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RESEARCH ARTICLE

EXPERIMENTAL STUDIES ON BIODEGRADATION DOMESTIC WASTE USING THERMOPHILIC FUNGI

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ABSTRACT

Fungi play an important role in the degradation of dead leaves and other plant detritus in aquatic environments. Aquatic fungi secrete enzymes that degrade polysaccharides and other polymers. Fungi are also efficient in the biodegradation of recalcitrant compounds like xenobiotics, lignin and dye stuffs by their extra cellular ligninolytic enzyme system. Hence, the present study was aimed at using aquatic fungi in the bioremediation of waste water. The selected species were *Aspergillus niger*, *Rhizopus arrhizus*, *Penicillium citrinum* and *Fusarium oxysporum* which were isolated from the River Cauvery collected from Upper Anicut (Mukkombu). Results indicate that all the four fungal species helped in decreasing the levels of BOD, COD and nutrients like PO_4 and nitrate. However, each species responded differently. Nevertheless, among the four fungal species studied, *Aspergillus niger* showed the most promising results and it appears to be the best candidate for bioremediation.

INTRODUCTION

Fungi play an important role in the degradation of dead leaves and other plant detritus in aquatic environments. Aquatic fungi secrete enzymes that degrade polysaccharides and other polymers (Suberkropp and Klug, 1980; Singh, 1982; Chandrasekar and Kaveriappa, 1988; Mansfield, 2005; Sangar Rao, 2013). These changes in the substrate composition and the concurrent increase in furgal biomass 'condition' plant activities for invertebrate consumption by raising its nutritional value (Barlocher, 2005). Fungi are also efficient in the bio degradation of recalcitrant compounds like xenobiotics, lignin and dye stuffs by their extra cellular ligninolytic enzyme system (Hippen *et al.*, 1997; Boulanger *et al*., 2000). In addition, the extracellular enzyme system also enables fungi to tolerate high concentrations of pollutants (Knapp *et al*., 1998). Hence it is apparent that fungi can play an important role in degrading materials in the ecosystem and that they have the potential for remediating contaminated soils and waters. Cooke (1976) advocated the use of fungi in waste water 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 biosorption of toxic compounds during waste water treatment (Kshirsagar, 2013). Hence, the present study was aimed at using aquatic fungi in the bio remediation of waste water.

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MATERIALS AND METHODS

Collection of waste water

The domestic waste water samples used in this study were collected from Tiruchirappalli city.

Analytical methods

The waste water samples were analysed for p^H using a p^H 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 the River Cauvery. The experiments described in this study were carried out using four different species of fungi. *Aspergillus niger*, *Rhizopus arrhizus*, *Penicillum citrinum* and *Fusarium oxysporum* were used as test organisms for the treatment of waste water.

Fungal Medium Culture

For isolation of fungi from the polluted water, 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^oC. 100ml of malt extract broth (MEB) was prepared and dispensed into 250ml conical flasks. On cooling, a loopful of individual fungal stains was inoculated in MEB under sterile condition and kept at 25 - 27°C for 7 days on a rotary shaker at 100 rpm. 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, the whole mass was mixed in a blender to make a homogenous mixture. In order to obtain sufficient biomass for the subsequent growth and waste water treatment experiments, all the fungi were cultured in MEB and grown for a week before using for the experiment.

Experimental Set-up

The selected species of fungi were used for waste water treatment. The waste water samples collected from Tiruchirappalli city were used for the fungal assays. The waste water was filtered using Whatman No.1 filter paper to remove suspended solid particles. To study the role of fungi in waste water treatment, the waste water was treated with selected species of fungi. Waste water without fungi was used as a control. Experiments were conducted in triplicates. Flasks with 200ml of waste water were used for the treatments. Two ml of uniform suspension of *A. niger*, *F. oxysporum, P. citrinum* and *R. arrhizus* were used as initial inoculums (7 days old culture) in each of the four flasks. The experiment was conducted for a total duration of 16 days within a temperature range of 30 ± 2°C. Samples were periodically (every 4 days) analyzed for physico-chemical parameters such as p^H, phosphate, nitrate, BOD and COD using standard methods (APHA, 1998).

