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Improving the biotreatment of hydrocarbons-contaminated soils by addition of activated sludge taken from the wastewater treatment facilities of an oil refinery

Pierre Juteau*, Jean-Guy Bisaillon, François Lépine, Valérie Ratheau, Réjean Beaudet & Richard Villemur

*INRS – Institut Armand-Frappier, Université du Québec, Laval, Québec, Canada, H7V 1B7 (*author for correspondence: e-mail: pierre.juteau@inrs-iaf.uquebec.ca)*

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Abstract

Addition of activated sludge taken from the wastewater treatment facilities of an oil refinery to a soil contaminated with oily sludge stimulated hydrocarbon biodegradation in microcosms, bioreactors and biopile. Microcosms containing 50 g of soil to which 0.07% (w/w) of activated sludge was added presented a higher degradation of alkanes (80% vs 24%) and polycyclic aromatic hydrocarbons (PAHs) (77% vs 49%) as compared to the one receiving only water, after 30 days of incubation at room temperature. Addition of ammonium nitrate or sterile sludge filtrate instead of activated sludge resulted in a similar removal of PAHs but not of alkanes suggesting that the nitrogen contained in the activated sludge plays a major role in the degradation of PAHs while microorganisms of the sludge are active against alkanes. Addition of sludge also stimulated hydrocarbon biodegradation in 10-kg bioreactors operated during 60 days and in a 50-m^3 biopile operated during 126 days. This biopile treatment allowed the use of the soil for industrial purpose based on provincial regulation ("C" criteria). In contrast, the soil of the control biopile that received only water still exceeded C criteria for C_{10}–C_{50} hydrocarbons, total PAHs, chrysene and benzo[*a*]anthracene. The stimulation effect of sludge was stronger on the 4-rings than on 2-rings PAHs. The soil of the biopile that received sludge was 4–5 times less toxic than the control. These results suggest that this particular type of activated sludge could be used to increase the efficiency of the treatment of hydrocarbon-contaminated soils in a biopile.

Introduction

Petroleum transport, storage and refining have often been a source of soil contamination. In some cases, this is due to accidental events (spills) but in others, it is a consequence of negligent disposal practices of residues like the oily sludge that accumulate in storage tanks. In many situations, soil rehabilitation can be accomplished by bioremediation. As for other biological treatments, a key factor is the nutriment balance. This is generally adjusted with inorganic fertilisers, a practice called biostimulation (Alexander 1994). On the other hand, bioaugmentation, which consists in adding selected microorganisms, is highly controversial. Especially for petroleum contamination, bacteria and fungi capable of degrading hydrocarbons are widely distributed and, generally, just need to be stimulated (Atlas 1995a, b). Independent evaluations of certain commercial inocula failed to show their usefulness (Thouand et al. 1999; Venosa et al. 1992a, b). Consequently, bioaugmentation is generally not recommended, but microbial amendment is still studied and used for certain cases (von Fahnestock et al. 1998). For example, Mishra et al. (2001) described a field test in which bioaugmentation with an inoculum developed in their laboratory improved hydrocarbon degradation more than biostimulation.

Some authors used activated sludge from municipal wastewater treatment plant as a source of nutriments (mainly nitrogen) for biostimulation (Gallego et al. 2001; Maki et al. 1999). This practice represents a cost saving compared to inorganic fertiliser and a good way to valorise a waste that is generally disposed by landfilling or incineration. In this context, activated sludge is not considered as a source of competent microorganisms since they are not particularly adapted to hydrocarbon degradation. However, Thouand et al. (1999) showed that an activated sludge taken at a municipal wastewater treatment plant, after being adapted in laboratory to crude oil, had a biodegradation capability equivalent or superior to commercial bacterial formulations sold as soil amendment for bioremediation processes. In the present work, an activated sludge produced in wastewater treatment facilities of an oil refinery was used in laboratory and field experiments to improve the biodegradation of hydrocarbons in soil. Due to its origin, this activated sludge could be a source of microorganisms adapted to these particular pollutants. The relative contribution of nitrogen and microorganisms from the activated sludge to the hydrocarbon degradation was investigated. The soil to be treated was taken from a site that was used for oily-sludge disposal. We chose the biopile technology for the field trials. Biopile involves excavating the contaminated soil, making piles with it and using the appropriate equipment in order to aerate the soil and to stimulate the aerobic biodegradation of the contaminants (von Fahnestock et al. 1998).

