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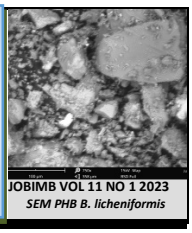

Biodegradation of Atrazine Herbicide: A Mini-review

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ABSTRACT

Atrazine herbicide is known to disrupt the endocrine system and is potentially carcinogenic. The long-term use of this herbicide results in high residue levels in soil, causing water contamination of agricultural land. Microbial degradation of herbicide represents a cost-effective way of eco-restoration compared to the more expensive physicochemical methods, especially in soil settings. Growth and degradation of atrazine by microorganisms are optimal at specific concentrations, temperature, pH, inoculum size and hours of incubation. Previously isolated microorganisms have demonstrated high efficiency for atrazine biodegradation with a broad optimum pH and temperature. The metabolic pathway for biodegradation has been elucidated and reveals important characteristics. These organisms as suitable candidates for bioremediation of atrazine-polluted sites have shown great potential for atrazine degradation. This review aimed to catalogue and update the characteristics of isolated atrazine-degrading microorganisms to date.

INTRODUCTION

The explosive rise of the global population has led to the increased exploitation of natural resources to respond to the high demands of the population for food, energy, and all other requirements. The industrial revolution, a response to these requirements, however, has resulted in the production of a huge number of organic and inorganic chemicals that have directly and indirectly led to the prolonged pollution of habitats [1]. Bioremediation is an increasingly popular alternative to conventional chemical methods for treating waste compounds with the possibility to degrade contaminants since it uses natural microbial activity mediated by different consortia of microbial strains. Many studies on bioremediation have been reported and the scientific literature has revealed the progressive emergence of various advances in bioremediation techniques [2].

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) is a triazine herbicide with the molecular formula $C_8H_{14}ClN_5$ [3]. It is often detected on surface and groundwater due to its long half-life of 13 – 261 days [4]. Atrazine can restrain and remove broadleaf weeds and some grass weeds that affect crop growth, and also inhibit some perennial weeds [3]. It is the most widely used herbicide worldwide because of its low cost and effectiveness, it is used on crops such as sorghum, maize, sugarcane and horticultural and forests as well [5].

Bioremediation as an alternative to conventional physicochemical strategies

Bioremediation techniques consist of natural processes capable of effectively biodegrading a lot of pollutants, including persistent ones. Therefore, they can be a viable and effective way of mitigating soil contamination. The choice of the most appropriate and feasible in-situ or ex-situ biological remediation techniques depends on preliminary analyses of the environmental conditions, type of pollutant, soil composition, removal costs and time available for treatment. However, the characterization of the contaminated site is the main step towards successful bioremediation [6]. Bioremediation as sustainable technology becomes important in analyzing the high release of anthropogenic chemicals into the environment [7]. There were few reports on physical processes as well as chemical processes which are engaged in the removal of atrazine. However, biodegradation is recognized as a suitable technique for the removal of atrazine from the soil. Few soil microorganisms can mineralize atrazine, and other soil microorganisms to form intermediates such as hydroxyatrazine, deethylatrazine, deisopropylatrazine, N-isopropylammilide, N-ethylammilide, and cyanuric acid [8].

To better reflect the usage of bacteria of atrazine, a Scopus keywords search focused on the topics of "biodegradation AND atrazine AND degrading AND bacterium" yielded 213 journal articles.

The VOSViewer software is used to create the keyword co-occurrence of the documents, which is displayed in **Fig. 1**. The study's goal is to conduct a complete bibliometric assessment of the research landscape of atrazine biodegradation by bacteria. Five clusters were discovered with the bacterial genus Pseudomonas followed by Arthrobacter occurring the most often indicating a predominant atrazine degradation within this genus. It appears that in recent years, atrazine biodegradation by the genus Bacillus is more often reported and atrazine in wastewater is more studied than in an agricultural setting (**Fig. 2**).

