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DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBON BY PURE STRAIN ISOLATED FROM MUNICIPAL SLUDGE: SYNERGISTIC AND COMETABOLISM PHENOMENA

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ABSTRACT

Polycyclic aromatic hydrocarbons are classed as potentially hazardous chemicals of environmental and health concern. Some PAHs exhibit carcinogenic and mutagenic properties and are listed by the USEPA as priority pollutants. 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 using microbial from petroleum contaminated area but limited information could be found from other medium. Thus, the previous studies have not investigated in detail the interaction among PAHs during biodegradation. This study was carried out to isolate PAHs degrading bacteria from municipal sludge and to determine the effect of enrichment substrate and PAH degradative bacteria activities during degradation of PAHs. Several microorganisms were isolated from municipal sludge for their ability to mineralize PAHs as sole carbon and energy source. Enrichment method process utilised single PAHs as a sole source of carbon. The pure culture was identified using the Biolog system. Biodegradation experiment was performed using 250 ml reactor flasks containing 50ml minimal media, 10% of bacterial inoculum and the specific PAHs. All samples were tested at an initial pH of 7.0 and temperature controlled at 30^{o}C. The PAHs in the samples were extracted using solid phase microextraction. Extracts were analyzed by Perkin Elmer Clarus 500 gas chromatography. The result of this study showed that isolation and identification process had sucessfully determined a few potential bacteria that able to degrade PAHs. Degradation trend observed in degradation study could be attributed to the different substrate provided during isolation process. Interaction through cometabolism and synergistic occurred for bacteria srains isolated from single substrate.

Keywords: Biodegradation; Degradative bacteria; Polycyclic aromatic hydrocarbon

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compound that originate from natural and anthropogenic pyrolysis of organic matter such as forest fire, automobiles exhaust, coal refining processes and oil industry (Bouchez *et al*., 1994). These compounds are widespread in environment and it is believed as the most abundant organic compounds in the universe that might pose a threat to the environment and mankind (Harvey, 1991; Salama & Allamandola, 1995; Mrozik *et al*., 2003). Significant levels are detected in air, water, soils and sediments (Harvey, 1991; Yang & Hildebrand, 2006). PAHs impact the aquatic environment by targeting the bottom feeder such as polycaetes, nematode, bivalves and crustaceans (Smith *et al*., 2007; Moreno *et al*., 2009), thus creating a disturbance in the ecological balance of the aquatic systems. PAHs may be released into the environment when

the soils or sediments are disturbed. Released PAHs become accessible to the benthic organisms and other aquatic life. PAHs are susceptible to bioconcentration or bioaccumulation in food chain.

PAHs can also be emitted to the environment through anthropogenic sources mainly involving combustion of fossil fuels. PAHs can also be introduced to the environment through natural sources such as volcanic eruptions and forest fires. In addition, smoked food or weathering of petroleum can also result in the formation of hydrocarbons and other byproducts (Wilson & Jones, 1993; Samanta *et al*., 2002). Another important source of PAHs is tobacco smoke which may induce carcinogen effects (Harvey, 1991; Samanta *et al*., 2002). Anthropogenic and natural activities lead to their ubiquitous environmental distribution, where their stability and persistence is governed by their chemical and physical properties on the contaminated sites.

Contaminated sites contain complex mixture of PAHs where interaction between the compounds can alter the rate and degree of their degradation. It is therefore important to look into and understand biodegradation of PAHs mixture for successful implementation of bioremediation technology. Biodegradation of individual and mixture of PAHs by PAHs degradative bacteria have been reported by several researchers (Yuan *et al*., 2000; Wong *et al*., 2002; Yu *et al*., 2005; Desai *et al*., 2008). Thus, these studies have not investigated in detail the interaction among PAHs during biodegradation. There is also lack of information on the effect of enrichment substrate and PAH degradative bacteria activities during degradation of PAHs.

Enrichment culture has long been a method of choice for isolating bacteria capable of degrading compounds such as PAHs (Bastiaens *et al*., 2001). The process involves providing condition suitable for the growth of microorganism capable of metabolizing the desired compounds (Gaskin & Bentham, 2005). The desired microorganisms can be obtained by providing a target substrate as the sole carbon source. A recent study demonstrated that the enrichment substrate can have profound effects on the PAH substrate degrading range of the bacteria (Kastner *et al*., 1994; Mesdaghinia, *et al*., 2005). Organisms isolated on naphthalene and fluorene as carbon sources were unable to grow on anthracene, phenanthrene, fluoranthene or pyrene, whereas organisms isolated on any of these other four compounds were able to grow at least on one of the other substrate. Similar findings were reported when several bacterial strains were isolated capable of using a variety of PAHs as sole carbon and energy source (Bouchez *et al*., 1995; Mohamed *et al*., 2006). *Rhodococcus* sp was isolated from a manufactured gas plant soil when pyrene was used as enrichment substrate.

