INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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Review Article

MICROBIAL DEGRADATION OF ATRAZINE, COMMONLY USED HERBICIDE

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ABSTRACT

Atrazine is one of the most environmentally prevalent s-triazine groups of herbicides used for the control of broad leaf weeds in corn and sorghum. One of the major environmental problems today is the release of atrazine which has led to the contamination of terrestrial ecosystems and has been detected in ground and surface waters in many countries beyond the permissible limits. The widespread use of atarzine and its toxicity necessitates search for remediation technology. A number of methods have been developed for the removal of atarzine from environment such as adsorption, incineration, reduction- oxidation, dechlorination, photolysis, reverse osmosis, chemical degradation etc. However, these methods are costly and produce many other toxic intermediates. Bioremediation is the promising technology for the treatment of the contaminated sites since it is cost-effective and will lead to complete mineralization. Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms. This paper presents an updated overview of atrazine degradation by microorganisms under different ecosystems.

KEYWORDS: Atrazine, Bacteria, Bioremediation, Biodegradation, Fungi, Microbial consortium

INTRODUCTION

Agrochemicals refer to a broad range of insecticides, fungicides and herbicides, and among them atrazine, a herbicide is extensively used in sugarcane, corn and sorghum cultures (Luciane *et al*., 2010). Atrazine is one of the most widely used herbicides all over the world. Nearly 34,000 metric tons of atrazine is used annually in the US alone. Although several countries gave up the use of atrazine because of its toxicity, it is still one of the most popular herbicide in many countries (Jin and Ke, 2002). Atrazine is still being used in India as the herbicide of choice and hence there is a high possibility of soils and water contamination in various parts of the country. Therefore, there is an urgent need to develop the soil cleanup methods for the remediation of atrazine. The US EPA has established 3 µg/L as the maximum contaminant level (MCL) for atrazine in drinking water, whereas other countries have adopted less than 3 µg/L concentration as MCL. According to World Health Organisation (WHO), the permissible limit is restricted to 2 µg/L. Atrazine is a pollutant of environmental concern due to its low biodegradability and its high potential to contaminate the surface waters and ground water (Chan and Chu, 2005).

Atrazine (2-Chloro-4-ethylamino-6-isopropylamino-s-triazine) is a chlorinated systemic selective herbicide widely used to kill weeds globally. It is used on crops such as sugarcane, pineapple, maize, conifers, chemical fallows, foresty, grassland, macadamia nuts etc. Atrazine is highly persistant in soil. The average half-life of atrazine in soil ranges from 13 to 261 days (US EPA, 2003), in river water more than 100 days (Seiler *et al*., 1992), in seawater is around 10 days (Armbrust *et al*., 1991) and nearly 660 days in case of anaerobic degradation. No degradation of atrazine was observed after incubation with adapted activated sludge (Freitag *et al*., 1984; Fulka *et al*., 1985).

Due to its high mobility and long half life in soil, residues of both the parent compound and its derivatives have been detected in soil, surface water and groundwater after year's application (Schiavon, 1988). Presence of atrazine at ppb level has shown to disrupt the sexual development in amphibians and thus may pose serious ecological risks (Rhine *et al*., 2003). As a principal process of atrazine mineralization, bioremediation has received attention throughout the world. Many microorganisms were isolated and studied for their abilities to mineralize atrazine including the members of genera *Pseudomonas, Acineto-bacter, Agrobacterium, Arthrobacter, Rastonia and Norcardioides* (Eaton and Karns 1991; Yanzekontchou and Gschwind 1994; Bouquard *et al*., 1997; Struthers *et al*., 1998; Strong *et al*., 2002). In some studies, soil fungi were found to be dominant causing the dealkylation of atrazine while bacteria were responsible for its further degradation and mineralization (Segula *et al*., 1993). Many Fungi belonging to genera such as *Aspergillus, Rhizopus, Fusarium, Penicillium, Trichoderma* and *Phanerochaete* have been found to be capable of degrading atrazine (Kaufman anb Blake 1970; Mougin *et al*., 1994).

