DJS Vol. 48(2)(2024) –pp.161-181 ISSN: 1012-5965

Delta Journal of Science
Available online at
https://djs.journals.ekb.eg/

Research Article

MICROBIOLOGY

Mycoremediation of industrial wastewater using *Aspergillus niger (EM1)* and *Fusarium proliferatum (EN1)* mats and pellets biomasses

Mohamed M. Gharieb[1], Nouran Y. Mohamed[2] and Engy Mustafa[2*]

[1]Botany and Microbiology Department, Faculty of Science, Menoufia University, Shibin Al Kawm, Al Menoufia, Egypt
[2]Sanitary and Environmental Institute (SEI), Housing and Building National Research Centre (HBRC), Giza, Egypt
***Corresponding author:** Engy Mustafa **E-mail**: engymustafa98@gmail.com

Received: 23/7/2024 **Accepted:** 7/8/2024

KEY WORDS

Aspergillus niger EM1, Fusarium proliferatum sp. *(EN1),* Mycoremediation, Mat and pellets biomasses, Tannery wastewater.

ABSTRACT

The study evaluated the efficacy of *Aspergillus niger* and *Fusarium proliferatum* as mat and pellet biomasses for wastewater treatment as a mycoremediation method. In synthetic wastewater, *A. niger* mat form demonstrated a maximum removal efficiency of (80.20[a] ± 0.16%) for organic pollutants under specific conditions (pH 6, 0.1 gm in 100 ml, 7 days at 150 rpm, 30°C). However, mycelial pellets of this fungus achieved a higher maximum removal efficiency of (86.96[a] ±0.05%) for organic pollutants (at a concentration of 65.20 ±0.23 mg/L) under different conditions (pH 7, 0.2 gm in 100 ml, 5 days at 150 rpm, 30°C). *F. proliferatum's* mycelial mat demonstrated a maximum removal efficiency of 48.07a ±0.08% for organic pollutants under specific conditions at a concentration of 259.65 ± 0.42 mg/L, pH 6, 0.2 gm in 100 ml, 7 days at 150 rpm, 30°C), Mycelial pellets demonstrated maximum removal efficiency of 33.57a ± 0.11% for organic pollutants under various conditions (pH 6, 0.2 gm in 100 ml, 7 days at 150 rpm, 30°C). The study found that *A. niger* pellet had significantly higher removal efficiency than mat form, while *F. proliferatum* mat and pellet forms showed similar differences but lower values. *A. niger* is generally more effective in decomposing water pollutants, offering scalability in bioremediation applications, ease of production in labs, and pH tolerance. The study suggests that fungal biomass, particularly pellets, can effectively and eco-friendly treat wastewater with variable acidic or alkaline conditions through mycoremediation.

Introduction

Water is an essential resource for life, and its importance in maintaining good health cannot be overstated. It plays a crucial role in various bodily functions, including hydration, digestion, detoxification, nutrient transport, and regulation of body temperature **(Rathi *et al.*, 2021)**. Water also supports the proper functioning of organs such as the kidneys and liver, aids in digestion and prevents constipation, and acts as a solvent for essential nutrients, distributing them throughout the body **(Wang & Yang, 2016)**. Additionally, maintaining adequate hydration is vital for optimal physical performance, brain function, and weight management **(Libey *et al.*, 2020).**

Tannery wastewater is a significant environmental issue due to its high concentration of pollutants and contaminants **(Hansen *et al.*, 2020)**. It contains toxic chemicals like chromium, heavy metals, sulfides, and organic matter, which can lead to water pollution and pose risks to human health and aquatic organisms **(Sivagami *et al.*, 2018)**. Tannery wastewater can also contaminate soil, affecting crop growth and irrigation, and produce foul odours that can impact local communities and property values **(Korpe & Rao, 2021)**. To mitigate these disadvantages, tanneries need to implement effective wastewater treatment processes, adopt sustainable practices, and comply with environmental regulations **(Yadav et al., 2019)**.

Fungi have been emerged as valuable organisms in bioremediation, which is the process of using biological organisms to eliminate or neutralize pollutants from the environment **(Wet & Brink, 2021)**. Fungi employ various techniques and mechanisms to contribute to water bioremediation **(Lin *et al.*, 2022)**. These include biodegradation, biosorption, extracellular enzyme production, mycoremediation, pollutant transformation, symbiotic relationships, and bioremediation consortia **(Liu *et al.*, 2017)**.

Biodegradation involves the enzymatic breakdown of complex organic pollutants in water, with fungal enzymes like ligninases, peroxidases, and laccases **(Dave & Das, 2021)**. Biosorption allows fungi to adsorb and absorb pollutants onto their cell walls or mycelium, thereby reducing pollutant concentrations. Extracellular enzyme production involves fungi secreting enzymes outside their cells, facilitating the breakdown of complex pollutants into simpler compounds that can be metabolized by fungi or other

microorganisms **(Marco-Urrea *et al.*, 2015)**. Mycoremediation involves using fungi to remediate contaminated environments, such as employing white rot fungi and oyster mushrooms. Fungi can also transform pollutants through processes like oxidation, reduction, methylation, and demethylation, leading to the detoxification or mineralization of pollutants and reducing their harmful effects on the environment **(Pal *et al.*, 2020)**. Symbiotic relationships between fungi and other organisms can enhance bioremediation processes by combining the metabolic capabilities of multiple organisms. Bioremediation consortia work synergistically to degrade a wide range of pollutants in water, utilizing the complementary metabolic pathways of different microbial species **(Kumar *et al.*, 2021)**.

