Bioremediation of crude oil polluted soil as affected by sewage-sludge

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Abstract
During the Persian Gulf war in 1991 around eight million barrels of crude oil were spilled into water. This caused a large volume of contaminated water has to move to land in particular, Khuzestan's soil. A real danger of oil pollution would be an environmental calamity, and therefore, reclamation practice on the spoil soil area to expel the oil is essential. Bioremediation methods will be surveyed. In this study the soil artificially polluted with 1% density of crude oil and sewage sludge were applied at 3 levels of 0, 50 and 100 ton/h equivalent to the field application. These soils were kept at 30°C and 60 percent of F.C. for 5 to 10 weeks. The soils were then analyzed for hydrocarbon-degrading, heterophic bacteria count and some other soil chemical properties. Oil degradation was measured by a Soxhlet extraction method, and gas chromatography. Data indicated that treatments caused the degradation of 45% to 60% of oil in contaminated soil. The results showed that the degrading heterotrophic bacteria population increased from 6×10^3 cfc/gr in control soil to 2×10^{10} cfc/gr in treated soil and C/N ratio decreased from 6 to less than 3. Gas chromatography results also showed a decrease in normal paraffin and isopernoids, like phytane and pristine.

Key Words
Bioremediation, heterotrophic bacteria, gas chromatography, isopernoids.

Introduction

In recent year there has been a high demand for crude oil as a primary source of energy in civilized societies. Its market value and consumption of its end products have caused a huge increase in production of crude oil. This further encouraged more refineries and photo chemical industries and the need to exploit and drill for more oil to some extent ignoring the consequence of such activities on the environment and human life. Bioremediation is a useful method for improving oil contaminated soils. In this method living microscopic creatures use the hydro carbonic materials as food and energy resource and then change them easily to non poisonous materials like water and carbon-dioxide.

The result of this process is to reduce the oil hydrocarbons in soil (Espinoza and Dendooven 2003). Bioremediation is intermediate between engineering and natural methods and involves human interference like agricultural practices (plowing, fertilizing and etc) in order to promote suitable living conditions for microscopic soil creatures and increases the degradation of soil pollutants. Also, use of palliative materials like applying organic matter with nitrogen and phosphorus and plowing in order to ventilate the soil in a better way are beneficial. This may help to increase the microbial activity by bacteria and fungi. Namkoong *et al.* (2002) in bioremediation of diesel oil contaminated soil found that mixing the polluted soil with sewage-sludge on a volume of 0.5:1 showed the highest palliative degradation. Gogoi *et al.* (2003) in bioremediation of crude oil polluted soil have observed that ventilation, using organic matters with nitrogen and phosphorus and microbes insemination has lead to the demolition of 75 percent of the contamination factors. Therefore, the aim of this research was to evaluate the effects of sewage sludge application as organic fertilizer on stimulating biological processes and crude-oil bioremediation oil polluted soil.

Methods

Uncontaminated soil was taken from field, air dried and passed through a 2mm sieve. Then, this soil was contaminated by crude oil at rate of 1% weight artificially. The crude oil which was delivered from well No. 69 of the Maroon oil field is paraffin oil (Figure 1). The soil samples were kept for two weeks then divided into 5 kg portions and stored in special containers. Dry sewage sludge was prepared from the south sewage refinery of Ahvaz and after crushing and passing through a 2 mm sieve was added to the soil at the rates of 0, 50, 100 ton/ha. The soil treatments were kept under controlled humidity 60 percent of F.C. and 30°C. In order to having aerobic soils conditions, they were stirred thoroughly twice a week for 5 to 10 weeks. At the end of this period the soil samples were removed, air dried and passed through a 2 mm sieve. The soils were analyzed for hydrocarbon-degrading, heterophic bacteria and some soil chemical properties. Oil remaining

was measured by a Soxhlet extraction method, and gas chromatography. Hydrocarbon-degrading heterotrophic bacteria were determined using a MPN method.

