Bioremediation of industrial waste through mushroom cultivation

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Abstract: Handmade paper and cardboard industries are involved in processing of cellulosic and ligno-cellulosic substances for making paper by hand or simple machinery. In the present study, solid sludge and effluent of both cardboard and handmade paper industries was collected for developing a mushroom cultivation technique to achieve zero waste discharges. Findings of present research work reveals that when 50% paper industries waste is used by mixing with 50% (w/w) wheat straw, significant increase (96.38%) in biological efficiency over control of wheat straw was observed. Further, cultivated basidiocarps showed normal morphology of stipé and pileus. Cross section of lamellae did not show any abnormality in the attachment of basidiospores, hymenal trama and basidium. No toxicity was found when fruiting bodies were tested chemically.

Key words: Mushroom cultivation, Pollution abatement, Handmade paper, Pulp and cardboard industries

Introduction

Handmade paper industries (HMPI) are involved in processing of cellulosic and ligno-cellulosic substances for making paper by hand or simple machinery and thought to be non-polluting whereas cardboard industries (CI) are also involved in processing of similar raw material as well as recycling of waste paper and boards having shortened fibres, and thought to be polluting industries. The pulp, paper and cardboard industries are also utilizing huge amount of water which is discharged as untreated effluent to natural water bodies and pollutes it.

Presence of dyes and chemicals, in handmade paper and pulp industries effluent reveals by its colour, low pH and high COD (Kulshreshtha et al., 2008). Secondly, problem also arises due to the repeated recycling of paper. In the last stage of recycling, cellulosic fibres become too short to be recycled and utilized for making cardboards. Paper can be recycled to 4-5 times, after that non-recycled pulp residues accumulated in the front of cardboard industries as solid sludge and produce enormous odour in the area due to its biologically degraded compounds. These industries are not only present in India but also in many other European, American and Asian countries. Hence, the problem is of global concern.

The present work is based on effluent and sludge utilization of handmade pulp, paper and cardboard industries situated around Amani Shah Drainage in Sanganer near Jaipur. These industries are utilizing cellulosic hosiery rags, cotton bags; mill broke, and lignocellulosic waste paper and cardboard for making paper and cardboards respectively.

Further, after making paper and boards, effluent is discharged to the Amani Shah Drainage which is then utilized for irrigating nearby agricultural fields. Solid sludge of non-recycled pulp residues is also accumulated in the front of these industries at the rate of 10 Kg day⁻¹.

The present research work was planned to protect environment by utilizing the pulp residues separated from effluent and solid sludge of both industries for mushroom cultivation because it is not only capable of bioremediation of waste but also provides a highly proteinaceous food (Mane et al., 2007; Kuforiji and Fasidi, 2009).

Cultivated fruiting bodies were collected for further analysis of toxicity, morphological and anatomical studies.

Materials and Methods

Sampling site and collection of sample: Sanganeri handmade pulp, paper and cardboard industries were selected for collection of solid sludge and effluent. Effluent and solid sludge of these industries were collected separately, in a clean plastic container, transferred to laboratory and stored at 4°C until use for analysis.

Physico-chemical analysis of effluent: Effluent samples were analyzed for physicochemical parameters such as pH, temperature, salinity, conductivity and total dissolved solids (TDS) by water analyzer kit. Biological oxygen demand (BOD) was analyzed by membrane electrode method and chemical oxygen demand (COD) was analyzed by closed reflux method as given in American Public Health Association (APHA) manual (1995). All these parameters were analyzed within 24 hrs. in each of three replicates.

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Mushroom culture and cultivation on waste: Mushroom (Pleurotus florida) culture and prepared spawn were obtained from National Research and Mushroom Center, Solan, Shimla, India. Strain was subcultured on Potato dextrose agar and maintained at 4°C.

The effluent was mixed with solid sludge. Due to mixing, thick slurry of composite waste was obtained; therefore no presoaking is required like wheat straw. 1.25 ml l⁻¹ of 40% formalin solution (v/v) and 0.75 g l⁻¹ (w/v) of anti-fungal substance bavistin were added to the slurry. After 18 hrs, slurry was filtered by a clean cloth and collected pulp fiber spread on a surface clean previously by formalin, to remove excessive water and maintain 80% humidity. Waste was then sterilized by steam for 1 hr. In control, wheat straw (WS) was soaked in water containing formalin and bavistin, for 18 hrs and sterilized before using it as a substrate. Pulp fibres, alone as well as in combination of wheat straw (1:1) was tested for mushroom cultivation.

After cooling, 1 Kg of wet waste alone and mixing with 50% (1:1, w/w) wheat straw was inoculated with spawn and packed in polythene bags possess holes at 3 cm distance. After inoculation, polythene bags were incubated at 22°C±1°C. When Pleurotus mycelium fully colonized the substrate, polythene bags were removed and substrate bundles were hanged. To maintain humidity bundles were sprayed with water twice a day. Six bundles of each combination of substrate were prepared. Each substrate bundle was observed daily and time was recorded for pinhead formation, immature fruiting body and mature fruiting bodies formation.

One bag of each substrate and its combination was dried to calculate the actual dry weight of the substrate. Biological efficiency of mushrooms were calculated by dividing weight of fresh mushroom yield (in Kg) by weight of air dried substrate (in Kg) and multiply by 100 (Banik and Nandi, 2004).

