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Full Length Research Paper

Biodegradation of used motor oil by single and mixed cultures of cyanobacteria

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This study was carried out to evaluate the potential of single and mixed cultures of *Nostoc hatei* and *Synechocystis aquatilis* in the biodegradation of 10% used motor oil. The rates of biodegradation of the oil were studied for a period of 21 days under laboratory conditions. Single cultures of *N. hatei* performed best in the biodegradation of the oil, showing dramatic reduction in total petroleum hydrocarbon with net loss of 13.0% within 14 days as compared to other treatments. First-order kinetic model revealed that *N. hatei* was the best microorganism in the biodegradation of used motor oil with biodegradation rate constant of 0.0667 day^{-1} and half-life of 10.39 days. The findings demonstrate the potential of cyanobacteria for oil bioremediation in the order: *N. hatei* > *N. hatei* + *S. aquatilis* > *S. aquatilis*.

Key words: *Nostoc hatei*, *Synechocystis aquatilis*, used motor oil, biodegradation.

INTRODUCTION

Motor oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents (Butler and Mason, 1997) that are used to lubricate the parts of an automobile engine, in order to keep the engine and its entire operation running smoothly (Hagwell et al., 1992). Used motor oil is a hazardous waste that contains more metals, and toxic and mutagenic polycyclic aromatic hydrocarbons (PAHs) that accumulate steadily with mileage because of direct leakage of fuel into the motor oil as well as the accumulation of incomplete combustion products (Keith and Telliard, 1979; Grimmer et al., 1981; Pruell and Quinn, 1988; Hagwell et al., 1992; Boonchan et al., 2000). Thailand is vulnerable to PAH contamination in aquatic environments due to a growing number of motor oil consumption with lack of proper treatment and disposal. Sewage effluents and urban runoffs with high concentrations of PAHs are due to the contamination by used motor oil (Tanecredi, 1977; MacKenzie and Hunter, 1979; Hoffman et al., 1980; Brown et al., 1985; Latimer et al., 1990) that leaks from automobile engines, dockyards and garages, including agricultural fields to street, and then gets washed from the street into the storm drain into lakes, rivers and streams. In addition, industrial discharge is a major source of toxic PAHs that contributes significantly to water contamination (Rehman et al., 2007).A number of innovative physical and chemical technologies such as soil washing, vapor extraction, encapsulation and solidification/stabilization are available to remediate hydrocarbon-contaminated environments. However, these methods are expensive and may only be partly effective. In addition, public pressures may restrict the field utilization of such intensive techniques (Dominguez-Rosado and Pichtel, 2004). Therefore, the utilization of microorganisms for bioremediation of hydrocarbon-contaminated environments through degrading and/or detoxifying organic contaminants is an alternative choice for bioremediation of hydrocarbon-contaminated environments as this means is an effective, economic, versatile and environmentally sound technology for treatment of hydrocarbon contaminants in the environments (Dominguez-Rosado and Pichtel, 2004; Singh and Lin, 2008).

This study reports on the biodegradation potential of single and mixed cultures of *N. Nostoc hatei* and

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Synechocystis aquatilis.

MATERIALS AND METHODS

Used motor oil was obtained from the garages in Muaeng Khonkaen, Thailand. Solvents were purchased from Lab-Scan, Gliwice, Poland.

The cyanobacterial strains *N. hatei* TISTR 8405 and *S. aquatilis* TISTR 8705 were obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. They were individually maintained in 50 ml BG-11 medium (BGM) in a 250 ml Erlenmeyer flask, and incubated at 28±1°C on a rotary shaker (120 rpm) in the light (light intensity of 3,000 lux) for 14 days before use. The BGM contained ($mg.l^{-1}$): $NaNO_3$ (1,500.0); $K_2HPO_4.3H_2O$ (40.0); $MgSO_4.7H_2O$ (75.0); $CaCl_2.2H_2O$ (36.0); citric acid (6.0); ferric ammonium citrate (6.0); ethylenediaminetetraacetic acid (EDTA (disodium magnesium)) (1.0); Na_2CO_3 (20.0); H_3BO_3 (2.86); $MnCl_2.4H_2O$ (1.81); $ZnSO_4.7H_2O$ (0.22); $Na_2MoO_4.2H_2O$ (0.39); $CuSO_4.5H_2O$ (0.079); and $CO(NO_3)_2.6H_2O$ (0.049). The pH of BGM was adjusted to 7.4 with 1 N HCl and 1 N NaOH prior to sterilization by autoclaving at 121°C for 20 min.

