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Original Research Paper

Biodegradation of Domestic Fuel Oil from Contaminated Soil Using Indigenous Microorganisms

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ABSTRACT

Biodegradation of hydrocarbons by natural population of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants are eliminated from the environment. The polluted soil was taken from a historically contaminated site which is located in Ghent, Belgium. The experiment was carried out under different temperatures and nutrient conditions and aerobic biodegradation was estimated by measuring CO_2 production from the microorganisms. The results clearly revealed that high temperature enhances the biodegradation of domestic fuel oil in comparison to the low temperature and also adding a low amount of nutrients having higher biodegradation efficiency.

INTRODUCTION

Petroleum is still the most important primary energy source; additionally petrochemicals that are derived from petroleum or natural gas, are used in a wide variety of areas for instance household goods, medicine, archaeology, and crime detection. The scale of transport and use of these chemicals causes considerable emissions to the environment by accidents, defects or handling losses. Tanker oil spills and well blow-outs can cause serious soil pollution. Fuel storage tanks, leaking pipelines, and handling of fuels are other sources of soil contamination by petroleum hydrocarbons. These cause petroleum to be a major pollutant of soil and water. Fuel contamination in soil is often a long-term source of groundwater contamination due to leaching of dissolved compounds, transport and dissolution of non-aqueous phase liquids, transport of dissolved organic matter or gaseous transport of volatile organic compounds (Pasteris et al. 2002).

The toxicity of petroleum pollution has been documented. There is inadequate evidence for association between cancer and exposure to fuel oils for humans. However, dermal exposure to fuels can produce skin or liver cancer for some species of animals. Some effects such as problem in breathing into the lungs, pulmonary oedema, and problem in nervous system can be seen if accidental ingestion of kerosene happens and also irritation of gastrointestinal system can happen due to acute exposure. Increasing blood pressure and eye and skin irritation in humans have been seen due to inhalation and dermal acute exposure (Ekanem et al. 2011, Lopes et al. 2010).

There is wide diversity of remediation techniques that can be applied for different contaminants and site conditions. Phytoremediation and bioremediation using plants and microbes to remove metals, organic matter, nitrite etc. pollutants from contaminated soil, water, waste water is being developed as new methods for the bioremediation of contaminated soil and water (Gupta et al. 2011, Singh et al. 2010, Tejo et al. 2010, Zhao 2009). Polycyclic aromatic hydrocarbons are found in soil and water, which occur as natural and anthropogenic activities, were biodegraded by *Streptomyces rochei* (Chaudhary et al. 2011).

In the USA the traditional method for remediation of leaking underground storage tanks was pumping up or heating for groundwater contamination and excavation and disposal for soil contamination. To get more effective and cheaper treatment technologies compared to the traditional methods, the US Environmental Protection Agency proposed some *in-situ* alternatives such as dual phase extraction, air sparging, bioventing, biosparging, soil vapour extraction, *in-situ* groundwater bioremediation, and monitoring natural attenuation. *Ex-situ* methods are land farming, biopiles and soil washing, and low temperature thermal adsorption.

Bioremediation is an efficient method for removal of petroleum hydrocarbons. In many cases, microorganisms can utilize pollutants as nutrient sources and degrade them to

environmentally harmless or benign compounds (Bruins et al. 2000). This technique uses the biodegradation of the pollutants to water and carbon dioxide. However, due to the variety of chemical, physical and biological factors, the overall process can be unpredictable. The rate of biodegradation of oil is strongly affected by environmental conditions such as oxygen, temperature, moisture content, nutrients, soil pH, and bioavailability (Cattaneo et al. 1997). Therefore, the rates of biodegradation of hydrocarbons from oil spills appear to be highly dependent on localized environmental conditions. It is apparent that the microbial degradation of oil pollutants is a complex process and that environmental factors have a great influence on the fate of spilled oil. The goal of this research work was to investigate the effect of nutrients and temperature on the aerobic biodegradation of aged domestic fuel oil in soil.

MATERIALS AND METHODS

The samples were taken from historically contaminated site in the Harelbekestraat 72 Ghent, Belgium. The pollution originated from two underground storage tanks containing domestic fuel oil. Both contaminated and uncontaminated soils were sampled.

