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PHYCOREMEDIATION OF SEWAGE WASTEWATER USING SELECTED SEAWEEDS FROM HARE ISLAND, TUTICORIN, TAMILNADU

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Abstract: Algal bioremediation is considered as an efficient and environmentally safe technology for inexpensive decontamination of polluted systems. Water is the most important resource for mankind existence and development. Therefore, maintaining the water to a high quality is crucial. Nowadays, the focus has shifted to the use of microalgal cultures, as an interesting step for wastewater treatments, which can improve the treatment process to get clean and healthy water.In the present study, seaweeds such as *Caulerpa racemosa* (Green algae), *Padina gymnospora* (Brown algae) and *Acanthophora spicifera* (Red algae) were used for the phycoremediation of sewage waste water in which *Caulerpa racemosa* played an important role of phycoremediation compared to other selected seaweeds. *Caulerpa racemosa* showed better effect in the reduction of BOD, COD, chloride content and removal of iron, chromium, copper (43.3%) in the waste water using the immobilized form of seaweeds in foam and agar method.The present investigation of phycoremediation was considered as an efficient and environmentally safe technology for inexpensive decontamination of polluted system to face the environmental challenge.

Index Terms - **Seaweeds, bioremediation, phycoremediation, *Caulerpa racemosa***

I. INTRODUCTION

Phycoremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. Bioremediation is a cost effective and efficient method of decontamination that has become increasingly popular now-a- days to reduce environmental pollution. In urban and semi-urban colonies, sewage disposal has become an ecological problem (Moore, 1998). Wastewater is a major problem faced by our environment. Wastewater causes water pollution, there by impacting biota death, driving the extinction of species and threatening human health. Depending on the source of the pollution, various indicators can be measured to determine the level of environmental pollution, including COD, BOD, nitrates, nitrites, phosphates, and ammonia (Shah *et al*., 2013).Alternative methods, such as adsorptive processes (including biosorption) and filtration membranes, have been proposed for the removal of metals in well-diluted solutions or as a complement to conventional treatments (Pozdniakova*et al.*, 2016).New technologies are being proposed to access the treatment of waste water. Algal form is considered as one of the components in new technology for waste water treatment. Algal bioremediation has been well studied over the past 40 years by Ryther (1972), Kuyucak (1988), Romero Gonzalez (2001) and Wang (2011).Considerable research efforts have been devoted to the development of algal biosorbents to remediate pollutants, particularly heavy metals (Hubbe,2011). Few species of marine algae such as *Ascophyllum* and *Sargassum*are effective in the biosorption of pollutants (Volesky and Fourest, 1996; Yu *et al*., 1999). The major advantage of this is that concentrations of heavy metals in the polluted environment are reduced to a very low level. In the past 20 years the use of immobilized enzymes or cell components for the production of a series of metabolites has become a branch of biotechnology of rapidly growing importance. The use of immobilized algae in the removal of heavy metal is efficient and offers significant advantages in bioreactors (Hameed and Ebrahim, 2007).The efficiency of removal is 80 to 90% for inorganic nutrients and nitrogen from the aquaculture wastewater. Depending on the culture system, the type of contaminant and environmental conditions, a diversity of seaweed genera can be used in bioremediation, and these include green seaweeds (Chlorophyta; predominantly *Ulva*), red seaweeds (Rhodophyta; predominantly *Gracilaria*), and brown seaweeds (Phaeophyceae; predominantly *Saccharina*, *Undaria*, and *Sargassum*). Concurrently, seaweed processing also produces high quantities of wastewater that contains residual chemicals that can pollute the environment, including NaOH, H_2O_2, KOH, and KCl (Sedayu *et al*., 2007). Research papers on biosorption have been mainly published regarding the synthetic metal solutions under laboratory conditions. However, the composition of real effluents is more complex than synthetic solutions, presenting light metal ions (e.g., Na+, K+, Ca_2+, Mg_{2+}) and other contaminants in large amounts. Thus, the biosorption from real effluents becomes highly competitive (Vijayaraghavan and Balasubramanian 2015; Pozdniakova *et al*., 2016). Thus, immobilization process in polymer

matrices, as alginate, can facilitate the bed packing and improve the distribution of biosorbent in the column. The present study was focused mainly on the bioremediation of wastewater and industrial effluent with the help of the few selected seaweeds.

