The 6th International Symposium on "Novel Carbon Resource Scienes"

Aiming toward Low-Carbon Society

November 12-13, 2010, Fukuoka, Japan

Venue: Chikushi Campus, Kyushu University

ABSTRACTS

ORGANIZED BY

Kyushu University GCOE program: Novel Carbon Resource Sciences

The 6th International Symposium on Novel Carbon Resource Sciences

Aiming toward Low-Carbon Society

November 12-13, 2010
Kyushu University
Fukuoka, Japan

http://ncrs.cm.kyushu-u.ac.jp

九州大学グローバルCOE
新炭素資源学
—— 石炭エコイノベーション
NOVEL CARBON RESOURCE SCIENCES : Coal-Based Eco-Innovations

Contents

Preface

On behalf of symposium chairs, I would like to express hearty welcome and thanks to all those who have gathered at Chikushi Campus of Kyushu University for the 6th International Symposium on Novel Carbon Resource Sciences.

Kyushu University and Fukuoka Women's University have established a Global Centre of Excellence (G-COE) in Novel Carbon Resource Sciences (NCRS) focusing on Coal-based Eco Innovations. The COE is financially supported by Ministry of Education, Culture, Sports, Science and Technology, Japan. It has started in July 2008 and will run for five years. The principal concerns of the NCRS G-COE are the development of new scientific frameworks and technologies in the processing and utilization of coal and other carbon resources. The NCRS G-COE tackles the problems of energy security and environmental protection in global priority by trans-disciplinary collaboration over materials, processes, as well as environmental and economic analyses. The aim of NCRS unquestionably contributes to the realization of low-carbon society.

The NCRS International Symposium aims to provide opportunities of exchanging and deepening ideas about NCRS through presentation and discussion as well as of cementing mutual understanding among partner organizations. The 1st Symposium was held in Fukuoka on October, 2008 as a kickoff of NCRS G-COE activity which was followed by the 2nd (March, 2009; Bandung, Indonesia), 3rd (November 2009, Fukuoka, Japan), 4th (December 2009, Shanghai, China) and 5th (April, 2010, Perth, Australia) Symposiums. The 6th Symposium is organized as a continuation of successful predecessor symposiums with the sub-title of "Aiming toward Low-Carbon Society". The symposium covers all aspects of novel carbon resource sciences, and the scientific program includes keynote lectures, invited lectures and contributed oral and poster presentations. In addition to scientific general sessions, we have planned two special sessions; "NCRS session" and "Student Session". Since the year of 2010 is the midterm of the project (2008-2012), it must be a good opportunity to evaluate the past activity and to exchange ideas of the future course of NCRS G-COE. The Student Session will be operated by G-COE students including the selection of the discussion subject. This is the first attempt in a series of the NCRS symposium. Symposium chairs believe that the Student Session must be of significance in G-COE activity, because G-COE is an education program to develop human resources to play active parts globally. All the participants are welcome to join actively in both general and special sessions.

Symposium chairs gratefully thank all participants for their contribution as well as students and staffs in Kyushu University to make the symposium possible.

Yasutake Teraoka

On behalf of Symposium Chairs

The 6th International Symposium on Novel Carbon Resource Sciences

Host Organization

Kyushu University GCOE program: Novel Carbon Resource Sciences

6-1 Kasuga Koen, Kasuga 816-8580, Japan

Tel. +81 92 583 7616, fax. +81 92 583 7619, e-mail. ncrs@cm.kyushu-u.ac.jp

Symposium Chairs

Prof Hideo Nagashima
Director, Institute for Materials Chemistry and Engineering, Kyushu University, Japan

Prof Yasutake Teraoka
Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Japan

Prof Akira Harata
Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Japan

A/Prof Michitaka Ohtaki
Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Japan

International Committee

Prof Doo-hwan Jung
Korea Institute of Energy Research, Korea

Prof Yanqing Wu
Shanghai Jiao Tong University, China

Prof Sudarto Notosiswoyo
Institut Teknologi Bandung, Indonesia

Dr Nitin Labhsetwar
National Environmental Engineering Research Institute, India

Prof Chun-Zhu Li
Curtin University of Technology, Australia

Organising Committee

Prof Hideo Nagashima
Director, Institute for Materials Chemistry and Engineering, Kyushu University, Japan

Prof Yasutake Teraoka
Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Japan

Prof Tsuyoshi Hirajima
Graduate School of Engineering, Kyushu University, Japan

Prof Kyuro Sasaki
Graduate School of Engineering, Kyushu University, Japan

Prof Seong-Ho Yoon
Institute for Materials Chemistry and Engineering, Kyushu University, Japan

Prof Katsuhiko Tomooka
Institute for Materials Chemistry and Engineering, Kyushu University, Japan

Prof Shiyoshi Yokoyama
Institute for Materials Chemistry and Engineering, Kyushu University, Japan

Prof Jun Fukai
Graduate School of Engineering, Kyushu University, Japan

Prof Norio Miura
Art, Science and Technology Center for Cooperative Research, Kyushu University, Japan