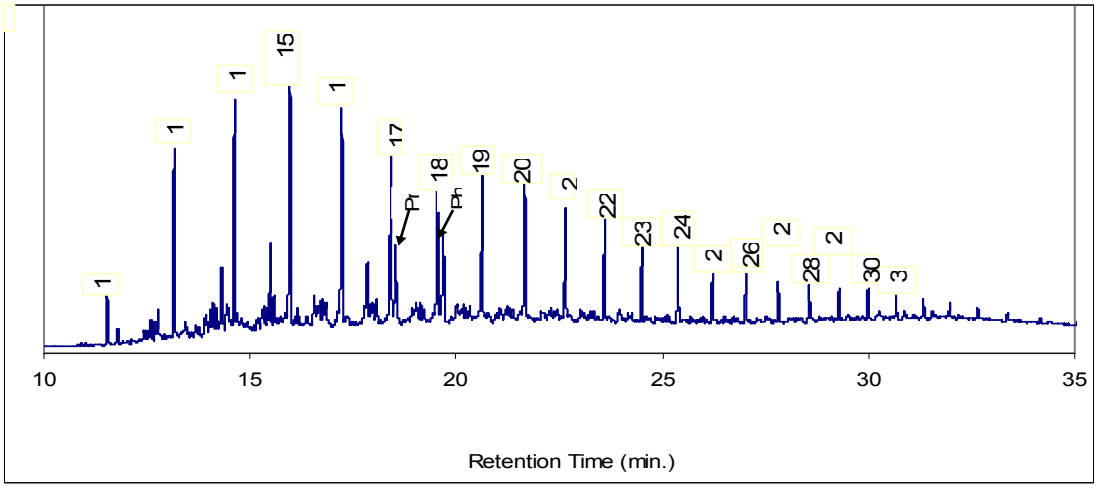

Figure 1. GC of crude oil sample from 69 well

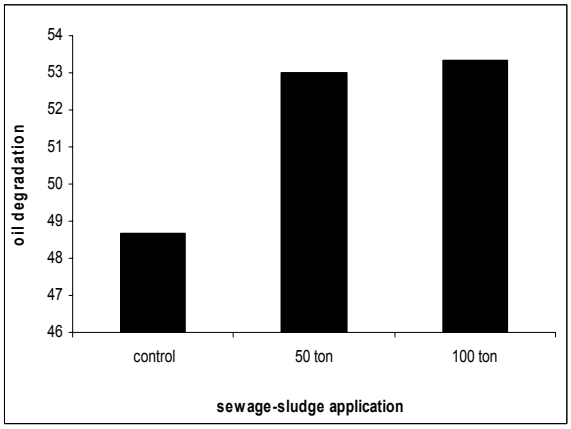

Figure 2. The effect of sewage-sludge treatments on oil degradation.

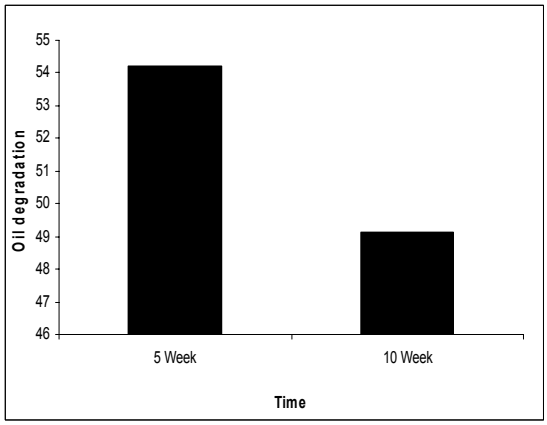

Figure 3. The effect of time on oil degradation.

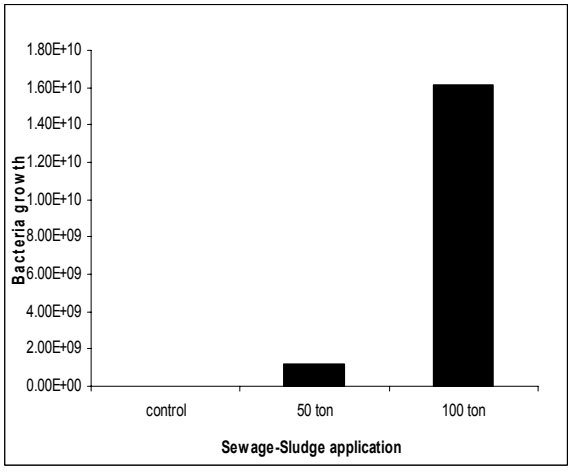

Figure 4. The effect of sewage-sludge treatments on bacteria growth.