II. METHODOLOGY

Seaweeds such as *Caulerpa racemosa*, *Padina gymnospora* and *Acanthophora spicifera* were collected from Hare Coast, Tuticorin. They were washed thoroughly and dried under shade and made into powder and stored. Waste water samples were collected in Waste Water Treatment Plant, Lady Doak College campus.

2.1 Physico-Chemical Analysis

The physico-chemical parameters of collected waste water and industrial effluent samples were determined before and after treatment by following the Standard Method Examination of Water given in APHA (2011). They are

2.1.1. Determination of pH

pH meter was switched on and left for 10 minutes to warm up. The electrode was rinsed with distilled water and wiped off with tissue paper. Electrode was dipped in standard buffer solution of pH- 7 for calibration. Again the electrode was rinsed with distilled water and dipped in standard buffer solution of pH- 4 and readings were noted.

2.1.2 Determination of Temperature

Water sample was taken in three different beakers and placed the thermometer in the first beaker for 2 minutes and the temperature was recorded in the file. This process was repeated with second and third beaker and the temperature reading were recorded. Average temperature was calculated.

2.1.3 Estimation of Total Solids

Previously dried empty porcelain dishes (W1) were already kept at 105^0C for 2 hours and in desiccator for 30 minutes. 100 ml of well mixed sample was taken in each of the porcelain dishes and placed the porcelain dishes in an oven at 105^0C carefully for 24 hours. After 24 hours, the dishes were taken out and kept in the desiccator for 30 minutes for cooling. Now the porcelain dishes were weighed and noted the final weight (W2). The process of weighing and drying was repeated till constant weight was achieved. TS (mg/l) = (W2-W1) x 106 / Sample volume (ml)

2.1.4 Estimation of Total Suspended Solids

The filter paper was dried in oven (105^0C) and cooled in desiccator and weighed. Then 300 ml of sample was filtered through filter paper and kept in oven for overnight, dried and then cooled it in a desiccator and weighed again. TSS was calculated using the formula- W2-W1 x 1000/V

2.1.5 Estimation of Total Dissolved Solids

The beaker was dried in oven (105^0C) and cooled in desiccator and then weighed. Then 300ml of filtered sample was taken in beaker and evaporated up to dryness on a hot plate, cooled in desiccators and weighed again. TDS was calculated using the following formula- W2-W1 x 1000/V

2.1.6 Determination of Biological Oxygen Demand (BOD)

Sample was taken in BOD bottle & 2 ml of $MnSO_4$ solution was added followed by the addition of 2 ml alkali iodide azide reagent. Appearance of yellow colour precipitate was obtained (if white precipitate appears it indicates that there is no DO). Put stopper carefully to exclude air bubbles and mix by inverting bottle 40-50 times. Allow to settle the precipitate. When precipitation has settled sufficiently add 2 ml conc. H_2SO_4. Mix by inverting bottle several times until precipitate dissolved completely. Titrate 50 ml sample with standard 0.025N sodium thiosulphate solution to a pale yellow color, add 1 ml starch indicator and continue titration until the disappearance of blue color. Note the reading and calculate DO by following formula
Amount of oxygen = Xml of Sodium thiosulphatex0.2x1000xcorrection factor/Volume of Sample
Correction factor = Volume of bottle/Volume of bottle-Reagents added for fixation
BOD (mg/L) = Initial DO in water sample – DO in water sample after 5 days of incubation

2.1.7 Determination of Chemical Oxygen Demand

50 ml of sample was taken into a 500 ml refluxing flask. 1 gm $HgSO_4$, glass beads and 5 ml of sulphuric acid reagent were added slowly with mixing to dissolve $HgSO_4$, while mixing to avoid possible loss of volatile materials. 25 ml $K_2Cr_2O_7$solution is added and mixed well. Flask is attached to condenser and cooling water was turned on. Remaining sulphuric acid reagents (70 ml) was added through open end of condenser. Reflux for 2 hours, cool, and wash down condenser with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with FAS using 0.1-0.15 ml (2-3drops) of ferroin indicator. COD (mg/l) = (A-B) x N x 8000/Volume of sample