Biodegradation study

Experiments were performed using 250 ml Erlenmeyer flasks containing 0.25 g fresh weight of cyanobacteria suspended in 25 ml BGM amended with 10% used motor oil. Used motor oil biodegradation was compared among three treatments: single cultures of *N. hatei*, single cultures of *S. aquatilis* and mixed cultures of *N. hatei* and *S. aquatilis*.

All cultures were incubated at 28±1°C on a rotary shaker (120 rpm) with light intensity of 3,000 lux for 21 days. Before sampling, each flask was shaken vigorously to ensure mixing. The residues of used motor oil in each culture were determined on days seven, 14 and 21. All experiments were performed in triplicate.

Laboratory analysis

Hydrocarbon content

Hydrocarbon contents at seven, 14 and 21 days were determined gravimetrically by toluene cold extraction method as described by Adesodum and Mbagwu (2008) with a slight modification. To a 25 ml volume of each culture sample contained in a 250 ml flask, 25 ml of toluene (AnaLar grade) was added. After shaking for 30 min on a rotary shaker, the liquid phase of the extract was measured at 420 nm using spectrophotometer. The total petroleum hydrocarbons (TPHs) in each sample were estimated with reference to standard curve derived from fresh used motor oil diluted with toluene.

Cyanobacterial growth

The growth of cyanobacteria was determined in terms of biomass and chlorophyll a content. Biomass was quantified by dry weight analysis based on the method described by Chrzanowski et al. (2006) and Mona et al. (2011) with a slight modification. Briefly, flasks were withdrawn from a rotary shaker on days 7, 14 and 21 followed by centrifuging flask content at 8,000 g for 10 min. Supernatant was discarded and the biomass obtained was washed twice with 10 ml of *n*-hexane to remove used motor oil. The pellet was then oven dried at 80°C to constant weight.

Chlorophyll a content was determined by spectrophotometry according to the method described by Meeks and Castenholtz (1971). Chlorophyll a was extracted from the cells with 90% methanol. Absorbance was determined at 665 nm, and the chlorophyll a content was calculated with an extinction coefficient of 12.7 $\mu g.ml^{-1}$.

Data analysis

The used motor oil degradation data gotten from this study fit well with first-order kinetics: $S = S_0e^{-kt}$, $t_{1/2} = \ln 2/k$, where S_0 is the initial substrate concentration, S is the substrate concentration at time t, t is the time period, and k is the degradation rate constant. The percentage of the residues of used motor oil was calculated from the concentration of residual used motor oil divided by the initial concentration of used motor oil. Statistical significance was accepted at $p<0.05$. All results were analyzed by One-way ANOVA using the Statistical Package for Social Sciences v17.0 software (SPSS Inc. IL, USA).

RESULTS

Biodegradation of used motor oil

The level of biodegradation of used motor oil throughout the study period is shown in Figure 1. There was a dramatic reduction in TPHs within the first 14 days of the study in all treatments as compared to those of control. At the end of 14 days, TPHs were reduced by 62.3, 58.1 and 53.2% observed in single cultures of *N. hatei*, mixed cultures of *N. hatei* and *S. aquatilis* and single cultures of *S. aquatilis*, respectively.

At the end of 21 days, *N. hatei* performed best in the bioremediation of used motor oil, showing a sharp reduction in TPHs (77.9%), followed by the mixed cultures of *N. hatei* and *S. aquatilis* (73.7%), while *S. aquatilis* showed 72.1% reduction at the end of 21 days.

The effectiveness of each treatment was compared by calculating the net percentage loss of used motor oil in the medium. The highest net percentage loss was observed 14 days in single cultures of *N. hatei* (13%), followed by the mixed cultures of *N. hatei* and *S. aquatilis* (8.8%) and single cultures of *S. aquatilis* (6.9%), respectively (Table 1).

