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Microbial Biodegradation of S-triazine Herbicides in Soil

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Abstract

S-triazine herbicides have been or are used worldwide as a herbicides to control broadleaf and grassy weeds in corn, sorghum, and sugarcane in agriculture. They have been detected in surface and groundwater at levels exceeding the Environmental Protection. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is the most popular among the s-triazine herbicides is still in use in many developing countries to improve crop yields. Thus, its residues and metabolites are frequently detected in soil, ground and surface water. Researchers have demonstrated that atrazine has toxic effects on algae, aquatic plants, aquatic insects, fishes and mammals. Biodegradation of atrazine is a complex process depends on nature and amount of atrazine in soil or water. The biodegradation of atrazine in environment is limited by microorganisms available. The present review aim to summarize the possibility of microbes to degrade the s-triazine herbicides (i.e. atrazine) in soil.

Keywords : Microbes; S-triazines; Biodegradation

Microbial Biodegradation

During the twentieth century, the explosive development of chemical industries has produced a bewildering variety of chemicals compounds that have led to the modernization of our lifestyles. The large-scale production of a variety of chemical compounds has caused global deterioration of environmental quality. Microbial biodegradation has concentrated in recent years to getaways to clean up contaminated environments. It is nature's way of recycling wastes that it can break down organic substance to smaller compound by enzyme produced by living microbial organisms such as (bacteria – fungi – algae) [1-3]. The substances degraded by microorganisms are used as a source of energy and carbon source to plants. Biodegradable substance is generally organic material such as plants, animal substance and other substances originating from living organisms or artificial materials.

Some microorganisms have degrading range of compounds such as Hydrocarbons (PAHS), Pharmaceutical substance, Radionuclides, Pesticides, Herbicides and Metals [4]. The important bacteria that used in the biodegradation process include Bacillus, Pseudomonas, Klebsiella, Actinomycetes, Nocardia, Streptomyces, Thermoactinomycetes, Escherichia coli, Azotabacter and Alcaligenes [2].

Microbial biodegradation can be aerobically or an aerobically degradation. Aerobic biodegradation occurs with presence of oxygen that produced carbon dioxide. Many organic contaminates are degraded by aerobic bacteria and this process called aerobes. Aerobe is known as cellular respiration use oxygen to oxidize substrates in order to obtain energy. The activity of aerobic microbes can be measured by the amount of oxygen that consume or the amount of carbon dioxide that produce [5,6]. The main characteristics of aerobic microorganisms degrading organic pollutants include: (1) Optimizing the metabolic processes via contact between microbial cells and the organic pollutants since the chemicals must be available for the organisms, (2) The oxidative process is the initial intracellular attack of organic pollutants since the incorporation of oxygen is the enzymatic key reaction by oxygenases and peroxidases that would be catalyzed, (3) Organic pollutants will be convert step by step into intermediates by peripheral degradation pathway, (4) The central precursor metabolites will be used in biosynthesis of cell biomass [5].

Anaerobic biodegradation process is occurring without oxygen and produce methane. It contains four key biological and chemical stages (Hydrolysis – Acidogenesis – Acetogensis – Methanogensis). Hydrolysis is process of breaking the chains and dissolving the smaller molecules into solution such as (sugar, amino acid, fatty acid). Acidogenesis is process break down of the emailing components by fermentative bacteria. Acetogensis is process occurs by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen. Methanogensis utilize the intermediate products of the preceding stages and convert them to methane, carbon dioxide and water [6,7].

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S-Triazine compounds

Herbicides are one of environmental pollutants. They are selective and kill unwanted plant matrices while leaving the desired crop relatively unharmed. The mechanism of this herbicide family kill plants by binding to the quinine – binding protein in photosystem II that inhibit the electron transport in the photosynthesis process. S- Traizine is a family of herbicides also called 1, 3, 5 s-triazine. In 1895 the first synthesized of 1, 3, 5-triazine was unknowingly by Nef by treating hydrogen cyanide with ethanol in an ether solution saturated with hydrogen chloride. It is an organic chemical compound with the formula $(HCN)_3$. The general structural composition of S-triazine molecule is a heterocyclic six membered ring composed of alternating carbon and nitrogen atoms joined by double bonds. This type of a ring system necessitates a numbering of nomenclature in which the nitrogen atoms are labeled as 1, 3 and 5 with the 2, 4, and 6 carbon atoms .S-Triazine herbicides have been used extensively for control broadleaf and grassy weeds in corn, sorghum and sugarcane crops in agriculture. S-Triazine herbicides are also used on residential lawns and golf courses.

