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Influence of inocula with prior hydrocarbon exposure on biodegradation rates of diesel, synthetic diesel, and fish-biodiesel in soil

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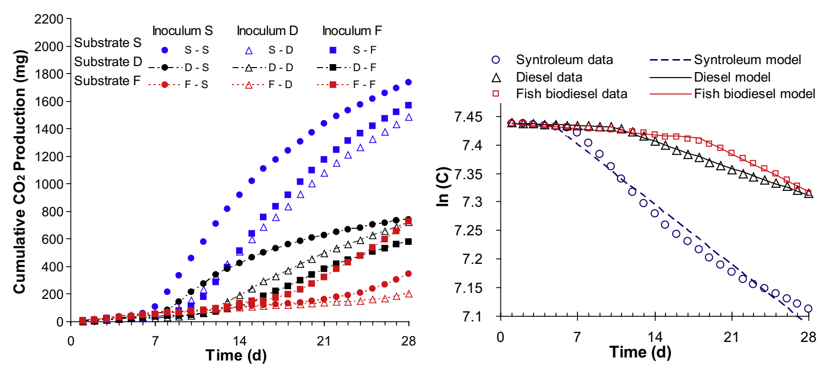
HIGHLIGHTS

- For Syntroleum, lag times were shortest and cumulative CO_2 production was highest.
- For fish biodiesel contamination, the lag time was longer than for other fuels.
- Matching inocula shortened lag phase but did not affect rate constants for 2 phases.
- Low respiration with the diesel-adapted inoculum was due to its lower microbial MPN.
- The difference between various inocula decreased after daily respiration peaked.

GRAPHICAL ABSTRACT



Inocula types affected the lag phase of diesel, Syntroleum or fish biodiesel biodegradation in soils

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ABSTRACT

To achieve effective bioremediation within short warm seasons of cold climates, microbial adaptation periods to the contaminant should be brief. The current study investigated growth phases for soil spiked with diesel, Syntroleum, or fish biodiesel, using microbial inocula adapted to the specific substrates. For modeling hydrocarbon degradation, multi-phase first order kinetics was assumed, comparing linear regression with nonlinear parameter optimization of rate constants and phase durations. Lag phase periods of 5 to >28 d were followed by short and intense exponential growth phases with high rate constants (e.g. from $k_{Fish} = 0.0013 \pm 0.0002$ to $k_{Syntr} = 0.015 \pm 0.001\ d^{-1}$). Hydrocarbon mineralization was highest for Syntroleum contamination, where up to three times higher cumulative CO_2 production was achieved than for diesel fuel, with fish biodiesel showing initially the slowest degradation. The amount of hydrocarbons recovered from the soil by GC–MS decreased in the order fish biodiesel > diesel > Syntroleum. During initial weeks, biodegradation was higher for microbial inocula adapted to a specific fuel type, whereby the main effect of the inoculum was to shorten the lag phase duration; however, the inoculum's importance diminished after daily respiration peaked. In conclusion, addition of an inoculum to increase biodegradation rates was not necessary.

1. Introduction

In the Arctic and subarctic environment, biodegradation can be one of the most suitable remediation processes for soil or water contaminations. In recent years, alternative fuels have become more widely used due to an increasing demand for renewable energy sources. When fuel spills occur in rural areas, where excavation and shipping to specialized treatment facilities can be cost prohibitive, natural attenuation and biodegradation might be the best and cheapest removal option. Hydrocarbon contaminant removal rates from soil and water vary widely both in laboratory

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and field scale studies. Laboratory studies have shown prominent total hydrocarbon degradation during short time periods, such as 27% diesel degradation after 1 d in an aquatic environment (Zhang et al., 1998), or up to 80% during 38 d in the terrestrial subsurface (Margesin et al., 2007). High diesel removal of over 90% within 11 d has also been reported (Moliterni et al., 2012). Compared to that, in field studies much less degradation was observed over a longer time period, even after years, such as 37% within 180 d (Fernandez et al., 2011) or up to 68% during 447 d (Margesin and Schinner, 2001). Opposite results have also been observed, for example microbial degradation of hydrocarbons, especially the volatile compounds, in unsaturated sandy soils in laboratory studies in cold environments was lower than in field studies (Hohener et al., 2006). Although good correlations between laboratory and field studies have been reported previously (Garcia Frutos et al., 2012), diesel contamination can persist in the environmental systems more than in the laboratory (Zytner et al., 2001).

In the current research, arctic grade Syntroleum was used, which was produced from natural gas and processed through the Fischer–Tropsch gas-to-liquid technique by the Syntroleum Corporation. A main advantage of the ultra-clean synthetic fuel (Syntroleum) compared with conventional diesel fuel is that it has a minimal content of aromatics, sulfur, and heavy metals (FTA, 2007; Corporan et al., 2011) and therefore is less toxic. Toxic effects of Syntroleum fuel on humans have not been widely investigated; however, animal research suggests negative effects on pulmonary health after continuous exposure (Wong et al., 2009). Therefore, fast remediation of contaminated areas is essential. Syntroleum can be used in its pure form or as a blend with conventional diesel fuel without any mechanical modification to an existing diesel engine (FTA, 2007). Since Syntroleum use in cold climates is still in the experimental phase, its environmental effects and biodegradability potentials are less known. Syntroleum could be more degradable compared with conventional diesel fuel due to its higher content of straight aliphatics (C_{8-28}) (Marchal et al., 2003; Penet et al., 2004), which are typically more easily degradable than branched or cyclic hydrocarbons that are more abundant in conventional diesel fuel. Prior research of the present authors demonstrated a generally faster degradation of Syntroleum compared with diesel fuel (Horel and Schiewer, 2009).

