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Degradation of Phenanthrene in Liquid Culture and Sand Slurry by Corynebacterium urealyticum

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Abstract
Most studies on PAHs biodegradation evaluated the degradation potential of microorganisms under indigenous condition in either liquid or solid. There are limited studies on evaluation of the same microorganisms in degrading PAHs under non-indigenous condition in both liquid and solid media. This study investigated the potential of the bacterium, *Corynebacterium urealyticum* isolated from municipal sludge in degrading phenanthrene in both liquid and solid media. Batch experiments were conducted over 20 days. Batch reactors containing artificially contaminated phenanthrene minimal media and sand slurry were inoculated with bacterium culture. Percentage phenanthrene degradation in liquid culture and sand slurry were found to be 87% and 29%, respectively. Apart of having higher rate in liquid culture, the degradation activity by bacterium remain active throughout the experiment. There were no significant differences on the degradation of phenanthrene at low and high initial phenanthrene concentrations in liquid cultures, which differed to the observation in sand slurry. From the viable count analysis, it was observed that this bacterium immediately adapted to the new environment. This study shows that *Corynebacterium urealyticum* show better capability in degrading phenanthrene in liquid culture compared to sand slurry.
Keywords: Bioremediation, Corynebacterium urealyticum, liquid cultures, Phenanthrene, sand slurry

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread and recalcitrant organic compounds that might pose a threat to mankind and the environment (Harvey, 1991; Mrozik *et al*., 2003). Significant levels of PAHs had been detected in air, water, soils and sediments (Harvey, 1991; Yang and Hildebrand, 2006). PAHs pollution in soil differs from water. In water, dilution of PAH may take place where large volume of water is available. PAHs may be transported to other parts of the environment, following the flow of water bodies. However, in soil, PAHs are generally adsorbed to soilparticles and degradation of PAHs is limited (Madsen, 2003). Phenanthrene is a three-ring PAH commonly found in water bodies and contaminated soil. This compound is normally utilized as a model compound in PAH biodegradation studies (Woo *et al*., 2004; Tang *et al*., 2005; Lors *et al.,* 2010).

Biodegradation appears to be the main process responsible for the removal of PAHs from the natural environment (Wilson and Jones, 1993; Mohan *et al*., 2006). Bacteria constitute one of the more frequently used microorganisms in biodegradation, where they transform PAHs to other organic or inorganic end products (Cerniglia, 1984; Rakesh *et al*., 2005). Many bacteria are capable of consuming PAHs as energy and carbon sources or cometabolism, which lead to the biodegradation and reduction processes (Atlas, 1995; Rakesh *et al*., 2005). Various studies had identified these bacteria to be mainly from the genera *Pseudomonas sp.*, *Rhodococcus sp*., *Mycobacterium sp*., *Sphingobacterium sp.*, *Sphingomonas sp., Bacillus cereus, Flavobacterium sp.*, *Beijerinckia sp.* and *Burkholderiacepacia* (Samantha *et al.*, 1999; Moody *et al.*, 2001; Abdul-Ghani, 2008; Chauhan*et al.*, 2008; Zhao *et al.*, 2008; Janbandhu and Fulekar, 2011).

In a more recent study, Janbandhu and Fulekar (2011) found a new bacterium, *Achromobacterinsolitus* MHF ENV IV capable of degrading phenanthrene. In addition to this, a new bacterium, *Corynebacterium*

urealyticum capable of PAH degradation was identified by Othman *et al.* (2010).The genus *Corynebacterium*, established in 1896 by Lehman and Neumann, belongs to the family *Corynebacteriaceae*. This genus is a gram positive bacterium, coccusbacillus-shape, catalase positive, non-spore forming and non-motile (Collin and Cummins, 1986).

Reports on use of bacteria strain in PAH biodegradation in either liquid or solid media are available in the literature. Studies in liquid medium have been reported by Moody *et al.* (2001), Abdul-Ghani*et al.* (2008), Zhao *et al.* (2008) and Janbandhu and Fulekar (2011). Bacteria used in these studies are capable of degrading more than 95% of PAHs in less than 14 days. However, for certain cases where the bacteria were exposed to high concentrations of PAH, less than 60% was degraded (Janbandhu and Fulekar, 2011).

