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Microbes as a potential bioremediation tool for atrazine-contaminated soil: A review

Chiranjib Mili[1]*, Sanjib Kalita[2], Subham Roy[3]

[1]Department of Botany, B. P. Chaliha College, Kamrup, Assam, India.
[2]Department of Botany, Gauhati University, Guwahati, Assam, India.
[3]Department of Botany, Rangapara College, Rangapara, Assam, India.

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ABSTRACT

Atrazine is a controversial and widely used herbicide to control weeds in both agriculture fields and residential sites. Instead of adopting manual weed control, atrazine is being used by people who resulted in a negative impact on the environment. Therefore, removing atrazine in soil has received considerable attention. Microorganisms have terrific potential for degradation of hazardous pollutants which always motivates continuous bioremediation-directed research. The objective of this review is to identify, analyze, and compile all the studies on atrazine–degrading microorganisms. Particular emphasis is made on the atrazine degradation pathways, a diverse group of bacteria, fungi, and yeast along with the genetics and enzymology aspects of degradation. The present review may act as a source of information for developing a cheaper and microbiological method for rescuing the atrazine-contaminated soil and water in the future.

1. INTRODUCTION

The widespread and long-term use of chemicals including atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) herbicide in both agriculture and non-agricultural field is still a severe concern today. These compounds have the potential to runoff and leach through the soil leading to surface and groundwater contamination [1]. Most attentively, atrazine can cause serious human health problems such as endocrine disruption, central nervous system, reproductive system, immune system, and carcinogenic disorders [2]. Atrazine inhibits photosynthesis efficiency, superfluous energy dissipation in electron transport, and destroys cellular structure which resulted in the inhibition of growth in algae [3]. Moreover, atrazine has a moderately persistent, long half-life, and high mobility in soil than some other herbicides. Due to its high toxicity, persistence, and mobility in the environment, atrazine was prohibited by the European Union in 2004 [4], but it is still one of the most extensively used herbicides against weeds today in several countries, for example, annually 23 million kg in the USA [5], 27 million kg in Brazil, 16 million kg in Argentina [6], and 3 million kg in India [1]. Therefore, for a safe and sound environment, the rapid abolition of atrazine from the contaminated site has become very crucial.

*Corresponding Author:
Chiranjib Mili,
B. P. Chaliha College, Kamrup, Assam, India.
E-mail: milichiranjib93 @ gmail.com

Microorganisms have tremendous potential for bioremediation and herbicide degradation due to the presence of various catalytic enzymes [7]. The presence of such characteristics, microorganisms can degrade atrazine into different metabolites that act as a source of energy for other organisms. Many strains have been reported for their abilities in atrazine mineralization including members of the genera *Pseudomonas*, *Bacillus*, *Burkholderia, Arthrobacter*, *Enterobacter,* and *Norcardioides* [8-11]. In addition, several fungal species belonging to the genera *Fusarium*, *Aspergillus*, *Penicillium*, and *Pleurotus* have also been isolated and studied for degradation of atrazine [2,12,13]. Therefore, microorganisms can be chosen for easy and better strategies for the rescue of atrazine polluted sites ecofriendly.

In recent years, several review papers have been published on the degradation of atrazine in different aspects such as the impact of atrazine in the aquatic environment, technologies used to reduce the toxicity of atrazine as well as advantage and disadvantages [14,15]. In 2021, a similar review was published by Abd Rani *et al*. [16] that focused on only bacteria while fungi and yeast are neglected. In contrast, this review is a humble attempt to accumulate all the microbes associated with atrazine degradation in a single article that has already been gathered through vigorous research. This article also presents the clear degradation pathways along with the genes and enzymes involved in atrazine-degradation. This review will help researchers to develop a cost-effective and efficient microbiological technology for the remediation of atrazine-contaminated soil.

2. DEGRADATION OF ATRAZINE

2.1. Pathways of Atrazine-degradation

Degradation pathways of atrazine occur through three major different pathways that channel into cyanuric acid metabolism [1]. The degradation pathway is generally initiated by two enzymes, that is, atrazine chlorohydrolase and atrazine monooxygenase. The first pathway is initiated by the enzyme atrazine chlorohydrolase catalyzes the hydrolytic dechlorination of atrazine and produces hydroxyatrazine (HA) which is further converted into N-Isopropylammelide by the activity of atrazine ethylaminohydrolase and finally into Cyanuric acid later on by N-isopropylammelide isopropylaminohydrolase [17].

