

Waste Engineering and Management

Proceeding

SIBE-2009

The 1st International Conference on Sustainable Infrastructure and Built Environment in Developing Countries

SABUGA ITB, Bandung - Indonesia
2nd - 3rd November 2009

Published by
Faculty of Civil and Environmental Engineering
Institut Teknologi Bandung - Indonesia

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SIBE 2009 published eight (8) volumes of proceeding as following :

Volume A : Structure and Material
Volume B : Transportation System and Engineering
Volume C : Water Engineering and Management
Volume D : Waste Engineering and Management
Volume E : Ocean Engineering
Volume F : Construction Management
Volume G : Geotechnical Engineering
Volume H : Environmental Protection and Management

PREFACE

The 1st International Conference on Sustainable Infrastructure and Built Environment in Developing Countries (SIBE) 2009 is aimed to provide a forum to discuss and disseminate recent advance in scientific research, technology, and management approach to obtain better environment quality.

Infrastructure that provides the basic need of a society and sustainable infrastructure system are essential for the survival, health and well-being of a society. In developing countries, civil and environmental engineers are at the epicenter in seeking means to enhance the quality of human life through modernization of infrastructure as evidenced by provision of shelters, water, and transport, amongst others. The current rate of urbanization and industrialization raises a number of environmental issues, often resulting in environmental mismanagement, especially in developing countries. The problems are further aggravated by environmental degradation such as soil erosion, depletion of water resources, etc. In order to meet these multifaceted challenges, proper planning followed by implementation and verification must be exercised, via an integrated, multi disciplinary and holistic approach.

The conference will provide an opportunity for professionals and researchers to learn, share and exchange about the latest development and research in civil and environmental engineering. The scope of the conference covers all aspect of civil and environmental engineering practices.

Participants of the conference include researchers, academic staffs, students, industries, public and local governments. The keynote presentations during the conference are as follows:

Keynote speakers:

- **Indonesian Government Representative**
  Minister of Public Works, Indonesia
- **Dr. Puti Farida Marzuki**
  Dean of the Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung, Indonesia
- **Dr. Tony Liu**
  National Taiwan University, Taiwan
- **Prof. Shunji Kanie**
  Hokkaido University, Japan
- **Prof. Syunsuke Ikeda**
  Tokyo Institute of Technology (AUN/SEED-Net), Japan.

Invited speakers:

- **Dr. Setiawan Wangsaatmaja**
  Environmental Protection Agency of West Java Province, Indonesia
- **Dr. Edwan Kardena**
  Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung, Indonesia
- **Prof. Harianto Rahardjo, Ph.D.**
  School of Civil and Environmental Engineering, Nanyang Technological University, Singapore
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The objectives of this conference are:

1. To provide a platform for exchange of ideas, information and experiences among academics, researchers, consultants, engineers, manufacturers and post graduate scholars in civil and environmental engineering.
2. To discuss and evaluate the latest approaches, innovative technologies, policies and new directions in infrastructure development, pollution prevention and eco-friendly technologies adapted to developing countries.
3. To promote cooperation and networking amongst practitioners and researchers involved in addressing infrastructure and built environment issues.

The oral and poster presentations are subdivided into 8 major sections, as following:

A. Structure and material
B. Transportation system and engineering
C. Water engineering and management
D. Waste engineering and management
E. Ocean engineering
F. Construction management
G. Geotechnical engineering
H. Environmental protection and management.

There are 174 contributors in oral presentation and 36 contributors for poster presentation.

Finaly, the Organizing Committee wishes that this conference is able to provide beneficial scientific information to the participants and other concerned readers.

