PCB DEGRADATION USING MICROORGANISMS AND ETHANOL AS A CO-SOLVENT

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Introduction

The study area is situated on the site of an old petrochemical landfill at the juncture of the river Estrela and Guanabara Bay in Rio de Janeiro. The study area has been identified as containing approximately 2000 litres of polychlorinated biphenyls (PCBs) buried at a depth of about 4 meters. The predominant soil type in this area is organic clay, with 22% organic matter - the low conductivity and high organic matter content of this soil has acted to retard migration of the PCBs plume. However, PCBs have been found at 50 meters from source at concentrations of between 35 ppb and 400 ppb [1]. Following removal of the contaminant source, there was a need to treat the PCBs plume. For this purpose the technique of soil bioremediation was considered because it has been proved effective at sites contaminated with a variety of organic compounds[2,3,4,5]. A preliminary series of laboratory tests showed that microorganisms isolated from the site were able to degrade 36% of a 150 ppm Aroclor 1242 solution in a period of 280 days[1], thus it was estimated that in order to achieve the statutory levels of PCBs concentration in the sediment at the site (i.e. less than 50 ppm), the in-situ bioremediation would need to be carried out for a period of at least 500 days. To achieve the most rapid and cost-effective rate of degradation it was necessary to increase PCBs bioavailability, which is recognised as a key factor to improve the degradation rate[6,7]. To this end it was decided to use ethanol as a co-solvent, which is known to increase the solubility of the PCBs in an aqueous medium and consequently increase biodegradation. In this paper the ability of two different pools of microorganisms to degrade PCBs were tested with ethanol in varying concentrations. One pool of microorganisms was isolated from the study area (S1), whereas the other pool was isolated from Praia da Ribeira (S2), a location which had been heavily contaminated by polycyclic aromatic hydrocarbons (PAHs).

Material and methods

The growth medium used in the experiments contained the following (g l^{-1}): 0.5 KH_2PO_4; 1.5 Na_2HPO_4; 1.5 $NaNO_3$, 1.0 sodium acetate, 0.9 NH_4Cl, 0.38 $Na_2S.10H_2O$; 0.1 $MgSO_4.7H_2O$, 0.03 $MnSO_4.H_2O$, 0.02 $CaCl_2.2H_2O$; 0.001 resazurin, 0.15 Aroclor 1242. Total cell counts were performed according to Kepner and Pratt [8]. Each bacterial pool sample was filtered through a 0.22 µm Black Polycarbonate membrane (Isopore Membrane Filters, Millipore). The stained bacteria were counted at a magnification of x1000 using epifluorescence microscopy, ten fold in triplicate (Axioskop 50, Zeiss). To examine the ability of the bacterial pools to degrade PCBs, 10^8cells of S1 and R were grown in triplicate in a 50 ml serum vial containing 30 ml of growth medium containing ethanol at 1.3% and 6.5%. The control for each condition was uninoculated medium. The vials were kept at room temperature (24ºC) for 15 and 30 days. To determine the remaining PCBs in solution, each vial was extracted 3 times using 10 ml of n-hexane after incubation. Extract purification and analysis methods are described elsewhere[9]

Results and discussion

S1 degradation results: The results obtained from the degradation tests using co-solvent and bacteria from the site contaminated with PCBs (S1) indicate that the addition of ethanol increases the degradation rate of almost all PCBs when compared to the experiment without co-solvent (see Table 1). The addition of 1.3% ethanol did not increase the degradation of PCB 28. However, for PCB 101 and PCB 118 the degradation rate increased more than 5 times. For PCB153, PCB 138 and PCB 180 the degradation rates were 0.1266, 0.0953 and 0.0072 ppb day^{-1} respectively, an increase of 6 to 12 times when compared with the experiment without co-solvent. A more significant increase on the degradation rate was observed with ethanol at 6.5% . The respective degradation rates of PCB 101, PCB 118 and PCB 153 were 0.8683, 0.6714 and 0.5563 ppb day^{-1}, which are between 24 and 30 times higher than the degradation rate without co-solvent. For PCB 180 the increase in degradation rate was even more marked, increasing to 0.0284 ppb day^{-1} which is47 times higher than without co-solvent. It indicates that in a system without a co-solvent the microorganisms will attack preferentially the PCBs which are more soluble in water and lower molecular weight (i.e. PCB 28). The data shows that by increasing the solubility of the PCBs in water, the microorganisms were able to attack more efficiently PCBs with higher molecular weight.

