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Feasibility of fungi bioaugmentation in composting a flare pit soil

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Abstract

The feasibility of fungi bioaugmentation in composting of a flare pit soil was studied in lab-scale composters. The preliminary screening tests, using a range of bulking agents and white rot fungi strains, were conducted to determine, best strain and bulking agent for the main experiments. The initial total petroleum hydrocarbon (TPH) of the flare pit soil was found to be 16%. The effect of moisture and bulking agent content and the fungi application on biodegradation of hydrocarbons were then evaluated based on a fractional factorial design over a 3-months period. Analysis of the TPH content of the soil after 98 days (using gravimetric method) showed an average of 29% reduction in most jars. Furthermore, gas chromatograph (GC) analysis of the oil extract from the samples showed 70–99% reduction in the peak area of the selected hydrocarbons. However, statistical analysis of the results did not show any significant effect due to the fungi application or the change in the moisture content (30–50% range). The results showed that the change in the bulking agent content was marginally significant for the hydrocarbon loss.

Keywords: Fungi bioaugmentation; Composting; Flare pit bioremediation; Hydrocarbon bioremediation; White rot fungi

1. Introduction

A major concern in most bioremediation work related to the petroleum hydrocarbons is the presence of heavy compounds such as polycyclic aromatic compounds (PAH), asphaltenes and many branched $C20^{+}$ compounds. The heavy hydrocarbon constituents are hard to bioremediate and are considered to be of potential health risk (possible carcinogenic and mutagenic activities). An example seen in Alberta is the flare pit sites from the oil industry, which has been used to store or burn produced fluids at oil and gas exploration sites [1]. These pits generally contain heavy hydrocarbons, metals and salts. Composting

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seems to be the most feasible way to treat the hydrocarbon content of these scattered and often relatively low-volume waste sites. However, bioremediation of contaminants with heavy hydrocarbon constituents can be problematic because these compounds have very low water solubility and are often tied to soil particles. Both the factors severely reduce the biodegradation rates [2,3]. Currently many efforts are being made to use the biodegradation potential of lignin-degrading fungi in bioremediation of such compounds. There have been numerous published researches on biotransformation of toxic and recalcitrant pollutants such as chlorinated phenols, pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) by lignin-degrading fungi [4–6]. The ability of the fungi to degrade such a wide variety of compounds has been attributed, at least in part, to the action of ligninolytic enzymes [7].

The main purpose of this study is to evaluate the feasibility of fungi bioaugmentation in composting of a flare pit soil of oil industry. Currently, there is not enough data to support the fungi bioaugmentation during field bioremediation processes. Additionally, most of the reported fungal bioremediation studies have been performed in laboratory conditions with sterilized media and very often low concentrations of hydrocarbons were employed in the tests.

2. Material and methods

2.1. Flare pit soil

The soil was from one of the Alberta flare pit sites, with 63% silt, 37% clay and 0% sand with a silty loam texture and very low amounts of salt and heavy metal contents. The initial hydrocarbon content was 15.9% (w/dry w); in which 46% was saturate, 28% aromatics, 18% resin and 8% asphaltenes. No PAH was detected in the soil.

2.2. Bulking agents

Peat moss, pine wood shavings and bran flakes (amendment), or a mixture of them were used as bulking agents. After some screening tests, a mixture of peat moss and bran flakes was selected for the main tests.

2.3. Fungi strains

Twelve strains of white rot fungi known for hydrocarbon biodegradation ability were obtained from Dr. M. Pickard, Department of Biological Sciences, University of Alberta, Edmonton, Alberta. Fungi were grown on potato dextrose agar plates (PDA, Difco) at 28°C for 5–7 days before being stored at 4°C and were transferred every 3 months. After screening tests for growth, sensitivity, and enzyme production, *Bjerkandera adusta* BOS 55 was selected for the main experiments.

2.4. Inocula preparation

Fungi strains were grown for 7 days in 50 ml of glucose-malt extract–yeast extract (GYM) media in a 250 ml flask, at 28°C [8,9]. The cultures were then homogenized for 20 s using

Table 1
Summary of the experimental design

Test no.	A	B	C
1[a]	1	1	1
2	0	1	1
3[a]	1	0	1
4	0	0	1
5	1	1	0
6	1	0	0

[a] With replicates. Extra jars were used as control for evaporation and adsorption.

a homogenizer (SPER Scientific Homogenizer) and 15 ml of fungi homogenate was used to inoculate the bulking agents.

2.5. Experimental design

The design was based on a fractional factorial design with three factors, each at two levels. The factors were (1) fungi application, (2) moisture content, and (3) bulking agent. The two levels of fungi were "no fungi application" and "fungi application". The moisture content was studied at two levels of 30 and 50% (w/w of dry soil) and the bulking agent was studied at two levels of 6 and 12% (w/w of dry soil). More details of the design are presented in Table 1. Factors are shown with uppercase letters, A for fungi, B for moisture content and C for bulking agent. The high levels of each factor are designated with 1 and the lower level with 0. No forced aeration was found to be necessary. Samples were taken every two weeks for hydrocarbon analysis (gravimetric and GC), and for ligninolytic enzyme activity and moisture content measurements. Two extra jars were used as control in which only the bulking agent is added to the soil.

