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Research Article

A STUDY ON THE ABILITY OF MIXED CULTURE IN BIOLOGICAL DEGRADATION OF DYES

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ABSTRACT

Acid violets are commercially used in industries as it is having high fixation property. Microorganisms are the nature's tools for cleaning the environment. Several physico-chemical methods are used for the removal of dyes. Among this, using of microorganism will show the better degradation and also it's a cheapest method. The study is mainly focused on degradation of acid violet dye by effect of pH, initial concentration, glucose concentrations and time variation. High dye reduction was achieved at pH 7 and 1 g/ l glucose concentration. On the other hand, mineralization study shows that maximum COD reduction was achieved at pH 2.5.

Keywords: Acid violet dye, mixed culture, bioremediation, mineralization

INTRODUCTION

Huge quantity of dyes (0.7 million tonnes) are produced annually. Dyes are highly toxic and cause kidney tumours and other harmful effects[1-2]. Food dyes, especially pose mutagenicity, genotoxicity, etc to aquatic organisms as well as to animals. The dyes are degraded by physico – chemical and biological methods, out of these biological methods are considered to be most economical and efficient. Various bacteria and fungi are analysed and mixed culture proved to be more efficient in treating dye effluents.

The decolorization of textile wastewater is still a major environmental concern because the synthetic dyes used are difficult to remove by the conventional wastewater treatment systems based on adsorption, oxidation, coagulation, flocculation, chemical degradation, photo degradation, aerobic and anaerobic biodegradation and bio sorption[3-6].

The decolorization process is affected by several factors, such as concentration of pollutants, dyestuff concentration, initial pH and temperature of the effluent. The treatments are not suitable for all dyes because some of them are recalcitrant to biological breakdown and are non-transformable under aerobic conditions[7].The present work is focussing on the effectiveness of mixed culture as an alternative for degradation for waste water treatment.

MATERIALS AND METHODS

The model compound acid violet 17 is obtained from local textile mill. The structure of acid violet is given in Fig. 1. The physical and chemical property of the dye is given in Table 1.

Glucose medium composition

The mixed culture was grown in a medium containing glucose and dye as the carbon source. The composition of this medium with 1% glucose is given in the below Table.2. The concentration of glucose and the nutrients were varied according to the experimental requirements, but the same relative concentration values were maintained for all the media components. The concentration of dye was varied according to experimental requirements and it was independent of glucose concentration.

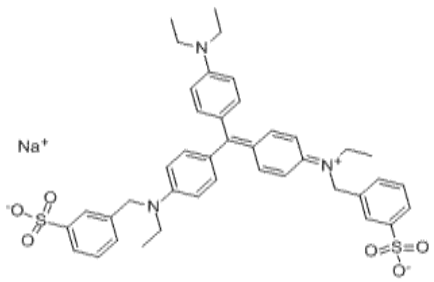

Fig. 1: Acid violet 17

Table 1: Physical and Chemical properties of acid violet 17

Chemical Name	Acid Violet 17
Molecular Formula	$C_{41}H_{44}N_3NaO_6S_2$
Form	Dustless powder
pH	6.0-8.0
Appearance	Dark blue
Odour	None
Specific gravity	~0.6 (loose) - 0.8 (packed) g/cc
Evaporation rate	Non-volatile
Solubility in water	Soluble
Vapour pressure	Not Applicable
Boiling point °F	Not Applicable
Melting point	Not available
Vapour density	Non-volatile

Table 2: Glucose medium compositions

Component	(g/l)
Glucose	1.000
Yeast extract	0.340
NH_4Cl	0.840
KH_2PO_4	0.134
K_2HPO_4	0.234
$MgCl_2.6H_2O$	0.084

Analytical Methods

The pH of the synthetic wastewater is fixed using Systronics water analyser 371. pH was adjusted using 0.1 N HCL and NaOH. The degradation was analysed using Systronics UV – Vis Spectrophotometer. The concentration of the Acid violet dye was measured at 550 nm. COD was analyzed using Hach COD colorimeter. The sample was digested with Hach LR COD solution for 2 hours. After cooling, the mixture was measured for COD in a colorimeter. Biomass measurement was done at 600 nm using Systronics UV – Vis Spectrophotometer.

