

AMERICAN UNIVERSITY OF BEIRUT

BIOREMEDIATION OF PAH-CONTAMINATED MARINE
SEDIMENTS USING SEDIMENT MICROBIAL FUEL CELLS

by
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A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science in Environmental Sciences
to the Interfaculty Graduate Environmental Science Program
Environmental Technology
of the Faculty of Engineering and Architecture
at the American University of Beirut

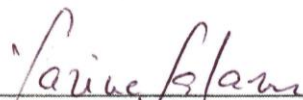
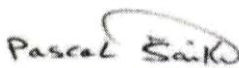
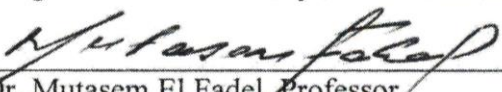
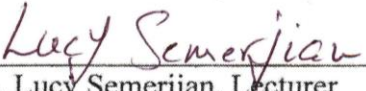
Beirut, Lebanon
August 2014

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ACKNOWLEDGMENTS

First of all I would like to show my sincere gratitude to my thesis advisor Dr. Darine Salam for all her guidance and support throughout my thesis research here at AUB. I have rarely seen someone who offers as much help as you and who cares for his/her student as much as you. Your support was essential for me to develop my potential as a researcher beyond what I thought would be possible.

Second of all I would like to thank Dr. Lucy Semerjian for all of her assistance and advice she provided at the Environmental Engineering Research Center at AUB and for her contribution to my research. Also, I want to thank Mr. Joseph Dawud for the help he provided in the lab.

Moreover, I want to thank Dr. Pascal Saikaly who was the one who came up with the idea of this research and who provided key notes to make this research possible.

Additionally, special thanks go to Dr. Mutasem El-Fadel who was my academic advisor when I started my graduate studies here at AUB and who was always available for any help when needed.

Last but not least, I would like to acknowledge the contribution of the Central Research Science Laboratory, especially Dr. Youssef Mouneimne and Ms. Rania Shatila for all their help and support.

Finally, I would like to thank my family, my friends and my colleagues (especially my EERC colleagues) for all their support throughout my last few years spent at AUB. In addition, I would like to give special thanks to my colleague Mr. Khaled Salam for providing support and guidance regarding various aspects of my research.

I also want to show my gratitude to Dr. Ahmad Hourri from LAU who was the reason why I applied to the graduate school in the first place, not to forget all his advice provided, which allowed me to face many of the hard obstacles I faced when I started my graduate studies.

AN ABSTRACT OF THE THESIS OF

Hamdan Zuheir Hamdan for Master of Science in Environmental Sciences
Major: Environmental Technology

Title: Bioremediation of PAH-Contaminated Marine Sediments Using Sediment Microbial Fuel Cells

Contamination of marine sediments with polycyclic aromatic hydrocarbons (PAHs) poses a significant concern due to the contaminants toxicity and resistance to biodegradation. Current bioremediation studies suggest that degradation of PAHs in marine sediments occurs under anaerobic conditions using terminal electron acceptors (TEAs) such as sulfate, nitrate or iron (III), with sulfate reduction being the most dominant pathway due to the sulfate abundance in marine sediments. Nevertheless, in addition to being a slow rate process, degradation of PAHs under anaerobic conditions is impeded by depletion of indigenous sulfate, which requires its frequent replenishment. Microbial Fuel Cells (MFCs) are controlled biochemical systems emerging as a viable alternative to traditional anaerobic processes with the benefit of direct electric power generation and enhanced contaminant removal efficiency.

In Sediment Microbial Fuel Cells (SMFCs), a conductive anode embedded in the marine sediment is perceived as an inexhaustible TEA that is thermodynamically favorable over sulfate and nitrate. This study evaluates the efficiency of SMFCs for enhanced in situ bioremediation of PAHs contaminated marine sediments. For this purpose, biodegradation of PAHs was tested in laboratory-scale marine sediment/seawater microbial fuel cells under different experimental conditions. SMFCs were operated under four operating conditions after spiking the sediments with three PAHs (naphthalene, 2-methylnaphthalene and phenanthrene). The change in the concentration of naphthalene and 2-methylnaphthalene did not vary appreciably among the SMFCs as they exhibited a similar fast drop in concentration aided by their volatility and not by microbial degradation. On the other hand, the drop in the concentration of phenanthrene, a more stable and less volatile PAH, showed a significant difference between the treatment conditions. Under controlled autoclaved SMFCs conditions, abiotic losses of phenanthrene contributed to a slight drop from 20 to 16 mg/Kg of dry sediments after 28 weeks. In the open circuit SMFCs phenanthrene concentration decreased to 12 mg/Kg of dry sediments, which was due to the activity of naturally occurring indigenous sulfate reducing bacterial community in the sediments. In the closed circuit SMFCs, the activity of the exoelectrogenic bacteria resulted in a significant drop in the concentration of phenanthrene from 20 mg/Kg to 1.1 mg/Kg in SMFCs treated with molybdate to inhibit sulfate reducing bacteria (SRB), and to 2.4

mg/Kg of dry sediment in SMFCs that were not treated with molybdate. Additionally, a significant utilization of sulfate as a TEA was noted in the case of open circuit SMFCs in which SRB were the main community involved in the anaerobic degradation of the contaminants. Last but not least, in closed circuit SMFCs, treatment with molybdate achieved the highest generated voltage.

Keywords:

Bioremediation, electrochemically active bacteria, marine sediments, polycyclic aromatic hydrocarbons, sediment microbial fuel cells, sulfate, terminal electron acceptor, sulfate reducing bacteria.

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CHAPTER I

INTRODUCTION

Among all pollutants that get adsorbed to the marine sediments, a special concern is given to Polycyclic Aromatic Hydrocarbons (PAHs) due to their mutagenic, carcinogenic and teratogenic potentials to humans, fauna and flora (Botsou and Hatzianestis 2011; Man et al., 2013; Thavamani et al., 2012). PAHs are organic pollutants composed of two or more fused aromatic benzene rings that are mainly present in petroleum and petroleum derived products such as asphalt and tar (Mahmoudi et al., 2013). These pollutants tend to be ubiquitous in the environment and they enter through natural (such as forest fires and volcanic eruptions releasing PAH into the atmosphere) and anthropogenic (such as accidental oil spills in aquatic environments and incomplete combustion of organic materials and fossil fuels) pathways (Mahmoudi et al., 2013; Ene et al., 2012). PAHs tend to adsorb readily to sediments and soils due to their hydrophobic chemical structure and low water solubility, which renders them stable and persistent in the environment (Mahmoudi et al., 2013; Ene et al., 2012). They also tend to accumulate in fat tissues of food chains such as fish and other aquatic organism, thus extending their effect to human consumers of polluted food (Ene et al., 2012).

