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Research Article

Filamentous Fungi for Bioremediation of Oily Effluents of a Local Ghee Industry in Pakistan: An Environmental Perception

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Abstract: Mycoremediation is emerging as a potential approach for eco-friendly, cost-effective, and the most natural attenuation due to the biodegradation of polluted effluents from oil effluents which affect human health and the ecosystem. This work dealt with the analyses of the biodegradation capability of some potential indigenous fungal isolates viz., *Aspergillus flavus*, *Aspergillus niger,* and *Rhizopus stolonifer,* against oil effluents collected from a local ghee industry in Pakistan. Percentage reduction potential in different parameters i.e., pH, Electrical Conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD), confirmed that these fungi had the potential to degrade oily effluents. *Aspergillus niger* showed the highest reduction potential, while *A. flavus* and *R. stolonifer* had the least reduction potential to treat oil pollution. This indicates the potential of these identified fungi as biosorbents for removing high oil contents from industrial and wastewater discharge.

Keywords: Micromycetes, Mycoremediation, Eco-friendly, Waste management, Biodegradation

1. INTRODUCTION

Our ecosystem is under constant threat from continuing anthropogenic activities on the water by domestic, industrial, agricultural, shipping, radioactive, and aquaculture wastes. Industrial effluents are the major cause of pollution of water and soil in which these are discharged. This discharge belongs to various classes such as pesticides, fertilizers, hydrocarbons, phenols, plasticizers, biphenyls, detergents, oils, greases, pharmaceuticals, etc. These industrial effluents can have negative environmental impacts, causing climate change, loss of natural resources, air and water pollution, and extinction of species. These threaten the global environment as well as economic and social welfare. Thus, disturbing the ecological balance of the environment [1].

Oil industries are not only a significant source of energy but also a major supplier of raw materials for numerous products made from petroleum. On the other hand, the most hazardous and pervasive result of oil industry activities is pollution from oil and grease as toxic organic wastes, which not only destroy ecosystems but also cause biodiversity loss [2]. The layer of these discharged effluents on the water and soil surface reduces the amount of dissolved oxygen [3]. Maximum pollution levels in water bodies increase total suspended solids, total dissolved solids, chemical oxygen demand, and biological oxygen demand. After entering the ecosystem, they interact intricately with a variety of organic and inorganic pollutants, including waste from paper mills, sewage from the food and pharmaceutical industries, leaks from septic tanks, pesticides from agricultural runoff, and heavy metals from the mining and metal industries [4] This

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increase makes water inappropriate for irrigation, drinking or any use for any other purpose [5].

Compared to traditional remediation methods, bioremediation (i.e., Mycoremediation) is more environmentally friendly. Fungal enzymes, in particular laccase and oxidoreductases, are widely used to remove contaminants from freshwater, marine, and terrestrial environments [6-9]. For instance, the ability of fungi to break down petroleum hydrocarbons, including alkanes, aromatic, and nitrogen-sulfur-oxygen-containing compounds, highlights the potential of fungi in bioremediation processes [10-13]. Fungi isolated from the environment of an oil spill can mitigate oil pollution. *Aspergillus* is naturally occurring in contaminated sites; its habitat's hazardous material levels are automatically reduced. As a result, *Aspergillus* strains might be thought of as organic cleansers that the environment uses to do bioremediation [14].

Due to their huge biomass and diversity, microbial communities play a vital role in the formation of activated sludge and serve as the main decomposers in wastewater treatment systems. They are particularly important in the processes of organic matter biodegradation and nutrient cycling [15]. The scope of the current study was to propose a suitable, cost-effective, and environment-friendly bio-treatment process for the small-scale biodegradation of ghee industry effluents, keeping in mind the significance of bioremediation. The objectives of the present investigation were the isolation and identification of some indigenous micromycetes of oily effluents and the evaluation of their potential efficacy in the remediation of pollutants.

