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A Review on Origin, Occurrence, and Biodegradation of Polycyclic Aromatic Hydrocarbon Acenaphthene

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Abstract Polycyclic aromatic hydrocarbons (PAHs) encompass a huge and diverse group of priority environmental pollutants, which are ubiquitous contaminants derived from both natural and anthropogenic processes. Their abundance in the environment is of immense concern because many of them are toxic, mutagenic and/or carcinogenic. Among them, acenaphthene has often been used as a model substrate to investigate the microbial metabolism of PAHs since its structural skeletons are found in many carcinogenic PAHs. The current article, in brief, describes the advances that have occurred in the area in terms of the origin, occurrence, and significance of acenaphthene found in the environment. The destiny of acenaphthene by various microorganisms in the environment is also discussed concisely in light of the degradation pathway depicting several metabolites and enzyme-substrate/metabolite relationships.

Keywords: *Polycyclic aromatic hydrocarbons (PAHs), environmental pollutants, carcinogenic, Acenaphthene, biodegradation*

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1. Introduction

In the recent times, with improved consciousness of the probable undesirable effects of environmental pollutants on human health and environment, remediation and reclamation of environment polluted with harmful materials have received growing attention. Among others, polycyclic aromatic hydrocarbons (PAHs) are considered as one of the major priority organic pollutants of critical concern owing to their toxic, genotoxic, mutagenic and/or carcinogenic properties [1,2]. PAHs constitute a family of hazardous compounds that are widely present as contaminants in the air, soil, and aquatic environments. PAHs are detected in the air [3,4], soil and sediments [5-10], surface water, groundwater, and road runoff [11-14]. PAHs have their origin in both natural and anthropogenic processes and enter the environment in many ways. Anthropogenic as well as natural sources of PAHs in combination with worldwide transportation phenomena result in their universal sharing, and subsequently, PAHs are spreaded from the ambiance to vegetation [15] and contaminate foods [16,17]. There is a grave alarm about their presence in the environment, especially for their potential of bioaccumulation in various food chains [18-21], and are therefore considered as substances of potential human health hazards [22,23,24]. Considering these facts, the US Environmental Protection Agency has listed 16 PAHs as priority pollutants (Figure 1) for remediation [25].

Compared to other organic compounds PAHs are less easily degraded in soils since they are moderately stable and recalcitrant in soils. Consequently, by using the conventional techniques for soil decontamination, PAHs are difficult to remove from the contaminated soils; and therefore, PAHs are considered as persistent environmental pollutants. Several sanitization techniques have been suggested in the past and various physicochemical methods have been used to remove these compounds from the environment, but they have many certain limitations. In many cases, by using the conventional techniques, the contaminated compounds can not be destroyed appropriately, rather they transport them from one environment to another. In contrast, employing bioremediation/biodegradation techniques, environmental pollutants can be detoxified into simpler and less toxic compounds using microorganisms. Therefore, biodegradation addresses the limitations by bringing about the absolute demolition of various organic contaminants at a lower cost and ambient conditions. As a result, over recent years, bioremediation has grown from virtually unknown technology to a technology that has gained acceptance and becomes an increasingly popular remedial alternative for pollutant removal. At present, it is well accepted that microbial degradation is a safer, more competent, and less costly choice to physicochemical methods for the decontamination of infected sites with organic pollutants [26].