The natural degradation of PAHs involves several processes, including volatilization, photo-oxidation, chemical oxidation, bioaccumulation, adsorption, and biodegradation (Balachandran et al., 2012). Low molecular weight PAHs have been reported to degrade readily by bacteria and fungi (Amezcuca et al., 2012) and even by algae (Mahmoudi et al., 2013) through a process called bioremediation. Taking advantage of such natural bioprocesses to enhance the degradation of PAHs is a

potential inexpensive method for increasing the rate of removal of these contaminants in polluted environments without producing toxic byproducts (Amezcuca et al., 2012). Yet, the non-polar nature of PAHs, their high affinity for organic matter and their low water solubility makes them poorly available to microorganisms, especially when they go through the process of aging by partitioning onto sediments or soil particles and diffusing into micropores (Mahmoudi et al., 2013).

Several studies have investigated PAHs biodegradation in sediments under anaerobic conditions (Chang et al., 2002; Langenhoff et al., 1996). These studies have shown that many PAHs, mainly those with lower molecular weights, can be anaerobically biodegraded using sulfate, nitrate or iron (III) as the terminal electron acceptor (TEA). Due to its abundance in marine sediments, sulfate has been found to be the predominant anaerobic TEA in the biodegradation of PAHs (Townsend et al., 2003), and high populations of sulfate reducers were noted in almost all cases (Lei et al., 2005). However, in heavily contaminated marine sediments, the biodegradation process is limited by the availability of indigenous sulfate that may be depleted rapidly and needs to be frequently replenished (Rothermich et al., 2002). On the other hand, the faster and more effective aerobic biodegradation is also limited by the low availability of oxygen in the marine sediments (Yan et al., 2012). Additionally, introducing oxygen into the sediments is costly and limited due to the low solubility of oxygen and its high volatility. Besides, it causes resuspension of sediments during the aeration process, which results in nutrients being released from the medium, limiting the microbial metabolism (Yan et al., 2012).

In 2001, a new system targeting increasing the rate of the anaerobic bioremediation was introduced and defined as Sediment Microbial Fuel Cells (SMFCs)

(Logan, 2008). It consists of an anode buried in the anaerobic marine sediments and connected to a cathode suspended in the overlying oxygen-rich water. Indigenous exoelectrogenic bacteria can grow attached to or in the vicinity of the anode to which they transfer the electrons generated from the oxidation of organic compounds (through nanowires). The electrons then migrate to the cathode producing an electric current (Logan, 2008). The high ionic strength of seawater-sediment medium allows cations (H^+) produced during organic matter biodegradation to migrate towards the cathode where they combine with oxygen and electrons to form H_2O (Hong et al., 2010). The overall performance of the SMFCs depends on several intrinsic parameters including the system design, electrodes material and depth, indigenous bacterial communities, and extrinsic parameters including the temperature and pH of the medium (Sonawane et al., 2013).

Reported literature on the use of SMFCs for the bioremediation of PAHs in aquatic sediments taking advantage of the intrinsic microbial communities is limited. Yan et al., 2012, used SMFCs for the bioremediation of PAHS in freshwater sediments. In this case, the sediments were spiked with phenanthrene and pyrene at 8 and 4 mg/Kg, respectively, and significant removal of both contaminants to less than 0.5 mg/Kg was achieved in about 6 months for phenanthrene, and 2 months for pyrene. The concentration of PAHs remained almost the same in the control SMFCs with no bacterial activity.

The objective of this study is to evaluate the feasibility of SMFCs as an efficient and cost-effective approach for the in situ bioremediation of PAHs contaminated marine sediments. For this purpose, SMFCs were tested for the removal

of lingering PAHs from the historically largest fuel spill which occurred along the Mediterranean coast in 2006.

CHAPTER II

MATERIALS AND METHODS

2.1 Sediments and Seawater

2.1.1 Collection

Anaerobic sediments were collected from near the Jiyeh power plant (Figure 1) (30 km south of Beirut, Lebanon) where the 2006 fuel spill occurred. This location was chosen because of the behavior of the spill in terms of its spatial distribution as the 18,000 tons of spilled fuel oil contaminated around 150 km of the coastline north of the spill site in Lebanon and Syria, and even threatened Cyprus (El-Fadel et al., 2012).



Figure 1. Sampling locations

Sediment samples were collected from 2 points about 100 meters apart from each other at about 1.5 Km north of the plant and fuel storage tanks. The first sample (point A, Lat: 33° 40' 1.0014", Long: 35° 24' 39.981") was collected at a depth of 7.4 meters below the water surface, while the second sample (point B, Lat: 33° 40' 0.8178", Long: 35° 24' 45.6264") was collected at a depth of 5.2 meters.

Another sample was collected from Beirut near Ain Al Mrayseh (about 30 Km North of Jiyeh) in order to check for contamination with PAHs at this location and for comparison of PAHs profile in the sediments in the two sampling sites.

The sediments were collected at a depth of 30 cm below the water-sediment interface to ensure anaerobic conditions. Seawater samples from each sampling point were also collected at 1 m above the water-sediment interface.

Both anaerobic sediments and seawater were collected in jars that were filled to the top and sealed directly under the water to avoid exposure to oxygen. The jars were transported within less than an hour to the laboratory and were stored at 4°C in the dark until further analysis was conducted.

2.1.2 Analysis of Background Parameters

Further sediment analysis and manipulation were conducted in an anaerobic glove box (Coy Scientific Products, United States) filled with 100 % nitrogen. Prior to their use, the top layer of the sediments was discarded for potential exposure to oxygen.

Anaerobic sediments were then sieved through 2 mm sieve to eliminate any coarse material which might interfere with the SMFCs operation. Finally, before proceeding with the analysis, a composite sediment sample and a composite seawater sample were prepared with a 1:1 ratio from the original A and B samples. Analyses of

several parameters were then conducted on all of the samples, A, B and the composite sample.

