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EVALUATION OF BIODEGRADATION OF DIESEL OIL BY FUNGI FROM SLUDGE

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Abstract

Microrganisms were isolated from diesel oil sludge, and their ability to degrade diesel oil was evaluated after growth for 30 and 60 days at 30ºC in mineral medium (modified BH) with diesel oil as sole source of carbon. The experiments were conducted with single species and with the consortium, *Aspergillus fumigatus*, *Hormoconis resinae* and *Candida silvicola*. Diesel fuel is a mixture of n-paraffins, branched paraffins, cyclic paraffins and aromatic hydrocarbons. *Aspergillus fumigatus* preferentially degraded aliphatic hydrocarbons of chain lengths C_{11}-C_{13}, producing 47,7%, 37,5% and 51% reductions in C_{11}, C_{12} and C_{13}, respectively. After 60 days, this fungus showed more degradation than the consortium. Probably the presence of other species in the consortium inhibited the growth of *Aspergillus fumigatus*, thus resulting in a lower rate of diesel fuel degradation.

Introduction

Since many naturally occuring microrganisms have the ability to utilize hydrocarbons as sole source of carbon and are widely distributed, the biodegradation of these natural compounds is common in nature (Richard & Vogel,1999). Some hydrocarbons like n-alkanes are easily degraded by a large number of microrganisms which use them as sources of carbon and energy. According Walker & Cooney (1975) monoterminal oxidation appears to be the major pathway of n-alkane oxidation; this involves conversion to the homologous primary alcohol, aldehyde and monoic acid, and the fatty acid is oxidized via β-oxidation rather than being incorporated directly into cell components. This process has been demonstrated for a number of bacteria and yeasts. Detailed biodegradation studies have demonstrated that some microbial populations will degrade both the saturated and aromatic hydrocarbon fractions contemporaneously. However, in addition to the structure of the compound , the composition of the hydrocarbon mixture also affects the degradability of individual components (Olson et al, 1999).

Diesel oil is a complex mixture of hydrocarbons, which varies according to the production process. Many publications describe the degradation of diesel oil by microorganisms (Wright & Ratledge,1988; MacCormack et al., 1998; Margesin & Schinner,1996; Richard & Vogel, 1999; Olson et al., 1999), but there have been few studies on the ability of fungi isolated in Brazil to degrade Brazilian grade diesel oil (Gaylarde et al,1998).

In this paper, we report the degradation activities of microrganisms isolated from sludge in diesel storage tanks in Brazil.

Materials & Methods

Microorganisms and Growth Conditions: Microorganisms (filamentous fungi, yeasts and bacteria) were isolated from diesel oil sludge. The microorganisms tested were *Aspergillus fumigatus, Hormoconis resinae* and *Candida silvicola*, in single and mixed cultures. The fungi were cultured on malt agar and incubated at 30° C for 7 days for filamentous fungi and 48 hours for yeasts. After the growth of filamentous fungi, sterile distilled water with 0.01% Tween was added to produce a spore suspension. The spores were counted in a Petroff-Hauser chamber. The yeast suspension (in distilled water) was counted by decimal dilution on spread plates.

Experimental Conditions: The experiments were carried out in 50 mL modified Bushnell-Haas mineral medium (BH* without chloride), at neutral pH (7.2), containing 5 ml diesel oil as the sole source of carbon. Five replicates were set up for each treatment. Diesel oil was sterilized through a 0.22 µm filter membrane and the mineral medium was autoclaved. The experiments were carried out with each microrganism separately and the mixed culture (consortium). Control samples were not inoculated and contained only diesel oil and mineral medium BH*.

Analytical Procedures: After incubation, the hydrocarbon layer was extracted with a separation funnel and to each 50 mL one mL of DCM (dichloro methane) was added .

Operating conditions for GC-MS analysis:

HP 5890II GC with HP5972 MS (Mass Selective Detector); Scan Mode: 35 to 450 AMU (atomic mass units); Column: SGE BPX5 25m x 0.2 mm, 0.25 µm film thickness, bonded phase capilary; Carrier gas: He @ 1 ml/min flow rate

Injector temp: 250°C, split injection @ 20:1. ; Injection size 1.0 µl; Type (MSD) Detector temp 280°C.; Oven program: initial temp 60°C, held for 3 min, then ramped at 7°C /min to 280°C and held for 10 min. Duration of the run: approximately 40 min.

The percentage degradation was calculated for each peak assigning 100% to C_{17}. We were not interested in quantifying peaks above C_{17} as they are resistant to biodegradation. The peak areas from the uninoculated flasks were compared with the peak areas from the inoculated flasks. Three samples were run and the values averaged.

