UNIVERSITI PUTRA MALAYSIA

BIOREMEDIATION KINETICS OF PYRENE BY MICROBIAL CONSORTIUM ISOLATED FROM LOCAL POLLUTED SOIL

BABA SHEHU UMAR IBN ABUBAKAR

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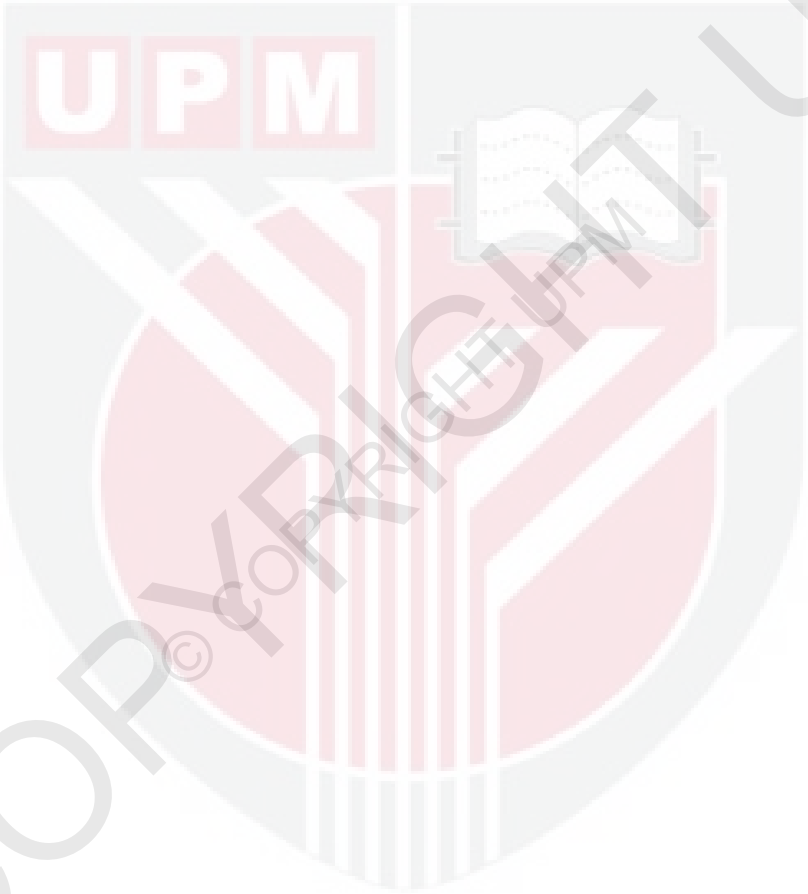
By

BABA SHEHU UMAR IBN ABUBAKAR

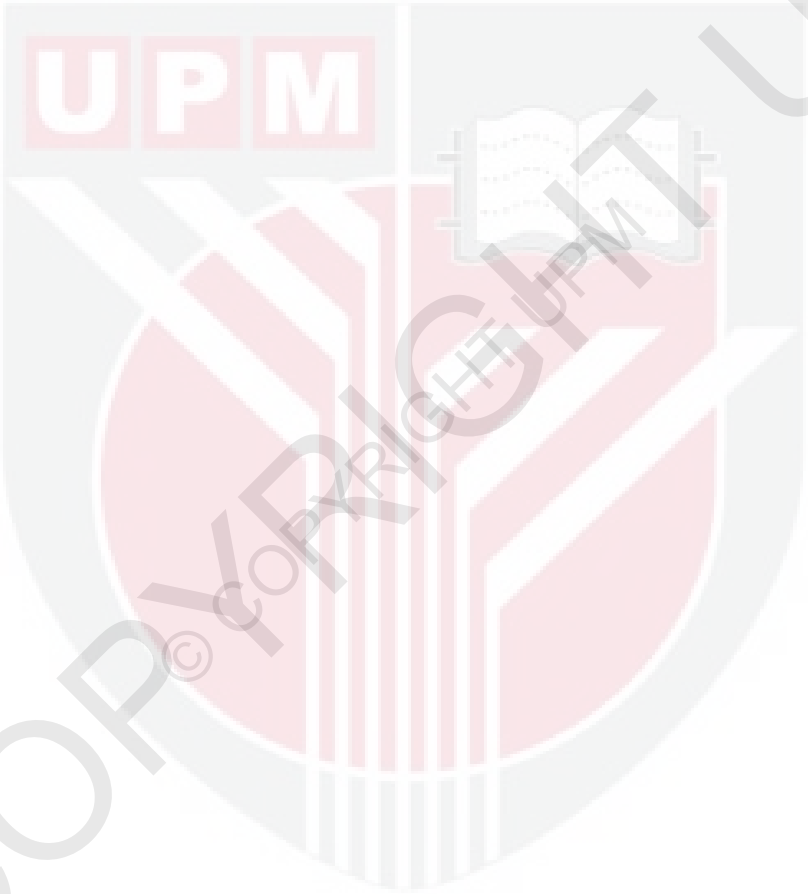
Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2014



