International Journal of Biological Research (IJBR)

Research paper

BIOREMEDIATION OF PAPER MILL EFFLUENT ON GROWTH AND DEVELOPMENT OF SEED GERMINATION (BLACKGRAM)

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ABSTRACT

Environmental pollution is one of the major problems of the world and it is increasing day by day due to urbanization and industrialization. Over the last few decades large scale usage of chemicals in various human activities has grown very fast, particularly in a country like India which has to go for rapid industrialization in order to sustain over growing large problem of population. The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The released organic compounds and heavy metals are one of the key factors that exert negative influences on man and environment causing toxicity to plants and other forms of biotics and abiotics that are continually exposed to potentially toxic heavy metals.

Keywords : Paper Mill, Blackgram

INTRODUCTION

Urban industrial activities have long been identified as a major source of environmental pollution. Waterborne infections are,the most common causes of morbidity and mortality in the under developed and developing countries and 80% of the infectious diseases are waterborne in India (Tambekar et al., 2008). Most of the rivers in urban areas of the developing world are the end points of effluents discharged from the industries Pulp paper industries are the sixth largest effluent generating industries of the world (Ugurlu et al., 2007), as these generates as low as 1.5 m3 of effluent per tone to as high as 60 m3 tone of paper produced (Asghar et al., 2007). These effluents have been found to contain more than 200- 300 different organic compounds and approximately 700 organicand inorganic compounds (Tambekar et al., 2008). Organic and inorganic contents of the effluent provide ample opportunity to flourishing a variety of pathogenic microorganism (Chandra et al., 2006).

Despite the considerable feats achieved such as water recirculation and reduction of pollutants prior to disposal, conventional treatments are limited by fouling of membranes and filters, poor dissolution of oxygen in wastewater and tremendous input of electricity in aeration tanks (Thompson et al., 2001; Ahn and Logan, 2010). On the other hand, the hallmark of anaerobic treatment of effluents has been the substantial reduction of sludge accompanied by a chemical reduction otherwise recalcitrant to aerobic treatment (Bogaert et al., 2010). Several methods have been described in the scientific literatures; however, these treatment methods do not solve the problem because of the transfer of contaminants from one phase to another. However, in biological treatment, the microorganisms degrade the organic pollutants using them as a carbon source to produce metabolic energy to survive. The effects of various industrial effluents, sludge materials and metal elements on seed germination, growth and yield of crop plants have captivated the attention of many workers (Rahman *et al*., 2002; Selivanorskaya and Latypova, 2006; Yu and Gu, 2007; Gannis *et al*., 2008; Sahu and Arora, 2008). However no detailed experiments have been performed on the germination and plant growth using treated and untreated tannery effluents. Hence an attempt has been made to study the effects of paper mill effluent (both treated and untreated) on the germination of blackgram.

MATERIAL AND METHOD:

Effluent source

The effluent was obtained from RAJAGANAPATHY PAPER MILL (Board mill private ltd) VADAMANGALAM, Pondicherry, A south Indian base integrated pulp and paper mill industry. The effluent was collected from outlet of primary settling tank were used for investigation. The sample was collected in a plastic container and transported to Annamalai University Microbiological Research laboratory situated in Chidambaram within 4 hours. The effluent was stored at 4^{o}C until further investigation.

EFFLUENT ANALYSIS

Physicochemical parameters in raw paper mill effluent analysis

To characterize the paper mill effluent, selected physiochemical characteristics were analyzed in the effluent samples.;

Colour: The colour of the effluent was observed visually.

Odour: It was categorized as objectionable or non-objectionable by direct smelling of the sample.

Temperature: It was measured by using a standard thermometer in the field itself.

pH: The pH of the effluent was determined by using a pH meter.

Electrical conductivity (EC):

EC is the measure of the ability of an aqueous solution to convey an electric current. This ability depends upon the presence of ions, their total concentration, mobility, valence and temperature. EC was determined by conductivity meter following the procedure of Richard (1954).

Total Hardness

Diluted 25ml sample to about 50ml with distilled water in a suitable vessel. 1 to 2ml buffer solution was added into it. Usually 1ml will be sufficient to give a pH 10 to 10.1. Then 1 to 2 drops of indicator solution were added. EDTA titrant was added slowly, with continuous stirring, until the last reddish tinge disappears. The last few drops were added at 3 to 5 seconds intervals. At the end point the solution was normally blue.