The second and third pathways are beginning with atrazine monooxygenase activity that degrades the atrazine into Deisopropylatrazine and Deethylatrazine, respectively [18]. In the second pathway, the enzyme s-triazine hydrolase transforms the Deisopropylatrazine into deisopropylhydroxyatrazine which is then converted into 2,4-Dihydroxy-6-(N'-ethyl)amino-1,3,5-triazine by 2,4-Dihydroxy-6-(N'- ethyl)amino-1,3,5-triazine aminohydrolase. Later, ethylaminohydrolase convert it into Cyanuric acid. In the third pathway, Deethylatrazine is transformed into deisopropyldeethylatrazine by deethylatrazine monooxygenase which is then converted into cyanuric acid through several steps [18] [Figure 1].

2.2. Atrazine-degrading Microorganisms

A large range of microorganisms involved in the degradation of atrazine leads to the production of metabolites while some other microorganisms derive their nutrients and energy by mineralizing them completely into CO_2 and NH_4^+ [19]. Atrazine degrading

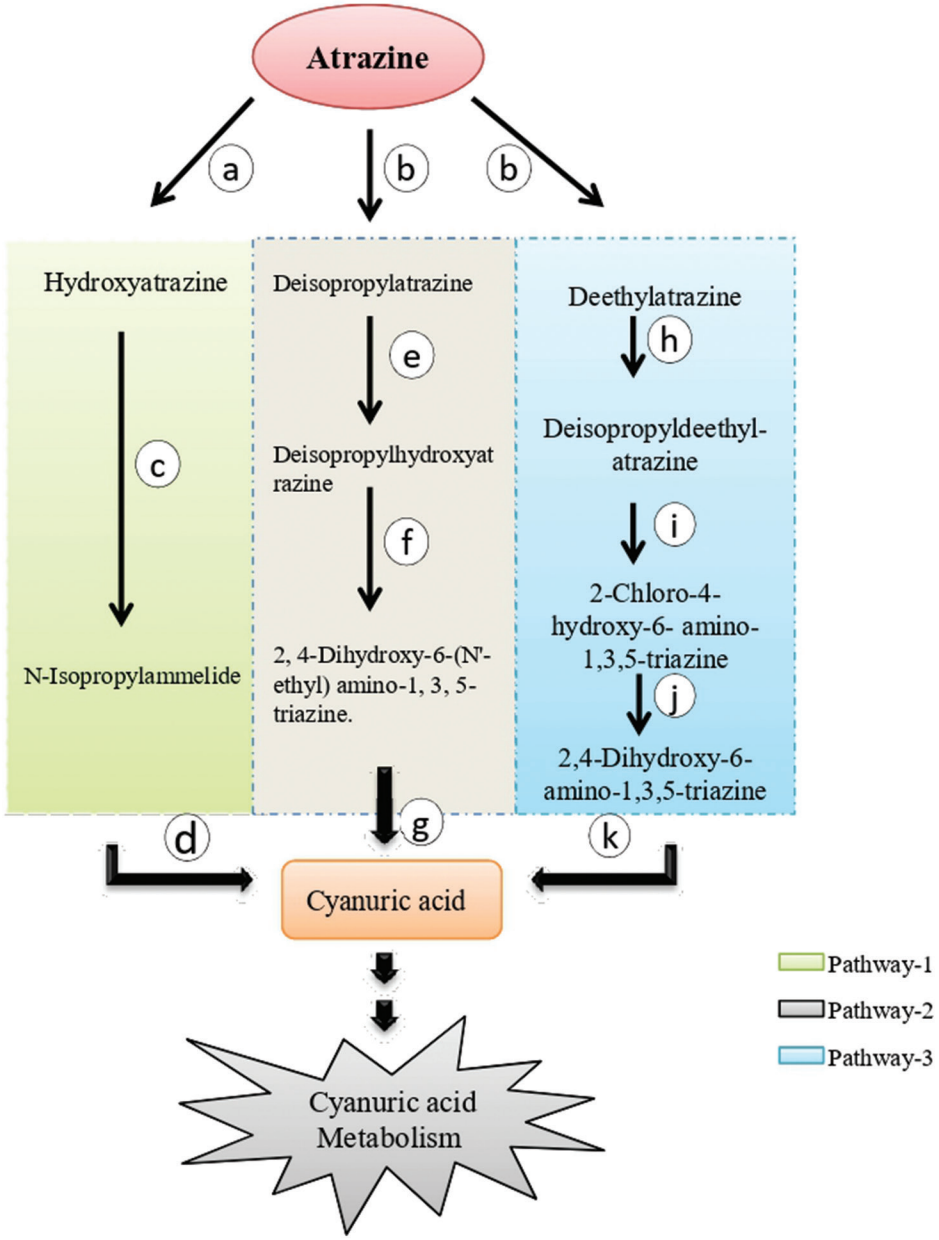

Figure 1: Atrazine-degradation pathways. a=atrazine chlorohydrolase, b=atrazine monooxygenase, c=Hydroxydechloatrazine ethylaminohydrolase, d=*N*-isopropylammelide isopropylamido hydrolase, e=s-triazine hydrolase, f=2,4-Dihydroxy-6-(N'-ethyl)amino-1,3,5-triazine hydrolase, g=ethylaminohydrolase, h=deethylatrazine monooxygenase, i=s-triazine hydrolase, j=hydroxychloroatrazine ethyaminohydrolase, k=N-isopropylammelide isopropylaminohydrolase.

microorganisms are not limited to only bacteria and fungi, but many microalgae; for example, *Chlamydomonas mexicana*, *Chlorella* sp., and *Selenastrum capricornutum* have also been reported by several researchers [20,21].

2.2.1. Bacteria

Bacteria are the most widely reported microorganism for atrazine elimination from polluted sites [22]. As the potential machines for bioremediation, a large variety of strains of Gram-positive and Gram-negative bacteria that degrade atrazine have been isolated and identified. Atrazine degrading bacteria produce various catalytic enzymes that break down atrazine (i.e., atrazine chlorohydrolase, allophanate amidohydrolase, HA hydrolase, N-isopropylammelide amidohydrolase, triazine hydrolase, 1-carboxybiuret