

ASSESSMENT OF INDIGENOUS MICROORGANISM FOR NATURAL ATTENUATION OF ABANDONED MINE SITE

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ABSTRACT

Soil samples taken from an abandoned gold mine site in East Kalimantan exhibited high concentration of manganese (9,830-13,190 mg/L), iron (340-8,060 mg/L) and arsenic (160-510 mg/L). However, the indigenous microbial population was found to be present at high level ($> 10^7$ CFU/g), indicated that the site matrix did not appear to be inhibitory and relatively favorable to microbial growth. Furthermore, laboratory microcosm studies showed that the indigenous microorganisms possessed a considerable bioremoval potential of contaminated metal at the site. Those results suggested that natural attenuation of metal bioremoval would occur under present site condition.

Keywords : Abandoned mine site, indigenous microorganism, natural attenuation, bioremediation, microcosm study

1. INTRODUCTION

Indonesia has many abandoned mine sites that located throughout the country. Clean-up of abandoned mine sites for redevelopment provide an opportunity to turn these sites into land that has beneficial uses. Mine sites have a variety of potential reuses depending on location including recreation, wildlife habitat, rangeland, historic and scenic preservation. A major challenge in cleaning up and redeveloping mine sites is finding resources that are needed to assess and address potential contamination at these large complex sites. Environmental contamination can come from mine drainage, waste rock, dump leaches, heap leaches, tailing, surface water run-off from open pits, ore stockpiles, etc. In addition, mine sites are typically characterized by abnormally low pH (highly acidic), acute toxicity of the metals in the soil, nutrient deficiencies and lack of vegetation.

There are several clean-up approaches commonly used at mine sites. For example, contaminated soil can be excavated for disposal at an offsite landfill. Conventional treatment technologies for soil, ground water, or surface water include chemical

treatment (such as use of lime to neutralize acid mine drainage and to precipitate metals), stabilization, solidification, and vapor extraction. Innovative and emerging treatment technologies include phytoremediation and amended bioremediation. Residuals from waste-water treatment can be used as a soil amendment to add organic matter and nutrients to the soil to create a fertile soil horizon with a reestablished microbial community, invertebrates, and plants. Amendments can also address metals toxicity and acidity (US EPA, 2006).

To remediate the contaminated land and water at such post mining site, bioremediation is one possible technique to be used. Bioremediation is the use of microorganism to detoxify and degrade environmental contaminants (Baker and Herson, 2004). Bioremediation is a site-specific process and its efficiency may be limited by microbiological and physical-chemical conditions of the soil. The limiting factors for soil bioremediation include the type and concentration of contaminants and the indigenous microbial population, availability of nutrients and electron acceptors, pH, temperature, moisture content of soil and substrate bioavailability

(Balba et.al, 1998). In situation where bioremediation may be a potential component of the remediation plan as the main technique or in concert with other techniques, some assessment of the site's microbial population should be made at a very early stage of the overall site assessment process (Anderson, 1995). Moreover, if bioremediation is to take place in situ using indigenous microorganism (intrinsic bioremediation, natural attenuation), it is recommended to do laboratory microcosm studies using sample collected from the site (Unterman, 1995; Lamar, et al., 1999).

This paper presents the results of a study to investigate the potential of indigenous microorganisms for their potential use in bioremediation. The study included evaluation of present condition of an abandoned gold mining site in East Kalimantan as a case study, determination of contaminated metals in soils, water and plant, and isolation of native microorganisms. The microorganisms have been tested for their bioremediation potential by measuring pollutant degradation.

2. METHODS

2.1 Sample Collection

The samples of soil, water and plant were taken at different points of site: Location I (upstream), Location II (middle stream), and Location III (down stream). The distance of each point was about 1 km. The substantial water and sediment samples from the streams were taken and stored in plastic containers. The pH was measured by pH meter. The biotic and abiotic characteristics of the location were also observed. The nearest plant materials from the streams, as well as algae, were removed, including roots with as little soil as possible, then stored in plastic containers. The surrounding soil was taken and stored in other plastic containers. Upon return to the laboratory, all samples were stored in a fridge at 0-4°C.

2.2 Sample Separation

The sediment and water samples were separated using centrifugation. The plant materials were separated from contaminating soil by gently washing using reverse osmosis (RO) water.

