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Changes in microbial diversity and methane yield caused by overloading in systems of chicken litter, microalgae oil-free and glycerol in co-digestion

Cambios en la diversidad microbiana y producción de metano causados por sobrecarga en sistemas de gallinaza, microalgas libres de aceite y glicerol en codigestión

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Abstract
The objective of this study was to observe the response of chicken litter (CL), oil-free microalgae (M), and glycerol (G) in disturbed systems with organic loading rate (OLR) in mono, and co-digestion. To elucidate the impact of the OLR on the methane yield, Volatile Fatty Acids (VFAs), and microbial communities. In this study, 3 treatments were performed CL, CL-M, CL-M-G, in different ratios 100: 0: 0 (CL), 70:30:0 (CL-M) y 67:30:3 (CL-M-G) which were based on the best substrates with the highest Biochemical Methane Potential (BMP) reported in our previous research. Our results indicated that the CL-M system had the highest methane yield (12,481.16 mL CH_4 $g_{vs\ added}^{-1}$) and a lower production of VFAs (70,842.07 mg L^{-1}) compared with the CL and CL-M-G systems. In addition, the microbial analysis revealed that each methanogen was more related to a system, *Methanoculleus* to the CL system, *Methanosarcina* to the CL-M system, and *Methanothrix* to the CL-M-G system. Although the systems were disturbed, did not inhibit the anaerobic digestion (AD). These findings have shown that disturbances acidify the environment, reduce the abundance of bacteria, and promote methane production in the hydrogenotrophic pathway.
Keywords: Disturbances; Organic loading rate; Mesophilic; Chicken litter; Microbial diversity; Methane yield.

Resumen
El objetivo de este estudio fue observar la respuesta que tuvo pollinaza (CL), microalga libre de aceite (M), y glicerol en sistemas perturbados con sobre carga de materia orgánica (OLR) en mono- y co-digestión. Para elucidar el impacto de la OLR sobre el rendimiento de metano, en los ácidos grasos volátiles (AGVs) y en las comunidades microbianas. En este estudio, se realizaron 3 tratamientos CL, CL-M, CL-M-G, en diferentes proporciones 100: 0: 0 (CL), 70:30:0 (CL-M) y 67:30:3 (CL-M-G) que se basaron en los mejores sustratos con el mayor Potencial Bioquímico de Metano (PBM) reportado en nuestra investigación anterior. Nuestros resultados indicaron que CL-M tuvo el mejor rendimiento de metano (12,481.16 mL CH_4 $g_{svalimentados}^{-1}$) y la más baja producción de AGVs (70,842.07 mg L^{-1}) comparado con CL y CL-M-G. En adición, el análisis microbiano reveló que cada metanógeno fue más a fin a cada sistema, *Methanoculleus* para el sistema CL, *Methanosarcina* para CL-M y *Methanothrix* para CL-M-G. A pesar de que los sistemas se encontraban perturbados no se inhibió la DA. Estos hallazgos han demostrado que las perturbaciones acidifican el medio, reducen la abundancia de bacterias y promueven la producción de metano por la vía hidrógenotrófica.
Palabras clave: Perturbaciones; Sobrecarga de materia orgánica; Mesofílico; Pollinaza; Diversidad microbiana; Rendimiento de metano.

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1 Introduction

Anaerobic digestion (AD) is one of the alternatives to produce clean energy, either in the form of heat, electricity, and/or biofuels (methane and hydrogen) (Kabeyi & Olanrewaju, 2022). AD can also contribute to reduce the greenhouse gas (GHG) emissions (Malet *et al*., 2023) through the use of digestate produced rich in carbon (C) and nitrogen (N), which, when used as fertilizer, helps to capture organic carbon in the soils, thus contributing to the elimination of atmospheric carbon dioxide (CO_2) and by replacing synthetic nitrogen fertilizers helps to avoid the emission of gases from of these synthetic fertilizers (Malet *et al*., 2023). Another use of digestate from AD is the phytoregulators; for instance, Castro-Sierra *et al*. (2024) reported that dairy cattle and swine manure digestates produce gibberellic acid, indoleacetic acid, and kinetin. The residuals are from diverse sources, such as food (Mahmudul *et al*., 2022) and algae (Rivera-Hernández *et al*., 2022) wastes. Each residual has its own characteristics and biodegradability, which can be used as a mono-substrate or by the co-digestion method, using municipal (Sanaye *et al*., 2022) to food waste (Oduor *et al*., 2022). Among several substrates, *Chlorella* sp microalgae have been used in the co-digestion with effluents from wastewaters (Solé-Bundó *et al*., 2019), cooking oil, maize silage (Wirth *et al*., 2019), cow manure (Alharbi, 2024), and chicken litter and glycerol (Meneses-Reyes *et al*., 2018).