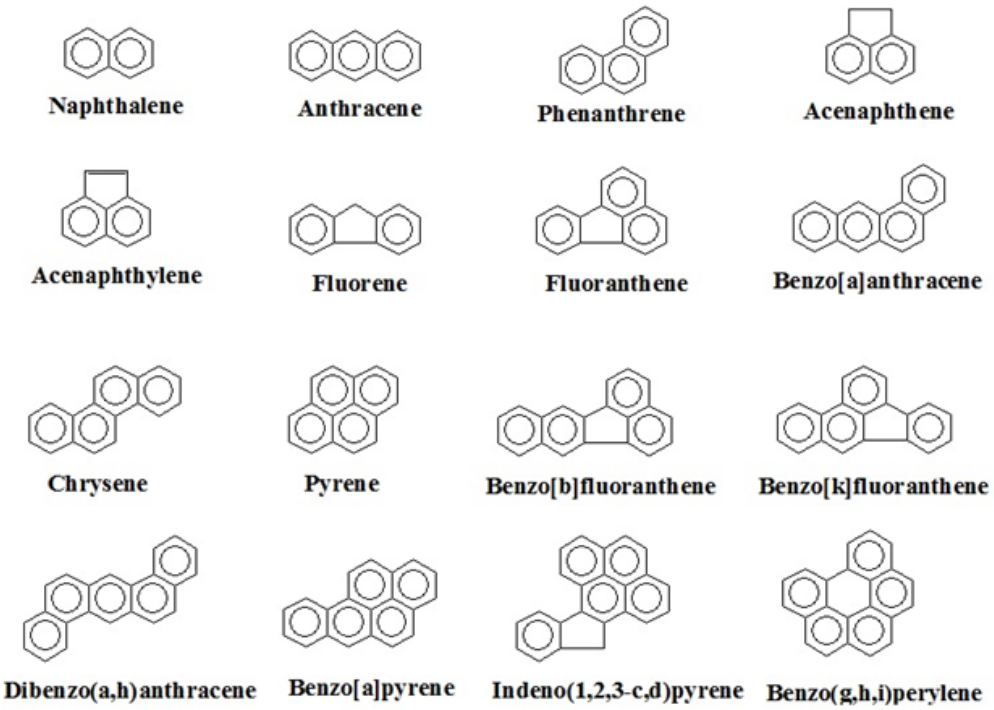

Figure 1. Structure of 16 PAHs listed as priority pollutants by US EPA

Acenaphthene, one of the abundant PAHs found in the environment, is considered as prototypic PAH because of its structural similarity with various carcinogenic PAHs such as in Fluoranthene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Indeno(1,2,3-c,d)pyrene, etc.; and seldom serve as signature compounds to identify different carcinogenic PAHs contamination. Since acenaphthene is the smallest PAH containing two condensed rings with two saturated methylinic carbon atoms joined therein, it is frequently used as a replica substrate for studies on the metabolism of High Molecular Weight carcinogenic PAHs [27]. Acenaphthene has also been used as a model PAH to establish factors that influence the bioavailability, biodegradation prospective, and rate of microbial deprivation of PAHs in the environment [28]. In comparison to other Low Molecular Weight PAHs such as naphthalene, and phenanthrene, biodegradation studies of acenaphthene are somewhat limited. In spite of that, there are several reports of assimilation of acenaphthene by various microorganisms, which cover a huge area of literature in the context of biodegradation of acenaphthene. However, no review article presents describe the detailed literature of acenaphthene biodegradation in a concise way. The present communication briefly summarizes the source, occurrence, and implication of acenaphthene found in the environment along with the diverse established metabolic pathways involving numerous novel metabolites, depicting the biodegradation potentials by the various microorganisms in the management of acenaphthene in the environment.

2. Discussion

2.1. Properties of Acenaphthene

Acenaphthene (1,2-dihydroacenaphthylene) is one of the simplest polycyclic aromatic hydrocarbon (PAH) having the empirical formula $C_{12}H_{10}$. It is an ethylene-bridged, three-ring unsaturated hydrocarbon derived from naphthalene. The physicochemical properties and some relevant information of acenaphthene are summarized in Table 1.

Table 1. Physicochemical properties and some relevant information of acenaphthene

Molecular Formula	$C_{12}H_{10}$
CAS Number	83-32-9
Molar Mass	154.212 g·mol^{-1}
Appearance	White or pale yellow crystalline powder
Density	1.024 g/cm^3
Melting Point	93.4°C
Boiling Point	279°C
Solubility in water	0.4 mg/100 ml
Vapor Pressure	2.5 × 10 −3 (mm Hg at 25°C)
Flash Point	135°C
Autoignition temperature	> 450°C
Log Octanol/water partition coefficient	3.92[a]
TEF[b]	0.001
IARC[c]	3
EPA[d]	D
Estimated half-lives (days)[e]	5.66
Measured half-lives (days)[f]	n.d.