Fusarium proliferatum and *Aspergillus niger* are fungal species that have been studied for their potential applications in bioremediation of wastewater. They produce enzymes that can degrade organic pollutants in wastewater, breaking down complex compounds into simpler substances **(bibi *et al.*, 2018)**. These fungi can also adsorb heavy metals and organic compounds onto their cell walls through biosorption processes, effectively removing contaminants from wastewater **(Shan *et al.*, 2022)**. Moreover, *Fusarium proliferatum* and *Aspergillus niger* have the ability to degrade hydrocarbons, such as petroleum products, in wastewater **(Rajendran *et al.*, 2017)**. They produce enzymes that break down hydrocarbons, contributing to the remediation of oil-contaminated water bodies **(Gulzar *et al.*, 2017)**. Additionally, they can aid in nutrient removal from wastewater by utilizing nitrogen and phosphorus compounds as nutrient sources, thereby improving water quality **(Salgado *et al.*, 2016)**. Furthermore, these fungi can detoxify pollutants by transforming them into less harmful forms through various metabolic processes **(Kumar & Dwivedi, 2019)**. Therefore, these fungal strains offer promising potential for the bioremediation of wastewater contaminated with organic pollutants, heavy metals, and other harmful substances. This is because their biodegradative, biosorptive, and detoxification capabilities make them valuable tools in environmental remediation efforts. However, further research and application development are necessary to optimize their use in wastewater treatment processes and enhance their efficiency in bioremediation.

Materials and Methods

Collection of samples

Sterilised glass bottles were used to collect wastewater samples from PIEL Colour Tannery and Union for Leather

SAE planet in Quesna, Egypt, the wastewater was not treated beforehand.

Fungal Isolation

Using potato dextrose agar (PDA) media, the collected wastewater samples were screened to determine if any fungus species could be isolated. A 1 mL sample of Tannery effluent was placed in 9 mL of distilled water and diluted up to 10^{-6} in steps. 0.1 millilitre of each dilution was inoculated onto PDA plates; 100 mg/L of ampicillin to media inhibit the growth of bacteria. The plates were incubated at 28ºC for five to seven days. the most isolates were taken and cultured in Dox agar medium, which was prepared without adding any carbon or nitrogen sources, and Tannery wastewater after sterilization was used as carbon and nitrogen sources in media. The plates were then cultured for five to seven days at 28°C to obtain the most fungal isolates that can grow in these media and be able to make treatment. Two fungal species were purified and identified morphologically and genetically, as shown in a and d in Fig. (1). Then preserved at 4°C for future experiments.

Fig. (1): (a) purified fungal colonies of *Fusarium proliferatum (EN1*), (b and c) mat and pellets of *F. proliferatum (EN1*), (d) Purified fungal colonies of *A. niger EM1*. (b and c) mat and pellets of *A. niger*

Molecular identification of fungal strains

Utilising internal transcribed spacer (ITS) amplified DNA and genomic DNA, the three most potent fungal isolates were identified genetically. 100 mg of mycelium was used to extract the genomic DNA for the ITS-based sequencing. QIAamp DNeasy Plant Mini kit Catalogue no. 69104 was used for DNA extraction. ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCC TCC GCT TAT TGA TAT GC) primers were utilised **(Luo and Mitchell, 2002)**. Conventional PCR

Master Mix preparation in the Biotechnology Unit, Reference Lab for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt, using the Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit. For the creation of the traditional PCR Master Mix, 12.5 µl of Emerald Amp GT PCR mastermix (2x premix), 4.5 µl PCR grade water, 1 µl each of the Forward and Reverse primers (20 pmol), and 6 µl of Template DNA were utilised. In a DNA Engine Thermal Cycler, the PCR was carried out with hot beginning at 94°C for five minutes, 35 cycles of 94°C for thirty seconds, 56°C for forty seconds, and 72°C for fifty seconds, and a final extension carried out at 72°C for ten minutes. Using an automated DNA sequencer from Applied Biosystems (ABI, 3130, USA), the samples were sequenced either forward or backward**.** Making use of a prepared response Bigdye cycle sequencing kit, Terminator V3.1. (Applied Biosystems/Perkin-Elmer, Foster City, CA), Cat. No. 4336817. Using the Basic Local Alignment Search Tool, or BLAST® analysis the first sequence identity to GenBank accessions seen at https://submit.ncbi.nlm.nih.gov/subs/?search=SUB14019645 was established **(Altschul et al., 1990)**. Phylogenetic analyses were performed using maximum likelihood, neighbour joining, and maximum parsimony in MEGA6 **(Tamura et al., 2013)** and the CLUSTAL W multiple sequence alignment programme, version 12.1 of the MegAlign module of Lasergene DNAStar software Pairwise (Madison, Wisconsin, USA), which was designed by Thompson et al., (1994).

Fungal inoculum preparation for bioremediation

Mycelial mats and pellets were created as the fungal inoculum for the bioremediation investigation on tannery effluent. Triplicates of Erlenmeyer flasks each containing 100 ml potato dextrose broth and were inoculated by mycelial discs and incubated for five days at 28^{o}C, these flasks were incubated in a static environment for production of mycelial mats. Another triplicate was incubated in an orbital shaker at 130 rpm for production of mycelial pellets (b, c, e and f in Fig.1) **(Sharma & Malaviya, 2014)**. Both kinds of mycelia were filtered using sterile filter layers and air dried. Before being used in treatment, the pellets were renewed by soaking them in a solution of 0.1% glucose and 0.1% ammonium sulphate for one hour, after which they were thoroughly washed with deionized water. The pellets were kept in a 0.8% NaCl solution until needed, where they can be

used for up to two months at 4°C **(Boujelben et al., 2022)**.

Analysis of untreated wastewater

Using a pH metre model Adwa AD 8000, the pH, total dissolved solids (TDS), and electrical conductivity (EC) were measured. A spectrophotometer and COD digestor were used to quantify the chemical oxygen demand (COD), and after five days, the biological oxygen demand (BOD5) was determined. The stannous chloride technique was used to measure the total phosphorus, whereas Kjeldahl-N was used to analyse the total nitrogen content (TKN) and N-NH4. Atomic absorption spectrometry (AAS) was used to measure the amounts of heavy metals. The 24th Edition of the Standard Methods for the Examination of Water and Wastewater was followed in all analyses.

