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Paper Mill Effluent Degrading *Streptomyces* sp. S1 from Marine Sediments

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ABSTRACT

Paper mills are foremost sources of industrial pollution worldwide. Paper industries generate great quantities of waste water, waste gases and solid waste. There has been considerable problem to the environment. In this study, evaluate the effluent odor (smell) removal and inorganic chemical degradation using marine Streptomyces. Totally 55 different kinds of marine Streptomyces isolates were obtained from mangrove soil. The selective potent Streptomyces sp. S1 showed better reaction for effluent degradation. The degradation capacities of Streptomyces sp. S1 were estimated on both sterilized and unsterilized samples under different time intervals (30 and 90 days). Streptomyces sp. S1 was provided good result for inorganic chemical degradation. The degradation of effluent odor both sterilized and an unsterilized sample was exhibited greater result. The results were studied at the combined effect of Streptomyces beside with natural microbial population enclosing unsterilized effluent and Streptomyces alone sterilized system were exhibited similar mode of degradation activity. Thus, there is great possibility for marine Streptomyces research in future is suggested based upon the deliberation of above issues.

Keywords: Effluent degradation, Odor removal, *Streptomyces*

INTRODUCTION

Paper industry is one of the major water and energy consuming industry on global level. Paper industries generate great quantities of waste water, waste gases and solid waste [1]. This industry uses the 5th largest energy consumer processes; approximately 4% of total energy is used worldwide. Most of the paper-producing units in India are integrated pulp and paper mills. Paper mills are believed to consume 230-500 m^3 of water per ton of paper production and contribute to one of the major source of waste water generation [2]. The production of high amount of effluent mixes into water leading to pollution of aquatic system and represent major environmental problems [3]. Some inorganic and organic compounds typically enter in to the environment through incidental or accidental releases during their use (or) disposal. They can also be released to the environment through improper waste management's and unpermitted discharge. Paper mills based on nonconventional agro residues are being fortified due to increased demand of paper and acute shortage of forest-based raw materials. Small-scale pulp and paper mills are generally situated in rural areas due to availability of raw materials (wheat straw, rice straw and baggase). The agro-based pulp and paper mills are highly water intensive, consuming tons of fresh water.

The paper manufacturing process involves three steps, pulping, bleaching, and finally paper making. Pulping can be done *via* chemical (Kraft pulping) or mechanical methods. Chemical pulping is the most commonly employed pulping technique in agro-based pulping mills. The effluent emanating from the pulping process is called black liquor and it contains inorganic chemicals, chlorophenolic compounds, and fiber residues which have characteristically extreme quantities of organic chemicals and high level of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids and organic compounds [4]. The discharge of black liquor from 30 ton per day agro-based pulp and paper mills without chemical recovery yields pollution load equivalent to a 100 tpd mill with chemical recovery. The harmful environmental effects of effluents and the stricter environmental norms compel the mills to reduce color, toxic compounds and other pollutants to safe disposal levels prior to effluent discharge. Generally 20,000 liter of spent wash is generated every day in each industry [5].

The agro based pulp and paper mills are highly water intensive, consuming 100-250 m^3 fresh water/ton paper produced [6]. These units correspondingly generate large quantities of waste water, approximately 150-200 m^3 effluent/ton of paper produced. The environmental impact of waste water emanated from small scale pulp and paper mills is therefore of particular concern [7]. Chemical pulping is the most commonly employed pulping technique in agro based pulping mills [8]. Recently *Streptomyces fulvissimus* CKS7 seem to be a promising application for contaminated wastewater [9].

Industries that were formally inaccessible are now closer to homes, parks and public areas. Odor has been proven to increase heart rate, respiration and blood pressure. Long term exposure to odor can cause atrophy and may cause the loss of anes ability to smell. As a result, odor nuisance complaints from local citizens and environmental groups have increased. Hence presently odor removal from effluent by a biological means is being explored.

