Bio-Degradation of Orange G Dye using Microbial Action

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***Abstract*- Degradation of dye is a problem in textile industries. This present study is mainly conducted to investigate the decolourisation and bio-degradation of orange G dye using Bacillus subtilis microbes , a type of Bacteria .The Bacterial Inocules were inoculated into a flask containing Orange G Dye with trace amount of Peptone and Glucose with other components like CaCl2, NaHNO3 , MgSO4,. This medium undergoes sterilization process and shaken in the shaker for a period of 24 Hrs. The percentage of Decolourisation and Degradation is calculated out for three days. The best decolourisation of orange G dye by Bacillus subtilis is noted .For the optimum period of 48 Hrs. The pH value is determined on the specimens . The Degraded product is examined by thin layer Chromatography and Fourier transformed IR Spectroscopy analysis and also by UV Spectrometer .**

Keywords: Decolourisation And Bio-Degradation , Peptone And Glucose , Bacillus subtilis, UV Spectrometer.

I. INTRODUCTION:

The textile industries in a country mainly generate a high volume of Wastewater. Which has high toxic effluents .This textile effluents when disposed in atmosphere and environment cause great damage. Dye significantly affect the environment and cause pollution to it. In many developing countries it is one of the real problem faced. In several developing countries minimum quantity of industrial effluent is treated and disposed. Textile industry form a backbone to many developing countries.

Considering India as an example it accounts for 30 %. The wastewater from industries has high coloring agents, large amount of suspended solids, high pH, salts , heavy metals, sulphates, chlorine etc., Normally dyes mainly contain Cd,Cr,Hg,Ni,Fe&Mn , sediments and suspended solids. These form a important comfor metals in wastewater. In many cases these toxic metals cause rapid depletion of dissolved oxygen and causing oxygen slag in the water and leads to damages of aquatic life. The untreated wastewater from textile account about 15 to 20 % of total wastewater in India. Azo Dyes are mainly used in industries which contribute 84 %. About 3500 dyes are used in practical. Effluents like orange G dye, Remazol Black B, Methyl Red, Methyl Blue etc. These untreated substrates are extremely toxic to both flora & fauna, also including human beings. Another criteria is that the surface and underground water get damaged due to the industrial effluent and the wastewater from the industries containing dye which contaminates the environment and aquatic life in water bodies. In developing countries they follow traditional method. The dye effluent may be treated by physical, chemical and biological methods. Now-a-days advanced physicochemical and biological methods have been adopted for the removal of these Dyes. In current situation bio technique improvements paved the way for biological methods for treating these effluents to decolorize and degrade. This biological method receives great attention among the countries all over the world as it is Eco friendly method.

The aim of our study is to decolorize and degrade the components present in the dye from textile industries by biological method using microbes.

II. MATERIALS AND METHODS

A. SAMPLE COLLECTION:

Orange G dye is collected form a small dyeing industry which is a major effluent in that industry. This Orange G dye is the basic source for this study. The samples are collected and preserved in a cooler box.The microbe Bacillus subtilis. was obtained from Centre for Bioscience and Nanoscience Research (CBNR) isolated and maintained under optimum circumstances.

B. ORANGE G DYE:

It is a synthetic azo dye which is also called as orange gelb. It is commonly used as a staining agent in textile industry .This dye available as powders and disodium salts.

C. DECOLOURISATION OF DYE:

In this study biological decolourisation of orange G dye is done. Biological decolourisation consumes less time than physical and chemical treatments and also found to be highly efficient.

D. IDENTIFICATION, ISOLATION OF BACTERIA:

Many microbes has an ability to degrade the effluents from various industries. In which Pseudomonas, Bacillus, Clamydomonas and Coccussp are used to degrade dye waste. In this paper the species in Bacillus family called Bacillus subtillis is used to degrade the Orange G dye.The bacteria is isolated and treated in buffer solution for further process.

E. GROWTH AND MAINTANANCE:

The microbe is cultivated in a culture medium at preferable temperature and buffer solutions to create 2-4 colonies of Bacillus subtillis. Kept at a suitable temperature.

E. MEDIUM PREPARATION:

A medium for 250ml is prepared for the growth of the bacteria and to perform the experiment.

TABLE **1** INGREDIENTS AND QUANTITY TAKEN FOR PREPARATION OF MEDIUM

S.NO.	COMPONENTS	QUANTITY (gms)
1.	$MgSo_4$	0.05
2.	K_2HPo_4	0.25.
3.	$CaCl_2$	0.005
4.	$FeCl_3$	0.0125
5.	NH_4No_3 or $NaNo_3$.	0.25
6.	Glucose	25
7.	Yeast or peptone	1.25
8.	Water	250ml.

