

Article

# Application of *Aspergillus Niger* Bioremediation for the Treatment of Industrial Wastewater

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**Abstract:** Bioremediation is a sector of biotechnology that utilizes living organisms, such as microalgae and fungi, to eliminate contaminants, pollutants, and toxins from soil, water, and various habitats. The current study aimed to evaluate some characteristics of industrial wastewater discharged through the collection of water samples, with one sample and three replicates. The physical, chemical, nutrient, and heavy metal properties were assessed, and fungal species were identified morphologically for treatment purposes. The evaluated physical properties included electrical conductivity and total dissolved solids. The chemical properties included total hardness, sodium ions, and potassium ions. The nutrients were chloride, sulfate, phosphate, nitrate, and active nitrite. The heavy metals were zinc, lead, iron, manganese, and aluminum. For physical properties, the removal rates for electrical conductivity reached 45.06%, while the total dissolved solids (TDS) rate was 43.93%. The efficiency of electrical conductivity was 31.89%, and for TDS, it was 58.66%. As for chemical properties of industrial wastewater treated by the treatment unit and *Aspergillus niger* fungus, they had varying percentages, with the highest removal rate of sodium ions being 89.81% and the lowest removal rate of calcium ions being 22.9%. The efficiency of sodium ions was 76%, and for total hardness, it was 54.46%, and the lowest was for potassium ions at 31.29%. While the nutrient was for nitrates (76.36%), the lowest was for phosphates (48.06%). The highest efficiency was for nitrate at 82.27%, and the lowest efficiency was for chloride at 51.36%. As for heavy metal, the highest treatment rates were achieved for manganese and lead at 99.77% and 99.66%, respectively. The remaining rates, from highest to lowest removal rate, were as follows: zinc, iron, and aluminum, at 89.2%, 85.92%, and 85.92%, respectively. The highest efficiency was for zinc at 99.78%, and the lowest efficiency was for aluminum at 45.83%.

**Keywords:** *Aspergillus Niger*, Physical Properties, Chemical Properties, Nutrient, Heavy Metal

## Introduction

Water is the foundation of life on Earth and a precious natural resource that plays a vital role in the lives of all living beings. Therefore, rationalizing water use has become crucial to meeting the

demands of ambitious development plans [1]. Water pollution is a form of corruption caused by humans, directly or indirectly, because despite knowing the importance of water, humans have chosen it as a dumping ground for harmful pollutants in various forms, which are transferred to them through the use of this contaminated water [2]. As societies become more advanced, the need for water increases, and consequently, the degree of pollution increases. This, in turn, leads to aquatic ecosystems being constantly threatened if they are not managed more effectively [3].

Pollution in general leads to changes in many of the physical, chemical, and biological properties of water. Discharging industrial wastewater into rivers, whether treated or untreated, causes immense damage to the aquatic environment due to the high concentrations of harmful environmental pollutants it contains. One of the damages caused by disrupting the ecological balance is the occurrence of methylation [4].

The presence of substances in relatively small concentrations that have a toxic effect on microorganisms poses a danger because of their ability to bioaccumulate within the organism's organs. Over time, the presence of these elements in high concentrations in the body leads to bioaccumulation [5]. The development of industrial processes today has led to water pollution with hazardous materials and heavy metal waste. This is a result of industrial activities such as mining, refining, electroplating, and some other industries [6]. The large quantities of industrial waste discharged from facilities without proper treatment lead to a decline in the quality of potable water and an imbalance in the ecosystem. Undoubtedly, the introduction of industrial waste, such as heavy metals, into the aquatic environment causes an imbalance in the system. These elements are highly toxic to humans when ingested [7]. Therefore, it is essential to treat industrial wastewater and liquid waste in general through integrated treatment [8].

Thus, the treatment of water containing industrial waste is crucial, and several treatment methods exist, including physical and chemical treatment systems. These systems must consider the quantity and quality of the polluted water and provide the necessary chemicals. However, their use is difficult due to high costs, and improper chemical reactions can produce other pollutants that are difficult to remove [9]. Due to the high economic cost of physical and chemical methods, alternative, low-cost, and efficient methods have been found for treating the toxicity of these elements. One such method is the biological method using biomass, which has the ability to remove heavy metals from aqueous solutions [10].

