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Research Article

Effect of carbon and nitrogen sources on biodegradation of textile azo dye Reactive Violet 5 by *Pseudomonas aeruginosa* GSM3

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Abstract: The aim of this work is to study the effect of various carbon and nitrogen sources on decolorization of Reactive Violet 5 by bacterial strain GSM3which was isolated from dye contaminated soil sample. This potential strain has ability to decolorize Reactive Violet 5 completely within 52 h using dye as a sole source of carbon under static condition at 37oC. The MSM supplied with various carbon and nitrogen sources, which may increase decolorization rate by enhancing the growth of dye degrading bacteria. Among all carbon and nitrogen sources, glucose and yeast extract were found to be the best carbon and nitrogen source in our study. In the presence of glucose (1 g/l) and yeast extract (1 g/l), organism had the ability to decolorize Reactive Violet 5 completely within 24 h and 16 h respectively by enhancing the growth rate of dye degrading bacteria. There is significant reduction in time required for complete decolorization of Reactive Violet 5 in the presence of co-substrates which indicates its commercial application in the treatment of textile industry effluents containing textile azo dyes.
Keywords: Carbon sources, Nitrogen sources, Biodegradation, Decolorization, Pseudomonas aeruginosa GSM3, Reactive Violet 5, Textile azo dye

INTRODUCTION

Textile azo dyes are diazotized amines coupled to an amine or phenol, with one or more azo bonds (–N=N–). They are synthetic aromatic compounds and account for more than 50% of all the dyes produced annually, showing the largest spectrum of colors [1]. These dyes are extensively used in the textile, leather, paper, food, color photography, pharmaceuticals and medicine, cosmetic, hair coloring's, wood staining, agricultural, biological and chemical research, light-harvesting arrays, and photoelectrochemical cells [2]. Since 1856, over 10^5 different dyes have been produced worldwide with an annual production of over 7×10^5 metric tons [3]. 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 [4]. The traditional textile finishing industry consumes about 100 L of water to process about 1 kg of textile materials [2]. 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, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries [5]. The presence of very small amount of dye in water (<1 ppm) is highly visible, affecting the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies [2]. Most synthetic dyes are highly resistant to degradation due to their complex chemical structures [6]. Improper textile dye effluent disposal in aqueous ecosystem leads to the reduction in sunlight penetration which in turn decreases photosynthetic activity, dissolved oxygen concentration, water quality and depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems worldwide. In addition to their visual effect, azo dyes also haveadverse impact in terms of total organic carbon (TOC)and chemical oxygen demand (COD). Many syntheticazo dyes and their metabolites are toxic, carcinogenic,mutagenic, leading to potential health hazard tohumankind [7]. The release of textile dyes may therefore present eco-toxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain [8]. Moreover, numerous reports indicate that textile dyes and effluents have toxic effects on the germination rates and biomass of several plants species which have important ecological functions, such as providing habitat for wildlife, protecting soil from erosion and providing the organic matter that is so significant to soil fertility [9]. Therefore, the treatment of industrial effluents containing such aromatic compounds is necessary prior to their final discharge into the environment [7].

In the last few decades, several physicochemical treatment methods have been developed, including flocculation combined with flotation, electroflocculation, membrane filtration, electrokinetic coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation and ozonation involving the use of activated carbon and air mixtures, but these technologies are generally expensive, are ineffective in color removal from textile dyestuffs, are not adaptable to a wide range of dye wastewaters, and produce a large amount of sludge or cause secondary pollution due to excessive chemical usage [4]. Conversely, biological processes provide an alternative to existing technologies because they are more cost-effective, environmental friendly and do not produce large quantities of sludge. Many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolorize azo dyes[10]. Pure fungal cultures have been used to develop bioprocesses for the mineralization of azo dyes, but the long growth cycle and moderate decolorization rate limit the performance of fungal decolorization system [11]. In contrast, bacterial decolorization is normally faster. Bacteria capable of dye decolorization/biodegradation either in pure cultures or in consortia have been reported [3, 7, 11−15]. However, comprehensive solutions for sulfonated azo dyes removal are far from reality, which calls for continued search for new organisms and technologies.

In the present investigation, we have reported the effects of various carbon and nitrogen sources on decolorization of Reactive Violet 5 in MSM under static condition. Optimizations of different concentrations of glucose and yeast extract have been carried out to make decolorization process more economical. The information obtained from this study is to develop practical bioremediation process for Reactive Violet 5 using bacterial strain GSM3 as biocatalysts.

MATERIALS AND METHODS

Dyes and Chemicals

The textile vinyl sulfone mono azo dye Reactive Violet 5 was generous gifts from Colors India Inc. Pvt. Ltd. Ahmedabad, India. It has been most widely used in textile and dyeing industries. Moreover, it is multisulfonated polycyclic aromatic compound and was used as a model azo dye in this study (Figure 1). All chemicals used during the study wereof analytical grade and were procured from S.D. Fine chemicals and Sigma-Aldrich, India.