Atrazine is slowly degraded in the soil, yielding metabolites such as desethylatrazine, desisopropylatrazine, desethyl-desisopropylatrazine, and hydroxyatrazine, which can be further, degraded (Baluch, 1993; Segula *et al*., 1993). The best studied atrazine-degrading bacterium is *Pseudomonas* sp. strain ADP which was isolated from a

herbicide spill site by Mandelbaum *et al*., 1995). For atrazine degradation, bacterial consortia appeared to be the more common and more efficient than individual species (Mandelbaum *et al*., 1993; Assaf and Turco 1994). Some microbial consortia were reported for their metabolic cooperative actions by investigating the individual's contribution in atrazine degradation (de Souza *et al*., 1998; Smith *et al*., 2005). The present article represents the degradation studies carried out by various microorganisms as potential agents for the removal of atrazine from environment towards the better understanding in bioremediation challenges.

Atrazine toxicology and health effects

The health effects of atrazine are classified in three categories such as developmental, reproductive and cancerous (U.S.D.H.H.S. 2003). In the first category, it causes post implantation losses, decrease in fetal body weight, incomplete bone formation, neurodevelopment effects, delayed puberty and impaired development of the reproductive system. The deleterious effects on reproductive system include pre-term delivery, miscarriage and various birth defects. The cancerous effects include Non – Hodgkin's lymphoma, prostrate, brain, testes, breast and ovarian cancer. The types of Atrazine toxicity and its effects have been shown in Table 1.

TABLE 1. Types of atrazine toxicity and its effects.

Types of toxicity	Effects
Acute toxicity	Atrazine has low acute toxicity and is a mild skin irritant. However, the acute toxicity of atrazine's metabolites such as desethyl- and deisopropylatrazine was found to be twice as that of atrazine. Acute toxicity of atrazine were shown to induce mammary gland tumors in Sprague-Dawley (SD) female rats (Stevens *et al*., 1994).
Chronic toxicity	
Teratogenicity	Atrazine has no significant teratogenic effect in rats, mice or rabbits, so it is not considered to be teratogenic (Pesticide news, 2002).
Reproductive Effects	Atrazine reduces the ability to reproduce and also found to be the reason for the premature birth, miscarriage, and various birth defects in humans (Pathak and Dikshit , 2011).
Mutagenecity	The US EPA and ACP evaluation both concluded atrazine does not have a mutagenic effect. The Northwest coalition for alternatives to pesticides (NCAP) found a significant increase in the percentage of chromosomal damage in the blood cells of workers in an atrazine production plant (Pesticide news, 2002).
Carcinogenicity	Several epidemiological cancer studies concerning atrazine and its possible association with carcinogenic effects in humans are being reviewed by the US EPA (Luciane *et al*., 2010).
Phyto toxicity	In agricultural soils, deethylatrazine and deisopropylatrazine, metabolites of atrazine, which retain the chlorine atom, are considered phytotoxic (Hounout *et al*., 1998).

REMOVAL TECHNOLOGIES

The widespread use of atrazine and its toxicity necessitates search for remediation technology (Parag *et al*., 2007). Several methods are available for atrazine removal from contaminated soil, water and wastewater. Among these, the most commonly used techniques are chemical treatment, incineration, adsorption, phytoremediation and biodegradation. Most commonly employed chemical methods for the remediation of atrazine bearing wastewaters are photolysis, hydrolysis, dehalogenation and oxygenation. Conventional methods of atrazine removal have some disadvantages (Table 2). Removal of atrazine from drinking water by adsorption using granular activated carbon or powdered activated carbon has been recognized as the best available technology. However, the feasibility of a specific adsorbent-adsorbate system must be examined in the laboratory and there are several reports on success and failure of adsorption process on atrazine removal from water.

TABLE 2. Physico-chemical methods for atrazine removal.