2.6. Hydrocarbon analysis

Both gravimetric and GC analysis was carried out. The hydrocarbon extraction was based on the modified EPA 3550B [14]. However, since asphaltenes block the GC column, the asphaltene fraction of the oil was removed after gravimetric analysis [10]. The extracted hydrocarbons were then deasphaltenated and diluted with DCM (dichloromethane). One microlitre of the solution was used for injection into the GC. The GC temperature ramping method used for this analysis was calibrated for normal C_7 to C_{40} hydrocarbons. The GC with FID had an initial temp of 300°C, increasing to 380°C in 15 min.

3. Measuring fungal ligninolytic enzyme activity

The poly R-478 decolorization assay was used to measure ligninolytic enzyme activity of the fungi. It has been shown that poly R-478 dye decolorization is correlated with the onset of secondary metabolism and ligninolytic activity [11]. The assay was slightly modified

to study the ligninolytic enzyme activity of fungi in the soil. To verify overall ligninolytic activity in the soil, 5 ml of dye poly R-478 in aqueous solution (0.02 g/l) was added to 1 g of the soil. The solution was kept under light for 24 h for the enzyme reaction to take place. Then the solution was centrifuged for 4 min at 5000 rpm (5804 Eppendorf Centrifuge) to separate the soil particles from the aqueous solution of dye and extracted enzymes. The absorbance ratio (A520/A350) of the decanted supernatant solution was then measured by the UV–VIS spectrophotometer (Cary 4E, Varian Inc., Canada).

4. Results and discussion

Preliminary screening tests using a range of bulking agents and white rot fungi stains were conducted to determine best strain and bulking agent for the main experiments. After screening the fungi strains for growth, sensitivity to the contaminated soil, and enzyme production, *Bjerkandera adusta* BOS 55 was selected for the main experiments. The best bulking agent for good growth and penetration of fungi into the soil was found to be a mixture of peat moss and bran flakes (about 5% bran flakes). Four litre glass jars with loose caps were used for setting up the tests. Provisions were made for aeration of the jars by placing an air sparger at the bottom; however, periodic analysis of the air at the bottom did not show significant oxygen depletion over a 3-months period. Table 2 shows the gravimetric analysis of the hydrocarbon content of the jars after 28 and 98 days. The TPH reduction data are corrected for adsorption and evaporation, based on the average loss from the control jars.

Statistical analysis of the results was performed to determine if any of the factors (fungi, bulking agent and moisture content) had significant effect on the outcome of the experiment. The summary of the main effect analysis, based on the Box–Hunter method [12], is shown in Table 3.

4.1. Fungi application

As can be seen from Table 3, the fungi main effects after 28 and 98 days are within the statistical error (≅95% confidence interval). This means that the fungi applications did not have any significant effect on the outcome of the experiments. This could be caused by the

Table 2
Results for TPH reduction from the test jars based on gravimetric analysis (±3% error)

Test no.	Corrected TPH reduction (%)	
	After 28 days	After 98 days
1, 2, 3	29.8, 34.1, 21	32.8, 31.6, 29.7
4	28.7	31.3
5, 6, 7, 8	27.8, 25.3, 26.8, 29	31.7, 27.1, 28, 32
9	27	32
10	15	25
11	29.7	29.1

Table 3
Results for the main effects of fungi (A), moisture content (B), bulking agent content (C) and the standard errors (σ) based on the Box–Hunter method [12]

Factor	After 28 days			After 98 days		
	Main effect	2σ	Significant	Main effect	2σ	Significant
A	−1.69	5.66	No	−2.2	2.9	No
B	−1.42	4.38	No	0.09	2.26	No
C	4.93	5.66	No	3.63	2.9	Yes

presence of native white rot fungi in all the jars, as neither the soil, nor the peat moss used as bulking agent were sterilized. On the other hand, since the soil and bulking agent were not sterilized, the bacterial activity was present in all test jars. In many cases it has been reported that the competition between the soil bacteria and white rot fungi for nutrients is an inhibitory factor for fungi [13,14].

4.2. Moisture content

The moisture content was either 30 or 50% (w/w dry soil) in different test jars. Analysis of the experimental data (Table 3) showed that the main effect of this change on the experimental results were within $\pm 2.0\sigma$ and therefore, did not have a significant effect on the hydrocarbon loss.

4.3. Bulking agent

As can be seen from Table 3, the main effect of bulking agent after 98 days is not within $+2\sigma$, which means that the bulking agent had a significant effect on the hydrocarbon loss of the test jars after 98 days. In other words, the variations in the 98-days results from changing the percent of bulking agent are not merely caused by experimental errors. The results showed that the change in bulking agent from 6 to 12% caused an increase of approximately 4–5% in hydrocarbon loss over 98 days. The increase could be due to the higher microbial activity or higher adsorption rates in the high bulking agent jars. However, the bulking agent had no significant effect after 28 days of growth in the soil. The increase could be due to the higher microbial activity or higher adsorption rates (time dependent) in the high bulking agent jars.