Bioremediation using microorganisms such as fungi or bacteria is an effective method for removing industrial waste from water because microorganisms require some of these elements [11]. Aquatic fungi play an important role in the balance of the aquatic environment, as they break down organic matter using enzymes they secrete, such as cellulase, and return it to its natural components. The bioremediation system is a natural, safe, low-cost, and easy-to-implement method [12]. Fungi are well-suited for the biosorption of minerals and the bioremediation of pollutants from industrial wastewater due to their unique characteristics that enable them to grow in highly polluted and nutrient-poor environments. Fungi exhibit remarkable tolerance to mineral pollutants [13].

Given the importance of fungi in addressing the environmental pollution caused by wastewater discharged from the industrial area of Tikrit, Iraq, this study aims to identify the role of fungi in water conservation by assessing the level and type of pollution in industrial wastewater discharged from the industrial area of Tikrit.

## Materials and Methods

**Sampling Method:** Industrial wastewater samples were collected from location within the study area between February 2025 to assess pollution in the industrial water and its subsequent treatment. Sterile 20-liter polyethylene containers were used, with one sample collected and three replicates per container. Each container was washed twice with sample water at each station, then sealed tightly and stored in a designated cork container for transport to the laboratory for physical, chemical, and heavy metal analyses, both before and after treatment. Samples for fungal isolation were collected in sterile 250 ml glass bottles, opened underwater to a depth of 10-20 cm to prevent contamination. The bottles were then sealed underwater. All samples were labeled with the date and

location numbers and transported to the laboratory in cork containers to preserve sample properties for physical and chemical analyses (APHA).

**Isolation and diagnosis of fungi:** Fungi were isolated from wastewater from the study area in Petri dishes containing potato dextrose agar medium with the antibiotic chloramphenicol added to prevent bacterial growth [14]. The sterile dishes were incubated at  $25 \pm 2^\circ\text{C}$  for 5-7 days, after which the fungi were purified and identified [15].

**Industrial Wastewater Treatment:** A two-stage treatment unit was prepared. In the first stage, a grit filter was prepared to remove sludge and large impurities. In the second stage, treatment was carried out using fungi, specifically several fungal species isolated from the wastewater itself.

**Biological Treatment of Industrial Wastewater Using Fungi:** *Aspegillus niger* was isolated from the water and cultured in solid PDA medium. It was then transferred to liquid PDB medium. 250 ml of the medium was added to the wastewater in a transparent glass bottle and incubated at  $25-28^\circ\text{C}$  for five days. The mixture was then filtered through  $0.45 \mu\text{m}$  filter paper, and physical, chemical, and heavy metal analyses were performed.

**Treatment Unit Efficiency Assessment:** The removal percentage was calculated using the following equation [16]:

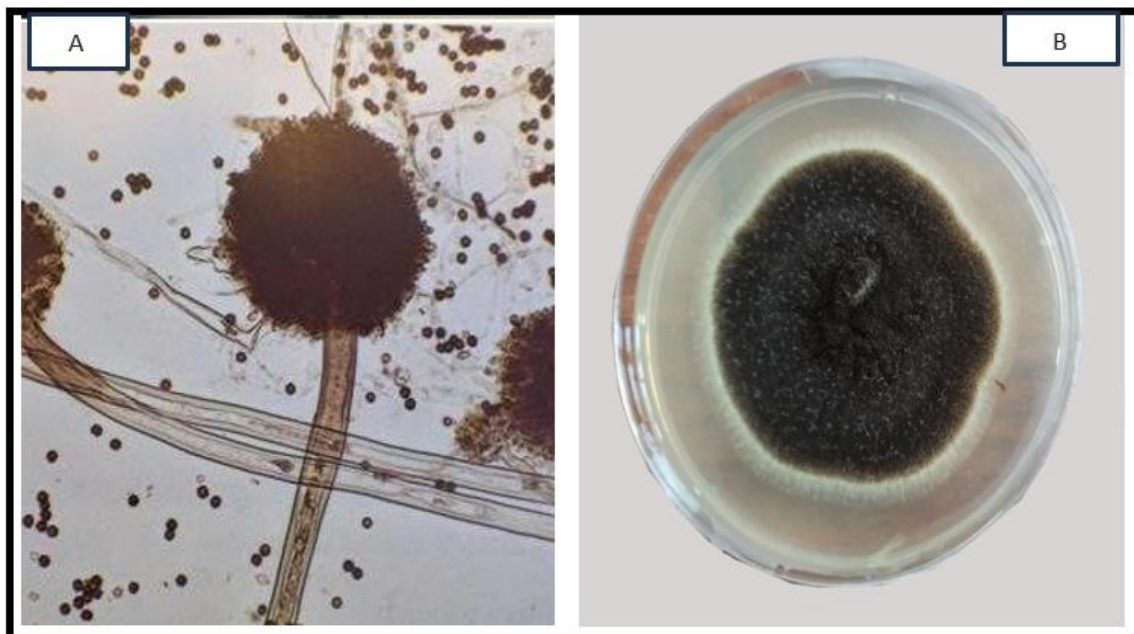
$$\text{Removal \%} = \frac{(\text{Con. of pollutant before treat.} - \text{Conc. of pollutant after treat.})}{\text{Conc. of pollutant before treatment}} \times 100$$

