Research Article

Effect of Carbon and Nitrogen Sources for the Degradation of Red 2G by Bacillus Sp.,

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ABSTRACT

Azo dyes are largely produced and used in dyestuff industries and textile industries. The waste water effluent from the textile industry is considered as one of the most polluting among all other industries. They cause serious health hazards to humans, animals and plants. In the present study eleven organisms were isolated using the pour plate technique on M9 media along with Red2G from effluent collected near Peenya industrial area and Magadi road .The physicochemical parameters such as temperature, colour, pH and odour were measured. Among eleven organisms, one organism shown more degradation (14.94%) by spectrophotometric method. The organism was identified as *Bacillus sp.,* based on morphological and biochemical tests. The decolorization was more at 72hrs at pH 9 and temperature 30^{o}C. The different carbon sources such as dextrose, sucrose, maltose, starch and cellulose were used for degradation, among all starch shown more degradation (25.61%). Among nitrogen sources such as peptone, tryptone, tyrosine, glycine and ammonium ferrous sulphate, tyrosine shown maximum degradation (45.47%) compared with other sources. The decolorization was confirmed by HPLC method, the results have resembled with spectrophotometric method. Hence for the degradation of Red 2G, *Bacillus sp*., was confirmed as a potentive organism. Thus organism can be used in textile industry for the treatment of effluent.

Keywords: Degradation, Decolorization, Red 2G, Bacillus Sp., Textile Industrial effluents.

INTRODUCTION

Textile dyes are chemicals with complex aromatic structures designed to resist the effects of laundering and the sunshine[1]. A great number of dyes and other chemicals are used in textile wet processing[2]. There are more than 105 commercially available dyes with over 70,00,000 tone of dyes stuff produced annually worldwide[3]. It is estimated about 10-15% of dyes are released into processing water during the procedure [4]. The textile industry covers two third of the gross dye stuff market. During manufacturing and usage, approximately 10-15% of the dye is lost directly to waste water that finds its way into the environment [5].

Impact of textile dyes

The waste water effluent from the textile industry is considered as one of the most polluting among all other industries. The environmental and health effects of textile industry wastewater have been a subject of scientific scrutiny for a long time. The chemical nature of the waste from textile industry ranges from organochloride based waste pesticide to heavy metals associated with dyes and dying process[6].The disposal of reactive dyes into the environment causes serious damage as they intensely affect the photosynthetic activity of hydrophytes by limiting the light penetration and their breakdown products may be toxic to some aquatic organisms [7]. It also affects water body's ecosystems integrity soil fertility, plant growth and productivity of plants and susceptibility of plant pathogens. Some parameters for testing plant response to pollutants are germination percentage, seedling survival, shoot and root length of the seedling etc., [8].

The colored effluents that enter into the water bodies can also cause water-borne disorders such as nausea, perforation of nasal septum, ulceration of skin and mucous membrane, renal damage cramps, dermatitis, haemorrhage, hypertension, sporadic fever, severe irritation of respiratory tract or cancer. The bioaccumulation of the dyes depends on the availability and persistence of water, food and physiochemical properties [9, 10]. In the year 1991 FDA certified the use of 3000 tonnes of azo dyes in food drugs and cosmetics. Some of the azo dyes induce liver nodules in experimental laboratory animals.[11] Microorganisms bacteria, actinomycetes fungi, and algae, have been shown to degrade and biotransform azo dyes [12]. The azo dyes structures are reductively cleaved into colourless amines by several bacterial species [10].

The present study deals with the isolation of azo dyes degrading bacterium from dyes contaminated the environment, its ability to degrade reactive dyes into non-toxic products and optimization of temperature and pH for maximum dyes degradation.

MATERIALS AND METHODS

Sample collection

Industrial effluents were collected in sterilized screw capped bottles from textile industries near Peenya industrial area and Magadi road, Bangalore. Then the samples were brought to the laboratory. Physicochemical parameters of wastewater such as temperature, pH, colour and odour were measured.

