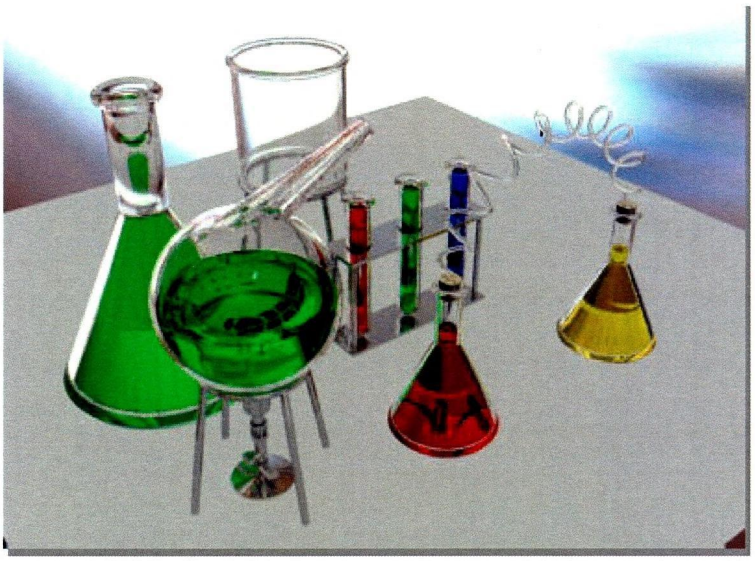


Methodology

3. EXPERIMENTAL PROCEDURE

The experimental procedure adopted for the present study "**Removal of Color and Odor from Textile Dye Effluent Using *Bacillus Subtilis* and *Thiobacillus Bacteria.***" is discussed under the following headings

PHASE I

3.1 Screening and Isolation of textile dye effluent decolorizing bacteria

3.2 Optimization of different parameters for textile dye effluent Decolorization using *Bacillus Subtilis Bacteria*

3.3. Analysis of textile dye Effluent and Treated Effluent

3.4. Organic pollutant measurement

PHASE II

3.5 Dye Degradation by *Bacillus Subtilis*

3.6 Odor degradation by *Thiobacillus Bacteria*

PHASE III

3.7 Utilization of Treated water for dyeing cotton fabric

3.8 Dyeing procedure

3.9 Nomenclature

PHASE IV

3.10 Evaluation

PHASE I

3.1. SCREENING AND ISOLATION OF TEXTILE DYE EFFLUENT DECOLORIZING BACTERIA

3.1.1. Effluents

Effluent is an out flowing of water or gas from a natural body of water or from a human-made structure. Effluent is defined by the United States Environmental Protection Agency (2010) as "wastewater - treated or untreated - that flows out of a treatment plant, sewer, or industrial outfall. Generally refers to wastes discharged into surface waters". Effluent is also defines as "liquid waste or sewage discharged into a river or the sea" (2010).

3.1.2. Selection of source

Samples were collected from a textile dyeing unit of effluent treatment plants located in Tirupur. The effluent sample was collected from the middle point of the area. The effluent was collected in a sterile polythene container. As per the suggestion given by Manivasagam (1995) the container was rinsed with the sample before collection. The sample was transported to laboratory at 40°C as in accordance with the standard methods (Plate I).

3.1.3. Isolation of *Bacillus Subtilis* from Textile Waste Effluent

Initially serial dilution plating technique was followed for the isolation of bacteria and then was cultivated on nutrient agar medium which consists of Peptone of 5gm, Yeast extract of 2gm, Beef extract of 3gm, NaCl of 5gm and Agar of 15gm. The plates were incubated at 30°C for 24 hrs. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation (Plate II).

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.

PLATE I

TEXTILE DYE EFFLUENT FROM DYEING UNIT

Preliminary Test

- **Gram Staining**

Bacterial smears of 16-18 hrs old cultures were made on clean grease free slides. The slide was flooded with crystal violet solution which contains crystal violet of 2g in 20ml of 95% ethanol and ammonium oxalate of 8g in 80ml of distilled water for a minute, then drained and rinsed with water. This was further followed by Grams iodine solution which consists of iodine of 1g, potassium of 2g in 300ml of distilled water for a minute, drained and rinsed with water. Decolorized was carried out in a solution contained 70ml of 95% ethanol in 30ml in distilled water for 30 Sec. This was then counter stained with 0.25g safranin and 10ml of 95% ethanol in 100ml of distilled water for one minute and observed under an oil immersion microscope.

- **Motility Test**

Motility Test was conducted by hanging drop technique to observe the motility of the organism. Observation was made under the microscope.

- **Catalase Test**

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 effervescence. The production of effervescence showed the ability to produce the enzyme catalase.

- **Oxidase Test**

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

➢ **Biochemical Test**

- **Indole Test:**

The culture was inoculated into indole medium which contains 10g of tryptone in 1000ml of distilled water, incubated at 37°C for 48 - 72 hours. About 0.2 - 0.3 ml of Kovac's reagent which contains 5g of para- dimethyl amino benzoldehyde, 75ml of butyl alcohol in 25ml of concentrated hydrochloric acid, was then added to the test tube, shaken and allowed to stand. The formation of red ring on the surface of the broth confirmed the production of indole.

- **Methyl Red Test:**

In order to conduct methyl red test culture was inoculated with Methyl red - Voges proskauer (MR-VP) broth which contains 7g of peptone, 5g of dipotassium phosphate, 5g of sodium chloride was mixed with 1000 ml of distilled water and incubated for 48 - 72 hrs at 37°C. pH was maintained with 7.2. The appearance of a red color on addition of methyl red solution was considered as positive.

- **Voges - Proskauer Test:**

In order to conduct 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 which contains 5g of 5% of alpha naphthol in 95ml of absolute ethanol and 1ml of Barrit's reagent B which contains 40g potassium hydroxide, 3g of creatine in 1000ml of distilled was added. The tubes were shaken and allowed to stand for 15 minutes and observed for color change. The development of pink color was considered as positive.

