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Bioremediation of cardboard recycling industrial wastewater using isolated native fungal strains

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ABSTRACT

Cardboard recycling industrials are one of the major wastewater-producing industries. Bioremediation processes are economical and eco-friendly alternatives for industrial wastewater treatment. This study aimed to evaluate the bioremediation of cardboard recycling industrial wastewater by isolated native fungal strains. At first, samples of the cardboard recycling industry wastewater were cultured on Sabouraud dextrose agar. After that, the grown colonies were identified using morphological characteristics and microscopic observation. In this study, fungi including *Aspergillus niger*, *Aspergillus flavus*, *Penicillium digitatum*, *Fusarium*, *Alternaria*, *Paecillomyces*, *Drechslera*, *Geotrichum*, *Mucor*, and *Absidia* in cardboard recycling industrial wastewater were identified. Considering the frequency of grown fungi, *A. niger*, *A. flavus*, and *P. digitatum* were selected for bioremediation of the cardboard recycling industry wastewater. Then a suspension containing 1×10^6 CFU mL^{-1} of fungal spore and the wastewater in dilutions of 25%, 50%, and 90% and pH levels of 5, 7, and 8 were prepared. Following that, 10 mL of fungal spore suspension was inoculated into the samples for decolorization and chemical oxygen demand (COD) removal and incubated for 10 d. The results of this study were analyzed by SPSS software and one-way ANOVA tests were performed and the significance level was considered $p < 0.05$. In this study, the maximum decolorization was obtained by *A. flavus* (50.58%) at pH of 7 and dilution 25% and the maximum removal efficiency of COD was obtained by *P. digitatum* (70.98%) at pH of 5 and dilution 25% for 10 d. This study confirms that native fungi have the potential to decolorize and remove COD from the cardboard recycling industry wastewater.

Keywords: Biodegradation; Cardboard recycling; COD; Color; Fungus; Wastewater

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1. Introduction

The wastewater resulting from production processes in industries is one of the main causes of environmental pollution, especially in developing countries [1]. Cardboard recycling industry is one of the fast growing global industries using recycled paper and cardboard for cardboard production. This industry is the third and sixth largest wastewater and pollution producer in the world, respectively [2,3]. Cardboard recycling industry in Yazd has a capacity of 55–60 tons of cardboard production per day. The industry uses about 150–200 m^3 of water per ton of produced cardboard, resulting in a high volume of wastewater. The two main sources of wastewater producers in the cardboard recycling industrial are pulp and cardboard making processes which a major part of the wastewater pollution is due to pulp making stage [4,5]. Properties of the wastewater produced from various processes of cardboard recycling industry depend on the used raw materials, types of processes and technologies applied for cardboard production, management methods, return of treated wastewater, and the amount of water used in production processes [6]. The wastewater of this industry is usually alkaline and has high chemical oxygen demand (COD) characteristics, suspended solids, odor, and light brown color, among which color and COD are of great importance [7].

Several studies have reported the complications associated with this type of wastewater, including carcinogenicity, mutation, endocrine disruption of fish, and other aquatic organisms [8]. The loss of oxygen in the receiving waters, mortality of aquatic plants, unpleasant odor in water, reduction of photosynthesis, esthetic problems, increasing toxic substances in receiving waters, and pollution of drinking and agriculture water are other problems associated with cardboard recycling industry wastewater [4,9,10]. Therefore, the treatment of wastewater from this industry is necessary before discharging into the environment [11]. Applying a suitable method for treatment of these wastewaters can both minimize environmental impacts and water consumption in this industry by reusing the produced wastewater [12,13].

For treatment of the cardboard recycling industry wastewater physical and chemical methods, such as electrical coagulation, ozonation, chemical oxidation, ion exchange, membrane filtration, and surface absorption have been used. Although physical and chemical methods have been effective in removal of contaminants in wastewater, they have limitations, such as high chemical consumption, sludge production, and high operating and investment costs. Therefore, for wastewater treatment of this industry, efficient, cost effective and adaptive methods are required [7,14,15].

