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Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge

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Abstract

Biodegradation is cost-effective, environmentally friendly treatment for oily contaminated sites by the use of microorganisms. In this study, laboratory experiments were conducted to establish the performance of bacterial isolates in degradation of organic compounds contained in oily sludge from the Jordanian Oil Refinery plant. As a result of the laboratory screening, three natural bacterial consortia capable of degrading total organic carbons (TOC) were prepared from isolates enriched from the oil sludge. Experiments were conducted in Erlenmeyer flasks under aerobic conditions, with TOC removal percentage varied from 0.3 to 28% depending on consortia type and concentration. Consortia 7B and 13B exhibited the highest TOC removal percentage of 28 and 22%, respectively, before nutrient addition. TOC removal rate was enhanced after addition of nutrients to incubated flasks. The highest TOC reduction (43%) was estimated after addition of combination of nitrogen, phosphorus and sulphur to consortia 7B.

A significant variation ($P < 0.005$) was obser0ved between the effect of consortia type and concentration on TOC% reduction. No significant variation was observed between incubation at 10 and 18 days in TOC% reduction. This is the first report concerning biological treatment of TOC by bacteria isolated from the oil refinery plants, where it lays the ground for full integrated studies recommended for the degradation of organic compounds that assist in solving sludge problems.

Keywords: Bioremediation; Oil sludge; Total organic carbons (TOC); Degradation

1. Introduction

Large quantities of organic and inorganic compounds are released into the environment every year as a result of human activities causing serious environmental problems. Among those problems are oil contamination of soil and water from industrial sources and other activity, which create a great environmental hazard. Also, accidents are likely to occur in the form of pipeline leaks, transport accidents, storage tank ruptures. Petroleum hydrocarbon continues to be used as the principle source of energy and hence a large global environmental pollutant. Apart from accidental contamination of ecosystem, the vast amounts of oil sludge generated in refineries from water–oil separation systems and accumulation of waste oily materials in crude oil storage tanks poses great problems because of the expensive disposal methods [1,2]. Petroleum is a complex mixture of non-aqueous and hydrophobic components like *n*-alkane, aromatics, resins and asphaltenes. Many of these components are toxic [3], mutagenic and carcinogenic [4,5]. Therefore, their release to the environment is strictly controlled and they are classified as priority environmental pollutants by the US Environmental Protection Agency, due to their adverse impact on human health and environment [6]. A variety of methods have

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been suggested to treat these materials. Physical or chemical methods such as incineration, chlorination, ozonation, and combustion are expensive, requiring elaborate equipment and substantial amounts of additional fuel. Biological treatment of organic pollutants is a promising field of research, which gives reliable, simple and cheap technologies over chemical and physical processes [7–9].

Several laboratory and field tests have demonstrated that bioremediation could be a cost-effective clean-up technology to treat oily sludge and sediments containing biodegradable hydrocarbons and indigenous specialized microorganisms [10–13]. Bioremediation has become a major method employed in the restoration of oil-polluted environments, and attempts to accelerate the natural hydrocarbon degradation rates by overcoming factors that limit bacterial hydrocarbon degrading activities [14,15]. Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and amount of oil or hydrocarbon present.

During the last 20 years, many bacteria capable of environmentally beneficial degradation properties have been isolated and investigated, but it should be noted that there is no single strain of bacteria with the metabolic capacity to degrade all the components found within oil sludge. In nature, biodegradation of a crude oil typically involves a succession of species within the consortia of microbes present. Degradation of petroleum involves progressive or sequential reactions, in which certain organisms may carry out the initial attack on the petroleum constituents. This produce intermediate compounds that are subsequently utilized by a different group of organisms, in the process that results in further degradation [16].

The indigenous populations of microorganisms, which are ubiquitous in soil and groundwater and self adapted to hard conditions, actually grow by using the carbon from the pollutants as energy source and cells building blocks. This breaks down the contaminants into carbon dioxide and water as end products [17]. Despite decades of research, successful biotreatment of petroleum hydrocarbon contaminated sites remains a challenge and several factors must be fulfilled and optimized to determine the outcome of the biodegradation process such as: biomass concentration, population diversity, bacterial growth, metabolic pathways, nature and concentration of pollutants, chemical structure of organic compounds, toxicity of contaminants, and presence of nutrients [17].

