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Application of two fungal strains *Aspergillus niger* and *Candida albicans* in wastewater quality improvement

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Abstract

The nutrients Phosphorous, Nitrogen and Carbon are essential for aquatic life. However, in excess they also cause serious problems. For this reason, wastewater treatment must meet nutrient effluent limits. A wastewater sample was taken near Dhahibah village from the Erbil wastewater channel in the north of Iraq. In this experiment, pure cultures of *Aspergillus niger* and *Candida albicans* were used to treat wastewater. Samples were measured for physicochemical parameters like EC, pH, Phosphate, Nitrate, Nitrite, and BOD5 using standard methods every third day during 21 days of experiment. The results revealed that *Aspergillus niger* had the maximum efficiency in removing BOD_5, NH_4, NO_3, and EC (87.27, 89.57, 83.52, and 78.49%) respectively. On the other hand, during the experimental period, *Candida albicans* had the maximum efficiency in decreasing PO4, and NO2, were (91.58% and 88.89%), respectively. Statistically, there were differences ($P \leq 0.05$) between the control sample and the treated wastewater sample for both fungal stains for all parameters during the experiment.

Keywords: Fungal remediation; *Aspergillus niger*; *Candida albicans*; Wastewater.

استخدام سلالتين فطريتين ***Aspergillus niger*** و ***Candida albicans*** في تحسين جودة مياه الصرف الصحي

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الملخص

الفسفور و النتروجين و الكاربون من المغذيات الضرورية للحياة المائية ، ومع ذلك زيادتها تسبب مشاكل خطيرة. لهذا السبب ، يجب أن تعالج مياه الصرف الصحي بحدود تدفق المغذيات. تم أخذ عينة من مياه الصرف الصحي بالقرب من قرية الذهيبة من قناة الصرف الصحي في أربيل. في هذه التجربة تم استخدام مزارع نقية لكل من *Aspergillus niger* و *Candida albicans* لمعالجة مياه الصرف الصحي. تم تحليل العينات للعوامل الفيزيائية والكيميائية مثل الأس الهيدروجيني والتوصيل الكهربائي والفوسفات والنترات والنتريت ، والمتطلب الحيوي للأوكسجين BOD_5 كل ثلاثة أيام باستخدام الطرق المتبعة لمدة 21 يومًا. أظهرت النتائج أن *Aspergillus niger* كانت لها أعلى كفاءة في إزالة BOD_5 و NH_4 و NO_3 و EC (87.27 ، 89.57 ، 83.52 و 78.49٪) على التوالي. من ناحية أخرى ،و خلال فترة التجربة ، كان ل *Candida albicans* أقصى كفاءة في خفض قيمة كل من PO_4 و NO_2 بنسبة (91.58٪ و 88.89٪) على التوالي. احصائيا لوحظ وجود فروقات معنوية ($0.05 \geq P$) بين معاملة المقارنة ومياه الصرف الصحي المعالجة لكل من الفطرين المستخدمين و لجميع العوامل المدروسة خلال التجربة

الكلمات المفتاحية: الفطريات كمعالجات *Candida albicans*; *Aspergillus niger*; مياه الصرف الصحي.

1. Introduction

One of nature's resources is water that is required for all living organisms to survive as well as the ecological syste

m [1]. Water has always been the foundation of many well-known civilizations, and it is currently an essential part of agricultural, commercial, and industrial activities, all of which aid societies and national development [2]. Natural water bodies receive a major proportion of various wastewaters. With the advancement in technology, there is an increase in the amount of waste generated by human activities, which negatively affects the environment, notably the quality of irrigation and drinking water [3, 4]. Water pollution has a significant impact on human health since water bodies serve as waste sinks [5]. Deliberate human waste discharge into water bodies has resulted in significant changes in environmental water quality. As a result, a large number of water bodies have become unsuitable for various uses [6].

Furthermore, water pollution is regarded as a major hazard to the food production process, causing both human health and environmental problems. One of the emerging environmental issues is the degradation of environmental quality as a result of continuous waste effluent discharge in many countries, particularly in developing countries [7]. Less studies have been done on fungi's ability to remove nutrients and organic load from non-sterile wastewater [8].

