



Proceedings of
Asia-Pacific Workshop
on Ecohydrology,
Indonesia, 2001

UTILIZATION OF BACTERIAL TABLET FOR BIOREMEDIATION OF PETROCHEMICAL AND AROMATICS CONTAMINATED SOIL

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ABSTRACT

The formation of bacterial tablet as microorganisms source for the biomineralisation of petrochemical and aromatics compound has done. The bacterial tablet were containing the mixed of mineral from the nature and activated carbon as carrier and additif. Physico chemical characteristic of the minerals and activated carbon support the microorganisms in the tablet form which are shown by the viability of the bacteria and they capabilities on the biomineralisation of petrochemical and aromatics compound after store for 14 months.

Petrochemicals and aromatics compound which are degraded by the cultures are benzene, p-nitrophenol, 2,4 dichlorophenol, phenol, p-chlorophenol, aniline and benzoic acids. The viability and capability of bacterial tablet shown tha the tablet have a great potential in the bioremediation of petrochemicals and aromatics contaminated site or soil, especially site which are near the aquiver or water body.

Key words: bacterial tablet; bioremediation

1. INTRODUCTION

Remediation is an important activity to solve the problem of contaminated or polluted area such as agricultural area, petrochemical industrial area, pesticide storage sites

and others. In the decade of 80's, remediation of polluted area was established. Remediation technology was by excavation of polluted soil's and substitution with unpolluted soils to become a clean and unpolluted

area. The excavate soil's later then washed or treated by thermal desorption. Such a technology has some problem namely the problem of liquid waste from washing of contaminated soils, emission of pollutant by thermal desorption and the technology consumed so much energy and known as intensive technology

Nowaday such treatments are inefficien meanwhile, contaminated area to be remediate are abundant separately through the nation and the industry as owner of contaminated area or who contaminated the environment are unable to remediate the sites. This is a big problem. Whenever intensive technology used to remediate the sites, the industries should spent a lot of money . It affect the competitiveness in the market significantly, their competitiveness would be lower. On the other hand, whenever the industries stop their operation, the oportunities to work and working place would be decrease or loosing. In the remediation activities, consumption of energy, emmitted of waste and water pollution have impact on the environmental merit. The performance of technology should be pointed out as critical criteria to be choosen and applied for remediation. Energy consumption by using fossil energy is problematic. Fossil energy emmit some substances in their use which are toxic and unfriendly to the environment beside the problem of economical cost. Minimization of the using fossil energy by the use of alternative energy such as solar energy and wind energy should be take as opportunities or by the changes the intensive energy process with the less intensive energy consumption such as biological process.

To solve the idustries problem on the remediation of contaminated sites are by the using of technology which are consume less energy and

less resources. By the use such technology, the industries could manage their competitiveness in the market, has good revenue and due their duty on environmental protection namely remediate all sites which potentially to be polluted. Technology to be used should be an extensive technology.

2. BIOREMEDIATION AS AN EXTENSIVE TECHNOLOGY

The aims of remediation of contaminated sites are to minimize environmental damaged by pollutants. In many case the purpose of remediation in certain area only to solve the problems without comperhensive analysis. Global evaluation on the activity is needed. The environmental merit analysis is more useful, while any activity on the remediaton have impact on the environment such as:

- 1.global warming
- 2.aquatic toxicity
- 3.terresterial toxicity
- 4.waste disposal
- 5.energy consumption.

The aims of remediation using environmental merit analysis is needed to decide which remediation should be in charge to overcome the problem. Environmental criteria which are important namely : Economical impact, social aspects and the perception of population (Heijnings *et al.*, 1993)

Generally, remediation should be; Minimize the risk such as minimisation the concentration and toxicity of pollutant, and limitation of pollutant mobilities, has minimal impact to the environment (environmental merit) and low cost.

The characteristic of extensive technology is that the needs technology on the resources is low which are the cutting edge to the intensive technology. In this case remediation

is a long term process. Long term process may be inappropriate for any location such as site which are immediately develop to a certain purpose, but the most important is the choosing of the treatment should be well planned and meet the need of environmental problem solving.

