

Down Regulating Colour and Toxicity of Effluent using Microaerophilic Bioreactor



Levin Anbu Gomez, Jesse Joel T, David Paul Raj R.S, Ramesh Siva R

ABSTRACT: Synthetic dye effluent is a major hazard to the surrounding environment. This hazardous condition is inevitable but the regulation of decolourizing and detoxifying could be done using reactors. In this study we have designed a microaerophilic bioreactor which incorporates a microbial consortium of *Pseudomonas stutzeri* and *Alcaligenes faecalis*. We had used the effluent from microbiological labs from our university and we treated them at the range from 20ppm to 60ppm of Crystal Violet dye. Standardization of dye removal was done primarily in small scale and later implemented in a large scale setup. Characterization studies for adsorbent was done using SEM analysis to get the nature of the pore size. In this study we chose 40ppm and 60ppm of dye effluent to be treated in lab scale setup with adsorbent and adsorbent along with microbial consortium and the treated effluent was analysed using UV-Visible spectrophotometry at 579nm and the efficiency of the percentage of removal was observed to be 99.7% and 99.1% for 40ppm with adsorbent and adsorbent with consortium respectively. Similarly for 60ppm it shows 99.8% and 99.4% removal respectively. The experiment done in small scale bioreactor with 60ppm shows 99.8% and 99.6% removal with adsorbent and adsorbent along with consortium and also the highest removal is achieved at 8pH. The adsorption isotherm was also studied.

Keywords: Crystal Violet, Decolourization, bacterial Consortium, SEM analysis, UV-Visible spectrophotometry.

I. INTRODUCTION

Dye is a coloured substance that has an affinity towards the substrate in which it is applied. Dyes are used in many industries such as textile, leather, paints, printing inks, coloration of plastics etc. The main application of dyes in medical field is for the staining process. Staining is an important technique that enhances contrast in the microscopic images.

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Dyes are also used in microbiology where it is applied to make microorganisms distinctly visible and to differentiate them. Crystal violet, malachite green, safranin are the important dyes that are used in the Gram's stain. Dyes have a systemic classification as natural or synthetic dye based on their chemical structure. Dye containing effluents are very problematic to the environment as it has high suspended solids and toxic compounds [1],[2].

Many bacteria, fungi, yeast have been used to decolourize and demineralize dyes [3],[4]. Nowadays bioreactors are configured and used to facilitate demineralization of dyes [5]. The objective of this study is to design a bioreactor which incorporates a microbial consortium of *Pseudomonas stutzeri* and *Alcaligenes faecalis* to decolourize and demineralize dyes.

II. MATERIAL AND METHODS

2.1 Design of the Downflow microaerophilic reactor

The design was done using autocad software and was outsourced for fabricating at Yuvaraj Engineering works, Coimbatore. Height of the Reactor is 90cm, Height of the bed is 28cm.

2.2 Activation of cultures from lyophilized form.

Lyophilized cultures of *Pseudomonas stutzeri* and *Alcaligenes faecalis* were bought from Microbial type culture collection and gene bank (MTCC) Chandigarh. These were activated by adding it into the Nutrient broth [10].

2.3 Sub culturing of microorganisms

After reviving of *Pseudomonas stutzeri* take 50ml of nutrient broth and add 2ml of activated *Pseudomonas stutzeri* into the nutrient broth. Now keep the broth in 25°C for 48 hours in shaker. Similarly add 2ml of activated *Alcaligenes faecalis* into 50ml of nutrient broth and keep the broth in 30°C for 48 hours [7].

2.4 Preparation of adsorbent

Sugarcane bagasse [8] was collected from a vendor in Karunya nagar Coimbatore. It was dried in sunlight and made into charcoal by flaming.

2.5 Preparation of Stock dye solution

Crystal violet dye powder was used to prepare the stock solution. 1 gram of crystal violet dye [9] powder was weighed and dissolved in 1000 ml of distilled water.

2.6 Preparation of dye with different concentration

To prepare 20 ppm concentration of dye, 2 ml of stock solution was made up to 100ml using distilled water. Similarly 40 ppm, 60 ppm, 80 ppm concentrations were prepared.

