ISOLATION AND OPTIMIZATION OF PHENANTHERENE DEGRADATIVE BACTERIA FROM MUNICIPAL SLUDGE FOR PAHs BIOREMEDIATION

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Isolation and Optimization of Phenanthrene Degradative Bacteria from Municipal sludge for PAHs bioremediation

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Abstract
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds in the environment that originate from natural and anthropogenic pyrolysis of organic matter. It also can cause great environmental concern because of their persistent, toxicity, mutagenicity and carcinogenicity. The USEPA has identified 16 PAHs compounds as priority pollutants whose level in industrial effluents require monitoring. Possible fates for PAHs released into the environment include volatilization, photo-oxidation, chemical oxidation, bioaccumulation and adsorption on soil particles. The principle process for the successful removal and elimination of PAHs from contaminated environment is microbial degradation. Many studies from temperate countries had reported on biodegradation of PAHs but limited information could be found on tropical region. This study is carried out to isolate phenanthrene degradative bacteria from municipal sludge and to optimize their degradation condition for bioremediation purposes. Selective enrichment method using shaken liquid media, namely, minimal medium was adopted to isolate bacteria capable of degrading phenanthrene. The process involves providing conditions suitable for the growth of bacteria capable of metabolizing the phenanthrene. Bacterial strains were enriched from municipal sludge sample in minimal media broth supplemented with 0.1 % phenanthrene as the sole carbon source. The mixed cultures were grown, at 30 °C on a rotary shaker at 150 rpm for two months. Four parameters including the concentration of bacteria, concentration of phenanthrene, pH and temperature were selected to determine their effects on the degradation of phenanthrene by the isolated bacteria strains. Several bacterial strains were isolated through enrichment and one strain, *Corynebacterium uroalyticum* that was tentatively identified by the Biolog system, demonstrated a high removal rate of phenanthrene over other strains. Following one day lag phase more than 85% of phenanthrene degraded after 2 weeks incubation. Maximum rate of phenanthrene removal occurred in the culture containing 100 mgL^{-1} of phenanthrene. Media at a pH 7.0 was more favourable for the degradation of phenanthrene by *Corynebacterium uroalyticum*. Optimal temperature was determined as 30°C. The isolated *Corynebacterium uroalyticum* demonstrated to be a feasible strain for degradation of phenanthrene at a neutral pH, 30°C even up to a phenanthrene concentration of 100 mgL^{-1}.

Keywords: Biodegradation, Bioremediation, Isolation, Optimization, Phenanthrene.

INTRODUCTION

PAHs constitute an enormously large and diverse class of organic compounds that are emitted into the atmosphere by a number of sources, anthropogenic and natural. They are thermodynamically and chemically stable, which distinguish them as persistent environmental pollutants (Juhasz and Naidu, 2000).

The fate of PAHs in nature is of great environmental concern due to their toxic, mutagenic and carcinogenic properties (Pahlmann and Pelkonen 1987; Weissenfels *et al.*, 1990; Wong *et al.*, 2002). For example, phenanthrene is known to be a human skin photosensitizer and mild allergen. It has also been found to be an inducer of sistem chromatid exchanges and a potent inhibitor of gap junction intercellular communications. PAHs can sorb to organic rich sediments, accumulate in fish and other aquatic organisms, and may be transferred to humans through seafood consumption.

Possible fates for PAHs released into the environment include photolysis, chemical oxidation, photo-oxidation, bioaccumulation, adsorption on soil particle and volatilization. The major decomposition processes for their successful removal are currently believed to be microbial transformation and degradation (Cerniglia, 1984). PAH degradation by bacteria occurs primarily under aerobic conditions involving oxygenase mediated ring oxidation and subsequent catabolic formation, ring fission and metabolism.

Studies on the microbial processes to degrade PAHs in several countries utilizing microbes from different sources have established information on the best microorganism to be used (Churchill *et al.*, 1998; Yuan *et al.*, 2000; Gaskin *et al.*, 2005; Yu *et al.*, 2005). Churchill *et al.*, (1998) studied riverine sediment sample in USA. Meanwhile, Yuan *et al.*, (2000) studied PAH degrading bacteria from petrochemical effluent in Taiwan. Beside that, Gaskin *et al.*, (2005) studied on biodegradation of PAHs using bacteria from soil sample taken from coal gasification site in South Australia. PAHs degradative bacteria isolated from mangrove sediment had been reported by Yu *et al.*, (2005). Very few researches have been reported on studies in tropical countries like Malaysia (Ghazali *et al.*, 2004 and Dzulkifli, 2007). It is noted that bacteria strain isolated from different sources, places and environment will show diverse potential in treating PAHs contaminated sites. Lack of information on microbial consortium to degrade PAHs in Malaysia currently impedes further development on bioremediation efforts to treat PAHs contaminated sites.