Biodegradation rate constant and half-life

First-order kinetics model of Yeung et al. (1997) was used to determine the rate of biodegradation of used motor oil in the three treatments. Table 2 shows the biodegradation constant (k) and half-life ($t_{1/2}$) for the different treatments on days seven, 14 and 21. At the end of seven days, single cultures of *N. hatei* exhibited high biodegradation rate of 0.0357 day^{-1} and half-life of 19.42 days, followed by mixed cultures of *N. hatei* and *S. aquatilis* performing the biodegradation rate of 0.0321 day^{-1} and half-life of 21.59 days, while single cultures of *S.*

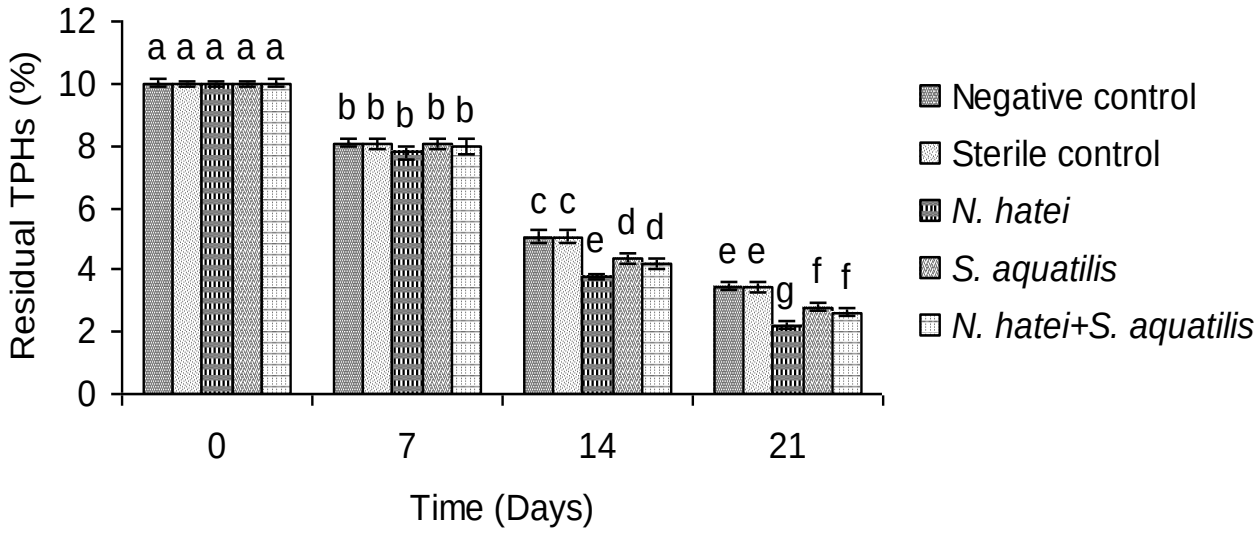

Figure 1. Residual TPHs in medium during bioremediation. Different letters indicate a significant difference (P<0.05) among the different treatments. TPH, Total petroleum hydrocarbons.

Table 1. Net percentage loss of used motor oil biodegraded by different types of cyanobacterial cultures.

Types of cyanobacterial culture	Net percentage loss (%)		
	Day 7	Day 14	Day 21
Single cultures of *N. hatei*	2.9±0.05^{a}	13±0.10^{a}	12.7±0.04^{a}
Single cultures of *S. aquatilis*	0.2±0.07^{c}	6.9±0.14^{c}	6.9±0.03^{c}
Mixed cultures of *N. hatei* and *S. aquatilis*	0.9±0.06^{b}	8.8±0.09^{b}	8.5±0.02^{b}

Net percentage loss = Percentage loss in TPHs of each treatment - percentage loss in TPHs of control. In each column, values followed by different letters (a, b or c) indicate significant difference at the P<0.05 level.

Table 2. Biodegradation rate and half-life of used motor oil biodegraded by different types of cyanobacterial cultures.

Types of cyanobacterial culture	Day 7		Day 14		Day 21	
	k (day^{-1})	$t_{1/2}$ (day)	k (day^{-1})	$t_{1/2}$ (day)	k (day^{-1})	$t_{1/2}$ (day)
Negative control	0.0205^{c}	33.81	0.0470^{c}	14.75	0.0479^{c}	14.47
Sterile control	0.0207^{c}	33.49	0.0473^{c}	14.65	0.0480^{c}	14.44
Single cultures of *N. hatei*	0.0357^{a}	19.42	0.0667^{a}	10.39	0.0674^{a}	10.28
Single cultures of *S. aquatilis*	0.0310^{b}	22.36	0.0568^{b}	12.20	0.0575^{b}	12.05
Mixed cultures of *N. hatei* and *S. aquatilis*	0.0321^{b}	21.59	0.0596^{b}	11.63	0.0600^{b}	11.55

In each column, values followed by different letters (a, b or c) indicate significant difference at the P<0.05 level.

aquatilis showed low biodegradation rate of 0.0310 day^{-1} and half-life of 22.36 days.

