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Methanogenesis in sediments of a tropical coastal wetland: a culture-dependent method

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ABSTRACT

Introduction: Methanogenic archaea (MA), participate in the anaerobic mineralization of organic matter in mangrove sediments, their activity is related to atmospheric warming due to the production of methane; several environmental variables can influence the presence of MA and methane production in these sediments.

Objective: To analyze, through culture-dependent techniques, viable methanogenic archaea (VMA) in the sediments, and the production of methane from acetate in different climatic periods in the mangrove El Morro-La Mancha, Veracruz, Gulf of Mexico.

Methods: From May to November 2019, following a salinity transect, sediment samples from El Morro-La Mancha mangrove were collected at three locations, in three different climatic seasons, dry (May), rainy (October) and northern (November) (N = 9). VMA in the sediments was quantified using the Most Probable Number (MPN) technique with acetate and methanol as substrates. The influence of sulfate on methane production was analyzed from acetate in microcosm by gas chromatography and the chemical variables of salinity, pH, Eh, carbohydrates, organic content, and carbonates in the sediments were evaluated.

Results: The abundance of VMA was 10^2 to 10^8 MPN/g of wet sediment, higher than that reported in other studies, this abundance was higher when methanol (10^4 - 10^8 MPN/g sediment) was used as substrate, compared to acetate (10^2 - 10^5 MPN/g sediment); methane production in the microcosms increased in sulfate-free conditions (29.78-929.75 nmol CH₄/month) and in the sediments of the rainy season.

Conclusion: The influence of the chemical conditions of the mangrove sediments on the methanogenic dynamics is highlighted, determining that in the rainy season, the decrease in salinity, more electronegative Eh, and the increase in organic fractions favored the methanogenesis.

Key words: acetate; climatic season; mangrove; methanol; microcosms; viable methanogenic archaea; sulfate.

RESUMEN

Metanogénesis en sedimentos de un humedal costero tropical: un método dependiente de cultivo

Introducción: Las arqueas metanogénicas (MA) participan en la mineralización anaerobia de la materia orgánica en sedimentos de manglar, su actividad está relacionada con el calentamiento atmosférico por la producción de metano; diversas variables ambientales pueden influir en la dinámica metanogénica y la producción de metano en estos sedimentos.

Objetivo: Analizar, mediante técnicas dependientes de cultivo, la abundancia de las arqueas metanógenas viables (AMV) y la producción de metano en diferentes épocas climáticas en los sedimentos del manglar El Morro-La Mancha, Veracruz, Golfo de México.



Métodos: De mayo a noviembre 2019, siguiendo un transecto de salinidad, se recolectaron sedimentos del manglar del Morro-La Mancha, en tres localidades, abarcando tres diferentes épocas climáticas, secas (mayo), lluvias (octubre) y nortes (noviembre) (N = 9), cuantificándose la abundancia de las AMV mediante la técnica del Número Más Probable (NMP) con acetato y metanol como sustratos; la influencia de los sulfatos en la producción de metano a partir de acetato en microcosmos, se analizó por cromatografía de gases y se evaluaron las variables químicas de salinidad, pH, Eh, carbohidratos, contenido orgánico y carbonatos en los sedimentos.

Resultados: Se determinó una abundancia de AMV de 10^2 a 10^8 NMP/g de sedimento húmedo, superior a la reportada en otros estudios, dicha abundancia se incrementó cuando se utilizó metanol (10^4 - 10^8 NMP/g sedimento) como sustrato, en comparación con el acetato (10^2 - 10^5 NMP/g sedimento); la producción de metano en los microcosmos aumentó en condiciones libres de sulfatos (29.78-929.75 nmol CH_4 /mes) y en los sedimentos de la temporada de lluvias.

Conclusión: Se destaca la influencia de las condiciones químicas de los sedimentos del manglar sobre la dinámica metanógena, determinándose que, en la temporada de lluvias, una disminución de la salinidad, Eh más electro-negativos y el incremento de las fracciones orgánicas favorecieron la metanogénesis.

Palabras clave: acetato; arqueas metanógenas viables; estación climática; manglar; metanol; microcosmos; sulfatos.

INTRODUCTION

Mangrove forests are coastal wetlands located in tropical and subtropical latitudes, adjacent to coastal lagoons and estuaries, which occupy an area of approximately $1.8 \text{ km}^2 \times 10^5 \text{ km}^2$ in tropical and subtropical latitudes (Alongi, 2002; Zhang et al., 2021). They are extremely complex ecosystems formed by woody trees that experience tidal and freshwater influence, and with numerous interactions between vegetation, animals, and microorganisms, Mangrove forests are considered among the most productive wetlands on Earth, with high rates of recycling of organic matter and nutrients. These forests harbor high biodiversity and provide several ecosystem services (Zhang et al., 2021).