Bandung, November 2009
Organizing Committee

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CONTENT

POSTER SESSION PAPERS

International Conference on Sustainable Infrastructure and Built Environment in Developing Countries
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The Application of Bioemulsifier Produced by *Azotobacter vinelandii* in Bioremediation Oil-Contaminated Soil

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Abstract
Emulsification properties of the bioemulsifier produced by Azotobacter vinelandii were stable at wide range of temperature, pH and salinity indicating advantages over its applications in the bioremediation of oil-contaminated soil and others purposes in oil industry. The addition of this bioemulsifier in a broth culture was able to reduce the surface tension from 72.67 to 45.85 dyne/cm. The addition of bioemulsifier in the bioremediation process of oil-contaminated soil could enhance the degradation process by making the crude oil more soluble and readily to petrophylic bacteria to attack this substrate as indicated with the bacterial growth. The TPH removal efficiency of reactors after running for about 3 days were 47.04% for reactor with addition of petrophylic bacteria only, whereas in the addtion of 5% bioemulsifier produced by Azotobacter vinelandii the removal efficiency reached 61.8%. TPH removal in the reactor control was about 4.86% for reactor control and 61.8%.

Keywords: bioemulsifier, *Azotobacter vinelandii*, TPH, removal efficiency.

1. Background

Bio-emulsifiers have been applied widely in the environmental protection, crude oil recovery, agriculture, mining, health care and food-processing industries (Desai and Banat, 1997). Bioemulsifiers have special advantage over the chemical surfactants, such as lower toxicity, higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity at extreme temperature, pH and salinity (Desai and Banat, 1997; Makkar and Cameotra, 1997a). As environmental compatibility becoming an increasingly important factor in the selection of industrial chemicals, the use of Bioemulsifiers in environmental application, such as biodegradation and dispersion of oil spill is increasing (Banat, 1995). In addition, Bioemulsifiers could also be used for others purposes in petroleum industry, such as enhancing oil recovery process and the transportation of crude oil. Other possible application fields are in the food, cosmetic and pharmaceutical industries. In these industries, most Bioemulsifiers are used as emulsifiers. The largest possible market for Bioemulsifier is in the oil industry both for petroleum production and for bioremediation of oil contaminated sites. The application of bioemulsifier in the bioremediation process would accelerate the natural biodegradation processes in contaminated environments. Bioemulsifiers are a structurally diverse group of surface-active molecules synthesized by microorganisms. These molecules reduce surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures. Bioemulsifiers activities can be determined by measuring the charge in surface and interfacial tensions, stabilization or destabilization of emulsion. The surface tension at the air/water and oil/water interfaces can easily be measured with a tensiometer. The surface tension of distilled water is 72 mN/m, and addition of surfactant lowers this value to 30 mN/m (Desai and Banat, 1997). An emulsion is formed when one liquid phase is dispersed as microscopic droplets in another liquid continuous phase. Bioemulsifier may stabilize (emulsifiers) or destabilizer (deemulsifiers) emulsion. The emulsification activity is assayed by the ability of the surfactant to generate turbidity due to suspended hydrocarbons such as a hexadecane or Kerosene in an aqueous assay system. Hydrocarbon (CxHy) degradation in soil has been extensively studied among many researcher. Degradation is dependent on presence in soil of hydrocarbon-degrading species of microorganisms, hydrocarbon composition, oxygen availability, water, temperature, pH, and inorganic nutrients. The physical state of CxHy can also affect biodegradation. Addition of synthetic surfactants or microbial emulsifier resulted in increased mobility and solubility of CxHy, which is essential for effective microbial degradation. Use of bioemulsifier in CxHy degradation has produced variable results i.e the fungus Cladosporium resiuae, grown on alkane mixtures, produced extracellular fatty acids and phospholipids, mainly dodecanoic acid and phosphatidylcholine. Supplement of the growth medium with phosphatidylcholine enhanced the alkane degradation rate by 30%. Emulsan, stimulated aromatic mineralization by pure bacterial cultures, but inhibited the degradation process when mixed cultures were used. Naphthalene was utilized in the first phase of CxHy degradation; other oil components were degraded during the second phase after the surfactants produced by concerned microorganisms lowered the interfacial tension. Addition of Bioemulsifiers, such as bacterial population was enhanced. Surfactants have been studied for their use in reducing viscosity of heavy oils, thereby facilitating recovery, transportation, and pipelining. Emulsan, a high molecular weight lipopolysacharide produced by *A. calcaoceticus*, has been proposed for a number of applications in the petroleum industry such as to clean oil and sludge from barges and tanks, reduce viscosity of heavy oils, enhance oil recovery, and stabilize water-in-oil emulsions in fuels. The specific solubilization of CxHy was strongly inhibited by EDTA which was overcome by excess Ca++. It was concluded that specific solubilization of CxHy is an important mechanism in the microbial uptake of CxHy (Karanth et al., 2005).

