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Degradation of polynuclear aromatic hydrocarbons by two strains of *Pseudomonas*

Obinna C. Nwinyi[a,d,*], *Oluseyi O. Ajayi*[b], *Olukayode O. Amund*[c]

[a] *Department of Biological Sciences, School of Natural and Applied Sciences, College of Science and Technology, Covenant University, Canaan Land, Ota, Ogun State, Nigeria*
[b] *Department of Mechanical Engineering, College of Science and Technology, Covenant University, Canaan Land, Ota, Ogun State, Nigeria*
[c] *Department of Microbiology, University of Lagos, Lagos, Nigeria*
[d] *Department of Biotechnology and Food technology, University of Johannesburg, Doornfontein, Johannesburg, South Africa*

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ABSTRACT

The goal of this investigation was to isolate competent polynuclear aromatic hydrocarbons degraders that can utilize polynuclear aromatic hydrocarbons of former industrial sites at McDoel Switchyard in Bloomington, Indiana. Using conventional enrichment method based on soil slurry, we isolated, screened and purified two bacterial species strains PB1 and PB2. Applying the ribotyping technique using the 16S rRNA gene analysis, the strains were assigned to the genus *Pseudomonas* (*Pseudomonas plecoglossicida* strain PB1 and *Pseudomonas* sp. PB2). Both isolates showed promising metabolic capacity on pyrene sprayed MS agar plates during the preliminary investigations. Using time course studies in the liquid cultures at calculated concentrations 123, 64, 97 and 94 ppm for naphthalene, chrysene, fluroanthene and pyrene, *P. plecoglossicida* strain PB1 and *Pseudomonas* sp. PB2 showed partial utilization of the polynuclear aromatic hydrocarbons. Naphthalene was degraded between 26% and 40%, chrysene 14% and 16%, fluroanthene 5% and 7%; pyrene 8% and 13% by *P. plecoglossicida* strain PB1 and *Pseudomonas* sp. PB2 respectively. Based on their growth profile, we developed a model $R^2 = 1$ to predict the degradation rate of slow polynuclear aromatic hydrocarbon-degraders where all the necessary parameters are constant. From this investigation, we confirm that the former industrial site soil microbial communities may be explored for the biorestoration of the industrial site.

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Introduction

Global industrialization is without consequences, particularly with the deposition of hydrophobic contaminants in sediments, surface soil and dump sites. Polynuclear aromatic hydrocarbons (PAHs), a hydrophobic contaminant have been listed as one of priority/toxic environmental contaminants. They are chemical compounds comprising mainly of carbon (C) and hydrogen (H); arranged in form of two or

* *Corresponding author.*
E-mails: nwinyiobinna@gmail.com, obinna.nwinyi@covenantuniversity.edu.ng (O.C. Nwinyi).
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more aromatic rings with various structural configurations.[1] PAHs can be classified as alternant (e.g., benzo[a]pyrene, benz]a]anthracene, chrysene, dibenz[a,h]anthracene) or non-alternant (e.g., fluoranthene, benzo[k]fluoranthene, benzol[j]fluoranthene, indeno[1,2,3-c,d]pyrene). The alternant PAHs comprises of aromatic rings with six carbon atoms while the non-alternant ones contain aromatic rings with less or more than six carbons. This peculiarity is based on the electron density associated with the molecule. Alternant PAHs have an equally distributed electron density, whereas non-alternant PAHs behave almost as if they were two different molecules because of an uneven distribution of electron density from one portion of the molecule to another. PAHs being a derivative of benzene, and thermodynamically stable, have two or more fused aromatic rings arranged in linear, angular, or clustered structures.[2,3] PAHs are ubiquitous environmental pollutant, entering the environment from natural and anthropogenic sources such as natural fires, volcanic eruptions, aluminum smelting, coke production, and creosote preservation. The anthropogenic sources typically are as a result of incomplete combustion of the organic substances that make up such substances.[4,5]

The human body could be exposed to PAHs through inhalation, dermal contact and ingestion. Within the body, PAHs being highly lipid-soluble are quickly absorbed by the fatty tissues such as the kidney, liver and gastro-intestinal tract of mammals. In humans, the highest metabolizing capacity present is the liver, then the lungs, intestinal mucosa, skin and kidneys. Metabolism may also take place in nasal tissues, mammary glands, spleen, brain follicles, erythrocytes, platelets, leukocytes, placenta and uterus. In the liver, PAHs are adapted by cytochrome P450 into epoxides a major intermediates that are reactive and enzymatically metabolized to dihydrodiols and phenols. The dihydrodiols and phenols react against DNA and proteins causing mutagenic damage to cells.[6]

Naphthalene has been noted to be a hazardous air pollutant.[7] When experimental organisms are exposed to naphthalene, it causes decrease in their hemoglobin concentration and inhibits their oxygen consumption. Naphthalene is used as raw chemical for industrial synthases of phthatic anhydride.[8] Naphthalene at high concentration may cause hemolytic anemia and some other conditions, whereas the tumorigenic potential is presently considered low.[9,10] Naphthalene has been often used as a model PAH due to high speed of utilization by microorganisms compared to other PAH and the relatively simple structure of the intermediates in the catabolic pathways. Thus information on bacterial degradation of naphthalene has been used to understand and predict pathways of most PAHs.

Pyrene is a hydrophobic compounds and its persistence within ecosystems is due to low water solubility, dense clouds of p-electrons on both sides of the ring structures, making them resistant to nucleophilic attack.[11]

Soil can act as a sink for carbon. It could receive considerable amount of PAHs that may likely remain persistent in the environment due to low solubility and sequestration in soil and sediments; to lack of versatile metabolic capacity of microorganisms to degrade these compound.[11] According to Regonne and co-workers, for effective cleanup of PAH-contaminated soils, cheaper and more ecologically friendly options are proposed over chemical and physical processes.[12] These options will involve greater understanding of the processes involved and factors that limit the degradation of high molecular and low molecular weight PAHs. Bioremediation is a proficient and safe method to clean up PAH from contaminated sites. It has been applied to both terrestrial and aquatic ecosystems; and may possibly provide a position in biorestoration of contaminated soils. Microorganisms transform the PAHs to CO_2 and water through metabolism or co-metabolism. They PAHs serve as carbon and energy sources, thus reducing the associated toxicity and co-metabolic substrates of PAH.[11] Since the 1950s efforts have been made to select microorganisms with ability to degrade PAHs from pure cultures.[13] Since then, many bacteria strains have been isolated for their ability to transform, degrade and utilize PAHs as a source of energy and carbon.[5,14–17] Hitherto, it has been recognized that few bacteria have been isolated that are capable of utilizing PAHs with four or more aromatic rings as sole sources of carbon and energy.[18,19] Consequently, it is of paramount importance to isolate and investigate versatile degraders of PAHs. In this study, we report for the first time the isolation and characterization of bacterial strains from a former industrial site in Bloomington in Indiana using conventional enrichment of soil slurry. The bacteria *Pseudomonas plecoglossicida* strain PB1 and *Pseudomonas* sp. PB2 were screened for their growth and degradation fluxes in naphthalene, fluoranthene, pyrene and chrysene. We proposed a mathematical model to assist in the simulation of the degradation rates of our bacterial species on the selected PAHs (naphthalene, fluoranthene, pyrene and chrysene).

Materials and methods

Chemicals

The naphthalene, fluoranthene, pyrene and chrysene of analytical grades were purchased from Sigma Aldrich Corp. (St. Louis, MO, USA). Sodium benzoate (99+% purity), 2,2,4,4,6,8,8-heptamethylnonane (HMN), and all other organic solvents were obtained from Fisher Scientific Co. (Springfield, NJ, USA). Hexane, a high purity solvent for GC-chromatograph was obtained from EMD Chemicals Inc. Merck. The PAH analytical standards were procured from Accustandard Inc. (New Haven, CT 06513). All other chemicals and reagents used were of reagent grade or better.

Stock solutions and media

For the enrichment and degradation experiments, chloride free minimal salts (MS) medium as described by[20–23,5] were used. The medium consisted of (g) $0.5(NH_4)_2SO_4$, $0.1MgSO_4{\cdot}7H_2O$, $0.076Ca(NO_3)_2{\cdot}4H_2O$ and 1.0 mL each of trace metal and vitamin solutions per liter of 40 mM phosphate buffer (pH 7.25). Naphthalene stock solution were prepared in HMN, a non-degradable carrier to provide an initial concentration of ca. 123 ppm. The concentration represents the total mass in both the aqueous and HMN phases, divided by the aqueous volume. The appropriate stock solution was added using a gas-tight syringe in 250-μL aliquots to provide test

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