Mixed Cultures

Mixed culture was prepared from the combination of Pseudomonas putita, Pseudomonas aeruginosa, Bacillus subtili and E. coli. Mixed cultures are kept in room temperatures with the required nutrient medium at regular intervals of time and are kept in shakers at 150 – 160 rpm for the good aerobic supply and good mixing of the nutrient medium in all sites of the reactor. The reactor used here is the standard conical flask.

Decolorization was studied using several concentrations of glucose at varying pH (2.5,7,10) and dyes concentrations (25,50,100 and 150 ppm).

RESULTS AND DISCUSSION

Effect of initial concentration

Rate of decolorization increased proportionately with increase in initial dye concentration up to 25ppm, 50ppm and 100ppm. The results are presented in figs 2-5. It can be seen that at an initial concentration of 100 ppm, 90-95% dye reduction was observed for all glucose concentrations. This is higher than that observed for 25 and 50 ppm initial concentration. As there is aqueous and solid phase involved, there will be mass transfer resistances. Higher concentration leads to higher concentration gradient across the phase, thus reducing the resistances. There could be better distribution of dye molecules yielding a higher contact time. All these factors can contribute higher decolorization at higher concentrations of 100 ppm. This is a good result as most of the dye effluents are in this range. There are but reports indicating otherwise[8-9].This is also observed for other toxic chemicals such as, toluene[10]and atrazine [11]. The toxic chemicals obviously have a deleterious effect on the microorganisms. However, these data are reported for pure culture. Even with cometabolic substrates[12], the outcome of the intrinsic toxicity of highly toxic chemicals is incomplete mineralization. Mixed culture on the other hand, utilizes the substrates efficiently because of a stable association of bacteria. The multiple bacteria undergo multiple exchanges and are likely to mineralize the target compounds. Mixed culture also have increased growth rates as well as reduced potential for phage infections[13].But, further increase in dye concentration resulted in slight reduction in decolorization rates. Lower decolorization rate at high dye stuff concentration was due to the inhibitory effects of high dyestuff concentration. Similar results are also observed by Khataee et al. (2009)[14].

Effect of pH

pH plays a crucial role in biological degradation. Microbes' production as well as dye degradation is influenced by pH. Figs 2-5 presents the effects of pH on removal of acid violet. Dye degradation increased from 70 to 90% on raising the pH from 2.5 to 7 for 25 ppm. However, further increase in pH reduces the dye reduction. Similar results were observed for other concentrations. Like any other substrate, dye molecules have to move across the cell membranes. The lipid bilayer is hydrophobic and dye molecules have hydrophilic moiety, the activity of which is a function of pH[15]. Chen et al., (2003)[16] and Guo et al., (2007)[17] have also noted that dye degradation normally takes place between pH 6 and pH 10. This fact was not supported by Aksu and Donmej (2003)[18], who observed maximum removal of Remazol Blue Reactive Dye at pH 12.

