Nigerian Research Journal of Engineering and Environmental Sciences 3(2) 2018 pp. 750-762

Nigerian Research Journal of Engineering and Environmental Sciences
Journal homepage: www.rjees.com

**Original Research Article**

# Bioremediation of Pyrene Contaminated Soil using Isolated Mixed Culture

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**ARTICLE INFORMATION**

*Article history*:
Received 03 August, 2018
Revised 29 September, 2018
Accepted 04 October, 2018
Available online 30 December, 2018

*Keywords*:
Pyrene
Soil-water ratio
Percentage degradation
Pyrene-soil ratio
Biomass growth

**ABSTRACT**

*In this research, bioremediation of pyrene (Pyr) contaminated soil using isolated mixed culture containing Bacillus cereus and Entrobacter aerogenes was investigated. A factorial design (FD) was employed to investigate and optimize the bioremediation of Pyr-spiked soils with various sets of operating conditions in soil-slurry batch reactors. From the results, biomass growth was dependent on Pyr concentration and slurry initial pH, but not soil/water ratio. Designed points of soil/water (13%), Pyr/soil (1000 mg/kg) with a pH of 6.5 gave about 90% removal of Pyr within the first six days of incubation. The chosen soil/water ratio might provide adequate space for mixing and microbial mobility within the soil-slurry reactors. A comparison of soil properties on biodegradation of Pyr in three different Pyr-spiked soils showed that soil A (Munchong series) had the highest rate of percentage degradation (97.6%) and soil B (Silty clay) had the lowest rate of percentage degradation (74.24%). Average percentage degradation for all the three soils A, B, and C (Clay loam) were 91.42%, 90.07% and 92.12% respectively. On the overall, there were no marked differences in percentage degradation of Pyr among all the soils examined. Therefore, careful determination of factors that control biomass growth, contaminant concentration and soil characteristics may provide efficient solutions to remediation projects.*

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## 1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are large groups of persistent organic pollutants (POPs) with at least more than two fused benzene rings variously arranged together (Cerniglia, 1992). They are formed

naturally under low-temperature and high-pressure reactions of organic matter, which are hydrocarbon derivatives. In addition, they are released to the world through various means such as burning of woods and forest fire, coal gasification, home heating, oil spills, vehicular traffic emissions, waste incineration and wastewater leakage (Wilson and Jones, 1993; Maliszewska-kordybach, 1999; Douben, 2003; Johnsen et al., 2005; Johnsen and Kerlson, 2007). PAHs are highly toxic and potential environmental hazard. They are lipophilic and easily adsorbed by mammals' metabolic system to produce characteristically mutagenic and carcinogenic products (Pereira Netto et al., 2000; Jacques et al., 2008). They exhibit both ecological and human health problems and could represent high risk to humans and ecosystem; consequently, ranked 9$^{th}$ in the 2001 Comprehensive Environmental, Response, Compensation, Liability Act (CERCLA) of the USA priority list hazardous substances (ATSDR, 2007)

Comparatively, plethora of works have been documented in the literature concerning the application of soil-slurry bioreactor for the remediation of PAH-contaminated soil (Robles-Gonz ´alez et al., 2008; Christodolatos and Koutospyros, 2006,). There are different reactor configurations that have been employed, namely soil column bioreactors and soil-slurry bioreactor. The soil slurry bioreactor facilitates an efficient contact with the water phase and enhances effective remediation of contaminated soil and sediments. The aqueous phase carries nutrients, carbon sources and oxygen under controlled environments so that it can facilitate effective contact between contaminant and microorganism (Venkata et al., 2004; Venkata et al., 2006; Kumada et al., 2005). The soil contents will also alter the distribution of target compounds between the water and the soil with different mass transfer properties while the inoculated strains having specific degradation capability would be responsible for biodegrading the target compound. Available reports indicated that soil slurry bioremediation for the cleaning up of PAH-contaminated soil has been carried out in many different process configurations under aerobic conditions (Lee et al., 2001; Kim et al., 2001; Woo et al., 2001; Garon et al., 2004; Lei et al., 2004; Seung et al., 2004; Biswas et al., 2005).