2. Methodology

Microbial Source & Medium

Azotobacter vinelandii and petrophylic bacteria were obtained from the culture collection of Environmental Biotechnology Laboratory-Environmental Engineering Department, Institut Teknologi Bandung, Indonesia. These microorganisms were maintained at 4^0 C on mannitol enrichment agar slants containing (g/L): 20 mannitol, 20 yeast extract, 20 tryptone, 15 agar. Sub-cultures were made to fresh agar slants every 2 month to maintain viability. All cultures were grown aerobically in Minimal Basal Medium (MB) which contained the following components (g/L) of distilled water: K_2HPO_4 1.5; KH_2PO_4 0.5; $MgSO_4$ 0.2; $(NH_4)_2$ SO_4 0.5; glucose 5. 10ml Trace Element solution was added per liter of MB medium, the composition of this trace element (g/L) is $Na_2EDTA_2.H_2O$ 12 ; $FeSO_4.7H_2O$ 2 ; $CaCl_2$ 1 ; $ZnSO_4.7H_2O$ 0.4 ; $NaSO_4$ 10 ; $MnSO_4.4H_2O$ 0.4 ; $CuSO_4.5H_2O$ 0.1 ; $Na_2MoO_4.2H_2O$ 0.5.

Microbial Growth

50 ml of MB was inoculated with 0.5 g of oil contaminated soil and oil sludge in a 250 ml erlenmeyer flask and incubated in an orbital shaker at 150 rpm. The inoculated flasks were incubated at room temperature for several days until visible growth was observed. 10 ml subcultures were used subsequently to inoculate fresh batches of MB medium which added with 1 % crude oil. The enriched flask were incubated until visible emulsification of crude oil layer was noticed. This procedure was repeated several times to ensure the capability of microbial culture in emulsifying crude oil in the medium.

Bioemulsifier Production

To produce crude bioemulsifier, an inocula was prepared by transferring the bacterial colonies into a MB media with selected carbon source, and the culture was incubated at room temperature in a shaking incubator at 150 rpm for 24 h. Then a MB media containing 2% inoculums and 2% carbon source was incubated at room temperature (28^0 C) under aerobic condition in a shaking incubator at 150 rpm for 48 h to obtain the highest microbial and surfactant concentration. After that, the solution was centrifuged at 4^0C and 13.000 rpm for 30 min in order to remove the bacterial cells. The obtained supernatant was further treated by acidification to pH 2.0 using 4 M hydrochloric acid solution, and the acidified supernatant was left overnight at 4^0C for the complete precipitation of the Bioemulsifiers (Pornsunthorntawee et al., 2007). After centrifugation, the supernatant was then dissolved in a 4 M hydrochloric solution, followed by the bioemulsifier extraction step with a chloroform solvent at room temperature. The organic phase was transferred to a round-bottom flask, yielding a viscous brown-colored crude bioemulsifier product and evaporated to remove the solvent.

Total Petroleum Hydrocarbon Extraction

A 5 ml of sample was mixed with 5 ml Hexane. This mixture was then double extracted by centrifuging at 3500 rpm for 10 minutes. The supernatant part was weighing after the Hexane was evaporated at 70°C.