Calculation
Hardness (EDTA) as mg $CaCO_3$/ L=AxBx1000/ml sample.
Where A= ml titration for sample
B=mg $CaCO_3$ equivalent to 1.00ml EDTA titrant.

Total Suspended solids (TSS):

Total suspended solids are the portion of solids that usually remains on the filter paper. Suspended solids consist of silt, clay, fine particles of organic and inorganic matter, which is regarded as a type of pollution because water high in concentration of suspended solid may adversely affect growth and reproduction rates of aquatic fauna and flora. For TSS analysis, known amount of sample was filtered through the previously weighed filter paper. Filter paper was then dried at 103-105° C. TSS was determined by using following formula (Anon, 1992).

TSS mg/L = (final wt - initial wt)/amount of sample taken * 1000

Total Dissolved Solids (TDS):

Total dissolved solids (TDS) are the measure of total inorganic salts and other substances that are dissolved in water. TDS was determined following the procedure of Richard (1954) by using Electrical Conductivity (EC) meter.

TDS (mg/L) = EC μs/cm *0.67

Estimation of Biochemical Oxygen Demand (BOD)

Winklers iodometric method (AOAC, 2005)

Preparation of dilution water: 1.0 ml of calcium chloride, magnesium sulphate, ferric chloride and phosphate buffer solutions were added to one litre of aerated distilled water and mixed thoroughly. This is the standard dilution water, prepared freshly every time. Freshly settled raw sewage at 2.0 ml was added as seeding to one litre of dilution water. The test water samples were diluted with seeded dilution water sample (1%, 5% and 10%). Each dilution sample was taken in two sets of BOD bottles. In one set of flasks DO was determined immediately while the other set was kept for incubation at 20º C for five days.

Determination of DO:

To the contents of the BOD bottle 2.0 ml of magnesium sulphate solution and 2.0 ml of alkali-iodide-azide solution were added and mixed thoroughly. A brown precipitate was formed, which was allowed to settle completely leaving a clear supernatant liquid. Then, 2.0 ml of concentrated

sulphuric acid was added along the sides of the bottle and mixed for complete dissolution. The contents were transferred to a 500ml conical flask and titrated immediately against 0.025N sodium thiosulphate using starch as an indicator.

Calculation for DO:

Volume of 0.025 N sodium thiosulphate used in the titration = DO in mg/L

DO at 0º C 760 mm pressure = DO x 0.07 mg/L

Calculation for BOD:

BOD (5 days at 20º C) = (DO_0 - DO_5 - BC) x100\ percent sample.

DO_0 = Initial DO

DO_5 = DO after 20º C incubation for 5 days

BC = Blank correction i.e., difference in DO of blank on the initial day and after 5 days incubation.

Estimation of Chemical Oxygen Demand (COD)

Titrimetric method (AOAC, 2005)

A refluxing flask of 250 ml capacity was used with a ground glass 24/40 neck fitted with a 300 mm double surface condenser to which, a glass cap was fitted. 50 ml of the sample was taken in the flask. Mercuric sulphate of suitable quantity was added such that the ratio of chloride content of the sample to mercuric sulphate was 1:10 (For this, chloride content of the sample was estimated). Then 5 ml of sulphuric acid-silver sulphate reagent was added, dissolved the mercuric sulphate and cooled in cold water while mixing. 25 ml of 0.125 N potassium dichromate was pipetted into the flask and mixed. Few porcelain bits were added and the condenser was attached. The water circulation was started and refluxed for two hours. After cooling the contents of the flask was transferred and diluted to about 350 ml with distilled water. Then 2 to 3 drops of ferroin indicator was added and titrated against 0.125N ferrous ammonium sulphate solution. The end point was the sharp colour change from blue- green to reddish brown. A blank was conducted using 50 ml of distilled water instead of the sample.

Calculation

COD in mg/L = (Blank titre value-sample titre value) x 0.125 x 1000 x 8 volume of the sample taken.

