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ENVIRONMENTAL SIGNIFICANCE OF ATRAZINE IN AQUEOUS SYSTEMS AND ITS REMOVAL BY BIOLOGICAL PROCESSES: AN OVERVIEW

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ABSTRACT

Atrazine, a chlorinated s-triazine group of herbicide is one of the most widely used pesticides in the World. Due to its extensive use, long half-life and various toxic properties, it has very high environmental significance. Up to 22 mg l^{-1} of atrazine was found in ground water whereas permissible limit of atrazine is in ppb level in drinking water. As per Indian standard there should not be any pesticide present in drinking water. Among many other treatment processes available, Incineration, adsorption, chemical treatment, phytoremediation and biodegradation are the most commonly used ones. 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 bacterial strain. Although, general atrazine degradation pathways are available, the specific pathways in specific conditions are not yet clearly defined.

In this paper extensive review has been made on the occurrence of atrazine in surface and ground water bodies, probable sources and causes of its occurrence in water environment, the toxicity of atrazine on various living organisms and its removal by biological processes.

INTRODUCTION

Modern agricultural practices often include the extensive use of a wide range of pesticides. Environmental contamination due to the excessive use of pesticides has become a great concern to the public and to environmental regulatory authorities. In 1995, conventional pesticide use in USA amounted to about 1.22 billion pounds, which was one fifth of the world's use of such chemical [1]. Herbicides are the pesticides used to remove weeds that would otherwise compete with the crop. At present, pesticides of 32 billion dollar have been marketed in the world [2], whereas in India it amounts Rs. 4,500 crore. Among all the pesticides, herbicide production and its use was more than any other pesticides in the world [1]. Though herbicide use in India is insignificant compared to other countries in the World, the total intake of pesticide containing chlorine by an Indian is the highest [3]. Atrazine is a chlorinated herbicide and at present, it has been used in more than 80 countries and probably it is the most commonly used herbicide in the world [4]. It exhibits acute, chronic and phytotoxicity. It has been proved that atrazine contains mutagenic and carcinogenic agents also.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a member of s-triazine group of herbicides. Atrazine is almost non-volatile and its half-life in neutral condition is about 200 days but varies from 4-57 weeks [5,6] depending on various environmental factors like pH, moisture content, temperature and microbial activity [7-12]. Atrazine is used mainly for pre and post emergence control of annual grass and broad-leafed weeds in Maize, Sorghum,

Pineapple, Sugarcane, Macadamia nuts and many other crops and have been sold in different commercial names like Aatrex, Aatratol, Bicep, Gasaprim etc. [13]. Its solubility in water is 33 mg l^{-1} at a temperature of 20 ^{0}C whereas, solubility in methanol, ethylacetate, dichloromethane, and chloroform are 18, 24, 28 and 52 g l^{-1} respectively [13].

Due to its excessive usage, high persistence and mobility, atrazine along with its metabolites are transported to surface and subsurface water bodies and had been found in ground water, rivers, high mountain lakes, drinking water supplies, rain water and even in fog [14]. The high mobility of atrazine in soil [15] and its potential contamination of ground waters [16] may represent a serious human health hazard because of the potential carcinogenic effects of s-triazines [17]. As high as 22 mg l^{-1} of atrazine residue levels was observed in the well water near the mixing / loading sites [18,19] whereas the permissible limit of atrazine in drinking water is in ppb (parts per billion) level. In Canada, the acceptable level of atrazine in drinking water is 0.06 mg l^{-1} [20]. According to World Health Organization (WHO), the permissible limit is restricted to 2 μg l^{-1} [29]. Under safe drinking water act, Environmental Protection Agency (EPA) establishes Maximum Contaminant Level (MCL) for atrazine as 3 μg l^{-1}, which took effect in 1992 [21]. European Economic Council (EEC) Legislation restricts the occurrence of individual pesticides in drinking water to 0.1 μg l^{-1} [22]. As per Indian Standards, no pesticide should be present in drinking water [23]. As such no guidelines exist till now on the permissible limit of atrazine concentration on effluent discharged into water bodies.

TOXICITY OF ATRAZINE

In most of the cases, toxicity effect of atrazine was observed at higher concentrations or doses, but detrimental effects on several species of algae were observed even at an atrazine concentration as low as 20 ppb [24]. Although it is less toxic to mammal, birds and fishes, many aquatic organisms are susceptible to atrazine at low levels [13]. Although atrazine is placed in toxicity class III by USEPA, which means it is slightly toxic, it has been classified as restricted use pesticide (RUP) due to its ground water contamination potential. Also exposure to atrazine may cause detrimental effects and irritation to eyes, nose and throat [25]. Due to the toxicity behavior of atrazine, German Government banned all atrazine-containing products in 1991 [26].

Acute Toxicity

Acute toxicity is the ability of a substance to cause harmful effects soon after a single exposure or dose or any severe poisonous effect resulting from a single short-term exposure to a toxic substance. LD_{50} (lethal dose 50) is defined as the dose that kills 50% of a population of test animals. The oral LD_{50} was observed to be 1869-3080, 1750-3992 and 750 mg of technical grade atrazine/kg for rats, mice and rabbits respectively [13]. The 1-hour and 4-hour inhalation LC_{50} (Lethal Concentration) was observed to be greater than 0.7 mg l^{-1} and 5.2 mg l^{-1} respectively in rats [25, 27]. Table 1 shows the LD_{50} of atrazine on various test species. It had been observed that after consuming a large oral dose, rat exhibits muscular weakness, breathing difficulty, prostration, convulsions and death [21].

Table 1. LD_{50} value of atrazine on various test species

Type	mode	Species	Amount	Units
LD_{50}	oral	Rat	672	mg kg^{-1}
LD_{50}	intraperitoneal	Rat	235	mg kg^{-1}
LD_{50}	oral	Mouse	850	mg kg^{-1}
LD_{50}	oral	Rabbit	750	mg kg^{-1}
LD_{50}	skin	Rabbit	7500	mg kg^{-1}
LD_{50}	oral	Humane	1000	mg kg^{-1}
LD_{50}	inhalation	Rat	5200	mg m^{-3} $4hr^{-1}$
LD_{50}	intraperitoneal	Mouse	626	mg kg^{-1}

Chronic Toxicity

Chronic toxicity is the capacity of a substance to cause long-term or delayed adverse health effects. Several reports showed the chronic toxicity of atrazine on various test organisms.

