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EXPERIMENTAL STUDY ON NON SPORULATING ESCHERICHIA COLI BACTERIA IN REMOVING METHYLENE BLUE

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ABSTRACT

World is ever witnessing the fashion revolution and youth passion, always lies in adopting and updating themselves with the recent trends and developments. Therefore, it creates a need for well designed and colorful clothing requirements day by day. Increasing the demand for clothing and simultaneous increase in production activities, largely responsible for the textile dye waste. In the present work methylene blue is biodegraded using Escherichia Coli to study the parameters influencing the dye degradation mechanism such as contact time, initial concentration of dye, pH of the solution, temperature, inoculum volume, and carbon and nitrogen sources. Dye degradation percentage of up to 89.52 has been achieved in 4 hrs of optimum contact time. Best results were observed at the temperature of 37^{o}C. Degradation percentage shoots up to 92.9% with an increase in inoculum volume to 8 ml. Glucose and ammonium sulphate are proven to be the best carbon and nitrogen sources respectively.

KEYWORDS: *Biodegradation; Methylene blue; Escherichia Coli bacteria; Carbon source; Nitrogen source*

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INTRODUCTION

Dyes vary in their adhering capacity to the fabric and the large amount of fabric reject dye results as waste in water. Triarylmethane (also called Triphenylmethane) dyes are one of the most commonly used dyes in textiles industries, with about 30%–40% of the total consumption of dyes and they are applied extensively on nylon, cotton, wool, and silk. They were also used for coloring food, oils, fats, waxes, varnishes, cosmetics, paper, leather, and plastics, etc.[1]. Amongst these, Azo dyes represent the largest and most versatile class of synthetic dyes. Due to the increase in environmental awareness among each and every human being, a strong and enforceable act have been developed and set a disposal limits on the waste discharge, making industries to practice a zero residual or minimum waste synthesizing techniques in their operations. In order to remove the dye from waste water a lot of conventional techniques are used namely adsorption on activated carbon[2], electro coagulation[3], flocculation[4], ion flotation[5], ion exchange[6], membrane filtration[7], ozonation[8] and reverse osmosis[9] have been used for decolorization of dyes in wastewater. However, these methods are less efficient, costly, of limited applicability, and produce wastes, which are even much more difficult to dispose off. On contrary to these conventional techniques, biological processes provide a low cost, environmentally benign, and efficient alternative for the treatment of dye wastewater. Biosorption involves the entrapment of dyes in the matrix of the adsorbent (microbial biomass) without destruction of the pollutant, whereas in biodegradation, the original dye structure is fragmented into smaller compounds resulting in the decolorization of synthetic dyes[10]. The use of microorganisms as biosorption agents in the removal of pollutants from wastewater has been suggested by many researchers[11, 12, 13]. However, due to operational ease and facile adaptability of microorganisms to a given set of conditions, the biodegradation mechanism using bacteria, fungi, algae, actinomycetes and other aerobic and anaerobic microorganisms is always considered efficacious in comparison to biosorption for treatment of dye wastewater. Between aerobic and anaerobic degradation, aerobic degradation is very effective. Since, it oxidizes the reduced components to further lesser toxic state than before. Hence, aerobic degradation is the safe method over anaerobic degradation[14]. Microbial decolorization and degradation is an environmentally friendly and cost-competitive alternative compared to the chemical/physical methods and have received more interest because of their cost effectiveness, lower sludge production and environmental friendliness in treating industrial effluents[15]. In the work presented by Gurulakshmi et.al [15], the Bacillus subtilisis was used to degrade the reactive dye –RED M5B and concluded that the decolorisation was due the action of enzyme peroxidase produced by the organisms during its growth. 95%degradation was observed within 40 h of inoculation. Vidhya et.al[16] had investigated the potential of the chosen bacterium, a natural isolate Bacillus subtilis isolated from textile industry effluent (textile effluent adapted bacterium) and the isolate was found to be more efficient in degrading Dimethy formamide (DMF) based on the assessment of physico-chemical parameters like pH, turbidity, carbon dioxide and ammonia released during the degradation process. Sarnaik et.al [17] used Pseudomonas mendocina MCM B-402 to utilize a triphenylmethane dye, methyl violet as the sole source of carbon when incorporated in synthetic medium. Almost complete decolorization of methyl violet by P. mendocina was observed within 48 h of incubation at ambient temperature (28.2°C) under aerated culture conditions, when the bacteria were inoculated into Davis Mingioli's synthetic medium at a concentration of 100 mg/l medium and concluded that the decolorization of the dye involved demethylation. Sapna Kochher et.al[18] studied physicochemical characterization of the textile industry effluent collected from Oswal Textile Industries, Ludhiana (Punjab.) India and the results showed that the temperature (40°C), pH (8.00), Biological Oxygen Demand (260 mg–1), Chemical Oxygen Demand (790 mg–1), Total Suspended Solids (2000 mg–1), Total Dissolved Solids (7000 mg–1 and colour over the prescribed fresh water limits. According to them decolorization was effective at pH 8, 35°C with starch and peptone as carbon and nitrogen sources and in static conditions.

