Removal of Atrazine by coupling Fenton reaction with bioreactor in series

Sachin Rameshrao Geed, Avinash Raj, Manish Kumar Kureel, Vijay Pratap Singh, Sumit Kumar, Balendu Shekhar Giri, Birendra Nath Rai & Ram Sharan Singh*

Department of Chemical Engineering & Technology, Indian Institute of Technology (BHU), Varanasi-221 005, Uttar Pradesh, India

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Atrazine is a commonly used weedicide in agriculture fields. Owing to its long half-life (125 days) and slow-biodegradability, it adds to problematic residues in the environment. It is known to disrupt endocrine and reproductive systems and has potential to damage vital organs such as liver, kidney and heart. While atrazine is banned in European countries, many countries, such as India, China, and the USA it is still in use widely. In atrazine biodegradation, batch bioreactors are most common cost effective alternative to conventional methods. However, it has only major limitation of slow rate of degradation. In this work, we explored coupling of UV-Fenton and biological method for atrazine removal and also optimized the process parameters. In the bioreactor, Loofa was used as the packing media on which consortia was immobilized. The performance of coupled system was studied with an initial atrazine concentration of 300 mg/L. Overall, maximum removal efficiency of 93% was achieved for the coupled system. GC-MS analysis of residual treated effluent sample was performed to identify the intermediate compound. Two metabolites biuret and urea were identified which confirmed the degradation of atrazine. The growth kinetic parameters $\mu_{\text{max}}$ (0.224 per day) and $K_S$ (106.64) were calculated using Monod model. The coupling method was found superior than individual chemical and biological methods for treatment of atrazine.

Keyword: Biodegradation, Bioleaching, Biuret, Pollution, Residues, Urea, Weedicides

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is an extensively used herbicide against weeds, particularly in agricultural fields. Production of atrazine at 1200 MT in 2014-15 in India vouches for its usage. Due to its long half-life (125 days) and slow-biodegradability in water, atrazine is often leached into the environment and observed in soil and water. It acts as an endocrine disruptor in human and animal cells and also affects the reproductive system in human. It has potential to damage vital organs such as liver, kidney, and heart in animals as well as human. According to EPA, the maximum allowable concentration of atrazine in drinking water is 3 ppb. Atrazine is banned in European countries while many countries, like India, China and the USA it is still used widely. The conventional methods of removal of atrazine by wastewater treatment processes, such as coagulation, sedimentation, filtration, reverse osmosis and electrochemical oxidation have been proved not effective.

Biodegradation of atrazine, particularly in waste streams usually by different bioreactors, more so by batch bioreactors, are most common for they are good and cost effective alternative to conventional methods. The only major limitation of biodegradation is slow rate of degradation for compounds like atrazine which makes it unsuitable for practical operations. To overcome the limitations of biodegradation, the process may be coupled with other available processes to make overall treatment of atrazine more efficient and suitable for practical applications. In this context, many conventional processes were studied and hydroxyl radical (HO$^-$) based advanced oxidation processes (AOPs) were found most suitable to couple with biodegradation. Stand alone AOPs have many limitations, such as requirement of strong dose of oxidizing agents, long treatment time, cost and formation of highly unstable intermediates of the reaction byproducts than the parent compound.

Some Efforts to evaluate performance of coupled system by combining AOPs (UV-Fenton) and biological system for removal of herbicides (atrazine, cypermethrin, chlorpyrifos and chlorothanoil)
and pesticides including atrazine\textsuperscript{16,19} are not uncommon. The performance of the coupled systems were better than standalone systems. The coupled system helped each other to overcome their limitations.

In the present study, we evaluated the performance of coupled system containing Fenton’s oxidation and bioreactor in series for treatment of waste water stream containing atrazine. Specific microbes isolated (consortia) from agricultural runoff wastewater was used in the bioreactor. In the stage-I, the sample was treated in Fenton reactor, and then effluent was fed to stage-II for neutralization of pH. Finally, the bioreactor was operated in stage-III to degrade the atrazine.

