THE 6TH INTERNATIONAL CONFERENCE ON MATHEMATICS AND NATURAL SCIENCES

ICMNS 2016

ON THE ROAD TOWARDS SUSTAINABLE DEVELOPMENT

BOOK OF ABSTRACTS

2-3 NOVEMBER 2016

INSTITUT TEKNOLOGI BANDUNG
BANDUNG, INDONESIA

ORGANIZED BY:

FACULTY OF MATHEMATICS AND NATURAL SCIENCES
SCHOOL OF LIFE SCIENCES AND TECHNOLOGY
SCHOOL OF PHARMACY

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ICMNS 2016

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Welcoming Remarks

On behalf of the Organizing Committee, I am very delighted to welcome all honorable speakers and participants to the 6th International Conference on Mathematics and Natural Sciences at ITB. It is wonderful to have over 400 speakers and participants representing various universities, research institutes, government agencies, and industries from Indonesia, Malaysia, Singapore, Korea, Japan, Australia, The Netherlands, Germany, and Kazakhstan.

We are very grateful to the Steering Committee who has provided tremendous supports and allowed us to develop an engaging program for this conference. The conference program will feature a Nobel Laureate Lecture, plenary sessions, parallel sessions, and poster sessions.

We would like to express our sincere gratitude to all honourable invited speakers who will share their knowledge and experiences of the frontiers research and recent developments in the field of chemistry, physics, mathematics, material science, computational science, medical and pharmaceutical sciences, life sciences, environmental sciences, science of renewable energy, as well as earth and space sciences.

We greatly thank the Scientific Committee who has done a terrific job in evaluating and selecting over 300 abstracts. We would like to express our great appreciation to the participants who will share their work in the parallel and poster sessions. Our very special thank goes to all moderators for their valuable time to chair the conference sessions.

We have been very fortunate to have a generous support from our sponsors to this conference. We would like to extend our sincere appreciation to PT Anugerah Inti Mulia, PT Bio Farma, PT Cayapata Citra Kemika, PT Seger Agro Nusantara, PT Teknolab, PT Unggul Cipta Teknologi, PT Kaltim Satria Samudra, PT Pupuk Kaltim, PT Paragon, PT ANTAM, IA Kimia ITB, BNI PTB, and LPPM ITB.

We hope that you will enjoy these two days of excellent talks and poster presentations, have stimulating discussions and build strong networks. Finally, we sincerely thank for your memorable contribution and participation in the 6th ICMNS.

Dessy Natalia

The 6th ICMNS Organizing Committee
Chairperson

Welcoming Remarks

It is our great pleasure and honor to welcome all of you to The 6th International Seminar on Mathematics and Natural Sciences. The 6th ICMNS is organized by the Faculty of Mathematics and Natural Sciences and supported by School of Pharmacy and School of Life Sciences and Technology ITB. This year conference is very special because it marks a decade of the biannual ICMNS which has been established since 2006.

This conference aims to disseminate research results in the fields of chemistry, physics, mathematics, material science, computational science, medical and pharmaceutical sciences, life sciences, environmental science, science of renewable energy, as well as earth and space sciences. In addition, the conference is also set up to promote multi- and inter-disciplinary researches in various domains of science and technology. Therefore, we hope that the intensive interactions among all distinguished speakers, presenters and participants will foster the advancement of science and technology which is essential to support sustainable development.

We are very honored to have Prof. Gerard 't Hooft, the 1999 Nobel Laureate in Physics, in this conference. Prior to the conference day, Prof. 't Hooft has delivered a series of lectures to ITB and Physics communities, and after this conference he will give a Grand Public Lecture entitled "A Road to Nobel Prize". The 6th ICMNS participants are welcome to join the Grand Public Lecture, which is going to be held on 4 November, 2016.

We would like to express our gratitude to all distinguished speakers, presenters and participants. We also sincerely thank the Steering Committee, the Scientific Committee, the Organizing Committee and all Sponsors for their great efforts and contributions to make this conference possible.

We wish you all having a fruitful and enjoyable conference.