2.3 Acid Digest

The type and concentration of metals in samples

were determined using acid digest method. Five grams of sample was put into a pyrex test tube, added by 8 ml of concentrated HCl, and boiled in hot plate for 10 minutes. The tubes were allowed to be cool, before added by 0.5 ml of concentrated HNO₃, and continued to boil for approximately 10 minutes until fuming ceased. The samples were then made up to a known volume in H₂SO₄ 0.01 M pH 2, and the concentration of metal ions were determined using AAS (Atomic Absorption Spectroscopy).

2.4 Degree of Binding in Soil Samples

One gram of soil sample was put into centrifuge tube, added by 40 ml of RO water, and then centrifuged at 5,000 rpm for 10 minutes. The washed water and the solid were weighed, then dried (as dry weight). The concentration of metals strongly bound to the soil and loosely bound in washed water was determined by acid digest and AAS.

2.5 Determination of Indigenous Microorganism

A one gram of solid sample from (2.3) was put into two conical flasks containing 100 ml of DM3 and 9K media respectively, and incubated at a rotary shaker at 30°C. DM3 medium consisted of (g/L): MnSO₄ 5.0, yeast extract 1.0, urea 2.0, glucose 25.8, KH₂PO₄ 0.05, MgSO₄.7H₂O 0.02, CaCl₂ 0.1, NaCl 0.2; and 9K medium consisted of (g/L): (NH₄)₂SO₄ 1.0, MgSO₄.7H₂O 0.2, KH₂PO₄ 0.5, FeSO₄.7H₂O 10.0. The number of viable cells in the samples was estimated using plate count method. The sample was diluted (serial dilution) using 0.9 % saline solution to 30-300 cell/ml. A 0.1 ml diluted sample was added to the agar plate and spread with an alcohol-flamed hockey glass stick. The plate was incubated for 24-48 hours at 30°C. The number of viable cells was calculated from the number of colonies and multiplying by the dilution factor. The number of viable cells was expressed as colony forming units per gram (CFU/g). For comparison with the plate count method, the number of total cells in the sample was counted in a cell counting chamber of known depth (0.02 mm) and etched with squares of known area (1/400 mm²). The cells were counted under phase contrast using a light microscope at 400 x magnification.

2.6 Laboratory Microcosm Study

A mixture of soil sample from all location (50 g)

was put into 2 conical flasks. Each flask was added by 50 ml of H₂O and 50 ml of DM3 medium, then shake by hand. The mixtures were then separated by filtration. The supernatant and the solid were transferred into 2 separate flasks. Each flask was added by 100 ml of DM3 medium, and inoculated by 30 ml of indigenous microbial culture (mixed culture of 3 types of microbes), and incubated at a rotary shaker at 30°C. Periodically (at 1, 3 and 7 day), 5 ml of sample from each flask was removed and analyzed for metal concentration using AAS.

3. RESULTS AND DISCUSSION

3.1 Present Site Condition

The activities of the gold mine in the past have resulted in some clearing of land and vegetation, lost of top soil, and it will remain sterilized from future use unless rehabilitation is done. The topography of the area featured an undulating peneplain. Soils include massive brown-red earth, red (non-cracking) clay. There were significant areas with truncated profiles, chemically affected soil or places with the surface of the land covered with waste rock and process tailing. The creek water was extremely acid, as low as pH 2. There was little vegetation around the creek and dominated by native pastures.

3.2 The type and Concentration of Contaminated Metals in Soil, Water and Plant

According to the mineralogy of the ore (the past history of the site), all of soil samples predictably contain manganese, copper, zinc, iron and arsenic. Therefore, the samples were analyzed to determine the concentration of those metals. When metals are present under a more unavailable form, more severe extraction methods are necessary. In this case, acid digest method was used to leach out all metals in the samples. The results are presented in Table 1, Figure 1, 2 and 3.

From Table 1 and Figure 1, it can be seen that all of soil samples contained manganese in high concentration, ranging from 9,830 mg/L (middle stream) to 13,190 mg/L (upstream), but contained copper and zinc at relatively low concentration. The iron concentrations were as the range between 340 – 8,060 mg/L and for arsenic were ranging

from 160 – 510 mg/L. Soil sample from upstream showed the highest concentration level of all metal compared to that of middle stream and down stream. The results suggest the heterogeneity of soil characteristic. The soil at different location might have different composition, particle size distribution, percent moisture content and different cation exchange capacity which will affect the amount of metal content in the soil. The manganese and iron concentration in washed water from middle stream soil were also high and exceed their level in the soil itself. This indicate that in that particular location, the metals were not strongly bound to the soil, and it may have advantages to the bioremediation because it is often assumed that bound substrates are not microbially available until desorption occurs (Morra, 2006).

