

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2017.602.017>**Biological Decolorization of Reactive Textile Dye Yellow CRG**Khan Mohd. Sarim^{1*}, Kamlesh Kukreja¹, Rakesh Kumar¹ and Ikbal²¹Department of Microbiology, CCS HAU, Hisar, India²Department of Biotechnology and Molecular Biology, CCS HAU, Hisar, India**Corresponding author***A B S T R A C T****Keywords**Decolorization,
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The extensive use of fascinating colorful dyes during textile manufacturing indirectly causes severe environmental problem. These dyes exert toxic effects on terrestrial and aquatic life forms, imparting photosynthesis and other metabolic processes. Color is an escalating problem in industrial wastewater. Hence, there is a pressing need to eradicate this contaminant for a sustainable environment. This investigation was directed to isolate and characterized potential bacteria which can efficiently decolorize common recalcitrant textile dye Yellow CRG. Out of 40 bacterial isolates obtained, an isolate was selected and screened out for the study of decolorization of textile dye Yellow CRG. Initial decolorization was recorded as 53.2% by selected bacterial isolate under shake culture condition at 30°C and pH 7.0. Optimization of culture conditions revealed significant increase in decolorization upto 78% with sucrose and ammonium chloride as carbon and nitrogen source. Optimum temperature and pH was 35°C and 7.0 respectively with 1% inoculum size. Assessment of toxic effect of treated dyes on two important agricultural crops (wheat and mung bean) ensured that bacterium was able to reduce the toxicity of dye through biosorption. Bacterial isolate exhibited potential to decolorize textile dye efficiently therefore can be used in wastewater treatment.

Introduction

Textile industries consume a wide variety of colorful dyes, and the delivery of such dyes onto the textile fibers is not an efficient process, because of this inefficiency, most of the wastewater produced from the textile industries is colored. It has been figured that about 10-15% of total dye directly release in the textile processing wastewater (Fu and Viraghvan, 2001). The color is generally the first contaminant to be discerned in wastewater because a small quantity (< 1ppm) of synthetic dye in water is highly visible. Discharging the wastewater without treatment affects the aesthetic merit, transparency and

gas solubility of water bodies and reflects the sunlight entering the water, thereby interfering aquatic species growth and hampering photosynthesis. Additionally, they can have acute and/or chronic effects on organisms depending on their concentration and length of exposure. Toxic compounds in textile industry effluent pass through the food chain and ultimately reach the man and cause various physiological disorders like hypertension, sporadic fever, renal damage, cramps etc (Novotny *et al.*, 2004). Removal of color from dye-containing wastewater is the first and major concern, it becomes more

important also, as most of the industrially discharged partially treated wastewater used for irrigation in agricultural practices. Among different sectors e.g. irrigation, drinking water, industry, energy and others, only irrigation need >80% of total water by all sectors (Kaur *et al.*, 2012). Conventional physical and chemical methods of wastewater treatments are effective but expensive and many times produce secondary pollutants. Biological methods are the alternative. Hence, the present study was carried out to find out efficient microorganism that can decolorize such contaminants efficiently for a safe environment.

Material and Methods

Dyes and chemicals

Dye used in present study was of industrial grade and procured from Laxmi Textile Mill, Kanpur, India. All the chemicals were of analytical grade. The bacterial culture was routinely grown in nutrient broth at 30°C and decolorization study was carried out in modified dye medium (glucose 5.0; NH₄Cl 1.5; KH₂PO₄ 0.5, K₂HPO₄ 0.5; MgSO₄ 0.1; CaCl₂ 0.1; and FeSO₄ 0.07 g L⁻¹). The final pH of the medium was adjusted to 7.0.

Isolation and identification of bacteria obtained from contaminated soil samples

Contaminated soil samples were collected from adjoining sites of the textile industry where the effluent was being discharged from textile processing units. The soil samples were used as inoculum for several round of enrichment in nutrient broth, from the final enrichment flasks, bacteria were isolated by serial dilution technique on nutrient agar medium.