Biological methods are preferable to physical and chemical methods due to their simplicity, cost-effectiveness, and lack of adverse effects on the environment [16]. In recent years, using bioremediation processes for wastewater treatment have been proposed as an economical and eco-friendly method containing various types of microorganisms, such as fungi, yeasts, bacteria, actinomyces, and algae [17,18]. In these processes, organic compounds are completely converted to mineral compounds such as H_2O and CO_2 [19]. Microorganisms used for bioremediation processes may be native to the contaminated environment or isolated from different sources and transported to contaminated sites [20]. Due to the wide variety of contaminants in the wastewater, the microorganisms that are able to adapt to these contaminants will dominate and play a major role in wastewater treatment. Among these microorganisms, fungi play a key role in treatment of colored wastewater [21]. Fungi contain a group of extracellular enzymes (lignin peroxidase, manganese peroxidase, and laccase) to break down the biological contaminants in the wastewater; moreover, they are more resistant to bacteria than inhibitors. In addition to the production of extracellular enzymes, the cell wall of fungi and its compounds play an important role in the biological absorption of contaminants in wastewater during the treatment process [22,23].

Contaminants, such as color and COD can be eliminated through biological absorption mechanisms, biodegradation, and fungi enzymatic system. Therefore, one or more mechanisms can be used for decolorization and COD removal, depending on the type of fungus. Based on the results of various studies, fungi, such as *Aspergillus niger*, *Schizophyllum commune*, *Coriolus versicolor*, *Phanerochaete flavidoalba* can decolorize and remove COD from paper and cardboard wastewater [8,24]. Approximately 61% of color and 58% of COD were removed from the paper and cardboard industry by *Nigrospora* sp. during 7 d [2]. Moreover, in another study, 78% of color and 89% of COD were removed from the cardboard industry wastewater by two anonymous fungi isolated from marine environments [25].

Many bioremediation studies have examined paper and cardboard wastewater by various fungal species. Considering that the cardboard recycling industry (which uses paper and cardboard as raw materials) has different production processes and wastewater properties compared with the paper and cardboard industry wastewater (which uses wooden materials as raw materials), the wastewater of this industry has not been studied using these fungal species. Therefore, in this study, due to the strong degrading enzymatic system in fungi and high contamination, especially the color of the cardboard recycling industry wastewater, bioremediation rate of one of the cardboard recycling industries wastewater in Yazd was investigated using native fungi isolated from the wastewater of this industry.

2. Materials and methods

2.1. Sample collection from cardboard recycling industrial wastewater

In this study, three samples were collected from the cardboard recycling industry wastewater treatment plant in Yazd to isolate and identify treating fungal species. The first sample was collected from Kraft tank inlet, the second sample from the Kraft outlet and the third sample from the wastewater storage tank outlet. Moreover, to perform bioremediation tests, a sample was collected from the output of the coagulation and flocculation unit of the wastewater treatment plant. Then all the samples were stored at 4°C and immediately transferred to the laboratory.

2.2. Isolation and identification of fungal species

One mL of the wastewater sample was diluted up to 10^{-9} using sterilized distilled water. Then, 0.1 mL of dilutions

10^{-5}, 10^{-6}, and 10^{-7} was cultured on Sabouraud Dextrose Agar supplemented with streptomycin through the pour plate method. The cultured media were kept at 28°C for 7–10 d. After that, sampling was carried out from grown colonies for purification. They were cultured in separate media of Sabouraud Dextrose Agar (Merck, Germany) at 28°C and incubated for 5 d [26].

2.3. Identification of fungal strains

Colonies appearing in culture media were identified using macroscopic characteristics (conic morphology, shape, tissue, growth rate, color, diameter, and appearance of colony) and microscopic observation (conidiophore shape and structure, reproductive structure, septated mycelium, conidia placement type on conidiophore, etc.) [26].