After the sign of respiratory distress and paralysis of the limbs, 40% rats were died at an atrazine oral dose of 20 mg kg^{-1} day^{-1} for 6 months. Structural and chemical changes in different parts of the body like brain, liver, kidney, ovaries etc. and growth retardation were also observed [25, 27]. In a 2-years study with dogs, 7.5 mg kg^{-1} day^{-1} atrazine dose caused decreased food intake and increased heart and liver weights. At a dose of 75 mg kg^{-1} day^{-1}, additional effects such as lowered blood cell counts and occasional tremors or stiffness in the rear limbs were noticed [27]. Diminished weight gain in rats and dogs was considered to be one of the symptoms of chronic toxicity of atrazine [28]. The No Observable Effect Level (NOEL) on rat was 70 mg kg^{-1} whereas in dog it was 15 mg kg^{-1} of body weight.

Carcinogenicity

International Agency for Research on Cancer (IARC) had concluded that there was inadequate evidence in human and limited evidence in experimental animals for the carcinogenicity of atrazine (Group 2B). Hence, Tolerance Daily Intake (TDI) approach was concluded to be suitable to calculate a guideline value of 2 $\mu g\ l^{-1}$ for atrazine in drinking water [29]. Atrazine is a xenobiotic compound that was recognized by EPA as Group C – "possible" human carcinogen [30]. Also, it had been observed that women previously exposed to triazines, developed tumor [31]. But reports by EPA on 2000 classified atrazine as a "likely" human carcinogen rather "possible" human carcinogen [32] on the basis of research results obtained during last 5 years. However, recently the international agency for research on cancer (IARC) has removed atrazine from its list of known or suspected carcinogens [33].

Mutagenicity

Although evidence on mutagenicity of atrazine has not been found [27], a water-soluble extract from maize plants grown in the presence of Aatrax 80W (with active ingredient of atrazine) contained mutagenic agents when tested on strains of yeast [34]. Another study showed that, at higher oral dose of atrazine, rats suffered DNA lesions in the stomach, kidney and liver [35].

Reproductive Effects

Stevens and Sumner [25] and Kidd and James [27] observed no adverse reproductive effects but inhibition of certain hormone secretion and hence, reduction of reproduction capacity on rat when treated with atrazine was observed [36]. The maternal NOEL on the rat mothers was estimated to be 10 mg kg^{-1} day^{-1} where as 78% maternal mortality dose of atrazine was 700 mg kg^{-1} day^{-1} [36].

OCCURRENCE OF ATRAZINE IN THE ENVIRONMENT

The problem of pesticide contamination of surface and ground water bodies are often results from non-point sources mainly agricultural runoff. Due to non-point sources, concentrations of atrazine in water bodies are generally in ppb levels [37]. Triplett *et al.* [38] reported the highest atrazine concentration of 0.48 ppm in the runoff from Ohio River watershed soon after its application, whereas, in Lake Erie and River Sundusky atrazine concentration of 0.5-3.5 and 5.92-11.3 $\mu g\ l^{-1}$ were observed respectively [39]. Many researchers detected atrazine concentration of about 100 $\mu g\ l^{-1}$ in surface and subsurface waters [40-41]. Although atrazine concentration was observed in ppb level, among all other pesticides, it was the most detected one in most of the cases as water contaminant. Out of 181 samples collected from 31 sample stations in Bayou Maringouin stream of upper Terrebonne basin, 82-samples had atrazine concentration more than the Maximum Concentration Level (MCL) of 3.0 ppb. The highest concentration detected was 216.2 ppb [40-41]. Widespread water contamination by atrazine is not only due to non point sources but also point sources like pesticide manufacturing industry, dumped site of pesticide wastes, warehouse fires, handling and distribution activities, wood treatment facilities, farms and agrochemical retailer and commercial

applicator facilities [42]. Ground water quality surveyed by the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) has indicated widespread contamination of private drinking water wells by atrazine residues due to point source pollution. Atrazine concentration of 25 $\mu g\ l^{-1}$ was observed in Minnesota because of spill [43]. The atrazine residue levels in the well water near the mixing / loading sites ranged from 0.024 to 22 $mg\ l^{-1}$ [18 – 19]. Metabolites of atrazine, which are equally toxic to that of the mother compound were found both in surface and ground waters [44].

Very few reports are available on the occurrence of atrazine in domestic or industrial wastewater. This may be due to the lack of regular monitoring mechanisms for pesticides in wastewater. But various point sources, mentioned above, can significantly contribute atrazine to existing wastewater treatment plants. People have a tendency to throw out the unused pesticide and allow the water after washing pesticide container, into existing sewer systems. By this way also atrazine can get mixed with domestic wastewater [45]. Pesticide rinsate generated from the rinsing and cleaning of pesticide container consists 1-1000 $mg\ l^{-1}$ of pesticide along with other formulating agents [46]. Gerecke *et al*., [47] monitored atrazine concentration in the effluent from existing wastewater treatment plants in Switzerland and found that in many cases concentration was more than the permissible limit.

Effluent from a manufacturing plant of chloro s-triazine compound is of great environmental importance. Such effluents contain high dissolved organic matter as that of atrazine contaminated wastewater, but the atrazine concentration is many fold more. Hogrefe *et al*. [48-49] carried out atrazine biodegradation studies using wastewater from a chloro-s-triazine synthesis plant, supplied by Ciba Gaige. It contains s-triazine herbicides measured as, Total Kjeldahl nitrogen (TKN) of 810 $mg\ l^{-1}$ N, total organic carbon content of about 1200 $mg\ l^{-1}$ and a biological oxygen demand after 5 days (BOD_5) about 530 $mg\ l^{-1}\ O_2$ [48-49].