Isolation of organism

The bacterial isolates were isolated by serial dilution method using pour plate technique on minimal agar medium [Potassium di hydrogen phosphate3.0g, Disodium hydrogen phosphate 6.0g, Sodium chloride 5.0g, Ammonium chloride 2.0g, Magnesium sulphate 0.1g, Glucose 8.0g, Agar 15g, Distilled water 1000L] along with dye in the concentration of 10mg/100ml. After incubation, the clear zone around the colonies were observed and streaked on LB agar slants and at stored 4^0C.

Screening for decolorization

The bacterial isolates were analyzed for the degradation of Red 2G dye in broth cultures. The flask containing Minimal broth medium [Potassium di hydrogen phosphate3.0g, Disodium hydrogen phosphate 6.0g, Sodium chloride 5.0g, Ammonium chloride 2.0g, Magnesium sulphate 0.1g, Glucose 8.0g, Distilled water 1000L] and dye was inoculated using loopful of isolated bacterial suspension. The culture flasks were incubated on an orbital shaker with 120rpm, at 37^0 C. The flasks without inoculation were kept as control. OD values and growth of organism were measured spectrophotometrically at 600nm to estimate the decolorization process. The rate of decolorization was calculated using the following formula.

$$\% \text{ Decolorization} = \frac{\text{Intial absorbance value} - \text{final absorbance value}}{\text{Intial absorbance value}} \times 100$$

Various factors were optimized to achieve highest degradation by the selected bacterial isolate.

Effect of Temperature

The bacterial culture was inoculated in 50 ml of Minimal broth medium with Red 2G dye at different temperatures ranging from 25˚C, 35˚C, 45˚C and 55˚C and these flasks were kept for incubation for 24 hrs, 48 hrs, 72 hrs and 96 hrs. These flasks were drawn at12 hours intervals for dye decolorization assay. Readings were taken after every 24 hours for 4 days.

Effect of pH

The bacterial culture was inoculated in 50 ml M9 medium with Red 2G dye at various pHs ranging from 5-9. The flasks for pH were kept in shaker incubator for 24 hrs, 48hrs, 72 hrs and 96hrs. These flasks were drawn at 24 hours intervals for dye decolorization assay. Readings were taken after every 24 hours for 4 days.

Effect of Incubation time

The incubation time varying from 24hrs to 120hrs were examined for the detection of optimum incubation time required for the degradation of dye by bacterial isolate. These flasks were drawn at 24 hours intervals for dye decolorization assay. Readings were taken after every 24 hours to 120 hours.

Effect of carbon sources on bacterial growth

The bacterial culture was inoculated in 50 ml M9 medium with Red 2G dye with different carbohydrates sources such as dextrose, sucrose, maltose, starch and cellulose. The inoculated flasks were kept at 37˚C in an orbital shaking incubator. These flasks were drawn at 24 hours intervals for dye decolorization assay.

Effect of nitrogen sources on bacterial growth

The bacterial culture was inoculated in 50 ml M9 medium with Red 2G dye with different nitrogen sources such as peptone, tryptone, tyrosine, glycine and ammonium ferrous sulphate. The inoculated flasks were kept at 37˚C in an orbital shaking incubator. These flasks were drawn at 24 hours intervals for dye decolorization assay. Readings were taken after every 24 hours for 4 days.

Enzyme for degradation

The bacterial degradation of Red2G dye by *Bacillus sp.,* was catalyzed by azo reductase enzyme (cytoplasmic enzymes with low specificity to the substrate) was analysed. The assay mixture contains 1mM potassium phosphate buffer (pH 7.0) 20 µM of azo dye and enzyme 0.1 µg. The reaction was initiated by the addition of 0.1mM NAD(P)H. The enzyme activity was measured by a decrease in absorbance over a 2 min period using a UV-visible spectrophotometer at 300nm. The enzyme activity was defined as the decrease in dye concentration (µM) per min (For the control, the enzyme was denatured by boiling for 15 minutes).