Estimation of Carbonate and Bicarbonate (Alkalinity)

Titrimetric method (Natarajan et al., 1988)

25ml of the sample and 25ml of distilled water in a 250ml conical flask were added. Phenolphthalein indicator solution was added. If no pink colouration, it indicated phenolphthalein alkalinity. If pink colour appeared then titrated with sulphuric acid (0.02N) until the solution became colourless. Added 3 drops of mixed indicator solution in which phenolphthalein alkalinity had been determined and titrated against sulphuric acid (0.02N) to light pink colour.

Calculation

Volume of the sample taken = 25ml
Volume of 0.1N sulphuric acid used up to phenolphthalein end point = A ml
Volume of 0.1N sulphuric acid used up to methyl orange end point =B ml
Volume of 0.1N sulphuric acid required up to neutralize bicarbonate alone = (B-A) ml.

Carbonate

1.0ml of 0.1N sulphuric acid=0.003g of CO_3
2 x A ml of 0.1N sulphuric acid =0.003 x 2 x Ag
Amount of carbonate per litre of sample=0.003 x 2 x 1000 x 1000/25 mg

Bicarbonate

1.0ml of 0.1N sulphuric acid = 0.0061g of HCO_3
(B-A) ml of 0.1N sulphuric acid = 0.0061x(B-A)g of HCO_3
Amount of bicarbonate per litre of sample = 0.0061 x (B-A) x 1000 x 1000/25mg

Estimation of Calcium

EDTA titrimetric method (APHA, 2005)

Pipetted out 50ml of the sample. Added 2.0ml of sodium hydroxide to it to produce a pH of 12-13 and mixed well. Added 0.1-0.2g of the indicator, titrated immediately with EDTA. The end point is from pink to purple.

Calculation

If the EDTA titrant is exactly 0.02N mg/l calcium (as $CaCO_3$) = ml EDTA titrant x 1x 1000ml sample taken for the titration.

Estimation of Magnesium

Calculation method (APHA, 2005)

Calculation

Mg/l magnesium (as $CaCO_3$) = mg/l total hardness (as $CaCO_3$) = mg/l calcium (as $CaCO_3$) mg/l.

Estimation of Chloride

Silver nitrate titrimetric method (Jackson, 1973)

Three ml of aluminum hydroxide was added to a measured volume of the sample in a beaker. Stirred well and allowed to settle. The precipitate was filtered, washed with chloride free distilled water. 100 ml of the sample was pipetted out into a porcelain dish and the pH was adjusted to be in the range of 7- 9.5 to which 1ml of potassium chromate indicator solution was added. This was titrated against standard silver nitrate solution with constant stirring until a slight precipitate reddish colouration persisted. A blank was set by placing 100 ml chloride –free distilled water instead of sample.

Calculations

If the silver nitrate solution is exactly 0.0282N,

Chloride mg/L = Vol of 0.0282 N consumed (sample- blank) × 1000

Estimation of Sodium and Potassium

Flame photometric method (Jackson, 1973)

The flame photometer was standardized before feeding the sample and zero reading was set using deionised water. Using the stock solutions of sodium and potassium, the reading was adjusted to 100 at their specific wavelengths. Then the samples were fed in the flame photometer and noted the readings to get the amounts of sodium and potassium directly as milligrams per litre, by referring to the appropriate calibration curve.

Estimation of Fluoride

Ion selective electrode method (APHA, 2005)

Prepared a series of standards by diluting standard fluoride solution 5.0, 10.0 and 20.0ml with 100ml distilled water, these standards are equivalent to 0.5, 1.0,and 2.0 mg fluoride/l. Added equal volume of fluoride buffer to samples were, standardized and immerse the electrode are measured.

Calculation

mg fluoride/l= µg fluoride/ml sample

Estimation of Nitrate

Nitrate electrode method (APHA, 2005)

Transferred 10ml sample to a 50ml beaker, added 10ml buffer solution and stirred with a magnetic stirrer. Measure standards and samples at about the same temperature. Read concentration from calibration curve.

Estimation of Nitrite

Colorimetric method (APHA, 2005)

Sample pH which was not between 5 and 9 was adjusted to that range. To 50.0ml of sample added 2.0ml of color reagent and mixed. After adding color reagent to standard and sample, the absorbance was measured at 543nm.