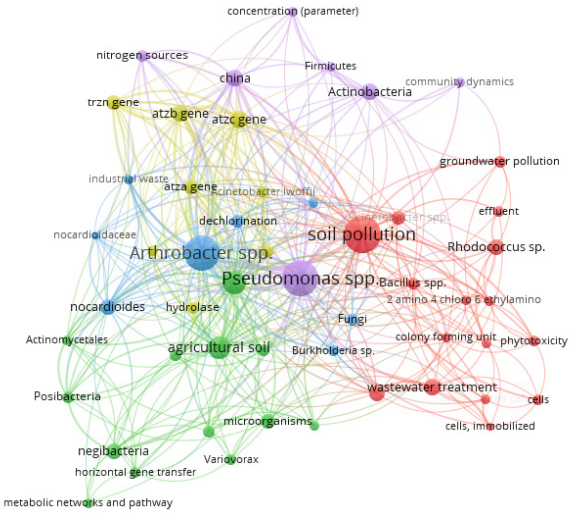

Fig. 1. Density visualization of bibliometric keyword search results with the topics of "biodegradation AND atrazine AND degrading AND bacterium".

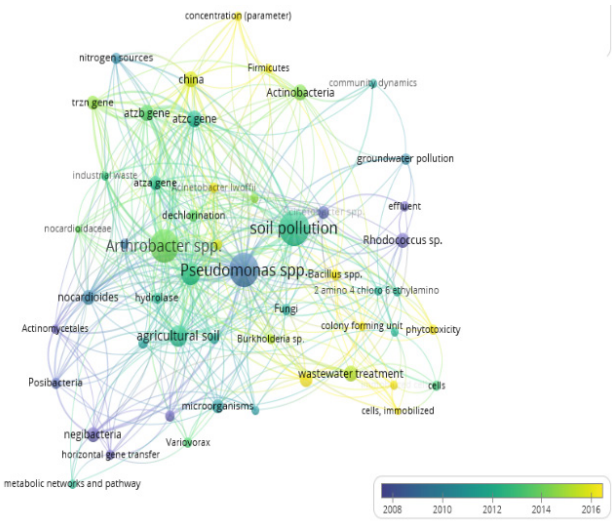

Fig. 2. Overlay visualization of bibliometric keyword search results with the topics of "biodegradation AND atrazine AND degrading AND bacterium".

The term bioremediation is made of two words: "bios" means life and refers to living organisms and "to remediate" means to solve a problem. "Bioremediate" means to use biological organisms to solve an environmental problem such as contaminated soil or groundwater [1]. Bioremediation is the use of living microorganisms to degrade environmental pollutants or prevent pollution. In other words, it is a technology for removing pollutants from the environment thus restoring the original natural surroundings and preventing further pollution [9].

Bioremediation could simply be defined as a biological process of the decontamination of a contaminated environment. The environment may be either terrestrial, aqueous, or both. However, a more comprehensive definition describes bioremediation as a means of cleaning up contaminated environments by exploiting the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (IV) oxide and water, or conversion into microbial biomass [1]. A point to emphasize here is that bioremediation and biodegradation should not be confused with each other. Bioremediation as a technique may include biodegradation as only one of the mechanisms involved or applied in the process of bioremediation [10].

Types of Bioremediation

Based on the place where wastes are removed, there are principally two ways of bioremediation: insitu and exsitu bioremediation.

In-situ Bioremediation

In-situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. It is a superior method for cleaning contaminated environments because it saves transportation costs and uses harmless microorganisms to eliminate chemical contaminations. These microorganisms are better if they present positive chemotactic affinity toward contaminants, so as to increase the probability of bioremediation in close points where bioremediants are not distributed. Also, the method is preferred as it causes the least disruption of the contaminated area. This would be of much relevance either where the least investment and pollution are favored (for example in factories) or in areas contaminated with dangerous contaminants (for example in areas contaminated with chemical or radioactive materials) [11].

Another advantage of in situ bioremediation is the feasibility of synchronous treatment of soil and groundwater. However, in situ bioremediation possess some disadvantages: it is more time consuming compared to other remedial methods, and it leads to a changed seasonal variation in the microbial activity because of the direct exposure to the variations in uncontrollable environmental factors as well as the use of additives may lead to additional problems. The yield of bioremediation is determined by the kind of waste materials, namely if wastes could provide the required nutrients and energy, then microorganisms would be able to bioremediate [12]. Following are some of the in-situ bioremediation techniques: Bioventing, biostimulation, air sparging, bioaugmentation, natural attenuation and phytoremediation.

Ex-situ Bioremediation

Ex-situ bioremediation techniques are those that are applied to soil and groundwater at the site which has been removed via excavation (soil) or pumping (water) and therefore require transportation of contaminated soil or pumping of groundwater to the site of bioremediation. This technique has more disadvantages than advantages [13]. Following are some of the

ex-situ bioremediation techniques: Land farming, Composting, Controlled solid phase treatment and Slurry phase biological treatment.

