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Evaluation of Paper Effluent with two Bacterial Strain and their Consortia

Priya Tomar
Ph.D Scholar from Shri Venkateshwara University, Gajraula, Amroha (Uttar Pradesh)

Pallavi Mittal
Sr. Lecturer, I.T.S Para Medical College, Muradnagar, Ghaziabad

Abstract:
As industrialization is inevitable and progress with rapid acceleration, the need for innovative ways to get rid of waste has increased. Recent advancement in bioresource technology paves novel ideas for recycling of factory waste that been polluting the agro industry, soil and water bodies. Paper industries in India are in a considerable number, where molasses and impure alcohol are still being used as raw materials for manufacturing of paper. Paper mills based on nonconventional agro residues are being encouraged due to increased demand of paper and acute shortage of forest-based raw materials. The colouring body present in the wastewater from pulp and paper mill is organic in nature and is comprised of wood extractives, tannin, resins, synthetic dyes, lignin and its degradation products formed by the action of chlorine on lignin which imparts an offensive colour to the water. These mills use different chemical process for paper manufacturing due to which lignified chemicals are released into the environment. Therefore, the chemical oxygen demand (COD) of the emanating stream is quite high. This paper presents some new techniques that were developed for the efficiency of bioremediation on paper industry. A short introduction to paper industry and a variety of presently available methods of bioremediation on paper industry and different strategies are also discussed here. For solving the above problem, two bacterial strains (Pseudomonas aeruginosa and Bacillus subtilis) and their consortia
(Pseudomonas aeruginosa and Bacillus subtilis) were utilized for the pulp and paper mill effluent. Pseudomonas aeruginosa and Bacillus subtilis named as T – 1, T – 2, T – 3, T – 4, T – 5, T – 6, for the decolourisation of paper industry effluent. The results indicated that a maximum colour reduction is (60.5%) achieved by Pseudomonas aeruginosa and COD reduction is (88.8%) achieved by Bacillus subtilis, maximum pH changes is (4.23) achieved by Pseudomonas aeruginosa, TSS reduction is (2.09 %) achieved by Bacillus subtilis, and TDS reduction is (0.95 %) achieved by Bacillus subtilis, When the wastewater was supplemented with carbon(glucose) and nitrogen(yeast extract) source and data revealed that efficiency of Bacillus subtilis, having more with glucose than the Pseudomonas aeruginosa.

Key Words - Key words 1; Bioremediation, Key words 2; Paper and Pulp Mill Effluent, Key words 3; Treated Effluent.

Introduction

Industrial effluents represent a significant environmental and economic problem. The pulp and paper industry typically generates large quantities of wastewater whose correct treatment prior to discharge into the environment is critical. These industries disturbing the ecological balance of the environment by discharging a wide variety of waste water. Although, paper factory effluent is one of the major pollutants on the earth. These effluent has recognized as environmental hazards and categorized one of the twelve most polluting industry in our country. World demand for paper has grown rapidly and was around 5-6% per year. The paper mills have a larger investment and provide employment to 2 lakh people. It is estimated that the capacity of the mills increases from 8.3 million tonnes in 2010 to 14 million tonnes in 2020. In India the total production 70% is from hardwood and bamboo fiber, agro-waste and other 30% is from recycled material. For paper, paperboard and newsprint production, 550 mills in India use wastepaper

(Kesalkar et al, 2012). Furthermore, the pulp and paper mill effluent is highly coloured. The chemical composition of such effluents depends on the nature of the feedstocks, as well as the treatment procedure. The paper mill wastewater characteristically contains colour, very high level of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD),due to presence of lignin and its derivatives from the raw cellulosic materials , chlorinated compounds, suspended solids (mainly fibers), fatty acids, tannins, resin acids, sulphur and sulphur compounds, etc. Most of these industries discharged their insufficiently treated waste into the rivers or streams, which makes serious problem to aquatic life and flora-fauna. Thus, it is necessary to develop an economical solution on the effluent discharged. The complex nature of such lignin compounds and their phenolic content make them extremely resistant to biological degradation. The physical and chemical treatment methods including ultra filtration, ion-exchange, and lime precipitation, are expensive and are also less efficient. Therefore, alternate low-cost biological treatment processes are now being considered as viable options. There have been several attempts to use biological methods to decontaminate effluent from paper mills. Several microorganisms (mainly Bacillus subtilis and Pseudomonas aeruginosa) have been used to decolorize effluents from different sources because of their ability to degrade lignin. The brown color of the effluent may increase water temperature and decrease photosynthesis, both of which may lead to decreased concentration of dissolved oxygen. These microorganisms are able to degrade cellulose and hemicelluloses as they oxidize and solubilize the lignin component. Physical and chemical methods undertaken to study colour removal from the effluent is not found to be cost-effective technology. Hence, biological treatment has been applied for the decolourization of effluent of pulp and paper mills. An important strategy for effluent treatment is the isolation and characterization of generally significant microorganisms together with designing and optimization of process parameter to deal with specific environment pollutants. Several strains have been proven to modify effluents which are produced during the chemical bleaching of pulps. Although, there is considerable potential for treating these effluents by biological methods. Therefore, present investigation is undertaken initially to collect bacterial strains for optimization of decolourization pulp and paper mill effluent in a laboratory which was further tested at large scale for removal of major contaminants from the effluent. In the present study, an attempt was made to find out efficiency of some selected bacteria and their consortia for decolourization of paper industry effluents.