The sediments of the mangroves are characterized by a high content of salts (20.8-22.8 PSU), hydrogen sulfide (1-25 mM) (Bhattacharyya et al., 2015), a redox values low (-150 to -200 mV), a low or no oxygen content and a high proportion of organic matter (8-30 %), mainly coming from the accumulation of leaf litter of the mangrove trees (Lyimo et al., 2002a; MacFarlane et al., 2007; Zhou et al., 2010). The development of anaerobic metabolisms is favored in these sediments, highlighting the sulfate reduction and methanogenesis in

the terminal phases of the mineralization of organic matter (Taketani et al., 2010).

Methanogenesis is a process carried out by the methanogenic archaea (MA), a group of prokaryotes of anoxic habitats (Conrad, 2020), due to the sensitivity of methyl-coenzyme M reductase to oxygen, which catalyzes the last reaction for the formation of methane (CH_4); however, methanogens that have genes against oxidative stress have been reported in arid areas (Lyu et al., 2018). Historically MA had been classified as a phylogenetically diverse group of the phylum Euryarchaeota (Methanococcales, Methanopyrales and Methanobacteriales). However, new phylogenetic analyzes have revealed greater diversity, which includes other phyla, Halobacteriota (Methanomicrobiales, Methanocellales, Methanonatronarchaeales, and Methamosarcinales), Thermoplasmata (Methanomassiliicoccales), and potentially Crenarchaeota, and this continues to be reviewed. In addition to methanogenesis carried out by archaea, it has been proposed that other metabolic pathways present in fungi, plants and animals can produce methane (Buono de Mesquita et al., 2023; Lyu et al., 2018; Zhang et al., 2021).

The energy metabolism of methanogenesis involves the formation of CH_4 from organic compounds such as acetate, formate, or short-chain methylated compounds like methanol;

or MA can also grow autotrophically, from the reduction of CO_2 by H_2 (Kurth et al., 2020; Thauer et al., 2008). In this sense, the acetate and the H_2 - CO_2 also represent important substrates for the sulfate reducing bacteria (SRB), so that a competition for such substrates can start between those and the MA (Conrad, 2020). However, in the mangrove sediments, the coexistence of both microbial groups has been demonstrated (Lyimo et al., 2002b; Taketani et al., 2010). In the mangrove sediments, it has been established that the abundance and richness of MA are related to several environmental factors, especially temperature, pH, oxide-reduction potential (Eh) and quantity and quality of organic matter (Euler et al., 2020; Yasawong et al., 2013).

The study of the methanogenesis process can be carried out using specific culture media that allow the growth and quantification of viable microorganisms, as well as laboratory experiments (microcosm) in which, under controlled conditions, the aim is to analyze the utilization of a substrate by MA or the effect of an electron acceptor on methanogenic metabolism (Bueno de Mesquita et al., 2023). One of the traditional microbiological methods used to study MA is MPN, which evaluates the size of a microbial population in a liquid culture medium and is useful for estimating the abundance of VMA from the formation of their product metabolic, CH_4 (Wagner et al., 2012). For the cultivation of VMA, a medium that contains minerals, trace metals, vitamins and methanogenic substrates is necessary, and these components are in Balch's culture medium.

The methanogenic activity is related to the global climate change through production CH_4 and CO_2 , important gases involved in the greenhouse effect, responsible for global warming (Conrad, 2020). Estuaries, coastal lagoons, and mangroves are the main marine ecosystems that emit methane into the atmosphere (Arai et al., 2021; Purvaja et al., 2004).

Globally, Mexico has an area of 51 610 km^2 of mangrove forests, occupying the fourth place in coastal wetlands extension, after Indonesia, Brazil, and Australia. The mangroves in Mexico are under the category of special

protection according to the Official Mexican Standard NOM-059-ECOL-2010 (2010), being threatened by the deforestation for agricultural activities and the urban development. It has been estimated that the mangrove deforestation generates about 10 % of the global carbon emissions per year (SEMARNAT, 2016).