In this study six PAHs namely napthalene, acenapthylene, acenapthene, fluorene, phenanthene and anthracene in mixtures was studied using PAH degradative bacteria isolated from municipal sludge. The experiments were carried out. using bacteria isolated from single PAHs as substrate. The main purpose of this paper is to study the interactions between these PAHs during their biodegradation by isolated strains from single PAHs.

MATERIALS AND METHODS

Chemicals. Tolune and n-hexane was obtained from Merck, Gemany. PAHs was purchased from Dr. Ehrensdorfer (Augsburg,Germany). The minimal media contained 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 $FeCl_3$. This medium was sterilized by autoclaving 30^0C at 121^0C.

Enrichment, Isolation and Identification of Microorganisms. Several microorganisms were isolated from municipal sludge for their ability to mineralize PAHs as sole carbon and energy source. Enrichment method process utilised single and mix PAHs as a sole source of

carbon. The enrichments adopted shaken liquid media method. Previous studies had shown that enrichment of bacteria able to utilize PAHs as carbon source mostly been done in shaken liquid media. As this method favors bacteria that grow well in suspension (Bastiaens *et al.*, 2001; Gaskin & Betham, 2005).

The mixed cultures were grown, at 30 °C on a rotary shaker at 150 rpm for two months. Erlenmayer flasks with sterile minimal medium supplemented with 0.1% of specific PAHs were used as control. These enrichment processes were done in triplicate for each hydrocarbon.

The isolation process involved the use of the streaking plate method. After two months of enrichment the flask which produced a turbid culture in minimal media was selected for isolation. 10 ml of culture from each flask was then aseptically suspended in 100ml Erlenmayer flask containing 90 ml of peptone water. The mixture then was homogenized by using a vortex mixer. Minimal media and nutrient agar plates were prepared and spread with 0.1% of PAHs. Each dilution was aseptically plated on minimal media and nutrient agar plates which were appropriately labeled. The plates were incubated at room temperature (about 30°C) for two weeks. Colonies appeared were picked and cultured in new minimal medium for seven days. Multi steps of purification were performed until a pure strain was obtained. Purification process involved streak plate method was done to ensure only single strain was obtain for each plating.

The pure culture was identified using the Biolog system (MicroLogTM System released 4.2). The Biolog is an advanced tool for identifying and characterizing microorganisms. Biolog's technology uses each microb abilities to use particular carbon sources to produce a unique pattern for that microbe. As a microorganism begins to use the carbon sources in certain well of the micro plate, it respires. For bacteria, this respiration process will reduce a tetrazolium dye and those well will change to purple color (Biolog , 2001).

Biodegradation Experiment and Analytical Procedure. The biodegradation experiment was performed using 250 ml reactor flasks containing 50ml minimal media, 10% of bacterial inoculum and the specific PAHs. All samples were tested at an initial pH of 7.0 and temperature controlled at 30^{o}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 PAHs in the samples were extracted using solid phase microextraction (SPME) optimized by Othman *et al*., 2008. The PAHs used in biodegradation study were the six PAHs and 99% of n-hexane was used as solvent to dissolve the PAHs. 20ml of the samples were aseptically transffered into 25ml glass bottle with septum cap. Then they were put in an ultrasonic water bath set at temperature 60^{o}C.

The SPME fibre holder assembly equipped with a 7µm polydimethylsiloxane (PDMS) SPME fibre (purchased from Supelco, Sigma-Aldrich Chemie) was used to extract PAHs. 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^{o}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^{o}C for 1 minute, increased to 150^{o}C at a rate of 15^{o}C/min and held for 1 min, and finally ramped at 5^{o}C/min to 300^{o}C

and held constant until 15 minutes of the total run time. Identification of analytes in the chromatograms was based on retention times.

RESULTS AND DISCUSSION

Isolation and Characterization of Pahs Degrading Strains. Isolation of PAHs degrading bacteria were performed as described in materials and methods. The isolated strains were tested for their ability to degrade PAHs and use different individual PAH. The clear zones on agar plates appeared as colonies were grown on PAHs coated agar plates and these phenomena indicated PAHs degradation. Cultures isolated were able to utilize single PAHs, such as, naphthalene, acenapthylene, acenapthene, fluorene, phenanthrene and anthracene. The culture were also performed positive result on their ability to degrade mix PAHs. All isolates were then prepared for identification process using Biolog.

The Automated Microbial Identification System developed by Biolog for rapid identification of laboratory cultures, has been shown to be useful in the characterization and classification of bacteria. Therefore, species isolated in this study were identified using Biolog Microbial identification system. The Microlog software was used to search for the appropriate microorganisms from its database. Using this software, the isolates were tentatively identified and presented in Table 1.