2.7 Experimental procedure

2.7.1 Effect of pH

The laboratory scale study was carried out in a burette. Dye with 40 ppm and 60 ppm with varying pH of 2, 4, 6, 8, 10 was prepared. The burette was filled with 1g of adsorbent and 10ml of dye was added into it and after half an hour the treated dye was collected. Then UV - visible spectrophotometric readings were taken before and after treatment of the dye. Similarly the same procedure was repeated but along with the adsorbent 10ml of broth was added into it; and then 10ml of dye was added. After 4 hrs the treated dye was collected and analysed using UV-visible spectrophotometry. Small scale study was carried out in the down flow microaerophilic reactor [6]. In this 5g of adsorbent, 800ml of broth and 1000ml of 60ppm concentration of dye were taken; and the treated dye was collected after 48 hrs and was analysed using UV-visible spectrophotometry at 579nm.

2.7.2 Effect of Contact time

The lab scale experiment: To a burette containing 1g of adsorbent, 20ml of dye was added and the samples were collected at 5 minutes interval till 30 minutes. The same procedure was repeated along with 10ml of broth and the samples were collected in 5 minutes interval of time. Then the collected treated dye samples were analyzed using UV-visible spectrophotometry at 579nm [11].

The small scale experiment was done using down flow microaerophilic reactor. In this 5g of adsorbent was taken and 1L of dye was added and the treated dye was collected for every 12hrs till 72 hrs. The same experiment was done along with adding 800ml of broth and similar step was repeated and the treated dye samples were analyzed using UV-visible spectrophotometry at 579nm [5].

2.7.3 Effect of varying the amount of adsorbent

The lab scale study was carried out in a burette taking the adsorbent in 1g, 2g, 3g. After taking the adsorbent in each burette, respectively 10ml of dye was added and the reaction was allowed to take place for half an hour. Then the treated dye was collected and the same procedure was repeated along with 10 ml of bacterial consortium and the treated dye was collected. Then these treated dye samples were analysed using UV-visible spectrophotometry at 579nm [8]. The small scale study was carried out in a down flow microaerophilic reactor ¹¹ and same procedure was carried out as lab scale but add 5g,10g,15g of adsorbent and 800ml of broth each and 1L of dye and the treated dye was collected after 2days and was analysed using UV-visible spectrophotometry at 579nm.

III. RESULTS AND DISCUSSION

As per Fig 1,2,3 The design was done using autocad software and the bioreactor [12] was fabricated at Yuvaraj engineering work Coimbatore and the front

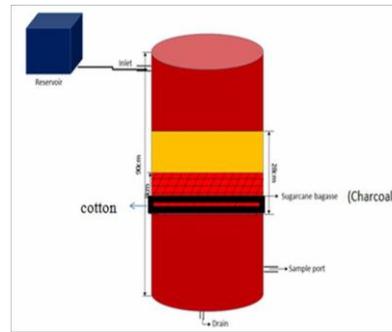


Fig 1: AutoCAD design of the bioreactor



Fig2: Frontview of the reactor



Fig-3 Filter bed front and inner view

view of the reactor [13] and filter bed, the inner view of the filter bed is shown along with the dimensions in Table 1.

Table 1: Dimensions of the bioreactor

Dimensions	Reactor	Filter bed
Height	90cm	28cm
Volume	59L	19L
Area	7174.84 cm ²	660.18 cm ²

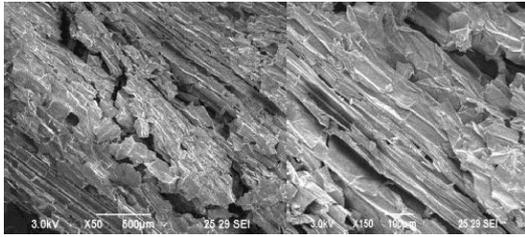
The commercially procured two microorganisms *Pseudomonas stutzeri* (MTCC no:101), *Alcaligenes faecalis* (MTCC no:2951) from MTCC Chandigarh and the subcultured microorganisms [14] [7] *Pseudomonas stutzeri*(MTCCno:101), *Alcaligenes faecalis* (MTCCno:2951) were kept in the nutrient broth at 25 and 35°C respectively for 48 hours in a shaker.