The extensive use of s-triazine herbicides in diverse countries causes environmental and health concern. The movement of s-triazine in soil has contributed to the contamination of surface- and groundwater in several countries [8]. S-triazine is toxic compounds acting as endocrine disrupters and cause potential human carcinogens. Therefore, they have environmental pollution and it has been classified as possible human carcinogen class by EPA [9]. The most common derivative of 1, 3, 5-triazine (S-Traizine) is 2,4,6-triamino-1,3,5-triazine, commonly known as melamine or cyanuramide. Another important derivative is 2, 4, 6-trihydroxy-1, 3, 5-triazine known as cyanuric acid. Cyanuric acid is only slightly water soluble and is thermally unstable. At high temperatures, it decomposes to form toxic cyanic acid. Trichloro-1, 3, 5-triazine (cyanuric chloride) is the starting point for the manufacture of many herbicides such as simazine and atrazine [10]. Atrazine and simazine are the most members of s-tiazine family among other less used compounds such as, dedthylhydroxysimazine, hydroxyatrazine, deethylatrazine, deisopropylatrazine, fluoroatrazine, propazine, terbutylazine, cyanuric acid and cyanazine[11]. Simazine (6-chloro-N, N'-diethyl-1, 3, 5-triazine-2, 4-diamine) is an herbicide used to control broad-leaf weeds and annual grasses in crop fields. Simazine was first introduced in 1956 by the Swiss company J. R. Geigy. Simazine uptake is via the roots of emerging seedlings. It inhibits the photosynthetic electron transport process in the plant leaves and causes to turn it to yellow and die [13] The general degradation pathways of Simazine (A) Where 2-hydroxy - 4, 6 Bis (ethlyamino)-s-triazine (B) is produced by hydrolysis. Photolytic loss of alkyl groups produces deisopropyl atrazine (C) and diamino chlorotriazine (D) [12,13].

Atrazine

Atrazine is the common name for an herbicide that is widely used to kill weeds. Atrazine (2-choloro - 4-ethylamino - 6 - isopropyl amino -1,3,5 – triazine) is an effective herbicide used worldwide for broadleaf weed control. Atrazine is used on crops corn, pineapples, sorghum, sugarcane, macadamia nuts, and on ever green tree farms and forever green forest re-growth. It used to keep weeds from growing on both high way and rail road rights of way. Atrazine sprayed on cropland's as forms liquid or granules or powder before grow [14,15]. The average half-life of atrazine in the soil ranges from 13 to 261 days, in river water more than 100 days or in sea water is around 10 days [16,17]. Atrazine is slowly degraded in the soil because there are several factors determine the persistence of atrazine in soil. Soil physical and chemical properties such a texture, organic matter content as well as the pH of the soil. In addition, chemical properties of the atrazine such as solubility, vapor pressure, and the susceptibility to chemical are affecting in its degradation.

Some trade names of atrazine are (Atazinax, Atranex, Candex, Cyazin, Fenatrol, Gesaprim, Primatol, Radazin, Strazine, Vectal, Zeapos). Some bacteria that able to degrade s-triazine have been isolated and characterized such as Pseudomonas, Arthrobacter, Chelatobacter, Agrobacterium, Rhodococus, Stenotrophomonas, Pseudominobacter and Nocardiodes [8,18]. In the 1980s Pseudomonas .sp strains A, D, and F and Klebsiella pneumonia strains 90 and 99 able to use s-triazine as nitrogen source. Pseudomonas sp strain ADP is able to mineralize atrazine [19]. Several gram negative and gram positive bacteria was isolated and used to degradation atrazine in USA [20,21]. Recently, Radosevich, Yanze-Kontchou, et al. [20,22] and Mandelbaum, et al. [19] have reported the isolation of pure bacterial cultures which have ability to use atrazine as sole N and/or C sources to support microbial growth under aerobic conditions.

Atrazine have effects on health that classified in three groups developmental reproductive and cancerous U. S. Department of Health & Human Services (U. S. D. H. H. S). In developmental causes post implantation losses, decrease in fetal body weight in complete bone formation, neuro development effects, delayed puberty and impaired development of reproductive system. The effects harmful on reproductive system include pre-term delivery, miss carriage and various birth defects. The cancerous effects include Non-Hodgkin's lymphoma, prostrate, brain, testes, breast and ovarian cancer [11].

Atrazine degradation

Atrazine used widespread and toxicity necessitates search for remediation technology. Several methods are available for remove atrazine from contaminated soil, water and wastewater such as chemical treatment, incineration, adsorption, phytoremediation and biodegradation [11].