2.1.8 Determination of Chloride

Take 100 ml sample in a conical flask and adjust the pH in the range of 7-10.Add 1 ml K_2CrO_4 indicator, titrate with $AgNO_3$ end point will be pinkish yellow. Note the reading repeat the titration with distilled water blank. Cl^-(mg/l) = (A-B) x N x 35.45 x 1000/ volume of sample

2.1.9 Test for Coliforms

Presumptive Test - Water samples were collected in sterilized bottles. 1.0ml of water sample was inoculated in sterilized lactose fermentation broth with Durhams tube. Incubate the tubes at 37^0C for 24 or 48 hours. If the presumptive test is positive, save the tubes showing the gas. Completed and Confirmed Test - Take the inoculum from the positive presumptive test was streaked onto the plates of EMB and Endo agar. Incubate the plates at 37˚C for two days and observe.

2.1.10 Analysis of Heavy Metals by Atomic Absorption Spectroscopy

The Copper ion, Chromium ion and Nickel ion present in the waste water sample before and after treatment were analyzed using Atomic Absorption Spectroscopy

2.2 Phycoremediation of Waste Water using Seaweeds

The waste water samples were inoculated with seaweeds such as *Caulerpa racemosa, Acanthophoraspicifera*and *Padina gymnospora* which were collected from Hare Island, Tuticorin, Tamilnadu, India. The seaweeds were dried and made into a powder. 15 gram of seaweed powder was taken and mixed with 100 ml of Guillard's F/2 medium and then centrifuged. The pure culture was obtained. The following treatments were applied for the phycoremediation of waste water and industrial effluent.

WATER SAMPLE (100% and 50%)	NAME OF THE SEAWEED
CR	*Caulerpa racemosa*
PG	*Padina gymnospora*
AS	*Acanthophoraspicifera*

2.2.1 Phycoremediation of Waste Water using Free Cells

To 100% and 50% of the waste water sample, 15ml inoculum of *Caulerpa racemosa, Acanthophoraspicifera*and *Padina gymnospora*were added. The treatments were studied for a period of 45 days.

2.2.2 Phycoremediation of Waste Water Using Immobilized Cells

2.2.2a Immobilization of Seaweeds in Foam

Polythene foam was cut into small cubes of the size 5mmx5mm. The cubes were then washed with distilled water and were dried in the oven. The foams were then transferred to the conical flask containing Guillard's F/2 medium and the corresponding seaweeds. About 20ml of the seaweed suspension were used the immobilization. The preparation was then incubated for a period, under agitation at 25˚c - 30˚c and an intensity of 3000 lux till the matrices got fully packed with the seaweed. Then the immobilized *Caulerpa racemosa, Acanthophoraspicifera* and *Padina gymnospora*were added into the respective effluents. The treatments were studied for a period of 45 days.

2.2.2b Immobilization of Seaweeds in Agar

Pure culture of *Caulerpa racemosa, Acanthophora spicifera* and *Padina gymnospora* were inoculated in 100ml of Guillard's F/2 medium in a 250ml conical flask and illuminated at ambient temperature. It was allowed to grow for 10-15 days, until it reached the exponential phase of growth. 2% agar was dissolved in the growth medium, sterilized and cooled at 35˚C - 45˚C. Concentrated seaweed culture to be immobilized was introduced and mixed for uniform distribution. This molten agar was poured to a glass plate and after solidification they were cut into cubes of the size 5mmx5mm.Kept under constant agitation till the cells in the pore spaces within the matrix. The matrix packed with the seaweed can be transferred to the water to be treated for the removal of metal ions.

3. RESULTS AND DISCUSSION

Bioremediation constitutes the use of natural biota and their processes for pollution reduction. It is a cost effective process and the end products are non-hazardous (Ahmedna *et al.*, 2004). Algae are important bioremediation agents, and are already being used in wastewater treatment. The potential for algae in wastewater remediation is however much wider in scope than its current role (Volesky, 1990; Wase and Forster, 1997).In the present study seaweeds such as *Caulerpa racemosa* (Green algae), *Padina gymnospora* (Brown algae) and *Acanthophora spicifera* (Red algae) were used for the phycoremediation of sewage waste water.