The introduction of isolated PAHs degradative bacteria at contaminated sites for bioremediation requires a specific set of abiotic factors, namely, pH, temperature, concentration of bacteria and concentration of substrate. Since pH and temperature in bioremediation system can significantly affect the activity and diversity of microbial, a better understanding of these factors would lead to improve process control of bioremediation system. The effects of concentration of bacteria and concentration of substrate have also being studied for improving the degradation of PAHs compounds.

In the present study, bacteria strains from municipal sludge will be isolated and the degradation condition will be optimized.

METHODOLOGY

Municipal sludge was collected from Mawar College, UiTM, Shah Alam, Malaysia. All samples were stored at 4°C prior to being used in laboratory tests. A set of triplicate samples were used for the enrichment process. Ten gram of municipal sludge was weighed and put into a 100ml conical flask and 50ml of autoclave minimal media (Na_2HPO_4, KH_2PO_4, NaCl, NH_4Cl, $MgSO_4.7H_2O$, $CaCl_2$, $CuSO_4$, KI, $MnSO_4.H_2O$, $ZnSO_4$, H_3BO_3, $FeCl_3$) was added into the flask containing municipal sludge samples. 0.1% phenanthrene was then inoculated into minimal media. The suspensions were incubated at 150 rpm on a reciprocal shaker at 30°C in the dark for two months. Samples which produced a turbid culture in mineral media after the enrichment process was selected for isolation.

The turbid samples were subjected to a series of dilution from 10^{-1} to 10^{-8} with peptone water. Minimal media and nutrient agar plates were prepared and spread with 0.1% of phenanthrene. Each dilution was aseptically plated on minimal media and nutrient agar plates which were appropriately labeled. The plates were incubated at room temperature (about 28 °C) for two weeks. Multi steps of purification were done until a pure strain was obtained. The pure culture was identified using Biolog system (MicroLogTM System released 4.2).

The biodegradation experiment was performed using 250 ml reactor flasks containing 50ml minimal media. Four parameters including the concentration of bacteria, concentration of PAH, pH and temperature were tested for investigating their effects on the degradation of PAHs of the isolated bacteria strains.

Amount of bacterial strains were varied from 1% to 12% (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%) from the total volume of minimal media broth. Concentrations of substrates were varied and value depended on characteristic study of sludge and previous researches (10ppm, 100ppm, 500ppm). The pH chosen were 5, 6, 7, 7.5 and 8 for minimal media broth. The pH solution was adjusted to the desired pH glacial acetic acid and ammonium hydroxide. Temperatures chosen were 20^{0}C, 25^{0}C, 30^{0}C, 35^{0}C and 40^{0}C.

The samples were then agitated in an incubated shaker at 150rpm for two weeks. Samples were periodically collected from reactor flasks for day 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, and 15 for the` purpose of measuring residual concentration of PAHs.

The phenanthrene in the sample was extracted using solid phase microextraction (SPME) (Othman *et al.*, 2008). 99% of n-hexane was used as solvent to dissolve phenanthrene. 20ml of the samples were aseptically transffered into 25ml glass bottle with septum cap. Then it was put in an ultrasonic water bath set at temperature 60°C.

The SPME fibre holder assembly equipped with a 7μm polydimethylsiloxane (PDMS) SPME fibre (purchased from Supelco, Sigma-Aldrich Chemie) was used to extract phenanthrene. The PDMS fibre was immersed in the sample for 60 minutes. 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.