Sediments and seawater samples were analyzed for sulfates, nitrates, sulfides, nitrites, total nitrogen and total phosphorus according to standard methods and using a HACH (HACH DR/2011) spectrophotometer. Sediment samples were first extracted with 50 mL of deionized water. Other sediment characteristics including pH and dry and wet density, were also determined.

2.1.3 Measurement of PAHs in the Sediments

An aliquot of 1 ml of wet sediments was extracted with 2 mL of methanol by ultrasonication (BRANSONIC 5) for an hour and a half. Extracted samples were centrifuged for ten minutes at 1500 \times g, and filtered through a 0.2 μ m filter. The filtrate was analyzed for PAHs using High Performance Liquid Chromatography (HPLC) equipped with a DAD detector (Agilent 1100) according to the method described by Yan et al. (2012). Chromatographic analysis was performed using a C18 column (DISCOVERY HS C18, 5cm, 4.6 mm, 5 μ m). An isocratic elution was carried out using 90% methanol and 10% water at a flow rate of 1 mL/min. The column temperature was maintained at 30°C, and PAHs were detected at 220 nm (naphthalene and 2-methylnaphthalene) and 251 nm (phenanthrene).

2.2 Experimental Setup

Laboratory scale SMFCs were constructed and consisted of 0.9 L Plexiglas reactors (10L x 6W x 15H cm). Marine sediments used in the SMFCs were spiked with

a solution of naphthalene, 2-methylnaphthalene, and phenanthrene prepared in methanol. Spiking of the composite sediment sample was performed under anaerobic conditions in an anaerobic hood filled with nitrogen while mixing mechanically at low speed for 1 hour to achieve a homogeneous distribution of PAHs in the sediments. Spiking was performed to achieve an initial concentration of 60 mg/Kg of dry sediments of naphthalene and 2-methylnaphthalene, and 20 mg/Kg of dry sediments of phenanthrene. However, due to their high volatilization enhanced by the mixing process, achieved concentrations of naphthalene and 2-methylnaphthalene were only 2.5 mg/Kg and 9 mg/Kg of dry sediments, respectively. The SMFCs were filled with spiked marine sediments to a depth of 7 cm. Seawater was then added to form a 7 cm column on the top of the sediments. The anodes and the cathodes were placed in the middle of the sediment layers and in the middle of the water layers at 3.5 cm below and above the sediment-water interface, respectively. Carbon fiber brushes that are twisted around a titanium wire were used as the anode and cathode materials because the electrode materials in MFCs must be highly conductive, non-corrosive, non-toxic to microorganisms and inexpensive (Rezaei et al., 2007). Also, it is reported that electrode materials that are mostly used in MFC applications are carbon-based electrodes (carbon or graphite) (Logan et al., 2010). The electrode brushes were 6 cm in length and 6 cm in diameter. Titanium was selected for being non-corrosive and non-toxic to microorganisms. The spacing of the electrodes in the SMFCs was most important for the anode because it must be at least 0.5 cm below the sediment water interface, given that oxygen is usually depleted at a depth of 0.5 cm below the sediment water interface in marine environments (An et al., 2013). With respect to the cathode placement, it is important to keep it completely submerged with water so that the maximum surface area

be in contact with the oxygen in the water, so it was also placed in the middle of the water layer. Finally, sediments were equilibrated with the overlying seawater for 1 day before connecting the anode to the cathode via a fixed resistor of 10 ohms. SMFCs were then operated at room temperature in the dark to prevent photolysis. The Setup of the SMFCs is shown in figure 2

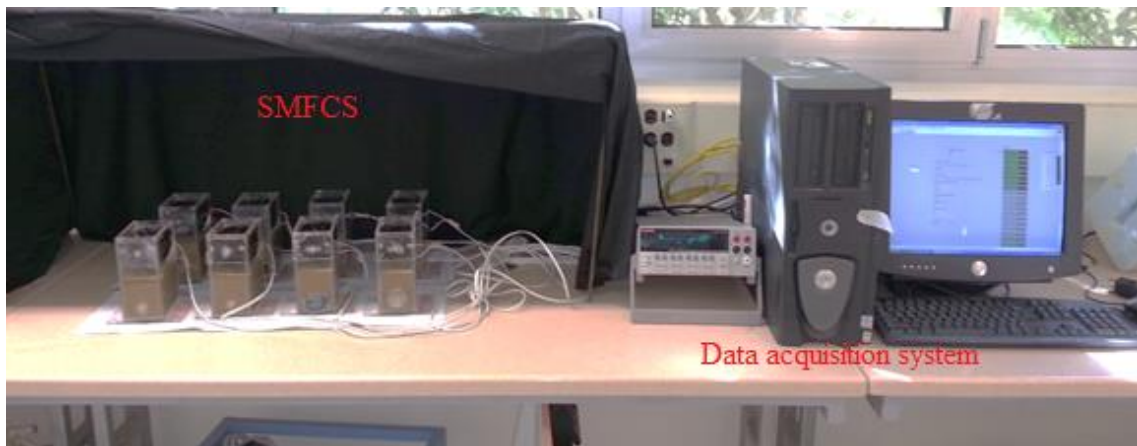


Figure 2. Experimental setup

2.3 SMFCs Operation

2.3.1 *Operation Conditions*

SMFCs were run under different operating conditions as presented in table 1 with corresponding rationale. For each operating condition duplicate SMFCs were run, with a total of 8 SMFCs tested.

Table 1. Operating conditions

SMFC operating condition	Description	Significance
1	SMFCs were operated under closed circuit condition.	To evaluate the potential of SMFCs in enhancing PAHs biodegradation. This is expected to occur mainly due to the activity of exoelectrogenic bacteria associated with the anode.
2	Control SMFCs (1). SMFCs were operated under open circuit condition.	Removal of PAHs in this case is expected to be mainly associated with the activity of sulfate reducing bacteria (SRB), and will be compared to the % removal achieved under operating condition 1.
3	Control SMFCs (2). Sediments were autoclaved twice for 30 minutes at 120°C to kill existing bacterial species. Closed circuit conditions were applied in this case.	To monitor potential non-biological processes involved in PAHs removal from the sediments. These might include photolysis and chemical oxidation.
4	Molybdate was added to inhibit sulfate-reducing bacteria (SRB), the main contributor to PAHs removal under anaerobic conditions. SMFCs were operated under closed circuit condition.	To evaluate PAHs removal in the absence of SRB activity, and assess the efficiency of electrode as the main terminal electron acceptor.

