air deposits, producing large volumes of methane and carbon dioxide in the atmosphere.

## CONCLUSIONS

An anaerobic digestion process was implemented at the pilot plant level, using organic waste generated at the central supply center of Mexico City as a substrate for biogas production. At the same time, value was given to the glycerol obtained as a by-product in the biodiesel plant of the same center, using it as a co-substrate to reduce the hydraulic retention time, increase the production volume, and improve the quality of the biogas. The organic matter removal efficiency obtained was 77.6 ± 0.3 %, achieving a biogas production of 0.576 ± 0.084 $m_3$ $d^{-1}$, with a methane composition of between 60 and 70 %, corresponding to 681 ± 0.098 L of $CH_4$ $kg^{-1}$ of volatile solids, with the prospect of its use in the generation of high-efficiency heating and electrical energy.

## ACKNOWLEDGMENTS

This work was funded by SIP-IPN project support; COFAA-IPN grants for Deifilia Ahuatzi-Chacón, Angélica María Salmerón-Alcocer, EDI-IPN grant for Deifilia Ahuatzi-Chacón, EDD-IPN grant for Angélica María Salmerón-Alcocer, and SECIHTI grant for Sergio Muñoz-Martínez.

## REFERENCES

Alves IRFS, Mahler CF, Oliveira LB, Reis M, Bassin JP. 2020. Assessing the use of crude glycerol from biodiesel production as an alternative to boost methane generation by anaerobic co-digestion of sewage sludge. Biomass and Bioenergy 143: 105831. https://doi.org/10.1016/j.biombioe.2020.105831

Andlar M, Belskaya H, Morzak G, Ivančić SM, Rezić T, Petravić TV, Šantek B. 2021. Biogas production systems and upgrading technologies: A review. Food Technology and Biotechnology 59 (4): 387–412. https://doi.org/10.17113/ftb.59.04.21.7300

Atelge MR, Krisa D, Kumar G, Eskicioglu C, Nguyen DD, Chang SW, Atabani AE, Al-Muhtaseb AH, Unalan S. 2020. Biogas production from organic waste: Recent progress and perspectives. Waste and Biomass Valorization 11 (3): 1019–1040. https://doi.org/10.1007/s12649-018-00546-0

Botero BR, Preston TR. 1987. Biodigestores de bajo costo para la producción de combustible y fertilizante a partir de la excreta. Manual para su instalación, operación y utilización. San José, Costa Rica. 20 p.

Butkowska K, Mikucka W, Pokój T. 2022. Enhancement of biogas production from cattle manure using glycerine phase as a co-substrate in anaerobic digestion. Fuel 317: 123456. https://doi.org/10.1016/j.fuel.2022.123456

Chilakamarry CR, Sakinah AMM, Zularisam AW, Pandey A. 2021. Glycerol waste to value added products and its potential applications. Systems Microbiology and Biomanufacturing 1 (4): 378–396. https://doi.org/10.1007/s43393-021-00036-w

de Mello BS, Pozzi A, Rodrigues BCG, Costa MAM, Sarti A. 2024. Anaerobic digestion of crude glycerol from biodiesel production for biogas generation: Process optimization and pilot scale operation. Environmental Research 244: 117938. https://doi.org/10.1016/j.envres.2023.117938

DOF (Diario Oficial de la Federación). 1978a. NORMA Mexicana NMX-F-066-S-1978. Determinación de cenizas en alimentos. Gobierno de México. Secretaría de Economía. Ciudad de México, México.

DOF (Diario Oficial de la Federación). 1978b. NORMA Mexicana NMX-F-312-1978. Determinación de reductores directos y totales en alimentos. Gobierno de México. Secretaría de Economía. Ciudad de México, México.

DOF (Diario Oficial de la Federación). 1978c. NORMA Mexicana NMX-F-317-S-1978. Determinación de pH en alimentos. Ciudad de México. Gobierno de México. Secretaría de Economía. Ciudad de México, México.

DOF (Diario Oficial de la Federación). 1980. NORMA Mexicana NMX-F-68-S-1980. Determinación de proteínas en alimentos. Gobierno de México. Secretaría de Economía. Ciudad de México, México.

DOF (Diario Oficial de la Federación). 1986. NORMA Mexicana NMX-F-083-1986. Determinación de humedad en productos alimenticios. Gobierno de México. Secretaría de Economía. Ciudad de México, México.

DOF (Diario Oficial de la Federación). 2015. NORMA Mexicana NMX-AA-034-SCFI-2015. Medición de sólidos y sales disueltas en aguas naturales, residuales y residuales tratadas. Gobierno de México. Secretaría de Economía. Ciudad de México, México.

Espinosa-Negrín AM, López-González LM, Casdelo-Gutiérrez NL. 2021. Pretratamiento de biomasas lignocelulósicas: breve revisión de los principales métodos utilizados. Revista Centro Azúcar 48 (3): 108–119.

González R, Peña DC, Gómez X. 2022. Anaerobic co-digestion of wastes: Reviewing current status and approaches for enhancing biogas production. Applied Science 12 (17): 8884. https://doi.org/10.3390/app12178884

González-Rentería SM, Soto-Cruz NO, Rutiaga-Quiñones OM, Medrano-Roldán H, Rutiaga-Quiñones JG, López-Miranda J. 2011. Optimización del proceso de hidrólisis enzimática de una mezcla de pajas de frijol cuatro variedades (Pinto villa, Pinto saltillo, Pinto mestizo y Flor de mayo). Revista Mexicana de Ingeniería Química 10 (1): 17–28.

