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BIODEGRADATION OF REACTIVE DYE REACTIVE VIOLET 5 BY BACTERIA ISOLATED FROM DYE CONTAMINATED SOIL

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ABSTRACT

The *Pseudomonas aeruginosa* GSM3 isolated from dye contaminated soil was able to degrade Reactive Violet 5 completely as a sole source of carbon (300 mgL^{-1}) within 20 h under static condition. The organism exhibited good decolorization ability in the pH ranges from 5.0 to 9.0 and temperature from 30 to 40^oC respectively. The optimum degradation was observed at 37^oC and pH 7. The maximum concentration of Reactive Violet 5 (800 mgL^{-1}) was degraded up to 58% within 68 h. The culture showed remarkable ability to decolorize repeated additions of dye, with decrease in time up to 10 h at fifth dye aliquots addition and also tolerate high salt concentration up to 6 gL^{-1} NaCl. Contineous decrease in optical density at 558 nm on incubation, increased the colorless bacterial biomass and no change in color on changing pH of decolorized filterate depicted that decolorization was due to biodegradation. Thus, the isolate *P. aeruginosa* GSM3 could be good candidate organism for the biotreatment of textile industry effluents.

KEYWORDS: **Reactive Violet 5, Reactive dye, Decolorization, Biodegradation and *Pseudomonas aeruginosa* GSM3.**

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INTRODUCTION

Synthetic dyes are most widely used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetic, and leather industries[1]. Since 1856, over 10^5 different dyes have been produced worldwide with an annual production of over 7×10^5 metric tons[2]. The textile industry is one of the greatest generators of liquid effluent pollutants, due to the high quantities of water used in the dyeing processes. It is estimated that 280,000 tonnes of textile dyes are discharged in such industrial effluent every year worldwide[3]. All dyes do not bind to the fabric depending on the class of the dye. Its loss in wastewaters could vary from 2% for basic dyes to as high as 50% for reactive dyes, resulting in severe contamination of surface and ground waters in the vicinity of dyeing industries[4]. Textile wastewaters are highly colored because they are typically discharged with a dye contain in the range 10–200 mgL^{-1} and many dyes are visible in water at concentrations as low as 1 mgL^{-1} [5]. Effluents from the textile industries are characterized by extreme fluctuations in many parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, color, and salinity. The wastewater composition will depend on the different organic-based compounds, chemicals, and dyes used in the industrial dry and wet-processing steps[6,7].

Discharge of textile industry effluents into rivers and lakes results into reduced dissolved oxygen concentration, thus creating anoxic conditions that are lethal to resident organisms. Many reports indicate that textile dyes and effluents have toxic effect on the germination rates and biomass of several plant species, whereas plant play many important ecological function such as providing the habitat for a wildlife, protecting soil from erosion, and providing bulk of organic matter that is so significant to soil fertility[8]. Many synthetic azo dyes and their metabolites are toxic, carcinogenic, and mutagenic[9]. Therefore, treatment of textile industrial effluents containing azo dyes and their metabolites becomes necessary prior to their final discharge to the environment. Existing physicochemical methods for color removal are expensive, commercially unattractive and greatly interfered by other wastewater constituents or generate waste products that must be difficult to handled[10]. The microbial decolorization and degradation of aromatic compounds has been of considerable interest since it is inexpensive, eco-friendly, and produces a less amount of sludge[11].

The decolorization potential of the microbes depends on the adaptability and the activity of selected microorganisms. Many microorganisms capable of degrading reactive dyes, including bacteria[11,12,13], fungi[14,15], actinomycetes[16], and algae[17]. Pure fungal cultures have been used for the treatment of textile industry effluent has some disadvantages, including long growth cycle, low pH requirement for the optimum activity of enzymes, the long hydraulic retention time for complete decolorization and requirement of longer time for decolorization[18]. In contrast, it is reported that many potential bacteria having ability to degrade or mineralize several textile dyes[11]. However, comprehensive solutions for Reactive azo dye removal are far from reality, which calls for continued search for new organisms and technologies. In this study a bacterium *Pseudomonas aeruginosa* GSM3, capable of degrading Reactive Violet 5 was isolated and the effects of different parameters (such as static, shaking, temperature, pH, initial dye concentration, salt concentration and repeated addition of dye aliquots) on dye decolorization by an isolated bacterium was investigated.

