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## BIOSORPTION OF CRYSTAL VIOLET DYE FROM AQUEOUS SOLUTIONS USING NATURAL BIOSORBENTS

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### Abstract

*Textile and dyeing industries are continuously increasing the pollution levels of water bodies regularly due to improper treatment and disposal of their effluents. The synthetic dyes are recalcitrant in nature and their intense color leads to the reduction in photosynthesis and raise temperature in the deeper layers of water bodies causing serious damage to the aquatic life. It is necessary to adopt eco-friendly treatment methods like bio sorption and microbial decolorization to decolorize the effluents before they are disposed into the environment. Batch mode bio sorption experiments were carried out using the Annona reticulata leaves powder and Cucurbita maxima seed powder. Optimization studies were carried out for the decolorization of crystal violet dye. Under optimized conditions, the percentage decolorization by bio sorbent, Annona*

*reticulata leaves powder (at pH = 8, temperature 400C) was 90.3% for 60 minutes. The amount of biosorbent dosage added for maximum color removal is 0.2g/ 100ml. In case of Cucurbita maxima seed powder, the percentage removal was 98.5% under same conditions. Effective removal was observed by Cucurbita maxima seed powder compared to Annona reticulata leaves powder. Toxicity assay showed that in the presence of biosorbent Annona reticulata leaves the growth of microbes was more favorable when compared to Cucurbita maxima seed powder. The present study reveals that the use of natural biosorbents in the decolorization of industrial effluents is an effective and commercially viable technique.*

### **Keywords**

Bio Sorption, Crystal Violet, Annona Reticulate, Cucurbita Maxima, Toxicity Assay

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## **1. Introduction**

Textile industry is one of the fastest growing industries in the world. The usage of synthetic dyes and the release of untreated effluents into natural water bodies poses serious problem of its entry into food chain. Azo dyes are widely employed in textile dyeing, paper printing food processing, pharmaceuticals, decolorization, and waste pulp removal, cosmetics and plastic industry (Nosheen et al., 2010; Mohanty et al., 2006). Approximately 10-15% of untreated dye effluents from the textile units are released into the environment every year (Spadary et al., 1994; He et al., 2004). The high capital investment and regeneration cost of conventional methods of dye treatment have led to the idea of using natural bio sorbents. The physical and chemical methods available are ineffective in dye degradation processes which include adsorption, concentration and chemical transformation. A combination of physical, chemical, and biological processes is efficient for dye degradation but requires highly skilled labor and presently there is an increasing need for a better and cost effective treatment.

The dye used for the present study is Crystal violet is a type of basic dye. It belongs to the group triarylmethane dye. IUPAC name of crystal violet is methyl violet and the chemical name is Tris 4- di methyl amino phenyl methylium chloride (molecular formula C<sub>25</sub>N<sub>3</sub>H<sub>30</sub>Cl). Crystal violet is not only used as a textile dye but also used to dye paper and as a component of navy blue and black inks for printing, ball-point pens and ink-jet printers and also used to colorize diverse products such as fertilizers, anti-freezes, detergents, and leather jackets.

## 2. Literature Review

Mohammad, et al., (2012) attempted to study the operating feasibility using reverse osmosis (RO) and nanofiltration (NF) membrane systems as an alternative treatment method for the dyes acid red, reactive black and reactive blue dyes ( Abid et al., 2012). Their study indicates that the use of NF membrane in dye removal from the effluent of Iraqi textile mills is promising. Similar studies were carried out by Nidheesh et al., (2012) using bottom ash (particle size of 75-150  $\mu\text{m}$ ) for three different concentrations of 10, 20 and 40 mg/L of textile effluent sample and maximum removal was observed at a 60 minute interval (Nidheesh, et al., 2012). Percentage removal was maximum at lower concentrations and as the initial concentration increased from 10 to 40 mg/L the percentage removal decreased from 70% to 30% but the adsorption capacity of bottom ash increased from 6.978 to 11.446 mg/g for the above condition. This is because at lower concentration the ratio of the initial number of dye molecules to the available surface area is low. Lakshmipathy R and Sarada NC (2013) used protonated watermelon rind to remove Methylene blue, crystal violet and Rhodamine B dyes for aqueous solutions (Tangappan, et al., 2012). Batch studies were carried out for various parameters like pH, adsorbent dosage and at saturation point, removal efficiency was found to be 83%, 92% and 57% for Methylene blue, crystal violet and rhodamine B dyes respectively. A comprehensive study of the bio sorption works carried out by various researchers and attempted to focus on the dye removal mechanism that uses natural sources as well as economically efficient to textile industries.