The oil sludge generated from the Jordanian Oil Refinery represent one of the most serious environmental problems in Jordan, and efforts started to develop a strategy to solve such problem, where small scale analysis has been conducted by the company laboratories. But the urgent need for a biotreatment approach has arisen to fully utilize the large number of microorganisms found in the oil-contaminated sites and to optimize the environmental conditions for biodegradation. However, before impacts at site can be approximately managed, the type, degree and location of the impacts must be determined for hydrocarbon site. This is typically done by sending numerous reports off-site for laboratory analysis of total petroleum hydrocarbons (TPH). If more than one TPH weight fraction (gasoline, diesel, residual) may be present at the site, multiple analysis will be required, resulting in high analytical costs that are further increased by the rapid turnaround times often needed to insure timely decision making during the sampling event. Total organic carbon (TOC), is a single analysis that can quantify all weight fractions of TPH and that can be performed quickly and easily on site. Determination of TOC values gives a gross measure of all forms of organic carbon including petroleum hydrocarbons and natural matter [18]. In this study our aim was to measure the TOC% reduction without having to go through special chemical analysis of the petroleum chemistry and fractionation. This could be recommended for future research.

Jordan is a country faced with significant volume of oil sludge being produced from the Jordan Oil Refinery and disposed into ponds causing environmental problems. Although, there is evidence that bioremediation can be used to treat oil sludge effectively, the main limitation is the difficulty in formulation treatment strategies that produce a specified outcome in term of degradation rate while residual contaminant concentration remains.

In this study, the ability of different consortia to degrade TOC in oily sludge was investigated for the first time. Efficiency of consortia supplemented with different nutrients was also compared.

2. Materials and methods

2.1. Microorganisms isolation and characterization

A number of nine bacterial isolates were selected by classical enrichment culture method and developed in minimal salt medium as described by Lal and Khanna [19]. These bacterial isolates were selectively obtained from naturally occurring microbiota of composite sludge samples collected from two locations at the effluent of Jordan Oil Refinery Plant. Morphological identification procedures of isolated strains were performed according to Buchanan and Gibbons [20] after the colour and shape of the colonies of bacterial isolates were determined, gram stain, shape of isolates were performed. Also physiological and biochemical tests using API 20NE (Bio-Merieux, France) were used for preliminary characterization.

2.2. Bacterial consortia preparation

Bacterial isolates were selected based on their ability of oil-sludge degradation and were grown at two temperatures 43 and 37 °C. The following consortia were prepared from the selected isolates harvested at their mid log phase (10^8 CFU/ml) and mixed in equal proportion. Three different bacterial consortia were prepared as follows.

(a) Bacterial consortia number 7 was prepared from isolates 1, 3, 4, and 5 with a corresponding cell density of 10^8 CFU/ml for each, and then all isolates were grown at 43 °C.
(b) Bacterial consortia number 13 was prepared from isolates 2, 3, and 7 with a corresponding cell density of 10^8 CFU/ml for each, and then all isolates were grown at 43 °C.
(c) Bacterial consortia number 16 was prepared from isolates 5 and 7 with a corresponding cell density of 10^8 CFU/ml for each, and then all isolates were grown at 37 °C.

These three natural bacterial consortia have been tested for their ability to utilize the oily sludge as their sole carbon source under aerobic conditions.

2.3. Biodegradation experiments

These experiments were conducted to examine the ability of consortium 7, 13, and 16 to degrade oily sludge collected from Jordan Oil Refinery treatment plant. The initial content of total organic compound in oily sludge was 83.3% estimated as described in Section 2.5, which was considered as base line control sample.

The laboratory tests were carried out in duplicate under aerobic conditions in Erlenmeyer flasks (250 ml) as incubation reactors. Flasks were shaken on a rotary shaker at 200 rpm at 43 and 37 °C (field temperature range of the studied area). The oily sludge-degrading efficiency of different consortia was screened on minimal salt medium as described elsewhere [19]. In three sets of Erlenmeyer flasks, oily sludge (100 ml sample) was inoculated with bacterial consortium prepared at different concentration of minimal salt medium (0.1, 1 and 0.05%) designated as A, B, and C, respectively. The total final volume of sludge, nutrient broth and consortia was 100 ml. The final consortia concentration was maintained as indicated. In addition control samples on bacterial cells-free basis were run in parallel. Flasks were incubated for 10 and 18 days. TOC% degradation was determined as mentioned in Section 2.5, which was considered as our base line control sample.

