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Effect of remediation strategy on crude oil biodegradation kinetics and half life times in shoreline sediment samples

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ABSTRACT: Bioremediation, the process by which microorganisms degrade organic compounds to non-toxic or less toxic substances, has been widely used for cleanup of coastal ecosystems after oil spills. In this study, the hydrocarbon degradation rate and half lives in three bioremediation strategies (natural attenuation, biostimulation, and bioaugmentation) were compared in weathered crude oil (WCO) contaminated sediment samples. Three initial WCO concentrations of 3, 30 and 60 g WCO per kg sediment were investigated. Kinetic evaluations were carried out in a 90-day laboratory scale experiment. All oiled sediments showed decreasing WCO concentrations over time. After two weeks, the rate of degradation in the natural attenuation experiments stayed approximately constant. Bioaugmentation demonstrated higher oil removal than biostimulation or natural attenuation. The results indicated that first order kinetics can describe bioremediation of crude oil in sediments. The values of R^2 (coefficient of determination) varied from 0.9552 to 0.9965. The first order kinetic constant for the reactors at different WCO concentrations was between 0.0014 and 0.0159/day. The half life of WCO in sediment reactors was different for each applied method. The minimum WCO half life for natural attenuation, biostimulation and bioaugmentation was 408, 69 and 44 days respectively.

Keywords: *Biodegradation; Petroleum; Nutrients; Kinetic; Half-life*

INTRODUCTION

When an ecosystem is contaminated by petroleum and/or oil products, a catastrophic change occur in the physical and biogeochemical characteristics of the environment. Both physical and chemical treatments can be applied for soil cleanup, but they are often extremely expensive. Moreover, they are harmful to the environment, as they may damage the structure of the soil (Aghamiri *et al.*, 2011; Wang *et al.*, 2011; Couto *et al.*, 2010).

Biodegradation occurs at slow rate, but it can be enhanced by introducing nutrients and inoculating bacterial consortium that can degrade hydrocarbons in the contaminated environment. Bioremediation technologies have been successfully used to remove crude oil from contaminated sediments (Mohajeri *et al.*, 2011; Kumar *et al.*, 2011, Prince, 2010; Yousefi Kebria *et al.*, 2009). Through the bioremediation process, under appropriate conditions, contaminants are converted to harmless products such as water and carbon dioxide by using the different metabolic abilities of microorganisms. The rate and extent of bioremediation are affected by the nature of the contaminated environment and the interactions between microorganisms (Di Toro *et al.*, 2006; Margesin and Schinner, 2001).

Since studies on biodegradation kinetics are critical for understanding the bioremediation process, a number of studies have been published regarding the effects of environmental factors on the bioremediation kinetics of crude oil. The factors influencing bioremediation are described by the amount of substrate removed with time and the yield curve representing the transformation of the compounds by the microbial culture in the laboratory and sometimes in the field (Roncević *et al.*, 2005). Chen *et al.*, (2008) investigated the effects of several factors on the biodegradation kinetics of polycyclic aromatic hydrocarbons (PAH). The results showed that salinity and inoculum size were significant factors while PAH concentration, nutrient addition, and temperature were insignificant. Another study showed that pH, soil nitrogen content, and airflow greatly influenced the biodegradation of oil in soil (Pala *et al.*, 2006). The kinetics of oil biodegradation in soil was studied by Abioye *et al.*, (2010). The rate of oil degradation enhanced with bioavailability of

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the nutrients to bacteria in the oil-polluted soil. In addition, low pH affects the growth and biodegradation activities of hydrocarbon-utilizing bacteria in soil.

The purpose of this study was to investigate the biodegradation rate in three bioremediation strategies – natural attenuation, biostimulation and bioaugmentation – for coastal sediment samples contaminated with weathered crude oil (WCO). The influence of factors affecting degradation rates and biodegradation half-lives were evaluated for these three techniques.

MATERIALS AND METHODS

Sampling

The sediment samples for both the acclimatization phase and the main research were obtained from Butterworth Beach, Malaysia. Coastal sediment samples were collected from a depth of 20 cm. The sediment contained 19.4% sand, 57.7% silt, and 22.9% clay. The bacterial inoculums and details of the experimental set up have been explained before (Mohajeri *et al.,* 2010a).