MATERIALS AND METHODS

Dyes and chemicals

Reactive dye Reactive Violet 5 has been most commonly used in dyeing and textile industries. Moreover, it is a sulfonated polycyclic aromatic compound and hence it was selected as a model dye (Figure 1). This dye was gifted from

Colors India Inc. Pvt. Ltd. Ahmedabad, India. All required chemicals were procured from Sigma-Aldrich, India and Hi-Media, Mumbai, India. All chemicals used were of the highest purity available and of an analytical grade.

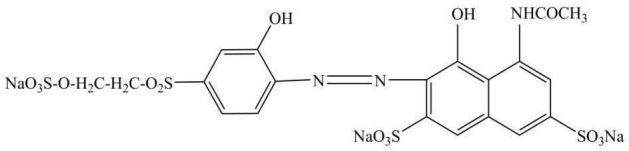

Figure 1
Structure of Reactive Violet 5.

Culture medium

The mineral salts medium (MSM)[19] containing (gL^{-1}): $Na_2HPO_4.2H_2O$ (12.0), KH_2PO_4 (2.0), NH_4NO_3 (0.50), $MgCl_2.6H_2O$ (0.10), $Ca(NO_3)_2.4H_20$ (0.050), $FeCl_2.4H_20$ (0.0075) and 10.0 mL of trace element solution containing (mgL^{-1}): $ZnSO_4.7H_2O$ (10.0), $MnCl_2.4H_2O$ (3.0), $CoCl_2.2H_2O$ (1.0), $NiCl_2.6H_2O$ (2.0), $Na_2MoO_4.2H_2O$ (3.0), H_3BO_3 (30.0), $CuCl_2.2H_2O$ (1.0); pH 7.0. The MSM was supplemented with 0.1% (w/v) of yeast extract was used to see the change in the degradation efficiency of bacterium. This was blended with different concentrations of Reactive Violet 5 was used throughout the study as a test and without culture as a control for decolorization studies. When required, 1.9% (w/v) of agar was added into the media to solidify. All the media were sterilized at 121°C for 20 min before use.

Isolation, screening and identification of dye degrading microorganism

The isolation, screening and identification of a dye degrading bacterium was done as earlier reported by Bheemaraddi *et al*. [20].

Decolorization studies of Reactive Violet 5 in liquid medium

The decolorization experiments were performed in 250 ml Erlenmeyer flasks containing 100 ml of sterilized MSM broth with yeast extract (0.1% w/v) and 100 mgL^{-1} Reactive Violet 5. They were inoculated with 5 ml of *P. aeruginosa* GSM3 culture broth in test and without inoculum acts as control. The flasks were incubated at 37°C under static as well as shaking (120 rpm) conditions. The 5 ml of cultures were withdrawn at different time intervals (4 h) from both flasks and supernatant was collected by centrifuging at 10,000 rpm for 15 min. The supernatants were used to check the optical density (OD) at 558 nm using UV–Vis spectroscopy (Systronics AU-2700).

Effect of different parameters on dye decolorization

Effects of various parameters were conducted using varying in initial pH values ranges from 4 to10 keeping temperature constant at 37°C. Similarly, calculate the optimum temperature for maximum dye decolorization using varied temperatures ranging from 20 to 50°C with 5°C intervals keeping optimum pH 7.0 and incubating under static conditions. By the optimization study optimum pH (7.0) and temperature (37°C) were selected for further studies using different physicochemical factors such as initial dye concentration (100–800 mg L^{-1}), NaCl concentration (1–6%) and continuous addition of Reactive Violet 5 on dye decolorization by *P. aeruginosa* GSM3.

Decolorization Assay

Decolorization of dyes by *P. aeruginosa* GSM3 was determined by measuring absorbance of culture supernatants at 558nm using UV-visible spectroscopy, and percent decolorization was calculated as mentioned by Dave and Dave[21,20].