The capabilities of bacteria to degrade PAHs in solid medium were investigated by Kwok and Loh (2003), Sheng and Gong (2006), Gottfried *et al.* (2010) and Karamalidis *et al*. (2010). Incomplete degradation of PAHs was observed in most of these studies, i.e., less than 85%. Moreover, a few of these studies required long duration, up to 191 days, to achieve high percentage removal. The lower removal and longer duration are due to complex processes involved such as solubility, physico-chemical sorption, concentration of PAH sand low bioavailability of PAHs in solid medium (Boopathy, 2000).

There are limited degradation studies on the capability of bacteria under non-indigenous condition in both liquid and solid media. A better knowledge on to what extent PAHs were biodegraded by bacterial in different media under non-indigenous condition could enhance understanding on the use of bacteria in bioremediation of PAH contaminated media. This will lead to better implementation of effective remediation strategies.

The specific aim of this present work is to evaluate the potential of *Corynebacterium urealyticum* in degrading phenanthrene in liquid culture and in sand slurry. This study also compares the trend of degradation at different initial phenanthrene concentrations in both media. In addition, the survival of bacterium in sand slurry during the phenanthrene degradation was also investigated.

2. Materials and Method

2.1 Materials

All chemicals used for extraction, preparation of minimal media and bacteria culture were of analytical grade and supplied by Merck, Germany. Phenanthrene standard for Gas Chromatography Mass Spectrometer (GCMS) was obtained from Dr. Ehrensdorfer (Augsburg, Germany). For extraction in liquid culture, Solid Phase Micro Extraction (SPME) fibre holder assembly equipped with a 7µm polydimethylsiloxane (PDMS) SPME fibre was purchased from Supelco, Sigma-Aldrich Chemie. Samples from sand slurry were extracted using the pressurized microwave extraction system (MAE) Multiwave 3000 (Rotor 8XF100 SOLV and solvent safety system).

2.2 Samples Preparation

Liquid cultures were prepared in 50 mL sterile minimal media. It has no carbon source, but contains trace elements, namely, 8.5g Na_2HPO_4, 3.0g KH_2PO_4, 0.5g NaCl, 1.0g NH_4Cl, 0.5g $MgSO_4.7H_2O$, 0.0147g $CaCl_2$, 0.0004g $CuSO_4$, 0.001g KI, 0.004g $MnSO_4.H_2O$, 0.004g $ZnSO_4$, 0.005g $H_3BO_3$and 0.002g $FeCl_3$. These chemicals were dissolved in 1L ultra-pure water (18MΩ). For sand slurries, pre-screened sand (less than 1mm) was washed in soil: water ratio of 1:2 (w:w) and dried at 60°C for 5 days.

Subsequently, minimal media and cleaned sand were autoclaved for 20 minutes at 121ºC and spiked with phenanthrene/hexane solution. Hexane was evaporated under continuous mixing to ensure homogenous distribution of phenanthrene in samples. Samples were stored under 4^0C until used in biodegradation studies. The initial concentration of phenanthrene was verified in triplicates.

2.3 Bacteria Strain Preparation

Corynebacterium urealyticum, used in this study was isolated from municipal sludge by Othman *et al*. (2010). The strain, which was preserved at temperature of -80^{o}C in microbeads (microbank^{TM}), was grown in nutrient broth. A series of dilution streaking was performed and the strain was sub-cultured three times to attain the active bacterium before it was used in biodegradation studies.

2.4 Biodegradation Study

The prepared liquid cultures were inoculated with 10% bacterial inoculums. On the other hand, sand slurry samples were prepared comprising artificial phenanthrene contaminated sand, 40% minimal media and 10% bacterial inoculums. Both liquid and sand slurry samples contain bacteria in the range of 10^7 - 10^8cfu ml^{-1}. All samples were tested at an initial pH and temperature of 7.0 and 30^{o}C respectively. These conditions were adopted from optimization studies by Othman *et al.* (2010).

After inoculation, all flasks were shaken in an incubation shaker at 150 rpm. Phenanthrene degradation was determined every day until the degradation is completed or the remaining concentrations stabilized over the

20-day study duration. This duration was determined from a preliminary study. Biodegradation studies were performed in triplicate. A control reactor flask without inoculum was also used during the study. Viable count analysis was conducted in all samples. In sand slurry, sterile water was supplied at 2% remaining weight every day. The water was supplied to compensate for drying effects on samples during the experiment. Two initial phenanthrene concentrations, namely, 350 ppm and 100 ppm were adopted.

