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RESEARCH ARTICLE

DEINKING OF NOTE BOOK PAPERS AND EFFUENT DEGRADATION USING ASPERGILLUS FUMIGATUS

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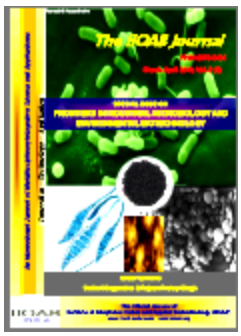
ABSTRACT



Aspergillus fumigatus was isolated from soil sample was efficient in degradation of ink , paper stained with water soluble fountain pen ink and also could be deinked by boiling for 5 minutes. The effluent obtained by deinking process could be effectively treated with Aspergillus fumigatus. The quality of the deinked paper is acceptable under paper recycling norms.

Key words: ***deinking, Aspergillus fumigatus, paper quality***

[I] INTRODUCTION

Writing is a fundamental need of education and schools insist on the use of fountain pens to improvise the handwriting of young children. Thus in every academic year papers stained with fountain pen inks are being thrown out and recycling of these papers stained with fountain pen inks becomes essential. Paper recycling is the process of recovering waste paper and remaking it into new paper products [1]. Today the strong market in ink-reservoir writing instruments is still dominated by the ball-ended pen, now with several ink options, but the fountain pen will not lie down, it is still highly valued in our electronic communication culture [2]. The paper has two main levels. The first level stores the ink that is 'floating' on the paper. This is wet ink that can be dragged across the page. The second layer is the ink that has dried – that is, that has been absorbed by the page and is no longer able to flow [3]. Carbon black now replaces spinel black, rutile black and iron black in nearly all black inks. In fact the ink industry is the second largest consumer of carbon black [4]. The protocol for the enzymatic deinking of laser printed waste papers on a laboratory scale using cellulase and hemicellulase of *Aspergillus niger* was developed as an effective method for paper recycling [5].

[II] MATERIALS AND METHODS

Approximately one kilogram of marine soil sample was collected from Chennai beach (east coast) and Trivandrum beach (west coast). This soil sample was spread neatly on separate trays. Another tray filled with soil from staining tray was also taken [6].

2.1. Isolation of bacteria and fungi capable of deinking

The method was followed as described in Zollner et al [7]. Three pairs of paper of size 3x3 was cut and dipped in ink. These papers were dried. One pair of the paper was buried in Chennai beach soil sample, other in Trivandrum beach sample and the third pair of paper was buried in the staining tray. This set up was left undisturbed for 7 days. On the 7th day 5 nutrient agar plates and 5 nutrient agar plates were prepared. The pair of papers buried in soil sample was carefully taken out using a sterile forceps and was used to inoculate one nutrient agar plate and one potato dextrose agar plate.This completes inoculation of one set of plates with papers from Chennai soil sample, other set with Trivandrum soil sample, the third with staining tray paper sample and the fourth set of plates was inoculated with 1g of soil sample collected directly from the staining tray. The fifth set is marked as control. These plates are incubated at 37°C for 24 hours.

2.2. Enrichment Culture

Minimal media was prepared and autoclaved. After cooling, ink mix was added at a concentration of 0.1ml/ml to the minimal media. The

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organisms from the nutrient agar plates and Czapek-dox agar plates were inoculated separately. It is incubated at 37^{0}C for 7 days. Lactophenol cotton blue mounting was used to identify the presence of characteristic mycelia and fruiting structures.

2.3. Cellulose assay and characterization of cellulose-related phenotype

Minimal salt broth was prepared [Glucose (0.1g), Dipotassium phosphate (0.7g), Monopotassium phosphate (-0.2g), Sodium citrate (-0.05g), Magnesium sulphate (-0.01g), Ammonium sulphate (-0.15g), Agar (-2.5g), Distilled Water (-100ml)], poured in 10 conical flasks (250ml) and sterilized by autoclaving. These flasks were arranged in two sets (each set containing 5 conical flasks).four conical flasks were marked as blue, black, red and green, the fifth was marked as control. 0.5ml of the respective ink was added to each of the flask separately, one set was inoculated with *Aspergillus fumigatus* and the other with *Penicillium* spp. These flasks were incubated at the room temperature and observed for color reduction. The color reduction was measured by visible spectrophotometer.

2.4. Degradation of gel ink

Minimal salt broth was prepared, poured in 6 conical flasks (250ml) and sterilized by autoclaving. These flasks were arranged in two sets (each set containing 3 conical flasks).two conical flasks were marked as, blue gel and black gel. The fifth was marked as control.0.5ml of the respective ink was added to each of the flask separately. One set was inoculated with *Aspergillus fumigatus* and the other with *Penicillium* spp These flasks were incubated at the room temperature and observed for color reduction.

