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Research Article

# Monitoring Atrazine Removal by *Aspergillus versicolor* via Electrochemical Techniques from Aqueous Solutions

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**Abstract**

A triazine group pesticide called Atrazine (ATR) is commonly used in agricultural activities, but ATR residues move underground and surface water from the soil, therefore it accumulates in water in high concentrations. ATR-contaminated water is toxic for living organisms, particularly at higher concentrations. The remediation of the ATR-contaminated aqueous environment becomes an important issue. Bioremediation is recommended as an eco-friendly way to treat contaminated water. Bio-removal and bio-sorption are the mechanisms of bioremediation technologies using growing and dried biomass, respectively. This study aims to investigate the ATR bioremediation by *Aspergillus versicolor* via electro-chemical methods using both growing and dried biomass. The optimal pH for ATR removal was deter-mined as 6 in both mechanisms. The growing fungal culture removed 66.67% of ATR after 3 days of incubation. The dried fungal biosorbent removed 57.14% of ATR after 24 hours. The results of this study showed that the fungal strain effectively removed ATR from pesticide-contaminated aqueous solutions in a short period. Using fungal strains, biological wastewater treatment technology is an effective approach to remove triazine group pesticides from pesticide-contaminated water.

**Keywords:** Atrazine (ATR), Bio-removal, Bio-sorption, Fungi

## Introduction

Atrazine (ATR), a triazine group herbicide, is commonly used for controlling the wide leaf and grass formation in corn and sugar beet farming in most countries [1, 2]. ATR is easily reached to surface and underground water because the water solubility of ATR is as high as 33 mg/L [3] and the soil adsorption ability of ATR was low [4]. The accumulation of pesticides by living organisms in under-ground and surface water causes significant health and environmental problems [5; 6]. It is reported that ATR causes health defects in the nervous and endocrine systems [7, 8]. It is important to remove pesticides from the aquatic environment near farming fields due to their toxic effects on living organisms especially, those accumulated from agricultural products [9]. Biological treatment is suggested as an eco-nomic and easy method to remove pollutants such as dyes and pesticides [10-13]. Recently, most research has focused on the bioremediation of pesticide-contaminated water [14-17].

The biological treatment technologies used different methods called bio-removal and bio-sorption. Bio-removal is defined as the removal of pollutants by actively growing living cells (Dönmez 2002). No need the separating steps for biomass production as harvesting and drying is an advantage of the bio-removal mechanism [18]. Bio-sorption is a removal technology based on the adsorption of pollutants on inactive or dead dried cells [19, 20]. Bio-sorption is advantageous because it is inexpensive, rapid, and has no nutritional requirements [21]. Both bio-removal and bio-sorption mechanisms are examined for the removal of pesticides separately in the literature [22]. Studies in recent years have focused on removing ATR by bio-sorption, especially using fungi [23, 24].

According to our present literature review on bio-removing ATR using growing fungi, a limited number of studies have been conducted [25, 26].

However, our current literature evaluation has no study about the application of growing *A. versicolor*, isolated from Turkey, for remediation of ATR contamination in water.

This study aims to determine the ATR removal performance of a wild filamentous fungal strain biomass comparing bio-removal and bio-sorption mechanisms. In addition to this, the concentrations of ATR in aqueous solutions were determined by electrochemical methods in this study. In previous studies, high-performance liquid chromatography (HPLC) and gas chromatography (GC) methods were used for pesticide detection [27, 28]. Monitoring ATR bioremediation by fungus in aqueous solutions via electrochemical techniques is a new approach, which is applied in this study.

**Materials and Methods**

***ATR solution preparation***

The ATR was obtained from Ciba Geigy and the stock solution was prepared with 50 mg of ATR dissolving in 50 ml methanol and water solution (25%:75%) as 1000 mg/ml. The appropriate solutions of ATR were prepared from the stock solution to dilute at the desired concentration.

***ATR analyse method***

The ATR concentrations in solutions were determined with BAS, Bioanalytical Systems, IN 47906, USA. A square wave voltammetric technique with a glassy carbon electrode (as a working electrode) was used in the electrochemical determination of ATR modified from [29]. To order to observe peak currents for monitoring ATR, the Britton Robinson Buffer (BR) [26] solutions were prepared at pH 2 containing 19.6, 38.46, and 56.6 mg/L ATR and analysed. The voltammograms obtained for ATR standard solutions including different ATR concentrations at pH 2 were given in Figure 1. The calibration graph was obtained from peak current (µA) versus concentration (mg/L). In the ATR removal experiments, 2 ml of samples from the experimental solutions were used in ATR analysis. The peak currents of ATR in the samples were measured electrochemically and, then ATR concentration was calculated from the calibration graph.

