Ingeniería Ambiental

Revista Mexicana de Ingeniería Química
Vol. 13, No. 2 (2014) 509-516

HYDROCARBON POLLUTION STUDIES OF UNDERWATER SINKHOLES ALONG QUINTANA ROO AS A FUNCTION OF TOURISM DEVELOPMENT IN THE MEXICAN CARIBBEAN

ESTUDIOS DE CONTAMINACIÓN POR HIDROCARBUROS EN CENOTES DE QUINTANA ROO RELACIONADA AL DESARROLLO TURÍSTICO EN EL CARIBE MÉXICANO

S.A. Medina-Moreno[1], A. Jiménez-González[1], M. Gutiérrez-Rojas[2], M.A. Lizardi-Jiménez[1]*

[1] *Universidad Politécnica de Pachuca, Carretera Pachuca-Cd. Sahagún, km 20, Ex-Hacienda de Santa Bárbara, Municipio de Zempoala Hidalgo*

[2] *Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco No. 186, Col. Vicentina, Iztapalapa, México, D.F. C.P. 09340, México.*

Received March 3, 2013; Accepted May 21, 2014

Abstract

This work studied in a wide geographical area during two vacational seasons, the presence of hydrocarbon contaminants in sinkholes along the Mexican state of Quintana Roo, an important caribeann tourism region. Phenanthrene, naphthalene and benzene derivates were found as the most common hydrocarbon contaminants present in underwater sinkholes located in Cancún and Playa del Carmen, two well-developed tourism poles. Other regions of Quintana Roo, with intermediate tourism development (Puerto Morelos, Tulum, Cozumel and Bacalar) show hydrocarbon presence too. In comparison, Holbox which is a recent touristic development where the use of motor transportation for tourists and locals is less common, has not reached the contamination level of Cancún or Playa del Carmen nor the pollution level of intermediate tourism development poles as Tulum or Cozumel. Concentration of hydrocarbons is related to vacational seasons, sinkholes during "High" season shows major hydrocarbon concentration and diversity than during "Low" season.

Keywords: airlift bioreactor, hydrocarbon, water pollution, tourism, sinkholes.

Resumen

En este trabajo se estudió, en una amplia zona geográfica y dos temporadas vacacionales, la presencia de hidrocarburos contaminantes en cenotes del estado de Quintana Roo, una región turística importante en el Caribe. Se encontró Fenantreno, naftaleno y benceno entre los hidrocarburos contaminantes más comunes presentes en los cenotes ubicados en Cancún y Playa del Carmen, dos polos turísticos bien desarrollados. Otras regiones de Quintana Roo, con desarrollo de turismo intermedio (Puerto Morelos, Tulum, Cozumel y Bacalar) muestran la presencia de hidrocarburos también. En comparación, Holbox, que es un reciente polo de desarrollo turístico, donde el transporte de turistas y lugareños es bajo, no presenta contaminación por los hidrocarburos estudiados en este trabajo. La concentración de hidrocarburos también está relacionada con la temporada turística, los cenotes durante la temporada "alta" mostraron mayor concentración y diversidad de hidrocarburos que en la temporada "baja".

Palabras clave: biorreactor airlift, hidrocarburos, contaminación del agua, turismo, cenotes.

1 Introduction

Environmental pollution with petroleum and petrochemical products (complex mixture of hydrocarbons) has been recognized as the most damaging on coastal environments (Davenport and Davenport, 2006). Accidental leakages from petroleum carrying ships, lead to oily layers over the water surface (Jain *et al.*, 2011), affecting the economic development of these coastal regions.

* *Corresponding author. E-mail*: chamarripas@yahoo.com.mx
Tel. Fax + (771) 2118365, Fax + (771) 2118365

Few regions of the world are directly dependent of their natural resources for economic development as the Caribbean (Dixon *et al*., 2001), where intensive development of tourism on the coast of the southern Mexican state of Quintana Roo, is causing pollution of aquifers and underwater sinkholes, specifically in two sites: Cancún (Lizardi-Jiménez *et al*., 2014) and Playa del Carmen, (Gold-Bouchot, 2009; Lizardi-Jiménez *et al*., 2014). As it is, although the water pollution due to hydrocarbons is well documented in Mexico (Scholz-Böttcher *et al*., 2008; Medina-Moreno *et al*. 2009), these kind of studies of pollution in tourism poles are scarce.