2.5. Deinking of papers directly using Aspergillus fumigatus

Trial 1: A paper dipped in ink was dried and placed on a sterile Petriplates. Spores of A*spergillus fumigatus* was collected and dispersed over the paper directly. The plates are incubated at 37ºC. **Trial 2:** A paper dipped in ink was placed on a sterile Petriplate and around 1 ml of minimal media was added to wet the paper. To this wet paper spores of *Aspergillus fumigatus* were added and incubated. **Trial 3:** A paper dipped in ink was placed on a sterile petriplate and was soaked in excess of minimal media. Spores of *Aspergillus fumigatus* was collected and dispersed over the medium. These plates are incubated at 37ºC. **Trial 4:** Sterile Minimal agar plates were prepared. A paper dipped in ink was placed over the medium. The organism was spread evenly on the plate using sterile peptone swabs and incubated at 37ºC.

2.6. Deinking of fountain pen inks

A mixture of ink was prepared by mixing equal volume of all the four colors (blue, black, red and green) of fountain pen ink. Using a dry cotton swab the ink mix was spread evenly on the small standard A4 paper (TNPL copier).The paper was dried, immersed in the beaker containing water, which was heated for 5 minutes.

2.7. Degradation of gel pen inks

A mixture of ink was prepared by mixing equal volume of gel blue ink and gel black ink. Using a dry cotton swab the ink mix was spread evenly on the small standard A4 paper (TNPL copier).the paper was dried and was immersed in the beaker containing water. It was heated for 5 minutes [8, 9].

2.8. Degradation of effluent from deinking trial

The quantity of effluent was measured as described by Scott and Ollis 1995 [10]. The minimal media components were added to the effluent based on the amount of effluent. It was sterilized by autoclaving. The fungal culture was then inoculated using a sterile inoculation loop. It was incubated at 37ºC and observed for color reduction. The color reduction was measured by visible spectrophotometer [11]. **Brightness:** Whiteness Meter was used to measure the brightness, yellowness and shade of the deinked paper. This whiteness meter is mainly used to measure the whiteness of paper, paperboard, paper pulps, pulps of chemical fiber, cotton, chemical fiber, textile, plastic, starch, salt, white cement, porcelain clay and talcum powder, etc. **Porosity:** Paper is a highly porous material and contains as much as 70 % air. Porosity is a critical factor in Printing Papers, Laminating Paper, Cigarette Paper and Bag Paper. The POROLOG MICRO 5000 is an instrument which is widely used to detect changes in porosity on the moving paper web [12]. **Smoothness**: Smoothness tester determines the smoothness and porosity of paper and board, based on rotameter airflow principle. It is provided with three imported variable area flow meters (Rotameter). **Caliper:** Caliper used to measure the thickness of a sheet of paper expressed in thousandth of an inch.

[III] RESULTS AND DISCUSSION

3.1. Isolation

Czepek-dox agar plates were inoculated with paper from staining tray paper and staining tray soil showed white and green color fungal colonies **[Figures–1 to 4]**. Nutrient agar plates showed white colonies **[Figure–2]**.

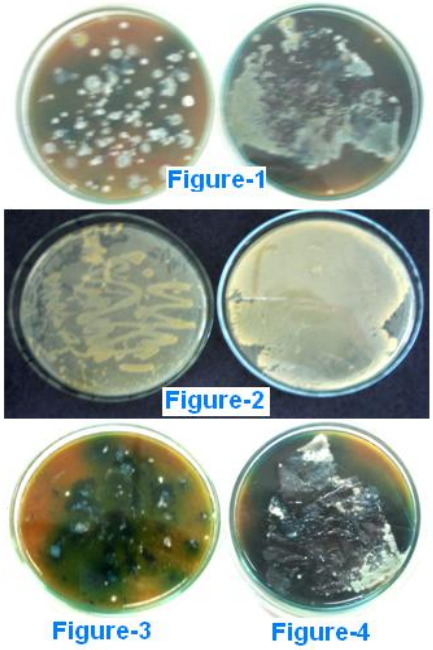

Fig: 1. Fungal colonies. Fig: 2. Bacterial colonies. Fig: 3. Growth of fungus. Fig: 4. Growth of fungus.

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3.2. Enrichment culturing

The isolated organisms were cultured on medium enriched with medium containing ink of which only the two fungal species showed considerable reduction in color [13].

3.3. Identification

Lactophenol cotton blue preparation of a slide culture showed a typical flask- shaped vesicles and characteristic rows of conidia on fruiting bodies and was found to be *Aspergillus fumigatus* **[Figure–5]**. Lactophenol cotton blue preparation of a slide culture showed dense brush-like spore-bearing structures with simple conidiophores terminated by clusters of flask-shaped phialides, was found to be *Penicillium* spp **[Figure–6]**.

