BIOREMEDIATION OF REACTIVE RED DYE BY *BACILLUS SUBTILIS.*

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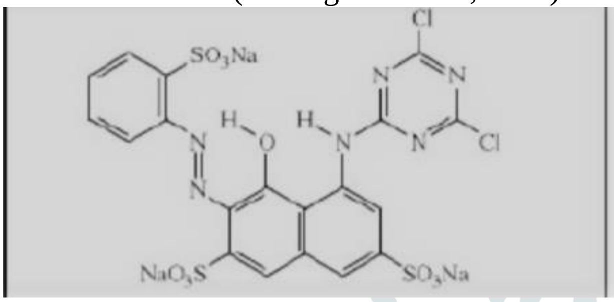
ABSTRACT
In the present study the untreated wastewater produce from the dye industry. In the textile industry azo dyes are used widely. Textile industry is removing the wastewater which is containing colour, and it is very serious issue. Manufacturing of different types of dyes are used for the study. For the control of this issue, the physic-chemical process is available and also biological process can be applied. In this task bacteria are used to degrade the reactive dye-Red M5B. During the procedure the bacterial organisms are grow and found the decolourization. By the using of surface methodology the optimum condition of dye degradation was identified. The wastewater analyzed for – pH, Toxicity, Nitrogen sources, Carbon sources and etc. After the treatment of wastewater the sludge are generated in high amount by the study. In industry many stages of manufacturing process are produced wastewater. This wastewater is not discharged properly by the norms of GPCB. Wastewater contains toxic substances. This toxic substance is harm to aquatic organisms as well as animals. Under the natural condition they are not easy degrade. By the treatment system the toxicity are removed by the waste.

KEYWORDS: Bioremediation, Isolation, Reactive textile dye, Dye decolourization.

INTRODUCTION

Without colour we cannot imagine our life. Colour can be using by the various types of dye. The dye is two types: - (1) Synthetic dye and (2) Natural dye. (R. Kant et al., 2012) Natural dyes which is made from plants, minerals and invertebrates. Other natural dyes are made from the plant roots, berries, organic sources, bark, leaves, wood and fungi. (Rajkumar et al., 2011) The dye materials are put into the water pot and textile dye are add into the pot, which are heated and stirred until the transferred colour. Many natural dyes required the chemical usage call mordents to bind the textile fibers. The man-made or synthetic dyes are made in mid-19th century. (Naik D.J. et al., 2013) World's first commercial dye was discovered by the William Henry perk in 1856. (Rai et al., 2005) Synthetic dye was applied to fibers to give them colour permanent. More than ten thousand synthetic dyes are delivered. (Robinson et al., 2011) Synthetic dyes are produced in large amount. In the India, dyestuff sector are one of the major chemical industry. (Hilden brand S. et al., 1999) In chemical industry they are second highest export segment. The widely use of dye in India - papers, plastic, textiles, printing ink and foodstuffs. The textile dye sector production nearly 80% for cotton, fiber and polyester. In the printing ink, plastic, papers and foodstuff the dyestuff are used. (R. Kant et al., 2012)

