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# Monooxygenase and dioxygenases by bacterial specific enzymes network

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***Abstract*---**Naphthalene, a polycyclic aromatic hydrocarbon (PAH), is present in different industrial sites, including combustion products of fossil products (such as coal tar and petroleum) and biofuels. It is also a chemical with a substantial quantity of commercial manufacturing. Moth repellents and toilet deodorant blocks are among the most common consumer items that include the chemical compound naphthalene. It is also widely used in the production of chemical intermediates (such as phthalic anhydrides) as well as pigments and surfactants, and in the tanning of leather. Naphthalene has critical health impact, inducing mutations and/or cancers. This review focuses on the bacterial enzymes used in the degradation of naphthalene. Naphthalene is an important contaminant of environment that can cause serious health problems. The compound can be bacterial-degraded used specific enzymes networks, such as monooxygenases, dioxygenases, dehydrogenases, etc.

***Keywords*---**Biodegradation, naphthalene, microbial remediation.

## Introduction

Natural sources of naphthalene, a PAH, include combustion products of fossil products (such as coal tar and petroleum) and biofuels. It is also a chemical with a substantial quantity of commercial manufacturing. Moth repellents and toilet deodorant blocks are among the most common consumer items that include the chemical compound naphthalene. It is also widely used in the production of chemical intermediates (such as phthalic anhydrides) as well as pigments and

surfactants, and in the tanning of leather (1). Naphthalene is predominantly released into the atmosphere through anthropogenic processes, open fires, vehicle smoke, and cigarette smoke, etc. Ingestion of mothballs or soil polluted with naphthalene may put children at greater risk of naphthalene exposure (2).

Investigations on animals and humans have shown that exposure to naphthalene causes cancer and non-cancerous health consequences. The National Toxicology Program's (NTP) 2-year inhalation laboratory studies in rodents are the primary source of data regarding naphthalene's carcinogenic activity. Nasal epithelial adenomas (benign), olfactory neuroblastomas (malignant), and pulmonary adenomas (benign) were all discovered to be induced in the NTP studied animals exposed to naphthalene (3). Two case reports have reported cancers linked to naphthalene compound exposure in humans: an elevated risk of laryngeal cancer in a German naphthalene filtration factory and a greater risk of colorectal cancer in Nigerian patients who had previously taken indigenous therapies that contained naphthalene for anorectal health issues. NTP 2016 categorized naphthalene as a fairly expected human carcinogen on the basis of these observations, and the International Agency for Cancer Research (IARC 2002) categorized it as possible carcinogenic compound (Group 2B) on the basis of these results; both statistics referenced appropriate proof in animal experiments and insufficient proofs in humans (4–7).

It is a biotechnological method that aims to restore the polluted environment and manage contaminants through decontamination and mineralization via bioremediation. The persistent organic pollutant (POP) treatment employing microbial enzymes is regarded environmentally benign, cost-effective, inventive, and encouraging. Bioremediation, on the other hand, has significant drawbacks (8). There have only been a few species of bacteria that have shown the capacity to breakdown contaminants via the production of specialized enzymes in the long-term. Due to their ability to produce required enzymes in high quantities and under optimal circumstances in genetically modified microbes, researchers favor them for use in bioremediation procedures. Agrochemicals, microplastics, polyhalogenated substances and hydrocarbons, as well as other xenobiotic pollutants, may be catalyzed or metabolized using genetically modified microbes that have the ability to produce appropriate enzymes. Toxic substances are converted to harmless ones, and new products are created in the process (9).

**Bacterial enzymes for the degradation of naphthalene**

Because the low molecular weight-PAHs (LMW-PAHs) are hydrophobic, reducing, and very sparingly soluble, they produce resistance to breakdown by natural processes. Molecular oxygen may be used by aerobic microbes in order to decompose them. They are mostly oxido-reductase enzymes, which execute diverse processes such as the hydroxylation of aromatic rings (mono- or di), dehydrogenative-based properties, and the cleavage of the aromatic rings. The products of these processes have a higher oxidation state, making them more amenable to the central carbon pathway's metabolic processing (10). Enzymes involved in the decomposition processes may be able to be induced. When organisms are cultivated on a single carbon supply, such as glucose or organic acids, the activity of these enzymes are extremely low or insignificant. Enzymes

that play a role in the breakdown and degradation of naphthalene (11) and related compounds are listed in Table 1.

