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ENVIRONMENTAL REMEDIATION FULL-SCALE IMPLEMENTATION: BACK TO SIMPLE MICROBIAL MASSIVE CULTURE APPROACHES

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Abstract

Using bioaugmentation and biostimulation approach for contaminated soil bioremediation were investigated and implemented on field scale. We combine those approaches by culturing massively the petrophilic indigenous microorganisms from chronically contaminated soil enriched by mixed manure. Through these methods, bioremediation performance revealed promising results in removing the petroleum hydrocarbons comparatively using metabolite by product such as biosurfactant, specific enzymes and other extra-cellular product which are considered as a difficult task and will impact on cost increase.

Keywords: bioremediation, environmental biotechnology, oil and gas industry

1. Introduction

Since early 1990s, waste biotreatment that contains the group of compounds known as petroleum hydrocarbons has been developed in Indonesia [1,2]. The negative effects of these compounds are well documented in terms of personal health and that of the environment. Bioremediation technology has enjoyed a growth in popularity over recent years, not least because it is the ‘greenest’ option for petrochemical waste disposal. Unlike chemical treatments, which produce their own waste bi-products, a bio-treatment involves microbes that simply die off once processing is complete. In addition to this, it requires a modest capital investment and low level energy input, is self sustaining and environmentally safe [3]. This paper describe over the time improvement basing on five platforms technology which have been commensurate for bioremediation research and development. To shed some light, we provided the highlighted of several full-scale implementation of the bioremediation conducted by our institutions.

2. Methods

Platforms Technology

Bioremediation agent. Bioremediation is the technology that utilizes the metabolic potential of microorganisms to clean up contaminated environment. This technologies share the advantages of *in situ* application and environmental acceptability compared with most physicochemical strategies. In this paper, we focus on bioremediation rather than other biological remediation techniques such as phytoremediation. Current targets include petroleum hydrocarbons products, especially for hazardous crude oil compounds such as BTEX (Benzene, Toluene, Ethylene dan Xylene) and PAHs (Polycyclic Aromatic Hydrocarbons). The technology enables detoxification of environmental areas where the bioremediatory organism is administered with a supplementary source of nutrients [4].

Bioproducts. Extra-cellular microbial product have been shown to play key roles in optimalization into the overall clean-up process a contaminated sites leading to cleaner, faster, cheaper by bioremediation efforts. By nowadays, we develop an *in situ* exploration and exploitation potential resources from many vulnerable ecosystem, notably for many microbial strains capable to reduce recalcitrant from petroleum hydrocarbons. We consider a deeper understanding of the regulation microbial extracellular production, fundamental relationship between biosurfactant, contaminant solubilization and biodegradation rates as our pillars strategy in integrated waste management technology.

Metabolic pathways for pollutants decomposition. Petroleum is a complex mixture of hydrocarbons, but it can fractioned into aliphatics, aromatics, asphaltics and small portion of non-hydrocarbons compounds [5]. The general outline bioremediation pathways for aliphatic and aromatic hydrocarbons have been formulated and continues to be developed in greater detail with time. Best practice of such concept was implemented by alternate aerobic-anaerobic modes, depend on the nature of the substances.

Chemical dispersant agent. We develop the application of the chemical dispersant agent, a surfactant-based compound in order to accelerate petroleum hydrocarbons degradation and other pollutants and facilitate their mineralization by forming more labile organic compound through the breakdown of intra-moleculars bonds. Our current application related to this section is to improve chemically, stabilization and/or solidification processes of pollutants prior to disposal in an off-site landfill.

Physical agent. Contaminant bioavailability to microorganisms is affected by sorption/desorption and also by grain size on the soils/sediments. That sorptive properties of soils greatly influence the kinetic behavior of their indigenous microbial communities. Other studies on some physical parameteres (*e,g.* cation, porosity, hydraulic conductivity, and grain size) were performed on representative soil/sediment samples to identify and quantify other parameters affecting in situ bioremediation of the soil/sediment.

