



**Characterization of sediments from the upper basin of the Lerma River, Mexico:
Microbiome and biomethane potential**

**Caracterización de los sedimentos en el curso alto del río Lerma, México: Microbioma y
potencial de biometano**

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Abstract

The Lerma River's high pollution has changed its environmental conditions. Therefore, in batch cultures, the present work characterized the sediments from the Lerma River, where the methane production and the structure of the microflora were evaluated in three sampling points. Biomass was taken from an anaerobic Waste Water Treatment Plant (WWTP) as a control sludge. Results showed that the glucose degradation rate of the control was 48 times faster than the degradation rate of the sediments; however, the substrate degradation rates presented by the three sediments were similar, with chemical oxygen demand (COD) removal efficiencies higher than 95%. Regarding the Biomethane Potential (BMP), the control and the three sediments presented high BMP. Finally, the sediments showed the potential to produce methane, and the main microflora identified in the sediments were *delta-proteobacteria*, *beta-proteobacteria*, *clostridia*, *bacteroidia*, and *methanomicrobia*; these classes are involved in each stage of anaerobic digestion.

Keywords: Anaerobic-sludge, sediments, BMP, microflora, methane, river.

Resumen

La alta contaminación del río Lerma ha modificado sus condiciones ambientales. Por lo tanto, en el presente trabajo se realizó la caracterización de los sedimentos del río Lerma, donde se evaluó la producción de metano y la estructura de la microflora en tres puntos de muestreo, en cultivos por lote. Como estudio control se utilizó biomasa de una Planta de Tratamiento de Aguas Residuales (PTAR) anaerobia. Los resultados mostraron que la tasa de degradación de la glucosa del estudio control fue 48 veces más rápida que la tasa de degradación de los sedimentos; sin embargo, las tasas de degradación del sustrato que presentaron los tres sedimentos fueron similares, con eficiencias de remoción de la demanda química de oxígeno (DQO) superiores al 95%. En cuanto al Potencial de Biometano (PBM), el control y los tres sedimentos presentaron valores altos de PBM. Finalmente, los sedimentos presentaron el potencial de producir metano, y la principal microflora identificada en los sedimentos fueron *delta-proteobacteria*, *beta-proteobacteria*, *clostridia*, *bacteroidia* y *methanomicrobia*; esta microflora participa en cada una de las etapas de la digestión anaerobia.

Palabras clave: lodo anaerobio, sedimentos, PBM, microflora, metano, río.

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1 Introduction

The Lerma River Upper Basin (LRUB) is the second longest river in Mexico, with 1281 kilometers. LRUB has become the central waste collector from anthropogenic activities, a receptor of wastewater from towns, industrial zones, industrial parks, and water treatment plants, which contains high contents of organic matter, heavy metals, and other complex pollutants (Barceló-Quintal *et al.*, 2013, González-Blanco., 2017, Mora *et al.*, 2021). The increased pollution exceeded its assimilation and dilution capacity throughout the Lerma River, showing accumulation of pollutants (e.g., 82.41 mg BOD₅/L, 0.2 mg dissolved oxygen (DO)/L, 18.3 mg NH₄⁺/L, and metals like Pb at 444.3 ± 13.3 µg/g) (Salinas Tapia *et al.*, 2015; Hernández-Mendoza *et al.*, 2018). In addition, the high pollution in the Lerma River significantly diminishes the dissolved oxygen concentration, propitiating anaerobic conditions and destroying aquatic life.

The Lerma River, being contaminated for a long time, allows microorganisms to adapt to these pollutants (Carreño *et al.* 2018). The characterization of sediments in terms of molecular biology and the degradation of organic matter to produce methane is scarce in the literature. In the present work, the sediments were studied as inoculum sources to evaluate their capability to produce methane, using glucose as carbon and energy source, in batch cultures. This information might be helpful to understand the influence of the anaerobic conditions in the microflora, sediments' participation in the water quality of the Lerma river, and their capability to degrade glucose and produce methane. In addition, these sediments might be an excellent inoculum source to inoculate biological reactors. Therefore, evaluating the sediments' biomethane potential and microbiota would indicate their metabolic potential. Thus, this work aimed to assess the BMP and characterize the microflora of the Lerma River sediments.

2 Material and methods

2.1 Sampling points

The sample collection sites are P_1 , P_2 , and P_3 , as shown in figure 1. 5 liters of simple samples were taken. The flow of water direction is from P_1 to P_3 . The first sample collection site (i.e., P_1) was taken one kilometer before WWTP (Reciclagua Ambiental), the second sample site (P_2) was 500 meters before WWTP, and the last sample site was taken 200 meters after the discharge point of the WWTP. Anaerobic sludge from the WWTP of an industrial candy producer was used as a control.

