Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.876670

54 (2015) 44–49
April

Taylor & Francis
Taylor & Francis Group

Airlift bioreactors for hydrocarbon water pollution remediation in a tourism development pole

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Received 12 July 2013; Accepted 9 December 2013

ABSTRACT

Underwater sinkholes in Quintana Roo, Mexican Caribbean, are affected by the run-off of nearby highways promoting the presence of aromatic and polyaromatic hydrocarbons. Phenanthrene, naphthalene, and benzene derivatives were found as the most common hydrocarbon contaminants present in underwater sinkholes located in Cancún and Playa del Carmen, two well-developed tourism poles. This confirms the impact of urban activities related with the increased transportation of locals and tourists around these areas, in comparison with Holbox which is a recent touristic development where transportation of tourists and locals is scarce; there is no hydrocarbon detectable pollution. Additionally, 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.

Keywords: Airlift bioreactor; Hydrocarbon; Water pollution; Tourism

1. Introduction

The tourism industry is one of the most important economic activities for many countries. Few regions of the world are as reliant on their natural resources for economic development as the Caribbean [1]. Caribbean countries are four times more dependent on tourism than any other area in the world [2,3]. Intensive development of tourism on the coast of the southern Mexican state of Quintana Roo, specifically Cancún and *Riviera Maya*, is causing pollution of aquifers which, in turn, impacts the marine ecosystems [4]. Environmental studies are primary focused on pharmaceutical and personal care products [5]; however, there is little published work about hydrocarbons pollution in this region. Environmental pollution with petroleum and petrochemical products (complex hydrocarbon mixture) has been recognized as one of the most important current problem in the southern region of Mexico such as Campeche, which is one of the adjacent states to Quintana Roo [6]. Accidental leakages from petroleum carrying ships lead to oily layers on the water surface [7]. The Yucatan peninsula, where the state of Quintana Roo is located, is a high permeability fractured limestone, a well-developed karst system of interconnected fractures, joints, and

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solution openings with little top soil which allows a quick transport of microbial and chemical contamination resulting in a significant potential increase of pollution affecting the ecosystems. Sinkholes are one of its many attractions of the area, as many have been enabled for snorkeling or swimming, and are also clear paths for contamination. The waste is leaking into these passages where contaminants flow through them and impact the groundwater. Recent studies have detected the presence of hydrocarbons on sinkhole surface water in the region [5]. The explanation offered is the presence of hydrocarbons from run-off cars driven on the roads nearby [5]. Since tourism resorts require effective transport links, the increase in the number of motor vehicles could aggravate the problem during the coming years. Many coastal roads were built simply to connect resorts and sight-seeing opportunities [3]; those structures change the current coastal systems and disturb the environmental patterns of these areas. So, it is necessary to create technologies that contribute to the remediation of this special concern. Hydrocarbons and its derivatives, are susceptible to microbial degradation processes [8] generating a large industrial and research interest in the development of technologies to recover water and contaminated soils such as excavation and landfill, steam stripping, stabilization and solidification, soil washing, chemical precipitation, vitrification, incineration, among other physicochemical methods [9]. Many of these treatments do not eliminate the pollutant compounds but transfer them from one place to another, in addition, some of these methods are expensive and sometimes generate more toxic products that require further treatment. The biotechnological alternative presented in this paper refers to the use of oil-degrading microbial consortium [8], an alternative that does not have the disadvantages of physicochemical methods. Microbial consortia hydrocarbon degraders can be grown in airlift bioreactors (ALBs). ALBs have important advantages over bubble-column and stirred bioreactors. ALB reduces cell damage, higher aeration rates, larger mass transfer capacity, higher liquid surface velocity and gas flow, simpler construction and lower energy costs [10]. Also, morphological and metabolic changes in cultured cells are limited in ALB, for that reason ALB are widely used in bioprocesses [10]. The number of applications of ALB in environmental bioremediation technologies has increased, establishing its role in bioprocesses requiring high oxygen transfer, low power consumption and no mechanical agitation [11].