Streptomyces dominate the microbial life in soil. They play a major role in decaying dead organic matter. Many of them have turned out to be the source of valuable antibiotics. Though, not much work has been undertaken towards paper mill effluent degradation by actinomycetes. In the present study, to determine the degradation capacity of *Streptomyces* capable of growing in waste water and their roles in both alone and in combination with natural populations of microbes on paper mill effluent.

MATERIALS AND METHODS'

Isolation and identification of *Streptomyces*

Soil samples were collected from Muthupet mangrove forest, located in south east coast of India. Soil sample were collected at a depth within 10 -15 cm from the surface of the soil. The texture of the samples was sandy with brown to blackish in color. The soil samples were packed in sterile polypropylene bags brought to the laboratory. Then, the collected samples were serially diluted using 50% sterile aged sea water. From 10 fold serial dilutions of the samples were prepared; As of the required dilutions 0.1 ml of suspension was spread over the surface of starch casein agar medium and incubating at 28°C for 2-3 weeks [10]. After incubation, the colonies of *Streptomyces* were recognized by their characteristic on the plates as colored, dried, rough, with regular or irregular margin was observed. The isolated *Streptomyces* were sub-cultured in SCA slants and then preserved using 20% glycerol stored at -80°C.

The microscopic characterization was done by cover slip culture method. The mycelium structure, color and arrangement of conidiophore and arthrospores on the mycelium were observed through the oil immersion (100X). The observed structure was compared with Bergey's manual of determinative bacteriology, and the organism was identified [11]. Various biochemical tests were performed for the identification of *Streptomyces* [12].

Effluent degradation

Paper mill effluent sample was collected from paper mill industry in India. The effluent sample was filtered through cotton to remove suspended solids and used as such without dilution. The treatment process was conceded with *Streptomyces* in two different ways. The *Streptomyces* sp. S1, were inoculated with unsterilized effluent sample (to study the role of *Streptomyces* along with natural population of microbes) and another one method the same *Streptomyces* culture was inoculated with sterilized effluent sample (to study the role of *Streptomyces* alone).

Experiments were conducted under controlled conditions with Erlenmeyer flask. 5 ml of uniform suspension of *Streptomyces* sp. S1 was separately inoculated in to both sterilized and unsterilized effluent sample. The inoculated *Streptomyces* containing effluent were incubated at 37°C for different incubation to obtain the effect of degradation. This experiments were estimated the degradation capacity of *Streptomyces* on 30, 60 and 90th day. Study the influence of *Streptomyces* were grown in the paper mill effluent in both (Sterilized and unsterilized) conditions and for the control. BOD, COD and DO procedure was carried out using standard method [13]. Inorganic chemical degradation procedure was followed according to the standard method [14].

RESULTS AND DISCUSSION

Streptomyces isolates were obtained from mangrove soil. Totally 55 different kinds of marine *Streptomyces* isolates were obtained from mangrove soil. 10 morphologically distinct cultures were used for primary effluent degradation process; from that potent *Streptomyces* sp. S1 exhibited better response for effluent degradation process. *Streptomyces* sp. S1 morphological, physiological and biochemical characteristics is shown in (Table 1). The present study determined the degradation capacity of *Streptomyces* sp. S1 on both sterilized and unsterilized paper mill effluent sample.

Table 1: Morphological, physiological and biochemical characteristics of effluent degrading *Streptomyces* sp. S1

Morphological/physiological characteristics	*Streptomyces* sp. S1	Bio chemical characteristics	*Streptomyces* sp. S1
Appearance	Sandal with white colonies	Gram staining	+
Aerial mycelium	Light white	Endospore staining	-
Substrate mycelium	Whitish grey	Glucose fermentation	-
Spore surface	Smooth	Sucrose fermentation	-
Size	5 mm	Starch hydrolysis	+
Texture	Powdery	Casein hydrolysis	+
Odor	Geosmin	Gelatin activity	+
Oxygen relationship	Aerobic	Citrate	+
Light microscope	Filamentous	Catalase	+
pH	7.2	Urease	+
Temperature	28°C	Nitrate reduction	+