In the present investigation, the suitability of *Annona reticulata* (custard apple leaf) powder and , *Cucurbita maxima* (pumpkin seed) powder as natural bio sorbents for the effective removal of crystal violet dye from aqueous solutions has been studied by considering crystal violet as dye as a bio sorbate. Among factors which can influence dye sorption, contact time, initial solution pH, initial dye concentration and bio sorbent dosage were examined and their effects were reported. (Table. 1)

**Table 1:** Operating Variables Studied and Percentage Removal of Crystal Violet Dye

Parameters	Custard apple leaf(CAL)				Pumpkin seeds(PS)			
	min	max	Constant	% Removal	min	max	Constant	% Removal
Contact time (min)	5	150	60	86	5	150	55	87.2
pH	2	10	8	89.4	2	10	8	93.4
Initial dye concentration (mg/L)	10	300	100	89.5	10	300	100	97.3
Temperature ( <sup>o</sup> C)	20	50	40	89.4	20	50	40	97.3
Adsorbent dosage (g)	0.02	0.5	0.2	90.3	0.02	0.5	0.2	98.5

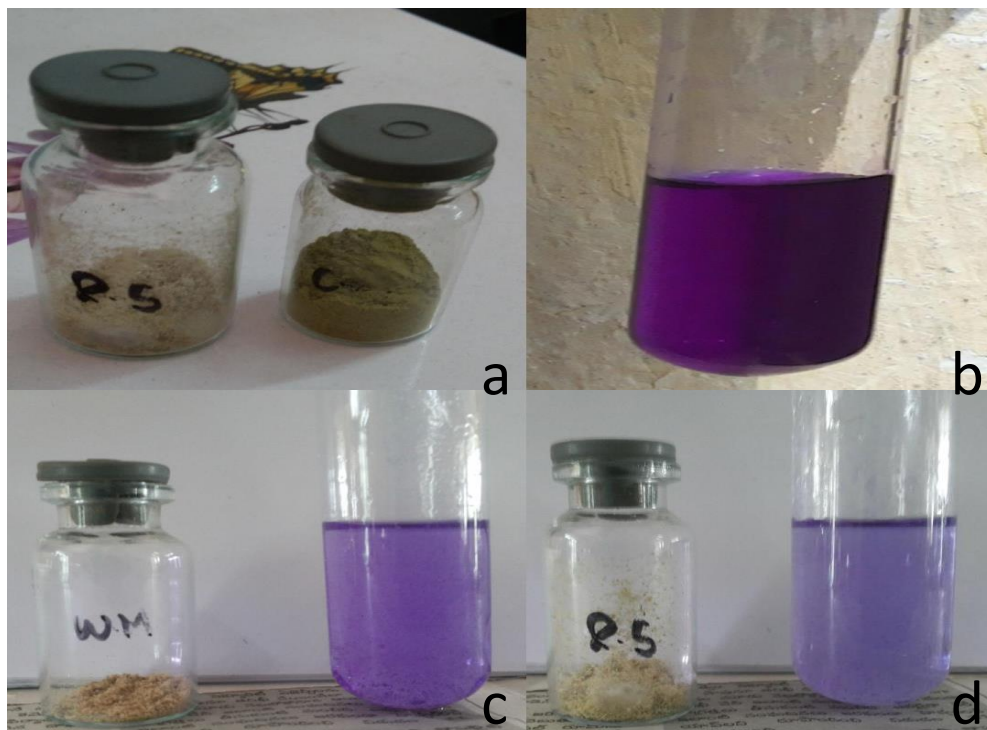
### 3. Materials and Methods

#### 3.1 Dyes and Chemicals

All the chemicals used in the present study were of analytical grade. The dye used was crystal violet dye (Merck Chemicals, AR Grade). Distilled water is used in preparing the stock solutions and also for parametric analysis throughout the experiment. Chemicals used for toxicity assay for preparation of nutrient broth and nutrient agar were yeast extract, peptone and agar agar which were of analytical grade.