Chlorella sp microalga biomass is rich in protein; it has been reported that its carbon-nitrogen ratio (C/N) ranges from 4.3 (Zhu et al., 2019), 4.86 (Ruirui Li *et al*., 2017) to 5.35 (Meneses-Reyes *et al*., 2017), which is less than the optimal range for AD (20 to 30) (Gil et al., 2019). Microalgae have been reported to be efficiently digested in co-digestion with carbon-rich raw materials, rebalancing the C/N and increasing methane production. The optimal C/N for co-digestion varies depending on the co-digestion material. Thus, for chicken manure was 6.75 C/N (Ruirui Li *et al*., 2017), for triple co-digestion with glycerol and chicken litter was 6.94 C/N (Meneses-Reyes *et al*., 2017), and for used cooking oil was 4.77 C/N (Rétfalvi *et al*., 2016).

It has also been reported that high loads of Organic Loading Rate (OLR) can cause a drop in pH due to the rapid generation of volatile fatty acids (VFA) (Magdalena *et al*., 2019). Slezak *et al*. (2017) studied the effect of OLR on VFA production in dark fermentation and identified the VFA concentration increases only up to the initial OLR of 48.2 g_{VS} L^{-1}. Increasing the OLR also effects the VFAs composition in the product stream. Wijekoon *et al*. (2011) identified that the predominant VFAs component changed from acetic acid to n-butyric acid with an overall increase in VFAs concentration when OLR was increased from 5 to 12 kg COD $m^{-3}d^{-1}$ in a two-stage thermophilic anaerobic membrane bioreactor. In a study by Babaee and Shayegan (2011) in a 70 L digester with vegetable wastes, when reaching OLR of 2.75 kg VS $m^{3\text{-}1}d^{-1}$, the degradation of volatile solids and biogas production decreased. Musa et al. (2018) reports a decrease in chemical oxygen demand (COD) from 50 % to an OLR of 15 g $L^{-1}d^{-1}$ of cattle slaughterhouse wastewater.

In fact, changes in OLR affect the AD process in terms of population dynamics and organic matter availability and, therefore, methane production yields (Li *et al*., 2022). The OLR is a critical operating parameter of AD that must be controlled to avoid disturbances in the process (Nkuna *et al*., 2022). An OLR shock generally causes an imbalance between the hydrolysis / acidogenesis and methanogenesis steps (Wu et al., 2021). The behavior of bacterial communities during disturbances due to organic overload is more complex due to the high number of species and their functional redundancy (Nguyen *et al*., 2019). Even when the overload is caused by the same co-substrate, the results of different studies are contradictory, so the response of the microbial community is not predictable by the raw material or the inoculum used in the reactors (Braz *et al*., 2019).

Most mono-substrate AD practices have poor digestion performance due to inherent substrate defects (Vivekanand *et al*., 2018) leading to different conversion rates and lower production efficiency methane (Wei *et al*., 2019). Anaerobic co-digestion of two or more organic wastes (Zhang *et al*., 2020) can overcome the inherent limitations associated with mono-digestion of an individual substrate by synergistic effect, resulting in increased biomethane yield specific or an increase in digestion kinetics (Kim *et al*., 2019). For example, Serna-García *et al*. (2020) evaluated the performance of biogas production and reported a methane production of 130 mL CH_4 $d^{-1}L^{-1}_{reactor}$, with microalgae (*Scenedesmus* and *Chlorella*) and primary sludge. With an increase in biodegradability of around 73 % compared to the mono-digestion of pristine algae. Another study conducted by Zhang et al. (2020) showed that microalgae *Chlorella vulgaris* in co-digestion with potato processing waste and glycerol (G) to an average OLR of 0.30 g COD per L per d improved the volumetric production of methane with an average production of 0.59 ± 0.08 L CH_4 per L per d. An additional study by R. Li *et al*. (2017) microalgae *Chlorella* 1067 was cultivated in chicken manure (CM) based digestate, and then algae biomass was used as co-substrate for AD with CM. They showed that co-digestion achieved the highest methane production of 238.71 mL g_{VS}^{-1} and the most

significant synergistic effect.