The mangrove El Morro-La Mancha is located around the coastal lagoon of La Mancha, in the state of Veracruz (Gulf of Mexico). In this ecosystem, as far as it is known, there is no information available on the methanogenic activity in sediments, although there is a study on the dynamics of MA and SRB in the estuarine sediments of the La Mancha coastal lagoon (Torres-Alvarado et al., 2016). Therefore, the objective of the present study was to analyze, using the cultivation dependent techniques, viable methanogenic archaea (VMA) in the sediments at different climatic periods, and the influence of sulfates on methane production from acetate in microcosms, in the mangrove El Morro-La Mancha, associated with an intermittent coastal lagoon and its possible relation to chemical characteristics of the sediments.

MATERIALS AND METHODS

Study site: The mangrove forest El Morro-La Mancha borders the coastal lagoon of La Mancha. It is located on the coastal plain of the Gulf of Mexico in the state of Veracruz, at $19^\circ 51' - 19^\circ 61' \text{ N}$ & $96^\circ 37' - 96^\circ 44' \text{ W}$. The climate of the region is warm-subhumid, with three climatic seasons: dry (March to May), rainy (June to October) and northern (November to February). The dry season has high temperatures and low rainfall ($44 \pm 37 \text{ mm}$), while maximum rainfall ($224 \pm 25 \text{ mm}$), as well as an increase in river volume and land runoff characterize the rainy season. In the northern season, cold fronts cause a temperature decrease and occasional rains.

The mangrove forest El Morro-La Mancha is influenced by the freshwater inflow of Caño Grande River, as well as the marine influence through the partial communication of the lagoon with the ocean, which generally is interrupted during the northern season. The process



of opening and closing of the inlet influences the dynamics of physicochemical parameters.

The Morro-La Mancha is a low tree mangrove, whose trees have an average height of 8.1 m, formed mainly by the basin mangrove and, to a lesser extent, by the fringe and riverside mangroves. Its characteristic species are *Avicennia germinans* L. (black mangrove), *Laguncularia racemosa* (L.) C. F. Gaertn (white mangrove), *Rhizophora mangle* L. (red mangrove) and *Conocarpus erectus* L. (button mangrove) (Moreno-Casasola, 2003). The four species are under the category of threatened in the Official Mexican Standard NOM-059-SEMARNAT-2010. Nevertheless, the mangrove in some areas is impacted by the livestock activities (paddock transformation) and the agricultural ones (sugar cane crop). The anthropogenic activities have caused the decrease of the mangrove forest extension from 430 ha in 1976 to 268 ha in 2010 (López-Portillo et al., 2009).

The mangrove species of El Morro-La Mancha are distributed in three hydrographic regions related to the salinity gradient: a) Oligohalin Region in the inflow of Caño Grande river, where *R. mangle* predominates with 73-76 % of the total abundance, followed by *A. germinans*; b) Oligohalin-Mesohalin Region, in the impacted area, with 64.4 % of *R. mangle*, and c) Mesohalin region, close to the communication with the sea, with a predominance of *L. racemosa*, which can reach up to the 89.8 % of the total of mangrove species.

Field work: Sediment samples were collected in three mangrove sites associated with the salinity gradient in the months of dry (May), rainy (October) and northern (November) seasons. Site one was in the influence of the *Caño Grande River* (oligohalin region), site two was located on La Pajarera island (oligohalin-mesohalin region) and site three was located near of communication to the ocean, where marine influence increases (mesohalin region).

The sediment was collected with a 4.5 cm diameter acrylic corer which was introduced to a depth of 20 cm and the temperature was

evaluated only in the superficial layer with a thermometer. Subsequently each core was stored vertically in a container until their processing in the laboratory.

Laboratory work: Vertical profiles of pH and Eh of each sediment core were evaluated with a Unisense brand Microprofiler (RD-N-5409/picoammeter PA 2000/ Reference-5160); for the concentration of hydronium ions, a Unisense-3390 pH sensor was used, and for the redox potential, an RD-N-5409 Unisense sensor and a reference one (5160).

Sample preparation: Every core had a total depth of approximately 14-15 cm and was divided into two strata of sediment in an atmosphere of nitrogen to carry out the microbiological and chemical analyses: the first layer (surface) was 0 to 6 cm deep and the second one (bottom) was 6 to 12 cm deep. The selection of the layer was related to the intervals of quantified Eh, slightly reducing from 0-6 cm (-10 to -100 mV) and totally reducing from 6-12 cm (-100 to -300 mV).

Quantification of viable methanogenic archaea: The technique of the Most Probable Number (MPN) was used, performing serial dilutions of each sample (10^{-1} a 10^{-10}), with three tubes per dilution and a control one.