3.1 PHYSICO CHEMICAL STUDY

Physico-chemical characteristics of the waste water and industrial effluent were analyzed. Temperature, pH, BOD, COD, Chloride content, Total Solids, Total dissolved solids and presence of heavy metals such as copper, chromium and iron were analyzed for both waste water and industrial effluent.

3.1.1 Temperature

Temperature plays an important role in controlling other physicochemical and biological parameters of water. It plays an essential role in water quality and also influences the aquatic environment (Singh and Mathur, 2005) as the temperature of the water rises, the rate of photosynthesis increases, providing adequate amount of nutrients. It affects the production rate of various aquatic bacteria and production of algal biomass in fresh water as well as marine water (White *et al*., 1991)

3.1.2 pH

pH is the scale of intensity of acidity and alkalinity of water. It measures the concentration of hydrogen ions. pH determines the life of aquatic plants and other living organisms living inside the water. They are affected by pH because most of their activities are pH dependent. pH can also affect the solubility and toxicity of chemical and heavy metals in the water. Even minor changes of pH can produce long-term effects. Optimal pH range for sustainable aquatic life is pH 6.5-8.2 (Murdoch *et al*., 2001). pH of the waste water and industrial effluent is 8 and 6, and they were within the permissible limits.

3.1.3 Total Dissolved Solids

Total Dissolved Solids (TDS) is a measurement of inorganic salts, organic matter and other dissolved materials in water. Total dissolved solid cause toxicity through increase in salinity, changes in the ionic composition of the water and toxicity of individual ions. Total dissolved solids are an extremely important cause of water quality deterioration leading to

aesthetic issues, a decline in the fisheries resource, and serious ecological degradation of aquatic environments (Billota and Brazier, 2008).

3.1.4 Chloride

Chloride content in water plays a vital role in water quality. The accumulation of chloride poses a risk to the water quality. Chloride content in industrial effluent is 204 mg/L and in sewage water 298.76 mg/L

3.1.5 Biological Oxygen Demand

Biological Oxygen Demand test is the most widely used parameter of water analysis. The BOD may be defined as the oxygen required for the microorganisms to carry out biological decomposition of dissolved solids or organic matter in the waste water under aerobic conditions at standard. Therefore, with an increase in the amount of organic matter in the water the BOD increases. BOD is one of the common measures of pollutant organic material in water (Queresimatava and Solanki, 2015). BOD of the collected waste water is 24.06 mg/L and in industrial effluent is 26 mg/L. it indicated that the amount of organic matter is low

3.1.6 Chemical Oxygen Demand

Chemical Oxygen Demand determines the available dissolved oxygen in water. High COD level decrease the amount of dissolved oxygen available for aquatic organism. COD of collected effluents ranges between 125 to 174.02 mg/L. Permissible limit of COD is 100 mg/L. high level of COD indicates low amount of dissolved oxygen available which has an adverse effect on aquatic flora and fauna

3.2 PHYCOREMEDIATION OF WASTE WATER BY FREE CELLS, IMMOBILIZED FOAM, IMMOBILIZED AGAR (DAY – 1)

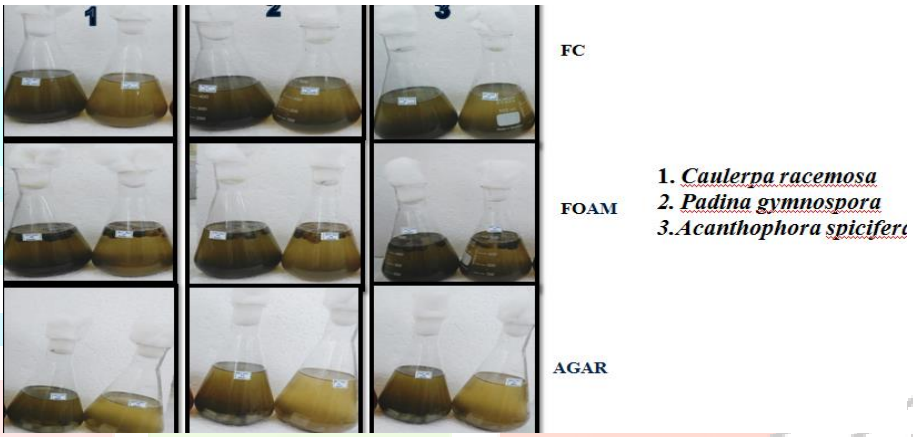

Figure 3.2.1 preparation of Free Cells, Immobilized Foam, Immobilized Agar (Day – 1)