Calculation

Prepare a standard curve by plotting absorbance of standard against NO_2-N concentration.

Estimation of Sulphate

Turbidimetric method (APHA, 2005)

Measured 100ml sample into an Erlenmeyer flask. Added 20ml buffer solution and mixed by stirring apparatus. Added a spoonful of barium chloride crystals. After stirring the readings are taken in a spectrophotometer at 420nm and measured turbidity at 5 ± 0.5 min.

Heavy metal analysis

Atomic absorption spectrophotometric method

The estimation of metals such as for Ni, Zn, Cu, Fe, Cd, Pb, Mn in the industrial effluent was performed as per Malik *et al*. (1984). Three concentrations of each standard metal solution were selected to find out the expected metal concentration of a sample. Then each standard was aspirated into flame and the absorbance was recorded. A calibration curve was prepared by plotting the absorbance of standards versus their concentrations. The estimations of Nickel, Zinc, Copper, Iron, cadmium, Lead and Manganese were done at the wavelengths of 232.0, 213.9, 324.7, 248.3, 228.8, 217.0 and 279.5 nm respectively.

Calculation

The concentration of each metal ion was calculated in milligrams per liter, by referring to the appropriate calibration curve.

METHODS

All the testing was performed according to standard microbiological method for the examination of water and wastewater as described (Washington, D.C., 1995).

Bacterial isolation

The sample was serially dilution using sterile pipettes from 10^{-1} and 10^{-8} dilution the bacterial strain capable of growing on nutrient agar medium. For enumeration of bacteria nutrient agar medium was used. To obtain pure culture were repeatedly streaked nutrient agar medium and incubated at 37^{o}C for 24 hours. The isolated bacteria were identified by colony morphology, gram staining, microscopic observation and confirmation test. The identified bacteria were,

shigella sonnei, Pseudomonas aeroginosa,,Bacillus subtilis,. The isolated fungal culture were identified as *Trichoderma reesei* using Lactophenol cotton blue staining method.

Biochemical identification of different bacteria:
The different bacterium was identified morphologically and biochemically using standard procedures (Barrowand Feltham 1993) **.**

Microbial indentification: Identification was done base on morphological, cultural, biochemical and physiological characteristics base on (Cappuccino *et.al*.1999) and (Schaad *et al*.,2001) and the result were checked with Bergey's Manual of determinative Bacteriology (Buchanan *et al*., 1974)

PHYSICO-CHEMICAL PARAMETERS ASSESSED IN BIOREMEDIATED PAPER MILL EFFLUENT

Based on the bioremediation process, effective method was used to treat the paper mill effluent.After remediation, the physico-chemical parameters were assessed followed by the earlier said effluent analysis procedures.

Preparation of inoculam:

A loopful of culture (*shigella sonnei., Pseudomonas aeroginosa.,Bacillus subtilis.)* was inoculated individually pre-sterilized 100ml nutrient broth.The flask was kept in a shaker at 120rpm for 16-18hrs at 30^0C. The culture broth was centrifuged at 1000rpm for 20 minutes.cell suspension was prepared using sterile distilled water and adjusted to 0.5 OD using UV-spectrophotometer,1% (10^5 CFU/ml) of the above suspension was used as inoculum for the bioremediation of paper mill effluent.

SEED GERMINATION AND PLANT GROWTH EXPERIMENTS

In order to check the plant growth ability of the raw and bioremediated paper mill effluent, the growth experiments were carried out with economical important cash Blackgram(ADT-3) crop (*Vigna mungo*) in laboratory conditions.

The present study various dilutions of the selected tannery effluent (both untreated and bioremediated) were used for plant growth study and observation were recorded.

Preparation of different dilutions of paper mill effluent

The collected Paper mill effluent. from discharge site and the bioremediated effluent were diluted as follows and used for the *in vitro*.

T_1: 0% - control (Bore water)
T_2: 20% - 20ml paper mill effluent. + 80 ml water

T_3: 40% - 40ml paper mill effluent. + 60 ml water
T_4: 60% - 60ml paper mill effluent. + 40 ml water
T_5: 80% - 80ml paper mill effluent. + 20 ml water
T_6: 100% - 100ml paper mill effluent.

