Summary and Conclusion

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The textile wet processing industries line up as processing, dyeing and finishing units. Amongst the various activities in textile wet processing industry, chemical processing contributes about 70% of water pollution. Water plays a very important role in all areas of textile wet processing such as heating, cooling and washing. The processes also produce considerable amount of waste water, due to the use of dyes and auxiliaries.

It is well known that cotton processing units consumes large volumes of water for various processes such as sizing, desizing, scouring, bleaching, mercerization, dyeing, printing, finishing and washing. These processes lay the foundation for colorful, comfortable, fashionable and value added products on the brighter side. Environmental concerns are not only threating the textile industry, but the entire chemical industry. The process being heterogeneous phase reactions, complete removal of dyes and chemicals is highly impossible.

Although decolorization of effluent is a challenge for textile industry, as well as waste water treatment systems, the literature suggest that there is a great potential for developing microbial decolorization systems, with total color removal in some cases within few hours. Biological treatment methods are attractive due to their cost effectiveness, diverse metabolic pathways and versatility of micro-organisms. Several chemical and physical decolorization methods that are available include, adsorption, precipitation, coagulation, flocculation, oxidation, and electrolysis and membrane extraction. They also concentrate the pollutants into solid or liquid side streams which require additional treatment or disposal thus escalating cost of effluent treatment. Biological decolorization is the most common and widespread technique used in textile effluent treatment. The two types of biological treatment are aerobic and anaerobic. Aerobic systems require oxygen for fungi and bacteria to perform the degradation process whereas, anaerobic operate in the absence of air and under static conditions.

Odor may be caused by a variety of odorous compounds that are released or generated by various waste water processes. Odorous waste gases are a special kind of air pollutants. Humans can perceive even extremely small amounts of an odorant. It is estimated that only 10^8 or 10^9 molecules of odorant vapor in the nose is enough to trigger detection.

A biological odor treatment system has many advantages compared to conventional physical and chemical treatment technologies. It is highly efficient in the treatment of waste gases characterized by high flow rates and low concentrations of contaminants and the pollutants are completely destroyed and its cost is also low. Considering these facts the research was planned with the following objectives.

The objectives of the study are:

- To collect the textile waste water effluent,
- To isolate the *Bacillus Subtilis* and *Thiobacillus Bacteria* by biochemical tests,
- To decolorize the textile effluent under different parameters,
- To optimize the decolorizing medium by using different parameter like pH, temperature, inoculums size and co-substrates,
- To measure the odor degradation by gas chromatography,
- To reuse the decolorized effluent water for dyeing,
- To evaluate the dyed samples.

Experimental procedure

Screening and Isolation of Textile Dye Effluent Decolorizing Bacteria

Effluent is an out flowing of water or gas from a natural body of water, or from a human-made structure. Generally it refers to wastes discharged into surface waters". Effluent is also defines as "liquid waste or sewage discharged into a river or the sea".

Samples were collected from a textile dyeing unit of effluent treatment plants located in Tirupur. The container was rinsed with the sample before collection. The effluent was collected in a sterile polythene container.

Initially serial dilution plating technique was followed for the isolation of bacteria and then was cultivated on nutrient agar medium. The plates were incubated at 30°C for 24 hrs. Isolated colonies were subjected to biochemical tests were performed to identify microbes such as standard catalase test, citrate utilization, coagulase, oxidase, Methyl red, Voges-Proskauer, Indole production, motility, Glucose, sucrose, maltose, lactose, Characterization and identification of the isolates was done.

Bacterial cultures were made on clean grease free slides. The slide was flooded with crystal violet solution for a minute, drained and rinsed with water, followed by Grams iodine solution for one minute, drained and rinsed with water. Decolourised with ethyl alcohol for 30 sec and later counterstained with safranin for one minute and observed under an oil immersion microscope.

The hanging drop technique was followed to observe the motility of the organism. Observation was made under the microscope.

A small amount of culture was placed over a clean slide. A drop of three percent hydrogen peroxide was placed over the culture and observed for effervescene. The production of effervescence showed the ability to produce the enzyme catalase.

The organism spotted on oxidase disc (HiMedia) the blue or purple colour change was observed within ten seconds.

Biochemical test

- **Indole test**

The culture was inoculated into indole medium and incubated at 37°C for 48 – 72 hours. About 0.2 – 0.3 ml of Kovac's reagent was then added. The formation of red ring on the surface of the broth confirmed the production of indole.

- **Methyl Red Test**

Culture was inoculated with Methyl red – Voges proskauer (MR-VP) broth and incubated for 48 – 72 hrs at 37°C. The appearance of a red colour on addition of methyl red solution was considered as positive.

- **Voges – Proskauer Test**

Culture was inoculated with MR - VP medium and incubated at 37°C for 24-48 hrs. After incubation, 3 ml of Barrit's reagent A and one ml of Barrit's reagent B was added. The development of pink color was considered as positive.

- **Test for H_2S Production and Glucose Utilization**

Culture was inoculated with Triple sugar iron agar slants and incubated at 37°C for 24 hrs. A blackening of the medium indicated production of H_2S. Breaks in the medium showed production of gas from glucose.

- **Urease Test**

Culture was inoculated with urease medium and incubated at 37°C for 24 hrs Phenol red which is incorporated in the medium changes its color from yellow to red in alkaline pH, thus indicating the presence of urease activity.

Dye Degradation by *Bacillus subtilis*

The *Bacillus subtilis* was used to decolorize the textile dye effluent maximum rate of decolorization was observed (90%) when starch & peptone was supplemented in the medium. The optimum dye decolorizing activity of the culture was observed at pH 7.0 and incubation temperature of 370°C. Maximum, dye-decolorizing efficiency was observed at 200 mg/1 concentration of Textile Dye Effluent. Decolorization was confirmed by UV-VIS spectrophotometer. The initial dye solution showed high peak at the wavelength of 560nm. The decolorized dye showed disappearance of peak, which indicated that the decolorization is due to dye degradation. Decolorization was carried out following the methods below.