2.3.2 Periodic Monitoring

Concentration of PAHs and sulfates was measured twice per week for the first four weeks and once per week thereafter. After 15 weeks of the SMFCs operation, monitoring was performed once every 2 weeks. Sulfate was selected for its high background concentration as compared to other potential TEAs and for its major involvement in anaerobic degradation of PAHs in marine environments (Townsend et al., 2003).

The electrical potential difference caused by the electrical current generated by the bacterial activity was also measured as voltage between the anode and the cathode. The voltage was monitored every 15 minutes across the electrodes using a multimeter

with a data acquisition system (model 2700; Keithley Instruments Inc., United States). Obtained data were then averaged on a daily basis.

An autoclaved phosphate buffer and nutrient solution ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.75 g/L; NH_4Cl , 0.31 g/L) was added periodically to the seawater to provide N and P source for bacterial growth and to compensate for the water lost through evaporation.

Aeration of the reactors was performed by using an air diffuser system to maintain a dissolved oxygen concentration above 2 mg/L at the level of the cathode. The SMFCs running under autoclaved conditions were not connected to the air diffuser to avoid potential contamination.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Background parameters of sediments and seawater

3.1.1 *Physiochemical characteristics*

The physiochemical characteristics of water and sediment samples are presented in tables 2 and 3.

For comparison reasons and to check for any significant variations in the characteristics of the samples, another seawater sample was collected from the sea surface at the Jiyeh sampling site and was also analyzed.

Table 2. Water parameters

Water	Sample A	Sample B	Composite sample	top water
pH	7.86	7.86	7.87	7.84
Sulfates (SO_4^{2-}) mg/L	4900	4700	5000	4400
Nitrates (NO_3^-) mg/L	ND	ND	ND	ND
Sulfides (S^{2-}) mg/L	0.003	0.004	0.003	0.011
Nitrites (NO_2^-) mg/L	0.003	0.003	0.003	0.003
Phosphates (PO_4^{3-}) mg/L	0.02	0.01	0.02	0.01
Total Nitrogen (N) mg/L	2	2.3	3	2.2
Total Phosphorous (P) mg/L	0.02	0.03	0.02	0.02

ND: Non-Detectable

Table 3. Sediment characteristics

Sediments	Sample A	Sample B	Composite sample
pH	8.34	8.32	8.3
Wet Density g/mL	2.03	2.01	2.00
Dry Density g/mL	1.59	1.55	1.55
Sulfates (SO ₄ ²⁻) mg/Kg dry	1099.7	1160.751	1132.75
Nitrates (NO ₃ ⁻) mg/Kg dry	6.2	7.0911	6.9553
Sulfides (S ²⁻) mg/Kg dry	0.0261	ND	ND
Nitrites (NO ₂ ⁻) mg/Kg dry	ND	ND	ND
Phosphates (PO ₄ ³⁻) mg/Kg dry	ND	ND	ND
Total Nitrogen (N) mg/Kg dry	12.57	11.95	12.08
Total Phosphorous (P) mg/Kg dry	0.22	0.19	0.20

ND: Non-Detectable

The results show a relatively high background concentration of sulfates in the marine water and sediments, confirming the general trend of having sulfate as the most abundant compound that is utilized as a final electron acceptor during the slow anaerobic bacterial activity below the sediment-water interface in marine environments (Lei et al., 2005).

3.1.2 PAHs Analysis in the Marine Sediments

Naphthalene, 2-methylnaphthalene, and phenanthrene were detected at high concentrations in the collected sediments from Jiyeh (Table 4). The same PAHs were also found in sediment samples collected from Beirut.

Table 4. Concentration of PAHs (mg/Kg of dry sediments) in collected sediments

PAH	Jiyeh	Beirut
Naphthalene	0.2	0.16
2-methylnaphthalene	0.04	0.03
Phenanthrene	0.06	0.057

This similarity in the background profile of these contaminants and their low concentration with respect to what was reported after the Jiyeh spill in 2006 suggest that

their current presence is most likely not related to the spill. The spill occurred eight years ago, so it might have been long enough for natural processes to mitigate the resulting PAHs contamination.

Following the Jiyeh fuel spill in 2006, different PAHs were found at several locations near the Alkaber Aljanuby river estuary at the northern boundary of Lebanon (Mohamad et al., 2011). These included 1,2,4-Triethylbenzene, 1,3,5-Triethylbenzene, 1-Methylnaphthalene, 1-Ethylnaphthalene, Acenaphthylene, Fluorene, Phenanthrene, Anthracene, 2-Methylphenanthrene, 1-Methylphenanthrene, 3,6-Dimethylphenanthrene, Fluoranthene, Pyrene, 1-methylpyrene, Chrysene, and Perylene, and were reported at different concentrations ranging from less than 5 mg/Kg to more than 130 mg/Kg of dry sediments. The total concentration of PAHs reached 365 mg/Kg of dry sediments. On the Lebanese side of the river estuary (in the sea), the most abundant PAHs that were detected in the sediments were mainly methylnaphthalene, fluorene, anthracene, and phenanthrene and its methylated derivatives. Phenanthrene and its methylated derivatives were also predominant in spilled oil samples collected from Batroun (about 80 Km North of Jiyeh), as reported by Khalaf et al. (2006) after 1 month of the spill, suggesting that detected PAHs in the sediments at the northern boundary of Lebanon result from the Jiyeh spill. Reported PAHs concentrations in the marine sediments in the Mediterranean Sea ranged from 108 to 26,633 mg/kg of dry sediment near Greece (Botsou et al., 2011) and from 1 to 20,500 mg/kg of dry sediment near the French Riviera, Corsica, and Sardinia (Baumard et al., 1998). Results from PAHs analysis in the collected samples are consistent with reported PAHs in the sediments after the 2006 spill, with the most prevailing contaminants being naphthalene, 2-methylnaphthalene and

phenanthrene (Mohamad et al., 2011). Accordingly, these PAHs were used in the SMFCs experiments.