With the following above treatments were used for *Vigna mungo* crop. The experiment was conducted in completely randomized block design with three replications.

Seed collection: The seeds of Blackgram (ADT 3,Duration: 75 days) seed were collected from experimental farm, Department of Agronomy, Faculty of agriculture, Annamalai University, Tamil Nadu, India.

Seed germination experiment under *invitro* condition

Germination studies were conducted under laboratory conditions. Six test solutions (T_0= 0%, T_1= 20%, T_2= 40%, T_3= 60%, T_4= 80% & T_5= 100% v/v) prepared by diluting paper mill effluent for both untreated and bioremediated with distilled water were used to investigate the effects of wastewater on germination of blackgram. The healthy and uniform sized blackgram (ADT-3) seed were surface sterilized with 0.1% mercuric chloride for 2- 3 minutes, washed in running tap water for 3 minutes and in distilled water for 2 minutes. They were thoroughly washed with tap water to avoid surface contamination. Ten healthy and undamaged seeds of equal size were evenly placed in each sterilized petridish which contained water soaked filter papers. The petridishes were arranged in completely randomized block design. Measured quantities of test solutions was added in each replicate and exposed for 8 hours. Seedlings were then grown in distilled water. Random samples were taken from each treatment after 7 days. Following growth parameters were measured and recorded.

Growth measurements under *in vitro* condition

- Germination percentage
- Total seedling length (cm)
- Vigour index
- Total Chlorophyll (mg/g)
- Carbohydrate (mg/g)
- Protein content (mg/g)

Germination percentage

The number of seeds which germinated after sowing was counted and the percentage was calculated.

Seedling length

On the eighth day, the seedlings from different concentrations were removed carefully and washed in water. The length of root and shoot was measured with the help of a wetted twine (for flexibility) and a scale.

Vigour index

Vigour index was calculated by the formula

V.I. = Germination percentage x total length of seedling (cm)

Total Chlorophyll

Chlorophyll content in the leaves was estimated by the method of Yoshida et al., (1971).

Procedure

1 gm of fresh leaves were cut into small pieces and homogenized in a mortor with pestle using 80% acetone. Decanted and filtered the supernatant through a funnel using Whatmann No.42 filter paper. Added sufficient quantity of 80% acetone and repeated the extraction. Transferred the contents from the motor to a funnel and washed the brei with acetone until it became colourless. Pooled the filtrates and made up the volume to 100 ml in a volumetric flask. Transferred, 5ml of the extract into a 50ml volumetric flask and diluted by making up the volumewith 80% acetone. Measured the absorbance at 645 and 663 nm for the determination of chlorophyll-a and chlorophyll-b and total chlorophyll.

The chlorophyll content was calculated on the fresh weight basis using the formula:

mg chlorophyll a /g tissue=12.7(A663) -2.69(A645) X V/1000 X W

mg chlorophyll a /g tissue=22.9(A645) -4.68 (A663) X V/1000 X W

mg total chlorophyll b /g tissue=20.2 (A645) +8.02(A663) X V/1000 X W

Carbohydrate

Carbohydrate contents in the leaves and seeds were estimated by the method of Dubois (1956).

PROCEDURE

100mg of the sample was weighed and it was hydrolysed by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N hydrochloric acid and cooled to room temperature. They were neutralized with solid sodium carbonate until the effervescence ceases. The volume was made upto 100ml and centrifuged. 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard were pipetted out into a series of test tubes. 0.2ml of the sample solution was pipetted out into another test tube. The volume was made up to 1ml with distilled water in all the tubes. 1ml of water was served as blank. 4 ml of anthrone reagent was added, heated for 8 minutes in boiling water bath, cooled rapidly. The blue colour developed was read at 630 nm.

Protein

Protein contents in the leaves and seeds were estimated by the method of Lowry et al., (1951).

Procedure

Extraction of protein from sample

Weighed 500mg of the sample and grinded well with a mortar and pestle in 10ml of the phosphate buffer. Centrifuged and used the supernatant for protein estimation.

Estimation of protein: Pipetted out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes. Pipetted out 0.1ml of the sample extract in another test tube. The volume was made up to 1ml in all the test tubes. A tube with 1ml of water served as a blank. Added 5ml of reagent C to all the test tubes including the blank. Mixed well and allowed to stand for 10 minutes then 0.5ml of reagent D was added, mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour was developed. Readings were taken in spectrophotometer at 660nm. Standard graph was drawn and the amount of protein in the sample was calculated.