#### Statistical Analytics

The results of this study were analyzed using SPSS software, and analysis of variance, Duncan's test, and the t-test for similar samples were performed at a probability level of  $p \leq 0.05$ . Pearson's correlation coefficient was also applied to some of the studied variables.

#### Results

**Isolation and diagnosis of the fungus *A. niger*:** The results of isolation and identification of *A. niger* from wastewater samples after incubation at  $25^\circ\text{C}$  on PDA medium. The colonies appeared on the culture medium as soft white at first and then turned black due to dark colored conidia with a velvety or cottony texture. The conidia heads were large and brown and were observed under the microscope as spherical, the conical heads were double brown and the phialides were spherical, dark brown and rough-walled. Then, based on the taxonomic keys mentioned by [17], it was found that the mold is *A. niger*, Figure.(1)



**Figure 1.** Microscopic examination of *A. niger* (40X), B: colony of *A. niger* r on PDA medium

The two-stage treatment unit, consisting of a sand filter as the first stage and treatment with *A. niger* fungus as the second stage, proved effective in removing and improving most of the physical properties of the studied industrial wastewater, as shown in Table.(1)

The removal rates for electrical conductivity reached 45.06%, while the total dissolved solids (TDS) rate was 43.93%. The efficiency of electrical conductivity was 31.89%, and for TDS, it was 58.66%. The physical properties of the water treated by the unit and *A. niger* fungus were within the Iraqi standards for drinking water, indicating the fungus's effectiveness in improving these physical properties. As shown in Table (1).

**Table 1.** Treatment physical proprieties by *A. niger* fungus

Parameters	Before Filtration	After Filtration	<i>A.niger</i>	Percentage	Efficiency
Electrical Conductivity	1174 a	947 ab	645 b	45.06 %	31.89 %
Total Dissolved Solids	1386 a	1022 ab	573 b	43.93%	58.66 %

Analysis of variance (ANOVA) tests for the means with respect to nutrients indicated significant differences at a significance level of 0.05%. With the exception of potassium ion, no significant differences were recorded at the 0.05% significance level. It was found that the chemical properties of industrial wastewater treated by the treatment unit and *A. niger* fungus had varying percentages, with the highest removal rate of sodium ions being 89.81% and the lowest removal rate of calcium ions being 22.9%. The efficiency for sodium ions was 76%, for total hardness it was 54.46%, and the lowest efficiency was for potassium ions at 31.29%. As shown in Table (2).

**Table 2.** Treatment by *A. niger* fungus

Parameters	Before Filtration	After Filtration	<i>A. niger</i>	Percentage	Efficiency
Total Hardness	661 a	475 b	301 c	36.63%	54.46%
Sodium Ion	33 a	28 b	7.9 c	71.79 %	76%
Potassium ion	14.7 a	13.1 a	10.1 a	22.9 %	31.29%

Analysis of variance (ANOVA) of the mean values for nutrients indicated significant differences at the 0.05% significance level, except for calcium ion, where no significant differences were recorded at the 0.05% significance level. The treatment unit and the *A. niger* fungus were found to be effective in treating industrial wastewater for nutrients. The highest removal rates for wastewater were observed for nitrates (76.36%), while the lowest were for phosphates (48.06%). The highest efficiency was for nitrate at 82.27%, and the lowest efficiency was for chloride at 51.36%. As shown in Table 3.