2. MATERIALS AND METHODS

2.1. Sample Collection

The samples for this investigation were taken at regular intervals of every two weeks from various distances of the direct discharge point of the selected factory in Lahore, Punjab, Pakistan (i.e., from the main point, at a distance of 2 and 4 meters). Three samples were collected at each sampling point as well and their physicochemical parameters were analyzed. Electrical conductivity is a physical parameter while pH, Total Suspended Solids, Total Dissolved Solids, Chemical Oxygen Demand, and Biological Oxygen Demand are chemical parameters. The samples were further divided into two groups for pre and post-treatment analysis and were stored in sterile glass bottles [16]. All of the samples were transferred to the lab for additional examination.

2.2. Fungal Isolation and Identification

The fungal strains were isolated from the samples that were collected by using the pour plate method. Each effluent was poured into a separate, empty Petri dish in an amount of 100 microliters. The samples in the Petri dishes were then covered with the autoclaved Malt extract agar, which had been cooled to 50 °C. To ensure proper mixing, the Petri dishes were immediately swirled. Each sample was divided into three samples, which were then incubated at 27 °C for a few days to allow the colonies to reach maturity after which fungal development could be seen. Each pure fungal isolate was then sub-cultured onto a new medium containing ampicillin to prevent bacterial contamination. Taxonomic identification was performed based on the standard morphological characteristics of the isolated fungi at the species level by Molla *et al.* [17].

2.3. Treatment of Samples with Fungal Isolates

Most dominant taxa isolated were then cultured on collected samples from the Shan Ghee Industry. Fifty milligrams per milliliter of sample was aliquot in a beaker followed by aseptic inoculation of each of the fungal cultures separately. To prevent any contamination, aluminum foil was used to cover the beakers [18].

2.4. Parameters Analyzed for Mycoremediation

The parameters i.e., pH, Electrical Conductivity (EC), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), and temperature were assessed in the laboratory. These parameters were selected for the analysis of effluents because the permissible limits of NEQS are available for them. Standard procedures were used to determine all the parameters [19]. All the indicated parameters were analyzed both at pre and post-fungal treatment.

2.5. Determination of Biodegradation Potential of Selected Fungi

The reduction potential (Percent) of *Aspergillus flavus, Aspergillus niger,* and *Rhizopus stolonifer* were noted from post-treatment reading differences. In this way, the most efficient fungal strain was selected for further analysis. All obtained data was analyzed statistically to determine the percent reduction.

3. RESULTS

3.1. Micromycetes Isolated from Effluents

Nineteen fungal species (Fig. 1) were recorded during this study of which only three taxa were selected based on their occurrence frequency. *Aspergillus niger* was observed as the most abundant species, followed by *Aspergillus flavus* and *Rhizopus stolonifer* (Fig. 2).

3.2. Efficiency of Fungal Species in Controlling pH

The pH measures the amount of free hydrogen and hydroxyl ions present in water, which serves as an indicator of acids and bases. The pretreated sample collected from a direct source point showed a pH of 8.84 (Alkaline). Notably, the pH remains alkaline even after treatment with fungi while post-treatment of the direct source sample with *A. niger* has exhibited maximum capability to reduce pH to 8.64 (Table 1, 2, and 3), for a period of 6 days, as per the permissible limits of the National Environmental Quality Standards (N.E.Q.S) standard (6-10). Similarly, the sample of 2 m and 4 m away showed a pH reduction to 8.76 (Table 1, 2, and 3) and 8.78 (Table 1, 2, and 3) after being treated with *A. flavus* and *R. stolonifer,* respectively as per limits indicated above. *A. niger* exhibited maximum pH reduction (2.26 %) followed by *A. flavus* (0.90 %) and *R. stolonifer* (0.67 %) (Table 4).

3.3. Efficiency of Fungal Species in Controlling Electrical Conductivity (EC)

EC measures how well water can carry an electric current, which in turn is influenced by the ion concentrations in the solution. The EC value shouldn't have exceeded 400 S/cm, as per WHO guidelines. The EC value of the pretreated sample collected from a direct source was observed to be 766 while the post-treatment with *A. niger* revealed a gradual decrease of EC values from day one to day six, respectively soon by average (Table 1, 2,