The results show that four different genus have been identified which include *Micrococcus, Rhodococus, Corynebacterium* and *Pediococcus*. Two of the isolates namely N1, and F1 yield the same identities. Out of six isolates identified, two of isolates are from genus *Corynebacterium* but they represent different species.

Table 1: Identification of Bacteria Using Biolog

Source of carbon	Organism	Identity
Napthalene	N1	*Micrococcus diversus*
Acenapthylene	AC1	*Rhodococus rhodochrous*
Acenapthene	A1	*Corynebacterium agropyri*
Fluorene	F1	*Micrococcus diversus*
Phenanthrene	P1	*Corynebacterium uroalyticum*
Anthracene	AN1	*Pediococcus pentosaceus*

Microorganisms with the ability to degrade a variety of PAHs compounds have been isolated and characterized by several researchers (Weissenfels *et al*., 1990; Yuan *et al*., 2000; Dean Ross *et al*., 2001; Wong *et al*., 2002; Prabhu and Pale, 2003; Santos *et al*., 2007; Zhoa *et al*., 2009). Previous studies reported that that most of PAHs degradative bacteria belong to *Pseudomon*as spp. This genus is a gram negative bacteria. This genus also is one of the most studied and reported as PAHs degrader as well as of other organic recalcitrant pollutants. Previous studies by Yuan *et al*., (2000); Juhasz and Naidu, (2000) and Dean Ross *et al*., (2001) had proved that *Pseudomonas spp* had an ability to degrade both low and high molecular weight PAHs if supplied with appropriate growth medium. In comparison with previous studies this study had discovered different genus of PAHs degradative bacteia as stated in Table 1.

This study also provides evidence that the communities of PAHs degrading bacteria in the municipal sludge were very diverse. Discovery on new species provided essential knowledge on PAH degrading bacteria. No evidence and descriptions have been found on the newly

isolated strains discovered in this study to date. Therefore, studies related on newly isolated strains are needed especially to verify the potential of particular strains on PAHs degradation.

Degradation Study by Bacteria Isolated from Single Substrate. This study looked into effect on using bacteria isolated from single PAH as substrate on PAHs degradation. Graphs on results during PAHs degradation study by bacteria strains isolated from naphthalene, acenapthylene, acenapthene, phenanthrene and anthracene as substrate are shown in Figure 1, 2, 3, 4, 5 and 6 respectively. Generally, degradation of PAHs varies according to isolates used.

In this study, degradation of PAHs are determined by the remaining concentration of PAHs in the culture broth because isolated bacteria can only degrade those PAHs that exist in soluble form. This study also looks into interactions of substrate and bacteria which include synergistic or antagonistic interaction. Interactions of PAHs were observed using different of degradation rate with high interaction. Detail description on interaction of substrates is shown in Table 2.

Table 2: Type of Interaction between Substrate and Bacteria

Type of interaction	Range of interaction (different between highest interaction)	Symbol
High (synergistic)	≤ 10%	++
Moderate (synergistic)	>10% and ≤ 98%	+
Low/No (antagonistic)	≥ 99%	-

(a) PAHs Degradation by *Micrococcus diversus* (Isolation Substrate-Naphthalene)

Figure 1 shows degradation of PAHs by *Micrococcus diversus.* The results show that the remaining PAHs in the samples decrease with time. *Micrococcus diversus* shows preference towards naphthalene. Thus, this strain is tested for the ability to utilise different individual PAH as it sole carbon and energy source. In the beginning of incubation time acenapthylene degrade slowly. However, after 11 days the degradation of acenapthylene is faster as naphthalene provided in sample has been utilized by *Micrococcus diversus.* In the case of acenapthene slow degradation process is observed. Other PAHs namely, fluorene, phenanthrene and anthracene did not decrease significantly.