**Material and Methods**

**Chemicals and mineral salt medium used**

Atrazine with more than 99.0% purity was purchased from Sigma Aldrich, India. The mineral salt medium (MSM) with composition in mg/L (KH\(_2\)PO\(_4\):840; K\(_2\)HPO\(_4\):750; (NH\(_4\))\(_2\)HPO\(_4\):474; NaCl: 60; CaCl\(_2\): 60; MgSO\(_4\)\(_7\)H\(_2\)O: 60; Fe(NH\(_4\))\(_2\)SO\(_4\)\(_6\)H\(_2\)O:20) and micronutrient solution with composition in mg/L of (H\(_3\)BO\(_3\):600; CoCl\(_2\):400; ZnSO\(_4\)\(_7\)H\(_2\)O:200; MnCl\(_2\):60; Na\(_2\)MoO\(_4\)\(_7\)H\(_2\)O:60; NiCl\(_2\):40; CuCl\(_2\):20)\textsuperscript{23} were purchased from Merck, Mumbai, India and used for grow and maintenance of microbial species in the bioreactor. MSM was autoclaved for 15 min at 121°C, and final pH was adjusted to 7.0±0.2. Further, bacterial strains (consortia) were inoculated for the experiment of atrazine degradation.

**Atrazine degrading potential microorganisms**

Atrazine contaminated soil samples were collected from Banaras Hindu University’s agricultural farm, Varanasi, India (25\textdegree19\textquoteleft N; 83\textdegree3\textquoteleft E, 129 m above mean sea level). The sampling site was used for intensive agricultural activity from several decades, and thus exposed to atrazine since long time. The enrichment of isolated bacterial consortia was done in MSM using atrazine as a sole carbon source. To analyze the morphology of consortia using scanning electron microscope (SEM QUANTA 200F, Netherland), the Loofa sample containing bacterium was cut into small pieces, dried overnight at 30°C in the oven (Model 159SS NSW, India) and finally coated with gold and carbon particles for better resolution. The SEM analysis was performed under low pressure\textsuperscript{21}.

**Parametric optimization in batch process**

*UV Fenton*

All the experiments were carried out in 1 L glass reactor as shown in Fig. 1 at room temperature (23±3°C) and stirred at 200 rpm for 1 h. The parametric effects such as mole ratio of H\(_2\)O\(_2\)/COD (0.25-1.5), H\(_2\)O\(_2\)/Fe\(^{2+}\) (5-50) and pH (1.0–5.0) on UV-Fenton reaction were studied in the Fenton reactor to obtain the optimum condition keeping the atrazine concentration fixed at 150 mg/L. The effect of H\(_2\)O\(_2\)/COD molar ratio was examined by varying the ratio from 0.25 to 1.5 keeping the other operating parameters fixed (pH 2.5, H\(_2\)O\(_2\)/Fe\(^{2+}\) 25, initial COD 2900 mg/L, reaction time 60 min and 200 rpm).

Similarly, the effects of pH and H\(_2\)O\(_2\)/Fe\(^{2+}\) molar ratio on the removal of atrazine were studied.
simultaneously by varying molar ratios from 5 to 50 and pH from 1 to 5. All experiments were conducted in triplicates to minimize the experimental error.

The optimum process parameters were used for removal of atrazine. Experiments were performed under optimum operating conditions to obtain the maximum removal by UV Fenton process to degrade atrazine.

**Biological process: bioreactor**

The schematic diagram of the bioreactor is shown in Fig. 1 with all the outlet sampling ports closed during the experimental period. The set of experiments has been performed to study the parametric effects such as pH (4.0 to 9.0) and DO (3.5 to 7.5 mg/L) on biodegradation of atrazine. The pH and DO were varied, keeping the atrazine concentration fixed at 150 mg/L to get their optimum values. Effect on atrazine concentration was studied at optimum values of pH and DO by varying the atrazine concentration from 50-500 mg/L. All experiments were performed in triplicate to minimize the error.

**Experimental setup**

The experimental setup of coupled system is as shown in Fig. 1. The experiments were carried out in a 1000 mL reactor filled with 500 mL of the sample mixed at 200 rpm using magnetic stirrer (ELICO; GI 631) at room temperature (23±3°C) in three different stages, where stage-I was UV Fenton process. The pH (2.5) of the solution was maintained by flow controller using 1N HCl and 1N NaOH. Afterwards, FeSO₄ salt 206g/L and 2.1 mL H₂O₂ (30% w/v) were added to the reactor. Then the experimental solution was irradiated with a UV Lamp (Mineralight; V41) for a reaction time of 60 min at 200 rpm. The Fenton effluent was filtered using filter paper (Ultrapore N66, Pall Life Sciences, India) with vacuum filtration Unit (Riviera Glass Pvt. Limited, India).