The Yucatan peninsula, where the state of Quintana Roo is located, is a high permeability fractured limestone land, a well-developed karstic system of interconnected fractures that allows rapid transportation of microbial and chemical contamination resulting in a significant potential increase of pollution affecting the ecosystem. Natural sinkholes are one of the many attractions of this area. As many have been enabled for snorkeling or swimming, leading a clear path for pollution. The waste is seeping into these passages where contaminants flow through them and impact the groundwater. The offered explanation to the presence of hydrocarbons is the run off from cars driven on the roads nearby (Metcalfe *et al*., 2011). This has been confirmed by recent studies that detected the presence of hydrocarbons in underwater sinkholes in Playa del Carmen (Gold-Bouchot, 2009; Lizardi-Jiménez *et al*., 2014). Recently, Lizardi-Jiménez *et al*. (2014) has been reported the use of airlift bioreactors with consortia isolated of one of these underwater sinkholes, for the remediation of environments coastal, polluted with polycyclic aromatic hydrocarbons (PAH). However, due to intensive touristic activity in the Caribbean (Quintana Roo) it is necessary to carry out studies of several sinkholes to determine with the best approach the degree of pollution of hydrocarbons on this entire region, considering their correlation associated to both high and low vacational seasons.

The aim of this work was to evaluate the hydrocarbon contamination in eleven sinkholes along of the entire Quintana Roo State as a result of tourism development intensity associated to both high and low season of vacation stations, and the proposal to use oil degrading microorganisms from the sinkholes themselves as a biotechnological alternative of bioremediation.

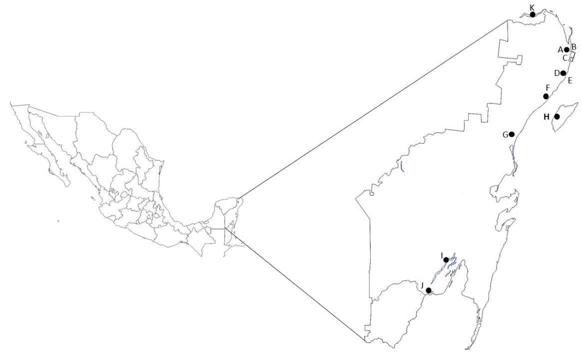

Fig. 1. Studied sinkholes in Quintana Roo state: Urban zone of Cancún City (A,B,C) (21°11’ 25.5” N, 86°50’ 14.9 ”W; 21° 9’ 41.3” N, 86° 51’ 0.81” W; 21° 8’ 19” N, 86° 51’ 39.35” W), Puerto Morelos (D,E) ((20º 50’ 53.7” N, 86º 52’ 33.98” W), Riviera Maya (F,G) (20°39’ 43.59”, 87°4’ 7.25” W; 20° 12’ 31.26” N, 87° 28’ 21.81” W); Cozumel (H) (20°26’35”N, 86°59’40”W); Chetumal (I,J) (18° 46’ 2.37” N, 88° 18’ 24.92” W; 18°30’ 50.98”N-88°25’ 27.29? W); Holbox (K) (21°32’ 16.0 ” N, 87°13’ 12.0 ” W).

2 Materials and methods

2.1 Sampling points

Figure 1 shows the study sinkholes in Quintana Roo: Urban zone of Cancún City (A,B,C) (21°11’ 25.5” N, 86°50’ 14.9 ”W; 21° 9’ 41.3” N, 86° 51’ 0.81” W; 21° 8’ 19” N, 86° 51’ 39.35” W), Puerto Morelos (D,E) (20º 50’ 53.7” N, 86º 52’ 33.98” W), Riviera Maya (F,G) (20°39’ 43.59”, 87°4’ 7.25” W; 20° 12’ 31.26” N, 87° 28’ 21.81” W); Cozumel (H) (20°26’35”N, 86°59’40”W; Chetumal (I,J) (18° 46’ 2.37” N, 88° 18’ 24.92” W; 18°30’ 50.98”N-88°25’ 27.29” W); Holbox (K) (21°32’ 16.0 ” N, 87°13’ 12.0 ” W). A total of 33 samples were taken.

The sample was taken from the upper water body from 1 to 1.5 m depth and deposited in amber glass containers. Vials filled without air bubbles. Samples were labeled and kept at low temperatures during transportation to the laboratory (4 °C ± 2 °C). The entire sampling procedure was performed according to the Mexican normative (NMX-AA-014-1980).