Soil samples pretreatment: After collecting, the samples were put in plastic bags and transferred to the lab. The soil was air-dried in aluminium dishes and grounded to break aggregated parts by manual grinding and kept in plastic bags at 4°C. For the determination of the proportions of sand, silt and clay, the soil samples were processed by sieving. Triangular coordinates provide a convenient method for condensing bulky tables on texture relationships. Texture of soil samples were classified as loamy sand. Soil moisture content of the air dried soil, was measured by the gravimetric method in triplicate. For the incubation experiment, 24 jar bottles (with 500 mL volume) were prepared. The bottles were washed with distilled water and acetone and dried in the oven at 105°C. The experiment was carried out under treatment of contaminated and uncontaminated soils, temperature 10°C and 20°C as well as nutrient with low fertilization and high fertilization in triplicates. The nutrient solution for the low and high fertilization provided was as in Table 1.

The bottles were filled with 50 g of air dried soil and an adequate amount of fertilizer solution and distilled water was added to reach a gravimetric moisture content of 20%. During incubation the gravimetric soil water content was maintained at 20% by periodically weighing and adding the appropriate amount of distilled water. For the loamy sandy soil, this amount of water is almost 60% of water filled pore space which was found by Linn & Doran (1984) to be the optimum water content for microbial activity. The soil and liquid inside the bottles were thoroughly mixed and allowed to equilibrate with the nutrient solution for two days. The small amount of soil (50 g) in the bottles resulted in a thickness of 3 cm for the soil layer, allowing for easy oxygen penetration and aerobic condition in the soil sample. All bottles were incubated at 20°C or 10°C for 63 days with periodic monitoring of CO_2 evolution and O_2 consumption. At the beginning of the incubation experiment, the rubber septum was removed briefly to equilibrate the sample with the atmosphere.

Table1: Domestic fuel oil and nutrient information.

Treated soil	Oil content (µg/g)	N (µg/g)	P (µg/g)	K (µg/g)	C/N/P
Low fertilization	3000	300	60	76	100/10/2/2.5
High fertilization	3000	600	120	152	100/20/4/5

Titration method: During incubation microorganisms use oxygen and produce CO_2. This CO_2 is captured by NaOH inside the cuvet, according to:

$$CO_2\ (gas) + 2\ OH^- \rightarrow CO_3^{-2} + H_2O \quad ...(1)$$

The CO_2 captured in the NaOH solution is determined by titration of the NaOH solution with HCl according to:

$$HCl + NaOH \rightarrow NaCl + H_2O \quad ...(2)$$

In this experiment, 3 mL of NaOH inside the cuvet were added to 40 mL of fresh NaOH that was kept in a closed barrel. This solution was titrated with 1N HCl by autotitrator machine. The solution containing 43 mL of NaOH was agitated with a magnetic stirrer during the determination. Separate acid (HCl) and base (NaOH) titrations were then performed to reach at the final pH of 8.5. The amount of CO_2-C produced during the incubation in a sample is calculated by:

$$CO_2\text{-}C\ (mg) = (B - S) \times M \times E \quad ...(3)$$

Where,

B is the amount of HCl added to the blank (mL)
S is the amount of HCl added to the sample (mL)
M is the molarity of HCL (1 Molar)
E is the equivalent mass of C in the reaction (6)

After calculation of the amount of CO_2 production in each flask in 50 g of soil, the amount of CO_2 production for 1 kg soil was calculated. This amount of CO_2 production is related to a time interval of 21 days so for every day the amount of CO_2 production was calculated being the rate of CO_2 production. The hydrocarbon degradation rates were calculated stochiometrically. The hydrocarbon degradation expressed as grams CO_2 produced per gram of hydrocarbon degraded, is fairly constant for different hydrocarbon compounds.

$$K_{HC} = K_{CO2} / 3.4 \quad ...(4)$$

Table 2: CO_2 production (mg/kg soil) measured with the titrimetric method at different treatments.