In stage-II, the effluent from UV-Fenton was fed to 1 L stabilization reactor. The effluent from UV Fenton was filtered, and pH was adjusted to neutral (7±0.2). Finally, this effluent was fed to the bioreactor (stage-III) for biodegradation of atrazine at room temperature (23±3°C). The bioreactor was made up of the borosilicate glass (cylindrical column) having length 55 cm, internal diameter 6.5 cm of 1000 mL working volume (1826 mL; total volume) as shown in Fig. 1. Loofa purchased from the local market Varanasi, Uttar Pradesh, India was used as a packing material for treatment of atrazine. The inlet (5 cm from bottom) and outlet (50 cm from bottom) ports were provided to facilitate entry and execution of reactants/products. The outlet was fixed with 0.2 μm filter to restrain the outflow of bacteria from the bioreactor. Silicon tubing was used to close all the sampling ports with pinch cork. Air from the compressor (Khosla, India S.N.65739) was supplied to the bioreactor to maintain the aerobic condition and proper mixing. The aeration rate was controlled by a pre-calibrated rotameter (Eureka, Pune, Model SRS-MG5) having a range of 5-35 L/min. Air sparger was used for uniform distribution of air and was placed 2.5 mm above the base of reactor upward direction to maintain the liquid in agitated state.

**Effluent analysis: COD, HPLC, GC/MS**

Chemical oxygen demand (COD) was determined according to the standard methods. To eliminate the interference of H₂O₂ and Fe²⁺ salt in COD analysis, samples were adjusted to pH 10 and stirred at 120 rpm for 30 min. The residual atrazine concentration and intermediate products from Fenton oxidation and biological processes were analyzed using HPLC (ELICO, India) with the C-18 column, UV detector (HD469) and pump (HD464). Atrazine and byproducts were determined at a wavelength of 210 nm using mobile phase (acetonitrile:water; 45:55) for 15 min, with a constant flow of 1.0 mL/min.

GC-MS analysis was performed to confirm metabolites formed during atrazine degradation. The samples were prepared by extracting residual atrazine with the addition of an equal volume of chloroform and degraded sample, and after vigorous shaking, the organic layer was separated. The extracted samples were analyzed using GC-MS-QP2010 Ultra (JNU New Delhi). The gas flow was at 1.21 mL/min with split ratio of 10, injection temperature at 260°C. MS measurements were done at a temperature of 230°C for MS ion source, 270°C for MS interference, total time 20 min, and solvent delay 3 min.

**Growth kinetics of atrazine in bioreactor**

For growth kinetic study, a set of experiments were performed. The kinetic model parameters estimated by various factors such as bacterial growth, substrate concentrations and time. Monod model best fitted for the obtained experimental data in the biodegradation process. The Monod model equation is as follows:

\[ \mu = \frac{1}{X \theta + K_x} \frac{dX}{dt} \]  \( \theta = \frac{S}{K_S + S} \)  \( \text{(i)} \)
where $\mu$ is specific growth rate (1/day), $\mu_{\text{max}}$ is maximum specific growth rate (1/day), $K_S$ is half-saturation rate constant (mg/L), $X$, $S$, and $t$ are microbial cell, initial substrate concentrations (mg/L), and time, respectively. Linear form of above equation was

$$\frac{1}{\mu} = \frac{S}{\mu_{\text{max}}} + \frac{1}{\mu_{\text{max}}} \quad \ldots \quad (2)$$

The value of $\frac{1}{\mu_{\text{max}}}$ and $\frac{1}{K_S}$ were calculated and plotted to obtained $\mu_{\text{max}}$ and $K_S$.

**Results and Discussion**

**Parametric optimization: UV Fenton**

The effect of $\text{H}_2\text{O}_2$/COD molar ratio on % removal was studied by varying $\text{H}_2\text{O}_2$/COD molar ratio. The % removal was found to be 55, 52 and 68% for $\text{H}_2\text{O}_2$/COD molar ratio of 0.25, 0.5 and 0.75, respectively. The $\text{H}_2\text{O}_2$/COD molar ratio was increased from 1 to 1.5, and corresponding % removal was decreased from 62 to 52%. The maximum removal was 68% at $\text{H}_2\text{O}_2$/COD molar ratio 0.75 (Fig. 2A). It was observed that the $\text{H}_2\text{O}_2$/COD molar ratio 0.75 was optimum for degradation of atrazine. An addition of excess $\text{H}_2\text{O}_2$ decreased the degradation of atrazine. Kavitha and Palanivelu\(^{27}\) reported that this might be due to scavenging of OH* by $\text{H}_2\text{O}_2$ as in reaction.