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

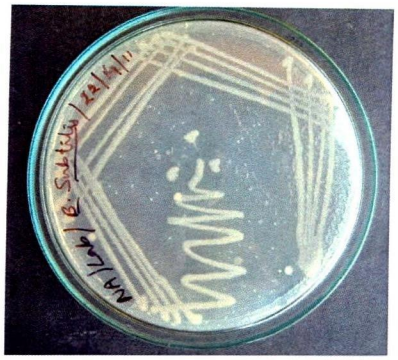
In order to conduct the Test For H_2S Production and Glucose Utilization, Culture was inoculated with Triple sugar iron agar slants which

contains 3g of Beef extract, 3g of Yeast extract, 20g of Peptone, 5g of Sodium chloride, l0g of Lactose, l0g of Sucrose, 1g of Dextrose, 0.2g of Ferrous Sulphate, 25g of Sodium thiosulphate, 15g of Agar, 0.024g of Phenol red was mixed with 1000ml of distilled water .pH was maintained at 7.2, and incubated at 37°C for 24 hrs. The change in color of the medium was noted from red to yellow which indicated the production of acid from glucose. A blackening of the medium indicated production of H_2S. Breaks in the medium showed production of gas from glucose.

- **Urease Test**

In order to conduct the Urease test, culture was inoculated with urease medium which contains 1g of Peptone, 1g of Dextrose, 5g of Sodium chloride, 1.20g of Dipotassium phosphate, 0.8g of Monopotassium phosphate, 0.012g of Phenol red, 20g of Agar was mixed with 1000ml of distilled water. pH was maintained with 6.8. Culture was inoculated with urease medium and incubated at 37°C for 24 hrs. Urea is a diamide of carbonic acid. Urease is the enzyme possessed by the bacterium which hydrolysis urea and releases ammonia and carbon-di-oxide, ammonia reacted in the solution to form ammonium carbonate which was alkaline leading to increase in the pH. Phenol red which was incorporated in the medium, which changed its color from yellow to red showing alkaline pH, thus indicating the presence of urease activity.

Bacillus Subtilis **on nutrient agar**



Biochemical test

Oxidase

Catalase

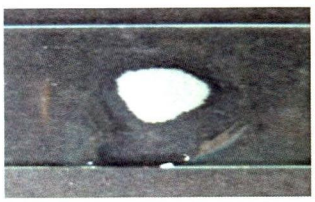
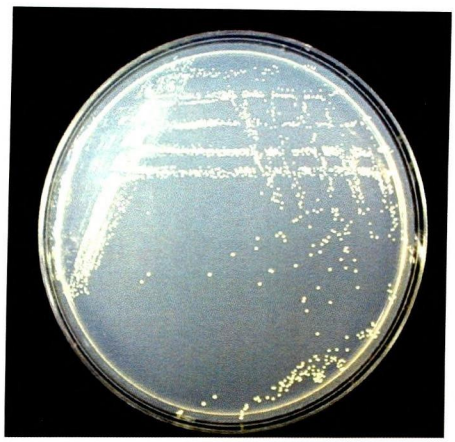


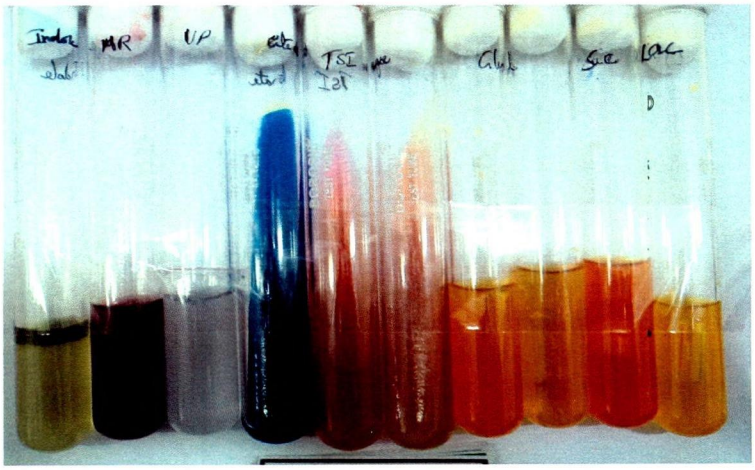
PLATE II

ISOLATION OF *BACILLUS SUBTILIS*

Thiobacillus Bacteria



Biochemical test



Oxidase

Catalase

PLATE III

ISOLATION OF *THIOBACILLUS BACTERIA*

3.1.4. Isolation of *Thiobacillus Bacteria* from textile dye Effluent

Isolation medium was *Thiobacillus* mineral salts medium (MSM) which was composed of 2g of KNO_3, 1g of NH_4C1, 2g of KH_2P0_4, 2g of $NaHCO_3$, 0.8g of $MgS0_4.7H_20$, 5g of $Na_2S_203.5H_20$ and 1ml of trace element in 1000 ml distilled water and the pH was adjusted to 6 with 1N KOH. The composition of trace element were as follows: 50g of Na_2- EDTA, 7.34g of $CaCl_2.2H_20$, 5g of $FeS0_4.7H_20$, 2.5g of $MnCl_2.4H_20$, 2.2g of $ZnS0_4.7H_20$, 0.5g of $(NH_4)_6$ $Mo_70_{24}.4H_20$, 0.2g of $CaS0_4.5H_20$ and 11g of NaOH dissolved in 1000ml of distilled water (DSMZ, 2002). And agar of 15g was added to solidly the medium as suggested by Kantachote and Innumwat (2004). Biochemical tests used were: catalase, oxidase, urease, MR (methyl red), VP (Voges-Proskauer), H_2S production, glucose (acid-gas), utilisation of thiosulphate and sugurs. (Plate III). These tests were conducted to identify, isolate and grown *Thiobacillus Bacteria.*

3.2. OPTIMIZATION OF DIFFERENT PARAMETERS FOR TEXTILE DYE EFFLUENT DECOLORIZATION USING *BACILLUS SUBTILIS* BACTERIA

3.2.1. Effect of Inoculum Concentration on decolorization of textile dye Effluent

Conical flask 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 (Plate IV). Five percent inoculums of the selected culture showing maximum decolorizing activity was added to nutrient broth flasks containing Textile dye effluent (250 mg /l). The flasks were covered with aluminum foils and incubated at 32°C for Three days. These flasks were observed for decolorization of the dye present in the medium.

