Effect of Various Environmental Parameters on Biosorptive Removal of Atrazine from Water Environment

Raj Kumar Pathak and Anil Kumar Dikshit Member, IACSIT

Abstract—Weeds are major threat to crop yield in face of global population growth and increasing food demand. These have significant impact on production of food, fiber and fuel. Atrazine is key component of proactive and reductive management system especially for managing herbicide resistant weeds and glyphosate resistant weeds. Approximately 32 million kg of atrazine are used each year for crop production in the United States. Recent reports have raised concern about continued use of atrazine and several other herbicides because of their negative impacts on aquatic life and potential endangerment of animal/ human health and environment. Identification of novel fungal species capable of degrading these herbicides provides evidence for the vast diversity in microbial communities that still remains to be explored. Present paper discussed an attempt to biosorb atrazine on an insolated fungal strain and to study the effect of various parameters on biosorption of atrazine.

Index Terms—Atrazine, bioremediation, biosorption, degradation.

I. INTRODUCTION

The availability of the triazine herbicides in the late 1950s revolutionized weed control in corn and grain sorghum. By the mid-1960s, atrazine had attained a record share of the corn herbicide acreage. Today, approximately 65–70% of US corn acreage is treated with atrazine. Atrazine is a selective systemic herbicide. The mode of action for atrazine is through inhibition of photosynthesis in the target plants. Atrazine is water-soluble and can be transported in dissolved form from one place to other [1]-[3]. Atrazine is quite susceptible to leaching and/ or runoff. Atrazine is commonly detected in soil, surface water supplies and groundwater [4-8]. There is little evidence that native microbial populations in estuarine sediments are capable of degrading atrazine.

Wicks et al. found that atrazine provides selective weed control by conserving moisture through superior weed control and reduced tillage, since tillage tends to dry out the soil in the tilled zone [9]. Earlier studies showed that atrazine was better degraded in anaerobic conditions than in aerobic ones and those which were earlier considered as recalcitrant in aerobic process, were successfully degraded in anaerobic conditions. Recent studies have shown that the aromatic structure of atrazine could be metabolized to gaseous end products in mixed cultures under methanogenic conditions [10]. Information concerning the anaerobic degradability and fate of atrazine in biologic reactors is limited, with only some kinetic studies [11] and some batch and continuous studies performed under anaerobic and aerobic conditions [12].

There are a number of options available for treatment of atrazine contaminated water. Removal of atrazine appears to be dependent on the treatment technology employed, source water and the presence of natural organic matter (NOM). Main methods used include physical, chemical, and biological treatments. Physical remediation technologies include incineration, air stripping, hydraulic destruction, aeration, foam fractionation and activated carbon absorption, soil flushing/ washing, ion exchange, and membrane separation. Remedial chemical treatments include neutralization, reduction-oxidation, photolysis, and dechlorination [13]-15]. Biological treatments primarily include bioaugmentation, biodegradation through the enrichment of soil indigenous microorganisms, and biostimulation through the inoculation of contaminated soils with microorganisms adapted for the degradation of a particular toxic chemical as well as biosorption [16].

Millner et al. [17] investigated the removal of eight pesticides (organochlorine and organophosphate) by the conventional methods such as alum coagulation, clarification, softening and chlorination, filtration and sedimentation and concluded that these methods are not effective for removing atrazine. The same results were confirmed by other groups of researchers [18],[19]. Coagulation with alum was attempted by Aly and Faust [20] and got little success.

Technologies that are reported to be at least partially effective include adsorption by activated carbon, ozonation, and membrane filtration. These may be used individually or combined with other treatment processes [21],[22]. Although granular activated carbon (GAC) is recommended as the best available technology for the removal of atrazine [23], studies have shown that other water treatment technologies may have better removal efficiencies. In early sixties, biological methods were attempted for the removal of pesticides. Other approaches that are currently being developed include application of immobilized enzymes and the use of plants to contain or transform pollutants through phytoremediation.