The success in implementing bioremediation approach can be determined by many factors, such as soil composition, water content, nutrient concentration, available oxygen content, intrinsic microbial characteristics-uptake and metabolism (Venkata et al., 2006). Other factors that determine the transport and fate of contaminant in the environment also must be taken into consideration in designing a bioremediation system.

Traditionally, bioremediation environmental factors can usually be optimized by employing the single-variable-at-a-time (SVAT) method, (the most common practice of holding all other variables constant and observing one other factor at a time) (Kaneco et al., 2009). However, this approach has its attendant pitfalls viz time-consuming, poor interactions or lack of it among many different variables, and difficulty in predicting the actual optimum conditions. Therefore, in this work, an ex-situ method for remediating PAH-contaminated soil using the isolated mixed microbial consortia in a soil-slurry batch system is presented. Optimization of pH, soil-water ratio, and Pyr-soil ratio against biomass growth and percentage degradation of Pyr was conducted. A comparison of three Malaysian soils was also tested using an optimized pH, soil-water ratio, and Pyr-soil ratio in order to determine the effect of soil type on biomass growth and percentage degradation. An efficient mixing was provided in the batch bioreactor system to ensure a homogeneous mixture between contaminant and microflora.

## 2. MATERIALS AND METHODS

### 2.1. Physiochemical Characterisation of Soil Samples

Uncontaminated soil samples were obtained from the soil bank of Department of Agriculture, Universiti Putra Malaysia, transferred to the laboratory and stored at 4$^{o}$C. Soil samples were pretreated by drying, crushing, homogenizing and sieved with 2 mm sieve to remove debris and pebbles and subsequently stored before use. To determine the physical and chemical properties of the experimental soils, a representative of

the three composite samples was oven dried at 60ºC, ground and then sieved through 2 mm mesh size and stored for further analysis. Soil particles size distribution was determined base on method described in (Teh, 2006). The pH, Carbon (C), organic Carbon (C), total Nitrogen (N), available Phosphorus (P), exchangeable Potassium (K), Calcium (Ca), Magnesium (Mg) and Cation Exchange Capacity (CEC) were determined based on standard methods. Total carbon was measured by the combustion technique using LECO carbon analyzer (Merry and Spouncer, 1988). Total N was determined using Kjeldahl method (Bremner and Mulvaney, 1982). Soil exchangeable K, Ca, Mg and CEC were determined using 1M $NH_4OAc$ (pH 7.0) method (Thomas, 1982), while P was extracted based on Bray II method (Bray and Kurtz, 1945).

**2.2. Design of Experiment**

The experiment was designed using JMP 9 statistical software (North Carolina, USA) to determine the effect of soil types on the removal of Pyr and biomass growth. The factors were selected between the highest and the lowest values: pH was kept between 6.75-7.25, while temperature was kept between 25ºC-30ºC. The values of soil-water ratio were selected based on the consideration of soil characteristics and degradation rates. Soil-water ratio is one of the key factors that can determine the mixing power requirement and aeration efficiency in aerobic slurry bioreactors. Reported pollutant mineralization from soil-water ratios of 5-10% and 5-20% was similar (Robles-González et al., 2008) and 1:20 was also reported to give efficient mineralization of contaminants in soil-slurry reactors (Mohan et al., 2007a). Concentration of contaminant to soil ratio has been determined based on consideration of heavy and light level of soil pollution. In order to create artificial contamination during soil-slurry reactor design in the laboratory, various ranges have been reported: 500 mg/kg of naphthalene (Wang and Vipulanandar, 2001); 3000 mg/kg of oil (Jonge and Verstrate, 1995) and as high as 10,000 to 20,000 mg/kg of creosote were also reported (Rutherford et al., 1998). Therefore, in this work the ranged of factors at a level between a minimum to a maximum ratios were adopted in order to determine the optimum removal rates and is presented in Table 1 (Abubakar et al., 2012). Consequently, a factorial design with three factors-two levels was used. The factors are: soil water ratio, pH and the concentration of Pyr to soil ratio that were previously optimised, while keeping the soil type as categorical factors (which are fixed variables). The design resulted in 24 runs of test. The reactors were monitored over a period of 20 days and sample were taken at the interval of 3 days to monitor biomass growth, but to determine the residual concentration of Pyr, samples were taken at the end of the 20-day period.