DEDICATIONS

My Mum Hajja Fatima Lawal
My Dad Late Sgt. Garba Kukawa



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

BIOREMEDIATION KINETICS OF PYRENE BY MICROBIAL CONSORTIUM ISOLATED FROM LOCAL POLLUTED SOIL

By

BABA SHEHU UMAR IBN ABUBAKAR

July 2014

Chair: Associate Professor Norhafizah Bt Abdullah, PhD

Faculty: Engineering

Pyrene (Pyr), a toxic four-ring polycyclic aromatic hydrocarbon (PAHs) pollutant, is often found at a relatively high concentration in soil sediments of polluted sites. It has been used as a model substrate for higher molecular weight PAHs bioremediation studies. In spite of abundant works on isolation, characterization and application of PAHs degrader, information on bioremediation kinetics and optimisation of Pyr remediation is still very rare. Consequently, the present research aims at isolation and identification of major consortia of Pyr-degrading bacteria from a local polluted site; develops biodegradation kinetics under different operating parameters and investigate their remediation capability using different Pyr-spiked soils. A mixed culture was isolated from a hydrocarbon-contaminated soil by enriching with 1.5 ppm of Pyr as sole source of carbon and energy. The phenotype of mixed culture was identified by screening and biochemical methods. Inoculum was grown in nutrient broth supplemented with 0.75 ppm of Pyr for aqueous degradation, and with 100 ppm of Pyr in a mineral salt medium supplemented with 1% of yeast extract for the development of kinetics. Investigation was conducted on the degradation of Pyr in aqueous medium using different range of Pyr concentrations (10 ppm-100 ppm and 100 ppm-700 ppm) as carbon source and monitored over the period of 15 days. The progress of pyrene degradation was quantitatively monitored using HPLC. Surface response methodology was employed as a design tool in optimizing bioremediation of Pyr-spiked soils with various sets of operating conditions in a soil-slurry batch reactor. The initial screening from the mixed-culture showed 14 types of microbial strains isolated: 12 strains were identified biochemically as *Bacillus cereus* and the remaining 2 were identified as *Enterobacter aerogenes*. Degradation of 1.5 ppm and 3.0 ppm of Pyr and biomass

