International Conference on Advancements in Engineering, Technology and Sciences
Dhaanish Ahmed College of Engineering, Chennai (ICAETS-2018)
(Approved by AICTE, New Delhi and Affiliated to Anna University, Chennai)
16th-17th March 2018 www.conferenceworld.in ISBN : 978-93-87793-11-8

A comparative assessment on efficiency of Aspergillus niger and Aspergillus flavus in treatment of pulp and paper mill effluent

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ABSTRACT

Pulp and paper mill is considered as an important industrial sector and fifth largest contributor to industrial water pollution. The waste water generated is having high levels of color, high levels of BOD, COD, turbidity, suspended solids, absorbable organic halides (AOX) and phenolic components (Selvam et al., 2011). Many treatment technologies 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. This technique requires suitable microbial strains which can undergo various physico- chemical reactions in the polluted water and during the metabolism the pollutants are degraded and removed. Research using different bacterial and fungal strains for bioremediation studies of paper mill effluent has been reported. In the present investigation, an attempt has been made to study the effluent treatment efficiency of Aspergillus niger and Aspergillus flavus. Results reveal that organisms have proved their bioremediation potency in treatment of pulp and paper mill effluent.

Key words: Bioremediation, Degradation, Strains, Metabolism, Phenols, Turbidity

I.INTRODUCTION

Waste water generated from industries is one of the main reasons for water pollution. Pulp and paper mill is considered as core sector and fifth largest contributor for industrial water pollution. The problems associated with pulp and paper mill effluents are pH, colour, high levels of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids (SS), Adsorbable Organic Halides (AOX) etc., [1]. The generated effluent is highly toxic and cause severe hazard to aquatic organisms. Furthermore, some compounds in the effluents are resistant to biodegradation and can bioaccumulate in the aquatic food chain, [2].

Treatment and disposal of paper mill effluent is found to be critical, so it is needed to develop cost effective technologies for effluent treatment. Many physical and chemical treatment technologies are already in practice but they have several drawbacks. A search is on to determine a method which is feasible, effective and ecofriendly. Hence, bioremediation method is being tried to treat the effluent.

Many microbial strains were already tested for their bioremediation potential. Mycoremediation is an economically sound alternative to extracting, transporting and storing toxic waste and reported in many research

works: wood rot fungi [3], *Fomes lividus* and *Trametes versicolor* [3], *Phanerochaete chrysosporium* and *Aspergillus fumigatus* [4], and *Phanerochaete chrysosporium* [5].

In the present study, a comparative assessment is being done to study the biodegradation potential of *Aspergillus niger* and *Aspergillus flavus* in treatment of bleach plant effluent from a large paper mill, in particular, its potential in reducing BOD and COD.

II. MATERIALS AND METHODS

2.1 Fungal isolation and screening

Fungal strains utilized for the study were isolated from the effluent by effluent enrichment technique. 1 mL effluent sample was inoculated into 25 mL 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. Fungal colonies which appeared on the media were sub- cultured to obtain pure isolates.

2.2 Identification of fungus

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* and *Aspergillus flavus* by following the culture identification technique [6].

2.3 Source of effluent

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 0.5mm sieve to remove suspended particles. The manufacturing unit generates enormous quantity of waste water. The most significant sources of pollution among various processing stages are wood preparation, pulping, pulp washing, screening, washing and bleaching [7]. For all these processes high amount of alkali and chlorine compounds are used. Hence the waste water obtained in these processes was dark in color with charring wood and chemical odour [8].

2.4 Experimental setup

The individual fungal isolates were 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 *Aspergillus niger* and *Aspergillus flavus* at different effluent concentration. The organisms were inoculated into each concentration and treatment was conducted in laboratory for 7 days.

2.5 Analytical methods for physico-chemical characterization of effluent

2.5.1 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.

2.5.2 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.

III. RESULTS AND DISCUSSION

3.1 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.

Table 1: Reduction in COD concentration after treatment with *Aspergillus niger*

Effluent concentration	COD (mg/L) removal by *Aspergillus niger*	COD (mg/L) removal by *Aspergillus niger*
	Before treatment	**After treatment**
Raw	742.4	347.17±0.29**
75%	547.9	238±0.20**
50%	368.3	147.6±0.47**
25%	184.2	71.3±0.35**

*Values are expressed as mean ± SEM (n=3), *p<0.05; **p<0.01, denotes significance with respect to initial values using one way ANOVA followed by Tukey's test.*

Table 2: Reduction in BOD concentration after treatment with *Aspergillus niger*

Effluent concentration	BOD (mg/L) removal by *Aspergillus niger*	BOD (mg/L) removal by *Aspergillus niger*
	Before treatment	**After treatment**
Raw	629.4	104.33±0.58**
75%	495.83	70.6±0.31**
50%	304.1	38.7±0.85**
25%	145.2	16.4±0.53**

*Values are expressed as mean ± SEM (n=3), *p<0.05; **p<0.01, denotes significance with respect to initial values using one way ANOVA followed by Tukey's test.*

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 184.2 mg/L to 71.3±0.35. BOD reduction by *Aspergillus niger* was appreciable during the treatment and the results are tabulated in Table 2. Maximum BOD degradation rate was observed in lower effluent concentration. In 25% concentration it was reduced from 145.2 mg/L to 16.4±0.53.

Table 3: Reduction in COD concentration after treatment with *Aspergillus flavus*

Effluent concentration	COD (mg/L) removal by *Aspergillus flavus*	COD (mg/L) removal by *Aspergillus flavus*
	Before treatment	**After treatment**
Raw	569.2	202.53±0.35**
75%	436.4	142.6±0.58**
50%	289.2	79.8±0.25**
25%	149.7	39.6±0.25**

*Values are expressed as mean ± SEM (n=3), *p<0.05; **p<0.01, denotes significance with respect to initial values using one way ANOVA followed by Tukey's test.*

Table 4: Reduction in BOD concentration after treatment with *Aspergillus flavus*

Effluent concentration	BOD (mg/L) removal by *Aspergillus flavus*	BOD (mg/L) removal by *Aspergillus flavus*
	Before treatment	**After treatment**
Raw	450.8	69.17±0.12**
75%	344.2	45.47±0.12**
50%	229.4	26.43±0.06 **
25%	118.3	11.10±0.10**

*Values are expressed as mean ± SEM (n=3), *p<0.05; **p<0.01, denotes significance with respect to initial values using one way ANOVA followed by Tukey's test.*

Aspergillus flavus has shown better performance in COD (mg/L) reduction in all the concentrations. However maximum reduction was seen in 25% concentration where it decreased from 149.7 mg/L to 39.6±0.25. BOD (mg/L) removal in all the concentrations was appreciable. In 25% concentration it has shown maximum degradation efficiency from 118.3 mg/L to 11.10±0.10.

These changes most probably occurred due to transformation of particulate chromophores and other colloidal substances in the effluent by the extracellular ligninolytic enzymes secreted by the fungal consortium, Malavia and Rathore (2007). Variations in the pH during treatment could have also contributed to these changes.

IV.CONCLUSION

Results reveal that *Aspergillus niger* and *Aspergillus flavus* have the potential to bioremediate paper mill effluent. Comparatively, *Aspergillus flavus* has shown maximum degradation potential with respect to COD and BOD. Hence, these isolated fungal strains can be recommended for bioremediation of paper mill effluent.

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