RESULTS AND DISCUSSION

The parameters that were analysed in the waste water using various fungi are presented in Tables (1-5). Table1 records the influence of fungi on the p^H levels for a period of 16 days. All the four species of fungi recorded a decrease in p^H levels till the 12th day followed by an increase on the 16th day. The p^H reduction in terms of percentage, ranged from 8.16 – 8.89%, while in the control there was a decrease of only 3.23%. Among the various fungi, *Aspergillus niger* recorded maximum reduction in p^H (8.89%) followed by *F. oxysporum* (8.6%), *P. citrinum* (8.20%) and *R. arrhizus* (8.16%). In general, there was a decrease in the p^H levels till the 12th day in all the experimental set ups as well as the control. Further, the p^H levels were also found to increase in all the setups as well as the control on the 16th day. The effect of using fungi on BOD levels is shown in Table 2. As seen from the table, while the control setup recorded only a decrease of 5.5% at the end of the 16th day, the experimental setups recorded a decrease in BOD levels ranging from 71.6 to 88.4%. Eventhough all the experimental setups recorded a decrease in BOD levels, the maximum reduction was recorded in the experimental setup using *Aspergillus niger* (88.4%) and the minimum using *F. oxysporum* (71.6%). The other two experimental set-ups showed values in between. Literature reveals that Azab (2008) recorded BOD values to decrease from 67.7 to 85% using fungi in sawdust mixture while Andleep *et al*. (2010) recorded BOD values to decrease by 66.5% during biological treatment of textile effiluents. Kshirsagar (2013) while studying the bioremediation processes using aquatic fungi recorded a decrease in BOD levels ranging from (66.98 – 82.46%). He recorded lowest BOD values using *Cunnighamella* species and the highest using *Aspergillus terreus.* Thus a comparison between these levels with those of the present study reveals a general agreement.

Table 1. Variation in pH content among the various experimental setups

Days	Control	*Aspergillus niger*	*Rhizopus arrhizus*	*Penicillium citrinum*	*Fusarium oxysporum*
0	8.21 ± 0.2	8.21 ± 0.14	8.21 ± 0.16	8.21 ± 0.8	8.21 ± 0.4
4	8.0 ± 0.32	7.84 ± 0.64	7.90 ± 0.02	7.85 ± 0.42	7.88 ±0.3
8	7.96 ± 0.4	7.56 ± 0.5	7.73 ±0.6	7.62 ± 0.3	7.67 ± 0.4
12	7.94 ± 0.6	7.48 ± 0.4	7.54 ± 0.2	7.53 ± 0.2	7.50 ± 0.3
16	8.11 ± 0.3	7.74 ± 0.21	7.83 ± 0.23	7.66 ± 0.26	7.92 ± 0.25
%		8.89	8.16	8.20	8.6

Table 2. Variation in NO_3 content among the various experimental setups

Days	Control	*Aspergillus niger*	*Rhizopus arrhizus*	*Penicillium citrinum*	*Fusarium oxysporum*
0	100	100	100	100	100
5	6.2	16.4	20.4	19.4	21.4
10	7.6	44.6	30.2	28.4	32.4
15	8.7	68.4	60.6	58.4	66.4
20	10.2	82.2	70.4	71.6	70.6

Table 3. Variation in PO_4 content among the various experimental setups

Days	Control	*Aspergillus niger*	*Rhizopus arrhizus*	*Penicillium citrinum*	*Fusarium oxysporum*
0	100	100	100	100	100
5	9.6	21.6	18.6	17.2	16.8
10	10.4	38.4	40.2	42.6	38.4
15	11.6	66.	68.4	70.2	72.4
20	12.4	79.2	81.4	83.4	82.4

Table 4. Variation in BOD content among the various experimental setups

Days	Control	*Aspergillus niger*	*Rhizopus arrhizus*	*Penicillium citrinum*	*Fusarium oxysporum*
0	100	100	100	100	100
4	2.2	18.4	17.4	16.2	15.6
8	3.4	45.6	36.6	34.2	33.4
12	4.6	75.6	72.4	65.4	64.6
16	5.5	88.4	79.4	72.4	71.6