Table 1: Selected levels of factors and treatment combinations

| Factor | Treatment | Low | High |
|---|---|---|---|
| A ($X_1$) | Soil/Water ratio | 0.1 | 0.2 |
| B ($X_2$) | Initial pH | 5 | 8 |
| C ($X_3$) | Pyrene/Soil ratio, mg/kg | 500 | 1000 |

**2.3. Preparation of Inoculum**

Inoculum was grown in nutrient broth supplemented with 0.75 ppm of Pyr and incubated at 30ºC with shaking speed of 180 rpm. After 48 hr of growth, when the bacteria had reached late exponential stage, the cells were harvested by a centrifuge at a speed of 8000 g for 10 min at 4ºC and rewashed with sterilized distilled water. Finally, the inoculum was resuspended in sterilized distilled water.

**2.4. Soil-slurry Bioreactor**

A 500 mL shake flask was used to emulate a laboratory bench scale bioreactor. An amount (200 mL) of the mineral salt medium (MSM) was prepared with various combinations of the designed factors of soil/water, Pyr concentration to soil ratio and pH values. After preparing the different combinations of the soil/water, the flasks were autoclaved and cooled, before spiking with Pyr (pre-dissolved in acetone) with an amount

according to the respective contaminant/soil ratio for the different combinations. Then, it was mixed thoroughly and left overnight for the acetone to evaporate in a fume hood cabinet prior to use for degradation studies. The mixed culture was allowed to grow in the MSM supplemented with Pyr and at the same time to biodegrade Pyr under incubation condition of 30$^{o}$C, 180 rpm for 20 days.

### 2.5. Extraction of Pyr

At the end of degradation time, the culture was centrifuged at 4500 rpm for 20 min. The supernatant (3 mL) was extracted three times (v:v) with equal volume of dichloromethane (DCM). The three extracts were combined and the aqueous phase was dried by filtering through a glass wool placed in a glass funnel containing a gram of baked anhydrous sodium sulphate at 200$^{o}$C for 2 hrs. Finally, the extracts were evaporated using a rotary evaporator at reduced pressure and concentrated to 1 mL under slow nitrogen purging. The biomass growth was quantified by plate count method. Subsequently, biomass growth was reported as colony forming unit per milliliter of a sample (CFU/mL).

### 2.6. Quantification of Pyr

The quantification of Pyr was conducted using a high-performance liquid chromatography (HPLC) (Shimadzu model) equipped with UV detector. The column was a Phenomenex Synergi 4µm Max-RP80A (250 x 4.6 mm) and the mobile phases consisted of water and acetonitrile in a gradient mode (65:35) for 2 min; (0:100) in 24 min, for 10 min and the flow rate was 1.5 mL/min under ambient temperature condition and detected at 254 nm. Finally, the unknown samples were quantified using standard Pyr correlation curve.