Decolorization mode of Pseudomonas aeruginosa GSM3 on Reactive Violet 5

Dye decolorization may take place by adsorption[22] or degradation[23]. In the case of adsorption, dyes are only adsorbed onto the surface of bacterial cells, whereas new compounds come into being when dyes are degraded by bacterial enzymes during the degradation process. In adsorption, examination of the absorption spectrum will reveal that all peaks decrease approximately in proportion to each other. If the dye removal is attributed to biodegradation, either the major visible light absorbance peak will completely disappear or new peaks will appear[24]. Dye adsorption can be also easily judged by an evidently colored cell pellet, whereas those retaining their original colors are accompanied by the occurrence of biodegradation[25].A bacterial cell suspension was withdrawn at regular intervals and supernatant were used to check the optical density (OD) at 558 nm using UV-visible spectroscopy and similarly cell pellet washed with methanol was used to confirm decolorization is not due to surface adsorption.

RESULTS AND DISCUSSION

Acclimatization studies on Reactive Violet 5 degradation by Pseudomonas aeruginosa GSM3

To enhance the bacterial growth and dye decolorization efficiency of *P. aeruginosa* GSM3, we added 0.1% (w/v) yeast extract to MSM broth as a co-substrate. We observed that complete decolorization of 100 mgL^{-1} Reactive Violet 5 in MSM medium within 16 hours as compared to 52 hours without yeast extract under static condition. Similar observations have been reported by Khalid *et al.*[26] However, they used 0.4% of yeast extract as a cosubstrate for the growth of azo dye decolorizing organisms. Earlier, Isik and Sponza[27] showed that yeast extract could be used as growth supplement for azo dye degrading bacteria. In our study 0.1% yeast extract enhances the dye degradation efficiency of this organism by three times and reduced the time period to one third. So, in this study further decolorization experiments was performed using MSM broth supplemented with 0.1% (w/v) yeast extract as a cosubstrate for *P. aeruginosa* GSM3 growth.

Optimization of abiotic parameters

We optimized the static/shaking, temperature, pH, and Initial dye concentration for the maximum decolorization of Reactive Violet 5 by *P. aeruginosa* GSM3. Figure 2 shows that complete decolorization of added 100 mgL^{-1} Reactive Violet 5 within 16 h under static condition as compared to only 18% decolorization in the culture flask incubated in the shaking condition, Hence, in this study static conditions were maintained to investigate bacterial decolorization.

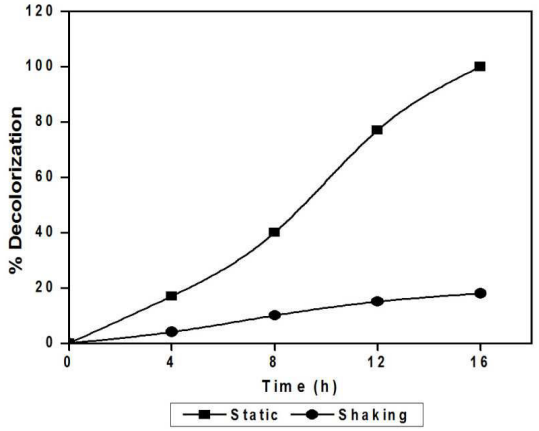

Figure 2
Effect of static and shaking conditions on decolorization of Reactive Violet 5.

In the temperature optimization study, dye decolorization activity of *P. aeruginosa* GSM3 was found to increase with increase in incubation temperature from 20 to 37°C. Further increase in temperature to 40°C, decolorization efficiency was decreased by 2% and 25% decreased at 45°C, and 31% at 50°C (Figure 3). Optimum temperature for this organism was in the range of 30 to 40°C and similar finding was reported by Sheth and Dave[12]. The decrease in decolorization at high temperature can be attributed to the decline in microbial activity that led to the inactivation of the enzyme and eventually the no decolorization of dye[28].

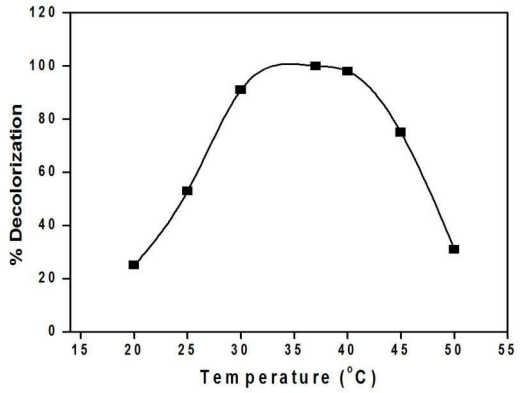

Figure 3
Effect of temperature on decolorization of Reactive Violet 5.