Textile effluents are polluting the water bodies. (Correia V. M. et al., 1994) Because of the effluent human health affected and effect on ecology and this is very serious issue. (Ponnusami V. et al., 2009**)** In the wastewater dye removing process are very expensive and difficult. The wastewater and water consumption of textile industry is depending on the processing of manufacturing. (Dhanve et al., 2008) Textile industries are one of the larger sources of liquid waste generation. (R.G. Saratale et al., 2010) Different methods for dye removal are included. Biological treatments are used in this method. The bacteria (Springer et al., 2010) enzymes, fungi, (Daniel D. et al., 2002) aerobic and anaerobic microorganisms are used. (Hassan E. et al., 2009) For azo dye degradation (Nachiyar and Rajkumar et al., 2005) the biological treatment contains two steps. (Mona E.M. et al., 2008) First is oxidation-reduction and second is oxidation of intermediates. By these steps products are less toxic, colourless and in acceptable state. This process are occurs because of microorganism growth. (V. Gunasekar et al., 2013) Reactive dye is synthetic dye. Reactive dye is synthetization by the chemical reaction of colour and reactive components. (Pearce CI. Et al., 2003) by the terms of chemical properties the reactive dye and natural dye are different. The chemical properties are water resistance, variety of colours and dyeing application process. (D.P. Chattopadhyay et al., 1997) The reactive dyes are mostly used in the cellulose fibers dyeing because the brightness and shades are depending on the nature of reactive dye. (Kamilaki A. et al., 2000) Recently the chemical properties of reactive dye are developed. They increase water solubility and reactivity to cellulose fibers. (R.M. Christie, et al., 2001) Most reactive dyes are from in power after the synthesis process. In this synthesis process the parameters of chemical properties of raw material controlled. (Thanapat S. et al., 2013) The visible spectrum to their chemical structure is absorbing light the azo dyes. Which are characterized one by one azo groups (-N=N-). (Chang, J.S.et al., 2000) Aerobic and anaerobic conditions the different bacteria group is decolorize azo dye. The bacterial decolourization of azo dye diverse the group of extensive studies. (R.G. Saratale et al., 2010)Reactive azo dyes are not degraded properly by the nature of xenobiotic. So, the chemical wastewater treatment is used. (Leisinger T. et al., 1981)



Mechanism of reactive red dye

And controls of the Parameters are depending on the reactive dye structure, synthesis operation and chemical properties. The special property of dye is key component. (Thanapat S. et al., 2013) Industrialization and Urbanization increasing day by day and pollute the environment. Without any treatment of effluent discharge into water, soil, aquatic organisms and ecosystem are damage.(Riu J. et al., 1998) The different industries are one of them textile industry, are discharge large volume of effluent, after the dyeing process.(Ameta C. et al., 2003) Textile dyes are largely creating azo dyes. Azo dyes are most important group of synthetic colours. (Zollinger H. et al., 1987) They are widely used in textile, pharmaceutical, and printing industry. They are most significant group of xenobiotic compounds and are inflexible in biodegradation process. (N. Puvaneswari et al., 2006)

Different factors affect the colour removal from dye effluent: (Temperature, pH (Chang J-S. et al., 2001), dye concentration (Dubin P. et al., 1975), dye structure and toxicity) was studied. (C.I. pearce et al., 2003)

MATERIALS AND METHODS

1. Dye sample:-Reactive dye: Reactive red M5B
2. Media:-The medium used for isolation and enrichment were nutrient agar Medium and complex medium of Glucose (0.1gm), peptone (0.5gm), yeast extracts (0.6gm) and Glucose peptone yeast agar.

1] Primary screening of dye decolorized microorganisms:

We take 10ml of G.P.Y. medium with 10 test tubes and added 1ml concentration of reactive dye.PH was balanced between the 7.0 and autoclave for 45min at 37◦c. for 48 hours.

2] Secondary screening of dye decolorized microorganisms:

We prepared G.P.Y. plate including 1ml of reactive dye. The culture was added in G.P.Y. plate and show maximum dye decolourization. The decolourization was observed after 48 hours and incubation process.

3] Optimization of dye decolorized at different dye concentration:

We take set of test tubes with 10ml of G.P.Y. medium contain and autoclave for 4min.After that old culture was added in all test tubes and incubated for 48 hours at 37◦c.

4] Optimization of different carbon sources-We prepared different sugar like lactose, maltose, starch, and dextrose and cellulose solution and also prepared control tubes for each sugar. We take a set of test tubes with 10ml of G.P.Y. medium and autoclave for 45min. After that old culture was added in all test tubes and incubated for 48 hours at 37◦c.

5] Optimization of different nitrogen sources-We prepared different nitrogen source like (organic source) urea, peptone and yeast extract. The inorganic nitrogen source like (inorganic source) NH4NO3, NACL and NH4CL. Control tubes are prepared for each source. We take a set of test tubes with 10ml of G.P.Y. medium and autoclave for 45min. After that old culture was added in all tubes and incubated for 48 hours at 37◦c.