3. Results and Discussion

Environmental Biotechnology Products

Base on the five platforms used as a technology development, from several treatability test on the laboratory, we can therefore grouping our biotechnological products as follows:

Bacterial strains cultures. Conducted research was made in order to isolate a maximum (cultivable and viable cells) strain potentially capable to degrade petroleum hydrocarbon compounds and then to stimulate theirs biosurfactant production. Some PAHs were used as model organic contaminants to study the effects of petroleum hydrocarbons on marine sedimentary bacterial compartment. The culture strains are then sublimized with the following PAHs: Phenothiazine, Fluorene, Fluoranthen, Dibenzothiophene, Phenanthre and Pyrene. Overall, our consortia collection was originally isolated chronically contaminated maine sediment, from crude oil contaminated soil (COCS), mud pit (MP), and compost's material. For instance, from a mangrove's sediment chronically contaminated by petroleum hydrocarbons, 16 different colonies have been isolated and then *Flexibacteraceae bacterium, Bacillus aquimaris, B. megaterium, B. pumilis, Halobacillus trueperi* and *Rhodobacteraceae bacterium* have been identified through 16s DNA amplification using primer 9F and 1510R [6].

Bioproduct (Metabolite by product). Metabolites by product of the microbial consortia culture was obtained and separated as a biological product containing surfactant and other unidentified presence of extra cellular product. They simply catalysts result in the acceleration of the rate of chemical reactions. Reference [7] reported that hydrocarbons have a low solubility in seawater but their bioavaibility can be increased by the extra cellular metabolite product (*i.e.* exopolysaccharides; surfactant) that would be produced by microorganisms. Since bacterial growth required a direct contact of cells with a solube and/or non-soluble substrate, stimulation by using chemical or biological dispersant (emulsifier, surfactant) will increase degradation rate for many petroleum hydrocarbons compounds such as paraffin [8]; Polycyclic Aromatic Hydrocarbons [9]; crude oil [10,11]. Reference [12] showed high capacity of microbial adhesion to an hydrophobic substrates. We suggest some specific enzymes have been induced and released into liquid media system. Our isolates bacteria may produce their own specific enzymes in order to facilitate the uptake of their sources of carbon and energy. So, by combining the proper bacteria strains with the appropriate bioproduct, Mixed formula have an immediate action simultaneously.

Advantages: 1) Applicable for oil contaminant/spills (in case of Marine Oil Spillage) on site; 2) Remediation of remote areas where mobilization of traditional equipment is not possible.

Surfactant (Chemicals agent). We develop a methodology which consist a mixture of chemical substance specifically designed to assist in the resolution of emulsions. Such mixture has proven efficacy in resolving oil in water emulsions and inverse emulsions.

The application of such mixture is highly effective in a variety of oil/water/solids. The mixture can help produce high clarity water, a dense rag layer and a clean, high quality oil phase. The mixture compounds are usually slug fed neat to a point of good mixing in the application.

Advantages: To reduce surface and interfacial tension, the mixture works by destabilizing oil-water or water-oil emulsion and then to precipitate oil compounds into organic phase (separation)

Full-Scale Implementation of the Bioremediation.

The main objective of a full-scale bioremediation process is to modify environmental conditions so that specific targeted contaminants are converted to those of lesser environmental concern at acceptable rate and cost.

Obviously, such technology is used by enhancing microbial activity in hydrocarbon-contaminated soil by maintaining certain parameters at optimum conditions. Those parameters include nutrients availability, oxygen levels, moisture content, pH, conductivity, temperature, absence of toxic or inhibitory substances as well as the presence of a consortium of microbes capable of affecting the desired conversion [3].