Batch study

In the given experimental setup, a batch approach was used to investigate the effects of chemical oxygen demand (COD) treatment on two fungal strains, *Aspergillus niger* EMI and *Fusarium proliferatum* (ENI). The goal was to examine the COD removal efficiency and assess the morphology of living cells in the mat and pellets. The initial COD concentration was set at 500 ppm. pH values of 4, 5, 6, 7, 8, 9, and 10 were adjusted before experimentations. Different amounts of fungal biomass were used in the experiment. The values tested were 0.1, 0.2, 0.5, 1, and 2 grams (fresh weight) in 100 mL of solution. Contact times of 1, 3, 5, 7, 9, and 11 days were examined and the shaking speed were 80, 100, 120, and 150 rpm. The experiment involved testing different temperatures to evaluate their influence on COD removal efficiency were 15, 20, 25, 30, 35, and 40°C. To measure the remaining COD in the solution, Whatman No. 41 filter paper was used to remove the adsorbent suspension after digestion using a COD digestor. The solution was then analyzed using a UV spectrophotometer at a wavelength of 600 nm. The equation provided (Equation 1) was used to calculate the percentage of removal efficiency (R):

$$\mathbf{R\ (\%) = [(Co - Ce) / Co] \times 100}$$

(Equation 1)

Where:

R: Percentage of removal efficiency

Co: Initial COD concentration

Ce: COD concentration after treatment

This analysis allows the assessment of the effectiveness of the treatment under different parameters and conditions.

Statistics

The results were represented as the average of three measurements or as the mean ± standard error. A one-way ANOVA test was used to identify significant differences, and Duncan's test

at P<0.05 (SPSS) was used after. IBM, NY, software version 22.

Results and Discussion

Fungal Isolation and Identification

The data indicated that the fungal isolates were *Fusarium proliferatum* coded as (EN1) belonging to *Fusarium* and *Aspergillus niger* coded as EM1 belonging to *Aspergillus*. ITS gene sequencing enabled the genetic identification of the most promising fungal isolates, EM1 and EN1 shown in Fig. (2).