RESULTS

Physico-chemical parameters of samples

The physical and chemical parameters *viz.,* colour, odour, temperature, pH, EC, total hardness, TSS, TDS, BOD, COD, carbonate, bicarbonate, calcium, magnesium, chloride, sodium, potassium, fluoride, nitrate, nitrite, sulfate, chromium, nickel, zinc, copper, iron, cadmium, lead and manganese were estimated and listed in the **Table-1**. The effluent released from the main outlet of Paper mill effluent is light brown in colour and has an offensive odour. The temperature is about 38° celsius. It is alkaline in nature and has a pH of 9.5. The Electrical conductivity of the sample was 30.7 (dsm^{-1}). The total hardness was 540 mg/l. The total suspended solids 750 mg/l and 906 mg/l total dissolved solids. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the effluent were found to be 1260 (mg/l) and 1765 (mg/l) respectively. It also contains carbonate (850 mg/l), bicarbonate (1423 mg/l), calcium (150 mg/l), magnesium (58 mg/l), chloride (1462 mg/l) and sodium (186 mg/l), potassium (600 mg/l), fluoride (6.0 mg/l), nitrate (46 mg/l), nitrite (32 mg/l), sulphate (345 mg/l).Nickel, Zinc, Copper, Iron, Cadmium, Lead and Manganese in the effluent were 58, 34, 2.8, 18, 5.0, 4.0 and 9 mg/l respectively.

Effect of untreated and bioremediated tannery effluent on seed germination, seeding length and vigour index of blackgram under *invitro* condition

The effect of untreated and bioremediated tannery effluent on seed germination, seedling length and the vigour index of blackgram given in **Table 2**. The seeds grown in control, 20 and 40%

untreated effluent showed the highest germination of 100% and its vigour index was 830, 920 and 900 respectively. Whereas in the bioremediated effluent except 100% concentration, other all the concentrations were recorded 100% germination was observed in all the dilutions. The vigour index at 20, 40 and 60 percent of the bioremediated effluent was increased over the control with 940, 960 and 850 respectively. Seeds of blackgram grown with 80 and 100% untreated effluent showed a reduction in vigour index (810 and 730) compared to the control plant. The seedling length showed an increase over control upto 60% concentration of bioremediated effluent and slightly decreased in the higher concentrations. The highest seedling length (9.6 cm) was recorded at 40% of the bioremediated effluent. The lowest seedling length (5.6 cm) was recorded at 100% of untreated effluent.

Effect of untreated and bioremediated Paper mill effluent on total chlorophyll, carbohydrate and protein content of blackgram under *invitro* condition

The total chlorophyll, carbohydrate and protein content of blackgram seedlings grown in different concentrations of untreated and bioremediated effluent are presented in **Table 3.** The total chlorophyll, carbohydrate and protein contents in untreated effluent showed a gradual decrease over control. In bioremediated effluent, the carbohydrate and protein contents were gradually increased over control upto 60% concentration. Total chlorophyll content was decreased from 80% and 100% concentration even in bioremediated effluent. The total chlorophyll, carbohydrate and protein contents were found maximum in the seedlings grown in 20% bioremediated effluent (0.843, 25.6 and 24.5 mg/g/plant) and minimum in 100 % untreated effluent (0.391, 12.4 and 11.6 mg/g/plant).