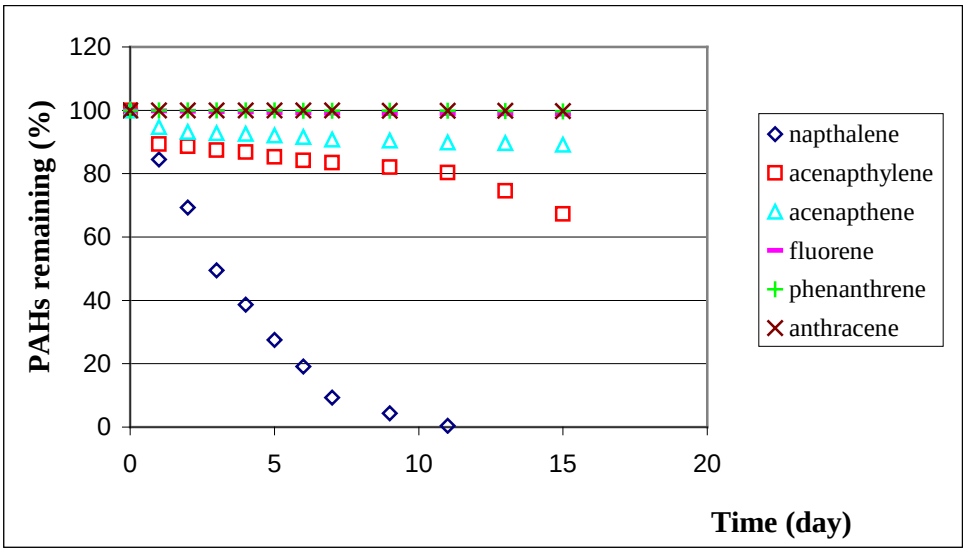

Figure 1: Pahs Degradation Using *Micrococcus Diversus* (Isolation Substrate-Napthalene)

The different degradation trend observed in this study can be attributed to the different substrate provided during isolation process. Interaction during PAH degradation by *Micrococcus diversus* is presented in Table 3. Using naphthalene as a reference, all co-substrates are found inhibitory, except weakly for acenapthylene and acenapthene which both cometabolise weakly with naphthalene. Thus, it is apparent that synergistic interaction via cometabolism is an important features in the degradation of PAHs, as it commonly occurs and widens the range of PAH attacked by a defined strain. It must be noted here, that only kinetic studies on PAH degradation could provide the detail description on this interaction. The present determinations allow identifications of general patterns interaction.

The degradation rate for naphthalene, acenapthylene, acenapthene, fluorene, phenanthrene and anthracene were 8.24ppm/day, 2.64ppm/day, 1.38ppm/day, 0.019ppm/day, 0.014ppm/day and 0.008ppm/day respectively. The results clearly show that *Micrococcus diversus* effectively degrade naphthalene. The results strongly showed that enrichment substrate can have profound effects on the degrading range of bacteria isolated.

Table 3: Interaction between Micrococcus Diversus and Pahs

Strain	PAHs used as substrate					
	NAP	ACE	AC	FLU	PHE	ANT
Micrococcus diversus	++	+	+	-	-	-
Different of degradation rate with high interaction	-	67.9%	83.3%	99.7%	99.8%	99.9%

(++: indicate high interaction; +: indicate moderate interaction if different with high interaction more than 10%; -: no interaction/low interaction if different with high interaction more than 99%)
(NAP-napthalene; ACE-acenapthylene; AC-acenapthene; FLU-fluorene; PHE-phenanthrene; ANT-anthracene)

Among PAH, naphthalene presented specific characteristics. Strains isolated with naphthalene as substrate do not use other PAH as carbon sources and show limited cometabolism capacities with other PAH. Another situation observed in this study, is antagonistic interaction through inhibition in degrading a defined PAH in the presence of other PAH using strain isolated from single source of PAH. Inhibition normally took place whether cometabolism occurred or not and it is related to the fact that PAH, as homologous

compounds, are susceptible to interaction at several levels. Competition at the active site of enzyme, in particular the initial oxygenase, has to be expected. When cometabolism occurs, a second possible cause of inhibition is the accumulation of toxic end product. Hence, interaction at the level of enzyme induction can also take place. It can also completely block off the induction of the initial oxygenase.

(b)PAHs Degradation by *Rhodococus rhodochrous* (Isolation Substrate-Acenapthylene)

The percentages of PAHs show a decreasing trend for acenapthylene and acenapthene. No obvious reduction of fluorene, phenanthrene and anthracene as shown in Figure 2 was observed. Positive interactions occur between *Rhodococus rhodochrous* and acenapthylene, acenapthene and naphthalene. Acenaptylene represents the highest degradation. Acenapthene and naphthalene show moderate interaction and rapid degradation occurred after day 9.

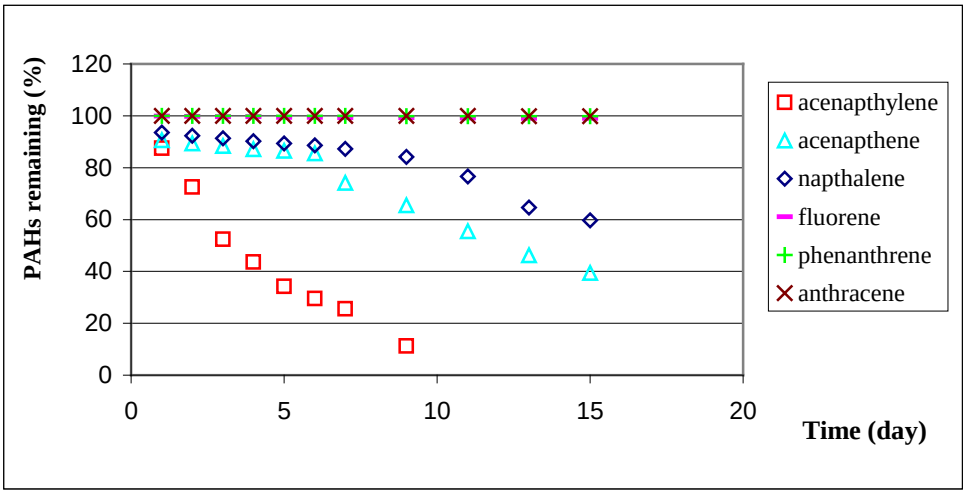

Figure 2: Pahs Degradation Using *Rhodococus rhodochrous* (Isolation Substrate-Acenapthylene)