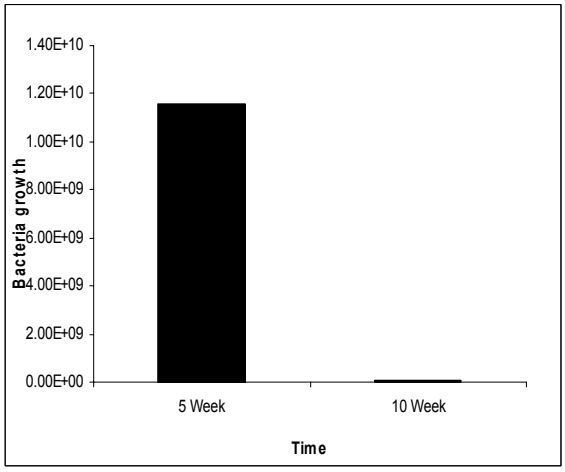

Figure 5. The effect of time on Bacteria growth.

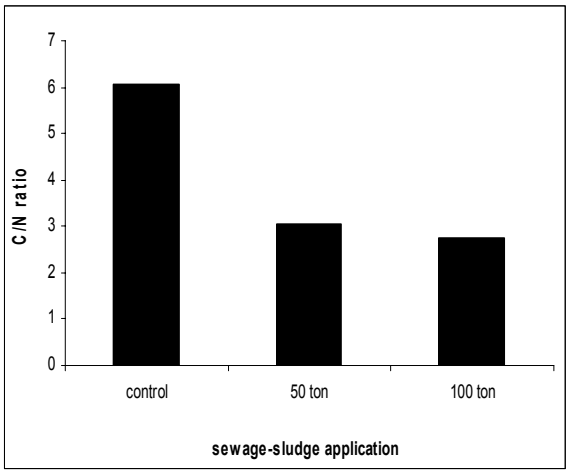

Figure 6. The effect of sewage-sludge treatments on C/N ratio.

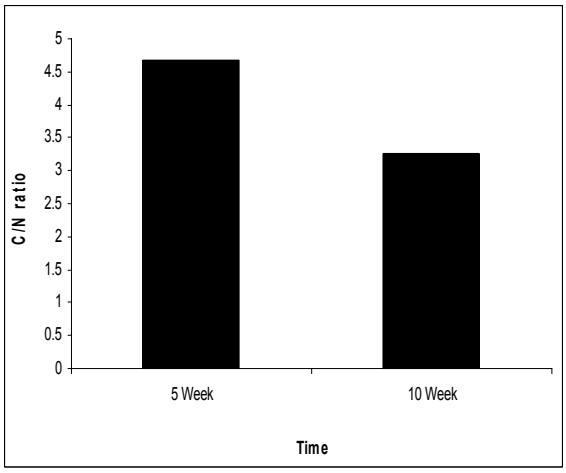

Figure7. The effect of time on C/N ratio.