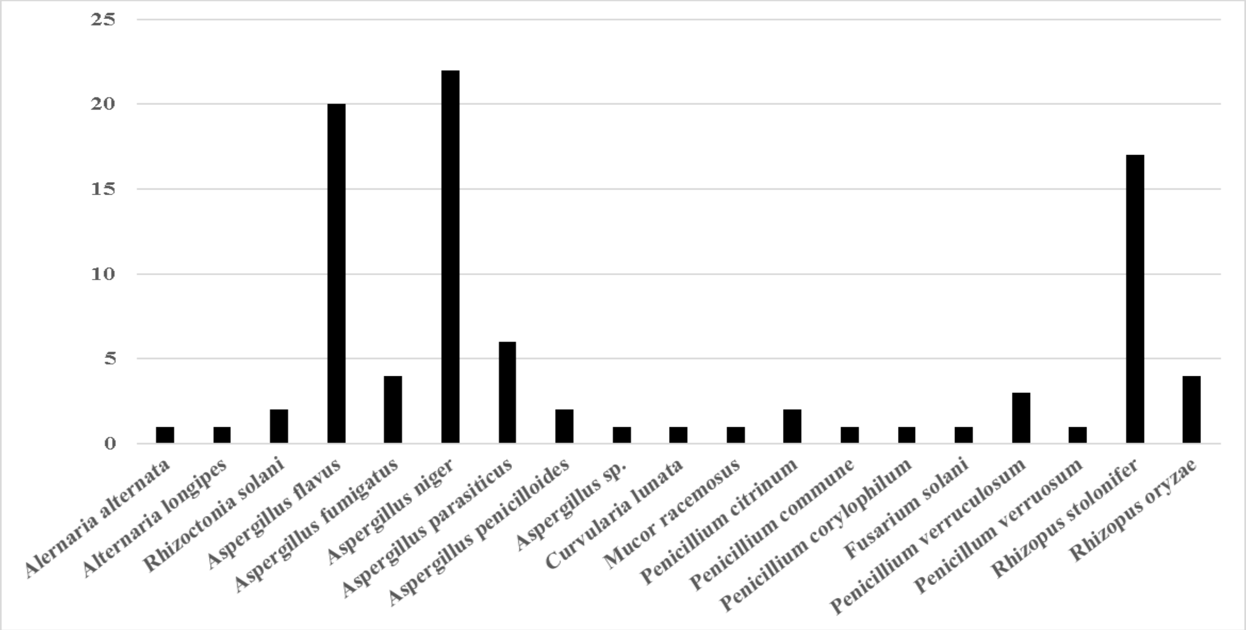

Fig. 1. Frequency of occurrence of fungal taxa isolated during this study from effluents.