2.2 Microbial consortium culture

A native sinkhole consortium obtained from the point located in urban zone of Playa del Carmen was cultured in diesel enriched liquor at 37 ºC and then isolated by cross plate. The identification of the mixed culture was done by biochemical test. Afterward, the native consortium was cultured and grown in a sequential batch airlift bioreactor (ALB) with a previously reported mineral medium (Lizardi-Jiménez *et al*., 2014) added with 13 g L^{-1} of diesel oil for 14 d. At the end of the culture time the airlift ALB was drained and the original conditions were restituted.

2.3 Microorganism isolation and identification

A preenriched culture with water samples from hydrocarbon-polluted sinkholes were used. Brain Heart Infusion, (BHI) and Lauril Sulfate Broth (LSB) were used as preenriched culture medium. BHI and LSB were inoculated with water samples from hydrocarbon-polluted sinkholes. Other series, as a repetition including diesel, were added with diesel 5% at 37 ºC 24 h.

Soil samples were collected in pre-sterilized glass bottles from various sinkholes in Quintana Roo, Mexican Caribbean, and transported to the laboratory for analyses. Enrichment and isolation of oil-degrading bacterial cultures were done using mineral salts medium (Kennedy *et al*., 1975) with diesel as a substrate and a serial dilution-agar plating technique on nutrient agar medium (Bhoosreddy, 1995), respectively. With the aim to isolate microbial strains by morphological characteristics the next solid culture medium were used: Xilose-Lysine-Desoxicolate (XLD), Salmonella Shigella (SS), Hektoen (HK), Sulfito Bismuto (SB), Bair Parker (BP). The isolated bacterial cultures were characterized by their morphological and biochemical characteristics (Holt *et al*., 1994). The biochemical tests used were solid culture medium: TSI, LIA, H_2S, GAS, Motility Indol Ornitine (M. I. O.), liquid: APA, Arginine, Lisine.

2.4 Bioreactor

A 1-Liter airlift bioreactor (ALB) was used in this work. ALB cylindrical vessel was made of Pyrex glass (6.8 cm diameter; 27 cm height) provided with a draft tube (4.5 cm diameter; 21 cm height) located 1.36 cm above the bottom; air was sparged through a L-shaped air diffuser (7 orifices; 1.0 mm diameter) stainless steel 1/4 inch internal diameter.

2.5 Analytical techniques

Biomass growth was followed by suspended solids (SS) technique. A sample of 10 mL of mixed liquor from ALB was centrifuged (J2-HS, Beckman, USA) at 4000×g for 30 min at 4 ºC. Three phases were formed: hydrocarbon, aqueous and solid. The SS, including the oil-degrading consortium, was determined in the solid phase after heating in a low-pressure oven at 60 ºC for 48 h (DuoVac, Lab-line Inc. Instruments, USA). The SS fraction that remained trapped in the hydrocarbon phase was recovered by three successive extractions, as described above. The organic phases, including residual hydrocarbons were pooled and stored at 4 ºC in 30 mL vials. Each biomass determination was done by triplicate.

Gas chromatography was used to analyze the presence of hydrocarbons in water samples. Several hydrocarbon standard references for polyaromatics hydrocarbons (PAHS's) and Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX) were used since those are the most representative of engine combustion. PAHS's standard used during the screening were Naphthalene, Acenaphtylene, Acenaphtene, Fluorene, Phenanthrene, Anthracene, Benzo (α) Anthracene, Fluorantene, Pyrene, Benzo-α-anthracene, Crisene, Benzo-β-Fluorantene.

3 Results and discussions

3.1 Tourism development level

Mass tourism is a modern phenomenon, stemming primarily from the introduction of personal vehicles and motorized mass transportation. The Mexican Caribbean became increasingly popular as a tourist destination. The number of tourists during a year in Quintana Roo depends on the month and two seasons could be identified. Figure 1 shows tourist number each month. Two seasons could be distinguished: "Low" and "High". The 7th month, July, could be representative of "High" season and the 10th month, October, could be representative of "Low" season. These seasons are related to the intensity of traveling from national tourists and tourists from developing countries (Burger, 2002). Figure 2 shows occupancy rates from the last 5 years, July and October are representative of "High" and "Low" seasons too. This work evaluated hydrocarbon presence along the most important Mexican tourism pole: the state of Quintana Roo.

