

**How to Cite:**

Esmaeel, J. R. ., & Alfatlawi, M. A. A. (2022). Effect of enzymes and metabolic pathway of genus *Clostridium* in forming simple molecules of different compound: A review. *International Journal of Health Sciences*, 6(S1), 10223–10231. <https://doi.org/10.53730/ijhs.v6nS1.7421>

## Effect of enzymes and metabolic pathway of genus *Clostridium* in forming simple molecules of different compound: A review

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**Abstract**---Naphthalene and derivatives, such as methylnaphthalene are polycyclic aromatic hydrocarbons (PAHs) with molecular weight of low levels that are utilized in variety of industrial sectors and have been shown to be toxic to nucleic acids, which can lead to the occurrence of mutation and/or cancer in living creatures. These are chemically synthesized substances (CSSs) or xenobiotics, which are considered as major contaminants to the environment and public health. The emissions from industrial sectors and tools, such as the gasification of coal, refining of petroleum, the exhaust of motor vehicles, and applications in the agricultural industry decide the concentration, transformational fate, and transmission of these substances. Bioremediation, which uses microorganisms that can breakdown CSSs entirely or transform them into non-toxic metabolites, has been shown to be a safe, not expensive, and attractive replacement to the conventional physicochemical techniques of cleaning and elimination of these harmful compounds. Soil microbes related to Proteobacteria, such as *Pseudomonas*; Firmicutes, such as *Bacillus*; and *Actinobacteria*, such as *Rhodococcus* were found to be capable of breaking down numerous CSSs. Naphthalene is a seriously dangerous compound that can be found in different environmental sites, such as oil refineries and contaminated water resources, which can induce mutations and/or cancer in animals and humans. There are different microbes that can degrade this compound to simpler molecules, such as species from the genus *Clostridium*, using various enzymes and metabolic pathways.

**Keywords**---Bacterial degradation, naphthalene.

## Introduction

There has been an increase in world economy and higher living quality as a result of the fast development of industrial sectors such as petroleum, agricultural, pharmacological, textiles and dyes, beauty, etc. As a consequence of this rapid growth, CSSs have been developed that are employed in the production of a wide range of items. PAHs, insecticides, herbicides, coloring dyes, medicines, organophosphorus compounds, volatile organic solvents are examples of CSSs or xenobiotics (1). Ecosystems that are exposed to these pollutants are affected in a wide range of ways, from changing physicochemical qualities to causing harm to a wide range of living things. Some of the most precious ecosystems, such as coral reefs, lakes, and deep water, have been adversely affected by a variety of aromatic pollutants. Stone sculptures and metal monuments, as well as art objects, have been found in modern geomicrobiological research to degrade faster when synthetic organics (such as aromatic pollutants) and equivalents accumulate on their surfaces (2). The biodegradation of historical buildings may be accelerated and worsened by human activities such as air contamination and climate changes. Using atmospheric moisture from the air, these organic contaminants interact with each other, causing physical and chemical degradation of the substance (3–7).