2.4. Preparation of fungal spore suspension

Among the identified fungi, *P. digitatum*, *A. flavus*, and *A. niger* were selected based on the frequency of the population of fungal species grown on the culture medium for bioremediation tests of cardboard recycling industry wastewater. Separate cultures were prepared from the three selected fungi in the SAD medium and incubated for 28 d at 28°C for 5 d. Then, using sterile physiologic serum, a suspension containing 1×10^6 CFU mL^{-1} spore was prepared from each fungus, separately. The suspension was used to perform wastewater bioremediation tests [27,28].

2.5. Sample preparation

In order to investigate the effect of dilution and pH on decolorization and COD removal efficiencies from cardboard recycling industry, the samples were tested in different dilutions (25%, 50%, and 90%) and pH values (5, 7, and also the initial pH of the wastewater [no pH adjustment]). In order to avoid interference of wastewater suspended particles in tests, the samples were centrifuged at 6,000 rpm for 15 min [17,28]. Total Kjeldahl nitrogen (TKN) and phosphate were measured in accordance with the instructions given in the standard methods in water and wastewater experiments. Furthermore, the carbon-to-nitrogen ratio in the samples was adjusted to 10–12 using ammonium chloride [29]. The samples were autoclaved for 15 min at 121°C and 1 atm pressure. To adjust the samples pH, 1 N sulfuric acid was used [21,30].

2.6. Bioremediation tests

Sterilized samples were inoculated with 10 mL of the suspension containing 1×10^6 CFU mL^{-1} spores of *P. digitatum*, *A. flavus*, and *A. niger*. The total volume of the tested samples was 100 mL which was transferred into the 250 mL Erlenmeyer and incubated in 140 rpm shaker at 30°C for 10 d. The tests were carried out with control samples containing wastewater samples without inoculation of fungal strains. All the tests were conducted with three repetitions [17,31,32]. Incubation sampling was done at intervals of 0, 3, 6, and 10 d.

Then, using a 3,500 rpm centrifuge for 5 min (to prevent the interference of fungal spores in measuring parameters), the supernatant was used to measure the color and COD [16,33]. The color and COD were measured using the American Dye Manufacturer Institute (ADMI) and closed reflex methods by the DR6000 spectrophotometer (Hach Co.), respectively, referred to the standard methods for the examination of water and wastewater book [34]. The results of decolorization and COD removal efficiencies were calculated and reported in accordance with Eq. (1).

$$E = \frac{(C_0 - C)}{C_0} \times 100 \quad (1)$$

E: Color or COD removal efficiency (%); C_0: the amount of color (ADMI) or COD (mg L^{-1}) before bioremediation; *C*: the amount of color (ADMI) or COD (mg L^{-1}) after bioremediation.

2.7. Statistical analysis

Data analysis was performed in Microsoft Excel and SPSS version 16 using Tukey's test as post-hoc and analysis of variance (ANOVA). In addition, *p*-value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Physical and chemical characteristics of cardboard recycling industrial wastewater

The cardboard recycling industry wastewater had light brown color, turbidity, high suspended particles, and an unpleasant odor. The data showed that the output wastewater from the industry has high COD and color, which is above discharge standards. The results of the physical and chemical characteristics of this wastewater are presented in Table 1.

3.2. Identification of native fungal strains in cardboard recycling industrial wastewater

A total of three species of *A. niger*, *A. flavus*, and *P. digitatum*, and seven genus, including *Fusarium*, *Alternaria*, *Paecillomyces*, *Drechslera*, *Geotrichum*, *Mucor*, and *Absidia* were identified from the cardboard industry wastewater. Among the identified fungi, due to the frequency of the population of isolated fungal species grown in the culture medium,

Table 1
Physical and chemical characteristics of the cardboard recycling industry wastewater

Parameter	Value
pH	10
Color, ADMI	865
TSS, mg L^{-1}	503.2
COD, mg L^{-1}	4,000
EC, ms cm^{-1}	11.43
Turbidity, NTU	73.48
Temperature, °C	26

P. digitatum, *A. flavus*, and *A. niger* fungi were selected to perform bioremediation tests of cardboard recycling industry wastewater (Table 2). The colonies that appeared in the culture medium were identified using macroscopic characteristics and microscopic observation [35]. Table 3 and Fig. 1 describe the macroscopic characterization and microscopic observation of selected fungi.