6] Optimization of environment condition for the dye decolourization: (pH)

We prepared 10ml of G.P.Y. medium with different concentration of pH like 3-11 contain. The all test tubes are put in autoclave for 45min. After that old culture was added in all tubes and incubated for 48 hours at 37◦c.

7] Optimization of different toxicity on dye decolourization:

We take different source of toxic component like zinc, lead, manganese and a cadmium solution was prepared and adds dye concentration. Put it on autoclave for 45min. After that old culture was added in all tubes and incubated for 48 hours at 37◦c.

RESULTS

1] Primary screening of dye decolourization for microorganism:

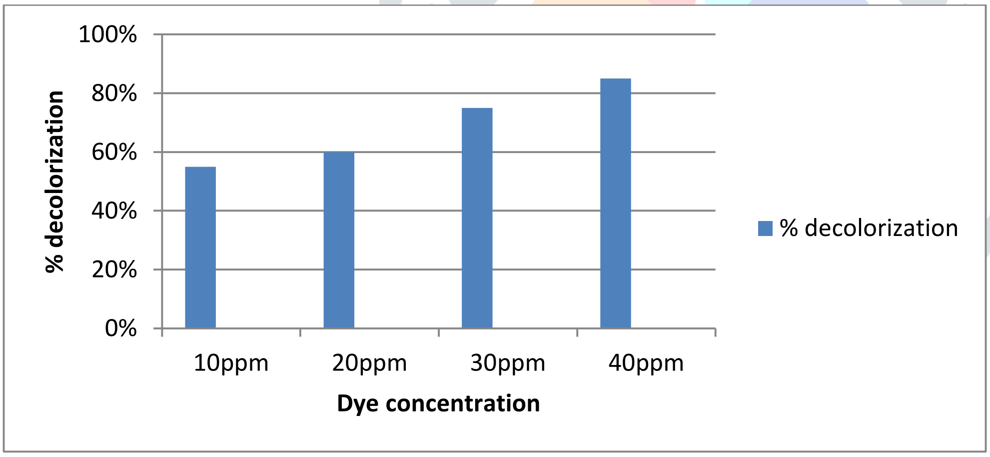
The results are shows in 10ml of G.P.Y. medium sample containing reactive red dye. The decolourization of dye is not seen another soil sample.

2] Secondary screening of dye decolourization of microorganism:

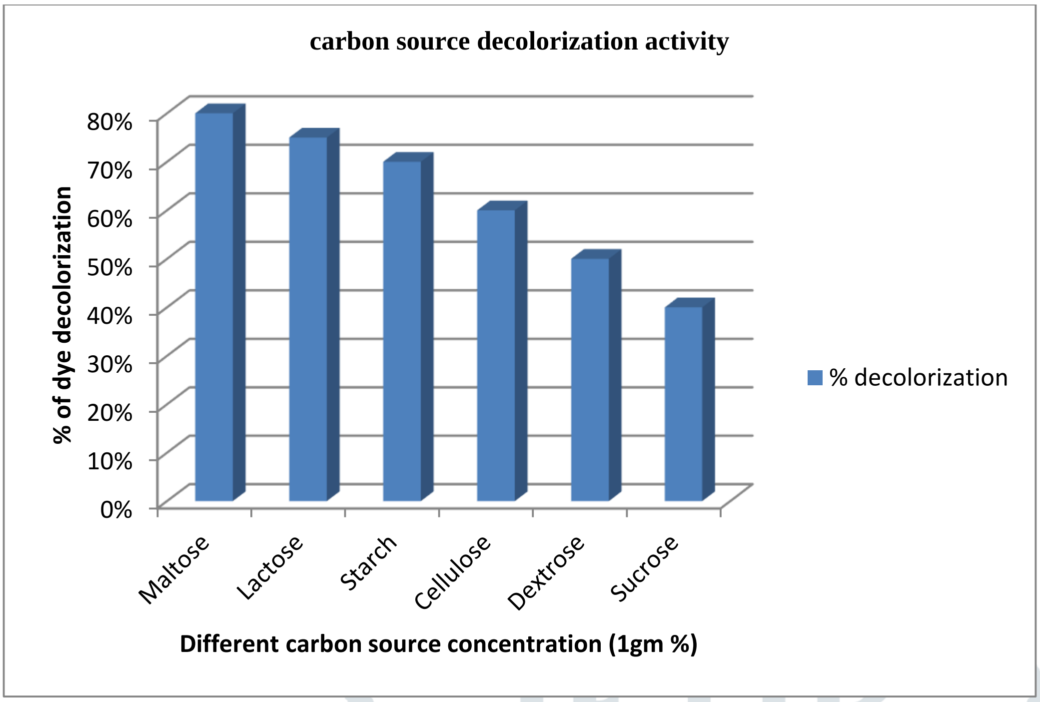
The growth of culture and decolourization was shows in G.P.Y. plate. In control Petri dish decolourization was not show.