5. Ligninolytic enzymatic activity

The enzymatic activity of fungi in each test jar was monitored regularly using the dyedecolorization method. The results for the enzyme activity measurements over 98 days are shown in Fig. 1. As can be seen from this figure, all of the test jars showed high activity, except the control jars 12 and 13. The enzyme activity in jar 10 was not as high as others, possibly because the soil was too wet and adhesive due to its high moisture content and low

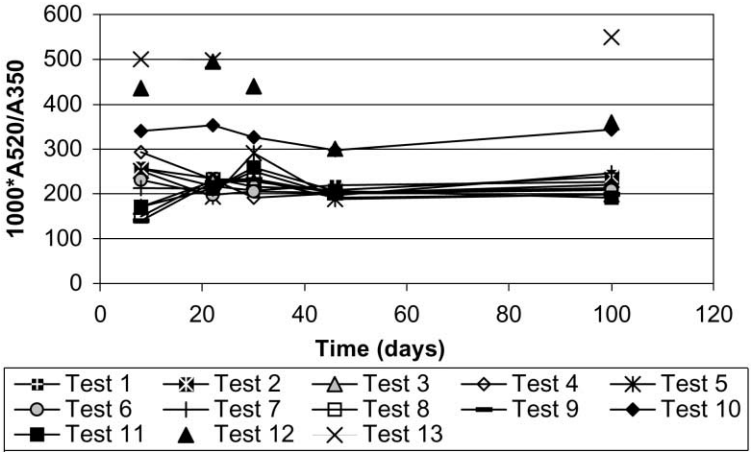

Fig. 1. Results for the ligninolytic enzyme activity in test jars over a 98-days period. Jars 12 and 13 are control jars (lower absorbance ratio means higher enzyme activity).

bulking agent. The ligninolytic enzyme activity in the jars with no fungi application could be mainly from the natural white rot fungi present in the bulking agent or the soil.

6. Gas chromatography analysis of the hydrocarbon loss in the test jars

In contrast to the gravimetric method, the GC analysis provides information on each individual hydrocarbon in the soil, except for the asphaltene fraction. The reduction in the

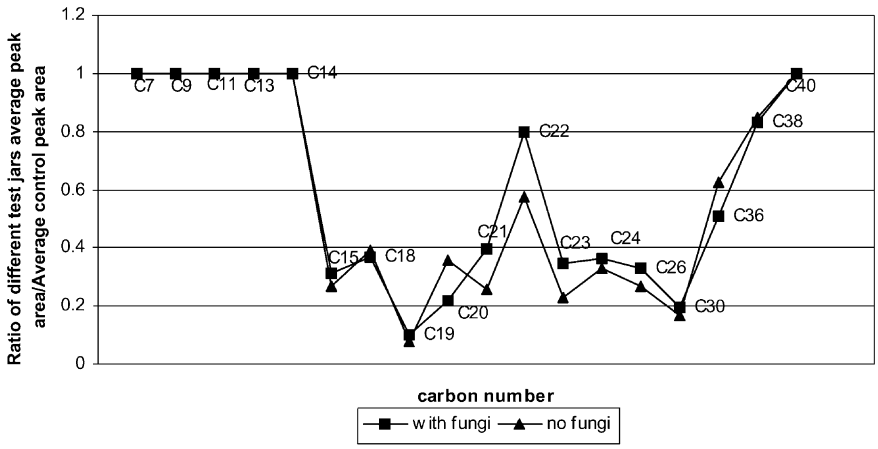

Fig. 2. The ratio of the peak area for “with” and “without fungi” to the peak area of the control jars for a number of calibrated hydrocarbons.

peak areas was very similar in all jars. To compare the changes in the hydrocarbon peak areas for jars with and without fungi application, the ratio of calibrated peaks to that of control were studied. A summary of the results for the reduction of the peak areas for jars with and without fungi application can be seen in Fig. 2.

In this figure, a ratio equal to 1 indicates that there has been an equal reduction in the compound, both in control and the test jar, and a ratio of less than 1 means higher reduction than the control jars. For C_7 to C_{14}, the ratio is 1, which is due to the total loss of compound both in control and other test jars. The ratio is less than 1 for C_{15}–C_{38}, which is an indication of higher loss of hydrocarbon in the main test jars. There had been very little loss of C_{40} in all the jars and therefore, the ratio is close to 1. It can also be seen that generally the "with fungi" and "no fungi" jars, followed the same pattern over C_{15}–C_{38} and achieved almost the same degree of hydrocarbon loss over the whole spectrum.

7. Conclusions

As can be seen from the results, fungi bioaugmentation did not have a significant effect on the bioremediation of the flare pit soil studied here. The fungi may play a role in the biodegradation process, however, fungi addition in nonsterilized systems does not necessarily enhance the biodegradation rates. This is in part due to the presence of natural strains of white rot fungi in most woody material used as bulking agents and in the soil. This study was conducted with only one type of flare pit soil, and further tests with other soils and different hydrocarbon contaminants could provide a better understanding of the role of fungi bioaugmentation.

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