Factors Affecting Bioremediation

The factors limiting the success of bioremediation include energy sources, bioavailability, bioactivity, pH, temperature, toxicity of compound, water content and geological character, nutrient availability and external electron availability.

Merits and Demerits of Bioremediation

The merits of bioremediation include being effective, economical, eco-friendly, less toxic byproduct generation, on-site treatment possible and does not affect natural flora. However, the demerits include being more effective for readily biodegradable compounds, the requirement for specific environmental conditions, specific microflora and often taking a long time to remove or transform contaminants.

Atrazine as Selective Herbicide for Pre and Post-emergence Weeds

Atrazine [ATR; 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine], is a broad-spectrum chlorotriazine herbicide that was first registered in 1958 by JR Geigy SA (currently known as Syngenta) (**Fig. 3**). It is a nonpolar and nonvolatile [13]. It is a white powdery solid and unstable at high temperature. Its melting point is between 173 °C – 175 °C, with 200 °C boiling point, the solubility in water is 33 mgL^{-1} at 20 °C, and it is easily soluble in organic solvents [14]. For more than half a century, it has been extensively used to control broadleaf weeds, and presently one of the two most widely used pesticides in the US [5]. Due to its widespread use, relative persistence in the water (half-life >6 months), and extreme persistence in the soil (detectable 22 years after application), atrazine has become a ubiquitous environmental contaminant. In places with heavy atrazine use, such as the Midwestern US, surface and drinking water atrazine concentrations (up to 224 and 34 μgL^{-1}, respectively) substantially exceed the current maximum contaminant levels (MCL) for both the US and Europe, which are 3 and 0.1 μgL^{-1}, respectively [3].

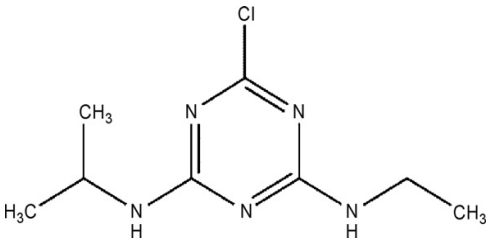

Fig. 3. Molecular Structure of Atrazine [15].

Metabolites of Atrazine

Chloro-s-triazine Metabolites

The toxicity profiles and mode of action of the chloro-s-triazine metabolites are similar to those of atrazine, and the potency of these metabolites concerning their neuroendocrine disrupting properties appeared to be similar to that of the parent compound [3]. Like atrazine, the chloro-s-triazine metabolites are of moderate or low acute oral toxicity in rats, with LD_{50} of 1110, 1240 and 2310 – 5460 mg kg^{-1} body weight for DEA, DIA and DACT, respectively. These chloro-s-triazine metabolites delayed the sexual development of male rats exposed on postnatal days 23 – 53 to atrazine molar equivalent doses of ≥25 mg kg^{-1} body weight per day (DEA, DIA) and ≥12.5 mg kg^{-1} body weight per day (DACT) [16].

Hydroxyatrazine

The metabolite hydroxyatrazine does not have the same mode of action or toxicity profile as atrazine and its chlorometabolites (**Fig. 4**). The main effect of hydroxyatrazine was kidney toxicity (owing to its low solubility in water, resulting in crystal formation and subsequent inflammatory response), and there was no evidence that hydroxyatrazine has neuroendocrine disrupting properties. Also, the acute oral toxicity of hydroxyatrazine in rats (LD_{50}>5050 $mgkg^{-1}$ body weight) was lower than that of atrazine or its chlorometabolites [17].

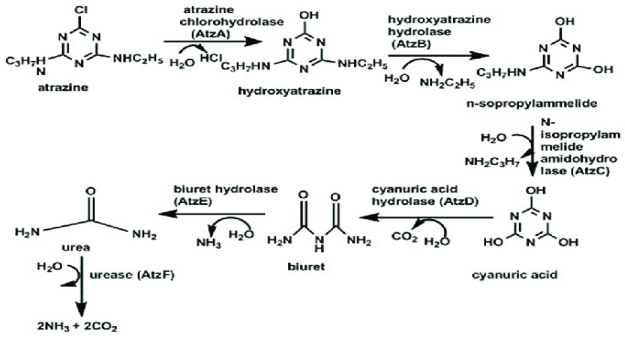

Fig. 4. Atrazine degradation pathway adapted from [18].