Extracts were analyzed by Perkin Elmer Clarus 500 gas chromatography. This unit, equipped with Elite column 5MS with 30m long x 0.25mm internal diameter x 0.25μm thickness, was used to separate the compounds. Flame ionization detector (FID) was adopted for the analysis. The injector was operated as follows; it was set at 250°C in the splitless mode with a 2 minute splitless period. Helium was used as the carrier gas with 1ml/min constant flow rate. The column temperature was initially set at 50°C for 1 minute, increased to 150°C at a rate of 15°C/min and held for 1 min, and finally ramped at 5°C/min to 300°C and held constant until 15 minutes of the total run time. Identification of analytes in the chromatograms was based on retention times.

RESULTS & DISCUSSIONS

After a series of enrichment and purification process three pure bacteria strains were isolated from municipal sludge. Using the MicroLog Station System, the isolates were tentatively identified as *Corynebacterium uroalyticum*, *Micrococcus diversus* and *Sphingomonas sanguinis*. *Corynebacterium uroalyticum* and *Micrococcus diversus* are coccusbcillus in shape and gram positive bacteria while the color are cream and slightly pink respectively. *Sphingomonas sanguinis* is a gram positive bacteria with coccusbacillus shape while the color is slightly pink.

Four parameters including the initial concentration of bacteria, pH, temperature, concentration of substrate were tested for investigating their effects on the degradation of phenanthrene using the isolated bacteria strains. Degradation of phenanthrene was found to vary according to isolates used.

A typical graph on the effect of bacteria concentration on phenanthrene degradation is shown in Figure 1. Figure 1 shows that after 14 days incubation, the degradation of phenanthrene increase as the initial concentration of bacteria increase. The PAHs degradation reaches the optimum level at 10% of bacteria concentration. The rate for 1% bacteria concentration is 3.9 ppm per day. The rate increased to 6.2 ppm per day, for 10% bacteria concentration and remains unchanged at 12% bacteria concentration.

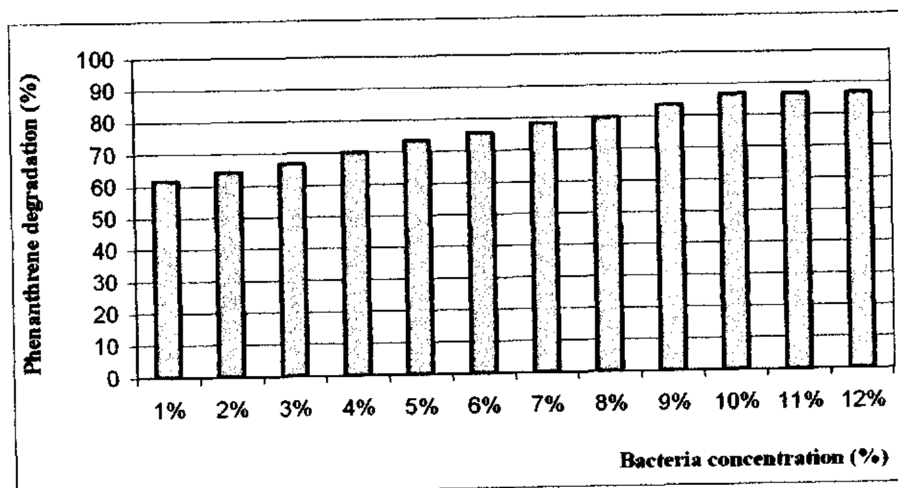

Figure 1: Phenanthrene degradation at different concentration of *Corynebacterium uroalyticum* after 14 days of incubation

More enzymes could be synthesized with the increase in bacteria concentration. Each enzyme has a certain area on its surface that is known as active sites. The active site is the region at which the enzyme forms a loose association with PAHs as its substrate. Therefore, the more enzymes are secreted the more active sites will be provided for PAHs. Enzymes remain unchanged while they speed up degradation of PAHs.

The percentage of degradation increased as the concentration of bacteria increased and finally reached a steady state known as chemical equilibrium. At this point the enzyme's active site or binding site is working at full capacity.

Romero *et al.* (1998) and Yuan *et al.* (2000) reported the same range of bacteria concentration used for biodegradation of PAHs. A summary of reports on effects of bacteria concentration for several bacteria capable of degrading phenanthrene is provided in Table 1. The table showed that the results for PAHs biodegradation by *Corynebacterium uroalyticum* in this study are comparable with other researchers on the influence of bacteria concentration.