Soil contamination by atrazine is a severe threat to environment. Excessive use of pesticide and its long persistence in neutral environment leads to the accumulation of atrazine and its toxic metabolites in soil and sometimes needs remediation. Accidental spill of pesticide and warehouse fires are the other major causes of soil contamination. The Wisconsin Department of Agriculture surveyed 20 of the approximately 650 commercial agrochemical applicator facilities located in the United States of America [19]. The highest pesticide concentrations were found in areas of known spills, burn piles, mixing/loading areas and discarded pesticide container storage areas. Herbicides such as alachlor, atrazine, cyanazine and metolachlor were the most prevalent. Leaks and spills during repackaging and storage tank filling operations contaminated the soils and gravel. In a report, median concentration of atrazine in stored rinse water, contained at 4 Illinoise agrochemical facilities was 14,100 ppm [50].

ATRAZINE REMOVAL TECHNOLOGIES

There are several technologies available for the removal of atrazine from water, wastewater and contaminated soil. Among these, the most commonly used techniques are chemical treatment, incineration, adsorption, phytoremediation and biodegradation.

Chemical Degradation

Most commonly employed chemical methods for the remediation of atrazine bearing wastewaters are photolysis, hydrolysis, dehalogenation and oxygenation. In natural soils, detoxification of atrazine occurs principally by chemical hydrolysis in the 2-position [8, 51-53]. Chemical hydrolysis of atrazine produces hydroxyatrazine in strongly acidic or basic solutions [8, 54-56]. Alkaline hydrolysis likely involves direct neucleophilic displacement of Cl^- from 2-position of atrazine by OH^- whereas acid hydrolysis may results from protonation of a ring or chain nitrogen atom followed by cleavage of the C-Cl bond by water [57]. That may be the cause of more rapid hydrolysis of atrazine in alkaline medium than in acidic medium [57]. It is also reported that the chemical degradation of atrazine was due to the removal of chlorine group by hydrolysis, catalyzed by clay and organic matter, which generated hydroxyatrazine [52,58]. Formation of hydroxy analogs of simazine, atrazine and propazine in five soils were not inhibited even by 200 ppm of sodium azide. But the rapid detoxification of atrazine at 95^OC in soil and slow detoxification in aqueous solution in absence of soil inorganic constituents showed the importance of inorganic soil constituents for non-biological

degradation of atrazine [51 – 52]. Blumhorst and Weber [59] reported that atrazine degradation was possible by chemical processes even in moderately acidic or neutral pH soil. Chemical hydrolysis is accelerated in the presence of humic materials in acidic or basic environments [60].

Various new techniques that have been proposed and explored for the destruction of chlorinated triazine herbicides, in particular atrazine included photocatalytic degradation [61], advanced oxidation [62-65], Fenton like reactions [66-67], electrocatalytic dechlorination [68] and reductive degradation by the use of zero-valent iron [69-70].
Chemical process using Fenton's reagent was tried to degrade atrazine. Ferrous salts and hydrogen peroxide (Fenton's reagent) was able to dealkylate atrazine [71]. A modified Fenton's system consisting of electrochemical generation of iron in the presence of hydrogen peroxide, around 90% of atrazine was removed [72] at a pH range of 5 to 7.5. Fenton's reagent ($FeSO_4$: H_2O_2) in a 1:1 ratio was capable of completely degrading ^{14}C atrazine to 27 % of 2-chloro-4,6-diamino-s-triazine and 28% of 2-acetoamido-4-amino-6-chloro-s-triazine in 30 seconds [73]. Dehalogenated s-triazines represented the balance of the remaining 14 carbons. Atrazine degradation decreased from 99% at pH 3 to 37% at a pH of 9. Atrazine could not be degraded by Fentons type reagent ($Fe(III)/H_2O_2$ system) while it was alone but degraded when simultaneous oxidation of pesticide and any phenolic compound (pHB, Ty, pCu) was carried out [74].

Hapeman and Torrents [75] have reviewed the effects of pretreatment by chemical methods on the mineralization of several pesticides for the subsequent biological treatment. Although chemical pretreatment enhanced the biodegradation and mineralization of 2,4-dichlorophenol, 2,4,5-trichlorophenol, organophosphate, and several other pesticides, it could not improve the mineralization potential of triazines [76]. Atrazine was also not mineralized by H_2O_2-UV but cyanuric acid was the final product [77].

Incineration

The US Environmental Protection Agency (EPA) has designated incineration as the Best Demonstrated Available Technology (BDAT) for many of the most toxic waste streams. An incinerator can destroy a waste or soil contaminant within a few seconds or minutes and reduces the waste volume significantly and the mineralized end product can be dumped in landfills. Incineration process is generally practiced for strong wastes of an industry. In optimum condition, more than 99.9% destruction of organic pesticide can be possible by this process [78]. One of the main disadvantages of this process is the formation of corrosive and toxic gases according to the component of the pesticide incinerated. For example, pesticide containing chlorine can produce Hydrochloric acid (HCl) and pesticide containing nitrogen can produce nitrogen oxide and nitrogen dioxide during incineration. All the above gases are acidic and corrosive. Hence, acid resistant materials should be used for the construction of incinerators treating these toxicants. Moreover, the toxic exhaust gases are to be treated before letting it out to the environment. Due to these reasons incineration technique is not widely practiced for pesticide bearing waste treatment.

Adsorption

Adsorption can be defined as the accumulation of a substance (pollutant) at the interface between two phases, usually a solid and a fluid (air or water). Removal of atrazine from drinking water by adsorption using granular activated carbon (GAC) or powdered activated carbon (PAC) has been recognized as the best available technology (BAT). However, the feasibility of a specific adsorbent-adsorbate system must be examined in the laboratory as the quantity of adsorbate that can be taken up by an adsorbent is a function of both the characteristics and concentration of adsorbate and the temperature. Generally the amount of material adsorbed is determined as a function of the concentration at a constant temperature, and the resulting function is called an adsorption isotherm. Various most commonly used adsorption isotherms are developed by Langmuir, Freundlich and Brunauer, Emmet, and Teller (BET isotherm). These three adsorption isotherms result in significantly different functional relations. Both, Langmuir and BET isotherm equations are developed on the basis of scientific assumptions of monolayer and multilayer adsorption theory respectively whereas,