Deans,

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TABLE OF CONTENTS

From Lab to Field:
Enhancing Bioremediation Process by Increasing Bioavailability of the Contaminant
(Case: Crude Oil Contaminated Soil Bioremediation)

Agus Jatnika Effendi
Faculty of Civil & Environmental Engineering – ITB
Paper of Invited Speaker on
6th International Conference on Mathematics & Natural Sciences
Bandung, 3 November 2016

Abstract
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). However, a low bioavailability of the contaminant, not only because of poor mixing, but also due to complexity and solubility of the contaminant, resulted in a very slow process of bioremediation. 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. By increasing the bioavailability of the contaminant and at the same time by optimizing the environmental condition such as porosity and hydraulic conductivity, it is expected to increase biomass concentration in oil-contaminated soil site resulting in faster bioremediation process. Some efforts had been made such as by the addition of surfactant to create micelle oil that can be easily attacked by microorganisms. Also, by combining with oxidants, such as Hydrogen Peroxide (H_2O_2) and Sodium Percarbonate that are expected to increase the bioavailability of oil-contaminated soil by oxidizing complex hydrocarbons into simpler forms that are going to be easily degraded by bacteria. This paper will demonstrate the preliminary efforts that had been made to increase the bio-availability of crude oil contaminated soil (COCS).
Keywords: bioremediation, bio-availability, surfactant, oxidants

Introduction
Remediation required when contamination occurred unintentionally. To prevent further transmission to the receptor (via 3 portal of entry: dermal absorption (because of direct contact), inhalation due to blowing dust and volatilization and ingestion via drinking water or food) as shown in figure 1, the contaminated site must be cleaned up, either in-situ or ex-situ (which means excavation will be required).

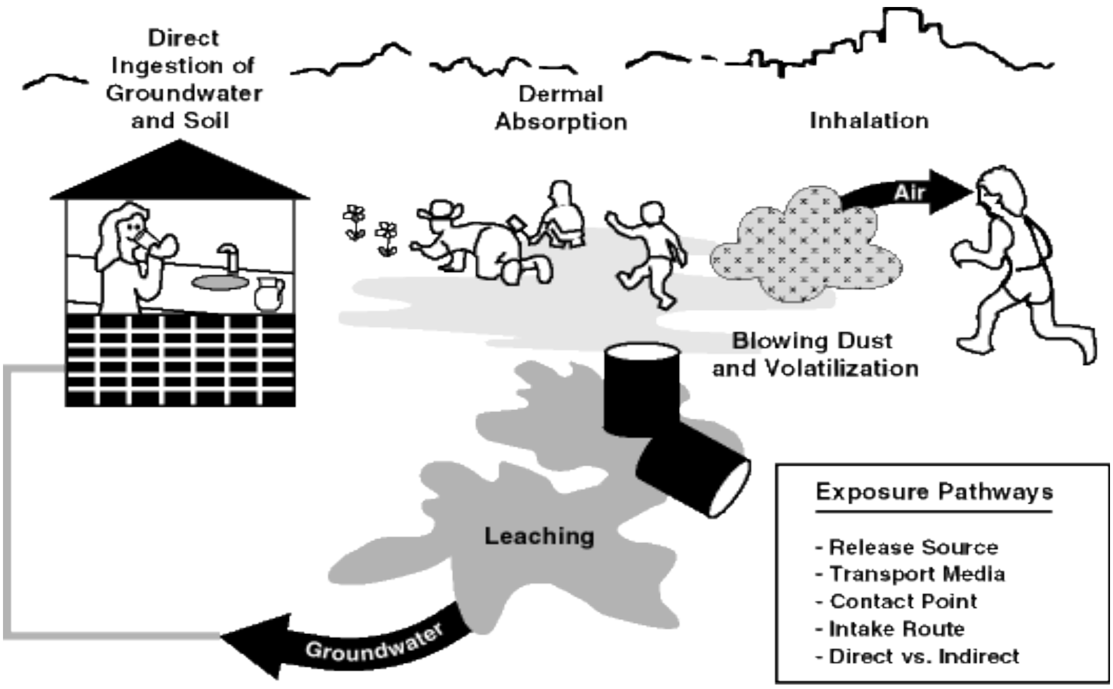


FIGURE 1 Major Exposure Pathways for Human Exposure to Contaminated Soils and Sediments. SOURCE: EPA Region 9 Preliminary Remediation Goals website (www.epa.gov/region09/waste/sfund/prg).