Decolorization assay

Decolorization activity was expressed in term of percent decolorization. Two ml of sample

was withdrawn aseptically at different intervals and centrifuged at 8000 rpm for 15 min. The clear supernatant of each isolate was taken and its optical density (O.D) was determined at λ_{max} of dye using UV-Vis spectrophotometer. The percent color removal of textile dye was determined by using ascertained following formula.

$$\text{Color removal \%} = \frac{C_o - C_e}{C_o}$$

C_o = initial color removal, C_e = final color removal

Determination of decolorization efficiency and mode of decolorization

To determine decolorization efficiency, bacterial isolate showing high decolorization was selected. Efficiency of isolate was determined against time and dye concentration. For this selected isolates were inoculated in liquid medium added with different concentration of dye and decolorization was studied at different time intervals. To study the mode of decolorization as whether decolorization of dye due to adsorption by the bacterial cells or by degradation, decolorization study was carried out with culture supernatant of selected isolate. Collected supernatant was used as inoculum and added into fresh dye medium and decolorization study was determined.

Antibiotic sensitivity assay

Bacterial isolate TS-2 was evaluated for its ability to tolerate inhibitory effect of various antibiotics. A total of 12 antibiotic Chloramphenicol (10µg/disc), Streptomycin (25µg/disc), Tetracycline (10µg/disc), Norfloxacin (10 µg/disc), Rifampicin (15µg/disc), Kanamycin (30 µg /disc), Neomycin (30 µg /disc), Penicillin (20 µg

/disc), Ampicillin (25 µg /disc), Nalidixic acid (30µg/disc), Tobramycin (µg/disc), Gentamycin (10 µg/disc) were studied for their resistance/susceptibility against potential bacterial isolate according to the Kirby-Bauer disc diffusion method given by Bauer *et al.* (1966). Bacterial culture was plated onto separate nutrient agar plates and antibiotic discs were placed over it. Plates were incubated at 35°C for 24 h to observe the bacterial tolerance against different antibiotics by measuring zone diameter.

Optimization of culture conditions for maximum decolorization

The isolate was grown for 48 h in conical flasks containing 100 ml modified dye medium (MDM) amended with 100 mg Yellow CRG dye. Decolorization was studied with various inoculum sizes, shake and static culture condition, carbon sources (Sucrose, glucose, fructose, lactose, maltose mannitol and starch) nitrogen sources (Yeast extract, urea, peptone, ammonium sulphate and ammonium chloride) different pH values (6.0-8.0) and temperature (25°C-45°C). Growth was monitored spectrophotometrically at 420 nm. An aliquot of culture medium was withdrawn after 48 h, centrifuged at 6000 rpm for 15 min in a refrigerated centrifuge to separate the bacterial cell mass. Decolorization percentage was determined as described earlier.

Effect of colored/decolorized dye on growth of crop plant seedling

Toxicity assay was carried out on two important agricultural crops seedlings (wheat and mungbean) and toxic effect was observed on the seed germination, plumule and radical length. Seeds of wheat (WH-711) and mungbean (Asha) were spread on petridishes lined with double layer of filter paper saturated with equal volume of (5ml) of

colored/decolorized dye water. For each treatment there were three replicate and for each replicate 15 seeds were taken. Data on per cent germination, length of plumule and radical was recorded. Germination percentage was calculated by the following formula. All analysis was conducted in triplicate and results presented here are the mean of triplicate \pm standard deviations (SD).

$$\text{Germination\%} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds used}} \times 100$$

Immobilization of whole bacterial cell for decolorization of Yellow CRG

Cell immobilization was carried out using 3% sodium alginate and 0.2 M of calcium chloride. Prepared beads diameter was found to be in the range of 3 mm to 4 mm and left into cross-linking solution of CaCl₂ to attend hardness. Repeated batch culture was performed with Yellow CRG to evaluate the efficiency of immobilized cells with a varying number of beads per ml (1 and 2 beads/ml). During sorption of dye, samples were withdrawn at every 2 h from all flasks and assessed for adsorption analysis using UV-Visible Spectrophotometer.

Results and Discussion

Isolation and identification of bacteria

A total of 40 bacterial isolates were obtained from contaminated soil samples by enrichment culture technique. Many bacterial isolates were having the ability to decolorize textile dye Yellow CRG. In view of the ability of bacterium TS-2 to decolorize textile dye more efficiently, it was exploited for decolorization of Yellow CRG and selected for further studies. On the basis of morphological and biochemical test TS-2 was tentatively identified as a member of genus *Pseudomonas*.