Some extensive technology which are appropriate in remediation namely: 1. in-situ soil flushing or in-situ soil washing. 2. bioventing, 3. bioventing and composting, and 4. utilization of microorganisms inoculum,

1. In-situ soil flushing or in-situ soil washing stressed on the washing contaminated soils on sites and collection of waste water. The waste water treated further more to eliminate the toxic substances. However, it is difficult to decide that all toxic substances was removed by washing, because each layer of soils and rocks has different pollutant absorption capacity
2. Bioventing is an integrated system with Soil Vapour Extraction method (SVE). The methods is appropriate to eliminate unstable organic compounds by microorganisms. The oxygen which are needed for microorganisms growth easier to supply compare to water. It based on the nature of water which has a limited on the oxygen transportation.
3. Bioventing and Composting. The process is by mixing of contaminated soils with organic compounds to produce compost. Supply some strain bacteria on the process is helpful on the biodegradation of toxic compounds and control on the process is significantly important to have an optimum product.
4. Utilization of microbe strain into contaminated soils. Contaminated soils in this process firstly analyze

and later then the microbes to be used in the process are selected. Availability of the capable microbes to degrade toxic compounds is the key of the process. The microbes to be used could be a mixed or pure culture, which are depend on the capability on the biodegradation of the inocula and their physiological characteristics.

The choose of a certain technology to be used not only from the view of cost, but the purpose of the activities and the environmental merit should be as central parameters. It is important to remember that there are no perfect technology hundred percents. Each technology has their limits. Thoroughness is the key word.

3. THE POTENTIALS OF BACTERIAL TABLET ON THE BIOREMEDIATION OF AROMATICS POLLUTED SITES

Bacterial tablet was consist of some minerals and mixed bacteria which are able to degrade phenolic compounds. The minerals use for tablet formation are zeolite, dolomite activated carbon and some additif as binder. The variation of minerals percentage was experimented and the best ratio was 60:30:10 for zeolite, dolomite and activated carbon. Using the ratio, it found tha the texture of tablet is stable in the form and rigidity but easy to dilute in the water or destroy by finger pressure. Such characteristics are appropriate for transportation and the end use of the tablet. Further more, calcination of zeolite in certain temperature found that calcination at 200 ° C have effect on the retaining of bacteria on the sedimentation as shown in table 1. The result, later then was used for the formula on the formation of tablet.

Table1: Bacterial content of tablet with variation of calcination temperature of zeolite

Nr.	Calcination of zeolite	Bacterial count in suspension (cells/ml)	Weight of tablet (mg)	Viable bacteria in the tablet (cells/mg)	Observed percentage (%)
1	100 ° C (Z ₁₀₀)	15	530	5.66 x 10 ⁶	20,2
2	200 ° C (Z ₂₀₀)	25	520	9,6 x 10 ⁶	34,29
3	400 ° C (Z ₄₀₀)	13	520	5 x 10 ⁶	17,86

Furthermore on the evaluation of viable cells in the tablet after keeping for 14 months in the refrigerator at 10 °C, found that the suspension of tablet using the 200 ° C calcinated zeolite was better than 100 °C and 400 °C calcinated zeolite. Therefore the using of 200 ° C calcinated zeolite with the ratio 60 % of the tablet is the preference on the production Table2. shown the viable cells from suspension of tablet in the series of dilutions. For higher dilutions found that growing cells was detected although it take longer time of incubation.

The capability of bacterial tablet on the degradation of phenol was reported elsewhere (Sembiring and Susilorukmi,1999a). The differentiation of mixed cultures using agar slant and/or agar plate methods was succeed on the isolation of 2 strain bacteria I₄ and I₈. The pure culture of isolates I₄ and I₈ are able to degrade aromatic compounds. Petrochemicals and aromatics compound which are degraded by the cultures are benzene, phenol, p-chlorphenol, p-nitrophenol, 2,4 dichlorphenol, anniline and benzoic acids.

Table 3 shown the petrochemical and aromatic compounds using bay

the strain I₄ and I₈ from bacterial tablet. The media for growth was minerals media (Henry and Grbic-Galic, 1990). The petrochemical compounds or aromatic compounds was using as singgle carbon source for bacterial growth. Meanwhile the biomineralisation of phenol and 2,4 dichlorphenol by strain I₈ shown that, the mineralisation of 2,4 dichlor phenol was only occur in the presence of phenol. In this case the function of phenol was as inductor for the strain on the biodegradation of 2,4 dichlorphenol. Figure 1. Shown the biomineralisation of phenol and 2,4 dichlorphenol.