HPLC Analysis

Decolorization was estimated by using HPLC. Water 510, C-18 column [4.6 diameter, 250mm length]. Mobile phase [acetone:water, 70:30], flow rate 1ml/min, pressure 1200psi, injection volume 20µltr, UV detector 282nm was used for analysis. Qualitative analysis was done based on RTvalue, quantitative analysis was done based on area.

RESULTS AND DISCUSSION

Physico chemical characteristics of effluent

Eleven soil samples were collected from an industrial area in Peenya industrial area and Magadi road Bangalore. Surveys of the government of India Ministry of Small Scale Industries, 2039 garment industries in Karnataka are largely located in Bangalore. After textile processing, the waste sends to the sewage and causes water pollution. Effluents were collected in sterilized screw capped bottles from textile industries near Peenya industrial area and Magadi road, Bangalore. [13] worked on the contribution of

electrochemical treatment in textile dye wastewater; they have also collected effluent from the textile processor, located at kumbalagodu, Bangalore they found industrial effluent was toxic due to the wide variation of waste water characteristics and heavy COD load. [14] worked on water quality assessment near Peenya industrial area, Bangalore, stated that the contamination of underground water around Peenya industrial area due to garment washing and textiles.

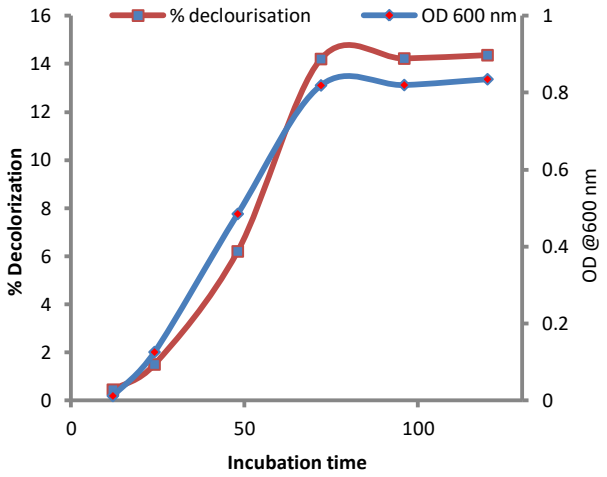
The samples were brought to the laboratory to isolate the microorganisms. The samples named as S1, S2, S3 and Physicochemical parameters of samples such as temperature, pH, colour and odour were analyzed. The temperature of S1, S2 S3 was 30^0 C, 32^0C, 28^0 C and pH was 7.9, 9.2, and 8.6 respectively. The colour of all the three samples was blackish blue and paint odour. The soil samples were subjected to isolate the organism by serial dilution using M9 media along azo dye. Dextrose is an energy source. Dipotassium and monopotassium phosphates provide buffering to the medium. Magnesium sulphate and ammonium sulphate are sources of ions that simulate metabolism.[15] used minimal agar medium for their work on bioremediation ability of individual and the consortium of non-immobilized and mobilized bacterial strains on industrial azo effluent. Another work has been done on biodegradation of azo dyes by using soil bacteria by[16].They have also used minimal agar for isolation work. After incubation at 37ºC for 48 hours found out clear zones surround the organism.

Isolation and identification of bacterial isolate

Total of 10 cultures of bacteria were isolated and screened for the degradation of azo dyes. Among the cultures, one bacterial isolate showing maximum decolorization based on the spectrophotometric method, the organism identified as *Bacillus sp.* on the basis of various physiological and biochemical tests as described in Bergey's manual of determinative bacteriology (1974)[17]. When it was subjected to Gram staining it shows gram-positive rods arranged in chains. It produces endospores gives the positive result of oxidase and catalase tests. The bacterial isolate also ferments carbohydrate dextrose and sucrose with the production of acid but no gas production. It unable to ferment mannitol and lactose as there was no acid and gas production.