[a] [29]; [b] Toxic equivalent factor relatively to Benzo[a]pyrene [30]; [c] 1, carcinogenic to humans; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, not classifiable as carcinogenic to humans; n.c., not classified [31]; [d] EPA carcinogenic classification: A, human carcinogenic; B1 and B2: probable human carcinogenic; C, possible human carcinogenic; D, not Classifiable as to human carcinogenicity; E, evidence of non-carcinogenicity for humans; [e] [32]; [f] n.d., not determined [33].

2.2. Acenaphthene in the Environment

Acenaphthene is a normal constituent of basic oil and coal-tar and is mainly dispersed in the ambiance by coal and oil burning activities, natural fires, ignition of woods, and discharge from diverse industries [34,35]. Release from petroleum refineries and coal tar distillation industries are the chief contributors of acenaphthene in the atmosphere. High-temperature coal tars contain on average 0.3% of acenaphthene. Acenaphthene is one of the few PAHs, along with naphthalene, acenaphthylene, and anthracene produced commercially at present. Acenaphthene is widely used in diverse industries for the production of numerous dyes, soaps, pharmaceuticals, insecticides, fungicides and plastics [35]. It is used on a large scale to prepare naphthalene dicarboxylic anhydride, which is a precursor to dyes and optical brighteners [36]. Acenaphthene has been found in gasoline and diesel exhaust, cigarette smoke, in the exhaust from automobiles and in wood preservatives. Since acenaphthene is used in the manufacture of different commodities used by human beings, the release of acenaphthene into the atmosphere also occurs via manufacturing effluents, disposal of manufacturing waste by-products, municipal wastewater management facilities and municipal waste incinerators. The general population may be exposed to acenaphthene by the motor exhaust, smoking cigarettes and burning oil, coal or wood. The common people may also be exposed to acenaphthene during eating grilled and smoked meat. If acenaphthene is released to the environment, it will be broken down in the air. Acenaphthene released to air will also be in or on particles that eventually fall to the ground. It is expected to be broken down by sunlight. It will move into the air from moist soil and water surfaces but may adsorb strongly to soil and particles in water. It is expected to move slowly through some soils.

2.3. Acenaphthene Adverse Effects

Despite the fact that acenaphthene has not been identified as carcinogen it has several adverse effects. Acenaphthene has an acute as well as chronic health effect on human beings. Acenaphthene can affect us during breathing or when it is bypassing through our skin. It can irritate the skin, mucous membranes, and eyes. Breathing acenaphthene can irritate the nose and throat, which causes wheezing and coughing. Acenaphthene can irritate the lungs, and repeated exposure may cause bronchitis to develop with cough, phlegm and/or shortness of breath. Acenaphthene may also affect the liver and kidneys [37]. The substance is very toxic to aquatic organisms and may cause long-term effects in the aquatic environment. Liver toxicity was observed in laboratory animals fed high doses of acenaphthene over time. The International Agency for Research on Cancer has determined that acenaphthene is not classifiable as to its carcinogenicity to humans due to lack of human data and inadequate studies in laboratory animals.