Additionally, [9] have shown that various fungal species are susceptible of denitrification and [10] have shown that depending on the type of fungus, nitrites, nitrates, ammonia, or organic nitrogen compounds like a yeast extract and peptone can serve as a source of nitrogen for fungi.

Two important procedures for removing phosphorous from municipal wastewater are chemical precipitation, such as the deposition of struvite and biological assimilation by fungi [11]. Fungal phosphorus removal is considered to be more environmentally friendly than chemical precipitation and a cost-effective method of removing and recovering P from wastewater [12]. Fungi might be found in water, soil, sea, and the surface as well as a body of microorganisms. In different habitats, different populations of yeast could be found, and changes in the community reflect changes in the environment [13]. It has been proposed that the fungus *Cryptococcus* sp., *Trichosporon* sp., *Candida* sp., and *Rhodotorula* sp. serve as indicators of water contamination [14]. Furthermore, yeasts are highly biodegradable to a variety of resistive and organic poisons [13]. When compared to bacteria, filamentous fungi have the benefit of being easier to harvest because of mycelium growth and having a higher tolerance to poisonous and inhibitory substances [12, 15]. As a result, in addition to being potential candidates for micropollutant removal, filamentous fungi could also be able to improve conventional biological wastewater treatment to reduce nutrient concentrations [16]. They comprise a considerable group of microbial communities involved in the treatment of wastewater [17], and assist in the degradation of organic substances, including endocrine-disrupting substances, pharmaceuticals, heavy metals, lipids, complex carbohydrates, proteins, and aromatic hydrocarbons, using a variety of intra- and extra-cellular enzymes as well as sorption processes [18-20]. This study aims to use fungal strains to reduce or remove pollutants by degrading pollutants biologically into non-toxic compounds and to improve wastewater quality.

2. Material method

2.1 Wastewater sample collection and experimental set-up

The domestic wastewater samples used in this study were collected from the Erbil wastewater channel near Dhahibah village. The experiments described in this study were carried out using two different species of fungi. The most dominant species such as *Aspergillus niger* (ATCC 1640), and *Candida albicans* (ATCC 10231), were utilized as test organisms for the treatment of wastewater. On a horizontal orbital shaker, experiments were conducted in Erlenmeyer glass flasks as batch reactors at 150 rpm and 27 °C remediation experiments. To obtain enough biomass for the subsequent growth and wastewater treatment experiment, *Aspergillus niger* were cultured in the Sabouraud Dextrose Agar (SDB) and incubated for 7 days, while *Candida albicans* were incubated for 24 hrs before applying to wastewater treatment.

2.2 Microbial cultivation

2.2.1 Mold culture *Aspergillus niger* preparation

The fresh culture of selected fungi *A. niger* in this study were sub-cultured individually onto slants containing solidified Sabouraud Dextrose Agar (SDA) and incubated for 7 days at 25±1°C. Spores of *A. niger* were suspended from each culture slant in 10 ml of sterile distilled water. Double-sterile water were used to dilute spore suspensions in order to obtain similar cell counts of 3×10^4 CFU/50 ml.

2.2.2 Yeast culture *Candida albicans* preparation

The fresh culture for *C. albicans* were sub-cultured individually onto slants containing solidified Sabouraud Dextrose Agar (SDA) and incubated for and 24 hrs at 37°± 1°C, yeast cells were suspended in 10 ml of sterile distilled water. Double-sterile water was used to dilute yeast cells to get similar cell counts of 2×10^4 CFU/50 ml.

2.3 Analytical methods

The wastewater samples were analyzed for pH, EC, TDS, BOD_5, PO_4, NH_4, NO_3, and NO_2. The BOD5 was calculated using standard methods in a certified laboratory [21]. All nutrient analyses used a spectrometer to be analyzed photometrically. To study the role of fungi in wastewater treatment, wastewater was treated with pure culture of *Aspergillus niger* (*A. niger*), and *Candida albicans* (C. *albicans*). At the beginning of each series of experiments, 1200 mL of wastewater was inoculated in the flasks with pre-cultured both species as mentioned above. For single cultures of 12 ml of a uniform suspension of pure cultures were used as initial inoculums added in each flask containing 1200 ml wastewater sample [22-24]. The experiment was conducted under controlled conditions (27 ± 2 ºC) for a total duration of 21 days. Samples were periodically (every 3rd day) analyzed for all parameters using standard methods [21].

