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Effect of bioadsorbents in removal of colour and toxicity of textile and leather dyes

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Abstract
The main aim of this study was to reduce the colour and toxicity of textile dye Levafix Blue CA and leather dye Brown VBR by using prawn shell waste, rice husk, poultry soil waste, apricot seed and tea powder waste as adsorbents. The textile dye Levafix Blue CA and leather dye Brown VBR were prepared to the concentration 500mg/L as stock solution. The different initial dye concentration as 100,200,300,400 and 500mg/L were prepared from stock solution by diluting with distilled water. bioadsorbents used in this study were mixed to each dye for every concentration, and were agitated then subsequently removed by centrifugation for decolorization. The adsorbents treated, untreated textile and leather dyes were tested for the growth of P.putida and B.subtilis to evaluate the toxic effect. The development of colonies on treated dyes were measured as cfu ml-1. The development of non colonies on untreated dyes was also measured. This positive result confirmed the toxicity reduction on treated dye. The result showed that adsorption and decolorization capacity of the adsorbents in the order prawn shell waste>rice husk>poultry soil waste>apricot seed>tea powder waste.

Keywords: *Bioadsorbents,LevafixBlue CA,Brown VBR,Decolourization, Pseudomonas putida and Bacillus subtilis*

INTRODUCTION

A variety of pollutants are discharged in the environment from large number of industries and mills, especially in the form of dyes. Dye stuffs are chemically inorganic pollutants and comprise traces of heavy metals, which are toxic and hazardous to the environment, as they are non-degradable and tedious to recycle.

Dyes are basically chemical compounds that can attach themselves to fabrics or surfaces to impart colour. Most dyes are complex organic molecules and are need to be resistant to many things such as the weather and the action of detergents. Dyes are widely used in industries, such as textiles, paper, plastics and leather, etc., for the coloration of products. The effluents emanating from these industries often contain high concentrations of dye wastesSynthetic dyes are extensively used in many fields of up-to-date technology, e.g., in various branches of the textile industry (Gupta *et al*., 1992; Shukla and Gupta, 1992 and Sokolowska-Gajda *et al*., 1996), of the leather tanning industry (Tünay *et al*., 1999 and Kabadasil *et al*., 1999) in paper production (Ivanov *et al*., 1996), in food technology (Bhat and Mathur, 1998 and Slampova *et al*., 2001), in agricultural research (Cook and Linden, 1997 and Kross *et al*., 1996), in light-harvesting arrays (Wagner and Lindsey, 1996), in photoelectrochemical cells (Wrobel *et al*., 2001), and in hair colourings (Scarpi *et al*., 1998).

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Two percent of the dyes produced are discharged directly in aqueous effluent, with a further 10% subsequently lost during the textile coloration process (Easton, 1995). It has been reported that over 100,000 dyes are commercially available, with a production of over 7×10^5 tonnes per year (Zollinger, 1987; Aksu, 2005). Dyes are generally believed to be toxic and carcinogenic or prepared from other known carcinogens (Banat et al., 1996). The discharge of these dye stuffs from industries into rivers and lakes results in a reduced dissolved oxygen concentration causing anoxic conditions, which subsequently affect aerobic organisms (Chander and Arora, 2007). Apart from the toxicological properties of dyes, their color is one of the first signs of contamination recognized in a wastewater. Since a very small quantity of dyes in water is highly visible, it often affects the aesthetic merit and water transparency (Banat et al., 1996).

Various techniques have been employed for the treatment of dye/metal bearing industrial effluents, which usually come under two broad divisions:abiotic and biotic methods.Abiotic methods include precipitation, adorption, ion exchange, membrane and electrochemical technologies.Much has been discussed about their downside aspect in recent years (Atkinson et al.,1998; Crini,2006),which can be summarised as expensive, not environment friendly and usually dependent on the concentration of the waste. Therefore, the search for efficant, eco-friendly and cost effective remedies for waste water treatment has been initiated.

In recent years, research attention has been focused on biological methods for the treatment of effluents,some of which are in the process of commercialization (Prasad and Freitas,2003).There are three principle advantages of biological technologies for the removal of pollutants; first, biological process can be carried out *in situ* at the contaminated site; second, bioprocess technologies are

usually environmentally bening(no secondary pollution) and third, they are cost effective. There are many physical, chemical and biological methods employed in dye removal and they are highly expensive. To overcome this, more economically alternative low cost bioadsorbents would be of great value. Among many new technologies, utilizing Plant residues as adsorbents for the removal of dyes and metal ions from waste water is a prominent technology. Removal of dyes by using these biosorbents follows the mechanism of reaction with proteinaceous cellular material. The presence of proteins and chrome chemicals in waste water lead to changes in the pattern of biodegradation.