#### 3.2 Preparation of Bio sorbents

Custard apple (*Annona reticulata*) leaves and pumpkin (*Cucurbita maxima*) seeds were taken as novel bio sorbents for the decolorization of crystal violet dye in aqueous solution [Figure 1(a),(b)]. They were purchased from local fruit vendor. The collected leaves and seeds were washed several times with distilled water until the wash contained no dirt. These leaves and seeds were completely dried under sunlight for 15 days and were cut into 1 cm pieces and dried in the oven at 60°C for 24 hrs before grinding using domestic mixer. The powdered biosorbents were sieved to get the 75-125 µm sized particles. These biosorbents were stored in tightly closed containers under room temperature.



**Figure 1:** (a). Fine powders of *Annona reticulata* and *Cucurbita maxima*; (b).Textile effluent sample containing crystal violet dye; (c).Biosorption using *Annona reticulata* sample containing textile effluent; (d).Biosorption using *Cucurbita maxima* sample containing textile effluent

### 3.3 Preparation of Dye Solution

Dye stock solution of crystal violet with a concentration of 1000mg/L was prepared by dissolving 0.5 g of crystal violet in 500 ml of distilled water. It was aliquoted to the 250ml Erlenmeyer flasks which were prepared in duplicates with distilled water to arrive at a final concentration range of dye solutions from 10 to 300 mg/L for batch experiments. The above media were prepared in duplicates and marked 'test' and 'control'.

## 4. Bio Sorption

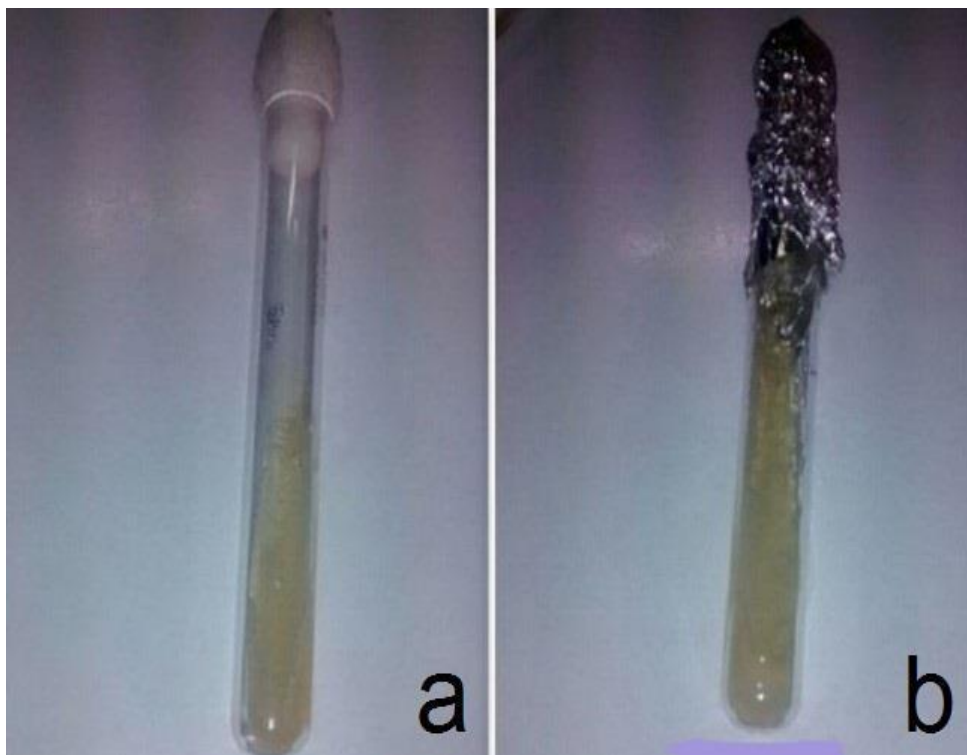
In accordance with the very abundant literature data, liquid-phase adsorption is one of the most popular methods for the removal of pollutants from wastewater since proper design of the adsorption process will produce a high-quality treated effluent. This process provides an attractive alternative for the treatment of contaminated waters, especially if the sorbent is inexpensive and does not require an additional pre-treatment step before its application. Adsorption is a well-known equilibrium separation process and has proven to be an effective

method for water decontamination applications (Dabrowski, 2001; Lakshmipathy & Sarada, 2014).