Prof Akira Harata
Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Japan

Prof Kikuo Matsui
Graduate School of Engineering, Kyushu University, Japan

Prof Kazuhide Ito
Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Japan

Prof Itsushi Uno
Research Institute for Applied Mechanics, Kyushu University, Japan

Prof Masaki Minemoto
Graduate School of Engineering, Kyushu University, Japan

Prof Katsuki Kusakabe
Faculty of Human Environmental Science, Fukuoka Women's University, Japan

Prof Jun-ichiro Hayashi
Institute for Materials Chemistry and Engineering, Kyushu University, Japan

A/Prof Michitaka Ohtaki
Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, Japan

A/Prof Shigeto Okada
Institute for Materials Chemistry and Engineering, Kyushu University, Japan

A/Prof Hiroki Ago
Institute for Materials Chemistry and Engineering, Kyushu University, Japan

A/Prof Toshihiko Takemura
Research Institute for Applied Mechanics, Kyushu University, Japan

A/Prof Masahiko Nakamura
Research Institute for Applied Mechanics, Kyushu University, Japan

A/Prof Toshiyuki Fujita
Graduate School of Economics, Kyushu University, Japan

Symposium Office

Miwa Hirashima

Yoshiko Kano

Yumiko Kodama

Masayoshi Makino

Eri Matsuo

Minako Matsue

Mina Nagato

Mami Naritomi

Junko Nose

Mari Wada

Keynote Speakers

Prof Yong-Gun Shul

Department of Chemical and Biomolecular Engineering
Yonsei University
Seoul 120-749, Korea

Dr Nitin Labhsetwar

Environmental Materials Division
National Environmental Engineering Research Institute (NEERI-CSIR)
Nagpur -440020, India

Invited Speakers

Dr Agus Jatnika Effendi

Faculty of Civil & Environmental Engineering
Institut Teknologi Bandung (ITB)
Bandung 40132, Indonesia

Dr Shan Wang

Curtin Centre for Advanced Energy Science and Engineering
Curtin University of Technology
Perth, WA 6845, Australia

Dr Ping Liu

School of Environmental Science and Engineering
Shanghai Jiao Tong University
Shanghai 200240, P. R, China

Dr Seongyop Lim

Fuel Cell Research Center
Korea Institute of Energy Research (KIER)
Deajeon 305-343, Korea

Dr Suresh Kumar

Environmental Materials Unit
National Environmental Engineering Research Institute (NEERI-CSIR)
Nagpur-440020, India

General Information

Social Program

Symposium Dinner

Date: Friday 12 November 2010

Time: 19:00 – 21:00

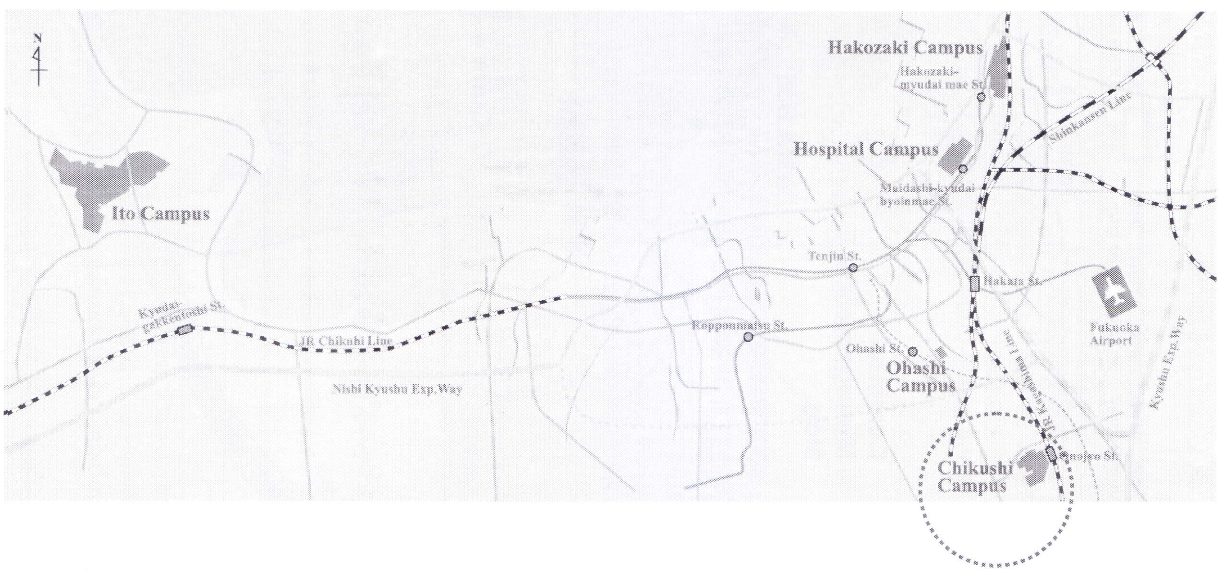
Venue: Ozen-ya,
2-5-19 Hakataeki Higashi, Hakata, Fukuoka 812-0013, Japan