Prior to 2004, Alaska generated 30 000 m^3 of fish oil per year as a byproduct of the Alaskan fish industry (Steigers et al., 2004), which increased up to 49 000 m^3 by 2007 (AEA, 2007). The type of fish biodiesel used in the current study was processed fish oil, where the raw fish oil was converted to biodiesel through the transesterification process by a commercial biodiesel plant in Hawaii (Witmer and Schmid, 2008). The proposed application of the fish biodiesel is use as heating fuel in rural Alaskan communities, mixed with high sulfur diesel fuel. Collection of raw fish oil is being organized from different parts of Alaska, from where it can be transported in large qualities (AEA, 2007). The fate of the fish oil and fish biodiesel needed to be investigated since its effects are relatively unknown in cold environments.

Previous exposure to hydrocarbons enhances the microbial communities' ability to degrade a substrate in the environment at a higher rate, compared with no prior exposure (Leahy and Colwell, 1990). That is because microbial communities previously exposed to hydrocarbons are already adapted to a specific carbon source and can respond to the presence of a contaminant in the field faster, even within hours (Atlas and Bartha, 1998). Soils with prior diesel pollution contain a diverse microbial consortium, which enables high hydrocarbon degradation (Vandecasteele, 2008). On the other hand, when cultivated bacterial cultures from a laboratory environment are applied, slow degradation might be observed due to competition for the substrate with indigenous species (Leahy and Colwell, 1990; Atlas and Bartha, 1998). The current authors propose that when bacterial cultures from native soil are used, laboratory results might be more applicable to the field. This study therefore investigated the degradation of different hydrocarbon fuels by naturally occurring microorganisms in Interior Alaskan soils. Prior research investigated the lag phases for a non-specific inoculum (Horel and Schiewer, 2009). One of the current study's main objectives was to determine if the lag phase or adaptation period can be considerably shortened by using naturally occurring microorganisms already adapted to a specific fuel type. Therefore, microorganisms adapted to specific substrates were introduced as inocula. To better assess the biodegradation effectiveness and estimate the time period required to achieve satisfactory contaminant removal, modeling of degradation rates was performed. Microbial growth phases were investigated, and degradation rate constants were determined.

2. Experimental section

2.1. Experimental setup

Hydrocarbon degradation was studied in small scale laboratory experiments as a function of time while varying the fuel (conventional diesel, Syntroleum, and processed fish biodiesel) and inoculum types. The experimental duration was 28 d. Abiotic conditions of the experiments were: a contaminant dosage of 2000 mg kg^{-1} dry soil, constant temperature of 20 °C, and nutrient dosage of 300 mg N kg^{-1} soil. For each experiment, 1 kg of soil (predominantly sand) was placed in an airtight 2.5 L container as previously described by Horel and Schiewer (2009). Fertilizer of the type 20–20–20 ($N–P_2O_5–K_2O$, where the total nitrogen ingredients were: 20% ammonia, 30% nitrate, and 50% urea nitrogen) was dissolved in water and added to the soil surface, achieving nutrient dosages of 300 mg N kg^{-1} dry soil, which ensured no nutrient limitation during the duration of the study in the soil matrices.

In the present experiments, soil from previous experiments containing microbial cultures already adapted to different fuel hydrocarbons was used as inoculum. This was done by adding a small amount of soil (2000 mg) with prior contamination of the respective fuel type to the mesocosms containing non-sterilized soil to simulate natural conditions. This enabled equitable comparison of different fuels under conditions (20 °C and 300 mg N kg^{-1} sand) determined to be favorable in earlier studies (Schiewer and Niemeyer, 2006; Horel and Schiewer, 2009).

The statistical analyses used to distinguish between contaminant types were two tailed, paired t-test and single factor ANOVA. The statistical significance in this analysis was defined at $p < 0.05$.

2.2. Soil analyses

The soil used in the experiments was not sterilized, and therefore contained naturally occurring microbial cultures that survive extreme temperature conditions (–50 to +30 °C) encountered in Interior Alaska. The purpose for using unsterilized soil was to further simulate natural degradation processes. Analysis for total nitrogen and total carbon using an elemental combustion system (Costech Instruments) showed that the average percent of total available nitrogen in the soil was 0.005% and the fraction of total carbon was 0.165%. The pH of the soil, which was analyzed using a Mettler Toledo pH meter at 22° C, was slightly above neutral, which is favorable for hydrocarbon degradation (Saadoun and Al-Ghzawi, 2005). The original water content before the start of the experiments was negligible, less than 1%, which was increased by addition of water and nutrient solution to achieve 4.8% overall gravimetric water content. Using ASTM C29 standard, the average soil bulk density was determined as 1.50 (±0.10) g cm^{-3} and the