Various remediation techniques have been developed to restore soil contaminated by various types of contaminants in an effort to minimize the negative impact on living things. Various remediation techniques of contaminated soils that are quite popular and have been widely used and proven to be an appropriate technology are (1) Soil Vapor Extraction, (2) soil washing, (3) stabilization / solidification, (4) electrokinetic remediation, (5) thermal desorption, (6) vitrification, (7) bioremediation and (8) phytoremediation (Sharma & Reddy, 2004). All of these techniques can be applied both in-situ and ex-situ with various modifications that will involve physical, chemical, thermal, electrical and biological processes.

Although there are many technology options available, the main alternative in choosing remediation technology is natural attenuation which is intended if the pollution that occurs has the potential for degradation, by naturally occurring microorganisms. This approach is the most less intervention approach compared to other technologies, so that it is in line with the goal of environmental remediation that is to return the polluted soil to the reference condition. In general, the bioremediation approach is the most economical technology when compared to other technologies, especially if the pollution is dominated by organic compounds and the soil has sufficient hydraulic conductivity to deliver nutrients and oxygen supply. According to some sources, the cost of the remediation process is in the range of USD30-USD750 per cubic yard.

Factors affecting the bioremediation process

Some factors that could affect the successful of bioremediation of contaminated soil process are as follows:

- Environmental factor; namely the factors that will affect the optimum conditions of microorganism growth achieved so that the bioremediation process can run well.

These factors include temperature, pH, availability of nutrients, oxygen as an electron acceptor and also soil moisture (can also be expressed as water content). Soil bacteria that generally exist in Indonesia, will work optimally at neutral pH and temperature in the range of 20-40^{0}C. Soil moisture is also an important parameter because bacteria will take nutrients for growth from solution, but if the humidity is too high it will cause low oxygen availability as electron acceptors due to low oxygen solubility in water. The water content should be maintained in the range of 15-20% so that oxygen will remain available in the air trapped in soil pores.

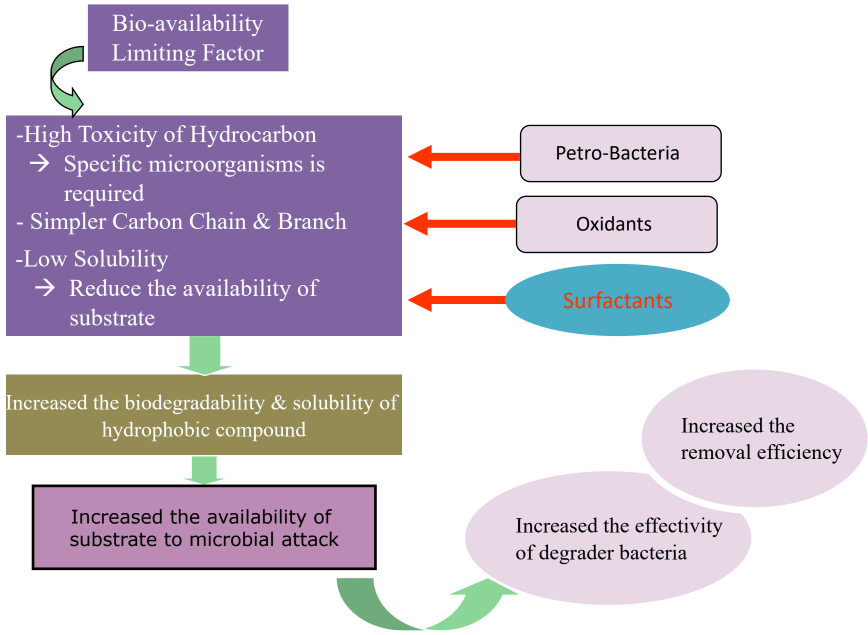
- Physical factors; The most important physical factor for the bioremediation process to occur is bioavailability of contaminants, adequate presence of water & adequate electron acceptor supply. Hydrocarbons are generally non-polar compounds, so they tend to be in a solid phase with large enough molecular size for bacteria to attack. Even this causes dissolved hydrocarbon concentrations in the liquid phase to be measured in low concentrations.
- Chemical Factors; namely the molecular structure of the contaminants that will greatly affect the physical & chemical properties and the biodegradability of the contaminants. Biodegradability is very closely related to solubility, carbon chain & branch and saturation of the hydrocarbons. The more saturated, the longer and more persist the existing carbon chains making the biodegradation process will be increasingly difficult, even though these compounds have the same empirical formula.