2.4. Effect of nutrients on biodegradation capacity of consortium 7B

Other experiments were carried out under aerobic condition to investigate the effect of supplementary nutrient such as nitrogen, sulphur and phosphorus on the TOC% degradation in different samples by consortium 7B. Four sets of experiments for each consortium were designed as follows:

(a) 0.005% potassium sulphate and 0.005% ferrous sulphate were added to sludge sample, bacterial consortium and nutrient broth were then added and all were incubated at 43 °C in 250 ml Erlenmeyer flask under moving condition on a rotary shaker at 200 rpm.
(b) Ten percent potassium hydrogen orthophosphate was added sludge sample, where bacterial consortium and nutrient broth were then added and all were incubated at 43 °C in 250 ml Erlenmeyer flask under moving condition on a rotary shaker at 200 rpm.
(c) Ten percent ammonia was added to sludge sample, where bacterial consortium and nutrient broth were added, then all incubated at 43 °C in 250 ml Erlenmeyer flask under moving condition on a rotary shaker at 200 rpm.
(d) 0.005% potassium sulphate, 0.005% ferrous sulphate, 10% potassium hydrogen orthophosphate, and 10% ammonia were added to sludge sample, where bacterial consortium and nutrient broth were then added, all incubated at 43 °C in 250 ml Erlenmeyer flask under moving condition on a rotary shaker at 200 rpm.

All samples were incubated for 10 days, the set of flasks were sampled and TOC% degradation was determined as mentioned in Section 2.5.

2.5. Extraction of total petroleum hydrocarbon (TOC) from oil-sludge sample

TOC determined by High-Temperature Combustion/(5310) Method, where 2 ml of oily sludge were filtered through 0.45 μm-pore-diameter. Filter and filtrate were heated in the oven to 600 °C for 90 min and then was cooled down to 25 °C [21].

2.6. Statistical analysis

One-way ANOVA and LSD tests were used to determine whether, TOC% degradation differs significantly according to type of consortium and nutrient. *P* value of less than 0.05 was considered to indicate statistical significance [22].

3. Results

3.1. Biodegradation experiments

Nine bacterial strains able to utilize oil-sludge as a sole carbon and energy source were isolated from enrichment aerobic cultures containing petroleum oil-sludge. Their taxonomic evaluation according to Berg's Manual [20] allowed the preliminary designation of isolates as *Bacillus* sp. and they were classified into two groups based on the growth temperature, group one was grown at 43 °C, while group two was grown at 37 °C. Selection of strains was made on preliminary estimation of their degrading activity and taxonomic distribution into strain 1, 2, 3, 4, 5 and 7 from group one, and strains 2, 3, and 7 from group two for designing degradation experiments (Section 2.2). The effect of the different consortia on TOC% reduction activity is shown in Table 1. The initial TOC% reduction activity before addition of consortium was 83.3%, which was considered as a baseline control sample.

Table 1
Effect of consortia type and concentration on TOC reduction after 10 days of incubation (initial TOC 83.3%) (blank)

Consortium	Final TOC% estimation
7A	62.3
7B	55.3
7C	65.3
13A	77.3
13B	61.3
13C	78.3
16A	79.6
16B	80.8
16C	83.0

A, B, C: bacterial consortia.

A significant variation ($P < 0.005$) was observed between the effect of consortia type and concentration on TOC% reduction. The highest TOC% reduction was achieved (83.3–55.3) after 10 days incubation period by consortium 7B, followed by consortium 13B (83.3–61.3). Moreover, the total TOC% reduction achieved by different consortia type and concentration was 0.3 and 28.0 (Fig. 1). The degradation of oil sludge as a whole was determined by measuring total organic carbon (TOC) percentage reduction by different consortia prepared from the isolated strains. The data in Fig. 1 revealed that the maximum TOC% removal achieved by consortium 7B followed by consortium 13B with a corresponding values of 28 and 22%, respectively. On the other hand, incubation of consortium 16C under the indicated experimental conditions resulted in negligible TOC reduction (0.3% TOC). Furthermore, a significant effect of temperature was displayed in Fig. 1.These results suggested that bacterial consortium prepared from isolates grown at 43 °C were more active in TOC degradation than consortium prepared from isolates grown at 37 °C. Addition of consortia 7A and 13A at concentration of 0.1% to the sludge sample (100 ml) provided a total TOC reduction of 21 and 6%, respectively. However, a total reduction of 3.0% was obtained by consortium 16A. Moreover addition of different consortia at 1% concentration revealed similar results where the highest and the lowest TOC reduction were 28.0 and 1.5% for consortia 7B and 16B, respectively.