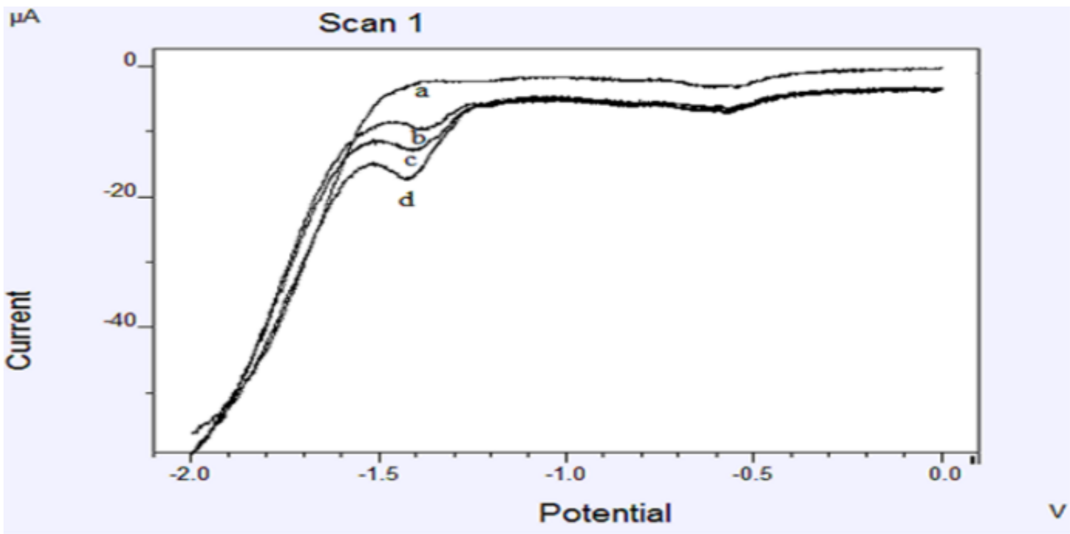


***Figure 1.*** *Voltammograms obtained for ATR standard solutions including different ATR concentrations at pH 2 (a: BRT buffer solution at pH 2 without ATR used as control; BRT buffer solutions at pH 2 with ATR concentrations of b: 19.6 mg/L; c: 38.46 mg/L; d: 56.6 mg/L)*

The removal percentage of ATR was calculated from Equation (1);

$$\text{ATR removal (\%)} = (C_o - C_f) / C_o \times 100$$

***Equation 1***

where $C_o$ is the initial ATR concentration (mg/L) and $C_f$ is the final ATR concentration (mg/L) in the ATR removal working solutions.

***Microorganism and growth conditions***

The cells of *A. versicolor* isolated by Tastan et al. (2010) in Turkey were used. The fungal cells acclimated in the ATR-containing media. After activation, the fungal culture (2 mL) was inoculated into Erlenmeyer flasks (250 mL) containing molasses medium (100 mL) with adequate ATR concentration. Sugar refineries' secondary low-cost product called molasses was used as a carbon source in fungal growth media. Gül and Dönmez (2014) [30] previously explained the ingredients of the growth molasses-containing medium used in this study. NaOH (0.1 M) was used to adjust pH. After autoclaving (121°C for 15 min) the medium, the desired concentration of ATR solutions was added to the growth medium.

In bio-sorption experiments, the dried biomass of fungal strain was used as a biosorbent. In order to prepare the biosorbent, 3 ml of *A. versicolor* culture was inoculated into molasses medium having 100 ml volume at pH 5. The fungus was filtered from the medium after 10 days, and then treated with distilled water and 1% formaldehyde, respectively. The biomass was dried at 80ºC for 24 hours, then smashed and used for biosorption studio use as biosorbent. The final biosorbent dosage was 1.0 g/L in all experiments. Bio-sorption experiments were conducted with 1 g/L dried fungal biosorbent in flasks (250 ml) containing 200 ml double distilled water and ATR. The effect of pH (2, 4, 6, 8, and 10) at 5 mg/L ATR concentration and ATR concentrations (5, 10, 15, 30, and 50 mg/L) on fungal biosorption capacities were investigated. All experiment series were carried out at 25 °C. Also, Freundlich and Langmuir models were calculated to define equilibrium data between ATR and fungal cell surfaces. The pseudo-first-order and second-order models were used to describe bio-sorption kinetics.