2.5 PAHs Extraction and Analysis

SPME method was adopted to extract PAHs in liquid culture. An innovative SPME method was optimized by Othman *et al.* (2010). The sample of 20 mL volume was withdrawn and transferred into 25 mL glass bottle with septum cap. The SPME fibre holder was immersed in the samples for 60 minutes in water batch under 60ºC. The fibre was then retracted and transferred to the heated injection port of the gas chromatograph unit for analysis. In this analysis, each sample was tested in triplicate and efficiency recovery from this method was 70%.

For sand slurries, 500 mg of the sand sample was dissolved in 25 mL of n-hexane and acetone 7:3 (*v/v*). The extractions were performed with the pressurized microwave extraction system (MAE) Multiwave 3000 (Rotor 8XF100 SOLV and solvent safety system). MAE was performed under controlled pressure of 10 bars for 40 minutes. When the extraction period was completed, 20 minutes was allowed for the equipment to cool down to room temperature. Subsequently, the samples were filtered with Whatman fibre filter with pore size of 11 µm. The samples were concentrated by means of a rotary evaporator to about 1 mL. The extraction method was modified from Gfrerer *et al.* (2007). This method had achieved high efficiency recovery for phenanthrene of 98.99%.

Extracted supernatant was analyzed by using GCMS (Perkin Elmer Clarus 600). Elite Column 5MS with 30m long, 0.25mm internal dimension and 0.25µm thickness was used to separate the compounds. The injector was operated at 250^{o}C in the splitless mode with 2-minute splitless period. Helium was used as the carrier gas with 1mL min^{-1} constant flow rate. The column temperature was initially set at 50^{o}C for 1 min, increased to 150^{o}C at a rate of 15^{o}C min^{-1} and held at 1 min, and finally ramped at 5^{o}C min^{-1} to 300^{o}C and held constant until the end of the 35 min total run time.

2.6 Survival of Bacteria in Sand Slurry

Concentration of bacteria from sand slurry were quantified by mixing 1g of sand with 9mL of sterile phosphate buffered saline and homogenized at high speed for 1 minute using a vortex mixer. Successive 1/10 dilutions were made by adding 1 mL of the sand suspension to 9mL of phosphate buffered saline. An aliquot (0.1 mL) of each dilution was transferred to nutrient agar on Petri dish. The dishes were incubated at 30°C at the inverted position. After 4 days, colonies of the bacterial strain grown and the numbers of colonies were counted using a plate counter. Plates with different dilutions were prepared and those with colonies in the range of 30 to 300 were used to estimate the number of bacteria. This number of colonies was then multiplied by the dilution factor to find the total number of bacteria per 1g of the sand. The numbers of colonies were expressed as colony-forming units per gram of soil (CFU g^{-1}). All tests were conducted in triplicates.

2.7 Statistical Analysis and Biodegradation Kinetic

The differences in concentrations of phenanthrene for both conditions were determined by using analysis of variance (ANOVA) test with time as co-variance. All statistical analyses were performed with Microsoft Excel software. The biodegradation kinetics of phenanthrene was described using the first order rate model (Eq. 1):

$$S = S_0 e^{-kt} \quad \text{(Eq. 1)}$$

where S is the phenanthrene concentration in the medium at time, t, S_0 is the initial phenanthrene concentration and k is the first order rate constants of degradation process. By using this model, values of first order rate constants (k), regression coefficient (R^2) and half-life ($t_{1/2}$) were determined. The half-life, $t_{1/2}$ is the time required for concentration of substrate to reach one-half of its initial value and calculated as Eq. 2:

$$\ln \frac{1}{2} \frac{S_0}{S_0} = -kt_{1/2}$$
$$ln \frac{1}{2} = -kt_{1/2}$$
$$t_{1/2} = -\frac{ln\, 1/2}{k} \quad \text{(Eq. 2)}$$

