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OPTIMIZATION OF HIGH-STRENGTH HYDROCARBON BIODEGRADATION USING RESPIROMETRY

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ABSTRACT: Laboratory respirometry experiments were conducted on mixtures of soil and oily sludge to estimate biodegradation rates by CO_2 production rates and determine optimum conditions for biodegradation of high-strength hydrocarbon waste products. These experiments were used to determine a suitable range of total petroleum hydrocarbon (TPH) concentration for biological treatment and to optimize for nutrient addition and moisture content. CO_2 production rates from biological respiration of hydrocarbon-contaminated soil were maximized at concentrations of 3-9% TPH (30,000-90,000 mg/kg TPH). CO_2 production rates decreased dramatically at concentrations above 9% TPH, indicating that either these concentrations are lethal to microbes present, or this high sludge content inhibits aeration of the soils. Addition of 120 mg/kg nitrogen, 40 mg/kg phosphorous, and 40 mg/kg potassium to the soils resulted in a three fold increase in CO_2 production rates. No significant increase in CO_2 production was observed when the nutrient addition was increased to 240 mg/kg nitrogen, 80 mg/kg phosphorous, and 80 mg/kg potassium. Maximum CO_2 production rates were observed at 15-20% moisture content. CO_2 production rates decreased significantly at and below 10% moisture and at and above 25% moisture. Maximum CO_2 production rates observed for soil with 50,000 mg/kg TPH, with added nutrients at optimum moisture content, were 30-35 μL CO_2/g/hr. Assuming all CO_2 was generated from hydrocarbon degradation, this maximum CO_2 production rate corresponds to a hydrocarbon biodegradation rate of approximately 500 mg TPH/kg/day (assuming 100% respiration for a conservative estimate). If ideal conditions are maintained and rates of respiration remain high, clay soil contaminated with 60,000 mg/kg TPH sludge could probably be remediated in 2 months.

INTRODUCTION

Current oil production results in the production of a sludge by-product which cannot be refined and must be treated and disposed. This sludge is an extremely thick, viscous mixture of sediments, water, various hydrocarbons and metals. Bioremediation by land treatment has been practiced for decades for the treatment of this type of waste (API, 1983). Admon et al. found that 70% - 90% of sludges can be effectively treated in 2 months at 9,000-60,000 mg/kg total petroleum hydrocarbon (TPH). Since produced sludge may contain greater than 60,000 mg/kg TPH, this research was undertaken to characterize and optimize the rate of microbial biodegradation of very high-strength petroleum hydrocarbon waste using closed-cell respirometry. To achieve this goal, TPH concentration, nutrient addition, and moisture content were varied to determine the appropriate TPH concentration and "formula" to achieve maximum respiration. Biodegradation rates were estimated by measuring the rate of CO_2 production using a Micro-Oxymax Respirometer.

MATERIALS AND METHODS

CO_2 production rates were measured using a Micro-Oxymax closed cell respirometer (Columbus Instruments, Columbus Ohio) with hardware version 6.0 and software version 6.06d and 6.09b. Individual soil/sludge samples ranging from 25-100 grams of sample were placed in 250 ml jars. Jars with contents were placed in a Precision 180 Series water bath and maintained at 30°C for all experiments. CO_2 production was monitored using an infrared detector. The respirometer measured CO_2 production every three hours and purged jar headspace every six hours.

Sludge was obtained from a Unocal oil production facility. The sludge contained 370,000 mg/kg TPH as measured by gas chromatography/mass spectrophotometry (GC/MS) (Zymax Analytical Laboratory, San Luis Obispo, CA). The soil used for mixing in these experiments was obtained from a former land treatment area and contained 15,000 mg/kg TPH as determined by GC/MS. Moisture content analysis per Standard Method 2540B was estimated to be 12% by weight. Total phosphorous, total Kjeldahl nitrogen, nitrogen (nitrate and nitrite), and potassium was measured according to their respective Environmental Protection Agency (EPA) protocols. Nutrients present in land treatments soil are presented below in Table 1.

TABLE 1. Nutrient analysis of contaminated soil. Concentrations listed as ND (not detected) were below the Practical Quantification Limit.

