Solid waste treatment by fungal enzymes in artificially constructed wetland

Nirmalkar Vaishali*, Khan Uquba, Ansari Shahana and Shaikh Asfiya
G.M. Momin Women's College, Bhiwandi, Maharashtra, INDIA
*nirmalkarvaishali@gmail.com

Abstract
Solid waste is normally defined as unwanted solid, semi-solid material ensuing from human or animal activities. The production of solid waste has become a common problem worldwide. A large number of enzymes from bacteria, fungi and plants have been reported to be involved in the biodegradation of toxic organic pollutants. Constructed wetland or artificial wetland are used here which are inexpensive, easy to operate and are effectively used to treat various kinds of waste water. In the present study artificial wetland was constructed and various parameters like BOD, COD, DO and Turbidity were measured. Fungi like Aspergillus sp. and Penicillium sp. were inoculated individually as well as in a mixture and production of amylase and cellulase by these fungi was measured.

It was found that these fungi produced the enzymes and degradation of solid waste was observed. It is suggested that such fungi would prove to be useful in the degradation of organic waste present in the natural wetlands. This would be economical, cost effective, simple and easy to operate, no complex technology is needed and will help in making the environment more safer.

Keywords: Artificial wetland, solid waste, fungi, enzymes.

Introduction

Modernization and progress had its share of disadvantages and one of the main aspects of concern is the pollution it is causing to the earth – be it land, air, or water. With increase in the global population and the rising demand for food and other essentials, there has been a rise in the amount of waste being generated daily by each household. Solid waste is normally defined as unwanted solid, semi-solid material ensuring from human or animal activities[9].

Solid wastes are the organic and inorganic waste materials formed through various forms in the society losing their value to the users. This waste is ultimately thrown into municipal waste collection centres from where it is collected by the area municipalities to be further thrown into the landfills and dumps[9].

However, either due to resource crunch or inefficient infrastructure, not all of this waste gets collected and transported to the final dumpsites. A large number of enzymes from bacteria, fungi and plants have been reported to be involved in the biodegradation of toxic organic pollutants[5].

Wastewater pollution has always been a major problem through the world. One of the main sources of the pollution is from municipal wastewater which comes from residential, industrial or agricultural area[10,16]. The wastes consist of organic and inorganic waste including food scarf, waste oil and detergent[24].

The ability of natural wetlands to improve water quality through its physical, chemical and biological processes and their interactions has been recognized for so many years. Wetland also provides a valuable aquatic habitat for a diverse species of flora and fauna. The value of the world's wetlands is increasingly receiving due attention as they contribute to a healthy environment in many ways. Constructed wetland known as an artificial wetland is one of the technology treatment system used internationally. Usually they consist of three elements which are water or waste water that needs to be treated; microorganism that can degrade all the contaminant or pollutant in the waste water[16,22].

Microorganisms play a main role in biochemical transformation of contaminants and their capability in removing toxic organic compounds added to wetlands has been reported [7,21]. Constructed wetland is less expensive and has low maintenance cost than traditional wastewater treatment systems. Additionally, these systems have more aesthetic appearance than traditional wastewater treatment systems [3,8].

Liquid waste treatment uses microbes to degrade organic matter, thereby reducing the biochemical oxygen demand (BOD). The ability of a microorganism to degrade an environment pollutant is highly dependent on the chemical structure of the pollutant. Basically, municipal wastewater contains high level of chemical oxygen demand (COD) and total suspended solid (TSS). This high amount of chemical oxygen demand (COD) results in low dissolved oxygen (DO) in water and this can lead to mortality of aquatic lives. In addition, suspended solid such as organic or inorganic material can cause dirt and odour to the water[16].

Bioremediation is a microorganism mediated transformation or degradation of contaminants into non-hazardous or less-hazardous substances[20]. It is a slow process and only few bacterial and fungal species have the ability of degradation[5,11-13]. In the present study, constructed wetland or artificial wetland is used which is inexpensive, easy to

operate and are effectively used to treat various kinds of waste water. The solid waste is tried to treat using fungal enzymes which would prove to be cost effective and potent.

Material and Methods

Collection of soil samples: The soil samples were collected from 6 different sites each from sizing industries. The soil sample was mixed well and processed next day [1].