Material and methods

Soil

A contaminated soil from a sandpit near Montreal (Province of Québec, Canada) was used. This location was used in the late sixties as a dumping site for oily sludge produced by some oil refineries located in the Montreal island. The soil was composed essentially of fine grain sand (based on a visual evaluation; particle size analysis has not been made). For the laboratory work, a composite reference lot of this soil was constituted and kept at 4 °C for two years, during which the experiments were conducted. Some characteristics of this soil are given in Table 1.

Table 1. Characterisation of the activated sludge and soil

Parameters (mg kg^{-1} dry weight, when applicable)	Activated sludge	Soil
% humidity	98.6	5.4
Heterotrophic bacteria (CFU g^{-1})	1.7×10^9	4.9×10^6
Oil and grease	75,797	6,782
Alkanes	366	113
MAH	51	31
PAH	155	416
Total carbon	4,400	5,337
Kjeldahl-nitrogen	91,000	96
Phosphorus	7,250	1,158
C:N:P	100:2,070:165	100:2:22
PH	6.90	6.98
Silver	<4	<2
Arsenic	5.1	0.7
Barium	130	2.3
Cadmium	<1	<2
Chromium	38	26
Cobalt	<5	5
Copper	92	6
Mercury	1.4	0.10
Molybdenum	6	<0.5
Nickel	53	9
Lead	26	20
Selenium	5.3	<0.1
Tin	19	<2
Zinc	175	28

Sludge

An activated sludge from the wastewater treatment facilities of an oil refinery was used. This plant treats the process water of the whole refinery. The treatment consists in a primary sedimentation tank, a dissolved-air flotation system, an aeration tank and a secondary clarifier. The withdrawn activated sludge is thickened and then dewatered on a belt filter press. The sludge used in this study was taken between the thickening unit and the belt filter press. For the laboratory work, a reference lot of this sludge was constituted and kept at 4 °C for two years. Some characteristics of this sludge are given in Table 1.

Microcosms

Microcosm experiments were performed in 500 ml Erlenmeyer flasks containing 50 g (wet weight) of soil. For each series, one microcosm was prepared for each sampling time, and all the content of the

microcosm was extracted for the determination of the hydrocarbons concentration.

In a first experiment, the soil was supersaturated by the addition of 27 ml of water or sludge or 2.7 ml of sludge plus 24.3 ml of water, which gave a final sludge concentration of 0, 0.7 and 0.07% w/w, respectively (percentage weights are given as dry weight). Microcosms were incubated at room temperature and agitated once a day at 400 rpm for 5 min. The pH of these cultures was adjusted to 7.0 and was subsequently maintained between 7.0 and 7.5 by addition of 1N sulphuric acid (Fisher Scientific, Montreal, QC, Canada). Each week, a given volume of water, determined by the loss of weight of the microcosm, was added to compensate for evaporation. Microcosms containing 0.25% (w/v) $HgCl_2$ (Anachemia, Montreal, QC, Canada) were used as abiotic controls.

In another experiment, the sludge was centrifuged at 10,000 $\times$ g for 30 min (model J-25 centrifuge, Beckman Coulter, Mississauga, ON, Canada) and the supernatant was sterilised by successive filtration on 5, 0.45 and 0.2 μm filter (Millipore, Bedford, MA, USA). The sterility was confirmed by plating 0.1 ml of the filtrate on nutritive agars (Plate Count Agar, Difco, Sparks, MD, USA) and incubating them at 30 °C for 10 days. The soil was supersaturated by the addition of 27 ml of sterile sludge filtrate or with 27 ml of a 1.3 mM ammonium nitrate (Aldrich, Mississauga, ON, Canada) solution.

For all the assays, the degradation of hydrocarbons was determined by making the difference between biotic and abiotic series. This ensures that the calculated degradation percentages do not include physical processes that could reduce the hydrocarbons concentration over time, like volatilisation.

Reactors

Polyvinyl chloride (PVC) tubular reactors, with their inner surface having been epoxy coated, were used. Their height was 55 cm by 14.5 cm internal diameter. They contained 10 kg (wet weight) of soil to which 1.2 l of water or sludge was added, which corresponds to 66% of the soil water retention capacity (final concentration of sludge = 0 and 0.18% w/w, respectively). The soil was thoroughly homogenised before being added into the reactors. The abiotic control reactors contained 0.25% (w/v) $HgCl_2$. A 0.5 M pH 6.5 phosphate buffer was also added to each reactor to better control pH fluctuations. The bottom of the reactor contained a metal wire mesh through which humid air was continuously pumped. The reactors were weighted at regular time intervals in order to determine evaporation and water was added to compensate losses. The reactors were incubated at room temperature. Hydrocarbons were analysed on composite samples of 250 g.