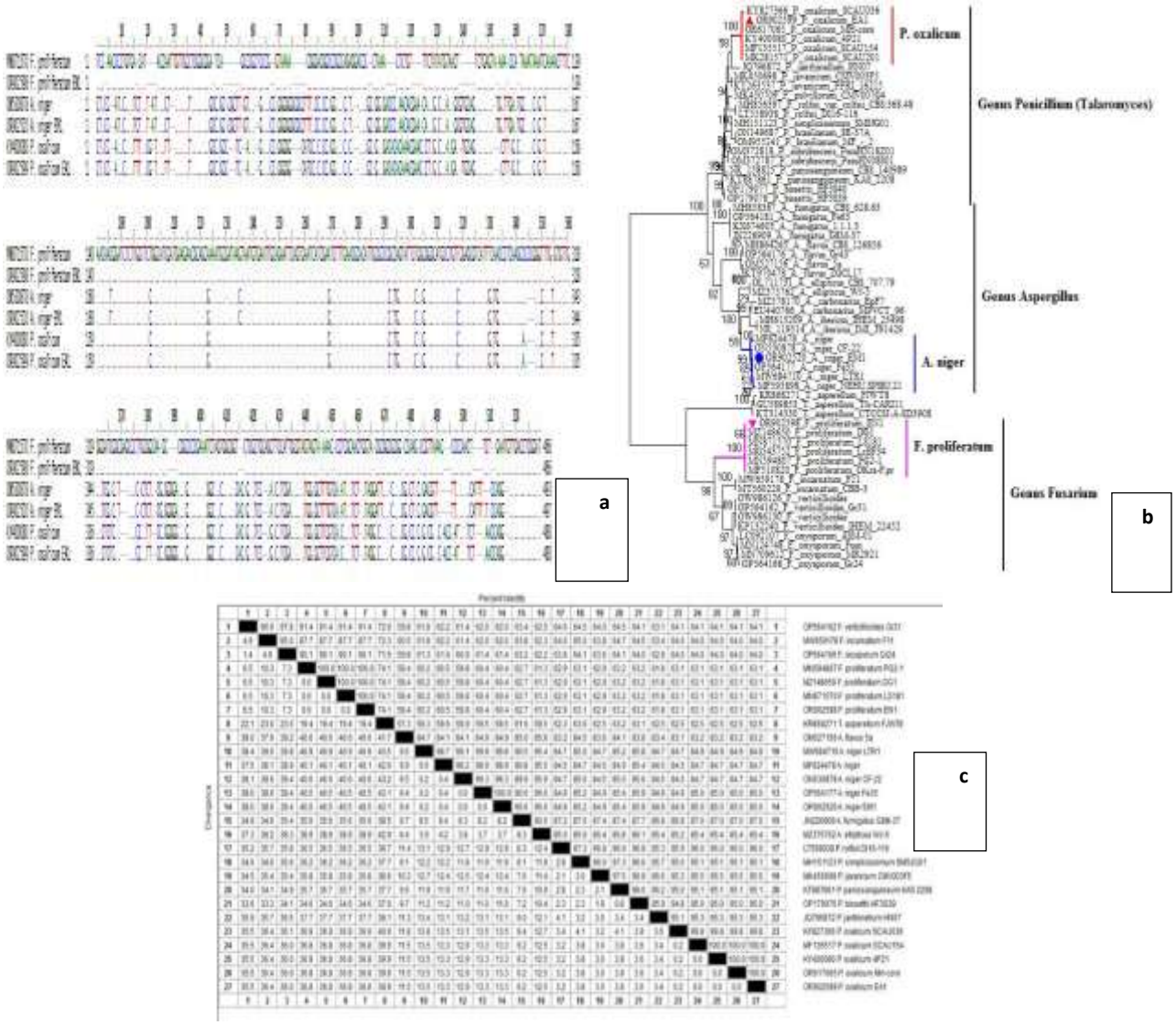

Fig. (2): (a) DNA alignment of fungus parasitic on Pulsatilla species' ITS region sequences. denotes nucleotide sequence indel or non-sequencing; indicates nucleotide sequence identity across all sequences examined for comparison. *Aspergillus niger EM1* and *Fusarium proliferatum* sp. (EN1) are two species. (b) ITS sequences from the fungal isolates A2 and G2-1, identified as *Aspergillus niger EM1* and *Fusarium proliferatum* sp. *(EN1)*, are arranged in a phylogenetic tree using sequences from the NCBI. (c) SUB14019630 *Aspergillus* and SUB14019645 *Fusarium*, with similarity percentages of 100%, respectively, were the molecular identities based on the ITS sequencing study for the accession numbers of *A. niger* EM1 and *F. proliferatum* EN1 (Fig. 3). The sequences that were acquired from this investigation were entered into Gene Bank with accession numbers OR902520 and OR902598 for *F. proliferatum* EN1 and *A. niger* EM1, respectively.

Effect of pH on Chemical oxygen demond (COD) removal

The results in Fig. (3) indicated that maximum removal efficiency of *A. niger* EM1 for mat form were (65.95[a]± 0.04%) (170.23 ±0.21mg/L) detected at (pH 6, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and for pellet form was (73.81a ± 0.08%) (131.33 ±0.33 mg/L) detected at (pH 7, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and the maximum removal efficiency of F. proliferatum EN1 for mat form were (41.34[a] ±0.06 %) (293.32 ±0.31 mg/L) detected at (pH 6, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and for pellet form was 26.30[a] ±0.13 % (368.52 ±0.64 mg/L) detected at (pH 6, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) as shown in Fig. (3).

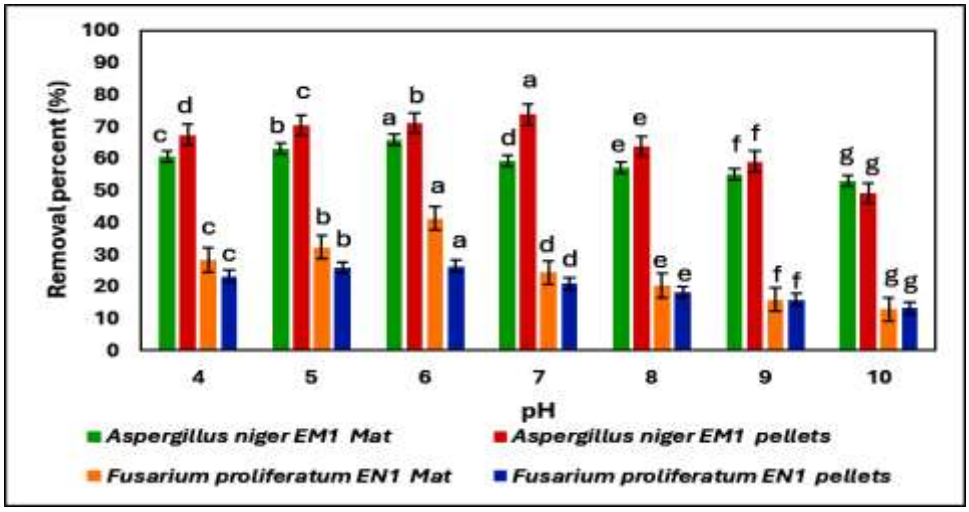

Fig. (3): Effect of pH on COD removal using mycelial mats and pellets of *A. niger EM1* and *F. proliferatum* mats and pellets, each value is the mean of three readings ± standard errors. Values with the same small letter in the same column show non-significant difference (at ≤ 0.05).

To preserve their internal pH levels, fungi employ a variety of adaptation strategies, such as controlling ion transporters, synthesising enzymes such as ATPases and proton pumps, releasing organic acids, controlling gene expression, and undergoing morphological modifications **(Misslinger et al., 2021)**. Even when there are pH variations outside the cell, ion transporters aid in preserving intracellular pH levels **(Skoneczny, 2018)**. Proton pumps and ATPases are examples of enzymes that assist in pumping protons out of cells and controlling pH levels inside cells **(Gleason et al., 2019)**. Organic acids can raise the pH of the surrounding medium to a more growth-friendly level by either acidifying or alkalinizing it **(Jiang et al., 2022)**. Fungal gene expression control enables the

production of proteins and enzymes required for survival under a variety of pH conditions. Extreme pH circumstances can also be accommodated via morphological modifications, such as changing the hyphal growth pattern or developing specialised structures **(Gostinčar et al., 2022)**. It has been shown that 5.5 to 7 is the ideal pH range for *F. oxysporum* development and sporulation **(Ajmal et al., 2023)**.

Effect of biomass dose on COD removal

The results in Fig. (4) indicated that maximum removal efficiency of *A. niger* EM1 for mat form was (65.93^{a} ± 0.07%) (170.23 ± 0.21 mg/L) observed at (pH 6, 0.1 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and for pellet form was (73.81^{a} ± 0.08%) (131.33 ± 0.33 mg/L) observed at (pH 7, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and the maximum removal efficiency of *F. proliferatum* EN1 for mat form were (41.34^{a} ± 0.06%) (293.32 ± 0.31 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and for pellet form was (26.30^{a} ± 0.13%) (368.52 ± 0.64 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) as shown in Fig. (4).