3.2 SMFCs Experiments

3.2.1 Degradation of PAHs in the SMFCs

All of the SMFCs exhibited a similar pattern of the naphthalene removal from the sediments (Figure 3). Within less than a week most of the naphthalene disappeared and its concentration dropped significantly from 2.5 to 0.5 mg/Kg of dry sediments in all SMFCs, even under autoclaved conditions. After that, the concentration continued to drop at a slower rate to reach about 0.2 mg/Kg of dry sediments during the fourth week, after which it continued to drop slowly and gradually to reach less than 0.1 mg/Kg of dry sediments in all SMFCs after about 28 weeks.

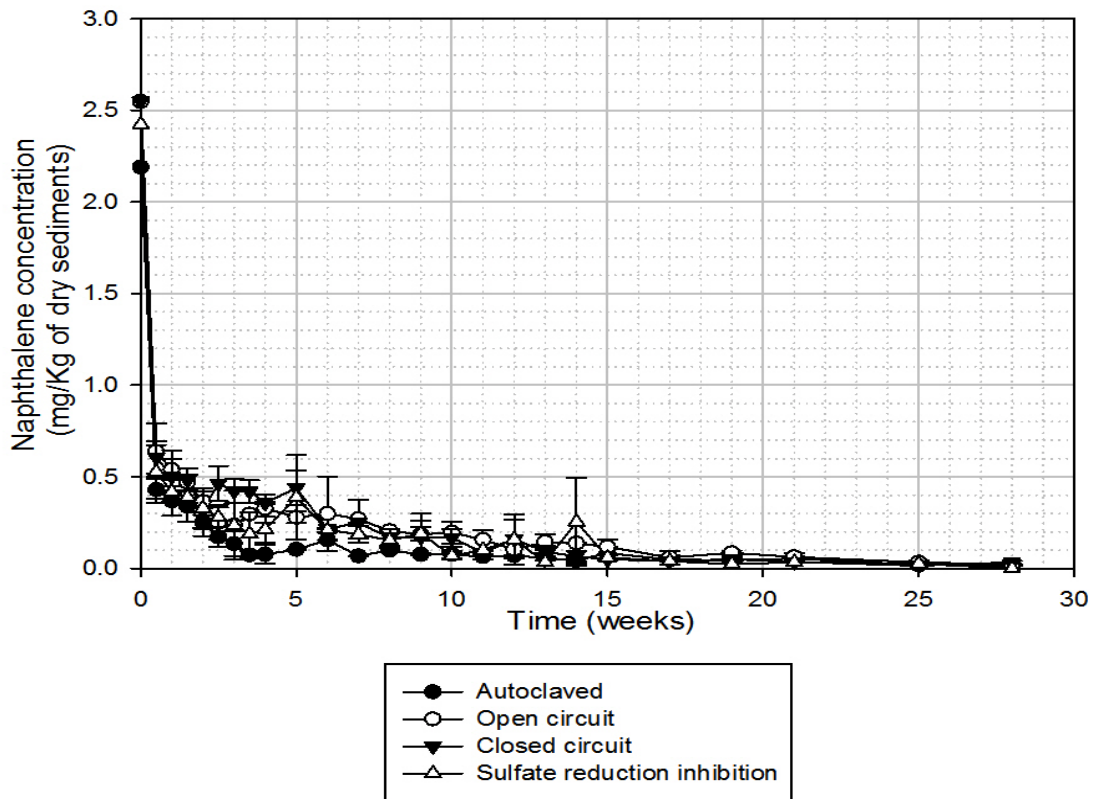


Figure 3. Naphthalene concentration profile in the SMFCs during 28 weeks

The similar pattern in the drop of the naphthalene concentration in SMFCs operated under different conditions suggests that its disappearance is related to abiotic processes, namely volatilization, rather than to biodegradation. Volatilization has been reported to be the major process involved in the removal of naphthalene and low molecular weight PAHs from contaminated sediments (Venkata Mohan et al., 2006). The steep drop in the naphthalene concentration observed during the first week and followed by a slower decrease in the contaminant level, indicates an initial loss of the loosely adsorbed molecules to the sediment and organic matter followed by the gradual loss of the strongly adsorbed (partitioned) molecules (Mohan, et al. 2006).

In the case of 2-methylnaphthalene (Figure 4), the contaminant level dropped from 9 mg/Kg to 2.5 mg/Kg of dry sediments in all SMFCs within the first 6 weeks of operation after which a slower degradation rate was observed. Also, no significant difference in the achieved 2-methylnaphthalene removal was observed among the different treatments.