−: Negative; +: Positive

The degradation analysis such as BOD, COD and DO reduction was conducted only with fresh effluent (unsterilized effluent) sample. BOD reduction was moderately observed on *Streptomyces* sp. S1 within 30 days and the reduction was around 40 percent beyond which there was only a slight variation for further reduction (60 and 90 days) (Figure 1a). COD reduction was observed within 90 days the reduction was around 60 percent (Figure 1b). DO reduction was exhibited very low range to degrade the Dissolved oxygen (Figure 1c). The correlation between the initial up to final decrease in BOD, COD and DO was observed in the study have the similar assessment by [15]. Efficiency of some actinomycetes isolates in biological treatment and removal of heavy metals from waste water was investigated [16]. Treatment of the paper factory effluent was done with free cells could bring a maximum 40% reduction in COD after 32 h treatment was investigated [17].

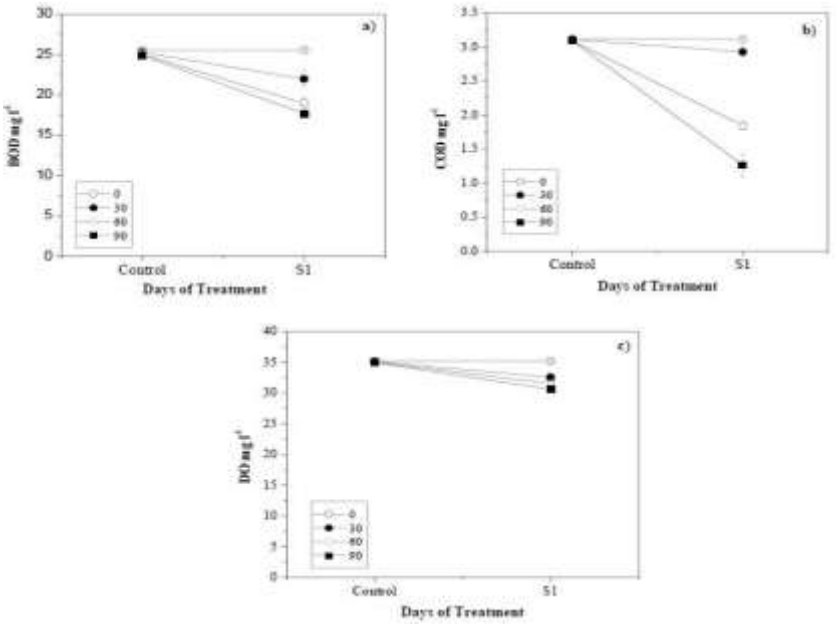

Figure 1: Treatment of paper mill effluent by *Streptomyces* sp. S1. The effluent treatment process was performed at 0, 30, 60 and 90 days. The BOD, COD and DO values are expressed mg/l^{-1}. All experiments were performed triplicates and the mean values along with standard error mean (n=3) are presented

In organic chemical degradation, the removal of calcium was well established in both sterilized and unsterilized effluent with *Streptomyces*. Calcium was removed gradually and steadily up to 90 days, beyond which it became almost stabilized in all treatments (Figure 2a). Magnesium reduction was able to degrade at higher level in sterilized effluent than compared to unsterilized effluent samples (Figure 2b). Similar mode of chloride reduction was observed in sterilized and unsterilized effluent (Figure 2c). The microbial conversion of chlorinated bleach plant effluents by actinomycetes was studied [18]. Reduction of ammonia was observed good effect in unsterilized effluent containing *Streptomyces* but the reduction was moderately observed in sterilized effluent (Figure 2d). Removal of phosphorous level was highly indicated by *Streptomyces* sp. S1 in unsterilized effluent than for sterilized sample (Figure 2e).

