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Degradation of Phenanthrene by Natural Consortia in Seawater

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Abstract

In this study 17 aerobic bacterial consortia were isolated from seawater and sediments; the culture medium was enriched with phenanthrene to evaluate the potential degradation of this pollutant. The consortia were evaluated for their ability to degrade phenanthrene as the only source of carbon and energy in synthetic seawater. The percentage degradation of the consortium S4 was 75% after 7 days of incubation (100mgL^{-1}), with a specific microbial growth rate of 0.031 h^{-1} at 33°C$\pm$ 3° and 150 r min^{-1}.

Keywords: *phenanthrene, consortia, seawater, biodegradation*

1. Introduction

The phenanthrene is a simple polycyclic aromatic hydrocarbon(PAHs), that is more distributed in nature, as it is a component of fossil fuels and which is formed by incomplete combustion of organic matter (JUHASZ AND NAIDU [5]). Therefore this compound has been used as a model for studies of biodegradation of polycyclic aromatic hydrocarbons by bacteria (MROZIK AND PIOTROWSKA-SEGET [9]). The phenanthrene contains in its structure three fused benzene rings and a region-bay and a region-K, which gives the characteristic of being highly reactive chemically and biologically, is considered a dangerous pollutant to aquatic life, plants and other organisms and has often been mentioned as one of the 16 PAHs, which may be carcinogenic. The degradation of this pollutant is necessary to preserve the environment and human. SCHVOERER & al. [13] presented an investigation on human health risk assessment, their results indicated that 7.5% of the individuals experienced some type of wound and 53% some health problem (30% lumbar pain, 22% migraine, 16% dermatitis) and in a smaller degree ocular irritation (9%), respiratory problems (7%) and nausea (6%).

Differentbacteria as *Sphingomonas sp. Pseudomonas sp. Staphylococcus sp. Bacillus sp. Nocardioides sp*. among others, are known as phenanthrene degraders, which can mineralize low molecular weight hydrocarbons (MALLICK & al. [8]; DODDAMANI AND NINNEKAR [2]; SAITO & al. [12]; TAO & al. [15]; PRABHU AND PHALE [10]). Bacteria are an essential component in the food chain, through their interaction with other organisms, result in changes in their environment and are able to grow in polluted environments (BOSCHKER & al. [1]). In general, biodegradation with pure strains is not the actual behavior of the microorganisms in the environment; bioremediation actually depends on the interaction of the metabolic activities

of mixed microbial populations. An advantage of using microbial consortia is that they have multiple metabolic interaction capacities that enhance the efficiency in the bioremediation process (GHAZALI & al. [3]).The objective of this study was to obtain and evaluate the potential degradation of isolated consortia of seawater and sediments to withstand the high temperatures that are on average in the region to study using phenanthrene as carbon and energy source, by comparing percentages of degradation of microbial consortia and their growth kinetics.

2. Materials and methods

Seawater (0-15 cm) and sediments samples (0-5 cm) contaminated with phenanthrene were collected at ten stations in North Beach, in Ciudad del Carmen, Campeche, Mexico (N 18°39´53.6–WO 91°50´22.2; N 18°39´53.6–WO 91°50´50.8; N 18°39´52.9–WO 91°50´20.7; N 18°39´54.3–WO 91°50´56.3; N 18°39´56.4–WO 91°50´28.7; N 18°39´58.7–WO 91°50´06.7; N 18°39´00.7– WO 91°50´42; N 18°39´06.4–WO 91°50´12; N 18°39´08.3–WO 91°50´03.5; N 18°39´13.7–WO 91°50´40) (Figure 1). The samples were placed into brown glass bottles sterilized and stored at 4°C until used.

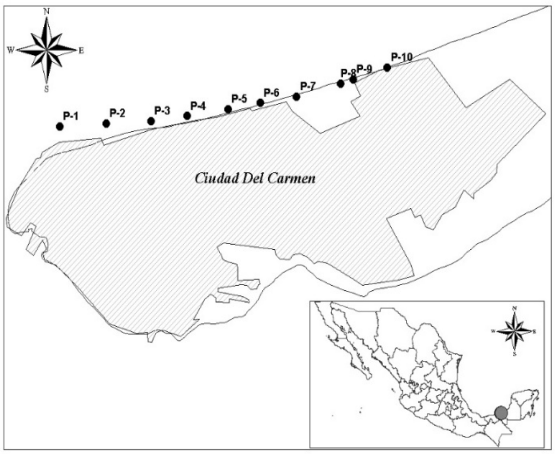

Figure 1. Sampling sites for the production of inoculum for isolation of consortia in Mexico.