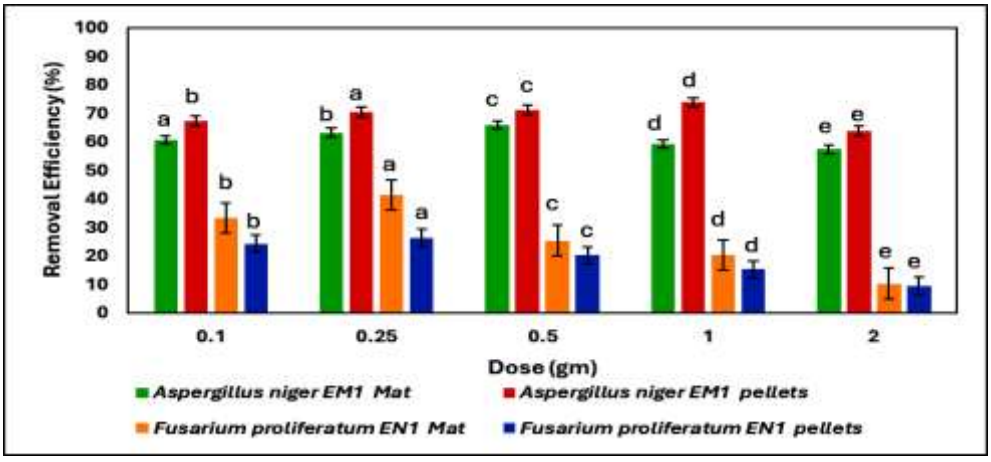

Fig.(4): Effect of biomass dose on COD removal using *A. niger EM1* and *F. proliferatum EN1* in mycelial mats and pellets, each value is the mean of three readings ± standard errors. Values with the same small letter in the same column show non-significant difference (at ≤ 0.05).

For the treatment of wastewater, finding out the appropriate fungal dose is essential. It guarantees balanced nutrient utilisation, maximum treatment efficiency, and lowest treatment costs **(Hassan et al., 2020)**. Fungi are essential for the breakdown of complex organic molecules into simpler forms, and the right amount of fungus helps to keep the process of nutrient utilization balanced **(Negi & Das, 2023)**. It lessens the impact on the environment by preventing the waste of biomass. A fungal overdose as 2gm in case of *F.*

proliferatum EN1 mat (10.23^{e} ± 0.06%) and pellet (9.34^{e} ± 0.04%) can cause problems such nutritional imbalance, overcrowding, higher operational expenses, and lower effluent quality. Low fungal dosages can lead to inadequate nutrient cycling, imbalance in the microbial community, slower reaction rates, decreased treatment efficiency, and higher risk of upset as shown in (Fig.5), decreased degradation of organic pollutants can be caused by insufficient fungal biomass, which is important for the breakdown of complicated chemicals **(Mohamed et al., 2021)**. This may lengthen the time needed for treatment and raise pollution levels. Furthermore, a tiny amount of fungus can upset the microbial community, increasing the system's vulnerability to changes in the composition of the influent **(Noman et al., 2020)**. Moreover, it may restrict the recycling of vital nutrients, which could have an impact on the efficacy of treatment. Thus, in wastewater treatment systems, it is crucial to maintain a balanced fungal dose **(Dasgupta et al., 2024)**.

Effect of contact time on COD removal

The results in Fig. (5) indicated that maximum removal efficiency of *A. niger* EM1 for mat form was (65.93^{a} ± 0.07%) (150.37 ± 0.37mg/L) observed at (pH 6, 0.1 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and for pellet form was (73.81^{a} ± 0.08%) (130.95 ± 0.40mg/L) observed at (pH 7, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and the maximum removal efficiency of *F. proliferatum* EN1 for mat form was (41.34^{a} ± 0.06%) (293.32 ± 0.31 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at ٧days, 150 rpm, 28°C) and for pellet form was (26.30^{a} ±0.13%) (368.52 ± 0.64 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) as shown in Fig.(5). The COD levels showed a little increase after the fungus eradication treatment's ideal day. This may be due to the depletion of nutrients and the metabolites and enzymes released by the fungi **(Akhtar & Mannan, 2020)**. As autolysis is usually caused by physical stress, carbon hunger, or nutritional shortages, the observed loss in biomass can be attributed to the mycelia's lysis caused by the lack of nutrients **(Kumar & Dwivedi, 2021)**. Because the fungus needs nutrients to survive, a lack of them could cause the biomass concentration, there is a study proved that in another strain such as *T. versicolor* grows until the ninth day, at which point it enters a stationary phase. Cellular lysis is therefore possible, which would increase the COD **(Esterhuizen et al., 2021)**.

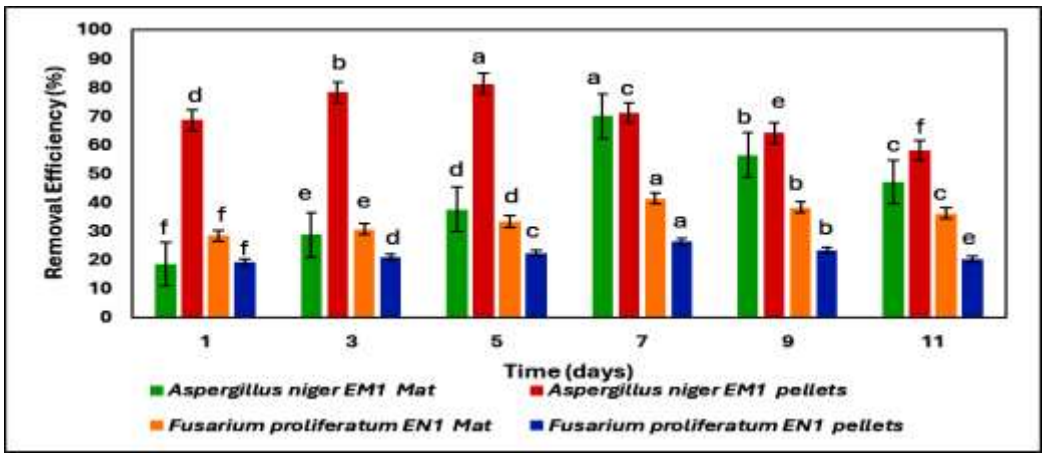

Fig. (5): Effect of contact time on COD removal using using *A. niger EM1* and *F. proliferatum EN1* in mycelial mats and pellets, each value is the mean of three readings ± standard errors. Values with the same small letter in the same column show non-significant difference (at ≤ 0.05).