Rhodococus rhodochrous effectively uses acenapthylene as substrate compare to other PAHs as shown in Figure 2 and Table 4. *Rhodococus rhodochrous* has an ability to use acenapthene and naphthalene as co-substrate with acenapthylene. In this situation both co-substrate are found cometabolize. Other substrate namely, fluorene, phenanthrene and anthracene are found inhibitory due to substrate competition. The low interaction or negative effects are due to competitive inhibition of multiple substrates or other means of retarding the degradation of one substrate in the presence of another. Competitive inhibition lowers the affinity of enzyme. In competitive inhibition, multiple substrates are transformed by a common enzyme system. Similar or identical enzyme system may catalyze the degradation of compounds or being exploited to the same active site.

Table 4: Interaction between *Rhodococus rhodochrous* and Pahs

Strain	PAHs used as substrate					
	NAP	ACE	AC	FLU	PHE	ANT
Rhodococus rhodochrous	+	++	+	-	-	-

Different of degradation rate with high interaction	54.1%	-	69.1%	99.8%	99.9%	9.99%

(++: indicate high interaction; +: indicate moderate interaction if different with high interaction more than 10%; -: no interaction/low interaction if different with high interaction more than 99%)
(NAP-napthalene; ACE-acenapthylene; AC-acenapthene; FLU-fluorene; PHE-phenanthrene; ANT-anthracene)

The degradation rate for acenapthylene, acenapthene, naphthalene, fluorene, phenanthrene and anthracena were 6.77ppm/day, 3.11ppm/day, 2.05ppm/day, 0.009ppm/day, 0.008ppm/day and 0.005ppm/day respectively.

In the case of *Rhodococus rhodochrous* isolated from acenapthylene, it has ability to use acenapthene which also has three benzene ring but different molecular weight ($MW_{acenapthylene}$:152g and $MW_{acenapthene}$:154g). Napthalene is possible to be degraded because it has lower molecular weight ($MW_{napthalene}$:128g). The degradable component of acenapthene by *Rhodococus rhodochrous* may has stimulated the expression of oxygenase enzyme system that could then act to degrade similar number of benzene ring with slightly higher molecular weight. Alternatively physiological properties of microorganism such as production of biosurfactant might have enhanced degradation activities.

(c) PAHs Degradation by *Corynebacterium agropyri* (Isolation Substrate-Acenapthene)

Degradation of PAHs by *Corynebacterium agropyri* is presented in Figure 3. The remaining PAHs show decreasing trend for acenapthene, acenapthylene and naphthalene.

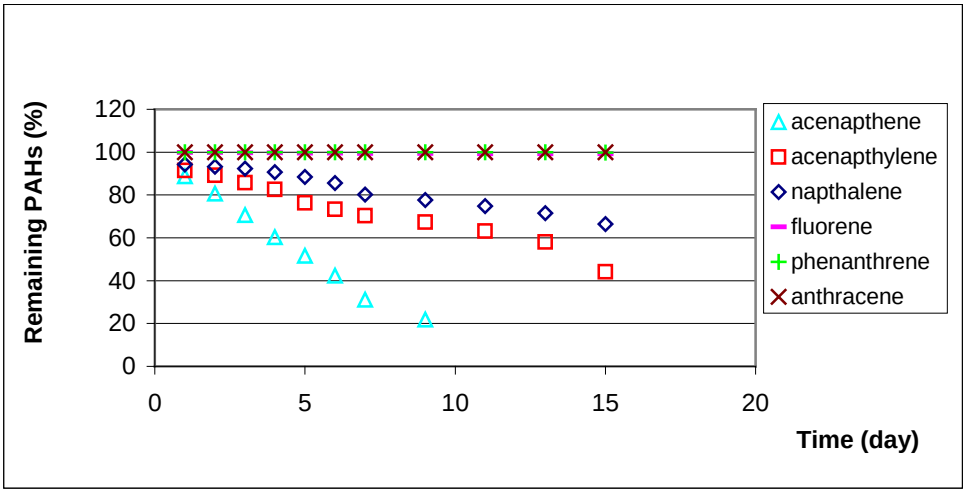

Figure 3: Pahs Degradation Using *Corynebacterium agropyri* (Isolation Substrate-Acenapthene)

Corynebacterium agropyri has an ability to degrade acenapthene at the fastest rate. Following that is degradation of acenapthylene and naphthalene. No reduction of other PAHs namely, fluorene, phenanthrene and anthracene. The results show that this strain only degrades lower molecular weight of PAHs. Synergistic interactions via cometabolism occur among acenapthene, acenapthylene and napthalene while fluorene, phenanthrene and anthracene show antagonistic interaction as shown in Table 5.