3.2.2. Effect of Incubation Period on decolorization of textile dye Effluent

To determine the effect of incubation period on decolorization, 100 ml of 0.02% textile dye effluent was taken in five different 250 ml Erlenmeyer flasks and were inoculated with *Bacillus Subtilis* at a concentration of five percent was incubated for different time intervals (1,2,3,4,5,6,7 days) and to determined the percent decolorization.

PLATE IV
AUTOCLAVE

PLATE V
NANO UV - VIS SPECTROPHOTOMETER

3.2.3. Effect of pH on Decolorization of textile dye effluent

To determine the effect of pH on decolorization 0.02% textile dye effluent was adjusted to 4,5,6,7 and 8 pH using IN HCL or IN NaOH. The dye solution adjusted with different pH was added with 20% of inoculums and incubated for optimized incubation period (four days) at room temperature. After incubation period, percent decolorization was calculated.

3.2.4. Effect of Temperature on textile dye effluent Decolorization

The dye decolorization activity of the culture was found to increase with increase in incubation temperature from 25°C to 37°C with maximum activity attained at 37°C (1.203mg/l/h). Further increase in temperature resulted in maginal reduction in decolorization activity of the bacterial culture *Bacillus subtilis*. Thus the bacterial culture *Bacillus subtilis* was more sensitive to temperature.

3.2.5. Effect of Co Substrate on Dye Decolorization

Bacterial culture *Bacillus subtilis* exhibited maximum decolorization of textile dye effluent when starch and peptone were supplemented in the medium. In absence of co-substrate the bacterial culture was unable to decolorize the dye, which indicates the availability of supplementary carbon source seems to be necessary for growth and decolorization of dyes, view Nigam et al., (1996). The ability of our culture to use starch and peptone as co-substrates was encouraging from a commercial point of view.

3.3. ANALYSIS OF TEXTILE DYE EFFLUENT AND TREATED EFFLUENTS

The raw effluent and treated effluent were analyzed for finding out the toxic substances present in them and to know the pollution load of the water besides the quality of water.

3.3.1. General physico chemical parameters

The major physico-chemical parameters that were taken into consideration for analysis were pH, chloride content, amount of total dissolved solids, total suspended solids, biochemical oxygen demand and chemical oxygen demand as suggested by Shukla (2005) and Quevauviller (2002).

3.3.1.1. pH

The intensity of acidity or alkalinity (pH) determines the concentration of hydrogen ions present in water, reports Ansari and Parmer (2008). It is the most important parameter as it indicates instantaneously, the acidic or alkaline condition of the effluent water, views Kenkel (2002). Litmus paper can be used to measure pH or with a pH indicator with a color scale or a specially designed voltmeter, called pH meter, expresses Alexander (2000).

The pH meter was first standardized using buffer solutions of pH 7.0 and pH 9.2. The electrodes were rinsed in distilled water, immersed in the untreated and treated effluent samples. Then the readings were noted in the digital display.

3.3.1.2. Total Suspended Solids

Suspended solids of the untreated and treated effluent samples were estimated by centrifugation method. Fifty ml of the sample was centrifuged and the residue was washed with distilled water. Later it was recentrifuged and the suspended solids in the centrifuge tube was transferred to a pre weighed silica dish and dried at 105°C. The increase in weight was equal to the amount of suspended solids. The suspended solids present in the sample were calculated by using the formula.

$$\text{Total suspended solids in mg/l} = \frac{\text{Final wt. - Initial wt. of the crucible}}{\text{Volume of the sample}} \times 1000$$

3.3.1.3. Total Dissolved Solids

Fifty ml of the untreated and treated effluent samples were taken in a preweighed silica crucible and the samples were evaporated to dryness using a water bath. After complete evaporation the final weight of the crucible was taken. The total dissolved solids present in the samples were calculated by using the following formula

$$\text{Total Dissolved solids in mg/l} = \frac{\text{Final wt. - Initial wt. of the crucible}}{\text{Volume of the sample}} \times 1000$$

3.3.1.4. Turbidity

The turbidity was estimated by Jackson candle method and was presented as number of Jackson turbidity units (Plate VI).

3.3.1.5. Electrical Conductivity

The electrical conductivity of the untreated and treated effluent samples was measured, using Conductivity Bridge and expressed in millisiemens (ms) (Plate VII).

3.4. ORGANIC POLLUTANT MEASUREMENT

3.4.1. Biochemical Oxygen Demand (BOD)

Reagents

- **Phosphate buffer solution**

Disodium hydrogen phosphate of 33.4g, 8.5g of potassium dihydrogen phosphate, 21.75g of dipotassium hydrogen phosphate, 1.7g of ammonium chloride in 1000 ml of distilled water in a volumetric flask and pH was adjusted to 7.2.

- **Dilution Water**

Double distilled water taken in a glass container was aerated for half an hour using an aerator. One ml of phosphate buffer, one ml of M_gSO_4 (22.5 g/l) one ml of $CaCl_2$ (27.5 g/l) and 1ml of $FeCl_3$ (0.25 g/l) were added.