Methods	Application	Disadvantage	References
Incineration	More than 99.9% destruction of organic pesticide is possible.	Formation of corrosive and toxic gases.	Ahalya *et al*., 2003
Reverse osmosis	Impurity is separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids.	Expensive.	Ahalya *et al*., 2003
Electrodialysis	Semi-permeable ion-selective membranes used. Electrical potential applied between the two electrodes causes a migration of cations and anions towards respective electrodes.	Formation of metal hydroxides, which clog the membrane.	Ahalya *et al*., 2003
Chemical Degradation	Includes photolysis, hydrolysis, oxygenation etc.	May result in formation of other toxic or unwanted products, costly	Ahalya *et al*., 2003

Immobilized enzyme based technology	Use of two fusion proteins which dechlorinate atrazine while being firmly bound to an insoluble cellulose matrix.	Costly, affects stability of enzymes and diffusion problems.	Ahalya *et al*., 2003
Phytoremediation	Poplar trees seemed to be effective in rapid assimilation of ring leveled atrazine (90%) from sandy soil in less than 9 days	Application limited to surface and subsurface soils, time consuming process.	Burken *et al*.,1996

Source: Pathak and Dikshit, 2011.

Recent methods for atrazine removal include 1) De-oiled two phase olive mill waste 2) Ultrasonic destruction 3) Photocatalytic degradation 4) Dissipation. Table 3 shows the applications of the recent methods used for atrazine removal.

TABLE 3. Recent methods for atrazine removal.

Method used	Applications	References
De-oiled two-phase olive mill waste	Effects of de-oiled two-phase olive mill applied to soil for sorption	Antonio *et al*., 2010
Ultrasonic Destruction	The use of high power ultrasound to destroy pesticide contaminants like DDT, chlordane, atrazine, 2,4,5-T and endosulfan in sand slurries	Collings *et al*., 2010
Photocatalytic Degradation	Photocatalytic degradation of pyrene on soil surfaces using nanometer anatase TiO_2 under UV irradiation. The organic contaminants destroyed in a relatively short time when the contaminated soils containing atrazine, 2-chlorophenol, 2,7 dichlorodibenzodioxin mixed with TiO2 and exposed to simulated solar radiation.	Dong *et al*., 2010
Dissipation	The dissipation of herbicide O-methyl-O-(2,4-dimethyl-6-nitrophenoxy)-N-isopropyl phosphoramidothioate (H-9201) in soil	Zhang *et al*., 2010

Source: Pathak and Dikshit, 2011

BIODEGRADATION OF ATRAZINE

Biodegradation of atrazine is a complex process that depends on the nature and on the amount of the atrazine present in the soil or water. One of the important factors that limit biodegradation of atrazine in the environment is their limited availability to microorganisms.There are reports on the biodegradation of atrazine. The ability of soil microorganisms to degrade atrazine partially or totally directing it to carbon dioxide and ammonia formation have been demonstrated (Mandelbaun *et al*., 1995; Rosseaux *et al*., 2003 and Sing *et al*., 2004). According to Rhine *et al*., (2003), the repeated exposure to atrazine can increase biodegradation, which may also be enhanced as a result of limited N availability. Hydrolysis, dealkylation, deamination and ring cleavage were proposed as the major steps of atrazine degradation pathway. But, still the pathway of atrazine biodegradation is not very clear. Many researchers believed that hydrolysis and dealkylation share the primary steps of chloro s-triazine herbicides degradation. Biological degradation of atrazine depends upon various factors like the operating environment, external carbon and nitrogen sources, carbon/ nitrogen ratio (C/N), water content and the microorganism involved in degradation (Pranab and Ligy, 2006). The stimulatory or inhibitory effects of additional carbon or nitrogen sources on atrazine degradation vary among the degraders used. Struthers *et al*., (1998) stated that additional carbon source such as sucrose did not significantly faster the mineralization rates or shorten degradation lag times in *Agrobacterium radiobacter J14a*. However, Mandelbaum *et al*., (1995) stated that glucose was used as a carbon source by *Pseudomonas Sp.* but fructose, sucrose, galactose, lactose, or maltose did not show encouraging results. The use of citrate and sucrose as mixed carbon sources have contributed to the success of studies for atrazine mineralization by stable bacterial mixed cultures for Mandelbaum *et al* (1993). Gschwind (1992) enriched a mixed microbial community by using atrazine as sole carbon source. Atrazine is used as an N source by atrazine-degrading microorganisms (Mandelbaum *et al*., 1995; Radosevich *et al*., 1995) and mineral N addition could inhibit atrazine mineralization by offering an alternative source of N. Nitrogen amendments have been shown to have a negative effect on atrazine biodegradation by indigenous populations in soils (Entry *et al*., 1993; Alvey *et al*., 1995 and Abdelhafid *et al*., 2000). The synthesis or the activity of the enzymes like peroxidases responsible for atrazine degradation was reported (Entry *et al*., 1993; Li *et al*., 1994; Kaal *et al*., 1995). The influence of nitrogen compounds on the efficiency of atrazine catabolism has been the focus of a number of studies, since most atrazine-degrading bacteria use it as a nitrogen source and agricultural soils are often rich in nitrogen due to routine fertilization. *Pseudomonas sp. strain ADP* has been shown to metabolize atrazine rapidly when previously grown on atrazine while degradation was significantly slower with cells grown on ammonium, nitrate, or urea (Bichat *et al*., 1999; Katz *et al*., 2001). Bacterial isolates have been found to use atrazine as a carbon source (Yanze-kontchou *et al*., 1994) and as a nitrogen source (Radosevich *et al*., 1995). Of the environmental conditions tested, soil pH was the most significantly related parameter for atrazine mineralization (Sabine *et al*., 2000). In soils with pH lower than 6.5, less than 25% of the initial atrazine was mineralized even after