2.2 Batch cultures

Batch studies were performed in serological 120 mL bottles of nominal volume and 80 ml of operating volume. The sludge and sediments were washed ten times with sodium chloride (9 g/L) to eliminate all residual contaminants or impurities. Batch cultures were inoculated with 4 g VSS/L and 1 g glucose-COD/L as a carbon and energy source. The culture medium was the following, in g/L: NH₄Cl (0.15), K₂HPO₄ (0.35), KH₂PO₄ (0.27), NaHCO₃ (1.5), CaCl₂ (0.01), and 0.5 mL/L of trace elements (Romualdo-Martínez *et al.*, 2022). The liquid culture medium and headspace were flushed with helium for 3 min to remove oxygen and ensure anaerobic conditions. Batch cultures were carried out at 30°C in an incubator in duplicate. An inverted column containing a 3% sodium hydroxide solution was used for measuring methane. This study evaluated the potential of the sludge and sediments through the following variable responses: the BMP, specific COD consumption rate, and COD removal. The Gompertz equation was used to compute the specific rates through the OriginLab program. In addition, the Tukey statistical test ($\alpha = 0.05$) was used for data analysis in the MiniTab 18 software.

Owen *et al.* (1978) mentioned that to evaluate the organic content in a liquid sample, the BMP should be expressed as L CH₄/ g COD_{removed}. So, according to the following equations, the theoretical BMP calculated according to the environmental conditions of batch cultures was 0.384 L CH₄ / g glucose-COD_{removed}.

$$BMP = \frac{\Delta COD}{K_t} = LCH_4 \quad (1)$$

$$k_t = \frac{p * L}{R * T} \quad (2)$$

Where Δ COD is 1 g of glucose-COD removed and converted to CH₄, R is the gas constant (0.082 atm-L/mol-K), T is the temperature of the experiment (303.15 K), p is the atmospheric pressure (1.01 atm), and L is 64 g COD/mol CH₄.

2.3 Microbial community analysis

A mass of 0.25 g of sediments and anaerobic sludge was used to extract DNA from an extraction kit (QUIAGEN, USA). The methodology detailed in Aguirre-Garrido *et al.* (2016) was employed for DNA extraction, PCR analysis, amplicon multiplexing, and sequencing. 16S rRNA data was processed in MOTHUR software (Schloss *et al.*, 2009). The number of OTUs, indices of Chao1 richness, inverse Simpson diversity, and Shannon diversity were calculated in MOTHUR. Finally, the composition and structure of the bacterial communities were determined with the RDP Trainset 14 Bayesian classifier (Wang *et al.*, 2007).



Figure 1. Sample collection sites in the Lerma River Upper Basin (taken from google earth).

3 Analytical methods

The soluble COD was determined with the closed reflux technique. The volatile suspended solids (VSS) were quantified by the gravimetric technique reported in the standard methods (APHA, 2005). CH_4 was detected by gas chromatography, with He as the carrier gas at a 20 mL/min flow rate and thermal conductivity detector; the injection port, oven, and detector temperatures were 80, 30, and 120 °C, respectively, using a stainless-steel column packed with Porapak T (60/80 mesh) (GOW-MAC Model 580 isothermal 120 V, 60 Hz). The pH was determined with a selective electrode (HANNA Instrument).

4 Results and discussion

Figure 2 shows the COD consumption profiles. The biomass from WWTP and sediments of P_2 took 32 days to remove the initial COD at 100%, whereas the sediments of P_1 and

P_3 took 40 days. The control study showed a fast drop from 0 to 6 days; the profile consumption was slow after that. However, the sediments P_1 , P_2 , and P_3 showed a linear consumption profile from the beginning to the end of the batch cultures. For instance, the samples collected from P_1 and P_2 were more influenced by municipal wastewater, whereas P_3 was more influenced by municipal wastewater, industrial wastewater, and treated water from the WWTP. The control study showed a COD consumption rate of 418.04 ± 88 mg COD/g VSS-d. P_1 , P_2 , and P_3 displayed a COD consumption rate of 7.35 ± 1.47 , 9.78 ± 1.49 , and 13.39 ± 7.93 mg COD/g VSS-d, respectively (Table 1). Statistically, the sediment studies did not show a significant difference.