3.3. Bioremediation tests

To investigate the ability of fungi in decolorization and COD removal from cardboard recycling industry wastewater, a laboratory-scale study was conducted. Three types of native fungi isolated from cardboard recycling industry wastewater, including *A. niger*, *P. digitatum*, and *A. flavus* for decolorization and COD removal at different pH (5, 7, and initial pH) and dilutions (25%, 50%, and 90%) were investigated for 10 d.

3.4. Effect of pH on decolorization and COD removal efficiencies by fungal species

The pH of the reaction medium is one of the most important factors that should be considered in the process of bioremediation by fungi. To evaluate the effect of pH, the decolorization by *A. niger*, *P. digitatum*, and *A. flavus* at pH 5, 7, and initial pH (8) was investigated at optimum dilution 25% for 10 d. Given the fact that the maximum decolorization was at dilution 25%, the effect of different pH on the decolorization was reported in this dilution.

Fig. 2 shows that the decolorization by *P. digitatum*, *A. niger*, and *A. flavus* was 20.83%, 23.26%, and 25.17% at pH of 5; 29.52%, 44.41%, and 50.58% at pH of 7 and 47.36%, 36.84%, and 26.31% at pH of 8, respectively. The results of this study showed that by decreasing pH from 8 to 7, the decolorization increased and the maximum decolorization was observed at pH 7 by *A. flavus*.

Fig. 3 shows that the removal efficiency of COD by *P. digitatum*, *A. niger*, and *A. flavus* was 70.98%, 53.86%, and 68.11% at pH of 5; 67.30%, 58.50%, and 63.12% at pH of 7 and 11.08%, 15.32 %, and 17.13% at pH of 8, respectively. The results showed that by decreasing pH from 8 to 5, COD removal efficiency increased and the maximum COD removal efficiency was observed at pH 5 by *P. digitatum*.

In this study, the maximum decolorization was observed at pH of 7 and the maximum COD removal efficiency at pH of 5, which could be due to the fact that the optimum growth for fungi was in acidic pH range close to neutral [16,29]. Biological absorption usually occurs under acidic or basic conditions [16]. Therefore, the environment with neutral and acidic pH was a good condition for the growth of fungal species and, consequently, the color and COD removal from the cardboard recycling industry wastewater. Based on the results of this study, the decolorization and COD removal efficiencies by the three species of fungi differed in various pH. Each microorganism has a minimum, maximum, and optimum pH for growth and metabolism; moreover, the optimum pH is different for growth and metabolism of fungal species [36]. The results of this study showed that fungal species were able to grow and decolorize in all the tested pH (5, 7, and 8), which showed that wastewater can be contacted with fungi without adjusting the pH for degradation of contaminants. Therefore, pH is not a limiting factor in the growth of fungi and decolorization and COD removal efficiencies from cardboard recycling industry wastewater.

The results of the present study were in line with the study of Rajwar et al. [1], who investigated the fungal mixture

Table 2
Frequency of fungus isolated from cardboard recycling industry wastewater

Fungal species	Frequency (CFU mL^{-1})
Absidia	15
Drechslera	2
Alternaria	2
Geotrichum	12
A. niger	38
Mucor	4
A. flavus	28
Fusarium	2
P. digitatum	18
Paecillomyces	2

Table 3
Macroscopic characterization and microscopic observation of fungi selected for biodegradation

Fungus	Macroscopic characteristics	Microscopic observation
P. digitatum	Powdery colonies with moderate growth rate, white color colony at and then greenish blue, reverse side the colony: yellowish white	Transparent and branched reproductive myceliums, with a straight and stretched conidiophore at the top with phialides which chains of circular or oval single cell conidias come out of it which create brush-like views
A. niger	Powdery colonies with rapid growth, colony surface at first white and then black, reverse side of the colony colorless to yellow	Transparent and branched myceliums, long nonseptate conidiophores ended with spherical vesicle covered with phialides and spores. The phialides on the vesicle were placed in two rows which the second row phialides were shorter and flask shaped
A. flavus	Powdered colonies with rapid growth, colony surface yellowish-green, reverse side of the colony colorless to slightly yellow	Long conidiophore, spherical-semi spherical vesicle surrounded by phialides in one or two rows. A chain of smooth or rough conidia was placed on phialides