Materials and Methods

Collection of Samples: The samples for the analysis were collected from the Shamli paper industry and the wastewater was collected from the inlet of the effluent treatment plant of the paper mill for the experiment. After collection, Samples were stored at 4°c till further use and Sampling was done over a period of 0, 3, 6, 9 and 12 days with in three replicates.

Preparation ff Media: For Bacillus subtilis, prepare the media according the composition as follows: Beef extract (1.0 g), yeast extract (2.0 g), peptone (5.0 g), NaCl (5.0 g) and distilled water (1.0 L) by sterilized it in an autoclave. After sterilization pouring into Petri plates and after solidification of the media inoculates the strain of Bacillus subtilis and then placed in incubator at 30°c. For Pseudomonas aeruginosa, prepare the NAM media by sterilized it in an autoclave. And after sterilization pouring into Petri plates and after solidification of the media inoculates the strain of Pseudomonas aeruginosa and then placed in incubator at 30°c.

Bacterial strain: - During the present study we are using Bacterial strain that is Bacillus subtilis and Pseudomonas aeruginosa.

Treatment
T 1 - 0.6 gm yeast + Bacillus subtilis
T 2 - 1% glucose + Bacillus subtilis
T 3 - 0.6 gm yeast + Bacillus subtilis + beef extract + peptone + NaCl
T 4 - 0.6 gm yeast + Pseudomonas aeruginosa
T 5- 1% glucose +Pseudomonas aeruginosa
T 6- 1% glucose + Bacillus subtilis + Pseudomonas aeruginosa

Carbon and Nitrogen Sources: - To check whether COD reduction could be enhanced by addition of glucose as a carbon source and yeast as nitrogen source as nutrients, the experiment was performed with and without carbon and nitrogen source. The experiment was performed by adding carbon and nitrogen source in paper effluent and the results revealed that COD reduction could be achieved in a range of 62-63%. The supplementation of carbon and nitrogen source enhanced the reduction in COD.

Methods
COD: - COD was determined by using closed reflux method published by the APHA standard method.
Chemicals used:-
Standard potassium dichromate 0.25 N
Concentrated sulphuric acid
Ferroin indicator solution
Standard Ferrous ammonium sulphate 0.25 N
Calculation
COD (mg /l) :- (A –B) *8000 * C / ml of sample used
Where, A = ml. of FAS used for blank
B = ml. of FAS used for sample
C = Normality of FAS solution

Colour: - The decolourisation was observed at 0, 3, 6, 9 and 12 days. For the decolourisation the sample was scanned in a spectrophotometer to ascertain the specific wavelength where maximum absorbance occurs. A maximum absorbance at 475 nm was observed. The rate of decolourization was monitored at this wavelength for change of colour determination. The effluent was centrifuged at 10000 rpm for 5 min to remove all the suspended particulate material.
Calculation:-
% Reduction = (Initial OD – Final OD) *100 / Initial OD.

pH:- According to the decolourisation we did pH study of the sample and that pH is determined by pH meter.

TSS: - TSS of a water sample is determined by pouring a carefully measured volume of water through a pre-weighed filter of a specified pore size, then weighing the filter again after drying to remove all water. Filters for TSS measurements are typically composed of glass fibres. The gain in weight is a dry weight measure of the particulates present in the water sample.
Calculation:-
TSS (grams):- B – A *100 / A
Where, A = Weight of filter paper with Petri plate
B = Dry weight of residue with filter paper

Total Dissolved Solid (TDS) :- Total Dissolved Solids is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized or micro-granular (colloidal sol) suspended form. The principal application of TDS is in the study of water quality for streams, rivers and lakes, although TDS is not generally considered a primary pollutant it is used as an indication of aesthetic characteristics of drinking water and as an aggregate indicator of the presence of a broad array of chemical contaminants.