The culture medium of Balch et al. (1979), with acetate and methanol as substrates, at a final concentration of 20 mM, was used. Acetate and methanol are important substrates for methanogenesis in mangrove sediments, acetate represent approximately 67 % of methane production, while methanol is an important non-competitive substrate in brackish environments with high organic matter content (Conrad, 2020). The pH and the salinity of the medium were adjusted to match those evaluated in the sediment samples. NaS-9H₂O at 2.5 % was added to decrease the Eh.

The incubation was carried out for 30 days with the temperature quantified in the surface layer of sediment, at the end of the incubation, the tubes that produced methane were

considered positive. Methane was detected with a GOW-MAC Series 580 GC Gas Chromatograph, equipped with a thermal conductivity detector (Torres-Alvarado et al., 2016).

Methane production experiments: The experiments consisted in inoculating 5 ml of wet sediment in serum bottles, with 45 ml of the basic culture medium of Balch et al. (1979), adding sodium acetate, as a substrate, to a final concentration of 20 mM. To evaluate the effect of sulfates on the production of methane from acetate, the experiments were carried out by duplicate both in the presence and in the absence of sodium sulfate at a final concentration of 20 mM. The bottles were incubated in the darkness at 32 °C and the mineralization was evaluated by measuring changes in the percentage of methane for 30 days, using a GOW-MAC gas chromatograph, equipped with a thermal conductivity detector (Torres-Alvarado et al., 2016).

Analysis of the chemical characteristics: 40 g of homogenized sediment were weighed and centrifuged at 3 600 rpm at a room temperature for 20 min, to separate the interstitial water from the sediment. In the interstitial water, the salinity was determined by a refractometer (Hanna Instruments) and the total dissolved carbohydrates were evaluated using the Strickland and Parsons' methodology (1972).

In the wet sediment samples, the volatile solids (VS, organic fraction) and fixed solids (FS, inorganic fraction) was quantified using the American Public Health Association methodology (APHA et al., 2005). The carbonate content was determined from the ignition of the sample at 990 °C.

Data analysis: The data matrix included the abundances of VMA, and the chemical variables. To meet the assumptions of normality, the data of the variables were logarithmically transformed (Zar, 1999). For the seasonal analysis, the variables were grouped into three seasons (dry, rainy, and northern); for spatial analysis, the data were grouped into two depth

categories (surface area, from 0 to 6 cm, and the bottom one, from 6 to 12 cm). An analysis of variance (ANOVA) was performed to test significant differences between stations, depth, and the behavior in methane production in the microcosmos. Subsequently, through the Tukey test, a multiple comparison of means was made (Zar, 1999). In addition, a principal components analysis (PCA) and a redundancy analysis (RDA) were made to investigate the relationship between the microbial density, and the environmental variables (Infante-Cangrejo & Donato-Rondón, 2017; Lozano et al., 2019; Van den Wollenberg, 1977). All the analyses were performed using the statistical package R (R Core Team, 2020).

RESULTS

Environmental characteristics: The chemical characteristics of the mangrove sediments in El Morro-La Mancha, presented changes related to the climatic season and the sediment depth (Table 1). In the dry season there was a greater salinity ($P < 0.05$) than in northern and rainfall due to a decrease in the freshwater inflow, while, the pH values were in the basic range, being higher in rainfall. The greatest variations were determined in the Eh and the VS concentration ($P < 0.05$). The Eh was less electronegative in the dry season, compared to the rainy and northern ones. A similar behavior was recorded in the concentration of the VS, with minimums in the dry climate and maximum values in rainfalls ($P < 0.05$). Likewise, a significant increase in the amount of carbohydrates was determined in the rainy season compared to the northern and dry ones ($P < 0.05$). There were no significant changes neither in the concentration of the FS nor the carbonates ($P < 0.05$). Spatially, no significant changes in the chemical characteristics were determined, an exception was the salinity ($P < 0.05$).

Structure of the abundance and the distribution of VMA: The abundance of two

Table 1
Environmental variables in the sediments of the mangrove La Mancha, Veracruz.