The synergistic effect of co-digestion generally demonstrates higher biomethane yields, accelerated biodegradation processes, higher hydrolysis rate, or a combination of these (Karki *et al*., 2021). In this sense, co-digestion is a strategy that consists of mixing a substrate with another carbon source, in order to improve operational parameters such as the C / N ratio, buffer disturbances and / or dilute inhibitors. As a result, higher process performance can be achieved. Furthermore, co-digestion allows the treatment of different wastes using the same facilities, and biofertilizers can also be generated (Pan *et al*., 2021), in fact, co-digestion has been proposed as a promising approach to improve the performance of methane and the general performance of digestion (Lv *et al*., 2021). To our knowledge, various substrates have been used in co-digestion. Our research team has worked with chicken litter (CL) -oil-free microalgae (M) - glycerol (G) as substrates, where Meneses-Reyes *et al*. (2018) proved that the Biochemical Methane Potential (BMP) and the Specific Methanogenic Activity (SMA) increased in CL-M-G, which shows that these systems improved AD. In the present study we introduce an additional level of complexity, that is, disturbing systems with OLR. Therefore, the objectives of this study were to observe the response that the systems had to the overloading and to elucidate the impact of the influence of OLR on microbial communities and the methane yield in mono, and co-digestion of three substrates from the CL, M, and G. For a better understanding of how this complex process is affected, including microbial interactions and methane production. So far, the response of how the microbiomes in the digesters respond to overloading is imperative both from the point of view of microbial ecology and the performance of biogas production.

2 Material and methods

2.1 Experimental procedure

The feedstock used in this work were chicken litter (CL, provided by a commercial poultry farm located in the municipality of Tepetlaoxtoc, State of Mexico, Mexico); oil-free microalgae biomass from Chlorella vulgaris powder (M, Future Foods Company, México); and glycerol (G, grade United States Pharmacopeial Convention, USP). Our research team reported the physical and chemical characteristics of these feedstocks in a previous report (Meneses-Reyes *et al*., 2017). Three different treatments were tested based on different ratios of CL, M, and G as shown: 100:0:0 (CL), 70:30:0 (CL-M), and 67:30:3 (CL-M-G), which were based on the best feedstocks ratios with highest BMP reported in our previous research (Meneses-Reyes *et al*., 2017). The digestion process was performed in a stainless-steel digester with a working volume of 10 L and a headspace of 3 L each. To achieve a steady state condition, two hydraulic retention times (HRT=30 d), 60 d, were used, and the end of this period was considered time 0. The disturbances were induced with two OLR shocks of 49.3 g_{VS} L^{-1} d^{-1} and 35.7 g_{VS} L^{-1} d^{-1} starting on day 0 and 301, respectively, and both were maintained for 11 consecutive days, the other day's food with 3 % TS. On days 297 and 360, samples from the digester were taken for the DNA for microbial communities' analysis, as shown in Figure 1.

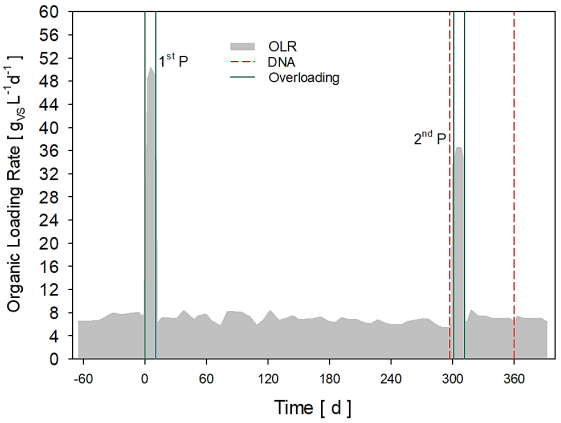

Figure 1. Organic loading rate and sample collection points for DNA analysis during the experiment. The green line ( ) indicated the first overloading period 49.3 g_{VS} L^{-1} d^{-1} (1st P), and the second overloading period 35.7 g_{VS} L^{-1} d^{-1} (2nd P); Red short dashed line (----) indicated dates of sampling DNA; Gray box indicates stable feeding of the 3 % TS system.

2.2 Analytical methods

The analytical methods employed in this study were previously described by our research group in Meneses-Reyes *et al*. (2017). To evaluate the profiles of volatile fatty acids (VFAs), including acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate, caproate, and iso-caproate, we followed the detailed procedure outlined by Meneses-Reyes *et al*. (2017) using gas chromatography (GC). Initially, VFAs were determined from a sample of effluent that had been acidified with HCl to achieve a pH of three after centrifugation at 14,500 rpm for 10 minutes to obtain the supernatant. The acidified samples were then injected into a gas chromatograph system (Clarus 500, Perkin Elmer, USA) equipped with a flame ionization detector (FID) and a capillary column (Elite-FFAP; 30 m x 0.32 mm; Perkin Elmer, USA). For analysis, we used the Volatile Free Acid Mix analytical standard (Supelco 46975-U, Sigma-Aldrich, USA). A potentiometer (Thermo Scientific

Orion 5 Star, Singapore) was employed to determine the pH. Biogas production was measured using a saline water displacement method in conjunction with a digital counter (LA8N-BN, Autonics, Korea). The methane content in the biogas was estimated using the same gas chromatograph (Clarus 500, Perkin Elmer, USA) based on a calibration curve established with a pure methane standard (HDSP No. P-4618-F, Praxair, México).