***FT-IR Analysis***

FT-IR analyses were done to explain interactions between ATR and fungal cell surfaces. The fungal biomass, which was grown in a molasses medium at pH 5 with 5 mg/L ATR after 7 days, was filtered with paper and washed two times with distilled water. The filtered biomass was dried for 80 °C at one night and, then was used in FT-IR analysis. The dried biomasses growing in the same medial conditions without ATR were used as a control. The changes in the functional groups on the A. versicolor surface were determined in the absence and presence of ATR by FT-IR (Perkin Elmer- Spectrum 100).

**Results and Discussion**

***The effect ATR on fungal growth***

The effect of ATR on fungal growth was tested. After the incubation period, the fungal biomass was filtered, then washed with distilled water and dried. The measured fungal dry weight is given in Table 1. The presence of ATR affected the fungal growth negatively (Table 1). Yada et al. (2019) [31] reported that pesticides such as insecticides inhibited fungal growth.

***Table 1.*** *The dry weight: D-W(g/L) of A. versicolor (Av) growing in the absence and presence of 5g/L Atrazine after 7 days of incubation at pH 5.*

| | **Growing in the presence of ATR** D-W (g/L) | **Growing in the absence of ATR** D-W (g/L) |
|---|---|---|
| *A. versicolor* | 3.75±0.18 | 2.40±0.11 |

***Bio-removal Experiments***

According to bio-removal experiments, after 3 days of incubation, maximum ATR removal was 66.67% by actively growing *A. versicolor* (Figure 2). A white-rod fungus removed 59% of ATR in 5 days [25]. Gül and Silah (2017) [26] showed that growing *R. arrhizus* performed maximum removal after 3 days as 57.45%. In this study, the fungal bio-removal percentage of ATR by *A. versicolor* was the highest as 66.67%, while comparing white-rod fungi and *R. arrhizus* published in literature before. Also, previous research showed that growing bacterial strains used ATR as a nutrient and removed ATR from the environment [32]. The results of this study supported that the fungal strain *A. versicolor* might use ATR as a nutrient or carbon source while actively growing and also removed from the medium.

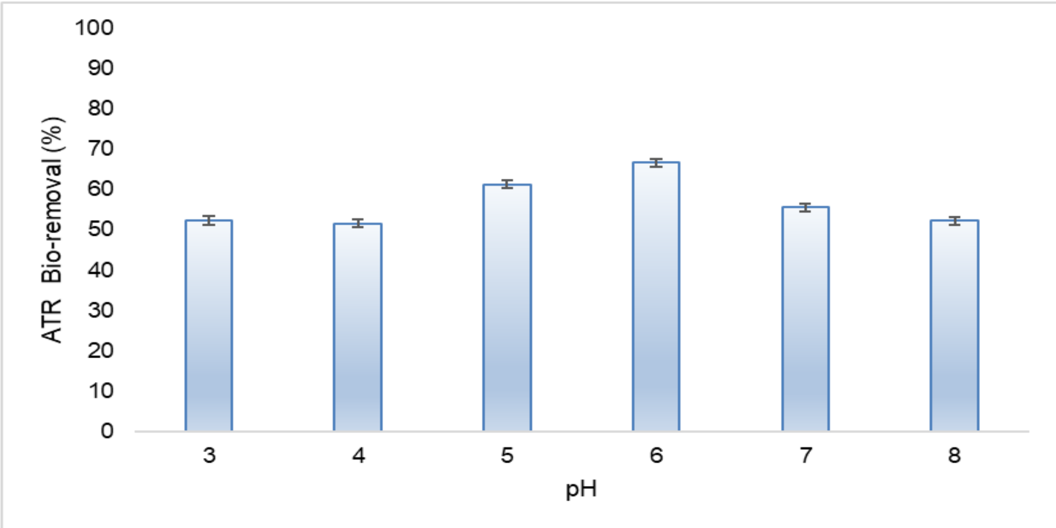


***Figure 2.*** *The effect of pH on ATR bio-removal by growing A. versicolor in molasses medium (Initial ATR concentra-tion: 5 mg/L; Incubation Time: 3 days)*