## 3. RESULTS AND DISCUSSION

### 3.1. Characterization of Soil Samples

The unpolluted soil A belonged to Munchong series, a member of the Munchong Family, which is described as very fine, kaolinitic, isohyperthermic, red-yellow (Table 2). Munchong was developed from fine-grained sedimentary rocks (shale) and low-grade metamorphic rocks. More description of this classification of Malaysian soil has been explained previously by Paramananthan (2000). All the three unpolluted soil samples were classified according to United States Department of Agriculture (USDA) soil classification triangle by tracing the percentage of clay, sand and silt representing each side of the triangle, and arriving at a meeting point on the triangle. The unpolluted soils A, B and C were classified as Silty clay and Clay loam respectively. The soil A (the Munchong Series) used in this study for the optimization had a high cationic exchange capacity (23.9 Cmol/kg) compared with the soils B and C. This allows it to bind with opposite charged PAH contaminants. It also had the highest carbon content (4.7%) and calcium content (1445.2 µg/g) than the other two soil samples. In addition, Soil A had an almost neutral pH of 7.22 and the other soil types (A and B) were acidic and their pH were 4.28 and 4.58 respectively. Soil A was chosen because of its presumable property of binding organic compounds and could thus be a sink for organic contaminants.

Table 2: Physiochemical properties of the unpolluted soils

| Parameters | Soil type | | |
|---|---|---|---|
| | Soil A | Soil B | Soil C |
| Cation exchanger capacity (Cmol/kg) | 23.90±0.033 | 14.10±0.005 | 14.30±0.057 |
| Carbon content (%) | 4.70±0.008 | 0.47±0.005 | 1.30±0.035 |
| Nitrogen content (%) | 0.11±0.001 | 0.11±0.001 | 0.11±0.003 |
| Phosphorus content (µg/g) | 36.70±0.033 | 28.50±0.001 | 73.20±0.088 |
| Potassium content (µg/g) | 29.70±0.033 | 65.70±0.057 | 5.70±0.035 |
| Calcium content (µg/g) | 1445.20±0.088 | 1076.60±0.357 | 399.40±0.088 |
| Magnesium content (µg/g) | 22.00±0.057 | 160.00±0.333 | 66.00±0.318 |
| pH | 7.22±0.006 | 4.28±0.005 | 4.58±0.003 |
| Soil classification (USDA) | *Silty clay | **Silty clay | ***Clay loam |

*Clay (<2µm) 46.32%; Silt (2-50µm) 50.86%; Sand (>50µm) 2.67%
**Clay (<2µm) 21.36%; Silt (2-50µm) 6.66%; Sand (>50µm) 71.81%
***Clay (<2µm) 33.58%; Silt (2-50µm) 24.90%; Sand (>50µm) 41.36%
Data represent mean±standard error

### 3.2. Comparison of Pyr Degradation in three Spike Soils

The three soil samples were used as categorical factors, and factorial design was done in order to find out, whether soil types will affect the rate of Pyr degradation in the reactors. The factors selected were soil-water ratio (10%-20%), pH (6.5- 7.0) and Pyr-soil ratio (750 mg/kg-1000 mg/kg), and 24 experimental runs were obtained. These experimental runs were monitored for a period of 20 days. From Table 3, soil A had the highest percentage degradation rate of 97.62% while soil B had the lowest percentage degradation of 74.21%. The average percentage degradation rate from all the three soils, A, B, and C were 91.42%, 90.07%, and 92.12% respectively. From the analysis of variance, (Table 4) comparing the percentage degradation rates of the three soils, the prob>F of 0.5217 shows that there was no clear difference or effect of soil type on the degradation rates. The comparison of actual and predicted percentage degradation rates from Figure 1 indicated that about 64% of data were linearly related. Overall, there were no marked differences in percentage degradation rates among all the three soils. In addition, Figure 1 also further confirms that there was no clear linear relationship among the three soils. However, the percentage degradation of three soils showed that soil A had the higher representation than the other three soils.

Soil characteristics do affect the rate and extent of degradation of organic compounds in soils. The bulk of PAH concentration in the environment resides in soils and sediments, where PAH could be primarily partitioned into organic matter (Jones and de Voogt, 1999). Sandy soil has a higher degree of degradation of pentachlorophenol (PCP) mineralisation by inoculated R. *chlorophenolicus* (Crawford and Crawford, 2005). Rich organic soil has strong adsorption to soil and slower release of organic chemicals. Therefore, soil properties are important factor that need to be taken care of during soil remediation designs.

