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Effects of Tween-80 on Bioremediation of Soil Contaminated with Resin and Asphalt

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Hydrocarbons (HC) of oil introduction into the soil environment can occur from pipeline blow-outs, road accidents, leaking of underground storage tanks, landfarming fields and uncontrolled landfilling. When released on the soil surface, HC adsorb on the organo-mineral matter (OMM) of the soil (Chaîneau et al. 2000). The self-remediation of a polluted environment is a very long process (Shtina and Nekrasova 1998). Recently, microbiological methods (bioremediation) are drawing more and more attention, especially as post-treatment or polishing steps, due to their economic attractiveness and ability to fulfill with the stringent legislation requirements concerning a permissible level of oil pollution (Murygina et al. 2000).

Until now, there are few reports on the bioremediation of soil contaminated with viscous-oil, because viscous-oil contains amount of resin and asphalt which have high molecular weight and complex structure, and these components are not available to microorganisms which make them the most difficult to be biodegraded. It is indispensable to make resin and asphalt more bioavailable by taking some measures to enhance the bioremediation. Lee et al. (1988) reported that if bioremediation was to proceed at meaningful rates in the soil, desorption of the target molecules would have to be enhanced to facilitate bioavailability and consequent biodegradation of the sorbed molecules.

Some studies showed that surfaceactive agents are thought to improve the stability of the emulsion; the surfactant desorbs a number of hydrophobic compounds sorbed to soil, for example, anthracene, phenanthrene, pyrene (Liu et al. 1991), and PCBs (McDermott et al. 1989). Therefore surfactants have been suggested as possible enhancers of desorption and possibly, biodegradation of PAHs and other organics in the soil environments (Sanseverino et al. 1994). The objective of this study is to investigate the effect of surfactant (Tween-80 was selected) in bioremediation of soil polluted by resin and asphalt of Liaohe oil field, and to observe the biodegradation activities of resin and asphalt by using fungi and actinomycetes screened from Liaohe oil fields.

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MATERIALS AND METHODS

Soil used in this experiment was collected from a farmland near Liaohe oil field in Liaoning Province, and sieved (2mm), some properties of the soil are presented in Table 1. Adding aether to viscous-oil collected from Liaohe oil field, after 12hr, resin and asphalt was obtained from the sediments.

Table 1. Physical and chemical properties of tested soil

samples	pH	TN %	TP %	CEC $mmol \cdot kg^{-1}$	OMM %	TPH %
Uncontaminated soil	9.0	0.18	0.05	8.46	1.80	0.11
Contaminated soil	7.4	0.14	0.04	12.40	3.30	1.26

The soil was steam sterilized and artificially contaminated by resin and asphalt (1%, w/w) before experiments. To provide a homogeneous distribution of resin and asphalt, 100g of dry, finely ground soil was treated with the resin and asphalt dissolved in 20ml chloroform. After evaporation of chloroform, the dry, contaminated soil was mixed with 400g (dry weight) soil. Fungi and actinomycetes used in this experiment were screened from soil contaminated by oil in Liaohe oil field. They are, fungi F4, F9902, F6, F2006, F2008, F9904, F2017; actinomycetes A2012, A2016, A2004, A2017, A2013. Autoclaved Tween-80 (purity 95%, Beijing) was selected for this experiment, 5g:500g (Tween-80:soil,w/w). Fungi were cultured in slants (Medium PDA) at 28 °C, after 72hrs, spores were washed down to flasks by asepsis water, then made into aqueous suspensions (5.5×10^5 cfu/ml). Actinomycetes were incubated in flasks with Gause I medium (without agar) in a shaking incubator at 150 rpm at 30 °C for 72hrs. Actinomycete spores were obtained by passing through filter papers (15cm diameter), then spores were washed into flasks by distilled water and counted by actinomycete plate count (APC) techniques (4.2×10^7 cfu/ml). Sterility checks were conducted in preparation of soil and cell cultures. 30ml suspensions were added into contaminated soils respectively and samples with or without inoculation were homogenized. Contaminated soils with or without Tween-80 were inoculated. Moisture was kept at approximately 30%; temperature range was 20-25 °C The control test was carried out without addition of resin and asphalt. Controls and all treatments (each with three replications) were analyzed every 14-days during 64days.The extraction and types analysis of hydrocarbons in contaminated soils were according to weight method by analytical balance with a detection limit of 0.1 mg (National EPA, 1986). Average recoveries from fortified samples were between 20.1% and 32.7%, and the relative standard deviations were from 3.2% to 4.1%. Data obtained were subjected to statistical analysis of variance (ANOVA) in the SPSS statistical package.