The degradation rate for acenapthene, acenapthylene, naphthalene, fluorene, phenanthrene and anthracena were 6.24ppm/day, 2.09ppm/day, 2.04ppm/day, 0.007ppm/day, 0.007ppm/day and 0.004ppm/day respectively.

Table 5: Interaction between *Corynebacterium agropyri* and PAHs

Strain	PAHs used as substrate					
	NAP	ACE	AC	FLU	PHE	ANT
Corynebacterium agropyri	+	+	++	-	-	-
Different of degradation rate with high interaction	67.3%	50.5%	-	99.8%	99.8%	99.9

(++: indicate high interaction; +: indicate moderate interaction if different with high interaction more than 10%; -: no interaction/low interaction if different with high interaction more than 99%)
(NAP-napthalene; ACE-acenapthylene; AC-acenapthene; FLU-fluorene; PHE-phenanthrene; ANT-anthracene)

(d) PAHs degradation by *Micrococcus diversus* (isolation substrate-fluorene)

Figure 4 shows that *Micrococcus diversus* isolated from fluorene degrades flourene compound immediately after exposure time. Degradations were observed on acenapthene, acenapthylene and naphthalene. Phenanthrene and anthracene as the heaviest molecular weight do not show obvious degradation.

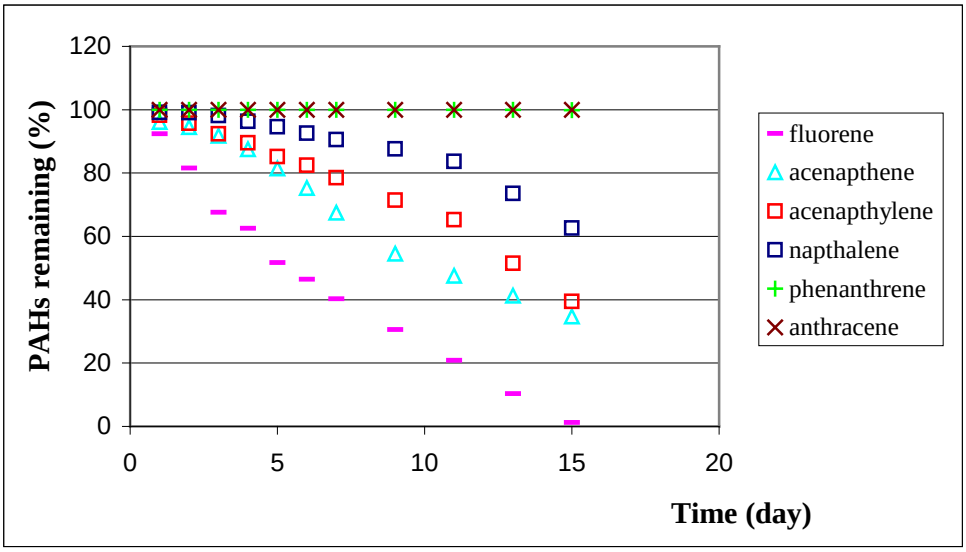

Figure 4: PAHs Degradation using *Micrococcus diversus* (Isolation Substrate-Fluorene)

The degradation rate for fluorene, acenapthene, acenapthylene, naphthalene, phenanthrene and anthracena were 6.17ppm/day, 2.90ppm/day, 2.14ppm/day, 0.93ppm/day, 0.003ppm/day and 0.002ppm/day respectively.

Positive interactions via cometabolism occur among fluorene, acenapthene, acenapthylene and naphthalene using *Micrococcus diversus* as shown in Table 6. Phenanthrena and anthracene show negative interaction or is known as antagonistic. Comparing with Figure 1 and Table 3, *Micrococcus diversus* also effectively degrade naphthalene.

The results show that this bacteria strain produces specific enzyme that have active sites to associate with substrate namely, naphthalene and fluorene. Like other molecule, substrates namely naphthalene and fluorene have kinetic energy and they collide with other molecule within cell. They are called enzyme-substrates complex when they collide with the same active site of enzyme. As a result of binding to the entire enzyme, some of the chemical bonds in the substrates are weakened. In this case, PAHs as substrate then undergo chemical change to form products.