Biopiles

Two 50-m^3 biopiles were made with the contaminated soil. One of them received the activated sludge while the other received only water. Perforated pipes covered by a gravel layer were installed at the bottom of the piles and connected to blowers that pulled air through the piles. The biopiles were covered with a plastic membrane. These experiments were performed from June to October. Initially, 6,000 l of sludge (0.2% w/w) or water were added to the soil to achieve 60–70% of the soil water retention capacity. To prevent drying, 3,000, 6,000 and 1,500 l of sludge (0.1, 0.2, and 0.05% w/w, respectively) or water were added to biopiles after 31, 66 and 98 days, respectively. The percentage of humidity was maintained in the biopiles at around 10% throughout the experiment. After each addition of liquid, the soil was mixed with a power shovel and a composite sample made from eight different samples was prepared for each biopile. The eight samples were taken randomly at different places and depths in the biopile and they were manually mixed. Parallel microcosm tests were performed in 500-ml Erlenmeyer flasks using composite soil samples (160 g). These microcosms were agitated manually twice a day. Sludge or water was added before each sampling to compensate the loss of weight of the microcosms. Abiotic control microcosms were used to roughly estimated hydrocarbon loss due to evaporation in the biopile.

Chemical analysis

For microcosms, chemical analyses were performed with the whole content of the Erlenmeyer flask, while 100 g (wet weight) of composite soil samples were used for monitoring reactors and the biopiles.

Percentage of humidity was calculated by determining the loss of weight of the sample after drying at 105 °C (method 209A; APHA et al. 1989).

Oil and grease was determined by a gravimetric method. The sample was suspended in water, acidified with hydrochloric acid (Fisher Scientific) and extracted five times with ethyl acetate (Accusolv grade, Anachemia). Extracts were dehydrated with anhydrous sodium sulfate (Fischer Scientific) and filtered.

An aliquot of this solution was placed in a preweighted flask and the solvent was evaporated with a rotary evaporator kept at 24 °C (Büchi, Labortechnik, Flawil, Switzerland). The flask was put under vacuum overnight and then weighted. The oil and grease content of the sample was calculated based on the difference in weight of the flask.

Petroleum hydrocarbons (C_{10} to C_{50}) were measured according to the method MA.410-HYD. 1.0 of the Centre d'Expertise en Analyse Environnementale du Québec (CEAEQ 1997). Briefly, the soil sample was first dehydrated with anhydrous magnesium sulphate, ethyl acetate was added and the mixture put in an ultrasonicator bath (model B-12, Branson, Shelton, CT, USA). The solvent was decanted, silica gel (Sigma, Oakville, ON, Canada) was then added to the extract and the supernatant analysed with a gas chromatograph model 5890A (Hewlett-Packard, Arondale, PA, USA) coupled to a flame ionisation detector (GC-FID) and equipped with a 30 m × 0.25 mm DB-5 capillary column (film thickness of 0.25 μm). Hydrocarbon concentration was determined by integration of all the peaks of the chromatograms located within the retention times of two n-C_{10} and n-C_{50} hydrocarbon standards.

Concentrations of individual alkane, monoaromatic hydrocarbons (MAHs) and polyaromatic hydrocarbons (PAHs) were determined by gas chromatography-mass spectrometry (GC-MS) using a GC model 3500 (Varian, Walnut Creek, CA, USA) coupled to an Ion Trap mass spectrometer (model 800, Finnigan, San Jose, CA, USA). The sample was extracted in the same manner as for the oil and grease analysis (see above). The residue was taken in ethyl acetate and analysed with by GC-MS. The column used was the same as for GC-FID analysis. One microliter of the sample was injected and the carrier gas was helium. The injector was at 250 °C and the detector at 270 °C. The oven was maintained at an initial temperature of 40 °C for 2 min, then increased by 10 °C/min to 120 °C, followed by 3 °C/min to 180 °C and by 6 °C/min to a final temperature of 310 °C, which was maintained for 8 min.

Heavy metal concentrations were determined by atomic absorption spectrometry (model AA-1275, Varian) using cold vapour for mercury and air-acetylene flame for the others (methods 3112 B and 3111 B, respectively, APHA et al. 1989). Total carbon was measured with a LECO (St-Joseph, MI, USA) apparatus (method 5310 B; APHA et al. 1989). Total nitrogen was determined by the Kjeldahl method (method 4500-Norg C; APHA et al. 1989). Total phosphorus was determined by the method 90.04/313-NTPT 1.1 (CEAEQ 1990), which consists in an acidic digestion and a colorimetric analysis at 880 nm.