Determination of decolorization efficiency and mechanism of decolorization

Decolorization efficiency of TS-2 with respect to time duration and with respect to maximum dye concentration was determined for Yellow CRG dye decolorization. It was observed that 48 h of incubation was necessary for the maximum decolorization of dye and also that TS-2 was able to decolorize as maximum as 80 mg L⁻¹, after that there was a gradual decrease in the dye decolorization. To study the mechanism of dye decolorization, addition of culture supernatant as inoculum in fresh medium didn't show any decolorization neither further addition caused a recognizable percent of color removal. Hence, mode of decolorization by the bacterial isolate was presumed as adsorption rather than degradation. This was also confirmed by the presence of colored pallet. These results provided obvious evidence of adsorption by selected bacterial isolate in the mode of decolorization. Adsorption of dye onto cell biomass is simplest mechanism of dye decolorization rather than degradation which need further assurance of non-toxicity of decolorized products, as various dyes after partial degradation produce aromatic amines which may be even more carcinogenic and harmful than parent compounds. Several studies have reported adsorption as potential mechanism of dye decolorization (Hu, 1996, Vander wall *et al.*, 1997 and Won *et al.*, 2005).

Antibiotic sensitivity assay

Pseudomonas sp. was found to be tolerant against different antibiotics *viz.* Neomycin, Norfloxacin, Nalidixic acid, Tobaramycin and Gentamycin (Table 2). Microbial community inhabiting in area where they encountered with plethora of pollutants, contaminants and mutagenic compounds, are more resistant to such compound as evident by many studies

(Bronstad *et al.*, 1996; Seiler and Berendonk, 2012).

Optimization of culture conditions for maximum decolorization

In order to enhance the decolorization efficiency of TS-2 by optimizing the culture conditions, several parameters (inoculum size, shaking and static condition, carbon, nitrogen, pH and temperature,) have been studied. Inoculum size is an important aspect so various inoculum sizes from 0.5% to 2.0% were tested and maximum dye decolorization was achieved with 1% of inoculum size, but reduced size delayed decolorization. Nikhil *et al.*, (2012) in their study prepared a consortium SpNb1 and tested the inoculum size that was sufficient for highest decolorization and reported that 3% inoculum size was necessary for maximum decolorization of reactive red dye M8B. In present study 1% inoculum size was sufficient to achieve maximum decolorization and is better for large scale operation (Fig 1). On the other hand, TS-2 exhibited highest decolorization when incubated under shake culture condition, whereas under static condition less decolorization activity was observed. Pradhan *et al.*, 2012, carried out a study for the decolorization of three dyes methyl orange, congo red and malachite green by *Bacillus* sp. and the rate of dye reduction was found higher when the culture was grown under shake condition.

In addition to this, initially glucose was given as sole carbon source which yield 52.2% decolorization by isolate. While, on the addition of different carbon sources other than glucose some sort of improvement in decolorization efficiency was observed and highest decolorization was achieved when cells utilized sucrose as carbon source (Figure 2). Praveen and Bhatt (2012) analyzed decolorization of azo dye Red 3BN by two

bacterial species *Bacillus cereus* and *B. megaterium*. Former decolorized maximum dye with 1% sucrose and later with 1% glucose. In present study sucrose (0.5%) was the preferred carbon source by bacterium which is a cheap carbon source. In order to find suitable nitrogen source, variety of such sources were provided in culture medium and surprisingly ammonium chloride was reported as best nitrogen source for the isolate. Interestingly, as the few carbon sources were utilized by the bacteria in previous testing but this was not true in case of nitrogen sources optimization, here isolate was able to utilize all the provided nitrogen source significantly but still when we consider the best nitrogen source, definitely it was ammonium chloride and it is good in respect to large scale process because ammonium chloride is cheaper than conventional yeast extract (Figure 3). Ammonium chloride was also reported as suitable nitrogen source in other studies (Carliell, 1995 and Darshan and Kirti, 2013).