Effect of stirring rate on COD removal

The results in Fig. **(6)** indicated that maximum removal efficiency of *A. niger* EM1 for mat form was (69.93[a] ± 0.07%) (150.37 ± 0.37 mg/L) observed at (pH 6, 0.1 gm fungal dose in 100 ml at 7days, 150 rpm, 28°C) and for pellet form was (81.21[a] ± 0.12%) (93.97 ± 0.62mg/L) observed at (pH 7, 0.2 gm fungal dose in 100 ml at 5 days, 150 rpm, 28°C) and the maximum removal efficiency of *F. proliferatum* EN1 for mat form was (41.34[a] ±0.08%) (293.32 ± 0.31 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at 7 days, 150 rpm, 28°C) and for pellet form was (26.30[a] ±0.13%) (368.52 ± 0.64 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at 7 days, 150 rpm, 28°C) as shown in Fig. (6).

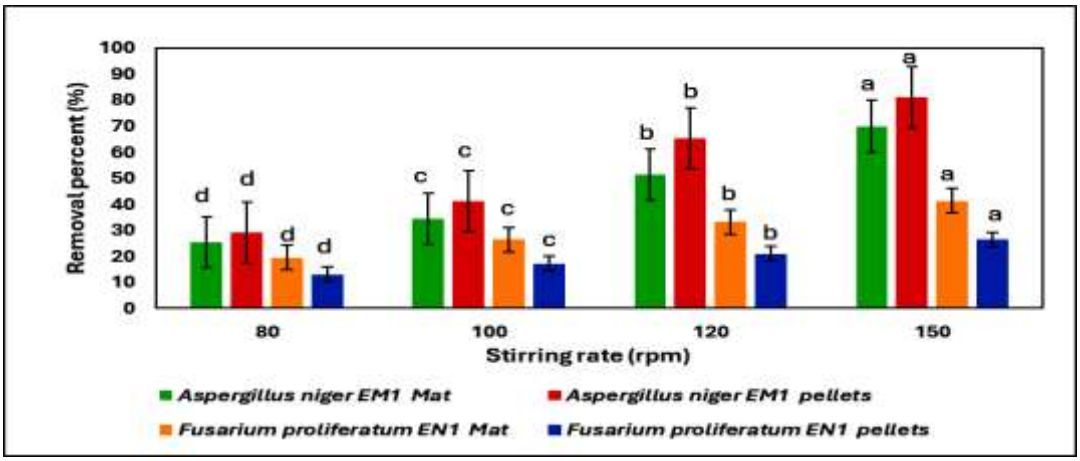

Fig. (6): Effect of stirring rate on COD removal using *A. niger EM1* and *F. proliferatum EN1* in mycelial mats and pellets, each value is the mean of three readings ± standard errors. Values with the same small letter in the same column show non-significant difference (at ≤ 0.05).

Through its effects on oxygen transfer, mass transfer of pollutants, nutritional distribution, biomass dispersal, fungal growth, and mixing efficiency, the stirring rate has a major influence on the treatment of fungal wastewater **(Silva et al., 2019)**. Sufficient stirring such as 150 rpm in Fig. (6) facilitates the flow of oxygen, which enhances the biodegradation of contaminants by aerobic fungus. In order to ensure that pollutants and fungal cells come into touch, it also affects the mass transfer of toxins from wastewater to fungal biomass **(Chu et al., 2021)**. In addition to maintaining the vitality and activity of fungus, proper stirring facilitates the dispersion of nutrients. Additionally, it affects biomass distribution, promoting effective biodegradation and fungal development **(Legorreta-Castañeda et al., 2020)**. Therefore, maximising the effectiveness and efficiency of fungal-based wastewater treatment procedures requires careful consideration of the stirring rate optimization **(Mooralitharan et al., 2023).**

Effect of temperature on COD removal

The results in Fig. (7) indicated that maximum removal efficiency of *A. niger* EM1 for mat form was (80.20^{a} ± 0.16%) (99.00 ± 0.78 mg/L) observed at (pH 6, 0.1 gm fungal dose in 100 ml at 7days, 150 rpm and 30°C) and for pellet form was (86.96^{a} ± 0.05%) (65.20 ± 0.23 mg/L) observed at (pH 7, 0.2 gm fungal dose in 100 ml at 5 days, 150 rpm and 30°C) and the maximum removal efficiency of *F. proliferatum* EN1 for mat form was (48.07^{a} ± 0.08%) (259.65 ± 0.42 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at 7 days, 150 rpm and 30°C) and for pellet form was (33.57^{a}± 0.11%) (332.13 ± 0.53 mg/L) observed at (pH 6, 0.2 gm fungal dose in 100 ml at 7 days, 150 rpm and 30°C) as shown in Fig. (7).

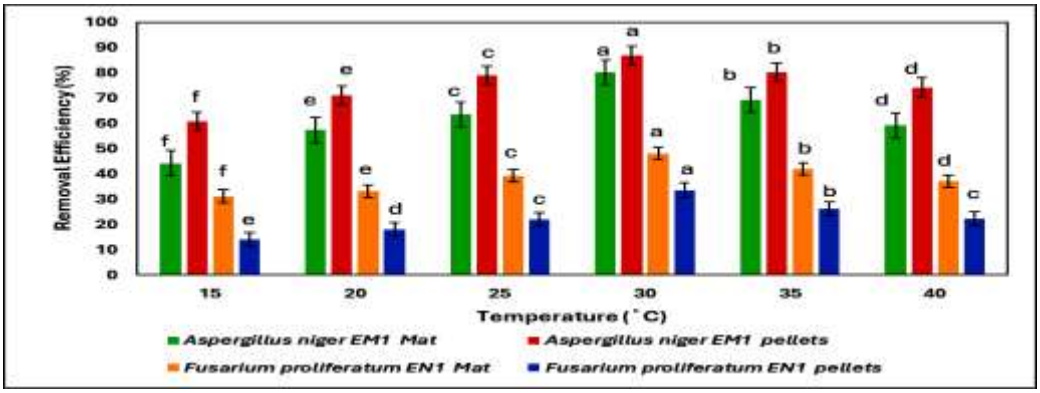

Fig. (7): Effect of temperature on COD removal using *A. niger EM1*and *F. proliferatum EN1* in mycelial mats and pellets, each value is the mean of three readings ± standard errors. Values with the same small letter in the same column show non-significant difference (at ≤ 0.05).