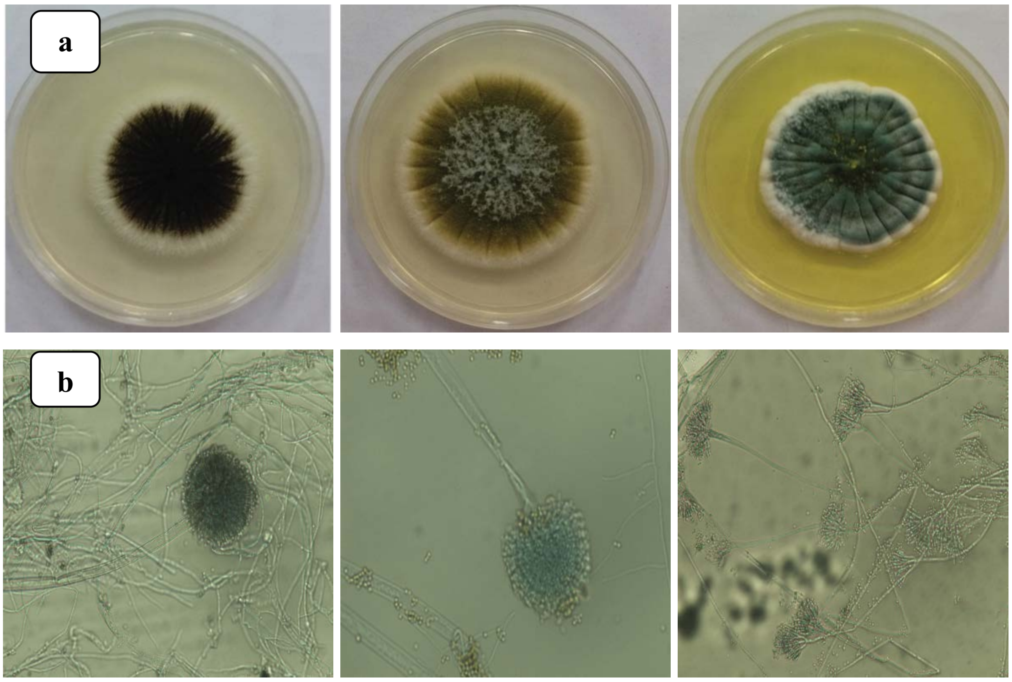

Fig. 1. (a) Macroscopic and (b) microscopic images of fungal species for bioremediation of cardboard recycling industry wastewater, *A. niger*, *A. flavus*, and *P. digitatum*, respectively.

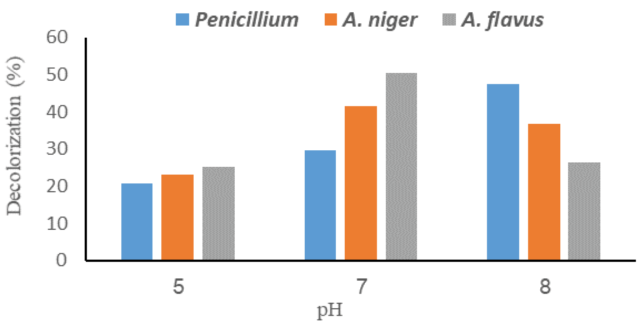

Fig. 2. Effect of pH on the decolorization by fungal species at dilution 25% in 10 d.