2.3 Total DNA isolation and sequencing

Total DNA was isolated using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, United States), following the manufacturer's protocol. The DNA integrity was checked by electrophoresis on agarose gel (1 % w/v), and the quantification of the extracted DNA samples was performed by using LabChip GX Touch Nucleic Acid Analyzer (Perkin Elmer).

For the preparation of libraries, the GenXpro (Frankfurt am Main, Germany) protocol was used, which consisted of after the fragmentation with directed ultrasound (Diagenode Bioruptor Pico), barcoding adapters included in UMIs - TrueQuant Adapters (patented by GenXpro) were added. All samples were sequenced by duplicate from the ends (2 × 150 bp) using the Illumina NextSeq500 platform (Illumina, USA).

2.4 Metagenomic analysis

Raw reds were quality checked by using FASTQC (Brown *et al*., 2017) and adapter-and low-quality-filtered (Q value ≤ 30) by using Trimmomatic (Bolger *et al*., 2014). Clean reads were used to perform the metagenomics assembly using MEGAHIT v1.0 (Li *et al*., 2016), and Kraken2 (Wood *et al*., 2019) on the Omics box platform (https://www.biobam.com/omicsbox). Subsequently, taxonomy classification and microbial abundance was assigned by MetaPhlAn v3.0 software through blasting marker genes with effective reads (Beghini *et al*., 2021).

The raw sequence data obtained from this study have been deposited into the NCBI sequence read archive (SRA) under the BioProject ID: PRJNA1183444.

3 Results and discussion

3.1 Methane yield

Figure 2 illustrates the effects of the overloading Organic Loading Rate (OLR) at 49.3 g_{VS} $L^{-1}d^{-1}$ and 35.7 g_{VS} $L^{-1}d^{-1}$ on the methane yield. Prior to the first disturbance, the three systems were in a quasi-study state. The average initial methane yield recorded was 218.17, 260.90, and 264.44 mL CH_4 $g_{vsadded}^{-1}$ for CL, CL-M, and CL-M-G systems, respectively.

During the first overloading of OLR (49.3 g_{VS} $L^{-1}d^{-1}$) phase, the impact on the methane yield was significant. The reductions observed were 59 %, 44 %, and 26 % for the CL, CL-M, and CL-M-G systems, respectively.

After the first disturbance, when the overloading of OLR pressure was alleviated, the systems exhibited partial recovery, displaying high methane yield values of 500 mL CH_4 $g_{vsadded}^{-1}$ for CL, 490 mL CH_4 $g_{vsadded}^{-1}$ for CL-M, and 700 mL CH_4 $g_{vsadded}^{-1}$ for CL-M-G. This recovery can be attributed to the residual organic matter available for methane processing after removing the OLR pressure overloading. The initial methane yield levels recovered to 164, 109, and 250, respectively.

Between the two disturbances, the CL-M system showed the most stability, suggesting that co-digestion aided in mitigating the impacts of organic matter overload.