3.2. Effect of nutrients on TOC reduction

Consortium 7B was selected for this study based on its demonstrated success for oil-sludge degradation. Experiments were conducted in 250 ml Erlenmeyer flask containing 100 ml sludge sample, incubated for 10 days at 43 °C on rotating shaker. The response of this consortium to the degradation of oil-sludge was positive and differed according to the type of nutrient added as illustrated in Fig. 2. The addition of either 10% potassium hydrogen orthophosphate or 10% ammonia to the incubation flask enhanced the ability of consortium 7B to reduce TOC percentage by 37 and 32%, respectively. This means an increase on TOC reduction percentage by 9% after P addition and 4% after N addition in comparison to the blank flask (no P or N added). The highest TOC removal (43%) was observed in the flask treated with combination of 0.005% potassium sulphate (S), 0.005% ferrous sulphate (S), 10% potassium hydrogen orthophosphate (P), and 10% ammonia (N), which is 15% higher than for blank flask. On

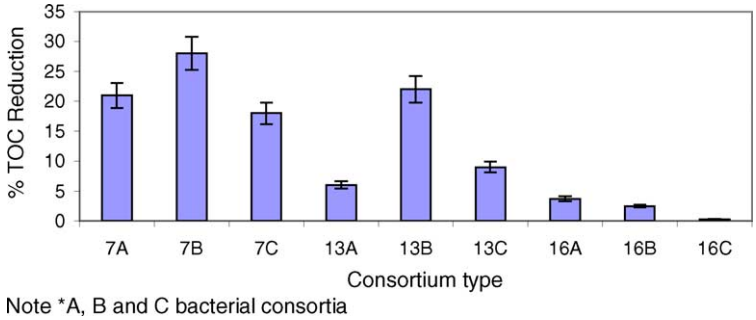

Fig. 1. Effect of consortia type and concentration on the total TOC% removal.

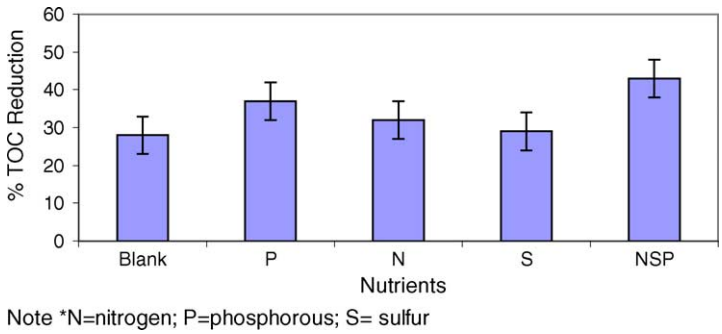

Fig. 2. Effect of nutrients addition on the total TOC% removal.