Symposium Venue

Chikushi Campus, Kyushu University

6-1, Kasuga Koen, Kasuga, 816-8580, Japan

Location Map



Oral Session

Improvement of Oil-Contaminated Soil Bioremediation by the Addition of Bio-surfactant Producing Bacteria Varied with Bulking Agents

Agus Jatnika Effendi[1] and Yodi Ilyas[2]
1) Water & Waste Research Group, Faculty of Civil & Environmental Engineering, ITB
2) Inter University Centre on Biotechnology, ITB

This paper was presented in
6Th International Symposium on Novel Carbon Resource Science
Fukuoka, Japan, 12th-13th November 2010
As Invited Speaker

Abstract
The isolation of bacteria capable of degrading oil as carbon source showed that bacteria isolated from treated soil had the best ability to degrade Total Petroleum Hydrocarbon (TPH). The value of Ks (0.010%) of these bacteria that was relatively small with a high growth rate, μ_{max} (0,022/hour). In the bioreactor where bio-surfactant-producing bacteria were added, to reach concentrations below 1% could be achieved with a relatively faster when compared with other variations. This indicated that the emulsification process of TPH gave a significant influence on the biodegradation process conducted by the isolated bacteria. To reach the concentration of 1%, which is a compliance point of bioremediation processes based on current regulations, it took less than a month. The result was better than reactors in which bioremediation processes only relied on isolated bacteria. Although the time required to reach 1% was longer, it indicated that the isolated bacteria had a strong ability to degrade TPH. The time required to reach 1% TPH concentration was less than 1.5 months. Another interesting outcome was that the addition of bulking agent to give a fairly significant influence on the bioremediation process. Result showed that the biodegradation process of TPH in the addition of palm shells bulking agent gave better results when compared with shredded grass was added as bulking agent. GC-MS analysis showed that the heavy fraction of TPH (C20-C25) were converted to medium fraction (around C18) within 0.5 month and then still left a small quantity of C12-C15 after running for 2 months.
Key Words: TPH, bio-surfactant, bioremediation, bio-augmentation

Introduction

Treating oil-contaminated soil using biological process (bioremediation) is basically a matter of biological process application in solid phase. The limitation of such process in most cases are caused by poor mass transfer and poor contact between substrate (pollutant) and biomass (bacteria). Not only because of poor mixing, but also a low concentration of biomass in the system resulted in a very slow process. It is common that bacterial population capable of degrading specific pollutant in the environment very low in concentration. From this point of view there is always a chance to enhance the speed of biological process that is by increasing the concentration of biomass. Increasing biomass in-situ may be possible by adding second more biodegradable substrate and other nutrients. However the effect of this technique may not be greater than adding bulk biomass directly. Some previous studies have successfully isolated local bacteria capable of degrading oil. This bacteria has been tested and posses a high activity in degrading crude oil. With this kind of bacteria, it will be even easier to increase biomass concentration in oil-contaminated soil site simply by producing these bacteria and inoculated back in to site (in this case, bioremediation area). It is also well known that the bioremediation process of oil-contaminated soil produced from various activities of mining and oil industry, is a time-consumed process. Some evident showed that it took more than 8 months to reach the end point criteria (1% v/v) that regulated by Indonesian Government. The addition of bio-augmentation bacteria and bio-emulsifier to the bioremediation process is expected not only to reduce the time of process but also economically and environmentally approved when discharged back to the original sites or used for others purpose.

Materials & Methods

- *Isolation of Petrophylic Bacteria*

The process of isolation of bacteria capable of using crude oil as carbon source was performed by taking inoculum sources originating from three sources, namely from the soil types existing in the storage (storage), soil to be treated and processed soil bioremediation processes. The following figure shows the process of direct isolation conducted on site. After several times being sub-cultured in the solid media that use crude oil as carbon sources, there were three dominant bacteria or bacterial consortia grown from each of the above inoculum source as in the picture below. Individually these bacteria showed their capability to growth on media containing petroleum hydrocarbon. It indicated that they use petroleum hydrocarbon as carbon source for growth. In order to determine its capability to use crude oil as carbon source, the isolated bacteria should also be maintained in liquid culture containing crude oil as its substrate. The following picture shows the process of bacteria maintenance in liquid phase.

- *Growth Kinetic Determination*

The aim of growth kinetic experiments is to determine the ability of isolated microorganisms in the degradation of petroleum hydrocarbon. The kinetics parameters to be determined are as follows:

- Specific Growth Rate, μ, 1/time
- Maximum Specific Growth Rate, μ_{max}, 1/time
- Half Saturation Constant, K_s, % (v/v)

Specific growth rate, μ, was determined by growing the isolated bacteria in various Total Petroleum Hydrocarbon (TPH) concentrations. The growth of bacteria was observed on daily basis in order to determine the growth pattern. The TPH concentration from each sources were significantly different. Thus, variations in the TPH concentration added to the culture would be different depending on the source of the bacteria isolated.