Fig4: Charcoal from sugarcane bagasse

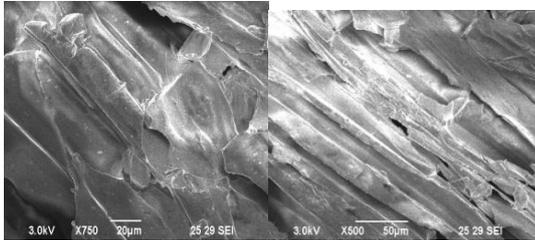
Fig 4 shows the charcoal prepared by flaming the sugarcane bagasse [8]

As per the Fig 5 The characteristic analysis of the sugarcane bagasse is done using SEM analysis at 50x, 150x, 500x, 750x and the size was found to be 500µm, 100µm, 50µm, 20µm respectively. Hence the increase in surface area provides more adsorbent capacity than that of sugarcane bagasse. The laboratory setup was done using a burette filled with layers of cotton, charcoal and microbial broth and dye with different concentrations [15].



@x50(500µm)

@x150(100µm)



@x500(50µm)

@ x75020µm

Fig 5: SEM analysis of sugarcane bagasse

Table 2 shows the OD value of the % removal of the dye and As the concentration increases the OD value also increases.

Table 2: OD value for % removal of dye.

Concentration (ppm)	OD (before treatment)	OD (after treatment with adsorbent)	% removal	rOD consortium	% removal after treatment with adsorbent
20	0.731	0.391	98.04	0.471	97.6
40	0.800	0.456	98.86	0.507	98.73
60	0.890	0.670	98.88	0.690	98.85
80	0.955	0.718	99.10	0.731	99.08

Laboratory scale results

In Fig 6 the graph shows the concentration with respect to the colorimetric reading as linear.

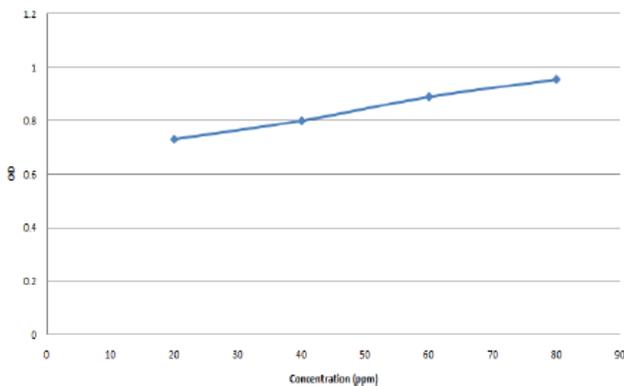


Figure 6: Graph for UV-Visible spectrophotometric analysis of crystal violet dye highest removal of dye is 99.1%.

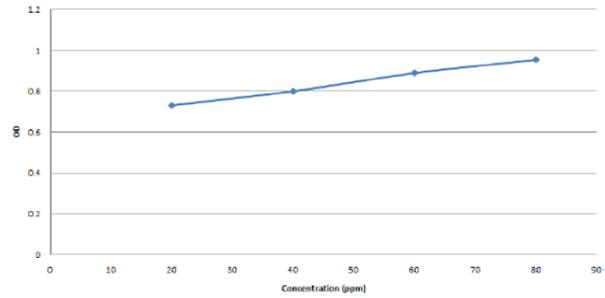
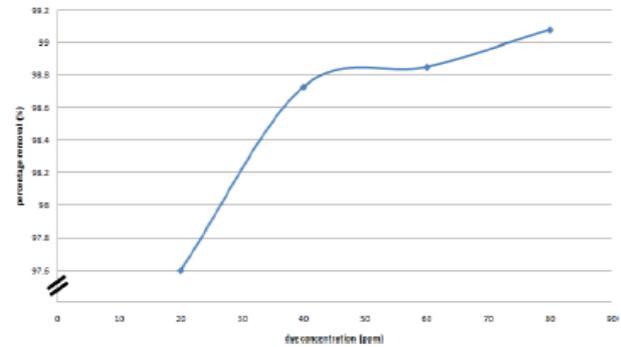


Figure 7 :Graph of the effect of dye concentration for % removal of dye after treatment with adsorbent



In Fig 8 the graph shows the % Removal of dye after treatment using adsorbent along with bacterial Consortium [5].The highest removal of dye is 99%.

Table 3 describes the effect of pH as an increase would proportionate to the % removal of dye as maximum for the highest pH.