Sorption is the common term used for both absorption and adsorption. These terms are often confused. Adsorption is the incorporation of a substance in one state into another of a different state (e.g., liquids being absorbed by a solid or gases being absorbed by water). Adsorption is the physical adherence or bonding of ions and molecules onto the surface of another molecule. It is the most common form of sorption used in cleanup.

Manuscript received February 6, 2012; revised February 27, 2012.
Raj Kumar Pathak is with Centre for Environmental Science and Engineering, Indian Institute of Technology Bombay, and Department of Biotechnology, Thadomal Shahani Engineering College, Bandra (W), Mumbai, India (email: rkpathak2k@iitb.ac.in).
Anil Kumar Dikshiti is with the Centre for Environmental Science and Engineering, Indian Institute of Technology Bombay, Mumbai, India (shabirami@iitb.ac.in; dikshit@iitb.ac.in).
Sorption by dead or alive cells through metabolism independent processes is termed biosorption. It is an energy-independent, growth independent and surface-binding phenomenon. The special surface properties of bacteria, yeasts, fungi and algae enable them to adsorb different kinds of pollutants from solutions. “Biosorption” is used to indicate a number of metabolism-independent processes (physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation, and micro precipitation) taking place essentially in the cell wall rather than oxidation though anaerobic or aerobic metabolism (biodegradation) [24]. Following are the important considerations in this regard:

Continuous supply of nutrients do not require for a dead cells. They can be stored or used for extended periods at room temperature without putrefactions occurring. High flow rates and biomass concentration without clogging of the system is possible. They are not affected by toxic wastes. Accumulation of pollutants onto dead cells has been found to be same or more as compared to live cells. Dead cells biosorbent can be more easily separated from the sorbate. They can be regenerated and reused for many cycles.

Raw materials for biosorption are either abundant (e.g. sea weeds) or wastes from other industrial operations (e.g. fermentation wastes, activated sludge process wastes). Removal of hazardous organics, both living and dead (heat killed, dried, acid and/or otherwise chemically treated) biomass can be used. In comparison to live cells, dead cells are more efficient in technical and economical aspect in most of the cases.

Lots of efforts have been made towards the development of biosorption technique for the uptake of heavy metals, dyes and phenols. But, so far uptake of pesticides by biosorption has not received much attention. There have been some studies [25,27] on the biosorption of pesticides by dead and live bacteria. However, in these studies the objective has been to assess pesticide (lindane, diazinon, malathion and 2-chlorobiphenyl) uptake potential of activated sludge and Rhizopus arrhizus.

The negative charge due to dissociation of the carboxylic group and due to electrostatic repulsion effects at pH 6.0 may be the reason for lower adsorption. It was concluded that dead biomass of microorganism could remove these molecules from the medium more effectively than live cells. It is reported that extracellular polymeric substances might also be involved in the biosorption process due to the attachment to the microorganisms itself.

Different kinds of biomass, such as fungal and yeast biomass [28], bacterial biomass [29,30], algal biomass [31] and so on have been studied for their biosorption. Biosorption performance has been affected by the species, growth conditions such as culture medium, physiological state and age of the organism [32].

Microbial biomass, such as fungi, would be particularly cost effective as there are many food-processing plants in many countries that could provide wastewater as substrate at a very low cost for the cultivation of these. Important fungal biosorbents include Aspergillus [33], Penicillium [34] and Rhizopus [35]. Even if the mechanisms regulating biosorption have not yet been fully explained, it seems to take place essentially at the cell wall level [24]. The cell wall of fungi consists of amino or nonamino-polysaccharides.

Fungal biosorption has been studied more extensively because of the amenability of the microorganisms to genetic and morphological manipulation [36]. Most fungi are robust organisms and are generally more tolerant to high concentrations of polluting chemicals than are bacteria.