- *Performance of Isolated Bacteria*

To determine the performance of isolated bacteria in degrading TPH, oil-contaminated soil was taken from an oil company's bioremediation site. The soil was then used as the target of this treatability test. The mix culture of isolated bacteria was used in this test. Laboratory scale of reactor for windrow technique has been developed and set up. There were five reactor conditions employed in this test. They were:

- Control reactor; indigenous bacteria (**A**)
- Reactor with the addition of Isolated Bacteria + Blotong Bulking Agent (**B**)
- Reactor running with the addition of Isolated Bacteria + Blotong Bulking Agent + Bio-surfactant (**C**)
- Reactor with the addition of Isolated Bacteria + Shredded Grass Bulking Agent (**D**)
- Reactor with the addition of Isolated Bacteria + Shredded Grass Bulking Agent + Bio-surfactant (**E**)

Result & Discussion

Maximum specific growth rate of isolated bacteria was determined in order to show the maximum rate of isolated bacteria in degrading TPH. This maximum rate could also indicate the time required to degrade TPH up to certain degree. The other kinetic parameter, half saturation constant, was observed in order to determine the affinity of the isolated bacteria to TPH as shown in the following table.

Table 1. The Maximum Specific Growth Rate & Half Saturation Constant of Isolated Bacteria

Isolates Source	μ_{max}, 1/hour	Ks, %
Storage	0,035	0,122
Un-treated Soil	0,018	0,010
Treated Soil	0,022	0,010

Based on the above results, it showed that bacteria that had the best ability to degrade TPH were bacteria that came from treated soil. It can be seen from the value of Ks of these bacteria that was relatively small with a large growth rate when compared with other isolates. These results were in accordance with the initial hypothesis in which the bacteria were grown in the treated soil had a long adaptation phase to allow the ability to use TPH as a carbon source for growth. Bacteria that were isolated from soil storage had a relatively high growth rate when compared with other isolates. However, to grow well, these isolates required TPH in a relatively high concentration when compared with other isolates. Thus, this bacterium had relatively a low affinity to TPH. The bacteria that isolated from un-treated soil seemed to have a long phase of adaptation. Therefore, when compared with bacteria from treated soil, the growth rate of these bacteria was slightly lower.

Bulking agents used in the developed laboratory scale windrows were Blotong (palm shells) and shredded grass with the composition of oil-contaminated soil : bulking agent : bacteria was 100:10:1. To the reactor C and E, bacteria *Azotobacter sp* which is isolated from other research, was added. In certain conditions, these bacteria have the ability to produce a kind of surfactant that is known as a bio-surfactant. Thus, with the addition of these bacteria occurred TPH emulsification process. Emulsified TPH will be easier taken by petrophylic bacteria because of the size of oil droplets are much smaller than before the addition of *Azotobacter sp*. Having been run for more than 3 months, it was clear that the isolated bacteria have capability to use TPH as carbon source. It could also indicate that the oil-contaminated soil used was treatable using bioremediation technique. Furthermore, the addition of bio-surfactant produced bacteria affected the biodegradation of TPH by the isolated bacteria. The following figure shows the performance of each reactor.