Unocal Indonesia (1999-2003). Two consecutive project collaboration with UNOCAL and Bogor Agricultural University have been done. Two projects respectively are (1). Hydrocarbons degradation with bioremediation technology sludge hydrocarbon at UNOCAL Santan Terminal Indonesia and (2). Integrated waste management for hydrocarbons sludge at Santan Terminal, UNOCAL Company Indonesia. Both project have done a good performance since TPH and some heavy metal clean-up has been achieved through bioremediation process. When the project is held, landfarming and static biopile were used in application.

The initial TPH concentration was 15-35 % and following the process (55 to 90 days), the TPH concentration was decrease signficanly to less than 2000 ppm. Biodegradation its self has been the major factor eliminating TPH. For this purpose, we have to improve certain environmental conditions such as soil texture, salinity, pH, temperature, moisture, nutrients and oxygen availability. All these environmental conditions can be manipulated relatively easily so that they are no longer limiting for bioremediation but enhance the degradation rate of the target contaminants.

During this task, we simply maintain the optimal growth condition for indigenous microorganisms without any complementari use of the Bioproduct or chemical agent to remediate the soil.

Brunei Shell Petroleum (2002-2006). Slope and Sludge tank cleaning for hydrocarbons sludge at Seria Crude Oil Terminal (2002-2006) is another bioremediation implementation project. By this time, we engaged to use slurry-phase treatment by using Bioreactor. Upon completion of the 4-6 processing bioreactor, about 8,000 m^3 of oil sludge was loaded on and spread out. By this project, we use for the frst time what we call. About 150 m^3 of *Bioenzymes 2003 1-30, SludgeBbreaker and Bonia* were added to improve the rate of biodegradation. The results was promoter, within 30 days 150.000 ppm of TPH can be removed to becoming less than 10.000 ppm. Does the bioproducts effectively work? Simple dilution has been made? This forum will shed some light. Infact, the availability of major nutrients, nitrogen and phosphorus were monitored and measured to assure their availability for the microbial growth.

Brunei Liquid Natural Gas (2004-2006). Bioremediation process for other type of waste: Oxazolidone. This compound is the byproduct of a washing process that use sulfonil to rid natural gas of carbon dioxide. With the similar process (bioreactor system), we demonstrated the important removal of derivd-oxazolidone whereas Dipa-oxa (74 to 12 %), Dipa (1.2 to 0 %), Sulfolane (4.5 to 0 %), DEA (3-0 %).

Exxonmobil Oil Indonesia (2006-2007). 6000 m^3 of oilly waste from mud pit has been treated and backfilled into land spread area. Simple methods by culturing hydrocarbonoclastic bacterium consortia massively prior for bioaugmentation of the bioremediation process are used in this project. The extent of Total Petroleum Hydrocarbons was lower than the threshold value administered from the Indonesian Environmental State Agency (KLH). During monitoring program three month after disposal), we observed more than 95 % of landspreading area was covered by the wild life habitats including grass, leguminaseae, shrub, trees and the animals [13].

Others Project. By using a combine techniques, through bioaugmentation (addition adaptive microorganisms) and biostimulation (addition of metabolites by products) as well as physico-chemical reaction, anothers bioremediation project have been done by our institutions. For instance, microbial addition and supplier for Landfarming program at Badak Field, Vico Indonesia Company in 2003, Mud drilling bioremediation project at East Kalimantan in collaboration with Baker Hughes and Total, and several simulating testing from local and regional oil and gas company.

4. Conclusion

Full-scale application of bioremediation technology has proved that, sophisticated treatment by separated treatment by treatment can be successfully utilized to decontaminate soil matrix. For Indonesian scientist, such challenge and opportunities are open in order to improve deployment of this technology. A microbial ecology study at the microcosm level, for instance, can be developed in this field. Specific activities (*i.e.*, photosynthesis, respiration, degradation) identified through microscale geochemistry (microcosms) may be correlated with changes in community structure, helping to define the actual degradation pattern. Nevertheless, base on our experiences, simple technique to maintain optimum growth condition of microbial massive culture is also prompted potential for reducing oily waste and its process and operational implicities make it attractive for further field application developments.

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