Calculation:-

TDS (grams):- B – A *100 / A

Where, A = Weight of filter paper with Petri plate

B = Dry weight of residue with filter paper

Results and Discussion

Paper industry effluents are highly water intensive units generating large volumes of high strength wastewater that poses a serious environmental concern. Hence, the present investigation was carried out with the objective of studying the decolourisation efficiency of bacterial strain Bacillus subtilis and Pseudomonas aeruginosa in the paper industry effluent.

Different combinations of bacteria along with different extra- metabolite carbon (glucose) and nitrogen (yeast extract) sources are designed for the analysis of paper industry effluent. The strains were applied for analysis of colour, pH, COD, TSS and TDS of pulp and paper mill effluent at 0, 3, 6, 9 and 12 days.

Six different treatments are designed for the analysis of the experiment. The strains were applied for analysis of decolourization of pulp and paper mill effluent after supplementation of carbon (glucose) and nitrogen (yeast extract) source. It was observed that bacterial strain Bacillus subtilis showed maximum potential to remove colour and showed maximum potential to degrade organic matter except COD. In organic matter (COD) case Pseudomonas aeruginosa having more efficiency to reduce it.

Colour Reduction

Effect of Bacillus subtilis on colour reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in colour with bacterial strain (Bacillus subtilis) in the presence of carbon and nitrogen source (Table 1) was 31.2 % and 34.45% respectively, in comparison to control. These results indicated that reduction was not appreciable in the presence of carbon source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of colour we noticed the it would enhanced more in the colour reduction as compare to the carbon source.

Effect of Pseudomonas aeruginosa on colour reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in colour with bacterial strain (Pseudomonas aeruginosa) in the presence of carbon and nitrogen source (Table 1) was 60.5 % and 39.9% respectively, in comparison to control. These results indicated that reduction was appreciable in the presence of carbon source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of colour we noticed that it would not enhanced more in the colour reduction as compare to the carbon source.

Effect of Consortia (Bacillus subtilis and Pseudomonas aeruginosa) on colour reduction at 0, 3, 6, 9 and 12 days

During the experiment performed reduction in colour with two bacterial strains (Bacillus subtilis and Pseudomonas aeruginosa) in the presence of carbon source i.e. glucose (Table 1) was 59.1 %, in comparison to control. These results indicated that reduction was not appreciable in Bacillus subtilis and Pseudomonas aeruginosa alone so, for the better result we are treat them as consortia of both in the reduction of colour we noticed that it would enhanced more in the colour reduction as compare to the alone.

COD Reduction

Effect of Bacillus subtilis on COD reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in COD with bacterial strain (Bacillus subtilis) in the presence of carbon and nitrogen source (Table 1) was 18.5 % and 62.9 % respectively, in comparison to control. These results indicated that reduction was not appreciable in the presence of carbon source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of colour we noticed the it would enhanced more in the COD reduction as compare to the carbon source.

Effect of Pseudomonas aeruginosa on COD reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in COD with bacterial strain (Pseudomonas aeruginosa) in the presence of carbon and nitrogen source (Table 1) was 33.3 % and 74.07 % respectively, in comparison to control. These results indicated that reduction was not appreciable in the presence of carbon source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of COD we noticed the it would enhanced more in the COD reduction as compare to the carbon source.

Effect of Consortia (Bacillus subtilis and Pseudomonas aeruginosa) on COD reduction at 0, 3, 6, 9 and 12 days

During the experiment performed reduction in COD with two bacterial strains (Bacillus subtilis and Pseudomonas aeruginosa) in the presence of carbon source i.e. glucose (Table 1) was 44.4 %, in comparison to control. These results indicated that reduction was appreciable more in Bacillus subtilis and Pseudomonas aeruginosa alone as compare to the consortia. For the better result we are treat them as consortia in the reduction of colour but we noticed that it would not enhanced more in the colour reduction as compare to the alone.

pH Changes

Effect of Bacillus subtilis on changes in pH at 0, 3, 6, 9 and 12 days

During the experiment performed, changes in pH with bacterial strain (Bacillus subtilis) in the presence of carbon and nitrogen source (Table 1) were 8 and 6 respectively, in comparison to control. These results indicated that changes was not appreciable in the presence of carbon source but after the carbon source when we added yeast as nitrogen source to see the better result in the pH changes we noticed the it would enhanced more in the pH changes as compare to the carbon source.