2.4 Statistical analysis

Statistical analysis was conducted for the data using a software program (SPSS version 25) and Excel spreadsheets. One-way ANOVA (Analysis of variance), a post hoc test (multi comparisons Duncan test) was applied to determine significant differences at 5%. All data are expressed as Mean ± SE [25].

3. Results and discussion

Wastewater samples were collected from Erbil wastewater channel near Dhahibah village. Some physicochemical parameters such as pH, EC, BOD_5, NO_3, NO_2, and PO_4 of wastewater were analyzed before treatment as control. The mean values of pH, EC, TDS, BOD_5, PO_4, NH_4, NO_3 and NO_2 were 8.2, 823 µS/cm, 487.2 mg/l, 982 mg/l, 29.7 mg/l and 76.05 mg/l, 68.27mg/l and 21.78 mg/l respectively before treatment(control) (Table1) and (Figure 1-6).

Table 1: Erbil wastewater characteristics and effect of *Aspergillus niger* and *Candida albicans* on their quality improvement, data represented as (Mean± SE).

Parameters	Control	*Aspergillus niger*	*Candida albicans*
pH	8.2 ± 0^a	8.38 ± 0.08^b	8.26 ± 0.08^b
EC ($\mu S.cm^{-1}$)	823 ± 0^a	319.8 ± 4.53^b	325.2 ± 4.59^b
TDS ($mg. l^{-1}$)	487.2 ± 0^a	197.8 ± 3.006^b	200.3 ± 3.10^b
BOD_5 ($mg. l^{-1}$)	982 ± 0^a	485.00 ± 6.3^b	488.5 ± 6.07^b
PO_4 ($mg. l^{-1}$)	29.7 ± 0^a	13.42 ± 0.242^b	12.53 ± 0.219^b
NH_4 ($mg. l^{-1}$)	76.05 ± 0^a	26.17 ± 0.399^b	27.68 ± 0.441^b
NO_3 ($mg. l^{-1}$)	68.27 ± 0^a	34.09 ± 0.53^b	33.65 ± 0.505^b
NO_2 ($mg. l^{-1}$)	21.78 ± 0^a	9.514 ± 0.225^b	9.542 ± 0.226^b

The pH value of wastewater at all batches steadily increased reaching 8.65, 8.52, and 8.45 after the 21st day of treatment. When the wastewater was treated with *A. niger*, and *C. albicans* the pH decreased initially and then increased (Figure 1). This is in agreement with the reports of [26]. It may be a result of the accumulation of organic acids and an indication of how well the microbes are able to biodegrade the effluent. Additionally, prior research has demonstrated that *T. versicolor* and *A. luchuensis* can reduce pH levels to 5 immediately following the incubation of fungal biomass in wastewater [27].

Yeasts grow in an acidic atmosphere with a pH of 5-6 and an optimal growth temperature of 25-30°C. As a significant microbial resource, they can adapt to a variety of conditions and have a healthy enzyme system in their bodies. Their characteristics include high metabolic efficiency, osmotic pressure resistance, acid resistance, and high temperature tolerance. As a result, it is extremely important to the biological treatment of wastewater. Remediation of electrical conductivity (EC) with *A. niger*, and *C. albicans* were 78.49%, and 78.12% respectively after 21st days of wastewater treatment, whereas TDS removals by the same fungal strains were, 79.76 and 79.47% respectively. During the present study, a single culture of *A. niger* showed the highest removal of EC and TDS from wastewater (Figure7). Total dissolved solids (TDS) refer to the amount of substances (salts, minerals, metals, calcium, and other organic and inorganic compounds) that have been dissolved in a liquid. TDS levels that are too high or too low might reduce the effectiveness of wastewater treatment plants and industrial activities that utilize raw water. TDS levels in wastewater typically varied from 250 to 850 $mg. l^{-1}$. TDS changes follow the same trend as EC changes [28]. As the concentrations of dissolved salts changes during wastewater treatment processes, the overall salt concentration leads to a change in the conductivity, which is a general measure of water quality [29].