Table 1
Microbial enzymes and encoding genes responsible for the degradation of naphthalene (11)

| Enzymes (genes) | Function via gene ontology |
|---|---|
| Ferredoxin reductase (*nah*Aa) | - Double iron and sulfur group binding<br>- Di-oxygenation (Transfer of electron)<br>- Binding of metal ions |
| 1,2-dioxygenase (*nah*Ab) | - Double iron and sulfur group binding<br>- Di-oxygenation (Transfer of electron) |
| Large subunit 1,2-dioxygenase (*nah*Ac) | - Double iron and sulfur group binding<br>- Di-oxygenation (Transfer of electron)<br>- Binding of metal ions<br>- Metabolism (Cellular) |
| Small subunit 1,2-dioxygenase (*nah*Ad) | - Di-oxygenation (Transfer of electron)<br>- Metabolism (Cellular) |
| Dehydrogenase: *cis*-Dihydrodiol (*nah*B) | - Metabolism (Cellular) |
| Dioxygenase:1,2-Dihydroxynaphthalene (*nah*C) | - Di-oxygenation (Transfer of electron)<br>- Metabolism (Cellular) |
| Isomerase: 2-Hydroxychromene-2-carboxylate (*nah*D) | - Isomerization: Disulfide oxido-reduction of protein<br>- Metabolism (Cellular) |
| Hydratase-aldolase: *Trans-O*-hydroxybenzylidene pyruvate (*nah*E) | - Lysis of Aldehyde<br>- Hydrolase of aldolase |
| Dehydrogenase: Salicylaldehyde (*nah*F) | - Dehydrogenation<br>- Metabolism (Cellular) |
| Hydroxylase: Salicylate (*nah*G) | - Mono-oxygenation (FAD-binding) Monooxygenase<br>- Metabolism (Cellular) |
| Catechol 2,3-dioxygenase (*xyl*E) | Di-oxygenation (Ion binding: Ferrous) |
| Dehydrogenase: 2-Hydroxymuconic semialdehyde (*xyl*G) | - Hydroxylation activity<br>- Oxido-reduction activity |
| Hydrolase: 2-Hydroxymuconic semialdehyde (*xyl*F) | - Hydrolase activity |
| Hydratase: 2-Oxopent-4-enoate (*xyl*J) | - Hydrolase activity |
| Aldolase: 4-Hydroxy-2-oxovalerate (*xyl*K) | - Binding of metals via aldolase |
| Dehydrogenase: Acetaldehyde (*dmp*F) | - Activity of dehydrogenase<br>- Binding of NAD |
| Decarboxylase: 4-Oxalocrotonate (*xyl*L) | - Activity of catalytic processes<br>- Binding of Metal ions |
| Tautomerase: 4-Oxalocrotonate (*xyl*H) | - Isomerization<br>- Metabolism (Cellular) |
| Catechol 1,2-dioxygenase (*cat*A) | - Activity of Oxido-reductase<br>- Binding of metal ions |
| Muconate cycloisomerase (*cat*B) | - Cyclo-isomerization activity<br>- Metabolism (Cellular) |

| | |
|---|---|
| | - Binding of metal ions |
| Delta-isomerase: Muconolactone (*cat*C) | - Delta-isomerization<br>- Metabolism (Cellular) |
| Hydrolase: β-Ketoadipate enol-lactone (*pca*D) | - Activity of Enol-lactonase function |
| α subunit-β-Ketoadipate succinyl-CoA transferase (*pca*I) | - Binding via CoA transferase activity<br>- Metabolism (Cellular) |
| β subunit-β-Ketoadipate succinyl-CoA transferase (*pca*J) | - Binding via CoA transferase activity<br>- Metabolism (Cellular) |
| Thiolase: β-Ketoadipyl-CoA (*paa*J) | - Activity of thiolase<br>- Metabolism (Cellular)<br>- Activity of catalysis<br>- Induction stimulation of DNA destruction |
| Large subunit salicylate-5-hydroxylase (*nag*G) | - Activity of Oxido-reductase<br>- Metabolism (Cellular)<br>- Binding of metal ions |
| Small subunit salicylate-5-hydroxylase (*nag*H) | - Activity of Oxido-reductase<br>- Metabolism (Cellular)<br>- Binding of metal ions |
| Gentisate 1,2-dioxygenase (*nag*I) | - Di-oxygenation activity<br>- Binding of metal ions |
| Isomerase: Maleylpyruvate (*nag*L) | - Isomerization activity<br>- Metabolism: Amino acids |
| Hydrolase: Fumarylpyruvate (*nag*K) | - Hydrolase processes<br>- Binding activity of metal ions |
| Carbaryl hydrolase (*mcb*A) | - Ingredients of integral membranes |
| 1-Naphthol 2-hydroxylase (*mcb*C) | - Metabolism (Cellular) |
| Oxidase: Formylsalicylate (-) | - Metabolism (Cellular) |