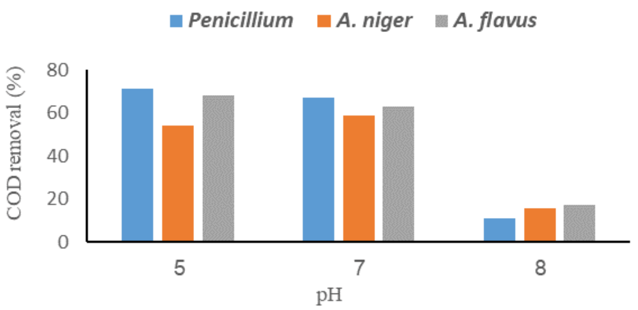

Fig. 3. Effect of pH on the COD removal efficiency by fungal species at dilution 25% in 10 d.

of *Nigrospora* sp. and *Curvularia lunata* for the degradation of paper and cardboard industry wastewater. In this study, the maximum COD removal efficiency (80%) was obtained at pH of 5. In the study of Bhattacharya et al. [36], the fungal species, including *A. niger*, *A. flavus*, *Cladosporium*, *Pleurotus ostreatus*, and *Penicillium chrysogenum* were isolated from soil and slime samples and analyzed for the degradation of Congo red colors at pH levels of 4–10. In this study, the maximum decolorization was found in pH of 7 by *A. flavus* (86.98%), which is consistent with the results of the present study. In the study of Kalaiarasi and Tamilselvi [37], the decolorization of casting wastewater was investigated by *A. niger*, *Aspergillus terreus*, *Penicillium purpurogenum*, and *A. flavus* fungi at pH levels of 5, 6, 7, and 8. In this study, the maximum decolorization (72.74%) was observed by *A. flavus* at pH of 7, which is in line with the results of the present study.

The results of ANOVA test showed that there was no significant difference between levels of decolorization and COD removal efficiencies between fungal species at different pH (5, 7, and 8) (p-value > 0.05). Therefore, the three fungi used in this test had the same performance in the decolorization and COD removal efficiencies.

3.5. Effect of dilution on the decolorization and COD removal efficiencies by fungal species

In order to investigate the effect of dilution, the decolorization and COD removal efficiencies by *P. digitatum*, *A. niger*, and *A. flavus* in different dilutions (25%, 50%, and 90%) were investigated at optimum pH levels of 7 and 5 for 10 d, respectively. Given the maximum decolorization at pH of 7 and the maximum removal efficiency of COD at pH of 5, the effect of different dilutions on decolorization and COD removal efficiencies was reported at this pH level.

Fig. 4 shows that the decolorization by *P. digitatum*, *A. niger*, and *A. flavus* was 29.52%, 41.44%, and 50.58% at dilution 25%; 35.75%, 33.34%, and 35.69% at dilution 50%;

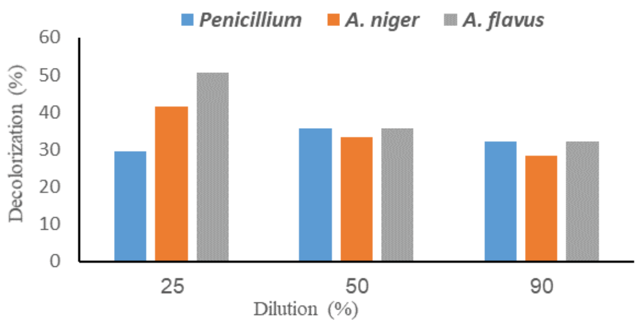

Fig. 4. Effect of dilution on the decolorization by fungal species at pH of 7 in 10 d.

and 32.07%, 28.30%, and 32.07% at dilution 90%, respectively. The results of this study showed that by decreasing the dilution from 90% to 25%, the decolorization increased and the maximum decolorization at dilution 25% was observed by *A. flavus*.

Fig. 5 represents that the removal efficiency of COD by *P. digitatum*, *A. niger*, and *A. flavus* was 70.98%, 53.86%, and 68.11% at dilution 25%; 65.53%, 48.69%, and 67.89% at dilution 50%; and 37.53%, 62.46%, and 38.12% at dilution 90%, respectively. The results of this study showed that by decreasing the dilution from 90% to 25%, the removal efficiency of COD increased, and the maximum removal efficiency of COD at dilution 25% was observed by *P. digitatum*.