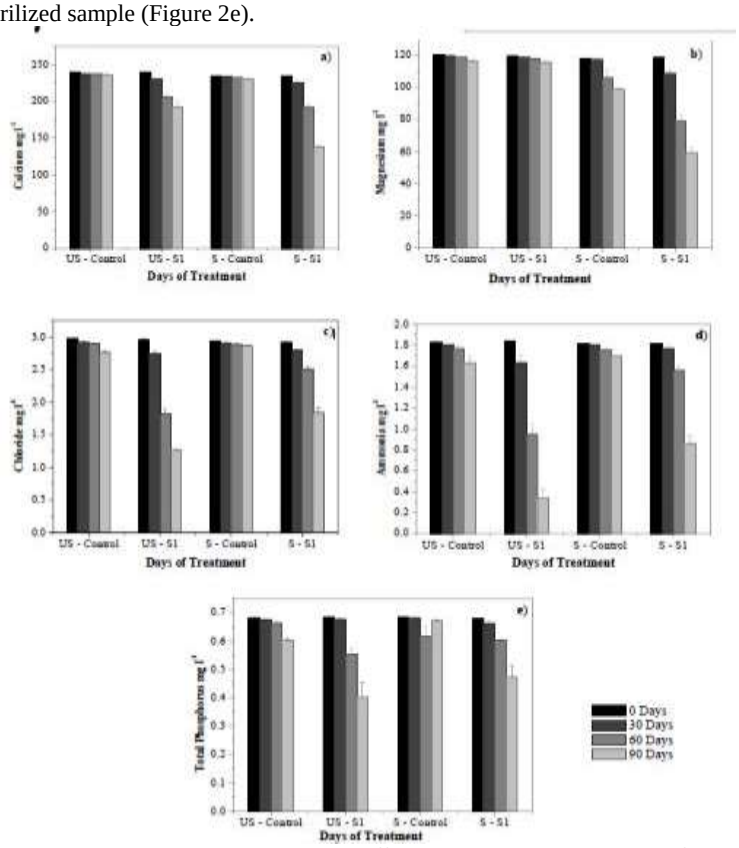

Figure 2: Inorganic chemical degradation by *Streptomyces* sp. S1. The effluent treatment process was performed at 0, 30, 60 and 90 days. The degradation of calcium, magnesium, chloride, ammonia and total phosphorous values are expressed as mg/l^{-1}. All experiments were performed triplicates and the mean values along with standard error mean (n=3) are presented

US-Control: Unsterilized control; US-S1-Unsterilized *Streptomyces* sp. S1

S-Control: Sterilized control; S-S1: Sterilized *Streptomyces* sp. S1

The overall experimental results showed predictable that the removal of all inorganic pollutants from effluent as well as odor removal by treatment of *Streptomyces* sp. S1. Reported microbial community processes for treating the same sewage [19]. Studied the degradation of textile dye reactive navy-blue RX (Reactive blue-59) by an isolated actinomycetes [20,21]. Bio degradation of azo dye reactive yellow was carried out enrichment method using *Streptomyces* sp [22]. *Gordonia terrae* strain was selected from activated sludge for its capacity to grow and degrade Ethyl t-butyl Ether (ETBE), reports on *Gordonia* species play an important role during waste water treatment was studied [23,24]. Biodegradation of effluent by microbial isolates, DSI1-3 [25]. The biodegradable potential of the *S. microflavus* CKS6 growing microbial cells for the colored waste water treatment. Hence, this study describe similar mode of degradation was observed both sterilized and unsterilized system.

CONCLUSION

The marine *Streptomyces* can be suited for biological treatment of effluent degradation. Investigations of effluent degradation in *Streptomyces* are very few reports than compare to other microbes such as bacteria and fungi. *Streptomyces* species itself is effluent (inorganic chemical) degrading capacity and clearly indicates the possibility of effective removal of pollutants from effluent combined with *actinomycetes* treatment. This study concludes that *Streptomyces* is new proficient than compare other microbes for effluent odor removal. Hence, the need of research is crucial for bio degradation of effluent using *Streptomyces.*

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