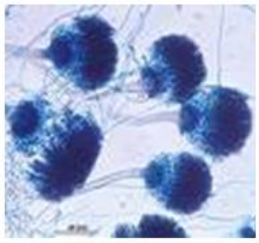
Figure-5

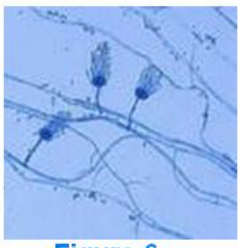
Figure-6

Fig: 5. *Aspergillus fumigatus.* **Fig: 6. *Penicillium spp.***

3.4. Visible spectrophotometeric analysis

The OD value taken for every four days for a period of 16 days was recorded **[Tables–1 to 4]**. *Aspergillus fumigatus* was found to be effective in degrading ink than *Penicillium* spp.

Table: 1. Blue ink degradation

Number of days	0	4	8	12	16
A. fumigatus	0.026	0.013	0.009	0.007	0.004
Penicillium sp	0.026	0.022	0.019	0.007	0.006

Table: 2. Black ink degradation

Number of days	0	4	8	12	16
A. fumigatus	0.091	0.065	0.051	0.042	0.03
Penicillium sp	0.091	0.091	0.088	0.076	0.062

Table: 3. Red ink degradation

Number of days	0	4	8	12	16
A. fumigatus	0.861	0.57	0.535	0.162	0.14
Penicillium sp	0.861	0.654	0.579	0.448	0.441

Table: 4. Green ink degradation

Number of days	0	4	8	12	16
A. fumigatus	0.048	0.044	0.037	0.032	0.023
Penicillium sp	0.048	0.048	0.046	0.04	0.035

3.5. Deinking of papers directly using Aspergillus fumigatus

All the four trials carried out to remove ink from the paper directly was found to be failure since the organisms stain the paper with its metabolic product after four days of incubation [14]

3.6. Deiniking of papers by boiling

The fountain pen inks are easily separated form the paper by boiling for 5 minutes as it does not fuse with the paper tightly but the gel pen inks fuse with the paper and cannot be removed from the paper by boiling.

3.7. Degradation of ink from effluent

Degradation of effluent from deinking trials using *Aspergillus fumigatus* for 16 days was carried out the initial and the final concentration of the effluent was measured in visible spectrophotometer and was ploted on a standard graph for ink mix dilution [15]**[Table–5]**.

Table: 5. Effect of degradation

Effluent	OD at 620 nm
Before treatment	1.79
After treatment	0.414

The effect of this deinking process can be calculated by calculating the loss of brightness

$$\text{Loss of brightness} = \frac{\text{Brightness of base paper - Brightness of the deinked paper x 100}}{\text{Brightness of base paper}}$$

$$= \frac{79.9\text{-}68.9 \times 100}{79.9}$$

$$= 0.1376 \times 100$$

$$= 13.76$$

Therefore the loss of brightness percentage is 13.76. According to Neal et al. treatment of news paper by Agglomerate Floatation using kerosene oil showed average the loss of brightness of 14.79 which is an acceptable standard for recycling of grade one papers [16].

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Table: 6. Quality of deinked Paper

Property	Base paper	Deink paper
Brightness [%]	79.9	68.9
Yellowness [%]	-5.5	-3.1
L* [%]	89.2	85.0
a*[%]	0.9	0.8
b*[%]	-0.3	-1.7
Smoothness [ml/inch]	Top-320 Bottom-220	Top-800 Bottom-600
Porosity [ml/inch]	100	130
Caliper [µ]	103	114

Since the porosity of the deinked paper is high compared to the base paper, it cannot be reused as writing material but can be used to make boards, boxes and tissue papers [17] **[Table –6]**.

Pulp recovery: The pulp weight was 0.271 before and after the treatment. There is no change in the pulp weight. Hence the recovery of pulp is 100%. According to Tradecom International Ltd, Mumbai, Recycled paper recovery in India is 22% of total consumption in comparison to 55-60% recovery in developed countries. In chemical deinking of newspaper waste, the maximum recovery is 60% [18]. In enzymatic deinking process 90-98% recovery is possible but it is an expensive process [19]. Hence this method of deinking using *Aspergillus fumigatus* is efficient as well as economical.

[IV] CONCLUSION

Aspergillus fumigatus isolated from soil sample is efficient in degradation of ink. Paper stained with water soluble fountain pen ink can be deinked by boiling for 5 minutes. The effluent from deinking process can be effectively treated with *Aspergillus fumigatus*. The quality of the deinked paper is acceptable under paper recycling norms.

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