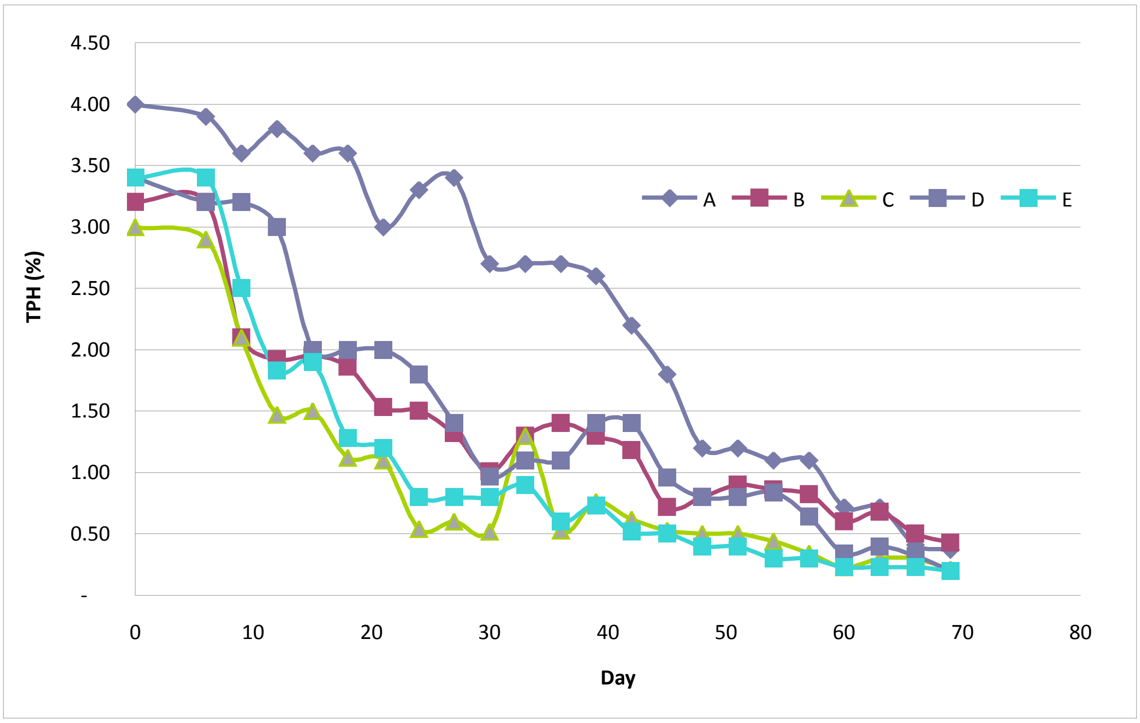
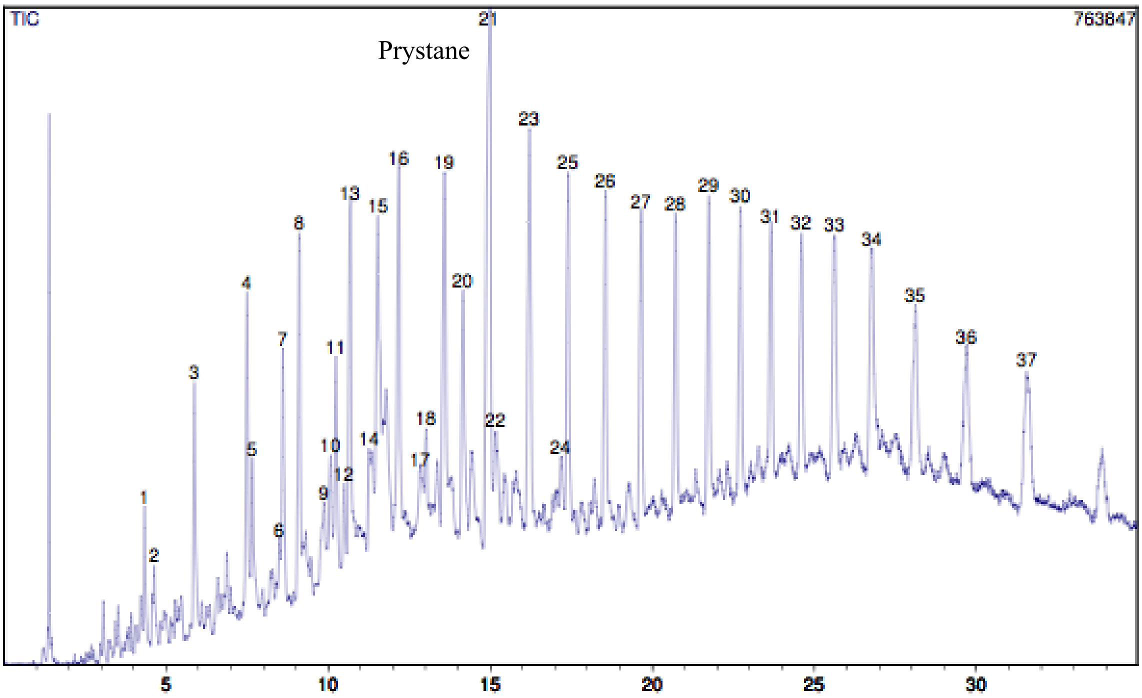
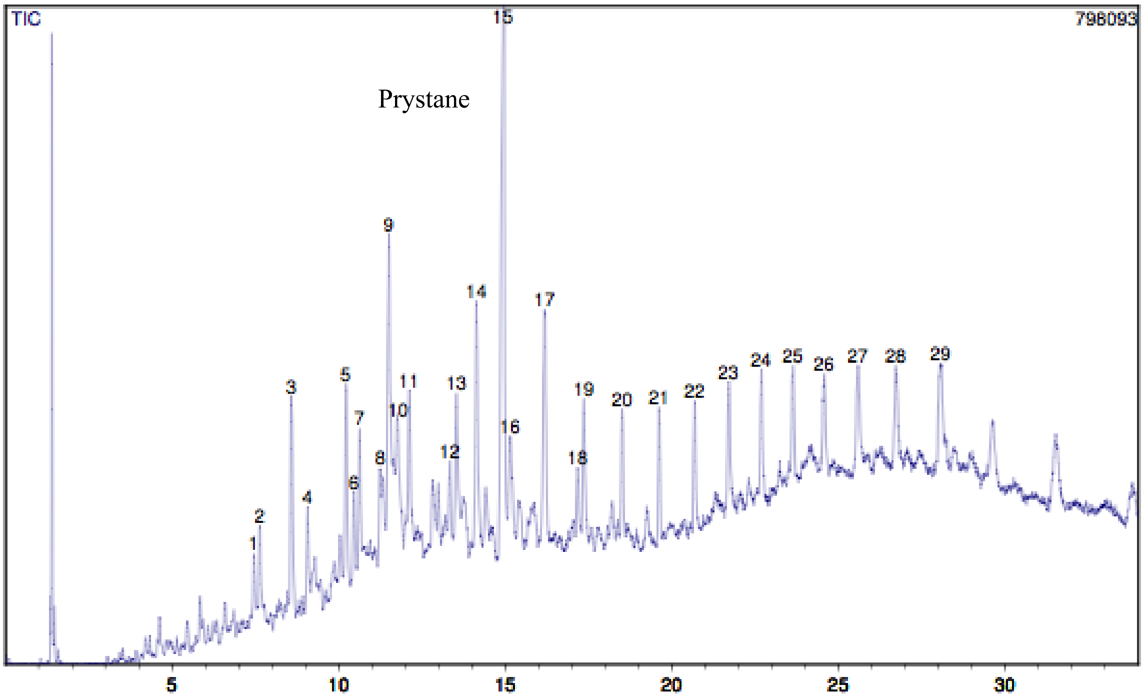