During bioremediation, microorganisms utilize chemical contaminants in the soil as an energy source, and through oxidation-reduction reactions, metabolize the target contaminant into usable energy to grow. In limited oxygen supply or lack of it, as in saturated soils or lake sediment, anaerobic respiration (without oxygen) prevails. Since, in the soil, microorganisms require moisture for cell growth and function, availability of water affects diffusion of water-soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. Anaerobic respiration produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation. Soil pH is another important factor because certain microorganisms' species may survive within a certain range of pH. Furthermore, soil pH can affect the availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the acceptable range is pH 6-8 (Braddock, et al., 1997).

Table 3. Comparison of treatment factors and % degradation of Pyr for the three soil types

| Runs | Soil Type | pH | Soil/water ratio (%) | Pyr/Soil ratio (mg/kg) | Degradation % |
|---|---|---|---|---|---|
| 1 | A | 7 | 10 | 1000 | 94.87 |
| 2 | A | 7 | 20 | 750 | 97.62 |
| 3 | A | 6.5 | 20 | 1000 | 97.37 |
| 4 | A | 6.5 | 20 | 750 | 87.84 |
| 5 | A | 6.5 | 10 | 750 | 93.00 |
| 6 | A | 7 | 10 | 750 | 84.90 |
| 7 | A | 7 | 20 | 1000 | 92.43 |
| 8 | A | 6.5 | 10 | 1000 | 83.31 |
| 9 | B | 7 | 10 | 1000 | 88.44 |
| 10 | B | 6.5 | 20 | 750 | 91.02 |
| 11 | B | 7 | 20 | 1000 | 94.57 |
| 12 | B | 7 | 20 | 750 | 93.40 |
| 13 | B | 6.5 | 20 | 1000 | 94.99 |
| 14 | B | 7 | 10 | 750 | 74.25 |
| 15 | B | 6.5 | 10 | 750 | 90.46 |
| 16 | B | 6.5 | 10 | 1000 | 93.44 |
| 17 | C | 6.5 | 10 | 1000 | 89.74 |
| 18 | C | 7 | 20 | 750 | 95.86 |
| 19 | C | 6.5 | 20 | 750 | 96.39 |
| 20 | C | 7 | 10 | 750 | 89.83 |
| 21 | C | 7 | 20 | 1000 | 95.59 |
| 22 | C | 7 | 10 | 1000 | 88.56 |
| 23 | C | 6.5 | 10 | 750 | 90.14 |
| 24 | C | 6.5 | 20 | 1000 | 90.88 |

Table 4: Summary of fit for comparing actual and predicted percentage degradation

| Parameter | Value |
|---|---|
| R Squared | 0.636839 |
| Adjusted R Squared | 0.001307 |
| Root Mean Square Error | 5.295875 |
| Mean Response | 91.08696 |
| Observations (or sum weights) | 23 |
| prob>F | 0.5217 |

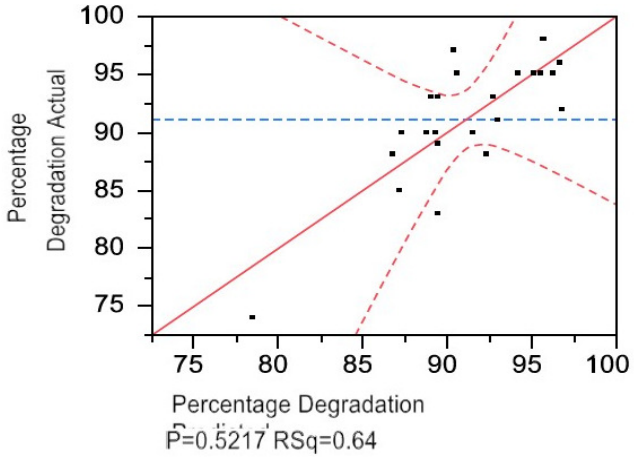


Figure 1: Comparison of actual and predicted percentage degradation

Contaminants can adsorb to soil particles, rendering some contaminants unavailable to microorganisms for biodegradation. Thus, in some circumstances, bioavailability of contaminants depends not only on the nature of the contaminant but also on soil type (Head and Swannell, 1999). Hydrophobic contaminant, like Pyr, has low solubility in water and tends to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants. Soil type is an important consideration when determining the best-suited bioremediation approach to a particular situation. For example, during in-situ bioremediation such as bioventing, soil texture directly affects the utility of bioventing. In as much as permeability of soil to air and water is a function of soil texture: fine-textured soils like clays have low permeability, which prevents bioventing oxygen and nutrients from dispersing throughout the soil (USEPA, 2006)