3. Result & Discussion

The bio-emulsifiers produced from *Azotobacter vinelandii* had been characterized in the previous study and presented in other paper. Result from the previous study indicated that the bioemulsifier was optimally produced after 72 hour of fermentation. Various factors affecting on growth and bioemulsifier production by *Azotobacter vinelandii* was studied. High concentration of bioemulsifier was achieved when the growth of *Azotobacter vinelandii* was supplemented with 0.25g/L Ammonium nitrate, 0.2 g/L Magnesium sulfate and in the absence of Ferrous sulfate. Higher nitrogen concentration resulted in higher yield of cell growth. In contrary, the increase of cell growth was followed by the reduction of bioemulsifier produced by *Azotobacter vinelandii.* The production of bioemulsifier decreased when nitrogen concentration was increased. The addition of metal cations was found to affect the bioemulsifier production. The presence of metal ions in high concentration inhibited the production of bioemulsifier from *Azotobacter vinelandii*. Under these conditions it was found that the production of bioemulsifier was yield up to 68.8 g/g biomass. Emulsification properties of the bioemulsifier were stable at wide range of temperature, pH and salinity. The bioemulsifier isolated from *A. vinelandii* under the conditions studied in this work,was not only being a good emulsifier, but also has a good tensio-active properties. Surface properties of several medium culture conditions after the addition the bioemulsifier produced from *Azotobacter vinelandii* compared to other references could be seen in Table 1. as follows:

Table 1 Surface activity of broth before and after inoculation with *A. vinelandii* compared with other references

Sampel	**Surface Tension (dyne/cm) at 25^0C**	**Reference**
Sterile Aquadest	71.91	Research data
Sterile Growth Medium	72.67	Research data
1% Sodium dodecyl sulphate	56,14	Research data
Cell free broth (Crude bioemulsifier)	45.85	Research data*
Cetyltrimethylammonium bromide	30	Christofi and Ivshina, 2002
Tween 20	30	Christofi and Ivshina, 2002
Linear alkylbenzene sulphonate	47	Christofi and Ivshina, 2002

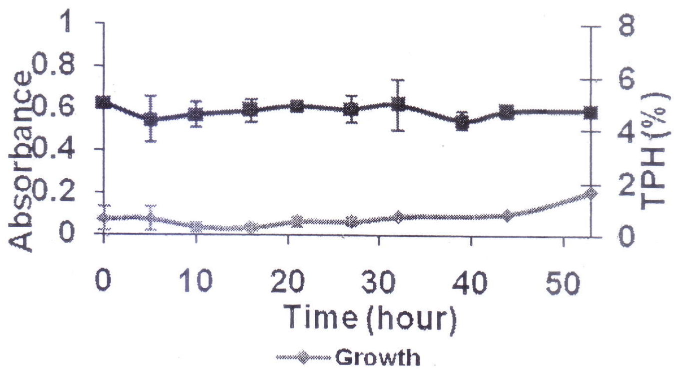
*Measurement after 48 hour incubation

From table 1 above, it could be seen that the surface tension of cell free broth (Crude Bioemulsifiers) showed relatively high surface properties compared to synthetic surfactant like Sodium Dodecyl Sulphate (SDS) as well as Linear Alkyl Benzene Sulphonate (LAS) in lowering the surface tension. This result suggest that bioemulsifier from *Azotobacter vinelandii* have a potential function as emulsifier/surfactant. Therefore, this bio-emulsifier could be utilized as surfactant in order to improve the biodegradation of petroleum oil process. The main physiological role of bioemulsifiers is to permit microorganisms to grow on water-immiscible substrates by reducing the surface tension at the phase boundary. Therefore, this role makes the substrate more readily available for uptake and metabolisms. In addition to emulsification of the carbon source, they are also involved in the adhesion of microbial cells to the hydrocarbon. The cellular adsorption of the hydrocarbon-degrading microbes to water-immiscible substrates, and the excretion of surface-active compounds together allow growth on such carbon sources. In general, negatively charged bioemulsifiers would inhibit the process, whereas positively charged bioemulsifiers promote microbial adhesion to hydrophobic phases. Furthermore, bioemulsifiers have been shown to be involved in antagonistic effect towards other microbes in the environment.