Rh (Rates of hydrocarbons in contaminated soils) = (TPH of different samples/dry weight of soil) ×100%

Biodegradation rates of strains o resin and asphalt in contaminated soils = (Rh of control-Rh of samples) ×100%

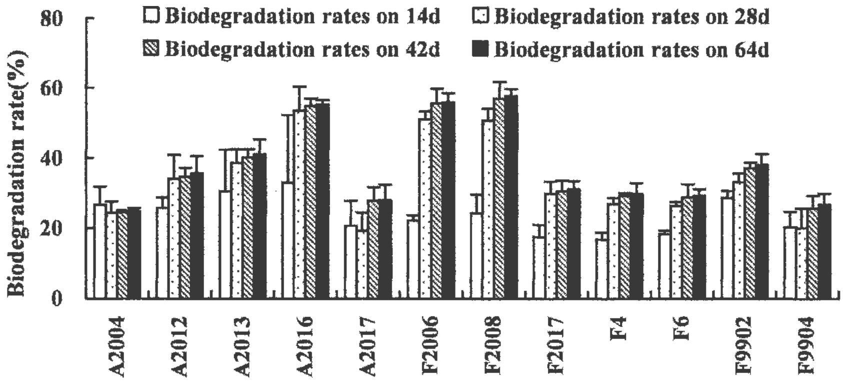

Figure 1. Comparison of biodegradation rates of different fungi and actinomycetes in treatments during 64days (T: Tween 80)

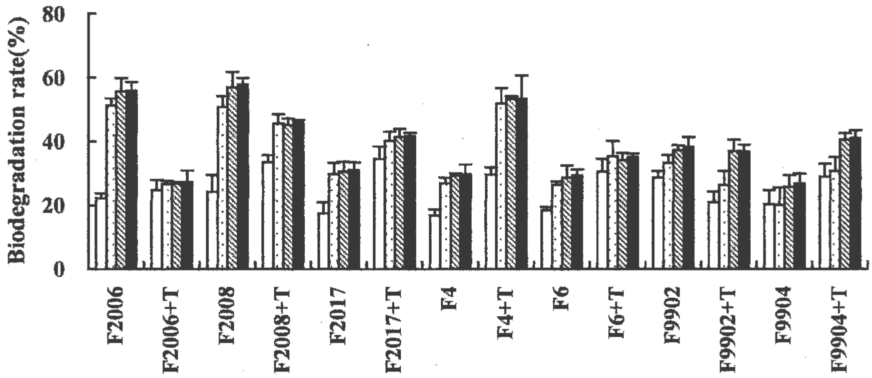

Figure 2. Comparison of biodegradation rates in treatments of utilizing fungi and utilizing fungi and Tween-80 during 64days (T: Tween 80)

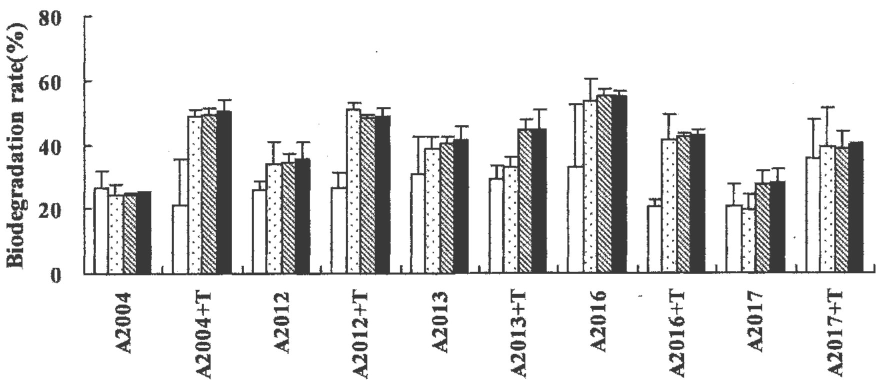

Figure 3. Comparison of biodegradation rates in treatments of utilizing actinomycetes and utilizing actinomycetes and Tween-80 during 64days (T: Tween 80)