Table 6: Interaction between *Micrococcus Diversus* and Pahs

Strain	PAHs used as substrate					
	NAP	ACE	AC	FLU	PHE	ANT
Micrococcus diversus	+	+	+	+ +	-	-
Different of degradation rate with high interaction	84.8%	66.1%	65.3%	-	99.9%	99.9%

(++: indicate high interaction; +: indicate moderate interaction if different with high interaction more than 10%; -: no interaction/low interaction if different with high interaction more than 99%)
(NAP-napthalene; ACE-acenapthylene; AC-acenapthene; FLU-fluorene; PHE-phenanthrene; ANT-anthracene)

This study provides evidence that *Micrococcus diversus* has potential enzymes in degrading both naphthalene and fluorene. No documented literatures have been found describing specific pathway on this strain and immobilization process of specific enzymes in degrading naphthalene and fluorene to date. This study offers beneficial information particularly for further research.

(e) PAHs degradation by *Corynebacterium uroalyticum* (isolation substrate-phenanthrene).

Figure 5 and 6 show PAHs degradation processes by *Corynebacterium uroalyticum* and *Pediococcus pentosaceus* respectively. The remaining PAHs in both graph show decreasing trend. It was interesting to note that both compounds have similar molecular weight with the value of 178.2g. However, they are different in molecule structure and aqueous solubility.

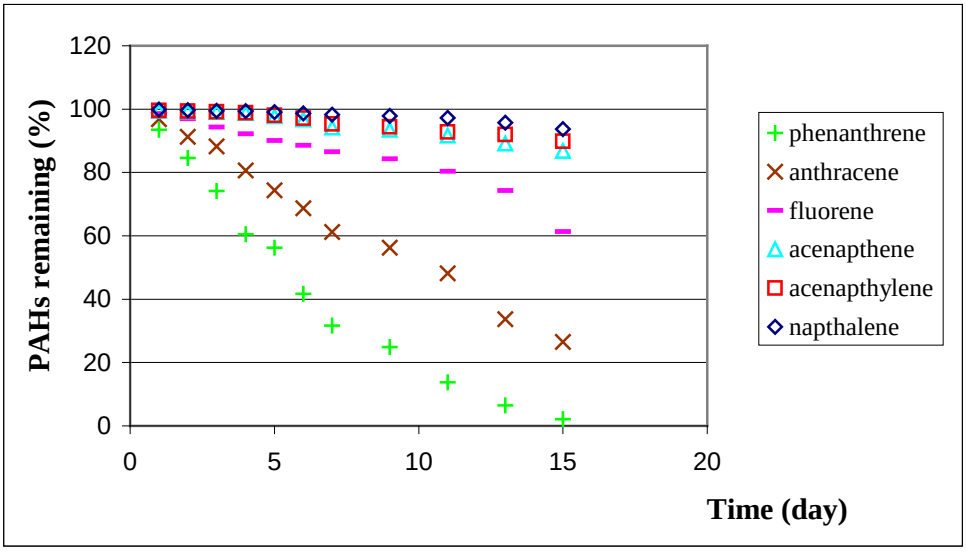

Figure 5: PAHs Degradation Using *Corynebacterium uroalyticum* (Isolation Substrate-Phenanthrene)

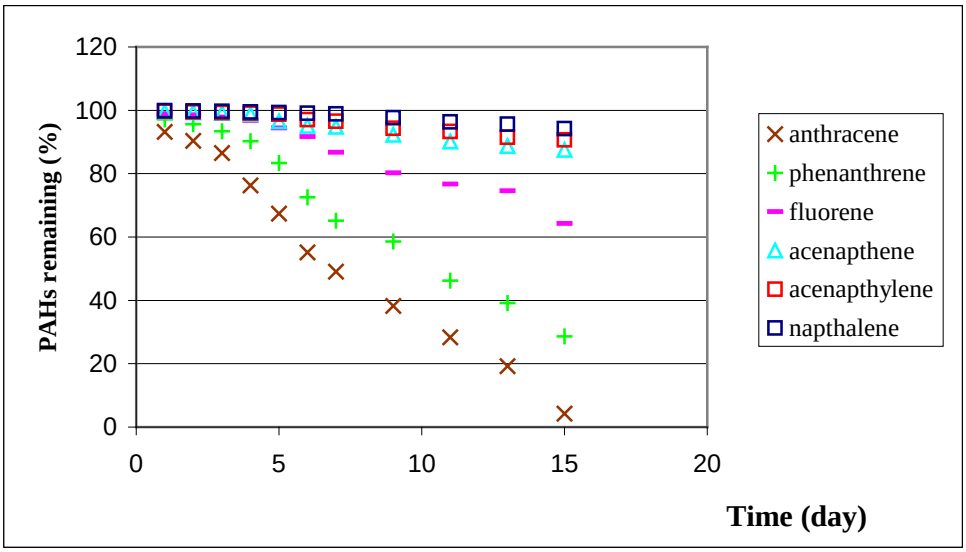

Figure 6: PAHs Degradation Using *Pediococcus pentosaceus* (Isolation Substrate-Anthracene)

The degradation rate for phenanthrene, anthracena, fluorene, acenapthene, acenapthylene and napthalene were 6.05ppm/day, 5.45ppm/day, 1.38ppm/day, 0.44ppm/day, 0.36ppm/day and 0.16ppm/day respectively by using *Corynebacterium uroalyticum*.