Plate Assay

Plate assay was performed for the detection of decolourizing activity of bacteria. The nutrient agar and textile dye effluent were autoclaved at 121°C for 15 minutes. *Bacillus subtilis* culture was plated on nutrient agar plates containing dye and incubated at 37°C for seven days.

Tube Method

The bacterial cultures were transferred to fresh nutrient medium (Casein, Yeast extract, Nacl) containing textile dye effluent (250 mg/l) and were incubated at 32°C, under static condition for three days and centrifuged at 10,000 g for ten minutes in a centrifuge at room temperature The supernatant was used for analysis of decolorization .

Conical Flask

Conical flask assay was performed for the detection of decolorizing activity of bacteria. The nutrient broth containing textile dye effluent was autoclaved at 121°C for 15 minutes. Five percent inoculums of the selected culture were added to nutrient broth flasks and were incubated at 32°C for three days.

Isolation of *Thiobacillus bacteria* from textile Dye Effluent

Isolation medium was *Thiobacillus* mineral N salts medium (MSM) which consisted of 1.0 ml trace element in 1000 ml distilled water. The pH was adjusted to 6. NaOH dissolved in 1000ml of distilled water (DSMZ, 2002). Then agar of 15 g was added to solidify the medium. Biochemical tests used were: catalase, oxidase, urease, MR (methyl red), VP (Voges-Proskauer), H_2S production, glucose (acid-gas) and utilisation of thiosulphate,and sugurs.

Odor Degradation by *Thiobacillus bacteria*

Into phosphoric acid-coated serum vials (68.4 ± 0.6 ml) was put 3% of 50mm Tris/HCl buffer containing 0.2mM EDTA. These were then sealed with Teflon-coated rubber stoppers. Three millilitres of headspace gas was removed and replaced by three ml of MM gas (1950 ppm). Vials containing H_2S were shaken for five minutes at 25°C and headspace gas concentration analyzed by injecting 50j.iL of gas into the gas chromatograph. To these vials were then added xml (0.05 to 0.5ml) of the crude bacterial sample.

Degradation of Odor Causing H_2S

The odor degradation was measured by Gas chromatography technique. After adding of bacterial cell culture, the H_2S level was reduced compared to control. Control has maintained without bacterial culture. Only very minimal degradation of H_2S was recorded.

Selection of Fabric

Cotton is one of the most commonly abundant natural fibre having various advantageous such as availability, comfortability, excellent heat conductivity and hydroscopic in nature. The fibre is most often spun into thread and used to make a soft, breathable textile. Their ravel was less than compared with the fabric of other weaves. Hence, cotton fabric made out of plain weave was selected for the present study.

Selection of Dye

Among the various fibers and dye classes, cotton and reactive dye system is the most popular due to their high wet fastness, brilliant color and variety of hue. To achieve good fastness properties of dyeing, reactive dye was selected for the study.

Dyeing Procedure

The desized cotton fabric was taken and weighed using an electronic balance and the dye solution was prepared based on the weight of the desized cotton fabric. The fabric to be dyed was immersed in the dye solution and the temperature was raised to 80°c and maintained for 15 minutes. Later the fabric was lifted and the common salt was added and stirred well. The fabric was put back and boiling was continued for another ten minutes. The fabric was lifted and sodium bicarbonate was added and stirred well. The fabric was put back and boiling was continued for another ten minutes. After the specified period the fabric was washed thoroughly by changing water thrice Acetic acid (four grams/liter) was added in the final rinse of water. Finally the fabric was taken out and squeezed and dried in shade. Following the same procedure, dyeing was performed in dye solution prepared with treated water for selected fabric. Thus the cotton fabric was dyed using treated effluent water and soft water with reactive dye.

Evaluation

Visual inspection was conducted for soft water dyed and treated effluent water dyed samples. The judges (25) comprised of PG students specializing in the field of Textile and clothing. General appearance, brilliancy of shade, and evenness of dyeing were the main aspects taken into consideration for visual examination.

Findings of the study

Isolation of bacillus subtilis from textile waste effluent

The biochemical test such as Catalase test, Voges proskauer test, Citrate utilization test, Starch hydrolysis test, Gelatin hydrolysis test, Caesin hydrolysis test, Nitrate reduction test, Sugar fermentation test D-Glucose, L-Arabinose, D-Mannitol and D-xylose showed positive result and Urease test, Indole test, Methyl Red test and Gas from Glucose showed negative result. Oxidase test showed result as d, which means variable (may be positive or negative).

Isolation of thiobacillus bacteria from textile waste effluent

The biochemical test such as Catalase test, Urease test, Methyl red test, citrate utilization test, sugar fermentation test D- Glucose, L-Arabinose, D-Mannitol and D-xylose showed positive result and Oxidase test, Indole test and Voges proskauer test showed negative result and TSI showed result as A/A, Gas, Acid sland and /acid bud, Gas produced in tube.

Optimization of Different Parameters for textile dye Effluent decolorization Using Bacillus subtilis Bacteria

- The increase in inoculum concentration from 5% to 20%, increased decolorization, with optimum decolorization at 3%. But an increase in inoculum concentration above 20 per cent results in decrease in decolorization.
- The third day showed the maximum decolorization. This was also considered as the optimum incubation period.
- The maximum decolorization is seen when the pH is 6.5. Thereafter an increase in the pH showed a decrease in decolorization.
- The optimum temperature for maximum decolorization was 37°C. There is a decrease in percentage decolorization after 37°C.