At the end of 14 days, there was a rapid increase in the biodegradation rate, resulting in a decrease in the half-life of used motor oil. The results reveal that single cultures of *N. hatei* performed best in the biodegradation of used motor oil, exhibiting the maximum biodegradation rate of 0.0667 day^{-1} and half-life of 10.39 days, followed by mixed cultures of *N. hatei* and *S. aquatilis* showing the biodegradation rate of 0.0596 day^{-1} and half-life of 11.63 days; whereas, single cultures of *S. aquatilis* had the biodegradation rate of 0.0568 day^{-1} and half-life of 12.20 days. This finding suggests that the length of time for the maximum biodegradation rate was 14 days. At the end of 21 days, there was a little elevation of the biodegradation rate; therefore, the half-life of the oil was slightly reduced. The findings hereby show that single cultures of *N. hatei* exhibited the biodegradation rate of 0.0674 day^{-1} and half-life of 10.28 days, whereas single cultures of *S. aquatilis* and mixed cultures of *N. hatei* and *S. aquatilis*

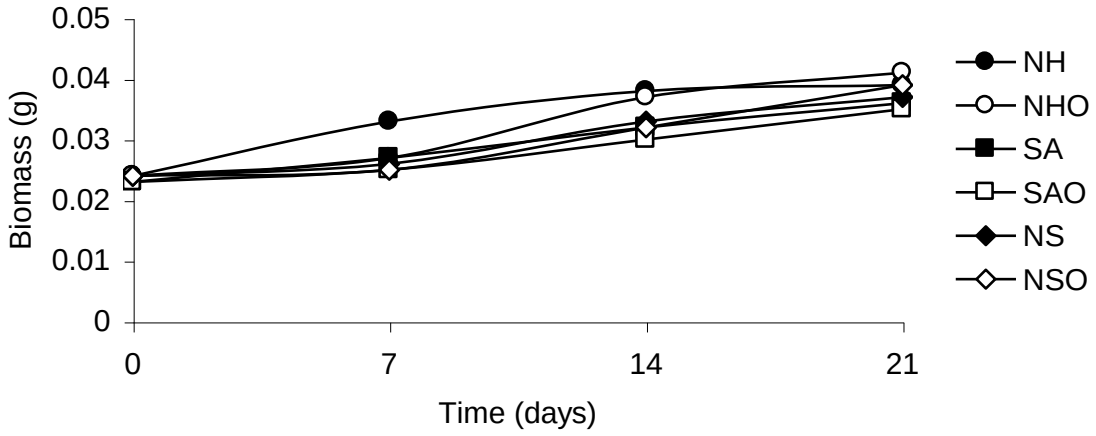

Figure 2. Biomass quantified by dry weight analysis during bioremediation; NH, *N. hatei*; NHO, *N. hatei* in used motor oil; SA, *S. aquatilis*; SAO, *S. aquatilis* in used motor oil; NS, *N. hatei* + *S. aquatilis;* NSO, *N. hatei* + *S. aquatilis* in used motor oil.