Based on the results of this study, fungal species were able to grow and decolorize and remove COD in all the tested dilutions. Moreover, by increasing the degree of dilution from 90% to 25%, the decolorization increased due to the fact that at dilution 25% the concentration of contaminants is lower; therefore, the fungi show a better removal efficiency of contaminants. In the present study, due to the high amount of COD (4,000 mg L^{-1}) indicating the high concentrations of organic contaminants in the wastewater, the samples required dilution to facilitate the treatment process. Dilution leads to a reduction in the concentration of contaminants in the wastewater and a better growth of fungi; therefore, it increases the decolorization and COD removal efficiencies from the wastewater [25,38]. Although the decolorization and COD removal efficiencies was higher at dilution 25%, in the sample with the lowest dilution (90% dilution), the fungi were able to grow, and decolorize and remove COD efficiencies indicating that the wastewater could be contacted with fungi without dilution to decompose the contaminants (Figs. 3 and 4). Therefore, high concentrations of contaminants in the wastewater are not a limiting factor in the growth of fungi, and decolorization and COD removal from the cardboard recycling industry wastewater.

The result of this study was in line with that of Kaurand and Chaman [39]. In this study, four dilutions of dairy wastewater (25%, 33%, 50%, and 100%) were used for bioremediation using *Aspergillus*, *Alternaria*, *Fusarium*, and *Penicillium* fungi. The results of this study showed that *Aspergillus* removed COD by about 80%–86% at dilution 25%. In Liqin et al. [40], the maximum COD removal efficiency was found by *Penicillium* fungus (62/38) which is consistent with the results of the present study. In a study by Verma et al. [41], bioremediation of textile industry wastewater was investigated by four wood destructive fungi, including *Cerrena unicolor*, *Coriolopsis byrsina*, *Diaporthe*, and *Pestalotiopsis* at three dilutions of 20%, 50%, and 90%. The results of this study showed the maximum decolorization at 20% dilution, which is consistent with the results of the present study.

The results of ANOVA test showed that there was no significant difference in the decolorization between fungus species in different dilutions (p-value > 0.05). Furthermore, according to the results of ANOVA, COD removal efficiencies rate was significant only at dilution 90% (p-value = 0.011), which indicates that *A. niger* fungus in this dilution had better COD removal efficiency than two other fungi.

The results of this study showed that at the beginning of degradation, decolorization and COD removal occurred without a delayed phase and the fungi quickly began to decompose the contaminants in the wastewater. Figs. 6 and 7 reveal a significant decrease in the decolorization and COD removal during 6 d of incubation by fungal species. It seems that this maximum decrease in the first days of incubation is due to the fact that the decolorization and COD removal occurs mainly in the growth stage of the fungus [29]. Moreover, the reduction in the decolorization and COD removal after 6 d may be due to poor fungal growth caused by shortage of nutrients over time [16]. In the study by Saritha et al. [25], the efficacy of two anonymous fungi isolated from marine environment and soil contaminated with wastewater and two fungi, including *Phanerochaete chrysosporium* and *T. hirsute* were investigated for bioremediation of paper and cardboard industry wastewater for 21 d. In this study, the removal trend of color and COD in all fungal was slow up to

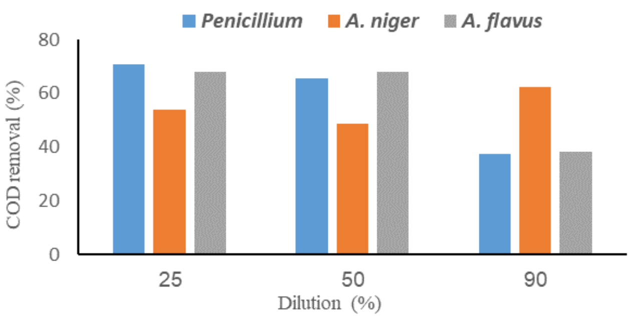

Fig. 5. Effect of dilution on the COD removal efficiency by fungal species at pH of 5 in 10 d.