***Bio-sorption Experiments***

Bio-sorption study results showed that after 480 minutes the fungal biosorbent performed maximum ATR removal at pH 6 as 57.14% (Figure 3). Pathak & Dikshit (2012) [33] showed that the optimal pH for the removal of ATR by different fungal biosorbents was pH 6. Another research reported that maximum ATR adsorption on amberlite occurred at pH 6.5 [34]. The results of this study were fitted with the literature and the optimal pH for ATR biosorption by *A. versicolor* biosorbent was found as 6 (Figure 3). Also, the changes of ATR concentration in control groups without microorganisms at the same pH values (2, 4, 6, 8, and 10) were monitored and there was not any significant change occurred in this experimental period.

To investigate the effect of ATR concentration on biosorption, various ATR concentrations as 5, 10, 15, 30, and 50 mg/L were tested. Augmentation of ATR concentration affected biosorption negatively (Figure 4). Recently, Gül & Silah (2017) [26] used the dried biomass of another filamentous fungus *Rhizopus arrhizus* as a biosorbent for removal of ATR. The results of their study showed that 63.16% of ATR (5 mg/L) at pH 6. A recent study showed that *Trametes versicolor*, white-rot fungi, removed 75.3% and 97.9% of ATR in 20 and 60 days of incubation [35].

In this study dried *A. versicolor* strain performed biosorption as 59.39 % of 10 mg/L ATR after 480 minutes which is acceptable as a successful performance compared a time-saving point of view.

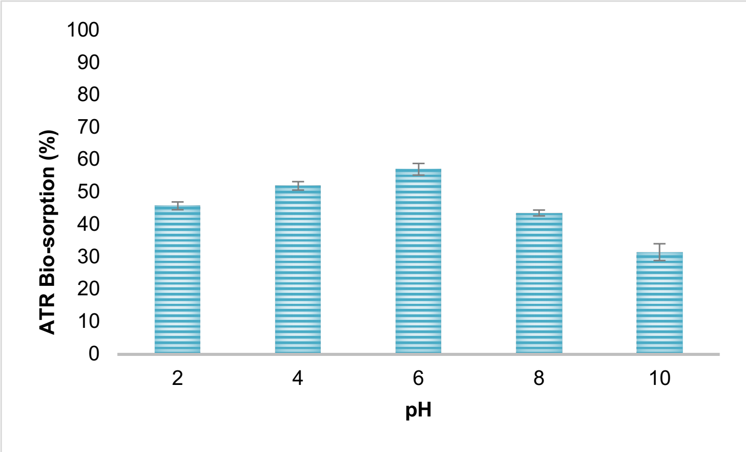


***Figure 3.*** *The effect of pH on ATR bio-sorption by dried A. versicolor biosorbent in ATR-containing solution (Initial ATR concentration: 5 mg/L, Incubation Time: 480 minutes)*

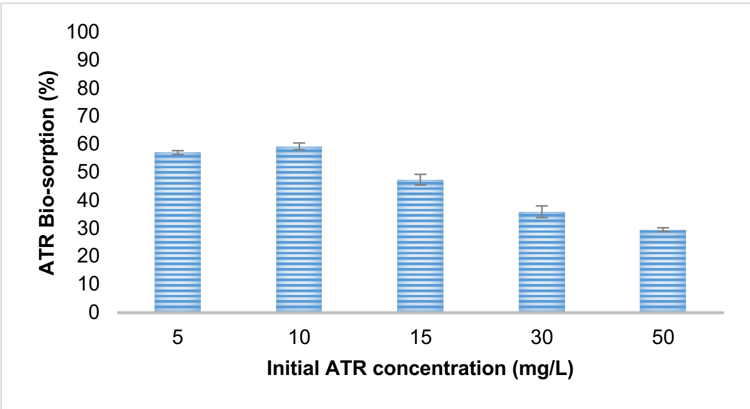


***Figure 4.*** *The effect of ATR bio-sorption by dried A. versicolor biosorbent in ATR-containing solution (pH:6; Incuba-tion time: 480 minutes)*

***Biosorption Isotherms***

The adsorption of ATR ions from the liquid phase to the fungal biomass surface can be described by Langmuir and Freundlich models. The Langmuir isotherm model assumes that a monolayer of adsorbate is adsorbed over a uniform adsorbent surface at a constant temperature. Langmuir isotherm model can be calculated by:

$$C_e/Q_e = (1/Q_m)C_e + 1/K_LQ_m$$

***Equation 2***

where $Q_m$ (mg/g) is the maximum capacity of adsorption, $C_e$ is the, and $K_L$ (L/mg) is a constant related to the affinity of the binding sites on the adsorbent surface.