Isolation of fungi: Fungal colonies were isolated from soil samples by serial dilution method where in SDA (Sabouraud dextrose agar) media was prepared, autoclaved and poured in sterile Petri plates. 50µl of soil samples diluted up to 10-5 dilutions were spread on respective solidified SDA plates with the help of sterile spreader. The inoculated Petri plates were incubated at 28°C for 48 hours. Two isolates were selected and further inoculated on SDA plates by point inoculation and incubated at 28°C for 48 hours in order to obtain pure fungal plates [6].

Identification of isolates: The isolated strains were carefully identified by morphological characteristics which include color of the colony and growth pattern studies as well as their vegetative and reproductive structures observed under the microscope [2]. Among the characteristics used were colonial characteristics such as surface appearance, texture and colour of the colonies. In addition, microscopy revealed vegetative mycelium including presence or absence of cross-walls and diameter of hyphae.

Construction of Wetland: Waste materials were collected from kitchen, market and road side. Horizontal subsurface artificial wetland was constructed thereafter. For construction of artificial wetland, first of all a layer of stones was spread at the base in plastic tubs, upper to the layer of stone, soil was spread up to 2 cm. Four liters of water were added after which collected solid waste materials were added. This was followed with the inoculation of fungal spore suspension. The inoculation was done by using both the fungal species individually as well as both were also mixed and inoculated so that synergistic effect could be observed if any.

This artificially constructed wetland was kept at suitable place at room temperature. Then the changes occurring in it were observed. The performance of the wetland was accessed from time to time by collection of sample and analyzing at predetermined interval to determine the effect of time on the treatment efficiency of the setup. The treated water was collected for continuous ten days from the effluent of the construed wetland and analyzed.

Chemical parameters of water sample: For determination of chemical parameters like COD, BOD, turbidity etc., water sample was collected daily from the constructed wetland. These characteristics were determined using the procedure mentioned in the standard methods (Wrinkler´s Titrimetric method).

Amylase activity was assayed by the dinitrosalicyclic acid (DNS) method given by Sadasivam and Manickam[14]. Amount of maltose released was determined by comparing the absorbance reading of the test enzyme at 560 nm with the standard graph plotted by reacting the known concentration of maltose ranging from 0.05mg/ml to 0.5mg/ml[6].

Cellulase activity was assayed by the dinitrosalicyclic acid (DNS) method given by Sadasivam and Manickam[14]. Absorbance at 540 nm was compared with the standard graph plotted by reacting known concentration of glucose (0.05 to 0.5mg/ml) with DNSA reagent[18].