Microbial analysis

The most probable number technique was used to determine the total heterotrophic bacteria in the sludge and in the soil. One volume of peptone water (Difco) was added to a 10 g-sample, the mixture was manually agitated and decimal dilutions were made in 24-wells sterile plates each containing 900 μl of Trypticase Soy Broth (Difco). The plates were incubated at 20–22 °C for 14 days.

Toxicity

The Microtox solid phase method with *Photobacterium phosphoreum* (reactors) and *Vibrio fischeri* (biopiles) was used to determine the toxicity of soil samples (Matthews & Hastings 1987) with a Microtox model m500 apparatus (Microbics, Carlsbad, CA, USA) following a standardised protocol (Environment Canada 1993). The EC_{50} values presented are those obtained after a 5 min contact. Lauryl sodium sulfate was the reference toxic compound.

Results

Characterisation of sludge and soil

The soil and the sludge used for the laboratory experiments was first characterised by analysing a sample of the reference lots (Table 1). The sludge contained only 1.4 percent solids. On a dry weight basis, heterotrophic bacteria and Kjeldahl nitrogen are about one hundred times higher in the sludge than in the soil. Phosphorous was in excess in both the soil and in the sludge.

Microcosms

Addition of sludge to the microcosms stimulated the degradation of alkanes and PAHs (Table 2). After 30 days of incubation, 80% of the alkanes and 77% of the PAHs found in the abiotic control were removed in presence of 0.07% (w/w) of sludge, as compared to 24% and 49%, respectively, for the microcosms to which only water was added. At higher concentration

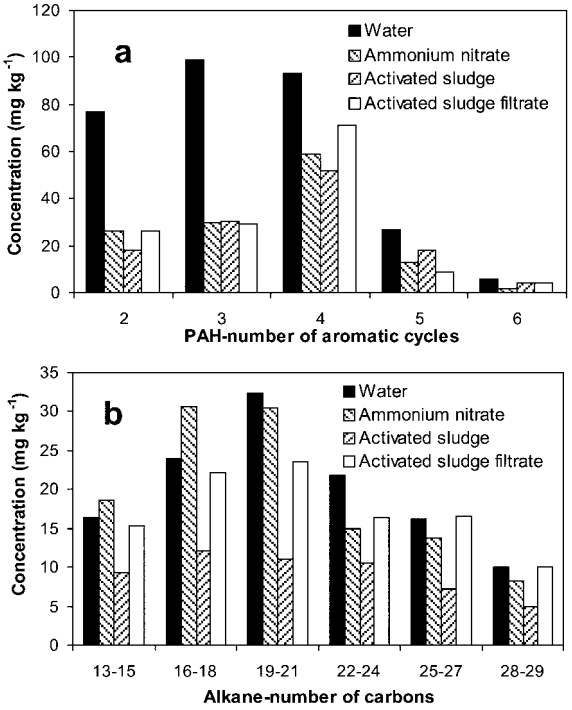

Figure 1. Concentration of hydrocarbons in microcosms after 15 days of incubation. Microcosms received sludge, sterile sludge filtrate, ammonium nitrate or water. (a) Concentration of PAHs. (b) Concentration of alkanes.

of sludge (0.7%), the alkanes were slightly more degraded (93%) but not the PAHs (70%). This increase in biodegradation in sludge-amended microcosms was observed on the majority of the individual alkane analysed and on 2-, 3- and 4-rings PAHs, whereas the higher molecular weight PAHs were in too low concentrations in order to assess their biodegradation (results not shown). The abiotic controls revealed that, after 30 days, alkanes were not volatilised while PAHs were partially volatilised.

Another experiment was designed to distinguish the role of nitrogen and microorganisms contained in the sludge. Microcosms received sludge (0.7% w/w), sterile sludge filtrate, ammonium nitrate or water. The nitrogen concentration was 91 mM in the activated sludge and 2.6 mM in the ammonium nitrate solution (the nitrogen concentration of the sludge filtrate was not analysed). The degradation of the 2-, 3- and 4-rings PAHs was similarly stimulated for all these three treatments after 15 days of incubation whereas no conclusion are made for the 5- and 6-rings PAHs due to their relatively low concentration (Figure 1a). However, clear stimulation of alkane degradation was only observed in presence of complete sludge, not just its liquid fraction (filtrate) nor with an inorganic nitrogen source (Figure 1b).