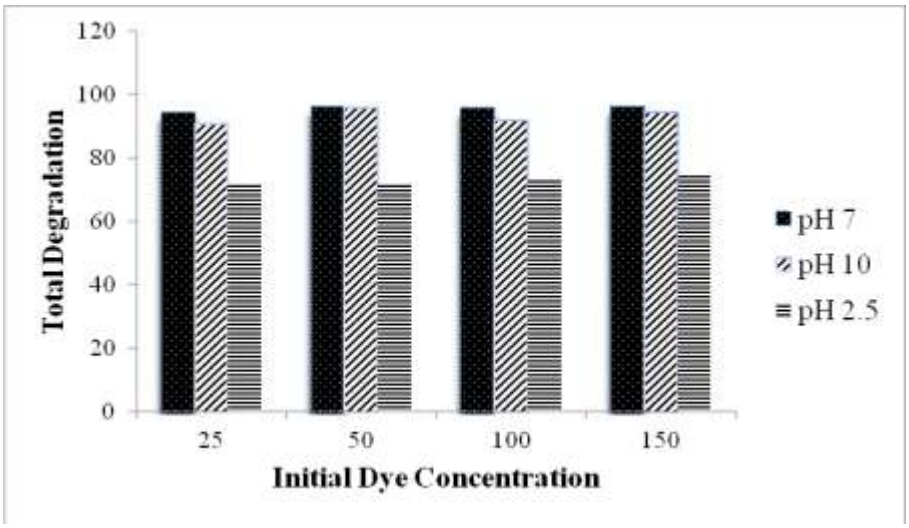

Fig. 2: Comparing dye degradation for different initial dye concentration and various pH's (0.5 g/l of glucose)

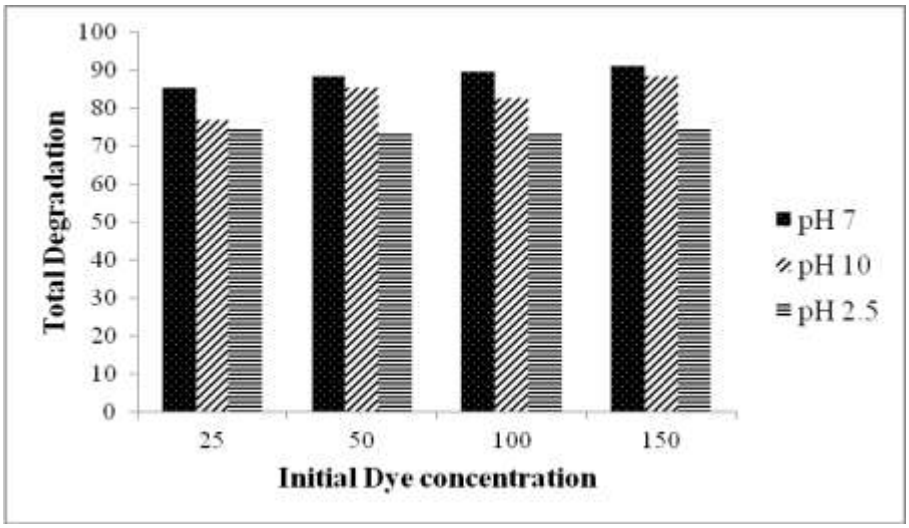

Fig. 3: Comparing dye degradation for different initial dye concentration and various pH's (1.0g/l of glucose)

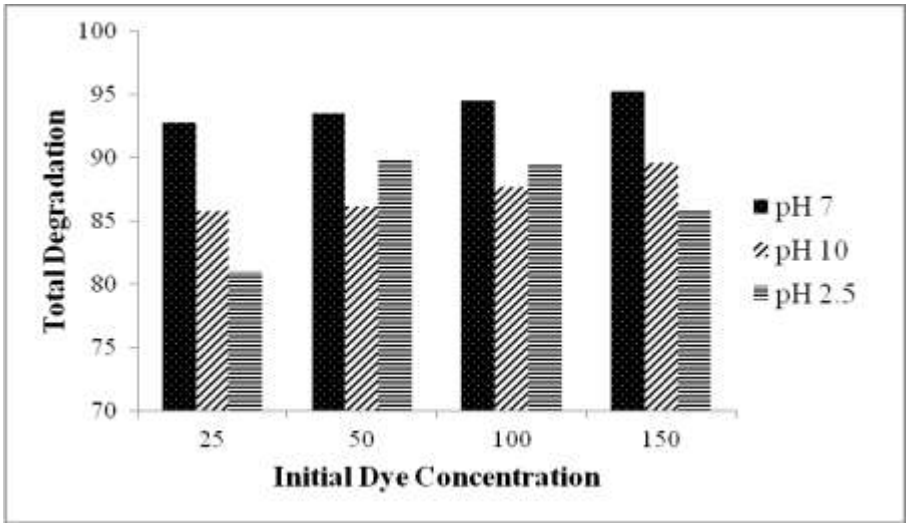

Fig. 4: Comparing dye degradation for different initial dye concentration and various pH's (1.5 g/l of glucose)