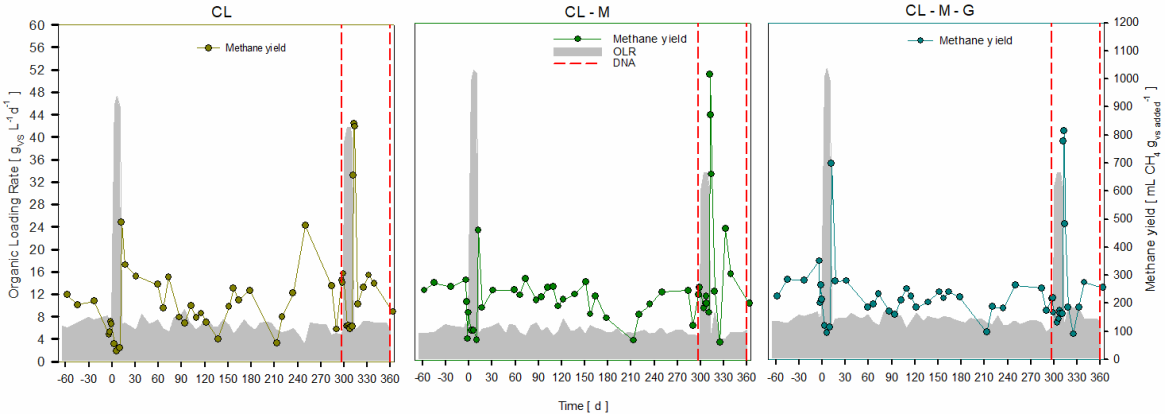

Figure 2. Changes observed in the methane yield and organic loading rate (OLR) during the experiment. CL: Chicken litter; M: Microalgae oil-free; G: Glycerol. The red short dashline (----) indicated DNA sample. Gray area ( ) indicated the organic loading rate.

During the second disturbance (35.7 g_{VS} $L^{-1}d^{-1}$), the decline in the methane yield was less severe than during the first disturbance, with the reductions being 44 %, 25 %, and 41 % for the CL, CL-M, and CL-M-G systems, respectively. The systems regained stability in 14, 7, and 28 days. This response may indicate that the systems were learning how to recover from disturbances, a phenomenon noted by other researchers who suggest that disturbed systems may have some degree of memory, allowing the microbiome to adapt to changes (Spirito *et al*., 2018).

After the second disturbance, upon stopping the OLR pressure overloading, the methane yield peaks were more pronounced in the systems. This could be due to the rapid degradation of substrates by resilient microorganisms that had been adapted following the first disturbance. In the CL and CL-M-G systems, the maximum peak in activity doubled, reaching around 800 mL CH_4 $g_{vsadded}^{-1}$, while in the CL-M system, it approached nearly 1000 mL CH_4 $g_{vsadded}^{-1}$. Although these measures are instantaneous reflections of methane production, they also indicate the potential development of specific microbial communities capable of efficiently processing acetate and propionate produced after the disturbances.

At the final observation point, all systems were in recovery, with the CL-M system demonstrating the best recovery. This success may be attributed to its higher abundance of microorganisms. The increased diversity has likely allowed for various microorganisms to fulfill different roles, adapting to the changed conditions.

In all cases, despite the disturbances, no process inhibition was observed. This suggests that there were microorganisms adapted to stressful overload conditions with rapid recovery, especially in the CL-M system, strengthening that co-digestion favors anaerobic digestion for biogas production.

3.2 Volatile fatty acid

fFigure 3 shows the volatile fatty acids (VFAs) in the order from greater to lower concentration as follows: acetate, propionate, iso-valerate, butyrate, iso-butyrate, caproate, valerate, and iso-caproate. In the 430-day study period, the highest total of the VFAs was obtained in the CL system with 214,591.11 mg L^{-1}, as observed in **Table 1**. In the VFAs generation, the opposite behavior was observed contrary to the methane yield (Figure 2). As expected in the first impact (49.3 g_{VS} $L^{-1}d^{-1}$), more significant concentrations of VFAs were generated concerning the second impact (35.7 g_{VS} $L^{-1}d^{-1}$).

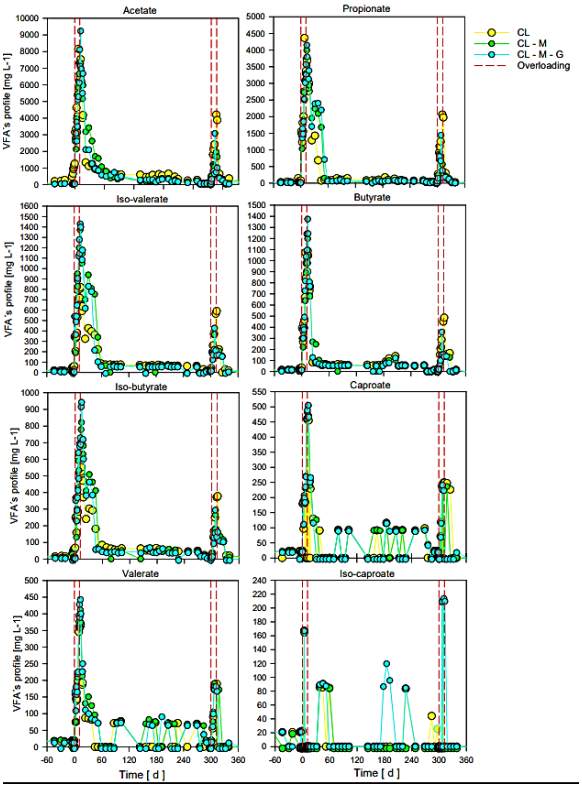

Figure 3. Volatile Fatty Acids profile during the experiment (VFAs). CL: Chicken litter; M: Microalgae oil-free; G: Glycerol. The red short dash line (----) indicated overloading periods.