Optimizing environmental factors

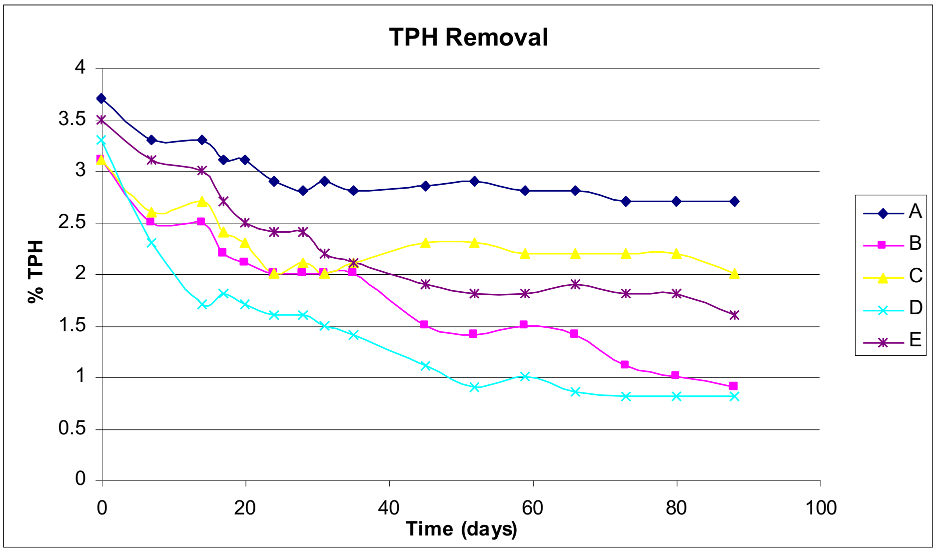
In the engineering of bioremediation process of hydrocarbon contaminated soils, those three factors mentioned above, are implemented simultaneously in an effort to obtain optimal operating conditions for the bioremediation process. An effort to optimize the three factors include:

- Mixing; This process will simultaneously increase the bioavailability of contaminants and increase hydraulic conductivity so that it has an impact on increasing available oxygen. Thus, although the hydrocarbons that have low solubility in water (especially for long chain hydrocarbons), the bioavailability of these compounds will increase through the mixing process. Through this stirring, the process of degradation of long chain hydrocarbon compounds will proceed well because the TPH degradation process which consists of highly complex hydrocarbons begins with the formation of the oxygenase enzyme whose activation depends on the presence of oxygen molecules (Atlas, 1995). Stirring will also result in agglomerated soil as a result of long chain hydrocarbons breaking into small sizes, which in turn will increase contact of soil contaminated with long chain hydrocarbons with existing bacteria.
- The addition of bulking agents can increase the porosity and hydraulic conductivity of oil-contaminated soils especially for soils generally dominated by clay soils tend to have low porosity and hydraulic conductivity. Thus, the addition of bulking agents will improve soil structure by increasing both physical factors. Therefore, oxygen molecules are available in sufficient quantities for the synthesis of oxygenase enzymes responsible for breaking the hydrocarbon chains into simpler chains. Various studies that have been conducted including at ITB, prove that the addition of bulking agents will increase the bioavailability of hydrocarbons.
- Dewatering and bio-surfactant addition; bioavailability of low long chain hydrocarbons can be increased through the dewatering process to maintain optimal humidity so that nutrients and oxygen can be used by bacteria easily. Addition of

auxiliary agents such as surfactants can increase the solubility of long chain hydrocarbons as shown in figure below. Thus, at an optimal water content, the addition of surfactants will result in hydrocarbons that originally had non-polar groups will turn into polar. Thus, long chain hydrocarbons that originally had a large size that is not possible to be adsorbed by bacteria, turned into micelle oil which is very small in size and ready to be used by bacteria. A pilot-scale study conducted at ITB (Helmy et al., 2015) shows that the addition of bio-surfactants, surfactants that is produced by bacteria of the Azotobacter sp. Group, has increased the bioavailability of long-chain hydrocarbons to enable the biodegradation of these compounds into compounds. simpler hydrocarbons with 2x removal efficiency and characterized by breaking long chains into simple chains both qualitatively and quantitatively.