The aim of this work was to evaluate the hydrocarbon contamination in sinkholes of a well-developed tourism poles, as Cancún and Playa del Carmen, and compare them with other low-developed pole like Holbox. The use of ALB with native microbial consortia as a remediation alternative for hydrocarbon water pollution in a tourism pole was also evaluated. Finally this work included a kinetic and stoichiometric characterization of a Cancún native sinkhole consortium degrading a synthetic media containing diesel oil.

2. Materials and methods

2.1. Sampling points

Fig. 1 shows the study area in Quintana Roo. Study sites were located in the urban zone of Cancún City, Playa del Carmen, and Holbox. A total of nine samples were taken. The sample was taken from the upper water body from 1 to 1.5 m depth and deposited in amber glass vials filled without air bubbles. Samples were labeled and kept at low temperatures during transport to the laboratory (4 ± 2˚C). The entire sampling procedure was following Mexican normative (NMX-AA-014-1980).

2.2. Microbial consortium culture

A native sinkhole consortium obtained from the sample point located in the urban zone of Cancún was cultured in diesel enriched liquor at 37˚C 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 ALB with a previously reported mineral medium [8] added with $13\,\mathrm{g\,L^{-1}}$ of diesel oil for 14 d. At the end of the culture time, the ALB was drained and the original conditions were restituted.

2.3. Micro-organism isolation and identification

Pre-enriched culture samples from hydrocarbon-polluted sinkholes were used. Brain heart infusion broth (BHI) and Lauryl sulfate broth (LSB) were used as pre-enriched 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˚C 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 [12] with diesel as a substrate and a serial dilution-agar plating technique on nutrient agar medium [13], respectively. With the aim of isolating microbial strains by morphological characteristics the

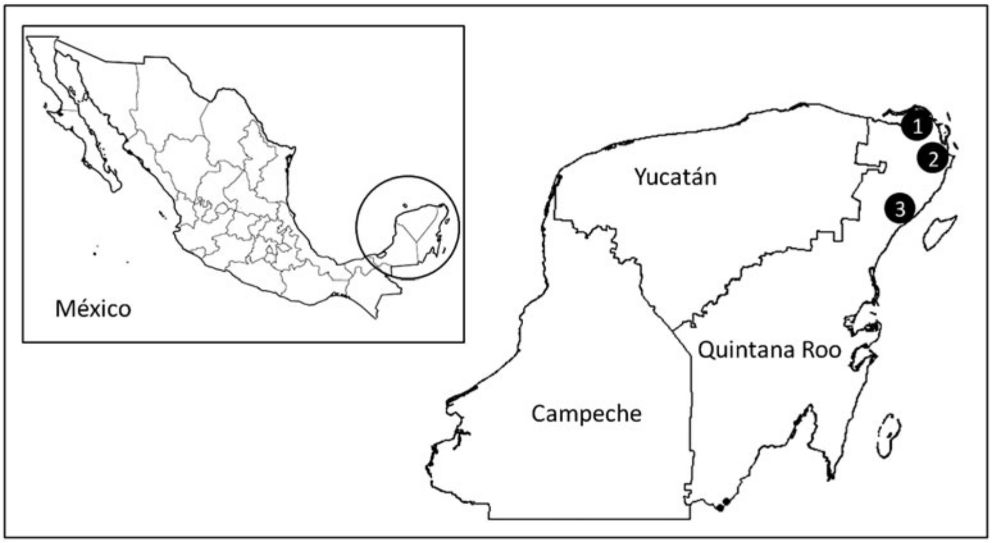

Fig. 1. Study area in Quintana Roo: Holbox (1), Cancún City urban zone (2), and Playa del Carmen (3).

next solid culture medium was used: xylose-lysine-desoxycolate, Salmonella-shigella, Hektoen, SulfitoBismuto, Bairr-Parker, TCBS. The isolated bacterial cultures were characterized by morphological and biochemical characteristics [14]. The biochemical tests used were solid culture medium: TSI, LIA, H2S, GAS, Motility Indol Ornitine (M.I.O.), liquid: APA, Arginine, Lysine.