### 3.3. Comparison of Viable cell Growth on three Soil Samples

#### 3.3.1. Effect of soil-water ratio on viable cell growth in Soil A

The results of the viable cell growth in Soil A is presented in Table 5. It indicates that at a constant pH of 6.5 and Pyr-soil ratio of (750 mg/kg), the viable cell growth was compared between 20% and 10% soil water ratios. There was a higher viable cell growth of (32 x $10^7$ CFU/mL and μ, 1.2926 CFU/mLh) with 20% soil-water ratio than the 10% soil-water ratio (7.2 x $10^7$ CFU/mL and μ, 0.7161 CFU/mLh). Nevertheless, both reached their exponential phase on the 6th day, and the reactor with 20% soil-water ratio growth remained constant for the remaining days. As compared to a reactor with a pH of 6.5 and Pyr-soil ratio (1000 mg/kg), the viable cell growth was higher (18 x $10^7$ CFU/mL and μ, 0.7316 CFU/mLh) at 10% than with 20% (2.5 x $10^7$ CFU/mL and μ, 0.6072 CFU/mLh); while both viable cell growth reached their highest growth (18 x $10^7$ CFU/mL and 2.5 x $10^7$ CFU/mL) on the same day. However, at constant pH of 7 and Pyr-soil ratio (750 mg/kg), at 10% soil-water ratio, the viable cell growth was bi-phasic at day 6 and day 12. However, at both 10 and 20% soil-water ratio, the viable cell growth was (12.5 x $10^7$ CFU/mL and μ, 0.5558 CFU/mLh and 12.5 x $10^7$ CFU/mL and μ, 0.9271 CFU/mLh respectively) and reached exponential phase on day 12; subsequently declined on day 15. But, when the reactor was at pH of 7 and Pyr-soil of (1000 mg/kg) however, there was a higher viable cell growth (42 x $10^7$ CFU/mL and μ, 1.2136 CFU/mLh) at 10% than 20% soil-water ratio (13 x $10^7$ CFU/mL and μ, 0.6587 CFU/mLh), and the growth declined on day 15. It also indicates that soil-water ratio has a minimal influence, or the chosen soil-water ratios were within the favorable condition. As can be seen in Table 5, at 10% soil-water ratio, (1000 mg/kg) and with pH of 7, there is a higher biomass growth (42 x $10^7$ CFU/mL) in soil A. However, the lowest growth was with 20% soil-water ratio and pH of 6.5 and Pyr-soil ratio of (1000mg/kg).

On the other hand, in soil B, the highest biomass growth was at 20% soil-water ratio, with pH of 6.5 and Pyr-soil ratio of (750 mg/kg), but lowest at pH of 7 and Pyr-soil ratio of (1000 mg/kg).

In soil C, however, the highest biomass growth was recorded at both Pyr-soil ratio of (750 mg/kg and 1000 mg/kg) with a pH of 7.0 and 10% soil-water ratio. This shows that even though there were significant growths at all level of treatment, the reactors with pH of 7, 10% soil-water ratio and (1000 mg/kg) Pyr-soil ratio indicated a favourable viable cell growth.