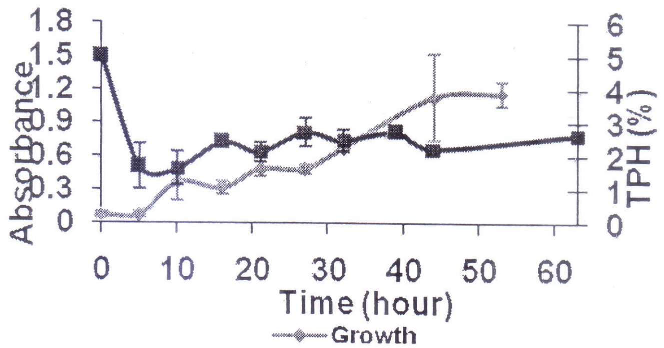
Bioemulsifiers are also used in emerging technologies like microbial remediation of hydrocarbon and crude oil–contaminated soils. Hydrocarbon contaminants are removed from the environment, primarily, as a result of their biodegradation, which is performed by native microbial populations. Such biodegradation is known to be time-consuming and new technologies have been developed; for example bioaugmentation, biosurfactants and etc. While, the effectiveness of enhancing hydrocarbon degradation through addition of microbial inocula of non-indigenous populations (bioaugmentation in essence) has been ambiguous, the use of a combination of new approaches has shown much success. For instance, the addition of bioemulsifiers help to stimulate the indigenous microbial population to degrade hydrocarbons at rates higher than those which could be achieved through addition of nutrients alone. A good example of this is the use of rhamnolipid from *Pseudomonas aeruginosa* to remove large quantities of oil from contaminated Alaskan gravel a decade back, in the Exxon Valdez oil spill.

A laboratory scale of bioemulsifiers enhanced biodegradation of crude oil was conducted. The effects of addition of bioemulsifiers from *Azotobacter vinelandii* in the biodegradation process are shown in Figure 1a-1e.

a)



b)



Continued............

c)

d)

e)