The biosorption process involves a solid phase (sorbent or biosorbent; adsorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (adsorbate, metal/dyes). Due to the higher affinity of the adsorbent for the adsorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound adsorbate species and its portion remaining in the solution. The degree of adsorbent affinity for the adsorbate determines its distribution between the solid and liquid phases

Recently,numerous approches have been made for the development of low-cost adsorbents from industrial and agricultural wastes and also various agricultural products and by products have been investigated to remove dyes from aqueous solution. These include crab shells (lee et al., 1997),cotton, rice husk, bark (McKay et al., 1999), rice straw (Abd El-Rahim, 2006), peanut hull (Gong et al., 2005;Duygu et al., 2007), orange peel (Namasivayam et al., 1996;Rajeshwari et al., 2001), sugar cane dust (Khattri et al., 1999), sugarcane begasse (Tsai et al., 2001), corncob (Preethi et al., 2006), barley husk (Robinson et al., 2002), egg shell (vijayaraghavan et al., 2005b), barley straw (Husseien et al., 2007), tree fern (Ho et al., 2005), coconut pollens (Agiri et al., 2007), coir pith (Namasivayam et al., 2001;Kavitha et al., 2007), banana pith (Namasivayam et al., 1992), de-oiled soya (Gupta et al., 2005;Alok Mittal et al., 2008), maize cobs (Abdel-Ghoni et al., 2007), jatropha husk (Namasivayam et al., 2007), kapok hull (Syed Shabudeen et al., 2008). In addition, hen feather (Alok Mittal, 2006), flyash (Manaskom et al., 2004), tamarind nut (Ramadevi et al., 2005), kiln dust-a cement factory waste (Abdel Monem et al., 1999), pomace-an olive oil industry waste (Emine Malkoc et al., 2008) etc., are also used as an effective low cost adsorbent for the removal of dyes and metal ions either by making as an activated carbon form or as raw biosorbent. Of these, crab shells (Lee et al., 1997), activated sludge (Al-Qodah, 2006), rice husks (Chuah et al., 2005), egg shell (Vijayaraghavan et al., 2005b) and peat moss (Sharma andForster, 1993) deserve particular attention

In this study, an attempt was made with textile dye- Levafix Blue CA, and leather dye-Brown VBR to reduce it's dye toxicity. The vinyl sufone group of the Levafix Blue CA and aromatic nitro group of the Brown VBR is considered to carry the risk of mutagenecity when inhaled or ingested. The removal of toxicity and decolorization was studied with bioadsorbents prawn shell waste, rice husk, poultry soil waste, apricot seed and tea powder waste. The main aim of decolourization is detoxification because dyes in textile and leather industry effluent water are very recalcitrant and may be inhibitory to microbial consortia of conventional treatment and the toxic effect of these adsorbents treated and untreated dyes was tested with the bacterial strains Pseudomonas putida and Bacillus subtilis.

MATERIALS AND METHODS

Sample collection

The textile dye sample Levafix Blue CA and leather dye sample Brown VBR were collected from the textile industry (Royal super fabrics pvt. Ltd.,Cuddalore,Tamilnadu,India) and leather industry(Krithika's leather finishers, Shaheen leathers, Ranipet, Vellore.Tamilnadu) respectively. The adsorbents prawn shell waste, rice husk, poultry soil waste, apricot seed and tea powder waste were collected from various local fields for this study.

Culture collection

The microbial culture *Pseudomonas putida* MTCC 102 and *Bacillus subtilis* MTCC 121 were obtained from Microbial Type Culture Collection Center, IMTECH, Chandigarh, India.

Preparation of adsorbents

The very cheap low cost adsorbents prawn shell waste, rice husk, poultry soil waste, apricot seed and tea powder waste were ground well in a powder form. The size distributions of the different adsorbents were determined using a variety of molecular sized sieves. Three different size as coarse, medium and fine with the size of 250 m,120 m and 75 m respectively were determined as well. Decolorization was done with both these size of adsorbents effectively.