Freundlich isotherm is an empirical model. The choice of best relation will depend on which one describes the data in a better manner . There are several reports on success and failure of adsorption process on atrazine and other pesticides removal from water phase. Adams *et al.* [79] studied the treatability of s-triazine herbicide metabolites in drinking water using PAC. It was found that the adsorptive capacity of PAC for atrazine was pH dependent and had the maximum capacity at a pH of 6.0. The data fit well with both Langmuir and Freundlich isotherms. Although it has been concluded that atrazine and its metabolites can be removed effectively by PAC process, the amount of adsorbent required for removing certain amounts of atrazine metabolites were about 3-4.5 times that required for parent atrazine. The effluent atrazine concentration was in between 1 and 3 μg l^{-1} where as the influent atrazine concentration varied from 5 to 20 mg l^{-1}. However, where PAC was unable to reduce pesticide level to the European Community's (EC) maximum admissible concentration i.e. 0.1 μg l^{-1} [80], GAC bed was able to reduce the effluent s-triazine concentration below 0.1 μg l^{-1} but regeneration frequency of activated carbon was 4-8 times more than that required for taste and odor control [81]. On the other hand, the adsorption study conducted with granular activated carbon (GAC) at Fremont, was not much effective. They could obtain only 47% of removal from wastewater containing a mean influent atrazine concentration of 4.83 μg l^{-1} [39]. Several researchers have assessed the effects of organic carbon in the adsorption of micropollutants. It has been reported that the presence of dissolved organic carbon (DOC) caused a capacity reduction on adsorption of trichloromethane and metolachlor [75]. A significant negative effect was observed on the adsorption capacity of activated carbon at an initial DOC concentration of 3.6 mg l^{-1} [82]. Adsorption of atrazine on the soil particles is one of the important factors, which decide the fate of atrazine in the environment. Many studies were conducted on the adsorption / desorption of atrazine in soil to know its fate in the environment [80,83-92]. Bailey *et al.* [93] studied the adsorption of atrazine on clay and observed that, regardless of chemical character, highly acidic H-montmorilonite gave better performance compared to near neutral Na-montmorillonite. Barriuso et al. [89] observed that atrazine adsorption was probably reversible and decrease with increase in surface charge density of the Smectites. However, small positive and negative affinity was observed on some clay. Roy and Krapac [94] reported that desorption of atrazine from low organic carbon sand, tile and alluvial samples were hysteretic. Deethylatrazine showed a lower affinity for the same adsorbents and desorption was reversible unlike atrazine. Chung *et al.* [95] reported that atrazine concentration on the sediment was linear with respect to the equilibrium aqueous atrazine concentration in the anaerobic sediment bioreactor. Hydroxyatrazine, the primary reaction product of atrazine was found to be strongly adsorbed on the sediments [95].

Although activated carbon is a costly material, it is quite cost effective for the removal of micro pollutant as a sole contaminant. But the process becomes uneconomical when the micro pollutant present along with dissolved organic carbon (DOC). Presence of dissolved organic matter in the wastewater hindered atrazine removal due to competitive adsorption, which in tern increases the treatment cost and frequency of carbon regeneration. These results forced the researchers to search out for low cost adsorbent for combined removal of atrazine as well as DOC. Keertinarayan [96] reviewed the literature on the behaviour of different materials as adsorbent for the removal of various pesticides and other pollutants. Wood charcoal was proved to be an effective low cost adsorbent for the removal of Lindane [97] and endosalfan [98] from water environment but was not suitable for the removal from wastewater containing high DOC [98]. Studies on atrazine adsorption on iron oxides [99], quartz, calcite, kaolinite and α-alumina [100] showed that only kaolinite was suitable for the adsorption of atrazine. It has been reported that waste activated carbon (WAC) from tap water purifier can be used for post treatment of upflow anaerobic sludge blanket (UASB) reactor effluent [101].

Phytoremediation

The insitu use of vegetation in bioremediation schemes is termed as phytoremediation. Phytoremediation is an emerging technology for the cleanup of contaminated environments such as soil, water and sediments. Different tolerant plants are planted in the contaminated sites which uptake the main pollutant along with other nutrients and thus changing the soil chemistry and increases microbial activity. Success of phytoremediation technique mainly depends upon the selection of proper tolerant plant and suitable soil [102]. Rice *et al.* [103],

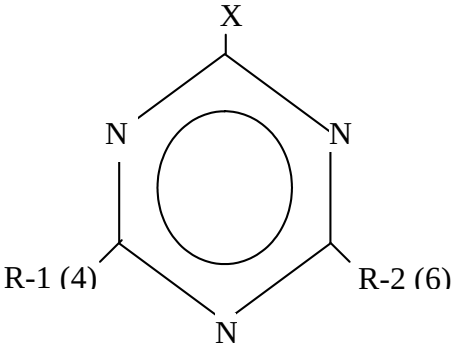
observed atrazine (^{14}C) removal of about 59% by submerged aquatic plant [103]. In a study, Bravo rhizosphere soil was found to be more suitable in supporting phytoremediation than other site soil [102]. Popler trees seemed to be effective in rapid assimilation of ring leveled atrazine (90%) from sandy soil in less than 9 days [104] whereas, in clayey soil the assimilation was very poor. Though phytoremediation is a promising treatment option for many pollutants like heavy metals and various pesticides, its application is limited to surface and subsurface soils. Remediation of soil or wastewater contaminated with herbicides, which can persist in the environment for long period, may not work on "hot spots" of very high contamination. Increasing the population of particular herbicide degrading pure culture bacteria by artificial means may solve such type of problem. Many researchers[105-108] isolated pure culture atrazine degrading bacteria and used for the remediation and treatment of atrazine contaminated soil as well as atrazine bearing wastewater.

Biodegradation

Mineralization is defined as the complete degradation of the parent compound to end products like CO_2 and H_2 (aerobic condition) or CO_2 and CH_4 (anaerobic condition), and biodegradation is defined as the alteration of the original substrate by biological process, but not necessarily to CO_2, H_2O or CH_4 [109]. Although there are several methods available for the control of atrazine, biological method is the only method that can mineralize atrazine.