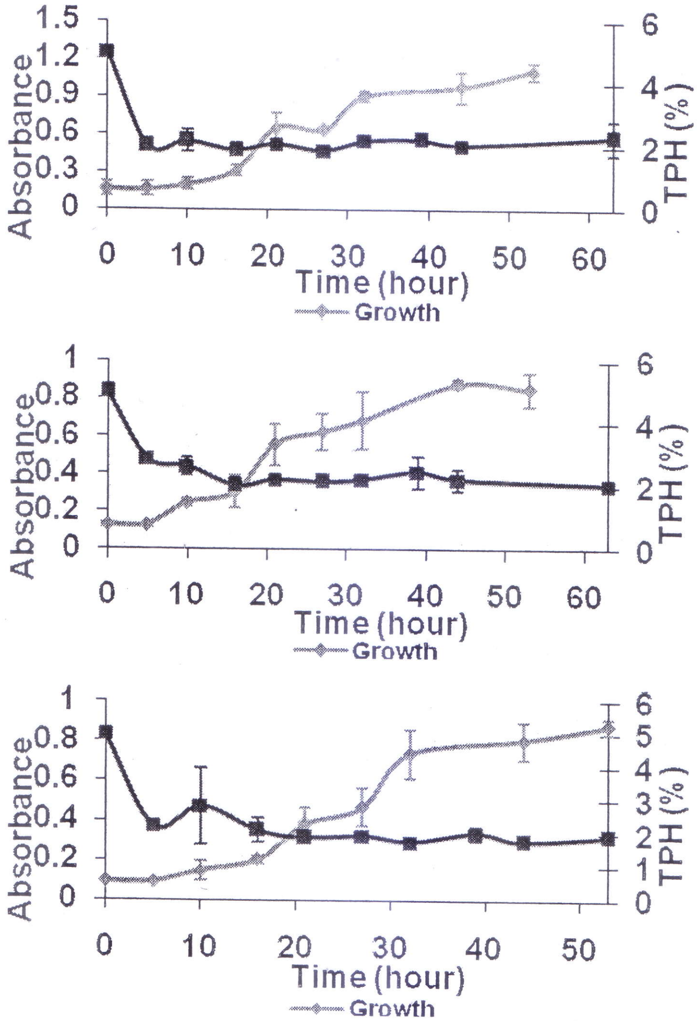


Figure 1 The biodegradation of petroleum oil in the presence of bio-emulsifier produced from *Azotobacter vinelandii* (a). Control, (b). 0%, (c). 1.5%, (d). 3%, (e). 5%

Figure 1 showed the performance of Petrophylic bacteria in degrading crude oil. There was no considerable decrease of Total Petroleum Hydrocarbon (TPH) concentration in the control reactor where was no addition of petrophylic bacteria in it. The reduction of TPH in this reactor was most likely due to physical influences such as temperature and volatilization of low molecular weight of hydrocarbon. In contrary, there was a significant decrease of TPH concentration that occurred in the reactor where petrophylic bacteria were added. These results suggested that the bio-augmented bacteria could degrade TPH significantly compared to control reactor. Physical and chemical transformation might also occur in the process as shown in reactor control. However, based on research result the biological transformation seemed to dominate the process. The growth of bacteria in the reactor was also observed during the process. This related to the biological transformation during the process in which the number of bacteria could be maintained during the degradation process. It was proved that the addition of bio-emulsifiers increased the biodegradation efficiency of petroleum oil removal markedly as showing in Table 2.

Table 2 TPH removal efficiency in Presence of Bio-emulsifier

Parameter	Initial TPH (%)	Final TPH (%)	Efficiency (%)
Control Reactor (- Petrophylic (P), - Bioemulsifier)	5	4.757	4.86
Reactor 1 (+ P, - Bioemulsifier)	5	2.648	47.04
Reactor 2 (+ P, + Bioemulsifier 1,5%)	5	2.296	54.08
Reactor 3 (+ P, + Bioemulsifier 3%)	5	2.042	59.16
Reactor 4 (+ P, + Bioemulsifier 5%)	5	1.910	61.80

(-): without; (+): with

It was evidence that bio-emulsifier could enhance the biodegradation process. The limiting factor in the degradation of hydrocarbon and other PAH is their insolubility, thus decreasing the efficiency and rate of degradation. This limitation can be overcome either by addition of surface-active compounds (surfactant) to the growing culture, thus making hydrocarbons more water-soluble and available for the cell to degrade, or by production of its own surfactant by the organism to facilitate the uptake process. Bio-emulsifiers are 'amphiphilic' molecules consisting of both hydrophobic and hydrophilic domains giving them the characteristic property of organizing at interfaces of different degrees of polarity such as oil/water or air/water and helping to lower the interfacial energy/tension. The addition of bioemulsifier could enhance the degradation process by making the crude oil more soluble and readily to petrophylic in attacking the substrates as indicated by its growth. The TPH removal efficiency of reactors after running for about 3 days were 47.04% for reactor with addition of petrophylic bacteria only and 61.8% for reactor with addition of petrophylic bacteria and 5% bioemulsifier, whereas the removal efficiency in the reactor control was 4.86% as shown in Table 2.

4. Conclusion

The bioemulsifier produced by *Azotobacter vinelandii* exhibited significant emulsification properties. The effect of addition of bioemulsifier in the crude oil biodegradation process indicated the enhancement of both degradation process and growth of petrophylic bacteria. TPH removal efficiency increased from 63.43% up to 82.04% as the addition of biosurfactant to the process.

5. Acknowledgement

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