Biodegradation of atrazine is a complex process depends on nature and amount of atrazine in soil or water. The biodegradation of atrazine in environment is limited by microorganism's available [19,23,24]. The major steps of atrazine degradation pathway are Hydrolysis, dealkylation, deamination and ring cleavage. Process dealkylation of amino groups to give 2–chloro 4–hydroxyl -6- amino– 1, 3, 5 triazine is unknown. In hydrolysis, atrazine degradation occurs by hydrolytic pathway is consist of three enzymatic steps catalyzed AtzA, AtzB and AtzC that hydrolysis the bound between c-cl and then Ethyl and isopropyl groups catalyzed and in the end of this process producing of cyanuric

acid that convert to ammonia and carbon dioxide by AtzD, AtzE and AtzF enzymes [25,26,27].

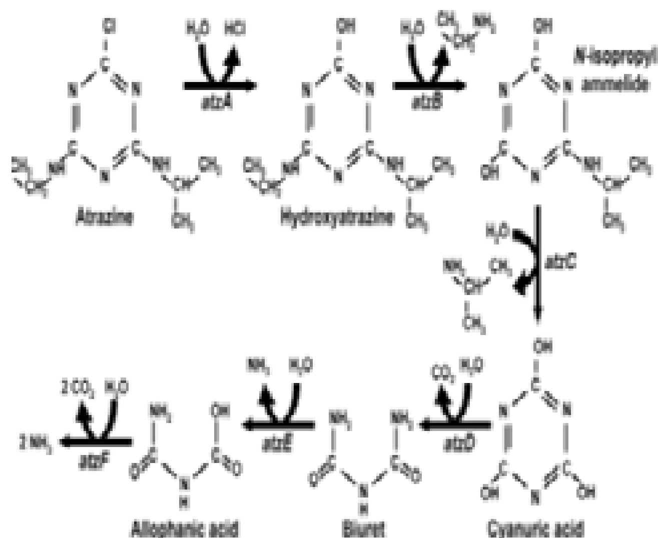

Figure 1: Schematic of the conserved hydrolyticatrazine-degradative pathway. Gene designations are those of Pseudomonassp strain ADP [28].

Some bacteria used to treatment or bioremediation of an environment contaminate i.e s–triazine compounds such as Pseudomonas sp strain MHP41, Pseudomonas sp strain ADP, Agrobacterium, Radiobacter strain J14a, Seudomonas sp, Rodococcus sp , Acinetobacter sp , Microbacterium sp, Bacillus sp, Micrococcus sp, Deinococcus sp, Delftia acidovorans, and Nocardia sp [29,19,30-32,24]. Pseudomonas sp. ADP is the best-characterized bacterial strain capable for degrading the herbicide atrazine. Enzyme response to dechloration atrazine is AtzA that converts atrazine to hydroxyatrazine. In a few Gram-positive strains, trzN gene is alternative for atzA. TrzN is a wide substrate range hydrolase that performs dechlorination of atrazine. In Pseudomonas sp ADP, the atzA, B, C genes are located on a large catabolic plasmid called p ADP-1 [33-36].

Figure 2: Physical circular map of the catabolic plasmid pADP-1 from Pseudomonas sp strain ADP [39].

The first three enzymatic encoded by genes atzA, atzB and atzC that transform atrazine to cyanuric acid. The atzA, atzB and atzC genes are not contiguous. These genes in Pseudomonas sp strain ADP have been localized to an approximately 100 kb plasmid (pADP-1) as shown in figure 2. Atrazine degradation generally initiate by a hydrolytic dechloration, catalyzed by enzyme atrazine chlorohydrolase (AtzA) encode by atzA gene. After wards two hydrolytic deamination reactions catalyzed by hydroxyatrazine ethlyamino-hydrolase (AtzB) encoded by atzB and N- isopropylammelide isopropyl-amino-hydrolase (AtzC) encode by atzC gene that produce cyanuric acid and respectively it converts to CO2 and NH3 by other three genes (AtzD, E, F) [37]. Cyanuric acid is intermediate pathways of atrazine biodegradation and its degradation occurs via hydrolytic cleavage of the atrazine ring and consecutive hydrolysis of biuret and allophanate to produce ammonium and carbon dioxide which is assimilated as a nitrogen source. The enzymes responsible for these reactions are encoded by the atzDEF operon. In Pseudomonas sp. ADP, the atzDEF operon encodes cyanuric acid amidohydrolase (AtzD), biuret amidohydrolase (AtzE), and allophanate hydrolase (AtzF) [26,28,38].