This indicates that the CL system remained acidified, which explains why it had the slightest methane yield compared to the CL-M system. These high levels of VFAs found in our systems could inhibit the process of methanogenesis (Serrano-Meza *et al*., 2020). However, these systems remained stable, and it is inferred that the microbiome adapts to these stress conditions. Braz *et al*. (2019) researched anaerobic reactors using sewage sludge, which produced 2,500 mg L^{-1} of VFAs. Basak *et al*. (2021) studied the effects of overloading digesters with food waste leachate, resulting in 11300 mg L^{-1} of VFAs. Both studies found that these conditions led to the acidification of the systems. It is well known that acidified environments inhibit biogas production because methanogens are sensitive to acidic conditions.

Table 1. Volatile Fatty Acids (VFAs) concentrations produced by each system.

System	Acetate [mg L^{-1}]	Propionate [mg L^{-1}]	Iso-valerate [mg L^{-1}]	Butyrate [mg L^{-1}]	Iso-butyrate [mg L^{-1}]	Caproate [mg L^{-1}]	Valerate [mg L^{-1}]	Iso-Caproate [mg L^{-1}]
CL	112,927.40	52,661.75	13,901.43	13,997.48	10,000.58	5,249.15	4,896.41	956.92
CL-M	103,575.49	49,677.13	19,149.85	12,295.13	11,853.58	6,211.71	5,998.41	546.34
CLM-G	98,259.82	52,560.35	18,672.62	13,726.77	11,900.30	6,350.18	5,961.98	1,516.77

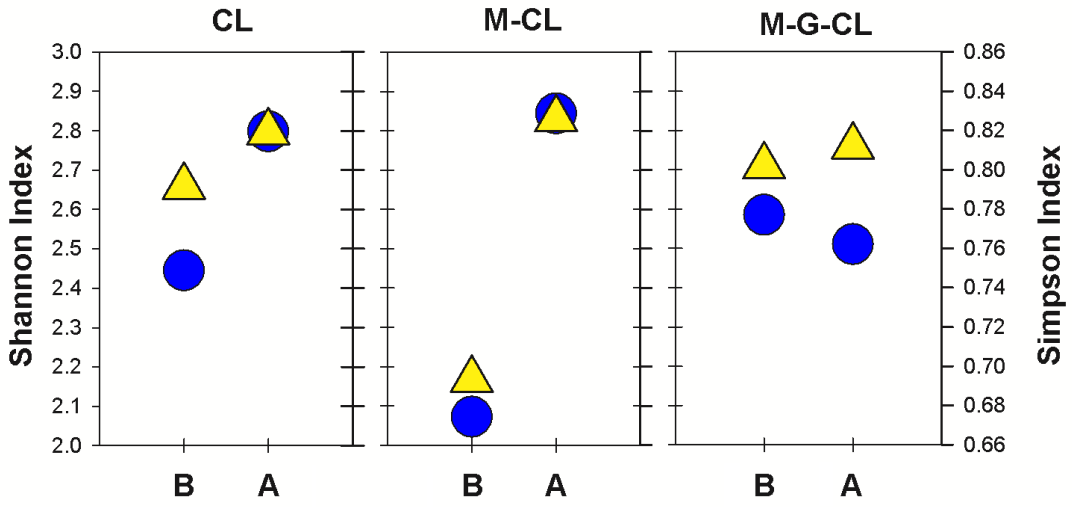

Figure 4. Shannon (●) and Simpson (△) index for each treatment. Letters B and A mean before and after the overloading period for each treatment, respectively.

3.3 Diversity index

Before the second disturbance, the Shannon and Simpson indices (Figure 4) for the CL, CL-M, and CL-M-G systems were recorded as follows: 2.44 and 0.79 for CL, 2.07 and 0.69 for CL-M, and 2.58 and 0.80 for CL-M-G. After the disturbance, the CL and CL-M systems showed an increase in diversity of 14.4 % and 37.3 %, respectively. In contrast, the CL-M-G system experienced a decrease in abundance of 2.9 %. The disturbances caused an increase in the diversity of microbial communities in the CL and CL-M systems, indicating that the CL-M system exhibited higher methane yield due to its greater species diversity. Cortez-Cervantes *et al*. (2024) and Rahman *et al*. (2021) reported that higher methane yields were associated with increases in microbial diversity.