Season	Northern		Dry		Rainy	
	6	12	6	12	6	12
Layer (cm)	6	12	6	12	6	12
Salinity	20 ± 0	22 ± 3.46	22.67 ± 2.08	25 ± 1.0	18.67 ± 0.58	20.33 ± 0.58
pH	7.59 ± 06	7.50 ± 0.09	7.69 ± 0.15	7.57 ± 0.09	7.89 ± 0.39	8.43 ± 0.44
Eh (mV)	-137.65 ± 104.64	-153.61 ± 120.24	-13.94 ± 8.18	-27.25 ± 23.54	-125.61 ± 78.5	-170.83 ± 109.1
FS (g/l)	345.5 ± 286.4	340.2 ± 299.2	170.8 ± 192.6	147.8 ± 128.8	179.4 ± 74.9	229.8 ± 113.3
VS (g/l)	188.61 ± 93.1	216.34 ± 186.6	7.16 ± 5.49	6.94 ± 4.02	270.78 ± 65.39	347.91 ± 49.6
Carbonates	6.53 ± 2.84	7.80 ± 4.41	4.83 ± 4.57	5.41 ± 4.96	12.23 ± 10.87	12.25 ± 11.46
Carbohydrates (g/l)	0.08 ± 0.03	0.09 ± 0.03	0.07 ± 0.08	0.04 ± 0.05	0.14 ± 0.01	0.13 ± 0.02

Mean ± Standard deviation.

nutritional groups of VMA was quantified: those that grow from the acetate, or acetoclastic (VMA-A) and those that use methanol, or methylotrophic (VMA-M). The global density of VMA was in an interval from 10^2 to 10^8 MPN/g of wet sediment. By physiological group, the abundance of VMA-A, had an interval from 10^2 to 10^5 MPN/g of wet sediment and, although their density was higher in rains (Fig. 1), with lower salinity conditions, no differences were registered between climatic seasons ($P > 0.05$). On the contrary, with respect to the vertical distribution, the highest abundance was determined by increasing the depth of the sediment, mainly in the rainy season ($P < 0.05$, Fig. 1).

In the case of VMA-M, their density had an interval from 10^4 to 10^8 MPN/g of wet sediment, higher than that of VMA-A and, contrary

to these, there were important changes in their abundance between the climatic seasons, reaching higher levels in rainy and northern ones than in the dry one ($P < 0.05$). However, its distribution by sediment layer was homogeneous ($P > 0.05$, Fig. 1). This study has revealed that apparently the effect of the depth is important in the changes of the density of the VMA-A, while for the group that grows from methanol their distribution pattern is more related to the weather season, regardless of the depth.

Methane production experiments: In all experiments, with sulfates or sulfate-free conditions, CH_4 production was quantified; however, in the case of microcosms with sediments from the rainy season, production began from day 6 while in the dry and northern samples, the formation of CH_4 was recorded until days 12-24.

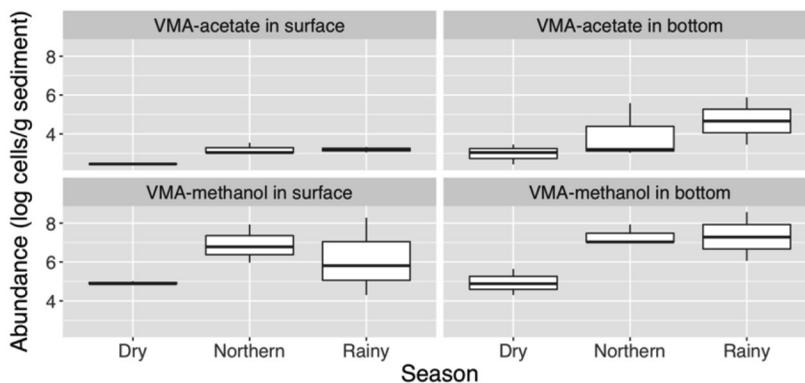


Fig. 1. Seasonal and spatial variation in the abundance of VMA in the sediment of the mangrove El Morro-La Mancha, Veracruz, Gulf of Mexico.

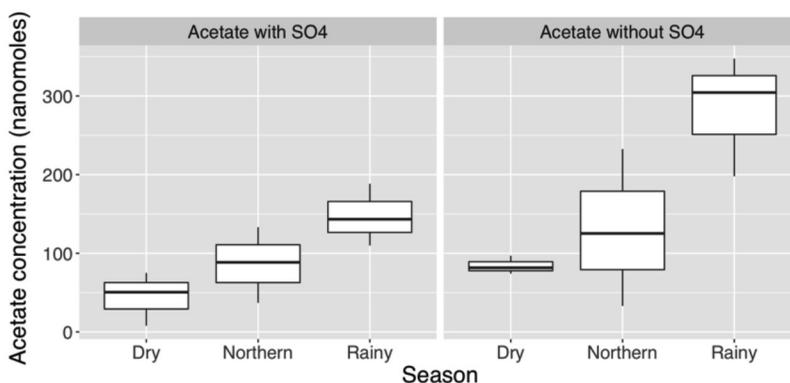


Fig. 2. Methane production from acetate, with sulfates and sulfate-free conditions.