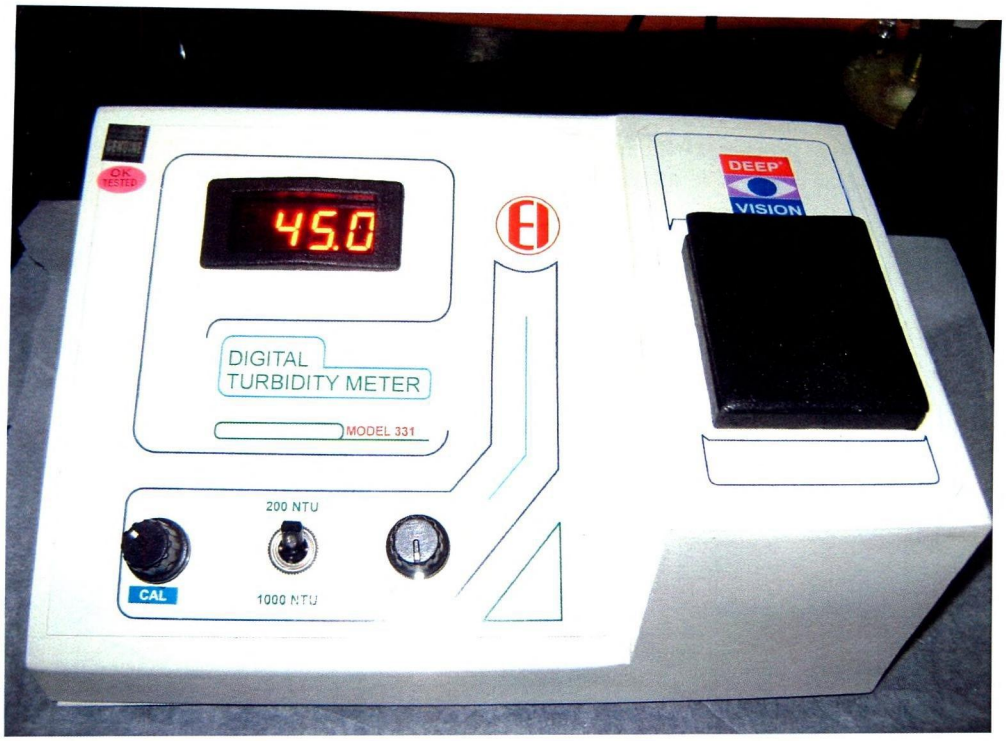


PLATE VI
TURBIDITY METER

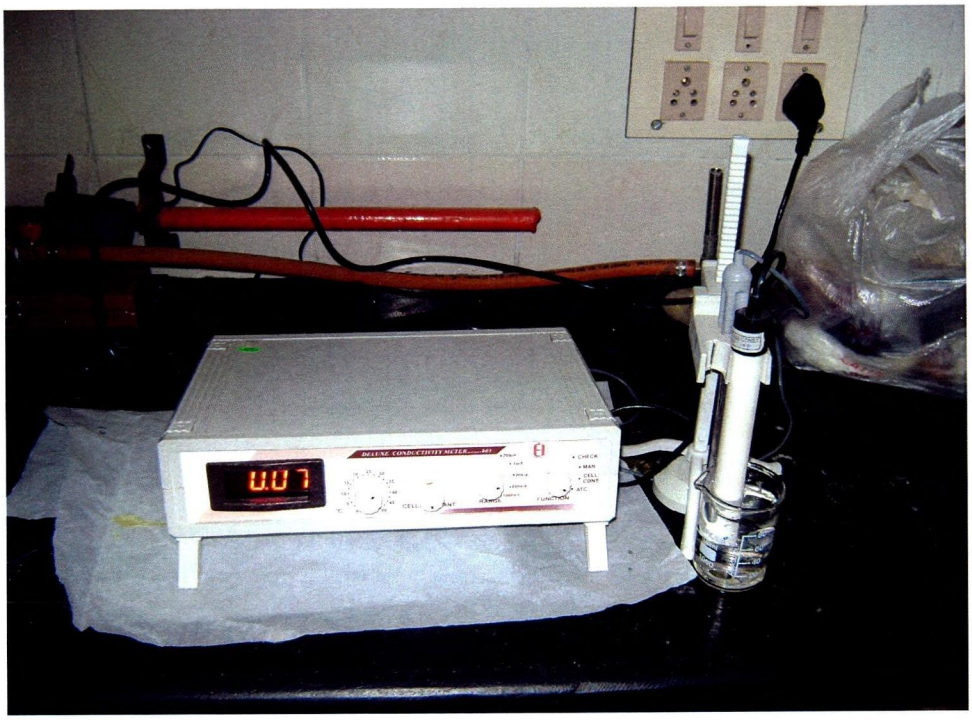


PLATE VII
ELECTRICAL CONDUCTIVITY METER

- **Procedure**

The raw and treated effluent samples were diluted (measured dilution) with dilution water (Dilution is not necessary for unpolluted waters and seeding is unnecessary for surface waters). The raw and treated effluent sample was taken in two BOD bottles. D.O content (D1) of one bottle was analysed and the other was incubated in BOD incubator (Plate XII) at 20°C for five days. Two other bottles were filled with dilution water D.O content was analysed immediately in one bottle and the other was incubated. D.O was analysed in the incubated water sample (D2) and dilution water after five days of incubation (Plate VIII).

- **Calculation**

$$\text{BOD (mg/l)} = \frac{(D1 - D2 - BC) \times 100}{\text{Percentage dilution of sample}}$$

BC = Blank Correction

3.4.2. Chemical Oxygen Demand (COD)

Reagents

- **0.25 N Potassium dichromate**

Potassium dichromate of 12.259 g in 1000 ml of distilled water.

- **0.1N Ferrous ammonium sulphate (FAS)**

Ferrous ammonium sulphate of 39.2 g and 20 ml of concentrated H_2SO_4 in 1000 ml of distilled water. The solution was standardised with 0.25N potassium dichromate solutions.

- **Ferroin indicator**

Phenanthroline of 1.485g and 0.695g of ferrous sulphate dissolved in 100 ml of distilled water.

PLATE VIII

BOD INCUBATOR

➢ **Procedure**

Ten ml of the raw and treated effluent samples were taken in a COD flask and 30 ml of conc. H_2SO_4 and ten ml of 0.25N potassium dichromate were added. The content was refluxed for two hours in a hot plate at 60° C, cooled, diluted with distilled water and made up to 140 ml. Two to three drops of ferroin indicator was added and titrated against 0.1 N FAS. The color change from blue green to reddish brown was observed. The entire procedure was repeated for blank.

COD of the raw and treated effluent samples were calculated using the formula.