MATERIALS AND METHODS

Materials

Methylene Blue was purchased from Finar reagents, Ahmedabad. Escherichia coli, Nutrient agar slants were collected from Council of Scientific and Industrial Research-Centre for Cellular and Molecular Biology (CSIR-CCMB), Hyderabad

Experimental Design

Methylene Blue is used to make solutions of different concentrations without further purification. 1g of accurately weighed methylene blue powder is dissolved in 1000ml of double distilled water collected from Andhra University distill water plant, to make a stock solution of 1000 ppm concentration and then this stock solution is further diluted to required concentrations from 10-50 ppm range using double distill water. Escherichia coli were grown on nutrient agar plates. The mother culture was then stored in refrigerator. The media used for sub culturing and decolonization contains 0.5% Peptone, 0.1% beef extract, 0.2%yeast extract, 0.5% NaCl in 1L distilled water. Nutrient Agar is prepared by adding 15g of Agar to the nutrient broth and heated till boiling. Further, it was cooled to room temperature using ice water and then the required quantities of solutions were transferred into test tubes and kept on a table with slight inclination. These Nutrient agar slants were used for subcultured and maintenance of the bacterial strains obtained from CCMB. To study the behavior of Escherichia coli in removing methylene blue, 50ml of solution containing nutrient broth and known concentration of dye (10-50 ppm) adjusted to pH-7 was autoclaved for 20 minutes at 15lb pressure and 121°C. It was then cooled and exposed to UV light in laminar flow for 15min. After UV irradiation, the samples are carefully inoculated with 5%

freshly prepared (6 hrs old) bacterial culture using a micropipette and incubated in an orbital shaking incubator at 37°C and 180rpm. Samples were collected at every 30 minutes interval of time and centrifuged at 5000 rpm for 15 minutes by in an Eppendorf tubes followed by analyzing using UV-Visible spectroscopy at 665 nm. The effect of different parameters such as initial dye concentration (10-50 ppm), pH of the solution (5-9), temperature (25, 30, 37 &40), inoculum volume (2-8%), carbon source (sucrose, glucose, lactose and starch were used as co-substrates), nitrogen source (urea, ammonium sulphate, ammonium chloride and ammonium nitrate were used as co-substrates) on %decolorization was studied individually. The standard graph was drawn between the concentration range studied and their absorbance. All the samples were analyzed using UV spectrophotometer (WSP-UV550, Mumbai) at a wavelength of 665nm. %decolorization is calculated using the formula[15].

$$\%\text{Decolorization} = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Finalconcentration}} \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

Growth curve of Escherichia coli

Freshly culture bacteria were used to obtain the growth curve of Escherichia coli at different time intervals and analyzed at 665 nm in UV-Visible spectrophotometer. A plot drawn between time on X-axis and absorbance on Y-axis is shown in the Figure-1.