Table 1: Previously reported optimum amount of various bacteria concentration in degrading phenanthrene

Reseachers	PAHs	Bacteria	Source of bacteria	Ranges of bacteria	Optimum bacteria concentration	Degradation observation
Romero *et al* (1998)	Phenanthrene	*Pseudomonas aeruginosa*	Refinery sediment	1-15%	12%	80%
Yuan *et al* (2000)	Phenanthrene	*Pseudomonas fluorescens*	Petrochemi - cal effluent	1-15%	10%	85%
This study	Phenanthrene	*Corynebacterium uroalyticum*	Municipal sludge	1-12%	10%	87%

Effect of pH on phenanthrene degradation was evaluated at pH 5, 6, 7, 7.5 and 8. Like other proteins, enzymes in bacteria are affected by extreme pH such as strong acid or alkali. Thus, this study evaluated the effect of pH on PAHs degradation at pH value that is mildly acid and mildly alkali. Typical graph on effect of pH on PAHs biodegradation by *Corynebacterium uroalyticum* is shown in Figure 2. Based on Figure 2, degradation of phenanthrene by *Corynebacterium uroalyticum* shows

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increasing trend as pH increase. The degradation reaches optimum level at pH 7. At pH 7.5 and 8 degradation processes decrease.

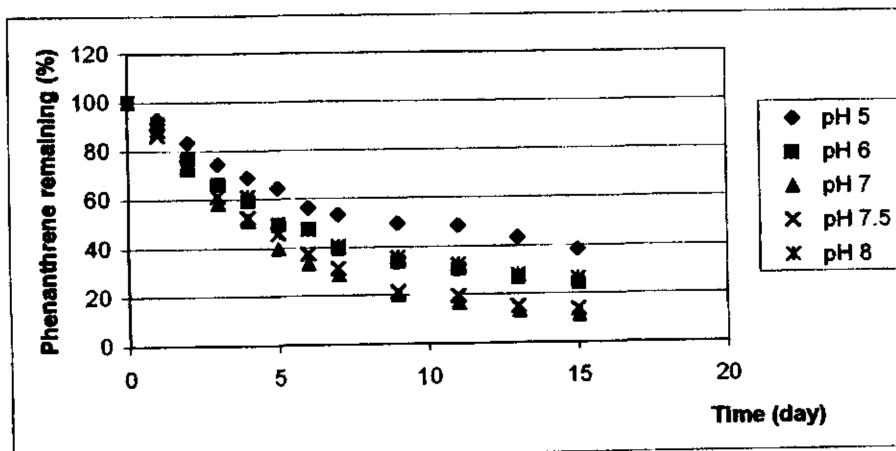

Figure 2: Effects of pH on phenanthrene degradation by *Corynebacterium uroalyticum*

At equilibrium, biodegradation of PAHs on *Corynebacterium uroalyticum* as a function of pH is shown in Figure 3, which indicated that at pH 5 and pH 6, slower biodegradation were observed with the range of phenanthrene degradation 61% to 75% respectively. While at pH 7, 88% of phenanthrene degraded. For the pH value higher than 7, biodegradation process is slower than optimum level. The result shows that the optimum degradation was accomplished at pH 7.