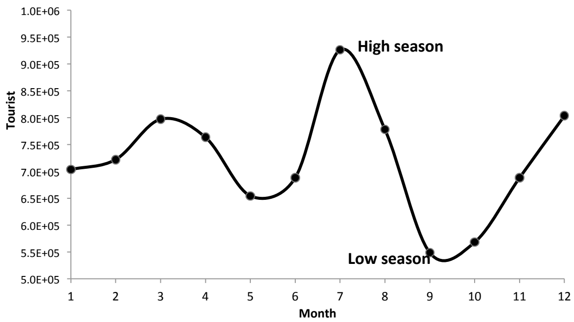

Fig. 2. Tourist number along 2012, two seasons could be distinguished: "Low" Season and "High" season.

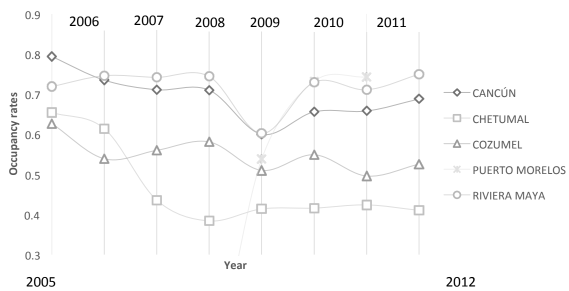

Fig. 3. Annual occupancy for different developed tourist pole. X Puerto Morelos, △ Cozumel, □ Chetumal, ◇ Cancún, o Riviera Maya.

Tourism activity not only could be related to a particular season, it also could be related to the occupancy of a particular place in the region. Figure 3 allows to separate Cancún (including Puerto Morelos since 2012), and *Riviera Maya* (Playa del Carmen and Tulum) as a pole with a larger tourist number occupancy and Cozumel and Chetumal (including Bacalar) as a minor occupancy region. Holbox shows scarce tourist activity. High tourist numbers implies generally high vehicles numbers across the highways. With the aim to study the impact of vehicles increment due to tourist activity on hydrocarbons concentration, several sinkholes along Quintana Roo were tested.

Table 1. Shows hydrocarbon presence in most of the sampling points in July of 2012, "High" season. Phenanthrene was the most common hydrocarbon detected. Phenantrene was detected in the north of the state (Cancún and Puerto Morelos) but not in the south. Although Phenantrene is the most common hydrocarbon, the benzo(a)Pyrene showed the largest concentration and was located in Playa del Carmen. Cancún and Playa del Carmen are the highest-developed tourism poles in the southern part of the state, Bacalar and Chetumal with intermediate development, presented lower hydrocarbon pollution (2.18 mg L^{-1} of naftalene and 1.14 mg L^{-1} of pyrene). Holbox sampling point did not show hydrocarbon concentration and could be related to scarce tourist activities. PAHs are environmentally significant because of their ubiquity and toxicity.

In brief, the most developed tourism poles, Cancún and Riviera Maya showed the most common contaminant and higher concentration of hydrocarbon. This result is novel for specific hydrocarbon-pollution but it supports all previous work which founded the impact of tourism and urbanization on the Caribbean (Holder, 1988).

Cancún, Playa del Carmen and Puerto Morelos were the most contaminated sites, Cozumel, Tulum, Bacalar and Chetumal show minor contamination and Holbox does not show any contaminant presence. This data could be explained for tourist development of the pole.

Our results suggest that the three kinds of tourism pole: "Low" pole (Holbox), "Medium" pole (Cozumel, Tulum, Bacalar and Chetumal) and "High" pole (Cancún, Playa del Carmen and Puerto Morelos) affect the hydrocarbon contamination levels: major development implies major hydrocarbon contamination.

Practically all the sampling points showed no hydrocarbon presence. The explanation offered could be that in these tourists poles there had been presented two vacational seasons "High" season and "Low" Season. In "High" Season the number of tourists is high while in Low Season the number of tourists is scarce. The difference between this two vacational seasons support our suggestion: concentration of hydrocarbons is related to the number of tourists. Tourism is now the largest single economic sector in the world. The impact of transportation and tourists in sinkholes environment are considerable. According with our results a previous work found impact of tourism in coastal ecosystems (Davenport and Davenport, 2006). Presence of hydrocarbons imposes the necessity of decontamination alternatives, use of native bacterial consortium could be quite important.

Table 1. Hydrocarbon presence in the sampling points in "High" season.