The extent of biosorption not only depends on the type of adsorbate, but also on the bacterial genus, due to variations in the cellular constituents. The bacterial cell wall provides structural integrity to the cell, but differs from that of all other organisms due to the presence of peptidoglycan (poly-N-acetylg glucosamine and N-acetylmuramic acid), which is located immediately outside of the cytoplasmic membrane [37]. Extracellular polysaccharides are also capable of binding organic matter or metals [37, 38].

Gram-positive bacteria are comprised of a thick peptidoglycan layer than Gram-negative bacteria. The anionic functional groups present in the peptidoglycan, teichoic acids and teichuronic acids of Gram-positive bacteria, and the peptidoglycan, phospholipids and lipopolysaccharides of Gram-negative bacteria were the components primarily responsible for the anionic character and organic/inorganic pollutant binding capability of the cell wall. Extracellular polysaccharides are also capable of binding organic matter or metals [38].

In the world, most easily available biomass is algae. Their ubiquitous distribution and their central role in the fixation and turnover of carbon and other nutrient elements, are responsible for their use as biosorbent material. There is no need for immobilization as the biomass particles from algae are large enough. Both electrostatic attraction and complexation play a major role in biosorption. Algae biosorption is mainly attributed to their cell wall. The most common type of algae that possesses a complete cell wall is Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyta (brown algae) showed different sorption behaviour, due to the different structures of the cell wall polysaccharides. Green algae are mainly cellulose, and a high percentage of the cell wall are proteins bonded to polysaccharides to form glycoproteins. These compounds contain several functional groups (amino, carboxyl, sulphate, and hydroxyl) which could play an important role in the biosorption process [39].

The aim of the present research work was to determine the performance of fungal isolate obtained from atrazine contaminated field on biosorption of atrazine from water environment and the impact of various parameters on the same.

II. MATERIALS AND METHODS

A. Glassware and Chemicals

All the experimental work was carried out using “Borosil” glassware. The glassware was soaked in dilute chromic acid overnight, washed thoroughly using Lyzol solution followed by washing with tap water. All chemicals and reagents used in this study were of analytical grade (AR). Technical grade atrazine (99.4%) was provided by M/s Divyalakshmi Chemicals Pvt. Ltd., Bangalore, India. Acetone and
ethyelacetate were purchased from Merck India Ltd. Mumbai. Nutrient agar and nutrient were procured from Hi Media Laboratories Pvt. Ltd., Mumbai. Other chemicals like concentrated sulphuric acid (98%), crystal violet, ethanol, gram’s iodine, potassium dichromate, saffrane, sodium sulphate, were analytical grade. The reagents were prepared in the lab using standard procedure.

B. Fungal Biosorbent

1 g of atrazine contaminated soil was weighed carefully and suspended in 10 mL of sterile physiological saline (0.85%). The suspension was mixed in a Vortex Mixer (Trishul Equipments Mumbai) thoroughly for about 5 min to resolve clotted clay particles and was allowed for the formation of a uniform suspension. The master suspension was serially diluted to 10⁻¹⁰ times. The last three dilutions (10⁻⁸, 10⁻⁹ and 10⁻¹⁰) were selected for pour plate isolation. 1 mL of each dilution was mixed with 20 mL of sterile molten potato dextrose agar (PDA) containing the selective dye, Rose Bengal. The mixture was poured into sterile petriplates and allowed to solidify. Each dilution was poured in triplicates.

The plates were incubated at 37°C in an inverted position till substantial growths of fungal colonies were observed. From the number of observably different fungal colonies that developed, 13 isolates were segregated and plated onto PDA without Rose Bengal. The process was repeated until all 13 isolates were obtained as pure colonies. One particular isolate was selected on the basis of swift growth and atrazine uptake capacity.

Above fungal culture grown in potato dextrose broth was filtered through Whatman filter paper no 1. Filter papers were washed with ultrapure water three times and dried (50°C for two hrs) before filtering the fungal culture. After filtration filter paper containing fungal culture was kept in hot air oven at 60 ± 2°C for 12-24 hrs. The dried fungal cultures was separated from filter paper. Dry mass was collected and stored in 125 mL Torsion air tight bottles. Dried fungal biomass obtained was ground with a laboratory grinder and sieved through 200 mesh to give particle size in the range of 0.15 to 0.3 mm. Sieved biosorbent was stored in air tight container for further experiments.