amidohydrolase, and cyanuric acid amidohydrolase) and enhance the metabolic mechanisms. They decrease the degradation half-life of atrazine by the different metabolic processes including dechlorination, dealkylation, hydroxylation, and ring cleavage [23]. The atrazine-degrading strains, for example, *Pseudomonas* strain ADP break down atrazine into cyanuric acid through three enzymatic steps, and cyanuric acid acts as a source of nitrogen for many other bacteria [24]. Moreover, some other bacteria belonging to the genera *Rhodococcus*, *Acinetobacter*, *Streptomyces*, *Pseudomonas*, *Clavibacter* [25], *Arthrobacter* [26], *Bacillus*, *Alcaligenes*, *Klebsiella*, and *Agrobacterium* [27], transform atrazine into cyanuric acid which further metabolized and produce carbon and nitrogen source compounds. However, in the past 10 years, among the most isolated atrazine-degrading bacteria only *Arthrobacter* sp., *Pseudomonas* sp., and *Bacillus* sp. are reported as capable of fully degrading atrazine into carbon dioxide and ammonia [16]. In a study, two atrazine degrading bacteria such as *Bacillus lichenoformis* and *Bacillus megaterium* were isolated from soil that showed 98.6% and 99.6% degradation efficiency of atrazine after 7 days [7]. At the same time, the degradation of atrazine was faster when two strains were used in combination under the same conditions. Based on the previous data, some of the bacteria and their producing metabolites are listed in Table 1.

2.2.2. Fungi

Fungi are another main element of soil microflora involved in atrazine degradation after bacteria. They degrade atrazine at different rates and produce different metabolites through N-dealkylation of either alkylamino group [28]. The application of fungi may be the most important way to remove atrazine from contaminated soil. They are very effective in bioremediation as they can use different carbon sources for metabolism by producing different enzymes which catabolize different steps during the transformation of chemicals [29].

Among fungi, wood-degrading basidiomycetes are also a key player in atrazine degradation. The ability of chemical degradation of white-

Table 1: List of some Atrazine-degrading bacteria.