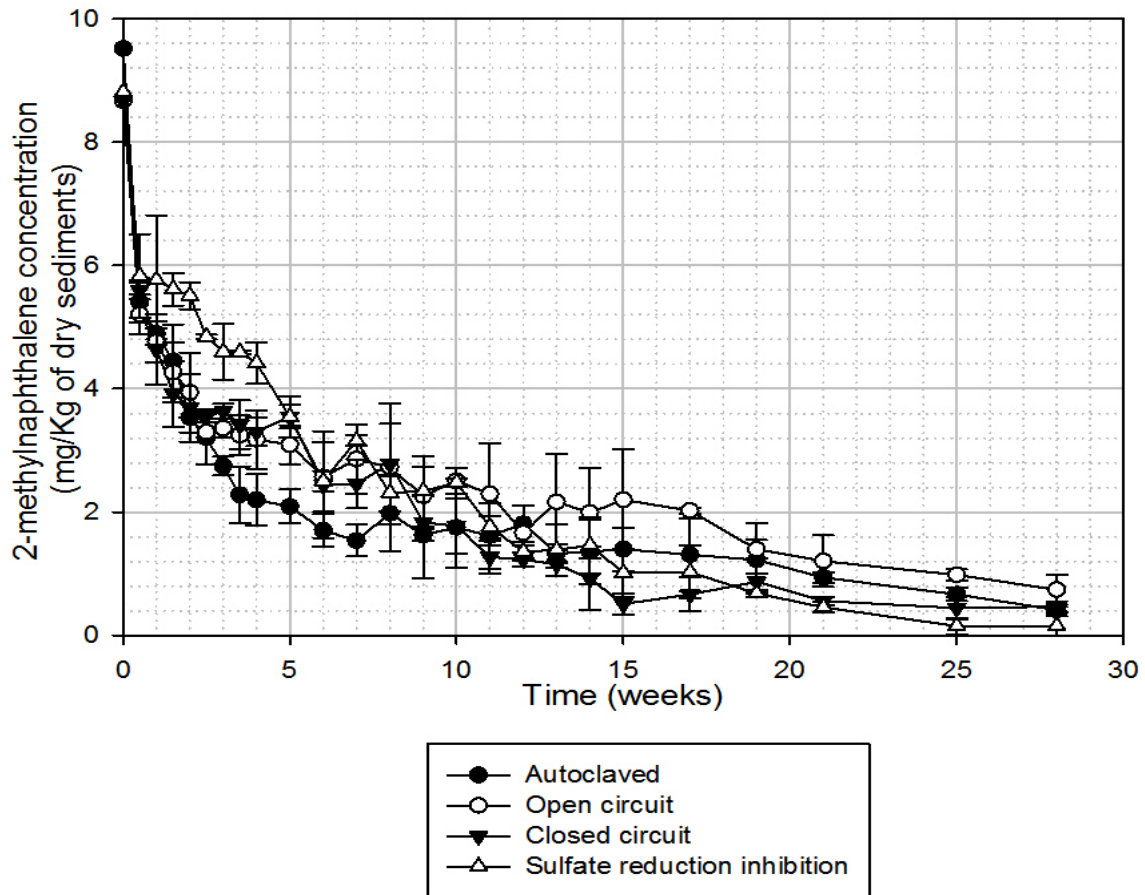


Figure 4. 2-methylnaphthalene concentration profile in the SMFCs during 28 weeks

These results suggest that the major process responsible for the removal of 2-methylnaphthalene from the sediment is also volatilization as in the case of naphthalene. This is consistent with the results reported by Mohan et al. (2006) whereby volatilization was found to be of more significance than biodegradation for the removal of 2-ringed PAHs from polluted sediments.

The effect of the different SMFCs treatments on the degradation of PAHs is better reflected with phenanthrene (Figure 5), a 3-ringed PAH that is much less volatile than the 2-ringed naphthalene and 2-methylnaphthalene (Guerin and Jones 1989).

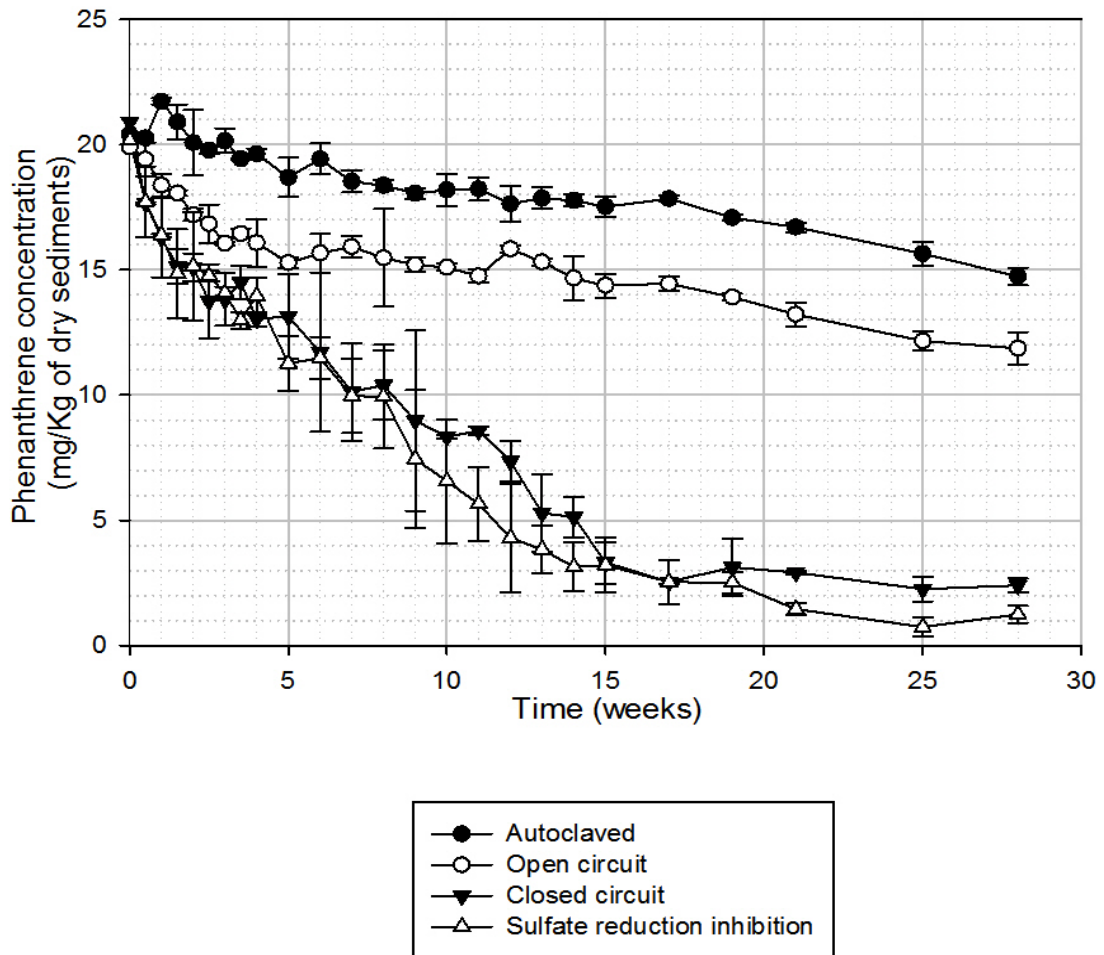


Figure 5. Phenanthrene concentration profile in the SMFCs during 28 weeks

In the open circuit SMFCs in which the indigenous microbial community naturally occurring in the sediments is involved in the biodegradation of the pollutant, phenanthrene concentration decreased to only 12 mg/Kg of dry sediments after 28 weeks. This indicates that the biodegradation of phenanthrene was limited, most probably due to the limited availability of the TEA (sulfate in this case) in the SMFCs for the anaerobic respiration, especially with no replenishment of the utilized sulfate and due to the drop in sulfate concentration that is noted in this case in which the sulfate

level reached at the end of the experiment was the lowest among the SMFCs (Section 3.2.2).