Reactors

Three reactors containing 10 kg (wet weight) of soil were operated. One of them received 1.2 l of sludge (corresponding to an addition of 0.18% w/w), another received water only and the third was an abiotic control. The degradation of alkanes and PAHs was higher in the reactor amended with sludge compared to the one that received water only (Table 3). After 30 days of incubation, the degradation was 82 and 7%, respectively, for the alkanes and 70 and 7%, respectively, for the PAHs, compared to the abiotic control. Addition of sludge increased the degradation of all the individual alkanes and of most of the 2- and 3-rings PAHs (data not shown). In the abiotic reactors, volatilisation of alkanes and PAHs amounted to 43 and 41%, respectively. Volatilisation of PAHs was not in direct relation with the molecular weight (volatilisation was 64, 14, 37, 42 and 0% for the 2-, 3-, 4-, 5- and 6-rings PAHs). Most of the volatilisation occurred during the first 30 days of the treatment, while it was negligible after that. The soil of the reactor that received the sludge was also about three times less toxic than the one that received water (EC_{50} = 37,284 vs 12,419 mg/l). Extending the incubation period to 60 days did not increase significantly the degradation of PAHs, but it permitted to reach 99% degradation of alkane in presence of sludge.

Biopiles

The addition of activated sludge to the biopile resulted in an increase in the degradation of all the measured parameters compared to the one that received only water (Figure 2). After 126 days of incubation, the C_{10}–C_{50} hydrocarbons were reduced by 75% (Figure 2a) and the PAHs by 95% in the sludge-amended biopile (Figure 2b), while they only showed 34 and 65% reduction, respectively, in the control biopile that received water only. The beneficial effect of sludge on PAH degradation increased with the number of aromatic rings up to 4 (Figure 3 and Table 4). For 4-rings PAHs, there was no degradation without sludge addition. Degradation of the higher molecular weight PAHs cannot be assessed because of their very low concentrations in the soil. Similar results were obtained in the corresponding microcosms (data not shown). Loss in abiotic microcosms was 39% for

Table 2. Biodegradation of hydrocarbons in microcosms

Material added to the soil		Hydrocarbon concentration					
Activated sludge (% w/v)	$HgCl_2$ (% w/w)	Alkanes (mg kg^{-1}) after days of incubation			PAHs (mg kg^{-1}) after days of incubation		
		0	15	30	0	15	30
0	0	nd[a]	134	124	nd	193	142
0.07	0.25	134	131	164	473	319	279
0.07	0	nd	55	33	nd	101	63
0.7	0	nd	20	11	nd	87	85

[a]Not determined.

Table 3. Biodegradation of hydrocarbons in microcosms

Material added to the soil		Hydrocarbon concentration					
Activated sludge (% w/v)	$HgCl_2$ (% w/w)	Alkanes (mg kg^{-1}) after days of incubation			PAHs (mg kg^{-1}) after days of incubation		
		0	30	60	0	30	60
0	0	115	69	46	413	228	222
0.18	0.25	131	74	98	416	244	220
0.18	0	141	13	1	442	73	89

C_{10}–C_{50} hydrocarbons and 10% for PAHs. These decreases were attributed to volatilisation. These values represent only an estimation since the environmental conditions in the flasks were not identical to those in the biopiles.

The removal of alkanes was initially faster in presence of sludge-amended biopile. However, the extent of this difference is hard to determine due to the high variability in one of the series, the alkane concentrations of the non-amended biopile (Figure 2c). After 126 days of incubation the residual concentration in each biopile was similar. This was not the case in the corresponding microcosms since a better degradation was obtained in presence of sludge (98 vs 82%). There was no volatilisation of alkanes in abiotic microcosms. Because the soil was weathered for more than three decades, the concentration of the very volatile monoaromatic hydrocarbons in the biopiles were too low to be measured accurately and no conclusion can be draw on the effect of sludge on these compounds.

In both biopiles, the pH became rapidly alkaline (from 6.7–6.9 to 7.4–7.7 after 31 days of incubation) and then stood at these values afterward. Before incubation, the toxicity of the soil in the biopile that received the sludge (EC_{50} = 4041 ± 699 mg/l) was similar to the one that received water (4113 ± 919 mg/l). After 126 days, the toxicity of the soil in the sludge-amended biopile was 4 to 5 time smaller (76152 ± 14673 mg/l) than the one that received water (16033 ± 2319 mg/l).

