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Reduction in physicochemical parameters in pulp and paper effluents by adapted bacterias Sanganer area

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Abstract
Environment biotechnology an important branch of biotechnology, deals with the detection of environmental contaminants contained in industrial, agriculture and domestic wastes and the remediation of the pollution caused by contamination. The study of this discipline primarily spans 2 main areas; environment science & biotechnology. Environment biotechnology involves applying the knowledge of biotechnology to solve environment problems. Environmental problems can be found in all areas of the world, and they affect land, air and water. Heavy metals are discharged from various industries such as electroplating, metal finishing, textile, storage batteries, mining, ceramic and glass. The present study was carried out to assess the quality of contaminated water of paper and pulp industry. The data obtained for the physico-chemical properties of the water sample, when compared, were not in the range of permissible limits indicating that the water was polluted.
Bacterial strains were isolated from effluent of paper and pulp industry of sanganer city, Jaipur by selective enrichment technique. Nutrient agar was used in enrichment technique supplemented with 0. 1% chromium metal. The data shows that isolated microorganisms belonged to sp., Micrococcus sp. and Staphylococcus sp. and were studied for their biodegradation potential for chromium. Most literatures found that effective indigenous isolates secreates organic acids and enzymes for utilization and degradation of xenobiotics compounds.

Keywords: Pollution, physico-chemical characterstics, DO, COD, chloride

Introduction
Modern paper is normally made from wood pulp. Wood is ground up and mixed with water and other chemicals to make a thin liquid called "paper pulp". Paper pulp can be bleached to make paper whiter, and dyes can be added to make colored paper. This pulp is pressed into sheets of paper. Printing is often done on paper before the paper is cut into sheets. Newsprint paper (newspaper) comes in a huge roll, and goes through the printing process as one continuous sheet. It is cut by a machine-drivenguillotine blade later. Folding comes last, then packing for distribution. Sometimes paper is made heavier and more glossy (shiny) by adding clay, and by 'milling' it. Milling is done by squeezing the paper through a series of rollers. Sometimes paper is made from used or waste paper: this is recycling. Not all paper is made from wood. Other kinds of fiber can be used. People still make paper from cotton, linen and hemp for special purposes. Bio means “life”. “Remediate” means to solve problem, and “bio-remediate” means to use biological organism to solve an environmental problem such as contaminated groundwater. Bioremediation uses living things to break down or remove toxic and harmful substance from water. Bioremediation is the process of using organisms to neutralize or remove contamination from waste water. Bioremediation is the use of living organisms, primarily microorganisms, to degraded environmental contaminants into less toxic forms. Bioremediation is a pollution control technology that use biological systems to catalase to degradation or transformation of various toxic chemical to less harmful from. The general approach to bioremediation are to enhance natural biodegradation by native organism to carry out environmental modification by applying nutrients or aeration or through addition of microorganism (K Murugesan *et al.* 2003). Bioremediation is the use of living organism, primarily microorganism, to degrade environmental contaminants into less toxic forms. Research has demonstrated that there are very few environments where microbes have not been able to survive, adapt & indeed, thrive.

Microbes are able to utilize a near infinite combination of electron donors & electron acceptors to drive their metabolism. The process of cleaning up the hazardous substances into non-toxic compounds is called the Bioremediation process. This process is majorly used for any kind of technology clean up that uses the natural microorganisms.

Materials and Methods

Chemicals

All the chemicals used in the study were of analytical grade. The laboratory glass wares used were washed with detergents and rinsed with distilled water then oven baked at 200 °C overnight, prior to use.

Sampling

1. Study area - Sanganer, Jaipur (Raj.)
2. Sampling site - century paper & pulp industry
3. Sampling - Grab sampling
4. Sample information - waste water

Methods

Physicochemical characterization

Temperature: Temperature is an important parameter. If affect water chemistry & important on biological activity of water. Temperature must be measured in situ because a water sample will gradually reach the same temperature as the surrounding air.

pH: pH may be measured accurately using a pH meter. The pH meter must be calibrated before making pH measurement. For calibration standard buffers of pH 4.00, 7.00 & 10.00 are used pH of water indicates the hydrogen ion concentration in water. The concept of pH was put forward by Sorenson in 1909.

