# Biodegradation of Atrazine (herbicide) using *Pseudomonas sp*.

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**ABSTRACT**

***Atrazine is one of the most environmentally prevalent s- triazine ring herbicide. The widespread use of Atrazine and its toxicity necessitates search for remediation technology. As Atrazine is still used in India as a major herbicide, exploration of Atrazine degrading microorganisms are of immense importance. Considering lack of reports on Bacteria as degrader of Atrazine, studies were carried out on Atrazine degradation using mixed culture of Pseudomonas, a Bacterial species isolated from contaminated agricultural soil. The influence of temperature on growth and Atrazine degradation were studied. The optimum growth conditions for Atrazine degradation Pseudomonas sp. was found to be at temperature 30 °C, shaking speed 120 rpm, inoculum size 10 % (w/v) with initial Atrazine concentration 50 ppm.***

***Keywords: Atrazine, Biodegradation, Temperature, Pseudomonas***

## 1. INTRODUCTION

Atrazine (2-chloro-4-(ethyl amino)-6-(isopropyl amino)-1, 3, 5-triazine is a selective, systemic triazine herbicide, belonging to the family of the s-triazines, widely used for the control of annual broadleaf and grassy weeds in corn, maize and low brush blueberries. Atrazine is worldwide used, often in combination with other herbicides (Cyclon *et al*., 2009), to control broadleaf and grassy weeds in agriculture, especially in corn, sorghum and sugar cane crops and in conifer reforestation planting (Rigas *et al*., 2005).

Although Atrazine is an effective herbicide, extensive toxicological investigations (Biradar and Rayburn, 1995; Allran and Karasov, 2000) have motivated and continue to motivate bioremediation directed research.

The degradation of Atrazine occurs through one of two pathways; it can be dehalogenated to form hydroxyAtrazine (HYA) or dealkylated to form deisopropylAtrazine (DIA) or deethylAtrazine (DEA). Without dehalogenation, the dealkylated metabolites still retain the phytotoxic properties and possibly the endocrine-disrupting potency of Atrazine, making further degradation or removal of metabolites desirable.

The toxicity of Atrazine has been researched in a variety of animals. Studies on Atrazine levels in fish species revealed that Atrazine does not tend to bioconcentrate, like the infamous pesticide DDT (dichlorodiphenyltrichloroethane). Male frogs in water contaminated with greater than 0.1 µg Atrazine /L show hermaphroditic and retarded gonadal development. In rodents, Atrazine is embryotoxic and embryolethal, but not teratogenic (Villanueva *et al*., 2005). In adult rats, Atrazine causes mammary gland tumors. Though this cancer mechanism is different in humans, it doesn't rule out the possibility of reproductive developmental effects by another mechanism. Health effects in humans from acute exposure to Atrazine levels above the maximum contaminant level include "congestion of heart, lungs and kidneys; hypotension; antidiuresis; muscle spasms; weight loss; adrenal degeneration".

Bacteria provide an alternative method for Atrazine removal, either by degradation or mineralization. Atrazine degradation is the disappearance of the parent compound, Atrazine, into intermediate compounds, or metabolites; Atrazine mineralization is the complete transformation of Atrazine and its metabolites into carbon dioxide.

## 2. MATERIALS AND METHODS

All the chemicals used in the present investigation were of analytical reagent grade (purity>98%). Rallis India Limited, Mumbai, India, supplied Atrazine of technical grade (purity=98%).

Different Solutions of Atrazine were prepared. Atrazine was dissolved in deionized water with 1% ethanol to a concentration of 20 mg/L.

The solutions was then carefully diluted with deionized water using pipettes, test tubes and graduated cylinders to obtain a series of different, but known concentrations in order to create a calibration curve. The curve establishes a relationship between aqueous concentration and absorbance measured by the UV spectrophotometer. A sample of each dilution series was pipette into a cuvette and placed in the UV spectrophotometer. A full scan was done to get the lambda maximum. It was found to be 225 nm.