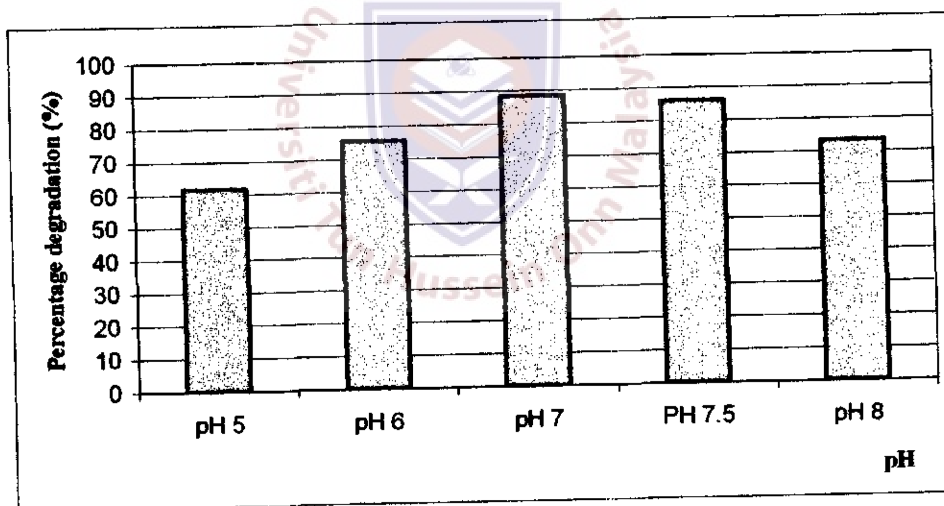

Figure 3: Phenanthrene degradation by *Corynebacterium uroalyticum*

Changes in pH can alter the electrical charge on various chemical groups in enzymes molecules. Changes in electrical charge probably alter the enzyme's ability to bind its substrate and catalyze a reaction. Imbalance of the electrical charges in very acidic and alkali condition can disrupt hydrogen bonds and other weak forces that maintain enzyme structure. Such disruption of enzyme structure is called denaturation. Thus, this phenomenon resulted in poor biodegradation process. This explanation was also offered by Yuan *et al.* (2000). They also had pointed out that microbial enzymes have an optimum pH at which they function most effectively, thus it is related to an organism normal environment.

The results from this study suggest that the biodegradation of PAHs strongly depends on pH, as it affects the ionization state of the main functional group in PAHs which is benzene on bacterial cell (Wong *et al.*, 2002). Several studies reported by Bouchez *et al.* (1995) and Yuan *et al.* (2000) indicated that optimum pH for PAHs biodegradation normally occurred at pH 7 as shown in Table 2. When compared to other studies, it was shown that optimum biodegradation by *Corynebacterium uroalyticum* was found to be at pH 7.0.

Table 2: Previously reported optimum pH for various PAHs degradation

Resaerchers	PAHs	Bacteria	Source of bacteria	Optimum pH
Bouchez *et al.*, 1995	Naphthalene, Fluorene, Phenanthrene	*Rhodococcus sp* *Pseudomonas stutzeri*	Soil	pH 7.0
Romero *et al.*, 1998	Phenanthrene	*Pseudomonas aeruginosa* *Rhodotorula glutinis*	Refinery sediment	pH 6.8
Yuan *et al.*, 2000	Phenanthrene	*Pseudomonas fluorescens* *Haemophilus*	Petrochemical effluent	pH 7.0
Wong *et al.*, 2002	Phenanthrene	*Pseudomonas versicularis* *Burkhalderia cocovenans*	Petroluem contaminated soil	pH 7.5
This study	Phenanthrene	*Corynebacterium uroalyticum*	Municipal sludge	pH 7.0

Degradation of phenanthrene using *Corynebacterium uroalyticum* as biodegrader is also carried out at different initial temperature. Degradation of phenanthrene using *Corynebacterium uroalyticum* was used for the purpose of discussion effect of temperature. The temperatures chosen for temperature dependence study were 20^{0}C, 25^{0}C, 30^{0}C, 35^{0}C and 40^{0}C. Figure 4 shows a typical graph on biodegradation of phenanthrene as a function of temperature.

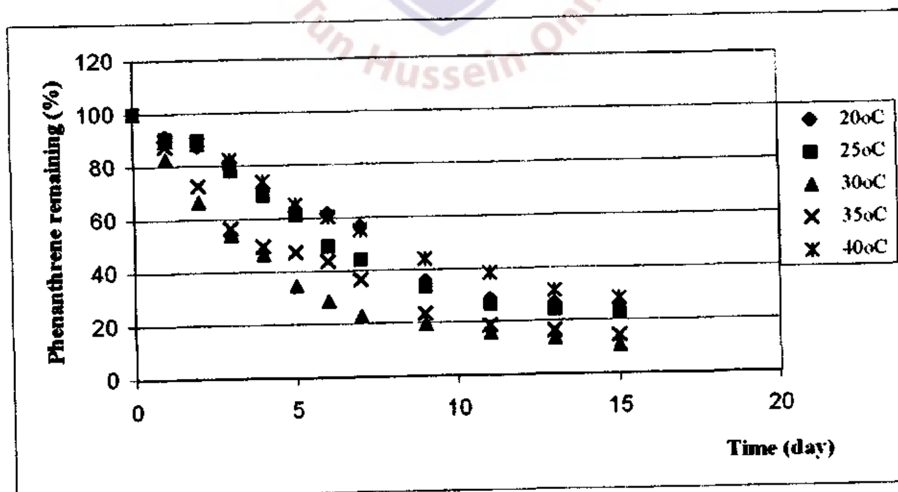

Figure 4: Effects of temperature on phenanthrene degradation by *Corynebacterium uroalyticum*

Figure 4 shows biodegradation of PAHs by *Corynebacterium uroalyticum* at equilibrium condition as a function of temperature. It is shown that at low temperature such as 20°C low degradation process is observed, while at 25°C the biodegradation process start to increase. The results show that the optimum temperature was attained at 30°C. The equilibrium PAHs biodegradation is 87.2% at this temperature value.