2.4. Bioreactor

A 1 L 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 an L-shaped air diffuser (7 orifices; 1.0 mm diameter) stainless steel with 1/4 inch internal diameter.

2.5 Analytical techniques

Biomass growth was followed with suspended solids (SS) technique. A sample of 10 mL of mixed liquor from ALB was centrifuged (J2-HS, Beckman, USA) at 4,000 g for 30 min at 4˚C. Three phases were formed: hydrocarbon, aqueous, and solid. The SS, including the oil-degrading consortium, were determined in the solid phase after heating in a low-pressure oven at 60˚C 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˚C 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 polyaromatic hydrocarbons (PAHs) and BTEX were used since those components are the most representative of engine combustion. PAH standard used during the screening were Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Benzo (α) Anthracene, Fluoranthene, Pyrene, Crisene, Benzo-β-Fluoranthene, Benzo (k) Fluoranthene, Benzo (α) Pyrene, Dibenzo (α,β) Anthracene, Benzo (ghi) perylene, and Indene (1,2,3 cd) Pyrene.

The residual hydrocarbons were determined from the same flasks prepared for the biotic experiments of emulsion characterization. Hydrocarbons in the pooled organic phases were measured by gas chromatography (Varian model 3900, USA) at 300˚C with a flame ionization detector, a DB-Petro narrow-bore column (30 × 0.00025 m; J&W Scientific), and helium as the carrier gas. The injector and detector temperatures were constant at 290 and 300˚C, respectively. The temperature program was: 120˚C for 1 min; increase by 10˚C min^{-1} until 150˚C (2 min); then by 15˚C min^{-1} until 170˚C (1.5 min). The detection limit for hydrocarbon determination method was 0.03 mg L^{-1}.

2.6. Biomass characterization

The growth yield ($Y_{X/S}$) was calculated from biomass growth and substrate uptake at the end of each experiment. The removal rate (R) was calculated using only data obtained at the beginning of each experimental condition.

2.7. Statistical method

The values obtained in triplicate for SRA, H_2S production, and TCE biodegradation during the experimental setup were compared using the Tukey–Kramer tests performed after analysis of variance ($\alpha = 0.05$) using the NCSS 2000 software (NCSS, Jerry Hintze).

3. Results and discussion

3.1. Evaluation of hydrocarbon contamination in Cancún and Riviera Maya Sinkholes

Table 1 shows the concentration of PAHs found in the studied areas. Cancún and *Riviera Maya* have presence of PAHs while hydrocarbon presence was not found in Holbox. Cancún and *Riviera Maya* as two important tourism development poles are highly visited by people that use cars to communicate, and consequently hydrocarbons spills from run-off cars driven on the roads nearby [5] are detected, while Holbox that has scarce tourism activity shows no hydrocarbon pollution. The presence of PAHs is, according with other studies [5], only focused in Playa del Carmen.

3.2. Native consortium identification and sequential batch culture

With the aim to obtain a native hydrocarbon-degrading bacterial consortium, native bacteria coming from hydrocarbon polluted sinkholes described in the previous section were isolated and cultured in a batch sequential ALB exposed to diesel to improve its hydrocarbon-degrading ability.

Table 1
Hydrocarbon concentration in sinkholes in Cancún, Playa del Carmen, and Holbox

Sampling points	Hydrocarbon	Concentration ($mg\,L^{-1}$)
Cancún	Naphthalene	5.94 ± 3.62
	Phenanthrene	0.90 ± 0.0
	Benzo-α-anthracene	0.03 ± 0.0
	Dibenzo (α,β) anthracene	0.82 ± 0.01
	Indene	0.11 ± 0.00
	Benzo-β-fluoranthene	0.07 ± 0.01
	Benzo (k) fluoranthene	0.32 ± 0.02
Playa del Carmen	Phenanthrene	1.54 ± 0.03
Holbox	ND*	ND*

*ND: not detected.