Preparation of dye solution

The dye stock solutions were prepared from the dye powder Levafix Blue CA and Brown VBR of textile and leather industry respectively. The stock solution was prepared in concentration of 500mg/L by dissolving the accurately weighed dye powders in distilled water. The different concentration as 100,200,300,400 and 500mg/L were prepared from the stock solution by further dissolving the stock solution in distilled water with required volume.

The accurate proportion of five different initial dye concentration for each adsorbent treatment vice versa totally 25 samples were prepared as experimental solution for each dye i.e. textile and leather dye. Finally 50 samples for both dyes, each sample with 50 ml in 250ml conical flask was prepared as well.

Decolorization experiment

Decolorization experiment was carried out with response to various factors like agitation speed, agitation time, pH, temperature, adsorbent size and adsorbent dosage.

The 250ml conical flasks containing 50ml of dye solution at natural pH were mixed with adsorbent dose of 0.5g and were agitated at 150rpm using rotary shaker for 30 minutes at 30 c. In later the agitation period, the flasks were removed from shaker and

the contents were centrifuged at 10,000 Xg for 5 min each. The pellet indicated itself the dye adsorbed waste, which was removed by transferring the supernatant to fresh test tubes.

The absorbance values of the supernatant were estimated at 480nm and 670nm for Levafix Blue CA and Brown VBR respectively. The residual dye concentration was determined using this absorbance values. The methods were replicated three times.

Toxicity assay

Pseudomonas putida and *Bacillus subtilis,* Bacterial strains were used to test the toxicity of treated and untreated dyes. For this, the subculture of *P.putida and B.subtilis* were separately prepared by inoculating these strains from master plates into fresh nutrient broth medium and were incubated for 18 hours at 30°C.

Each toxicity test was carried out in test tubes in a final volume of 5ml, containing 100 ml of bacterial suspension, 1ml nutrient broth and remaining of dye solution to be tested. The toxicity test was carried out for each dyes with different wastes, in different concentration. Totally 120 test tubes for both treated untreated dyes was carried out and this mixture was incubated for 24 hours at 37°C.

At the end of incubation period, 1ml of mixture was spread plated on nutrient agar plates and was incubated for further 24 hours at 37°C. Then the viable cells counted on nutrient agar plates using colony counter. The viable cell count was expressed as mean colony forming unit per ml (cfu ml-1).

RESULTS AND DISCUSSION

Effect of the initial dye concentration on decolorization

The effect of initial concentration of dye in solutions on removal of dye colour was studied. In previous study, the results shown that 0.5g of adsorbent dosage had highest dye removal capacity among various dose of 0.1, 0.2, 0.5 and 1.0g (Sibel Kahraman *et al.,* 2005). By support this study further experiments were done using 0.5g of adsorbent dosage. Rajeshwari *et al.,* (2001) reported that 0.6g/50 ml of adsorbent dosage (Orange peel) were effective in dye (Acid Violet 17) removal of 10mg/L dye concentration.

The experiments were carried out at fixed adsorbent dose(0.5g/50ml) in the test solution at 30°c temperature, natural pH,fixed agitation (150rpm) for 30 minutes time with adsorbent particle size of 75µm (fine), 120µm (medium), 250µm (coarse) for different initial concentration (100,200,300,400 and 500mg/L) of dye solution.

The result of centrifugation i.e., after the removal of pelletized supernatant showed that experiments with fine particle sized adsorbent had better decolorization visually. This results correlate with the study, the adsorption of dye (Safranin) on adsorbent (Corncob) was increased with increased finer mesh size (Preethi *et al.,* 2006).

The residual dye concentration of experimental solution dealt with fine particle sized adsorbents, were determined according to the absorbance values (Table 1&2) and further experiments were continued subsequently with this as an experimental solution.

The removal of dyes dependent on the initial concentration of the dye solution i.e., dye removal increased with decrease of initial dye concentration, was determined clearly as color difference (fig.6-9). In previous studies dye removal increased with increase in pH (Rajeshwari *et al.,* 2001), and temperature (Kavitha *et al.,* 2007). The textile dye Levafix Blue CA was decolorized, by prawn shell waste from 93% to 70% (fig.1); by rice husk from 91% to 67% (fig.2); by poultry soil waste from 88% to 61% (fig 3); by apricot seed from 90% to 58% (fig.4); by tea powder waste from 72% to 50% (fig.5).