3] Effect of dye decolourization at different dye concentration:

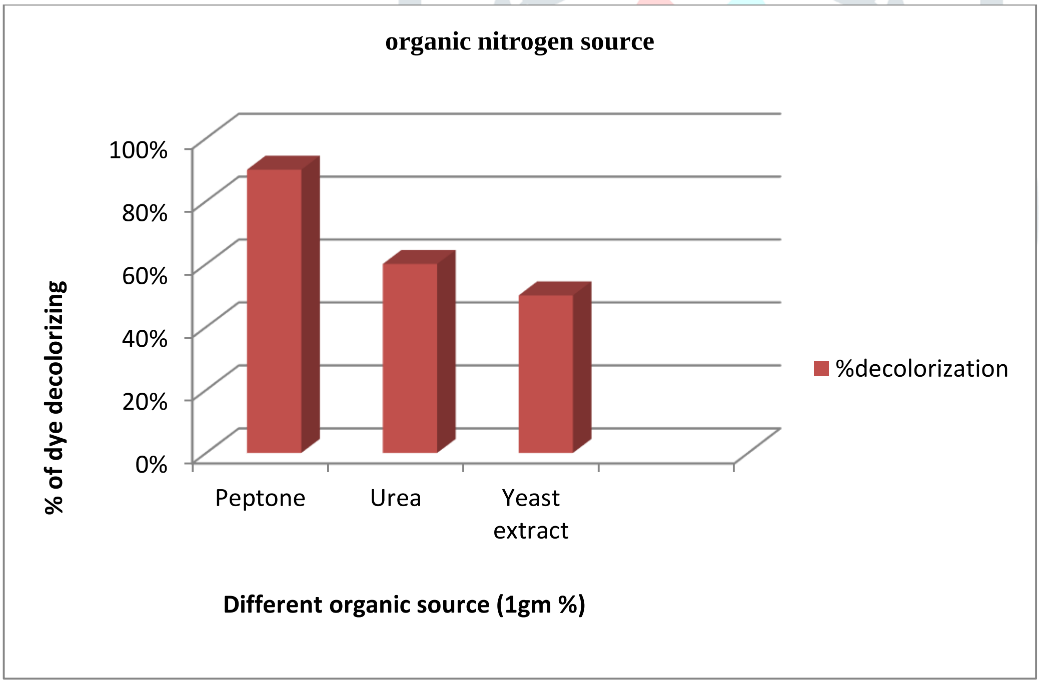
In 40ppm test tube the result was shows decolourization activity in 48 hours.



