A Study on Bioremediation Potency of Aspergillus Niger in Treatment of Pulp and Paper Mill Effluent and Evaluation of Phytotoxicity Effect of Treated Effluent on Vigna Radiata

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Abstract

Pulp and paper mill is considered as core sector industry and fifth largest contributor to industrial water pollution. The waste water generated is having intense color, high levels of BOD, COD, turbidity and phenols. Many physical, chemical and biological treatment technologies for effluent treatment are already in practice, among which biological method of effluent degradation is found to be efficient and cost effective involving the natural processes resulting in the efficient conversion of hazardous compounds into simpler ones. In the present investigation, Aspergillus niger is used as a test strain for pollution remediation. From the results it is observed that the organism proved its efficiency by reducing the COD, BOD and Lignin concentration of effluent.

Keywords: *COD, BOD, Lignin, Turbidity, Metabolism, Hazard*

I. INTRODUCTION

Industrial water pollution is a major issue being faced by the developing countries [7]. Rapid increase in industrialization leads to environmental degradation and the waste generated will contribute to pollution. Pulp and paper industry is one of the important industrial sector in India which ranks 15th among the paper producing countries of the world [3]. It utilizes huge quantity of water for industrial operations and waste water discharge is also voluminous and it is estimated that water required to produce 1 ton of paper is about 273-450 m^3 and waste water generated is about 60-300 m^3 [11]. Raw materials used in manufacturing process are rich in ligninocellulosic materials. The effluent is rich in biological oxygen demand (BOD), chemical oxygen demand (COD), toxic substances, recalcitrant organics, turbidity, high temperature and intense color [12]. Discharging untreated effluent to aquatic bodies will induce various effects on animals such as respiratory stress, liver damage, genotoxicity and induces health impacts like diarrohea, vomiting, headache, nausea, and eye irritation in children and workers exposed directly to paper mill waste water [4]. Furthermore, some compounds in the effluent are resistant to biodegradation and can bioaccumulate in the aquatic food chain.

Physical, chemical and biological treatment technologies were already in practice for effluent treatment. Physico-chemical method of effluent treatment is found to be quite expensive and not feasible. Biological treatment (secondary treatment) allows microorganisms to utilize the pollutants as nutrients and which lowers the pollutant concentration under aerobic or anaerobic condition [7].

In the present study fungal isolate *Aspergillus niger* was used to assess its bioremediation efficiency for treatment of pulp and paper mill effluent.

II. METHODOLOGY

A. Sample Collection

Samples were collected from the inlet of effluent treatment plant from a paper mill, in Karnataka, India, stored at 4^0 C and filtered through a 0.5 mm sieve to remove suspended particles.

B. Isolation of Fungal Strain and Screening

Fungus for bioremediation studies was isolated from the effluent by effluent enrichment technique. 1 mL effluent sample was inoculated into potato dextrose agar (PDA) media containing de-ionized water, potato extract, dextrose and agar. Media was kept undisturbed at room temperature for 48 hrs for spore formation [5]. Fungal colonies which appeared on the media were sub- cultured to obtain pure isolates.

C. Identification of Fungi

Fungal isolates were identified on the basis of morphology i.e hyphe, conidiophores, conida etc., Microscopic examination was done for isolates at 40X magnification. Isolated culture was identified as

Aspergillus niger by following the culture identification technique [2].

D. Experimental Setup

Aspergillus niger was tested for biodegradation ability under laboratory condition. For the treatment, effluent sample was diluted to 3 different concentration viz. 25%, 50% and 75%. The purpose of dilution is to study the degradation efficiency of the organism at different effluent concentrations. The organism was inoculated into each effluent concentration and treatment was conducted in laboratory for 7 days.

E. Analytical Methods for Physico-Chemical Characterization of Effluent

1) Biological Oxygen Demand (BOD)

BOD of effluent was determined by the standard dilution technique of APHA. The method consists of filling an air tight bottle with sample and incubating at 20^0 C for 5 days. The dissolved oxygen (DO) was measured before and after the incubation. The difference in DO was computed and BOD of effluent is calculated.

2) Chemical Oxygen Demand (COD)

COD of effluent was calculated using closed reflux unit by titrimetric method of APHA. The effluent is refluxed in strong acid (H_2So_4) solution with $K_2Cr_2O_7$, silver sulphate and mercuric sulphate. Oxygen consumed was measured by titrating the sample against ferrous ammonium sulphate (FAS) using ferroin indicator.