Two bacteria *Bacillus licheniformis* strain ATLJ-5 and *Bacillus megaterium* strain ATLJ-11, capable of efficiently degrading atrazine were isolated from soil in Nanjing City China. The degradation efficiency of atrazine reaches about 98.6%, and 99.6% after 7 days. The degradation of atrazine is faster when the two strains are used in combination [19]. An atrazine-degrading *Bacillus subtilis* strain HB-6 isolated from industrial wastewater has been shown through PCR assays to contain atrazine-degrading genes trzN, atzB and atzC. The strain HB-6 utilizes atrazine and cyanuric acid as the sole nitrogen source for growth. Atrazine is almost completely removed from the medium after 24 hours of incubation [20].

A strain of bacteria *Shewanella* sp. YJY4 which utilizes atrazine as its sole nitrogen source for growth was isolated from agricultural black soil in northeastern China. PCR analysis and sequencing confirmed that it contained atrazine-degrading atzA, atzB and atzC genes. After 36 hours, atrazine could not be detected by HPLC. This indicates that 100% of atrazine was degraded after 36 hours' incubation [21].

Nine selected bacteria species (*Ochrobactrum oryzae, Sphingomonas yanoikuyae, Bacillus* sp., *Serratia marcescens, Pseudomonas aeruginosa* (types I and II), *Acinetobacter radioresistens, Bacillus subtilis, and Paenibacillus lautus*) were studied in medium with atrazine. After 10 days of incubation periods, the results indicated that *Ochrobactrum oryzae* had the highest growth compared to the other bacteria and HPLC shows 31.46% degradation [22]. A study on *Arthrobacter* sp. strain LY-1 investigated the degradation effects of LY-1, as well as its capacity for soil remediation, under various conditions. The results of HPLC showed that the strain could degrade 99.5% of the atrazine within 48 hours [4].

Table 1. Characteristics of previously isolated atrazine-degrading isolates.