Under closed circuit conditions (in the absence and presence of molybdate as an inhibitor of the sulfate reducing bacteria), a significant removal of phenanthrene in the SMFCs was achieved. A relatively steep drop in the contaminant concentration was observed up to week 17 of incubation and leveled thereafter. This suggests that in the case of closed circuit conditions, the anode behaved as an alternative TEA enhancing the biodegradation of phenanthrene. The higher biodegradation rate under closed circuit conditions can be attributed to the faster utilization of the contaminant by the bacterial populations grown at the level of the anode.

Achieved phenanthrene removal under closed circuit conditions was slightly higher under the inhibition of sulfate reducing bacteria in the SMFCs treated with molybdate. In this case, phenanthrene concentration was equal to 1.1 mg/Kg of dry sediments after 28 weeks, while in the absence of molybdate, the contaminant concentration was 2.4 mg/Kg of dry sediment at the end of the incubation period. The higher remaining phenanthrene concentration in the absence of molybdate might be due to the competition between sulfate and anode reducing bacteria, inducing a lower biodegradation rate of the contaminant.

The drop in phenanthrene concentration in the autoclaved control SMFC from 20 mg/Kg to 16 mg/Kg of dry sediments after 28 weeks of the experiments is due to abiotic losses caused by its low volatility and its photosensitivity (although the SMFCs were operated in the dark, they were sometimes exposed to the light for brief times during sampling events).

3.2.2 Change in the Concentration of Sulfate as a Final Electron Acceptor

Sulfate is the major final electron acceptor involved in the biodegradation of marine sediments contaminated with PAHs due to its abundance in such environments (Townsend et al., 2003). However, in heavily contaminated sediments, sulfate tends to be consumed fast, and thus it will limit the anaerobic biodegradation of PAHs. Figure 6 shows the temporal variation in sulfate concentration in the different SMFCs.

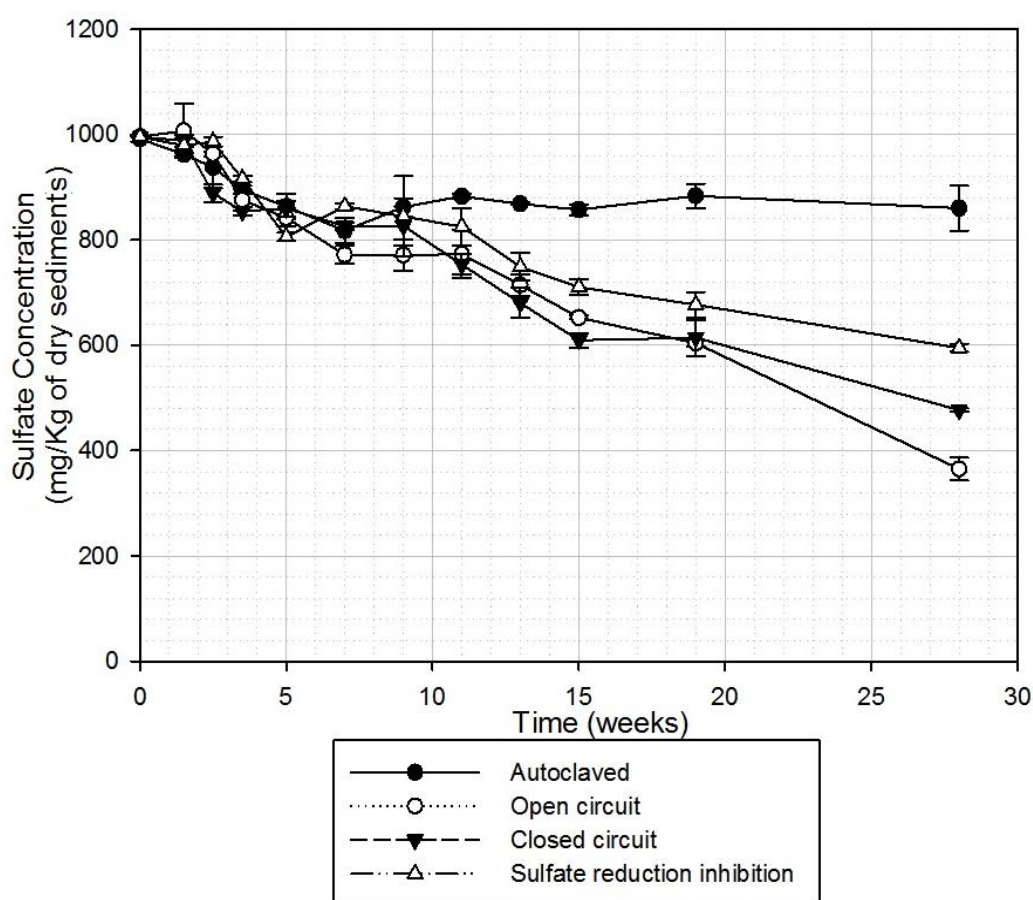


Figure 6. Sulfate concentration profile in the SMFCs during 28 weeks of operation

A significant sulfate reduction was observed in the open circuit SMFCs during the biodegradation of PAHs by the sulfate reducing bacteria. In this case, sulfate concentration decreased to 368 mg/Kg of dry sediments after 28 weeks of incubation. A higher sulfate concentration of 475 mg/Kg of dry sediments was measured under the

dual action of exoelectrogenic and sulfate reducing bacteria due to the simultaneous utilization of sulfate and the anode as TEAs. It is expected that, under both treatment conditions, all sulfate would be exhausted if the experiments time was prolonged. Sulfate was also consumed under SRB inhibition reaching a concentration of about 590 mg/Kg of dry sediments after 28 weeks. This decrease in sulfate concentration is mainly due to the sulfate diffusion from the sediment to the overlaying water and the utilization of sulfate during biomass production. Indeed, although the used concentration of molybdate exceeded the effective concentration for SRB inhibition in anaerobic sediments reported by Hansen et al (1998), complete bacterial inhibition might not have occurred probably due to the partial chelation of molybdate by the sediments rendering it unavailable for bacteria. Also, it is reported that bacteria may acclimatize to the presence of molybdate with time (Isa and Anderson, 2005), which might have happened in the case of this experiment with the SRB resuming their biodegradation activity and consuming sulfate as TEA.

Sulfate diffusion to the overlying water was also responsible for the drop in sulfate concentration in the autoclaved SMFCs to a concentration of about 850 mg/Kg of dry sediments. Cross contamination of the autoclaved control through weekly sampling was eliminated by running additional control where no sampling was taking place. Sulfate concentration in this control was only measured at the beginning and the end of the experiments, and the measured drop in sulfate concentration was thus exclusively related to the mineral diffusion from the sediments to the water column.