Biodegradation pathway

Understanding the biodegradation pathway of any pollutant in a specific condition is useful for better application and control. Atrazine degradation can occur via biotic and abiotic processes. Atrazine biodegradation had been reviewed extensively by Kaufman and Kearney[9] and Erickson and Lee [110] whereas, the abiotic detoxification and degradation had been reviewed by Jordan *et al.* [111]. Structure of various intermediates, which could be produced during the biodegradation process, is described in Figure 1.

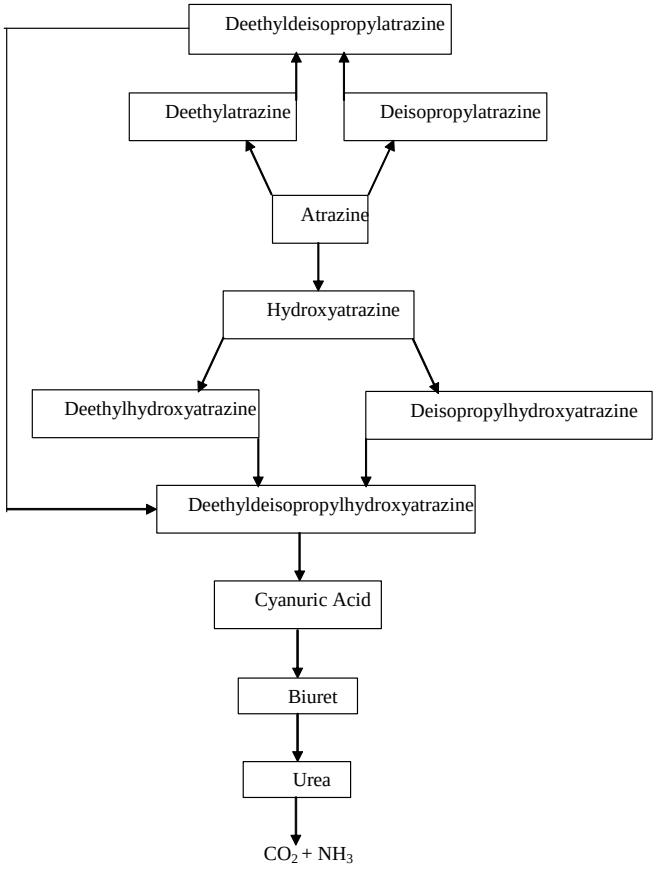


X (2), R-1(4) and R-2 (6) are the substituted groups in s-triazine ring in 2, 4 and 6 positions respectively.

Common Name	X	R-1	R-2
Atrazine	Cl	C_2H_5NH	CH_3CHCH_3NH
Hydroxyatrazine	OH	C_2H_5NH	CH_3CHCH_3NH
Deethylatrazine	Cl	NH_2	CH_3CHCH_3NH
Deisopropylatrazine	Cl	C_2H_5NH	NH_2
Deethyldeisopropylatrazine	Cl	NH_2	NH_2
Deethylhydroxyatrazine	OH	NH_2	CH_3CHCH_3NH
Deisopropylhydroxyatrazine	OH	C_2H_5NH	NH_2
Deethyldeisopropylhydroxyatrazine	OH	NH_2	NH_2
Cyanuric acid	OH	OH	OH
Urea			
Biuret			

Figure 1. Structure of atrazine and its transformed products

Erickson and Lee [110] proposed a degradation pathway for the s-triazine herbicides on the basis of literature. 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 [105-108] Hydrolysis of atrazine produces hydroxyatrazine substituting chlorine atom from its 2-position. It is widely accepted that hydroxyatrazine formed by dechlorination reaction, is due to chemical process while dealkylation reactions are biologically mediated [8,52-53,55-56,110,112-116]. Although hydrolytic dechlorination of atrazine was believed to be chemically mediated, it occurred by biological enzymatic action also [105]. N-dealkylation resulted the formation of deethylatrazine, deisopropylatrazine and deethyldeisopropylatrazine [108,110,117-118], which provided energy for growth and reproduction of microorganisms [110]. But whether deethyl- or deisopropylatrazine formation occurs first, depends upon the bacterial strain. Skipper and Volk [113] observed that the mineralization rate of ethylatrazine was 8 times faster than that of isopropyl atrazine. Adams and Thurman [119] also concluded that utilization of ethyl side chain was more rapid than isopropyl side chain and hence, the isopropylatrazine was more frequently found in surface and ground water bodies as contaminant than ethylatrazine. In contrary to this, Behki and Khan [108] observed that an aerobic bacterial isolate *Pseudomonas* preferentially utilize isopropyl side chain than ethyl side chain. Next step to dealkylation was deamination in which Cyanuric acid was formed. Consequent steps were the conversion of cyanuric acid to biuret, decomposiotn of biuret to urea by urease enzymes and the degradation end products were CO_2 and NH_3 [120]. Complete degradation of atrazine was observed through continued hydroxylation of the triazine ring and the formation of anniline, ammelide and cyanuric acid prior to ring cleavage, and finally to CO_2 and NH_3 [117]. Considering all the above results a proposed pathway of atrazine biodegradation is shown in figure 2.



Structure of different components has been illustrated in Figure 1.