Table 2. List of bacterial strains involved in the degradation of Acenaphthene

Bacterial Species	Strain	Acenaphthene Metabolism	References
Acinetobacter sp.	AGAT-W	Sole carbon source	[38]
Aeromonas hydrophila	AH	Sole carbon source [a]	[51]
Alcaligenes eutrophus	-	Sole carbon source	[39]
Alcaligenes paradoxus	-	Sole carbon source	[39]
Bacillus megaterium	DB	Sole carbon source [a]	[51]
Bacillus sp.	PD5	Sole carbon source	[50]
Halomonas sp.	PD4	Sole carbon source	[50]
Bacillus sp.	-	Sole carbon source	[52]
Beijerinckia sp.	B8/36	Co metabolism [b]	[34]
Beijerinckia sp.	-	Co metabolism [b]	[34]
Burkholderia cepacia	F297	Biotransformation [c]	[48]
Corynebacterium sp.	-	Sole carbon source	[52]
Cycloclasticus pugetii	PS-1	Sole carbon source	[40]
Micrococcus Luteus	-	Sole carbon source	[52]
Neptunomonas naphthovorans	NAG-2N-113	Co metabolism [d]	[53]
Neptunomonas naphthovorans	NAG-2N-126	Co metabolism [d]	[53]
Pseudomonas aeruginosa	PAO1	Biotransformation	[47]
Pseudomonas sp.	-	Sole carbon source	[52]
Pseudomonas sp.	-	Sole carbon source [e]	[41]
Pseudomonas sp.	A4	Sole carbon source	[43]
Pseudomonas sp.	KR3	Sole carbon source	[42]
Pseudomonas sp.	BC	Co metabolism [f]	[49]
Pseudomonas sp.	BR	Co metabolism [f]	[49]
Pseudomonas sp.	A2279	Sole carbon source	[49]
Raoultella ornithinolytica	DR	Sole carbon source [a]	[51]
Serratia marcescens	SE	Sole carbon source [a]	[51]
Sphingobacterium sp.	RTSB	Sole carbon source	[43]
Sphingomonas aromaticivorans	B0522	Sole carbon source [g]	[44]
Sphingomonas aromaticivorans	B0695	Sole carbon source [g]	[44]
Sphingomonas sp.	A4	Sole carbon source	[45]
Sphingomonas stygia	-	Sole carbon source [g]	[44]
Staphylococcus epidermidis	-	Sole carbon source	[52]

[a] In presence of bulking agents, biosurfactants or any other form of biostimulants; [b] In presence of succinic acid as growth substrate; [c] Fluorene-induced cells; [d] On incubation with a mixture of other seven PAHs; [e] Naphthalene grown cell; [f] In presence of naphthalene; [g] In presence of Tween 80.