3. Result and Discussion

3.1 Comparison of Phenanthrene Degradation in Both Media

Fig. 1 shows phenanthrene degradation by *C. urealyticum*in liquid culture and sand slurry at initial phenanthrene concentration of 350 ppm. In both media, the degradation was observed in three phases with Phase 1, characterised by rapid degradation, Phase 2 with slow degradation and Phase 3 with very slow or almost no degradation. Rapid degradation of phenanthrene in Phase 1 shows that the bacterium easily adapted to the new environment, artificially phenanthrene-contaminated media. Degradation rate in liquid cultures was determined to be 24.80 ppm day^{-1}, 13.94 ppm $day^{-1}$and 5.94 ppm $day^{-1}$for Phases 1, 2 and 3 respectively. These degradation rates were higher than degradation rate in sand slurry. In sand slurry, the degradation rate was determined to be 14.30 ppm day^{-1}, 2.19 ppm $day^{-1}$and 0.02 ppm day^{-1}respectively. At the end of the 20-day experiment, 12.85% (45 ppm) of phenanthrene remained in liquid cultures whereas 69% (245 ppm) remained in sand slurry.

In this study, the bacterium was inoculated in nutrient broth, which is a liquid medium. In the liquid cultures, phenanthrene was dissolved in minimal media during sample preparation. As the liquid culture is a homogenous media, phenanthrene is readily available to the bacterium. Phenanthrene in sand slurry was entrapped and had strong adsorption to sand particles, contributing to low bioavailability of phenanthrene to the bacterium.

PAHs degrading bacteria are able to degrade PAHs by the action of intracellular dioxygenases, meaning those PAHs and both atoms of molecular oxygen (O_2) must be transferred into the cell to begin PAHs degradation (Johnsen *et al.*, 2005). Thus, the low bioavailability in sand slurry slowed and to some extent limited the transfer of phenanthrene into the cell and subsequently retarded the degradation process. PAHs can form strong bonds with solid particle (Pizzul *et al*., 2007). Furthermore, PAHs are highly hydrophobic and have low solubility leading to limited microbial degradation of PAHs in solid media (Johnsen *et al*., 2005). In general, the results show that *C. urealyticum* was capable of degrading phenanthrene better in liquid culture compared to in sand slurry medium.

3.2 Effect of Different Phenanthrene Concentration in Both Media

Phenanthrene degradations at different initial concentrations in both media are shown in Fig. 2a and 2b. In both media, the same trends of degradation were observed at both initial phenanthrene concentrations. More than 98% phenanthrene was degraded at 100 ppm, while only 87% phenanthrene was degraded at 350 ppm in liquid cultures. In sand slurry, 69% and 29% of phenanthrene was degraded at 100 ppm and 350 ppm, respectively. Overall, the remaining phenanthrene was found lower at 100 ppm compared to 350 ppm. These results show that the performance of the bacterium was better at 100 ppm compared to 350 ppm. In liquid culture, almost complete degradation was observed at both initial phenanthrene concentrations. However in sand slurry, very slow degradation was observed after day 6 and day 12 at initial phenanthrene concentrations of 100 ppm and 350 ppm, respectively.The results showed that the degradation trend in both media was indifferent whether at low or high initial phenanthrene concentration.

Generally, bacteria consume organic carbon as substrate to produce energy in metabolism process (Boopathy, 2000). However, when the substrate is present in very high concentrations, the bacteria cell will be saturated with the substrate. The high concentration of substrate may become toxic to the bacteria and thus reduce the efficiency of the degradation process. In a separate but related study, Othman *et al.* (2010) conducted biodegradation study using the same bacterium at initial phenanthrene concentration less than 100 ppm and found that the percentage phenanthrene remaining was lower compared to the results of this present study. Thus, degradation process of phenanthrene by the bacterium was optimum or attained steady state at 100 ppm. At this point the strain has sufficient carbon sources for energy and growth. After the steady state, the substrate saturation occurs and degradation process starts decreasing and resulted in lower percentage of degradation. Similar observations were reported by Bouchez *et al*. (1995) and Romero *et al*. (1998).