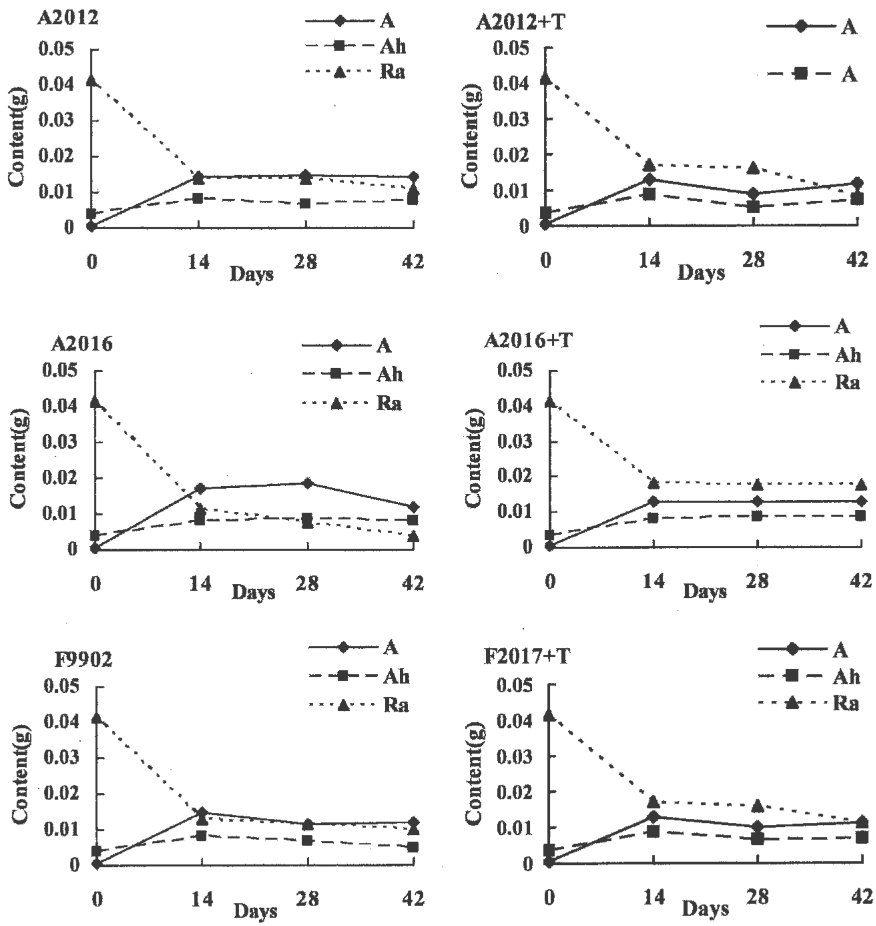

Figure 4. Types analysis of residual oil in soils after 42-days (T: Tween 80; A: Alkanes; Ah: Aromatic hydrocarbons; Ra: Resin and asphalt)

RESULTS AND DISCUSSION

After 64 days treatments, the degradation rates of resin and asphalt showed that fungi F2006, F2008 and actinomycete A2016 could degrade resin and asphalt more efficiently (55.97%, 57.89%, 55.30%, respectively) ($P < 0.05$) (Figure.1). Biodegradation rates varied in different fungi and actinomycetes. During 0-day to 14-days degradation rates in all treatments ranged from 16.9% to 33.01%; in the period of 14-days to 28-days, degradation rates of fungi F2006 and F2008, actinomycete A2016 increased obviously, while after 28-days biodegradation rates in all treatments began to slow down.