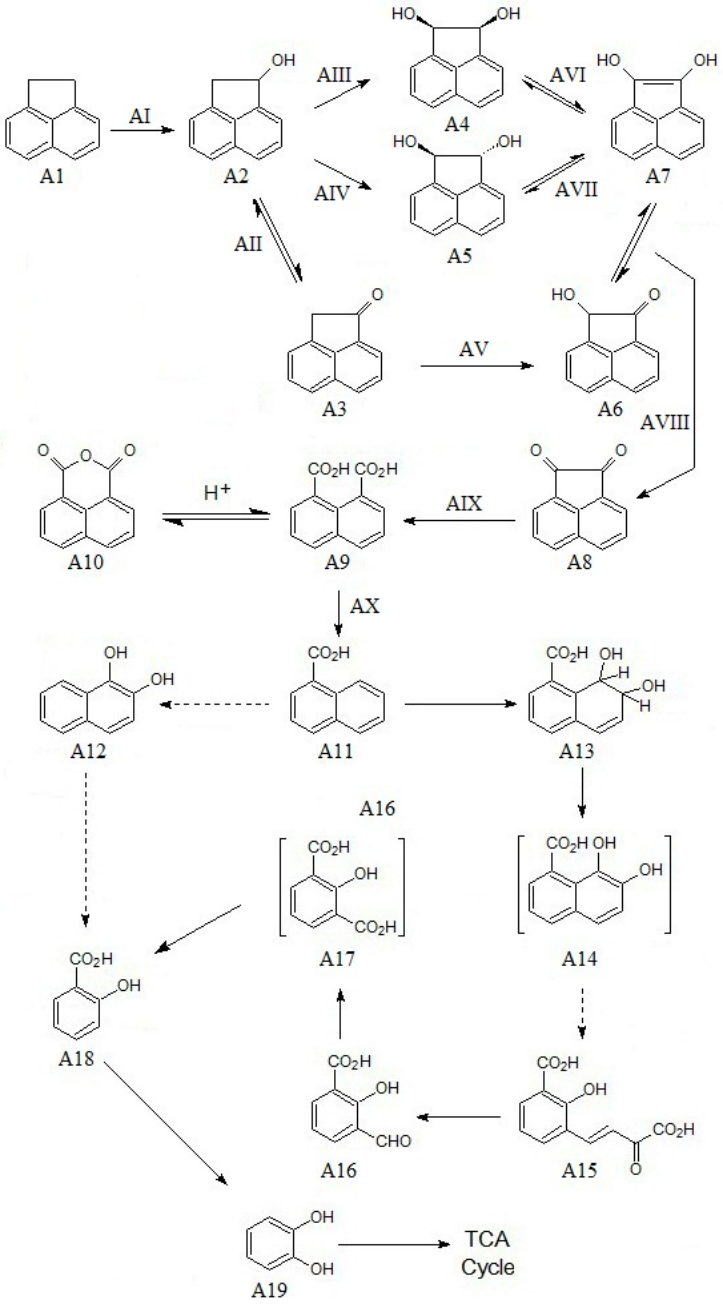

Figure 2. Catabolic pathways in the bacterial degradation of acenaphthene. Chemical designations: Acenaphthene (A1), 1-acenaphthenol (A2), 1-acenaphthenone (A3), *cis*-1,2-acenaphthenediol (A4), *trans*-1,2-acenaphthenediol (A5), 1-hydroxy-2-ketoacenaphthene (A6), 1,2-dihydroxyacenaphthylene (A7), acenaphthenequinone (A8), naphthalene-1,8-dicarboxylic acid (A9), 1,8-naphthalic anhydride (A10), 1-naphthoic acid (A11), 1,2-dihydroxynaphthalene (A12), 1,2-dihydroxy-l,2-dihydro-8-carboxynaphthalene (A13), 1,2-dihydroxy-8-carboxynaphthalene (A14), *trans*-3-carboxy-2-hydroxybenzylidenepyruvic acid (A15), 3-formyl salicylic acid (A16), 2-hydroxy isophthalic acid (A17), salicylic acid (A18), catechol (A19). Enzyme designations: Acenaphthene monooxygenase (AI), 1-acenaphthenol dehydrogenase (AII), 1-acenaphthenol monooxygenase (AIII, AIV), 1-acenaphthenone monooxygenase (AV), 1,2-dihydroxyacenaphthylene dehydrogenase (AVI, AVII), 1-hydroxy-2-ketoacenaphthene dehydrogenase (AVIII), acenaphthenequinone dioxygenase (AIX), naphthalene-1,8-dicarboxylate decarboxylase (AX)

2.4. Biodegradation of Acenaphthene

Over the last two decades, biodegradation of acenaphthene has received considerable attention to the environmental scientists and a number of bacterial species have been isolated capable of degrading acenaphthene as the sole growth substrate. Different bacterial species such as *Acinetobacter* sp., *Alcaligenes* sp., *Beijerinckia* sp., *Cycloclasticus* sp., *Pseudomonas* sp., *Sphingobacterium* sp., *Sphingomonas* sp., etc. [34,38-46] were reported to degrade acenaphthene in recent years. Table 2 represents the well-studied bacterial species involved in the acenaphthene degradation either solely or in a co-metabolic way. There are different reports of assimilation of acenaphthene by a single isolate capable of degrading acenaphthene as the only carbon source [38,39,43,44,46] along with co-metabolism studies [34,41,47], and several metabolic pathways are available depending upon the bacterial species under investigation. The detailed scheme depicting the diverse pathways involved in the assimilation of acenaphthene by different bacterial species is documented in Figure 2.

In general, the metabolic pathway is initiated by monooxygenation at 1-position of acenaphthene (A1) by