Experiment was repeated thrice. The data was pooled and ANOVA was used to test the significance of biological efficiency of mushroom.

Chemical toxicity test of mushroom: A drop of juice was pressed out of the fresh fruit body on a piece of newspaper. After the spot had dried, hydrochloric acid was dropped on it. A blue spot indicated the presence of toxins (Svrcek, 1998).

Anatomical studies: Anatomical structures were analyzed in cross-section of lamellae which was stained with lactophenol cotton blue staining technique and then observed under light microscope at 10x10x and 10x45x magnification.

Results and Discussion

Physicochemical characteristics: The pulp, paper and cardboard industries are major industrial sector which are polluting natural water bodies by its discharges (Malviya and Rathore, 2007). The generation of waste water and characteristics of pulp, paper and cardboard industries effluent depends upon the type of manufacturing process adopted and the extent of reuse of water employed in plant (Singh and Thakur, 2006) and it should be checked before it is used for the cultivation of mushrooms (Gray, 2000).

In this context, the physico-chemical characteristics of waste effluent of Sanganeri handmade paper and cardboard industries were carried out and results are shown in Table 1.

![Fig. 1: Fruiting bodies appeared on the coloured pulp residues of handmade paper industries when mixed with 50% wheat straw](image1)

![Fig. 2: Spore print of fruiting body](image2)
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Salinity, conductivity, and total dissolved solids were not found to be very high. Temperature and BOD of effluent of both industries is in acceptable range but pH and COD were found to be above the limits described by Central Pollution Control Board (MINAS, 2000). Low pH was also observed by Saakshi (2005). The reason of low BOD and high COD is the presence of high molecular weight compounds such as cellulose and lignin. This finding was evidenced by the work of Raj et al. (2007).

Handmade paper industries are using different dyes for making coloured papers revealed by the presence of colour in the effluent whereas brown or black colour of cardboard industries effluent is due to ink, dyes or chemicals released during pulp making process. Hence, effluent is not suitable for discharge in untreated form.

Growth characteristics and biological efficiency: A number of research reports have been published for biological treatment of kraft pulp and paper industries (Ragunathan and Swaminathan, 2004; Singhal et al., 2005; Bishnoi et al., 2006; Singh, 2007; Kousar and Puttaiah, 2009; Satyawali et al., 2009). However, no work has been done on treatment of solid as well as effluent wastes of handmade paper and cardboard industries.

Although effluent having low pH, high COD and colour yet it can be utilized for the production of mushroom because mushrooms have ability to degrade dyes (Espíndola et al., 2007), xenobiotics (Eggan, 1989), lignin and cellulose containing sawdust and wastepaper (Chang and Chiu, 1992; Baysal et al., 2003).

In the present study, composite waste (effluent + solid sludge) of handmade paper industries was found to be good support in the formation of fruiting bodies of *P. florida* (ANOVA, p<0.5) when mixed with 50% wheat straw (Table 2). The results are consistence with...
Baysal et al. (2003) who reported increase yield of P. ostreatus on mixing paper waste with rice straw. Biological efficiency of P. florida is not decreasing significantly over control in case of combination of cardboard industrial waste with wheat straw.

When waste was used alone significant decrease in biological efficiency of P. florida was observed. Spawn running time of P. florida was found to be highly co-related with 50% HMPI+50% WS, followed by 50% CI+50% WS, 100% HMPI and then control (Table 3). This may be due to the higher CO₂ concentration in the center of substrate bundles which might have resulted from the growth of mycelium.

Due to recycling, fibres of cardboard industry pulp residues are finer than that of paper industry, resulted in over compaction of substrate bundles. This resulted in lower yield due to high time requirement for complete fruiting body formation. This reasoning is evidenced by the work of Zhang et al. (2002).

Morphological studies of Pleurotus: Fruiting bodies of P. florida appeared in groups from a same point on each substrate, were normal with white colour. Content of fruiting bodies were found to be fleshy and odour fungoid. Oyster shape pileus was found to be spatulate when young, convex (Fig. 1) then later becoming concave on maturity, have smooth surface and white colour.

Lamellae were long-decurrent, crowd, white coloured and having smooth edge when young, later become undulating. Stipe was short and having solid context, longitudinally stritate surface, and white in colour. Presence of white coloured spores confirms by spore print (Fig. 2).

Hence, presence of chemicals in the effluent did not found to be inducing any morphological dissimilarity as no significant differences were observed in measurement of mature fruiting bodies (Table 4).

Anatomical study of Pleurotus: Cross-section of Pleurotus lamellae shows no abnormality (Fig. 3). Unstained preparation of gills, stalk and pleus mycelium of P. florida reveals no absorption of dyes on it. Spores were cylindrical, smooth and hyaline. Four spored, slenderly clavate basidia were found to be attached to irregular, hymenophoral trama and showed normal morphology.

Chemical toxicity test: P. florida cultivated on each of above substrate, showed no blue spots on reacting with HCl on lignin containing paper, revealing the presence of non-toxic compounds.

The present study deals with an approach that integrates biotechnology with waste management to achieve zero waste and zero discharges from handmade paper and cardboard industries. Cultivated mushrooms were not only found to be normal but also non-toxic.

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References

MINAS: For large pulp and paper mills with chemical recovery. CPCB, (2000).