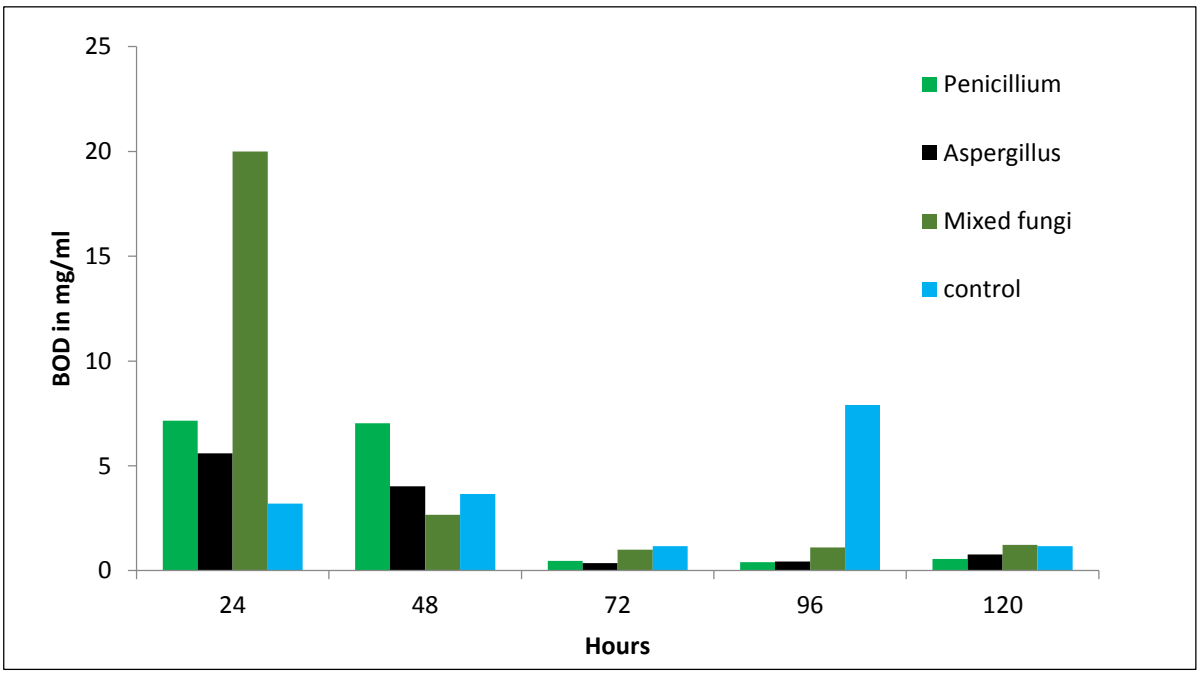

Fig. 1: BOD values for both the fungi

Results and Discussion

The fungal samples were isolated from soil. The fungal isolates were identified as *Aspergillus sp*. and *Penicillium sp*. The physicochemical analysis of water samples like DO, BOD, COD and Turbidity was carried for ten days. It was observed that as the retention time increased, there was generally a continuous decrease in values of all the parameters. At 24 hours the level of the BOD in artificially constructed wetland was higher; then at 48 to 120 hours, it was observed that the level gradually decreased (Fig. 1).

It was indicated that *Penicillium* and *Aspergillus* both are capable to degrade the organic pollutants in the wetland. But it was observed that *Aspergillus sp.* was more potent to degrade organic pollutants than *Penicillium sp.* At 24 hours the level of the COD in artificially constructed wetland was higher; then at 48 to 120 hour it was observed that the level of the chemical oxygen demand gradually decreased (Fig. 2).

The activity of the mixed fungus was not found to be significantly different in comparison with control and both the fungal strains. Turbidity values were found to decrease but most potential was *Aspergillus* sp. (Fig. 3). Thus the BOD, COD and turbidity values decreased gradually after inoculation of the fungi. Thus both the isolated fungi were capable to degrade the organic pollutants and hence could be used in biodegradation of the organic pollutants of wetlands. Also they belong to biological origin instead of using synthetic purifier which may be chemically originated and having a harmful impact on biology of wetland.

Both the fungal isolates showed good amylase activity at 48h of incubation. At 72h the mixed fungi showed the higher activity. At 120 h, activity of mixed fungi was lower than the activity of the *Penicillium species*. At 168 h, *Aspergillus species* showed higher activity and then the activity of the fungi gradually decreased. On the basis of these results, it could be concluded that mixture of the *Aspergillus* and *Penicillium* was more potent (Fig. 4).

The cellulase activity of *Aspergillus species* was higher at 48h while the activity of the mixed fungus was lower than the *Aspergillus species* at 48h. The activity of both fungus gradually decreased till 96 h and then activity of the *Penicillium* species increased at 120 h and here the *Aspergillus* species showed lower activity than *Penicillium*. At 144 h mixed fungus showed the activity but individual fungi showed marked reduction in the activity (Fig. 5).

Fungi are well known agents of decomposition of organic matter in general and of cellulosic substrate in particular. Even though there are many reports on fungi producing amylases[15] and cellulases[17], only a few have proved high activities for commercial success[4,19]. A wide range of *Aspergillus sp.* has been identified to possess all components of cellulases complex[19,23].

On the basis of these observations, it could be said that even the individual fungal species are capable to produce the enzymes which degrade the organic pollutants in wetland. If such fungi are inoculated, then it may prove to be helpful in the degradation of organic waste present in the wetlands and make environment more safe.