Isolate	Optimum Condition's	Manuscript Title	Ref
Bacillus licheniformis *Bacillus megaterium*	Con 200 mgL^{-1}, Temp. 30 ˚C	Study on isolation of two atrazine-degrading bacteria and the development of a microbial agent	[19]
Bacillus subtilis	Conc. 200 mgL^{-1}, Temp 30 ˚C, pH 7.0, Time 72 h	Isolation and characterization of atrazine mineralizing *Bacillus subtilis* strain HB-6	[20]
Shewanella sp.	Conc. 100 mgL^{-1}, Time 36 h	Isolation and characterization of atrazine-degrading Strain *Shewanella* sp. YJY4 from cornfield soil	[21]
Ochrobactrum oryzae	Conc. 50 mgL^{-1}, Time 120 h	Isolation atrazine-degrading bacteria in semi-salinity Medium	[22]
Arthrobacter sp.	Conc. 100 mgL^{-1}, Temp 30 ˚C, pH 7.0, Time 48 h	Isolating and identifying the Atrazine-Degrading Strain *Arthrobacter* sp. LY-1 and applying it for bioremediation of atrazine-contaminated soil	[4]
Nocardioides sp.	Temp. 37 ˚C, pH 7.0, Time 72 h	Influence of pH, temperature and nutrient addition on the degradation of atrazine by *Nocardioides* sp. isolated from agricultural soil in Nigeria	[23]
Arthrobacter sp.	Temp. 30 ˚C, pH 7.0, Time 72 h	Influence of soil pH, and Temperature on Atrazine Bioremediation	[24]
Arthrobacter sp.	Conc. 50 mgL^{-1}, pH 9.0, Time 14 h, Inoculum size 100 µL	Optimization of culturing conditions for isolated *Arthrobacter* sp. ZXY-2, an effective atrazine-degrading and salt-adaptive bacterium	[25]
Bacillus badius	Conc. 200.09 ppm, Temp. 30.04 ˚C, pH 7.05	Optimization studies on biodegradation of atrazine by *Bacillus badius* ABP6 strain using response surface methodology	[26]
Raoultella planticola	Conc. 35 ppm, Temp 28 ˚C, pH 7.0, Time 6 h	Atrazine Biodegradation by a Monoculture of *Raoultella planticola* Isolated from an Herbicide Wastewater Treatment Facility	[27]
Arthrobacter sp.	Conc. 80 mgL^{-1}, pH 7.2, Time 48 h	Atrazine degradation pathway and genes of *Arthrobacter* sp. FM326	[28]
Rhodococcus sp.	Conc. 100 mgL^{-1}, Temp 30 ˚C, pH 7.0, Time 120 h	Biodegradation of atrazine by *Rhodococcus* sp. BCH2 to N-isopropylammelide with subsequent assessment of toxicity of biodegraded metabolites	[8]
Bacterial consortium	pH 7.0, Time 24 h	Study of the Bioremediation of Atrazine under variable carbon and nitrogen sources by mixed bacterial consortium isolated from corn field soil in Fars province Iran	[29]
Klebsiella variicola	Conc. 50 mgL^{-1}, Temp. 30 ˚C, pH 7.0, Time 48 h	Biodegradation of atrazine by the novel *Klebsiella variicola* Strain FH-1	[30]
	Conc. 0.1 mgL^{-1}, pH 11, Time 120 m	optimization of atrazine degradation in the aqueous phase titanium catalyst doped with Iron (Fe^{+3}-TiO_2) process	[31]
Pseudomonas sp. *Bacillus* sp. *Micrococcus* sp.	Conc. 100 mgL^{-1}, Temp. 35 ˚C, Time 20 days	Biodegradation of atrazine by bacteria isolated from lotic water	[14]
Pseudomonas sp.	Conc. 0.06 mM, Temp 35 ˚C, pH 5.0, Time 20 days	Factors affecting potentials of certain bacterial isolates for atrazine bioremediation	[32]
Pichia kudriavzevii	Conc. 500 mgL^{-1}, Temp 30 ˚C, pH 7.0, Time 48 h, Inoculum size 3% (v/v)	Atrazine degradation in liquid culture and soil by a novel yeast *Pichia kudriavzevii* strain Atz-EN-01 and its potential application for bioremediation	[33]
Microbacterium sp. *Arthrobacter* sp.	Conc. 100 mgL^{-1}, Temp. 30 ˚C, Inoculum size 3%	Isolation of two atrazine-degrading strains and their degradation characteristics	[34]
Arthrobacter sp.	Conc. 50 mgL^{-1}, Temp. 34 ˚C, pH 9.0, Time 48 h, Inoculum size 10% (v/v)	Characterization of an efficient atrazine-degrading bacterium *Arthrobacter* sp. ZXY-2: An Attempt to Lay the Foundation for potential bioaugmentation application	[35]
	Conc. 12 mgL^{-1}, Temp. 29.3 ˚C, pH 6.7, Time 48 h, Inoculum size 5%	Optimization and kinetics studies on biodegradation of atrazine using mixed microorganisms	[36]
Bacillus safensis strain BUK_BCH_BTE6	Temp. 35 ˚C, pH 7.5,	Optimizing the Effect of pH and Temperature on Atrazine Degradation by Bacillus safensis strain BUK_BCH_BTE6 an Efficient Atrazine Tolerating Bacteria from an Agricultural Soil in Kura Local Government Area of Kano State, Nigeria	[37]

Studies on Atrazine degradation by *Nocardioides* sp. EAA-3 and *Nocardioides* sp. EAA-4 shows it is pH and temperature dependent, and requires no additional sources of carbon and nitrogen. *Nocardioides* sp. EAA-3 completely degraded atrazine whereas, *Nocardioides* sp. EAA-4 took a longer time to completely degrade atrazine [23]. A study was conducted with *Arthrobactor* sp. strain DNS10 to clarify soil pH and temperature influence on different atrazine bioremediation techniques, sawdust and animal manure. Atrazine remediation in soil with no additional amendment was only 34%, while in soil treated with sawdust, DNS10, sodium citrate and animal manure were 75.17%, 89%, 74.17% and 76.83% at optimized pH and temperature [24].

Results from atrazine-degrading *Arthrobacter* sp. strain ZXY-2 isolated from industrial wastewater indicated that the strain showed a high salinity tolerance. The strain was able to degrade more than 99% of atrazine within 20 hours [25]. A study on the optimization of environmental factors such as pH, temperature, agitation speed and atrazine concentration on atrazine degradation by *Bacillus badius* ABP6 strain, has been done through response surface methodology (RSM). The observed maximum atrazine degradation was 89.7% under the optimized conditions [26].