From the viable cell growth analysis, with consideration to pH, soil-water ratio, and Pyr-soil ratio, there was variability of viable cell growth due to influence of those factors. However, within this set up, pH 7 has been the most consistent with respect to its influence on the viable cell growth. That was why Robles-González et al. (2008) reported that pH in soil-slurry was usually kept between 6.75-7.25. However, Chen et al. (2010) found pH not significant using soil slurry reactor to treat (1,2,3-cd) Pyr. Interestingly, it is very important to monitor the biomass population in soil-slurry reactors to improve selectivity or manipulation of the reactor performance. In this study, the monitoring of the biomass population was done by the plate counting methods and enough information was obtained for examining the effect of those factors on viable cell growth. Using soil slurry treatment would enhanced removal and extent of bioremediation (Venkata et al., 2008; Doick and Semple, 2003). Since, soil-water ratio affects the rate of mass transfer, higher soil-water ratio may hinder the rate of mixing thereby reducing the rate of mass transfer and microbial mobility ( Mohan et al., 2007b; Chen et al., 2010) On the other hand, very low soil-water ratio may not be favorable for microbial oxygen uptake. Therefore, for efficient removal of contaminant in soil slurry, suitable soil water ratio is very important. In this study, 10% soil water ratio has been found to be very ideal in all the combinations of factors assessed and this has been confirmed to give highest mineralization rate in a well-mixed reactor (Jee et al., 1998).

Amount of contaminant-soil ratio will also influence the performance of soil-slurry remediation of PAH. Prasanna et al. (2008) reported that substrate-loading rate (16.66 kg soil/$m^3$/day) influenced the performance of bio-slurry in the degradation of Antracene. Venkata et al. (2009) reported about 90% efficiency of Pyr degradation with a substrate-loading rate of 0.36 g Pyr/kg of soil per day in a batch bio-slurry reactor inoculated with domestic sewage. In addition, Villemur et al. (2000) spiked Pyr at a concentration of 196 mg/kg and reported degradation of Pyr at the rate of 19 mg/(L day). Castaldi, (2003) used 680 mg/kg of 2-3 ring PAH and 38 mg/kg of 4-6 ring PAH in multi-stage continues flow soil-slurry reactor with over 90% removal efficiency. In this study, higher concentration of Pyr-soil ratio has a negative effect on the biomass growth in all the conditions operated.

Even though, this work was conducted using already isolated consortium of bacteria, isolation of bacteria or microorganisms capable of degrading PAH from a contaminated site is the first step in the application of bioremediation (Cordova-Rosa et al., 2009) . This will allow the isolation of effective aromatic-degrading bacteria. Pyr-degrading bacteria that were isolated were usually from hydrocarbon-contaminated site (Boonchan et al., 1998; Kazunga and Aitken, 2000; Kim and Freeman, 2005; Uyttebroek et al., 2007; Wang et al., 2008). However, several reports contended that even though isolated pure culture could degrade PAH in the laboratory, they usually failed to perform when inoculated into the field site. Because of competition between indigenous and inoculated pure cultures for limited carbon sources, as well as antagonistic interaction, and predation by protozoa or bacteriophages may also reduce the efficacy of pure culture (Mrozik and Piotrowska-Seget, 2010).

Another alternative approach to remediation of site contaminated with PAH is used of microbial consortia consisting many PAH-degrading microorganisms. In several attempts, consortia were more effective than single strain or pure culture. Because of the synergistic or intermediates of catabolic pathway of one strain

may be further degraded by another strains with suitable catabolic pathway (Heinaru et al., 2005; Mrozik and Piotrowska-Seget, 2010). In this study, the synergistic degradative strength of B. *cereus* and E. *aerogene* has resulted in higher degradation of Pyr (90% within just six days of incubation).