Treatments	21 days		42 days		63 days	
	10°C	20°C	10°C	20°C	10°C	20°C
Uncontaminated soil	139.64	214.75	79.21	159.89	78.92	80.38
Contaminated soil	249.25	408.02	117.05	244.32	146.8	231.29
LF* contaminated soil	267.9	499.2	145.22	296.07	122.33	271.07
HF* contaminated soil	201.02	468.74	119.69	239.04	104.38	240.08

*LF: Low Fertilization; HF: High Fertilization

Where,

K_{HC} is hydrocarbon degradation rate (mg/kg soil) per day
K_{CO2} is CO_2 production rate (mg/kg soil) per day.

RESULTS AND DISCUSSION

The mean CO_2 produced during the incubation for the different treatments is shown in Table 2. It is obvious that the degradation of the domestic fuel oil shows faster rate in the beginning up to 21 days, and then it gets slower. Firstly, an exponential increase until day 21 can be seen and afterwards a stagnation of the CO_2 production until day 63. Table 2 shows that the degradation of the domestic fuel oil decreases during the incubation. The observed stagnation of biodegradation is due to limited substrate transport that slows down the activity of the microorganisms. Many authors also observed decreasing hydrocarbon degradation during incubation. They found an initial exponential CO_2 production (Xin Lin et al. 2009) and suggested this to be due to the specific growth of the microbial biomass.

Effect of nutrients: Table 2 shows the effect of nutrients on biodegradation of domestic fuel oil at 10°C and 20°C. In addition, the total degradation for different treatments at 10°C and 20°C is shown (Fig. 1) which is calculated by eq. 4.

As expected, the CO_2 production is strongly dependent on oil concentration. From Table 2, it can be seen that the uncontaminated soil has the lowest CO_2 production compared to the other soil treatments. It is concluded that degradation of indigenous organic matter that produces CO_2 is much lower in comparison with the CO_2 production from the degradation of domestic fuel oil.

The results show that it is possible to enhance degradation of oil by adding fertilizers to the soil and using indigenous microorganisms for the low fertilization treatment (Jin et al. 2010). However, at 10°C adding the fertilization treatment is not much effective for degradation of domestic fuel oil compared to the unfertilized soil. The reason for the negligible effect of this higher fertilizer level can be a toxic effect on the microorganisms at this temperature. This hindrance shows that the toxicity of high fertilization is more at 10°C than 20°C (Lin et al. 2009). In this experiment biodegradation rate of low fertilization amendments is higher than the biodegradation in all of the treatments at two temperatures (Fig. 1). Biodegradation of domestic fuel oil at high fertilization treatment is greater than unfertilized soil at 20°C and 10°C. The increase in the CO_2 production for the high fertilization and low fertilization treatments at 20°C shows that adding fertilizers stimulated biodegradation. However, the high fertilization treatment was less efficient than low fertilization treatment at these temperatures. The total CO_2 production for the low fertilization treatment was higher than the total CO_2 production for the high fertilization treatment at both temperatures. This shows biodegradation can be stimulated by adding an effective level of nutrients to the contaminated soil. Therefore, high levels of nutrient have a reverse effect on oil biodegradation. The results suggest that biodegradation of the domestic fuel oil is not only affected by C/N/P ratios, but other factors such as temperature are also important, since the effect of nutrients is different at 10°C and 20°C. Many scientists report that changing the amount of nutrients due to fertilizer addition may affect microbial community in the polluted soils, which are able to degrade pollutants. A low fertilizer level can cause a dominance of some species that are more effective for degradation of domestic fuel oil, while a high fertilizer level can cause a dominance of species in communities which are less effective for biodegradation (Chaineau et al. 2000).