growth in aqueous medium occurred within six days with a short lag period followed by log phase from day 2- day 6 with moderate specific growth rate, μ at 0.07 h^{-1} and 0.024 h^{-1} respectively. For both cultures, more than 90 % of Pyr was degraded during this log phase growth period and thus can be classified as "growth associated" degradation kinetics. Investigation on environmental factors showed that they grew well at mild acidic to neutral pH of 4 to 7 and did not grow at pH 8. In addition, the effect of temperature indicated that the culture grows favourably at a range of temperature of 20^oC and 30^oC and not with temperature of 40^oC. Moreover, preliminary investigate from the culture media using GCMS revealed the metabolites of naphthalene, benzenepropanl, 1,4-benzenediol, benzoic acid, ethanone, 2(3H0)-Furanone, 1,2-benzenedicarboxylic acid, dibutyl phthalate and Di-n-octyl phthalate. From the results of the soil-slurry batch reactor, biomass growth was dependent on Pyr concentration and slurry's initial pH, but not soil/water ratio. A ratio of Pyr to soil of 1000 mg/kg and the initial pH of 5 resulted in the highest percentage of Pyr removal. There is linear relationship between initial pH and final pH, while the soil/water has no effect on the growth of the biomass in the reactor. Probably due to the chosen range of soil/water ratio of (0.1-0.2) might provides adequate space for mixing and microbial mobility within the soil-slurry reactor. Initial screening of concentration shows that, the mixed culture could not grow above concentration range of (100 ppm-700 ppm). Subsequently, the concentration ranges of between (100 ppm-700 ppm) and (10 ppm-100 ppm) were used for the development of the kinetics. The result of degradation kinetics developed were fitted into Monod equation, with r^2 0.67, and r^2 0.68, respectively. However, degradation models of Haldane, Webb, Yano and Aiba could not describe the degradation kinetics of the mixed culture. Consequently, there is non-conformity of the mixed culture to Monod model, although the correlation coefficient shows 67% and 68%, and fairly described by the Monod model, which describes microbial growth with respect to substrate depletion. Therefore, and probably, the mixed culture growth differently within the culture medium or perhaps their synergy on the growth substrate depends on different metabolites within the culture medium.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOPEMULIHAN KINETIK DARI PIRENA OLEH KONSORTIUM MIKROB PENGASINGAN DARIPADA TEMPATAN CONTOH TANAH TERCEMAR

Oleh

BABA SHEHU UMAR IBN ABUBAKAR

Julai 2014

Pengerusi: Professor Madya Norhafizah Bt Abdullah, PhD

Fakulti: Kejuruteraan

Pirena (Pyr), bahan pencemar hidrokarbon aromatik polisiklik (PAHs) empat cincin yang bertoksik, sering dijumpai pada kepekatan yang agak tinggi dalam enapan kawasan tanah tercemar. Ia telah digunakan sebagai lapisan substrat model untuk kajian biopemulihan bagi berat molekul PAHs yang lebih tinggi. Walaupun terdapat banyak langkah-langkah pengasingan, pencirian dan aplikasi penguraian PAHs, maklumat mengenai kinetik biopemulihan dan pengoptimuman untuk pemulihan Pyr masih sangat jarang dijumpai. Oleh yang demikian, kajian ini bertujuan untuk pengasingan dan pengenalpastian kumpulan utama bakteria pengurai Pyr dari kawasan tanah tercemar tempatan, membangunkan kinetik biodegradasi di bawah fungsi parameter yang berbeza dan menyiasat keupayaan penguraian bakteria tersebut dengan menggunakan tanah yang tercemar oleh Pyr dengan kepekatan yang berbeza.

Satu kultur campuran telah diasingkan daripada tanah yang tercemar dengan hidrokarbon dan diperkayakan oleh 1.5 ppm Pyr sebagai sumber tunggal karbon dan tenaga. Fenotip kultur campuran tersebut telah dikenalpasti dengan kaedah pemeriksaan dan biokimia. Inokulum telah dibekalkan didalam cecair nutrien yang diletakkan 0.75 ppm Pyr untuk penguraian berair, dan dengan 100 ppm Pyr didalam medium garam mineral yang diletakkan 1% ekstrak yis untuk pembangunan kinetik. Siasatan telah dijalankan keatas penguraian Pyr dalam medium berair menggunakan kepekatan Pyr yang berbeza (10ppm 100 ppm dan 100 ppm 700 ppm) sebagai sumber karbon dan dipantau sepanjang tempoh 15 hari. Penguraian pirena telah dipantau secara kuantitatif menggunakan HPLC. Metodologi

tindakbalas permukaan telah digunakan sebagai alat rekabentuk dalam mengoptimumkan biopemulihan tanah yang tercemar oleh Pyr dengan set keadaan operasi yang dipelbagaikan dalam reaktor tanah separa cecair.