DISCUSSION

In developing as well as underdeveloped countries, the industrial effluents are released directly or indirectly into natural water resources, mostly without proper treatment, thus posing a serious threat to the environment (Altug and Balkis, 2009). Environmental pollution is an extremely important issue today, affecting all of us in one way or the other. Due to rapid increase in human population and industrialization, the demand for natural raw materials and source of energy are increasing day by day (Abhay and Rajput, 2009). Many rivers of the world receive flux of sewage, domestic waste, industrial effluents and agricultural waste which contain substances varying from simple nutrients to highly toxic chemicals (Benazir et al., 2010). Paper mill industry contributes significantly towards exports, employment generation and occupies an important role in Indian economy. But these waste waters are disposed into nearby water bodies and they are being used for irrigation. The discharge of this effluent into water bodies or on soil is causing a serious problem of water pollution resulting in severe damage to the flora and fauna and environmental degradation (Pande, 2005). Besides the heavy metals are non- biodegradable and persist for longer periods in aquatic as well as terrestrial environments thus they can exert detrimental effect on human health and environment due to the toxicity of heavy metals (Maliwal *et al*., 2004b). They interfere with physiological activities of plants such as photosynthesis, gaseous exchange and nutrient absorption and cause reduction in plant growth, dry matter accumulation and yield (Sharma and Agrawal, 2005). They cause direct toxicity, both to human

and other living beings due to their presence beyond specified limits. Heavy metal pollution of soil and waste water is a significant environmental problem and has a negative impact on human health and agriculture (Michalak, 2006).

The utilization of bioremediated industrial effluent for irrigation of crop plants is one of the highly beneficial proportions of safe effluent waste disposal. It not only prevents the waste from being an environmental hazard but also serves as an additional potential source of fertilizer for agricultural use (Swaminathan and Vaidheeswaran, 2005). With increasing awareness of public on the use of chemicals, the focus is now shifted towards the use of microorganisms for the treatment of the effluents which would be eco-friendly and cheap over the use of chemicals. Earlier several microbes were also reported to take effective part in bioremediation of industrial wastes (Moreira *et al*., 2004b; Margesin and Schinner, 2001a; Hanife Buyukgungor, 2000a).

With this background in mind, the present study was planned and conducted (i) To collect the Paper mill effluent sample and analyze the physico-chemical properties (ii) To isolate, characterize and identify effective indigenous bacterial strains for bioremediation of Paper mill effluent (iii) To study the effect of bioremediated effluent on growth and yield of blackgram (iv) To study the various biochemical, enzyme and heavy metal content of blackgram grown in different dilutions of raw effluent and bioremediated effluent. The results of the various experiments conducted on the above lines in *in- vitro*, pots and field conditions are discussed in the following pages.

Characterization of Paper industry effluent

Paper industry contains several organic and inorganic chemicals, which are toxic metals and they cause soil and ground water pollution. These chemicals cause adverse effect on plant growth and the health of animals and people living in that area.Chemicals are added in excess and are only partly taken up by the Paper mill and the remaining is released in the effluent (Scholz and Lucas, 2003).

Heavy metals can pose health hazards if their concentrations exceed allowable limits. Even when the concentration of metals does not exceed these limits there is still a potential for long-term contamination, and heavy metals are known to accumulate within biological system (Altaf et al., 2008). Hence the effluent released is expected to have a higher amount of chemicals and toxic metals. In this view, in the present study, the effluent was collected and characterized for certain physicochemical parameters.

The effluent released from Paper mill industry was brown in colour and had an offensive odour. The colour of the effluent might be due to the presence of biodegradable and nono biodegradable high molecular weight organic compounds and high amount of chemicals used during the processing and the odour may be due to the processing of skin and hides by soaking and liming. The yellowish brown colour might be hindering the penetration of sunlight causing depletion in the rate of oxidation process (Ravibabu et al., 2007) and this colour might be due to physico-chemical treatments (Zahid et al ., 2006).

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (APHA, 2005). The turbidity of the effluent might be due to the discharge of high concentrations of

carbonate, bicarbonate, chloride, calcium, magnesium and sodium used in Paper mill industry (Chakrapani, 2005).

In the present study, Paper industry effluent of hundred liters of effluent sample was collected in the plastic containers from the outlet of a Paper industry in RAJAGANAPATHY PAPER MILL (Board mill private ltd) VADAMANGALAM, Pondicherry. The pH of sample was recorded as 9.5, The higher pH of the effluent indicates the basic nature of the effluent. The pH of waste water could vary due to the presence of various Paper mill and colouring materials. Total hardness of the Paper effluent in the present study was found to be 540 mg/l. Hardness is an indication of calcium and magnesium found in higher concentration in effluent.