Nutrient	Method	PQL (mg/kg)	Concentration (mg/kg)
Total Phosphorus as P	EPA 365.2	50	390
Total Kjeldahl Nitrogen	EPA 351.3	0.5	590
Nitrate as Nitrogen (NO_3-N)	EPA 300.0	5	ND
Nitrite as Nitrogen (NO_2-N)	EPA 300.0	5	ND
Potassium	EPA 6020	10	190

Samples prepared for moisture content experiments were mixed to achieve desired TPH concentration and then dried for 24 hours at 110ºF. Water was added after drying to achieve the desired moisture content. Nutrient addition was provided using Miracid®, an acidic form of Miracle-Gro® with an NPK ratio of 30-10-10. The bulk of N in Miracid® comes in the form of urea nitrogen.

RESULTS AND DISCUSSION

Effect of TPH concentration. To determine the effect of TPH concentration on biodegradation, CO_2 production was measured over TPH concentrations ranging from 20,000 mg/kg TPH to 370,000 mg/kg TPH (pure sludge). Jar contents and results are given in Table 2. Maximum CO_2 production rates were observed between 30,000 mg/kg and 90,000 mg/kg TPH. CO_2 production rates decreased rapidly at TPH concentrations of 100,000 mg/kg and greater. Very low CO_2 production rates were observed at the higher TPH concentrations, including the 370,000 mg/kg sludge. The sudden drop in the rate of CO_2 production from 60,000 mg/kg TPH to 100,000 mg/kg TPH is most likely due to toxicity, but it may also be due to the lack of porosity of these samples. Higher concentration samples have a greater amount of liquid sludge present, which may limit the amount of aerobic respiration in the samples because of decreased air permeability.

CO_2 production was measured with respirometry for 144 hours to determine if the rates change over this time period. Based on cumulative CO_2 production, the rate of CO_2 production appears to be constant for this time period (Figure 2). Therefore, subsequent experiments were run for 72 hours.

TABLE 2. Samples contents and results for respirometry measurements for 20,000 to 370,000 mg/kg TPH range.

TPH (mg/kg)	Time (hr)	Sample size (g)	CO_2 production rate (μL/g/hr)	Accumulated CO_2 production (μL/g)
0	144	0	-0.01	-0.8
0	72	0	0.00	-0.1
20,000	72	50	6.03	425
30,000	72	50	11.3	791
40,000	72	50	12.0	842
50,000	72	50	11.6	811
60,000	144	50	10.4	1481
60,000	72	50	11.8	822
70,000	72	50	11.6	806
80,000	72	50	11.6	812
90,000	72	50	10.9	755
100,000	144	100	5.6	795
200,000	144	100	2.7	381
228,000	144	125	2.1	296
280,000	144	100	1.6	223
370,000	144	100	1.0	146

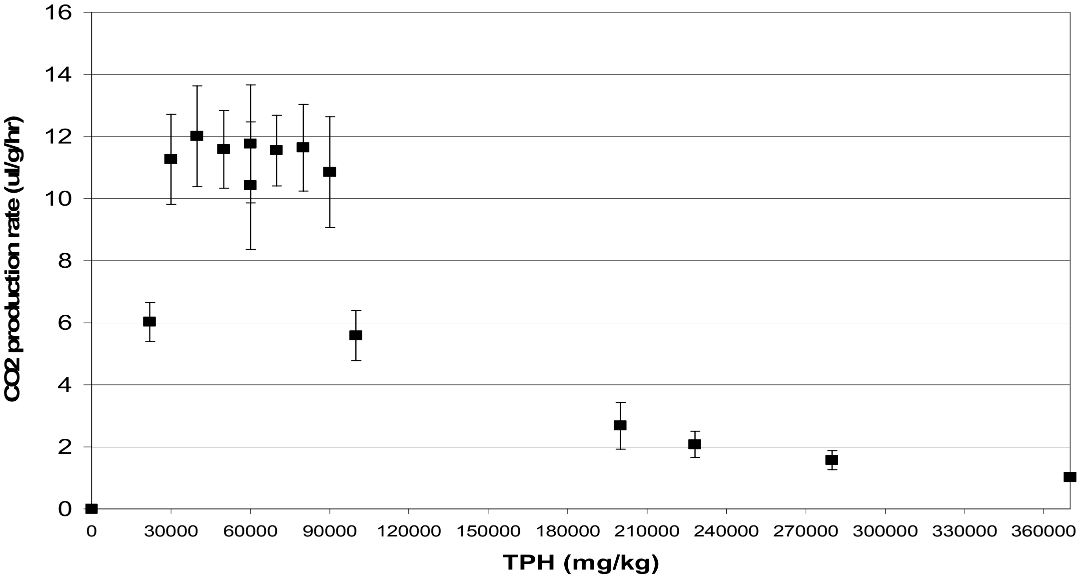

FIGURE 1. CO_2 production rates for soil/sludge mixtures over entire TPH range.