The Instrument was set to at 225 nm. A cuvette with deionized water was used as a blank to zero the spectrophotometer before measurement of each group of samples. Using aqueous solution of Atrazine of concentration 20 mg/L stock solution were prepared with varying volumes. Different concentrations of solutions were then prepared to take absorbance at 225 nm in which the U V. spectrophotometer was set. Figure 1 shows the calibration of Atrazine.

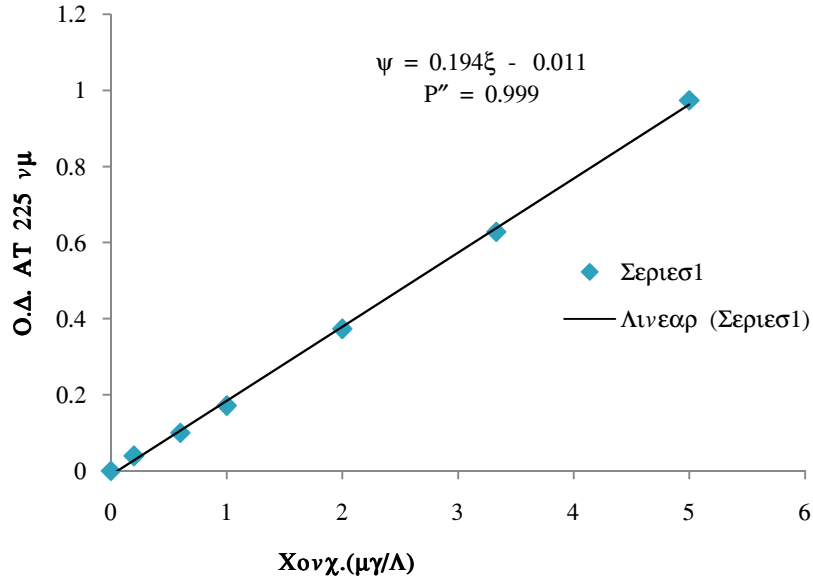


**Figure 1: Atrazine calibration curve.**

## 3. RESULTS AND DISCUSSION

To investigate the effect of growth parameters on Atrazine degradation, *Pseudomonas* isolate (10 % (w/v)) was grown in Atrazine (50 ppm) containing liquid medium of pH 6.0 maintained in 100 ml Erlenmeyer flask for 25 days on an orbital shaker at 120 rpm. The effects of growth parameter i.e. temperature (20- 30°C), on Atrazine degradation were studied in the medium containing Atrazine as sole carbon and nitrogen source. The Atrazine degrading efficiency of the isolated species was tested by varying growth temperature in the range of 20-30°C. During the experiment, samples were collected periodically at different intervals of time for estimation of biomass and Atrazine degradation. All the experiments were done in doublets to reduce experimental errors.

Atrazine degradation in media containing Atrazine as sole carbon and nitrogen source showed the maximum degradation at different temperature range. It reaches maximum degradation more early (16th day) in case of temperature 30°C (Figure 4) as compared to 25°C (Figure 3).The degradation rate was more at 25°C(Figure 3) but less than at temperature 30°C.

Laboratory and field studies by Shapir *et al*. (1998) and Katz *et al*. (2000) showed increased population sizes and herbicide degradation with the provision of tri-sodium citrate as a supplementary carbon source. However, several authors (Grigg *et al*. 1997; Ames and Hoyle 1999; Gebendinger and Radosevich, 1999) have reported that the concentrations and forms of C and N are major determinants of Atrazine mineralization. For example, inorganic nitrogen rather than