Degradation trend in liquid culture from this study was consistent with results from a study conducted by Abdul-Ghani *et al.* (2008). Table 1a and 1b show the performances of PAHs degrading bacteria in liquid culture and solid media, respectively. Abdul-Ghani *et al.* (2008) spiked their samples with mixed PAHs, whereas samples from this study were only spiked with individual PAHs. Irrespective whether single or multi PAHs was used, the degradation trends were observed to be similar in both studies. Meanwhile, effect of different initial phenanthrene concentrations established in this study was consistent with the study conducted by Janbandhu and Fulekar (2011), which found that percentage degradation were reduced when initial phenanthrene concentrations exceed 100 ppm. In using a bacteria consortium, Janbandhu and Fulekar (2011) reported degradation of 56.9% (from 250 ppm) phenanthrene. In contrast, this study used only a single bacterium, namely, *C. urealyticum* which degraded 80.5% of 350 ppm. Percentage degradation in this study was higher compared to that reported by Janbandhu and Fulekar (2011). Thus, this study had found *C. urealyticum*to be a more potential bacterium as phenanthrene degrader. Study conducted by Zhao *et al.* (2008) also discovered a bacterium, namely *Sphingomonas sp.* that degraded 100% of 250 ppm phenanthrene.

In sand slurry or solid medium, the degradation trend established in this study was found consistent with the study reported by Sheng and Gong (2006), which also found that the degradation rate decreased with increased duration. In addition, complete degradation was not achieved even though over an extended duration. Similar findings from studies conducted by Kwok and Loh (2003), Gottfried *et al.* (2010) and Karamalidis *et al.* (2010) have been reported. Results on effect of initial phenanthrene concentrations from this study concurred with findings reported by Sheng and Gong (2006), where it was observed that, as the initial concentration increased, the percentage of degradation was reduced. In general, it is observed that percentages of degradation in studies conducted in solid media are lower compared to liquid culture studies. These showed that complete degradation was not achievable in solid media.

3.3 Growth of C. urealyticum in Sand Slurry

Based on discussion in section 3.1, incomplete degradation in sand slurry cannot be achieved due to low bioavailability. Therefore, accessibility of phenanthrene becomes limited and subsequently interferes with the growth of the bacterium. Therefore, experiments on growth curve were conducted to determine the survival of the bacterium in sand slurry.

The growth curves of the bacterium in sand slurry at different initial phenanthrene concentrations are shown in Fig. 3. For the sample with an initial concentration of 100 ppm, the number of colonies increased from the average of 1.1 x 10^7 CFU g^{-1} at day 0 to 27-fold (3.13 x 10^8 CFU g^{-1}) at day 24. Furthermore, the colonies increased 13-fold (1.5 x 10^8 CFU g^{-1}) in the sample with initial concentration of 350 ppm. The bacterium continues to multiply for both concentrations until an early stationary phase was achieved at day 3 and day 2 for 100 ppm and 350 ppm, respectively.

Then, the bacterium concentration began to reduce until the end of the experiment. This reduction shows that the bacterium was unable to survive longer in sand slurries and subsequently retard the degradation process. Overall, Fig. 3 shows that the bacterium can grow better in 100 ppm compared to 350 ppm. This result showed that substrate saturation affect the growth of bacterium as discussed in section 3.2. The bacterium growth was optimum at substrate concentration of 100 ppm. This result was also supported by Boopathy (2000), which stated that one of the factors that influence the survival of bacteria in soil is the concentration of substrates.

Findings on growth of the bacterium from this study contradicted observations made in liquid culture (Zhao *et al.*, 2008; Janbandhu and Fulekar, 2011). Janbandhu and Fulekar (2011) found the bacteria concentration increased within 14 days of experiment and remained constant towards the end of the experiment. Although samples were spiked with high initial phenanthrene concentration, no reductions were detected in the bacteria concentration. Findings from Janbandhu and Fulekar (2011) are consistent with study in liquid culture conducted by Zhao *et al.* (2008).

3.4 Statistical Analysis and Biodegradation Kinetic

Statistical analysis using ANOVA were conducted to establish the correlation between the degradation of phenanthrene at different initial phenanthrene concentration. From ANOVA, at 5% level of significance, it was found that percentage of remaining phenanthrene for both concentrations in liquid culture were not significance (p>0.05). The poor correlation in the result confirms that the different concentrations of phenanthrene in liquid cultures from this study did not affect the degradation process. Significant correlation was determined (p<0.05) from percentages of phenanthrene degradation in sand slurry for both concentrations from ANOVA analysis. This analysis shows that the degradation of phenanthrene by C. *urealyticum* in sand slurry was influenced by initial phenanthrene concentration.