Figure 2. Atrazine biodegradation pathway

Biodegradation of atrazine in aerobic condition

Most of the research works on atrazine biodegradation are conducted on pure culture aerobic bacterial isolates. Mineralization of atrazine in soils under aerobic condition is reported by many researchers [53,121]. A soil bacterium, *Nocardia* [122] was able to utilize atrazine as the sole source of carbon and nitrogen to form dealkylated and deaminated metabolites. Dealkylation precedes deamination by that species and the final product observed was 2-chloro-4-amino-s-triazine which was reported as a non phytotoxic new metabolite. No chemical degradation of atrazine occurred on incubating the solution of herbicide in abiotic condition, whereas in biotic condition, within 6 days 60% reduction of atrazine was observed and only 10% of 4-amino-2-chloro-1,3,5-triazine was formed along with some other metabolites which could not be identified. In this investigation, formation of deethylatrazine, deisopropylatrazine and deethyldeisopropylated atrazine was not detected. Initial atrazine concentration employed for the above studies were 30 mg l^{-1}. A bacterial isolate *Pseudomonas* [107] was reported to utilize atrazine as sole source of carbon. With an initial atrazine concentration of 50 mg l^{-1}, maximum of 45% atrazine degradation was observed after an incubation period of 5 weeks. But when cyclohexaheximide (used for controlling fungal growth) was added, the degradation observed was only 31%. This shows the importance of fungi in degrading atrazine. Dealkylation of both side chains were observed with preferential utilization of the isopropyl side chain but hydroxylation by dechlorination was not observed in pure culture so long as both the alkyl side chains were remaining. It was suspected that the presence of both the alkylated groups might be inhibitory for bacterial dechlorination but mono-dealkylated atrazine could be dechlorinated by the *pseudomonas* bacterial isolate. Atrazine could not be mineralized by this bacterial isolate. In contrary to the above observations, Adams and Thurman [119] detected deethylatrazine more at different soil depth than that of deisopropylatrazine and concluded that soil bacteria used ethyl side chain of atrazine more preferentially than that of isopropylatrazine side chain. This explains the frequent occurrence of deethylatrazine in unsaturated soils under agricultural fields. In another study the mineralization rate of the isopropyl side chain was only slightly faster than the mineralization of the atrazine ring [122]. But Skipper and Volk [113] showed that mineralization of ethyl side chain were about 8 times faster than the isopropyl side chain of atrazine. Radosevich *et al.* [124] isolated an atrazine degrading bacterial culture capable of using atrazine as the sole source of nitrogen and carbon in aerobic condition. This bacterium was claimed to be the first pure culture capable of partial mineralization of atrazine through ring cleavage. The organism degraded atrazine aerobically in the presence or absence of any external carbon and/or nitrogen source. *Rhodococcus* strains *TE-1* [125] and *B-30* [126] degraded the herbicide EPTC (Ethyl dipropylthiocarbamate), metabolized atrazine to deethyl- and deisopropylatrazine in aerobic condition. Those metabolites were not degraded further. Among atrazine, propazine and simazine, atrazine degradation rate to its dealkylated products was more rapid than the other two. A *Pseudomonas* sp. Strain *Yaya 6* was able to rapidly mineralize atrazine from soil [127]. *Agrobacterium Radiobactor, J14a,* grown in nitrogen free medium with citrate and sucrose as carbon sources mineralized 94% of 50 mg l^{-1} of ^{14}C atrazine in 72 hours [128]. Protzman *et al.* [129], observed that *Agrobacterium Radiobactor J14a* degraded atrazine with an initial concentration of 30 mg l^{-1} to less than 1 mg l^{-1} within 12 hours from a sequencing batch biofilm reactor (SBBR) with a hydraulic retention time of 2 days.

Atrazine biodegradation in oxygen deficient conditions

Pesticide degradation by anaerobic process, especially chlorinated pesticides has been proved to be more effective than that of aerobic process. Many pollutants, which were considered to be recalcitrant in aerobic activated sludge process, were possible to degrade in anaerobic process. Guenzi and Beard [87] observed that normally persistent DDT was converted to DDD relatively faster in an anaerobic environment. Several researchers [87,130-131] emphasized the need for assessing the importance of microbial degradation of pesticides with limited oxygen supply. Seldick [132] observed that sewage sludge degrades [^{14}C] cyanuric acid, under anaerobic condition, but not in aerobic condition. Atrazine and other s-triazine biodegradation in different food and environmental conditions have been extensively reviewed [84,110,133] . There are several reports on the atrazine biodegradation

in oxygen deficient condition, which suggest that atrazine biodegradation was quite possible in facultative, anoxic and anaerobic conditions. Jessee *et al.* [118] observed that a facultative anaerobic bacterium could degrade about 40 mg l^{-1} out of 75 mg l^{-1} atrazine in one week time under anaerobic conditions. For first three days, rapid degradation of atrazine was observed. After 3 days, the bacteria reached stationary phase. In the above investigation, the inoculum used was cyanuric acid (CA) degrading microorganisms collected from a pond receiving wastewater from CA manufacturing industry. Chung et al. [95] studied the anaerobic biotransformation of atrazine in wetland sediments receiving wastewater from a local sugar mill with an initial atrazine concentration of 10 mg l^{-1} by co-metabolic process using various carbon and energy sources like methanol, sodium acetate, acetic acid and glucose to get assimilative organic carbon of 145 mg l^{-1}. About 20% of total atrazine was transformed into non-triazine species after an anaerobic incubation period of 38 weeks. This 20% reduction was assumed to be the mineralization of atrazine to end products like NH_3 and CO_2. Hydroxyatrazine was detected as the only primary metabolite produced that is non-phytotoxic in nature. The phytotoxicity of atrazine get destroyed by hydroxylation in 2-position but not by dealkylation of the two alkylamino groups [112]. Thus, hydroxyatrazine that was detected as the major degraded metabolites of atrazine [93,134] is nonphytotoxic in nature whereas deethylatrazine and deisopropylatrazine are phytotoxic. In co metabolic process where dextrose was used as external carbon source, mixed microbes in UASB reactor could remove up to about 50% of atrazine from wastewater [135], whereas about 65% of atrazine removal was achieved in a sequential batch hybrid reactor [98]. Higher reduction in hybrid reactor was due to combined removal of atrazine by wood charcoal (used as supporting material) and mixed microbial anaerobic bacteria. Stucki *et al.* [134] has observed rapid biotransformation and mineralization of atrazine by enriched microbial culture under denitrifying condition. With an initial atrazine concentration of 7.5 mg l^{-1}, within 30 minutes the effluent atrazine concentration was 10-100µg l^{-1}. Hydroxyatrazine was the main primary metabolite with a concentration range of 250-780 µg l^{-1}. Bacterial isolate *M91-3* [136] was able to utilize atrazine as its sole source of carbon and nitrogen under anoxic conditions. The metabolite detected was hydroxyatrazine. In another study it was observed that Cyanazine competitively inhibited *M91-3* bacterial isolate in atrazine degradation [137]. *Pseudomonas sp.* Strain *ADP* could mineralize 55% and 75% of atrazine in 2 and 4 days respectively under denitrifying conditions [138].