PH	40ppm				
	2	4	6	8	10
% removal (With adsorbent)	99.4	99.54	99.67	99.79	99.70
% removal (With adsorbent and bacterial consortium)	98.25	98.81	98.84	99.17	98.87
PH	60ppm				
	2	4	6	8	10
% removal (With adsorbent)	99.5	99.62	99.75	99.85	99.7
% removal (With adsorbent and bacterial consortium)	98.9	98.99	99.3	99.4	99.2

Table 3: Effect of pH on % removal of dye (40ppm& 60ppm)

In Fig 9 the Graph shows the Effect of pH for % removal of dye (40ppm) after treatment with adsorbent and adsorbent along with consortium in lab scale and the result shows that the highest removal is seen at pH 8.

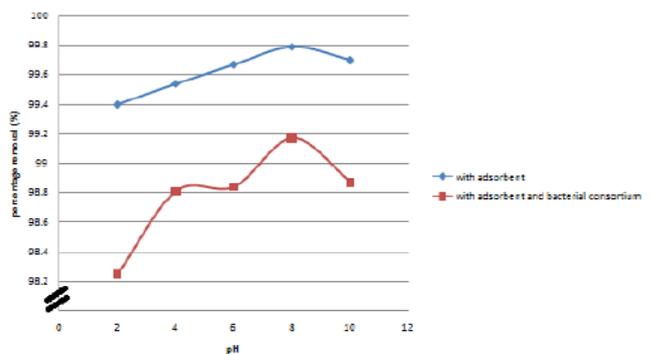


Fig 9: Graph for the Effect of pH for % removal of dye (40ppm) after treatment

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In Fig 10 the Graph shows the Effect of pH for % removal of dye (60ppm) after treatment with adsorbent and adsorbent along with consortium in lab scale and the result shows that the highest removal is seen at pH8.

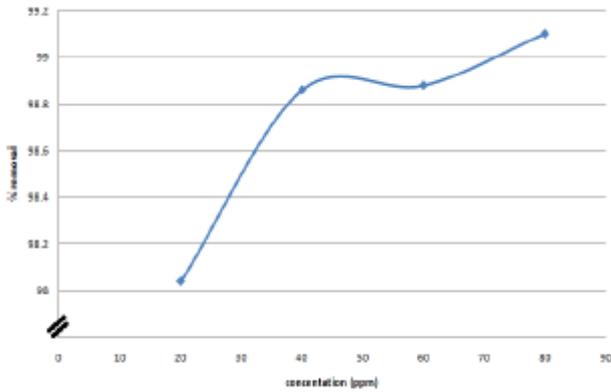


Fig 10: Graph for the Effect of pH for % removal of dye (60ppm) after treatment

Table 4 describes the contact time increases as the time increases.

Contact time (minutes)	5	10	15	20	25	30
40ppm						
% removal (with adsorbent)	99.35	99.41	99.5	99.64	99.79	99.79
% removal (with adsorbent and bacterial consortium)	98.5	98.8	99.10	99.15	99.22	99.22
60ppm						
% removal (with adsorbent)	99.55	99.61	99.68	99.81	99.85	99.85
% removal (with adsorbent and bacterial consortium)	98.9	99.13	99.2	99.3	99.45	99.45

In Fig 11 the Graph shows the Effect of contact time for % removal of dye (40ppm) after treatment with adsorbent and adsorbent along with consortium in lab scale [14] and the result shows that the removal of dye becomes constant at 25 minutes.

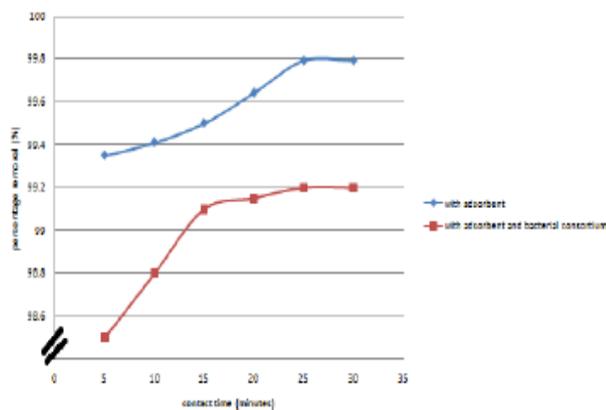


Figure 11: Graph for the Effect of contact time on % removal of dye (40ppm) after treatment

In Fig 12 the Graph shows the Effect of contact time for

% removal of dye (60ppm) after treatment with adsorbent and adsorbent along with consortium in lab scale and the result shows that the removal of dye becomes constant at 25 minutes.