Figure 3-A shows the methane production profiles; for example, the control study achieved almost 120 mL of CH_4 , but the sediments from P_1 , P_2 , and P_3 produced less, reaching about 72 mL of CH_4 at the end of the batch cultures. The BMP of all studies, including the control, showed an over methane production regarding the theoretical methane production (i.e., $0.384 \text{ L CH}_4 / \text{g glucose-COD}_{\text{removed}}$), as seen in figure 3-B.

According to the COD removed, producing that amount of methane is impossible, so there was another carbon source or the participation of endogenous metabolism. The time of batch culture suggested that anaerobic digestion of the sediments took place, so a control test was carried out without glucose to verify the methane production via anaerobic sludge digestion. The experimental results showed a methane production of 40 mL via anaerobic digestion of the sediments (Figure 3-A). These experimental results justify the over-methane production.

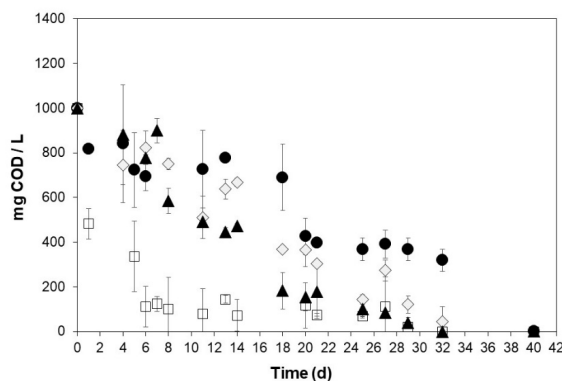


Figure 2. COD consumption profiles. (\square) control, (\bullet) P_1 , (\diamond) P_2 , (\blacktriangle) P_3

Table 1. Kinetic parameters of the batch cultures.

Batch culture	mg COD/g VSS-d	COD removal efficiency (%)	BMP ($\text{L CH}_4 / \text{g glucose-COD}_{\text{removed}}$)
Control (WWTP)	418.04 ± 88	100 ± 0.01	1.49 ± 0.05
P_1	7.35 ± 1.47	99.45 ± 0.07	0.91 ± 0.05
P_2	9.78 ± 1.49	95.41 ± 6.49	0.93 ± 0.05
P_3	13.39 ± 7.93	99.95 ± 0.07	0.90 ± 0.04

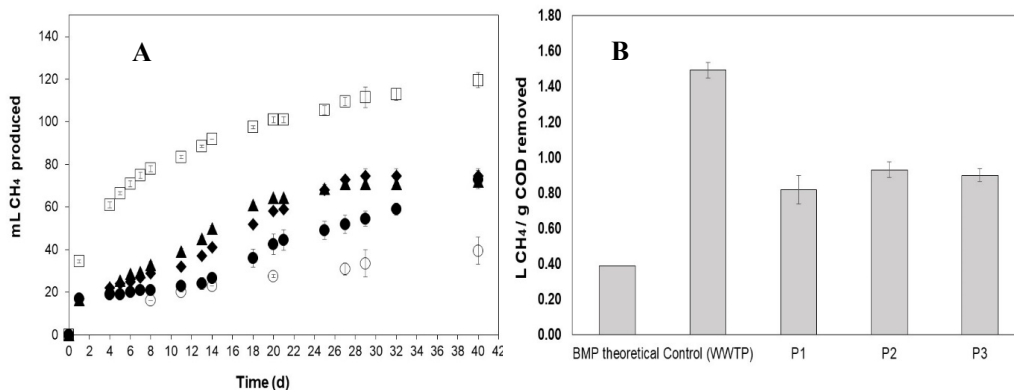


Figure 3. Methane production profiles and BMP. A) Methane production: (□) control, (●) P₁, (◇) P₂, (▲) P₃, (○) digested sediments. B) BMP of the kinetic cultures

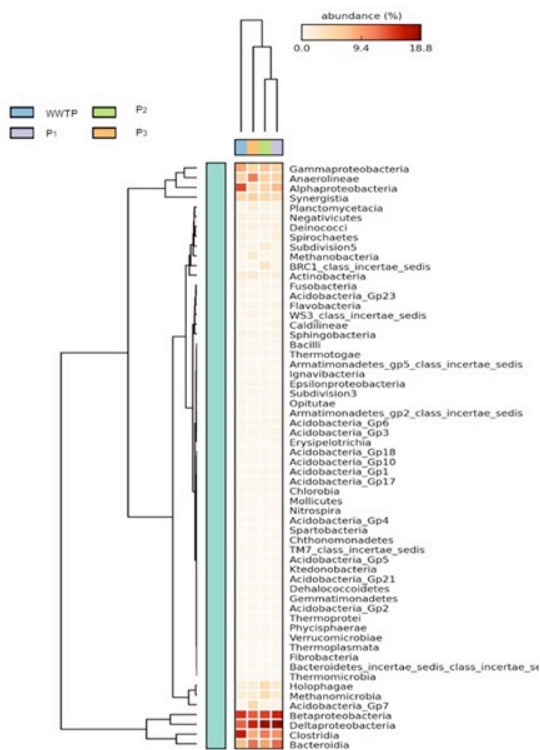


Figure 4. Relative abundance of main bacterial or archaea classes in the samples.