Research on *Raoultella planticola* bacterial cells isolated from a wastewater treatment plant of a herbicide factory reported that the degradation rates reached 50% depletion of atrazine, indicating that the strain can be added to the arsenal of atrazine-degrading bacterial cells that have the ability to degrade this substance under unfavourable conditions, such as those existing in the sludge of herbicide factories [27]. A study on *Arthrobacter* sp. strain FM326, revealed that it is a highly efficient atrazine-degrading bacteria, which expresses atrazine-degrading genes and could completely degrade atrazine into CO_2 and NH_3 and did not accumulate cyanuric acid in culture [28].

A study on *Rhodococcus* sp. strain BCH2 isolated from soil, long-term treated with atrazine showed that the bacterium was capable of degrading about 75% atrazine in a liquid medium under aerobic and dark conditions within 7 days [8]. The effects of carbon and nitrogen sources on atrazine biodegradation by mixed bacterial consortium were asses Sodium citrate and sucrose had 87.22% biodegradation rate and urea 26.76% after 30 days of incubation, the per cent of atrazine reduction rates were significantly enhanced in the inoculated soils 60.5% as compared to uninoculated control soils 12% [29].
A study on *Klebsiella variicola* strain FH-1 separated by means of enrichment culture from the soil applied with Atrazine for many years shows that the degradation rate of Atrazine reached 81.5% in 11 days of culture [30].

The results of a study focusing on evaluating the feasibility of using a titanium catalyst doped with iron (Fe^{+3}-TiO2) to remove atrazine shows 41.72% rate of atrazine removal. It can be concluded that the Fe^{+3}-TiO is an appropriate method for reducing atrazine in polluted water resources [31]. Bacteria capable of degrading atrazine were isolated from the lotic water using an enrichment technique. *Pseudomonas* sp. gave the highest atrazine degradation rate of 82.67% followed by *Bacillus* sp. 75.33% and *Micrococcus* sp. 69.33% at the end of 30 days [14]. Different bacterial isolates from two types of soil with different atrazine applications history were propagated. The isolates showed good capabilities for degrading 47.97% of atrazine after 48 hours. These percentages were found to be correlated to isolates counts in the soil. [32].

A novel yeast *Pichia kudriavzevii*. strain, Atz-EN-01 isolated from contaminated agricultural soil was found to be highly effective in degrading atrazine in liquid culture and soil. Atrazine was reduced by 50% after 2.2 days under optimal conditions The analysis of the metabolites using GC-MS identified the formation of 3 intermediates viz. hydroxyatrazine, N-isopropylammelide and cyanuric acid [33]. Two atrazine-degrading bacteria *Microbacterium* sp. strain Z9 and *Arthrobacter* sp. strain Z42, were isolated from black earth in a cold area with a long-term application of atrazine by standard enrichment techniques. The atrazine degradation rates of the two strains reached 77.7% and 65.6%, respectively after 14 days of culture in a liquid medium [34].

A study on *Arthrobacter* sp. strain ZXY-2, displayed its strong capacity to degrade atrazine, making it a potential candidate for bioaugmentation. Real-time quantitative PCR (RT-qPCR) results showed a positive correlation between the atrazine degradation rate and the expression levels of three functional genes (trzN, atzB, and atzC), which helped elucidate the role of strain ZXY-2 in bioaugmentation [35]. A study was carried out on atrazine degradation in batch reactors using mixed microorganisms obtained from pharmaceutical wastewater sludge. It was found that an increase in atrazine concentration decreases the degradation efficiency. The maximum atrazine degradation was found to be 94.4% [36]. Most recently, *Bacillus safensis* strain BUK_BCH_BTE6 locally isolated from active agricultural soil in Kano Northwestern Nigeria, efficiently degrade atrazine at optimized pH 7.5 and temperature of 35 °C.

CONCLUSION

This review has provided an update on the current knowledge regarding the characteristics of microbial degradation of atrazine herbicide isolated to date. Widespread and long-term usage of atrazine results in high residue levels in soil, which further causes water contamination. There is significant diversity in the genera of bacteria capable of degrading atrazine, the optimum condition required to achieve maximum degradation, and the relative efficiency with which microbes degrade atrazine. Further, knowledge of the genes recruited to organize the catabolic pathways will be the prospect of the future.

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