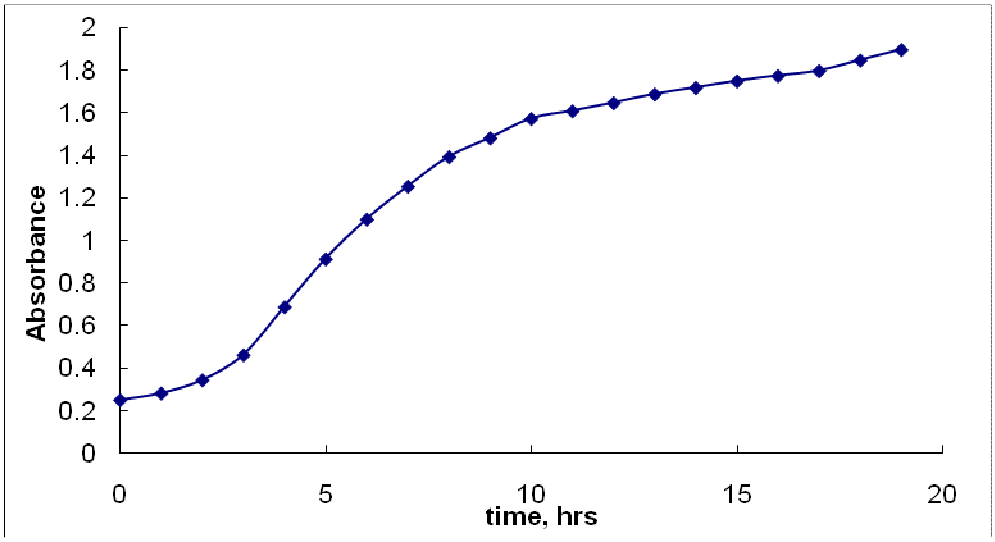

Figure 1
Effect of time on growth of E.coli

Effect of contact time on dye reduction

Experiments were carried out to study the effect of contact time on the removal of methylene blue concentration range of 10-50 ppm and also to find the equilibrium contact time, where no considerable reduction can be observed. From Figure-2, it can be noted that increasing the contact time increases the percentage decolorization until the equilibrium is reached and by observation the equilibrium time of 4 hrs is more than enough to saturate the E.coli and 4 hrs of contact time was chosen to study other parameters.