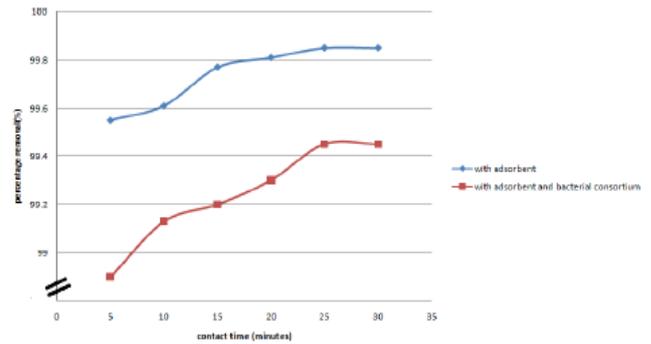


Fig 12: Graph for the Effect of contact time on % removal of dye (60ppm) after treatment
Table 5: Indicates the varying amounts of adsorbents increases the percentage removal of dye.

Amount of adsorbent (g)	1	2	3
40ppm			
% removal (With adsorbent)	99.37	99.55	99.7
% removal (With adsorbent and bacterial consortium)	98.26	99	99.17
60ppm			
% removal (With adsorbent)	99.7	99.78	99.84
% removal (With adsorbent and bacterial consortium)	99	99.17	99.41

In Fig. 13 the Graph shows the Effect of varying the amount of adsorbent for % removal of dye (40ppm) after treatment with adsorbent and adsorbent along with consortium in lab scale and it shows 99.72% removal of dye using only adsorbent and 99.1% removal of dye using adsorbent along with consortium.

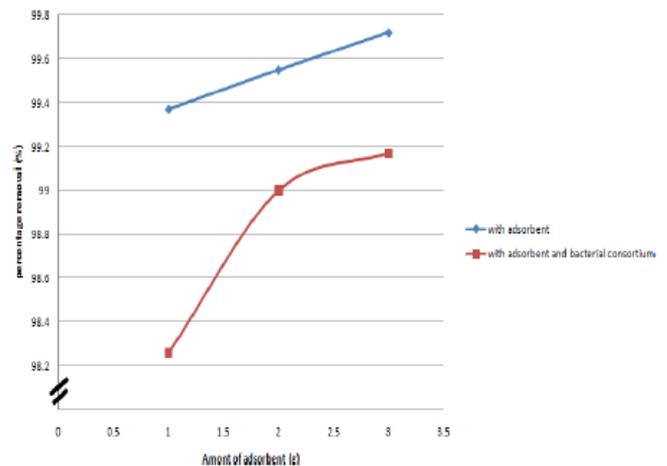


Figure 13: Effect of varying the amount of adsorbent for % removal of dye (40ppm) after treatment



In Fig. 14 the Graph shows the Effect of varying the amount of adsorbent for % removal of dye (60ppm) after treatment with adsorbent and adsorbent along with consortium in lab scale and it shows 99.84% removal of dye using only adsorbent and 99.4% removal of dye using adsorbent along with consortium.

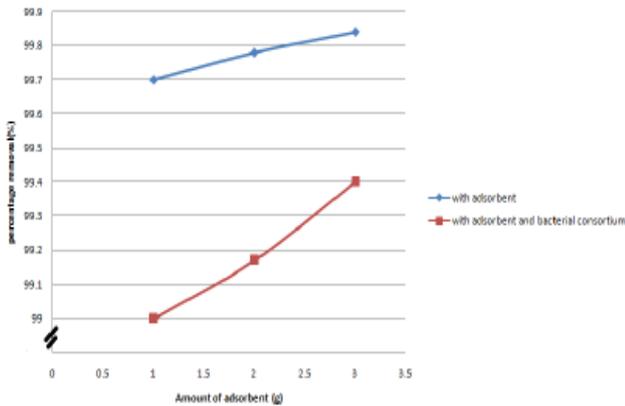


Figure 14: Effect of varying the amount of adsorbent for % removal of dye (60ppm) after treatment

Figure 15 and 16 show the packing of cotton and charcoal into the filter medium of the bioreactor [16].