the enzyme acenaphthene monooxygenase (AI) to form 1-acenaphthenol (A2). Then 1-acenaphthenol is converted to 1-acenaphthenone (A3) by 1-acenaphthenol dehydrogenase (AII) as well as to *cis*- and *trans*-1,2-acenaphthenediols (A4 and A5) in the presence of 1-acenaphthenol monooxygenase (AIII, AIV). After that there occurs the second hydroxylation of 1-acenaphthenone by 1-acenaphthenone monooxygenase (AV) to form 1-hydroxy-2-ketoacenaphthene (A6) followed by tautomerisation to yield 1,2-dihydroxyacenaphthylene (A7). Formation of 1,2-dihydroxyacenaphthylene in some cases may also occur via dehydrogenation of *cis*- and *trans*-1,2-acenaphthenediols in presence of 1,2-dihydroxyacenaphthylene dehydrogenase (AVI, AVII). Subsequent dehydrogenation of 1,2-dihydroxyacenaphthylene then yields acenaphthenequinone (A8). Nonspecific dehydrogenase activities present in some strain also lead to the conversion of either *cis*- or *trans*-1,2-acenaphthenediol to acenaphthenequinone. Acenaphthenequinone is supposed to be metabolized further via the formation of naphthalene-1,8-dicarboxylic acid (A9), where there occurs ring cleavage of acenaphthenequinone in presence of the enzyme acenaphthenequinone dioxygenase (AIX). In some cases, 1,8-naphthalic anhydride (A10) is reported to be formed under the acidic condition of extraction and was found among the products of biotransformations of acenaphthene. Subsequently, naphthalene-1,8-dicarboxylic acid gets decarboxylated to furnish 1-naphthoic acid (A11), which is either metabolized via salicylic acid (A18) to catechol (A19) through 1,2-dihydroxynaphthalene (A12) following naphthalene degradation pathway; or there occurs ring cleavage of 1-naphthoic acid to form *trans*-3-carboxy-2-hydroxybenzylidenepyruvic acid (A15), which is further processed via 3-formyl salicylic acid (A16), salicylic acid and catechol ultimately leading to TCA cycle intermediates.

Acenaphthene metabolism was reported earlier by a naphthalene-grown *Pseudomonas* sp., where acenaphthene was transformed into 1-acenaphthenol and 1-acenaphthenone [41]. Later cometabolism study of acenaphthene was carried out by Schocken and Gibson [34], where *Beijerinckia* sp. as well as its mutant strain *Beijerinckia* sp. B8/36 was found to metabolize acenaphthene through 1-acenaphthenol, 1-acenaphthenone, 1,2-acenaphthenediol, acenaphthenequinone and 1,2-dihydroxyacenaphthylene when grown in succinic acid. Oxidation of acenaphthene by recombinant strain *Pseudomonas aeruginosa* PAO1 was observed by Selifonov *et al.* [47], in which case 1-acenaphthenone as well as *cis*- and *trans*-1,2-acenaphthenediols were noticed to form from acenaphthene and subsequently converted to naphthalene-1,8-dicarboxylic acid via acenaphthenequinone. *Sphingomonas* sp. strain A4, previously recognized as *Pseudomonas* sp. strain A4, was reported to degrade acenaphthene as the sole growth substrate; and the assimilation took place through 1-acenaphthenol and 1-acenaphthenone [45]. It had been recommended that the organism has novel degradative enzyme coordination competent of cleaving acenaphthene, even though the whole degradation pathway had not been examined in this study. Ghosal *et al.* [38] narrated the assimilation of acenaphthene by *Acinetobacter* sp. strain AGAT-W, where the strain converted acenaphthene to 1-acenaphthenol and further degraded to catechol via acenaphthenequinone, naphthalene-1,8-dicarboxylic acid, 1-naphthoic acid, and salicylic acid. This was the first report on the complete degradation of acenaphthene individually by strain AGAT-W belonging to the genus *Acinetobacter*. However, this study did not highlight how 1-naphthoic acid was transformed into salicylic acid. *Sphingomonas aromaticivorans* strain B0695 and B0522, and *Sphingomonas stygia* had also been described to degrade acenaphthene as the sole carbon source but no detailed degradation pathway was mentioned [44]. *Burkholderia cepacia* F297 was reported not to grow on acenaphthene, but when incubated with the fluorene grown washed-cells of the organism, it transforms acenaphthene to 1-acenaphthenone, acenaphthenequinone and naphthalene-1,8-dicarboxylic acid [48]. Recently, Mallick [43] described a detailed acenaphthene degradation pathway, in which the *Sphingobacterium* sp. strain RTSB was found to assimilate acenaphthene via 1-acenaphthenol, acenaphthenequinone and naphthalene-1,8-dicarboxylic acid in the upper pathway of degradation. In the lower lane, 1-naphthoic acid was further processed through the formation of a novel metabolite *trans*-3-carboxy-2-hydroxybenzylidenepyruvic acid, and then to salicylic acid and catechol entering into the TCA cycle intermediates. Besides, Selifonov *et al.* [49] reported acenaphthene oxidation by *Pseudomonas* sp. strain BC and BR when naphthalene was added as a cosubstrate. They showed the presence of diverse metabolites such as 1-acenaphthenol, 1-acenaphthenone, *cis*- and *trans*-1,2-acenaphthenediols and 1,8-naphthalic anhydride with the help of [1-^{13}C]acenaphthene. Acenaphthene degradation by *Pseudomonas* sp. strain A2279 able to utilize acenaphthene as the sole carbon and energy source was also reported via naphthalene-1,8-dicarboxylic acid and 2-hydroxybenzene-1,3-dicarboxylic acid [49]. Besides these, a variety of microorganisms like *Alcaligenes* spp. [39], *Pseudomonas* sp. KR3 [42], *Bacillus* sp. PD5, *Halomonas* sp. PD4 [50], *Raoultella ornithinolytica*, *Serratia marcescens*, *Bacillus megaterium*, *Aeromonas hydrophila* [51], *Bacillus sp.*, *Corynebacterium sp.*, *Micrococcus Luteus*, *Staphylococcus epidermidis [52]*, *Neptunomonas naphthovorans* [53] were also reported to assimilate acenaphthene, but these studies did not reveal any detailed degradation pathway. Microbial degradation of acenaphthene under denitrification conditions was also identified in soil-water systems by denitrifying organisms [54].