Initial cultures were established by inoculating 1 g of sediment and 30 mL of sea water in 300 mL of mineral salts medium (M9) (JIRASRIPONGPUN [4]) . The composition of M9consisting of the following (per kg): Na_2HPO_4, 6000mg; K_2HPO_4, 3000mg; NaCl, 500mg; NH_4Cl, 1000mg, 2 mL $MgSO_4$ solution (120,000mg Kg^{-1}), 0.1 mL $CaCl_2$ solution (111,000mg Kg^{-1}). The pH was adjusted to 7 with HCl and NaOH solutions. A stock solution of phenanthrene in acetone (10000mg Kg^{-1}) was added to a sterile liquid medium in a concentration of 40 mg Kg^{-1}. All flasks were incubated at room temperature (33 ° C $\pm$ 3°) in a rotary shaker at 150 rpm for two weeks in darkness. The temperature is much higher than the real temperature of the environment reported in other work, because the average temperature in the state is 37°C. Phenanthrene degrading consortia were obtained after four transfers in series of intervals of approximately two weeks, by transferring 10 mL of active culture to a new flask containing the same culture medium. The consortia were isolated by enrichment cultures to increasing phenanthrene to a final concentration of 100 mg Kg^{-1}. Phenanthrene (98% pure) was obtained from Sigma Chemical (St. Louis, MO USA).

The next step was to adapt the consortia to artificial seawater (AS) 100% (LYMAN AND FLEMING [7]). This was in order to accustom the consortium to high salt concentrations. As composition per kg: NaCl, 24500mg, $MgCl_2 \cdot 6H_2O$, 11,100mg; Na_2SO_4, 4100mg, $CaCl_2$,

1540mg; KCl, 700mg; $NaHCO_3$, 200mg; KBr, 100mg; H_3BO_3, 30mg; $SrCl_2 \cdot 6H_2O$, 20mg; NaF, 3 mg. The pH was adjusted to 7.5. All media and solutions were prepared with distilled water and autoclaved at 121° C for 15 min.

1.1. Biodegradation experiments. To determine the ability of phenanthrene degradation of isolated consortia of micro scale, first, 100 ppm of the solution of phenanthrene in 50 mL sterile flasks, after vaporized acetone was added 14.5 mL of AS, and finally inoculated with 0.5 mL of each microbial consortium. The treatment was carried out with three replications of each consortium and uninoculated control. Flasks were incubated with shaking (150rpm) for 7 days at room temperature. Samples were taken after 1, 3, 5 and 7 days. Then the residual phenanthrene was extracted and quantified.

Simultaneously, the kinetics growth of each consortium were determined through plates microbial counts (CFU mL^{-1}) on nutrient agar. An aliquot of 1 mL of the medium was added in 9 mL of sterile saline solution. Dilutions were made in saline solution containing 0.85% of NaCl. Stirred in a vortex and corresponding dilutions performed. All plates were incubated at room temperature for 3 days.

1.2. Analytic method Quantitative analysis of phenanthrene was carried out in a GC-MS (TRACE GC ULTRA-ITQ). A TG-SQC capillary column (30 m length, 0.25 mm I.D., and 0.25 µm film thickness) was used. The temperatures of the injector, detector and transfer line were 250, 270, 270°C, respectively. The condition used in the oven ramp was: initial temperature of 50°C for 1 min with increase of temperature of 15°C /min to 225°C and heated until 300°C at 30°C/min and kept for 1 min. Split less mode injection were carried out with an injection volume 1µL. Helium was used as gas carrier at a flow rate of 1 mL/ min.

The recovery of phenanthrene was carried out based on the method 610 (US-EPA [17]) of dichloromethane, and allowed to stand for 10 minutes to separate the organic solvent from the aqueous phase, three times. The three organic extracts were combined in a 30 mL vial for analysis by GC-MS.

The quality was examined to analysis the validation of parameters: the range of linearity of this method in 100-1000 µg mL^{-1} is quite good since the correlation coefficient of the concentration of phenanthrene was always >0.999. Control test phenanthrene were determined to assess the efficiency of recovery under extraction conditions, was > 92.2 ± 2.86%, the detection limit (MDL) was 9.28 µg mL^{-1}.

1.3. Determination of growth parameters. The microbial growth rate (µ) was calculated in the exponential growth phase and obtained for each consortium. The equation is the Monod:

$$\mu = \frac{\left(ln\frac{X}{X_0}\right)}{t}$$

where: "X", presents the (CFU/ml); "Xo", microbial concentration at t = 0; and "t", time (h).