Bioremediation test

Crude oil was obtained from the Shell Refining Company in Port Dickson, Malaysia. The characteristics of crude oil are presented in Table 1. The oil sample was artificially weathered and reduced by 25% (Mills *et al.,* 2004). Experiments were performed in 30 cm height and 15 cm diameter Plexiglas reactors, each containing 1 kg of sediment. A control reactor, similar to other reactors but without WCO, was sterilized at 105˚C and operated separately (Prasanna *et al.,* 2008). Three types of operations were tested viz. natural attenuation (ATT), biostimulation (STM) and bioaugmentation (AUG). The bioaugmentation reactors were augmented by 2.4 $\times 10^6$ cell mL^{-1} bacteria inoculums. Reactors were maintained at room temperature, kept moist, and manually mixed to enhance oxygenation. Three concentrations of oil (3 g, 30 g and 60 g) were selected for bioremediation treatments, and total petroleum hydrocarbons (TPHs) were measured on days 0, 7, 14, 28, 50, 70 and 90, after introduction of nutrients and inoculated bacteria. The C:N:P ratio was adjusted to 100:10:1. The specification of each method is summarized in Table 2.

Chemical analysis

The concentration of inorganic nutrients was determined with a HACH DR2000 spectrophotometer (Hach Company, Loveland, CO) and TPHs were measured by the US-EPA (1999) method. GC/FID (GC 2000 Series, Fisons Instruments, Milan, Italy) analysis was then used to confirm the results. The instrument's details are given by Mohajeri *et al.,* (2010a) and Zahed *et al.,* (2010). The average recovery for total hydrocarbons was 87.19 ± 12.31%. Method Detection Limit (MDL) was 5 mg/kg sediment.

Table 1: The characteristics of crude oil used in this study

NO.	Characteristics	Unit	Tapis	Bintulu	Miri Light	Sutu den
1	Density at 15˚C	kg/L	0.798	0.842	0.864	0.849
2	API	API gravity	45.8	36.5	32.3	35.1
3	Sulphur content	Wt%	0.03	0.06	0.08	0.05
4	Viscosity at 20 ˚C	Pa.s	3.51	4.08	4.69	73.6
5	Crude oil components	%	54	17	5	24

Table 2: The sampling days and conditions for the bioremediation of WCO

Strategy	Oil (g)	N (g)	P (g)	Inoculum (cell/mL)	Residual in different days (g)						
					0	7	14	28	50	70	90
ATT	3	-	-	-	3	2.89	2.84	2.78	2.71	2.61	2.54
STM	3	0.3	0.03	-	3	2.86	2.63	2.36	1.82	1.59	1.44
AUG	3	0.3	0.03	2.4×10^6	3	2.62	2.31	1.78	1.13	0.91	0.78
ATT	30	-	-	-	30	28.57	28.36	27.85	27.37	26.03	25.36
STM	30	3	0.3	-	30	28.48	26.72	23.70	19.94	14.50	12.49
AUG	30	3	0.3	2.4×10^6	30	26.91	23.83	18.55	11.60	8.88	7.87
ATT	60	-	-	-	60	58.06	57.06	56.14	54.95	53.00	52.20
STM	60	6	0.6	-	60	57.59	53.77	49.47	44.60	38.67	34.19
AUG	60	6	0.6	2.4×10^6	60	54.75	49.56	44.35	35.64	30.33	25.02
Control	-	-	-	-	-	-	-	-	-	-	-

RESULTS AND DISCUSSION

Bioremediation performance

Pollutant concentrations play a critical role in biotransformation processes. The degradation rate was investigated under different initial concentrations of WCO. The residual on each sampling day and conditions for the bioremediation of WCO are presented in Table 2.

All oiled sediments showed a trend of decreasing WCO concentrations over time. After two weeks, the rate of degradation by natural attenuation stayed approximately constant. No TPH was observed in the control reactor.

For all initial oil concentrations tested, the percentage WCO removal followed the order AUG > STM > ATT. Mahanty *et al.,* (2010) demonstrated that the bioavailability of petroleum hydrocarbons to microbes is one of the main factors affecting bioremediation. The bioremediation rate was considerably greater in the reactors supplemented with nutrient (biostimulation) or nutrient and bacteria (bioaugmentation) compared to the non nutrient supplemented oiled sediments (Table 2). The efficiency of biostimulation depends on contact between the added nutrients, bacteria consortia, and the oil.

As mentioned by Chang *et al.,* (2011), Barrios San Martín (2011), and Padayachee and Lin (2011) supplementation of nutrient is essential for enhancing bacterial growth and degradation activity. Hence, inoculation with population of degrading strains or an active strain of bacterial isolate is an option for increasing the biodegradation rate and reducing acclimation period, when the indigenous population of degraders is small.

The maximum percentage of WCO removal in the 90 day experiment was 15.3%, 52%, and 74% for ATT, STM, and AUG, respectively – each obtained when the initial oil concentration content was 3 g. There was a trend of decreasing percent of oil removal as the initial oil concentration was increased. High WCO concentrations increase the toxicity of oil, inhibit bacterial growth, and reduce the oil degradation rate.