DO: DO is one of the most important parameter. Its correlation with water body gives direct and indirect information e.g. bacterial activity, photosynthesis, availability of nutrients, stratification etc. (Premlata Vikal, 2009) [10]. In the progress of summer, dissolved oxygen decreased due to increase in temperature and also due to increased microbial activity (Moss 1972, Morrissette 1978, Sangu 1987, Kataria, 1996) [11-14].

BOD: The biochemical oxygen demand is a way of expressing the amount of organic compounds in sewage as measured by the volume of oxygen required by bacteria to metabolize it under aerobic conditions. It is a good index of the organic pollution. If the amount of organic matter in sewage is more, the more oxygen will be utilized by bacteria to degrade it.

Alkalinity: It is composed primarily of carbonate and bicarbonate, alkalinity acts as a stabilizer for pH. Alkalinity, pH and hardness affect the toxicity of many substances in the water. It is determined by simple dil HCl titration in presence of phenolphthalein and methyl orange indicators. Alkalinity in boiler water essentially results from the presence of hydroxyl and carbonate ions.

TDS (Total Dissolved Solids): TDS refer to material that are completely dissolved in water. These solids are filterable in nature. It is defined as residue upon evaporation of filterable sample. The term total suspended solids (SS) can be referred to materials which are not dissolved in water and are non-filterable in nature. It is defined as residue upon evaporation of non-filterable sample on a filter paper.

Acidity: Acids contribute to corrosiveness and influence chemical reaction rates, chemical speciation and biological processes. Acidity of water is its quantitative capacity to react with a strong base to a designated pH. The measured value may vary significantly with the end point pH used in the determination.

Hardness: To measure the hardness of water, either titrate a sample of the water with an ethylene di amine tetra acetic acid, or EDTA, solution or use a test strip kit. Hardness refers to the amount of minerals in the water, such as calcium, magnesium and iron. Natural water always contains a variety of dissolved ions, including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cl^{-}, HCO_3^{-}, and some other. If concentrations of Ca and Mg ions are relatively large, the water is called *hard*. Hard water may cause many problem.

Chloride: Chloride are widely distributed as salts of calcium, sodium and potassium in water and wastewater. In potable water, the salty taste produced by chloride concentration is variable and dependent on the chemical composition of water. The major taste producing salts in water are sodium chloride and calcium chloride. The salty taste due to chloride anions and associated cations in water.

Test for bacteriological analysis

Isolation of isolates

The isolates were isolates by serial dilution followed by streaking.

Biochemical characterization

Gram staining

Gram staining, also called Gram's Method, is a method of staining used to differentiate bacterial species into two large groups (Gram-positive and gram-negative). Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in the cell wall of gram-positive bacteria. [1] Gram-positive bacteria retain the crystal violet dye, and thus are stained violet, while the gram-negative bacteria do not; after washing, a counterstain is added (Commonly safranin or fuchsine) that will stain these gram-negative bacteria a pink color.

Starch hydrolysis test: Some bacteria have the ability to hydrolyse starch, as they can produce the saccharolytic enzyme. While the starch forms dark blue color with iodine, its hydrolysed end products do not acquire such dark blue color with iodine. In the starch hydrolysis test, the test bacteria are grown on agar plates containing bacteria. After colonies of the bacteria have the ability to hydrolyse the starch its colonies hydrolyse the starch in the medium in the areas surrounding them, while the rest of the areas of the plates contain unhydrolysed starch.

Catalase test: The test performed for determination of those microorganism that can produce catalase enzyme that breaks hydrogen peroxide (H_2O_2) and O_2. Presence of gas bubble during the test indicated a positive result.