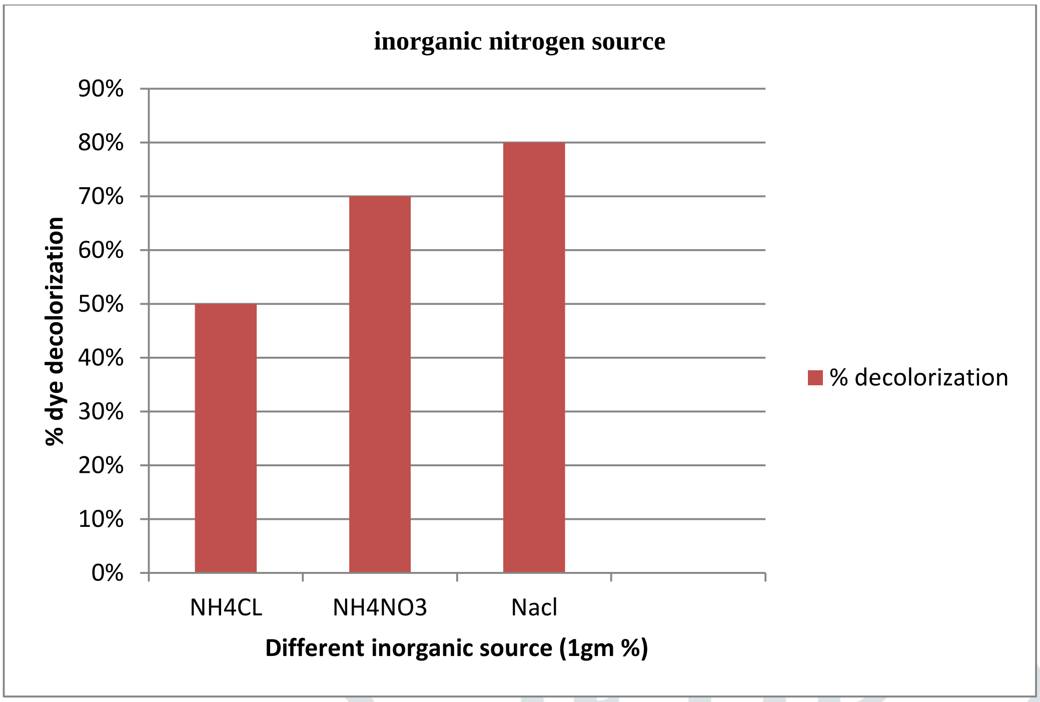
4] Effect of different carbon source on dye decolourization:



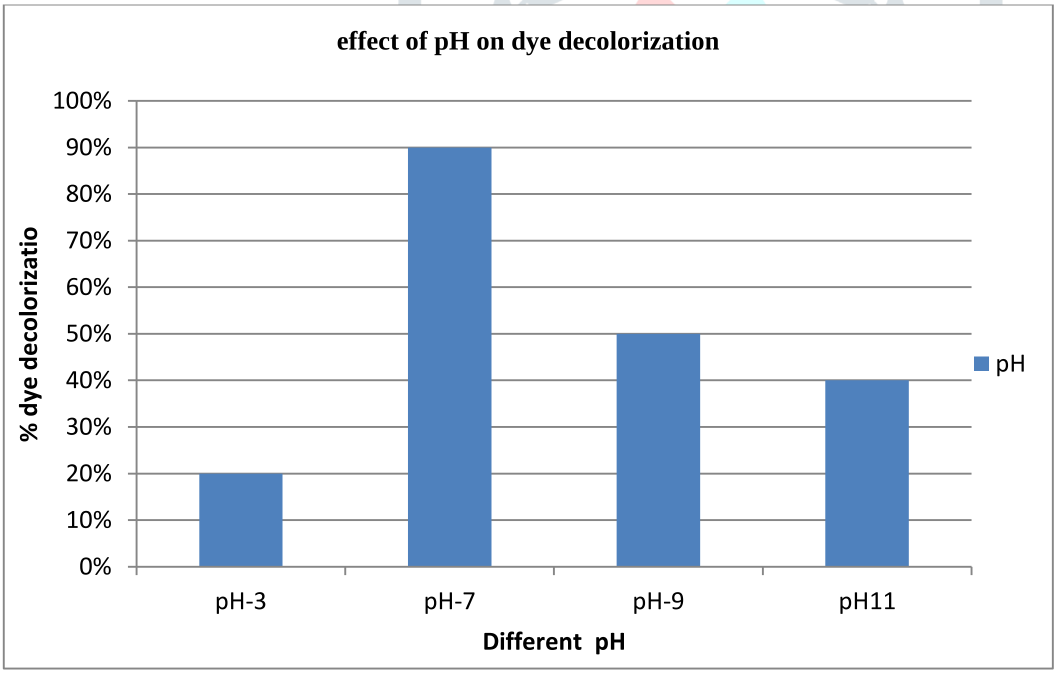
5] (A) Effect of different organic nitrogen source:



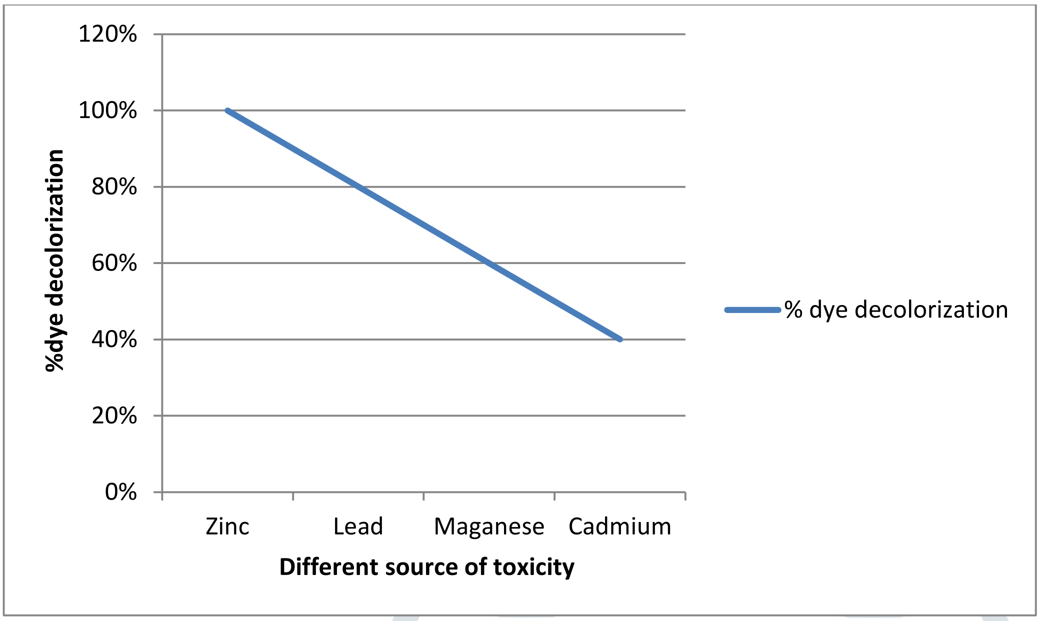
5] (B) Effect of different inorganic nitrogen source:



6] Effect of different pH dye concentration:



7] Effect of different toxicity on dye decolourization:



DISCUSSION

Optimization of carbon source: We studied the effect of different sugar like sucrose, maltose, lactose, starch, cellulose and dextrose taken 1gm %. The maximum dye decolourization observed in Maltose. The best sugar was Lactose at 1gm % of concentration.
Optimization of organic nitrogen source: We studied the effect of different organic nitrogen source like peptone shows high concentration of dye decolourization compare to the urea and yeast extract in 48 hours. So, 1gm % peptone was used as nitrogen source for any experiment.
Optimization of inorganic nitrogen source: We studied that effect of different inorganic source like Nacl shows high concentration of dye decolourization with 1gm % of concentration in 48hours.
Effect of initial dye concentration on decolourization: The different dye concentration like 10ppm, 20ppm, 30ppm and 40ppm are decolorized with increasing aye concentration with decreased rate of decolourization we have studied.
Decolourization of different pH: We studied that increasing decolourization with increasing of pH. In acidic medium the dye decolourization is very low but in alkaline medium highest pH-11 observed in 48 hours.
Decolourization at static and shaking condition: In static condition dye decolorized occurred only 40% and in shaking condition 80% of dye decolourization was observed by the study.

CONCLUSION

The complete decolourization of dye occurs in 48 hours. In natural condition the culture can decolorized on 40ppm. For efficiency of decolourization of dye occurs on 2ml culture of bacteria required. Maltose (1gm %) are found in carbon source and in organic nitrogen source peptone (1gm %) and inorganic source of nitrogen are Nacl (1gm %) observed by the experiment. The maximum decolourization was shows in all. Reactive red dye decolorized in 48 hours to 72 hours. On 11-pH dye decolorized maximum by the study. The shaking concentration flask is shows high decolourization compare to static condition.

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