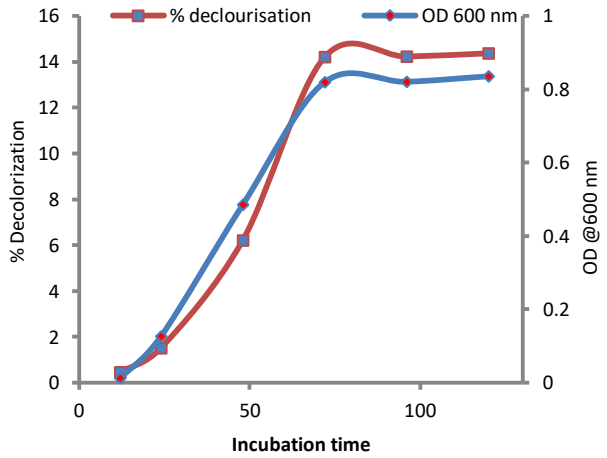
Growth curve

The logarithmic growth phase of the isolate, *Bacillus sp* was exhibited at 0.819 O.D against 600 nm after 72hr incubation. The O.D values were taken every 12 hr intervals up to 120 hr. the rate of degradation increased with the incubation period reaching maximum O.D of 0.819 at 385nm using spectrum as shown in the figure 1 below.



Effect of Incubation time

The incubation time varying from 24hrs to 120hrs were examined for the detection of optimum incubation time required for the degradation of Red 2G dye by bacterial isolate. These flasks were drawn at 24 hours intervals for dye decolorization assay. Readings were taken after every 24 hours to 96 hours. The bacterial isolate shown degradation at 24hr 8.07%, 48hr 12.06%, 72hr 13.68%, 96 hr 12.01%, and 120hr 10.59%. Studies have shown that 72 hr incubation was the ideal time to achieve maximum degradation. Fig 2 .



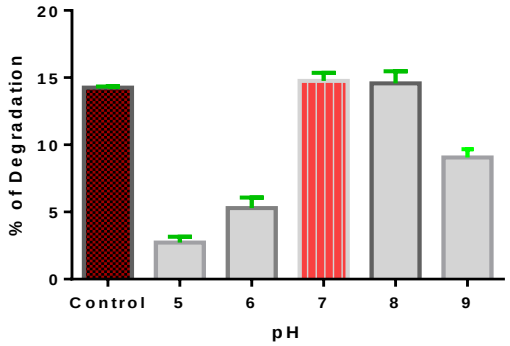
Effect of pH and temperature

The pH and temperature are the important factor for the optimal physiological performance of microbial cultures and decolorization of dyes. These factors affect the cell growth and various biochemical and enzymatic mechanisms.

Effect of pH on bacterial growth

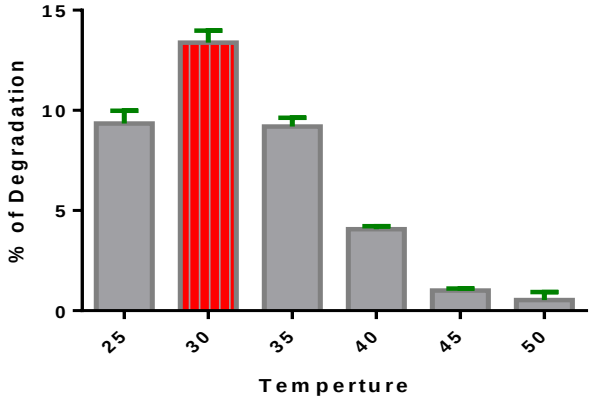
The bacterial culture was inoculated in 50 ml M9 medium with Red 2G dye at various pH ^{ranging} from 5-9. The flasks for pH were kept in shaker incubator for 24 hrs, 48 hrs, 72 hrs and 96 hrs. Studies have shown that P^H 9 of 96 hrs shows more degradation than other P^H. The decolorization of Red 2G dye by *Bacillus sp* strain was found in the pH range of 5-9. The maximum decolorization was observed at pH 9.0. A further increase

or decrease in pH from the optimum value decreases the decolorization rate. Our results are in good agreement with [18] who achieved maximum decolorization of Methyl Red by *Micrococcus* strain R3 in pH range of 6.0–8.0[19] reported that the decolorization of Remazol Black B by *Bacillus* sp. ETL-2012 was found in the pH range of 5.0–8.0. The optimum pH for decolorization of dyes is often at a neutral pH value or slightly acidic/alkaline pH. The rate of dye decolorization tends to decrease rapidly at strongly acid or strongly alkaline pH values. Fig 3.