Tourism pole	Sampling point	Hydrocarbon	Concentration (mg L^{-1})
Cancún	Talleres	Naftalene	5.94 ± 3.62
		Phenantrene	0.09 ± 0.02
Cancún	Rancho Viejo	Hexadecane	2.02 ±2.09
Cancún	R-510	NP	
Puerto Morelos	Mojarras	Pyrene	4.96 ± 0.10
		Phenantrene	0.53 ± 0.19
Puerto Morelos	Siete bocas	Hexadecane	3.18 ± 0.02
		Phenantrene	2.54 ± 0.02
Riviera Maya	Xca - ha (Playa del Carmen)	Bencene	1.00 ± 0.01
		Benzo (a) Pyrene	9.67 ± 0.02
		Decane	1.33 ± 0.07
		Hexadecane	5.87 ± 0.23
Riviera Maya	Chaac - mol (Tulum)	Naftalene	2.57 ± 0.11
Cozumel	Chanka - nab	Naftalene	3.48 ± 0.09
		Hexadecane	2.15 ± 0.19
Chetumal	Bacalar Lagoon	Naftalene	2.18 ± 0.54
Chetumal	Milagros Lagoon	Pyrene	1.14 ± 0.11
Holbox	Ojo de agua	NP	

*July of 2012

Table 2. Hydrocarbon presence in the sampling points in "Low" season.

Localization	Sampling point	Hydrocarbon	Concentration (mg L^{-1})
Cancún	Talleres	Phenantrene	0.07 ± 0.02
Cancún	Rancho Viejo	NP	
Cancún	R-510	Naftalene	0.12 ± 0.01
Puerto Morelos	Mojarras	NP	
Puerto Morelos	Siete bocas	NP	
Riviera Maya	Xca - ha (Playa del Carmen)		
Riviera Maya	Chaac - mol (Tulum)	NP	
Cozumel	Chanka - nab	NP	
Chetumal	Bacalar Lagoon	NP	
Chetumal	Milagros Lagoon	NP	
Holbox	Ojo de agua	NP	

*October of 2012

3.2 *Native consortium identified*

With the aim to obtain a native hydrocarbon-degrading bacterial consortium, native bacteria coming from hydrocarbon polluted sinkhole of Playa del Carmen, Xca-ha, described in the previous section as the most polluted sinkhole, was isolated and cultured in a batch sequential ALB exposed to diesel to improve their hydrocarbon-degrading ability.

Fifteen (15) pure cultures able to grow in mineral salts medium with crude diesel as carbon source were identified through enrichment and isolation procedure. Table 3 shows microorganisms identified. The isolated pure cultures were identified to belong to the genera *Pseudomonas, Vibrio, Diplococcus*. Other work founded *Pseudomonas* (Chayabutra and Ju, 2000) and mixed culture (Martínez-Trujillo and García-Rivero, 2012; Tanase *et al*., 2012) genera working with oil-contaminated soil.

Differential biochemical characteristics as determined in this study have been widely used to differentiate between species of microorganisms.

Table 3. Differential biochemical characteristics, as determined in this study. The biochemical test allows identifying species 1 as *Pseudomonas*, species 2 as Vibrio and species 3 as *Diplococcus*.

Test	1	2	3
TSI	K/A	K/K	A/A
H_2S	-	-	-
GAS	+	-	+
LIA	+	+	+
M	+	+	+
I	+	-	-
O	+	+	+
Urea	-	+	-
Arginina	ND*	ND*	ND*
Gramm	Negative	Negative	Negative

*ND=Not detected

However, further DNA analysis is required to carry out an accurate identification of the specie of the microorganisms. Due to their chemical stability and high recalcitrance properties, the evaluation of different strategies for degradation of PAH is matter of global concern. Their prolonged persistence in environments is related to the low water solubility (Luning Prak and Pritchard, 2002), and limiting their availability to be bio-degraded by microorganisms (Cerniglia, 1992).

With the aim to contribute to hydrocarbon remediation a sequential batch culture was carried.

3.3 Sequential batch cultures

Native bacteria were cultured in a batch sequential bubble column bioreactor (BCB) exposed to diesel to improve their hydrocarbon-degrading ability during 8 batch culture of 14 d each.

Figure 4 shows total diesel biodegradation profiles as a function of culture time when three initial values of diesel concentration: 1.3, 3 and 13 g L^{-1}. Figure 4 shows that diesel was 98.47 ± 0.38 % degraded in only 4 days at all initial diesel concentrations; diesel was practically exhausted within 2 days at lower initial diesel.