3.4 Microbial community

The most representative microbial community in this study is shown in Figure 5, composed of 2 kingdoms, 8 phyla, and 32 genera. Regarding the archaea, the genus *Methanosarcina,* the disturbances did not affect their abundance in the three systems. Otherwise, Methanothrix was affected. However, the CL-M-G system increased significantly. *Methanoculleus* was only favored in the CL system; it is worth mentioning that this archea increased its abundance in CL and CL-M systems after the system was disturbed, except in the CL-M system. Regarding bacteria, 3 genera were not affected by the disturbances; these were, *Fermentimonas, Petrimonas, Proteiniphilum, Erysipelothrix, Jeotgalicoccus,* and *Aminobacterium*, from higher to lower abundance, respectively. In the remaining 26 genera, their abundance is relatively low compared to the previous ones.

The Heatmap (Figure 6) showed us four separations: the most abundant bacteria, the medium ones, the low ones, and the methanogens. In the high abundance at *Fermentimonas,* disturbances increased its abundance, and *Proteiniphilum* was affected along with *Erysipelothrix*. *Petrimonas* only remained in the CL system. In the medium abundance, the disturbances favored the CL-MG system since it promoted the appearance of 15 genera (as shown in figure 6), otherwise to the CL system that disappeared, the CL-M system the disturbances promoted the increase and decrease of what already contained apart the appearance of 6 genera. Regarding the low abundance, the CL-M system was the only one favored with the disturbances activating *Acetomicrobium,* and *Bacteroidales*. *Methanosarcina mazei*, *Methanothrix soehngenii*, and *Methanoculleus bourgensis* were the representative methanogens in this study.

The CL-M System had the highest methane yield because the microorganisms involved allowed degradation of the VFAs, avoiding the medium's acidification. Each methanogen has a substrate to grow and tolerate disturbances. The microorganisms in AD systems change according to the fed substrate and the environmental conditions under which the digester operates (Karki *et al*., 2021). However, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are usually present in most cases (Pei *et al*., 2022); this may be because they are hydrolytic fermentative bacteria responsible for decomposing macromolecules (Menzel *et al*., 2020).

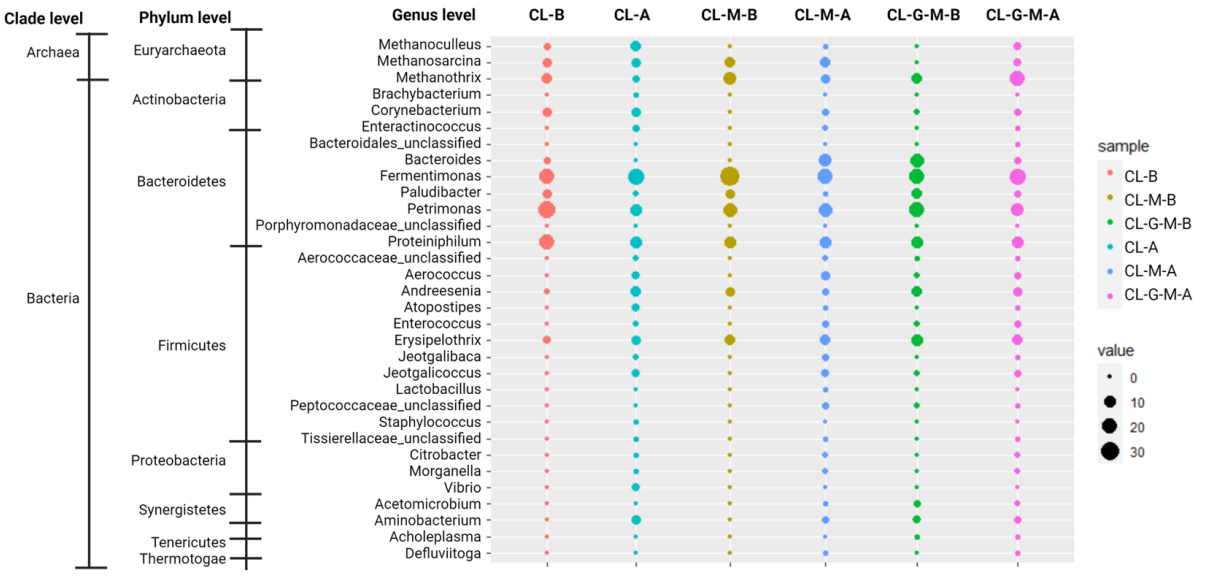

Figure 5. Relative abundance of main genera in CL-B: chicken litter before disturbance. CL-A: chicken litter after disturbance. CL-M-B: chicken litter-microalga before disturbance. CL-M-A: chicken litter-microalga after disturbance. CL-G-M-B: chicken litter-microalga-glycerol before disturbance. CL-G-M-A: chicken litter-microalga-glycerol after disturbance.