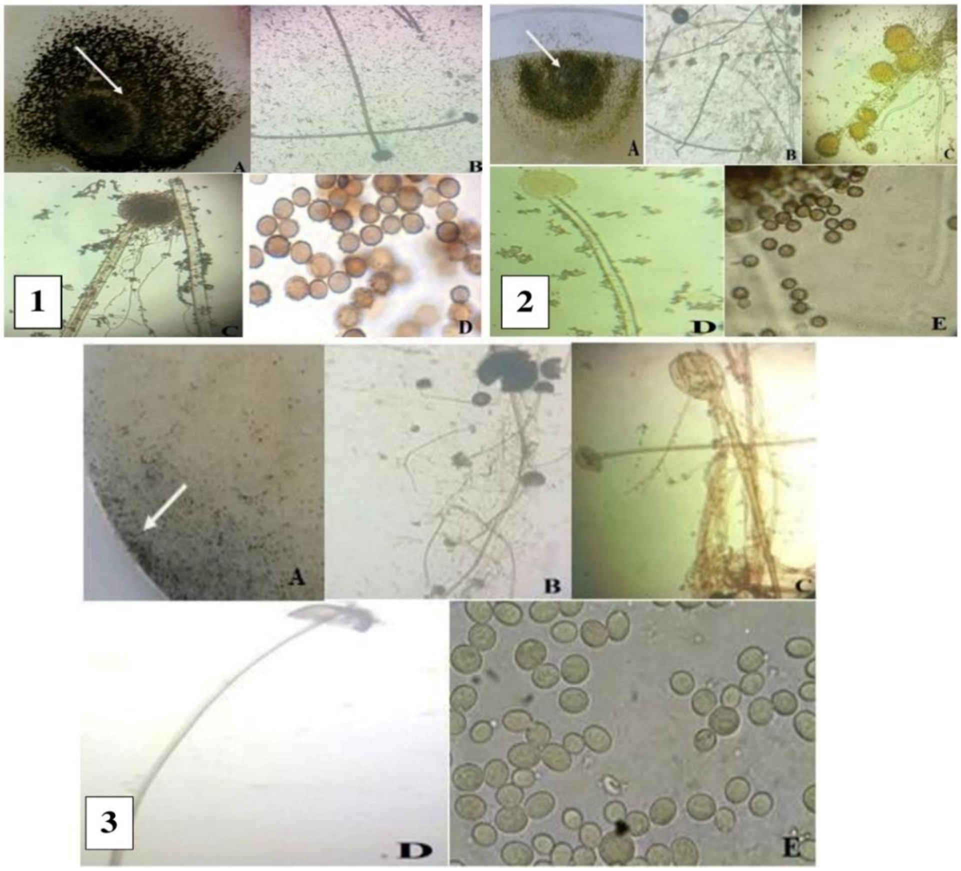

Fig. 2. Morphological characters of (1) *Aspergillus niger Colony,* (2) *Aspergillus flavus,* and (3) *Rhizopus stolonifer*

Table 1. Variation in all parameters in the direct source, 2 and 4 meters away from effluents treated with *Aspergillus niger* (Average of 6 days)

Aspergillus niger												
Parameters	**pH**		**EC**		**TDS**		**TSS**		**BOD**		**COD**	
N.E.Q.S	6-10		680 µS/m		3500 mg/L		150 mEq/L		80 mg/L		150 mg/L	
Effluents Collection	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.
Direct Source	8.84	8.46	766	435	633	150	505	281	485	426	471	421
2 Meter	8.89	8.56	810	508	686	293	523	349	480	233	485	237
4 Meter	8.80	8.52	857	491	706	366	546	409	496	325.	490	330

Table 2. Variation in all parameters in the direct source, 2 and 4 meters away from effluents treated with *Aspergillus flavus (Average of 6 days)*

Aspergillus flavus												
Parameters	**pH**		**EC**		**TDS**		**TSS**		**BOD**		**COD**	
N.E.Q.S	6-10		680 µS/m		3500 mg/L		150 mEq/L		80 mg/L		150 mg/L	
Effluents Collection	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.
Direct Source	8.84	8.76	766	410.17	633	216.7	505	357.17	485	433.67	471	448.33
2 Meter	8.89	8.66	810	635.67	686	275.2	523	380.33	480	313.83	485	313.83
4 Meter	8.80	8.62	857	491.33	706	366.5	546	409.83	496	325.67	490	330.33

Table 3. Variation in all parameters in the direct source, 2 and 4 meters away from effluents treated with *Rhizopus stolonifer (Average of 6 days)*

Rhizopus stolonifer												
Parameter	**pH**		**EC**		**TDS**		**TSS**		**BOD**		**COD**	
N.E.Q.S	6-10		680µS/m		3500 mg/L		150mEq/L		80mg/L		150 mg/L	
Effluents Collection	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.	Bf. Trt.	Af. Trt.
Direct Source	8.84	8.78	766	532.67	633	273.17	505	370.00	485	450.83	471	445.5
2 Meter	8.89	8.797	810	618.83	686	332.17	523	381.17	480	469.83	485	448.7
4 Meter	8.80	8.688	857	671.83	706	419.67	546	387.17	496	333.83	490	349.5

and 3). Similarly, the samples treated with *A. flavus* and *R. stolonifer* showed a constant decrease of EC values on a regular basis and were noted within the acceptable limit of NEQS (680 micro-Siemens/cm). *Aspergillus niger* exhibited maximum EC reduction (27.8 %) followed by *A. flavus* (19.8 %) and *R. stolonifer* (12 %) (Table 4).

3.4. Efficiency of Fungal Species in Controlling Total Dissolved Solid (TDS)

Total dissolved solids (TDS) is a measurement of the total amount of inorganic and organic compounds that have been dissolved and are suspended as molecules, ions, or microscopic granules (colloidal sol) in a liquid. *A. niger* has the maximum capability to reduce TDS followed by *A. flavus*, while *R. stolonifer* showed the minimum ability to reduce TDS.