$$\text{OH}^* + \text{H}_2\text{O}_2 \rightarrow \text{HO}_2^+ + \text{H}_2\text{O} \quad \ldots \quad (3)$$

Increasing $\text{H}_2\text{O}_2$/Fe$^{2+}$ molar ratio from 5 to 25 increased the degradation of atrazine, but there was a decrease in degradation for $\text{H}_2\text{O}_2$/Fe$^{2+}$ molar ratio 25 to 50. The maximum removal was 62% at $\text{H}_2\text{O}_2$/Fe$^{2+}$ molar ratios of 25 as shown in Fig. 2A. The decrease in the degradation of atrazine at increased Fe$^{2+}$ concentration was presumably due to direct reaction of OH* radical with Fe$^{2+}$ at high concentration as in reaction\(^{28}\).

$$\text{Fe}^{2+} + \text{OH}^* \rightarrow \text{Fe}^{3+} + \text{OH}^- \quad \ldots \quad (4)$$

when pH increased from 1 to 2.5, atrazine removal was increased up to 60%, but beyond pH 2.5, removal decreased as shown in Fig. 2A. The maximum removal was observed at pH 2.5.

Further experiments were conducted at optimum operating conditions. The ANOVA analysis results showed that $\text{H}_2\text{O}_2$/COD, $\text{H}_2\text{O}_2$/Fe$^{2+}$ and pH had a significant effect ($P < 0.05$) on the % removal of atrazine.

**Parametric optimization: bioreactor**

The parameters for bioreactor were optimized by varying one parameter and keeping all other parameters fixed. The effects of pH and DO for the removal of atrazine were investigated at constant atrazine concentration 150 mg/L as shown in Fig. 2B. The experiments were conducted with pH range (4.0-9.0) and DO (3.5 -7.5). The effects were studied, and maximum removal of 69% was found at pH 7.0. At alkaline pH, there was a decrease in atrazine degradation rate which is similar to the observation of Singh et al.\(^{29}\) who reported the inhibitory effect of pH in the alkaline range (pH >8) on the metabolism of microorganisms. The effect of DO level was studied, and a maximum removal of 68% was observed at DO 6.0 mg/L. DO level is an important parameter for consumption or utilization of substrates at high loading rate. As low DO level in bioreactor results in low oxygen concentration, which may be the reason for the decrease in growth of microbes and removal of atrazine\(^{30}\). The findings were similar to the results obtained by Mohan et al.\(^{30}\). The ANOVA analysis showed that the pH and DO had a significant effect ($P < 0.05$) on the removal of atrazine. The present result was in line with results obtained by various researchers for other pollutants\(^{31,32}\).

![Fig. 2 — Parameter optimization in (A) UV-Fenton process; and (B) packed bed bioreactor.](image)
Performance evaluation of coupled process: UV Fenton and bioreactor

The experiments were performed with combined treatment plant (three stages) including UV-Fenton (advanced oxidation process), pH neutralization (stabilization) and biodegradation process. In stage-I (UV-Fenton process), the experiments were carried out at 300 mg/L of atrazine at optimum process parameters pH, molar ratios of H₂O₂/COD and H₂O₂/Fe²⁺ and room temperature. The removal of atrazine was found to be 65% in 60 min which was indicated by a reduction in concentration up to 100 mg/L (Fig. 3). Garza-Campos et al.¹⁹ reported that 65% removal of atrazine concentration at 20 mg/L. In the present study, the maximum removal of atrazine at 300 mg/L by Fenton reactor was 65% in 60 min as shown in Fig. 3. Affam et al.¹¹ also reported a maximum removal of 64.8% at H₂O₂/COD molar ratio 2, H₂O₂/Fe²⁺ molar ratio 25 and pH 3.

In stage-II, the effluent from UV-Fenton process was transferred through filtration unit (0.2 µm). The pH was adjusted (by HCl/NaOH) to nearly neutral (7±0.2) with holding time 60 min and fed to the bioreactor in stage-III for the microbial treatment. The pH as appears to be one of the most important parameters for affecting the degradation of atrazine while applying advanced oxidation processes like UV Fenton, Photo-Fenton, etc.³³,³⁴.