organic nitrogen (plant debris/in situ soil organic matter) stimulated pesticide catabolism and glucose addition of ≤16gCkg$^{-1}$ soil facilitated the formation of bound Atrazine residues and, thus, reduced its bioavailability while ≥16gCkg$^{-1}$ soil enhanced dealkylation (Abdelhafid *et al*., 2000). Further work is, therefore, required before nitrogen and/or carbon supplementations can be introduced on a routine basis into site bioremediation strategies. In soils, the half-life of Atrazine is 15-20 days with little mineralization of the s- triazine ring by indigenous bacteria (Topp, 2001). The time required for indigenous bacteria to mineralize the s-triazine ring, thereby degrading Atrazine into less toxic metabolites, has been estimated to be 20-60+days. Complete mineralization is estimated to occur only to less than 40% of applied Atrazine. However, more rapid mineralization of Atrazine has been reported in agricultural soils that frequently come in contact with Atrazine. Repeated dosing of Atrazine naturally selects bacteria with an enhanced ability to degrade Atrazine. Isolation of some of these indigenous Atrazine -degrading bacteria began in the nineties and continues to the present day. Various temperature ranges were studied for the degradation and it has been found that the optimum degradation of Atrazine is at temperature 30°C. Further studies are required to determine the effect of other growth parameters such as concentration of Atrazine and pH on the degradation of Atrazine and *Pseudomonas* growth.