The degradation rate when using *Pediococcus pentosaceus* are 4.62ppm/day, 4.22ppm/day, 1.10ppm/day, 0.46ppm/day, 0.33ppm/day and 0.15ppm/day for anthracene, phenanthrene, fluorene, acenapthene, acenapthylene and napthalene respectively.

Interaction between PAHs and isolated strains are presented in Table 7 and 8. Both *Corynebacterium uroalyticum* and *Pediococcus pentosaceus* show positive interaction with PAHs. It can be observed that high interaction occur for phenanthrene and anthracene. Thus, naphthalene, acenapthylene, acenapthene and fluorene show low interactions.

Table 7: Interaction between *Corynebacterium uroalyticum* and PAH

Strain	PAHs used as substrate					
	NAP	ACE	AC	FLU	PHE	ANT
Corynebacterium uroalyticum	+	+	+	+	++	++
Different of degradation rate with high interaction	97.4%	94%	92.7%	77.2%	-	9.9%

(++: indicate high interaction; +: indicate moderate interaction if different with high interaction more than 10%; -: no interaction/low interaction if different with high interaction more than 99%)
(NAP-napthalene; ACE-acenapthylene; AC-acenapthene; FLU-fluorene; PHE-phenanthrene; ANT-anthracene)

In general, the results (as shown in Figure 1, 2, 3, 4, 5 and 6 present several strains isolated from single source of PAHs as growth substrate. These strains were tested for their ability to use different individual PAHs as their carbon and energy source. Table 9 shows interactions among isolated strains with PAHs. Positive and negative interactions represented with high or moderate and low interaction respectively. Thus, positive and negative interactions are known as synergistic and antagonistic effect respectively.

Table 8: Interaction between *Pediococcus pentosaceus* and PAH

Strain	PAHs used as substrate					
	NAP	ACE	AC	FLU	PHE	ANT
Pediococcus pentosaceus	+	+	+	+	++	++
Different of degradation rate with high interaction	96.8%	92.8%	90%	76.2%	8.7%	-

(++: indicate high interaction; +: indicate moderate interaction if different with high interaction more than 10%; -: no interaction/low interaction if different with high interaction more than 99%)
(NAP-napthalene; ACE-acenapthylene; AC-acenapthene; FLU-fluorene; PHE-phenanthrene; ANT-anthracene)

Table 9: Interactions of Isolated Strains with PAHs

Strains	PAHs used as substrate					
	NAP	ACE	AC	FLU	PHE	ANT
Micrococcus diversus (N1 & F1)	++	+	+	++	-	-
Rhodococus rhodochrous (AC1)	+	++	+	-	-	-
Corynebacterium agropyri (A1)	+	+	++	-	-	-
Corynebacterium uroalyticum (P1)	+	+	+	+	++	++
Pediococcus pentosaceus (AN1)	+	+	+	+	++	++

(++: indicate high interaction; +: indicate moderate interaction if different with high interaction more than 10%; -: no interaction/low interaction if different with high interaction more than 99%)
(NAP-napthalene; ACE-acenapthylene; AC-acenapthene; FLU-fluorene; PHE-phenanthrene; ANT-anthracene)

In comparison to other strains, *Micrococcus diversus* showed high interaction with naphthalene and fluorene. It was apparent that in some cases antagonistic phenomena might occur and resulted in blocking respective degradation pathways for other PAHs. When more than one compound was present in the same sample, the degradation rates for the compounds are delayed significantly reflecting an antagonistic effect on the degrading abilities of isolated strains. However, certain PAHs show positive interaction which could cometabolize with other PAHs.

The interactions among PAHs used in biodegradation study using isolated bacteria from single PAH have proved that enrichment and isolation technique had significantly affect biodegradation of PAHs. This study is timely to be conducted because lack of information on this interaction from previous studies. Hence, many of brownfield or polluted areas have been reclaimed for construction or other beneficial purposes and the pollutants from that areas might come from PAHs family. Knowing on interactions of PAHs will result in better biodegradation of PAHs.

CONCLUSIONS

The result of this study showed that isolation and identification process had sucessfully determined a few potential bacteria that able to degrade PAHs. Four different genus of bacteria had been identified based on Biolog Microbial identification system which include *Micrococcus, Rhodococus, Corynebacterium* and *Pediococcus*. Degradation trend observed in degradation study could be attributed to the different substrate provided during isolation process. For bacteria isolated from single PAHs as substrate, interaction of substrate could either be synergistic through cometabolism or antagonistic.

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