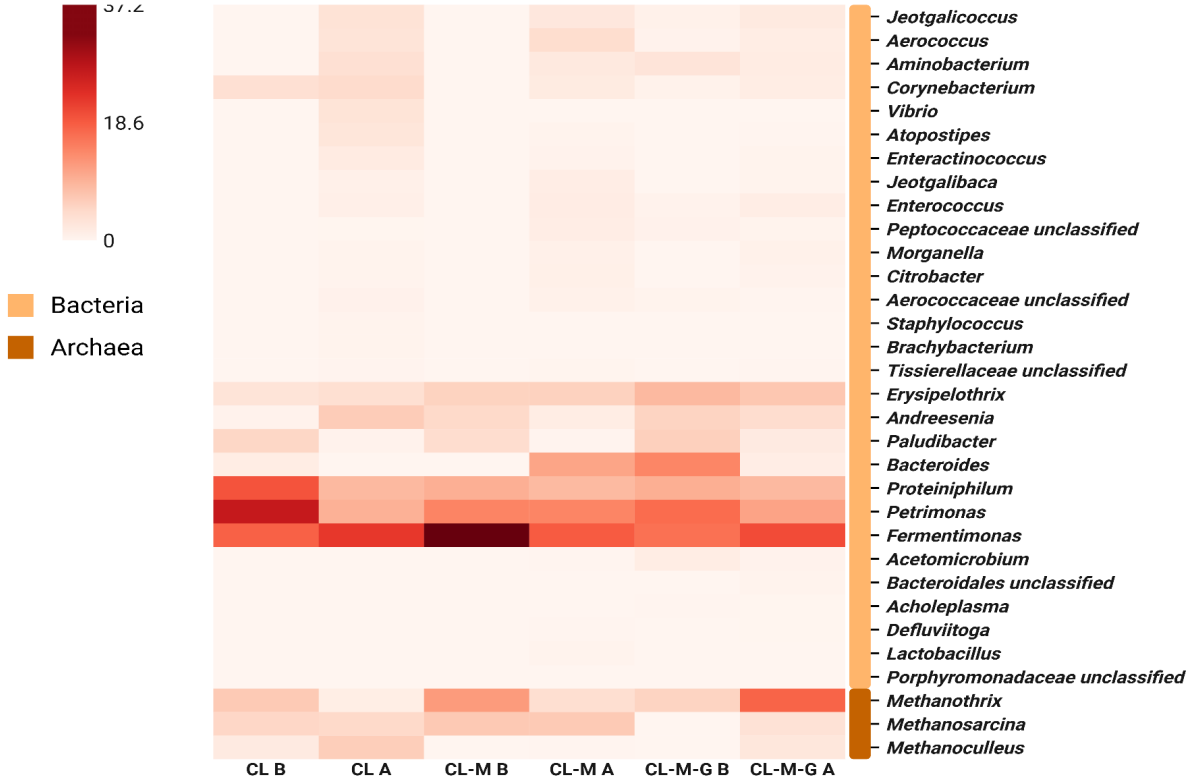

Figure 6. Heatmap of taxonomic abundance genus level found in systems.

Conclusions

The CL-M system exhibited the highest methane yield due to the buffering effect of co-digestion,

which mitigated disturbances caused by fluctuations in organic loading rates. This environment allowed for the rapid degradation of volatile fatty acids by bacteria such as *Jeotgalicoccus, Aerococcus, Aminobacterium, Corynebacterium, Enterococcus, Acetomicrobium, Erysipelothrix, Proteiniphilum, Petrimonas,* and *Fermentimonas*. As a result, the medium remained non-acidic, enabling *Methanosarcina* and *Methanothrix* to carry out the methanogenesis process without being inhibited.

Methanoculleus was linked to the CL substrate, *Methanosarcina* was found in both the CL-M and CL-M-G substrates, and *Methanothrix* was predominantly associated with the CL-M-G substrate. Notably, *Methanosarcina* was generally resilient to disturbances caused by organic matter overload across all three systems.

The disturbances negatively affected the bacterial populations, leading to a decrease in their abundance. Despite the overload of organic matter, the anaerobic digestion process remained stable across all three systems, as they continued to demonstrate specific methanogenic activity even after the disturbances.

Acknowledgements

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Data availability

The datasets generated and/or analyzed during the current study are available in the sequence read archive (SRA, https://ncbi.nlm.nih.gov/bioproject/?term=PRJNA1183444).

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