repeated application in field conditions. The optimum pH for atrazine degradation was found to be between 7 and 9 (Clotaire *et al*., 1994). As in natural environments, when atrazine was present as the sole carbon source, the s-triazine ring was not degraded in studies performed with specially selected microorganisms, and the organisms did not degrade atrazine when it was present at concentrations below 1 mg/liter. In soil, complete mineralization of atrazine was reported as the result of concomitant activity of fungi and bacteria (Wolf and Martin, 1975; Cook, 1987; Levanon, 1993). An understanding of these factors is necessary in order to improve the effectiveness of microorganisms in bioremediation applications

Atrazine degradation by bacteria

Among gram negative bacteria, complete degradation of atrazine has been limited to the genera, e.g., *Pseudomonas* (Mandelbaum *et al*., 1995; Hernandez *et al*., 2008), *Agrobacterium* (Struthers *et al*., 1998; Devers *et al*., 2007; *Pseudaminobacte*r (Topp *et al*., 2000), *Chelatobacter* (Rousseaux *et al*., 2001), *Delftia* (Vargha *et al*., 2005), and unidentified genera (Radosevich *et al*., 1995; Iwasaki *et al*., 2007). Of these, *Pseudomonas* sp. strain ADP (Mandelbaum *et al.*, 1995) has become a reference strain and has been used to elucidate the sequences of the catabolic enzymes atzA, atzB, atzC and atzD involved in aerobic degradation pathway and develop probes for the genes which encode these enzymes.

Atrazine-degrading gram-positive bacteria are distributed within the limited genera, e.g., *Rhodococcus* (Behki *et al*., 1993; Nagy *et al*., 1995 and Fujii *et al*., 2007;), *Arthrobacter* (Rousseaux *et al*., 2001; Strong *et al*., 2002; Cai *et al*., 2003; Aislabie *et al*., 2005 and Vibber *et al*., 2007), and *Nocardioides* (Topp *et al*.,, 2000; Piutti *et al*., 2003; Satsuma 2006; Vibber *et al*., 2007 and Yamazaki *et al*., 2008).Among these, *Arthrobacter* (Rousseaux, 2001) and *Nocardioides* (Topp *et al*., 2000) have been shown to grow on atrazine, the former using atrazine as the sole source of carbon and nitrogen for growth. *Streptomyces* strain PS1/5 was also shown to metabolize several s-triazine herbicides in the presence of additional carbon and nitrogen in the growth medium (Shelton *et al*., 1996). Atrazine is converted by bacteria to cyanuric acid through a series of hydrolytic reactions starting with dechlorination and then the sequential removal of the two alkylamino chains (Mandelbaum *et al*., 1995; de Souza *et al*., 1995 and BoundyMills *et al*., 1999). The intermediates of atrazine biodegradation through the formation of cyanuric acid are believed to be common, but the sequence of pathway steps varies among the known degrader (Ellen *et al*., 2002). Genes encoding enzymes that are involved in atrazine biodegradation have been identified and cloned from *Pseudomonas* and *Rhodococcu*s spp. (Eaton and Karns, 1991; Mulbry, 1994; Shao *et al*., 1995; de Souza *et al*., 1996 and BoundyMills *et al*., 1997). The genes atzABC from *Pseudomonas* sp. ADP have been found in several other atrazine-degrading bacteria (de Souza *et al*., 1998).