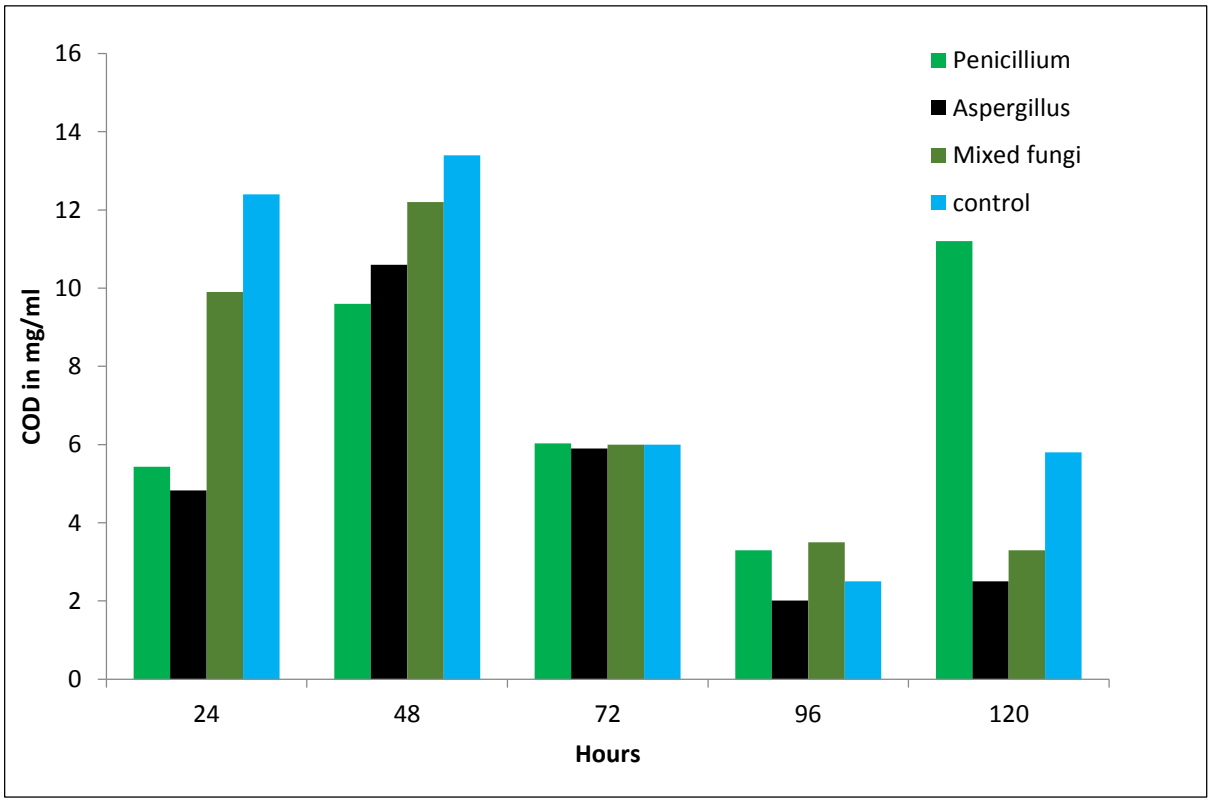

Fig. 2: COD values for both the fungi

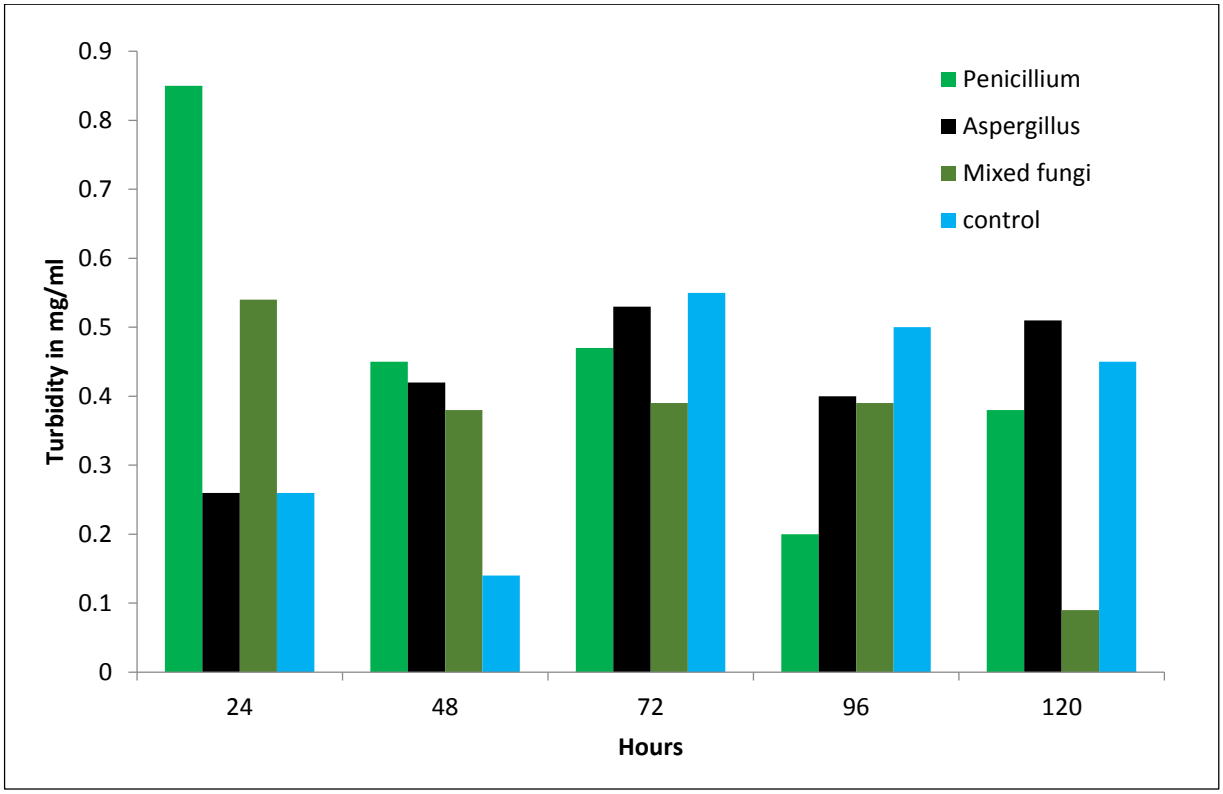

Fig. 3: Turbidity values for both the fungi

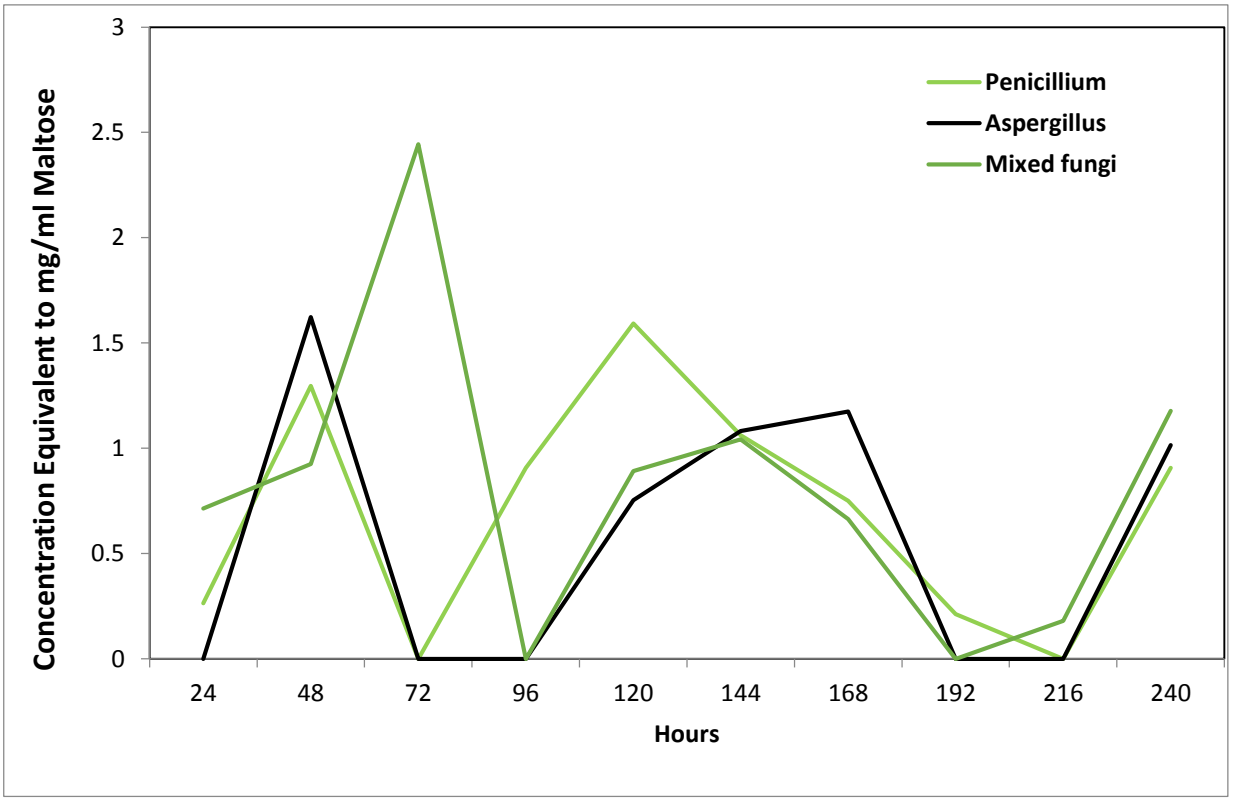

Fig. 4: Comparison of amylase activity of fungi

Conclusion