S2 degradation results

The pool of bacteria isolated from a site chronically contaminated by hydrocarbons (S2) demonstrates ability to degrade PCBs. However, the degradation rate was below the results obtained with S1 with and without ethanol (Table 1). The degradation rate of PCBs using 1.3% ethanol and S2 was minimal when compared with the experiment using the S1 pool without ethanol. For PCB 28 the degradation rate decreased 45%. By increasing the ethanol concentration to 6.5%, the degradation rate also had a positive response similar to S1 experiment. The degradation rate of PCBs 101 to 153 was between 17 and 21 times higher than the degradation rate without co-solvent. Nevertheless, the average results were almost two times lower than the results using 6.5% ethanol and S1. The only exception was PCB 180, whose degradation rate was very similar.

Factors influencing PCB degradation

Comparing the results from both experiments indicates that bacteria isolated from a site contaminated by a different compound have to adapt to a new carbon source. Figure 1 shows PCB consumption by S1 and S2 with time using 6.5% ethanol. PCBs consumption using local bacteria (S1) has no acclimation period to the carbon source. However, the acclimation period for S2 lasted for approximately 9 days. After that, PCB consumption increased exponentially until the end of the experiment. The degradation curve for S2 also indicates that at the end of the experiment the degradation was still in the exponential phase, while for S1 it was turning to asymptotic. It would be helpful to carry out the experiment for a longer period of time to get the total PCBs kinetic degradation by S2 and S1.

Figure 2 compares the effect of ethanol on the total PCBs degradation by S1 and S2 after 30 days of incubation. In the experiment using 1.3% ethanol the total degradation of PCBs was 10% and 25% for S2 and S1, respectively. For the experiment using 6.5% ethanol the total PCBs degradation was 55% for S2 and 68% for S1. The addition of ethanol in the medium had a greater effect on S2 than S1. S2 was able to degrade more than 5 times the amount of PCBs when ethanol concentration in the medium was increased by a factor of 5.

PCB bioavailability can be maximised by increasing its solubility in the medium. It could be achieved by using a co-solvent or a surface active agent. Silvester[10] compared AROCLOR 1242 degrading performance of *Comomonas testosteroni* B-356 in soil microcosm with and without a biosurfactant producer *Acinetobacter faecalis* B-566. After 22 days of incubation, the soil microcosm containing the biosurfactant producer strain degraded 28% of the AROCLOR 1242 mixture, compared to one without, which degraded 22%. Kastanek et al.[11] used a bioreactor in methanogenic conditions to clean up a soil contaminated with PCB. Ammonium sulphate was used to reduce the surface tension between PCB and water and butyric acid (co-substrate) to activate the decolourisation process. Our findings for R using 6.5%ethanol were similar to the results reported by Kastanek et al (Table 2). Though S1 was able to degrade a higher percentage of all the PCBs congeners analysed.

Data obtained in this research shows that the two specialized bacteria pools were able to degrade 5.1ppm (S1) and 4.1pm (S2) of AROCLOR 1242 in 30 days. We believe that if the laboratory conditions were applied on site

the PCBs present in the plume would be reduced to statutory levels in a short period of time. However, the same conditions should be applied in column tests using soil samples to identify further interactions between soil, PCB and ethanol before it can be applied on site.

All the results indicate that specialized bacteria can be used with success to degrade persistent organic pollutants. Bacteria isolated from the site contaminated with PCBs were able to degrade PCBs more efficiently than bacteria isolated from a different site. Inoculation of allochthonous bacteria (S2) could enhance the degradation process. Nonetheless, further tests should be conducted to demonstrate that S2 would compete successfully with the autochthonous community (S1) during treatment. It is also important to highlight that PCB bioavailability is a key factor in the biodegradation process. The use of ethanol at 6.5% in solution proved to have a positive effect in the PCB degradation process.

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Table 1: PCB degradation rate (ppb day^{-1}) by S1 and S2 bacteria pool.