Biomineralisation of phenol and benzene by the strain I₄ were very fast and the growth of bacteria in both substances were better then in the p-chlor phenol, anniline, p-nitrophenol and benzoic acids. The strain of I₈ could also use glucose and alcohols as carbon source for the growth. (Sembiring and Susilorukmi, 1999b) The ability of strain I₄ and I₈ on the mineralisation of aromatic compounds as shown by the experiment is potentially for the using of bacterial tablet in the bioremediation activity.

Table 2. Viable cells for each dilution of tablet suspension using zeolit with variation of calcinated temperature

Days	Viable cells of bacterial from tablet suspension														
	Z_0					Z_{200}					Z_{400}				
	10^0	10^1	10^2	10^3	10^4	10^0	10^1	10^2	10^3	10^4	10^0	10^1	10^2	10^3	10^4
0	9-10	6-7	b.t.	b.t.	b.t.	8-9	5-6	b.t.	b.t.	b.t.	6-7	4-5	b.t.	b.t.	b.t.
4	t.d.	t.d.	5-6	b.t.	b.t.	t.d.	t.d.	t.d.	6-7	b.t.	t.d.	t.d.	t.d.	6-7	b.t.
6	t.d.	12	8-9	7-8	4-5	t.d.	10-11	6	5-6	3-4	t.d.	9-10	7-8	6	b.t.
12	t.d.	14-15	10-11	7-8	5-6	t.d.	10	5-6	6	2-3	t.d.	9-10	6-7	5-6	3-4
22	t.d.	t.d.	26	22-23	16-17	t.d.	30	16	9-10	3-4	t.d.	31-32	10-11	6-7	3-4

Legends :

t.d. : no calculation (clogging)

b.t. : still no growth

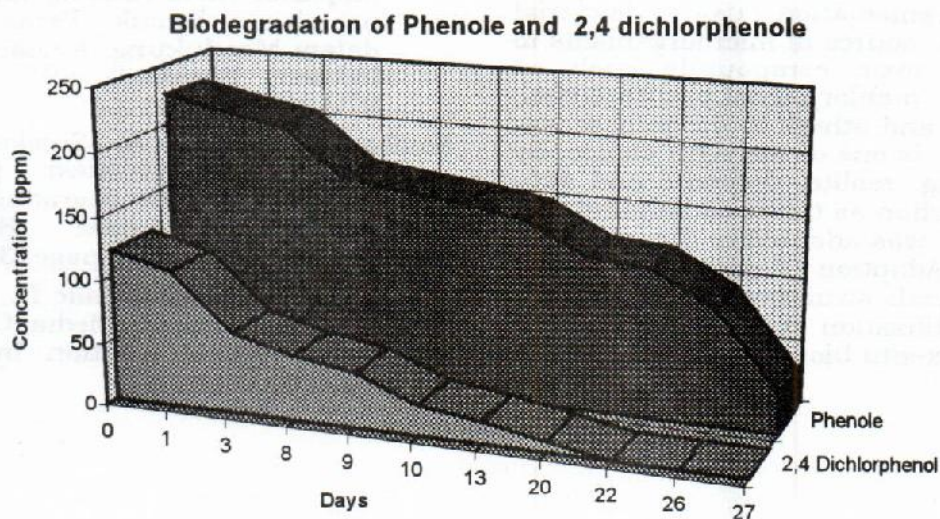


Figure. Biomineralisation of Phenol and 2,4 dichlorphenole at room temperature

Table 3. Aromatic compounds using as carbon sources for cultivation of strain I4 and I8

Nr	Aromatic compound	Strain I4	Strain I8
1	phenol (10 mM)	++++	++++
2	p-klorophenol (0.4 mM)	+++	+++
3	p-nitrophenol (0.25 mM)	+++	+++
4	2,4-dichlorphenol (0.50 mM)	+++	++
5	Aniline (1.0 mM)		++
6	Benzene(10 %)	++++	-
7	Benzoic acid	+++	+++

4. SUMMARY

Contamination of industrial sites especially by aromatic and petrochemical compounds are occurs in the petrochemical industries and petroleum company areas. Bioremediation of such areas is very important to have a clean environment. Extensive technology is the right choice to have an effective and efficient process. Bioremediation using bacterial tablet as source of microorganisms to degrade toxic compounds such as phenol, p-chlorophenol, p-nitrophenol, benzene and others in the soils or water body is one of the right choice. By the using, zeolite, dolomite and activated carbon as tablet constituent, the bacteria was adapted to the minerals sistem. Adaption of microorganism in the minerals structure has the benefit in the utilisation of the tablet for in-situ or ex-situ bioremediation.

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