Discussion

The addition of activated sludge taken from the wastewater treatment facilities of an oil refinery stimulated the degradation of hydrocarbons in a contaminated sandpit soil in microcosms, bioreactor and 50-m^3 biopile. The activated sludge from an oil refinery water treatment plant was chosen because it represented an inexpensive source of nutriments and also because microorganisms present might have been adapted to hydrocarbon degradation. There is no information in the literature on the use of this particular type of activated sludge for soil amendment in bioremediation processes. There is however some papers that report the use of activated sludge from municipal wastewater treatment plant. Maki et al. (1999) compared

Table 4. Monitoring of some targeted PAHs in biopile. Before treatment, these hydrocarbons were exceeding the criteria allowing the industrial use of the soil based provincial regulation (see text for details)

Name	Number of ring	Concentration (mg kg^{-1})		
		Before treatment	After 126 days of treatment	
			Without sludge	With sludge
1-methyl naphthalene	2	11.2	0.0	0.0
2-methyl naphthalene	2	11.5	0.1	0.0
phenanthrene	3	87.4	0.5	0.2
chrysene	4	12.6	13.0	1.8
benzo[*a*]anthracene	4	12.6	12.0	1.2

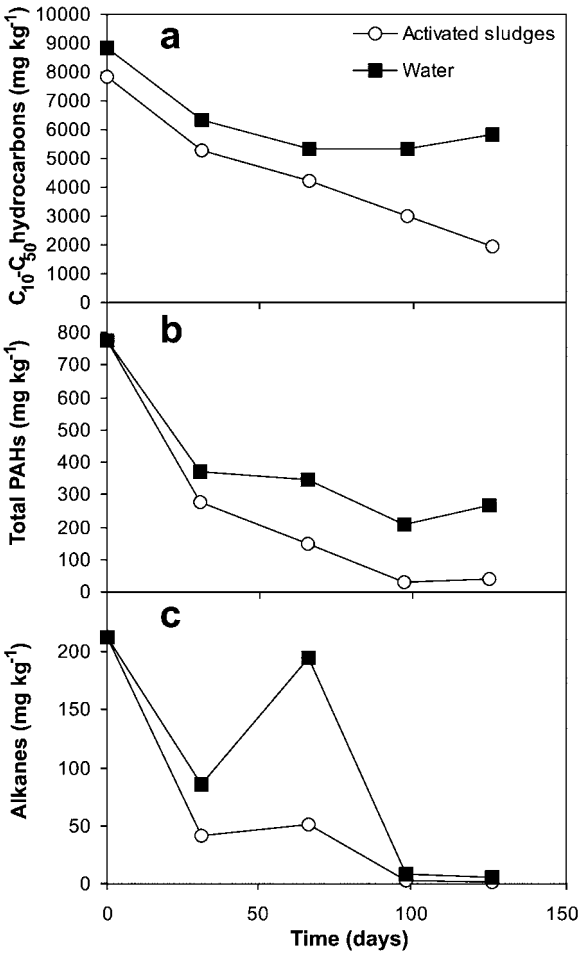

Figure 2. Monitoring of hydrocarbons in biopiles. Each biopile received activated sludge or water. (a) C_{10}-C_{50} hydrocarbons. (b) Total PAHs. (c) Alkanes.

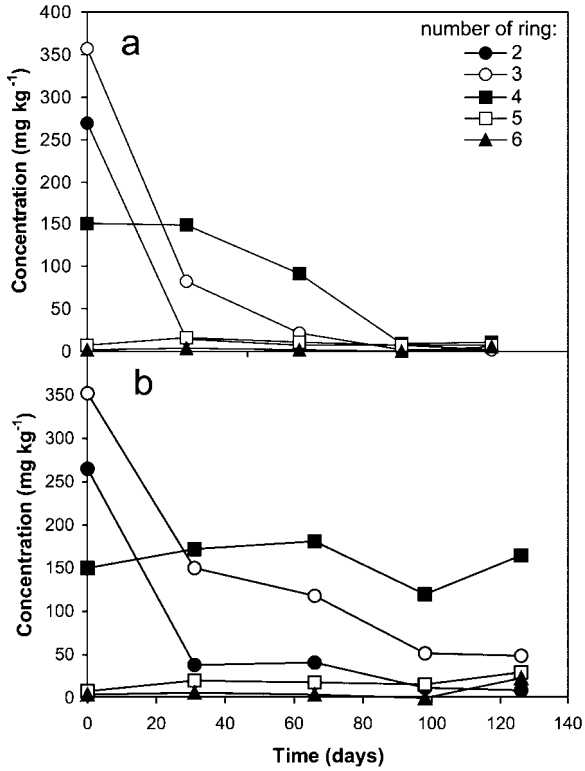

Figure 3. Monitoring of PAHs in biopile as function of their number of cycle. (a) Biopile that received activated sludge. (b) Biopile that received water only.