Atrazine biodegradation under various environmental and operating conditions are listed in Table 2.

EFFECTS OF VARIOUS FACTORS ON ATRAZINE BIODEGRADATION

There are several factors, which play important roles on atrazine biodegradation. These are external carbon source, external nitrogen source, initial concentration, carbon/nitrogen ratio and water content (for soil treatment).

Effects of External Carbon Source on Atrazine Biodegradation

Diverse background organic matter may provide the carbon and energy necessary for a mixed microbial population to metabolize trace (0.5-100 mg l^{-1}) xenobiotic compounds [139-140]. In presence of primary carbon sources, combination of bacterial species was found to be more effective in biodegrading atrazine than individual organisms. Microbial mineralization of atrazine was directly related to the fraction of organic matter (f_{om}) in the contaminated soil [12,141]. Insufficient total assimilable organic carbon and or inorganic nutrients had negative effect on s-triazine degradation by co-substrate or co-metabolic [142] processes. *J14a* cells grown in nitrogen free medium with citrate and sucrose as carbon sources mineralized 94% of 50 μg (^{14}C leveled) atrazine ml^{-1} in 72 hours whereas in medium without additional carbon and nitrogen sources, the microbes degraded atrazine, but the cell numbers did not increase. The degradation rate of atrazine by *J14a* was enhanced when a supplemented carbon source was added. Armstrong[8] observed that the disappearance rate of atrazine was more with higher organic matter content. Mineralization rate of ring-leveled atrazine was slow in the soil with lower organic matter content and low clay content [143]. Atrazine mineralization was enhanced after soil had been amended with 1000 mg/kg of mannitol [134].

Table 2. Work on biodegradation of atrazine

Ref. No.	Operating condition/ microorganism type	Performance			Remarks
		Initial atrazine	*Final atrazine or percentage removal*	*After*	
95	Anaerobic/mixed culture/co-metabolic process	10 mg l^{-1}	20%	38 weeks	Anaerobic sediment batch bioreactor/dextrose as external carbon source
98	Anaerobic/mixed culture	1.0 mg l^{-1}	40%	5 days	Sequential mode of operation/ dextrose as external carbon source
		1.0 mg l^{-1}	61.8%	34 days	Batch reactor/ No external carbon
		1.0 mg l-1	42%	150 days	Batch reactor/ No external carbon and nitrogen
107	Aerobic/ pure culture/ pseudomonas	Wide range	Up to 99.9%	3 weeks	Various combination of initial atrazine concentration in several culture media and operated in various mode
118	Facultative anaerobic bacterium	75 mg l^{-1}	40 mg l^{-1}	1 week	Experiment in test tube
122	Aerobic/ pure culture/ Nocardia	30 mg l^{-1}	60%	6 days	-
127	Aerobic/pseudomonas sp strain *Yaya6*	6 mg kg^{-1} of soil	Complete elimination	1day	0.3 g dry weight inoculant biomass/kg of soil
		6 mg kg^{-1} of soil	Complete elimination	5days	0.003g dry weight inoculant biomass/kg of soil
128	Aerobic/agrobacterium radiobactor, *J14a*	50 mg l^{-1}	94%	72 hrs	
129	Aerobic/agrobacterium radiobactor, *J14a*	30 mg l^{-1}	<1mg l^{-1}	12 hrs	
134	Denitrifying condition/ atrazine degrading enriched culture	7.5 mg l^{-1}	0.01-0.1 mg l^{-1}	30 min	
135	Anaerobic/granular sludge/ mixed culture/ Up-flow anaerobic sludge blanket (UASB) reactor	1-15 mg l^{-1}	45.2 % (Maximum)	8 hrs	Dextrose varying from 150 -2000 mg l^{-1}. Maximum degradation when atrazine = 5 mg l^{-1} and dextrose = 150 mg l^{-1}
136	Anoxic/ pure culture M91-3	21.6 mg l^{-1}	60%	6 days	Fixed film batch column system

The degradation rate of atrazine in a Sequential Batch Biofilm Reactor (SBBR) by *Agrobacterium radiobactor* strain *J14a* was enhanced when a supplemental carbon source was added [129]. Obiena and Green [53] had observed that hydrolysis of atrazine in Kappa soil (85%) was more than in Molokai soil (48%). Similar trend was observed by Goswami and Green [144] on atrazine mineralization in Kappa soil (0.59% of total atrazine as $^{14}CO_2$) and in Molokai soil (0.02% of total atrazine as $^{14}CO_2$). The high organic matter content and high microbial population of the Kappa soil probably enhanced degradation and mineralization of atrazine.

Some reports showed no effect of external carbon source on atrazine biodegradation. An increase in organic substance and incubation temperature did not influence the mineralization rate of benzene ring carbon of anilazine and the triazine ring carbon of dehydroxyanilazine significantly [145]. Even in the absence of organic amendments, as high as 73% of the atrazine was mineralized after 11 weeks by atrazine degrading enrichment culture in aerobic condition [146]. Initial atrazine concentration was 33 mg l^{-1}.

An important factor affecting the total microbial community and degrader population was the form of C-addition, which was more important than the C/N ratio [146]. Glucose, PTYG (Peptone, Trypton, Yeast extract and Glucose) and humic acid were reported to be effective as primary substrates for the cometabolism of atrazine in the Basalt Salt Medium, whereas sodium acetate and Mannitol were not effective in enrichment media [147]. Studies conducted to enhance the degradation rate by addition of readily biodegradable organic amendments showed that, although acetic acid enhanced the biotransformation from the parent compounds to hydroxyatrazine, only glucose improved the removal rate of the triazine species [95]. Ghosh and Philip [101] observed the effects of external carbon source on atrazine degradation by mixed culture anaerobic bacteria. In co-metabolic process (dextrose was the primary source of carbon and energy), there is certain ratio of primary substrate and the toxic compound, at which the degradation of atrazine was the maximum. When COD was about 2000 mg l^{-1}, atrazine degradation reduced drastically. This might be due to the inhibition on the secretion of atrazine degrading inducible enzyme. When the concentration of secondary component (atrazine) was very high (about 15 mg l^{-1}), inhibition, lack of energy and carbon source reduces the performance of the UASB reactor [135].