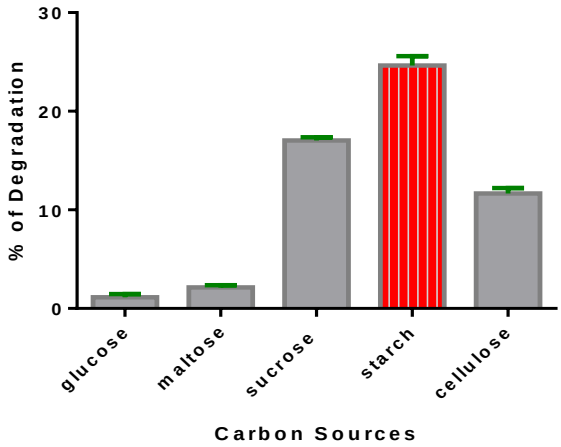
Effect of temperature on bacterial growth

The bacterial culture was inoculated in 50 ml of Minimal broth medium with Red 2G dye at different temperatures ranging from 25˚C, 30˚C, 40˚C, 45˚C, and 50^{0}C and these flasks were kept for incubation at 72 hrs. Similar to our result, [20] reported that the maximum decolorization of Reactive Red dye by mixed cultures was observed at 35°C. *A. hydrophilla* decolorize Red RBN dye in the range of 20–35°C.[21] found that bacterial consortium JW-2 showed maximum 93% decolorization of Reactive Violet 5R at 37°C. They also reported that further increase or decrease in temperature from optimum decreases the decolorization rate of Reactive Violet 5R dye. In many bacterial systems, the decolorization rate of azo dyes increases with increasing temperature up to the optimal temperature, within a defined range. Afterwards, there is a marginal reduction in the decolorization activity. The reduction in decolorization rate at a higher temperature may be attributed to thermal deactivation of azoreductase enzymes or loss of cell viability [22]. Fig 4.



Effect of different carbon sources

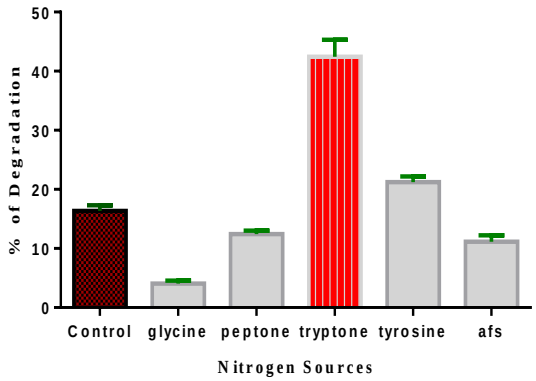
The bacterial culture was inoculated in 50 ml M9 medium with Red 2G dye with different carbon sources such as glucose, maltose, sucrose, starch and cellulose. The inoculated flasks were kept at 37˚C in an orbital shaking incubator. Studies have shown that starch shows more degradation than other carbohydrates. Azo dyes are deficient in carbon sources, and microbial degradation of dyes without any supplement of carbon or nitrogen sources is very difficult [23].The *Bacillus sp* showed maximum decolorization of Azo dye in the presence of carbon and nitrogen source as co-substrate. Among different carbon sources tested for efficient decolorization of azo dye, starch was found to be better carbon source with maximum decolorization (25.61%) whereas cellulose, glucose, maltose and sucrose was observed as poor carbon source showing only 12.06%, .51%, 2.38% and 17.42% decolorization of Red 2G dye, respectively. Several reports are available for the decolorization of dye in the presence of additional carbon sources. [24] reported that the addition of glucose to the medium enhances the decolorization rate of Reactive Red 3B-A and Reactive Black 5 by *Clostridium bifermentans*strains.[25] reported that in the presence of glucose, 90% decolorization of Reactive Red 180 dye by *Citrobacter sp*. CK3 was observed whereas in the absence of glucose only 26.72% decolorization was found. Carbon sources provide energy for the growth and survival of the microorganisms and as electron donors, which are necessary for the breakage of the azo bond [26] these sources generate reducing equivalents which are transferred to the dye during decolorization process. In the electron transport chain of the bacterial metabolism, these reducing equivalents such as flavin nucleotide (FAD) works as an electron shuttle between a dye and an NADH-dependent azo reductase. Fig 5.