Similarly the optimization of pH for maximum decolorization of Reactive Violet 5 by this bacterial strain was determined over a wide range of pH 4.0 to 10.0 with an interval of pH 1. The isolate showed maximum of 100% decolorization at neutral pH 7.0 at 37°C (Figure 4). Increase in the either side of neutral pH, the percentage of decolorization was decreased steadily from 93% to 31% on alkaline side from pH 8 to 10 however, steep decline in percent decolorization from 90 to 33% on acidic side at pH 6 and 4 respectively. More than 50% of decolorization was observed in the pH range of 5 to 9. Sheth and Dave[12] also found that pH 7.0 was optimum for the decolorization of Reactive Red BS (C.I.111) by *Pseudomonas aeruginosa* NGKCTS. Chan and Kuo[29] reported that the neutral pH would be more favorable for decolorization of the azo dyes and is suitable for industrial applications.

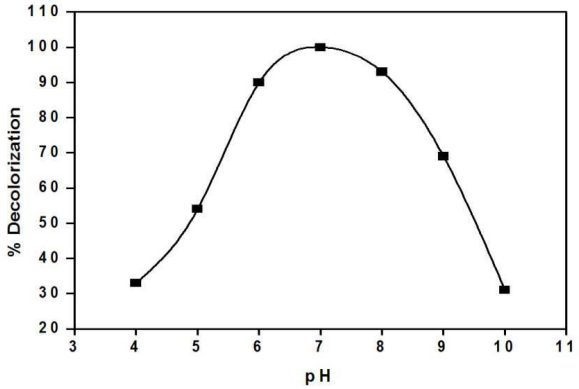

Figure 4
Effect of pH on decolorization of Reactive Violet 5.

The decolorization efficiency of Reactive Violet 5 by *P. aeruginosa* GSM3 was studied by increasing initial dye concentration (100–800 mgL^{-1}). We observed that the percentage of decolorization was decreased slowly with increasing dye concentration (Figure 5). It could effectively decolorize up to 300 mgL^{-1} Reactive Violet 5 (100%) within 20 h and is decreased to 58%, when dye concentration increased to 800 mgL^{-1} and decolorization time increases from 20 to 68 h respectively. The decrease in percentage of decolorization and enhanced time period at high dye concentration may be attributed to the inhibitory effects of high dye concentration and azo dyes usually contain one or more sulfonic acid groups on aromatic rings, which act as detergents to inhibit the growth of microorganisms[11]. Similar type of observations was reported by Jain *et al*[30] for same dye Reactive Violet 5 using bacterial consortium SB4.

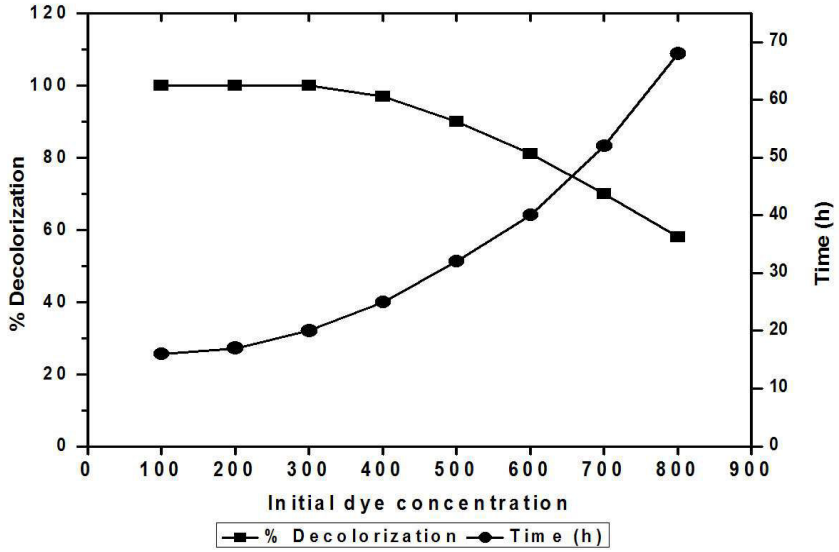

Figure 5
Effect of initial dye concentration on decolorization performance of Pseudomonas aeruginosa GSM3.

