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Bacteriological Analysis of Pulp and Paper Mill Effluents

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Abstract

The increase in BOD and bacterial level has been considered inactive of increasing pollution, a condition similar to observed in the studies. The high concentration of coliform bacteria is probably as a result of agricultural runoff and faecal contamination making water not fit for agricultural as well as industrial use. The COC values recorded were higher than BOD values clearly established that the non-biodegradable and oxygen demanding pollutants are present in the effluents which come in the system as industrial discharge. Studies on the distribution of bacterial population and their isolation have also been made in respect to total coliform and faecal coliform organism.

Keywords: Biodegradable, Contaminated Impregnated.

Introduction

Material and Method

For bacteriological analysis, phrogmets and cyperus plants were collected from the effluent channel. The following parameters were analysed form the collected sample.

1. Isolation of Bacteria

Bacteria were isolated from the upper and lower zone of soil of plant I and Plant II. For the isolation of rhizospere microorganism the rhizosphere soil was taken from bolt the plant upper and lower root zone, further the isolation of bacteria was done by serial dilution techniques.

2. Serial Dilution Techniques

In this techniques test tubes containing 9.0 ml 0.85% of saline water were prepared and autoclaved as per dilution technique. 1gm of soil was dissolve in 1ml of saline. This 1ml of saline was added to the first test tube and shake well 1ml effluent from the test tube transferred to the next tube and so an. various dilution were plated on presterlised petridish containing nutrient Agar purification of isolated bacteria strain by streak plate method. In the petriplates inoculated by dilution technique mixed culture were obtained. To obtained pure culture streak plate method was done by drawing a small amount of bacterial growth lightly across the surface of Agar plate in a pattern with an inoculating needle or loop. This inoculum become progressively diluted with each successive streak and eventually single cell were deposit on the Agar surface after 24 hours of inoculation isolated colonies develop in the plate of streak.

3. Identification of Isolated Bacterial Strains

The purified isolated strain from various stages of treatment system of distillery and laboratory experimental set up was studied of its morphology culture, characteristics like gram staining, catalase, oxidase, glucose acid molality and test by colour. Further bacterium genus was identified by other biochemical secondary character as follows:

4. Primary Character

(a) Gram Staining of Bacteria

The gram staining was done according to Hucker's modification as follows:

1. Smear on the slide was first stained with ammonium oxlyate crystal vilet solution for 1 minute.
2. Washed with top water for 2 second
3. Lugol's iodine was immersed on the slide
4. Washed with top water and dried
5. With 95% alcohol on smear was decolourised for 30 second
6. Counter strained with safarnine solution for 10 to 15 second
7. Washed with tap water
8. Dried and examine under oil emersion uses in Olympus microscope.

Blush violet coloured cell = Gramtive
Red or pink coloure cell = Gramtive

5. Oxidise Test

The production of oxidase enzyme by some bacteria are characteristics character which is done by oxidationof oxydase reagent i.e. 1% tetramethyl-p-phynelenedyamine aqueous solution. Test was performed or a piece of filter paper in a petridish plant 2.3 drops of oxydase reagent smear the culture under test across the impregnated paper with a platinum loop of positive reaction is indicated by the appearance of dark purple colour with in 10 sec.

Control Positive - Colony dedposite
Without inoculums - No purple colour develop

(c) Catelase

The culture was grown on a slope of nutrient suitable medium and run 1ml 3% H_2O_2after 4 min for bubble of gas.

Control Positive - Evaluation of gas bubble
Negative - No gas bubble

(d) Acid from Carbohydrate

Pure culture examined daily for 7 days and gas production of of peptone water and nutrient broth sugar dissolve the solids in the water, add the indicator and adjust to pH 7.1-7.2 sterilized at 115^0C for 20 min. A septically add 1% of the appropriated carbohydrate mix distribute into sterile tubes inverted Durhaln's tube steam for 30 min

Motility

Pure young broth culture of the organism incubated at the below the optimum temperature (eg 37^0C and 22^0C) microscopically in a hanging drop preparation and also observing the growth in semi-solid medium.

(i) Hanging Drop Preparation

1. Test culture inoculated into 5 ml sterile nutrient bath.
2. Incubated at 30^0C for 10h.
3. A drop of culture was transferred into thin coverslip.
4. Petroleum wax (very small amount) was applier at the corner of coverslip.
5. The concave area of cavity slide carefully inverted on to the coverslip with the culture and gently pressed to seal the coverslip.
6. Examine the slide with oil emersion under reduce illumination. Motile bacteria showed rapid movement with tumbling from one end to other end. Non motile bacterial showed braownian movement or no movement.

(ii) Motility on Semisolid Agar

The test culture was stabbed with straight platinum wire into semisolid motility medium upto depth of 5mm and incubated at 30^0C. The cloudy appearance around the stable after overnight incubation indicated motility of organism.