TDS levels within the 50–150 range are often thought to be the most suitable and acceptable range. TDS levels of approx.1000 PPM indicates that the water is hazardous and unfit for human consumption. The standard TDS (Table 1, 2, and 3) value according to N.E.Q.S is 3500 mg/L. The decrease in TDS value that was seen after being treated with *A. niger, A. flavus*, and *R. stolonifer* was within allowable ranges of standard. Similarly, the sample that was 2 meters away from direct discharge showed the reduction (Table 1, 2 and 3); 4 meters away samples reduction values were also within the permissible limits of standard (Table 1, 2 and 3). *A. niger* exhibited maximum TDS reduction

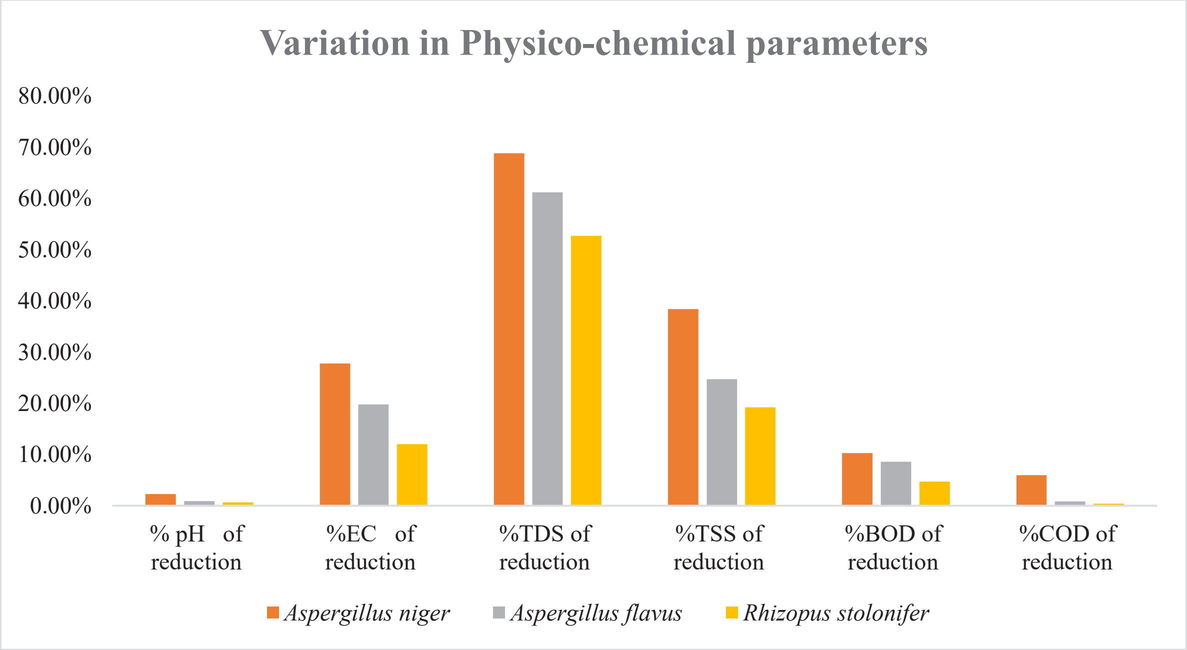

Figure 3. Variation in Physio-chemical parameters of *Aspergillus flavus, A. niger,* and *R. stolonifer*

(68.8 %) followed by *A. flavus* (61.2 %) and *R. stolonifer* (52.7 %) (Table 4).