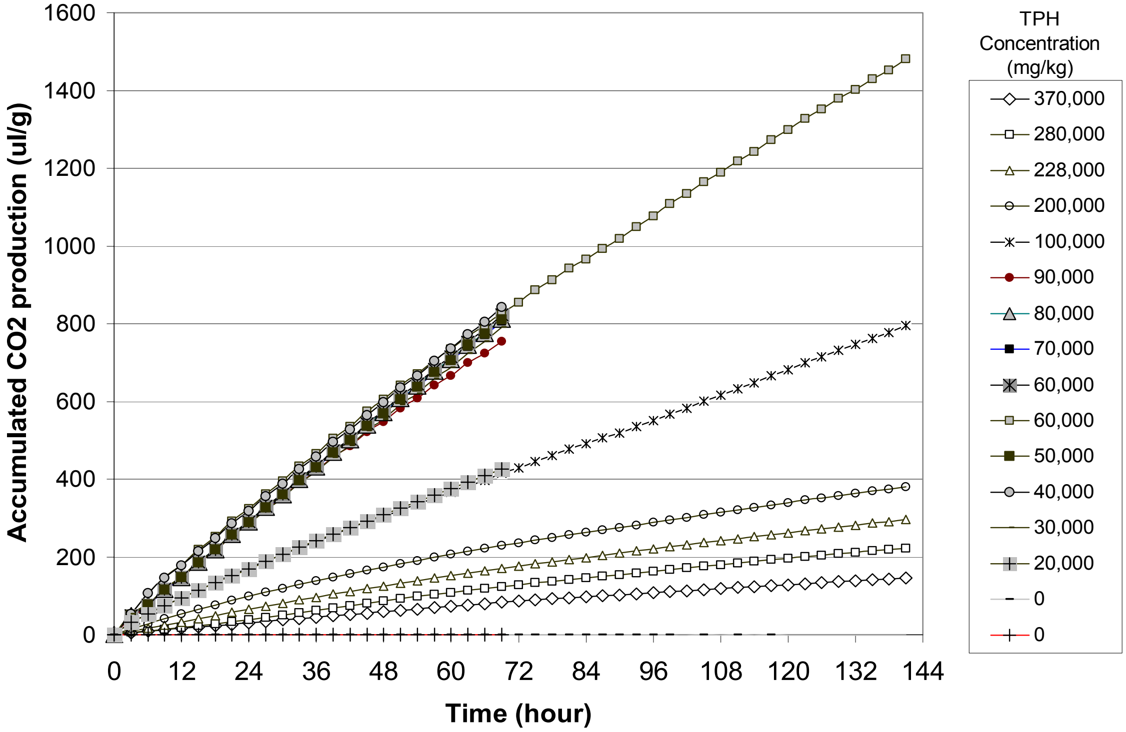

FIGURE 2. Time-course plot of accumulated CO_2 production for soil/sludge mixtures with a range of TPH concentrations.

Effect of nutrient addition. CO_2 production rates were measured with added nutrients to determine if rates could be increased. Miracid was added in solid form to the soil prior to sludge addition. The Miracid® has an NPK ratio of 30-10-10. Miracid was added at three levels: Level 0 (0-0-0 mg/kg NPK), Level 1 (120-40-40 mg/kg NPK), and Level 2 (240-80-80 mg/kg NPK). Nutrients were added to samples with 50,000 mg/kg to 200,000 mg/kg TPH. Contents of the sample jars used in the nutrient experiments are described in Table 3.