Significant differences ($P < 0.05$) were determined in methane production between treatments, under sulfate-free conditions the production of methane was higher than in the experiments with sulfates, quantifying an interval from 29.78-929.75 nmol CH_4 /month, on the contrary, an interval of 7.88-347.38 nmol CH_4 /month was determined in the experiments with sulfates. Likewise, the differences in methane production were significant ($P < 0.05$) between the samples from the different climatic periods, an increase in the methane production can be observed in the absence of sulfates in rainfall, compared to northern and the dry seasons (Fig. 2).

Environmental variables and VMA: The diagram obtained by the PCA allowed to quantify the effect of the environmental variables regarding the seasons and the structure of the VMA. For the analysis of the abundances, two components were obtained: the first one explained the 44.80 % of the total variance and the second one the 21 %, which, together, explain an accumulated variance of 65.80 % (Fig. 3). The variables with the highest participation in the definition of the first component were the salinity, pH, Eh, VS and carbohydrates; they contributed to the presence of the VMA of both nutritional groups. The second component was defined by the FS and the carbonate content, with little effect on the VMA. It

was also observed that the chemical conditions in the rainy season contribute to increasing the density of the VMA (Fig. 3).

Considering the redundancy analysis (RDA), the component 1 showed that the environmental variable which had a greater direct influence on the VMA-A was the Eh, contrary to the VS and the carbohydrates. In addition, considering component 2, the environmental

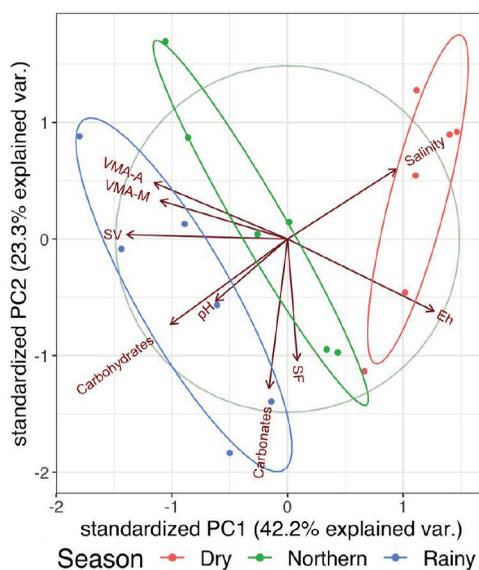


Fig. 3. PCA showing the relationship between VMA and environmental variables in the sediment of the El Morro-La Mancha mangrove, Veracruz, Gulf of Mexico.

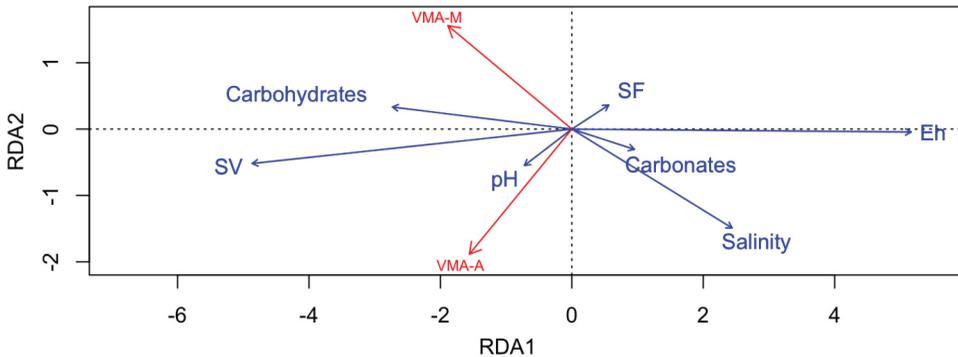


Fig. 4. Redundancy analysis of the relationship between environmental variables and MA in the sediment of the mangrove El Morro-La Mancha, Veracruz, Gulf of Mexico.

variable that had the greatest inverse influence on the VMA-M was the salinity (Fig. 4). Likewise, Eh and the salinity contribute to characterize the dry season, the FS characterizes the northern, while the VS, carbohydrates and pH characterize the rainy season (Fig. 4).