IMVIC test: The test was specified for the identification of enteric bacilli. It included

a) Indole test: It was done for differentiation of gram negative bacteria that can split tryptophan into indole and pyruvic acid using a hydrolase called tryptophase. The presence of red layer at the top on addition of Kovacs reagents indicated a positive result.

b) Methyl red test: It was done for differentiation of gram negative bacteria by the products produced during fermentation of MRVP medium. The presence of yellow layer on addition of yellow layer indicates a negative result.

c) Voges proskauer test: It was done for identification of bacteria that can ferment glucose. The presence of cherry red colour on addition of Barrett's reagent indicated a positive result.

Result

A) Physicochemical in water

Table 1: Results. Physicochemical properties of chromium affected effluent

Parameter	Units	Results for paper and pulp effluent	Standard per CPCB
pH	-	6.5	5.5-9.6
Temperature	°C	28.5	45
COD	mg/l	.281	350
BOD	Mg/l	90	30
Acidity	Mg/l	196	Na
Alkalinity	Mg/l	80	200
Salinity	Mg/l	356	500A
Oil grease	Mg/l	115	10
Hardness	Mg/l	998	Na
DO	Mg/l	1.9	4
TDS	Mg/l	0.5122	200

Table 2: Results for Physicochemical Analysis after the Bioremedial Setup

Parameter	Units	Result for paper and pulp effluent(1% inoculum)	Standard as CPCB
pH	-	6.5	5.5-9.0
Temperature	°C	28.5	45
COD	Mg/l	88.72	350
BOD	Mg/l	90	30
Acidity	Mg/l	190	Na
Alkalinity	Mg/l	80	2000
Salinity	Mg/l	356	500A
Oil and grease	Mg/l	115	10
Hardness	Mg/l	998	Na
DO	Mg/l	1.9	4
TDS	Mg/l	0.5122	200

Isolation, identification and biochemical characterization of isolated bacteria

The water sample collected was subjected to bacteriological analysis using culture dependent approach. The sample was screened with Chromium metal. The bacterial isolates which showed growth in the presence of Chromium were selected for their biodegradation potential as they had the ability to degrade Chromium. A total of 2 isolates were screened and were designated as CH1 and CH2. They were identified according to culture dependent approach. A series of biochemical tests were used to identify them.

Confirmation test for indigenous bacterial strain

Gram Staining

It is the first test which was used to classify the screened bacteria based on their gram staining characteristics and was classified as gram positive (streptococcus) gram negative (micrococcus).

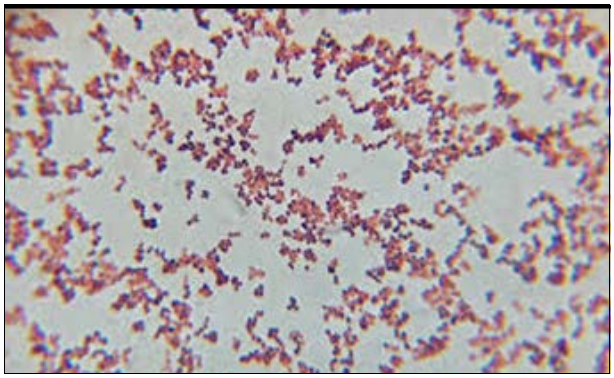

Fig 1: Gram positive (streptococcus)

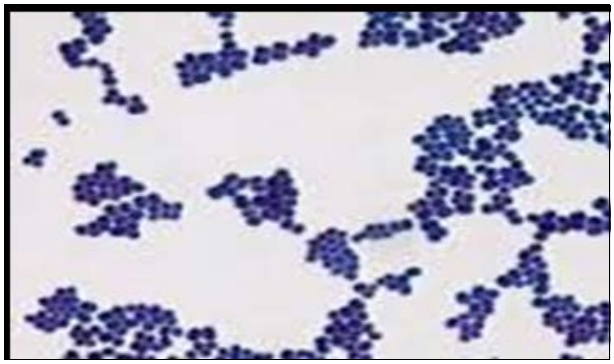

Fig 2: Gram positive cocci (Micrococcus)