Atrazine degradation by fungi

Atrazine degrading fungi are limited to the genera, eg. *Aspergillus, Rhizopus, Fusarium, Penicillium*, *Trichoderma and Phanerochaete* (Kaufman and Blake, 1970; Mougin *et al*., 1994). Soil Fungi was found to be dominant in dealkylation of atrazine. The fungus *Phanerochaete pulmonarius* demonstrated atrazine side chain dealkylation and hydroxylation via the constitutive metabolic activities of natural molecules present in soil. This fungus could also N dealkylate the atrazine in both of the alkyl groups. It has been suggested that N dealkylation is effected by enzymatic systems, such as P-450 monooxygenases and chloroperoxidases, as well as by chemical ozonization and photooxidation (Rejto *et al*., 1983; Kearney *et al*., 1988 and Okazaki *et al*., 1993) and its ability to dealkylate and hydroxylate atrazine was not dependent on previous incubation with the compound. Unlike *Phanerochaete pulmonarius*, which exhibited isopropyl hydroxylation, another white rot, *Phanerochaete chrysosponum*, could only N dealkylate the parent molecule, did not hydroxylate it. *Trametes versicolor* was able to grow and actively degraded atrazine in non-sterile soil under low water availability conditions (Bastos *et al*., 2009) contradicting the earlier findings by Tornberg *et al*., (2003).

Atrazine degradation by microbial consortia

It has been postulated that mixed cultures are likely to have a larger capacity to deal with a range of substrates by virtue of increased catabolic capabilities (Slater and Lovatt, 1984). Therefore, microbial communities may be capable of degrading all, rather than part, of a given atrazine and they appear to be more common in soils than individual species. Although several atrazine- degrading bacteria have been isolated and their individual catabolic pathways extensively studied, the cooperative metabolism of atrazine is yet poorly understood. In a microbial consortium, after the dechlorination of atrazine by *Nocardia* sp., the resulting hydroxyatrazine was afterwards degraded in two different ways. In one of them, *Nocardia* sp. converted hydroxyatrazine to N-ethylammelide via an unidentified gene product, whereas in the other, hydroxyatrazine generated by *Nocardia* sp. was hydrolyzed to N-isopropylammelide by *Rhizobium* sp., which contained the gene atzB. All the members of the consortium contained gene atzC, responsible for the cleavage of the ring, besides the gene trzD. However, none of the microorganisms showed to carry atzD, E or F genes (Smith *et al*., 2005). By a four member bacterial community along with *Clavibacter michiganese,* atrazine was converted to N-ethylammelide and was further degraded by *Pseudomonas* sp. CN 1, ensuring the complete atrazine degradation (de Souza *et al*., 1998). A similar example of cooperative metabolism was shown to occur within a complex eight-member atrazine-degrading community with two pathways of atrazine degradation (Smith *et al*., 2005). A four-member atrazine-mineralizing community enriched from an agrochemical factory soil was characterized which was found to be capable of rapidly mineralizing atrazine. The analysis of the genetic potential of individual community members revealed that two *Arthrobacter* strains, named ATZ1 (carrying trzN and atzC genes) and ATZ2 (carrying trzN, atzB and atzC genes) might be involved in the upper pathway producing cyanuric acid, and the other

two members, *Ochrobactrum* sp. CA1 and *Pseudomonas* sp. CA2 (both carrying trzD gene), were responsible for cyanuric acid catabolism (Kolic *et al*., 2007).

CONCLUSION

Cleaning up of atrazine in the environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants. Microbial degradation process aids the elimination of atrazine from the environment in a cost effective way. This is possible because microorganisms have enzyme systems to degrade and utilize herbicides as a source of carbon and energy. The knowledge of microbial degradation of atrazine may serve as a basis for the use of bioremediation systems for the removal of other herbicides in near future.

ACKNOWLEDGEMENT

Financial assistance from VIT University is gratefully acknowledged by the first author.

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