Table 5. Variation in COD content among the various experimental setups

Days	Control	*Aspergillus niger*	*Rhizopus arrhizus*	*Penicillium citrinum*	*Fusarium oxysporum*
0	100	100	100	100	100
4	3.2	20.4	19.6	28.6	17.4
8	5.6	46.7	45.6	45.6	32.4
12	7.6	70.2	68.7	72.4	61.4
16	8.2	82.4	76.4	84.2	70.6

The effect of fungi on COD levels in the various experimental setups are shown Table 3. As evident from the Table, all the experimental set ups including the control recorded a decrease in COD level till the 16th day. While the control recoded a decrease in COD level of only 8.2% on the 16th day, experimental setups recorded reduction in COD levels raising from 70.6 to 84.2%. While the minimum decrease in COD level was noticed in the experimental setup using *Fusarium oxysporum,* the maximum was noticed in the set up using *P. citrinum* with *R. arrhizus* recording 76.4% and *A. niger* recording 82.4%. Literature reveals that Hamdi *et al*. (1991) recorded a decrease in COD levels of 52.5% using *A niger* while Hamdi and Radhouane (1992) recorded a decrease of 60% using A. niger. Roux – Van der Merwe *et al*. (2005) while studying the use of various fungal species in treating oil effluents recorded a COD reduction of 98% using *Cunninghamella* species. Emtiazi *et al*. (2001) recorded a COD reduction of 52.5% using *A. niger* while Cereti *et al*. (2004) observed a COD reduction of 35 - 64%. However, Azab (2008) recorded a decrease in COD level of 65.7 – 79% using *A. tereus* and *A. niger* while Andleeb *et al*. (2010)recorded a decrease of 75.24% using *A. terreus*. Recently, Kshirsagar (2013) while studying the bioremediation of waste water using fungi recorded a COD reduction ranging from 71 - 85.88%. Thus the above results are in general agreement with the studies made by other workers.

The removal of nitrates in the various systems using the different fungal species are recorded in Table 4. As evident from the Table, nitrate reduction was found to range from 70.4 to 82.2% in the various setups. Among these, the lowest reduction was found in the set up using *Rhizopus arrhizus* (70.4%) and the highest in the set up using *A. niger* (82.2%). Kshirsagar (2013) also reported the highest reduction in nitrate level using *A. niger* during the bioremediation of waste water treatment. Earlier, Hwanga *et al*. (2007) also documented a similar observation while studying the removal of nitrogenous substances in a continuously stirred tank reactor system. Phosphate removal among the various experimental setups in tabulated in Table 5. It is clear that among the various setups, the reduction in phosphate level ranged from 79.2 – 83.4%. While the lowest phosphate reduction was found in the experimental set up using *A. niger*, the maximum reduction was found in the set up using *P. citrinum*, Nevertheless, there was no major variation among the four fungal species with regard to bioremediation of phosphates. The present study clearly indicates that fungi can be used in the bioremediation process for treating waste water. In the present study, the hierarchy in descending order with regard to p^H was *A. niger* > *P. citrinum* > *R. arrhizus* > *F. oxysporum* while for BOD the order was *A. niger* > *R. arrhizus* > *P. citrinum* > *F. oxysporum* and for COD it was *P. citrinum* > *A. niger* > *R. arrhizus* > *F. oxysporum*. With regard to nutrient nitrate, the order was *A. niger* > *F. oxysporum* > *P. citrinum* > *R. arrhizus* while for phosphate it was *P. citrinum* > *F. oxysporum* > *R. arrhizus* and *A. niger*. Thus, the results clearly reveal that all the species show a reduction in all the parameters studied. However, each fungal species behaved in a different manner with regard to each parameter. However, among the four candidate species, *A. niger* appears to be the best as it had the best reduction percentage among the five parameters (p^H, BOD and nitrate) analyzed.

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