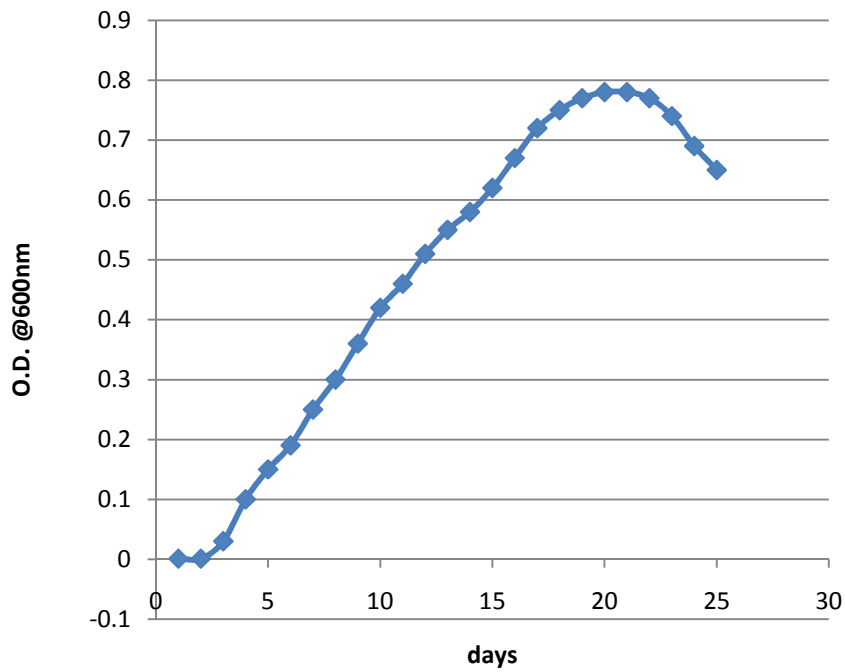


**Figure 2: *Pseudomonas* Growth Curve utilizing Atrazine at 20 °C.**

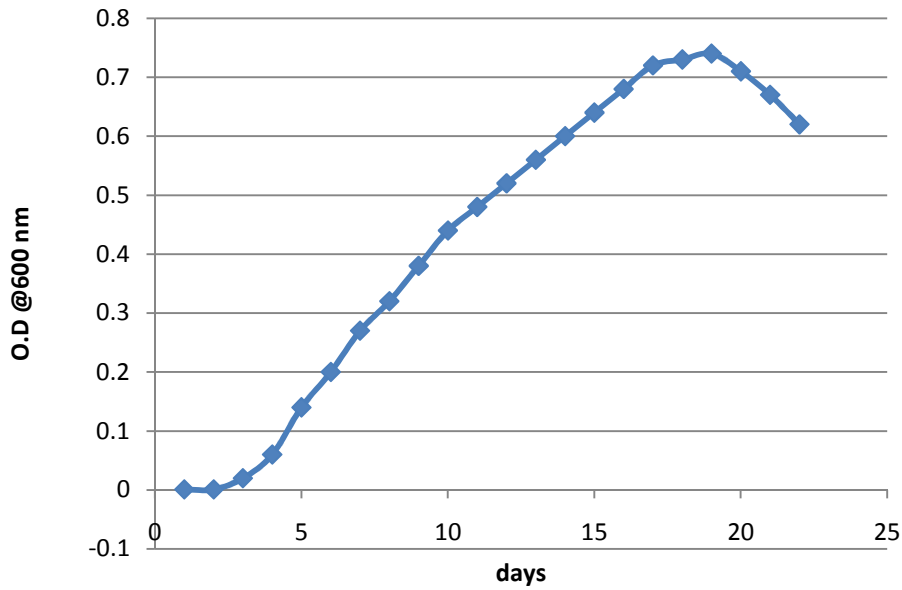


**Figure 3: *Pseudomonas* Growth Curve utilizing Atrazine at 25°C.**

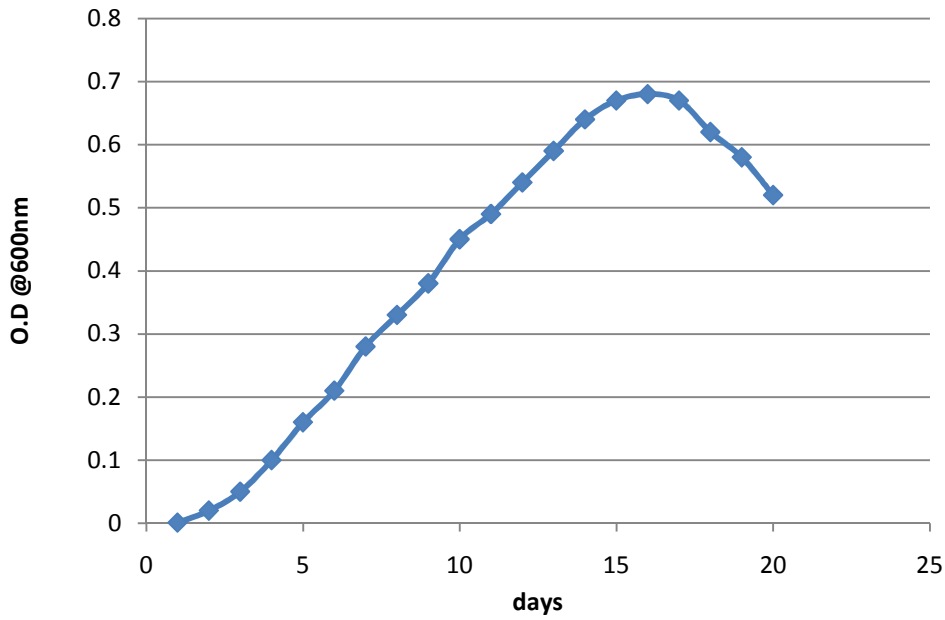


**Figure 4: Pseudomonas Growth Curve utilizing Atrazine at 30°C.**

## 4. CONCLUSION

The biodegradation of Atrazine and other herbicides in the environment is a complex process. Microorganisms such as bacteria and fungi are the key agents of bioremediation, with bacteria assuming the dominant role and fungi becoming more significant. However, factors influencing the degradation, such as the content and concentration of the Atrazine present, the physical and chemical environmental conditions and the composition of the microbial consortia, decide the rate of the overall microbial degradation processes.

Although natural environment is capable of degrading these toxic pollutants, but the long time scales involved to treat them make the process to be enhanced or creating new approach. The new approach involves the pollutants to be treated by mankind.

Considering that bioremediation technology has proven to be effective, economic and eco-friendly for the treatment of Atrazine -contaminated soils, focused research in some specific areas is desired for more effective treatment design and performance. Atrazine degradation in media containing Atrazine as sole carbon and nitrogen source showed the maximum degradation at different temperature range. From the experiments, It is found that the optimum temperature for degradation of Atrazine by *Pseudomonas sp*. is 30°C.

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