Freundlich model is applied to describe the sorption characteristics for the heterogeneous adsorbent surface.

The Freundlich equation is given by:

$\ln(Q_e) = \ln(K_F) + 1/n \ln(C_e)$ ***Equation (3)***

where $Q_e$ (mg/g) is the capacity of adsorption, $C_e$ (mg/L) is the concentration of ATR, and $K_F$ (L/mg) is a Freundlich constant.

Table 2 shows the isotherm model calculations for ATR biosorption onto dried A. versicolor biosorbent derived from Langmuir and Freundlich equations. The results indicated that ATR biosorption by the fungus was fitted with the Freundlich isotherm model in this study (Table 2).

***Table 2.*** *Langmuir and Freundlich isotherm calculations for ATR onto Aspergillus versicolor (A.v.)*

| | **Langmuir isotherm constants** | | | **Freundlich isotherm constants** | | |
|---|---|---|---|---|---|---|
| | $Q_m$ (mg/g) | $K_L$ (L/mg) | $R^2$ | $K_F$ (L/mg) | n | $R^2$ |
| *A. v.* | 19.23 | 0.085 | 0.965 | 3.11 | 2.34 | 0.986 |

***Biosorption Kinetics***

The kinetic data can be investigated using different types of mathematical equations and these data give useful information related to the efficiency and feasibility of the ongoing process for biosorption studies. The kinetic biosorption data were processed to investigate the dynamics of the process in terms of the theoretical $Q_e$ values and order of rate constant. The pseudo first-order and pseudo second-order kinetic data obtained for ATR uptake were calculated from equations given in Equations 4 and 5.

Pseudo first-order kinetic model equation:

$$\log (Q_e-Q_t) = -k_1/2.303t + \log Q_e$$

***Equation 4***

Pseudo second-order kinetic model equation:

$$t/Q_t = 1/k_2Q_e^2 + 1/Q_e.t$$

***Equation 5***

where $Q_e$ is the adsorption capacity ($Q_{ecal}$: The theoretical adsorption capacity; $Q_{eexp}$: The experimental adsorption capacity), $k_1$ is the rate constant of the pseudo first-order kinetic model, $k_2$ is the rate constant of the pseudo second-order kinetic model.

The theoretical $Q_e$ (cal)) and rate constants ($k_1$ and $k_2$) were obtained for the ATR biosorption employed using the equations for different initial ATR concentrations. Table 3 shows the rate constant of the pseudo first-order kinetic model increased with increasing the initial pesticide concentration for 10 and 15 mg/L ATR. The theoretical $Q_e$ is closer to experimental $Q_e$ for the pseudo first-order kinetic model than that for the second-order kinetic model. Although the correlation coefficient of the pseudo second-order kinetic model is slightly higher than the pseudo first-order kinetic model, the ATR biosorption kinetic data can be fitted more appropriately with the pseudo first-order kinetic model because the calculated $Q_e$ and experimental $Q_e$ values are closer than according to this model.

***Table 3.*** *The pseudo first and pseudo second-order biosorption rate constants and the experimental ($Q_{e\ exp}$) and calculated $Q_e$ (shown as $Q_{e\ cal}$) values for biosorption of different initial ATR concentrations onto dried A. versicolor biosorbent (I.c.: Initial concentration)*

| **I.c.** (mg/L) | | **First-order kinetic model** | | | | **Second-order kinetic model** | |
|---|---|---|---|---|---|---|---|
| | $Q_e$ exp (mg/g) | $k_1$ ($min^{-1}$) | $Q_e$ cal (mg/g) | $R^2$ | $k_2$ | $Q_e$ cal (mg/g) | $R^2$ |
| 10 | 5.94 | $1.15x10^{-3}$ | 4.16 | 0.944 | $3.54x10^{-4}$ | 7.36 | 0.964 |
| 15 | 7.13 | $2.65x10^{-3}$ | 6.68 | 0.993 | $3.36x10^{-4}$ | 8.81 | 0.998 |

