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Bioremediation of pulp and paper mill effluent using isolated *Bacillus* strain and its impact on the pH of effluent

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ABSTRACT : This study focussed on the effect exerted by *Bacillus* strain on the pH of the pulp and paper mill effluent. The study aimed at bioremediation of paper mill effluent using *Bacillus stratosphericus*, the result of the experiment indicated that after 48 hours of treatment a reduction in colour (47%), COD (73%) and lignin (33%) was observed suggesting its potential tool for treatment of wastewater. In addition, the pH of the effluent was varied from 4-9 at regular interval of 1 unit and was inoculated with bacterial strain and was kept at 35°C at 200 RPM for 48 hours. The study indicated that *Bacillus* possessed inherent capacity to adjust pH favourable for its growth ranging from pH 7.4-8.7.

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Key Words :
Bioremediation, Colour, Lignin, COD

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In India, there are about 813 paper mills, which is amongst water intensive industry (Jain *et al.,* 2015). In India typically 75 per cent freshwater supplied to paper industry is released as wastewaters. With the increasing demand of paper, the production has been up scaled leading to large amount of wastewater generation. Physico-chemical methods for the treatment of wastewaters are costly and energy extensive and generate toxic by-products (Kumar *et al.*, 2014). Bioremediation, has garnered lot of attention as it is cost effective, environmental friendly and easily adaptable for the already existing industries without much of infrastructural changes or construction. Most of the study on degradation of wastewater pollutants focuses on fungi, however, industrial application of fungi has its disadvantages and lack ability to grow at high pH, oxygen limitations and high lignin concentrations (Hooda *et al*., 2015). For this reason, studies on bacterial degradation of wastewater pollutants have garnered the attention of researchers. Recently various species of bacteria have been explored for their ability to degrade pollutants from pulp and paper mill effluent. The objective of this research is not only to find a bacterial strain isolated from pulp and paper mill sludge capable degrading pollutants *i.e.* colour, lignin and COD but to also establish the relationship between the isolated strain and its effect on the pH of the effluent sample.

EXPERIMENTAL METHODOLOGY

Isolation, identification and maintenance of bacterial strain :

The bacterial strain was isolated from sludge sample collected from a wood-based paper mill and stored at 4°C till further use. The sample was enriched and then was serially diluted to plate on MSM media (in g/

lit K_2HPO_4 0.5g, KH_2PO_4 0.5g, $MgSO_4.7H_2O$ 0.5g, Agar 20g and Lignin 0.1% - 1%). The isolated strain was screened on MSM medium consisting of Remadazol Brilliant Blue-R. The strain was identified using 16S rDNA studies at Xcelris Labs Ltd. Ahmedabad, India. Isolated bacterial strain, an indigenous isolate was maintain on nutrient agar media (in g/lit Beef extract 3g, Peptone 5g, NaCl 5g and Agar 15g) at 4°C by transferring into fresh media after every 4-5 days. The bacteria for study were up scaled in a nutrient broth (in g/lit Beef 3g, Peptone 5g, Sodium chloride 5g), the samples were centrifuged and bacterial pellet was obtained (Kumar *et al.,* 2012).

Bioremediation of paper mill effluent :

The bacteria for study were up scaled in a nutrient broth (in g/lit Beef 3g, Peptone 5g, Sodium chloride 5g) until O.D. 1, the samples were centrifuged and bacterial pellet was obtained. The effluent samples were treated for bioremediation, where the effluent sample was taken in a flask and inoculated with bacterial pellet. The samples were kept in a shaking incubator at 35°C, 200 RPM and pH 8. After 48 hours, the flasks were allowed to settle at room temperature, the clear supernatant was analyzed for various pollution parameters. In another experiment, the pH of the wastewater was manually set from pH 4-9 and was further inoculated with bacterial pellet and kept for treatment for 48 hours at 35°C and 200 RPM. The samples were allowed to settle at room temperature for 1-2 hour final pH was recorded (Singh *et al.,* 2011 and Dhall *et al.,* 2012).

Analysis of effluent sample:

The analysis for COD was done using standard open reflux method, APHA, 5220B; Lignin using UV method, CPPRI and colour using standard ASTM method.