Effect of temperature on the decolorization rate was evaluated and for this a range of temperature was evaluated 25°C, 30°C, 35°C, 40°C and 45°C was evaluated and among this 35°C temperature was found suitable to accomplish maximum decolorization by this isolate. TS-2 showed high decolorization (77.9%) when grown at 35°C temperature whereas slow decolorization was reported at 25°C and 45°C (Figure 4). Saharan and Ranga (2011) studied the *Bacillus subtilis* under different temperature for enhanced decolorization of Congo red dye in submerged fermentation. The cultured *Bacillus subtilis* under different temperature (25°C, 37°C and 45°C) and observed that highest decolorization was achieved when bacterium was grown at 37°C. The ionic concentration play an important role in the any process, a slight variation in pH can make big differences. In present investigation, a range of pH 6.0, 6.5, 7.0, 7.5 and 8.0 was

tested and highest decolorization was achieved at pH 7.0 (Figure 5). A progressive decline from pH 6.0 to 7.0 and then gradual decline was observed upto pH 8.0. Neutral pH for maximum decolorization has been reported by different researchers (Tripathi and Srivastava, 2011; Kannan *et al.*, 2013 and Neelambari *et al.*, 2013). After all the parameters optimization practices highest decolorization by TS-2 was recorded as 80.9% (data not shown).

Effect of decolorized dye on growth parameters of crop plant seedling

A small amount of dye can cause serious problems in plant and animal growth, hence, phytotoxicity on two common agricultural crops (wheat and mungbean) was tested. Germination percentage in both the crops was measured as 100% when treated with distilled water. In treated dye solution it was measured as 75.3% and 77.7% in mungbean and wheat crop seedlings respectively. A significant reduction in plumule and radical size of the seeds was also been measured when germinated in untreated dye solution. Results clearly indicated that dye possesses toxic effect on plant seed germination, and a bacterium used in this study has potential to efficiently reduce such toxic effect by decolorizing dye through biosorption (Table 1). Toxicity of textile dyes and effluent have been reported in several studies (David Noel and Rajan, 2015; Rehman *et al.*, 2009; Yousaf *et al.*, 2010; Divyapriya *et al.* 2014).

Immobilization of whole bacterial cell for decolorization of Yellow CRG

After immobilization, bacterial isolate exhibited efficient percent of decolorization as compared to free cells. The amount of dye adsorbed on the surface of biobeads was increased when time proceeded in experiment.

Table.1 Phytotoxicity assay of treated and untreated Yellow CRG dye

Parameters	Mungbean (Asha)			Wheat (WH-711)		
	Distilled water	Treated	Untreated	Distilled water	Treated	Untreated
Germination (%)	100.0 ± 0.00	75.3 ± 0.47	28.3 ± 0.67	100.0 ± 0.00	77.7 ± 0.46	30.7 ± 0.73
Plumule (cm)	4.2 ± 0.02	1.1 ± 0.07	0.8 ± 0.03	5.4 ± 0.17	5.1 ± 0.13	3.8 ± 0.04
Radicle (cm)	1.2 ± 0.02	1.1 ± 0.07	0.8 ± 0.03	6.7 ± 0.10	6.2 ± 0.05	4.5 ± 0.24

Values are mean of three replicates, Standard error (\pm), and seed germination in treated effluent and individual dyes differ significantly.

Table.2 Antibiotic sensitivity of TS-2 (*Pseudomonas* sp)

Antibiotic	<i>Pseudomonas</i> sp.
Chloramphenicol (10 μ g/disc)	Sensitive
Streptomycin (25 μ g/disc)	Sensitive
Tetracycline (10 μ g/disc)	Sensitive
Norfloxacin (10 μ g/disc)	Resistant
Rifampicin (15 μ g/disc)	Sensitive
Kanamycin (30 μ g /disc)	Sensitive
Neomycin (30 μ g /disc)	Resistant
Penicillin (20 μ g /disc)	Sensitive
Ampicilin (25 μ g /disc)	Sensitive
Nalidixic acid (30 μ g/disc)	Resistant
Tobramycin (10 μ g /disc)	Resistant
Gentamycin (10 μ g /disc)	Resistant