In this study, correlation between phenanthrene concentration and bacterium concentration in sand slurry was also evaluated by using ANOVA. At 5% level of significance, it was determined that concentration of phenanthrene and bacterium for both concentrations were significance (p<0.05). As a result, bacterium concentration had influenced the degradation of phenanthrene.

Experiments in liquid culture well fitted to first order rate model ($R^2_{100\ ppm}$ = 0.91 and $R^2_{350\ ppm}$ = 0.99). Fig. 4 shows the plots of biodegradation data as fitted to the first-order kinetics model. First-order rate constant for low initial concentration, 100 ppm (k = 0.159d^{-1}) were higher compared to high initial concentration, 350 ppm (k= 0.106d^{-1}). The half-life values for low initial concentration, 100 ppm ($t_{1/2}$ = 5.1) were significantly shorter than high initial concentration, 350 ppm ($t_{1/2}$ = 6.54). Thus, faster degradation occurred at 100 ppm compared to 350 ppm.

Experiments in sand slurry well fitted to first order rate model in the phase 1 (as discussed in section 3.1) with $R^2_{100\ ppm}$ = 0.98 and $R^2_{350\ ppm}$ = 0.97, as shown in Fig. 5. For phase 2 and 3, degradation of phenanthrene in sand slurry is very slow, almost no degradation occurred, and this trend does not fit to any rate law reaction. The reaction rate constant in sand slurry at 100 ppm (k = *0*.192 d^{-1}) were higher compared to 350 ppm (k = 0.047 d^{-1}), similar observation in liquid culture. The phenanthrene reduced to half of its initial concentration at day 3.61 for 100 ppm. Meanwhile, at 350 ppm of initial phenanthrene concentration, the bacterium unable to degrade

half of it initial concentration. Therefore, the half-life of 350 ppm phenanthrene cannot be determined by using first order rate model.

The degradation of phenanthrene in liquid culture is well fitted to first-order rate model throughout the experiments. This was consistent with the findings made by Okpokwasili and Nweke (2005) and Kwon *et al.* (2009). Meanwhile, studies conducted in solid media such as in sediment slurry (Chen *et al.,* 2008) and PAHs contaminated soil (Yang *et al.,* 2011) were consistent with result in sand slurry from this study. First order was well fitted after a certain period such as 24 hours by Chen *et al.* (2008) and 8 days by Yang *et al.* (2011).

4. Conclusions

As conclusion, the performance of *Corynebacteriumurealyticum* was better in liquid culture compared to sand slurry. It was observed that phenanthrene degradation at high initial concentration was lower compared to low initial concentration in both media. Nevertheless, the degradation trends in both media were similar at both initial concentrations. Degradation trends in both media established in this study were consistent with those reported in the literature. Complete degradation was observed in liquid culture, whereas in solid media such was not the case. The bacterium was unable to survive longer in sand slurry and thus affected the degradation process. Percentages of phenanthrene remaining for both initial concentrations in liquid culture were not significant. This is in contrast with observations in sand slurry where remaining phenanthrene is significantly higher. Bacterium concentration had influenced the degradation of phenanthrene in sand slurry at 5% level of significance. Phenanthrene degradation in liquid culture fitted well to the first-order rate model throughout the experiment. Whereas, in sand slurry only fitted to the first-order rate model in phase 1.

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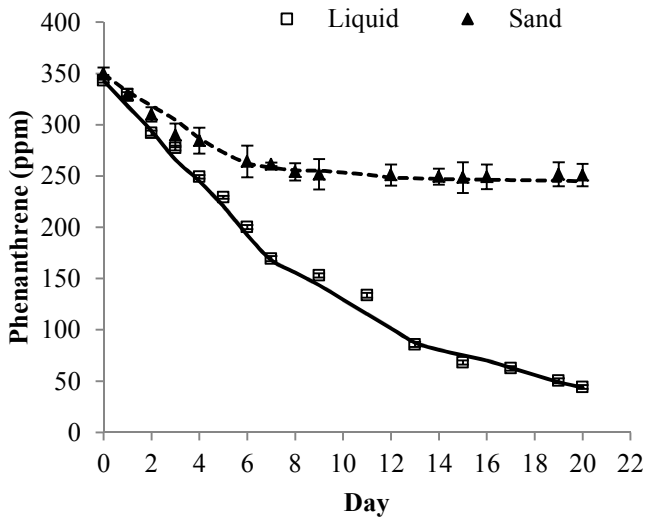