3) Lignin

Total lignin content of effluent was measured by following the method of Pearl and Benson. In this method, effluent sample (50 mL) was mixed with 1 ml of CH_3COOH (10%), 1 mL of $NaNo_2$ (10%) and pH was adjusted to 7. Then 2 mL of NH_4OH was added and absorbance was measured at 430 nm. The absorbance value was transformed into lignin content (ppm) using the following formula

$$\text{Lignin (ppm)} = \frac{\text{Absorbance}}{0.000247}$$

4) Phytotoxicity Studies

Phytotoxicity studies were conducted to check the toxic effects of treated effluent. Experiment was performed under room temperature using pre-sterilized 10 *Vigna radiata* seeds. For the experiment 10 mL of treated and untreated effluent samples were used per day. A control set was also maintained. Treatment was conducted for a week and results were noted in terms of seed germination index (GI), relative seed germination (RSG) and (RRE) relative root elongation [10].

$$\text{RSG}(\%) = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} \times 100$$

$$\text{RRE}(\%) = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \times 100$$

$$\text{GI}(\%) = \frac{(\%\text{Seed germination}) \times (\%\text{Root elongation})}{100}$$

III. RESULTS

A. Data Analysis

Results are expressed as mean ± SEM. The statistical analysis was carried out using one way ANOVA followed by *Tukey's* t-test. The difference in values at $p<0.05$ or $p<0.01$ were considered as statistically significant. Statistical analysis was performed using ez ANOVA 0.98 version.

B. COD

From the results it is observed that *Aspergillus niger* has shown COD reduction in all treatment systems and are tabulated in Table 1. COD reduction in 25% concentration was 184.20±0.35 to 88.20±0.35, in 50% concentration 368.30±0.36 to 179.37±0.47, in 75% concentration 547.90±0.26 to 279±0.20 and in raw effluent 742.40±0.53 to 347.17±0.29. Percentage of COD reduction in raw effluent was about 53.23. A study by Luis *et al.*, (2014) has reported 89% COD reduction.

Table 1: Reduction in COD Concentration After Treatment with A. Niger

Concentration	Before treatment	After treatment
Raw	742.40±0.53	347.17±0.29
75%	547.90±0.26	279±0.20
50%	368.30±0.36	179.37±0.47
25%	184.20±0.35	88.20±0.35

C. BOD

Results revealed reduction of BOD by *Aspergillus niger* during the treatment. In 25% concentration it was reduced from 145.20±0.62 to 60.20±0.53, in 50% concentration 304.10±0.56 to 81.87±0.85, in 75% concentration 495.83±0.45 to 94.07±0.31 and in raw effluent 629.40± 0.53 to 104.33±0.58. Percentage of BOD degradation in raw effluent is about 83.42 and Luis *et al.*, (2014) achieved 95% in their study.

Table 2: Reduction in BOD Concentration After Treatment with A. niger

Concentration	Before treatment	After treatment
Raw	629.40±0.53	104.33±0.58
75%	495.83±0.45	94.07±0.31
50%	304.10±0.56	81.87±0.85
25%	145.20±0.62	60.20±0.53

D. Lignin

Aspergillus niger has potency in lignin degradation. Degradation of lignin in 25% concentration was 1008.03±0.05 to 578.50±0.50, in 50% concentration 1587.02±0.02 to 788.67±0.58, in 75% concentration 1603.15±0.13 to 1141.50±0.50 and in raw effluent 1999.33±1.15 to 1347.50±0.50. Percentage of lignin degradation by A. niger was 32.60.

Table 3: Delignification of Effluent using A. niger

Concentration	Before treatment	After treatment
Raw	1999.33±1.15	1347.50±0.50
75%	1603.15±0.13	1141.50±0.50
50%	1587.02±0.02	788.67±0.58
25%	1008.03±0.05	578.50±0.50

E. Phytotoxicity Studies

The phytotoxicity test was conducted to analyze the toxic effect of effluent after treatment with *A.* niger using Vigna radiata. Seed germination test on different parameters like the percentage of seed germination, root and shoot length and GI index is presented in Table 4. It was observed that seeds grown in control and treated sample shows high germination percentage and high root and shoot growth. Seeds grown with untreated effluent shows reduced seed germination and low shoot and root growth. GI index was found to be low in untreated effluent. The effluent treated with A. niger shows high GI (83.07%). The samples having GI index (50-90%) indicates low phytotoxicity. Barapatre, A. and Jha, H. (2016) have made a similar observation in their studies. This indicates that the biological method of treatment using microorganisms is effective in reducing the pollution load of the effluent thereby facilitating the growth and germination of certain edible plants.

IV. CONCLUSION

Results reveal that Aspergillus niger has potency to biodegrade the pulp and paper mill effluent. From the phytotoxicity studies it was concluded that the effluent has low phytotoxicity and suitable for plant growth. Hence, this fungal isolate can be recommended for bioremediation of paper mill effluent.

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Table: 4 Effect of Effluent Treated with A. Niger on The Germination and Growth of Vigna Radiata.

Sample	Shoot Length (cm)	Root Length (cm)	Germination %	GI Index %
Control	21.5	3.9	100%	100%
Raw	15.7	3.2	90%	73.84%
Treated	17.2	3.6	90%	83%

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