Effects of External Nitrogen Source on Atrazine Biodegradation

One molecule of atrazine contains five atoms of nitrogen. Atrazine may be utilized as a nitrogen source for bacterial growth [105]. *J14a* cells grown in nitrogen free medium with citrate and sucrose as carbon sources mineralize 94% of 50 μg ^{14}C leveled atrazine ml^{-1} in 72 hours [128]. Theoretically, addition of high C/N organic materials should induce nitrogen limitation and increase selective pressure for utilization of recalcitrant N sources like s-triazine, which contains N that can be used by certain bacteria and microbial consortia [105,147]. Exogenous inorganic nitrogen inhibited atrazine degradative activity suggesting that regulation of s-triazine and N-metabolism is linked in the bacterial isolate *M91-3* [137]. Many researchers reported the degradation of atrazine by various bacterial isolates using it as the sole carbon and nitrogen source. Cook and Hutter [148] isolated three strains of *Pseudomonas* and two strains of *Klebsiella pneumoniae* those were able to use s-triazines as sole and limiting source of nitrogen for growth. But, Alvey and Crowley [146] observed no effect of C/N ratio in the soil on atrazine mineralization. Nitrogen concentration exceeding 1 mg l^{-1} inhibited bacterial degradation of low concentration of xenobiotics [149]. Similar results were observed in case of atrazine biodegradation also. The presence of nitrogen more than 1 mg l^{-1} in Minimal Salt Nutrient (MSN) inhibited the biodegradation of atrazine by bacterial isolate *SL3* [140]. Chung *et al.* [150] observed the inhibition in atrazine biodegradation in soil treated with 2.0 mg l^{-1} NH_4NO_3. All treatments receiving supplemental inorganic nitrogen had a considerable lower rate of atrazine mineralization [146] by soil microbes. Ro and Chung

[151] observed inhibition of atrazine degradation in aerobic condition and suspected that high level of nitrogen source in the Basalt Salt Media (BSM) might be the cause of inhibition.

Contrary to the above results, several researchers observed no effect or enhanced biodegradation of atrazine by increasing the external nitrogen supply. Supplemented ammonia nitrate did not inhibit atrazine mineralization in aerobic condition [152]. Chung *et al*. [150] observed that BSM enhanced the atrazine degradation rate in anaerobic condition. Even at a concentration of 400 mg l^{-1} of ammonia nitrate no inhibition on atrazine biodegradation was observed under aerobic condition [113]. Donnelly *et al.* [109] reported that the total herbicide (2,4-D and atrazine) degradation by *mycorrhizal* fungi increased with an increase in nitrogen concentration. Simazine, having a very similar structure to atrazine, was degraded even in presence of 30 mg-N l^{-1} in aerobic condition[140].

Effects of Water Content on Atrazine Biodegradation

Hurle and Kibler [153] found that atrazine disappearance in sandy loam soil was affected by soil water content and that the half-life of atrazine increased with dry soil. Dry soil (15% field capacity) showed a very low rate of mineralization. Mineralization rate was more with the increase of water content up to a certain limit (40% of field capacity) and then remained almost same (up to 100% field capacity) [143]. The rate of atrazine removal by *Pseudomonas* species strain *Yaya6* from soil slurry was proportional to the water content of the soil and the amount of bacteria added to the soil [127].

BIO-REACTORS USED ON ATRAZINE BIODEGRADATION

Most of the earlier studies were conducted with the aim to understand the mechanism of atrazine degradation, isolation of bacterial strains having potential of atrazine degradation/mineralization and to find out the optimum conditions for the degradation. Not many researchers tried to develop a reactor system for the treatment of atrazine bearing wastewaters or the effect of atrazine on the existing biological wastewater treatment systems. Atrazine concentrations used in most of those cases were many folds more than the actual concentration observed in surface and subsurface water near the pesticide handling and distribution sites. Most of such studies were performed in aerobic suspended growth batch process [106-107,124-125,128,140,152]. Nair and Schnoor [122] studied the mineralization rate of atrazine in soil using aerobic and anoxic batch bioreactor and soil microcosm. Maximum water applied was in anoxic bioreactor, which was having 100% of the field capacity of the soil. To remove atrazine from drinking water by aerobic process, Feakin *et al.* [140] used a hybrid reactor with pure bacterial culture along with Granular Activated Carbon (GAC) as carrier material. Very recently, continuous flow aerobic biofilm reactor was used to assess the microbial reaction kinetics of the atrazine degraders [147]. Yenze-Kontchou and Gschwind [107], used batch and sequencing batch reactors for the treatment of atrazine bearing wastewater in aerobic process using *Pseudomonas* strain *yaya6*. Atrazine biodegradation in oxygen limiting conditions were performed in Fixed Bed Reactor (FBR) [134], Anaerobic Sediment Suspended Growth Reactor [95] and Anaerobic Fixed Film Reactor (FFR) [136]. Glass beads were used as inert material in FBR [134] and FFR [136]. Recently Ghosh and Philip [154] and Ghosh *et al.* [135] have reported the performance of a mixed microbial consortium on atrazine degradation in anaerobic sequential batch reactor and UASB reactor respectively. Various other reactors, like soil slurry reactor [155], soil microcosm [105,122,146] and soil perfusion system [8] were used to study the fate of atrazine in aerobic condition in soil. Moteith *et al.* [45] studied the fate of various pesticides, including atrazine in Activated Sludge Process (ASP).

CONCLUSION

Atrazine is a toxic and persistent organic matter in neutral condition. It is a common pollutant in surface and ground water and as soil contaminant. Among various treatment technologies, adsorption by activated carbon is the most suitable technique for the removal of atrazine from drinking water, whereas, biodegradation is the most suitable technique for its removal from wastewater and soil. Use of pure culture microorganism enhanced the biodegradation rate of atrazine. Although the biodegradation pathway of atrazine is not yet fully understood, presence of other biodegradable organic matter, nitrogenous source, carbon/nitrogen ratio and water content are other key factors, which control the rate of biodegradation. A little effort has been given so far on the development of a suitable bioreactor for the treatment of atrazine bearing wastewater.

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