Batch mode bio sorption studies for crystal violet dye were carried out in 250 ml Erlenmeyer flasks at a constant agitation speed of 150 rotations per minute to investigate the effect of different parameters such as contact time, solution pH, initial concentration, biosorbent dosage and temperature. The same protocol was followed each time to experiment the effect of various parameters including contact time (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 90, 120, 150 min), pH (2.0 to 10.0 with 1.0 variation), concentration of dye (10, 20, 30, 40, 50, 100, 150, 200, 250 and 300 mg/L), temperature (200C, 300C, 400C, 500C) and biosorbent dosage (0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5 g) on dye decolorization. The concentration of dye solution before and after biosorption was determined using UV-Visible Spectrophotometer (Optizen 3220U) by monitoring the absorbance of the dye used.

#### **4.1 Microorganisms Used for Toxicity Studies**

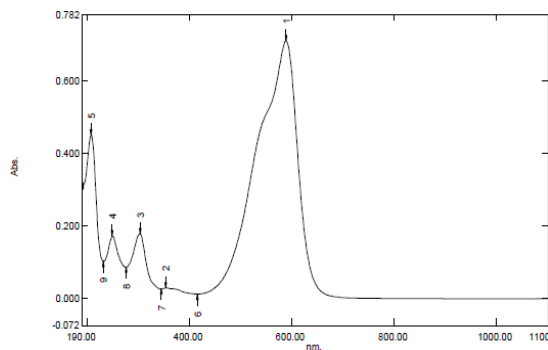
For the present work, the microorganisms i.e., the bacterial strains *Pseudomonas putida* and *Staphylococcus aureus* were maintained on nutrient agar slants and preserved at 40C and used for toxicity assay. This assay was performed to ensure that these two powders (*Annona reticulata* & *Cucurbita maxima*) are eco-friendly. These strains were procured from Biogenei laboratory, Ameerpet, Telangana [Figure 2].



**Figure 2:** (a) Agar slant with *Pseudomonas putida*; (b) Agar slant with *Staphylococcus aureus*

## 5. Results and Discussion

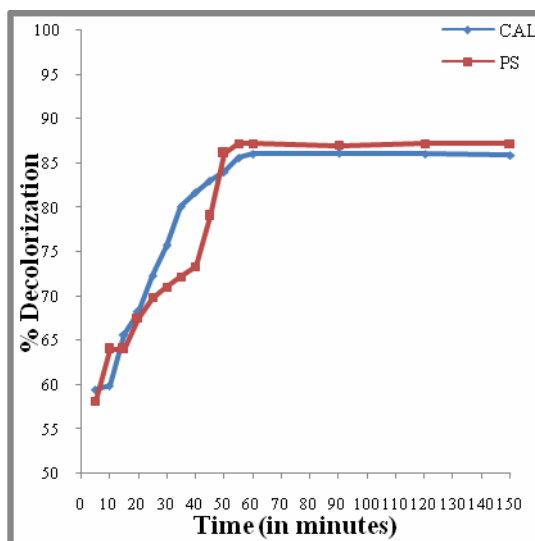
In the present investigation biosorbents that were selected for batch adsorption studies were prepared from natural sources. The effects of various physical parameters were determined by adding 0.1 gram each of the biosorbents with 100ml crystal violet solution of (10 mg $l^{-1}$ ) concentration in 250ml conical flasks. The mixture was shaken in a rotary shaker at 150 rpm and then the adsorbent was removed by filtration. [Figure. 1(c) 1(d)] The supernatant was analyzed for the residual concentration of crystal violet, spectrophotometrically at 580 nm wavelength [Figure 3].