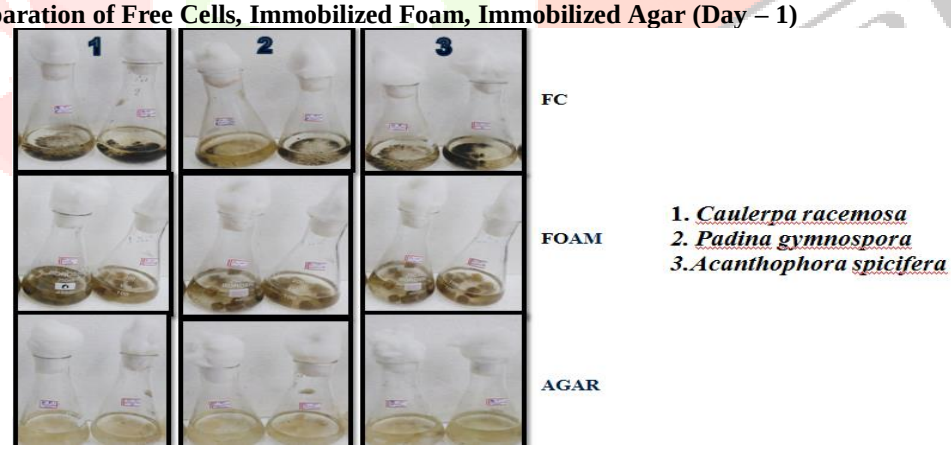

Figure 3.2.2 Preparation of Free Cells, Immobilized Foam, Immobilized Agar (Day – 45)

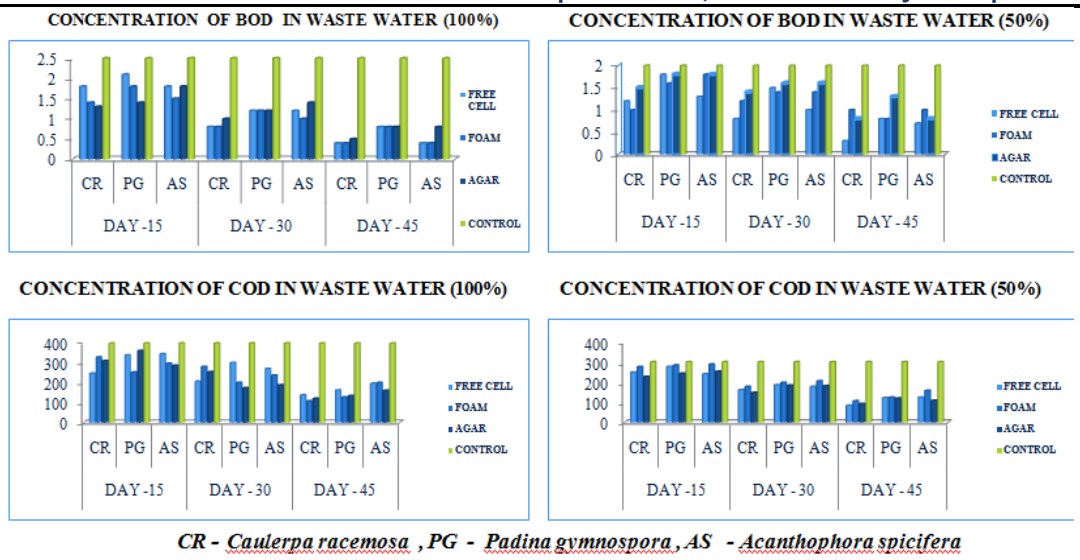

Figure: 3.2.3 Efficacy of Seaweed bioremediation in the concentration of BOD and COD in waste water

The inoculation of the effluent with the seaweed strains led to the decrease in both BOD and COD at all the tested incubation conditions and concentrations compared to the initial BOD and COD values in both the samples. Decrease in BOD and COD levels is suggestive of the fact that the process of remediation is in progress, as earlier studies have shown that the high BOD and COD levels are often indicated in waste water containing substances that can be biologically degraded. (Ademorotti *et al.*, 1992, Pathe *et al.*,1995). However, greater reduction potential was observed in the immobilized cells (Agar) when compared to free cells and also in the immobilized cells (Foam). Immobilized living cells have some advantages over free cells as they provide simple treatment for liquid, final separation of cells not required and metabolic activities remain constant for longer periods (Lukarsky *et al.*, 1996). The most commonly used matrices are Agar, Alginate beads, Polyurethane and polyvinyl foams.