C. Gas Chromatography (GC) Analysis

The sorption analysis was done using GC model 2014 (Shimadzu, Japan) with the injection port SPL1 of split type with injector temperature of 280°C and carrier gas as N₂. The column (Restek RXI-17) had a temperature range of 120-220°C (atrazine boiling point at 178°C) with an inner diameter of 0.25 mm, thickness 0.25 µm and length of 15 m. The ECD detector was used with detector temperature 300°C.

D. Effect of Dose

The effect of different doses of adsorbent was studied to find the optimum dose. The doses were selected from 100 mg to 1000 mg/L in different flasks. The samples were removed after 24 hr and analyzed for atrazine. Biomass concentration plays an important role in biosorption process.

E. Effect of pH

The removal of atrazine was studied at different values of pH. The pH was adjusted with HCl and NaOH. The pH value of aqueous phase, number arises from a measure of the activity of hydrogen ions or their equivalent in the solution, is important criteria for biosorption.

F. Effect of Temperature

The removal of atrazine was studied at three different temperatures 30, 40 and 50°C. The flasks were kept in shaker at 250 rpm at different temperatures and samples were removed, filtered, extracted and analyzed for presence of atrazine.

III. RESULTS AND DISCUSSIONS

The results of various observations are shown below.

A. Effect of Dose

Effect of dose on the removal of atrazine was studied. The result is shown in Fig. 1.

It can be seen that as the dose was increases, the amount of atrazine removal also increased from 50% to 95%. The optimum dose was found to be 600 mg/L at which removal was maximum. As concentration of biomass is increases removal of adsorbate is increases due to more biosorption sites are available. The result is similar to Ju et al. [30], who had studied the effect of initial biomass concentration of Bacillus megaterium for the biosorption of lindane at an initial lindane concentration of 4 mg/L. The results obtained showed that increased biomass concentration from 2 to 16 g/L had increase lindane biosorption capacity from 340 to 700 mg/g.

B. Effect of pH

Effect of pH was studied in the range of pH 2 to 12 and it is shown in Fig. 2. The maximum removal was obtained at 6 pH. Several studies have shown that in organic and inorganic compound biosorption by biological materials, pH is an important factor. There is an observed relationship between the organic and inorganic compound biosorption and the magnitude of negative charge on the surface of the biosorbent, which is related to the surface functional groups. Ju et al. [40] studied the effect of pH between 2 and 9 on the biosorption of lindane by Escherichia coli, Zoogloea ramigera, Bacillus megaterium and Bacillus subtilis. They found the isoelectric points of all bacteria at pH 2.0, except for Escherichia coli whose isoelectric point was at 3.0 and suggested that all cells were negatively charged under neutral conditions as well, the order in the magnitude of negative zeta potential at pH 7.0 will be Escherichia coli > Bacillus...
megaterium > Bacillus subtilis > Zoogloea ramigera. They observed higher biosorption under lower pH. The present study shows the similar trend.

![Effect of pH on removal of atrazine](image1)

**Effect of Temperature**

The removal of atrazine was studied at three different temperatures 30, 40 and 50°C. The results are shown in Fig. 3. The optimum removal was obtained at 30°C. Temperature is a crucial parameter in biosorption reactions as activation energy and thermodynamics properties of the process can be calculated on effect of temperature basis. It has been mentioned in the adsorption theory that adsorption decreases with increase in temperature and at elevated temperatures, molecules adsorbed earlier on a surface tend to desorb from the surface. However, temperature has not been studied as relevant variable in biosorption experiments.