Regulation of atrazine degradation

Regulation of atrazine degradation in Pseudomonas sp. strain ADP is affected by nitrogen availability, since atrazine is used primarily as a nitrogen source by degrading strains. The atzR, encoding the LysR-type transcriptional regulator, regulates transcription of the cyanuric acid degradative operon atzDEF which catalyze degradation of cyanuric acid to ammonia and carbon dioxide. Six genes are co-transcribed from the PatzR promoter to form the atzRSTUVW operon to regulate atzDEF operon action. Briefly, NtrC gene activates PatzT transcription through DNA looping whereas AtzR gene works as anti activator. The atrazine mineralization depends on regulatory phenomena in response to nitrogen limitation and transcriptional activation by LysR- transcriptional regulators [39,28,40].

AtzR is a LysR-type transcriptional regulator (LTTR) that is the most transcription regulator protein in bacteria. Proteins from this family activate promoters transcribed from their own gene that response to catalyst to small molecule [41,26,42]. AtzR Transcription itself is dependent on the nitrogen control protein NtrC and the alternative s factor σN. Its activate transcription of the atzDEF operon and also acts as the repressor at its own promoter. Furthermore, AtzR has reacted with a binding region in the PatzDEF at one binding site called activation binding site (ABS) which located between the RBS and activated promoter and often located around -35 region of the PatzDEF. There are three sequences of ABS named as ABS1, ABS2 and ABS3. RBS is called (repression binding site) that is in site overlapping PatzR. It is generally recognized as the primary LTTR binding site and involved in atzR auto repression (negative autoregulation) [41]. The mechanism of regulation, under regular conditions, AtzR binds to ABS2 and ABS3 sequences that cause extended form of the protein, while the cyanuric acid is added, AtzR binds to ABS1 and ABS2 that compact the form of protein,

this structural change allows the RNA polymerase access to the promoter, thus enhancing gene transcription. AtzR binding on the atzR-atzDEF Promoter region marked by interaction of AtzR with these areas and reveals the structural transition in AtzR-DNA complex in response to the presence of cyanuric acid. Expression of both the atzDEF operon and atzR is increased under nitrogen limitation that requiring mediation of the activator of σN promoter NtrC [26,38,28].

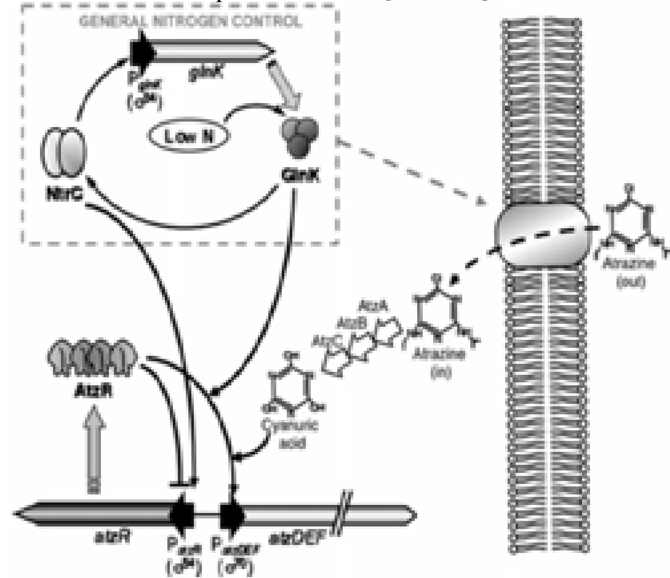

Figure 3: The Pseudomonas sp. ADP atrazine degradative gene occur by some elements of general nitrogen control such as (GlnD and NtrB) that used to regulate atrazine degradation in the cell [26].

Analytical methods for S-triazine herbicides in soil

Contaminated stream sediments are will be persistent for a longtime and a serious problem because of contaminant residues. Herbicide occurrence in the crop soils and especial attention should be paid to the presence of paraquat (PQ) in the studied vineyard-devoted soils due to the farmer application inputs. The solid state organic fraction with the clay mineral content considers the major factor governing the sorption of PQ and also making a significant contribution. The transport and mobilization of quats-enriched particles of soil may generate downstream and downslope environmental problems that affect detritivore ecology mainly [43,44]. paraquat can be separated by Liquid chromatography system including a P200 binary pump, a TSP SCM 1000 vacuum membrane degasser, an AS 1000 autosampler, and a UV 2000 detector. Separations can be done on a Luna C18 column (150 mm long 9 4.60 mm i.d., 5 lm particle size) obtained from Phenomenex (Madrid, Spain) and a guard column (4 mm long 9 2 mm i.d., 5 lm particle size) packed with the same material.) together with water containing 100 mM ammonium formate/formic pH 3/15 mM HFBA (A) and methanol (B). The gradient was: 90% A for 3 min, change to 90% B 10% A in 3 min, hold 5 min, change to 90% A and 10% B in 0.1 min and hold 9 min. The total analysis time was 20 min. The wavelengths used for detection were 258 nm for PQ. The injected volume was 50 lL and the LC flow-rate 0.7 mL/ min [44].