Table 5. Effect of treatment factors on biomass growth

| Treatments | | | | Biomass growth | |
|---|---|---|---|---|---|
| Soil type | Soil/Water ratio (%) | pH | Pyr/Soil ratio (mg/kg) | Viable cell (CFU/mL) x$10^7$ | Specific growth rate (μ) CFU/mLh |
| A | 20 | 6.5 | 750 | 32 | 1.2926 |
| | 10 | 6.5 | 750 | 7.2 | 0.7161 |
| | 20 | 6.5 | 1000 | 2.5 | 0.6072 |
| | 10 | 6.5 | 1000 | 18 | 0.7310 |
| | 20 | 7.0 | 750 | 12.5 | 0.9271 |
| | 10 | 7.0 | 750 | 12.5 | 0.5558 |
| | 20 | 7.0 | 1000 | 13 | 0.6587 |
| | 10 | 7.0 | 1000 | 42 | 1.2136 |
| B | 20 | 6.5 | 750 | 23 | 0.2979 |
| | 10 | 6.5 | 750 | 5.5 | 0.0406 |
| | 20 | 6.5 | 1000 | 2.1 | 0.1209 |
| | 10 | 6.5 | 1000 | 0.75 | 1.3319 |
| | 20 | 7.0 | 750 | 6.0 | 0.0503 |
| | 10 | 7.0 | 750 | 12.5 | 0.5558 |
| | 20 | 7.0 | 1000 | 0.65 | 0.4558 |
| | 10 | 7.0 | 1000 | 5.5 | 0.4121 |
| C | 20 | 6.5 | 750 | 0.5 | 0.2786 |
| | 10 | 6.5 | 750 | 1.8 | 0.0221 |
| | 20 | 6.5 | 1000 | 0.38 | 0.0512 |
| | 10 | 6.5 | 1000 | 0.8 | 0.1074 |
| | 20 | 7.0 | 750 | 1.5 | 0.2355 |
| | 10 | 7.0 | 750 | 2.5 | 0.6334 |
| | 20 | 7.0 | 1000 | 1.5 | 0.2355 |
| | 10 | 7.0 | 1000 | 2.5 | 0.6334 |

Even though in the real environment microbial communities exposed to PAHs contaminated site require time to adapt before degradation occur, the period is considerably important as it is the period of acclimatization. This period depends on several environmental factors such as: contaminant concentration, bioavailability, pH, temperature, level of nitrogen and phosphorus present, aeration level, and prior exposure of microbial communities to PAH or Pyr (Alexander, 1999; Doyle et al., 2008). Among the physical factors, temperature plays an important role in biodegradation of PAH contaminated site. Because it directly affects the chemistry of the PAH as well as the physiology and diversity of the microbial flora. For example, at low temperature, viscosity will increase while the volatility of the LMW PAH was reduced, thereby delaying the starting up of biodegradation (Das and Chandran, 2010). Temperature affects the solubility of Pyr, even though degradation occurs at a wide range of temperature. The rate of degradation is decreases with increasing temperature (Abubakar, et al., 2012)

Comparatively, many works have been documented in the literature regarding the application of slurry-soil bioreactor technology for the cleaning up of PAH- contaminated soil. Slurry-soil reactor facilitates effective degradation of soil bound contaminants by bringing about increased contact with the water phase that carries nutrients, additional carbon source and oxygen under controlled and optimized conditions (Mohan et al., 2006). Nevertheless, in the sediment slurry experiment described here, Pyr degradation was strongly influenced by pH, higher Pyr-soil ratio and 10% soil-water ratio indicating that slurry-soil reactor has the potential to enhance the biodegradation.

## 4. CONCLUSION

The results of this work have revealed that microbial growth factors such as pH, soil-water ratio and contaminant to soil ratio depend on soil types and on different ranges. Moreover, at higher concentrations of Pyrene-soil ratio, there was negative effect on the biomass growth, while at 10% soil-water ratio, there was significant positive influence on the biomass growth on all the three types of soil examined. This has implication on design of remediation projects. Careful determination of factors that control biomass growth, contaminant concentration and soil characteristic may provide efficient solutions to remediation problems.

## 5. ACKNOWLEDGMENT

The authors wish to acknowledge the assistance and contributions of the laboratory staff of Department of Chemical and Environmental Engineering and Department of Agriculture, Universiti Putra Malaysia. We also wish to acknowledge postgraduate grant for funding this work.

## 6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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