Figure 15: Packing of filter bed with cotton

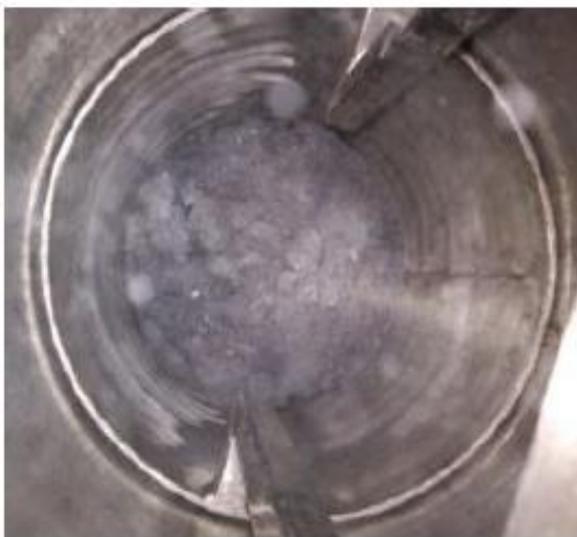


Figure 16: Packing of filter bed with charcoal

Small scale experimental results

Table 6 : Indicates the effect of pH at 60ppm on the percentage removal, with and without the adsorbent.

pH	2	4	6	8
% removal (With adsorbent)	99.5	99.6	99.8	99.88
% removal (With adsorbent and bacterial consortium)	99.1	99.17	99.36	99.6

In Fig. 17 the Graph shows the Effect of pH for % removal of dye (60ppm) after treatment with adsorbent and adsorbent along with consortium [17] in small scale bioreactor and the result shows that the highest removal is seen at pH 8.

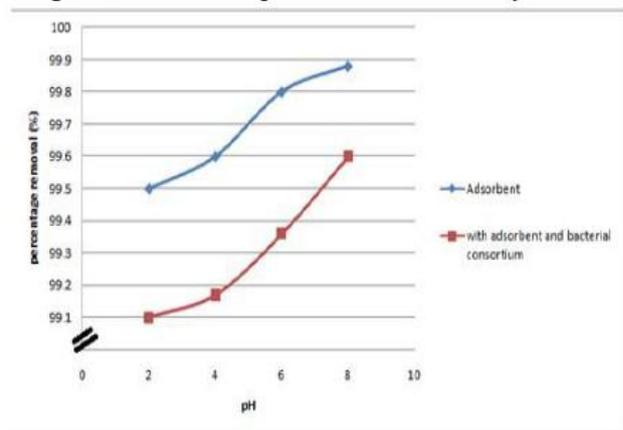


Figure 17: Effect of pH on % removal of dye after treatment with adsorbent and treatment with adsorbent and bacterial consortium

Table 7 indicates the effect of contact time on percentage removal as it increases proportionally as the contact time is increased.

Table 7: Effect of contact time on % removal of dye in the reactor (60ppm)

Contacttime (hours)	12	24	36	48	60	72
% removal (With adsorbent)	99.58	99.62	99.68	99.85	99.87	99.87
% removal (With adsorbent and bacterial consortium)	99.4	99.5	99.55	99.6	99.64	99.64

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Table 8 indicates the percentage removal in the reactor, which shows a linearity with and a decrease without the adsorbent.

Table 8: Effect of amount of adsorbent on % removal of dye in the reactor (60ppm)

Amount of adsorbent (g)	5	10	15
% removal (With adsorbent)	99.7	99.75	99.86
% removal (With adsorbent and bacterial consortium)	99.1	99.44	99.63

In Fig 18 the Graph shows the Effect of contact time for % removal of dye (60ppm) [12] after treatment with adsorbent and adsorbent along with consortium in small scale bioreactor and the result shows that the removal of dye becomes constant at 48 hours.

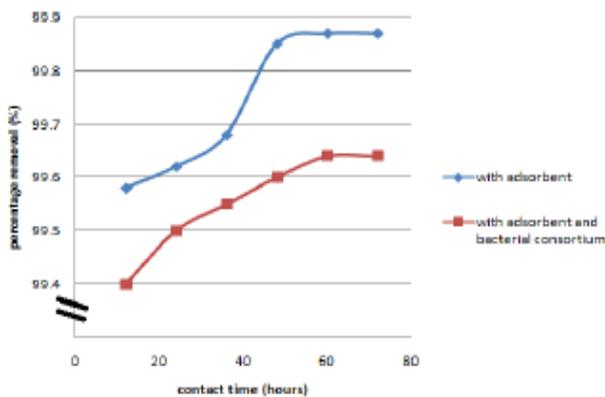


Figure 18: Effect of contact time on % removal of dye after treatment with adsorbent and treatment with adsorbent and bacterial consortium

In Fig 19 The Graph shows the Effect of varying the amount of adsorbent for % removal of dye (60ppm) after treatment with adsorbent and adsorbent along with consortium in small scale bioreactor and it shows 99.86% removal of dye using only adsorbent and 99.63% removal of dye using adsorbent along with consortium at the amount of 15g of adsorbent.