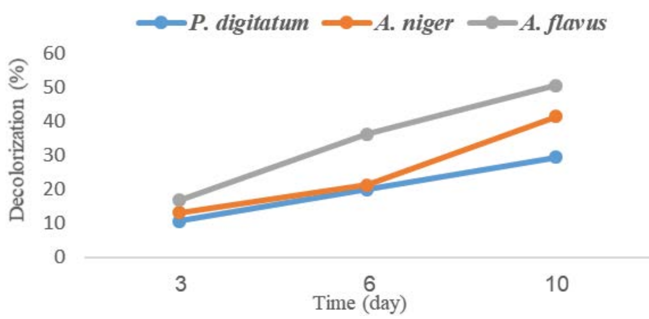

Fig. 6. Decolorization by fungal strains in dilution 25% and pH of 7.

5 d (25%), while after 5 d, a rapid trend was observed. This time was considered necessary for fungal species to adapt to the wastewater samples. The results of this study were not consistent with the results of the current study due to the absence of a delayed phase. In the present study, native fungal species isolated from the cardboard recycling industry wastewater were used which had been already adapted to the wastewater.

The results of this study were in line with the results of Taskin and Erdal [18]. In this study, the decolorization (60.68%) and COD removal efficiencies (89.48%) were observed by *A. niger* for 21 d in the paper industry wastewater. A significant reduction in these parameters occurred after 5 d of incubation.

Figs. 6 and 7 indicate that *A. flavus* at dilution 25% and pH of 7 had the maximum decolorization (50.58%) and *P. digitatum* at dilution 25% and pH of 5 had the maximum COD removal efficiency. In this study, the decolorization and COD removal efficiencies varied by three different fungal species which could be due to the type of enzyme secreted by each fungus [41]. The difference in the production of the enzyme depends on the genetic diversity of the fungal species [16]. In this study, no changes were observed in the control samples indicating the high potential of fungal species in the decolorization and COD removal. Moreover, the results showed that the fungal species had the potential for simultaneous decolorization and COD removal.

Reduction of decolorization and COD removal may be due to high concentrations of soluble nutrients and organic materials in the wastewater. Fungi use the contaminants as a source of carbon and energy for growth, and remove them from wastewater; therefore, they play a major role in bioremediation of wastewater [20,42]. It seems that color the enzymatic activity of the selected fungi is a possible mechanism for removing contaminants, such as and COD from the cardboard recycling industry wastewater [43]. Enzymes are produced throughout the life cycle of fungi. In addition to the production of extracellular enzymes, the cell wall of fungi and their compounds play an important role in biological absorption of contaminants in the wastewater [22]. Fungi reduce these contaminants by using biological absorption, biodegradation, and enzymatic systems, such as lipase, manganese peroxide, and laccase. Therefore, one or more mechanisms can be used to decolorize and remove COD, depending on the type of fungus [44].

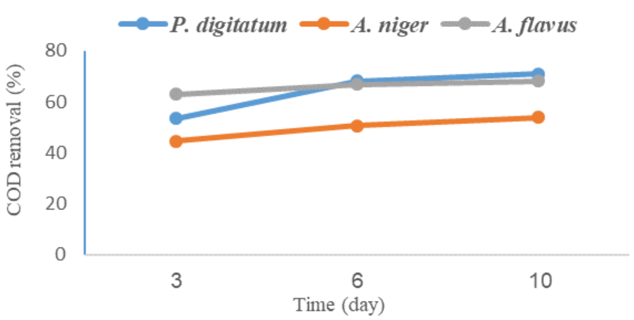

Fig. 7. Removal efficiency of COD by fungal strains in dilution 25% and pH of 5.

4. Conclusion

There are various native fungi according to the nature of the cardboard recycling industry wastewater. *A. flavus*, *P. digitatum*, and *A. niger* fungi were identified as the most common isolated species in the present study. The maximum decolorization by *A. flavus* was observed at dilution 25% and pH 7, and the maximum COD removal efficiency was obtained by *P. digitatum* at dilution 25% and pH 5. The results of this study confirm the growth potential of the native fungal strains of the cardboard recycling industry wastewater, as well as the ability of these fungi to decolorize and remove COD contaminants from the wastewater.

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