**Table 3.** Treatment by *A. niger* fungus

Parameters	Before Filtration	After Filtration	<i>Aspergillus niger</i>	Percentage	Efficiency
Chloride (Cl <sup>-</sup> )	25.7 a	24.9 a	12.5 b	49.8%	51.36%
Nitrate (NO <sub>3</sub> <sup>-</sup> )	44 a	33 b	7.8 b	76.36%	82.27%
Nitrite (NO <sub>2</sub> <sup>-</sup> )	9 a	8.7 a	3.7 b	57.47%	58.9%
Phosphate (PO <sub>4</sub> <sup>3-</sup> )	5.4 a	3.1 b	1.61 c	48.06%	70.19%
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	151 a	101 b	49 c	51.49%	67.55%

The treatment rates for *A. niger*, with respect to heavy metals in industrial wastewater after passing through the sand filter, showed high efficiency in the treatment unit at this stage. Manganese and lead achieved the highest treatment rates at 99.77% and 99.66%, respectively. The remaining rates, from highest to lowest removal rate, were as follows: zinc, iron, and aluminum, at 89.2%, 85.92%, and

85.92%, respectively. The highest efficiency was for zinc at 99.78%, and the lowest efficiency was for aluminum at 45.83%. As shown in Table (4).

**Table 4.** Treatment by *A. niger* fungus

Parameters	Before Filtration	After Filtration	Aspergillus niger	Percentage	Efficiency
Zinc (Zn)	0.33 a	0.24 a	0.082 a	89.2%	90.68%
Aluminum (Al)	0.029 a	0.021 a	0.01 a	40.90%	45.83%
Iron (Fe)	2.1 a	1.22 b	0.09 c	85.92%	88.88%
Manganese (Mn)	4.4 a	2.8 a	0.07 b	99.77%	99.78%
Lead (Pb)	0.085 a	0.05 a	0.001 b	99.66%	99.67%

## Discussion

The current investigation proved the efficacy of *A.niger* in eliminating and enhancing the majority of the physical qualities of the analyzed industrial effluent. In the study by [18] fungal diversity revealed that *Aspergillus* represented the highest prevalence at 60%. This genus has a diverse range of species that can adapt to various environmental conditions and extreme habitats, including water contaminated with hazardous compounds. *Aspergillus* produces numerous asexual conidia that can endure stress and creates sexual ascospores that aid in its widespread distribution [19]. The fluctuations in the prevalence of fungal genera can be ascribed to their resilience and adaptability to extreme conditions, their tolerance to varying temperature ranges, their ability to secrete enzymes that aid in the decomposition of diverse substrates for energy and growth, and their abundant production of reproductive units that enhance environmental dispersal [20].

The present study agrees with [21] which showed the capacity of *Aspergillus terreus* serve as a cost-effective and eco-friendly alternative for heavy metal remediation in wastewater treatment. The present study agrees with [22] which showed efficient treatment with *A. niger* in the removal of heavy metal.

Heavy metals can adhere to microorganisms via functional groups present on their cell walls. The fungal group possesses the highest surface area, resulting in the most cell wall material with functional groups capable of binding a bigger quantity of metals [23, 24]. Fungi play a crucial role in diminishing and eliminating heavy metal concentrations in soil and aquatic environments due to various mechanisms that enhance their efficacy in this process, including external fungal adsorption on cell walls and the formation of heavy metal complexes that are sequestered and stored within their cells [25], [26]. A study by Pande *et al.* [27] demonstrated that fungi have the ability to efficiently absorb mercury, cadmium, and lead in aquatic environments, highlighting the role of various fungi in the bioaccumulation of heavy metals. Fungi comprise various components, including polysaccharides and proteins. The latter consists of phosphates and amino acids, together with secondary groups such as carboxyl and hydroxyl hydroxyl [28], [29]. All of these substances influence the binding of heavy element molecules [30][31].

## Conclusion

To summarize, results of this study showed that the use of *Aspergillus niger* in a two-stage treatment system represents an efficient and economically reasonable method for the treatment of industrial wastewater. The results show reductions in several indicators of contamination, with high heavy metal (manganese (99.77%) and lead (99.66%)) removal efficiencies as well as significant reductions in various physicochemical and nutrient parameters, leading to treated water easily within the acceptable limits of Iraqi drinking water quality standards. These results further highlight the biodegradation potential and versatility biosorption capacity for *A. niger* in contaminated environments that can be a green alternative to traditional wastewater treatment methods. The findings of this study indicate that a fungal-based bioremediation can be widely applicable in the field of industrial wastewater management to minimize environmental pollution and to decrease the

operational cost. Nevertheless, these processes need to be investigated further for large-scale application and stability over time, operating under diverse environmental conditions and exploring co-culture with multiple microorganisms to achieve higher treatment efficiency and greater pollutant removal spectrum.

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