3.2.3 Voltage Generated from the SMFCs

MFCs in general can be used to recover energy from bacterial substrates in the form of electrons flowing from the anode to the cathode. In this study, where biodegradation was taking place under closed circuit conditions, an electrical current was generated creating an electrical potential difference measured as voltage between the anode and cathode.

Figure 7 shows the voltage profile in the SMFCs. In the closed circuit SMFCs, a similar pattern of voltage generation over 28 weeks was observed in the presence and absence of molybdate.

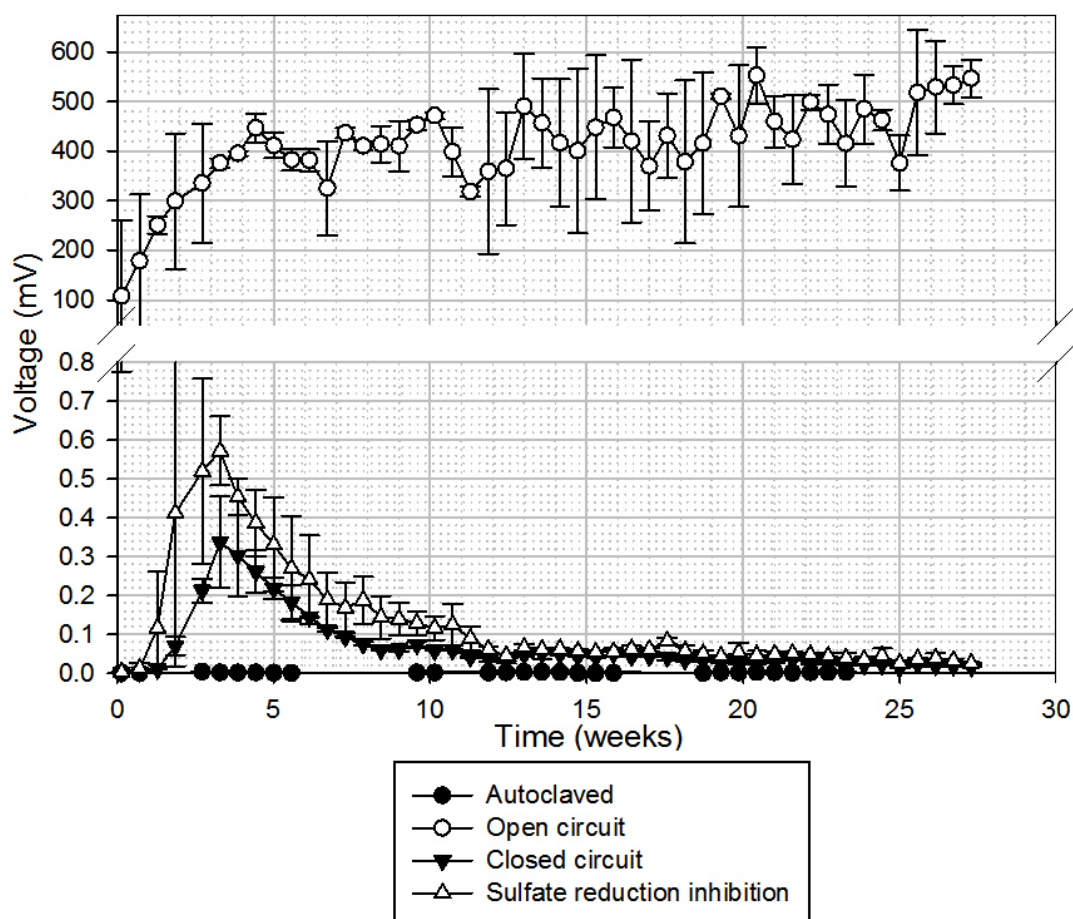


Figure 7. Voltage profile of the SMFCs over 28 weeks

The voltage initially increased rapidly from 0 mV to reach a maximum value (0.57 mV and 0.33 mV in the presence and absence of molybdate, respectively) after 3 weeks of operation, then decreased gradually to reach almost zero at the end of the experiments.

This confirms the gradual consumption of PAHs by the SRB and exoelectrogenic bacteria under closed circuit conditions. The lower voltage obtained in the absence of molybdate is due to the competition between anode reducers and sulfate reducers, which decreased the overall rate of PAHs biodegradation. It is important to note that in the closed circuit SMFCs, the period during which they generated most of the electrical activity was the same period when most of the degradation of the PAHs took place, which was from the start until the end of the 12th week of operation.

Under open circuit condition, the potential difference increased after launching the SMFCs from 100 mV to reach an average value of 450 mV after 35 days. The increase is most probably due to the duration during which indigenous bacterial populations within the sediments established themselves on the anode, after which stable conditions were reached. This is consistent with the reported open circuit voltage in SMFCs and even in other types of MFCs, in which the open circuit voltage increases gradually and can reach up to 700 mV depending on the type and the configuration of the MFC and the electrodes (Sajana et al., 2013; Larrosa-Guerrero, et al., 2010).

In the case of the autoclaved control SMFCs, no voltage was measured at any time of the experiments due to the absence of any microbial activity inducing the generation of electrical current.

CHAPTER IV

CONCLUSION

There are many studies targeting SMFCs performance; however, studies about their efficiency in the removal of PAHs are still limited. In this study, SMFCs were demonstrated to be successful systems for enhancing the natural attenuation of PAHs from contaminated sediments in marine environments. High PAHs removal was observed under closed circuit conditions when compared to open circuit conditions, suggesting a more efficient use of the anode as TEA in the biodegradation of PAHs in contaminated sediments. This was most prominent in the case of SRB inhibition with the anode being the main TEA used in the biodegradation of PAHs. Furthermore, the effect of the SMFCs is significant on higher molecular weight PAHs as compared to the volatile low molecular weight PAHs such as naphthalene.

The results from this study revealed that SMFCs offer a promising future for treating polluted sites from various contaminants including PAHs, although their use for this purpose is still relatively a new method for pollution control. Furthermore, the energy recovered from these systems can be used for useful applications such as powering up remote data collection devices in marine environments. So, further studies must be performed to analyze the effect on other high molecular weight PAHs and to optimize the system so that it can be applied directly in the environment.

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