The leather dye Brown VBR was decolorized, by prawn shell waste from 95% to 72% (fig.1); by rice husk from 92% to 69% (fig.2); by poultry soil waste from 90% to 60% (fig.3); by apricot seed from 89% to 55% (fig.4); by tea powder waste from 72% to 47% (fig.5).

The result showed that adsorption and decolorization capacity of the adsorbents in the order prawn shell waste>rice husk>poultry soil waste>apricot seed>tea powder waste.

Antibacterial effect of untreated and treated dyes

Dyes can be toxic to many organisms in the aquatic and soil environment. Therefore, the elimination of the dyes in waste water is an important objective in the search for a method to eliminate its pollution properties.

Antibacterial effects of the treated dyes on different bacterial strains, *pseudomonas putida* and *Bacillus subtilis* were tested to evaluate the toxicity after decolorization. In this study, it was shown that the dyes used were toxic and their toxic effects increased with an increase in concentration (Sibel Kahraman *et al.,* 2005). It appeared that the treated dyes were less toxic. The removal of Levafix Blue CA and Brown VBR by prawn shell,rice husk,poultry soil,apricot seed and tea powder adsorbents, reduced the toxic effects on *pseudomonas putida* and *Bacillus subtilis.*

As the development of colonies on treated dyes (Table.4-8 & fig.10), and non colonies on untreated dyes (Table.3 & fig.11) indicated clearly the toxic reduction on dye when treated with adsorbent. Moawad *et al*., (2003) reported that high concentration of dyes eliminated microbial colonies due to high frequency of mutation. This reduction in toxic effect is important both in respect of environmental biotechnology and detoxification.

Table 1. Absorbance of the treated Levafix Blue CA at 480 nm.

Dyes mg/L	Prawn shell waste	Rice husk	Poultry soil waste	Apricot seed	Tea powder waste
100	0.305	0.380	0.219	0.312	0.811
200	0.384	0.449	0.429	0.487	1.057
300	0.789	0.299	0.669	0.782	1.337
400	0.385	1.076	0.895	0.989	1.541
500	0.530	1.102	1.105	1.188	1.767

Table 2. Absorbance of the treated Brown VBR at 670 nm.

Dyes mg/L	Prawn shell waste	Rice husk	Poultry soil waste	Apricot seed	Tea powder waste
100	0.160	0.112	0.082	0.099	0.214
200	0.212	0.172	0.156	1.169	0.269
300	0.307	0.237	0.260	0.241	0.288
400	0.233	0.331	0.381	0.337	0.383
500	0.284	0.367	0.484	0.429	0.443

Table 3.The effect of untreated textile and leather dye on the growth of *P.putida* and *B.subtilis*

Viable cell number (cfu ml^{-1})

Dyes mg/L	Effect on *P.putida*		Effect on *B.subtilis*	
	Untreated Levafix Blue CA	Untreated Brown VBR	Untreated Levafix Blue CA	Untreated Brown VBR
100	8.6×10^2	7.9×10^2	7.7×10^2	5.2×10^2
200	6.4×10^2	4.8×10^2	4.2×10^2	3.5×10^2
300	2.8×10^2	3.6×10^2	2.5×10^2	1.7×10^2
400	0	0	0	0
500	0	0	0	0

Table 4.The effect of prawn shell waste treated textile and leather dye on the growth of *P.putida* and *B.subtilis.*

Viable cell number (cfu ml^{-1})

Dyes mg/L	Effect on *P.putida*		Effect on *B.subtilis.*	
	Untreated Levafix Blue CA	Untreated Brown VBR	Untreated Levafix Blue CA	Untreated Brown VBR
100	5.9×10^3	6.8×10^3	6.7×10^3	6.1×10^3
200	4.1×10^3	4.3×10^3	4.6×10^3	5.4×10^3
300	3.6×10^3	4.1×10^3	3.2×10^3	2.7×10^3
400	2.7×10^3	2.2×10^3	1.3×10	9.1×10^3
500	9.5×10^2	4.9×10^2	7.1×10^2	6.3×10^2

Table 5.The effect of rice husk treated textile and leather dye on the growth of *P.putida* and *B.subtilis.*

Viable cell number (cfu ml^{-1})