Figure 4 shows Suspended Solids (SS) profiles as a function of culture time when three initial values of diesel concentration: 1.3, 3 and 13 g L^{-1}. We considered it pertinent to compute batch culture productivity at 13 g L^{-1} when the instant productivity, defined as the derivative of SS (g L^{-1}) with respect to time, was zero (day 6).

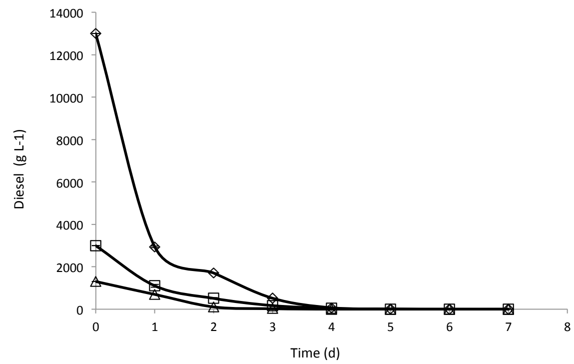

Fig. 4. Diesel uptake from: ◇ 13 g L^{-1}, □ 3 g L^{-1}, △ 1.3 g L^{-1}.

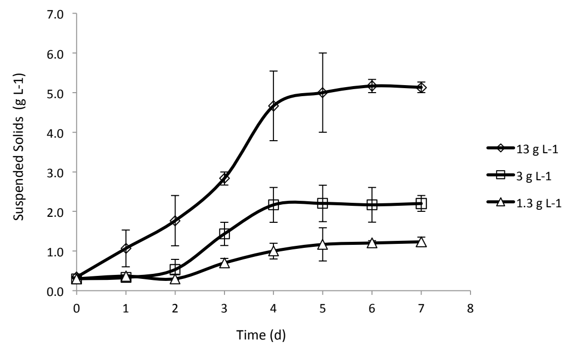

Fig. 5. SS production from: ◇ 13 g L^{-1}, □ 3 g L^{-1}, △ 1.3 g L^{-1}.

In the case of 3 g L^{-1} and 1.3 g L^{-1}, the derivative never was strictly zero and productivity was computed at the end of the batch. SS yield were 0.40 ± 0.01 g SS (g diesel) $^{-1}$, 0.13 ± 0.01 g SS (g diesel) $^{-1}$ and 0.07 g SS (g diesel) $^{-1}$ respectively. Productivity were 1.0 ± g SS (L d) $^{-1}$, 0.4 and 0.2 g SS (L d)$^{-1}$, respectively. Productivity was evaluated at day 5 when diesel was exhausted. Previous work (Kim *et al*., 2009; Isaac *et al*., 2003) reported the biodegradation of phenentrene and HAP by bacterial consortia respectively.

Conclusions

Most of the sampling sinkholes studied in our work in Mexican state of Quintana Roo show hydrocarbon presence. Aliphatic and aromatic hydrocarbons were detected in most of the sampling points in tourist pole development (Cancún and Playa del Carmen). In comparison Holbox which is a recent touristic development where transportation of tourist and locals has not reached the numbers of Cancún or Playa del Carmen. Other regions of Quintana Roo, with intermediate development of tourism (Puerto Morelos, Tulum, Cozumel and Bacalar) show hydrocarbon presence too. Additionally our work found that concentration of hydrocarbons is related to vacational season, sinkholes during "High" season shows major hydrocarbon concentration and diversity than during "Low" season.

Furthermore, the development of a new water cleaning approach by airlift bioreactors, using indigenous microbial consortium, shows promising results and may be used in future *ex-situ* remediation technique in tourism poles.

References

Bhoosreddy, W. (1995). *Manual of Diagnostic Microbiology*. pp. 185-193. Himalaya Publishing House, Bombay.

Burger, J. (2002). Tourism and ecosystems. In: Douglas I. (Ed), Causes and consequences of global climate change. *Encyclopedia of global environmental 3*, 597-609.

Cerniglia, C.E. (1992). Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation 3*, 351-368.

Chayabutra, C., Ju, L-K. (2000). Degradation of n-hexadecane and its metabolites by Pseudomonas aeruginosa under microaerobic and anaerobic denitrifying conditions. *Applied and Environmental Microbiology 66*, 493.

