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Biodegradation of hexadecane using sediments from rivers and lagoons of the Southern Gulf of Mexico

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ABSTRACT

The Southern Gulf of Mexico is an area highly impacted by crude oil extraction, refining activities and the presence of natural petroleum seepage. Oceanic currents in the Gulf of Mexico continually facilitate the transport of hydrocarbons to lagoons and rivers. This research evaluated hexadecane (HXD) degradation in marine sediment samples from lagoons and rivers that are fed by the Southern Gulf of Mexico, specifically six samples from rivers, three samples from lagoons, and one sample from a marine outfall. The highest rates of biodegradation were observed in sediments from the mouths of the Gonzalez River and the Champotón Lagoon. The lowest consumption rate was found in sediment from the mouth of the Coatzacoalcos River. With regards to the Ostión Lagoon and the Grijalva River, there was a low rate of consumption, but a high efficiency of degradation which took place at the end of the experiments. No correlation was found between the consumption rate and the environmental physicochemical parameters.

1. Introduction

The largest and most important base of the petroleum industry in Mexico is located in the Southern Gulf of Mexico, which is comprised of the coastal regions of the states of Campeche, Tabasco and Veracruz, where the extraction and maritime transportation of hydrocarbons occurs (García-Cruz and Aguirre-Macedo, 2014). This area also includes commercial tourism and various maritime activities, including the operation of artisanal fisheries. Accordingly, these coastal waters are continuously exposed to compounds such as gasoline, diesel and other kinds of hydrocarbons. The ocean currents intensify problems and can transport and concentrate the pollutants on the coast (Martínez-Lopez and Parés-Sierra, 1998).

The Gulf of Mexico has been affected by notable petroleum spills, including an incident that occurred in 2010 in the Macondo well (Deepwater Horizon oil spill), which discharged an estimated of 795 million L of oil into the Ocean (Kleindienst et al., 2015). In 1979, the Ixtoc well suffered a spill which caused a massive discharge of approximately 317 million L of crude oil. After the spill, oil from the Ixtoc well washed onto the shores of the state of Campeche, causing a major ecological impact in the area (Wang, 2011). Ixtoc petroleum contains hydrocarbons with > 20 carbon atoms (Boehm and Feist, 1982), thereby making it insoluble and non-volatile in the environment. However, these hydrocarbons lead to problems of toxicity and bioaccumulation in organisms living on the coast, which has been found to cause similar problems with regards to compounds like phenanthrene and anthracene (Michel, 1992). It has also been reported that some petroleum compounds can affect marine organisms by inhibiting metabolic processes and may eventually lead to the death of such organisms. In some cases, polycyclic aromatic hydrocarbons (PAHs) could seriously disrupt the food chain, causing even more serious ecological problems. With regards to microorganisms, these PAHs can destabilize the cellular membrane and can affect the metabolic pathway of carbon, nitrogen or sulfur, which also affect the geochemical cycles on the coast (Doyle et al., 2008).

Hydrocarbons from spill can be adsorbed tightly in sediments. So that, they can be potentially bioavailable to sediment microorganisms, as well as to higher benthic and water column organisms, representing a threat to the marine environment. Hydrocarbons can be covalently bonded to sediment humic substances or physically sorbed, favoring

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their accumulation. After long equilibration times, hydrocarbons sorbed by natural sediment organic matter become tightly bound to the sediment organic fraction and are no longer available to soil organisms, decreasing the rate of consumption (Seymour and Geyer, 1992).

In marine and freshwater sediments, the presence of microbial communities capable of degrading some petroleum has been reported (Fuentes et al., 2014; Yanggou et al., 2014). Bacosa and Inoue (2015) and Bacosa et al. (2013), reported the presence of bacteria of the genera *Burkholderia* and *Pseudomonas* in marine sediments and mangroves, respectively. These specific bacteria have the capacity to degrade polycyclic hydrocarbon compounds. Additionally, bacteria consortia can consume heavy oil. After the Macondo oil spill, bacteria of the genera *Alcanivorax*, *Marinobacter*, *Pseudomonas*, and *Acinetobacter* were reported on shorelines of the Gulf of Mexico and were impacted or contaminated by hydrocarbons (Kostka et al., 2011).

Commonly, hexadecane (n-C_{16}) is used as a reference to evaluate the biodegradation of aliphatic compounds and metabolic studies (Dombrowski et al., 2016). Fuels such as the gasoline and diesel used in most combustion engines are dominated by aliphatic compounds (TOXNET, 2016; ITRC, 2014; Chenier et al., 2003). Microbial degradation is an alternative that has been applied as a remediation technique in hydrocarbon-polluted sites. The rate of petroleum degradation depends on the microbiological and physicochemical properties of the specific site (Varjani and Upasani, 2017).

In the marine environment, it is important to examine how environmental factors and endemic microbial communities can affect the degradation of hydrocarbons. There appear to be no studies evaluating hydrocarbon degradation using marine sediments from the Southern Gulf of Mexico. Therefore, this study evaluated the capacity for hydrocarbon degradation within ten sediment samples from rivers and coastal lagoons from Southern Gulf of Mexico using hexadecane (HXD) as a model compound.