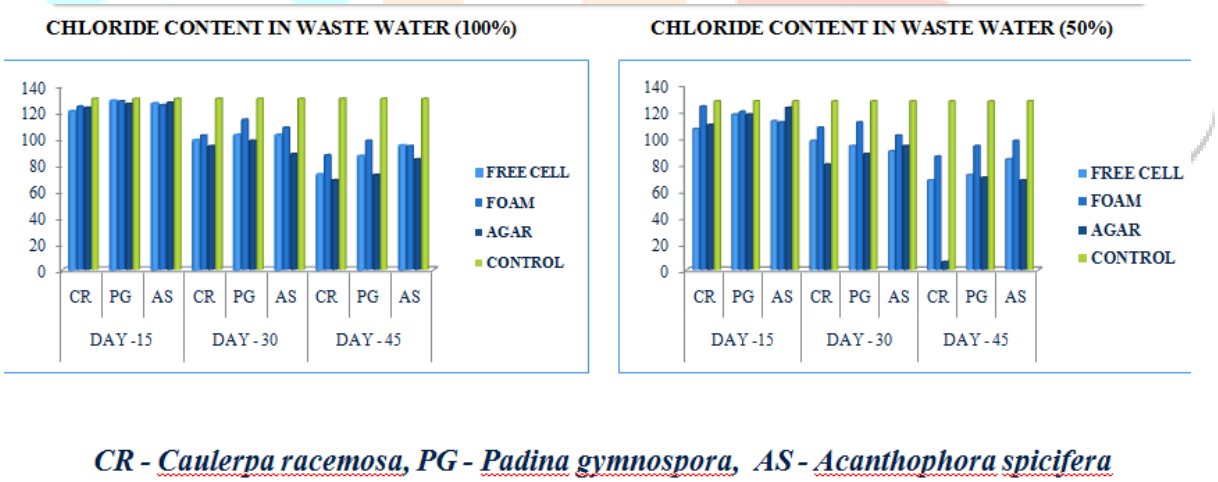

Figure: 3.2.4 Different ratio of efficiency of seaweed in removing the Chloride content

The above figure represents the removal efficiency of chloride by seaweeds. Depletion of chloride content in the treated samples by Agar and free cells of *Caulerpa racemosa* is comparatively higher than the *Padina gymnospora* and *Acanthophoraspicifera.* Chloride level is gradually reduced after 30 and 45 days of incubation respectively.

3.3 HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROSCOPY

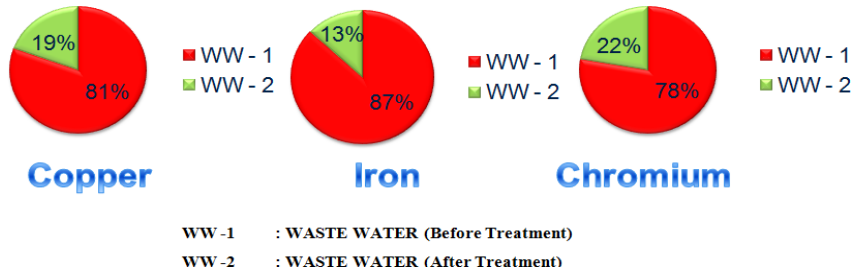

Figure: 3.3.1 Concentration of Copper, Iron, Chromium in waste water before and after treatment

The above figure represents the removal ability of metal ions by seaweeds (*Caulerpa racemosa, Padina gymnospora, Acanthophora spicifera*). Found 43.3% of decrease in copper biosorption by esterifying biomass of the species *Durvillaea antarctica* using methanol in acid media. (Mata *et al.,)* determined the effect of the immobilization of *Fucus vesiculosus* with alginate xerogels in the biosorption of Cd, Cu, and Pb. Copper, Iron and Chromium was found to be present in both the waste

water and industrial effluent. Metal ion concentration was reduced effectively when compared before treatment and after treatment of the seaweeds.