TABLE 3. Samples contents and results for nutrient experiments. Level 0 is 0-0-0 mg/kg NPK, Level 1 is 120-40-40 mg/kg NPK, and Level 2 is 240-80-80 mg/kg NPK. All samples are 50 grams. Respiration rates at 50,000, 65,000, and 86,000 mg/kg TPH with Level 2 nutrients not determined.

	CO_2 production rate (μL/g/hr)		
TPH (mg/kg)	Level 0	Level 1	Level 2
0	0	0	0
50,000	11.6	35.1	-
60,000	11.1	33.7	33
65,000	11	33.9	-
70,000	11.55	31.8	33.4
86,000	10.8	25	-
100,000	7.5	16.3	21.4
200,000	2.68	7.4	7.7

Addition of Miracid® to provide 120-40-40 mg/kg NPK greatly increased the rate of CO_2 production in the samples (Figure 3). Rates were tripled by this nutrient addition at TPH concentrations of 50,000 mg/kg and 65,000 mg/kg (Figure 3), and significantly increased at all TPH concentrations. Insignificant changes in CO_2 production rates were observed by increasing nutrient addition from 120-40-40 mg/kg NPK to 240-80-80 mg/kg NPK. Since 120-40-40 mg/kg NPK results in an increase in the rate of CO_2 production, and limited benefit is achieved using 240-80-80 mg/kg NPK, future long term and larger scale studies should include 120-40-40 mg/kg NPK addition.

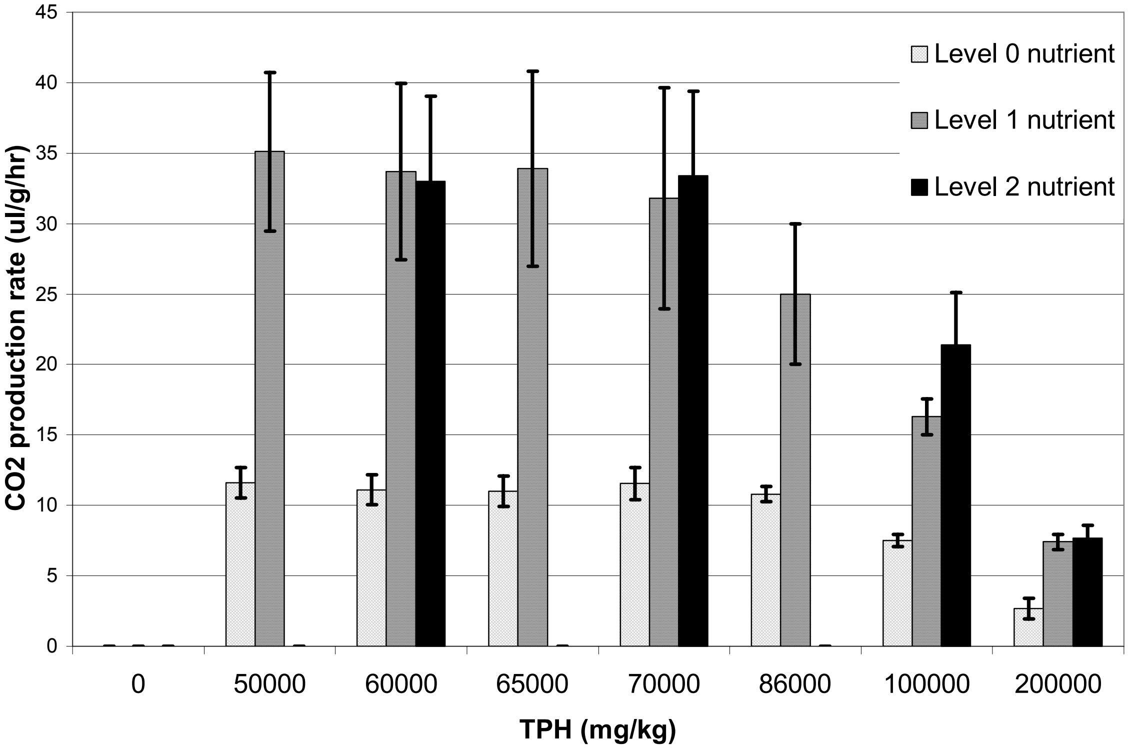

FIGURE 3. CO_2 production rates for varying nutrient levels. . Level 0 is 0-0-0 mg/kg NPK, Level 1 is 120-40-40 mg/kg NPK, and Level 2 is 240-80-80 mg/kg NPK.