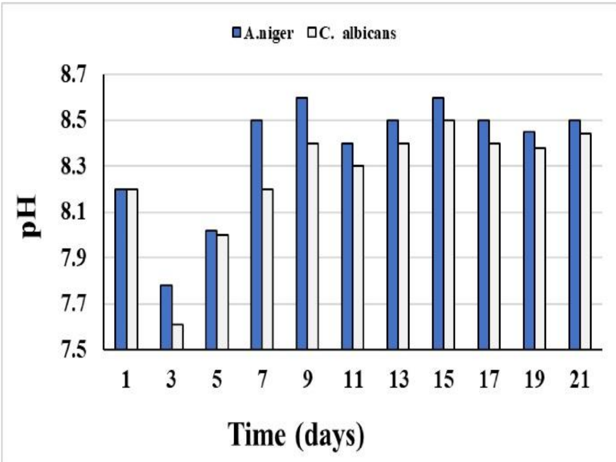

Figure 1: Effect of fungal strains on pH values of wastewater.

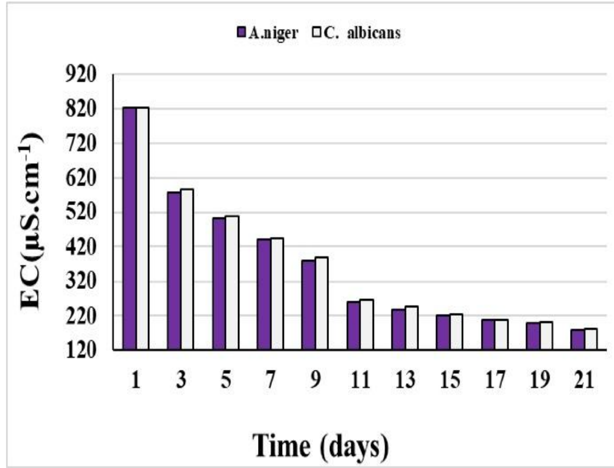

Figure 2: Effect of fungal strains on electrical conductivity of wastewater.

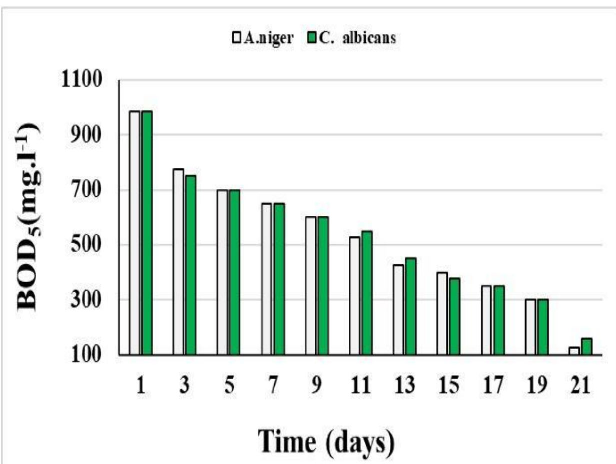

Figure 3: Effect of fungal strains on BOD_5 values of wastewater.