The sediment shows a slight lower metal concentrations than that of soil, except for arsenic. This high content of arsenic may be due to the difficulties in separation of algae from the sediment. The arsenic content in algae was found to be high (1,150 mg/L), and a small amount of algae possibly contaminated the sediment in its metal measurement.

The creek waters from all location had high concentration of manganese, copper, especially of iron (very high), but relatively low concentration of zinc, while for arsenic was undetectable (Table 1, Figure 2). The water was extremely acid, as low as pH 2. These results serve as evidence that leaching process by percolating rain and surface water was occurring at the site.

An interesting result was given by the level of metal concentration in algae and plant (Table 1, Figure 3). Algae contained 1,220 mg/L of manganese and 1,550 mg/L of arsenic. This was another indication that the water has been contaminated because sometimes the level of metal found in algae/plant biomass serve as index to detect the water contamination by metallic species (Paknikar et al., 1998). Furthermore, the plant from Location III (down stream) accumulated large amount of manganese, copper and arsenic in its roots and concentrated these heavy metals to levels far exceeding those present in soil and water. The plant from Location I seem to have ability to transport the metals to shoots, even to leaves since the metal contents in those plant's materials were also relatively high. It suggests that this plant potentially might play an important role in soil

Table 1. Concentration of selected metals in the samples

| Type of sample | Location | Metal concentration (%) | | | | |
|----------------------------|----------|-------------------------|---------|---------|---------|-------|
| | | Mn | Cu | Zn | Fe | As |
| Soil | I | 1.319 | 0.00097 | 0.0006 | 0.806 | 0.051 |
| | II | 0.983 | 0.00038 | 0.0003 | 0.034 | 0.016 |
| | III | 1.309 | 0.00076 | 0.0004 | 0.103 | 0.031 |
| Washed water | II | 1.059 | 0.00013 | 0.0001 | 0.514 | 0.003 |
| Sediment | I | 0.979 | 0.00023 | 0.00084 | 0.401 | 0.115 |
| Algae | III | 0.122 | 0.00011 | 0.00107 | 0.001 | 0.155 |
| Plant's roots | II | 0.353 | 0.00993 | 0.00034 | 0.210 | 1.012 |
| | III | 2.680 | 131.007 | 0.00613 | 0.271 | 1.518 |
| Shoots | I | 0.594 | 0.10749 | 0.00038 | 0.032 | 0.021 |
| Leaves | I | 0.162 | 0.05465 | 0.00014 | 0.64588 | 0.325 |
| Metal concentration (mg/L) | | | | | | |
| Creek water | I | 553 | 503 | 7.1 | 1,083 | 0 |
| | II | 651 | 444 | 2.6 | 661 | 0 |
| | III | 664 | 499 | 3.2 | 739 | 0 |