Electrical conductivity of the sample is 30.7. The higher electrical conductivity value of the effluent indicates that the discharge of chemicals as cations and anions were higher in the waste water. The higher conductivity alters the chelating properties of water bodies and creates an imbalance of free metal availability for flora and fauna reported by Akan et al., 2008. Venkatesh et al. (2009) also recorded that the electrical conductivity, pH, chloride, sulfides, biological oxygen demand and chemical oxygen demand in Paper mill effluent were much higher than the tolerance limits for industrial effluent discharged into land surface.

Levels of total suspended solids (TSS) found in the effluent (215mg/l) were greater than that of the permissible limit (100 mg/l). Somnath (2003) reported that larger solid particulate matter remains suspended as a result of charges on the surface of small particles in the effluent.

The effluent showed a higher level of total dissolved solids (TDS) (406 mg/l). Total dissolved solids are mainly due to carbonates, bicarbonates, chlorides, sulphates, phosphates, nitrates, nitrogen, calcium, sodium, potassium and iron already reported by Kannan et al., 2009. In the liming section of tanning process, protein, hair, skin and emulsified fats are removed from the hides, which are released in the effluent and therefore increase the total solids (Bhalli and Khan, 2006).

The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the effluent were found to be 1260 (mg/l) and 2035 (mg/l). BOD is the parameter which is widely used to determine the pollution load of waste water (Chavan and Wagh, 2005). It is the amount of organic matter in the water and the amount of oxygen required by the micro organisms to stabilize the biologically decomposable organic matter in wastes under aerobic conditions (Bhalli and Khan, 2006). Chemical oxygen demand (COD) is the test used to measure pollution of domestic and industrial waste (Chavan and Wagh, 2005). It is the amount of oxygen required for the oxidation of inorganic matter using a strong chemical oxidant. COD is tested to determine the degree of pollution in the effluent samples (Bhalli and Khan, 2006). The BOD and COD values in the effluent were found to be higher than that of the tolerance limits. The same results were also reported by Akan et al. (2007) in tannery effluent.

Industrial effluents and municipal sewage carry organic and inorganic substances which utilize dissolved oxygen resulting in oxygen depletion (Ravindran and vasudevan, 2008). Nearly 70% of the emission loads of biochemical oxygen demand (BOD), chemical oxygen demand (COD)

and total dissolved solids (TDS) emanate from the pretanning operations (Calherios et al., 2008b).

High carbonate (850 mg/l) and bicarbonate (1423 mg/l) in our sample contribute to the total alkalinity of the effluent. The similar findings were also reported by Balakrishnan and Karruppusamy, 2005. Usage of sodium bicarbonate during the process of pickling in tannery industry might have caused the excessive amounts of these in the effluent. The cations calcium and magnesium present in the effluent were found to be at higher levels (150 mg/l and 58 mg/l respectively) compared to BIS limits. Calcium and magnesium contribute to the hardness of the water and it imparts unpleasant odour, when present in higher levels also reported by More et al., 2002. The tannery effluent contains fairly large amount of calcium and magnesium because lime is used for loosening the hair.

Chloride is an indicator of pollution when present in higher concentrations (Singh et al., 2009). Sodium chloride used as a dehydrating and antiseptic agent is the source of chloride (Mehdi, 2005). The level of chloride in the effluent (1462 mg/l) was higher than that prescribed by BIS (2009). The presence of very high amounts of chloride and sulphate is responsible for high hardness and further it increases the degree of eutrophication. The similar study was done by Kannan et al., 2005.

The level of sodium and potassium in the effluent were 186 mg/l and 600 mg/l respectively. Sodium sulphide is used in the liming process of hide and skin. The residual sulphide in the range of 100 – 200 mg/l goes in the discharge and causes serious environmental problem. The higher concentrations of sodium and potassium in tannery effluent were also reported by Ram and Roger. (2004).

Fluoride levels in the effluent were also higher (6.0 mg/l) than that of Bureau of Indian Standards (2.0 mg/l). Less nitrate content (46 mg/l) was present in tannery effluent compared to standard value. Nitrite content in tannery effluent (32 mg/l) was also above the permissible limits (10 mg/l). Waste generated from tanning generally contains much higher concentration of total dissolved solids (TDS), total suspended solids (TSS), phenols, chromium, chlorides, nitrates, nitrites, ammonia and heavy metals also early reported by Das et al. (2010).

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