**Figure 3:** Spectroscopic Peak for Crystal Violet

### 5.1 Effect of Contact Time

The effect of contact time on bio sorption was studied by adding 0.1g of *Annona reticulata* leaf powder as biosorbent in Erlenmeyer flasks 100 ml of aqueous solution with an initial concentration of crystal violet 10 mg/L at known pH 7.0 and the content was shaken well in an orbital shaker. At time intervals of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 90, 120, 150 min at a constant speed of agitation (RPM=150) sample was withdrawn. This sample was filtered from the bio sorbent and analyzed in UV- Vis spectrophotometer for the determination of left out crystal violet concentration in the filtrate. The given procedure was repeated with *Cucurbita maxima* seeds powder decolorization of crystal violet. The experiment was done in duplicates and the mean of the two replicates were considered to find out percentage removal. The result of contact time (min) vs. percentage removal of crystal violet dye is graphically represented [Figure 4].



**Figure 4:** Effect of contact time (min) vs. percentage removal of crystal violet dye

### 5.2 Effect of Solution pH

The effect of initial pH of the solution on equilibrium uptake of crystal violet for both the biosorbents was studied over a pH range of 2.0 to 10.00. The pH of the solution was adjusted using 0.1N NaOH and 0.1 N HCl solutions. In this study 100 ml of crystal violet solution of initial concentration 10mg/L was agitated with 0.1 g of *Annona reticulata* leaf powder as biosorbent at room temperature (30±10°C) for equilibrium contact time at a constant agitation speed (150 RPM). The procedure was repeated separately for decolorization of crystal violet



using Cucurbita maxima seeds powder. The graphical representation of pH of the solution vs. percentage removal of crystal violet dye is shown [Figure 5].

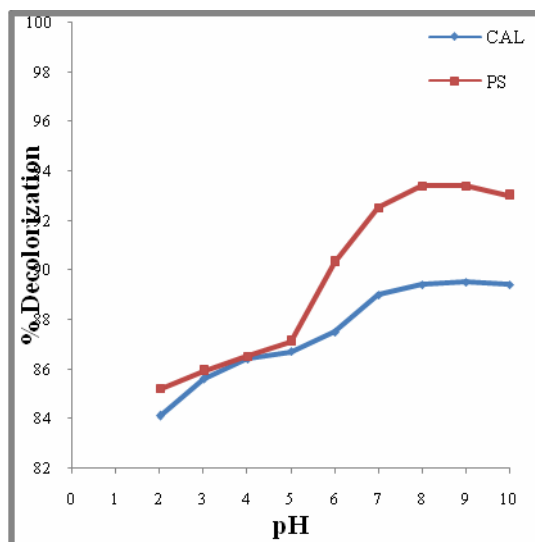


Figure 5: Effect of pH of the solution vs. percentage removal of crystal violet dye

### 5.3 Effect of Initial Dye Concentration

The influence of initial dye concentration was determined by adding 0.1 g of bio sorbent (Annona reticulata leaf powder) to 100 ml of crystal violet initial concentration of 10, 20, 30, 40, 50, 100, 150, 200, 250 and 300 mg/L keeping the contact time, solution pH and speed of agitation constant. The procedure was repeated for the bio sorption of crystal violet using Cucurbita maxima seeds powder. The mean of the two replicates were used to calculate the percentage decolorization of crystal violet and results of initial dye concentration (mg/L) vs. percentage removal of crystal violet dye is graphically represented [Figure 6].

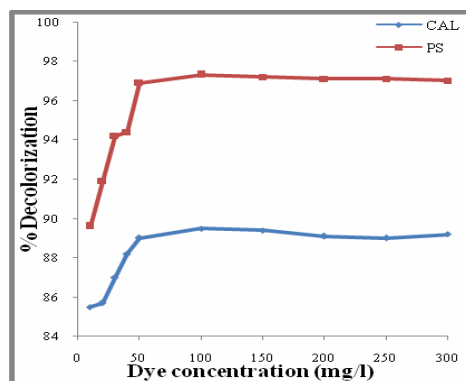
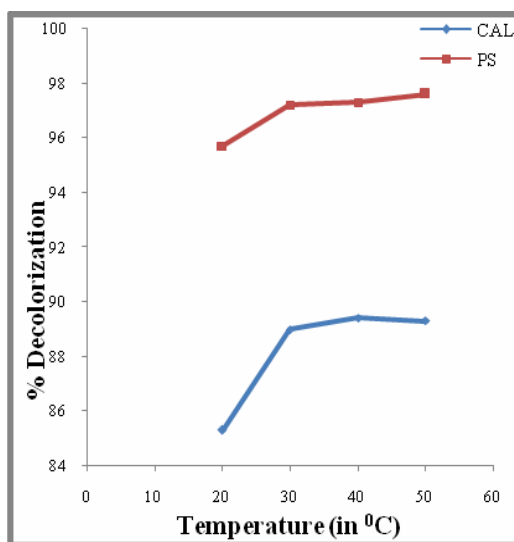