Low degradation process at low temperature suggests that this temperature is not sufficient for the enzyme to speed up the reaction rate. Therefore, increased in temperature will result in faster reaction rate in degrading PAHs. In general enzymes as catalysts have certain temperature that it withstands and normally at optimum temperature will catalyze reaction most rapidly. Microbial enzymes likewise function best at optimum temperature which related to an organism's normal environment. Thus, reaction such as degradation process is normally optimum at mild temperature in microbial cell. Above 40°C, however enzyme is rapidly denatured, and its activity decreased accordingly.

The results from this study suggest that the degradation of PAHs strongly depend on temperature. It affects the reaction rate to cleave binding in benzene as the main functional group. These observations were also reported by previous studies that indicated the optimum temperature for PAHs biodegradation occurred at 30°C as shown in Table 3. The results from this study are comparable with those of other reported studies.

Table 3: Previously reported optimum temperature for various PAHs degradation

Researchers	PAHs	Bacteria	Source of bacteria	Optimum temperature
Romero *et al.*, 1998	Phenanthrene	*Pseudomonas aeruginosa* *Rhodotorula glutinis*	Refinery sediment	30°C
Yuan *et al.*, 2000	Phenanthrene	*Pseudomonas fluorescens* *Haemophilus*	Petrochemical effluent	30°C
Wong *et al.*, 2002	Phenanthrene	*Pseudomonas versicularis* *Burkhalderia cocovenans*	Petroluem contaminated soil	30°C
This study	Phenanthrene	*Corynebacterium uroalyticum*	Municipal sludge	30°C

The experiment on effect of initial substrate was conducted at optimized parameters, 10% bacteria concentration, pH 7.0 and temperature at 30°C. The initial PAHs concentrations were concentration adopted from characteristic study, 10ppm, 50ppm, 100ppm and 500ppm. The lowest concentration was choosed based on PAHs concentration in sludge sample analysed. Other PAHs concentrations were choosed based on previous studies as reported by Romero *et al.*, (1998) and Wong *et al.*, (2002). PAHs degradation by *Corynebacterium uroalyticum* is affected by the change in phenanthrene concentration as shown in Figure 5. The percentages of degradation increase as concentration of substrate increase up to 100ppm of substrate concentration (as shown in Figure 5). Thus, degradation process is optimum at 100ppm of substrate or is known as a steady state. At this point the PAHs degrading bacteria have sufficient carbon sources for energy and growth.

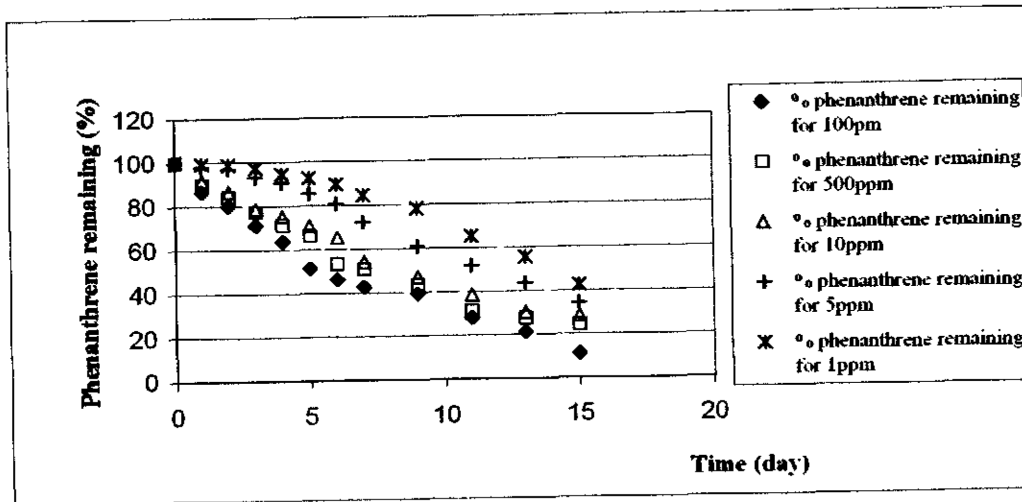

Figure 5: Effects of substrate concentration on phenanthrene degradation by *Corynebacterium uroalyticum*