1.4. Statistical analyses. The mean and standard deviation values of phenanthrene were calculated of three independent samples extractions. Statistical significance of the differences compared with reference the consortium was determined by a parametric one-way ANOVA

test ($p<0.05$). Statistical analyses were performed using the Statgraphics Centurion XV (StatPoint, Inc).

2. Results and discussion

2.1. Phenanthrene degrading abilities of isolated consortia. The sampling area is characterized by intense industrial expansion, which includes industrial, fishing ports and offshore crude exploitation. This place was studied to evaluate the potential phenanthrene degrading consortia. 17 consortia were obtained; ten were from seawater (A1, A2 A3, A4, A5, A6, A7, A8, A9, and A10) and seven from sediment (S2, S3, S4, S5, S7, S8, and S10).

All consortia obtained were used to assess their ability to use phenanthrene at concentrations of 100 mg /L in synthetic seawater. The ranges of phenanthrene degradation in seawater were 33% for the consortium A9 and 61% of the consortium A8, after 7 days of incubation (Figure 2). In sediment degradation ranges were 28% for the S7 consortium and 75% in the consortium S4 (Figure 3), which showed the highest degradation of all consortia isolated.

The control without inoculum was observed a phenanthrene decrease of 11%; this is due to the loss of the substrate likely by volatilization or chemical degradation.

However, the statistical analysis showed no significant difference between the mean of 16 consortia but it was significant between these 16 and the consortium S4, with $p<0.05$.

Researchers report 40% degradation of phenanthrene by consortia in 6 days in synthetic seawater, due to metabolic activity of the consortia (TAM & al. [14]). This indicates that the metabolites produced during phenanthrene degradation with various members of the consortium may facilitate the complete removal of the contaminant. Phenanthrene biodegradation is influenced by a number of environmental factors such as salinity, phenanthrene concentrations, and additions of coal and nutrients.

2.2. Microbial growth

Microbial growth consortia A8, A9, A5, A10, A6 and A7, no has differences in reaching a stationary phase (Figure 2), which showed very similar degradation rates. In contrast consortia A2, A3, A1 and A4 increased the percentage of degradation during the incubation time. This demonstrated that microbial growth in its exponential phase, indicates that degradation will increase until they reach the stationary phase of microorganisms.

The microbial consortium growth S4showed in its exponential phase, reached levels similar to other consortium (Figure 3), although this consortium was inoculated with a lower initial microbial load compared to other consortia. Therefore it indicates of a better assimilation of phenanthrene as carbon and energy source. However for terms of comparison with other researches in this study was calculate the specific microbial growth rate (μ), for all consortia (Table 1). In this study the degradation rates of different consortium vary with their respective growth rate of degradation, to the consortium S4 was 0.0312 $h^{-1,}$ and for S10 was 0.0180 h^{-1} compared with the consortia A7 and A8 of 0.018 h^{-1} and 0.015 h^{-1} respectively. Researches, shows μ of 0.028$h^{-1}$and 0.033h^{-1}, for 30 days treatment with *Rhodotorula glutinis* and 2 days with *Pseudomona*s, respectively (ROMERO & al. [11]; TIAN & al. [16]). The values obtained show that the isolated consortium has a high percentage of degradation and can be considered efficiently in phenanthrene degradation.

However, take note that those results were obtained using 100% seawater, thus, salinity is a factor affecting the adaptation process of consortia for degradation, high concentrations will alter in the cell membranes and cause their death (KARGI AND DANCER [6]). In the present study various consortia obtained were able to survive and maintain biodegradation rates in high salinity conditions.