RESULTS AND DISCUSSION

The main aim of the present work was to determine if diesel is degraded by yeasts and filamentous fungi isolated from storage tank sludge. The three fungi selected were able to degrade hydrocarbons, as shown in tables 1 and 2. The yeast *Candida silvicola* produced a low degree of degradation after 60 days in comparison with the other microrganisms, but secreted a bioemulsifier (Table 1) and formed emulsions in the oil phase, suggesting that it could, under the right conditions, increase the bioavailability of the hydrocarbons. Surface tension and pH at 60 days was reduced for all cultures, suggesting the production of surfactant and organic acids derived from hydrocarbon degradation. *Aspergillus fumigatus* produced the greatest reduction in pH and surface tension (Table 3), and was able to degrade hydrocarbons in BH* with diesel oil more efficiently than other microorganisms tested (Figure 1), while the fungus *Hormoconis resinae* showed low degradation activity at 30 days, but at 60 days the % degradation, mainly for C11, was highest 65.8% (Table 2). Reductions were observed in control values (not shown) due to abiotic processes like inorganic oxidation, transformation, sorption to glass walls, interactions with medium components and volatilization (Batts & Fathoni,1991; Margesin & Schinner, 1996). After 60 days incubation, substances above C_{17} were seen to be more resistant to biodegradation

(Figure 1). The accumulation of toxic products that takes place during the growth of microrganisms on fuels in closed systems may be responsible for the limited degradation of these hydrocarbons, but it is more likely that these components are naturally more recalcitrant.

Mixed populations meet the conditions of natural life where pure cultures do not. Microrganisms may complement each other with different metabolic activities, increasing their overall capabilities. The utilization of mixed microbial populations for bioremediation has already been reported by many authors (Wright & Ratledge,1988; MacCormack et al., 1998; Richard & Vogel, 1999). Using pure cultures in degradation experiments is far from representative, but in order to analyse the results such simplifications are necessary. Indeed it would be difficult to prepare a representative mixed culture since there would be so many interacting variables to consider. However the results presented in this work show that single microbial cultures may induce higher degradation than mixed cultures. The consortium did not efficiently reduce the level of any hydrocarbon detected in the diesel oil.

The results showed the high biodegradability of aliphatic and slow biodegradability of aromatic hydrocarbons. Above C_{17} the hydrocarbons appear to be resistant to degradation and the area under this peak does not vary significantly between samples (control diesel versus diesel inoculated with microorganisms). This suggests that after significant depletion of the alkane compounds, the microrganisms were unable to degrade the aromatic compounds.

These preliminary studies carried out in the laboratory indicate that the strains studied are able to grow on diesel oil and that these isolates could have a potential interest for the bioremediation of hydrocarbon polluted areas.

Conclusions

1. The microorganisms isolated from diesel oil storage tanks sludge were able to degrade the hydrocarbons of diesel oil.
2. *Aspergillus fumigatus* produced the greatest reduction in pH , surface tension and was able to degrade hydrocarbons in BH* with diesel oil more efficiently than other microorganisms tested, after 60 days.

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Table 1. Degradation of diesel oil after 30 days in mineral medium Bushnell-Haas with microrganisms (%).

Carbon chain length	*Aspergillus fumigatus*	*Hormoconis resinae*	*Candida silvicola*	Consortium
C_{11}	15.69	22.62	-5.32	14.18
C_{12}	31.32	2.80	24.33	9.12
C_{13}	42.10	6.16	26.61	-2.37
C_{14}	29.84	5.38	8.97	-3.26
C_{15}	18.28	3.69	12.68	-0.43
C_{16}	13	1.22	1.81	1.45

Table 2. Degradation of diesel oil after 60 days in mineral medium Bushnell-Haas with microrganisms (%).

Carbon chain length	*Aspergillus fumigatus*	*Hormoconis resinae*	*Candida silvicola*	Consortium
C_{11}	47.72	65.87	28.12	36.28
C_{12}	37.48	44.18	32.64	14.03
C_{13}	51.06	32.76	33.29	17.26
C_{14}	35.22	16.77	19.20	14.18
C_{15}	25	12.01	13.82	9.22
C_{16}	14.7	12.82	14.55	3.56

Table 3. pH and surface tension in aqueous phase after 60 days

Microorganisms	pH Initial: 7,0	Surface tension (mN/m) Initial: 74
Aspergillus fumigatus	4.8	48.5
Hormoconis resinae	6.4	52.5
Candida silvicola	6.6	60
Consortium	6.4	51
Control	6.9	70

Figure 1.Chromatogram shown degradation of diesel oil after 60 days of incubation with *Aspergillus fumigatus* , Control (uninoculated) and Consortium.