There was about 87.3% phenanthrene degradation for cultures with the addition of 100 ppm PAHs but only 80.5% phenanthrene degradation at 500 ppm. The average degradation rate of phenanthrene is 6.00 ppm/day at 100ppm, 3.28 ppm/day at 500ppm, 0.98 ppm/day for 50pm, 0.45 ppm/day at 10ppm, 0.12 ppm/day for 5ppm and 0.01 ppm/day at 1ppm (as shown in Figure 6). 100ppm phenanthrene shows a higher efficiency to remove phenanthrene compare to 500ppm phenanthrene culture. This may be due to the fact that too high the concentration of phenanthrene would saturate the bacteria cell with the substrate. Therefore, the optimum degradation on phenanthrene at 86.3% was obtained at phenanthrene concentration of 100ppm.

Degradation of phenanthrene by *Corynebacterium uroalyticum* increased with substrate concentration indicates a great potential in application of *Corynebacterium uroalyticum* as a biodegrader to the treatment of sludge containing PAHs at high concentration.

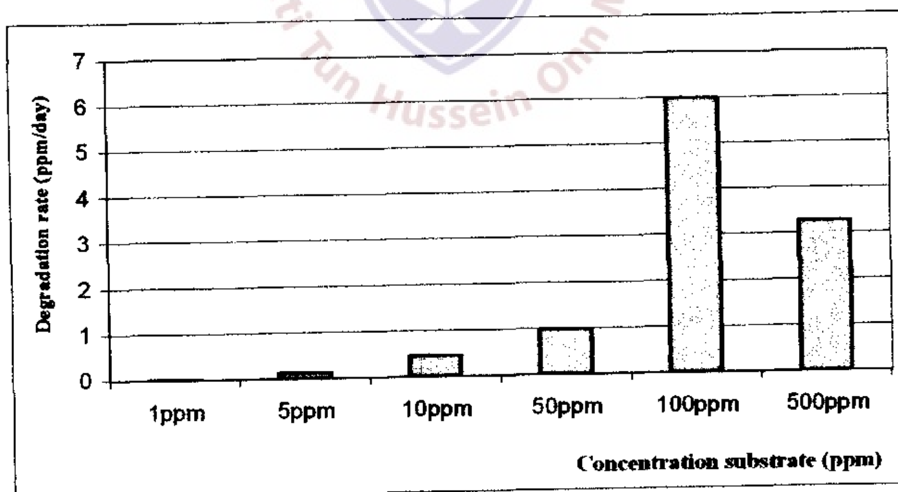

Figure 6: Average degradation rate for different concentration

Other researchers also reported that high concentration of PAHs were beneficial to the growth of isolated bacteria because PAHs could acts as a sole carbon source for bacterial growth. Therefore, low concentrations of PAHs would become a limiting factor for the bacterial culture to grow.

The degradation of PAHs at higher PAH concentration level shows an increasing trend because the increase of PAHs will increase the affinity of substrate toward formation of product. When neither substrate nor products are removed from the system, the reaction will ultimately reach a steady state known as chemical equilibrium. At equilibrium no net change was observed in the concentration of substrate or product. After the steady state, the substrate saturated and degradation process start decreasing and resulted in lower rate of reaction as shown in Figure 6 for 500ppm phenanthrene concentration. Similar observation had been put forward by Bouchez *et al.* (1995) and Romero *et al.* (1998).

The influence of initial concentration of PAHs by various studies is shown in Table 4. The results from this study are comparable with other studies reported in literature. In this optimization study phenanthrene acts as a sole of carbon and energy for bacterial growth. Thus, high concentration of substrate is valuable to cell development of the culture. The results from this study and previous studies also showed that too high concentration of substrate would become a limiting aspect for culture expansion.