F. GROWTH IN SAMPLES:

The prepared medium is equally shared and poured in 3 medium size conical flask. About 20ml of the Dye is taken in 2 conical flask and 40ml of Dye is taken in 1 conical flask which contain the medium. On taking these ratio the flask are sterilized for 30min in Autoclave at 121^0 C. After this the flask are cooled for two hours and the Bacillus subtillis is taken out from the petrish dish and put into the T_1 and T_2 flask and it is closed using cotton crock. The flask are placed in a shaker for 3 days, after every 24hrs the growth and degradation in T_1 and T_2 are noted by doing centrifuge and U.V. Spectrophotometer analysis

G. CENTRIFUGE AND ANALYSIS OF SAMPLES:

The samples are taken in 1ml FN Truff tube by using pipette. Then the samples are placed in Centrifuge machine,Which rotates in 8,500 RPM. It helps to separate and precipitate the pellets (Bacteria and Dye Effluent Degraded). After centrifuge process the sample is tested in UV spectrometer and the readings are obtained .From which the degraded value can be calculated.

DecolorizationRate =((IOD-FOD)/(IOD))x100.

IOD-Initial Observed value .

FOD-Final Observed value.

I. T.L.C. PAPER TEST:

Mercon Plate is used to do the TLC paper test. The substrates which are used to do the test are:

TABLE 2 PREPARATION OF TLC SOLUTION

SUBSTRATES	QUANTITY (ml)
Methanol	1
Ethyl Acetate	2
n Propanol or n butyl acid	3
Acetyl acetic acid	0.5

III. RESULT AND DISCUSSION

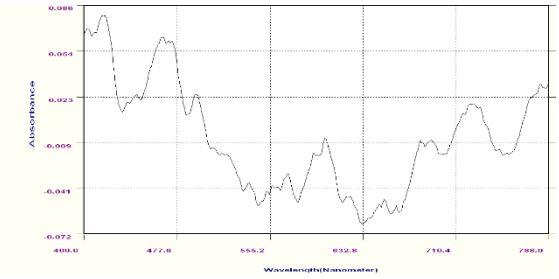
The degradation of the dye is calculated from the readings obtained from the UV spectrometer. The degradation values are tabulated for 2 daysand the graph is ploted.

A. DAY 1:

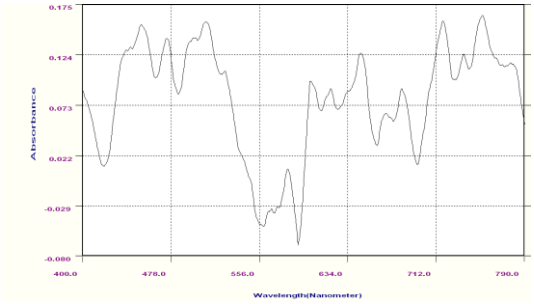
TABLE 4 DETERMINATION OF DEGRADATION RATE BY UV SPECTRO PHOTOMETER:

MEDIUM	ADSORBENT VALUE	DEGRADATION RATE (%)
CONTROL	0.37	-
TREATED-1 (T_1)	0.202	36.27
TREATED-2 (T_2)	0.159	49.84

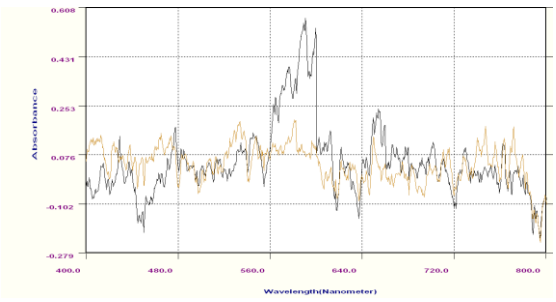
GRAPH 1 THE INITIAL SAMPLE (CONTROL)AT 550NM