The comparison of treatments by fungi and fungi with Tween-80 was showed in Figure.2. After 64 days, in treatments with fungi and Tween-80, the most effective are F2008+T and F4+T (45.81%, 53.43%, respectively) ($P < 0.05$). But the surfactant Tween-80 showed different effect on fungi. Compared with the treatments without Tween-80, adding Tween-80 to treatments of F2017, F4, F6 and F9904 could significantly increase the biodegradation rate of pollutants in soil ($P < 0.05$), about 10.48%, 23.65%, 5.79% and 14.19%, respectively. While the biodegrading abilities of F2006+T and F2008+T were lower than F2008 and F2006 ($P < 0.05$); there were no obvious difference between treatments by F9904 and by F9904+T. According to Figure.3, Tween-80 could boost the biodegrading of resin and asphalt in treatments by A2004, A2012, A2013 and A2017, while in the treatment of A2016+T, Tween-80 inhibited the biodegradation ($P < 0.05$).

42-days later, soils treated by A2012, A2012+T, A2016, A2016+T, F9902, F2017+T were randomly selected to analyze. The results showed that the biodegradation of resin and asphalt varied with different species (Figure.4). In the first 14-days the content of resin and asphalt reduced greatly in these treatments, but contents of alkanes and aromatic hydrocarbons increased; in the period of 28-days to 42-days, decompounding of resin and asphalt in soils slowed down, which resulted that the contents of alkanes and aromatic hydrocarbons fluctuated little.

The biodegrading of resin and asphalt with complex structure is the key to bioremediate soils contaminated with viscous-oil in Liaohe oil field. The results of our study together with other researchers have shown that resin and asphalt can be biodegraded by utilizing fungi and actinomycetes which are screened from oil-contaminated soil of Liaohe oil field. However, biodegrading abilities of different microorganisms change a lot in different periods of bioremediation of resin and asphalt. Facundo et al. (2001) reported that the extent of hydrocarbon biodegradation in contaminated soils was critically dependent upon four factors, namely the creation of optimal environmental conditions to stimulate biodegradative activity, the predominant petroleum hydrocarbon types in the contaminated matrix and the bioavailability of the contaminants to microorganisms. During the bioremediation of resin and asphalt, the changes of environment conditions (such as nutrient, moister, pollutants and so forth) may make biodegradation rates of resin and asphalt decrease after 14-days. Meanwhile, the petroleum hydrocarbons degradation is affected by the molecular composition of the hydrocarbons, characteristic which is directly related with the bioavailability of these compounds, and as a consequence, the biodegradation rate may be altered (Huesemann 1995). In this experiment, resin and asphalt could be metabolized into kinds of alkanes and aromatic hydrocarbons or even CO_2 and H_2O by fungi and actinomycetes, some alkanes and aromatic hydrocarbons might be much more toxic, which made the environment conditions not suitable for microorganisms or decreased the number of microorganisms that resulted biodegradation slowing down.

The effect of Tween-80 on the biodegradation of resin and asphalt was different in this experiment. The molecular structure of surfactants consists of a hydrophobic group which has little affinity for the aqueous phase and a hydrophilic group which is readily soluble in the aqueous phase (Atagana et al. 2003). For metabolism of a compound to proceed at an appreciable rate, the compound has to be dissolved in a medium (Guerin and Boyd 1992). Oberbremer et al. (1990) observed that surfactants could stimulate the degradation of a defined mixture of aliphatic and aromatic hydrocarbons in a soil suspension. Our results showed that Tween-80 could enhance the process of bioremediation by increasing the bioavailability of the resin and asphalt compounds to some fungi and actinomycetes. While Tween-80 can also inhibit the biodegrading abilities of some fungi and actinomycetes such as F2008, F2006 and A2016, it has been reported (Hughes et al. 1997) that the presence of non-ionic surfactants may decrease the rate of PAH degradation in soil.Tween-80 belongs to chemical dispersants which may have different effects on the colonizing organisms and may disperse the hydrocarbons differently. Cooney (1984) found that some chemical dispersants may enhance the oxidation of a particular hydrocarbon, while others will inhibit it. However, if there are no toxic effects on the degradative population, dispersion could accelerate microbial degradation (Atlas 1981).

In order to bioremediate soils contaminated with viscous-oil which has amount of resin and asphalt, suitable microorganisms can be applied, and measures should be taken during the bioremediation. Surfactant Tween-80 can enhance the biodegrading of resin and asphalt, but it must be considered to the variety of microorganisms. Further study is needed to uncover the effect of surfactants to biodegrading of resin and asphalt and to find microorganisms which have more effective biodegrading ability for bioremediation.

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