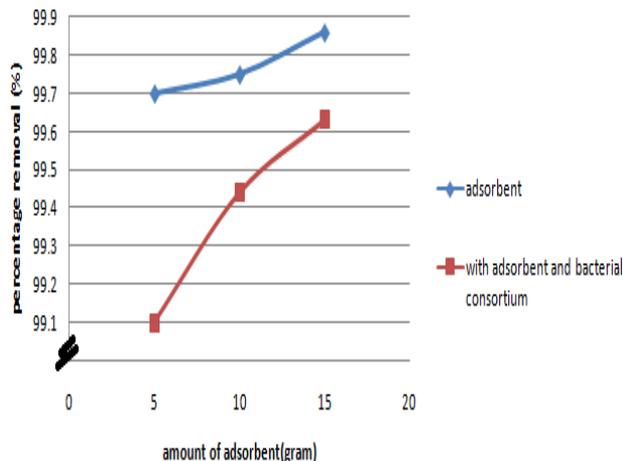


Figure 19: Effect of varying the amount of adsorbent on % removal of dye after treatment with adsorbent and

treatment with adsorbent and bacterial consortium Equilibrium models for the adsorption of crystal violet dye

Figures 20 and 21: Adsorption isotherms show the relation between the amounts of metal adsorbed per unit weight of adsorbate remaining in a test medium at equilibrium [18]. The capacity of charcoal was evaluated using Langmuir and Freundlich isotherm equations [15]. The Langmuir equation is used to estimate the maximum adsorption capacity corresponding to complete monolayer coverage on the adsorbent surface and is expressed by

$$1/q_{eq} = (1/Q_{max}b) + (1/ceq)$$

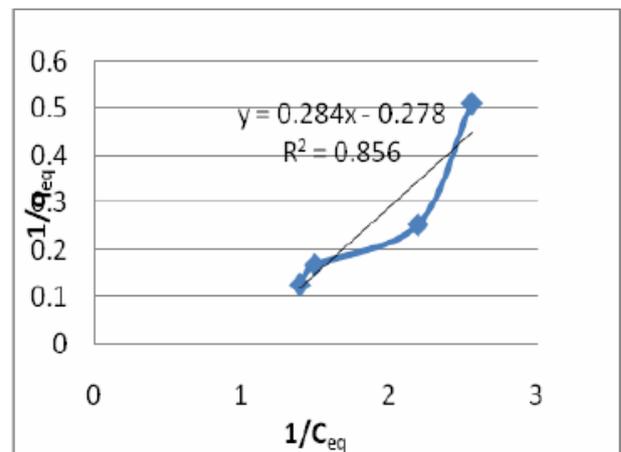


Figure 20: Langmuir Isotherm plot for dye adsorption into charcoal.

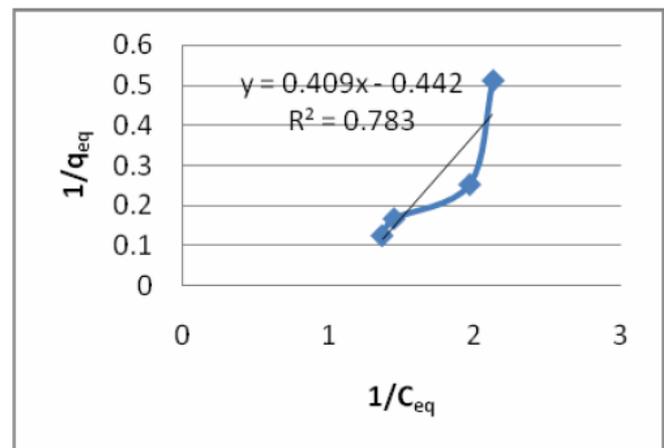


Figure 21: Langmuir Isotherm plot for dye adsorption into charcoal with bacterial consortium
Where, q_{eq} is the amount adsorbed at equilibrium (mg/g), ceq is the equilibrium concentration of dye (mg/L) and b is a constant related to the energy of adsorption

Table 9 indicates the Kinetics of the isotherm , Langmuir and the constants indicates the maximum removal with the adsorbent.