Dyes mg/L	Effect on *P.putida*		Effect on *B.subtilis.*	
	Untreated Levafix Blue CA	Untreated Brown VBR	Untreated Levafix Blue CA	Untreated Brown VBR
100	5.9×10^3	6.8×10^3	5.5×10^3	5.8×10^3
200	4.1×10^3	4.3×10^3	4.2×10^3	3.9×10^3
300	3.6×10^3	4.1×10^3	2.3×10^3	2.1×10^3
400	2.7×10^3	2.2×10^3	8.5×10^2	1.1×10^3
500	9.5×10^2	4.9×10^2	4.6×10^2	7.2×10^2

Table 6.The effect of poultry soil waste treated textile and leather dye on the growth of *P.putida* and *B.subtilis.*

Viable cell number (cfu ml^{-1})

Dyes mg/L	Effect on *P.putida*		Effect on *B.subtilis.*	
	Untreated Levafix Blue CA	Untreated Brown VBR	Untreated Levafix Blue CA	Untreated Brown VBR
100	3.6×10^3	2.9×10^3	3.5×10^3	4.2×10^3
200	3.1×10^3	2.4×10^3	2.8×10^3	2.7×10^3
300	2.3×10^3	1.9×10^3	3.1×10^3	2.5×10^3
400	7.2×10^2	5.5×10^2	1.6×10^3	1.2×10^3
500	5.8×10^2	3.1×10^2	6.2×10^2	6.4×10^2

Table 7.The effect of apricot seed treated textile and leather dye on the growth *P.putida* and *B.subtilis.*

Viable cell number (cfu ml^{-1})

Dyes mg/L	Effect on *P.putida*		Effect on *B.subtilis.*	
	Untreated Levafix Blue CA	Untreated Brown VBR	Untreated Levafix Blue CA	Untreated Brown VBR
100	2.8×10^3	3.1×10^3	3.1×10^3	2.8×10^3
200	2.5×10^3	2.7×10^3	2.2×10^3	3.6×10^3
300	1.3×10^3	2.0×10^3	1.7×10^3	2.1×10^3
400	4.9×10^2	1.1×10^3	8.5×10^2	1.4×10^3
500	3.9×10^2	6.3×10^2	6.7×10^2	6.3×10^2

Table 8.The effect of tea powder waste treated textile and leather dye on the growth of *P.putida* and *B.subtilis.*

Viable cell number (cfu ml^{-1})

Dyes mg/L	Effect on *P.putida*		Effect on *B.subtilis.*	
	Untreated Levafix Blue CA	Untreated Brown VBR	Untreated Levafix Blue CA	Untreated Brown VBR
100	1.5×10^3	1.8×10^3	2.2×10^3	1.8×10^3
200	1.1×10^3	1.3×10^3	1.4×10^3	1.2×10^3
300	8.5×10^2	5.6×10^2	9.6×10^2	8.9×10^2
400	2.8×10^2	2.1×10^2	4.7×10^2	6.1×10^2
500	0.8×10^2	0.5×10^2	1.1×10^2	2.3×10^2

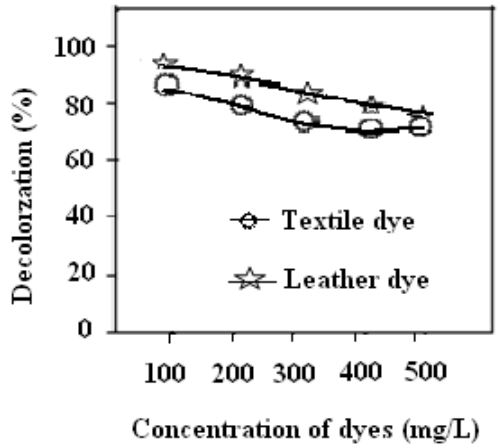

Fig 1. Effect of initial dye concentration on decolorization of Levafix Blue CA and Brown VBR by prawn shell waste.

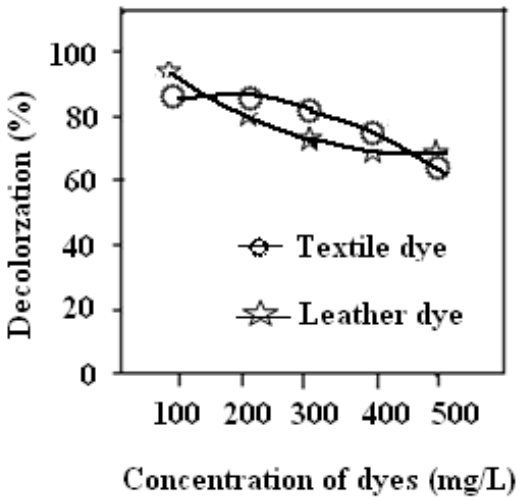

Fig 2. Effect of initial dye concentration on decolorization of Levafix Blue CA and Brown VBR by rice husk.