The ideal temperature range for treating wastewater is between 25 and 35°C, as this creates an atmosphere that is ideal for fungal metabolism and effective pollutant biodegradation **(Rico-Munoz *et al.*, 2019)**. *Aspergillus*, *Penicillium*, and *Fusarium* are examples of mesophilic fungi that are employed in biocontrol, industrial microbiology, and food production. *Fusarium* growth in between 25–30°C, while *A. niger* grows best at 20 to 40°C **(Ajmal *et al.*, 2023)**.

Application on real wastewater

The real wastewater treatment of *A. niger EM1* mat in chemical oxygen demand (COD), biological oxygen demand (BOD_5), total phosphorus (TP), total nitrogen (TN), chromium (Cr), hydrogen sulphide (H_2S) and Total organic carbon (TOC) 50.6%, 50.7%, 89.1%, 26.7%, 47.8%, 100% and 51.2% respectively. The real wastewater treatment of *A. niger EM1* pellets in chemical oxygen demand (COD), biological oxygen demand (BOD_5), total phosphorus (TP), total nitrogen (TN), chromium (Cr), hydrogen sulphide (H_2S) and Total organic carbon (TOC) 59.5%, 59.9%, 88.0%, 61.6%, 28.1%, 100% and 60.2% respectively. The real wastewater treatment of *F. proliferatum EN1* mat in chemical oxygen demand (COD), biological oxygen demand (BOD_5), total phosphorus (TP), total nitrogen (TN), chromium (Cr), hydrogen sulphide (H_2S) and Total organic carbon (TOC) 55.1%, 55.2%, 87.9%, 58.1%, 51.4%, 100% and 55.8% respectively. The real wastewater treatment of *F. proliferatum EN1* pellets in chemical oxygen demand (COD), biological oxygen demand (BOD_5), total phosphorus (TP), total nitrogen (TN), chromium (Cr), hydrogen sulphide (H_2S) and Total organic carbon (TOC) 54.2%, 54.1%, 86.3%, 62.0%, 51.6%, 100% and 54.8% respectively as shown in Table (1, 2) and Fig. (8).

Table (1): Efficiency of *A. niger EM1* mat and pellets forms in removing from real tannery wastewater

Test	Tannery wastewater	*A. niger* (EM1) mat	Removal efficiency (%)	*A. niger* (EM1) pellets	Removal efficiency (%)
pH	11.9	8	-	7.9	-
DO (mg/L)	0.08	1.35	-	2.27	-
TDS (mg/L)	5830	4800	17%	5430	6.9%
Color (Pt/Co)	605	218	64.0%	197	67.44%
Turbidity (NTU)	200	33.4	83.3%	19.5	90.3%
COD (mg/L)	4115	2032	50.6%	1668	59.5%
BOD (mg/L)	2460	1214	50.7%	998	59.4%
TOC (mg/L)	1355	661	51.2%	540	60.2%
NH_3 (mg/L)	40	28.6	28.5%	14.7	63.3%
NO_3 (mg/L)	0	2.87	0.0%	1.24	0%
TKN (mg/L)	86	61	29.1%	32	62.8%
TN (mg/L)	86	63	26.7%	33	61.6%
TP (mg/L)	4.1	0.448	89.1%	0.49	88.0%
H_2S (mg/L)	372	0	100%	0	100%
Cr (mg/L)	10	5.2	47.8%	7.19	28.1%

Table (2): Efficiency of *F. proliferatum EN1* mat and pellets forms in removing from real tannery wastewater

Test	Tannery wastewater	*F. proliferatum EN1* mat	Removal efficiency (%)	*F. proliferatum EN1 pellets*	Removal efficiency (%)
pH	11.9	7.9	-	7.2	-
DO (mg/L)	0.08	0.78	-	0.53	-
TDS (mg/L)	5830	5120	12.2%	5100	12.5%
Color (Pt/Co)	605	137	77.4%	98	83.8%
Turbidity (NTU)	200	29.5	85.3%	19.4	90.3%
COD (mg/L)	4115	1846	55.1%	1885	54.2%
BOD (mg/L)	2460	1102	55.2%	1129	54.1%
TOC (mg/L)	1355	599	55.8%	612	54.8%
NH_3 (mg/L)	40	15.8	60.5%	13.87	65.3%
NO_3 (mg/L)	0	2.82	0%	2.85	0%
TKN (mg/L)	86	34	60.5%	29.8	65.3%
TN (mg/L)	86	36	58.1%	32.65	62.0%
TP (mg/L)	4.1	0.497	87.9%	0.562	86.3%
H_2S (mg/L)	372	0	100%	0	100%
Cr (mg/L)	10	4.86	51.4%	4.84	51.6%

