



APPLICATION OF BIOREMEDIATION PROCESS FOR WASTEWATER TREATMENT USING
AQUATIC FUNGI

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ABSTRACT

A fungal isolate *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. previously isolated from the Mula river, Pune were efficiently utilized for the remediation. The domestic wastewater samples used in this study were collected from sewage wastewater treatment plant Bopodi from Pune city. To study the role of fungi in wastewater treatment, the following method was employed, i) Wastewater treated with *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. and ii) Wastewater treated without *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. (Control). Wastewater was analyzed for pH, BOD, COD nitrate and phosphate before and after wastewater treatment using fungi at various time intervals for 5th, 10th, 15th and 20th days wastewater samples were analysis using standard methods. Result revealed that *Aspergillus terreus* and *Aspergillus niger* which showed excellent pollutant removal capabilities. *Aspergillus terreus* were showed the best removal of nitrate and BOD while *Aspergillus niger* showed best removal of phosphate and COD capacity from wastewater. Present investigation focuses on the bioremediation of wastewater by using aquatic fungi.

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INTRODUCTION

Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area. The primary role of fungi in the ecosystem is decomposition, which is performed by the mycelium of fungi. The fungi are unique among microorganisms in that they secrete a variety of extracellular enzymes. The decomposition of lignocelluloses is rated as the most important degradative event in the carbon cycle of earth (Bennett and Faison, 1997). The mycoremediation is determining the right fungal species to target a specific pollutant. The importance of fungi in the environment is to decomposition and transformation of both organic and inorganic substrates. Nutrient removal using fungal species has been studied by various researchers. Cooke, (1976) advocated the use of fungi in wastewater treatment because fungi appeared to show higher degradation rates of organic matter. In addition to extracellular enzyme production, fungal cell walls and their components play a major role in bio sorption of toxic compounds during wastewater treatment.

A detailed review of heavy metal bio sorption by fungi can be found elsewhere (Kapoor and Viraraghavan, 1995). Aksu, (2005) reported biosorption of organic pollutants by various types of fungal species. Denizli *et al.*, (2004) found that fungal biomass removes considerable amounts of organic pollutants from aqueous solution through adsorption. Edible and/or medicinal fungi also play a role as natural environmental remediators (Pletsch *et al.*, 1999), as do aquatic fungi (Hasija, 1994). Present study particularly focusing on removal of pollutants from wastewater during wastewater treatment. Important parameters such as pH, BOD, COD, nitrate and phosphate were used to understand the role of aquatic fungi in remediation.

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MATERIALS AND METHODS

Collection of wastewater

The domestic wastewater samples used in this study were collected from sewage wastewater treatment plant Bopodi from Pune city. The maximum capacity of the plant is the received wastewater about 18 MLD.

Analytical methods

The wastewater samples were analyzed for pH using a pH meter. The biochemical oxygen demand (BOD), chemical oxygen demand (COD), phosphate and nitrate were determined as per standard methods (APHA, 1998).

Microorganism selection

The fungi used in this study were isolated from water from river Mula, Pune. The experiments described in this study were carried out using four different species of fungi. Firstly most dominant fungal strain was selected. The most dominant species such as *Aspergillus terreus* Thom, *Aspergillus niger* Tiehhem, *Rhizopus nigricans* Ehrenb and *Cunninghamella* sp. (Kshirsagar and Gunale, 2011; Kshirsagar *et al.*, 2012) were used as test organisms for the treatment of wastewater.

Fungal Medium and culture

For isolation of fungi from the polluted water the PDA and malt extract broth medium were used. The pure fungal cultures had been inoculated and grown in the test tube containing PDA medium maintained at 4°C. 100 ml of malt extract broth (MEB) was prepared and dispensed into 250 ml conical flasks. On cooling, a loopful of individual fungal strains was inoculate in MEB under sterile condition and kept at 25-27°C for 7 days on a rotary shaker at 100 r.p.m. In the culture for experimental purposes, cultures were prepared using mycelia from shaker flasks. The mycelium culture was pretreated by homogenization at maximum speed (3000 rpm) for 3 min. After that

whole mass was mixed in a blender to make a homogenous mixture. Homogenized fungal culture was then used as inoculums for remediation experiments. In order to obtain sufficient biomass for the subsequent growth and wastewater treatment experiment, *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. were culturing in the MEB and grew for 7 days before applying for wastewater treatment.