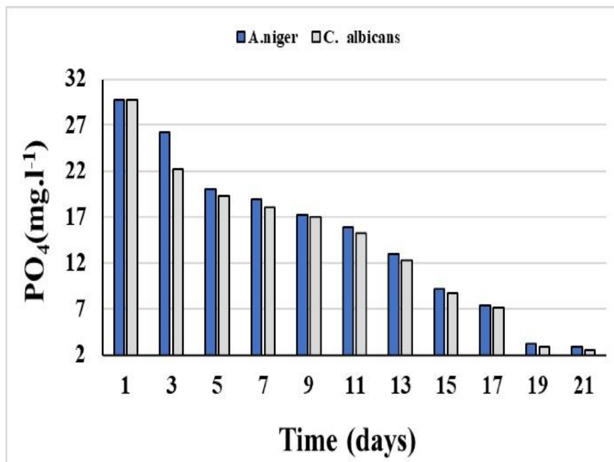

Figure 4: Effect of fungal strains on phosphorous content of

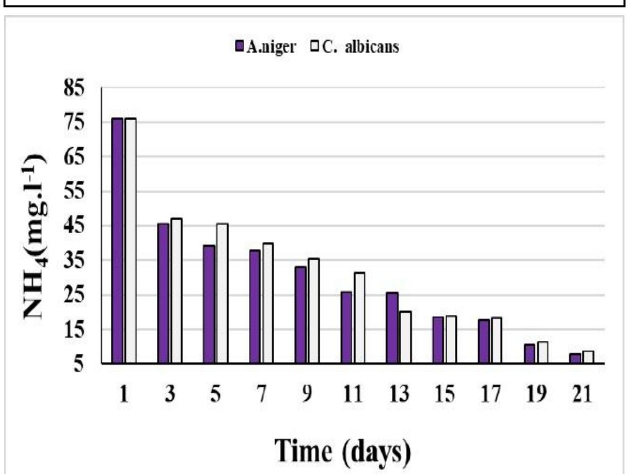

Figure 5: Effect of fungal strains on ammonium content of wastewater

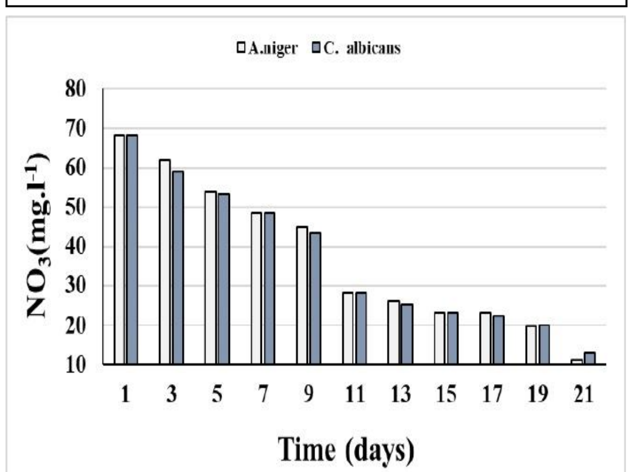

Figure 6: Effect of fungal strains on nitrate content of wastewater.

BOD_5 in the present study reduced when wastewater was treated with *A. niger*, (87.27%), and *C. albicans* (79.63%) (Figure 7). Organic contaminants are broken down by microorganisms as they consume them for growth and reproduction. The microorganism obtains energy by catalyzing chemical reactions that produce energy, and this energy is then used to produce a new cell [30]. Fungal communities serve as the main decomposers in wastewater treatment systems as a result of the size and diversity of the fungal biomass. They are particularly crucial for the biodegradation of organic matter and the cycling of nutrients [31].

The percentages of Phosphate (PO_4) reduction using *A. niger*, and *C. albicans* were 90.23, and 91.58%, respectively (Figure 7). Orthophosphate or organically bound phosphates are the major forms of phosphorus in natural water and wastewater. [32]. Thanh and Simard (1973) demonstrated the capacities of seventeen fungal biomasses to remove phosphates (84.1 %) [33]. *Aspergillus terreus* separately and successfully removed phosphorus (58 %) [34]. To remove phosphorous from the ore, *Aspergillus niger* was used, and after 21 days, 13.8 and 33.2 %, respectively, were removed [35].

Fungi are potentially effective candidates for removing nutrients and organic and inorganics from municipal wastewater [36]. Researchers that have previously used batch experiments to study the removal of phosphorus (P) from domestic wastewater have also shown that fungi can remove P, with removal efficiencies ranging from 12 to 100% [10, 11, 37]. The pH has a significant influence in the removal of P by the use of fungus in synthetic brewery wastewater [38]. Overall, in the future, a wastewater treatment system and reduction methods may greatly benefit from phosphorus reduction by fungi.