Figure 1. Degradation of phenanthrene in liquid culture and sand slurry at initial phenanthrene concentration of 350 ppm. Data plotted are mean of three replicates; error bars represent SD

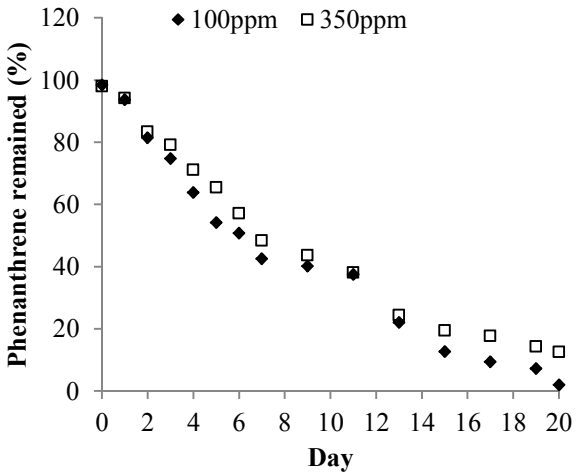

Figure 2a. Phenanthrene degradation by *Corynebacteriumurealyticum* in liquid cultures

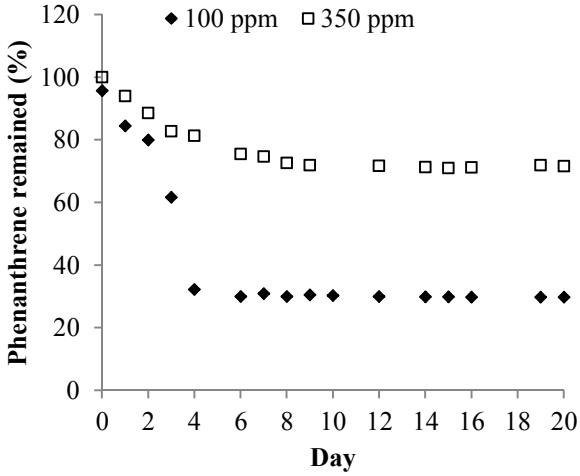

Figure 2b Phenanthrene degradation by *Corynebacteriumurealyticum*in sand slurry

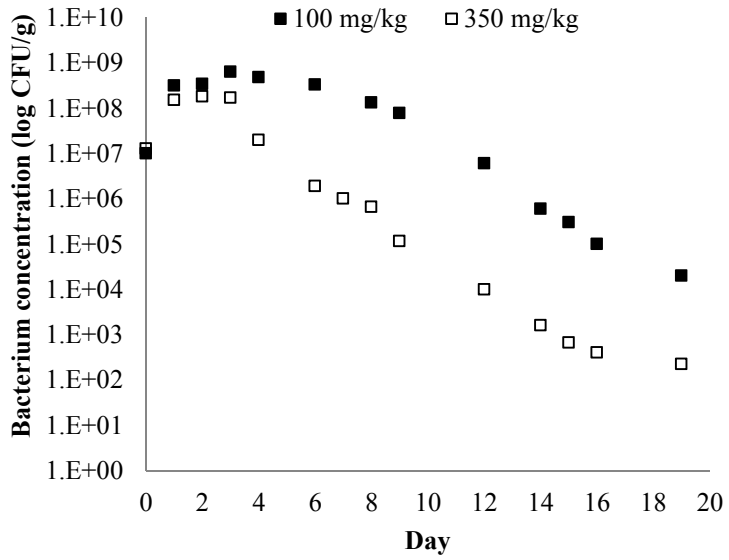

Figure 3. The growth of the bacterium in sand slurry

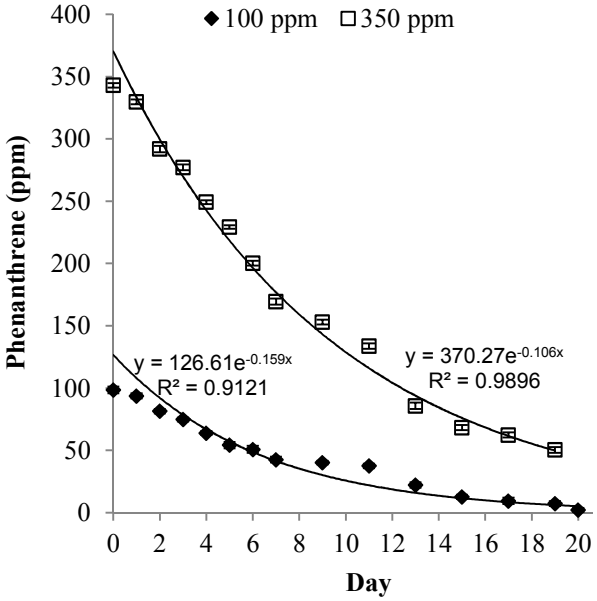

Figure 4. First order biodegradation kinetic in liquid culture. Data plotted are mean of three replicates; error bars represent SD

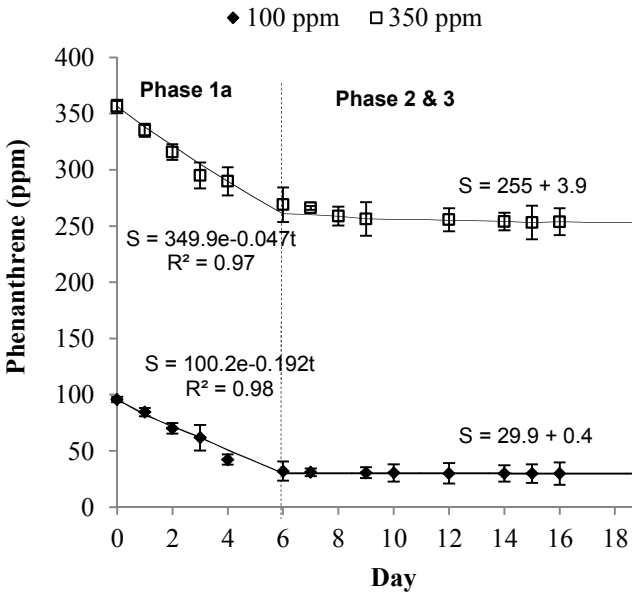

Figure 5. First order biodegradation kinetic in sand slurry Data plotted are mean of three replicates; error bars represent SD

Table 1a. Degradation of PAHs by Different Bacteria in Liquid Culture

References	Bacteria	PAHs	Initial conc. (ppm)	% degradation	Duration (day)
