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Bioremediation and toxicity determination of natural seawater polluted with weathered crude oil by salt-tolerant consortia in a SBR

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Abstract

The aim of this research was to compare the bioremediation treatment of seawater polluted with two different concentrations of weathered crude oil (0.2 for experiment (a) and 1.14% for experiment (b), v/v) by salt-tolerant consortia enriched from the sludge of a refinery wastewater treatment facility, in a SBR. The use of a commercial bioremediation stimulant (S200®) was also evaluated as an alternative to the traditional nitrogen and phosphorus supplement.

Keywords: Weathered crude oil; Biodegradation; Bioreactor; Toxicity; *Brachionus plicatilis*

1. Introduction

Oil spills are currently considered a major problem on the environment (Kingston, 2002; Wei et al., 2005). Bioremediation, defined as the use of microorganisms to degrade environmental contaminants (Atlas and Cerniglia, 1995; Boopathy, 2000) has proved to be a useful tool in removing oil. Since crude oils contain such a wide range of molecular structures, it was postulated that mixed cultures capable of rapidly degrading crude oil might have broader applications in the general biotransformation of hazardous hydrophobic environmental contaminants. Their use eliminates the high cost implications of pure-culture installations, provides greater metabolic diversity and a process able to degrade a variety of oily waste sludge (Ward et al., 2003).

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2. Materials and methods

2.1. Inoculum preparation

Seawater (3.5%) was periodically collected from Chania beaches, stored at 4 °C and filtered (0.45 μm) prior to use (Mielbrecht et al., 2005).

The crude oil was submitted to natural weathering process, resulting in weight loss of 28% ($\rho = 0.8202$ g/ml) and 27.1% ($\rho = 0.8166$ g/ml) for crude oil A and B, respectively. This weight loss, similar to that reported by Croft et al. (1995) and Wei et al. (2005), was achieved through continuous shaking (200 rpm) at constant temperature (30 °C).

The bacterial consortia used came from sludge from a refinery wastewater treatment facility in Athens (Greece). It was enriched in seawater with 1% (v/v) weathered crude oil as the only source of carbon for four weeks.

The following solutions were used as inoculum for the bioreactor:

(a) enriched culture (9 ml), 180 ml filtered sterilised natural seawater, 0.44 ml (0.2%, v/v) weathered crude oil A, and NH_4NO_3 and K_2HPO_4 in concentrations to keep a C:N:P ratio of 100:10:1 (Guerin, 2002) assuming that 85% of the crude oil is carbon.
(b) enriched culture (9.5 ml), 190 ml filtered sterilised natural seawater, 1.9 ml (1%, v/v) weathered crude oil B and 0.95 ml microbial stimulant S200® (not sterilised due to its thermal decomposition at >40 °C).

Solutions were kept at 27 °C in a shaker incubator (150 rpm) for four days, and then inoculated into the bioreactor: 148 ml mixture (a) for the first experiment (3.7×10^6 MPN/ml) and 190 ml (OD660 = 0.14725) mixture (b) for the second experiment. Adding this volume to the seawater in the bioreactor (1.8 and 1.9 l for experiment 1 and 2, respectively), the inoculum was close to 10% (Schmid et al., 1998; Cruz et al., 1999).

2.2. Bioreactor set-up

Experiments were carried out in a batch mode in an autoclavable 3L, BioFlo 110 bioreactor (New Brunswick Scientific) equipped with two six-bladed impellers, a sparger, a thermowell RTD, a harvest tube, an exhaust condenser, a sampler, and dissolved oxygen and pH electrodes, all mounted through the head plate. The experimental initial conditions were

(a) sterilised natural seawater (1800 ml) supplemented with C:N:P in a 100:10:1 ratio (Guerin, 2002), 27 °C, 300 rpm, 1.8 vvm aeration flow rate, pH 7.71 and 100% DO;
(b) natural seawater (1900 ml), 27 °C, 400 rpm, 2 vvm aeration flow rate, pH 7.64, and 100% DO.

When the conditions were stable, first the preculture and afterwards the crude oil (4 ml in experiment (a) and 21.6 ml in (b)) were added near the rotating impeller shaft (Fuller et al., 2003). The appearance of foam after the first 17 h in experiment (a) was controlled adding 1.8 ml Dow Corning® 2210, being initially added (1.9 ml) in experiment (b). Finally, S200® (0.95 ml, 0.05% v/v) was added in experiment (a). The bioreactor was then run as a closed system, with the exception of regular sampling for crude oil concentration analysis (6 ml, two replicates), bacterial growth estimation (2 ml) and acute toxicity determination (3 ml). Once the stationary phase of the bacterial growth was reached (88.5 in experiment (a) and 93.5 h in experiment (b)), 900 ml of the culture were removed and 900 ml fresh media added. The second batch was maintained for 82.5 and 92 h in both experiments, and 900 ml of broth were exchanged. The third runs were concluded after 63 and 95 h, respectively.

2.3. Analytical methods

Microbial population was determined by three methods. First, by the most-probable number (MPN) method for hydrocarbon degraders (Wrenn and Venosa, 1996), where results were registered visually and calculations performed using MS-Excel® (Briones and Reichardt, 1999). Second, cell densities were estimated based on optical density at 660 nm (OD660) measurements (Purwaninsdih et al., 2002). Third, samples transferred to 2 ml weighed Eppendorf tubes were centrifuged (15 min, 13,000 rpm) and the cell pellets dried to constant mass at 80 °C (Wubbolts et al., 1996).

Samples for crude oil concentration (stored at 4 °C) were extracted by liquid–liquid extraction, with an 87.6% extraction efficiency. The IR spectra were obtained by FT-IR on a Perkin Elmer Spectrum 1000 with variable path length liquid cell (Graseby Speac ZnSe 7009). The path length used was 0.05 cm. For experiment (a), the maximum corrected height was recorded between 3100 and 2700 cm^{-1}. For experiment (b), the corrected height at 2855 cm^{-1} was recorded between 3200 and 2600 cm^{-1}, due to the high concentration of the samples.

To estimate the toxicity of the treated seawater, 24 h acute toxicity tests, with a second scoring at 48 h, were carried out with the rotifer *Brachionus plicatilis* (experiment (b)). Following the ASTM Standard Guide E1440-91 (ASTM, 1998), cysts were hatched (28 h, 25 °C and 4000 lux). Then, the multiwell test plates were filled with samples collected from the bioreactor not earlier than 36 h (EPA, 2002a) and stored at 4 °C. Results were analysed according to the procedure suggested by EPA (2002b).

3. Results

The bioreactor was operated under controlled constant operational conditions (data not shown). The DO was maintained above a non-limiting level of 30% air saturation (Preusting et al., 1993; Schmid et al., 1998).

Fig. 1 shows the growth curves for each batch run. In the first run, the lag-phase was visible in experiment (a) (19 h), whereas in (b) the exponential growth phase was directly exhibited, until 25 h when the stationary phase was achieved. In (a), the consortia grew from 19.5 to 66.5 h, when it reached the stationary growth phase. During the second batch, the bacteria showed lag-phase in none of the experiments, but rapid growth after the exchange of media: 89.5 h and 93.5 h for (a) and (b), respectively. In the third batch, the consortia grew more in (a) than in (b), possibly due to the inhibition caused by the high concentration of crude oil (1%, v/v)

The isolated consortia has proved to be considerably effective on the removal of hydrocarbons (HC) from seawater polluted with weathered crude oil in a SBR, especially at 0.2% (v/v), although partial degradation was also