Table 4: Previously reported optimum concentration on PAHs for various biodegrader

Researchers	PAHs	Biodegrader	Ranges	Optimum concentration on PAHs	Observation.
Romero *et al.*, 1998	Phenanthrene	*Pseudomonas aeroginosa*	25-200ppm	100ppm	80%
Wong *et al.*, 2002	Phenanthrene	*Pseudomonas versicularis Burkhalderia cocovenans*	100-1000ppm	500ppm	85%
This study	Phenanthrene	*Corynebacterium uroalyticum*	1-500ppm	100ppm	87%

CONCLUSION

This study established that *Corynebacterium uroalyticum* has the capability to degrade phenanthrene in a range varying depend on environmental conditions provided. Maximum rate of phenanthrene removal occurred in the culture containing 100 mgL^{-1} of phenanthrene. Media at a pH 7.0 was more favourable for the degradation of phenanthrene by *Corynebacterium uroalyticum*. Optimal temperature was determined as 30°C. The isolated *Corynebacterium uroalyticum* demonstrated to be a feasible strain for degradation of phenanthrene at a neutral pH, 30°C even up to a phenanthrene concentration of 100 mgL^{-1}.

REFERENCES

Bouchez, M., Blanchet, D. & Vandecasteele, J.P. (1995). Degradation of polycyclic hydrocarbons by pure strains and by defined strains associations: Inhibition phenomena and cometabolism. *Applied Microbial Biotechnol*, 43, 156-164.

Cerniglia, C.E. (1984). Microbial metabolism of polycyclic aromatic hydrocarbon. Advance Applied Microbiology, 30, 31-71.

Churchill, S.A., Harper, J.P., & Churhill, P.F. (1998). Isolation and characterization of *Mycobacterium* species capable of degrading three and four ring aromatic and aliphatic hydrocarbon. *Applied and Environmental Microbiology*, 65, 549-552.

Dzulkifli N. (2007). Bioremediation of pertoluem sludge from petroleum refining plant. Master Dissertation Faculty of Civil Engineering, University Teknologi Mara. Unpublished.

Gaskin, S. & Bentham, R. (2005). Comparison of enrichment methods for the isolation of pyrene degrading bacteria. *International Biodeterioration and Biodegradation*, 56, 80-85.

Ghazali M.F., Zaliha N.R., Salleh, B and Basri, M. (2004). Biodegradation of hydrocarbon in soil by microbial consortium. Biodeteroration and Biodegradation, 54, 61-67.

Juhasz A.L. and Naidu R. (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of microbial degradation of benzopyrene. International Biodeterioration & Biodegradation, 45, 57-88.

Othman. N., Hussein. N., Abdul-Talib. S. (2008). Extraction technique for determination of PAHs in municipal sludge. The 2nd KAIST-UiTM Symposium on Urban Engineering and Sustainability. March 30th-April 1st 2008. KAIST, Daejon, Korea.

Pahlmann R., and Pelkonen O. (1987). Mutagenicity studies of different polycyclic aromatic hydrocarbon: the significance of enzymatic factors and molecular structure. Carcinogenesis, 8, 773-778.

Romero, M.C., Cazau, M.C., Giorgieri, S. & Arambarri, A.M. (1998). Phenanthrene degradation by microorganism isolated from a contaminated stream. *Environmental Pollution*, 101, 355-359.

Weissenfels W.D., Beyer. M., and Klein. J. (1990). Degradation of phenanthrene, fluorine and fluoranthene by pure culture bacterial strains. Applied Microbiology and Biotechnology, 32, 479-484.

Wong, J.W.C., Lai, K.M., Wan, K.K.M.A. and Fang, M. (2002). Isolation and optimization of pah degradative bacteria from contaminated soil for pahs bioremediation. *Water, Air and Soil pollution*. Vol. 139: 1-13.

Yu, S.H., Ke, L., Wong, Y.S. & Tam, N.F.Y. (2005). Degradation of polycyclic aromatic hydrocarbons (PAHS) by a bacterial consortium enriched from mangrove sediments. *Environment International*, 31(2), 149-154.

Yuan, S.Y., Wei, S.H. Chang, B.V. (2000). Biodegradtion of polycyclic aromatic hydrocarbons by a mixed culture. *Chemosphere*. Vol. 41: 1463-1468.