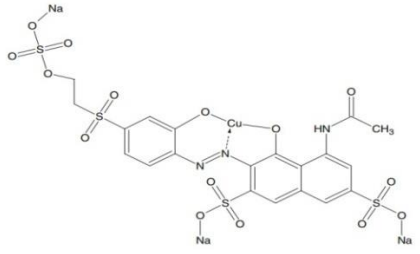

Fig. 1: Structure of Reactive Violet 5

Culture Medium

The Mineral Salts Medium (MSM) was prepared as per Brilon *et al.*[16] with some modifications. The MSM contained of following constituents (g/l): $Na_2HPO_4.2H_2O$ (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) with 10 mL of trace element solution per liter. The trace element solution was prepared as follows (mg/l): $ZnSO_4.7H_2O$ (10.0), $MnCl_2.4H_2O$ (3.0), $CoCl_2.6H_2O$ (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). Further, MSM was blended with different concentrations of Reactive Violet 5 and used throughout the study as a test medium and uninoculated flasks were also incubated as control. The final pH of the medium was adjusted to 7.0 ± 0.2. The MSM with agar (1.9% w/v) was used for isolation and maintenance of pure culture. 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 dye degrading microorganism was done as previously reported [17]. The 16S rDNA sequence was initially analyzed at NCBI server (http://www.ncbi.nlm.nih.gov) using BLAST (blastn) tool and corresponding sequences were downloaded. Phylogenetic tree was constructed by Neighbor-Joining method [18], using the MEGA5 software [19].

Decolorization assay

Decolorization of Reactive Violet 5 by *Pseudomonas aeruginosa*GSM3 was determined by measuring absorbance of culture supernatants at 554 nm. Percentage of decolorization was calculated as mentioned by Dave and Dave [20].

$$Decolorization\ (\%) = \frac{I - F}{I} \times 100$$

Where I = Initial absorbance and F = Absorbance of decolorized sample.

Effect of carbon and nitrogen sources on decolorization of Reactive Violet 5

Biodegradation of textile azo dyes is often limited and slow due to limited availability of carbon and nitrogen from complex structure of dyes. The addition of nutrients like carbon and nitrogen may increase the dye degradation efficiency [21]. Keeping in view the importance of carbon and nitrogen supply, this part of study was undertaken. MSM was used supplemented 100 mg/l of Reactive Violet 5 for studying the effect of various carbon and nitrogen sources on dye decolorization under static conditions. It was further incorporated with different carbon and nitrogen sources (1% w/v) such as glucose, sucrose, starch and lactose, yeast extract, peptone, beef extract, potassium nitrate and sodium nitrate respectively. The MSM was inoculated with 5% of inoculum and incubated at 37°C. All experiments were carried out in flask containing 100 ml of sterilized MSM medium and incubated under static conditions. The

percentage of decolorization was measured by UV-Vis spectrophotometer. Uninoculated controls were used to compare abiotic color loss during the decolorization studies.

Glucose and yeast extract at different concentrations (0.1-2 g/l) were supplemented in MSM to optimize their concentrations for efficient decolorization of Reactive Violet 5.

RESULTS AND DISCUSSION

Effect of different carbon sources

In order to minimize the time required for decolorization of Reactive Violet 5 by the strain GSM3, extra carbon and nitrogen source supplied to MSM. Isolate has ability to decolorize Reactive Violet 5 completely within 52 h without any carbon and nitrogen source, whereas supplementing different carbon and nitrogen sources in a MSM, time required for decolorization of Reactive Violet 5 was decreased significantly.

The influence of different carbon sources on Reactive Violet 5 decolorization was studied in MSM under static condition. Organism showed complete decolorization of Reactive Violet 5 in the presence of all carbon sources selected, but only the time required for decolorization was varied (Table 1). When lactose and sucrose were used as carbon sources, time required for 100% decolorization decreased significantly i.e. 30 and 28 h respectively. Strain GSM3 exhibited complete decolorization in the presence of starch and glucose within 26 and 24 h respectively. Among all carbon sources, glucose being the simplest carbohydrate was preferred carbon source in Reactive Violet 5 decolorization by GSM3. Similar finding was reported by Moosvi *et al.* [11].

Table 1: Effect of supplementation of different carbon and nitrogen sources on the decolorization of Reactive Violet 5 by bacterial strain GSM3

Medium	Decolorization (%)	Incubation time (h)
MSM	100	52
MSM + Glucose	100	24
MSM + Sucrose	100	28
MSM + Starch	100	26
MSM + Lactose	100	30
MSM + Yeast extract	100	16
MSM + Peptone	100	18
MSM + Beef extract	100	20
MSM + Potassium nitrate	100	30
MSM + Sodium nitrate	95	38