DISCUSSION

Environmental characterization: The changes observed in chemical characteristics of the mangrove sediments along the climatic season and the depth, were similar those reported in other studies (Bhattacharyya et al., 2015; Dias et al., 2011; Taketani et al., 2010). The changes in the interstitial water salinity were caused mainly by the influence of the freshwater contributions. It has been established that, on a seasonal scale, in the rainy season, when the volume of the river discharge and precipitation increases, there is a decrease in the salinity, while during the months of northern the salinity is the result of the dilution effect caused by the rains of the previous season (Díaz et al., 2017). On the contrary, the increase in the salinity during the dry season is caused by a lower inflow of freshwater, zero or low precipitation and an increase in the evaporation rate (Lara-Domínguez et al., 2006).

In the coastal ecosystems the variations of pH depend on the marine influence, precipitation, the amount of runoff, the origin of the

soils, the removal of the sediments by currents and the biological activity of the organisms. Biological processes, such as photosynthesis, respiration, and mineralization of the organic matter, are the ones that most influence pH, due to the changes they cause in the concentration of the carbon dioxide (De La Lanza Espino, 1994; Torres-Alvarado et al., 2016). In the lagoon of La Mancha to which the mangrove forest studied is associated, the changes in the pH have been related to the cycle of high and low tides: the first ones cause an increase of pH due to the adding of the alkaline seawater, while the latter cause the opposite process.

The sediments presented reducing characteristics ($Eh < 0$). The vertical changes observed could be related to a lower diffusion of the oxygen in the sediments, which causes a decrease of the Eh with the depth, forming a strong gradient of the redox potential that influences the sequence of metabolic reactions that occur during the degradation of the organic matter (Stolzy et al., 1981). It has been established that the sediment reducing characteristics favor the production of methane (Li, 2000).

Carbohydrates constitute a fundamental fraction of the organic carbon, they contribute between 14 % and 21 % of it to the coastal ecosystems (Børsheim et al., 1999; Preston & Prodduturu, 1992). In the mangrove of El Morro-La Mancha, the highest concentration of carbohydrates was determined in the rainy

season, when the freshwater inflow increase. Carbohydrates can be added by rivers and by vegetable matter, roots, and leaves of the mangroves; although they can be also originated by the agricultural activities of the cane industry and the discharge of the wastewater (Paez-Osuna et al., 1998).

Methanogenic dynamics: The VMA were present in an interval higher than that reported by Lyimo et al. (2009), who quantified an abundance of 10^5 - 10^6 MPN/g cells in the sediments of the mangrove of Mtoni, Dar-ES-Salaam, Tanzania. The seasonal and spatial variations observed in the abundance of the MA of the nutritional groups analyzed were related to the changes in the chemical characteristics of the sediments. The relative abundance of different types of methanogens in mangrove sediments may be regulated by the availability of substrates (quantity and quality of organic compounds), as well as by various environmental variables, including temperature, salinity, pH, and oxide-reduction potential of sediment (Bueno de Mesquita et al., 2023; Liu & Whitman, 2008).

In the present study, the abundance of the VMA in the rainy season was favored by the existing environmental conditions, mainly salinity (18 to 21), alkaline pH conditions (7.8 to 8.4) and Eh reducers (-125 to -250 mV), like those reported in other studies in mangrove sediments (Lyimo et al., 2009). The presence of MA in mangrove sediments has been associated with pH values of 6.6 to 7.2 (Mohanraju & Natarajan, 1992). Also, in the rainy season, the increase of VS and carbohydrates was important for the development of the MA. Taketani et al. (2010) mention that the presence of the methanogenic community in mangrove sediments is favored by organic matter, since its abundance and variety increase with a greater concentration of organic components.

Although the presence of the MA was determined in the two layers of the sediment, as it has been reported in other studies, in which an active methanogenic community and the production of methane have been detected at

depths of the sediment ranging from 0 to 30 cm (Lyimo et al., 2002b), it is evident that there were spatial changes, where the greatest abundance of the MA was found at greater depth. In several studies, the greatest abundance and methanogenic activity have been recorded in deep layers of the sediment, where the redox potential is more electronegative and where the concentration of sulfates decreases and the content of organic matter increases (Jing et al., 2016; Wilms et al., 2007). In the sediments of mangrove forests, with a high contribution of organic matter, methanogenesis can become the predominant terminal process in the anaerobic food chain, once the sulfate concentration decreases (Parkes et al., 2007).