**Monooxygenase and Dioxygenases**

The insertion of molecule $O_2$ by oxygenases into the aromatic ring is the greatest critical stage in stimulating the molecule for additional microbial breakdown, according to experiments that used ($^{18}O2$). An internal or external monooxygenase catalyzes the integration of a single oxygen atom of a molecular oxygen into the target (also known as hydroxylase activity) (12,13). H2O is formed from the other atom of oxygen. There are two types of monooxygenases: internally and externally derived enzymes. External monooxygenases employ NADH or NADPH for the reduction of flavin, whereas internal monooxygenases utilize the targeted substrate to reduce flavin. As an instance, salicylate 1-hydroxylase converts salicylate to catechol by hydroxylating it at the C1 location. Salicylate 5-hydroxylase, which is a group of a reductase, ferredoxin, and a subunit oxygenase on the contrary, accomplishes the hydroxylation activity at the position C5 of the salicylate to produce the gentisate (14–16).

Both O2 atoms are incorporated into the targeted substrate by dioxygenases. Depending on the end output, ring-hydroxylation or ring-cleavage, for which dioxygenases are sub-classified. Bacteria have been reported to have a ring-hydroxylating dioxygenase that transforms aromatic substrates (such as the

naphthalene) into cis-dihydrodiols (17,18). There have been enzymes, which have been demonstrated to develop on a wide variety of aromatic carbon donors, and these proteins have been categorized as naphthalene dioxygenase (NDO), toluene dioxygenase (TDO), and biphenyl dioxygenase (BPDO) (19–21). In order to catalyze the dioxygenation and side-chain activity of hydroxylation of a wide range of PAHs (such as toluene, nitrotoluene, and acetophenones) using NDO and BPDO both work as catalysts, it is necessary to combine the two methods. An oxygenase element with the active site is part of the NDO's multi-component network of enzymes, which contains oxido-reductase, a ferredoxin, and an oxygenase. In the NDO, the major and minor components (α and β), respectively, are stacked in a α3β3 arrangement to form the catalytic component (22–26).

Mononuclear non-heme iron NDO belongs to the wide category of oxygenases featuring Rieske [2Fe-2S] centers, which defines the selectivity of NDO for its target substrates. At the catalytic sites of the of the enzymes, two electrons are transported from the reduced form of pyridine nucleotide to Fe(II) ion through reductase, ferredoxin, and the center of Rieske. Because of the presence of reducing equivalents, molecular oxygen may be activated, allowing for dihydroxylation of the substrate (27,28).

Some NDOs have been isolated and thoroughly described from several types of microorganisms, and the genetic regulation of the processes engaged in naphthalene breakdown has been investigated in depth. Hydroxylated aromatic substances are targeted by ring-cleaving dioxygenases.

**Hydrolases and Dehydrogenases**

Other groups of covalent bond-cleaving enzymes, including hydrolases (esterase and amidase enzymes), use water to do their task. These hydrolases have a wide range of substrate specificities. It has been postulated that Gram-negative bacterial hydrolases, such as carbaryl hydrolases, are transmembrane proteins. Hydrolysis of carbaryl (with a bond of either ester or amide) may be carried out either through esterase or amidase to produce 1-naphthol. *Rhizobium* sp. and *Arthrobacter* sp. strains of (AC10023 and RC100, respectively) have the esterase and amidase activity, respectively. *Pseudomonas* sp. is documented to have esterase carbaryl and 1-naphthylacetate (at 100% and 36%, respectively) activity (11). Another significant enzyme in metabolism is NAD+/NADP+-dependent aromatic dihydrodiol-, alcohol-, and aldehyde-dehydrogenases that employ NAD+/NADP+ as an electron acceptor in a dehydrogenation process (29).

**Conclusion**

Naphthalene is an important contaminant of environment that can cause serious health problems. The compound can be bacterial-degraded used specific enzymes networks, such as monooxygenases, dioxygenases, dehydrogenases, etc.

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