PCB	S1	S1 1.3% EtOH	S1 6.5% EtOH	S2 1.3%EtOH	S2 6.5% EtOH
28	0.3005	0.2712	2.1827	0.1668	1.6876
52	0.1315	0.1708	1.2207	0.1424	0.8987
101	0.0359	0.1893	0.8683	0.0715	0.6234
118	0.0245	0.1313	0.6714	0.0374	0.4938
153	0.0190	0.1266	0.5563	0.0369	0.3948
138	0.0111	0.0953	0.3397	0.0268	0.2352
180	0.0006	0.0072	0.0284	0.0081	0.0246

Figure 1: Percentage of PCB degraded with time by S1 and S2 using 6.5% ethanol.

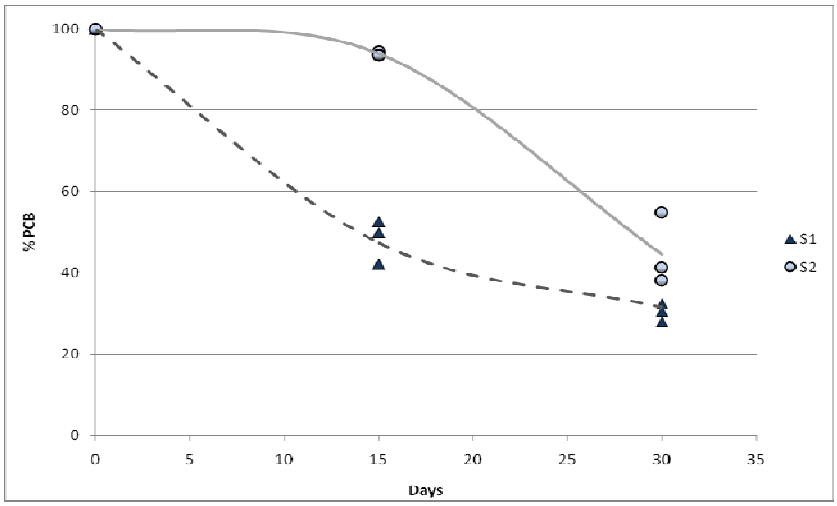

Figure 2: PCB degradation results by S1 and S2 after 30 days using 1.3% and 6.5% ethanol.

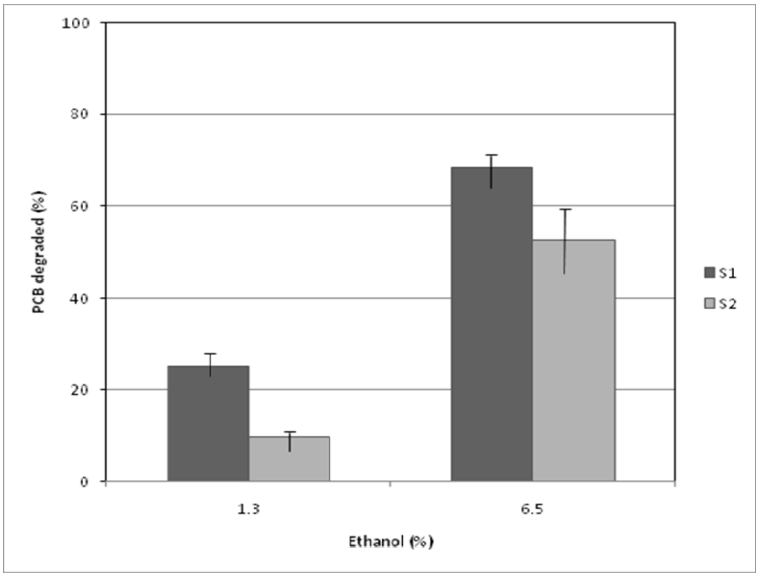

Table 2: PCB biodegradation results after 30 days using ammonium sulphate[6] and 6.5% ethanol.

PCB	% Degraded Kastank et al	% Degraded S2	% Degraded S1
28	60.4	49.1	63.4
52	54.0	47.9	65.0
101	49.0	50.4	70.1
118	54.4	53.6	72.9
153	46.0	51.5	72.6
138	50.0	49.7	71.
180	30.0	58.1	67.0