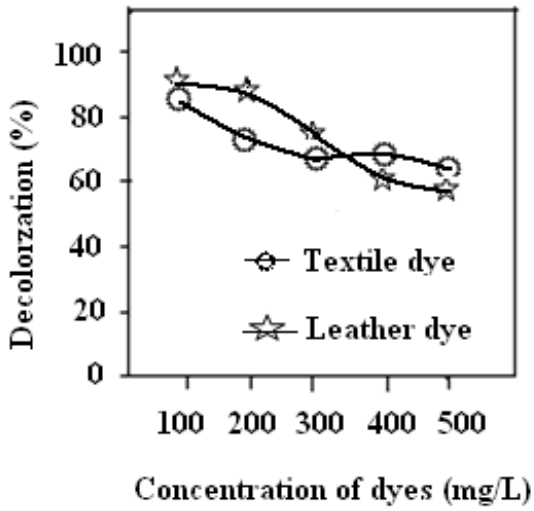

Fig 3. Effect of initial dye concentration on decolorization of Levafix Blue CA and Brown VBR by poultry soil waste.

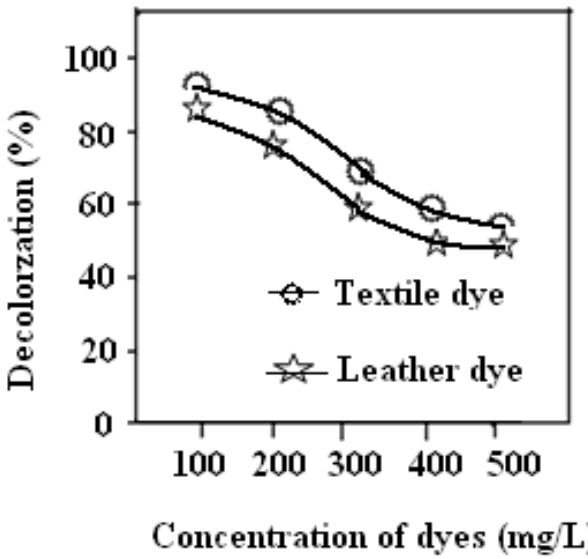

Fig 4. Effect of initial dye concentration on decolorization of Levafix Blue CA and Brown VBR by apricot seed.

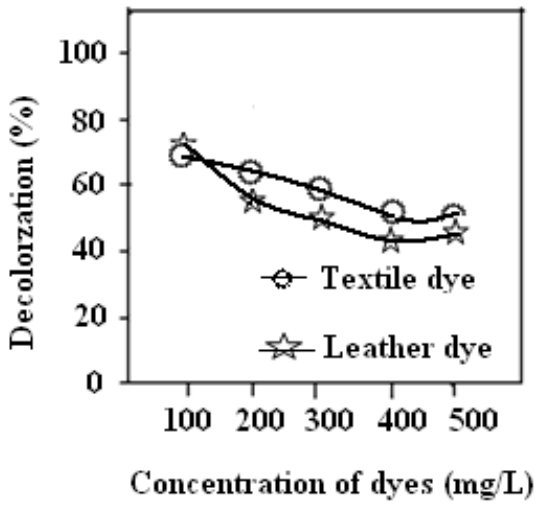

Fig 5. Effect of initial dye concentration on decolorization of Levafix Blue CA and Brown VBR by tea powder waste.