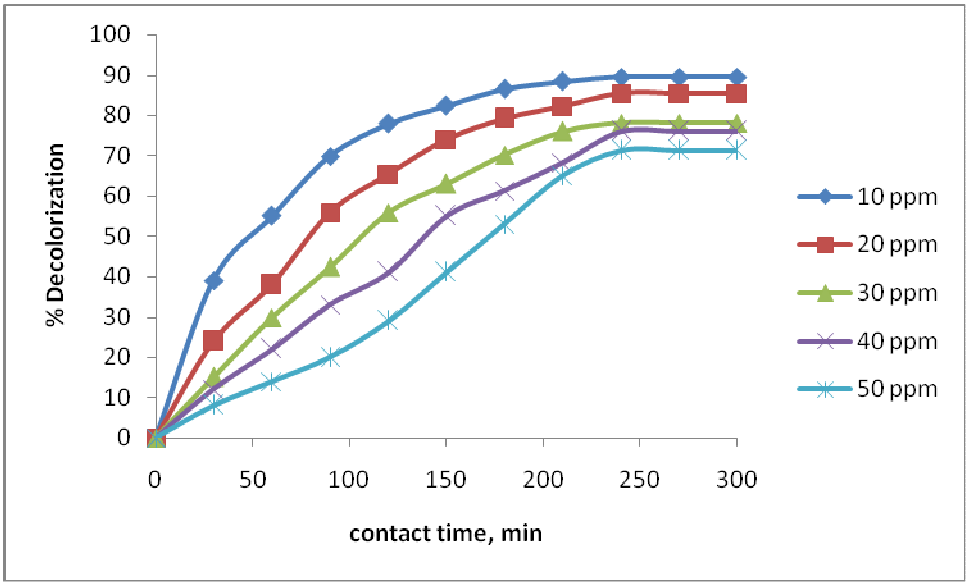

Figure 2
Effect of contact time on percentage decolorization

Effect of initial dye concentration

The results obtained are shown in Figure-3. The obtained plots show that the percentage of decolorization decreased with an increase in initial concentration of dye. The percentage of decolorization decreased from 89.52 to 76.02% with an increase in the initial concentration of dye from 10 to 50 mg/l respectively and the values are shown in Table-1. Similar results were observed for the bacterial decolorization of various azo dyes reported by Jadhav et.al[19]. In most cases the higher concentration of azo dyes inhibits the nucleic acid biosynthesis and cell growth [20]; hence the effect of dye concentration on growth of organisms is an important consideration for its field application. The work carried out by Lin-Na Du et.al [21] suggested that the decrease in decolorization efficiency might be due to increase in the toxic effect of dyes, with an increase in dye concentration.

Table 1
Effect of initial dye concentration on %decolorization

S.No	Initial concentration (mg/l)	% Decolorization
1	10	89.52
2	20	85.4
3	30	78.08
4	40	76.02
5	50	71.26

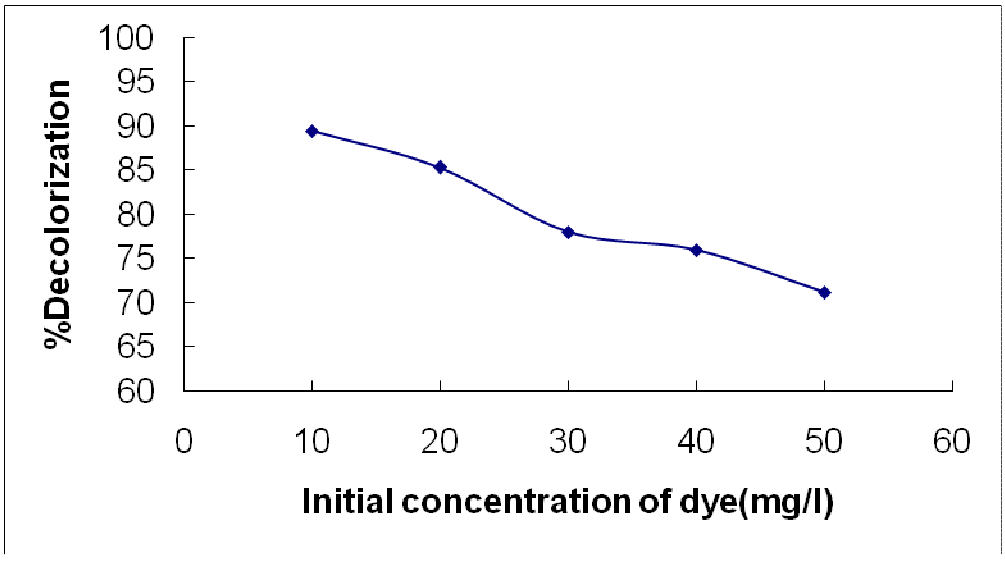

Figure 3
Effect of initial concentration of dye on %decolorization

Effect of pH

Experiments were conducted to study the effect of pH on the removal of methylene blue from its aqueous solution using E. coli and the results obtained are shown in Figure-4 and table-2. The percentage of decolorization of methylene blue first increased from 58.9 to 74.96% with an increase in pH from 5 to 7 respectively and then decreased from 74.96 to 45.09% with an increase in pH from 7 to 9 respectively. This result shows that the pH of the medium is also an important factor in decolorization. The rate of color removal is higher at the optimum pH-7, and tends to decrease rapidly above pH-7. Thus, maintaining the operational pH-7of the solution, against the operational (optimal) pH-8 concluded by V Sridevi[22], in her study on methylene blue doesn't require any additional investment case of E.Coli. The E.coli grows actively at pH-7 and also showing maximum dye degradable ability at that pH. Decolorizing ability of bacteria depends on cell growth and active metabolism of culture. According to the results the organism used has actively degrading the dye at neutral pH-7 rather than in acidic or alkaline conditions.