S-triazine herbicides in medium supernatant were, analyzed by reverse-phase high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) and was, extracted with equal volume of ethanol and to identify the potential metabolic products. A Luna C18 (2) 5μm, 100A column (150 × 2 mm, Phenomenex) with a mobile phases containing 5 mM acetic acid (pH 4.5) (A) and acetonitrile (B) at 35°C. A gradient was run at 0.5 mL min-1 from zero to 60 % B within 12 min. Spectra was recorded in the range from 190 to 800 nm at ca 1 Hz. For quantification the UV absorption signals at λ = 264 nm was applied. HPLC method was standardized using standard atrazine and intermediate metabolites. Mass spectra were recorded from 100 to 300 m/z. Vaporisation was achieved with 3.1 bar (45 psi) nebulizer pressure and 10 mL min-1 drying gas flow at 350°C. The atmospheric pressure electrospray ionisation (AP-ESI) interface was operated in negative mode with a capillary voltage of 5500 V [27].

Degradation the intermediate cyanuric acid metabolite by AtzD Enzyme

In Pseudomonas sp. ADP, atzD gene, the part of an operon that encodes for cyanuric acid degradation into carbon dioxide and ammonia, releases ammonia to support cell growth. AtzD (cyanuric acid hydrolase) initiates the degradation by opening the s-triazine ring to yield biuret and carbon dioxide, which represents an apparent cleavage of two carbon to nitrogen bonds within the six-membered ring of cyanuric acid. The AtzD enzyme is a member of a family of ring-opening amidases which first identified from Acidovorax citrulli strain 12227 and Enterobacter cloacae strain 99 [45].

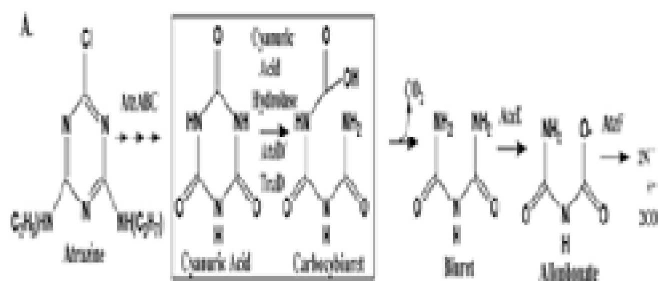

Figure 4: Metabolic pathways showing the reactions catalyzed by cyanuric acid hydrolase [45].

AtzD gene is exist in so many bacterial strains belong to many genera including Frankia bacteria such as (Pseudomonas sp ADP, accession AAK50331), (Arthrobacter sp AD25, accession ABK41866), (Moorella thermoacetica ATCC 39073, accession YP430955), (Bradyrhizobium sp. YR681, accession ZP10581004), (Clostridium asparagiforme DSM 15981, accession ZP03758143), (Methylobacterium radiotolerans JCM 2831, accession YP001757420), (Acidithiobacillus ferrivorans SS3, accession YP004783181), (A. caulinodans ORS 571, accession YP001526119), (Agrobacterium vitis S4, accession YP002547456), (Rhizobium sp. CCGE 510, accession ZP10837886), (R. leguminosarum bv. trifolii WSM2297, accession EJC83804), (Hydrogenophaga sp. PBC, accession ZP10152173), (Gordonia sp. KTR9, accession YP006671162), (S. acidophilus TPY, accession YP004719285), (Rhodococcus sp. Mel, accession AEX65082), ,(Micromonas sp. RCC299, accession XP002503480), (Rhodococcus erythropolis, accession CAC86669), (Frankia sp. EuI1c, accession YP004017027), (Bacillus cellulosilyticus DSM 2522, accession YP004094229), (R. erythropolis PR4, accession YP002769329), (Nocardioides sp. JS614, accession YP922706),

(Saccharomonospora viridis DSM 43017, accession YP003133937), (Streptosporangium roseum DSM 43021, accession YP003341997), (Bacillus alcalophilus ATCC 27647, accession ZP10819722), [39,45-48].

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