➢ **Calculation**

$$\text{COD (mg/l)} = \frac{\text{V x Normality of FAS x 8 x 1000}}{\text{Volume of the sample}}$$

PHASE II

3.5. DYE DEGRADATION BY *BACILLUS SUBTILIS*

The *Bacillus Subtilis* was used to decolorize the textile dye effluent. The bacterial culture exhibited 90% decolorization ability within 50 h. Maximum rate of decolorization was observed (90%) when starch & peptone was supplemented in the medium. Decolorization of textile dye effluent was monitored by TLC, which indicated that dye decolorization was due to its degradation into unidentified intermediates. The optimum dye decolorizing activity of the culture was observed at pH 7 and incubation temperature of 370°C. Maximum, textile dye effluent decolorizing efficiency was observed at 200 mg/1 concentration of textile dye effluent. A plate assay was performed for the detection of decolorizing ability of bacteria. Clearing zone (decolonization) was formed surrounding the bacterial culture. Decolorization was confirmed by UV-VIS spectrophotometer (Plate V). 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. The dye decolorization was further confirmed by COD & BOD Analysis, express Gurulakshmi et al., (2008).

3.5.1. Plate Assay

Plate assay was performed for the detection of decolorizing 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. The plates were wrapped with parafilm and were incubated at 37°C for seven days. The plates were observed for clearance around the colonies, state Gurulakshmi *et al.*, (2008) (Plate IX).

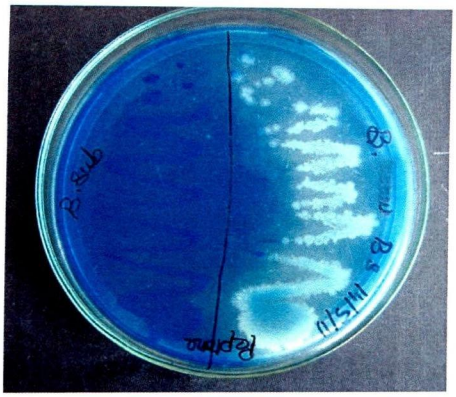
3.5.2. Tube Method

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. After which, aliquots (five ml) of the culture media were withdrawn, centrifuged at 10,000 g for ten minutes in a centrifuge at room temperature to separate the bacterial cell mass. The supernatant was used for analysis of decolorization and all the experiment was repeated in triplicates. Absorbance of the supernatant were withdrawn at different time intervals and the absorbance maximum wavelength for the textile dye effluent (λ max =480 nm) in the visible region on a UV-VIS spectrophotometer (UV 1601) viewed by Tripathi and Srivastava (2010) (Plate IX).

The percentage of decolorization was calculated from the difference between initial and final values using the following formula:

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance value-final absorbance value x100}}{\text{Initial absorbance value}}$$

Plate assay



Tube assay

Conical flask assay

PLATE IX

TEXTILE DYE EFFLUENT DECOLORIZATION BY *BACILLUS SUBTILIS*

3.5.3. Conical Flask Assay

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 inoculum of the selected culture was added to nutrient broth flasks containing textile dye effluent (250 mg /l). The flasks were covered with aluminum foils and were incubated at 32°C for three days. The flasks were observed for decolorization of the textile dye effluent present in the medium (Plate IX).

3.6. ODOR DEGRADATION BY *THIOBACILLUS BACTERIA*

The degradation was measured by Gas chromatography technique (Plate XX). Into phosphoric acid-coated serum vials (68.4 ± 0.6 ml) three percent of 50mM Tris/HCl buffer containing 0.2mm EDTA was added. 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 was analyzed by injecting 50ml of gas into the gas chromatograph. Later 0.05 to 0.5ml of *Thiobacillus Bacteria* were added.

3.6.1. Degradation of H_2S

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. The degradation of H_2S by a strain of *T. thioparus* has been reported previously by Smith and Kelly (1988) and Adoki (2007).

Compared with chemical or Physical methods, biological processes have received more interest because of their cost effectiveness, lower sludge production and environmental friendliness. The bacterial isolate can be exploited for bioremediation of textile dye effluent containing wastes, since it seems to have the potential to degrade the toxic reactive dyes into non-toxic

product form. Knowledge of biodegradation is important for the evaluation of the persistence of organic pollutants and the design of biodegradation facilities. Therefore, further detailed research is needed to quantify these substrates interactions in the degradation of dyes and its derivatives.

PHASE III

3.7. UTILIZATION OF TREATED WATER FOR DYEING COTTON FABRIC

As the cost of water supplied to industry keeps increasing, recycle schemes become more attractive with good pay back periods, say Rani and Nevi (2007). In increasing demand for water in industry and to control the pollution of environment, recycling or reuse of water is essential. Therefore in the present study the treated textile dye effluent was utilized for dyeing.

3.7.1. Selection of Fabric

Cotton is one of the most commonly abundant natural fiber having various advantageous such as availability, comfortability, excellent heat conductivity and hydroscopic in nature. The fiber is most often spun into thread and used to make a soft, breathable textile. It is natural fiber chemically known as cellulose. It has high degree of acceptability to wear next to skin such as shirts, blouses, trousers because of its high adsorption and swelling capacity regarding aqueous solution, informs Gienandt (2006). Plain weave is relatively inexpensive for construction and can be extensively used for cotton fabrics. Their ravel was less than compared with the fabric of other weaves, views Kaplan (2002). Hence, cotton fabric made out of plain weave was selected for the present study.

3.7.2. 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, reveal Molla *et al.,* (2004). Reactive dyes are a class of highly colored organic substances, primarily used for coloring textiles that attach themselves to their substrate by a chemical reaction that forms a covalent bond between the molecule of dye and that of the fiber. The dye stuff thus becomes a part of the fiber and is much less likely to be removed by washing than are the dye stuffs that adhere by adsorption, explains Shenai (2004). To achieve good fastness properties of dyeing, reactive dye was selected for the study.