The term "biodeterioration," which refers to changes in a substance's surface and qualities caused by biological processes, has long been generally accepted. Substances' architectural integrity, storage value, and cultural significance are all threatened by additional microbial impacts (metabolism). The development of biofilms and other defensive encrustations, on the contrary side, has been proven to be advantageous in a few circumstances, decreasing the decay/decomposition rate (8,9). Appropriate conservation solutions for stone, metals, and wood statues need a thorough knowledge of the major mechanisms engaged. Anthropogenic activity (industrial pollution), in contrast to natural activities, such as geological functions, fires of the wild forests, eruptions of volcanos, plant and microbial responses), releases vast amounts of PAHs and other CSSs into environments. DDT, atrazine and carbaryl, as well as pentachlorophenols and polychlorophenols (PCB) utilized in agriculture; petroleum-based plastics, polychlorinated biphenyls, detergents in industry; sunscreens in cosmetic care items; and 2,4,6-TNT in ammunition are all examples of PAHs that may pose a risk to human health (10). There has also been an increase in the anthropogenic pollutant emissions, such as CO<sub>2</sub> and other greenhouse gases, during the last several decades. Despite this, human activity has a far greater impact on the environment than does nature (11). Many ecological compartments include a wide range of CSSs, which have been identified as emerging pollutants because of their detrimental impacts on the health of the living population. As a result of their cytotoxic, genotoxic; mutagenic and/or carcinogenic properties, they have been designated as priority pollutants by agencies, such as the United States Environmental Protection Agency (USEPA). As a result, tight criteria for their disposal and efficient procedures for their removal from contaminated environments are required. As a result of inadequate and costly physical and chemical cleaning approaches such as pyrolysis, oxidative thermal, air-sparging, and land-filling have generated a wide range of toxic chemicals. Microbes that can digest these contaminants and their derived compounds (such as halo-, nitro-, alkyl-, and/or methyl-) have

acquired more interest as environmental consciousness has spread around the globe (12,13).

In respect of ecological security, affordability, productivity, efficacy, and durability, the utilization of these indigenous microorganism species alone or in mixed cultures to remove aromatic contaminants has proved beneficial. Several degrading microorganisms' genetic control, proteomic reactions, and fluxomic reactions are now well understood thanks to the introduction of genomic sequencing and omics techniques (14). We are learning more about how to choose and fine-tune microorganisms' targeted catabolic pathways (called metabolic designing) for productive and successful biodegradation by combining these technologies with system biology. Effective bioremediation procedures require an in-depth knowledge of an organism's biochemical capacity, metabolism complexity, genetic composition, and ecology in order to find acceptable microbiological candidates (10).

### **Biological impacts, sources, and fate of aromatic Compounds**

Different environmental niches, such as air, sediment, soil, and water from the surface and the ground, are in serious danger due to the prevalence of poisonous and dangerous aromatic chemicals. Because of their hydrophobicity and reduced state, these chemicals have exhibited long-term durability in the ecosystem. They are more persistent, with greater affinity for macromolecules and bioaccumulation properties in living organisms, when methyl ( $-CH_3$ ), carboxyl ( $-COOH$ ), hydroxyl ( $-OH$ ), or sulfonate group ( $-HSO_3$ ) are added to the aromatic. According to the USEPA, too many of these low molecular weight polycyclic aromatic hydrocarbon (LMW-PAH) compounds, including naphthalene and its derivatives, such as methyl naphthalenes, naphthalene sulfonates, naphthoic acids, and 1-naphthyl N-methyl-carbamate, have been regarded to cause mutations and/or cancer. Such LMW-PAHs are released and bioaccumulated in the food chain at different stages, resulting in a negative impact on environmental quality (10,12).

Biota's exposure to PAHs is mostly facilitated by the movement and interactions of PAHs amongst diverse ecosystem constituents, such as soil. PAHs are deposited on surfaces as a result of environmental pollution and transport caused by vehicle emissions, emission by industrial of coal gasification and coke manufacturing. Fabricated textiles, coloring dye and paint synthesis; wood protection; rubber preparation; cement production; insecticide manufacturing and usage in agriculture are the principal sources of PAHs in land and water systems. PAHs may also be found in sewage and groundwater. Due to emissions from electricity reactors, domestic heating, air/road transportation, and building operations are particularly susceptible to PAHs (15).

Chlorination of drinking water has also been linked to the creation of more harmful oxygenated and chlorinated PAHs. Toxic PAHs have been detected in many aquatic species such as fish as well as shellfish and shrimp, which are ingested or absorbed via the skin. PAHs are also a substantial byproduct of a variety of other food preparation processes such as frying and grilling. The food's physicochemical qualities influence the PAHs' deposition, while the toxicity of

these compounds is determined by the body's physiology, metabolism, absorption, distribution, and fractionation within the body (16–18).