2. Material and methods

2.1. Sediment sampling

Sediments were collected at the mouths of the principal rivers and coastal lagoons of the Southwestern Gulf of Mexico because of the high level of anthropogenic activities, as some rivers are transit routes for petroleum tankers and some coastal lagoons host significant fishing activity (Fig. 1, Table 1). Sampling was done in (DRY or RAIN SEASON), on YEAR. Six sediment samples were collected from the Gonzalez (RG), Coatzacoalcos (RC), Grijalva (RGR), San Pedro-San Pablo (RSS), Tonala (RT) and Papaloapan (RP) rivers, as well as three sediment samples from the Mecoacan (LM), Ostion (LO) and Champotón (LC) coastal lagoons, and one additional sediment sample from a marine outfall (PCM). All samples were taken from river or lagoon deltas, except for the marine outfall, where the sample was collected at the end of an oil pipeline in the ocean. The river and lagoon samples were collected with a Smith-McIntyre dredge at depths between 2 and

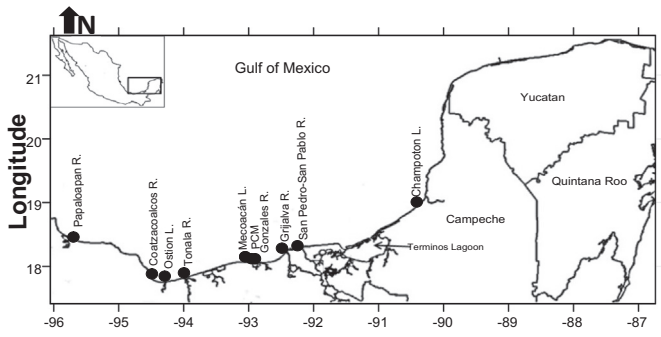

Fig. 1. Map of the sampling sites in the Southern Gulf of Mexico (R = river, L = lagoon).

7 m, while the PCM sediment sample was obtained at 27 m depth. The sediments were treated as follows: once the sediments were collected with the dredge, the water was drained and the first 5 cm of sludge was removed from the top, leaving the next ~5 cm of sludge (approx. 0.5 L of sediment) for collection using a sterile spatula. Those samples were placed in sterile bags, sealed immediately and kept refrigerated (4 °C) until used for testing in the laboratory.

For the quantification of polycyclic aromatic hydrocarbons (PAHs) in the sediments, a sediment sample was taken at each of the sampling points. The sediments were collected using a polytetrafluoroethylene (PTFE) spatula and were placed in glass vials, sealed and preserved under refrigeration (4 °C) until laboratory analysis. A different spatula was used for each sampling point.

2.2. Baseline properties of the sediment samples (day 0)

Oxygen, redox potential (E°) and pH were measured in-situ. Dissolved oxygen and redox-potential were determined using a YSI 5000 oximeter. pH was measured with a Hanna model HI98127 potentiometer and total carbon and nitrogen were determined following the methodology of Strickland and Parsons (1960). Turbidity was measured with an ICM, model 11150 turbidimeter. Hydrocarbonoclastic and heterotrophic bacteria counts, along with the quantification of hydrocarbons (polycyclic aromatic hydrocarbons, aliphatics, unresolved complex mixture (UCM), and hexadecane), which was carried out in the laboratory.

2.3. Identification and quantification protocol for hydrocarbonoclastic bacteria (HCB) and heterotrophic bacteria (Het)

For the detection and quantification of HCB and Het, a modification of the protocol reported by Lizárraga-Partida et al. (1991) was used. A dilution of 1:10 was made with 10 mL of the sample and 90 mL of Ringer solution (per 100 mL: 0.85 g sodium chloride, 0.04 g potassium chloride, 0.034 g calcium chloride dihydrate, to an osmolarity of 312 mOsm/L). For HCB determination, each test tube was immediately inoculated, containing Bushnell-Haas (Difco™) medium, with the addition of light crude oil at a concentration of 0.178 mg/L as the sole source of carbon and energy. For the Het determination, TSA (DIBICO™) was used as a medium. The tubes were incubated for 24 h at 36 °C and quantified following the most probable number methodology (Collins et al., 2004). The identification of gram-negative bacteria was achieved using the Analytical Profile Index Micromethod (bioMérieux) equipped with an API®/ID32 GN system. The gram-positive bacteria were detected using a Biomic V3 equipped with a BBL CRYSTAL Gram-positive ID system.

2.4. Quantification of polycyclic aromatic hydrocarbons (PAHs), total hydrocarbons (HCs) and hexadecane

The PAH extraction from 20 g of sediment was made using the Soxhlet technique with hexane and recirculating for over 8 h. Silica gel column chromatography was used for the separation of aromatic and aliphatic fractions. Samples were then concentrated to a volume between 0.5 and 1.0 mL. Finally, the extracts were analyzed by a Perkin Elmer Clarus 500 gas chromatograph coupled to a mass spectrometry in electron ionization mode (GC-EI-MS) at 70 eV, equipped with a J&W Scientific HP-5MS (30 m × 0.25 mm × 0.25 μm) column. Helium was the carrier gas at a flow rate of 1.5 mL/min. A 2 μL extract was injected in splitless mode (0.5 min). The acquisition was performed in the selected ion monitoring (SIM) mode using retention time windows, with one identification and two confirmation ions. Quantification of compounds was made by a 5-point calibration curve with real standards. The concentration numbers were added and reported as total PAHs (the PAHs detected are reported in the Supplementary material).

Aliphatic and unresolved complex mixture (UCM) were analyzed