Abdul-Ghani *et al.* (2008)	*Pseudomonas StutzeriBacterium* HS-D36 Uncultured *Pseudomonas sp.* clone 2-A *Pseudomonas Stutzeri bacterium* LS401	dibenzothiophene phenanthrene anthracene (Mixed)	100	95	14
Zhao *et al.* (2008)	*Sphingomonas sp.*	phenanthrene	250	100	8
Janbandhu and Fulekar (2011)	*Sphingobacterium sp.*, *Bacillus cereus* *Achromobacterinsolitus*	phenanthrene	100 250 500	100 56.9 25.8	14
This study	*Corynebacterium urealyticum*	phenanthrene	100 350	98 87	20

Table 1b. Degradation of PAHs by Different Bacteria in Solid Media

References	Bacteria	PAHs	Initial conc. (ppm)	% degradation	Duration (day)
Kwok and Loh (2003)	*Pseudomonas putida* (ATCC 17484)	phenanthrene	300	70	20
Sheng and Gong (2006)	*Pseudomonas sp. GF3* + wheat (planted)	phenanthrene	100 200	84.8 70.2	80
Karamalidis *et al.* (2010)	*Pseudomonas aeruginosa*	16 PAHs (mixed)	58	70	191
Gottfried *et al.*(2010)	*Pseudomonas putida* (ATCC 17484)	phenanthrene	32	68	10
This study	*Corynebacterium urealyticum*	phenanthrene	100 350	69 29	20

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