Results

The results showed that addition of sewage sludge and soil ventilation caused an increase heterotrophic growth and petroleum degradation (Figures 2 and 3). The effect of the 100 ton/ha sewage-sludge application on petroleum degradation was more than 50 ton/ha. Under controlled environment when soil has more ventilation normal condition suitable for bacteria activity were observed (Figures 2 and 3). Espinosa and Dendooven (2003) showed that the added organic matter like sewage sludge to the polluted soil would quicken bioremediation of diesel oil and TPH because of interring a great deal of nutrition materials. The effect of time on petroleum degradation was significant and this observation corresponded with the bacteria growth results (Figures 4 and 5). The highest bacteria growth was achieved after 5 weeks, the presence of paraffin and organic nutrition materials, result in decomposing petroleum and enhanced bacteria activity to a maximum rate. The results showed a significant difference between the various treatment effects of sewage-sludge on soil C/N ratio. As is observed in Figure 6, C/N ratio of 6 in control samples changed to 3. C/N ratio initially decreased but by the fifth week the proportion was higher than the 10^{th} week (Figure 7), because in the fifth week there was suitable feeding materials available for bacteria, and with passing of time the level of organic matters appear to lower little by little and would limit bacteria growth and oil degradation. Though this procedure of organic matter sewage sludge would release and become feeding materials in the dissolved material that it is creating a suitable nutrition condition which cause the increment of oil degrader heterotrophic bacteria. Ramsy *et al.* (2000) observed that continual ventilation and fertilizer increment will have a considerable effect on the growth of hydro carbonic degrader bacteria in soil. In the present research during 5 weeks the presence of normal paraffin and environmental conditions with appropriate feeding, bacteria growth and oil degradation were high, but in 10 weeks aromatic and remaining asphalted component with no nutrition elements caused to decrease the bioremediation process (4). Lee *et al.* (2007) showed that fertilizing effect on biological stimulate of local bacteria soil and process, decreases with time. In order to facilitate the normal paraffin change survey, on the base of their chemical nearness combination they are divided in to 6 classes as follows: <C13, C13-C16, C17-C21, C22-C25, C26-C29, C30-C36. Paraffin's class C13-C16, C26-C29, C30 were much degraded by heterotrophic bacteria in comparison to control sample (Figures 8 and 9). Changes in the medium chain paraffin like treatment required 50 tones of sewage sludge showed about 50% decrease in comparison to control which expresses the low tendency of hydro carbonic degrader heterotrophic bacteria for the degradation of singular normal paraffin's in these paraffin's which resulted of their primary source. With passing of 10 weeks of sewage sludge application as organic fertilizer for microbial degradation, class C30-C36 had increased significantly from 2% to 62% that showed the microbes tendency for decomposing high chain normal paraffin's (Figure 9). Classes C17-C21, C22-C25, and C26-C29 hydrocarbon degraded 37%, 22% and 69% respectively.

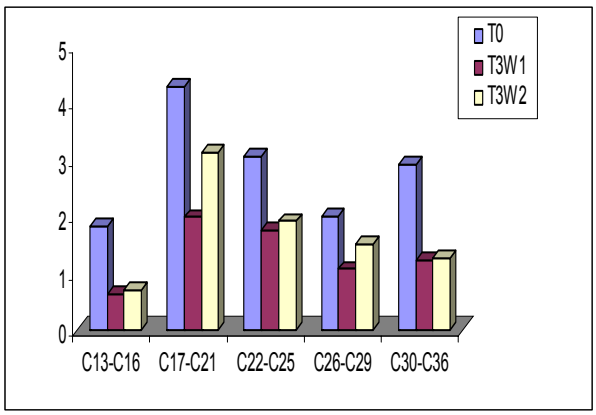

Figure 8. Comparison of percentage normal alkenes classes after 50 ton/ha Sewage-sludge application of 5/10 weeks as T3W1/T3W2.

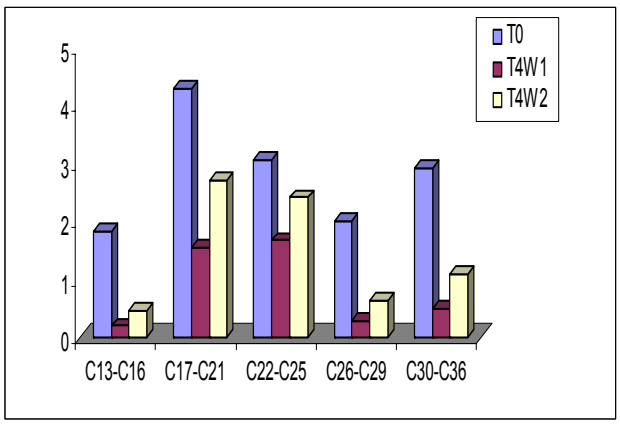

Figure 9. Comparison of percentage normal alkenes classes after 100 ton/ha Sewage-sludge application of 5/10 weeks as T4W1/T4W2.

Conclusions

- Sewage-sludge as a feeding source for soil micro organisms caused the degradation of about 45 to 60% of oil in a polluted soil.
- Hydrocarbon degraders of the heterotrophic bacteria population according to the applied nutrition treatments in this survey had a remarkable increase from 6×10^3 colony in unit/gr soil in the control sample increased to 2×10^{10} in sewage sludge treatments sample.
- C/N ratio 6 in control sample decreased to about 3 in sewage sludge treatments.
- Oil-degradation and microbe population increased in the treatments in the 5 week rather than the 10 week.

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