Effect of temperature: Fig. 2 shows total CO_2 production measured with the titrimetric method under treatment of two different temperatures. It can be seen that the biodegradation rate of domestic fuel oil is highly affected by temperature. At a higher temperature (20°C) more CO_2 is produced. The reason for the higher CO_2 production at 20°C is higher microbial activity. The amount of biodegradation for all the treatments at 20°C is more than at 10°C. At 20°C the HF treatment has a higher CO_2 production than the contaminated soil. This means at higher temperature microorganisms are able to assimilate mineral nutrients more than at lower temperature. In addition, the availability of domestic fuel oil at 20°C is higher than at 10°C, which is more favourable for microorganisms to degrade domestic fuel oil. According to Atlas & Bartha (1972), at low temperature low molecular weight hydrocarbons are not removed abiotically and some of these components are toxic to microorganisms. So the rate

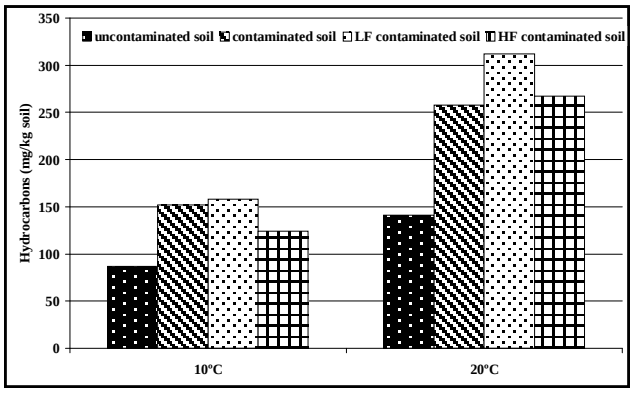

Fig. 1: Total degradation of hydrocarbons under different treatments.

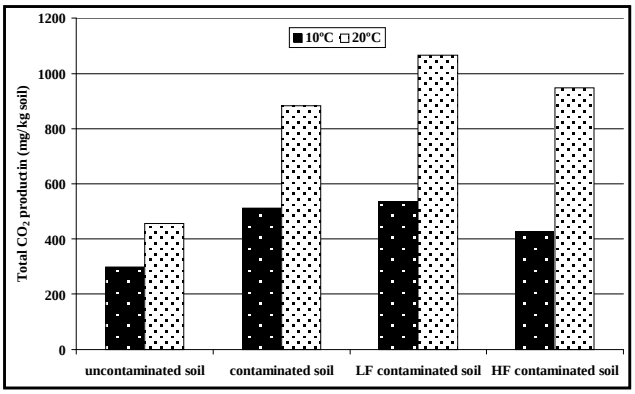

Fig. 2: Total CO_2 production for the different temperatures.

of microbial activity decreases at low temperature as well as biodegradation is inhibited by toxic components.

SUMMARY AND CONCLUSION

The results show that indigenous microorganisms are able to degrade domestic fuel oil pollutant if some limiting conditions improve. Enhancing suitable conditions for microorganisms causes an increased population and activity of microorganisms for degradation of pollutants. From the results it was shown that adding a low amount of nutrients has a positive effect on biodegradation rate. So it can be concluded that indigenous nutrients for degradation of oil are not enough because biodegradation for the contaminated soil is less than low fertilization treatment at two temperatures. The comparison of unfertilized and fertilized soil showed that adding nutrients to contaminated soil stimulated biodegradation of domestic fuel oil. Adding fertilizer above a favourable amount to the contaminated soil has less effectiveness for domestic fuel oil degradation. It is also demonstrated that a high temperature enhances the biodegradation of domestic fuel oil in comparison to low temperature. The results show that if temperature increases without adding nutrients to the contaminated soil, biodegradation is high in comparison to the condition of low temperature and nutrient addition. So in the study, temperature was more important than nutrients for increasing bioavailability of domestic fuel oil. However, in the case of high temperature and suitable concentration of nutrients, synergy effects will cause biodegradation to improve considerably. More investigations are necessary to understand the interaction between microorganisms and domestic fuel oil in the environment due to different conditions on this specific site. It can be proposed that when the behaviour of microorganisms is known on a specific site, it is possible to use models for prediction the fate of domestic fuel oil and improve strategies for using microbial activities to clean up the polluted site. Stagnation of biodegradation can be due to unsuitable indigenous community of microorganisms, since the results obviously demonstrated that CO_2 was quite well produced, therefore, the microbial community was suitable for domestic fuel oil degradation. Therefore, indigenous microorganisms can be used for degradation of domestic fuel oil pollutant if some limiting conditions improve.

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