Hach. 2024. Water analysis handbook. Loveland, CO, USA. https://www.hach.com/resources/water-analysis-handbook (Retrieved: March 2024).

Herrera-Uchalin MG, Valiente-Saldaña YM, Garibay-Castillo JV, Herrera-Cherres S. 2023. Manejo de residuos sólidos en la gestión municipal: revisión sistémica. Revista Arbitrada Interdisciplinaria Koinonía 8 (16): 150–170. https://doi.org/10.35381/r.k.v8i16.2540

Jameel MK, Mustafa MA, Ahmed HS, Mohammed AJ, Ghazy H, Shakir MN, Lawas AM, Mohammed SK, Idan AH, Mahmoud ZH, Sayadi H, Kianfar E. 2024. Biogas: Production, properties, applications, economic and challenges: A review. Results in Chemistry 7: 101549. https://doi.org/10.1016/j.rechem.2024.101549

López C, Martínez F, Paredes O. 2016. Automatización de un proceso de biodigestión anaeróbica. Revista Cubana de Ciencias Informáticas 10 (1): 1–16.

Martínez-Prats G, Chacón-Lozano LE, Silva-Hernández F. 2022. Análisis del sector agroindustrial de México 2020-2021. Revista de Investigaciones Universidad del Quindío 34 (S5): 133–138. https://doi.org/10.33975/riuq.vol34nS5.1089

Matiacevich S, Soto MD, Gutiérrez CM. 2023. Economía circular: obtención y encapsulación de compuestos polifenólicos provenientes de residuos agroindustriales. RIVAR 10 (28): 77–100. https://doi.org/10.35588/rivar.v10i28.5343

Mejías-Brizuela N, Orozco-Guillen E, Galáan-Hernández N. 2016. Aprovechamiento de los residuos agroindustriales y su contribución al desarrollo sostenible de México. Revista de Ciencias Ambientales y Recursos Naturales 2 (6): 27–41.

Olugbemide AD, Lajide L, Likozar B, Ighodaro A, Omunagbe OC, Ifijen IH. 2023. Biogas production through anaerobic co-digestion of rice husk and plantain peels: Investigation of substrate mixing ratios, digestate quality, and kinetic analysis. Brazilian Journal of Chemical Engineering 42 (1): 83–94. https://doi.org/10.1007/s43153-023-00415-x

Pérez-Contreras S, Hernández-Martínez R, Hernández-Rosas F, Herrera-Corredor JA, Varela-Santos EC. 2023. Enzymatic saccharification of pretreated sugarcane bagasse by hydrogen peroxide for bioethanol production. Tropical and Subtropical Agroecosystems 26 (2). http://doi.org/10.56369/tsaes.4831

Prasertsan P, Leamdum C, Chantong S, Mamimin C, Kongjan P, O-Thong S. 2021. Enhanced biogas production by co-digestion of crude glycerol and ethanol with palm oil mill effluent and microbial community analysis. Biomass and Bioenergy 148: 106037. https://doi.org/10.1016/j.biombioe.2021.106037

Ruvalcaba-Gómez JM, Arteaga-Garibay RI, Domínguez-Araujo G, Galindo-Barboza AJ, Salazar-Gutiérrez G, Martínez-Peña MD, Delgado-Macuil RJ. 2019. Uso de bacterias ácido lácticas para descontaminación de estiércol porcino mediante ensilaje experimental. Revista Internacional de Contaminación Ambiental 35 (1): 247–257. https://doi.org/10.20937/rica.2019.35.01.18

Sarandón SJ. 2020. Biodiversidad, agroecología y agricultura sustentble. Editorial de la Universidad Nacional de la Plata: Buenos Aires, Argentina. 429 p.

SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2020. Diagnóstico básico para la gestión integral de los residuos. Ciudad de México, México. 272 p.

Singh R, Das R, Sangwan S, Rohatgi B, Khanam R, Ghouse Peera SK, Das S, Lyngdoh YA, Langyan S, Shukla A, *et al.* 2021. Utilisation of agro-industrial waste for sustainable green production: A review. Environmental Sustainability 4 (4): 619–636. https://doi.org/10.1007/s42398-021-00200-x

Statista Research Department. 2023. El sector agrícola en México. Datos estadísticos. https://es.statista.com/temas/7029/el-sector-agricola-en-mexico/#topicOverview (Retrieved: March 2023).

Theuretzbacher F, Lizasoain J, Lefever C, Saylor MK, Enguidanos R, Weran N, Gronauer A, Bauer A. 2015. Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic digestion. Bioresource Technology 179: 299–305. https://doi.org/10.1016/j.biortech.2014.12.008

Tippens PE. 2011. Física, conceptos y aplicaciones (Séptima edición). McGraw Hill: Lima, Perú. 828 p.

Zhang Y, Lin J, Song T, Su H. 2022. Anaerobic digestion of waste for biogas production. *In* Abomohra AEF, Wang Q, Huang J. (eds.), Waste-to-Energy. Springer: Cham, Switzerland. https://doi.org/10.1007/978-3-030-91570-4_6