Location : I = upstream
 II = middle stream
 III = down stream

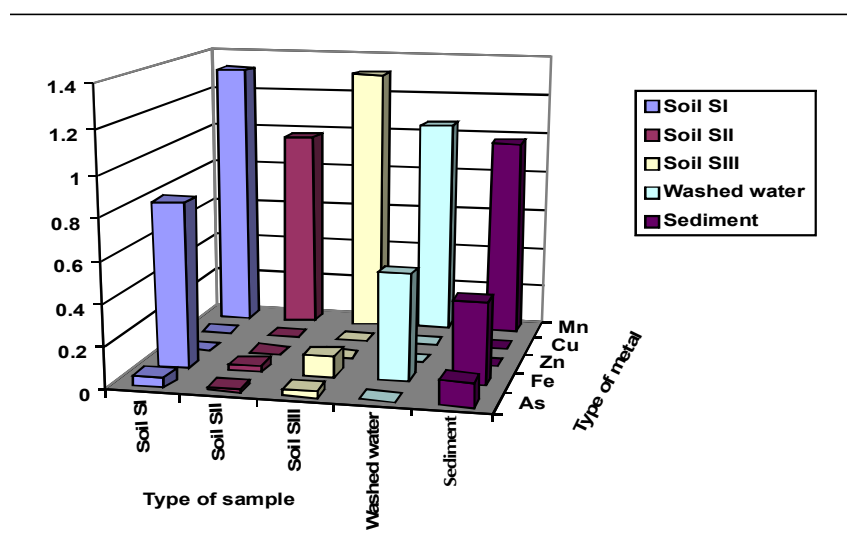


Figure 1. Metal concentration in soil, washed water and sediment

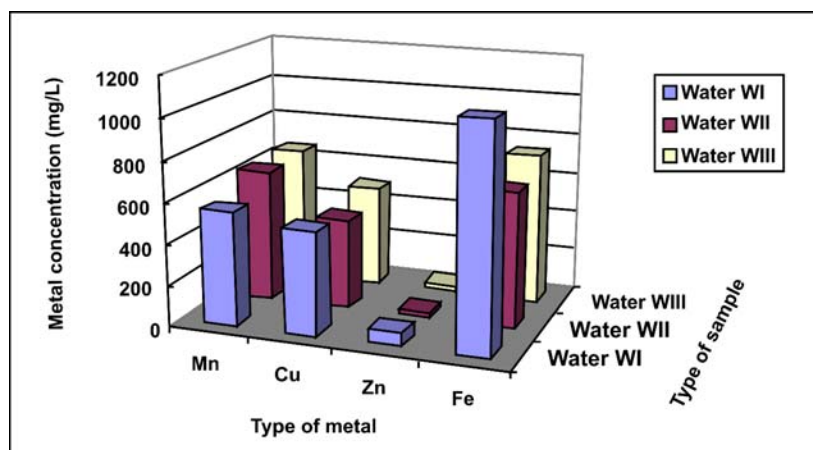


Figure 2. Metal concentration in creek waters

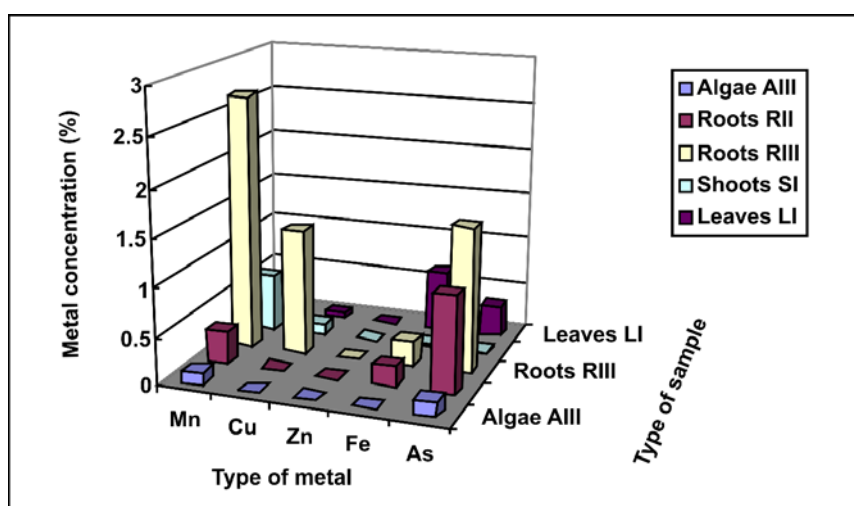


Figure 3. Metal concentration in algae and plant's materials

bioremediation (phytoremediation). This property perhaps can be exploited for soil reclamation if it is easily cultivated.

3.3 Microbial Enumeration

The microbial investigation was firstly concentrated on the quantitative determination of the types and numbers of indigenous microbial population present at the site using viable cell count and total count methods. The results are presented in Table 2.

The first impression is that the total amount of microorganism Type 1 was almost the same in all of soil sample (about 10^9 CFU/g). It was about a

hundred times higher than the values cited in the literature ($10^4 - 10^7$; Skladany and Baker, 1994). The results obtained indicate that the site possessed a rich microorganism population and might gave a signal that the environmental conditions were relatively favorable to microbial growth, and this population might sufficient to support bioremediation of the contaminant. However, the diversity of microorganism in the samples was a bit low because there was only three types of microorganism found although they had a variety of microbial colony in shapes, colors, and sizes. It is important to be noted that no single type of agar and nutrient would support the growth of all types of microorganisms. Viable counts on the same sample using different media can vary considerable