Our results on optimization studies revealed that *P. aeruginosa* GSM3 is a mesophilic (optimum of 37°C), facultative anaerobe (static incubation) and also decolorized Reactive Violet 5 more efficiently at neutral pH 7. These optimum parameters were maintained throughout this study on decolorization of Reactive Violet 5 using an isolate *P. aeruginosa* GSM3. Jain *et al*[30] reported that complete decolorization of dye Reactive Violet 5 at 37°C and pH 7 under static condition, in case of bacterial consortium SB4. Similar types of cultural conditions were adopted for the degradation of azo dyes using mesophilic bacterial strains such as *Pseudomonas* sp. SUK1, *Pseudomonas aeruginosa* NGKCTS and *Pseudomonas aeruginosa* BCH[11,12,13]. The reason for decreased decolorization under shaking condition was due to most of the azo dye degrading bacteria required reduced oxygen tension may be the mechanism of microbial degradation of azo dyes involves the reductive cleavage of azo bonds (–N=N–) with the help of azoreductase, while the presence of oxygen usually inhibits the azo bond reduction activity since aerobic respiration may dominate utilization of NADH; thus impeding the electron transfer from NADH to azo bonds[11].

Decolorization of repeated addition of dye aliquots

The ability of *P. aeruginosa* GSM3 to decolorize repeated additions of Reactive Violet 5 (100 mgL^{-1} each time) up to ten cycles was tested. There was 100% decolorization up to 5th aliquots with decreasing time taken for respective percent decolorization from 16 h to 10 h respectively. From the 6th cycle onwards percent decolorization is also reduce gradually from 95% to 58% (10th cycle). Similarly time taken for respective percent decolorization was increases from 14 h for 7th cycle to 42 h for 10th cycle (Figure 6). Repeated use of microbial cells for decolorization has been reported by other Researchers[12,13]. Our observations and earlier reports made by others, the decrease in the efficiency might be due to nutrient depletion, decrease in viable cells and inhibition of enzyme systems gradually[11]. Thus, *P. aeruginosa* GSM3 shows the ability to decolorize repeated addition of dye aliquots, which is significant for its commercial application.

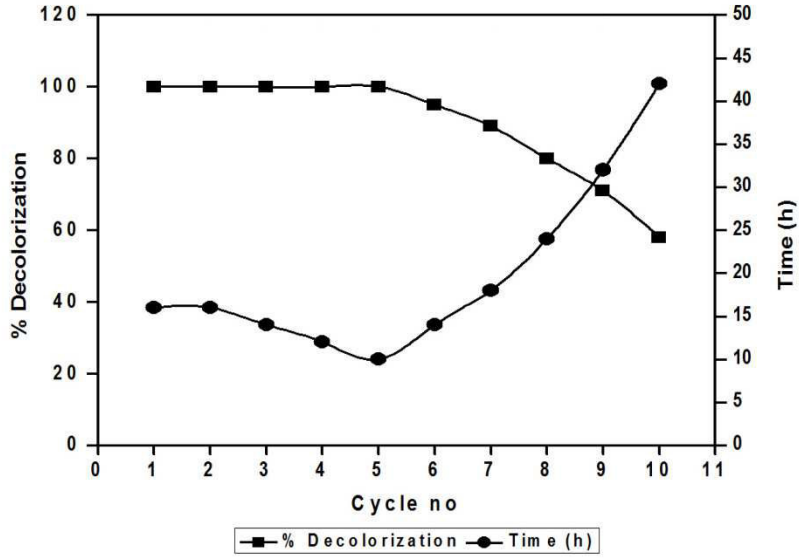

Figure 6
Repeated use of Pseudomonas aeruginosa GSM3 in mineral salts medium for decolorization of Reactive Violet 5.

Effect of salt concentration on decolorization of Reactive Violet 5

Effluents from the both dye manufacturing and dye consuming industries generally contains chloride salts of sodium and potassium which are most widely used for salting out of dyes and discharged along with unused dyes. Hence, the present investigation was undertaken to study the effect of salt concentration (1–6%) on decolorization of Reactive Violet 5 by the *P. aeruginosa* strain GSM3 was carried out. Isolate showed significant decolorization in the presence of all salt concentrations selected. At 1%, 2%, 3%, 4% of salt concentration, the percentage of decolorization of Reactive Violet 5 was of 100%, 100%, 98% and 92% respectively after 16 h of incubation. Further, increase in salt results in decreased percentage of decolorization. Furthermore, it was stated that sodium concentration higher than 3 g/L can cause inhibition of most the bacterial metabolism[31]. But on contradictory, in the presence of 5% and 6% salt drops dye decolorization to 81% and 67% respectively. Inhibition to microorganisms by high salt concentration may cause plasmolysis or loss of activity of cells[32]. Hence, *P. aeruginosa* GSM3 has great potential application in the bioremediation of azo dyes at higher salt concentration.