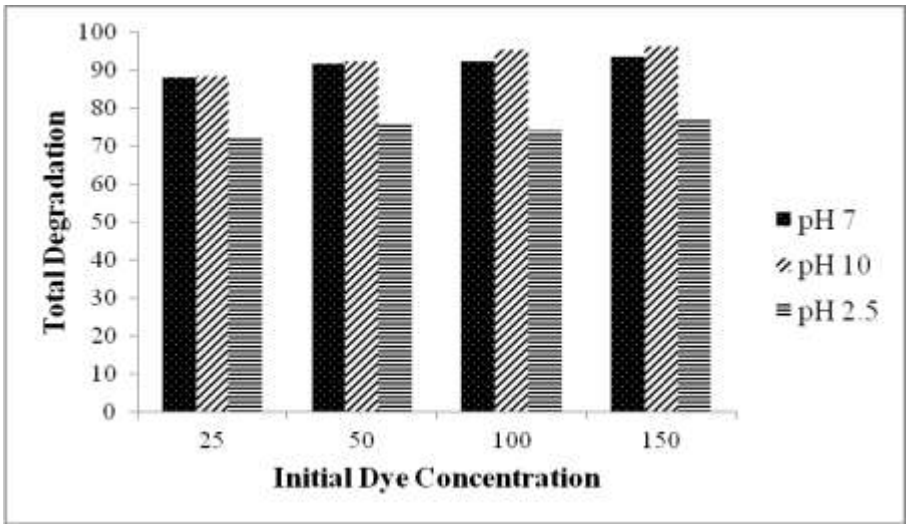

Fig. 5: Comparing dye degradation for different initial dye concentration and various pHs (2.0g/l of glucose)