Figure 6: Effect of Initial Dye Concentration (mg/L) on Percentage Removal of Crystal Violet Dye

## 5.4 Effect of Temperature

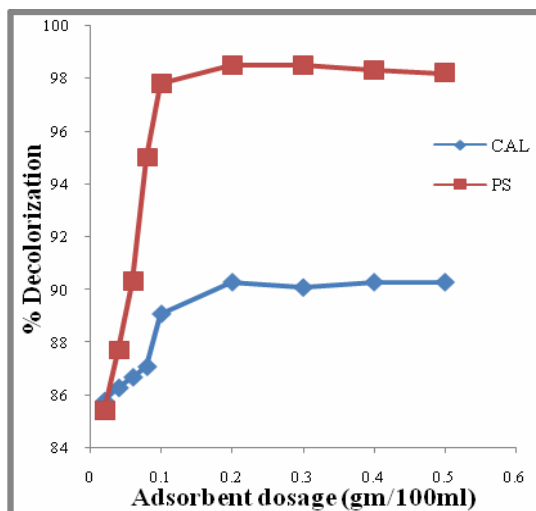
To study the effect of temperature, 100 ml of crystal violet solution of equilibrium initial concentration was taken and 0.1 g of bio sorbent (*Annona reticulata* leaf powder) was added. The solution pH was adjusted to optimum value using 0.1N NaOH and 0.1 N HCl solutions. The solution was agitated with constant agitation speed for equilibrium contact time of 60 min at different temperatures (20oC, 30oC, 40oC, 50oC). The mean of the two replicates were used to calculate the percentage decolorization of crystal violet and graph between temperatures (oC) vs. Percentage removal of crystal violet dye is represented [Figure 7].



**Figure 7:** Effect of Temperatures (0C) on Percentage Removal of Crystal Violet Dye

## 5.5 Effect of Bio Sorbent Dosage

The effect of bio sorbent dosage on the removal of dye crystal violet was determined by agitating 100 ml of solution of equilibrium initial dye concentration with 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5 g of bio sorbent dosage (*Annona reticulata* leaf powder) at optimum temperature, pH, and contact time at 150 rpm agitation speed. The experiment was done in duplicates and the mean of the two replicates were considered to find out percentage removal. The results of bio sorbent dosage (g) vs. percentage removal of crystal violet dye are graphically represented [Figure 8].



**Figure 8:** Effect of Biosorbent Dosage (g) on Percentage Removal of Crystal Violet Dye

## 5.6 Separation

The percentage decolorization of dyes by bio sorption of natural biosorbents was analyzed after every 5 minutes up to 2 hours of incubation. Similarly, other parametric variations were also analyzed in traditional stepwise protocol. 3 ml of solution was filtered and the supernatant was collected separately. The supernatant samples were carefully transferred and OD values were measured using a spectrophotometer.

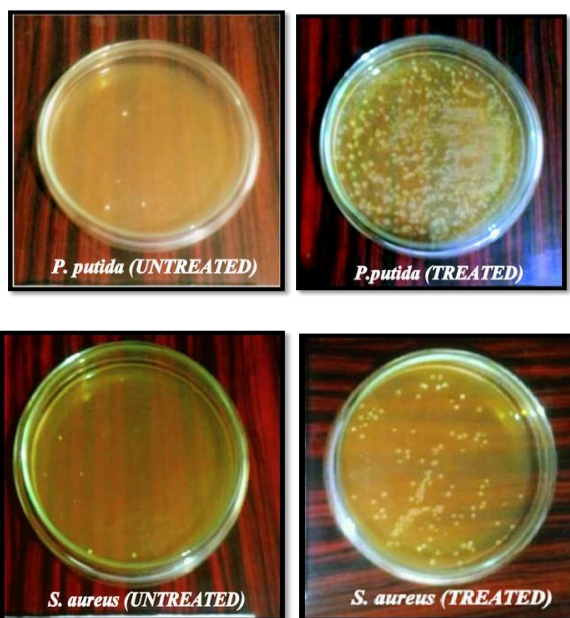
## 6. Conclusion

The following conclusions are drawn from batch experiments for both the natural biosorbents conducted for Biosorption studies:

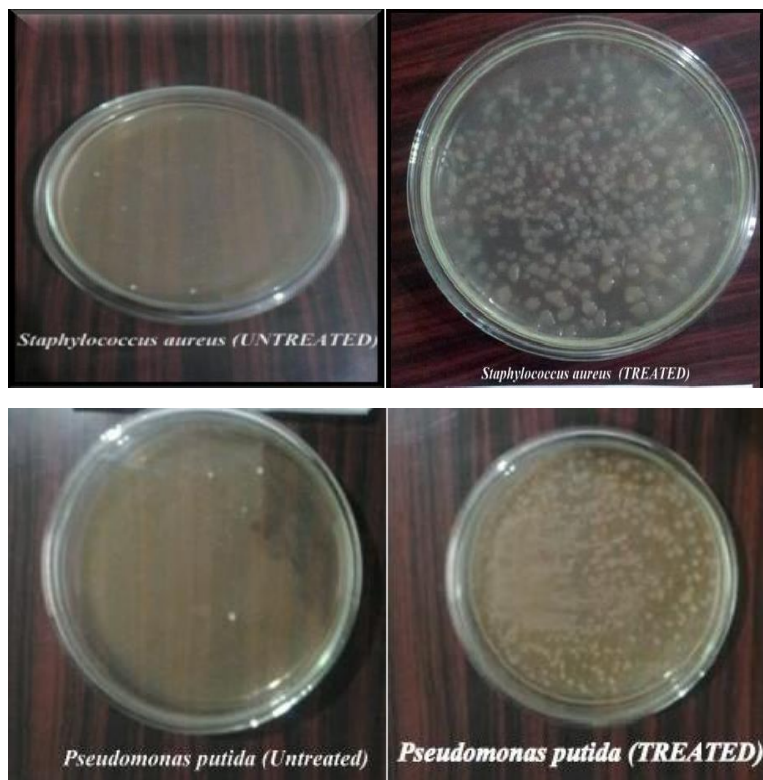
- The rate of adsorption increases when the pH is increased to 8.0 and remained constant, at the adsorbent dose 0.2 g / 100 ml; at 150 rpm of shaker for initial time of 55 minutes, with initial dye concentration of 25mg/L.
- It was found that the optimum pH of 8.0 has best to obtain maximum sorption of dye from the solution, the maximum adsorption was 98.5% and 90.3% for Cucurbita maxima seeds and Annona reticulata leaves respectively. This adsorption ability of these natural powders can be attributed to the fine particle size and large surface area of the pores.
- It is clear from the obtained results that the dye concentration is directly proportional to rate of adsorption and removal percentage, as dye concentration is increased from 10 mg/L to 100 mg/L and beyond which it maintains equilibrium, the maximum color removal was found at

100 mg/L that is 89.5% and 97.3% for *Annona reticulata* leaves and *Cucurbita maxima* seeds respectively. Toxicity assay showed that in the presence of bio sorbent *Annona reticulata* leaves powder the growth of microbes was more favorable when compared to *Cucurbita maxima* seed powder. [Figure. 9 & 10]

- Bio sorption technology has proven to be effective in adsorbing crystal violet dye from aqueous solution using of two natural bio sorbents *Annona reticulata* leaves and *Cucurbita maxima* seeds. Bio sorption technology has proven to be effective in adsorbing crystal violet dye from aqueous solution using of two natural bio sorbents *Annona reticulata* leaves and *Cucurbita maxima* seeds. Therefore it can be concluded that the two low cost bio sorbents are effective in removal of crystal violet dye and its harmful effects from the aqueous solutions.



**Figure 9:** The Treated (by Pumpkin Seed Powder) and Untreated Textile Dye (CV) on the Growth of *Pseudomonas putida* and *Staphylococcus aureus*



**Figure 10:** The Treated (by Custard Apple Leaf Powder) and Untreated Textile Dye (CV) on the growth of *Staphylococcus aureus* and *Pseudomonas putida*

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