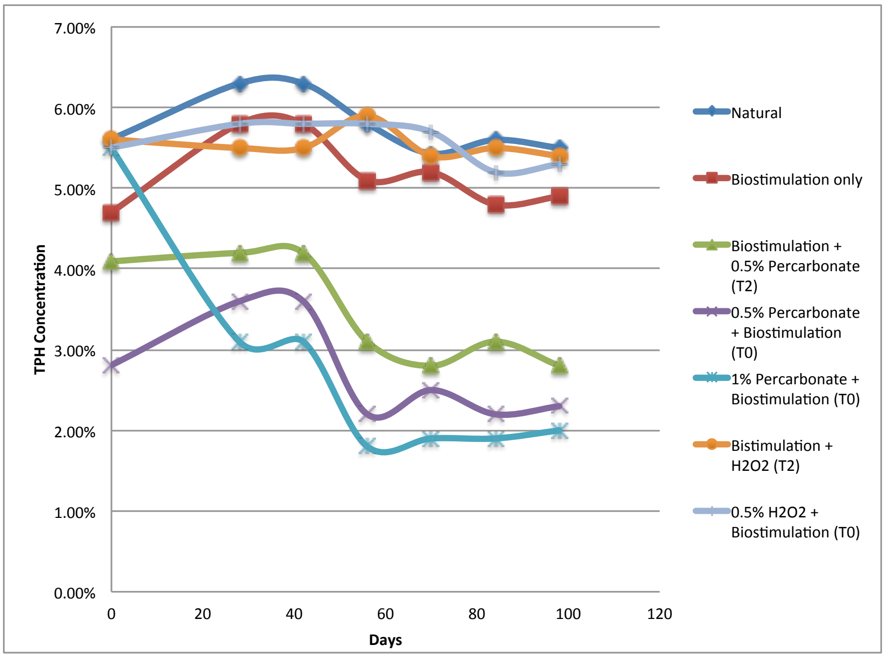


- Oxygen supply as electron acceptors; so that oxygen can always be available in sufficient quantities, the oxygen supply can be done in various ways. Passively can be done with a windrow system or actively by doing aerobic static biopile processes either by positive or negative pressure. Thus, the continuity of the availability of oxygen molecules needed by the oxygenase enzyme for hydrocarbon degradation will always be maintained.
- Bioaugmentation; hydrocarbons with carbon chains > C16 have recalcitrant characteristics and are less biodegradable. However, through a biological engineering process, it is evident that some long chain hydrocarbon compounds can undergo biodegradation. The bacteria responsible for terminating these long chain hydrocarbons have been widely isolated and used in various bioremediation processes for petroleum-contaminated soils. So, to increase the rate of bioremediation process of soil contaminated by long chain petroleum can be done by adding these petrophylic bacteria or referred to as the bioaugmentation process. The following figure shows a success example of bioaugmentation process experiment that performed in our laboratory.



- Control reactor; No bacteria were added (A): **Natural Attenuation**
- Reactor with the addition of Isolated Bacteria + Bulking Agent (B): **Bio-augmentation**
- Reactor running with Indigenous Bacteria + Bulking Agent (C): **Bio-stimulation**
- Reactor with the addition of PETREA + Bulking Agent (D)
- Reactor with the addition of Isolated Bacteria without Bulking Agent (E)

- The addition of oxidizing agent; by combination between oxidation & biodegradation process, it is expected to al so increase the bio-availability of hydrocarbon. Oxidation will form simpler compounds allowing bacteria to attack the product in performing biodegradation. Oxidized products is expected to be more degradable & higher solubility (Goi, 2005 & Brame et al, 2013), thus it will increase the hydrocarbon bio-availability. The following figure indicated the performance of oxidation-biodegradation process in laboratory scale.



In addition to the engineering process above which basically aims to increase the bioavailability of long chain hydrocarbons, the addition of N and P in optimal amounts is also essential (Rosenberg & Ron, 1996). Theoretically, it would take approximately 150 mg of N and 30 mg of P to convert 1 gram of hydrocarbons into bacterial cells. Generally, soils polluted by hydrocarbons, especially petroleum hydrocarbons, contain very low N & P elements. So, so that the bioremediation process runs well, the addition of N & P elements plays an important role.