PAHs' carcinogenicity and other health effects have long been established. Two- to three-ringed LMW-PAHs may bind covalently to a wide range of macromolecules such as DNA, RNA, and proteins, and this can lead to the occurrence of cancer. The hydrophobic nature of these molecules causes them to be segregated into lipid membranes. Cyp450 monooxygenase in the human body is crucial for the conversion of healthy cells into malignant ones by producing extremely reactive PAH epoxides from PAHs, which have been shown to be very toxic (19). Even more dangerous than the parent chemicals are the PAH transition products such as diols. Disruption of growth hormones and numerous metabolic processes by a number of PAHs and their metabolic byproducts leads to deleterious consequences on development, the central nervous system, reproduction, and the immune functions. In animal experiments, PAH exposure has been linked to reproductive and developmental problems, as well as eye cataracts, and renal and hepatic impairment (10).

DNA and other macromolecules may be harmed by reactive oxygen species (ROS) generated by quinones and free radicals, as examples. This has a detrimental effect on the tissue functionality and viability. Experimental animals have developed cancer after being exposed to low amounts of naphthalene over an extended period of time. The removal of these PAHs from damaged and contaminated places is a top priority because of their deadly and harmful impacts (20).

Several physical and chemical procedures have been used to remove PAHs from polluted areas. In contrast, bioremediation, which includes the use of microbes, either as a single culture or in combination with other organisms, is a potential option. Unlike to physical and chemical approaches, this technology is environmentally friendly, non-invasive, not expensive, and durable. Ecologically, bioremediation is a better option than traditional physical and chemical techniques since it may be done at the affected site (in situ) or in a properly designated location (3,12).

### **Naphthalene and derivatives**

Among a number of aromatic contaminants, naphthalene and derivatives are the major targets for this review. They are discovered to be the major elements of petroleum-derived fuels, textile coloring dyes, everyday items, such as clothing, insecticides, and tanners, and hence widespread in many habitats. In studies of PAH metabolism, genetics, and metabolic diversity, naphthalene and its derivatives are often utilized as model substrates because they are more water soluble than other PAHs with greater molecular weight (18,21). Countless microorganisms are capable of metabolizing them, thanks to detailed knowledge of metabolic pathways, enzymatic components, and regulatory mechanisms. Because of their greater quantities and bioavailability, these as prototypic chemicals are used to evaluate environmental contamination. It has been discovered that naphthalene has toxicity the respiratory system and carcinogenicity that are species-, regionally- and sex-selective (12,22).

The International Agency for Research on Cancer (IARC) has categorized naphthalene as a "likely human carcinogen" depending on information from animal experiments. Inhalation or oral route of naphthalene derivatives is the primary mode of exposure, resulting in lung tissue destruction and a higher likelihood of lung tumors in mice and rats. Acute exposure might cause symptoms such as nausea, vomiting, stomach discomfort, diarrhea, headaches, disorientation, excessive sweating, febrile reaction, and tachycardia. One of the most widely used insecticides in the world, carbaryl (1-naphthyl N-methylcarbamate), is hazardous to aquatic invertebrates such as frogs and bees as well as to people and has been proven to block acetylcholine esterase resulting to paralysis in animals and humans (Smulders et al., 2003; Bulen and Distel, 2011). For bioremediation from a polluted environment, then, it is essential to comprehend the processes of biodegradation, genetic controls, enzymatic and cellular response (23).

### **Microbial diversity of naphthalene-degrading bacteria**

Water-insoluble and lipid-soluble aromatic contaminants in polluted niches have different impacts on the environmental microbiome, such as modifying the membranous fluidity and permeability, thickening of lipid bilayers, disturbances of cellular energy transdifferentiation, and membrane-linked protein interaction. A number of soluble intermediates, including as the catechols and the quinones, also create ROS and generate complexation with DNA and proteins. To be able to breakdown a molecule efficiently at several physiological domains, including uptake/transport, internal conversions, assimilation/utilization and encapsulation are all influenced by the amount of the chemical in the environment (24).