3.7.3. Dyeing of cotton with reactive dye using treated and Soft Water dyeing procedure

Dyeing are used to add color to textile and may take part in the process chain at different stages of production either fiber, yarn or fabric (piece) dyeing, remark Lens *et al.,* (2002). Dyeing of textiles is considered as the most important and expensive step in manufacturing of textile fabric and garments. The process for reactive dyeing of cotton can be divided into three steps and the dyeing parameters are presented in

- Pre-treatment
- Dyeing
- Rinsing after dyeing

TABLE II

DYEING PARAMETERS

Fabric weight	1 meter
Shade	4%
Material: liquor ratio	1:30
Caustic soda	2%
Sodium chloride	20%
Time	35 minutes
Temperature	80°c.

3.8. DYEING PROCEDURE

During the pretreatment, (Desizing) one meter of cotton fabric was taken, weighed in electronic balance. Depending upon the fabric weight water was taken in a clean stainless steel vessel. Two gm of detergent was added and mixed well. The fabric was immersed in the solution and boiled for 30 minutes. Then the sample was taken from the vessel and rinsed in soft water and dried.

Then 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 gram /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.

3.9. NOMENCLATURE

The nomenclature of the samples thus selected for the study is given in Table III.

TABLE III

NOMENCLATURE OF SAMPLES

Samples	Code
Original Fabric	0
Soft water Dyed Sample	SWD
Treated Water Dyed Sample	TWD

PHASE IV

3.10. EVALUATION

3.10.1. Subjective Evaluation

The soft water dyed and treated effluent water dyed samples were evaluated visually by the panel members. The panel members which consisted of 25 judges comprising 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

3.10.2. Objective Evaluation

Textile testing as a whole refers to the vigours testing done on textile materials which may be inside the laboratory as well as in natural setting, says Paul and Jewel (2005).

3.10.2.1. Fabric Weight

Fabric weight (Plate X) is the relative weight of the fabric and expressed as the weight of a particular size of piece as grams/ square meter or ounces / square yard, view Angappan and Gopalakrishnan (2006).

Fabric weight of the original and dyed samples was determined using GSM cutter. It is a device to cut circular specimen of 100 square centrimeters of a fabric very accurately. It has four blades that cut the fabric when the hand weight is rotated by applying light pressure. The samples were cut and weighed accurately using digital balance having 0.01sensitivity (Plate XI). The value in gms multiplied by 100 gives grams / square meter of the fabric.

The samples were weighed for five times and the mean value was calculated and recorded.

3.10.2.2. Fabric Thickness

Based on BS: 2544:1954 the principle of fabric thickness is the determination of the thickness of a compressible material such as textile fabrics of the precise measurement of the distance between two plane parallel plates.

Fabric thickness gauge (Plate XII) was used to measure thickness of the sample. It has two parts the anvil and pressure foot. Pressure was given to the foot to make the gauge dual zero. The sample was placed between the cleaned pressure foot and anvil without any pressure. The reading shown by the dial was noted. For a single sample thickness was determined at five different places away from two inch of the selvedge.

3.10.2.3. Strength and Elongation

The tensile or breaking strength of the fabric is a measure of its resistance to a tensile load or stress in either warp or weft directions, define Singh *et al.,* (2004). Elongation as the increase in length of a specimen during tension test, expressed in units of length of the fabric when loaded reports AATCC (1995).

The samples of original and dyed fabrics were tested for tensile strength using Eureka cloth tensile strength tester (Plate XIII). Specimen of 12 inch x 2 inch from each samples was cut both in warp and weft direction of the fabric two inches apart from selvedge. The specimen was placed between the upper and lower clamp. The dial reacting was set to zero by adjusting the pendulum over the quadrant scale. The elongation pointer was checked for its position in zero. Before stating the machine the pendulum lock was released and machine was switched to run. At the point which the fabric started to break the machine was switched off the dial reacting in kg was taken.

Elongation reacting was noted from the elongation scale. The specimen was removed and the machine positioned back to original and the five specimens of both directions from each sample were tested and readings were noted.

3.10.2.4. Fabric stiffness

Fabric stiffness indicates the resistance of the fabric to bending and it is a key factor in the study of handle and drape describes Arun (2001). Shirley stiffness tester was used to test the stiffness of the fabric (Plate XIV).The sample A was cut to the size of 15cm x 2.5 cm using the template. The sample was placed on the platform with the template at the top of it, so that the leading edges coincide. Both were slowly pushed forward until the leading edges of the sample and the template projected beyond the edge of the platform. The sliding of the sample was stopped when it cut both the index lines. Then the bending length of the sample read from the scale opposite a datum line engraved on the side of the platform. Four readings were taken for sample A. Mean values of the bending length in warp and weft wise direction was calculated. Similarly other samples were calculated.

3.10.2.5. Abrasion Resistance

The ability of material to resist the action of abrasive forces is clearly one of the major criteria to take into account when assessing durability, states Basu (2001).Abrasion is just one aspect of wear and is the rubbing away of the component fibres and yarns of the fabric.

The Eureka Martindale abrasion resistance tester (Plate XV) was used to determine the fabric resistance to friction. The severity of abrasion varies with the nature of the abradant. Ten samples were cut at random from each of the dyed materials and original using a template. The initial weight of each sample was measured using an electronic balance. The samples were mounted on sample holders. The sample holders with 200 grams weight were used for this purpose. The rubs were standardised to 30 rotations. After 30 rotations, the samples were removed and the final weight of each sample was found out. Weight loss due to abrasion was calculated. The same procedure was repeated for all other samples and the mean value was calculated. Each time, a fresh abradant was used. Similarly the mean value of the ten readings for each of the sample was calculated and thus the loss in weight of each material was recorded separately.

3.10.2.6. Drapability

Brand (2003) defines the drape as the manner in which a fabric falls when hung on a form and it is one of the most important fabric aesthetic properties. Eureka Drape meter (Plate XVI) was used for this study. A circular specimen of diameter 25 cm was supported on a circular disc of diameter 12.5 cm measuring the following areas, the drape coefficient, F was calculated.