From the present findings it could be said that even the individual fungal species are capable to produce the enzymes which degrade the organic pollutants in wetland. If such fungi are inoculated, then they will prove to be helpful in the degradation of organic waste present in the wetlands. It would be economical, cost effective, simple and easy to operate; no complex technology is needed and could help in making the environment safer. However, more enzymes need to be explored with variety of fungi.

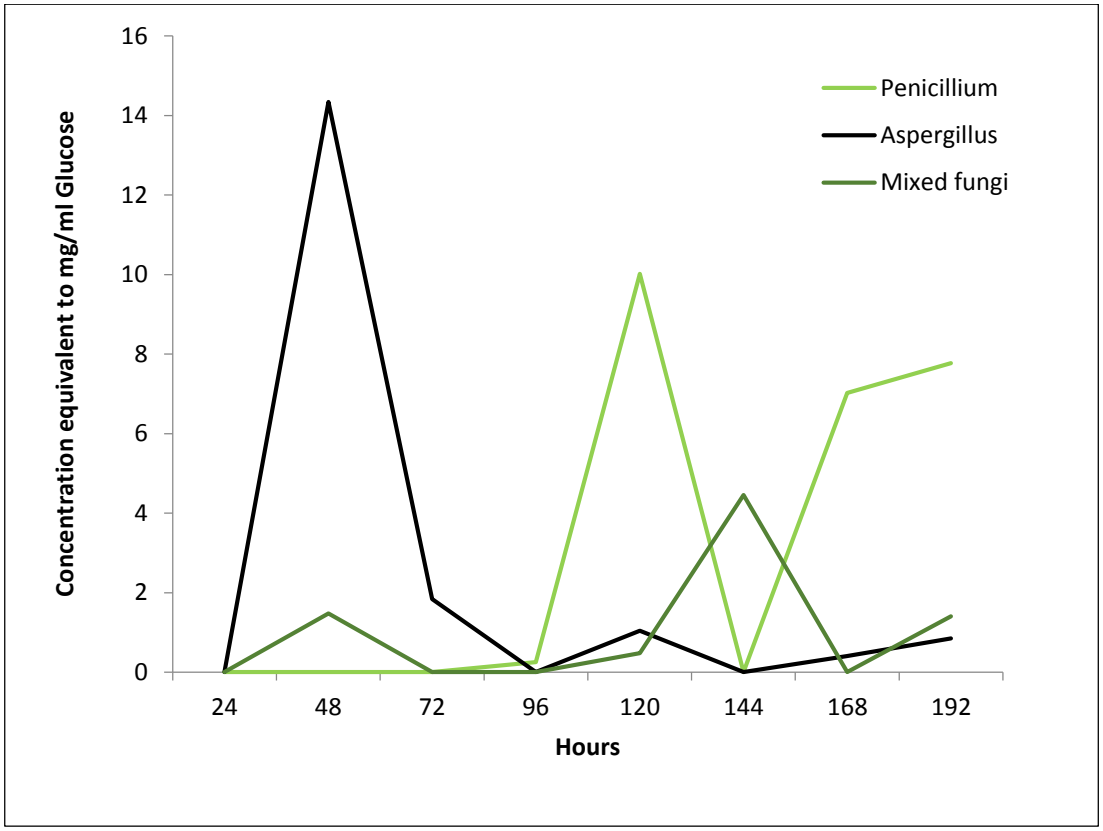

Fig. 5: Comparison of cellulase activity of fungi

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