Bell and Tsezos [25] studied the effect of temperature at 5 and 20°C for lindane, diazinon and malathion biosorption using activated sludge and Rhizopus arrhizus as a biomass. They observed that the biosorption of lindane was exothermic and the negative value of heat of biosorption was in the range where physical adsorption rather than chemisorption was the dominant mechanism, on both type of microorganisms. The present study shows the same trend.

![Effect of pH on removal of atrazine](image2)

**C. Effect of Temperature**

The removal of atrazine was studied at three different temperatures 30, 40 and 50°C. The results are shown in Fig. 3. The optimum removal was obtained at 30°C. Temperature is a crucial parameter in biosorption reactions as activation energy and thermodynamics properties of the process can be calculated on effect of temperature basis. It has been mentioned in the adsorption theory that adsorption decreases with increase in temperature and at elevated temperatures, molecules adsorbed earlier on a surface tend to desorb from the surface. However, temperature has not been studied as relevant variable in biosorption experiments.

Bell and Tsezos [25] studied the effect of temperature at 5 and 20°C for lindane, diazinon and malathion biosorption using activated sludge and Rhizopus arrhizus as a biomass. They observed that the biosorption of lindane was exothermic and the negative value of heat of biosorption was in the range where physical adsorption rather than chemisorption was the dominant mechanism, on both type of microorganisms. The present study shows the same trend.

![Effect of pH on removal of atrazine](image3)

**IV. CONCLUSIONS**

A particular fungal isolate could remove atrazine effectively. For its maximum removal, the biosorption system should maintain a dose of 600 mg/L at pH 6 at 30°C. The maximum achievable removal of atrazine could be 96% at the contact period of 8 hours.

**ACKNOWLEDGEMENT**

Authors thank M/s Divyalaxmi Chemicals Pvt Ltd., India for providing technical grade atrazine.

**REFERENCES**


293

Raj Kumar Pathak was born on 5th September, 1963 at district Jaunpur, Uttar Pradesh, India. He B.Sc. from Gorakhpur University in 1982. He did B.Tech. in Biochemical Engineering from H.B.T.I. Kanpur in 1985 and M. Tech in Biochemical Engineering from same institute in 1987. After M.Tech., he worked in various chemical industries as shift engineer. He joined Thadomal Shahani Engineering College Bandra as Lecturer and presently continuing the same college as associate professor. He is heading biotechnology department and currently pursuing Ph.D at Center for Environmental Science and Engineering at Indian Institute of Technology under supervision of Dr. Anil Kumar Dikshit. His work is on Biosorption of Atrazine from Wastewater. His field of specialization is Heat and Mass Transfer, Transport Phenomena and Enzyme Engineering. He has authored two books on Industrial Chemistry, a vocational course sponsored by UGC programme at B.Sc. level. He was a member for syllabus committee for Industrial chemistry ad also a very active committee member for setting the Syllabus for Biotechnology at BE level in Mumbai University. He has been also coordinator for central assessment system in University examinations. He has arranged several conferences, technical workshops and industrial tours. He is life member of ISTE and IICHE.


The areas of his interest in teaching are environmental systems planning and management; environmental impact assessment; water supply and wastewater engineering; environmental engineering; and solid waste management while the areas of research interest include water and wastewater treatment; environmental modelling and optimization; and GIS applications to water and environment related problems.

He is honorary professor of School of Civil Engineering, Survey and Construction, University of KwaZulu-Natal, Durban, South Africa. Also he is visiting professor of School of Civil and Environmental Engineering, Nanyang Technological University, Singapore.

He has guided 31 masters' and 8 doctoral scholars while 9 research scholars are working on their Ph.Ds. He has also worked on 30 national and international research and consultancy projects. He is having research collaboration with German, French, US, Singapore and South Africa Universities and Institutions. The results of academic research work has provided one patent, three books, 7 chapters in edited books, 91 publications in international and national journals, 98 in international and national conferences while many more papers are being reviewed. In addition, academic versions of a number of environmental modeling and simulation models, and a number of GIS applications on environmental management problems have been developed under his guidance. He is associate editor, reviewer or editorial board member for many environmental journals.