Bacteria	Gram status	GenBank accession no.	Detected metabolites	References
Bacillus licheniformis ATLJ-5 *Bacillus megaterium* ATLJ-11	+ +	MH879786 MH879805	Hydroxyatrazine and N-isopropylammelide	[7]
Pseudaminobacter sp. *Nocardioides* sp.	- +	nd	Hydroxyatrazine and N-ethylammelide	[51]
Bacillus atrophaeus	+	MH685187	nd	[43]
Paenarthrobacter sp. W11	+	nd	nd	[52]
Arthrobacter sp.C2	+	MF405158	Hydroxyatrazine, N-isopropylammelide, cyanuric acid, and deisopropylhydroxyatrazine (DIHA)	[26]
Klebsiella variicola FH-1.	-	nd	nd	[53]
Pseudomonas spp.strains ACB and TLB	-	nd	nd	[10]
Variovorax sp.strain 38R *Arthrobacter* sp.strain TES *Chelatobacter* sp.strain SR38	- + -	CP062121 CP062235 CP062112	nd	[11]
Myriophyllum spicatum *Acetobacter* sp.	- -	nd	Hydroxyatrazine (HA), deelthylatrazine (DEA), didealkylatrazine (DDA), cyanuric acid (CYA), and biuret	[22]
Arthrobacter sp.strain HB-5	+	nd	nd	[23]
Ensifer sp.	-	nd	N-isopropylammelide, Cyanuric acid (CA)	[54]
Nocardioides *Arthrobacter,* *Bradyrhizobium,* *Burkholderia,* *Methylobacterium* *Mycobacterium,* *Clostridium.*	+ + - - - + +	nd	N-isopropylammelide (IPA), ammelid, biuret, and cyanuric acid	[55]
Rhodococcus sp. strain MB-P1	+	FN357284	De-ethyl de-isopropyl atrazine, De-isopropyl atrazine, De-ethyl atrazine	[56]
Citricoccus sp. strain TT3.	+	nd	nd	[57]
Klebsiella variicola Strain FH-1	-	MH250202	2-hydroxyl-4-ethylamino-6-isopropylamino-1,3,5-triazine (HEIT) 2-hydroxyl-4,6-bis (ethylamino)-1,3,5-triazine (MEET), and 4,6-bis (ethylamino)-1,3,5-triazin-2 (1H)-one (AEEO)	[58]

In the table, nd=no data obtained in the cited reference.

rot fungi is due to the existence of the ligninolytic system [30]. Fungi belonging to basidiomycetes and ascomycetes produce both extracellular and intracellular enzymes that biotechnologically and industrially valued molecules are responsible for herbicides and pesticide degradation [31]. The purpose of white-rot fungi in atrazine degradation may be advantageous because they can tolerate a wide range of environmental circumstances, including varying temperature, moisture, and nutrient contents. For example, *Trametes versicolor* belonging to basidiomycetes can actively grow and degrade atrazine in nonsterile soil under low water availability conditions [32]. A well-known white-rot fungus *Phanerochaete chrysosporium* has been reported to degrade a large variety of environmentally persistent chemicals.

The potential roles of mycorrhizal fungi in the degradation of atrazine have been addressed by several authors. Axenic cultures of ectomycorrhizae fungi can degrade atrazine, and degradation was increased when they were full-fledged in symbiosis with plants [33]. Besides, ericoid mycorrhizal fungi have also been reported to degrade atrazine when they are axenically cultured [34]. Moreover, arbuscular mycorrhiza fungi have remarkable potential for atrazine degradation. They enhance soil microbial activity and increase the activities of soil enzymes [28]. *Glomus caledonium* and *G. etunicatum* can accumulate in fugal hyphae or the associated roots and atrazine dissipation in the near rhizosphere and bulk soils [35]. Some of the fungi associated with atrazine degradation are listed in Table 2.

2.2.3. Yeast

Apart from bacteria and filamentous fungi, yeast also has atrazine-degrading potential. A novel yeast strain *Pichia kudriavzevii* strain Atz-EN-01 was isolated from atrazine-contaminated soil which showed the efficient degradation in liquid culture media and soil [36]. This strain breaks atrazine down into three intermediates such as HA, N-isopropylammelide, and cyanuric acid. Another species of *Pichia* has (*Pichia pastoris* strain X-33) also been reported as the ability to transform atrazine into hydroxylisopropylatrazine, atraton (2-methoxy-4-ethylamino-6-isopropylamino-1,3,5-s-triazine), demethylated atrazine, HA [37], Hydroxy-dehydrogenated atrazine, and 2-OH-isopropyl-IPU [38]. Moreover, a yeast species called *Cryptococcus laurentii* was isolated from atrazine-contaminated agricultural soil and GC-MS analysis showed several metabolites such as HA, deethylatrazine, deisopropylatrazine, and deethyldeisopropylatrazin during atrazine degradation when conducting an *in vitro* experiment [39]. The role of *Saccharomyces cerevisiae* in atrazine degradation was also reported by Zhu *et al*. [40]. However, in recent times, most of the research is focused on bacteria and filamentous fungi while little information is available on the role of yeast in atrazine degradation.