Figure 1. Treatability Test under Various Conditions

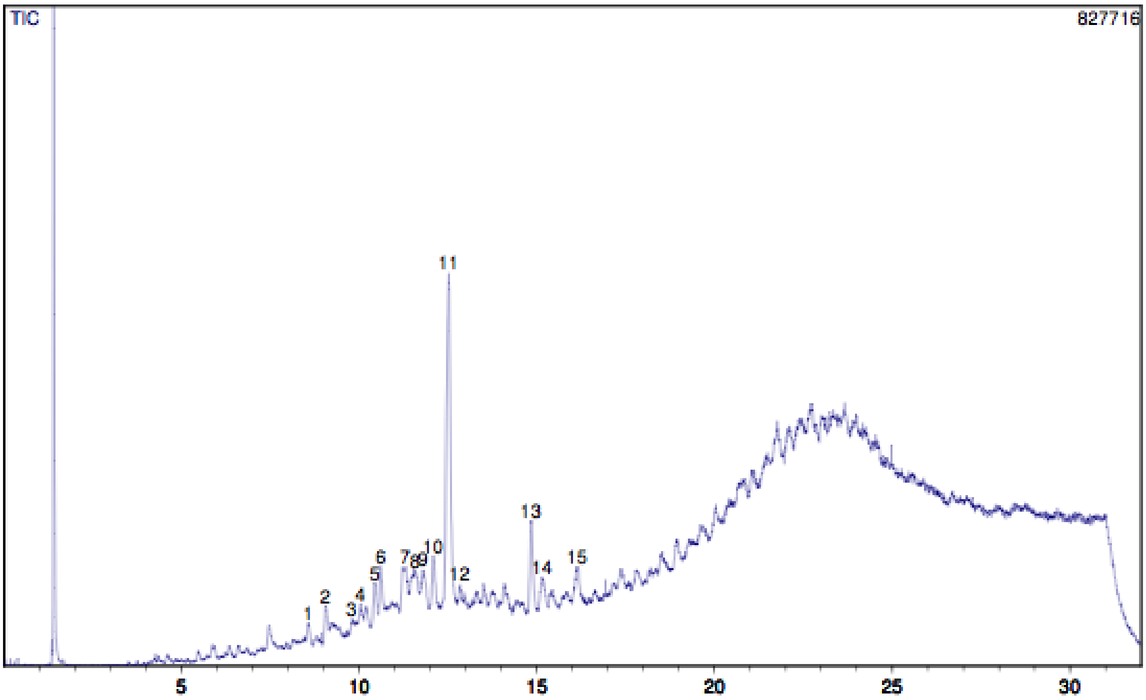
In the reactors where bio-surfactant-producing bacteria were added, namely the reactor C and E, to reach concentrations below 1% could be achieved with a relatively faster when compared with other reactors. This indicated that the emulsification process of TPH gave a significant influence on the biodegradation process conducted by the isolated bacteria. To reach the concentration of 1%, which is a compliance point of bioremediation processes based on current regulations, it took less than a month. The result was better than reactor B and D in which bioremediation processes only relied on isolated bacteria. Although the time required to reach 1% was longer, it indicated that the isolated bacteria had a strong ability to degrade TPH. The time required to reach 1% TPH concentration in reactor B & D was less than 1.5 months. Another interesting outcome was that the addition of bulking agent to give a fairly significant influence on the bioremediation process. In reactor A, which represented the reactor without the addition of bulking agent and bio-surfactant-producing bacteria and also bioremediation process that solely relies on indigenous bacteria, the biodegradation lasted longer (more than two months) when compared with other reactors. Moreover, the palm shells bulking agent gave better results when compared with shredded grass. The following figure shows an example of the Gas Chromatography/Mass Spectrophotometer (GC/MS) analyses of TPH for the best performance reactor (reactor C). These figures show in general that in the first month the heavy fractions of TPH slowly disappeared to become lighter fractions. Although TPH had reached 1% after 1 month for reactor C, there were still some fractions remained in the reactor. It was most likely that these were the aliphatic or aromatic compounds.