In stage-III, after the immobilization (15 days), the atrazine was treated in the bioreactor. The bacterial species immobilized on Loofa and biofilm formations is shown in Fig. 4. The lower concentration of atrazine (100 mg/L) was transferred to bioreactor phase-III next to phase-II. Several researchers have reported the biotreatment of atrazine favors at lower concentration²⁴,³⁴,³⁵. Biodegradation of atrazine was conducted in bioreactor and atrazine concentration was monitored day to day during the operation. The removal efficiency enhanced from 65 to 93% in continuation with the Fenton reactor during 12 days of operation excluding immobilization period. The performance of coupling method was studied as

![Fig. 3 — Three stage coupling method for the removal of atrazine.](image1)

![Fig. 4 — SEM analysis of Loofa and biofilm of immobilized bacterial species.](image2)
shown in Fig. 3. Atrazine degradation was carried out at pH 7.0±0.2 in a bioreactor using mixed consortia immobilized on Loofa. Atrazine removal was increased to 74% on the 3rd day, and corresponding atrazine concentration was 78 mg/L. On 4th day, a slight decrease in % removal was observed which may be due to the substrate deficiency and formation of fouling which causes the oxygen limitation as reported by Kureel et al.36 and Geed et al.26. Removal efficiency was increased again upto 93% on the 9th day of operation. Further, it became constant to 93% upto 12th day with the corresponding atrazine concentration of 21 mg/L. Du et al.37 reported 74.09% removal in 15 days and Sanchis et al.38 reported 80% atrazine removal by coupling of Fenton and biological oxidation processes.

**Analysis of residual atrazine: HPLC and GC-MS**

HPLC analysis was performed to find out the concentration of atrazine before and after the experiment. The control atrazine sample was run separately, and the peak was found at 2.5 min time interval as shown in Fig. 5A. Similar analysis was done for treated effluent sample. The atrazine (2.5 min) and its intermediates were found at different time interval as shown in Fig. 5B in treated effluent sample. Further, the intermediates were confirmed by GC-MS analysis.

The GC-MS analysis of control atrazine sample and treated effluent was performed. The GC-MS fragmented pattern of atrazine showed parent ion peak at m/z 43, 58, 92, 187, 200 and 215 as shown in Fig. 6A. The GC-MS result confirmed that the atrazine degraded during the treatment process. The Fig. 6B showed a fragmented pattern of biuret parent ion peak at m/z 42,44,59,70 and 102. Similar fragmented pattern for urea showed (Fig. 6C) m/z ion peak at and 26, 28, 44 and 60. In the present work, atrazine was degraded during the treatment process and converted to two metabolites (biuret and urea) as confirmed by GC-MS result, which is in agreement with previous work25,39. Vargha et al.39 reported that the isolated cultures was capable of utilizing atrazine as sole carbon and nitrogen source efficiently degrade atrazine and biuret were produced during the treatment.

**Monod growth kinetics for Atrazine biodegradation**

In the present study, the growth kinetic followed Monod model as shown in Fig. 7. The values of specific growth rates ($\mu_{max}$) and half saturation rate constants ($K_s$) were found to be 0.224 per day and 106.64 mg/L, respectively. It is better to combine the two kinetic constants into a single parameter $\frac{\mu_{max}}{K_s}$ and consider this parameter as a useful index for biological degradation11,31. In the present case the value of $\frac{\mu_{max}}{K_s}$ (0.0021 L/mg/day) was found. $K_s$ value normally depends on the nature of substrate provided to the biomass. Generally, substrate with both readily and slowly biodegradable organic matter will lead to higher $K_s$ value compared to substrate associated with a single compound. Apart from that, the morphology of

![Fig. 5 — HPLC analysis of control (A) Atrazine; and (B) effluent sample after treatment.](image)

![Fig. 6 — GC-MS analysis of control (A) atrazine and effluent sample after treatment shows intermediates; (B) Biuret; and (C) Urea.](image)
biomass could influence the substrate diffusivity into the biomass and affect the $K_s$ value. Geed et al. who reported $K_s$ and $\mu_{MAX}$ for malathion biodegradation was 80.69 mg/L and 0.312 per day, respectively at 125 mg/L for Pseudomonas putida.

Conclusion

The coupled system consisting UV Fenton and bioreactor was effectively used for the treatment of atrazine up to concentration of 300 mg/L. The bioreactor was packed with Loofa sponge immobilized with consortia and the potential microbes obtained from atrazine contaminated site. The performance of the coupled system was found superior than stand alone systems. The important process parameters were optimized and under optimum condition the maximum removal efficiency of coupled system was found to be 93%. The high removal at higher concentration of atrazine was found as compared to other researcher may be due to use of packed system in the batch reactor whereas most of other work has been done in batch system with free cell.

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