GRAPH 2 TREATED SAMPLE



GRAPH 3 COMBINED ANALYSIS

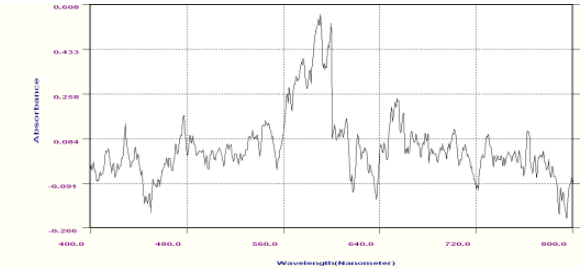


B. DAY 2:

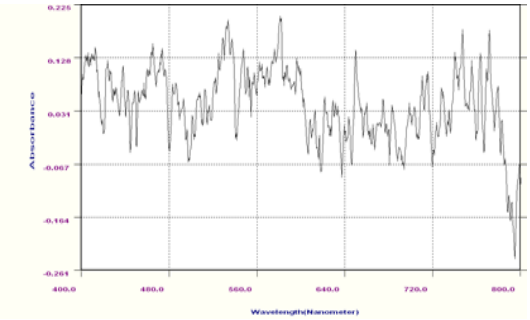
TABLE 5 DETERMINATION OF DEGRADATION RATE BY UV SPECTRO PHOTOMETER

MEDIUM	ADSORBENT VALUE	DEGRADATION RATE (%)
CONTROL	0.098	-
TREATED-1 (T1)	0.043	56
TREATED-2 (T2)	0.011	88

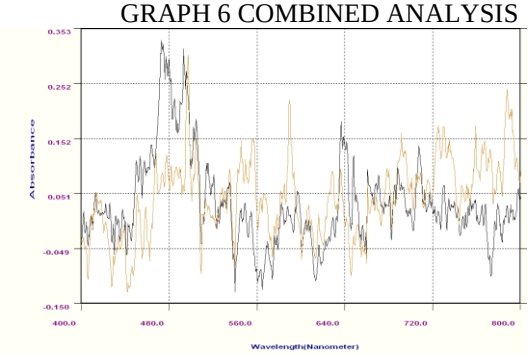
GRAPH 4 THE INITIAL SAMPLE (CONTROL) AT 550NM



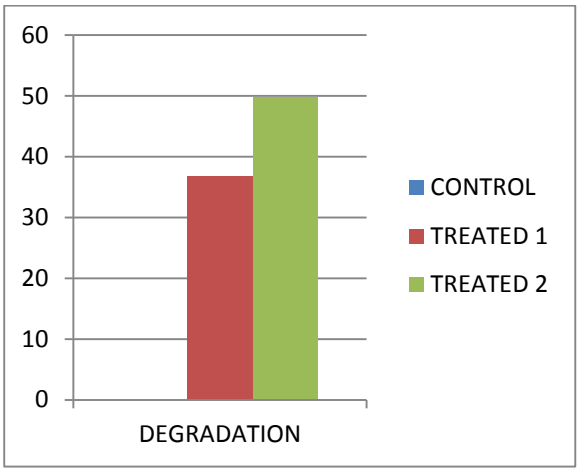
GRAPH 5 TREATED SAMPLE



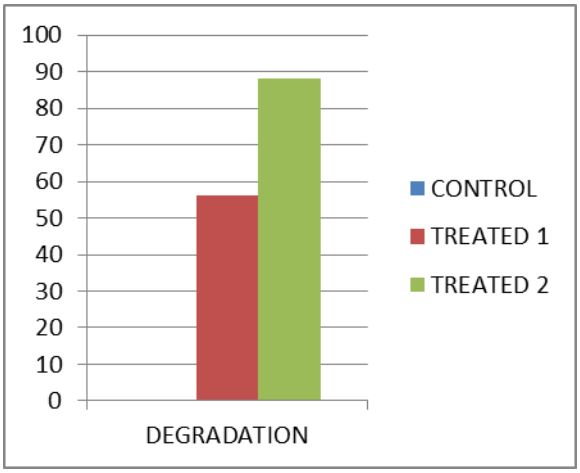
GRAPH 6 COMBINED ANALYSIS



GRAPH 7 DAY 1 DEGRADATION



GRAPH 8 DAY 2 DEGRADATION



DISCUSSION:

By analyzing the above charts and the results of degradation it is found that the Orange G dye gets biologically degraded by 88% at an optimized conditions within a period of 48 hrs.so further proceeding this experiments the degradation valve can be increased and also proper circumstances must be adopted while maintaining the test.the T.L.C test reports also give a good sign of perfect degradation further proceeding this test by adopting high concentration of microbes at suitable conditions the results will be perfect.

IV. CONCLUSION:

Although decolourisation forms a challenging process in the field of textile industries as well as waste water treatments. The results of this findings give a great potential for the bacteria to remove the colour from dye waste water. Interestingly, the bacteria used in carrying out the decolorisation of orange G in this study was isolated from the textile industry waste effluent. The bacteria

Bacillus spp. Showed good decolorisation and biodegradation at the rate of 88 % at an optimum period of 48hrs. This observation shows that the bacteria can be adopted for the degradation of dyes and its contaminants.

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