Regarding the dynamics of nutritional groups, the lower density of VMA-A, compared to the VMA-M, may be a result of acetate also being used by the SRB, although in this study the presence of the SRB was not analyzed, Torres-Alvarado et al. (2016) demonstrated the presence of the SRB and the MA, which used acetate in the sediments of La Mancha lagoon. The acetate is a product that is released during the fermentation processes of organic matter and, being a common source of carbon for SRB and MA, there may be competition between both microbial groups (Holmer & Kristensen, 1994). The sulfate reduction process from acetate is favored for thermodynamic reasons, since it produces more energy per mole of acetate (ΔG° of -43.8 kJ/mol), while the formation of methane produces a ΔG° of -19.9 kJ/mol (Canfield et al., 2005; Howarth, 1993).

The influence of the sulfate in the production of methane from acetate was observed in microcosm experiments, where methane production decreased in culture media where sulfates were added. In laboratory experiments it has been shown that methane production decreases when more energetically favorable electron donors are added, such as sulfates (Achnich et al., 1995) and although with the presence of sulfates it dominates sulfate reduction, methanogenesis can continue at the expense of other substrates, such as different methylated compounds. In addition, it



has been demonstrated in experiments with mixed pure cultures, that the MA and the SRB can coexist in syntrophic relationships due to the release and consumption of hydrogen (Ozuolmez et al., 2015).

Methylotrophic methanogenesis is common in saline environments, such as coastal wetlands, where AM convert so-called non-competitive substrates such as methanol, methyl and trimethylamines, and methyl sulfides to CH_4 and CO_2 . Methanol is a fraction of plant organic matter, it is released as a product of the degradation of pectin and lignin, being important in mangrove areas and favors the growth and development of the MA in the presence of the SRB (Lyimo et al., 2002b), which would explain the dominance of the VMA-M. In the sediments associated with mangroves, the MA that use methanol, methylamines and trimethylamines are an important methanogen component (Bueno de Mesquita et al., 2023; Mohanraju et al., 1997).

Although in the present study a higher MPN of VMA-M was quantified compared to VMA-A, it is important to consider that some studies report that their contribution to methane formation is reduced, between 1-10 % of the total, since it can be used as a carbon source in other anaerobic metabolisms such as denitrification (Conrad & Claus, 2005). In this sense, it is mentioned that, in marine environments and coastal wetland sediments, methylamine could represent a more important methylated substrate than methanol. Methylamine is formed from the production of glycine betaine, an excretion product of marine organisms and is an easily degradable substrate through methanogenesis and, like methanol, is a “non-competitive” substrate (Conrad, 2020; Bueno de Mesquita et al., 2023); therefore, it would be of interest to consider the use of this substrate in subsequent studies of methanogenesis in mangrove sediments. The use of specific inhibitors for methanogenesis (2-Bromoethanesulfonic acid or chloroform) or sulfate reduction (sodium molybdate), could also provide more detailed information on the importance of acetate in metabolic processes.

The objective of this manuscript was to analyze the presence of viable methanogenic archaea in mangrove sediments in different climatic periods, as well as the influence of sulfates on the production of methane from acetate in laboratory experiments. However, although methanogenesis can be analyzed using culture-based methods, the use of molecular analyzes could provide important information related to the composition of MA, the genes and biochemical pathways involved in methane production with different environmental conditions. In this regard, studies based on independent cultivation techniques have reported a number of archaeal 16S rRNA genes ranges from 10^7 to 10^{10} copies/g dry sediment (Zhou et al., 2017), and a diversity of acetoclastic MA that includes members of the families Methanosarcinaceae and Methanotrichaceae, while methylotrophic methanogens have a phylogenetic affiliation with the genera *Methanococcus* and *Methanometylovorans* (Conrad, 2020; Kurth et al., 2020; Lyimo et al., 2009; Yasawong et al., 2013).

It was identified that salinity, pH, redox potential, volatile solids, and carbohydrates were the main variables that were influenced by the seasonality in the area, and the seasonal variation of the chemical characteristics of the sediments of the mangrove of El Morro-La Mancha, influence the presence and distribution of the VMA-A and VMA-M. In the rainy season, the viable methanogenic archaea increased when lower salinity, more electro-negative oxide-reduction potentials and a higher concentration of organic components were recorded. These characteristics would determine that rains are an important factor related to the climate change. The production of methane from acetate in the presence of sulfates and the quantification of VMA indicates that the processes of sulfate reduction and methanogenesis can coexist in mangrove sediments of El Morro-La Mancha. However, due to the lack of molecular analysis, diversity of methanogenic communities cannot be draw, further research is necessary at respect.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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