Percentage reduction of nitrate was 83.52%, and 82.35% respectively after 21 days of the experiment, whereas ammonia reduction with *Aspergillus niger*, and *Candida sp*. was 89.75%, 88.46% respectively. Nitrite in the present study reduced when wastewater was treated with *A. niger* (88.79%), and *C. albicans* (88.98%) (Figure 7). According to [39], reduction rates in nitrite concentration was 97% when *A. niger* and *A. terrues* treated the wastewater. In contrast, *P. digitatum* caused an 86% reduction in nitrite content over the same time period. According to research, ammonium ion (NH_4^+) and free ammonia (NH_3) in wastewater are the two reversible forms of NH_4-N [40]. The pH level of wastewater has a significant impact on the composition ratio of NH_3 to NH_4^+[41]. The proportion of NH_3 increases with pH level; conversely, the proportion of ammonium ions increases with low pH [41, 42]. According to [43] NH_4-N removal increased linearly as pH increased, demonstrating the significancy of pH for the stability of the wastewater treatment system. Nitrogen, is a critical component of growth n which is used by fungi and employed for building many biomolecules, including amino acids, vitamins and proteins [44], that are generally involved in the formation of cellular organelles. Both inorganic and organic nitrogen is vital for fungal growth and regulating the process of nutrients moving through the cytoplasmic vascular system [45].

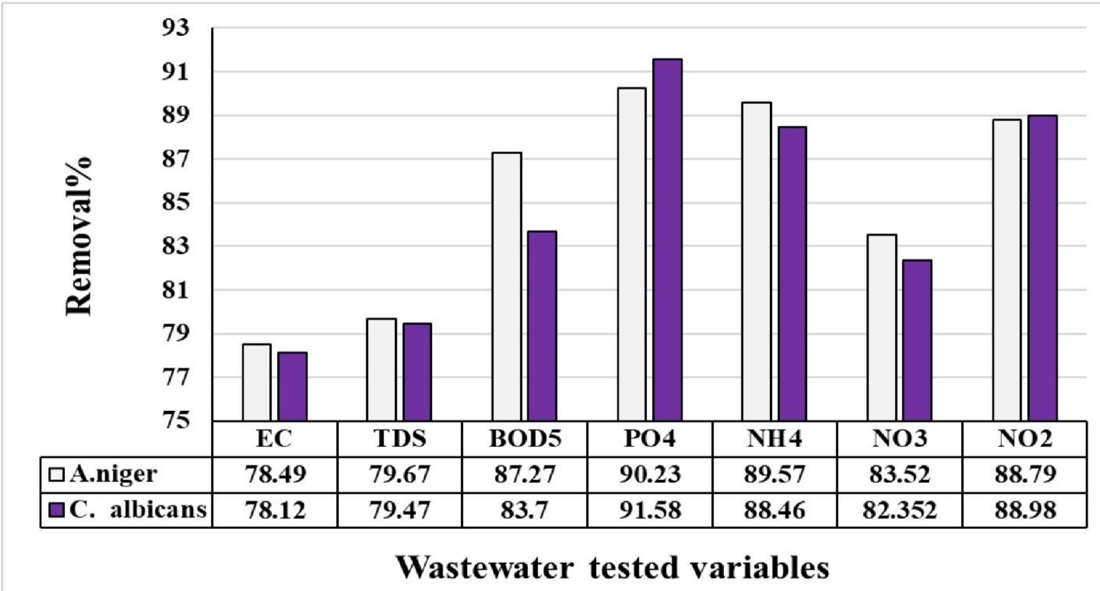

Figure 7: Removal percent of waste water tested variables by *Aspergillus niger* and *Candida albicans*.

Conclusion

The experiment revealed that both fungi lead to raised pH. *Aspergillus niger* is more effective to remove BOD_5 than *Candida albicans* in addition to NH_4, NO_3, and EC in 21 days of experiment. While *Candida albicans* shows the higher removal percent of PO_4, and NO_2.

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