Davenport, J., Davenport, J.L. (2006). The impact of tourism and personal leisure transport on coastal environments: A review. *Estuarine Coastal Shelf Science 67*, 280-292.

Dixon, J., Hamilton, K., Pagiola, S. and Segnestam L. (2001). *Tourism and Environment in the Caribbean: An Economic Framework*. Environment Department Papers. The World Bank, Washington, DC.

Gold-Bouchout, G., Metcalfe, D., Drouillard, K. (2009). Contaminantes tradicionales y emergentes, un amenaza para el acuífero. En Libro de resúmenes del seminario: *Análisis de la vulnerabilidad y riesgo de la contaminación de las aguas subterráneas en la península de Yucatán* (F. Bautista *et al*., eds), Pp 35. CIGA-UNAM, Mérida, Yucatán.

Holder, J.(1988). Pattern and impact of tourism on the environment of the Caribbean. *Tourism Management 9*, 119-127.

Holt, J.G., Kreig, N.R., Sneath, P.H.A., Stanely, J.T., Williams, S. T. (1994). *Bergey?s Manual of Determinative Bacteriology*. Williams and Wilkins, Maryland.

Isaac, P., Sanchez, L.A., Bourguignon, N., Cabral, M.E., Ferrero, M.A. (2013). Indigenous PAH-degrading bacteria from oil-polluted sediments in Caleta Cordova, Patagonia Argentina. *International Biodeterioration and Biodegradation 82*, 207-214.

Jain, P.K., Gupta, V.K. Gaur, R.K. Lowry, M., Jaroli, D.P., Chauhan, U.K. (2011). Bioremediation of contaminated soil and water. *Research Journal of Environmental Toxicology 5*, 1-26.

Kennedy, R. S., Finnerty, W. R., Sudarsanan, K., Young, R.A. (1975). Microbial assimilation of hydrocarbons. *Archives of Microbiology 102*, 75-83.

Kim, Y.M., Ahn, C.K., Woo, S.H., Jung, G.Y., Park, J.M. (2009). Synergic degradation of phenanthrene by consortia of newly isolated bacterial strains. *Journal of Biotechnology 144*, 293-298.

Lizardi-Jiménez, M. A., Leal-Bautista, R. M., Ordaz A. and Reyna-Velarde R. (2014). Airlift bioreactors for hydrocarbon water pollution remediation in a tourism development pole. *Desalination and Water Treatment 1-6*, DOI: 10.1080/19443994.2013.876670

Luning Prak, J.D., Pritchard, P.H. (2002). Degradation of PAHs dissolved in Tween 80 surfactant solutions by Sphingomonas paucimobilis EPA 505. *Canadian Journal of Microbiology 48*, 151-158.

Martínez-Trujillo, M.A. García-Rivero, M. (2012). Environmental applications of immobilized microorganisms. *Revista Mexicana de Ingeniería Química 11*, 55-73.

Medina-Moreno, S.A., Huerta-Ochoa, S., Lucho-Constantino, C.A., Aguilera-Vazquez, L., Jimenez-Gonzalez, A. and Gutierrez-Rojas, M. (2009). Biodegradation modeling of sludge bioreactors of total petroleum hydrocarbons weathering in soil and sediments. *Revista Mexicana de Ingeniería Química 8*, 245-258.

Metcalfe, C.D., Beddows, P.A., Bouchot, G., Metcalfe, T.L., Li, H., Van Lavieren, H. (2011). Contaminants in the coastal Karst aquifer system along the Caribbean coast of the Yucatan Peninsula, Mexico. *Environmental Pollution 159*, 991-997.

Ruiz-Marín, A., Zavala-Loria, J. C., Canedo-López, Y. and A. V. Cordoba-Quiroz. (2013). Tropical bacteria isolated from oil-contaminated mangrove soil: Bioremediation by natural attenuation and bioaugmentation. *Revista Mexicana de Ingeniería Química 12*, 553-560.

Scholz-Böttcher, B.M., Ahlf, S., Vazquez-Gutierrez, F., Rullkötter, J. (2008). Sources of hydrocarbon pollution in surface sediments of the Campeche sound, Gulf of México, Revealed by biomarker analysis. *Organic Geochemistry 39*, 1104-1108.

Tanase, A., Ionescu, R., Chiciudean, I., Vassu, T., Stoica I. (2012). Characterization of hydrocarbon-polluted bacterial strains isolated from oil-polluted soil. *International Biodeterioration and Biodegradation 84*, 1-5.