IMVIC test

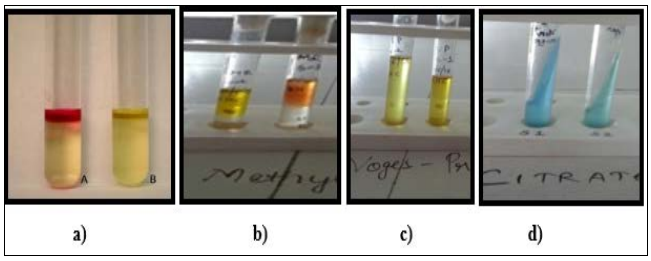

Fig 3: A) Indole test- negative for both the Isolates, B) MR test-positive for isolate 1, negative for isolate 2, C) VP test- Positive for both the isolates, D) Citrate utilisation test-Negative test for both isolates

Discussion

The present study was carried out to assess the quality of contaminated water of paper and pulp industry. The data obtained for the physico-chemical properties of the water sample, when compared, were not in the range of permissible limits (Richa *et al.* 2012) [15] indicating that the water was polluted.

Bacterial strains were isolated from effluent of paper and pulp industry of sanganer city, Jaipur by selective enrichment technique. Nutrient agar was used in enrichment technique supplemented with 0. 1% chromium metal.The data shows that isolated microorganisms belonged to sp., *Micrococcus* sp. and *Staphylococcus* sp. and were studied for their biodegradation potential for chromium. Most literatures found

that effective indigenous isolates secreates organic acids and enzymes for utilization and degradation of xenobiotics compounds. (Monica *et al*. 2011) [5]. Virendra Kumar (2011) [16] also found similar results with *Klebsiella* sp. (99%, accession no NR_074913.1), *Alcaligenes* sp. (99%, accession No. NR_025357.1) and *Cronobacter* sp. (97%, accession No.NR_102490.1). The results of survivability showed that the bacteria were not only effective but also dominant irrespective of the other strains present in the effluent. Intense research in this area confirms that besides bacteria, other microorganisms, including fungi and algae, can be used. According to Indhumathi P. (2014) [9], *Chlorella vulgaris* is a cheap and effective adsorbent for the removal of chromium ion from wastewater without requiring any pretreatment

Conclusion

Contamination is correlated with the degree of industrialization and intensity of chemical usage. The concern over water contamination stems primarily from health risks, from direct contact with the contaminated soil, vapours from the contaminants, and from secondary contamination of water supplies within and underlying the soil. Petroleum hydrocarbons are toxic and potent carcinogenic to human health as well as environment Utilization of chemical contaminant present in the soil as source of carbon and energy by different bacterial communities leads to ameliorate a wide range of contaminants like petroleum and polyaromatic hydrocarbons. Prevailing environmental conditions are among the most important limiting factors for optimum bioremediation. The factors affecting the success and rate of microbial bioremediation are nutrient availability, moisture content, soil reaction (pH), temperature, C/N ratio, soil texture etc. Oil adapted bacterial genera were isolated and identified from the test water sample: they were *Pseudomonas* sp., *Bacillus* sp. and *Staphylococcus* sp. It is therefore recommended that ability of the isolated and identified bacteria to bio remediate or utilize hydrocarbons, especially used petroleum oil should be investigated. We found that maximum biodegradation of oil was 95% in *Pseudomonas* sp. with 0.1% of inoculum, 95% in *Bacillus* sp. with 0.5% of inoculum and 95% in *Staphylococcus* sp. with 0.1% of inoculum using gravimetric analysis. We hereby suggest from the present investigations that diversity of indigenous microbes could be exploited using high throughput screening methods and the possible role of enzymes involved must be studied.

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