Sixteen pure cultures able to grow in mineral salts medium with crude diesel as carbon source were identified through enrichment and isolation procedure. Table 2 shows the identified micro-organisms. The isolated pure cultures were identified to belong to the genera *Pseudomonas*, *Vibrio*, *Enterobacter aerogenes,* and *Escherichia coli*. Differential biochemical characteristics as determined in this study have been widely used to distinguish between species of micro-organisms. However, further DNA analysis is required to carry out an accurate identification of the micro-organism species. Due to its chemical stability and high recalcitrance properties, evaluation of different strategies for degradation of PAHs is matter of global concern. Its prolonged persistence in environments is related to the low water solubility [15], and limiting its availability to be biodegraded by microorganisms [16].

Recent studies report *Pseudomonas* genera [17] and mixed culture [18] as hydrocarbon degrading strains.

3.3. Evaluation of ALBs as hydrocarbon contamination degrading

The potential of the native consortium to degrade a complex hydrocarbon mixture was tested in an ALB. Naphthalene, phenanthrene, and benzene, ratio of 1:1:1 w/w, were chosen as the model mixture fed to the ALB, since those are typical hydrocarbons found in Cancún and Playa del Carmen. Fig. 2 shows three kinetic studies done at 1.3, 3, and $13\,g\,L^{-1}$ of the mixture. As it is observed in Fig. 2, all the hydrocar-

Table 2
Differential biochemical characteristics, as determined in this study. The biochemical test allows identifying species 1 as *Pseudomonas*, species 2 as *Vibrio*, species 3 as *Diplococcus,* and species 4 as *Enterobacter*

Test	1	2	3	4
TSI	K/A	K/K	A/A	A/A
H_2S	−	−	−	−
GAS	+	−	+	−
LIA	+	+	+	+
M	+	+	+	+
I	+	−	−	+
O	+	+	+	+
Urea	−	+	−	ND*
Arginina	ND*	ND*	ND*	−
Gramm	Negative	Negative	Negative	Negative

*ND: not detected.