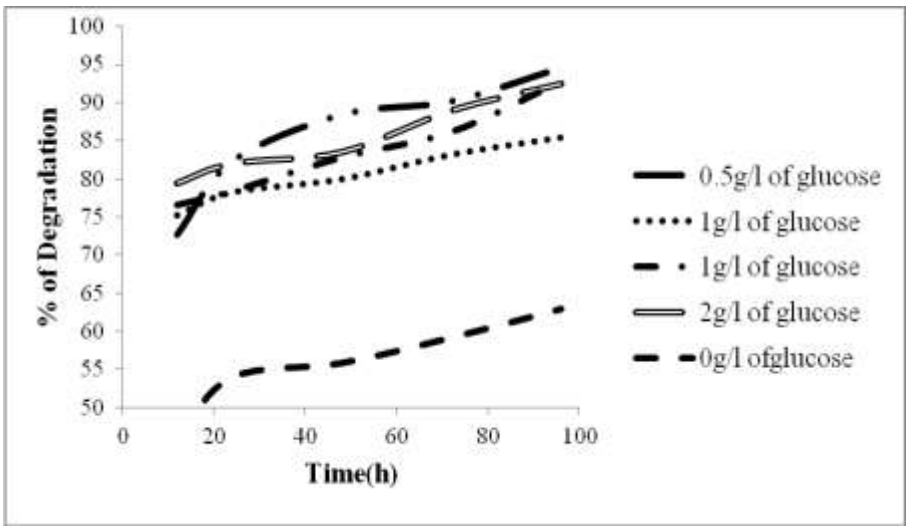

Fig. 6: Comparing the degradation with dye concentration of 25ppm at pH 7

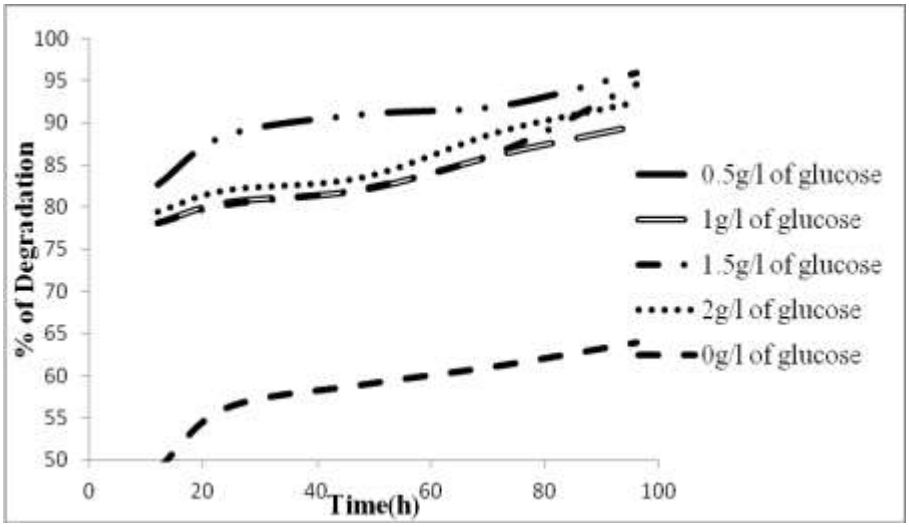

Fig. 7: Comparing the degradation with dye concentration of 100ppm at pH 7

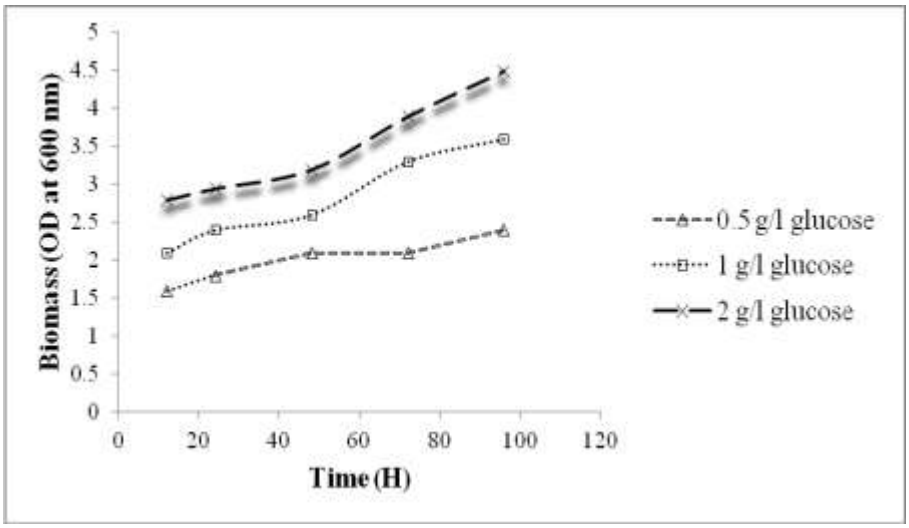

Fig. 8: Biomass variation with glucose concentration

Effect of glucose concentration

Dyes are toxic in nature and are not easily consumed by microbes. Moreover they are deficient in carbon content and hence cannot provide sufficient carbon on their own to the microbes. A biogenic substrate therefore seems to be essential requirements of such microbial degradation of dye effluents. Biogenic substrate such as glucose results in higher growth of microorganisms, probably leading to accelerated degradation of toxic compounds. Fig. 6 and 7 shows the effect of glucose concentration on dye degradation. It can be easily noted that there is increased degradation when glucose is present. The cultures without glucose have yielded only 25-30% degradation for both 25 ppm and 100 ppm initial concentrations of dye. Maximum reduction is however achieved at 0.5 g/l glucose for 25 ppm initial concentration and 1 g/l for 100 ppm initial concentration. This study is important since effluents normally contain the mixture of substrates. Sahinkaya and Dilek (2006)[19] also noted that concurrent degradation of multiple substrates may not be efficient. So increase in glucose concentration, though increasing the biomass, may not efficiently increase the degradation rate. It is apparent from the Fig. 8 that higher glucose concentration leads to high biomass concentration. Since toxic compounds are more a function of effective biomass that can consume the chemicals and hence degrade it, higher biomass may not be so useful in degrading these chemicals. There are however, reports on the usage of similar substrates resulting in increased degradation of toxic compounds[20]

Mineralization Study