3.4 Tests for Coliforms in Waste Water:

Reduction in Total Coliforms in pond were observed based on the 99% removal of all fecal indicator bacteria and similar result was observed by Winfrey *et al.*,(2010). In the presence of sunlight, fecal coliforms removal increased because sunlight promoted their inactivation. Attachment of fecal coliforms along with other bacteria on the surface was also reported by Awuah, (2006). The presumptive test is the screening test performed in the samples to observe the presence of coliform bacteria present in water samples, the coliform bacteria utilize the lactose and produces gas and acid which was observed in Durham's tube. It was confirmed using Endo agar and EMB media, the colonies show greenish metallic sheen in Eosin Methylene Blue agar media and pink color in Endo agar medium. Present study revealed that the utilization of seaweed for wastewater and industrial effluent treatment are very effective in reducing the total coliform.

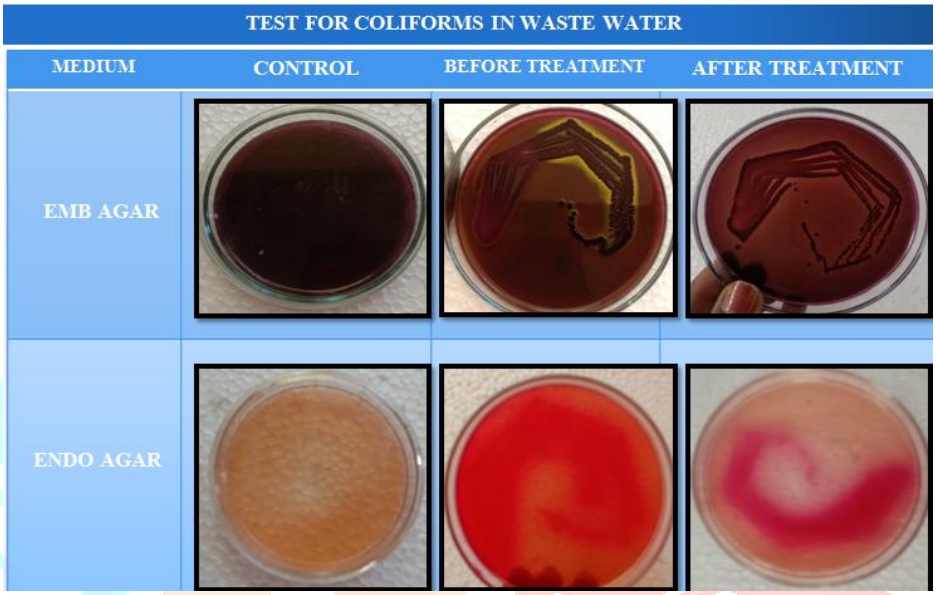

Figure 3.4.1 Test for coliforms in waste water

4. CONCLUSION

Algal bioremediation is considered as an efficient and environmentally safe technology for inexpensive decontamination of polluted systems. It is widely used for heavy metal removal from waste water. Water is the most important resource for mankind existence and development. Therefore, maintaining the water to a high quality is crucial. Wastewater treatment technologies are constantly evolving. Nowadays, the focus has shifted to the use of microalgae cultures, an interesting step for wastewater treatments, which can improve the treatment process in order to get clean and healthy water. Seaweeds can be used in waste water for a range of purpose including, reduction of BOD and COD, removal of metal ions, reduction of chloride content and for the removal of coliform bacteria. In the present study, seaweeds such as *Caulerpa racemosa*, *Padina gymnospora* and *Acanthophora spicifera* were used for the phycoremediation of sewage waste water and industrial effluent in which *Caulerpa racemose* played an important role of phycoremediation compared to other selected seaweeds. Cells immobilized in agar were more effective than the process of immobilization in Foam.The treated samples also enhanced the growth of *Vigna radiata*, thus the treated samples can also be used as a fertilizer for the growth of crops. The potential for algae in heavy metal removal from waste water and industrial water wastewater and remediation is however much wider in scope than its current role. The present investigation of phycoremediation was considered as an efficient and environmentally safe technology for inexpensive decontamination of polluted system to face the environmental challenge.

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