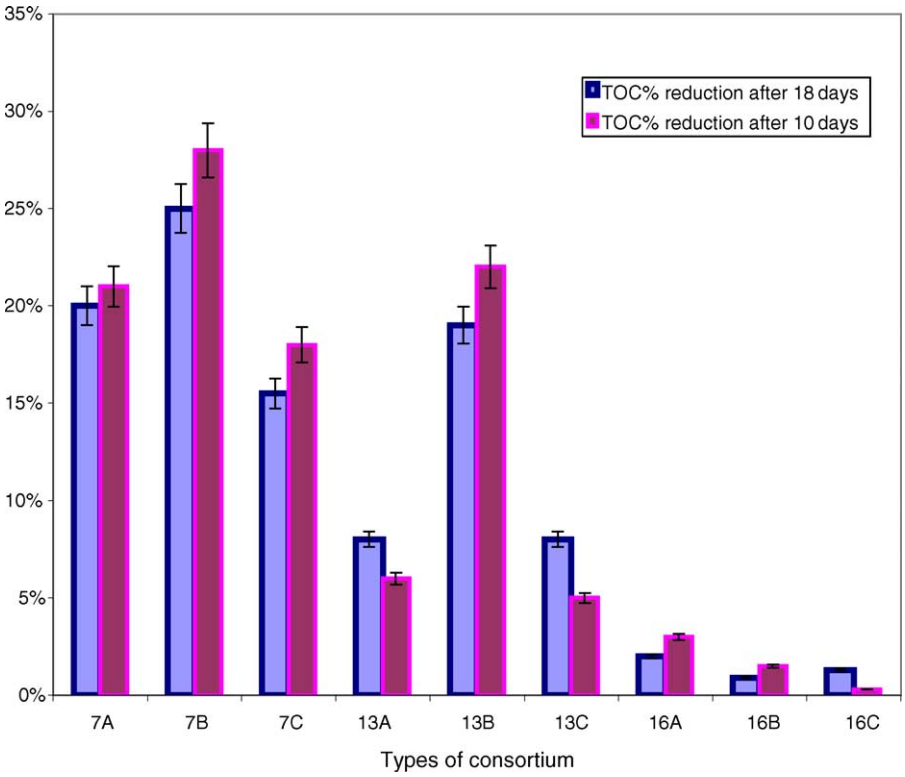

Fig. 3. Effect of incubation time on TOC% removal.

the other hand, less significant reduction on TOC percentage was noticed after S addition with corresponding value of 29% (1% increase over blank flask).

3.3. Effect of time on TOC degradation

Although bacterial consortia were incubated with the oily sludge aerobically for 10 and 18 days, a noticeable TOC reduction was observed only after 10 days and was lowered to nearly 57% of is initial level by consortia 7B (Fig. 3). On the other hand, after 18 days of incubation no further significant decrease in TOC was observed. And the same trend was observed for the remaining consortia (Fig. 3). This suggests that for this period of time (10 days) the microorganisms accomplished the process.

4. Discussion

This study was carried out to investigate for the first time the ability of different bacterial consortia isolated from the Jordanian Oil Refinery treatment plant to metabolize petroleum oily sludge as a source of energy and carbon for growth under aerobic conditions. These isolates have been characterized with the standard API 20NE identification system, which allowed preliminary designation of the isolates as *Bacillus* species. Further studies could be carried out in the future to fully identify the bacterial strains including sequencing of the 16S rDNA genes, DNA–DNA hybridization, DNA base ratio determinations, whole cell protein and fatty acid analysis and extensive biochemical characterization could be beneficial [23]. As it might appear, this work is the first study to be conducted in Jordan in this regard which could lay the ground for future researchers to conduct an integrated study including a full analysis using CS-MS techniques that could be acquired thought funded projects or other international assistance. Furthermore, the finding of this study could assist decision makers in establishing a baseline or a benchmark for TOC-associated pollution.

The biodegradation experiments carried out in this study have assessed the efficiency of three different consortia based exclusively on the percentage of TOC removal from the oily sludge sample, but we did not monitor other variables that could influence TOC biodegradation such as pH, or dissolved oxygen [24], which could be investigated in future studies. The treatment carried out by applying enriched specific populations isolated from the sludge to the contaminated sample, where the population of oil-degrading microorganisms was low [25]. However, the degradation capacities of the three consortia were studied in the presence of initial TOC (83.3%) concentration in the oily sludge samples. Consortium 7B provided the highest removal percentage of 28.0%, while consortium 16C showed the lowest degradation percentage of 0.3% after 10 days of incubation (Fig. 1). Both the effect of consortia type and concentration has been investigated, and it was observed that increasing the inoculums concentration would enhance the TOC degradation rate. Increasing inoculums concentration from 0.05 up to 1% lead to four-fold