Table 2. Population of three types of microorganisms found in the soil samples (CFU/g)

| Location | Microorganism Type 1 | Microorganism Type 2 | Microorganism Type 3 |
|--------------------|----------------------|----------------------|----------------------|
| I (upstream) | 2.25×10^9 | 1,16 | 1.20×10^8 |
| II (middle stream) | 6.80×10^9 | - | - |
| III (down stream) | 1.00×10^9 | 150 | - |

Type 1 : small-white colony (\pm 1 mm)

Type 2 : very small-white colony (\pm 0.5 mm)

Type 3 : big-yellow colony (\pm 2-3 mm)

rably (Skladany and Baker, 1994). For that reason, the results of this plate count numbers should be interpreted as the minimum rather than the actual number of viable microorganism present in the samples.

3.4 Assessment of Natural Attenuation (Laboratory Microcosm Studies)

The laboratory microcosm studies were designed to determine whether the indigenous microorganisms possessed the appropriate biodegradative potential for the contaminants of concern. The results of this assessment are shown in Table 3, Figure 4 and 5.

From Table 3 and Figure 4, it can be seen that the indigenous microorganisms could remove 24.5 % of manganese, 62.6 % of copper and 4.4 % of arsenic from the soil within 7 days. During the first three days, there was a considerable decrease in manganese concentration from 526 mg/L to 299

mg/L, but then it increased again to 397 mg/L. This metal uptake trend possibly can be explained by a theory proposed by Diels (1997) who mentioned that many bacteria can solubilize metals in the soil via the production of siderophores (an iron complexing agent), and then adsorb the metals in their biomass. In this experiment, manganese might be highly solubilized but not readily adsorb by microorganisms within 7 days of culture, and might need more time to do so. Another possibility, the microorganisms may dead in this period of time because of the toxicity of manganese in high concentration (lethal dose), so this metal was released back to the environment

Meanwhile, in washed water (Table 3, Figure 5), there was 70.5 % reduction in manganese concentration, 42.6 % of arsenic and 32.2 % in copper concentration during 7 day period of incubation. This may imply that metal biosorption was occurring in the system. The final level of manganese and copper after 7 days of culture were 397 mg/L of manganese and 181 mg/L of copper in

Table 3. Uptake of manganese, copper and arsenic in the soil and washed water by mixed culture of indigenous microorganisms

| Type of culture | Time (days) | Metal concentration (mg/L) | | |
|-----------------|-------------|----------------------------|-----|------|
| | | Mn | Cu | As |
| Soil | 1 | 526 | 484 | 58.6 |
| | 3 | 299 | 307 | 57.1 |
| | 7 | 397 | 181 | 56.0 |
| Washed water | 1 | 217 | 374 | 40.4 |
| | 3 | 143 | 244 | 23.2 |
| | 7 | 64 | 254 | 23.2 |