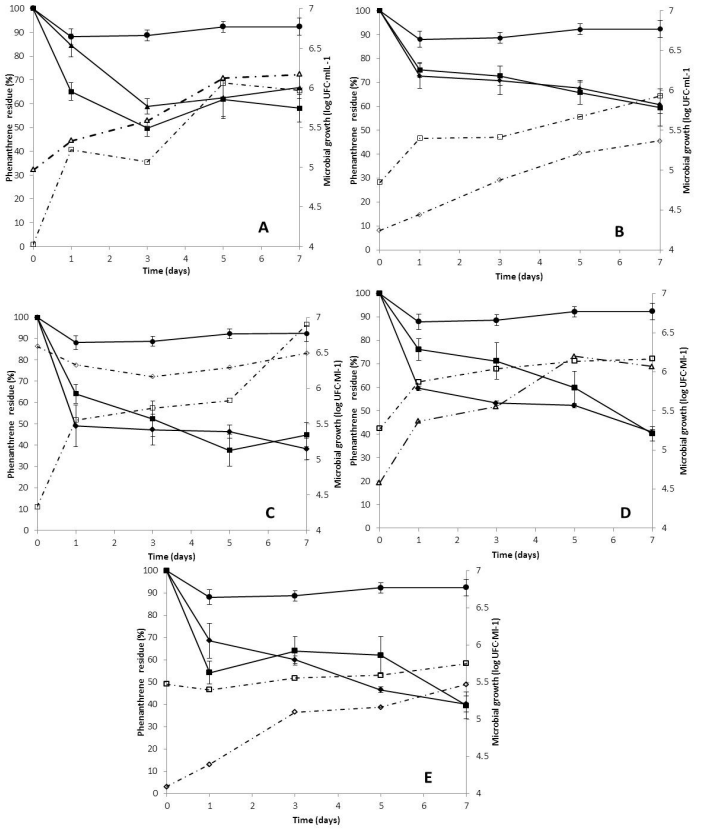

Figure 2. Degradation of phenanthrene by consortia (▲, ■) and microbial growth (Δ, □). Control (●). A) A9, A5; B) A2, A3; C) A8, A1; D) A10, A6; E) A4, A7. Mean and standard deviation of three replicates are shown.

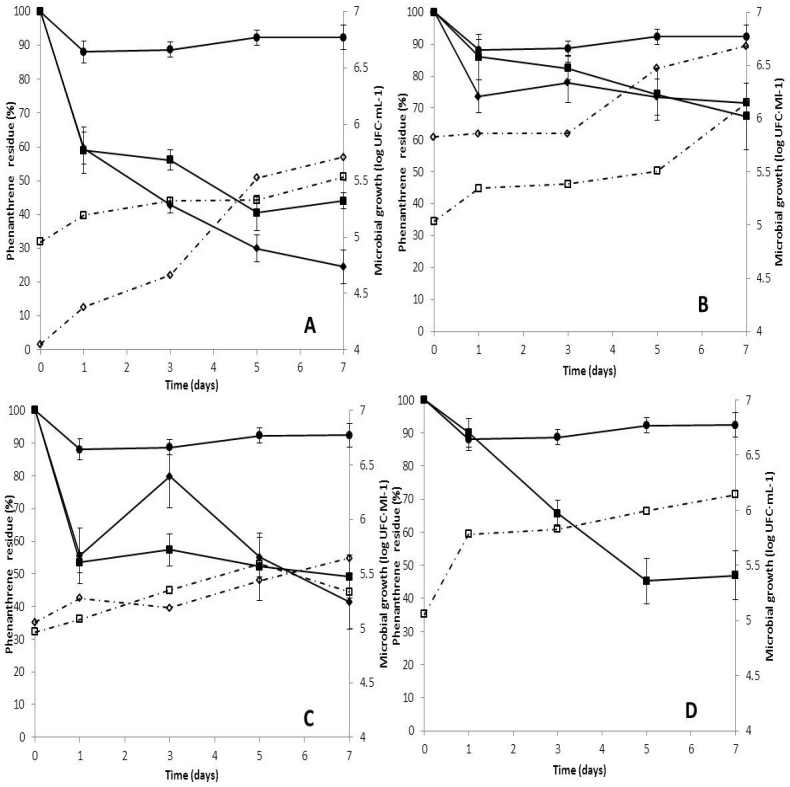

Figure 3. Degradation of phenanthrene by consortia (▲, ■) and microbial growth (Δ, □). Control (●). A) S4, S8; B) S7, S2; C) S10, S3; D) S5.Mean and standard deviation of three replicates are shown.

Table 1. Specific growth rates (μ) of isolated consortia

Consortium isolated	μ (h^{-1})	Consortium isolated	μ (h^{-1})	Consortium isolated	μ (h^{-1})
S2	0.015	S10	0.018	A4	0.018
S3	0.019	A8	0.015	A5	0.026
S4	0.031	A10	0.020	A6	0.012
S5	0.014	A1	0.035	A7	0.018
S7	0.012	A2	0.015	A9	0.016
S8	0.018	A3	0.014		

3. Conclusions

During the present work, 17 phenanthrene degrading consortia were obtained using synthetic seawater. The percentage of the S4 consortium after 7 days of incubation was 75%, with a specific microbial growth rate of 0.031 h^{-1} under a temperature of 33 $\pm$ 3°Cand 150 r/min. Although the natural consortium was evaluated in a batch system, the results could be used in a continuous system on a pilot scale to find the operating characteristics of the consortium, using a higher concentrations of phenanthrene.

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