EXPERIMENTAL FINDINGS AND DISCUSSION

It was hypothesized that the bacteria isolated from their native habitat have an inherent ability to utilize pollutants and incorporate it into its metabolic machinery (Dhall *et al.,* 2012). The bacterium was identified as *Bacillus stratosphericus,* which was tested for its ability to degrade pollutants from the pulp and paper mill effluent. The results of the experiment showed that, after 48 hours of incubation, the isolate exhibited 73 per cent reduction in the COD of the effluent sample. There was also a considerable amount of reduction in color (47%) and Lignin (33%) in the effluent sample (Fig.1). A consortia of *Klebsiella* sp., *Alcaligens* sp. and *Cronobacter* sps. was utilized for the treatment of paper mill effluent and 72.3 per cent reduction in COD, 55 per cent reduction in colour was observed (Kumar *et al.,* 2014).

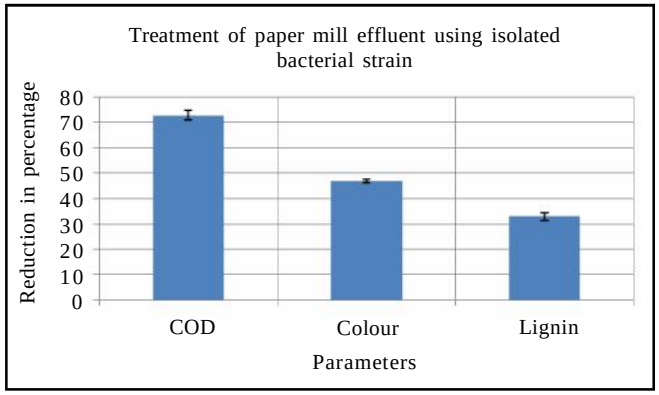

Fig. 1 : Reduction in COD, colour and lignin after bacterial treatment

The identified strain was further tested for its ability to affect the pH of the paper mill effluent. The results of the experiment indicated that after 48 hours of treatment the pH of the sample was in the range of 7.4-8.7, indicating the nature of bacterial strain to alter the environment favourable to its growth (Fig. 2). This shift in pH could be due to the denitrifying nature of the bacteria, it increases the pH of a biological treatment unit through the release of hydroxyl ions (OH^-) (Gerardi, 2006). *Bacillus* is considered to the prominent denitrifiers in various environments, thus, this supports the nature of the isolated microbial strains where they alter pH of the

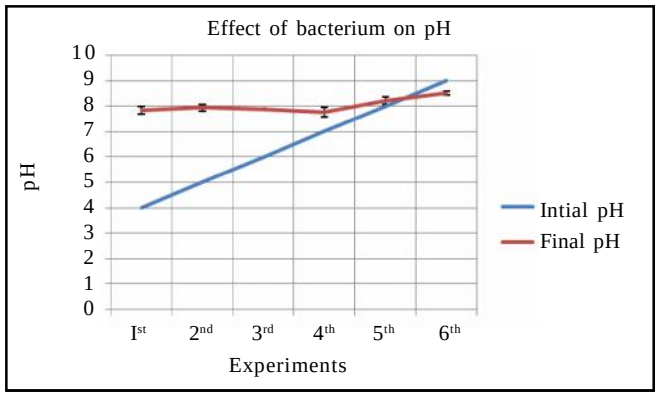

Fig. 2 : Effect of *Bacillus stratosphericus* on pH of the effluent sample

effluent on treatment (Fuka *et al*., 2007).

Conclusion :

Micro-organisms able to grow in complex paper mill effluent may be effectively utilized as a potential tool for bioremediation. The isolated microbial strain *Bacillus stratosphericus* was able to degrade the easily assimilable organic compounds and reduce the pollution load from the effluent. A reduction of 73 per cent in COD was observed, with 47 per cent reduction in colour and 33 per cent reduction in the lignin content of the wood-based paper mill effluent. In addition, the study establishes the relationship between bacterium and pH, helping in proper utilization of this microbe for treatment of paper mill effluent.

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