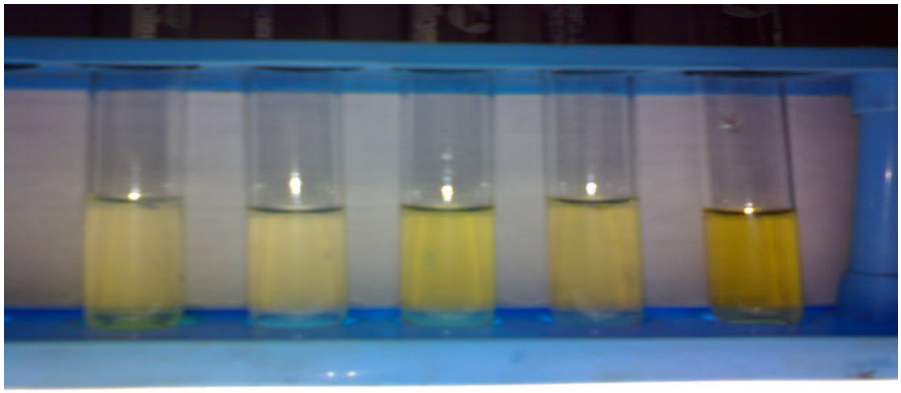

Fig 6. Decolorized levafix blue CA by prawn shell waste treatment

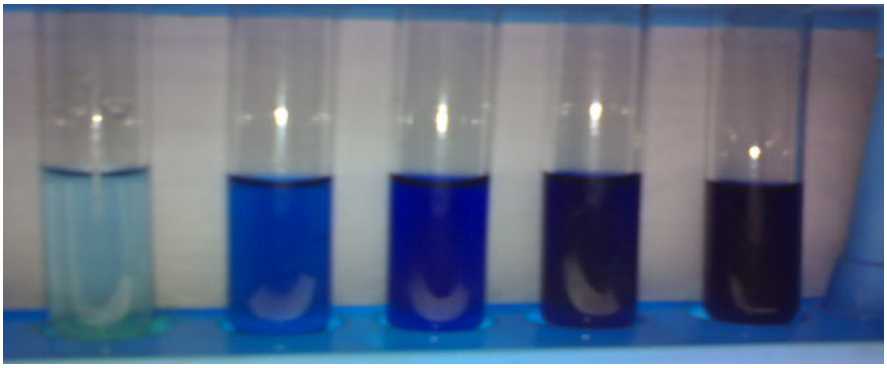

Fig 7.Decolorized levafix blue CA by rice husk treatment

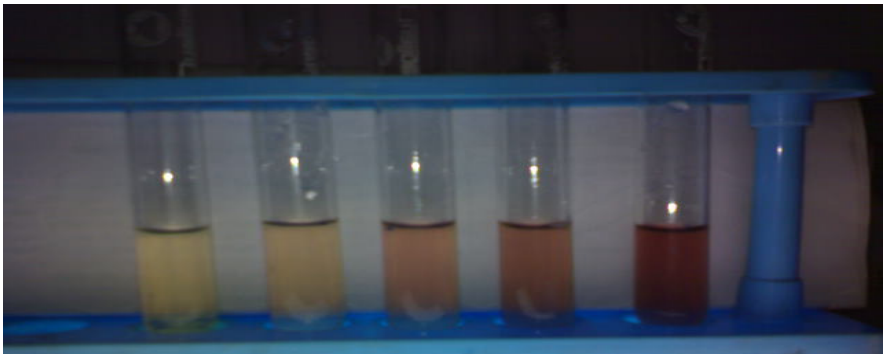

Fig 8. Decolorized brown VBR by prawn shell waste treatment

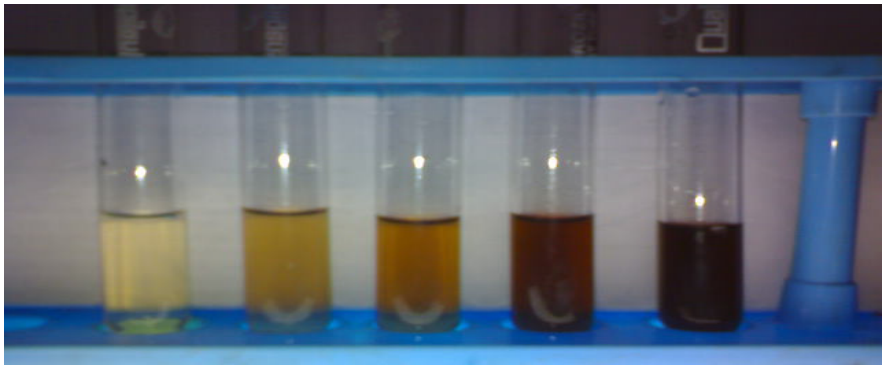

Fig 9.Decolorized brown VBR by rice husk treatment

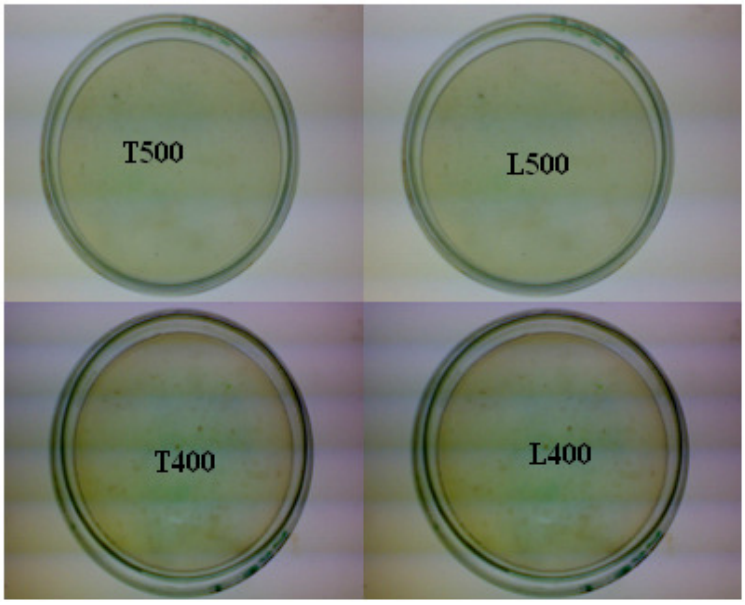

Fig 10. Development of non-colonies on untreated dyes

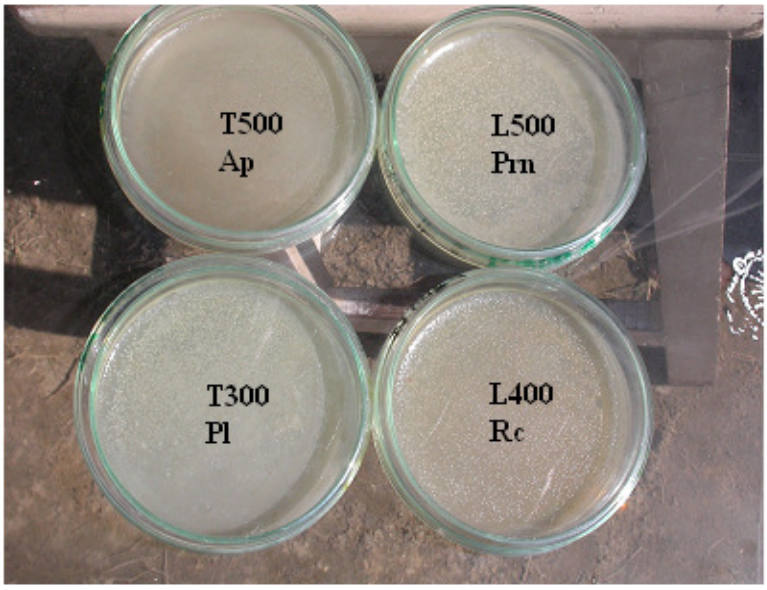

Fig11. Development of colonies on treated dyes

CONCLUSION

The present study showed that the prawn shell waste is more effective adsorbent than others and this is due to the polysaccharide chitoson content of the prawn shell waste which is the key factor for the reflection of this effective result. Presently, chitosan is attracting and increasing amount of research interest, as it is an effective scavenger for heavy metals also. Chitosan is produced by alkaline N-deacetylation of chitin, which is widely found in the exoskeleton of shellfish and *crustaceans*. It was estimated that chitosan could be produced from fish and crustaceans (Rorrer and Way 2002). The growing need for new sources of low-cost adsorbent, the increased problems of waste disposal, the increasing cost of synthetic resins undoubtedly make chitosan is one of the most attractive materials for wastewater treatment.

The other adsorbents such as rice husk, poultry soil waste and apricot seed except tea powder waste also behaved as better adsorbent in removing dyes from aqueous solution in our study. Therefore this agricultural and other waste can be used as cheap and promising adsorbents for the dye colour removal from waste water of textile and leather industries.

Because, most physico-chemical techniques used earlier have several shortcomings which include excess amount of chemical usage or sludge generation with obvious disposal problem, costly plant requirements or operating expenses. The results obtained in this study for the mechanism involved in dye removal can be considered as a fundamental step for the representation of the experimental behavior and for development of process design. In addition decolorization and detoxification ability of this agricultural, prawn shell and poultry soil wastes could be advantageous to integrate decolorization process prior to conventional process.

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