MSM: Mineral Salts Medium

Effect of different concentrations of glucose

The potential of *Pseudomonas aeruginosa* GSM3 has ability to decolorize added Reactive Violet 5 (100 mg/l) efficiently in the presence of yeast extract as a cosubstrate. Effect of different concentrations of glucose (0.1-2.0) g/l in MSM on the decolorization efficacy of GSM3 was evaluated. On addition of 1 g/l of glucose in MSM exhibited complete decolorization of Reactive Violet 5 within 16 h. Further increase in glucose concentration to 2 g/l efficiency of Reactive Violet 5 decolorization was decreased and only 95% of dye was decolorized within 24 h (Figure 2). Jain *et al.*[15] observed that the consortium SB4 fail to decolorize same dye Reactive Violet 5 in the presence of 5 g/L glucose and only 85% decolorization was observed even at high glucose concentration. The reason for decreased decolorization at high glucose concentration may be due to the metabolic regulation known as glucose/catabolic repression. Further, they added that during such repression there is a high possibility of inhibiting the transcription of cyclic-AMP-dependent genes (due to presence or at higher concentration of glucose), few of them might be involving in dye decolorization, encoding for azoreductase [22].

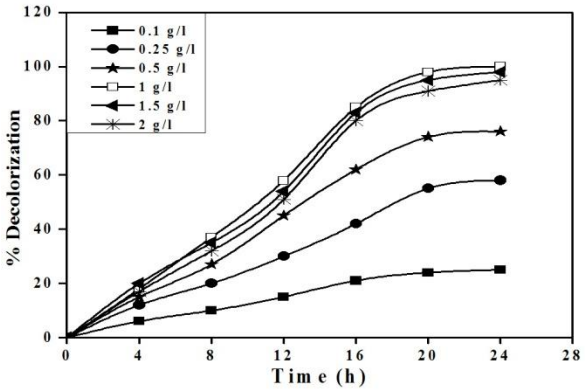

Fig. 2: Effect of different concentrations of glucose on decolorization of Reactive Violet 5 under static condition at 37^{o}C

Effect of different nitrogen sources

The dye decolorization efficiency of GSM3 in the presence of various nitrogen sources was studied in MSM at 37◦C under static condition. Isolate GSM3 showed more than 95% decolorization of Reactive Violet 5 in the presence of all nitrogen sources selected, but only the time required for decolorization was varied (Table 1).Organism showed complete decolorization of Reactive Violet 5 within 16 h in the presence of yeast extract and same response was shown in presence of peptone within 18 h under static condition. Likewise in the presence of beef extract and potassium nitrate, organism showed complete decolorization within 20 and 30 h respectively. Less decolorization (95%) within 38 h in the presence of sodium nitrate was observed in our study. Among all other nitrogen sources, only yeast extract served as better nitrogen source for decolorization of Reactive Violet 5 within less time was selected for further experiments.

Similar findings were also reported by other researchers [11, 15].

Effect of different concentrations of yeast extract

Pseudomonas aeruginosa GSM3 was able to degrade Reactive Violet 5 (100 mg/l) efficiently in the presence of yeast extract as a cosubstrate. Effect of different concentrations of yeast extract (0.1-2.0) g/l in MSM on the decolorization efficacy of GSM3 was evaluated (Figure 3). On addition of 1 g/l of yeast extract in MSM exhibited complete decolorization of Reactive Violet 5 within 16 h. Further increase in yeast extract concentration has no effect on decolorization activity. Thus, to make the process economical 1g/l of yeast extract concentration was found to be optimum. Similar results were reported by Jain *et al.* [15] where their findings proposed that yeast extract was essential for regeneration of NADH which acts as an electron donor in azo bond reduction.

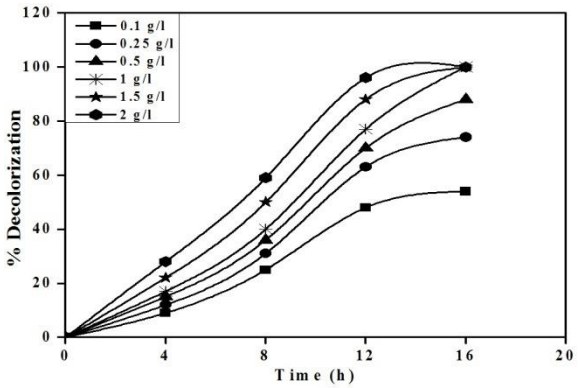

Fig-2: Effect of different concentrations of yeast extract on decolorization of Reactive Violet 5 under static condition at 37^{o}C

CONCLUSION

Rapid decolorization of Reactive Violet 5 was observed in the presence of glucose (1 g/l) when compared to other carbon sources like sucrose, starch and lactose. It might be due to the fact that presence of glucose in the media enhances the bacterial growth rate anddye decolorization efficiency.

Rapid decolorization of Reactive Violet 5 was observed in the presence of yeast extract (1 g/l) when compared to other nitrogen sources like peptone, beef extract, potassium nitrate and sodium nitrate. It might be due to the fact that Organic nitrogen sources such as YE are considered essential for regeneration of NADH which act as electron donor in azo bond reduction[15]. Hence, rapid decolorization of dye was observed only in the presence of glucose and yeast extract in the medium. Thus, a commonly available carbon and nitrogen source is imperative to enhance the bioremediation activity of this bacterium which has been the most suitable for textile industry wastewater treatment.

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