Kinetic study

Kinetics modeling of bioremediation of contaminated soils is very complex due to the number of factors involved. First order kinetics (Equation 1) was used to describe petroleum hydrocarbons biodegradation (Mohajeri *et al.,* 2010 b; Adesodun and Mbagwu, 2008; Venosa and Holder, 2007):

$$C = C_0 e^{-kt} \tag{1}$$

Where C denotes the concentration of hydrocarbons (g/kg) at time t, t is the bioremediation time (days), C_0 is the initial concentration of hydrocarbons (g/kg), and k is the rate constant for the change in hydrocarbon content of the sediment (day^{-1}).

The experimental data are shown graphically in the linearized plots, Fig. 1(a-c). The model fits are based on different times and starting concentrations (3, 30 and 60 g) for each bioremediation process.

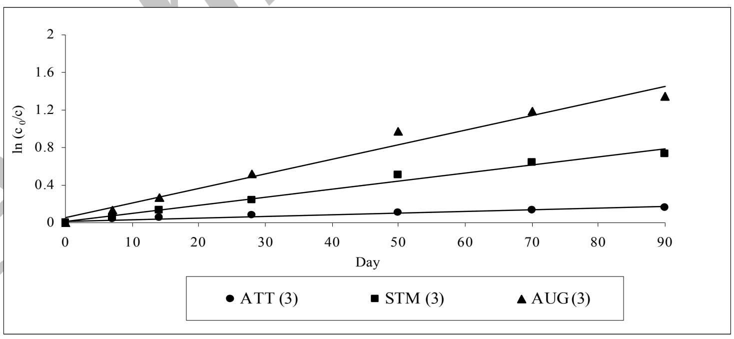

Fig. 1 (a)

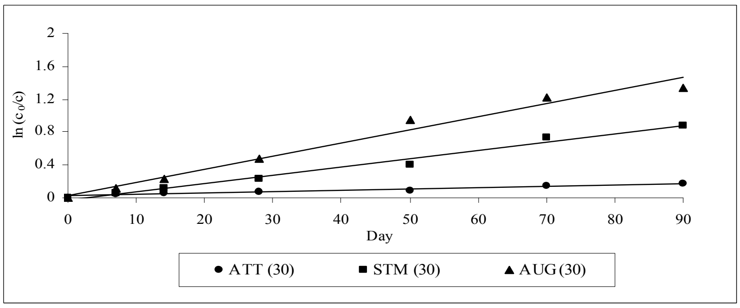

Fig. 1 (b)

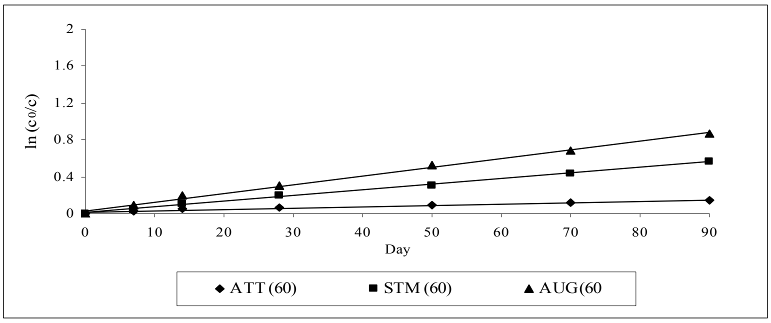

Fig. 1 (c)

Fig. 1: Kinetic evaluation of different remediation strategies for weathered crude oil concentrations of (a) 3g/kg, (b) 30g/kg, (c) 60g/kg.

Table 3: Kinetic equation and half life times for different remediation strategies and concentrations

No	Remediation strategy	Oil (g)	Kinetic equation*	R^2	$t_{1/2}$ (days)
1	ATT	3	Y_{ATT} = 0.0017x+0.0195	0.9692	408
2	STM	3	Y_{STM} = 0.0086x+ 0.0088	0.9860	81
3	AUG	3	Y_{AUG} = 0.0156x+0.0557	0.9767	44
4	ATT	30	Y_{ATT} = 0.0016x+ 0.0224	0.9461	433
5	STM	30	Y_{STM} = 0.0101x-0.0245	0.9884	69
6	AUG	30	Y_{AUG} = 0.0159x+0.029	0.9784	44
7	ATT	60	Y_{ATT} = 0.0014x+ 0.0189	0.9552	495
8	STM	60	Y_{STM} = 0.0061x+ 0.0072	0.9965	114
9	AUG	60	Y_{AUG} = 0.0095x+ 0.03	0.9964	73

*Y = $\ln(C_0/C)$, x = time (days)

Increases in the WCO removal with time are observed, in the sediment samples, at all oil concentrations tested. The results show that the experimental data of WCO degradation are in agreement with the first-order kinetic model, exhibiting an R^2 value of 0.9552 to 0.9965 (Table 3). This indicates that first-order kinetics is an appropriate model for WCO degradation. The equations listed in Table 3 show that the general trend of k values is to decrease with the increase in initial WCO concentration. The first order kinetic constants for the different reactors at different WCO concentrations are between 0.0014 and 0.0159/day.