Experimental set-up

The test species of fungi, *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. were used for wastewater treatment. The wastewater samples collected from Bopodi wastewater treatment plant were used for the fungal assays. The wastewater samples were analyzed for nitrate, phosphate, BOD COD and pH. The wastewater was filtered using Whatman No. 1 filter paper to remove suspended solids particles. COD, BOD, phosphate and nitrate reduction studies were made using pollution tolerant fungal culture from wastewater. To study the role of fungi in wastewater treatment, the following method was employed. i) Wastewater treated with *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. and ii) Wastewater treated without *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. (Control). Experiments were conducted in triplicates. Flasks with 200 ml of wastewater were added. They were used for the treatments and rest was used for initial analysis of the wastewater. Two ml of uniform suspension of *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp were used as initial inoculums (7 days old culture) in each flask containing 200 ml wastewater sample. The experiment was conducted under controlled conditions (temp. 27 ± 2 °C) for a total duration of 20 days. Samples were periodically (every 5th day) analyzed for physico-chemical parameters such as pH, phosphate, nitrate, BOD and COD using standard methods (APHA, 1998).

RESULT AND DISCUSSION

Wastewater was analyzed for pH, BOD, COD nitrate and phosphate before and after wastewater treatment using fungi. At various time intervals for 5th, 10th, 15th and 20th days wastewater samples were analysis as shown in Table-1 and Figure 1 and 2. When the wastewater treated with *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp, the pH was decreased initially and then increased (Table-1).

Table 1. Analysis of pH from wastewater using *A. terreus*, *A. niger*, *R. nigricans* and *Cunninghamella* sp.

Initial pH	7.41±0.10				
Days	Control	<i>A. terreus</i>	<i>A. niger</i>	<i>R. nigricans</i>	<i>Cunninghamella</i>
5 th	7.69±0.07	6.70±0.16	7.02±0.12	7.09±0.10	7.33±0.18
10 th	7.08±0.06	7.15±0.12	7.61±0.07	7.28±0.07	7.59±0.05
15 th	7.66±0.14	7.99±0.21	7.48±0.08	7.63±0.16	7.17±0.06
20 th	7.73±0.06	7.81±0.22	7.65±0.14	7.89±0.10	7.68±0.15

Values represent the mean ± S.E of the three replicates

Initial pH of control was 7.41±0.10 while pH of wastewater when treated using *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp were 7.15±0.12, 7.61±0.07, 7.28±0.07 and 7.59±0.05 respectively upto 10th day. In a study (Emtiazi, 1999) it was shown that the enzymes of *Aspergillus terreus* could degrade fiber in pulp and sugar beet wastewater of pH 5-7. BOD in the present study was reduced when wastewater treated with *Aspergillus terreus* (82.46%), *Aspergillus niger* (77.11%), *Rhizopus nigricans* (73.33%) and 66.98 % with *Cunninghamella* sp. respectively after 15th days. Where as in control set of experiment 18.22% reduction of BOD take place of wastewater (Figure-1). *Aspergillus terreus* and *Aspergillus niger* shows best reduction capacity of BOD during wastewater treatment. The parameters of the waste water treated *A. niger* were removed by 66.7 – 85.0 %, 65.7 - 79.0 % for BOD and COD (Azab, 2008). BOD and COD in the Stirred

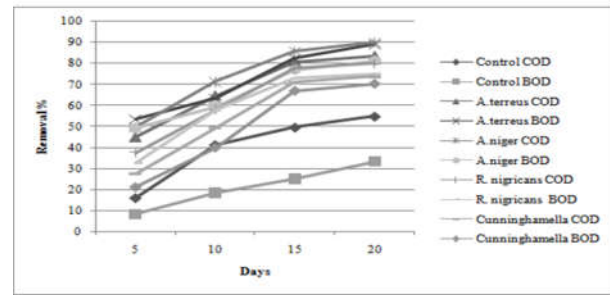


Figure 1. Removal % of BOD and COD of wastewater using *A. terreus*, *A. niger*, *R. nigricans* and *Cunninghamella* sp.