Observation and Result

The root may be treated as a solid cylinder and the most actively absorbing zone is surrounded by a holo of root hairs which are sufficiently crowed to diffusively interfere with each other. Roots are associated with a spectrum of microorganism ranging from loos associations with the rhizosphere zone, through ectomycorrhizas, endomycorrhizas to root nodule organism and finally on to the more extreme interactions with invading pathogens. All of these associations have consequences which influence vegetation ecology. There is now strong evidence that mycorrhizae increase the efficiency of nutrient absorption. Ectomycorrhizar, mostly of the class basidimycetes, form a sheath, external to the root with hyphae radiating into the surrounding soil. They are associated with a limited number of plates mostly trees and at least in some cases of host plants fail to thrive in the absence of the fungal symbiont. Endomycorrhizas may belong to any fungal lass are more common but different to absorb for which reason they were formerly neglected group. The most wide spread are the vesicular arbuscular mycorrhizas which, apparently infect most of the world's vegetation. There is no external sheath; the hyphae form vesicles and arbuscles within the root cortical cells and remify several cms into the surrounding soil, often forming connections between adjacent roots. Most of the early work on nutrient uptake was with *Fagus sylvatica* (beech) ectomycorihizas. The infected roots are considerably more effective in phosphorus uptake, partly because their life is prolonged possibly because P may be obsorbed and transported to the root through the external hyphae and possible by i\direct mobilization of P from organic sources. It has also been suggested that ectomycorrhizal infection may assist water uptake in different environment.

During the microbial analysis from the rhizosphere soil of growing plant i.e. *Phragmites* and *Cyperus* sp. ten different bacterial species were isolated. These bacterial species were isolated from upper root zone of *Phragmites* rhizosphere (i.e. A1, A2 and A3). Out of three, two were spherical and one rod shaped while the two bacterial species (A4 and A5). Similarly, two bacteria were isolated from upper zone of *Cyperus* rhizosphere soil (B3 and B4) as shown in Table 2. For the detail biochemical characterisation the isolated strains were tested for the Gram's reaction. Motility, Catalase, Oxidase, O-F test on basis of characterisation the isolated strains (A1, A2, A3, A4 and A5).

Table-1: Seasonal Variation in Total Coliform Index/100 ml. at Three Sampling Sites

Season	Months	Study sites		
		Site-I	Site-II	Site-III
Summer	March-2013	110000	12000	5000
	April-2013	98000	10000	9000
	May-20013	130000	18000	18000
	June-2013	145000	9000	7000
Rainy	July-2013	58000	9000	7000
	August-2013	70000	15000	9000
	September-2013	75000	12800	8500
	October-2013	73000	11500	6700
Winter	November-2013	53000	17000	6000
	December-2013	55000	14000	5000
	January-2014	77000	11000	5500
	February-2014	90000	15000	5000

Table-2: Seasonal Variation in Faecal Coliform Index/100 ml. at Three Sampling Sites

Season	Months	Study sites		
		Site-I	Site-II	Site-III
Summer	March-2013	93000	3500	3000
	April-2013	97000	2900	2700
	May-20013	95000	2800	3800
	June-2013	91000	2000	4000
Rainy	July-2013	82000	2700	4000
	August-2013	73000	5000	4000
	September-2013	77500	4800	5000
	October-2013	60800	4300	3700
Winter	November-2013	87000	4100	4100
	December-2013	91000	2900	3900
	January-2014	90500	3500	3000
	February-2014	90000	3700	2200

Table-3: Identification and Isolation of Bacteria

Type	Shape	Gram's reaction	Motility	Growth in air	Catlase	Oxidase	O.F. test
A1	S	+	+	+	+	+	O
A2	R	+	+	+	+	+	O
A3	S	-	-	+	+	-	F
A4	S	+	+	+	+	+	F
A5	R	-	-	+	+	-	F
B1	S	+	+	+	-	-	F
B2	S	+	-	+	+	+	O
B3	R	+	+	+	-	-	F
B4	S	+	-	+	+	-	F

Detail about result

A1=Bacteria isolated from upper zone of *Phragmites* rhizosphere.

A2=Bacteria isolated from upper zone of *Phragmites* rhizosphere.

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A1=Bacteria isolated from upper zone of *Phragmites* rhizosphere.

B1=Bacteria isolated from upper zone of *Cyperus* Rhizosphere.

B2=Bacteria isolated from upper zone of *Cyperus* Rhizosphere.

B3=Bacteria isolated from upper zone of *Cyperus* Rhizosphere.

B4=Bacteria isolated from upper zone of *Cyperus* Rhizosphere.

S= Spherical; R=Rod.

Discussion

Total and faecal coliform indices were higher during summer season at study site I while they were every low at study site III, the increase in BOD and bacterial level has been considered indicative of increasing pollution, a condition. A condition similar to observe in the studies of Basu *et al.,* (1997), Goyal (1991) and Kokulinew and Salonen (1982). The high concentration of coliform bacteria is probably as a result of agricultural runoff and faecal contamination making the water not fit for agricultural as well as industrial use. The COD values recorded were higher then BOD values clearly established that the non biodegradable and oxygen demanding pollutant are present in the industrial discharges.

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