Maletić *et al.,* (2009) studied biodegradation kinetics of an aged hydrocarbon-contaminated soil. They reported that the rate constant was in the range of 0.58×10^{-3} to 1.32×10^{-3} /day for mineral oil and total hydrocarbons, and was 6.7×10^{-3} to 8.8×10^{-3} /day for PAHs. The first order rate model also described the biodegradation of PAH's by both *Sphingomonas sp.* and the indigenous microorganisms in the sediment with R^2 values ranging from 0.69 to 0.99 (Chen *et al.,* 2008).

To decide whether a substance should be categorized as persistent, it is recommended to use the half-life as the principle standard. Substances with a half-life of more than 40 days are considered persistent (Dimitrov *et al.,* 2007). The half-life time ($t_{1/2}$) was calculated in accordance with Onwurah and Alumanah (2005) using Equation 2:

$$t_{1/2} = \ln(2)/k \qquad (2)$$

Half-life is defined as the time taken for half of the original amount of substrate to be converted. Half-life of WCO in sediment reactors, measured as described by Abioye *et al.,* (2010) is different for each applied treatment. The results indicate that half-lives in all reactors are more than 40 days. Therefore, WCO can be considered to be a persistent substance in sediment. Its biodegradation is enhanced by biostimulation and bioaugmentation strategies. The comparison of three strategies is summarized in Fig.2.

Couto and co-authors (2010) examined different bioremediation strategies for contaminated soil from a petroleum refinery. Among the treatments tested, only a combination of bioaugmentation with inorganic nutrients and surfactant amendments enhanced petroleum hydrocarbon degradation compared to natural attenuation. They suggested periodic revolving of the soil in order to expose the different layers of contaminated soil to the atmosphere as a cost effective way of reducing half-life for degradation of petroleum hydrocarbon.

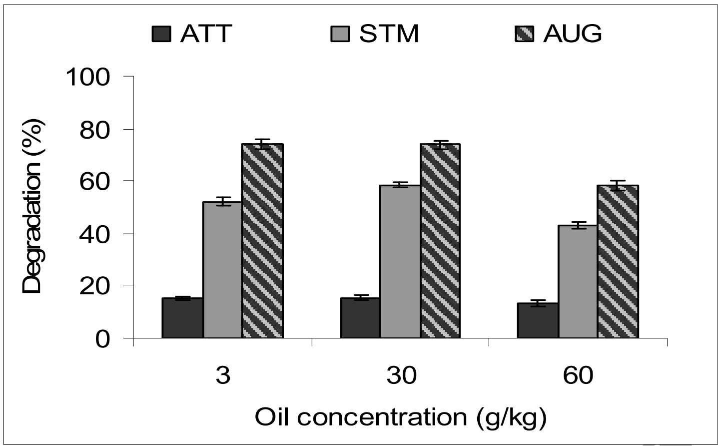

Fig. 2: Comparison degradation percentage in different remediation strategies

Adesodun and Mbagwu (2008) applied first-order kinetics in biodegradation of petroleum hydrocarbons. Since petroleum degradation is a natural process limited by temperature, pH and lack of nutrients such as N and P, a higher rate of total hydrocarbon reduction was observed with high organic wastes addition. Therefore, supplementary N and P (and also the C: N ratio) can affect the rate of total hydrocarbon bioremediation. Pala *et al.,* (2006) showed the influence of organic carbon content on microbial growth for a bioremediation process treatment. The amount of nitrogen was the variable that most affected the value of k in the bioremediation of a petroleum-contaminated sediment. High levels of nitrogen resulted in a low value of the kinetic constant and low organic carbon removal. However, Abbassi and Shquirat (2004) demonstrated that the value of the kinetic constant is greatly related to the microorganism concentration. An increase in microorganism concentration results in an increase in kinetic constant value.

CONCLUSION

Kinetic evaluation plays an important role for bioremediation understanding, modeling, and scale-up. The effect of weathered crude oil concentration on biodegradation kinetic behavior was investigated for different bioremediation strategies. The trends of rate constant and half-life variations with time decreased with the increase in initial WCO concentrations. The highest WCO removal observed for of natural attenuation, biostimulation and bioaugmentation was 15.5%, 58.4%, and 73.8% respectively. The half-life of WCO in sediment reactors was related to the treatments applied. A minimum half-life time of 44 days was obtained for WCO concentrations of 3 and 30 g/kg sediment using bioaugmentation technique.

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