2.3. Genes Involved in Atrazine-degradation

Atrazine a commonly known herbicide is used as a carbon and nitrogen source by different soil microflora by breaking it into CO_2 and NH_4^+ [41]. The degradation and utilization of atrazine by microflora are possible because of the complex catabolic pathway mediated by a diverse array of enzymes encode by a series of genes [16]. Different genes are involved in different steps throughout the degradation pathways that lead to the transformation of atrazine to its intermediate cyanuric acid [42]. To the best of our knowledge, the total number of eight genes involved in the atrazine metabolic pathway has been reported such as *atzA*, *atzB*, *atzC*, *atzD*, *atzE*, *atzF*, *trzN*, and *trzD*. The genes *atzABC* identified from *Pseudomonas* sp. strain ADP that homology to five atrazine-degrading microbial

Table 2: List of some atrazine-degrading fungi.

Fungi	Division	GeneBank accession no.	Metabolite produced	References
Anthracophyllum discolor	Basidiomycota	nd	nd	[59]
Glomus caledonium	Glomeromycota	nd	Deethylatrazine (DEA) (2-amino-4-chloro-6-isopropylanine-striazine) and deisopropylatrazine (DIA) (2-amino-4-chloro6-ethylamino-s-triazine)	[35]
Trametes versicolor	Basidiomycota	nd	nd	[32]
Fusarium sp. CCLM_DF *Fusarium sp*. CCLM_GU *Fusarium sp*. CCLM_GW *Fusarium sp*. CCLM_IB	Ascomycota	MT062480 MT062481 MT062482 MT062483	Deisopropylatrazine (DIA) and deethylatrazine (DEA)	[2]
Aspergillus niger	Ascomycota	nd	nd	[60]
Pleurotuso streatus INCQS 40310	Basidiomycota	nd	Deisopropylatrazine (DIA) and deethylatrazine (DEA)	[12]
Pluteus cubensis SXS320, *Gloelophyllum striatum* MCA7, and *Agaricales* MCA17	Basidiomycotina	nd	nd	[61]
Bjerkandera adusta	Basidiomycota	EF441742	nd	[62]
Metarhizium robertsii	Ascomycota	nd	2-hydroxy atrazine and desethylatrazine	[63]
Aspergillus niger AN 400	Ascomycota	nd	Deethylatrazine (DEA), deisopropilatrazine (DIA), hydroxyatrazine (HA)	[64]
Penicillium chrysogenum NRRL 807	Ascomycota	nd	nd	[13]
Saccharomyces cerevisiae	Ascomycota	nd	Hydroxyatrazine, deethylatrazine, deisopropylatrazine	[22]
Aspergillus fumigatus *Penicillium citrinum*	Ascomycota	nd	nd	[19]

In the table, nd=no data obtained in the cited reference.

Table 3: Microbial genes involved in atrazine-degradation.

Gene	Enzyme encoded	Step catalyzed	References
atzA	Atrazine chlorohydrolase	Atrazine → Hydroxyatrazine (HA)	[63]
atzB	Hydroxydechloroatrazine ethylaminohydrolase	Hydroxyatrazine (HA) → N-isopropylammelide	[64]
atzC	N-isopropylammelide isopropylamido hydrolase	N-isopropylammelide → Cyanuric acid+isopropylamine	[16]
atzD	Cyanuric acid amidohydrolase	Cyanuric acid → Biuret	[42]
atzE	1-Carboxybiuret hydrolase	Biuret → Allophanic acid	[42]
atzF	Allophanate hydrolase	Allophanic acid → CO_2+NH_4^+	[42]
trzD	Cyanuric acid amidohydrolase	Cyanuric acid → Biuret	[42]
trzN	Atrazine chlorohydrolase	Atrazine → Hydroxyatrazine (HA)	[65]

isolates which gives a piece of strong evidence for the genes are widespread. Some other bacteria such as *Arthrobacter agilis* and *Nocardioides nanhaiensis* are harbored *atzA/trzN* genes that code for atrazine chlorohydrolase that catalyze the dechlorination of atrazine into HA [42]. The same authors stated that *atzD/trzD* was involved in the conversion of cyanuric acid into biuret through ring cleavage by encoding an enzyme called cyanuric acid amidohydrolase. The other genes like *atzB/atzC* are associated with the dealkylation catabolic step while *atzE* and *atzF/trzF* are involved in biuret deamination and hydrolysis of allophanate, respectively. Different genes and their encoded enzymes involved in different steps in atrazine degradation are shown in Table 3.