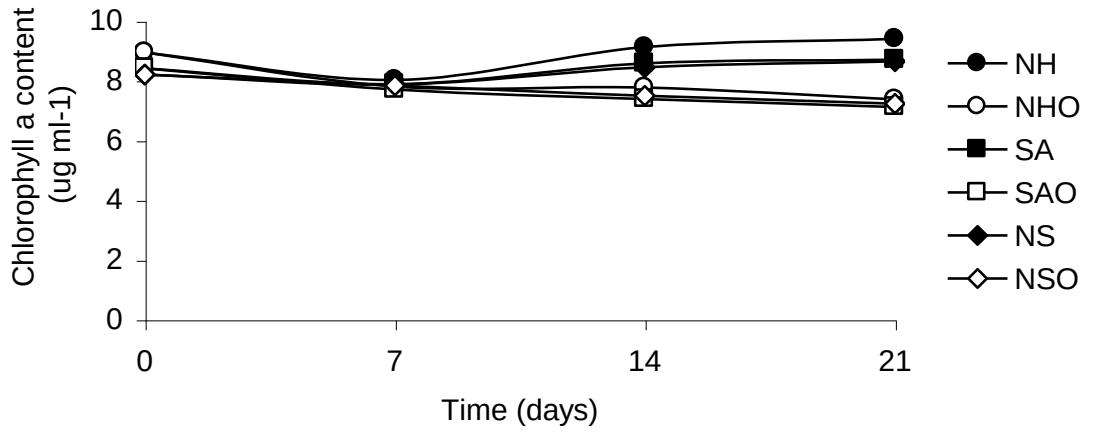

Figure 3. Chlorophyll a content during bioremediation; NH, *N. hatei*; NHO, *N. hatei* in used motor oil; SA, *S. aquatilis*; SAO, *S. aquatilis* in used motor oil; NS, *N. hatei* + *S. aquatilis*; NSO, *N. hatei* + *S. aquatilis* in used motor oil.

showed the biodegradation rates of 0.0575 and 0.0600 day^{-1}, and half-lives of 12.00 and 11.55 days, respectively. Based on the results, it was clearly shown that used motor oil can be naturally degraded by chemical and physical factors.

The biodegradation rates and half-lives of used motor oil amended in the medium in the absence of cyanobacteria were 0.0205 day^{-1} and 33.81 days at the end of seven days, 0.0470 day^{-1} and 14.75 days at the end of 14 days, and 0.0479 day^{-1} and 14.47 days at the end of 21 days, respectively.

Cyanobacterial growth

The growth of cyanobacteria under conditions of used motor oil contamination is shown in Figures 2 and 3. All treatments subjected to oil contamination showed that single and mixed cultures of *N. hatei* and *S. aquatilis* successfully withstood the toxicity of used motor oil, exhibiting a gradual increase in biomass throughout the study period. In such conditions, the biomass of single cultures of *N. hatei* ranged between 0.024 and 0.041 g, while that of single cultures of *S. aquatilis*, and mixed cultures of *N. hatei* and *S. aquatilis* ranged from 0.023 to 0.035 g and from 0.024 to 0.039 g, respectively. However, there was a decrease in chlorophyll a content in both single and mixed cultures of *N. hatei* and *S. aquatilis* exposed to used motor oil. The chlorophyll a content of single cultures of *N. hatei* dropped from 0.95 to 7.37 $\mu g.ml^{-1}$, whereas that of single cultures of *S. aquatilis*, and mixed cultures of *N. hatei* and *S. aquatilis* reduced from 8.41 to 7.12 $\mu g.ml^{-1}$ and from 8.20 to 7.23 $\mu g \cdot ml^{-1}$, respectively.

DISCUSSION

The results reveal that *N. hatei* and *S. aquatilis* were capable of withstanding the toxicity of used motor oil. These cyanobacteria successfully grew under this extreme condition, exhibiting an increase in biomass. However, the chlorophyll a content in cultures of cyanobacteria exposed to the oil was reduced. The reason for a reduction in chlorophyll a content may be because of an abundant carbon source as a source of energy from used motor oil; therefore, cyanobacteria did not carry out photosynthesis, thus leading to a decrease in chlorophyll a content.

In this study, the highest biodegradation rate of used motor oil was observed at the end of 14 days. Reduction of used motor oil was determined in terms of total petroleum hydrocarbon in this study rather than individual petroleum hydrocarbons because it is highly variable and has altered structure due to combustion process (Tauscher, 1988; Adesodun and Mbagwu, 2008).

The first-order kinetics was reported in many studies on petroleum hydrocarbons (Adesodun and Mbagwu, 2008; Abioye et al., 2009). The kinetics observed in this study suggested the maximum biodegradation rate of used motor oil degraded by single cultures of *N. hatei* at the end of 14 days. However, the biodegradation rate was not as high as that reported by other studies, which used bacteria isolated from oil-polluted sites to degrade the oil with varied concentrations in soil amended with organic wastes or animal droppings (Adesodun and Mbagwu, 2008; Abioye et al., 2009), inferring that the biodegradation rate depends upon microorganism species, oil concentrations, types of substrates and other amendments.In conclusion, the bioremediation method adopted in this study is simple and inexpensive. The biodegradation rate and half-life parameters obtained from the study demonstrated that the bioremediation of the oil depends on a variety of factors, such as concentration of the oil used, time and cyanobacterial species, as well as types of cyanobacterial cultures.

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