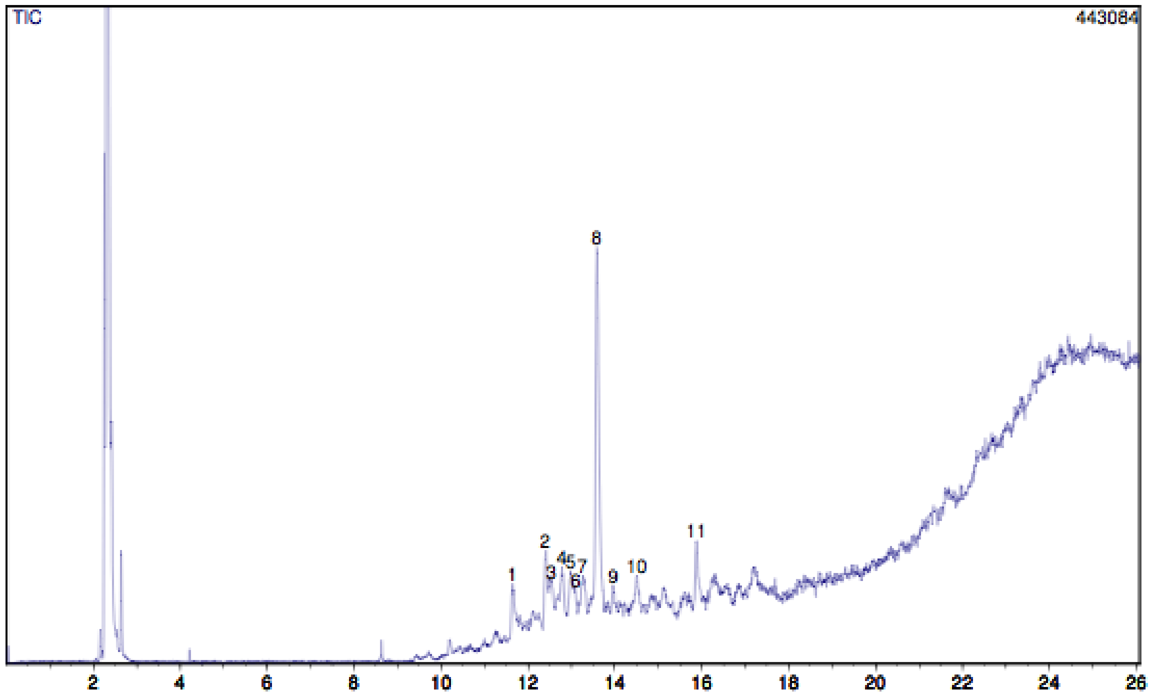
(a) t = 0 days



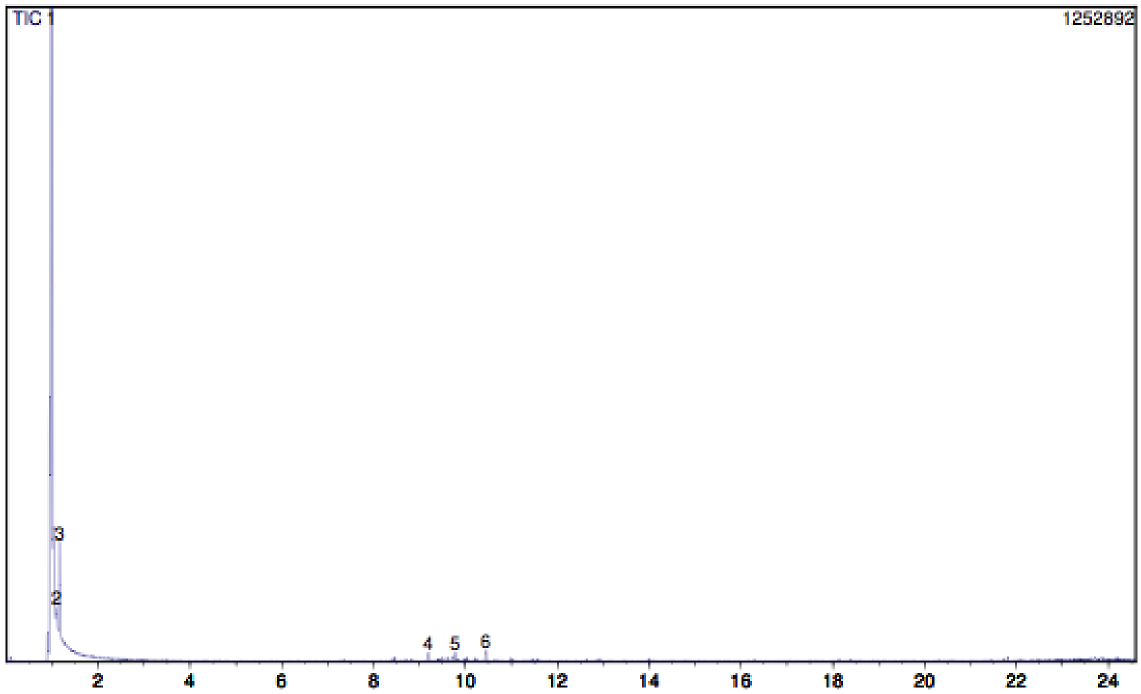
(b) t = 15 days



(c) t = 1 month



(d) t = 2 months



(e) t = 3 months

Figure 2. GC-MS Results

The number of bacteria in the reactors was also observed during the process. This is related to the biological transformation during the process. It was expected that the number of bacteria could be maintained in the same number as an indication of steady state process. Although so far there were no literature mentioned the optima number of bacteria involved in the process, based on experience normally it is around 10^4 to 10^7 colony per milligram soil. The number of bacteria in reactor during the process was around 10^7 colonies/mg soil. The pH of soil taken from oil-contaminated soil site was found to be relatively low. It was around pH 6. On average, after mixing with bulking agent, the pH during the bioremediation in each reactor was neutral (between pH 7 – 8). Since most hydrocarbon-degrading bacteria are classified as neutrophylic bacteria, these pH conditions supported the growth of these microorganisms under optima environmental conditions. Besides the availability of main substrate as carbon source that was TPH in this study, the growth of microorganisms also depends on the presence of Nitrogen (N) as nutrient. In this study, to provide nitrogen in a sufficient concentration, NPK fertilizers with a concentration of 0.5% was added to the reactors. Figure 3 shows the presence of N during the process. It could be seen that decrease of TPC was followed by the reduction of N. The presence of N in the bioreactors was also contributed from the soil itself as well as from the addition of bulking agent. The result indicated that N from blotong bulking agent was higher than from shredded grass. Also, it could be seen that N from blotong was more readily to be up-taken by microorganisms as indicating by rate of biodegradation and time consumed.