Pemeriksaan awal tentang kultur campuran menunjukkan kehadiran 14 tompokan mikrob; 12 kelompok dikenalpasti sebagai Bacillus cereus and selebihnya dikenalpasti sebagai Enterobacter aerogenes. Penguraian Pyr 1.5 ppm dan 3.0 ppm dan pertumbuhan biomas dalam medium berair berlaku dalam tempoh enam hari dengan jarak masa yang singkat, diikuti oleh fasa log dari hari kedua hingga hari keenam dengan kadar pertumbuhan tertentu sederhana, masing-masing pada kadar 0.07 h^{-1} dan 0.024 h^{-1}. Bagi kedua-dua kultur, lebih dari 90% Pyr telah terurai dalam tempoh pertumbuhan fasa log ini, maka ia boleh diklasifikasikan sebagai kinetik penguraian yang berkaitan dengan pertumbuhan. Siasatan keatas faktor persekitaran menunjukkan bahawa bakteria-bakteria tersebut berkembang dengan baik dalam keadaan rendah asid hingga neutral, dengan pH 4 hingga 7, dan tidak berkembang pada pH 8. Tambahan lagi, kesan suhu menunjukkan bahawa kultur tersebut bertumbuh dengan baik pada julat suhu 20^oC hingga 30^oC, dan tidak berkembang pada suhu 40^oC. Selain itu, penyiasatan awal daripada medium kultur menggunakan GCMS menunjukkan terdapat kehadiran naftalena, benzenepropanil, 1,4-benzenediol, asid benzoic, ethanona, 2(3HO)-Furanona, asid 1,2-benzenedikarboksilik, dibutil ftalat dan Di-n-oktil ftalat.

Daripada keputusan reaktor tanah separa cecair, pertumbuhan biomas bergantung kepada kepekatan Pyr dan pH awal tanah., tetapi tidak bergantung kepada nisbah tanah/air. Nisbah Pyr kepada tanah sebanyak 1000mg/kg dan pH awal 5.0 menyebabkan penyingkiran Pyr dalam peratusan tertinggi. Terdapat hubungan linear antara pH awal dan pH akhir, manakala nisbah tanah/air tidak memberikan kesan dalam pertumbuhan biojisim dalam reaktor. Kebarangkalian kerana julat tanah/air yang dipilih memberikan ruang yang mencukupi untuk pencampuran dan pergerakan mikrob di dalam reaktor tanah separa cecair. Pemeriksaan awal menunjukkan bahawa kultur campuran tidak boleh bertumbuh pada kepekatan melebihi (100 ppm 700 ppm). Selain itu, kepekatan kultur antara (100 ppm 700 ppm) dan (10 ppm 100 ppm) telah digunakan untuk pembangunan kinetik. Hasil penguraian kinetik yang telah dibangunkan dipakai dalam persamaan Monod, dengan r^2 masing-masing 0.67 dan 0.68. bagaimanapun, model penguraian Haldane, Webb, Yano dan Aiba tidak dapat menhuraikan kinetik penguraian kultur campuran tersebut. Oleh itu, Kultur campuran tersebut tidak mematuhi model Monod, walaupun ia adalah 67% dan 68% hampir sama dengan model tersebut, yang menggambarkan pertumbuhan mikrob dengan penggunaan substrat. Oleh itu, terdapat kemungkinan bahawa kultur campuran bertumbuh secara berbeza didalam medium kultur atau mungkin tindakbalas kultur tersebut terhadap pertumbuhan substrat bergantung kepada metabolit yang berbeza dalam medium kultur.

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I may not be able to forget the contributions of my peer colleagues in the de-

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I certify that a Thesis Examination Committee has met on 9th July 2014 to conduct the final examination of Baba Shehu Umar Ibn Abubakar on his thesis entitled "Bioremediation Kinetics of pyrene by microbial consortium isolated from local polluted soil " in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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Arbakariya B Ariff, PhD
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Date: 18 August 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of philosophy. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS