A Review of Pyrene Bioremediation Using *Mycobacterium* Strains in a Different Matrix

Mohammad Qutob 1, Mohd Rafatullah 1,* , Syahidah Akmal Muhammad 1,* , Abeer M. Alosaimi 2, Hajer S. Alorfi 3 and Mahmoud A. Hussein 3,4

1 Division of Environmental Technology, School of Industrial Technology, Universiti Sains Malaysia, Penang 11800, Malaysia; mohammadisamqutob@student.usm.my
2 Department of Chemistry, Faculty of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; a.alosaimi@tu.edu.sa
3 Chemistry Department, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia; halorf@kau.edu.sa (H.S.); maabdo@kau.edu.sa (M.A.H.)
4 Chemistry Department, Faculty of Science, Assiut University, Assiut 71516, Egypt
* Correspondence: mrafatullah@usm.my (M.R.); syahidah.muhammad@usm.my (S.A.M.); Tel.: +60-46532111 (M.R.); Fax: +60-4656375 (M.R.)

Abstract: Polycyclic aromatic hydrocarbons are compounds with 2 or more benzene rings, and 16 of them have been classified as priority pollutants. Among them, pyrene has been found in higher concentrations than recommended, posing a threat to the ecosystem. Many bacterial strains have been identified as pyrene degraders. Most of them belong to Gram-positive strains such as *Mycobacterium* sp. and *Rhodococcus* sp. These strains were enriched and isolated from several sites contaminated with petroleum products, such as fuel stations. The bioremediation of pyrene via *Mycobacterium* strains is the main objective of this review. The scattered data on the degradation efficiency, formation of pyrene metabolites, bio-toxicity of pyrene and its metabolites, and proposed degradation pathways were collected in this work. The study revealed that most of the *Mycobacterium* strains were capable of degrading pyrene efficiently. The main metabolites of pyrene were 4,5-dihydroxy pyrene, phenanthrene-4,5-dicarboxylic, phthalic acid, and pyrene-4,5-dihydridol. Some metabolites showed positive results for the Ames mutagenicity prediction test, such as 1,2-phenanthrenedicarboxylic acid, 1-hydroxyppyrone, 4,5-dihydropyrene, 4-phenanthrene-carboxylic acid, 3,4-dihydroxyphenanthrene, monohydroxy pyrene, and 9,10-phenanthrenequinone. However, 4-phenanthrol showed positive results for experimental and prediction tests. This study may contribute to enhancing the bioremediation of pyrene in a different matrix.

Keywords: pyrene; toxicity; pathways; mycobacterium; metabolites; biodegradation

1. Introduction

The demand for water and energy is increasing, putting additional strain on water and environmental resources. Water scarcity has been identified as a socioeconomic and environmental problem that challenges the world in the twenty-first century, affecting approximately four billion people worldwide at least one month per year [1–3]. The continuous release of harmful chemicals such as persistent organic pollutants (POPs) is considered one of the most threatening environmental problems, as mentioned in the Stockholm convention, 2004, Basel convention, 1989, the Rotterdam convention, 1998, Barcelona resolution, 1995, 8 Aarhus Protocol, 1998, and the Arctic environmental protection strategy, 1991 [4]. According to the listed protocols and conventions, harmful chemicals should be eliminated, or their production decreased. POPs are a group of toxic chemicals that stay in the environment long-term and resist natural degradation. There...
are two major sources of POPs, (i) natural sources, including forest fires, volcanic eruptions, and biogenic sources (microbial metabolites, plants, and algae), and (ii) anthropogenic sources, including incomplete combustion (oil, wood, petroleum, and coal), synthetic fertilizers, pesticides formulations, and industrial process [5]. POPs groups include personal care products, polychlorinated compounds, dibenzo-p-dioxins and dibenzofurans, and polycyclic aromatic hydrocarbons (PAHs) [6]. The increasing discharge of POPs into the environment leads to them bioaccumulating and becoming biomagnified until they reach a specific concentration leading to bio-toxicity. POPs can cross boundaries, move freely away from their original sources, and be absorbed by soil particles; they are volatile in the atmosphere, can run off into water bodies, enter into the food chain, be uptaken by plants, or leach into groundwater. PAHs are compounds that contain two or more benzene rings. The United States Environmental Protection Agency (USEPA) classified 16 PAHs as priority pollutants due to their low solubility, non-polarity, hydrophobicity, high boiling point, high melting point, corrosion resistance, conductivity, heat resistance, light-sensitive, bioaccumulation, biomagnification, and bio-toxicity [7,8]. Many adverse effects of PAHs have been reported on human health, aquatic organisms, and wildlife, such as genetic mutation, endocrine disruption, cardiovascular disorders, hypertension, immune system suppression, and birth defects. In this work, pyrene was the main target pollutant and was detected at higher concentrations than the standard values or maximum contaminant levels [9]. According to the United States Environmental Protection Agency (USEPA), the standard values of pyrene for human health for the consumption of water and organism is 20 μg/L, while for human health for the consumption of water is only 30 μg/L [10]. Many biological, physical, and chemical treatment techniques have been used to eliminate pyrene from different mediums. The advanced oxidation process is one of the most efficient treatment methods capable of oxidizing pyrene [11]. However, advanced oxidation processes need to inject a large number of chemicals to complete mineralization, which increases the treatment costs and the discharge of chemicals into the environment [12]. Bioremediation approaches have gained attention due to their advantages, such as being cost-effective and environmentally friendly. Biological treatment is a biological process that uses target pollutants as a source of energy and carbon to degrade, mineralize, transform, and detoxify the target pollutant in a specific medium. Bioremediation can use indigenous biological agents (biostimulation) or external biological agents (bioaugmentation) and can be applied either in situ or ex situ based on many factors such as, (i) type of pollutants, (ii) cost of treatment, (iii) geological site, (iv) pollutants’ concentration, and (v) depth of pollution. The most popular treatment techniques use in situ natural attenuation, bio-sluiping, bioventing, disparaging, and phytoremediation. Many ex situ techniques are used to treat many pollutants, including POPs, such as landfarming, bioreactor, windrow, and biopile windrow [13]. Quintella et al. [14] applied a strengths, weaknesses, opportunities, and threats (SWOT) analysis for the study of bioremediation technologies. They revealed that most of the studies have been conducted in the United States of America and China, and the most common biological agents used were bacteria, enzymes, fungi, algae, plants, and protozoa, with percentages of 57%, 19%, 13%, 6%, 4%, and 1%, respectively. Water, soil, and sludge were the most common degradation matrixes that were treated, with percentages of 53%, 36%, and 11%, respectively. They reported that the target pollutants that were degraded via biological agents the most were oil, metals, organic waste, polymers, food, and cellulose, and their percentages were 38%, 21%, 21%, 10%, and 5%, respectively. Recently, the degradation of pyrene via isolation of bacterial strains has increased. Mycobacterium strains and Rhodococcus strains were the most dominant bacterial species used for PAHs degradation; these strains were enriched and isolated from different sites contaminated with petroleum products, such as fuel stations [15,16]. Figure 1 represents the number of documents by year and the percentage of each type of document found using keywords (oxidation of pyrene by bacteria) through the Scopus database. In 1995, the number of studies related to the biodegradation of pyrene for each type of document was
5, while in 2021, the number of studies was 55, which means that this topic has been gaining researchers’ attention. More than 94% of these studies were articles, 2.3%, 2.0%, 0.6%, 0.5%, 0.5, and 2.0% were reviews, conference papers, notes, book chapters, short surveys, and errata, respectively. In this review, the main objective is to collect and organize the scattered information related to the studies that investigated the degradation of pyrene by *Mycobacterium* strains. The major topics that are investigated in this review are degradation efficiency, pyrene metabolites, bio-toxicity, and the proposed degradation pathways.

Figure 1. (A) represents the number of documents by year, and (B) the percentage for each type of document when using keywords (oxidation of pyrene by bacteria) on the Scopus database.

2. Degradation of Pyrene by *Mycobacterium* sp.

Several bacterial strains have been isolated to use pyrene as a sole carbon and energy source; most of them are Gram-positive, such as *Mycobacterium* and *Rhodococcus* [17,18]. *Mycobacteria* are catalase-positive, non-motile, non-spore-forming, rod-shaped bacteria (0.2–0.6 mm wide and 1.0–10 mm long). The colony morphology of *Mycobacteria* varies, with some species growing as rough or smooth colonies. Colony color ranges from white to orange or pink [19]. It has been reported that the first isolation of a bacterial strain to mineralize pyrene was in 1988 [20]. *Mycobacterium* was the most dominant strain to mineralize pyrene [21]. The successful mineralization of pyrene by *Mycobacterium* strains refers to their ability to produce several functional enzymes capable of metabolizing high molecular weight polycyclic aromatic hydrocarbons, such as pyrene. Dioxygenase is a complex, multi-component enzymatic system containing iron sulfur-containing terminal oxygenase, reductase, and ferredoxin [22]. It has been reported that hydroxylation is the initial biochemical step in the pyrene degradation process. It introduces a couple of oxygen atoms into aromatic pyrene rings [23]. The complete mineralization of pyrene occurs through different enzymatic reactions such as dioxygenase, dihyrogendiol, dehydrogenase, ring cleavage dioxygenase, epoxide hydrolase, alcohol dehydrogenase, acet-aldehyde dehydrogenation, and decarboxylation [24]. Figure 2 illustrates the biodegradation of pyrene by *Mycobacterium*. 
Figure 2. Illustrated the biodegradation of pyrene by Mycobacterium.

Many functional genes have been identified in the Mycobacterium strains, such as NidA, NidB, NidAB, NidA3B3, PhdA, PhdB, PdoA, PdoB, and PdoAB. Among Mycobacterium strains, the vanbaalenii PYR-1 strain has many functional genes capable of degrading pyrene and its metabolites. Table 1 includes some of the enzymes produced by different Mycobacterium strains during pyrene degradation. Miller et al. [25] identified NidB and NidA genes that are responsible for producing dioxygenase enzyme when Mycobacterium sp. JLS is used to catabolize pyrene. Zeng et al. [26] reported that the PdoAB gene is responsible for encoding a dioxygenase capable of oxidizing pyrene. Costa et al. [27] observed that PhdA and PhdB are the main genes of the dioxygenase enzyme in the Mycobacterium fortuitum strain.

Table 1. Summary of the functional genes for each Mycobacterium strain.

| Strain                          | Functional Genes                          | Reference |
|---------------------------------|-------------------------------------------|-----------|
| Mycobacterium sp.               | NidA, NidA3                               | [15]      |
| Mycobacterium sp. 6PY1          | PdoB2, PdoA1, PdoA2                       | [24]      |
| Mycobacterium sp. JLS           | NidB and NidA                             | [25]      |
| Mycobacterium sp. MCS           | NidA and NidB                             |           |
| Mycobacterium sp. NJS-P         | PdoAB                                     | [26]      |
| Mycobacterium sp. S65           | PdoAB                                     |           |
| Mycobacterium fortuitum         | PhdA and PhdB                            | [27]      |
| Mycobacterium sp. PO1 and PO2   | NidA, PhdA, and NidA3                     | [28]      |
| Mycobacterium sp. AP1-PYR       | NidAB and PdoA2B2                         | [29]      |
| Mycobacterium sp. MHP-1         | NidAB                                     | [30]      |
| Mycobacterium sp. RJII-135      | NahAc, BphA1, OrtolC1C2                   | [31]      |
| Mycobacterium sp. KMS           | PdoF                                      |           |
| Mycobacterium vanbaalenii PYR-1 | NidB, NidA, NidB2, PhdF, PhdG, NidD, PhdJ, PhtAb, PhtAc, PhtAd, PhtB, NidA3, NidB3 | [32]      |
| Mycobacterium gilvum PYR-GCK    | AraC                                      |           |
| Mycobacterium sp. gilvum PYR10  | NidAB and NidA3B3                         | [33]      |
| Mycobacterium sp. Pallens PYR15 | NidAB and NidA3B3                         |           |
In this review article, more than 40 studies related to pyrene degradation via *Mycobacterium* strains or consortium culture were collected. In general, *Mycobacterium* strains showed high degradation efficiency, most of them 80–100%. There are numerous *Mycobacterium* strains that can degrade pyrene. The phylogenetic tree of *Mycobacterium* strains is depicted in Figure 3. The *Mycobacterium* sequences were collected using the NCBI gene bank (Home Nucleotide—www.ncbi.nlm.nih.gov (accessed on 30 December 2021)). The sequences were assembled, aligned, and analyzed with MEGA software version 11.0.

![Figure 3. Phylogenetic tree for *Mycobacterium* strains that have been used for pyrene degradation.](image)

Wanapaisan et al. [28] used a consortium culture containing five bacterial strains (Mycobacterium sp. PO1, PO2, Bacillus sp. FW1, Ochrobactrum sp. PW1, and Novosphingobium pentaromativorans PY1). The result showed that 100 mg/L of pyrene was completely eliminated within 6 days of incubation. In addition, the Mycobacterium sp. NJS-1 strain was used to mineralize pyrene on metal-modified montmorillonite. This study revealed that around 93.6% of 15 mg/L of pyrene was degraded within 3 days at neutral pH conditions, and the degradation rate was first-order kinetics 0.62 k/d [34]. Additionally, Zhang et al. [35] applied a consortium of bacterial strains (Micrococcus sp. PHE9 and Mycobacterium sp. NJS-P) to decompose pyrene. About 58% of 100 mg/L of pyrene was removed after 18 days of incubation, and the degradation rate was 3.24 mg/L × day. Sun et al. [36] isolated the Mycobacterium sp. WY10 strain to oxidize 50 mg/L of pyrene in a mineral salt medium. Around 3 × 10⁸ CFU/mL was inoculated, and the degradation was 83% after 72 h of treatment. Xiaoning Li et al. [37] examined the Mycobacterium sp. NJS-1 strain to treat and remove high molecular weight polycyclic aromatic hydrocarbons, such as pyrene. The author used a mineral medium, and around 1.6 × 10⁷ CFU/mL was inoculated to degrade 200 mg/L of pyrene; the degradation was 90% of pyrene in the presence of humic acid, while about 10.5% was in the absence of humic acid within 7 days of incubation. The Mycobacterium gilvum CP13 strain was isolated for oxidizing pyrene in a mineral salt medium at alkaline conditions. The bacteria were inoculated at an optical density of 600 nm = 0.5, and 95% of 50 mg/L of pyrene was oxidized after 7 days of degradation treatment [38]. Furthermore, Chen et al. [39] applied biotechnology to treat agricultural and industrial soils contaminated with 16 priority polycyclic aromatic hydrocarbons, including pyrene. The Mycobacterium strain was capable of removing 85% of 100 mg/kg of pyrene during 35 days of treatment in both soils. Also, Terzaghi et al. [40] examined the Mycobacterium gilvum VM552 strain to degrade pyrene suspended on the leaf surface of holm oak (Quercus ilex). The results indicated that after 2 weeks of treatment, the removal was only 17%. Chen et al. [15] attempted to stimulate a microbial degradation approach for soil-containing pyrene. In this study, the active bacterial strains were identified; among them, Mycobacterium strains were the most dominant, and the degradation was 80% of 60 mg/kg within 35 days; the experiment was conducted at pH 8. Sarma and Pakshirajan [41] isolated the Mycobacterium frederiksbergense strain to mineralize pyrene using a batch shake flask reactor. After 200 h of incubation, the pyrene was totally eliminated at neutral pH conditions. Moreover, Peng et al. [17] reported that approximately 81% of 50 mg/kg of soil-containing pyrene was oxidized after 60 days of bioremediation under acidic conditions using the Mycobacterium strain. They pointed out that the NidA gene in Mycobacterium was responsible for generating the dioxygenase enzyme. In addition, the Mycobacterium vanbaalenii PYR-I strain was used in a phosphate-based mineral medium, and 25 µM of pyrene was completely oxidized after 24 h of treatment [18]. Table 2 provides a summary of the studies that used Mycobacterium strains to degrade pyrene in a different medium, pH, optical density, degradation efficiency, incubation time, and initial concentration.
Table 2. Summary of the studies that used *Mycobacterium* strains to treat pyrene in a different medium.

| Strains | Accession No./or Reference No. | Biodegradation Matrix | Degradation % | Incubation Time | pH | Temperature (°C) | Concentration of Pyrene | OD<sub>600</sub>/Number of Cells | References |
|---------|--------------------------------|-----------------------|---------------|-----------------|----|-----------------|-------------------------|-------------------------------|------------|
| *Mycobacterium* strains | * | Pyrene-containing soil | 80 | 35 days | Around 8 | 25 | 60 mg/kg | Ranging from 8.9 × 10^9 to 1.9 × 10^10 copies/g | [15] |
| Myco66F/Myco600R | FN690762 and FN690936 | Pyrene-spiked soils | 81 | 60 days | 5.84 | 25 | 50 mg/kg | * | [17] |
| *Mycobacterium vanbaalenii* PYR-1 | NR_074572.1 | Phosphate-based minimal medium | 100 | 24 h | * | * | 25 μM | OD<sub>600</sub> = 1.0 | [18] |
| *Mycobacterium* sp. KR2 | * | Mineral salts medium | 60 | 8 days | 7 | 20 | 0.5 mg/mL | OD<sub>600</sub> = 0.5–0.6 | [21] |
| *Mycobacterium* sp. PO1 and PO2 | | | | | | | | | |
| *Novosphingobium pentaromativorans* PO1 | (NZ_BLTG00000000.1) | Carbon-free mineral medium (CFMM) culture | 100 | 6 days | * | 30 | 100 mg/L | 10<sup>8</sup> CFU/mL | [28] |
| 3-Ochrobactrum sp. PW1 | (NZ_BLTH00000000.1) | | | | | | | | |
| 4-Bacillus sp. FW1 | | | | | | | | | |
| *Mycobacterium* sp. NJS-1 | AB548662 | Metal-modified montmorillonite | 93.6 | 3 days | 7 | 28 | 15 mg/L | 1.6 × 10<sup>8</sup> CFU/mL | [34] |
| *Micrococcus* sp. PHE9 | | Biofilms extracellular polymeric substances-extracted bacteria | 58 | 18 days | * | 28 | 100 mg/L | 1.6 × 10<sup>8</sup> CFU/mL | [35] |
| *Mycobacterium* sp. NJS-P | AB548663 | | | | | | | | |
| *Mycobacterium* sp. WY10 | NZ_CP018043.1 | Mineral salts medium | 83 | 72 h | * | 28 | 50 mg/L | OD<sub>600</sub> = 1.0 | [36] |
| *Mycobacterium* sp. NJS-1 | AB548662.1 | Mineral medium | 90 | 7 days | Acidic condition | 28 | 200 mg/L | 1.6 × 10<sup>5</sup> CFU/mL | [37] |
| *Mycobacterium gilvum* CP13 | KF378755 | Mineral salts medium | 95 | 7 days | Alkaline environment | 35 | 50 mg/L | OD<sub>600</sub> = 0.5 | [38] |
| 2-*Mycobacterium* sp. denovo930873 | * | Agricultural soil | 80 | 35 days | * | 25 | 100 mg/kg | * | [39] |
| *Mycobacterium gilvum* VM552 | ATCC 43909 | Pyrene present on the leaf surface of holm oak (*Quercus ilex*) | 17 | 2 weeks | * | 23±2 | * | 10<sup>4</sup> cells/g | [40] |
| *Mycobacterium frederiksborgense* | Taxonomy ID: 117567 | Batch shake flask experiments | 100 | 200 h | 7 | 28 | 1000 mg/L | * | [41] |
| Taxonomy ID/Strain Name | Description | Time (Days) | Results | References |
|-------------------------|-------------|-------------|---------|------------|
| Mycobacterium frederiksenbergense | Slurry phase and surfactant-aided systems | 100 | 6 days | 7 | 28 | 400 mg/L | * | [42] |
| Mycobacterium sp. flavedens | Mineral salts medium | 38.8 | 2 weeks | Natural and 4 | 24 | 50 µg/ml | 2.2 × 10⁷ cells/mL | [43] |
| Mycobacterium sp. AP1 | Pyrene-mineral salts medium | Decreased from 180 to 50 µg/mL around 72 | 6 days | * | 25 | 180 µg/ml | * | [44] |
| Mycobacterium sp. A1-PYR | PYR in liquid medium | 33 | 7 days | * | 30 | 10 mg/L | OD₆₀₀ = 1.0 | [45] |
| Selenastrum capricornutum | Soil extract (SE) medium | 100 | 14 days | 7 | * | 10 mg/L | 1.0 × 10⁷ CFU/mL | [46] |
| Mycobacterium sp. A1-PYR | Pyrene-mineral salts medium | 50 | 7 days | * | 30 | 10 mg/L | OD₆₀₀ = 1.0 | [47] |
| Mycobacterium gilvum | VM0442 | AF544636.1 | | | | | |
| Mycobacterium gilvum | VM0552 | AF544635 | | | | | |
| Mycobacterium gilvum | VM0504 | AF544634 | | | | | |
| Mycobacterium gilvum | VM0505 | AF544633 | | | | | |
| Mycobacterium chlorophenolicus | PCP-1 | X79094 | | | | | |
| Mycobacterium petroleiphilum | GCK | UEGS0100001.1 | | | | | |
| Mycobacterium sp. PYR | GCK | AY694989 | | | | | |
| Mycobacterium sp. PYR | GCK | AY694989 | | | | | |

*OD₆₀₀ refers to Optical Density at 600 nm.
| Strain | Accession Number |
|--------|------------------|
| 2-Mycobacterium gilvum VM0883 | AF544637.1 |
| 3-Mycobacterium gilvum VM0442 | AF544636.1 |
| 4-Mycobacterium gilvum VM0852 | AF544635 |
| 5-Mycobacterium gilvum iVM0504 | AF544634 |
| 6-Mycobacterium gilvum VM0505 | AF544633 |
| 7-Mycobacterium sp. BB1 | X81891 |
| 8-Mycobacterium sp. HE5 | AJ012738 |
| 9-Mycobacterium mucogenicum | AY457073.1 |
| 1-Mycobacterium sp. JLS | AF387804 |
| 2-Mycobacterium monacense B9-21-178 | AF107039.2 |
| 3-Mycobacterium vaccae VM0588 | AF544639.1 |
| 4-Mycobacterium vaccae VM0587 | AF544638.1 |
| 5-Mycobacterium sp. KMS | AY083217 |
| 6-Mycobacterium sp. MCS | AF387803.1 |
| 7-Mycobacterium doricum DSM 44339 | AF547917.1 |
| 8-Mycobacterium doricum AF264700.1 |  |
| 9-Mycobacterium duvalii NR_026073.1 |  |
| 10-Mycobacterium duvalii CIP 104539 | AF547918.1 |

**Minimal basal salts medium**

| Strain | Accession Number | Time | CFU/mL |
|--------|------------------|------|--------|
| Mycobacterium sp. RJGII-135 | AY216464.1 | 50 | 4–8 h | * | 0.5 μg/mL | * | [49] |
| Mycobacterium sp. AB180481 | Carbon-free minimal | 50 | 7 days | 9 | 30 | Final | 3.9 × 10^6 CFU/mL | [30] |
| Strain                  | Medium                                      | Concentration at 0.1% [w/v] |
|------------------------|---------------------------------------------|-----------------------------|
| 1-Mycobacterium sp. gilvum PYR10 | * Minimal media containing pyrene          | 100 mg/L                    |
| 2- Mycobacterium sp. palens PYR15 | *                                           |                            |
| 1-Mycobacterium         | * Bio-electrokinetic remediation            | 120.2 ± 1.76 mg/kg          |
| 2-Aeromicrobium         | * Soil placed into culture dishes          | 54.3 ± 1.7                  |
| 3-Arenimonas            | * Saline alkaline soils                    | 54.3 ± 1.7                  |
| 4-Bacillus              | * Mineral salts medium by LBL bio-microcapsules | 54.3 ± 1.7                  |
| 5-Hydrogenophaga        | * Pyrene-basal salts medium                | 54.3 ± 1.7                  |
| 6-Azoarcus              | * Soil pH 6.6                               | 54.3 ± 1.7                  |
| 7-Luteimonas            | * Soil pH 8.75                              | 54.3 ± 1.7                  |
| Mycobacterium sp. B2    | * Saline alkaline soils                    | 54.3 ± 1.7                  |
| Mycobacterium gilvum CP13 | * Mineral salts medium by LBL bio-microcapsules | 54.3 ± 1.7                  |
| Mycobacterium gilvum IPF | * Mineral salts medium                     | 54.3 ± 1.7                  |
| Mycobacterium frederikshergense | * Slurry phase system                      | 54.3 ± 1.7                  |
| Mycobacterium gilvum VM552 | * Aqueous medium                           | 54.3 ± 1.7                  |
| Mycobacterium gilvum CP13 | * Aqueous solution + modified peanut hull powder | 54.3 ± 1.7                  |
| 1-Mycobacterium barrassi | * Aqueous solution + sediments             | 54.3 ± 1.7                  |
| 2-Dyella ginsengisoli   | *                                           | 54.3 ± 1.7                  |
| 3-Rhodococcus equi     | *                                           | 54.3 ± 1.7                  |
| 4- Bacillus pumilus     | *                                           | 54.3 ± 1.7                  |
| 5-Bacillus weihenstephanensis | *                                           | 54.3 ± 1.7                  |
| 6-Labrys sp.            | *                                           | 54.3 ± 1.7                  |
| Mycobacterium strains (AB548662) for NJS-1 | * Liquid culture minimal                | 54.3 ± 1.7                  |
| Fermentation | 2022, 8, 260 |

| (NJS-1 and NJS-P) and (AB548663) for (NJS-P) and NJS-P, respectively. |

| 1-Mycobacterium fortuitum | 2- Bacillus cereus | 3-Microbacterium sp. | 4- Gordonia polisoprenivorans | 5-Microbacteriaceae bacterium |
|---------------------------|-------------------|---------------------|-----------------------------|-----------------------------|
| U92089.1 Pyrene-containing soil | 96.3 70 days 7 30 962.7 mg/kg 2.0 × 10^8 CFU/g | 60 96 h 30 1 mg/L 1.0 × 10^7 CFU/mL | 11.5 30 days 26 0.20 nmol/mL | 96.27 mg/kg 2.0 × 10^8 CFU/g |

| Mycobacterium sp. PYR-1 | Mycobacterium sp. S65 | Mycobacterium sp. API | Mycobacterium sp. KMS | Mycobacterium gilvum PYR-GCK | Mycobacterium sp. PYR-1 | Mycobacterium sp. API | Mycobacterium sp. * | MYCOBACTERIUM gilvum PCNR1 | MYCOBACTERIUM gilvum PCNR1 | MYCOBACTERIUM gilvum PCNR1 |
|-------------------------|-----------------------|----------------------|------------------------|---------------------------|-----------------------|----------------------|----------------------|-----------------------------|-------------------------|-----------------------------|
| Experimental Microcosms | Mineral salts medium | Mineral medium | Microcosm system | Mineral salt medium | Aqueous pyrene solution | Marine medium | Mineral salts solution, | NCBI Taxonomy ID | NCBI Taxonomy ID | NCBI Taxonomy ID |
| 74 mixture of PAHs including pyrene | 6 days | 24 | 1.0 M | OD545 = 2.95 | 24 | 1.0 × 10^7 CFU/mL | * | 26 | 0.20 nmol/mL | * |
| 916.7 μg/400 μl | 4.5 × 10^7 cells/mL | 1.0 × 10^7 CFU/mL | 2.0 × 10^7 CFU/mL | 200 mg/L | 2.0 × 10^7 CFU/mL | 200 mg/L | 2.0 × 10^7 CFU/mL | 250 μg/mL | 250 μg/mL | 250 μg/mL |

| 1-Sphingomonas | 2-Mycobacterium | 3-Rhodococcus | 4-Paracoccus | 5- Pseudomonas |
|----------------|----------------|--------------|-------------|-------------|
| | | | | |

[* Data unavailable].
3. Identification of Pyrene Metabolites Degraded by *Mycobacterium* sp. and Their Biotoxicity

The specific aim of the remediation is to achieve complete mineralization or convert the target pollutant into harmless products. Metabolites (by-products, also called intermediate products) are products that are partially degraded and are generated during and after the treatment process. Some metabolites could be more toxic to public health and the environment than the original pollutant. It has been illustrated that the risk of the pollutant’s metabolites is like an iceberg. The pollutants themselves are just the tip of the iceberg, while the metabolites’ products represent the majority of the iceberg, which is hidden underwater. The researchers monitor the metabolites for many reasons: (i) to examine the effectiveness of the treatment approach, (ii) to detect any ecotoxic by-products after the end of treatment operation, and (iii) to build an oxidation pathway based on detected by-products. Many metabolites have been detected during and after the remediation process. The studies that investigated the degradation of pyrene via the *Mycobacterium* strains detected many metabolites. For example, Seo et al. [70] pointed out that phenanthrene-4,5-dicarboxylic acid and naphthalene-1,2-dicarboxylic acid were the major intermediate products when *Mycobacterium aramativorans* strain JS19b1 was applied for pyrene degradation. Sun et al. [36] observed many metabolites produced by *Mycobacterium* sp. WY10 during pyrene degradation, such as cis-pyrene dihydrodiol, cis-pyrene-4,5-dihydrondiol, dihydroxy pyrene, methylated-phenantherene-4,5-dicarboxylic acid, 4-phenanthrene-4-carboxylic acid, phenanthrene-4,5-dicarboxylic acid, and phenanthrene-4-carboxylic acid. In addition, the *Mycobacterium* sp. flavescus PYR-1 strain was used for pyrene degradation. The major by-products were 4,5-dihydroxy-4,5-4,5-dihydropyrene, 4-phenanthonic, phthalic acid, and 4,5-phenanthrenedioic acid [43]. *Mycobacterium* sp. AP1 grew with pyrene as a sole carbon and energy source. The identified metabolites were trans- or cis-4,5-dihydroxy-4,5-4,5-dihydropyrene, phenanthrene-4,5-dicarboxylic acid, phenanthrene-4-carboxylic acid, and 6,6-dihydroxy-2,2-biphenyl dicarboxylic acid [44]. Additionally, Zhong et al. [45] mentioned that the by-products of pyrene were dihydroxy phenanthrene, monohydroxy pyrene, dihydroxy pyrene, 4-phenanthrene-carboxylic acid, and 4-phenanthonic when *Mycobacterium* sp. A1-PYR was applied. Rehmann et al. [21] used *Mycobacterium* sp. KR2 to remove pyrene. After 8 days of incubation, the metabolites were cis-4,5-pyrene dihydrodiol, 4,5-phenanthrene dicarboxylic acid, 1-hydroxy-2-naphthoic acid, 2-carboxybenzaldehyde, phthalic acid, and protocatechuic acid. Also, pyrene cis-4,5-dihydrondiol and dihydroxy pyrene were the main metabolites produced after 24 h of incubation of *Mycobacterium vanbaalenii* PYR-1 [18]. Furthermore, Luo et al. [46] used synergistic microbes (*Selenastrum capricornutum* and *Mycobacterium* sp. A1-PYR) to oxidize pyrene, and the metabolites were dihydroxy pyrene, 1-hydroxyxpyrene, 4-phenanthrol, 4-phenanthrene-carboxylic acid, hydroxyphenyl acetic acid, phenylacetic acid, salicylic acid, and benzoic acid. Kim et al. [71] observed 1,2-dicarboxynaphthalene, phenanthrene and pyrene-diols, and cis-4-(1-hydroxynaphth-2-yl)-2-oxobut-3-enoic acid. Liang et al. [72] detected pyrene-4,5-dione, cis-4,5-pyrene-dihydrodiol, phenanthrene-4,5-dicarboxylic acid, and 4-phenanthonic acid as a metabolite when *Mycobacterium* sp. strain KMS was applied. Moreover, Zhong et al. [47] examined a bacterial culture (*Mycobacterium* sp. A1-PYR and *Sphingomonas* sp. PheB4) for pyrene decomposition. The metabolites in this system were monohydroxy pyrene, pyrene diol, and dihydroxy pyrene. Xiaoqing Li et al. [37] used *Mycobacterium* sp. NJS-1 to oxidize pyrene in the presence of and without humic acid. The by-product in the absence of humic acid was phenanthrene 3,4-diol, while 1,2-dimethoxy pyrene was detected in the presence of humic acid. In addition, Wu et al. [38] studied the metabolites of *Mycobacterium gilvum* CP13 when pyrene was used as a sole carbon and energy source. The major metabolites were 4-phenanthrene carboxylic acid, 4-phenanthrenol, 1-naphthol, and phthalic acid. Also, phthalic acid, naphtha-
lere-1,8-dicarboxylic acid, diphenic acid, 6,6’-dihydroxy-2,2’-biphenyl dicarboxylic acid, Z-9-carboxymethylenefluorene-1-carboxylic acid, and phenanthrene-4,5-dicarboxylic acid were detected by [48]. Many metabolites were detected by [49] when the *Mycobacterium* sp. strain RJGII-135 was isolated to degrade pyrene. The metabolites were 4,5-phenanthrene dicarboxylic acid, 4-phenanthrene-carboxylic acid, and 4,5-pyrene-dihydrodiol. In conclusion, according to these studies, phenanthrene-4,5-dicarboxylic acid, dihydroxy pyrene, phenanthrene-4-carboxylic acid, phthalic acid, and pyrene-4,5-dihydrodiol were the most frequent metabolites that were detected when *Mycobacterium* sp. strains were used for pyrene degradation. Table 3 represents the metabolites of several organisms, such as plants, algae, earthworms, bacteria, and fungi, that have been utilized to remove pyrene from different mediums.

**Table 3.** Summary of the metabolites of pyrene from many species used for pyrene oxidation.

| Scheme | Metabolites | References |
|--------|-------------|------------|
| Lecieria adecarboxylate PS4040 | 1,2-phenanthrenedicarboxylic acid <br> 2-carboxybenzaldehyde <br> *Ortho*-phthalic acid <br> 1-hydroxypyrene | [73] |
| Fire Phoenix plant (*Festuca* spp.) mediated microbial | Phthalic acid <br> dehydroxylated pyrene <br> 1-hydroxypyrene <br> 1-hydroxy-2-naphthoic acid <br> Salicylic acid <br> Benzoic acid | [74] |
| Coriolopsis byrsina strain APC5 | Pyruvic acid <br> Benzoic acid <br> Benzoic acid 2-hydroxy pentyl ester <br> *Phenanthrene* diisopropylester <br> 4,5 dihydroxy pyrene | [75] |
| Fusant bacterial strain F14 fusion between *Sphingomonas* sp. GY28 and *Pseudomonas* sp. GP3A | 4,5-dihydroxy pyrene | [76] |
| *Hortaea* sp. B15 | Phthalic acid 1-Hydroxy-2-naphthoic acid | [77] |
| *Pseudomonas* sp. strain Jpyr-1 | Phthalate 3,4-dihydropyridol <br> Phthalate 1-hydroxy-2-naphthalene carboxylic acid <br> 4-phenanthrene-carboxylic acid | [78] |
| *Shewanella* sp. ISTPL2 | 4,5-dihydroxy pyrene <br> 2-carboxybenzalpyruvate <br> Phthalic acid <br> Salicylic acid | [79] |
| *Pseudomonas* sp. ISTPY2 | Pyrene 4,5-Dihydroxy pyren. <br> 1,2-dihydroxynaphthalene <br> 2,3-dihydroxybenzoate <br> Phthalate <br> Catechol | [80] |
| *Pseudomonas* sp. ISTPY2 | Phthalate 4,5-dioxygenase <br> Aldehyde dehydrogenase | [81] |
| *Pseudomonas* sp. JPN2 | 4,5-dihydroxy-4,5-dihydropyrene <br> 4-phenanthrol <br> 1-hydroxy-2-naphthoic acid <br> Phthalate | [82] |
| *Pseudomonas* putida G7. | 1-hydroxy pyrene <br> Phthalic acid <br> Benzoic acid <br> Silylated derivatives | [83] |
| *Candida tropicalis* MTCC 184 | *Menthyl salicylate* (methyl ester of salicylic acid) | [84] |
| *Pseudomonas* aeruginosa strain RS1 | *Phenanthrene* 4,5 dicarboxylate <br> 4-oxa-Pyrene-5-one | [85] |
| Organism                  | Metabolites                                                                 |
|--------------------------|-----------------------------------------------------------------------------|
| *Achromobacter xylosidans* PY4 strain | Dihydroxypyrene, 4-Phenantronic acid, 4,5-Dihydroxypthalate, 2,2-Dicarboxy-6,6-dihydroxybiphenyl, 4-Phenantronic acid, 3,4-Dihydroxyphenanthrene |
| *Enterobacter* sp. MM087 (KT933254) | 1-methoxyl-2-H-benzo[h]chromene-2-carboxylic acid, 9,10-phenanthrenequinone, 1-methoxyl-trans-2-carboxybenzalpyruvate, Dibutylphthalate |
| *Pseudomonas aeruginosa* RS1 | Maphthalene, 1-methylnaphthalene.                                             |
| *Acinetobacter baumannii* BJ5 | Benzyl benzoate, Butyl octyl phthalate, Phenol –2,4-bis(1,1-dimethylethyl), Phenol, 2,4-di-tet-butyl-Ethyl benzoate, n-Propyl acetate |
| *Sphingomonas* sp. YT1005 | 4-phenanthrenol, Protocatechuic acid, Phthalic acid, 1-hydroxy-2-naphthoic acid, 2-methylnaphthalene, 2-hydroxy-2-H-benzo[h]chromene-2-carboxylic acid, Dihydroxyphenanthrene, cis-4,5-pyrene dihydrodiol, Salicylic acid, trans-2-carboxybenzalpyruvate |
| *Earthworm Eisenia fetida* | Pyrene-4,5-dione, Phenanthrene-4-carboxylic acid, Phenanthrene-4,5-dicarboxylic acid, Phenanthrene-4-carboxylic acid, Protocatechuic acid |
| *Klebsiella* sp. LZ6 | 4,5-dihydro-phenanthrene, Dibenzo-p-dioxin, 4-hydroxycinnamate acid |

It should be noted that phthalic acid, 1-hydroxypyrene, 1-hydroxy-2-naphthoic acid, 4,5-dihydroxy pyrene, phenanthrene 4,5 dicarboxylate, and pyrene-4,5-dihydrodiol are the most frequent metabolites in the last table. It has been observed that the metabolites of *Mycobacterium* sp. strains and the species mentioned in Table 3 share 4,5-dihydroxy pyrene, phenanthrene-4,5-dicarboxylate, phthalic acid, and pyrene-4,5-dihydrodiol as the most frequent metabolites. That may be attributed to the enzymes that are shared between them, which in turn, leads to shared degradation pathways of pyrene.

The mass consumption of petroleum products and increase in their demand around the world leads to an increase in the opportunity for pyrene leakage into the environment and increases the opportunity for exposure to pyrene by organisms and humans. Frequent and long-term exposure to pyrene leads to bioaccumulation and biomagnification in the organism cell, which increases the possibility of carcinogenicity and mutagenicity. Many studies have mentioned the negative impacts of pyrene and its metabolites on animals and humans. The toxicity evaluation of pyrene metabolites is important to increase system efficiency. The toxicity assessment of pyrene and its metabolites was carried out using the United States Environmental Protection Agency’s software, called Toxicity Estimation Software Tool (TEST) version 5.1. This software is capable of apply-
ing mathematical models to predict pollutant toxicity based on Quantitative Structure-Activity Relationship (QSAR) methodology. The data were introduced by inputting the name of each by-product. The lethal concentration of 50% (LC50) (96 h) in fathead minnow and Ames mutagenicity were the considered toxicity for pyrene metabolites using *Mycobacterium* strain and other biological agents, represented in Table 4. Some metabolites showed positive results for the Ames mutagenicity prediction test, such as 1,2-phenanthrenedicarboxylic acid, 1-hydroxypyrene, 4,5-dihydropyrene, 4-phenanthrene-carboxylic acid, 3,4-Dihydroxyphenanthrene, Monohydroxy pyrene, and 9,10-phenanthrenequinone. However, 4-phenanthrol showed positive results for experimental and prediction tests.
Table 4. Summary of the results of LC₅₀ (96 h) fathead minnow and the Ames mutagenicity test for the main pyrene metabolites after treatment by using Mycobacterium strain and other biological agents.

| Metabolites                        | Fathead Minnow LC₅₀ (96 h) | Ames Mutagenicity | Prediction Result |
|------------------------------------|----------------------------|-------------------|-------------------|
|                                    | Prediction Value:          |                   |                   |
|                                    | Prediction Value:          | Prediction Value: |                   |
|                                    | Prediction Value:          | Prediction Value: |                   |
|                                    | ~/log (mol/L)              | (mg/L)            | Log10 (mol/L)     |                   |
| 1,2-phenanthrenedicarboxylic acid  | *                          | *                 | 0.86              | *                 |
| 2-carboxybenzaldehyde              | 4.30                       | 7.49              | 0.29              | *                 |
| 1-hydroxypyrene                    | 5.45                       | 0.77              | 0.76              | *                 |
| Phthalic acid                      | 3.69                       | 34.15             | 0.14              | Mutagenicity Negative |
| Benzoic acid                       | 3.21                       | 75.43             | −0.05             | Mutagenicity Negative |
| Salicylic acid                     | 3.34                       | 63.62             | −0.08             | Mutagenicity Negative |
| 1-hydroxy-2-naphthoic acid         | 3.77                       | 31.97             | 0.17              | Mutagenicity Negative |
| Pyruvic acid                       | 2.08                       | 734.13            | 0.41              | Mutagenicity Negative |
| 4,5 dihydroxy pyrene               | 5.13                       | 1.73              | 0.50              | Mutagenicity Negative |
| 4,5-dihydroxypyrene                | 6.33                       | 9.50 × 10⁻²       | 0.98              | *                 |
| n-Propyl acetate                   | 3.06                       | 89.38             | 0.19              | Mutagenicity Negative |
| 4-phenanthrene-carboxylic acid     | 4.52                       | 6.65              | 0.71              | *                 |
| Protocatechuic acid                | 3.73                       | 28.53             | 0.30              | Mutagenicity Negative |
| 1,2-dihydroxynaphthalene           | 4.55                       | 4.49              | 0.28              | Mutagenicity Negative |
| 2,3-dihydroxybenzoate              | 3.71                       | 29.86             | −0.04             | Mutagenicity Negative |
| Dibenzo-p-dioxin                   | 4.53                       | 5.40              | 0.23              | Mutagenicity Negative |
| Catechol                           | 3.81                       | 17.19             | 0.29              | Mutagenicity Negative |
| 4,5-dihydroxy-4,5-dihydroxypyrene  | 5.03                       | 2.21              | 0.15              | Mutagenicity Negative |
| 4-phenanthrol                      | 5.80                       | 0.31              | 0.76              | Mutagenicity Positive |
| Phenanthrene 4,5 dicarboxylate     | *                          | *                 | 0.22              | Mutagenicity Negative |
| 4-oxa-Pyrene-5-one                 | 5.01                       | 2.18              | 0.22              | Mutagenicity Negative |
| 4,5-Dihydroxypthalate              | 3.58                       | 51.61             | 0.47              | Mutagenicity Negative |
| 3,4-Dihydroxyphenanthrene          | 6.05                       | 0.19              | 0.60              | Mutagenicity Positive |
| Monohydroxy pyrene                 | 5.45                       | 0.77              | 0.76              | Mutagenicity Positive |
| 9,10-phenanthrenequinone           | 4.30                       | 10.47             | 0.52              | Mutagenicity Negative |
| Dibutyl-phthalate                  | 5.30                       | 1.40              | 0.18              | Mutagenicity Negative |
| Compound                                      | Initial | Final  | Mutagenicity | Notes               |
|-----------------------------------------------|---------|--------|--------------|---------------------|
| Naphthalene                                   | 4.20    | 8.15   | *            | Mutagenicity Negative |
| 1-methylnaphthalen                            | 4.32    | 6.74   | *            | Mutagenicity Negative |
| Benzyl benzoate                               | 4.86    | 2.92   | 0.05         | Mutagenicity Negative |
| Butyl octyl phthalate                         | 4.70    | 6.68   | 0.03         | Mutagenicity Negative |
| Phenanthrene-4,5-dicarboxylic acid            | *       | *      | 0.22         | Mutagenicity Negative |
| Naphthalene-1,2-dicarboxylic acid             | 3.93    | 25.69  | 0.10         | Mutagenicity Negative |

[* Data unavailable.]*
4. Proposed Biodegradation Pathways

Many bacterial strains have been applied to degrade pyrene in a different medium. Some bacterial strains share the same functional enzymes, which leads to the same degradation pathways, as shown in Table 5. Some genes in the *Mycobacterium* sp. strain produce enzymes capable of oxidizing pyrene. There are numerous advantages to determining the degradation pathway, including the ability to control the effectiveness of remediation systems, eliminating the influence of degradation on analytical results, and knowledge of degradation pathways for specific compounds can facilitate the assessment of environmental pollution with POPs based on the presence of degradation products. In addition, identifying the degradation pathway is useful for the future development of bioremediation [93, 94].

The following studies are examples of the degradation of pyrene by using the *Mycobacterium* sp. strain. Yuan et al. [29] proposed a detailed pyrene degradation pathway via *Mycobacterium* sp. strain A1-PYR. The first step of pyrene degradation was hydroxylation using *NidAB* and *PodA3B3*, leading to forming cis-4,5-dihydroxy-4,5-hydropyrene, then *PhdE* acting to convert cis-4,5-dihydroxy-4,5-hydropyrene into 4,5-dihydroxypyren, then phenanthrene-4,5-dicarboxylate via *PhdE*, further degradation leading to form phenanthrene-4-carboxylate. *PodA2B2* enzyme works to produce cis-3,4-phenanthrene-dihydrodiol-4-carboxylate, then *PhdE* acts to generate 3,4-dihydroxy-phenanthrene. More decomposition of 3,4-dihydroxy-phenanthrene via *PhdF* leading to form 2-hydroxy-2H-benzo[h]chromene-2-carboxylate then cis-4-(1'-hydroxy-naphth-2'-yl)-2' oxobut-3-enoate. *PhdG* leading to form 1-hydroxy-2-naphthaldehyde → 1-hydroxy-2-naphthoate, further degradation of 1-hydroxy-2-naphthoate leading to produce 2-cis-2'-carboxy-benzalpyruvate. Additionally, the *PhdI* enzyme converts 2-cis-2'-carboxy-benzalpyruvate into phthalate then the ring cleavage via *PhtC* results to form carboxylic acids compounds. The final metabolite step was that the small carboxylic acids enter the tricarboxylic acid cycle to produce energy, H₂O, and CO₂. In addition, Krivobok et al. [95] proposed the degradation pathway of pyrene by *Mycobacterium* sp. Strain-6 PY1. They observed that *PhdABCD, PhdE, PhdF, PhdG, PhdH, PhdI*, and *PhdK* enzymes were detected in the *Mycobacterium* sp. Strain-6 PY1. The degradation of pyrene started with the hydroxylation process of C4 and C5 positions to form pyrene cis-4,5-dihydrodiol then 4,5-dihydroxypyrene, further oxidation of 4,5-dihydroxypyrene generates 4,5-phenanthrenedioic → 4-phenanthrene acid → phenanthrene-3,4-diol → phenanthrene → cis-3,4-phenanthrene-dihydrodiol → 3,4-dihydroxy-phenanthrene → 2-hydroxy-2H-1-oxa-pyrene-2-carboxylic acid → 2-Hydroxy-2H-benzo[h]chromene-2-carboxylate → 1-Hydroxy-2-naphthaldehyde → trans-2'-carboxybenzal pyruvic acid → 2-2-Carboxybenzaldehyde → O-phthalic acid → tricarboxylic acid cycle. Wu et al. [38] studied the degradation of pyrene via *Mycobacterium gilvum* and the proposed the degradation pathway as the following: pyrene → 4-phenanthrenecarboxylic acid → 3,4-dihydroxy-phenanthrene → 2-Hydroxy-2H-benzo[h]chromene-2-carboxylate → 1-naphthol → phthalic acid. A simple degradation pathway of pyrene through *Mycobacterium* sp. is shown in Figure 4. The most common transformation metabolites that have been proposed to build degradation pathways are shown in Table 6, while Table 7 shows an example of pyrene degradation pathways via different microbial species.
Table 5. Summary of the main genes of *Mycobacterium* sp. strain that responsible in pyrene degradation and their functions.

| Primers | Sequences | Probable Functions | References |
|---------|-----------|--------------------|------------|
| NidA3   | Forward 5'-CTGTATGCGACGCAACTG-3' | Fluoranthene/pyrene ring-hydroxylating oxygenase, α subunit | [15] |
|         | Reverse 5'-GCAACCCTAGCGACCTCTT-3' | | |
| NidA    | Forward 5'-TTCCCGATACGAGGAGGATAC-3' | α Subunit pyrene dioxygenase | [17] |
|         | Reverse 5'-TCAGTTGATGAAACGCCCAAA-3' | | |
| NidB2   | Reverse 5'-CCTGATGCGACGACAATG-3' | Pyrene/phenanthrene ring-hydroxylating oxygenase, β subunit | [18] |
|         | Forward 5'-GCAACCCTAGCGACCTCTT-3' | | |
| NidB3   | Reverse 5'-GCCGAGCTGAAATCCGATCTTAGATCCAGAAATGACAG-3' | Fluoranthene/pyrene ring-hydroxylating oxygenase, β subunit | |
| PdoAB   | Forward 5'-CTGTATGCTACTTGCGTGAAGA-3' | α Subunit pyrene dioxygenase | [26] |
|         | Reverse 5'-CCCGATCTCTCATCGGACACCCGCGGGAATG-3' | | |
| PdoA1   | Forward 5'-GGCATATGCAAACGGAACGACCC-3' | α Subunit pyrene dioxygenase | |
|         | Reverse 5'-GGCATATGCTCAAACGACCAGCGGT-3' | | |
| PdoA2B2 | Forward 5'-GGCATATGCTACTTGCGTGAAGA-3' | α Subunit pyrene dioxygenase | [95] |
|         | Reverse 5'-CCCGATCTCTCATCGGACACCCGCGGGAATG-3' | | |
| PdoB1   | Forward 5'-GGCATATGAAACGCGGTCGGA-3' | Pyrene/phenanthrene ring-hydroxylating oxygenase, β subunit | | |
|         | Reverse 5'-GGCATATGCTACTTGCGTGAAGA-3' | | |
| PdoA2B2 | A2-Forward 5'-GGCATATGCTACTTGCGTGAAGA-3' | Catalysis of hydroxylation of HMW and LMW polyaromatic hydrocarbons including pyrene. | |
|         | B2-Reverse 5'-CCCGATCTCTCATCGGACACCCGCGGGAATG-3' | | |
| TolC1C2 | Forward 5'-GCAACCCTAGCGACCTCTT-3' | Small subunits of toluene dioxygenase | [62] |
|         | Reverse 5'-TGTTAACCAGCACCAGCCTC-3' | | |
| NahAc   | Forward 5'-GCCAAAAGCGCAACTG-3' | Naphthalene dioxygenase | | |
|         | Reverse 5'-CGTTTACAGCACCAGCCTC-3' | | |
| BphA1   | Forward 5'-GCCGATCCCGAGATGGAAC-3' | Large subunit of biphenyl dioxygenase | | |
|         | Reverse 5'-CGTGGCTCGGAGATGGAAC-3' | | |
| PdoB2   | Forward 5'-GCCGATCCCGAGATGGAAC-3' | β Subunit dioxygenase | [63] |
|         | Reverse 5'-CGTGGCTCGGAGATGGAAC-3' | | |
| PdoF    | Forward 5'-GCCGACACTCTTCTGACCTAA-3' | Putative extradiol dioxygenase | [65] |
|         | Reverse 5'-TTGGGTAGTGGGGAATTC-3' | | |
| PhdI    | Forward 5'-TGACGAAATGATGGGCTC-3' | 1-Hydroxy-2-naphthoate dioxygenase | |
| Reverse 5'-AGTCCGCTGTATTTCGTCG-3' | Forward 5'-ACATATGGCGCCTGATGCGACGACAATG-3' | NidAB | α Subunit pyrene dioxygenase |
|---|---|---|---|
| Reverse 5'-AAAACGAGATTCACATGACACAGCC-3' | Reverse 5'-TGCGATCCCAACTTTCAAGT-3' | NidB | β Subunit of arene dioxygenase |
| Reverse 5'-GCAGGAGATATATGTCGACAGGC-3' | Forward 5'-GGACTACCTCGGCGATATGA-3' | AraC | Transcriptional regulatory protein, AraC family |
| Reverse 5'-AGTGCCGTGTATTTCGTCG-3' | Reverse 5'-TTGAGACGTGCTCTCCATAG-3' | PdoA2 | Phenanthrene ring-hydroxylating oxygenase, subunit [97] |
| Reverse 5'-CAAGCTTTTAGATCCAGAATGACAGGTT-3' | Reverse 5'-AAAACTGCAGATTCACATGATCAGGGCGAGGTTGTGTCTATT-3' | NidA3B3 | Fluoranthene/pyrene ring-hydroxylating oxygenase [98] |
| Reverse 5'-TCGTCACCAACTTCAAGTC-3' | Reverse 5'-GCATTATGCGGCCGCAAGCTTTCATTCGTCTACGACTTC-3' | PhtAb | Oxygenase reductase component |
| Reverse 5'-ACGCAGAACTCCACAAGCTC-3' | Reverse 5'-TTCCCCATCGTGTCGTATAG-3' | PhtB | Phthalate 3,4-dihydrodiol dehydrogenase [99] |
| Reverse 5'-ACATATGGCGCCTGATGCGACGACACATG-3' | Reverse 5'-AGTGCCGTGTATTTCGTCG-3' | PhtAd | Oxygenase reductase component |
| Reverse 5'-ATCGGATCTCTCTTTCAGAAGATGTTGTGGAGACTTAAAGCAGAATG-3' | Reverse 5'-ACAAGCTTTTAGATCCAGAATGACAGGTT-3' | PhtAc | Oxygenase ferredoxin component [100] |
| Reverse 5'-ATCGGATCTCTCTTTCAGAAGATGTTGTGGAGACTTAAAGCAGAATG-3' | Reverse 5'-ACAAGCTTTTAGATCCAGAATGACAGGTT-3' | PhtJ | trans-2-Carboxylbenzalpyruvate hydratase-aldolase [101] |
| Reverse 5'-ATCGGATCTCTCTTTCAGAAGATGTTGTGGAGACTTAAAGCAGAATG-3' | Reverse 5'-ACAAGCTTTTAGATCCAGAATGACAGGTT-3' | PhdA | Hydratase-aldolase [102] |
| Reverse 5'-ACATATGGCGCCTGATGCGACGACAATG-3' | Reverse 5'-AGTGCCGTGTATTTCGTCG-3' | PhdB | α and β subunits of other ring-hydroxylating dioxygenases [103] |
| Reverse 5'-ATCGGATCTCTCTTTCAGAAGATGTTGTGGAGACTTAAAGCAGAATG-3' | Reverse 5'-AGTGCCGTGTATTTCGTCG-3' | PhdJ | Catechol O-methyltransferase |
| Reverse 5'-ACATATGGCGCCTGATGCGACGACAATG-3' | Reverse 5'-AGTGCCGTGTATTTCGTCG-3' | PhdA | Hydratase-aldolase |
| Reverse 5'-ACATATGGCGCCTGATGCGACGACAATG-3' | Reverse 5'-AGTGCCGTGTATTTCGTCG-3' | PhdB | Catechol O-methyltransferase |

[Data unavailable].
Figure 4. Proposed degradation pathway of pyrene by *Mycobacterium gilvum*.

Table 6. Summary of the most frequent transformation metabolites of pyrene.

| Metabolite | MW | Molecular Formula |
|------------|-----|------------------|
| P1         | 90.12 | C₆H₁₀O₂           |
| P2         | 142.15 | C₇H₁₀O₃           |
| P3         | 142.11 | C₇H₁₂O₄           |
| P4         | 141.10 | C₆H₁₂O₄           |
| P5         | 250.29 | C₁₄H₁₈O₄          |
| P6         | 88.06  | C₆H₈O₃            |
| P7         | 166.13 | C₆H₁₀O₄           |
| P8         | 194.18 | C₁₆H₁₀O₄          |
| P9         | 122.12 | C₇H₁₀O₂           |
| P10        | 206.28 | C₁₂H₁₈O₂          |
|     | **MW**  | **Formula**   |
|-----|----------|--------------|
| P11 | 190.19 C<sub>13</sub>H<sub>10</sub>O<sub>3</sub> |              |
| P12 | 150.13 C<sub>8</sub>H<sub>6</sub>O<sub>3</sub> |              |
| P13 | 110.11 C<sub>6</sub>H<sub>6</sub>O<sub>2</sub> |              |
| P14 | 154.12 C<sub>7</sub>H<sub>6</sub>O<sub>4</sub> |              |
| P15 | 154.12 C<sub>7</sub>H<sub>6</sub>O<sub>4</sub> |              |
| P16 | 232.23 C<sub>13</sub>H<sub>12</sub>O<sub>4</sub> |              |
| P17 | 198.13 C<sub>8</sub>H<sub>6</sub>O<sub>6</sub> |              |
| P18 | 220.18 C<sub>11</sub>H<sub>8</sub>O<sub>5</sub> |              |
| P19 | 138.12 C<sub>7</sub>H<sub>6</sub>O<sub>3</sub> |              |
| P20 | 122.12 C<sub>7</sub>H<sub>6</sub>O<sub>2</sub> |              |
| P21 | 170.25 C<sub>13</sub>H<sub>14</sub> |              |
| P22 | 160.17 C<sub>10</sub>H<sub>8</sub>O<sub>2</sub> |              |
| P23 | 172.18 C<sub>11</sub>H<sub>8</sub>O<sub>2</sub> |              |
| P24 | 200.15 C<sub>8</sub>H<sub>6</sub>O<sub>6</sub> |              |
| P25 | 170.16 C<sub>4</sub>H<sub>10</sub>O<sub>4</sub> |              |
| P26 | 142.20 C<sub>11</sub>H<sub>10</sub> |              |
| P27 | 220.18 C<sub>11</sub>H<sub>8</sub>O<sub>5</sub> |              |
| P28 | 178.23 C<sub>11</sub>H<sub>14</sub>O<sub>2</sub> |              |
| P29 | 128.17 C<sub>10</sub>H<sub>8</sub> |              |
| P30 | 302.24 C<sub>15</sub>H<sub>16</sub>O<sub>7</sub> |              |
| P31 | 242.23 C<sub>14</sub>H<sub>10</sub>O<sub>4</sub> |              |
| P32 | 242.23 C<sub>14</sub>H<sub>10</sub>O<sub>4</sub> |              |
| P33 | 270.24 C<sub>15</sub>H<sub>15</sub>O<sub>5</sub> |              |
| P34 | 216.19 C<sub>12</sub>H<sub>8</sub>O<sub>4</sub> |              |
| P35 | 242.23 C<sub>14</sub>H<sub>10</sub>O<sub>4</sub> |              |
| P36 | 244.24 C<sub>14</sub>H<sub>12</sub>O<sub>4</sub> |              |
| P37 | 188.18 C<sub>14</sub>H<sub>12</sub>O<sub>4</sub> |              |
| P38 | 210.23 C<sub>14</sub>H<sub>10</sub>O<sub>2</sub> |              |
| P39 | 278.30 C<sub>15</sub>H<sub>15</sub>O<sub>5</sub> |              |
| P40 | 274.23 C<sub>14</sub>H<sub>10</sub>O<sub>6</sub> |              |
| Compound | MW   | Structural Formula |
|----------|------|--------------------|
| P41      | 216.19 | ![Structure1](image) |
| P42      | 208.21 | ![Structure2](image) |
| P43      | 214.26 | ![Structure3](image) |
| P44      | 222.24 | ![Structure4](image) |
| P45      | 270.24 | ![Structure5](image) |
| P46      | 210.23 | ![Structure6](image) |
| P47      | 266.25 | ![Structure7](image) |
| P48      | 266.25 | ![Structure8](image) |
| P49      | 210.23 | ![Structure9](image) |
| P50      | 267.27 | ![Structure10](image) |
| P51      | 178.23 | ![Structure11](image) |
| P52      | 194.23 | ![Structure12](image) |
| P53      | 238.24 | ![Structure13](image) |
| P54      | 255.25 | ![Structure14](image) |
| P55      | 256.25 | ![Structure15](image) |
| P56      | 234.2  | ![Structure16](image) |
| P57      | 220.27 | ![Structure17](image) |
| P58      | 250.2  | ![Structure18](image) |
| P59      | 241.22 | ![Structure19](image) |
| P60      | 242.23 | ![Structure20](image) |
| P61      | 202    | ![Structure21](image) |
| P62      | 238.2  | ![Structure22](image) |
| P63      | 234.2  | ![Structure23](image) |
| P64      | 238.2  | ![Structure24](image) |
| P65      | 270.28 | ![Structure25](image) |
Table 7. Proposed degradation pathways and the active genes during pyrene oxidation.

| Organism                        | Active Enzyme/Gene | Proposed Pathways                                                                 | References |
|---------------------------------|-------------------|-----------------------------------------------------------------------------------|------------|
| Mycobacterium vanbaalenii PYR-1 |                   | (1) Pyrene → P64 → P56 → P58 → P66.                                               | [18]       |
|                                 |                   | (2) Pyrene → P62 → P63 → P48 → P44 → P55 → P46.                                   |            |
|                                 |                   | → P36 → P23 → P37 → P18 → P12 → P7 → P24 → P15 → Crboxylic acid → Tricarboxylic acid cycle |            |
| Mycobacterium vanbaalenii PYR-1 | PhdE, PhdG, PhdI, NidA3, NidAB, NidD, PhtAa, PhtB, and PhtAc | Pyrene → P62 → P63 → P48 → P44 → P55 → P46 → P36 → P23 → P37 → P18 → P12 → P7 → P24 → P15 → Crboxylic acid → Tricarboxylic acid cycle | [22]       |
| Mycobacterium vanbaalenii PYR-1 | NidAB, PhdE, PhdC, PdoA2B2, PhdF, PhdG, NidD, PhtAa, PhtB, and PcaGH | Pyrene → P62 → P63 → P58 → P48 → P38 → P7 → P15 → Crboxylic acid → Tricarboxylic acid cycle | [24]       |
| Coriopolis byrsina APC5          |                   | Pyrene → P57 → P62 → P63 → P51 → P5 → P72 → P73 → P6 → Tricarboxylic acid cyclic acid |            |
| Mycobacterium spp. PO1 and PO2  | NidA, PhdA, and NidA3 | Pyrene → P62 → P63 → P48 → P44 → P55 → P46 → P60 → P7.                             | [28]       |
| Mycobacterium sp. flavescens PYR-1 |                   | Pyrene → P62 → P63 → P48 → P44 → P53 → P46 → P16 → P36 → P23 → P37 → P18 → P12 → P17 or P24 → P15 → Crboxylic acid → Tricarboxylic acid cycle | [43]       |
| Many bacterial strains including Mycobacterium |                   | Pyrene → P62 → P63 → P56 → P58 → P58 → P66 → P36 → P23 → P37 → P18 → P12 → P17 or P24 → P15 → Crboxylic acid → Tricarboxylic acid cycle | [45]       |
| Mycobacterium aromatovorans Strain JS19b1 |                   | Pyrene → P48 → P44 → P46 → P22 → P13 or P7.                                          | [50]       |
| Lecateria decarboxylata PS4040  |                   | Pyrene → P69 → P48 → P12 → P7 → P13.                                              | [70]       |
| Coriopolis byrsina strain APC5   | laccase, LiP and MnP | Pyrene → P62 → P63 → P51 → P5 → P72 → P73 → P6 → Tricarboxylic acid cycle → CO_{2}. | [73]       |
| Halophilic Hortaea sp. B15       | Dioxygenase        | Pyrene → P49 → P35 → P7 → Tricarboxylic acid cycle                                | [75]       |
| Pseudomonas sp. ISTPY2           |                   | Pyrene → P63 → P68 → P48 → P44 → P55 → P46.                                       | [80]       |
5. Future Perspectives and Challenges

The current techniques for the biodegradation of pyrene by *Mycobacterium* strains still need further investigation for future works.

1. A knowledge gap between pyrene oxidation at the field site compared to laboratory conditions needs to be addressed for each product seeking commercial success.

2. The degradation of pyrene by *Mycobacterium* strains generates many metabolites. Some of the metabolites and their bio-toxicity have been identified, while most of them need bio-toxicity assessment.

3. The main biodegradation drawback is the limitation of the bioavailability of the target pollutant. Therefore, it is highly recommended to add a biosurfactant to increase the bioavailability.

4. The literature revealed that the biodegradation of pyrene via consortium microbial gives a better result than a single strain. That is referred to diverse enzymes capable of oxidizing pyrene and its metabolites.

5. There are several studies that applied successful synergetic biodegradation systems for pyrene degradation, such as biofuel cells and coupling of the advanced oxidation process and biodegradation system.

6. Conclusions

This article attempted to provide a review of pyrene bioremediation using *Mycobacterium* strains in various biodegradation mediums. This study’s findings are summarized as follows:

- *Mycobacterium* strains are efficient biological agents to degrade pyrene, that is, referring to their ability to produce many functional enzymes able to metabolite pyrene and its transformation molecules.

- Phenanthrene-4,5-dicarboxylic acid, dihydroxy pyrene, phenanthrene-4-carboxylic acid, phthalic acid, and pyrene-4,5-dihydrodiol were the most frequent metabolites.
that were detected when *Mycobacterium* sp. strains were used for pyrene degradation.

- Some metabolites showed positive results for the Ames mutagenicity prediction test, such as 1,2-phenanthrenedicarboxylic acid, 1-hydroxypyrene, 4,5-dihydropyrene, 4-phenanthrene-carboxylic acid, 3,4-Dihydroxyphenanthrene, Monohydroxy pyrene, and 9,10-phenanthrenequinone. However, 4-phenanthrol showed positive results for experimental and prediction tests.

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