rise on TOC percentage removal A similar observation was also reported by other researchers [26,27], where removal of crude oil degradation by mixed microbial consortium isolated from oil-contaminated soil samples, was observed. Other researchers have described the ability of mixed bacterial consortia to degrade 28–51% of saturates and 0–18% of aromatics present in crude oil [28–30]. On the contrary, Venosa et al. [11] showed that microbial inoculation did not enhance the removal of hydrocarbons from soil contaminated with crude oil, which might be due to other environmental parameters [31]. The significance of consortium concentration on TOC% removal obtained in this study is in agreement with other study by Forsyth et al. [25] who showed that biodegradation would not occur at a significant rate if population of indigenous microorganisms is less than 10^5 CFU/g of soil. It was also observed that TOC% reduction obtained by consortium 7B after10 and 18 days incubation were 28 and 25% (Fig. 3), which might be due to environmental parameters [31]. Similarly, Aldrett et al. [32] observed that the highest aromatic petroleum hydrocarbons degradation was observed after 7 days. The same observation reported by other researchers [33], where the disappearance of TOC from treated plots was faster during the first 3 months of inoculation. This observation is in agreements with our study where incubation time plays an important role in TOC% removal rate.

Temperature influences petroleum biodegradation by effects on the, physical, chemical properties of the oil, where rate of hydrocarbon metabolism by microorganisms and composition of the microbial community were reported [14,34]. In our study, it was observed that consortia prepared from isolates grown at 43 °C were more active than consortia prepared from group 2 (isolates grown at 37 °C). The same observation reported by other researchers during biodegradation of crude oil by a mixed bacterial consortium [27,35]. They found that percentage of degradation decreased with decreasing temperature, where higher temperature increased the rate of hydrocarbon metabolism to a maximum, typically in the range of 30–40 °C, which agree with results obtained in this study.

The highest TOC% reduction rate obtained in this study was under aerobic condition, which is in agreement with other reported studies [36–38] where aerobic conditions are generally considered necessary for extensive degradation of hydrocarbons in the environment. Many studies have shown that oxygen depletion leads to sharply reduced biodegradation activities in marine sediments and soils [39,40]. Another factor evolved in this study was the influence of the availability of nutrients on the biodegradation process of microorganisms [40]. The importance of nutrients to microbial processes has long been known, nitrogen (N) is required in amino acids and phosphorus (P) is involved in energy transport as adenosine triphosphate. Compositional analysis of microbial biomass indicates that C, N and P are present in the ratio of 106:16:1, respectively [41]. Contaminated soils that have intrinsically low N and P will require nutrient additions to allow a sufficient increase in biomass for environmentally significant hydrocarbon degradation to occur.

In this paper, the effect of nutrients addition such as N, P, and S individually and in combination on TOC removal by the bacterial consortia was investigated. Improvement on removal rate was noticed after adding all nutrients together. The highest TOC removal was 43% (Fig. 2). Also, it was noticed that addition of N and P individually resulted in more significant improvement on biodegradation rate that the addition of S. The effect of nutrients addition on biodegradation have been studied extensively [42–45], and it was concluded that different organisms have different requirements for N and P, and provision of these nutrients at different concentration (both absolute and relative to each other) will differentiate for the organisms most able to utilize the nutrients at levels provided in the oiled habitat. Thus, addition of N and P at different concentrations should be considered for different groups of organisms [46,47]. Additionally, other study indicated that addition of $NaNO_3$ had beneficial effects on hydrocarbon degradation [46], which is in agreement with our study.

In summary, the performance of hydrocarbon degradation bacterial consortia selectively isolated from naturally occurring microbiota of the oily sludge could be utilized for future oily sludge bioremediation application. No previous reports were published concerning the biotreatment of oil sludge in Jordan, and the results obtained in this study represented a support for developing investigations concerning the use of such bacteria consortia for degradation of petroleum hydrocarbons from the Jordan Oil Refinery. To fully explore the potential use of such bacteria for the treatment of the waste effluents, future studies should be carried out to optimize the different factors that influence the rate of biodegradation. This could be achieved by increasing the financial and technical support from funding agencies, companies, decision makers and governments.

5. Conclusions

Based on the results obtained from laboratory study, biodegradation could be considered as a key component in the clean-up strategy developed in the future for treatment of oil-sludge contaminants. In addition, evaluation of environmental conditions and optimization of biodegradation process based on several factors such as biomass type and concentration, temperature, nutrients, and pH are areas where further research is necessary.

Unlike the conventional treatment technologies, bioremediation technique must be tailored specifically to each polluted site. Each waste site has unique characteristics, and thus requires individual attention, so an official criterium for evaluating the success or failure of a particular strategy is needed. In addition, a successful biodegradation program requires a multidisciplinary approach, integrating fields such as microbiology, engineering, geology, soil science, and project management.

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