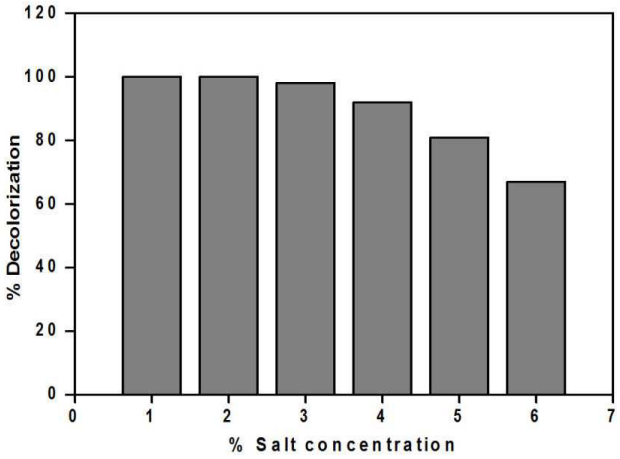

Figure 7
Effect of salt concentration on decolorization of Reactive Violet 5.

Decolorization mode of Pseudomonas aeruginosa GSM3 against Reactive Violet 5
Spectrophotometric analysis (400–800 nm) of supernatants at different intervals of incubation time showed visible decolorization and decrease in the dye concentration from batch culture. Reduction in the optical density of decolorized media observed at 558 nm as compared to the no change in the peak of the control medium through the period (16 h) of incubation (Figure 8). The significant changes in the visible spectra showed that the molecular structure of Reactive Violet 5 changed evidently after decolorization.

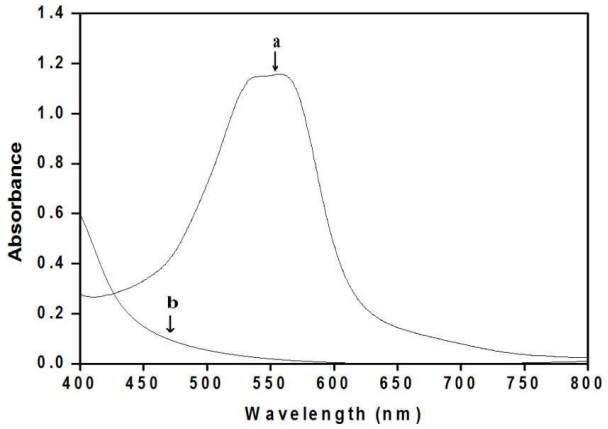

Figure 8
UV–Vis spectra of Reactive Violet 5 before and after decolorization by Pseudomonas aeruginosa GSM3 (a, 0 h; b, 16 h).

The present results indicate that the color removal by *P. aeruginosa* GSM3 may be due to biodegradation not by adsorption and it was confirmed by colorless cell pellet obtained upon centrifugation. Further colorless cell pellets were dissolved in methanol and analyzed by UV-visible spectroscopy. There is no absorbance at the 558 nm of methanol dissolved cell pellets indicates that decolorization was due to biodegradation and also we are not providing carbon source in media so that our organism can able to utilize dye as a sole source of carbon. These results provided the obvious evidence of

biodegradation of Reactive Violet 5 by an isolate *P. aeruginosa* GSM3 and also supported the earlier conclusion that decolorization by bacteria is mainly due to biodegradation, rather than inactive surface adsorption[25].

CONCLUSION

An isolate *P. aeruginosa* GSM3 has potential to degrade Reactive Violet 5 as a sole source of carbon under static condition. This isolate also showed decolorization of Reactive Violet 5 under continuous addition of dye fractions in ongoing decolorization experiments and also withstand the higher salt concentration up to 4% without change in efficiency of percent degradation of dye. Therefore, this isolate may prove to be a candidate organism for the treatment of textile industrial effluents containing reactive dyes especially Reactive Violet 5. However the complete degradation pathway of Reactive Violet 5 by this bacterium is in progress.

CONFLICT OF INTREST

Conflict of interest declared none.

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