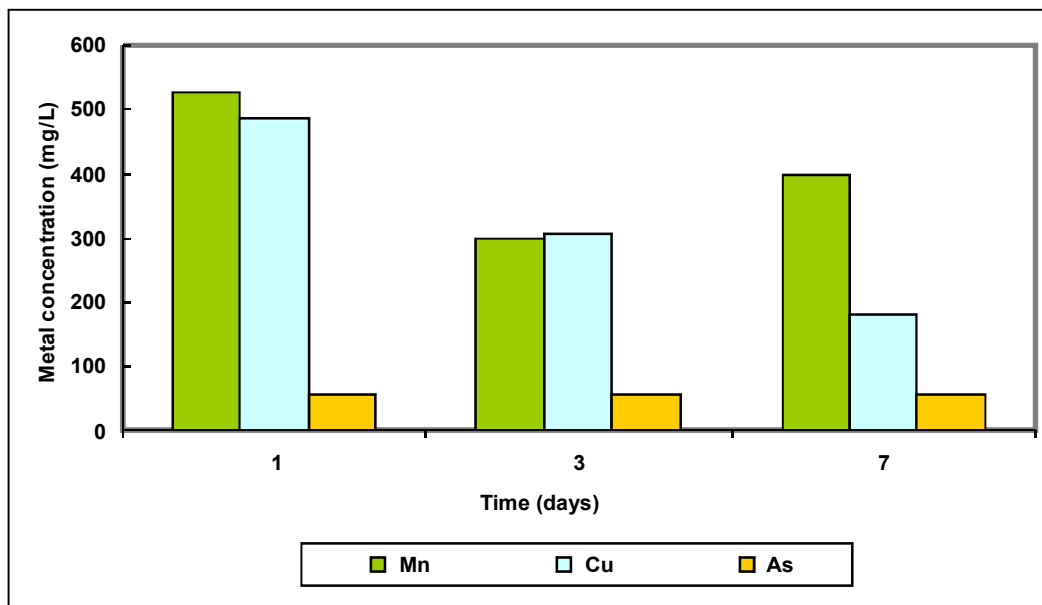


Figure 4. Uptake of manganese, copper and arsenic from soil by indigenous microorganism

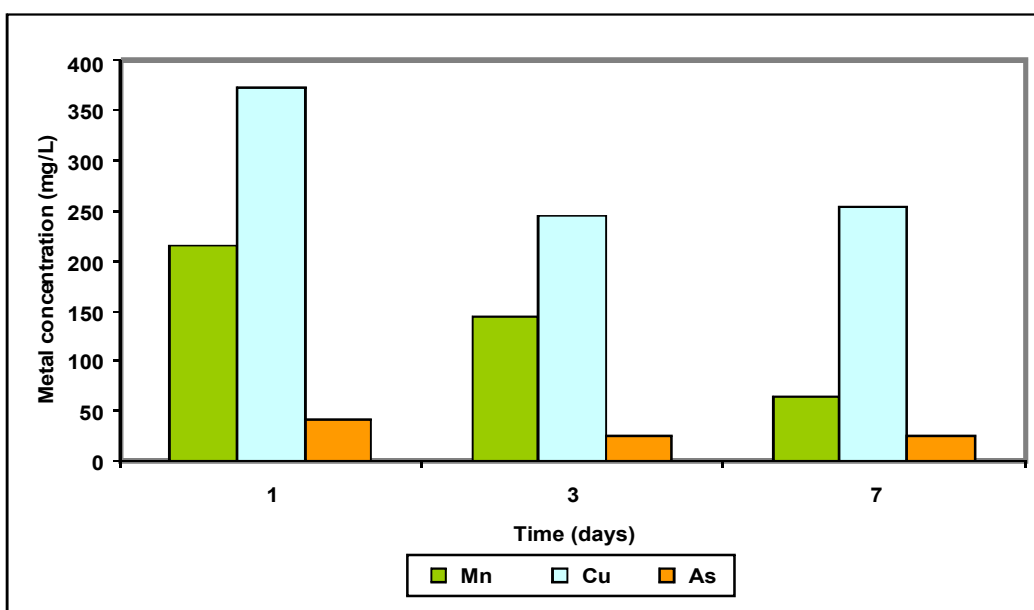


Figure 5. Uptake of manganese and copper from washed water of soil by indigenous microorganism

soil; 64 mg/L of manganese and 254 mg/L of copper in washed water. Those level were definitely still high. Nevertheless, these results seem to be attractive since the percent contaminant reduction were more than 20 %, except for arsenic in soil (4.4 %). Skladany and Baker (1994) proposed a “success” standard for biotreatability studies, that is the average contaminant concentration should be reduced by at least 20 % during a 6 to 8 week study to conclude that aerobic biological technique is a potentially suitable treatment for use at the site. They argued that this 20 % contaminant reduction “success” level is arbitrary to maximize the chances of the techniques in remedy screening evaluation. Therefore, a longer testing period is needed to be conducted in this experiment for biological processes to act sufficiently; to find out in what period of time the metals can be removed completely (duration of bioremediation) and what final concentration can be attained.

4. CONCLUSIONS AND RECOMMENDATIONS

These preliminary findings may suggest that the indigenous microbial population was found to be present at high level ($> 10^7$ CFU/g), indicated that the site matrix did not appear to be inhibitory and might support bioremediation of contaminated metal at this site. Those indigenous microorganisms possessed the bioremoval potential of contaminated metal at the site. It might provide a means of bringing this technique for next level of biotreatability testing such as toxicity testing and heavy metal tolerance, temperature tolerance, microbial inhibition testing (by oxygen uptake test), aerobic bioremediation studies to quantitative bioremediation (kinetics studies), and furthermore, if necessary, to enhance bioremediation rate by modifying present site condition (pH, oxygen, nutrient) with long-term monitoring.

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