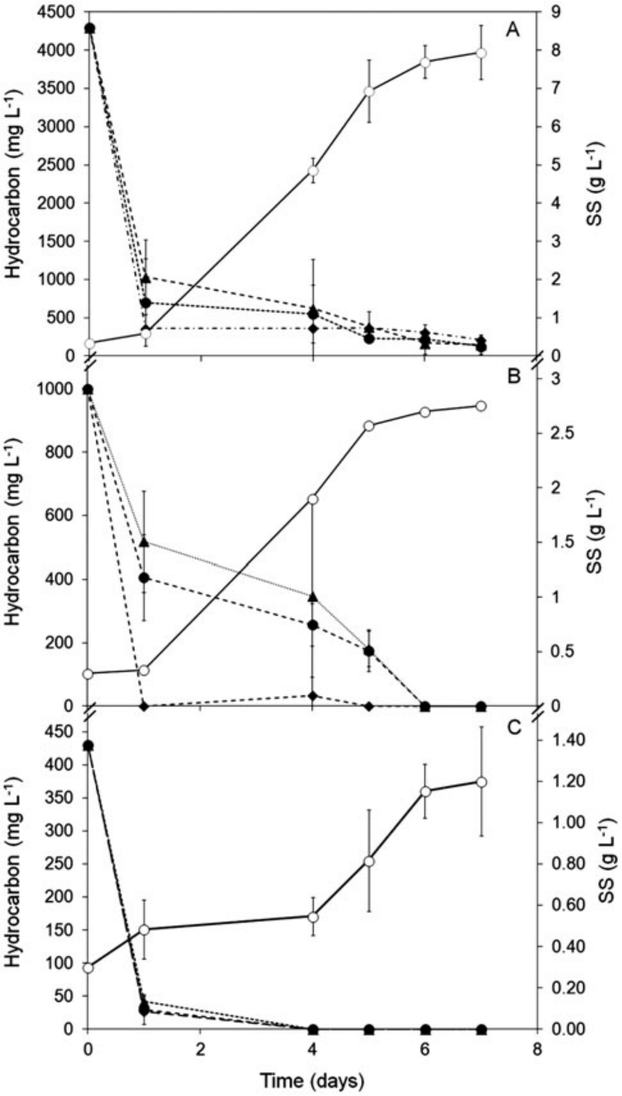

Fig. 2. Experimental data observed during the biodegradation of 13 (A), 3 (B), and 1.3 (C) g L^{-1} of hydrocarbon mixture. Biomass (○), naphthalene (▲), phenanthrene (♦), and benzene (●).

Table 3
Growth yield ($Y_{X/S}$) and hydrocarbon removal rate (R) were observed during the biodegradation studies performed at different initial hydrocarbon concentrations (C_0). Letters in parenthesis are Tukey–Kramer test results ($\alpha = 0.05$). Values with the same capital letter are not significantly different

C_0 (mg L^{-1})	$Y_{X/S}$ (mg SS mg^{-1} HC's) (A)	R (mg L^{-1} h^{-1})
1.3	0.698 ± 0.205	50 ± 3 (B)
3	0.819 ± 0.071	87 ± 10 (C)
13	0.614 ± 0.072	450 ± 35 (D)

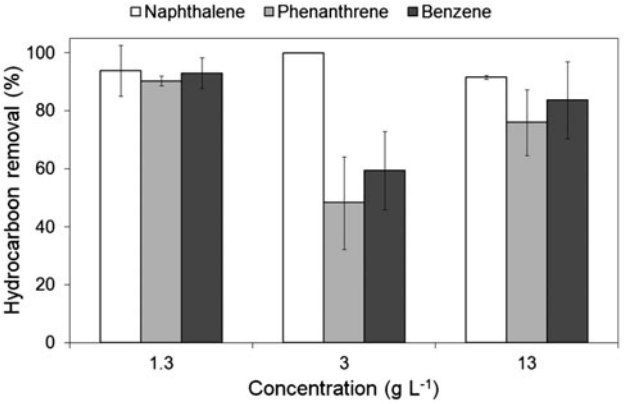

Fig. 3. Hydrocarbon removal (%) was observed after 24 h for all the experiments.

bons were practically biodegraded within the first day. However, important biomass growth was only observed from fourth day on ahead. Delay in the biomass growth during hydrocarbon consumption was attributed to an affinity of the biomass for short carbon intermediates produced during the biodegradation of the hydrocarbon mixture. Recent studies reported the biodegradation of phenanthrene [19] and PAHs [20] by bacterial consortia. It can also be observed that the three hydrocarbons were biodegraded almost at the same velocity during studies performed at 1.3 and 13 g L^{-1}; however during studies at 3 g L^{-1} ,phenanthrene was biodegraded very fast compared with anthracene and benzene. Table 3 shows that the $Y_{X/S}$ calculated during each kinetic study was not significantly different; also the observed values were close to others author's works [8]. A linear correlation was observed between the removal rate (R) and hydrocarbon concentration ($R = 34.37\ C_0$, $R^2 = 0.997$).

Fig. 3 shows the hydrocarbon removal (%) observed within the first day for all the experiments. The experiment carried out at 1.3 g L^{-1} showed 92.37 ± 5.46% of hydrocarbon removal during the first day while the experiment at 13 g L^{-1} showed 83.73 ± 11.09% of hydrocarbon removal; both data were not significantly different.

4. Conclusions

Water in sinkholes is affected by the run-off of nearby street and highways promoting the presence of PAHs, which are mostly related to combustion fuels. This confirms the impact of urban activities related with the increased transportation of locals and tourism around these areas. 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. Furthermore, the development of a new water cleaning approach by ALBs, using an indigenous microbial consortium, shows promising results and may be used in future *ex situ* remediation technique in tourism poles.

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