Effect of Pseudomonas aeruginosa on pH changes at 0, 3, 6, 9 and 12 days

During the experiment performed, changes in pH with bacterial strain (Pseudomonas aeruginosa) in the presence of carbon and nitrogen source (Table 1) were 4 and 6.6 respectively, in comparison to control. These results indicated that changes was appreciable in the presence of carbon source but after the carbon source when we added yeast as nitrogen source to see the better result in the pH changes we noticed the it would not enhanced more in the pH changes as compare to the carbon source

Effect of Consortia (Bacillus subtilis and Pseudomonas aeruginosa) on pH changes at 0, 3, 6, 9 and 12 days

During the experiment performed in pH changes with two bacterial strains (Bacillus subtilis and Pseudomonas aeruginosa) in the presence of carbon source i.e. glucose (Table 1) was 4.2, in comparison to control. These results indicated that changes was not appreciable in Bacillus subtilis and Pseudomonas aeruginosa alone so, for the better result we are treat them as consortia of both in the pH changes we noticed that it would enhanced more in the pH changes as compare to the alone.

TSS Reduction

Effect of Bacillus subtilis on TSS reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in TSS with bacterial strain (Bacillus subtilis) in the presence of carbon and nitrogen source (Table 1) was 0.11 % and 0.02 % respectively, in comparison to control.

These results indicated that reduction was appreciable in the presence of carbon (glucose) source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of TSS we noticed that it would not enhanced more in the TSS reduction as compare to the carbon source.

Effect of Pseudomonas aeruginosa on TSS reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in TSS with bacterial strain (Pseudomonas aeruginosa) in the presence of carbon and nitrogen source (Table 1) was 0.87 % and 0.21 % respectively, in comparison to control. These results indicated that reduction was appreciable in the presence of carbon (glucose) source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of TSS we noticed that it would not enhanced more in the TSS reduction as compare to the carbon source.

Effect of Consortia (Bacillus subtilis and Pseudomonas aeruginosa) on TSS reduction at 0, 3, 6, 9 and 12 days

During the experiment performed reduction in TSS with two bacterial strains (Bacillus subtilis and Pseudomonas aeruginosa) in the presence of carbon source i.e. glucose (Table 1) was 0.09 %, in comparison to control. These results indicated that reduction was appreciable more in Bacillus subtilis and Pseudomonas aeruginosa alone as compare to the consortia. For the better result we are treat them as consortia in the reduction of TSS but we noticed that it would not enhanced more in the TSS reduction as compare to the alone.

TDS Reduction

Effect of Bacillus subtilis on TDS reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in TDS with bacterial strain (Bacillus subtilis) in the presence of carbon and nitrogen source (Table 1) was 0.18 % and 0.15 % respectively, in comparison to control. These results indicated that reduction was appreciable in the presence of carbon (glucose) source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of TDS we noticed that it would not enhanced more in the TDS reduction as compare to the carbon source.

Effect of Pseudomonas aeruginosa on TDS reduction at 0, 3, 6, 9 and 12 days

During the experiment performed, reduction in TDS with bacterial strain (Pseudomonas aeruginosa) in the presence of carbon and nitrogen source (Table 1) was 0.35 % and 0.02 % respectively, in comparison to control. These results indicated that reduction was appreciable in the presence of carbon (glucose) source but after the carbon source when we added yeast as nitrogen source to see the better result in the reduction of TDS we noticed that it would not enhanced more in the TDS reduction as compare to the carbon source.

Effect of Consortia (Bacillus subtilis and Pseudomonas aeruginosa) on TDS reduction at 0, 3, 6, 9 and 12 days

During the experiment performed reduction in TDS with two bacterial strains (Bacillus subtilis and Pseudomonas aeruginosa) in the presence of carbon source i.e. glucose (Table 1) was 0.29 %, in comparison to control. These results indicated that reduction was appreciable more in Bacillus subtilis and Pseudomonas aeruginosa alone as compare to the consortia. For the better result we are treat them as consortia in the reduction of TDS but we noticed that it would not enhanced more in the TDS reduction as compare to the alone.