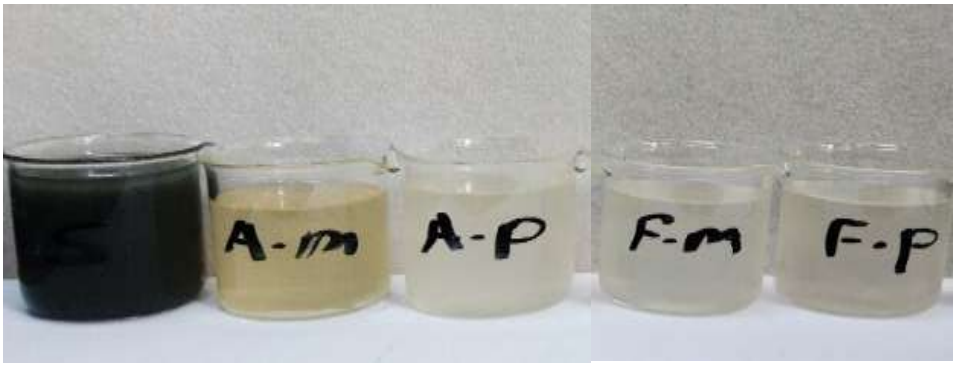

Fig. (8): Treatment of tannery wastewater (a), by *A. niger EM1mat* (b) at (pH 6, 1g/L, 7 days at 150 rpm, 30°C), *A. niger EM1* pellets (c) at (pH 7, 2g/L, 5 days at 150 rpm, 30°C), *F. proliferatum EN1* mat (d) at (pH 6, 2g/L, 7 days at 150 rpm, 30°C), and *F. proliferatum EN1* pellets at (pH 6, 2g/L, 7 day s at 150 rpm, 30°C).

Conclusion

Tannery wastewater, if not treated properly, can cause significant environmental damage, including water pollution, soil contamination, air pollution, biodiversity loss, and human health risks. Mycoremediation is a bioremediation method that uses fungal mycelia to degrade, absorb, or neutralize contaminants in the environment, including wastewater treatment. The present results indicated that *Aspergillus niger* EM1is more effective than *Fusarium proliferatum* (EN1). Its tolerance to a wide variety of pH values makes it suitable for treating wastewater with different acidic or alkaline conditions. Additionally, its simple lab growth requirement enabling possible scaling up in bioremediation applications. On the other hand, fungal pellets are more successful in treating wastewater because they have a larger surface area, greater control over the growth of fungus, are easier to handle, and have enhanced biodegradation. These components offer the ideal environment for the interaction of fungi with wastewater toxins, enabling the breakdown of contaminants and the resolution of environmental issues.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The author acknowledges the help received from Menoufia University and Housing and Building National Research Centre.

Author Contributions Statement

Each author contributes to the manuscript's writing, editing, and review.

Data availability

The corresponding author may provide the data supporting the study's conclusions upon request.

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معالجة الفطريات لمياه الصرف الصناعي باستخدام الاشكال المختلفة المستوية mat والكريات الحيوية pellets *Aspergillus niger* (EM1) و *Fusarium proliferatum* (EN1)

ا.د. محمد مدحت غريب[١]، ا.م.د. نوران يسري محمد[٢]، انجي مصطفي[٢*]

[١]قسم النبات والميكروبيولوجي – كلية العلوم – جامعة المنوفية
[٢]قسم الهندسة الصحية والبيئية - المركز القومي لبحوث الاسكان والبناء

قيمت الدراسة فعالية *Aspergillus niger* و*Fusarium proliferatum* في شكل مستوي وكرات لمعالجة مياه الصرف الصناعي، أظهر الشكل المستوي *A. niger* كفاءة إزالة قصوى تبلغ (٨٠.٢٠%) للملوثات العضوية في ظل ظروف محددة (درجة الحموضة ٦، ٠.١ جم في ١٠٠ مل، ٧ أيام عند ١٥٠ دورة في الدقيقة، ٣٠ درجة مئوية). ومع ذلك، حققت الكريات الفطرية لهذه الفطريات كفاءة إزالة قصوى أعلى بلغت (٨٦.٩٦%) للملوثات العضوية (بتركيز ٦٥.٢٠ مجم / لتر) في ظل ظروف مختلفة (درجة الحموضة ٧، ٠.٢ جم في ١٠٠ مل، ٥ أيام عند ١٥٠ دورة في الدقيقة، ٣٠ درجة مئوية). أظهرت الاشكال المستوية الفطرية الخاصة ب *F. proliferatum* كفاءة إزالة قصوى تبلغ ٤٨.٠٧% للملوثات العضوية في ظل ظروف محددة بتركيز ٢٥٩.٦٥ مجم / لتر ، درجة الحموضة ٦ ، ٠.٢ جم في ١٠٠ مل ، ٧ أيام عند ١٥٠ دورة في الدقيقة ، ٣٠ درجة مئوية) ، أظهرت الكريات الفطرية أقصى كفاءة إزالة تبلغ ٣٣.٥٧ ٪ للملوثات العضوية في ظل ظروف مختلفة (درجة الحموضة ٦ ٠.٢ جم في ١٠٠ مل ، ٧ أيام عند ١٥٠ دورة في الدقيقة ، ٣٠ درجة مئوية) ، أظهرت الكريات الفطرية أقصى كفاءة إزالة تبلغ ٣٣.٥٧٪ للملوثات العضوية في ظل ظروف مختلفة (درجة الحموضة ٦ ، ٠.٢ جم في ١٠٠ مل ، ٧ أيام عند ١٥٠ دورة في الدقيقة ، ٣٠ درجة مئوية). وجدت الدراسة أن حبيبات *A. niger* لديها كفاءة إزالة أعلى بكثير من شكل المستوي، بينما أظهرت أشكال مستوية *F. proliferatum* وأشكال الحبيبات اختلافات مماثلة، ولكن قيما أقل. يعتبر النيجر بشكل عام أكثر فعالية في تحلل ملوثات المياه، مما يوفر قابلية التوسع في تطبيقات المعالجة الحيوية، وسهولة الإنتاج في المختبرات، وتحمل الأس الهيدروجيني. تشير الدراسة إلى أن الكتلة الحيوية الفطرية، وخاصة الكريات، يمكنها معالجة مياه الصرف الصحي بشكل فعال وصديق للبيئة بظروف حمضية أو قلوية متغيرة من خلال المعالجة الفطرية.