In contrast to bacterial degradation, information about the fungal metabolism of acenaphthene is limited. The fungal metabolism of acenaphthene was similar to bacterial and mammalian metabolism since the primary site of the enzymatic attack was on the two carbons of the five-member ring. The filamentous fungus *Cunninghamella elegans* ATCC 36112 was observed to metabolize 64% of the acenaphthene within 72 h of incubation. *Cunninghamella elegans* metabolized acenaphthene to 6-hydroxyacenaphthenone (24.8%), 1,2-acenaphthenedione (19.9%), *trans*-1,2-dihydroxyacenaphthene (10.3%), 1,5-dihydroxyacenaphthene (2.7%), 1-acenaphthenol (2.4%), 1-acenaphthenone (2.1%), and *cis*-1,2-dihydroxyacenaphthene (1.8%) [55].

The non-lignolytic filamentous fungus *Penicillium* sp. CHY-2 was reported to degrade acenaphthene at low concentration (100 mg l^{-1}) when 10.0% of acenaphthene was degraded; however, at high concentrations (500 mg l^{-1}) acenaphthene was not degraded by the strain [56]. Laccase of *Trametes versicolor* in combination with 1-hydroxybenzotriazole was reported to metabolize acenaphthene totally after 70 h incubation, where the major products detected were 1,2-acenaphthenedione and 1,8-naphthalic anhydride. However, only 3% of the acenaphthene was oxidised by the laccase alone [57]. Acenaphthene was also reported to oxidise to several mono- and dioxygenated products by human P450s. When incubation was done in a standard reaction mixture with human P450s 2A6, 2A13, 1B1, 1A2, 2C9, and 3A4, 1-acenaphthenol was obtained as the major product in acenaphthene oxidation [58].

3. Conclusion

In the present study, the occurrence and environmental significance of polycyclic aromatic hydrocarbon molecule acenaphthene and the biodegradation of the said molecule by various microorganisms is discussed. The diversity of pathways of degradation of acenaphthene molecule by bacterial species so outlined in the present article is more informative and detailed in the context of the present state of the knowledge in the field. It appears that the diverse metabolic pathways of the degradation are generally varies depending upon the bacterial species under study. In spite of the limited reports of fungal metabolism, several acenaphthene metabolites found indicates metabolic diversity in the acenaphthene degradation by fungi. Moreover, a thorough analysis of pathways, based on the structure of metabolites, indicates possible involvements of some unusual catabolic enzymes that remain to be characterized. Nevertheless, microbial degradation reduces the risk of such chemicals although further research in this direction is needed to assess the biodegradation potential and the environmental impact.

Statement of Competing Interests

The author has no competing interests.

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