2.4. Factors Affecting Microbial Degradation of Atrazine

Several factors influence the microbial degradation of atrazine. The microbial population is influenced by both biotic and abiotic factors in the soil and they directly or indirectly affect the rate of degradation of atrazine.

2.4.1. Abiotic factors

Temperature, pH, depth from the soil surface, and oxygen content of the surrounding matrix are the main abiotic factors that can influence atrazine degradation [43]. The mineralization rate of atrazine is slower in anaerobic or denitrifying conditions than in aerobic environments [44]. The water content of soil and temperature has a significant impact on atrazine degradation. The atrazine degradation is directly proportional to the temperature of its surrounding [45]. A study on the half-life of atrazine in clay soil was carried out and the results showed that the average half-life of atrazine degradation was 62 days when the water content of the soil was 20–40%. Nevertheless, when the water content of the soil was decreased by 8%, the half-life was increased up to 338 days [46]. Moreover, the half-life was increased from 44 days to 206 days while the soil temperature was decreased from 30°C to 5°C. In addition, soil layers also have a great significance in a variation of atrazine degradation rate. The occurrence of atrazine in different soil layers also influences the rate of degradation. The rate of atrazine degradation is slower on the subsurface horizon while increasing in soil depth in silty clay loam [47].

2.4.2. Biotic factors

Crops and soil management systems have a significant impact on the rate of herbicide degradation [48]. An experiment was carried out and found that in a site where corn is cultivated for several years and treated with atrazine, the adaptation of atrazine degrading microorganisms was colonized more than where alfalfa was cultivated for 4 years with no application of atrazine [49]. In addition, the role of earthworms is also not the least in atrazine degradation. Two earthworm species such as *Eisenia foetida* and *Amynthas robust* have been reported to enhance the degradation rate of atrazine [50]. However, our literature survey revealed that the effect of biotic factors on the degradation of atrazine has not been extensively studied yet.

3. CONCLUSION AND FUTURE PROSPECTS

The environment is constantly being harmed by the extended use of toxic chemicals. Several hundreds of herbicides including atrazine have been used by farmers and non-farmers to kill weeds in crop fields or resident campuses. Such type of practice has been done for many couples of the year all over the world. However, bioremediation is the soundest and default method to rehabilitate polluted sites with cost efficiency and environmental friendliness by applying microorganisms. Through the discovery of atrazine-degrading soil microorganisms, the disposing of hazardous chemicals has gained credence. In this regard, microbes can tolerate a high range of toxic chemicals and transform them into non-toxic forms by biochemical reactions, more often contaminants serve as a source of energy. Removal of persistent herbicides using microorganisms has received attention as an outstanding option. Microorganisms could be applied in different strategies. Some of the recommendations are cited below:

- The gene editing and application of system biology on different microbial metabolic pathways are very important. The gene-editing tools such as clustered regularly interspaced short palindromic repeats (CRISPR-Cas), transcription activator-like effector nucleases, and zinc-finger nucleases can make it possible to design microbe with a functional gene of interest for degradation of atrazine for improved bioremediation. This will lead to optimizing the existing metabolic pathways toward the increased and efficient microbial remediation of herbicides. Genetic engineering can also open a new door for the degradation of herbicides by enhancing the capability of microorganisms. In this regard, the genetic transfer of degrading potential from one to another microbe can be a tremendous approach toward bioremediation.
- Both bacteria and fungi are the main dominant degraders of atrazine. However, there are not many influential studies that have been carried out on the application of the organisms as consortia. By screening their biocompatibility, consortia can be designed that could be more effective towards bioremediation. More research on the biochemical pathways related to the catabolism of consortia could allocate for more efficient remediation and narrative applications.
- Enzymes are always a major talking point in bioremediation research due to their inherent capability to degrade complex metabolites present in the pollutants. Enzyme technology could be one of

the excellent techniques for the improvement of bioremediation practices. Microorganisms harbor a wide range of catalytic enzymes including chromium reductase, alkane hydroxylases, laccase, carboxylesterases, peroxidases, phytase, haloalkane dehalogenases, phosphotriesterases, and horseradish peroxidase. Using biotechnological approaches enzymes can be formulated from microorganisms for direct application in the rehabilitation of the polluted site. Moreover, with the help of enzyme engineering, the enzyme can be modified to improve its properties such as activity, stress tolerance, temperature, and pH for bioremediation.

4. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

7. ETHICAL APPROVALS

This study does not require any ethical approval.

8. DATA AVAILABILITY

Data will be made available as per the journal policy.

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