3.5. Efficiency of Fungal Species in Controlling Total Suspended Solid (TSS)

Total suspended solids (TSS) is the dry weight of undissolved suspended particles that can be measured using a filtering system and captured by a filter. Higher concentrations of bacteria, minerals, pesticides, and metals in the water are frequently indicative of high TSS in a water body. The reduction in TSS values for the direct source sample was observed for a period of 6 days with *A. niger* exhibiting maximum capability to reduce TSS followed by *A. flavus* while *R. stolonifer* showed the minimum ability to reduce TSS. The reduction was noted within the permissible limits and according to the N.E.Q.S standard TSS value (150 mEq/L) (Table 1, 2, and 3). Similar observations in TSS reduction were noted with the sample 2 meters (Table 1, 2, and 3) and 4 meters (Table 1, 2, and 3) away as per the permissible limits of the standard indicated above. *Aspergillus niger* exhibited maximum TSS reduction (38.4 %) followed by *A. flavus* (24.7 %) and *R. stolonifer* (19.2 %) (Table 4).

3.6. Efficiency of Fungal Species in Controlling Biological Oxygen Demand (BOD)

When organic matter is decomposed aerobically (in the presence of oxygen) at a specific temperature, the term “Biochemical Oxygen Demand” (BOD) is used. So, BOD refers to how much oxygen is consumed by bacteria and other microorganisms. Relative reduction of BOD values was observed with a direct source sample for 6 days post-treatment with *A. niger* accounting for the maximum capability to reduce BOD followed by *A. flavus* >*R. stolonifer*. The BOD reduction was noted within the permissible limits and in accordance with the N.E.Q.S standard BOD value (80 mg/L), value means that effluents are severely polluted. Similar observations in BOD reduction were noted with samples at 2 meters (Table 1, 2, and 3) and 4 meters (Table 1, 2, and 3) away following the permissible limits of standards. *A. niger* exhibited maximum BOD reduction (10.3 %) followed by *A. flavus* (8.6 %) and *R. stolonifer* (4.7 %) (Table 4).

3.7. Efficiency of Fungal Species in Controlling Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) determines the amount of oxygen required to chemically oxidize the organic and inorganic nutrients found in water, such as ammonia and nitrate. Relative reduction of COD values of direct source samples was observed for a period of 6 days with *A. niger* exhibiting maximum COD reduction (5.94 %) followed by *A. flavus* (0.84 %) and *R. stolonifer* (0.42 %) (Table 4) which was within the permissible limits and in accordance to the N.E.Q.S standard COD value (150 mg/L). Similar observations in COD reduction were noted with sample 2 meters (Table 1, 2, and 3) and 4 meters (Table 1, 2, and 3) away in accordance with the permissible limits of standards as indicated above. *A. niger* exhibited maximum COD reduction (5.94 %) followed by *A. flavus* (0.84 %) and *R. stolonifer* (0.42 %) (Table 4).

3.8. Variation in Physico-Chemical Parameters

There is a relative reduction in all the observed parameter values following the treatment with fungal species. The pH reduction was exhibited highest for *A. niger* (2.26 %) followed by *A. flavus* (0.90 %) > *R. stolonifer* (0.67 %). Similar reduction profile of EC was observed with *A. niger* (27.8 %) > *A. flavus* (19.8 %) > *R. stolonifer* (12 %). *A. niger* also exhibited the highest reducing ability of TDS, TSS, and BOD of 68.8 %, 38.4 %, and 10.3 %, respectively in comparison to the other two fungal isolates, where *A. flavus* accounting for a reduction of 61.0 % (TDS), 24.7 % (TSS), and 8.6 % (BOD) followed by the least reducing values observed of 52.7 % (TDS), 19.2 % (TSS), and 4.7 % (BOD) for *R. stolonifer.* (Fig. 3)

4. DISCUSSION

Micromycetes are involved in bioremediation, which is useful for the removal of wastes and harmful materials because these fungi naturally have the potential to decompose many materials [20]. The identification, individual characterization, and use of this fungus in bioremediation were the main reasons for the isolation of these organisms from samples. Fungi are more effective natural degraders than conventional bioremediation methods, such as bacteria, as demonstrated by Batelle [21].

Aspergillus species were the most prevalent fungus that were successfully isolated from all the samples of fungal isolates. The filamentous fungi are the most frequently reported that can thrive on hydrocarbons. [22-24]. Oil effluents from the ghee industry in Pakistan showed higher BOD, COD, TSS, and TDS levels (above the acceptable limit of the National Environment Quality Standard) [25]. Notably, high BOD and COD values in the effluents cause a huge depletion of DO (Dissolved oxygen) in water which in turn, affects the aquatic life [26-28]. The current study demonstrated that treatment of the oil effluents with fungal isolates lowers the above-indicated parameters within the safety limits of NEQS and WHO [29].