### *FT-IR Analyzes*

The comparative infrared spectra of fungal biosorbent before and after ATR biosorption is given in Figure 5. The comparative infrared spectra of fungal biosorbent before and after ATR biosorption is given in Figure 5. The broad and strong bands between 3500 and 3000 $cm^{-1}$ are attributed to the overlapping of -OH and -NH stretching. There is a slight shift in the band at $cm^{-1}$ after ATR biosorption. The position of the band at 3271 $cm^{-1}$ was assigned to the stretching of N-H groups. The band seen at 2994.2 $cm^{-1}$ before ATR biosorption shifted to 2992.3 $cm^{-1}$ after ATR biosorption. The FT-IR bands present between 2850 and 3000 $cm^{-1}$ revealed the existence of –CH bonds in amino acids and aliphatic acids present in the cell membrane [36]. FT-IR spectrum of *A. versicolor* biosorbent after ATR bio-sorption displayed many changes of band such as a shift in the band position, the decreases/increases in the band intensity. As shown in Figure 5 the shifting of the peaks (from 1641 $cm^{-1}$ to 1634 $cm^{-1}$, 1544 $cm^{-1}$ to 1551 $cm^{-1}$, 1033 $cm^{-1}$ to 1025 $cm^{-1}$) were observed presence of ATR. The strong peak at the bands at about 1451 $cm^{-1}$ and 1399 $cm^{-1}$ in the spectra is attributed to the carboxylate group on the biomass [37]. Also, the bands at 1456 and 1393 cm-1 were the absorption peaks of variable angle vibration of the C-H bond [38]. According to the results obtained, it has been found that these functional groups may be effective in the ATR removal process.

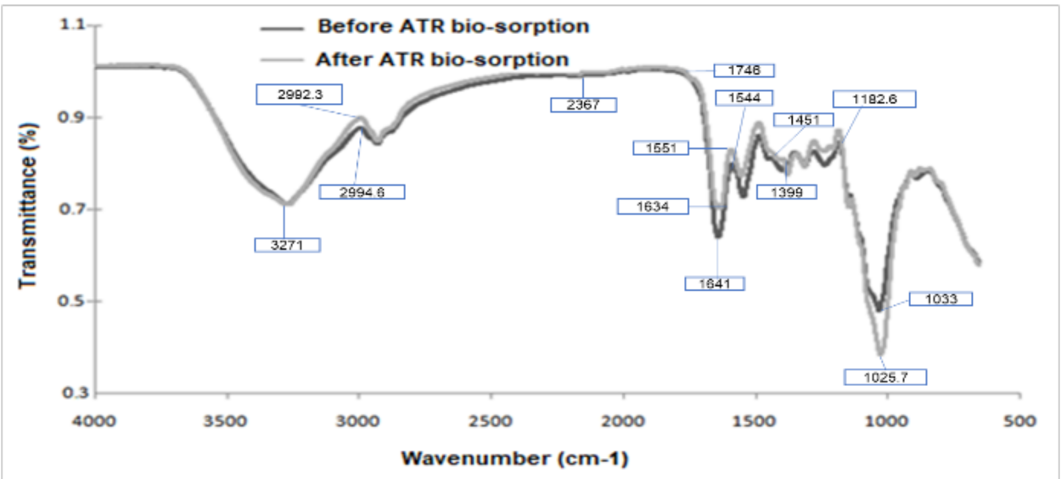


***Figure 5.*** *Infrared spectra of A. versicolor biosorbent before and after ATR bio-sorption*

## Conclusion

This study focused on the ATR removal performance of a fungal strain called *Aspergillus versicolor* via bio-removal (with actively growing fungal biomass) and bio-sorption (with dried fungal biosorbent) processes. Dried biosorbent removed 59.39% of ATR (at 10 mg/L concentration) after 480 minutes and growing fungal biomass bio-removed 66.67% of ATR (at 5 mg/L concentration) after 3 days at pH 6. Also, it was observed that the presence of ATR in the medium inhibited fungal growth. Langmuir and Freundlich isotherm models were calculated and Freundlich isotherm was found suitable for ATR biosorption by fungal biosorbent. The FT-IR analyses showed that some functional groups on the surface of fungal biosorbent had an important role in the removal of ATR. In addition, another important feature of this study is the monitoring of fungal ATR removal by electrochemical methods. In this case, the results of this study show that future-oriented electrochemical analyses can be used effectively in environmental biotechnology studies. To sum up, the results obtained from this study showed that *A. versicolor* strain can be effectively used in wastewater treatment systems to remove pesticides like ATR.

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