Effect of different nitrogen sources

The bacterial culture was inoculated in 50 ml M9 medium with Red 2G dye with different nitrogen sources such as peptone, tryptone, tyrosine, glycine and ammonium ferrous sulphate. The inoculated flasks kept at 37˚C in an

orbital shaking incubator. The decolorization efficiency of *Bacillus sp.,* in the presence of different nitrogen source (glycine, peptone, peptone, tryptone, tyrosine and ammonium ferrous sulphate) was studied. Studies have shown that tryptone shows more degradation than other nitrogen sources.The maximum decolorization (45.47%) was observed in the presence of tyrosine, whereas in the presence of glysine , peptone, tryptone ,ammonium ferrous sulphate only 4.59%, 13.06%, 22.26% and 12.24% decolorization of Red 2G dye was observed respectively. Similar to our finding, [27]reported that yeast extract was effective co-substrates for decolorization of Reactive Red 141 by *Rhizobium radiobacter* which is in agreement with our studies.[24] reported that maximum decolorization of Reactive Violet 5 by bacterial consortium RVM 11.1 was observed in the presence of peptone and yeast extract as co-substrate. Moreover,[28] used inorganic nitrogen source (NH_4 Cl) in anaerobic treatment of azo dye Acid Orange 7 under fed-batch and continuous culture conditions. The metabolism of organic nitrogen sources regenerates NADH which acts as an electron donor for the reduction of azo dyes by the bacterial system. Fig 6.

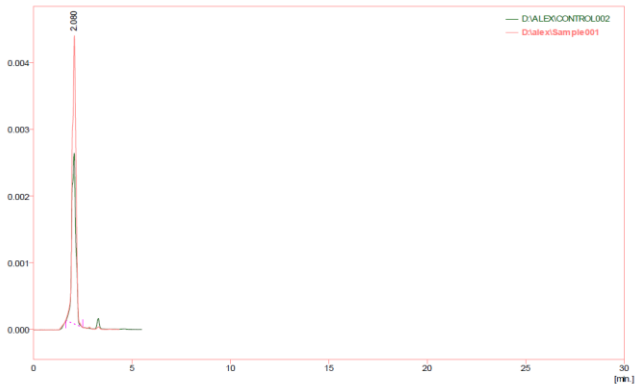


Azoruductase

Azo reductase usually acts on a wide range of substrate based on their chemical nature and size.Using NADH as an electron donor azo reductase was showed to exploit some azo dyes. The specific activity of cell free media obtained from *Bacillus sp.,* is 2.70 units.

HPLC

Based on the HPLC graph RT of the control was 2.07 and decolorized sample was 2.08. RT of the sample also resembles with the RT of control. The area of the standard is 57.14mV and decolorized sample is 40.77. Based on the area percentage of degradation shows 40%. Fig 7.



CONCLUSION

The current study has isolated, identified the Bacillus sp., and proved the decolorization activity of textile azo dye, Red 2G and optimized the physicochemical parameters for the maximum decolorization. Bacillus sp., shown maximum degradation of Red 2G in the presence of carbon source starch at pH 9 and 72 hours of incubation. This suggests that the addition of specific carbon source i.e. starch to the medium and optimization of pH at 9 and 72 hrs of incubation can enhance the degradation of Red 2G by bacillus sp. Thus Bacillus sp can be used as bioagent for the degradation of textile azo dye, Red 2G effectively.

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