the effect of activated sludge (liquid and dehydrated) and an inorganic fertiliser added to beach simulating tanks that contained gravel contaminated with crude oil. They find no significant difference between these treatments. In a laboratory study, Gallego et al. (2001) showed an increase of biodegradation of diesel fuel in a contaminated soil by adding an activated sludge, but the improvement was smaller than the one observed

with an inorganic fertiliser. The beneficial effect of this activated sludge was only due the nutriments that it contained, not the microorganisms, since a heat-sterilised sludge produced a better improvement of the biodegradation than a non sterilised sludge. The authors hypothesised that the microflora of the activated sludge, when active, consumes the carbon matter from the sludge and competes with the indigenous microorganisms of the soil (including the hydrocarbon degraders) for the inorganic nutriments. However, Thouand et al. (1999) showed that an activated sludge, taken from a municipal wastewater treatment plant and pre-adapted in laboratory to crude oil during 43 days, had a significant degradation activity (up to 19% degradation) on crude oil in liquid culture medium after 28 days. Guiot et al. (1995) showed that addition of activated sludge to a bioreactor containing a suspension of chlorobenzene-contaminated soil improved four-fold the rate of dichlorobenzene degradation.

As it is normally the case with hydrocarbon-contaminated soils, the soil used here contained a low nitrogen to carbon ratio of 2:100. Addition of 0.07% (w/w) sludge to the soil increased the nitrogen content up to 3:100, which stimulated hydrocarbon (PAHs and alkanes) degradation. Addition of 0.7% (w/w) sludge boosted the N:C ratio to 13:100 but did not improved the degradation of PAHs further (the case of alkanes will be discussed farther) even if this value is much closer to the 10:100 ratio generally considered to be optimal (USEPA 1995). Dibble and Bartha (1979) also reported an optimal N:C ratio relatively low for oily sludge (1.7:100). This can be explained by the fact that, for this type of contaminant, not all the carbon present can be assimilated by microorganisms, the heavier fractions being very recalcitrant to biodegradation. In these circumstances, applying the N:C ratio of 10:100 results in an overestimation of the nitrogen needed since the ratio refers to the material that bacteria consume. This suggests that the use of total organic carbon (TOC) values may not be a good basis for determining the amount of nitrogen that has to be added to a soil containing this type of pollutants. Case by case evaluation of optimal dosage has to be done by small-scale assays.

Replacing the activated sludge with ammonium nitrate or sterile activated sludge filtrate led to a similar increase of the PAHs degradation, suggesting that nitrogen is the main factor contributing to PAHs degradation in sludge-amended soils. In contrast, alkanes were considerably less degraded in presence of ammonium nitrate or sterile activated sludge filtrate compared with activated sludge, suggesting that the microorganisms contained in the sludge are involved in alkane degradation. This is different from authors who reported no effect (Maki et al. 1999) or a detrimental effect (Gallego et al. 2001) of the microflora from activated sludge from municipal wastewater treatment plant when used as soil amendment for hydrocarbon degradation. This support our starting hypothesis, namely that the source of the activated sludge that we used, the wastewater treatment facilities of an oil refinery, is well suited to produce hydrocarbon degrading microorganisms. Knowing that, we would expect that the extent of the alkane degradation would have been very different in the microcosm experiment in which two sludge concentrations were tested (0.07 and 0.7% w/w). There was a difference, but not by a factor of 10 (58% degradation compared to 85% with 0.07% and 0.7% of sludge, respectively, after 15 days, and 80% compared to 93% after 30 days). This means that there was another limiting factor. This was not the availability of nitrogen, as we discussed above. It could be the desorption or the dissolving rate of the alkanes, especially considering that the soil with 0.7% of sludge had reached relatively low concentrations (20 and 11 mg/kg after15 and 30 days, respectively).