Tank Reactor system were removed by 66.50 % and 75.24 %, respectively by the fungal strain *A. terreus* (Andleeb *et al.*, 2010). During present study, COD reduction was observed up to 80.83 %, 85.88 %, 77.66 % and 71.00 % for *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp respectively after 15th day wastewater treatment (Figure-1). Whereas *Aspergillus niger* was showed best result as compared to other for COD reduction from wastewater. The removal of COD showed by order, *Aspergillus niger* > *Aspergillus terreus* > *Rhizopus nigricans* > *Cunninghamella* sp > Control. Hamdi *et al.*, (1991) showed reduction the COD by 52.5% using *Aspergillus niger* in aerobic condition. Anaerobic digestion after pretreatment with *Aspergillus niger* removed over 60% of COD (Hamdi and Radhouane, 1992). Roux-van der Merwe *et al.*, (2005) treated oil-processing plant effluent with different fungal species showed that maximum COD reduction of 98 % was achieved using *Cunninghamella* sp. *Aspergillus terreus* reduced the COD of sugar wastewater and pulp industry by 40-80 %. The effects of crude enzyme of *Aspergillus terreus* for COD removal were studied (Emtiazi *et al.*, 2001). *Aspergillus niger* effectively removed 52.5% of COD in one study (Hamdi and Ellouz, 1992) and 35-64% in another study (Cereti *et al.*, 2004). Remediation of nitrate was 49.46% in control while with *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp were 64.82%, 54.93%, 49.45% and 45.04% respectively after 10th days of wastewater treatment (Figure-2). During present study *Aspergillus terreus* was showed highest removal of nitrate from wastewater. The removal of nitrate showed by order, *Aspergillus terreus* > *Aspergillus niger* > *Rhizopus nigricans* > *Cunninghamella* sp > Control. *Aspergillus niger* effectively removed nitrogen, could be maintained as a dominant

status and sufficiently efficient to be used in a small reactor (Hwang *et al.*, 2007). *Rhizopus* sp (Preetha and Viruthagiri, 2005) have been reported to be important adsorbents for the removal of metals and organics from municipal and industrial wastewater. Percentage of Phosphate reduction was 23.74 % for control after 10th days while using *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans* and *Cunninghamella* sp. were 50.83 %, 52.57 % and 42.40 % respectively after 10th days of wastewater treatment (Figure-2). *Aspergillus niger* resulted in highest 52.57 % of phosphate removal after 10th days. Percentage of phosphate reduction order in wastewater were *Aspergillus niger* > *Aspergillus terreus* > *Rhizopus nigricans* > *Cunninghamella* sp > Control. Thanh and Simard, (1973) demonstrated the capacities of seventeen fungal biomasses to remove phosphates (84.1 %) and chemical oxygen demand (39.3 %). *Aspergillus terreus* have separately and successfully removed phosphorus (58 %) (Anyakwo and Obot, 2010). *Aspergillus niger*

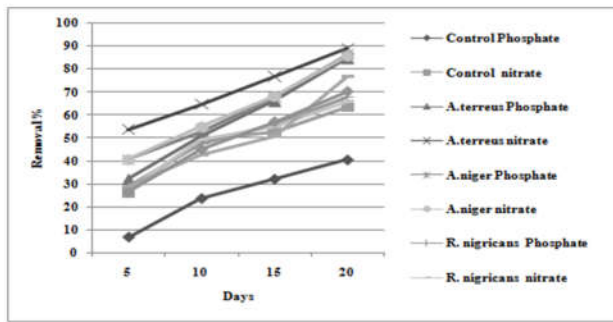


Figure 2. Removal % of Nitrate and phosphate of wastewater using *A. terreus*, *A. niger*, *R. nigricans* and *Cunninghamella* sp.

isolated from the Brazilian high phosphorus ore was employed to remove phosphorus from the ore and again in 21 days (13.8-33.2 %) (Pedro *et al.*, 2007). During present study, *Aspergillus terreus* and *Aspergillus niger* which showed excellent pollutant removal capabilities. Whereas, *Aspergillus terreus* showed the best result for removal of nitrate and BOD while *Aspergillus niger* showed best removal of phosphate and COD from wastewater.

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