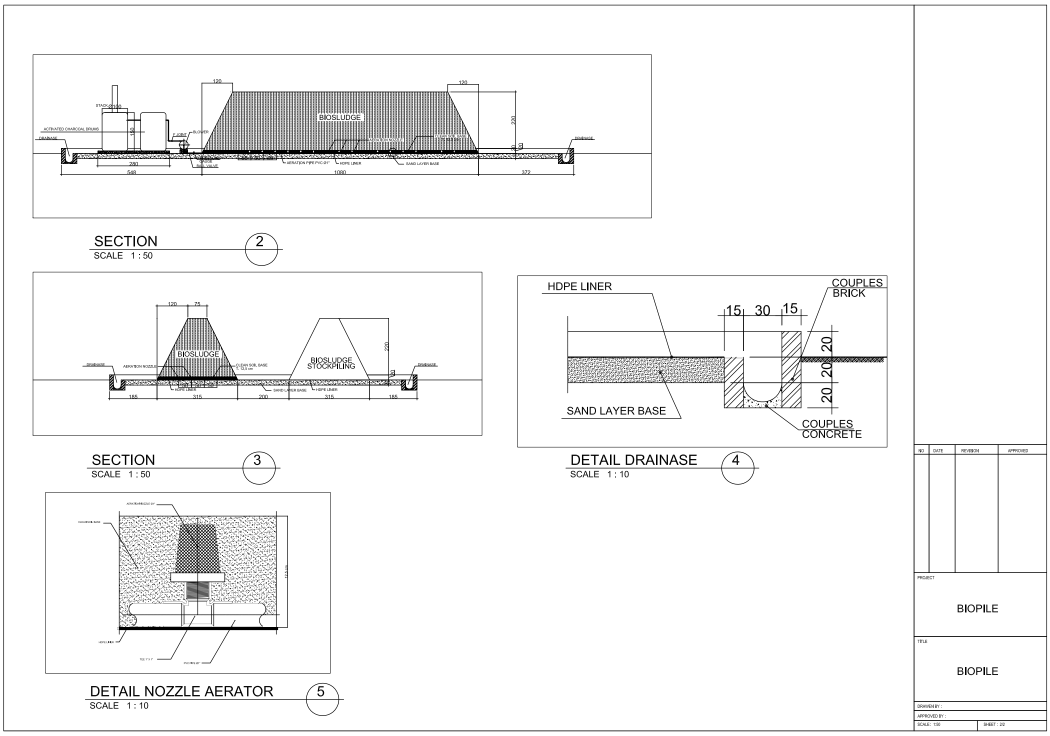
Various studies have been conducted to examine the bioremediation process of hydrocarbon polluted soils, especially those related to TPH. The complexity of the hydrocarbon fraction present in petroleum hydrocarbons makes the TPH degradation process an interesting research object. Eweis et al., 1998 and Cookson Jr., 1995, demonstrated the biodegradability of various TPH fractions carried out by various researchers. Hydrocarbons in TPH generally consist of alkanes, cycloalkanes, aromatics, polycyclic aromatics, asphaltines and resins, most of which are long chain hydrocarbons. Various species of bacteria and fungi have been successfully isolated and proven to be able to process part of a long chain hydrocarbons both through aerobic and anaerobic processes. A study conducted at ITB showed that petroleum-contaminated soil in PT CPI has a carbon chain dominated by the fraction > C16 has undergone a process of degradation into a simpler hydrocarbon compound, which is between C11-C15 within 80 days where at the same time its TPH concentration has decreased from 2% to 0.5%. From the results of the study it was also seen that there was a symbiosis between the petrophylic bacteria and the *Azotobacter* bacteria which are biosurfactant-producing bacteria. The biosurfactant produced by *Azotobacter* bacteria will increase the solubility of long-chain hydrocarbons.

Lab to Field: Stages of Bioremediation Process

To ensure the success of the bioremediation process implementation, several stages need to be carried out. Thus, the choice of treatment technology that will be applied in accordance with the needs and conditions in the field. These stages are as follows:

1. Site characterization; Things that need to be done at this stage include identification of the distribution of contaminants, background & site/site history, and the initial transformation of pollutants that have already taken place. Tracing can be done through collecting secondary and primary data (taking measurements in the field for delineation of vertical and horizontal pollutant distribution and also the physical characteristics of the soil to be recovered). The final goal of this stage is to see the feasibility of implementing the bioremediation process. This stage is the beginning of the screening process of bioremediation technology choices that will be applied (ex-situ or in-situ).
2. Treatability Study; This stage is done in the laboratory to ensure the level of biodegradability of the contaminants/pollutants. At this stage various laboratory tests are carried out including the bacterial growth kinetics involved in the bioremediation process, soil toxicity testing before processing and its processed products, analysis of product metabolites, various bioremediation techniques that may be carried out, and others. At this stage also the application of the process of bio-stimulation and bio-augmentation can be tried. So, on a field-scale application it has been decided which technique is best to remediate the contaminated soil. Similar to the site characterization stage, the treatability stage is only done at the initial stage of the bioremediation process. Treatability studies need to be done again if there is a fundamental change in the characteristics of the site which includes changes in the type and composition of contaminants/pollutants as well as the physical characteristics of the polluted soil.