Microcosm assays showed that relatively small amounts of activated sludge added to the contaminated soil was effective in stimulating biodegradation. This was compatible with the limit of sludge that can be added to a biopile in order to keep moisture at optimal level, that is between 40 and 85% of the water retention capacity of the soil (USEPA 1995; von Fahnestock et al. 1998). We chose to use 0.18% (w/w) sludge, which increased the moisture content to 66% of its water retention capacity. We tested the process with 10-kg bioreactors to better mimic the biopile conditions. The degradation observed after 30 days was similar in the bioreactor as it was in microcosms (82% and 79% for alkanes, 70% and 78% for PAHs, respectively), with respect to their corresponding abiotic control. PAHs volatilisation was similar in microcosms and bioreactors while alkanes, which were not volatilised in microcosms, suffered some volatilisation in the reactors, meanly in the first 30 days (Table 3). The decrease of volatilisation after that could be due to a stronger sorption of a fraction of the hydrocarbons to the soil particles. It could also be due to some hydrocarbons trapped in soil aggregates that are less aerated. Nevertheless, the major part of these non volatilised hydrocarbons is available to microorganisms for biodegradation since the remaining concentrations

after 60 days were 1 and 89 mg/kg for alkanes and PAHs, respectively, in the biotic series containing the activated sludge compared to 98 and 200 mg/kg in the abiotic series. In the case of PAHs, the fact that there was no degradation between 30 and 60 days indicates that the residual quantity is highly recalcitrant to biodegradation, a phenomenon that could be due to very strong sorption to the soil or to the nature of the remaining molecules (less biodegradable).

The laboratory works have been done with soil and sludge kept at 4 °C for a long period (up to two years). This could have affected the microflora and, hence, the results. However, for the soil, the indigenous microorganisms are adapted to an environment in which the temperature shows an important variation. The normal atmospheric temperature for the Montreal region goes from −18 °C in February to +27 °C in July. The temperature of the soil depends of the depth, but we can state that 4 °C is normal for the winter period. Consequently, the storage conditions should not have impaired the activity of this microflora. On the other hand, the storage could have reduced the activity of the activated sludge.

The soil used for the biopiles was taken at the same location than the soil used for the microcosms and bioreactors but contained much more hydrocarbons than the latter. It contained twice the amount of oils and greases and twice the total PAH concentration. Nevertheless, the degradation of alkanes and PAHs in the sludge-amended biopile after 30 days was 83 and 65% respectively. These values are close to those observed in the corresponding microcosms and bioreactors, when taking into account the rather small volatilisation of alkanes and PAHs observed after 126 days in the abiotic microcosms derived from these biopiles.

Before the biopile treatment, the soil was exceeding seven threshold values in order to be considered safe for industrial use, according to regulations (these "C" level values are given in parenthesis as mg/kg): C_{10}–C_{50} hydrocarbons (3500), total PAHs (200), phenanthrene (50), 1-methyl naphthalene (10), 2-methyl naphthalene (10), chrysene (10) and benzo[*a*]anthracene (10). Without sludge addition, only phenanthrene and methyl naphtalenes were depleted below the C level. With sludge addition, all other targeted parameters fell below C levels. These objectives were reached after 66 days in the case of chrysene and benzo[*a*]anthracene and after 98 days for C_{10}–C_{50} hydrocarbons and total PAHs. Also, the better reduction of toxicity observed with the sludge is another indication that the amendment strategy was beneficial.

Alkanes were removed faster in the amended biopile. However, since the treatment was prolonged up to 126 days to insure that all parameters were at least depleted to C values, alkanes in the control biopile were as low as the amended one at the end of the treatment. In this situation, the advantage of using an activated sludge containing microorganisms capable of alkane degradation is less marked. The overall effect of using this particular activated sludge is still highly positive: it is an inexpensive source of nitrogen, it is effective for biostimulation and it valorised a product that would otherwise had to be disposed of. The presence of alkane degraders in the activated sludge could be more beneficial for contaminated soils in which alkanes represent a higher proportion of total hydrocarbons present.

These results emphasise also the difficulty to use small-scale experiments to predict what will exactly happen at full scale. The biggest problem here was the fact that the soil used for the laboratory experiments, even if it was carefully taken at different places of the contaminated area, had a lower content in hydrocarbons than the soil treated in the biopiles. Consequently, the biopiles had to be maintained in operation for a longer period of time than expected. It is also advisable to use replicates in order to make statistical analyses, an approach that has not been used here due to some resource limitation. Nevertheless, trends have been identified with enough accuracy in order to highlight the usefulness of this particular activated sludge for bioremediation of hydrocarbon-contaminated soil.

Conclusion

The addition of activated sludge taken from the wastewater treatment facilities of an oil refinery to hydrocarbon-contaminated soils did enhance the biodegradation of these pollutants in microcosms, in bioreactors and in biopiles. This sludge is not only a good source of nitrogen but it also contains microorganisms adapted to the degradation of alkanes, which could accelerate the destruction of these particular hydrocarbons. This practice is a way to valorise a sludge that would be otherwise dispose by landfilling.

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