Effect of moisture content. To determine the ideal moisture content for biodegradation, CO_2 production rates were measured for contaminated soil with a range of moisture content. For this experiment, clay soil and sludge were mixed at an 18:3 soil:sludge ratio to achieve an initial TPH concentration of 65,000 mg/kg TPH. The mixture was dried for 24 hours at 110º F until the soil/sludge mix was completely dry. Deionized water was then added to sub-samples of the mixture to create samples with known moisture content ranging from 0% moisture to 35% moisture. Contents of each sample are listed below in Table 4.

Increasing moisture content from 5% to 20% increased the rate of CO_2 production dramatically (Table 4). Samples with a moisture content of 25% and above display a significant decrease in the rate of CO_2 production, probably due to a lack of O_2 permeability in the wet soil matrix. Maximum rates of CO_2 production occur in samples with 15-20 wt. % moisture.

TABLE 4. Sample contents and results for moisture experiments. All samples were 50 grams and contained 120-40-40 mg/kg NPK and 65,000 mg/kg TPH.

Water added (g)	Added Moisture Content (wt. %)	CO_2 production rate (μL/g/hr)
Empty	Empty	0.03
0	0%	0.03
2.5	5%	3.68
5.5	10%	12.05
8.5	15%	18.65
12.5	20%	19.49
16.5	25%	7.52
21.5	30%	5.95
27.5	35%	7.68

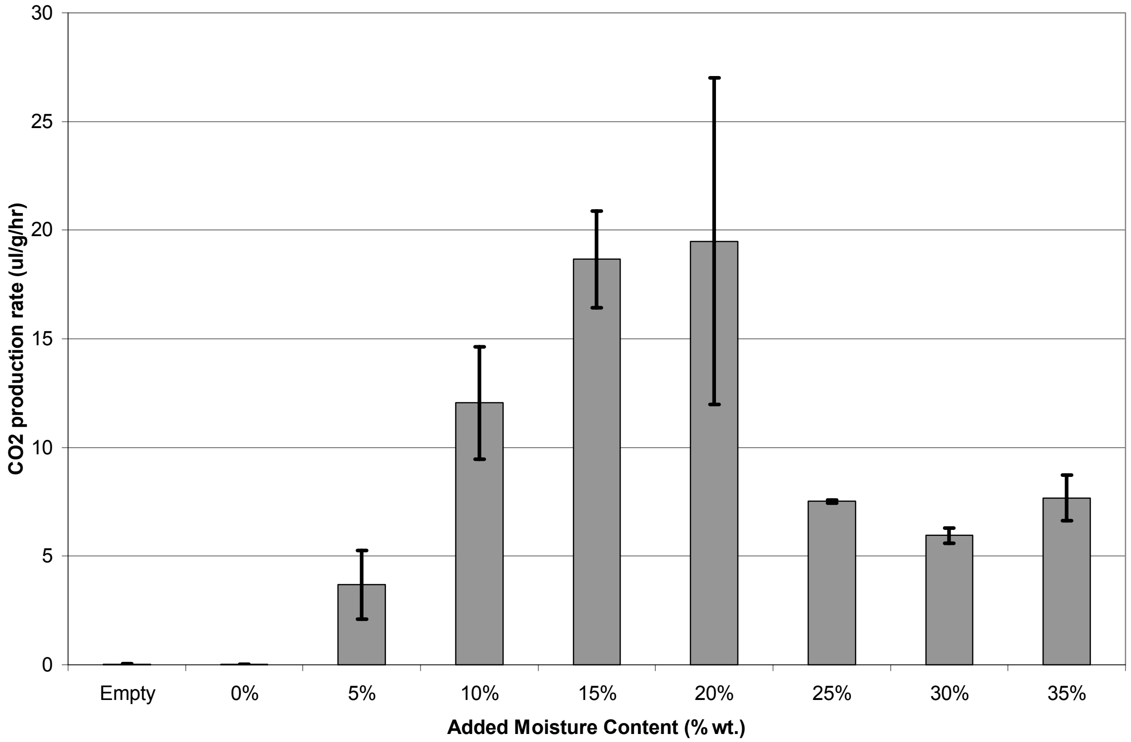

FIGURE 4. CO_2 production rates with varying moisture content. All samples contained 65,000 mg/kg TPH.