All of the fungal species (tested) were able to degrade the effluents to varying degrees. Notably, *A. niger* was observed to be most abundant and efficient in the degradation of the oil effluents among the three tested fungi. The higher biodegradation efficiency of *A. niger* in comparison to the other two species, is consistent with a previous study [30-32] where the isolated fungal species from the tainted soil were used to bio-remediate crude oil from contaminated environments. Furthermore, the biodegradation capability of *A. niger* was also tested in another study [33] where it has been observed that it can degrade effluents up to 38.0 % in comparison to *A. flavus* and *A. foetidus* that degrades up to 31.20 and 26.1 %, respectively following 60 days' treatment of the effluents. Similar results revealed from another study by Buvansewari *et al*. [34] showed the degradation of sugar mill effluents by *Aspergillus* was the highest in comparison to *Penicillium* and *Rhizopus*.

The outcomes of this are coherent with those of Keren et al. [35], who found that the presence of oil in the soil increased the fungal population. Similar findings were reported at the same time by Jawhari [36], which showed that more frequency of *A. niger* with 100 % in all samples, but *A. fumigatus* and *Penicillium funiculosum* were 83 %. Similar research reports have shown an increase in microbial variety and population.

One of the key factors controlling the composition and activity of fungi is the hydrogen ion concentration [37]. Organic acids and other metabolic products are frequently produced as a result of the microbial degradation of hydrocarbons [38]. The chemistry of the pollutants was directly impacted by temperature, which also had an impact on the diversity of the microbial flora and the biodegradation of hydrocarbons [39]. Oil-contaminated soil and wastewater can be recovered quickly by the culture of fungus (*A. flavus, A. fumigatus*, *A. niger,* and *Penicillium* sp.) which reduces oil pollution to levels that permit the reuse of land and water [36].

The processes used by different refineries result in effluents with various chemical compositions, which depend on the type of treatment they have undergone. [40-43]. In the aquatic ecosystem, fungi and other microorganisms can degrade various pollutants, including crude oil, and use them as a source of nutrients. [41]. *A. flavus, A. fumigatus*, *A. niger,* and *Penicillium* sp. were among the oil-degrading fungi that were isolated from the soil and wastewater, and their density was greater than that of other fungi. These fungi were ideally adapted to break down and make use of both raw and refined oil [36].

The current findings demonstrated that pH remains alkaline both at the pre- and post-fungal treatment with a regular decrease of pH from 8.84 (pre-treatment) to 8.76 and these results are similar to findings [44] where refinery effluents were analyzed. Furthermore, this study indicates a decrease in both COD and BOD values after treatment which corresponds to the literature [45]. Moreover, degradation of TSS and TDS occurs as demonstrated by earlier studies [46-47]. Similarly, the degradation of TDS in crude oil was tested by three fungi individually where *A. niger* has the highest degradation of 53.7 % followed by *Candida* sp. (45 %) and *R. stolonifer* (35 %) [46-47]. To sum up, the current study exhibited that filamentous fungal species, viz., *A. flavus, A. niger, R. stolonifer* contributed efficiently to the reduction of pH, EC, TDS, TSS, BOD, and COD in the oil effluent samples, confirming remediation of effluents.

5. CONCLUSION

It is concluded from our research that *Aspergillus* is the main dominant genus in oily effluent samples which indicates its resistance and highest tolerance index towards oily effluents. The tolerance and the resistance of the isolates depended much more on the fungus tested than on the sites of its collection. This study recommends that the species of *Aspergillus* and *Rhizopus* isolated from effluents should be utilized for the bioremediation process. Fungi have been widely used in bioremediation of industrially polluted soils and waters. The results obtained confirmed the response of isolates towards biodegradation potential, its concentration in the medium, and the isolate under consideration. The results encourage future studies to optimize the tolerance and degradation assay using the isolates that showed the best results, as well as studies on the treatment of environments contaminated with different types of pollutants, including oily effluents.

6. CONFLICT OF INTEREST

All the authors have no conflict of interest.

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