In order to better understand the degradation process of dye effluents, total mineralization study was also carried out for some of the experiments. Effect of pH for different glucose concentrations were studied for 100 ppm initial concentration of dye. Mineralization indicates complete conversion of effluents to innocuous products. The results of mineralization are presented in Fig. 9. It can be noted that maximum COD reductions were observed at pH 2.5 and 1 g/l glucose. This result is interesting as is noted in section 4.2 that maximum dye removal was observed at pH 7. The microbial reduction of dye goes through intermediate stages. The parent compound may be eliminated, but there are possibilities of intermediate compounds in the system, increasing the COD. Hence mineralization study is important to properly asses the degradation potential of microbial cultures. Since 1 g/l glucose concentration has given higher COD reduction, which conforms to the results obtained for dye removal. This is a significant finding as it eliminates the necessity of high biogenic substrate for dye degradation. High biogenic substrate leads to higher cost thus making the process uneconomical.

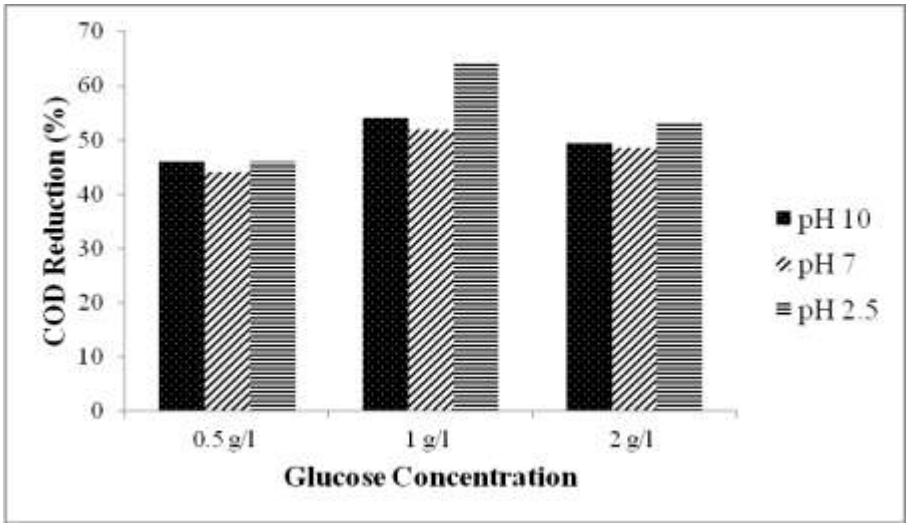

Fig. 9: Mineralization study of the effluents (effect of pH)

CONCLUSIONS

Removal of acid violet dye by degradation with mixed culture has been experimentally determined and the following observations are made:

1. Highest dye degradation was achieved at pH 7.
2. Dye removal was varying with initial concentration of dye. Comparing to the lower concentration (25ppm & 50ppm), the results are found to be better in higher concentration (100 & 150ppm).
3. There is higher degradation in the presence of glucose, however, maximum dye removal was observed at 1 g/l glucose concentration compared to 2 g/l glucose concentration.
4. Mineralization study was also done to assess the extent of mineralization. Contrary to dye removal, maximum COD reduction was achieved at pH 2.5.

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