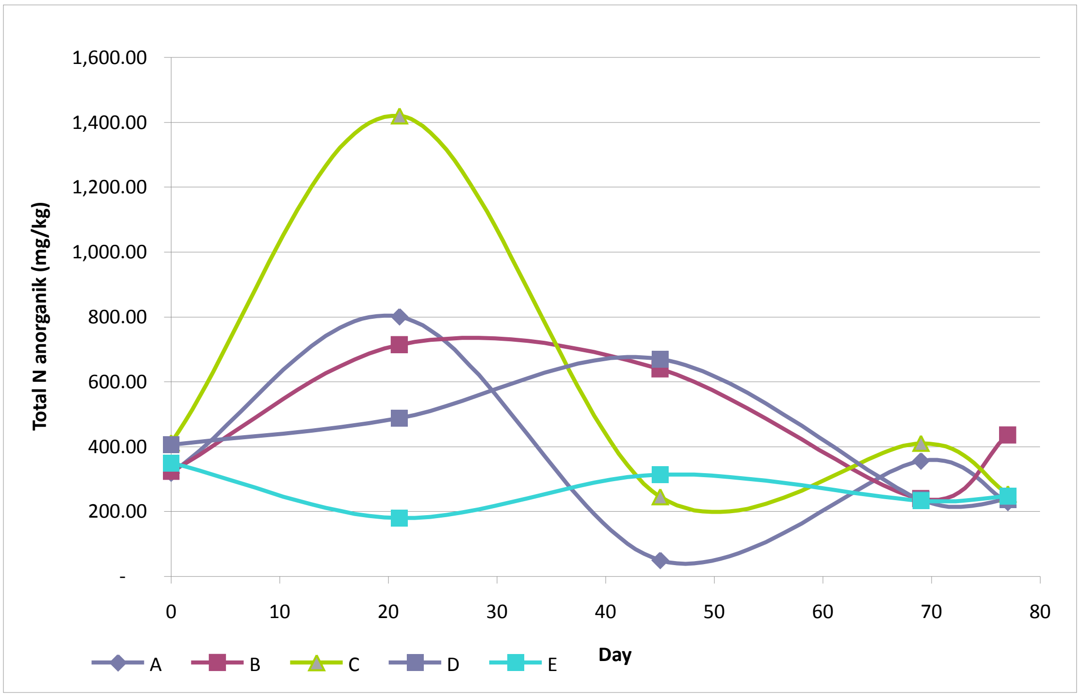


Figure 3. The presence of N-inorganic during Bioremediation Process

Conclusion

- There were at least 3 consortiums of bacteria successfully isolated and bio-augmented from contaminated soil storage, un-treated soil and treated soil
- Growth kinetics experiment of these isolates demonstrated that these bacteria had ability to degrade and utilize TPH as carbon source at low concentration
- This result indicated that the affinity of these bacteria to TPH were relatively high
- Using oil-contaminated soil for treatability test, the mix culture of those isolates demonstrated its ability to degrade TPH. Within less than 1 month the overall TPH concentration could reach below 1% by the addition of bio-surfactant-producing bacteria. The rate of biodegradation in this study was also affected by the type of bulking agent used

References

1. Eweis JB, Ergas SJ, Chang DPY, & Schroeder ED (1998): *Bioremediation Principles*, McGraw Hill, Boston, USA
2. Anderson, WC ed. (1995): *Innovative Site Remediation Technology*, American Academy of Environmental Engineers, USA
3. Cookson Jr, TC (1995): *Bioremediation Engineering: design and application,* McGraw-Hill, Inc, New York, USA
4. Crawford, RL, Crawford, DL (1996): *Bioremediation:: Principles and Application,* Cambridge University Press, Great Britain
5. Norris RD et al (1993): *In-situ Bioremediation of Groundwater and Geological Materials: A Review of Technology*, US Environmental Protection Agency, Cincinnati, USA
6. Mitchell WJ, Slaughter JC (1989): *Biology and Biochemistry for Chemists and Chemical Engineers*, Ellis Horwood Limited, Cichester, UK