On the other hand, the relative abundance of bacteria was more significant than in archaea in all samples. For instance, the control, P₁, P₂, and P₃ showed a relative abundance of 98.6, 97.25, 96.94, and 96.731% for bacteria, respectively. The relative abundance for archaea was less, 1.397, 2.048, 3.051, and 3.269%, respectively.

The class scheme shows a relative abundance according to the proportion of sequences ranging from 0 to 18.8% (Figure 4). The predominant microbial community was

delta-proteobacteria, *beta-proteobacteria*, *clostridia*, and *bacteroidia*. The microbiome detected is involved in various stages of methane production, such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis. For instance, the group of bacteria belonging to *Proteobacteria* degrades all kinds of carbohydrates by the presence of alpha, beta, gamma, and delta groups (Ariesyady *et al.* 2007). The *clostridia* class belongs to the phylum *Firmicutes*, responsible for hydrolysis, organic matter fermentation, and acetate formation (Fernández *et al.*, 2008, Ali *et al.*, 2020). The class of *Bacteroidetes* plays an essential role in hydrolysis and in producing volatile fatty acids, carbon dioxide, and molecular hydrogen (Traversi *et al.* 2012). *Bacteroides* were also associated with EPS secretion used for granulation or biofilm formation (Yu *et al.*, 2012). Also, in the present work, the class *methanomicrobia* (domain archaea) was identified in all samples. For example, Tabatabaei *et al.* (2010) observed in an anaerobic reactor that all the clones belonging to *methanomicrobia* were associated with the genus *Methanosaeta*, an essential acetoclastic methanogen for methane production in high-strength organic wastewater. On the other hand, *Anaerolineae* was identified in more relative abundance in P₃ than the other samples; for example, in P₃, the chemical composition of the Lerma River is more complex. *Anaerolineae* play a vital role in hydrocarbon-degrading environments under methanogenic conditions (Liang *et al.*, 2017).

Table 2 summarizes the results obtained from the analysis in Mothur. In the control test, a lower number of observed species was found compared to the samples of the Lerma River sediments. These results indicated a more extraordinary richness in the sediments of the Lerma River, which can be due to the diversity of substrates from wastewater discharges. Contrary, the anaerobic reactor (i.e., the WWTP) operates under-regulated operating conditions defined by hydraulic residence times, stable pH, organic load rates without much variation, and defined substrates. The latter could be one of the leading indicators of why there is fewer species diversity in the control sludge.

Table 2. Richness and diversity estimators of bacteria and archaea phylotypes.

Batch culture	No. Sequences	Sobs	Good's coverage	Invsimpson	Shannon	Chao1
Control (WWTP)	8659	3112	0.706	144.75	6.7	25134.04
P_1	8659	4006.99	0.611	284.23	7.22	27040.60
P_2	8659	4122.64	0.594	313.93	7.27	32169.23
P_3	8659	4302.39	0.578	336.74	7.44	36776.89

These experimental results evidenced that the microbiome of the Lerma River sediments showed that the prevailing environmental conditions favored the anaerobic conditions. Sediments exposed to wastewater discharges of chemical complexity make these sediments very attractive for evaluating their metabolic capacity to degrade recalcitrant compounds in future studies. In addition, these sediments might be an excellent inoculum source to inoculate reactors at the laboratory scale or even at the industrial level for inoculation or reinoculation since an inoculum source requirement is one of the main problems in startup wastewater treatment plants under anaerobic conditions because the inoculum is scarce or difficult to obtain, or sometimes it is for sale at a high cost.

Conclusions

Characterizing the sediments from the Lerma River displayed an excellent biochemical methane potential during glucose degradation. Sediments displayed microflora involved in anaerobic digestion, such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The main classes identified were *delta-proteobacteria*, *beta-proteobacteria*, *clostridia*, *bacteroidia*, and *methanomicrobia*. Finally, this work showed evidence of the sediments' high potential to produce methane, and they might be an excellent source of inoculum for wastewater treatment plants.

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