CONCLUSIONS

Biological respiration estimated by CO_2 production rates of hydrocarbon-contaminated clay soil was most efficient at TPH concentrations of 3-9% (30,000-90,000 mg/kg TPH).Only very limited CO_2 production was observed at higher TPH concentrations. CO_2 production rates decreased dramatically at concentrations above 9% TPH, indicating that either these TPH concentrations are lethal to microbes present or this high sludge content inhibits O_2 permeability in the soils. Addition of 120-40-40 mg/kg NPK to the soils resulted in a three to four fold increase in the rate of CO_2 production at 6% TPH. No further increase in CO_2 production rates was observed when the nutrient addition was increased to 240-80-80 mg/kg NPK. Maximum CO_2 production rates were observed at 15-20% moisture content. CO_2 production rates decreased significantly at and below 10% moisture and at and above 25% moisture. Maximum CO_2 production rates observed for soil with 50,000 mg/kg TPH, with added nutrients at optimum moisture content, were 30-35 μL CO_2 per g soil per hr.

It would be useful to translate the observed maximum CO_2 production rate (35 μL/g/hr) to a rate of hydrocarbon biodegradation in units of mg TPH/kg soil day. To estimate this biodegradation rate, we performed a series of calculations based on certain assumptions. Using the ideal gas law (PV=nRT) at 30°C,

$$1\ \mu\text{L}\ CO_2 = 4.5 \times 10^{-5}\ \text{mmol}\ CO_2 = 0.002\ \text{mg}\ CO_2$$

To convert this CO_2 evolution into an amount of hydrocarbon degraded, the following assumptions were made:

1) All CO_2 was generated from hydrocarbon biodegradation. This assumption neglects the possibility of CO_2 evolution from natural organic material in the soil. This is a fairly safe assumption because easily biodegraded organic material in the soil is likely to have biodegraded in the field before the samples were collected. Furthermore, CO_2 evolution was observed to increase with increasing added hydrocarbon in the laboratory experiments.
2) For a conservative estimate, we are assuming that bacteria used the hydrocarbons entirely for respiration. Since the bacteria are likely to convert some of the carbon in the hydrocarbon to cell mass rather than CO_2, the actual amount of hydrocarbon degraded would be greater than that estimated using this assumption.
3) 80% of the hydrocarbon mixture is carbon.

Employing these assumptions,

$$1\ \mu\text{L}\ CO_2\ \text{evolved} = 0.002\ \text{mg}\ CO_2\ \text{evolved} = 6.36 \times 10^{-4}\ \text{mg carbon} = 7.95 \times 10^{-4}\ \text{mg TPH degraded (80\% carbon)}$$

Then, converting units:

$$35\ \mu\text{L}\ CO_2\ \text{per gram soil per hour} = 667.8\ \text{mg TPH per kilogram soil per day.}$$

This calculation provides a rough biodegradation estimate of ~650 mg TPH per kg soil per day. If ideal conditions are maintained, the repeated soil/sludge mixtures could potentially be completely biodegraded in about 120-180 days. These calculations are very rough and assume zero-order degradation kinetics. Actual degradation rates are likely to be first order and may even reach an asymptotic level at low TPH concentrations. This could result in a significantly longer estimate for the time required to achieve complete biodegradation.

The above results were obtained under controlled laboratory conditions which may differ from field conditions. Respiration rates were measured immediately upon mixing, and were for relatively small samples (50-100 grams). Aeration of small laboratory samples may be greater than that achieved in the field. Therefore, actual rates achieved in the field will likely be different than those observed in the lab.

Data collected from these experiments can be used to guide future large scale land treatment processes to remediate produced oil sludges at oil production sites. If optimum conditions are maintained, soils contaminated with 60,000 mg/kg TPH could potentially be remediated within two months. Further applications of sludge can be added to the contaminated soil since a consortium of hydrocarbon degrading microorganisms will be thriving.

A major issue of concern for future experiments is to determine the volatilization rates of hydrocarbons in soil. Volatilization of hydrocarbons occurs after cultivating soils, but low levels of volatilization occur throughout the land-treatment process (Asuma, 2002).

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