Naphthalene or its compounds have been found to be abundant with 926 bacteria, in which 755 were from protobacteria members according to the Ribosomal Database Project-II (RDP-II). Firmicutes (52 members), Bacteroidetes (43 members), Actinobacteria (39 members), Tenericutes (10 members), and unclassified bacteria (8 members) were also present in that environment. There are 54% of all Gram-negative  $\gamma$ -proteobacteria, rich G+C composition groups that include Pseudomonadales and Xanthomonadales members, while 30% of all Gram-positive, lower G+C composition groups have members (Clostridiales and Bacillales) (25–27). For the decomposition and removal of the naphthalene and its methyl compound derivatives, the most common species is the pseudomonas (a maximum of 338 species), which is found in a wide range of polluted and natural environments. It has also been shown that uncultivable *Legionella* and *Clostridium* species may be capable of degrading these compounds, emphasizing the necessity to cultivate these bacteria in order to better comprehend new pathways and physiological variety in these organisms (28–31).

### **Degrading metabolic pathways**

Naphthalene is a primary supplier of carbon and energy for most microorganisms that breakdown aromatics, according to reports from the literature. NCIB 9816-4, G7, AK-5, PMD-1 and CSV86 strains of the *Pseudomonas putida* bacterium have all been shown to be engaged in the naphthalene metabolic pathway. *P. stutzeri*

AN10, *P. fluorescens* PC20, as well as other strains (ND6 and AS1) have also been shown to be associated (32).

NDO (naphthalene dioxygenase), RHDO (ring-hydroxylating dioxygenase) are the enzymes that initiate the metabolism by oxidizing one of the naphthalene aromatic rings utilizing  $O_2$  as a further substrate. This reaction is followed by the conversion of naphthalene compound to cis-naphthalene dihydrodiol. There are enzymes, such as dehydrogenase, that convert cis-dihydrodiol to 1,2-dihydroxynaphthalene. Then, 1,2-dihydroxynaphthalene dioxygenase (12DHND) is a ring-separating dioxygenase that produces 2-hydroxychromene-2-carboxylic acid from 1,2-dihydroxynaphthalene. In the presence of a hydratase-aldolase, trans-o-hydroxybenzylidene pyruvate is converted to salicylaldehyde and pyruvate and previously preceded by a cis-trans conformational change (10).

Pyruvic acid is the first C3 chemical produced from naphthalene's backbone chain and directed into the core carbon pathway. Salicylaldehyde dehydrogenase (NAD<sup>+</sup>-based), on the other hand, transforms salicylaldehyde to salicylate. The "upper pathway" for the breakdown of naphthalene refers to the metabolic process up to this point. Almost all naphthalene degraders choose this path. A few outliers exist; for instance, in *Bacillus thermoleovorans* Hamburg 2, the naphthalene compound breakdown begins with naphthalene 2,3-dioxygenase to produce the resulting 2,3-dihydroxynaphthalene (10).

Salicylate 1-hydroxylase (S1H) or salicylate 5-hydroxylase (S5H) subsequently breakdown the produced salicylate, however this depends on the microbe and its genomic set. It is common to refer to the stages from salicylic acid to TCA precursors as a "lower pathway" since salicylate is an essential component in the naphthalene synthesis (upper pathway). Both the upper and lower pathway based *nah* and *sal* operons, respectively, of naphthalene metabolism are often shown to be controlled by the same regulator, for instance, *NahR* and salicylate (12,22).

## Conclusion

Naphthalene is a seriously dangerous compound that can be found in different environmental sites, such as oil refineries and contaminated water resources, which can induce mutations and/or cancer in animals and humans. There are different microbes that can degrade this compound to simpler molecules, such as species from the genus *Clostridium*, using various enzymes and metabolic pathways.

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