In this study sampling was carried out for analysis of various parameters at 0, 3, 6, 9 and 12 days. There was significant change in colour reduction within 12 days. Therefore, sampling was performed for days however; data are presented in Fig. on the basis of days.The result suggest that Bacillus subtilis is able to secrete extracellular catalytic enzyme that can degrade colouring in 12 days incubation period and some amount of colour reduces due to adsorption of bacterial strain. This strain is also efficient in removing

pollution load by decreasing COD, pH, TSS and TDS.Similar result was observed material in the studies performed by Saritha, et al (2010). They showed that a maximum of 73.54, 79.6, 66.4 and 47.6 % of COD reduction in Phanerochaete chrysosporium. And K. Selvam, et al (2011).They showed 43 % reduction in COD and 63.6 % reduction in colour. Prabhu et al. (2005) reported that 84% effluent decolourization along with 79% COD reduction by P. chrysosporium. Anil Kumar, et al (2012) reduces the COD value up to 86.5% (back water) and 65% (80: 20, back water and black liquor mixture) of effluent generated from small (agro-based) pulp and paper mills. Wibowo Mangunwardoyo, et al January (2013). They showed 67% reduction in COD and 70% in BOD. After comparing the results with previous studies, the time taken to COD and colour reduction process was very less in our case.

Conclusion:

The paper mill is growing fast and produces different varieties of paper. The biological treatment of effluent from this mill revealed that the effluent is light brown in colour, and shows 60.5% reduction in colour and 88.8% reduction in COD, 2.09% reduction in TSS, 0.95% reduction in TDS and also pH shows alkaline nature of the treated effluent with Pseudomonas aeruginosa. These are the parameters which we are treated by utilized the Bacillus subtilis, Pseudomonas aeruginosa and their consortia.

References

1. V.P. Kesalkar, Isha.P.Khedikar, A.M.Sudame. "Physico-chemical characteristics of wastewater from Paper industry". International Journal of Engineering Research and Applications. Vol. [2], Issue[4], July-August [2012], pp.[137]-[143].
2. K.Murugesan."Bioremediation on paper and pulp mill effluents". Indian Journal of Experimental Biology. Vol.[4], November [2003], pp.[1239]-[1248].
3. Pratibha Singh and Indu Shekhar Thakur. "Removal of colour and detoxification of pulp and paper mill effluent by microorganisms in two step bioreactor". Journal of Scientific & Industrial Research Vol.[63] November [2004], pp.[944]-[948].
4. Manuel Hernandez, Juana Rodriguez, Juan Soliveri, Jose L. Copa, Maria I. Perez, and Maria E. Arias." Paper Mill Effluent Decolorization by Fifty Streptomyces Strains". Applied And Environmental Microbiology, Vol. [60] no.11 Nov. [1994], pp. [3909]-[3913].
5. Gomathi, V Cibichakravarthy, B Ramanathan, A Sivaramaiah Nallapeta, Ramanjaneya V, R Mula, Jayasimha Rayalu, D." Decolourization of Paper Mill Effluent by Immobilized Cells of Phanerochaete chrysosporium ".International Journal of Plant ,Animal and Environmental Sciences".Vol.2.[2], issue-1 ,Jan- Mar-[2012], ISSN [2231]-[4490]
6. Panda Sunakar, Panigrahi Jagadish Chandra and Tripathy Upendra Prasad." A Comprehensive Approach for the Characterization of Pulp and Paper Industry Post Oxygen Stage Effluent". Research Journal of Chemical sciences. Vol. 2[7], 41-46, July [2012].
7. Vara Saritha, Y.Avasn Maruthi and K.Mukkanti."Potential Fungi for Bioremediation of Industrial Effluents".BioResources [1], [8-][22]. [2010].
8. K.Selvam, M.Shanmuga Priya and C.Sivaraj." Bioremediation of Pulp and Paper Mill Effluent by Newly Isolated Wood Rot Fungi from Western Ghats Area of South India". International Journal of Pharmaceutical & Biological Archives 2011; 2[6]:[1765]-[1771].
9. L.Christov and B Van.Driessel."Waste Water Bioremediation in the Pulp and Paper Industry". Indian Journal of Biotechnology. Vol 2, July-2003, pp. [444]-[450].
10. Shweta Kulshreshtha, Nupur Mathu, Pradeep Bhatnagar and B.L. Jain. "Bioremediation of industrial waste through mushroom cultivation". Journal of Environmental Biology July 2010, 31, [441]-[444].
11. Virendra Kumar, Purnima Dhall, Rita Kumar, Yogendra Prakash Singh, and Anil Kumar. "Bioremediation of Agro-Based Pulp Mill Effluent by Microbial Consortium Comprising Autochthonous Bacteria ".The Scientific World Journal Volume [2012], Article ID [127014], 7 pages
12. Mangunwardoyo Wibowo, Tony Sudjarwo & Mufti Petal Patria."Bioremediation Effluent of Wastewater Treatment Plant Bojongsoang Bandung Indonesia Using Consorium Aquatic Plants and Animals". IJRRAS 14[1] January [2013].