	Slope	intercept	Q _{max} (mg/g)	b (L/mg)	R ²
With adsorbent	0.284	0.278	0.978	3.597	0.856
With adsorbent and bacterial	0.409	0.442	1.080	2.262	0.783

Figures 22 and 23: The Freudlich model is an empirical equation used to estimate the adsorption intensity of the sorbent towards the adsorbate and is given by

$$\ln q_{eq} = \ln k_f + (1/n) \ln c_{eq}$$

where, q_{eq} is the amount adsorbed at equilibrium (mg/g), c_{eq} is the equilibrium concentration of dye (mg/L), n is indicative of bond energies between metal ion and the adsorbent and k is related to bond strength.

The R² value is found to be 0.911, 0.869 respectively.

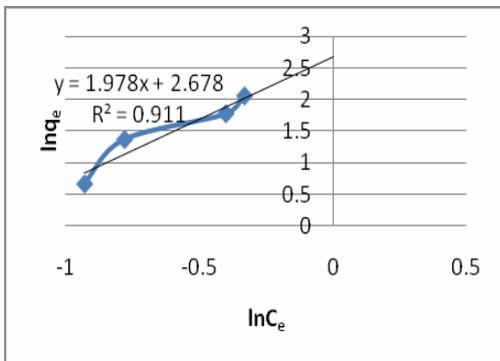


Figure 22: Freundlich Isotherm plot for dye adsorption into charcoal

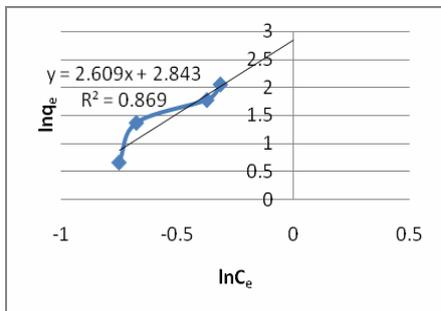


Figure 23: Freundlich Isotherm plot for dye adsorption into charcoal with bacterial consortium

Table 11 indicates the similar of Langmuir, the Freundlich isotherm constants with and without the adsorbents.

Table 11: Freundlich isotherm constants.

	K _f (mg/g)	R ²
With adsorbent	14.55	0.911
With adsorbent and bacterial consortium	17.16	0.869

IV. CONCLUSION

The down regulation of colour and toxicity of crystal violet dye was carried out in a down flow microaerophilic bioreactor. The bioreactor was designed using an AutoCAD software and was fabricated. Before treating the dye in the bioreactor, lab scale study was done using burette. Microbial consortium of *Pseudomonas stutzeri* and *Alcaligenes faecalis* was commercially procured and was activated using nutrient broth and was sub cultured. The adsorbent used is charcoal from sugarcane bagasse and the characterization of adsorbent was done using Scanning electron microscope. We had used the effluent from microbiological labs from our university and we treated them at the range from 20ppm to 60ppm of Crystal Violet dye. Standardization of dye removal was done primarily in small scale and later implemented in a large scale setup. In this study we chose 40ppm and 60ppm of dye effluent to be treated in lab scale setup with adsorbent and adsorbent along with microbial consortium and the treated effluent was analyzed using UV-Visible spectrophotometry at 579nm and the efficiency of the percentage of removal was observed to be 99.7% and 99.1% for 40ppm with adsorbent and adsorbent with consortium respectively. Similarly for 60ppm it shows 99.8% and 99.4% removal respectively. The experiment done in small scale bioreactor with 60ppm shows 99.8% and 99.6% removal with adsorbent and adsorbent along with consortium and also the highest removal is achieved at 8pH. The adsorption isotherm was also studied and the R² value for dye removal using adsorbent in small scale bioreactor was found to be 0.911. The cost of the bioreactor is Rs.10000 and the reactor can be used for further studies on waste water treatment and it has been dedicated to the Bioprocess lab of the Biotechnology department, Karunya Institute of Technology and Sciences. The adsorbent, culture, cotton used in the reactor can be reused twice.

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