Fig.1

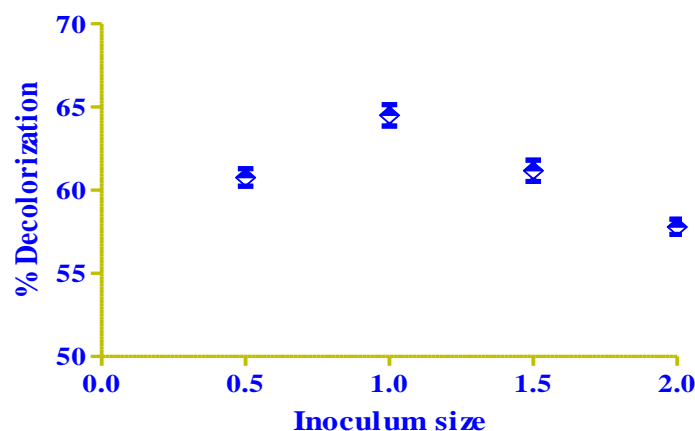


Fig 1: Effect of inoculum size on decolorization of Yellow CRG

Fig.2

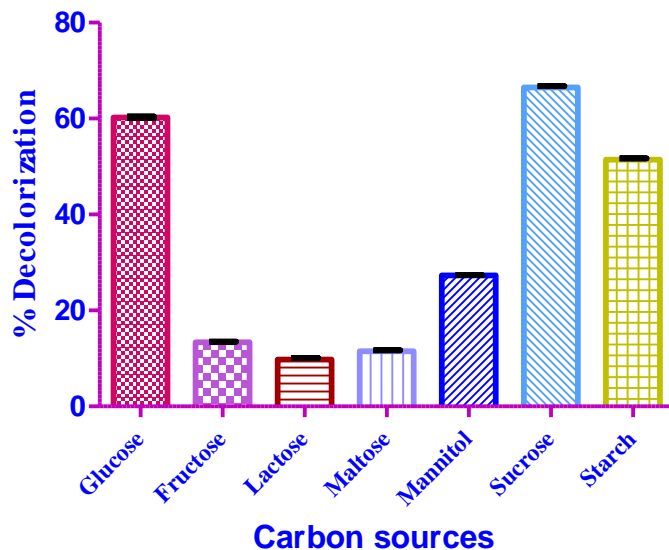


Fig 2: Effect of different carbon sources on decolorization of Yellow CRG

Fig.3

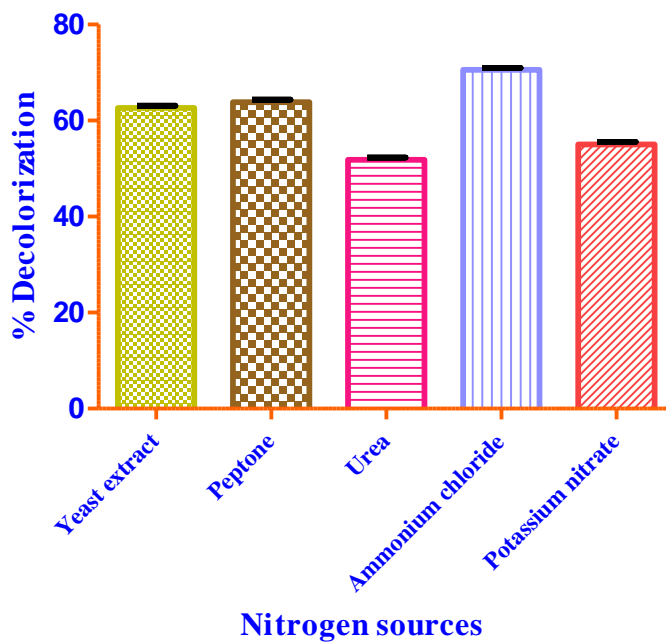


Fig 3: Effect of nitrogen sources on decolorization of Yellow CRG

Fig.4

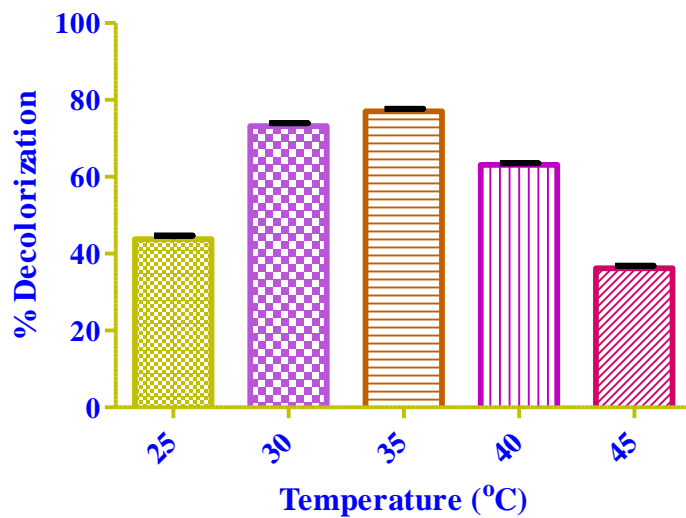


Fig 4: Effect of temperature on decolorization of Yellow CRG

Fig.5

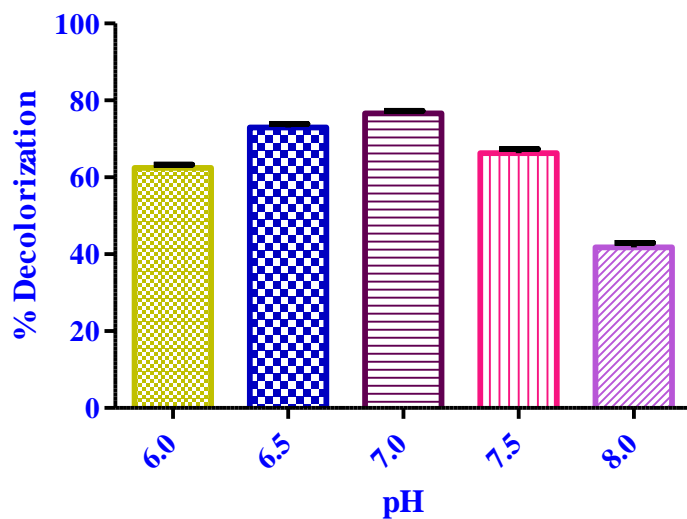
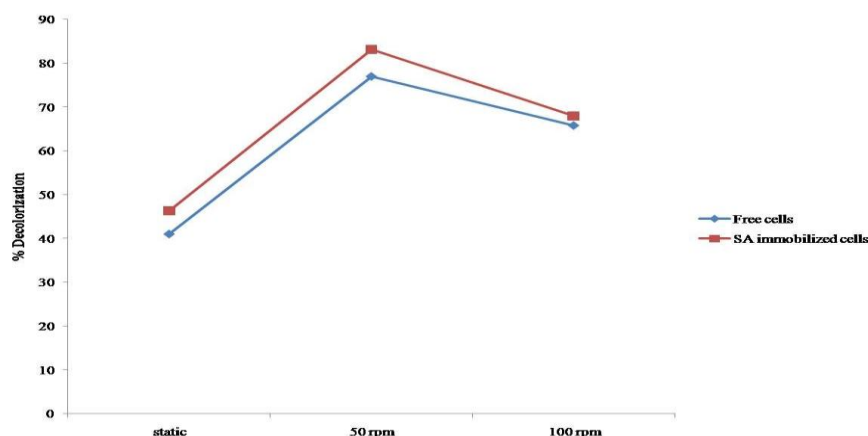


Fig 5: Effect of pH on decolorization of Yellow CRG

Fig.6 Decolorization of Yellow CRG dye by free cells and SA immobilized cells



Bacterial isolate TS-2 efficiently (83.2%) decolorized Yellow CRG dye at 50 rpm on orbital shaker. While at 0 rpm and 100 rpm dye decolorization was 46.3 and 68.0% respectively, which suggests that decolorization by immobilized TS-2 needs mild agitation and, higher agitation may cause disintegration of biobeads. Figure 6 represents the potential of immobilized cells over free cells and show higher decolorization was achieved under mild agitation by TS-2. Immobilization of bacterial cells on the solid support has several advantages such as enhanced efficiency and ease of biomass recovery as evident by Dorthy *et al.*, 2012; Illanjiam and Arunachalam 2012)

A potential bacterium TS-2 was identified as *Pseudomonas* exhibited capability to decolorize Yellow CRG dye efficiently under aerobic condition in 48h. On the optimization of process parameters, the efficiency of the bacterium for dye decolorization further improved and maximum dye removal (80.5%) was obtained when the medium was supplemented with sucrose and ammonium chloride as carbon and nitrogen source at pH 7.0 and 35°C temperature. One per cent inoculum was sufficient to decolorize high concentration (80 mg L⁻¹) of dye under shake culture condition. Bacterial isolate, TS-2 represents decolorizing activity through

adsorption of dye onto cell biomass. Decolorization of dye through bio beads offered culture stability as immobilized culture is more tolerant to pH, and temperature changes. The ability of the bacterial isolate to decolorize Yellow CRG dyes at high concentration gives an advantage to use it in the treatment of textile industry wastewaters. However, the potential of the bacterial isolate needs to be demonstrated for its application in the treatment of dye containing waste waters using appropriate bioreactors.

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