- area of the specimen, AD
- area of the supporting disc, Ad
- actual projected area of the specimen, As

The degree co-efficient, F is the ratio between the projected area of the draped specimen and its undraped area, after the deduction of the area of the supporting disc, view Angappan and Gopalakrishnan (2006).

$$\text{Thus, } F = \frac{As\text{-}Ad}{AD\text{-}Ad}$$

PLATE X
GSM Cutter

PLATE XI
Electronic balance

PLATE XII
Thickness Gauge

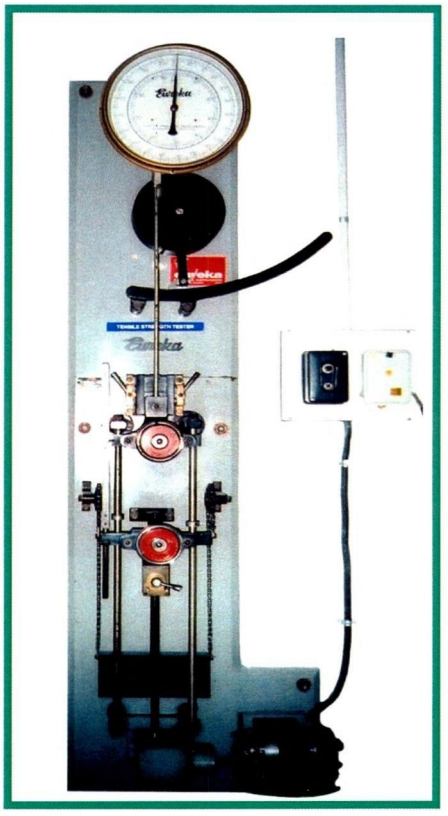


PLATE XIII
Strength and Elongation

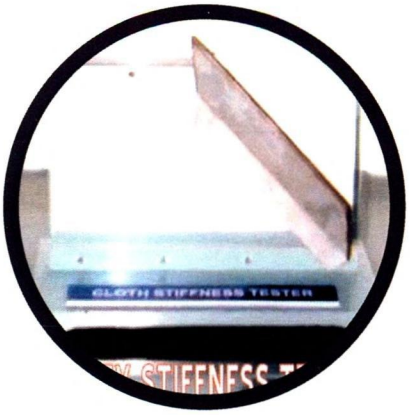


PLATE – XIV
Stiffness Tester

3.10.3. Absorbency test

3.10.3.1. Drop test

Absorbency is defined as the propensity of a material to take in and retain a liquid, usually water, in the pores and interstices of the material reports AATCC Technical Manual (2008). Wettability is defined as the time in seconds for a drop of water to sink into the fabric (Plate XVII).

A burette filled with distilled water was clamped in a stand. Sample A was mounted in an embroidery frame and was placed at the base of the stand. The distance between the sample and burette nozzle was kept constant. The nozzle of the burette was opened just to allow a drop of water to fall on the sample. The stop watch was started simultaneously and it was stopped when the drop of water fully sank into the material. The time taken for this was noted. Same procedure was repeated for ten times and the mean value was calculated. Similarly the water drop absorbancy time was recorded for samples B and C and their mean values were calculated.

3.10.3.2. Capillary rise test

The capillary travel method measures the rapidity of absorption. Five pieces of the sample A were cut measuring cut 15 cm length and 2.5 cm width. One end of the sample strip was pasted with a glass rod which was placed on heavy wooden blocks and at the other end, two grams weight was attached to keep the sample straight. At the weighed end two cm of the sample was allowed to immerse in a tray of distilled water. The rise of the water level in the strip was noted by keeping time as constant (one minute). The same procedure was repeated for the samples B and C and the mean value was calculated and recorded as viewed by Paul and Jewel (2005) (Plate XVIII).

3.10.3.3. Sinking time test

Sinking test is a simple test that helps to measure the wettability of a fabric reports AATCC Technical Manual (2008). In this method, conditioned sample A was cut into five equal sized squares of 1" x 1" and added to a 1000 ml beaker filled with distilled water. The stop watch was started when the fabric struck the surface of water and stopped when the last corner sank below the water surface. The test was carried out for all the samples and the mean time for sinking was calculated and recorded (Plate XIX).

3.10.4. Color Fastness Test

Dye-fiber interactions are varied and their strength or combined strength determines both the outcome and performance of the dyeing. Dyeing does not mean only to impact attractive on the fiber bat to obtain fast color on it.

Color fastnesses measure the resistance of the textiles when they are exposed to various agencies. The usual practice to apply the dye on the fabric in specified strength and subjected to the various agencies. The grey scale employed for color fastness test is 1-5 grades. In this scale one means poor fastness and five represent excellent color fastness, report Smith (2006) and Nimkar *et al.*, (2006). Here in the study, four color fastness testes were carried out. They are color fastness to sunlight, washing, wet and dry crocking, wet and dry pressing.

3.10.4.1. Color Fastness to sun light

The color fastness of textile material to day light is very important property, say Alikan et al. (2006). Color fastness as the resistance of a material to a change in its color characteristics as a result of exposure of the material to sunlight and an artificial tight source [AATCC, 2007].

PLATE XV
MARTINDALE ABRASION RESISTANCE

PLATE XVI
EUREKA DRAPE METER

PLATE XVII
DROP TEST

PLATE XVIII
CAPILLARY TEST

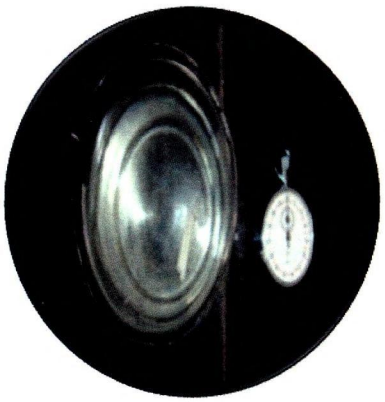


PLATE XIX
SINKING TEST

To test the fastness to light the specimens of 16 cm x 5 cm were cut from each sample of dyed fabrics and divided into eight equal parts measured as 20m each the specimens were covered with black chart. For the successive seven days the specimens were exposed to direct sunlight. First day first portion of the specimens were exposed and accordingly seven portions are exposed for 60 light the first portion was exposed for seven days and the 7^{th} portion was exposed for a day. The last portion was not exposed to sunlight and considered as standard for comparison were done using grey scale and the specimens were rated.