3. Pilot Study; This stage aims to scale up the treatability studies that are still in the laboratory scale. This process is important before full scale application because it is a miniature of the bioremediation process that will be applied. In the treatment study stage, most of the parameters determining the success of bioremediation are fully controlled. However, at the pilot scale stage some parameters will be greatly influenced by environmental conditions (for example temperature). So, it is expected that this stage is a representation of the field scale application. The parameters managed during this pilot study are all physical, chemical and environmental parameters that will affect the success of the bioremediation process.
4. Good engineering plan. Having followed the stages of bioremediation planning, one of the most important product is the engineering design of bioremediation. Engineering drawing of the plant should be produced in order to make sure that the bioremediation process following the engineering design criteria. The following figure shows an example of a biopile engineering drawing.



Bioremediation Process Application

As mentioned above, the process of bioremediation of contaminated soil can be done in-situ (no removal of contaminated material / soil) and ex-situ (involving removal of contaminated material or soil):

- In-situ bioremediation techniques are carried out with the aim of improving the biodegradation process at the place of contaminants originating from the topsoil (sub-surface) or at the location of existing contaminated soil. In-situ techniques have several advantages, including reducing the disturbance to the location of contaminated soil; exposure to contaminants or pollutants is very minimal and reduces transportation costs. In-situ techniques require more detailed and comprehensive exploration of contaminant sites. This is especially necessary to ensure that the treatment process does not result in expansion of the pollution area due to the movement or contamination of contaminants to other areas through groundwater flow. Therefore, it is often necessary to have many monitoring wells to check the treatment efficiency and the possibility of contaminant spillage or movement. In addition, for in-situ techniques, adding nutrients or oxygen to the soil will require further engineering considerations. Unsaturated soil conditions will provide different alternative options for delivery to the contaminated soil. Examples of bioremediation techniques in-situ are soil venting and bioventing. Soil Venting is used if the dominant contaminant is volatile. This method is done by supplying oxygen to the uncontaminated soil and evacuating the gas produced due to decontamination which is carried out and then subsequently treated. Bioventing is applied if the dominant contaminants are semi-volatile and non-volatile. This method is done by supplying oxygen to the contaminated area and injecting or infiltrating nutrients.

- Unlike the in-situ technique, in the ex-situ technique, contaminated soil will be collected (excavated) and then transported to the treatment site. Therefore, this technique will require costs for transportation and other areas specifically for treatment locations. Because of the ex-situ nature, the types of treatment that can be done are more diverse. Contaminated soil can be treated in the form of solid, slurry or maybe liquid. The treatment process can be in the form of landfarming, composting (windrow and biopile) and bioreactors for the slurry phase. Landfarming is usually carried out on open ground surfaces, where contaminated soil is spread and the process is carried out in open spaces. The aeration process is carried out by tilling. The composting method can be done either in open air or in a reactor/enclosed space where the aerating system uses active aeration which is to provide sufficient air supply into the contaminated soil through a piped system (aerated static biopile) or by passive aeration that is done routinely reversal (windrow). Whereas slurry-reactors are carried out in ponds or special bioreactors. The advantages of ex situ bioremediation include: enabling optimization of treatment conditions and process control; the fastest bioremediation technique; specific bacteria can be added. While the disadvantage of ex situ bioremediation techniques is that the most expensive bioremediation approach and can result in the release of volatile materials.

Conclusion

Efforts have been made in order to increase the rate of bioremediation process by optimizing some environmental factors that affecting the bioavailability of the contaminants. Laboratory scale results demonstrated that by optimizing environmental factors as well as the addition of some biological or chemical substances resulted in a good bioremediation performance. Since the process involving the addition of chemical substances, toxicity of treated soil must be further examined. However, in order to apply this laboratory scale results to field scale, some works should be done. Well prepared and engineered field scale in order to reduce the gap with laboratory results is a must.

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