3.10.4.2. Color Fastness to wet and dry crocking

Crocking is the rubbing fastness of dyes. AATCC (1995) describe crocking as the transfer of colorant from the surface of the colored fabric to an adjacent area of same fabric or to another surface, principally by rubbing action. Fastness to crocking is important in both apparel fabric as well as upholstery. Crocking test determines the extent to which color may be transferred from the surface of the dye fabric to another by rubbing states Adannur (1995).

Sasmira crock meter was used to determine the fastness to crocking. Each of dyed samples was cut in the measurement of 25 x 20 cm and mounted on flat base. The desized white cotton fabric was mounted in a ring on the rubbing finger. Each sample was given ten rubs based on standardization. The color transfer from the dyed sample to the white material was used for wet crocking. The procedure adopted was same as that of dry crocking. The color transfer from the dyed sample to the white material was assessed using grey scale.

3.10.4.3. Color Fastness to washing

Major loss of color from the fabric is due to washing and results in staining over the adjacent fabric. This phenomenon is used in wash fastness of color.

3.10.4.4. Color Fastness to Wet and dry pressing

Color fastness of the samples to pressing was measured following the specifications of Bureau of Indian standards (2000). Two specimens measuring 10 cm x 10 cm from each dyed samples were cut and one set of specimens covered at either side with 5 cm x 5 cm of desized white fabric. The prepared specimens were pressed for five seconds to assess its color fastness to dry pressing while the others were covered with wet white cloth and pressed for 5 seconds to assess its color fastness to wet pressing. The same procedure was repeated for five specimens. The color change in the dyed fabrics was graded using grey scale.

3.10.5. Gas chromatography analysis for H_2S gas from effluent

Compounds with primary amino groups react with o-phthalaldehyde in solution to yield highly fluorescent products. This reaction is now in wide use for detecting amino acids and amines in liquid-chromatography effluents. The effluent gas, nominally 20 ml/min, is delivered to a scrubber consisting of a small-bore, simulated capillary chromatographic column that is simultaneously supplied with one ml/min of the reagent solution. The liquid effluent of the scrubber, separated from the gas, is drawn through the flow cell of a fluorometer. Short-chain amines and ammonia were quantitatively scrubbed. The response of the fluorometer was directly proportional to the number of nanomoles injected into the column. Less than a nanomole of amine was detectable. Comparison of results with those from a hydrogen flame-ionization detector showed minimal additional peak broadening and compromise of resolution. These results demonstrate the feasibility of using highly specific, as well as sensitive, liquid-chromatographic detection methods for gas-liquid chromatography, view Chow and Karmen (1980).

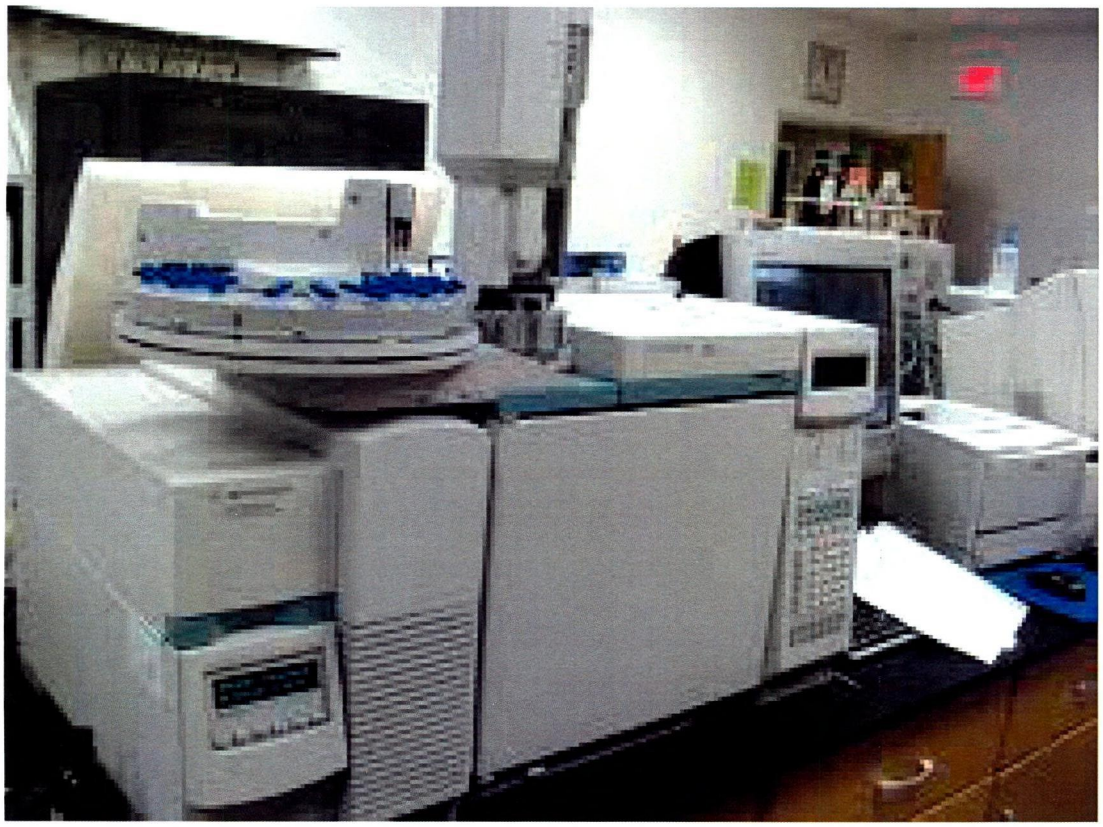


PLATE XX

GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC-MS)

3.11. STATISTICAL ANALYSIS

The results of the tests were analyzed statistically by selecting appropriate tests. The difference between the samples and within the samples was analyzed during "ANOVA" test. This analysis will help in exact results arrived in this study.