Table 2
Effect of pH on %decolorization

S.No	pH	%Decolorization
1	5	58.8
2	6	65.1
3	7	74.96
4	5	52.25
5	9	45.09

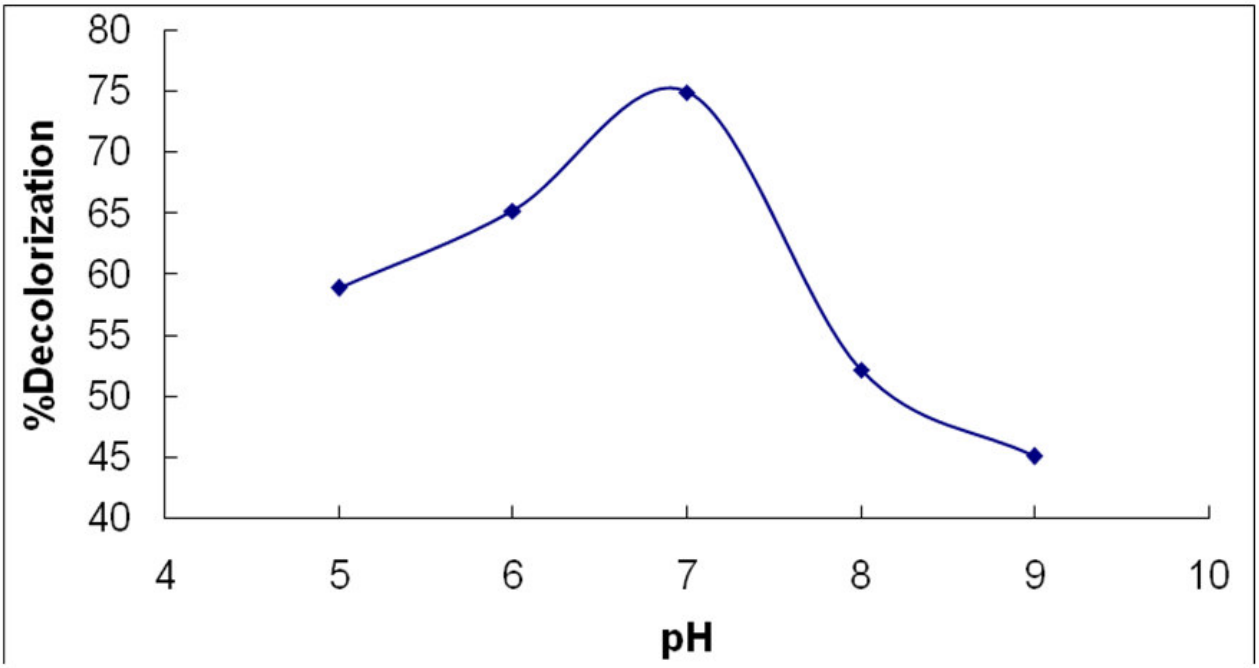

Figure 4
Effect of pH of dye on %decolorization

Effect of temperature

Experiments were conducted to determine the effect of temperature on percentage of decolorization of dyes at contact time (4h) as a function of temperature (25 to 45^{o}C) with other parameters, initial dye concentration of 30 mg/l, 5% inoculum volume and pH 7 keeping constant. The results obtained are shown in Figure-5 and Table-3. The % decolorization firstly increased from 59.35 to 77.5% with an increase in temperature from 25 ^{0}C to 37^{0}C respectively and then decreased from 77.5 to 57.5% with an increase in temperature from 37 ^{0}C to 45^{0}C respectively. In case of microorganisms, the environmental temperature establishes a direct relationship with microbial activity, as the microbial cell actively responds to temperature changes by adaptation via biochemical or enzymatic mechanisms. It was observed that the dye decolorization activity of the culture was found to be maximum at 37^{0}C. Cells may become metabolically active and capable enough to produce the required enzymes needed for degradation. Further increasing the temperature resulted in marginal reduction in decolorization ability of the bacterial culture E. coli. This might have occurred due to adverse effect of high temperature on the enzymatic activities and most of the enzymes cannot sustain at higher temperatures. Similar results were observed for the bacterial decolorization of various azo dyes by Guru Lakshmi et.al[15].

Table 3
Effect of temperature on % decolorization

S.No	Temperature, ºC	% Decolorization
1	25	59.35
2	30	65.11
3	37	77.5
4	45	57.5

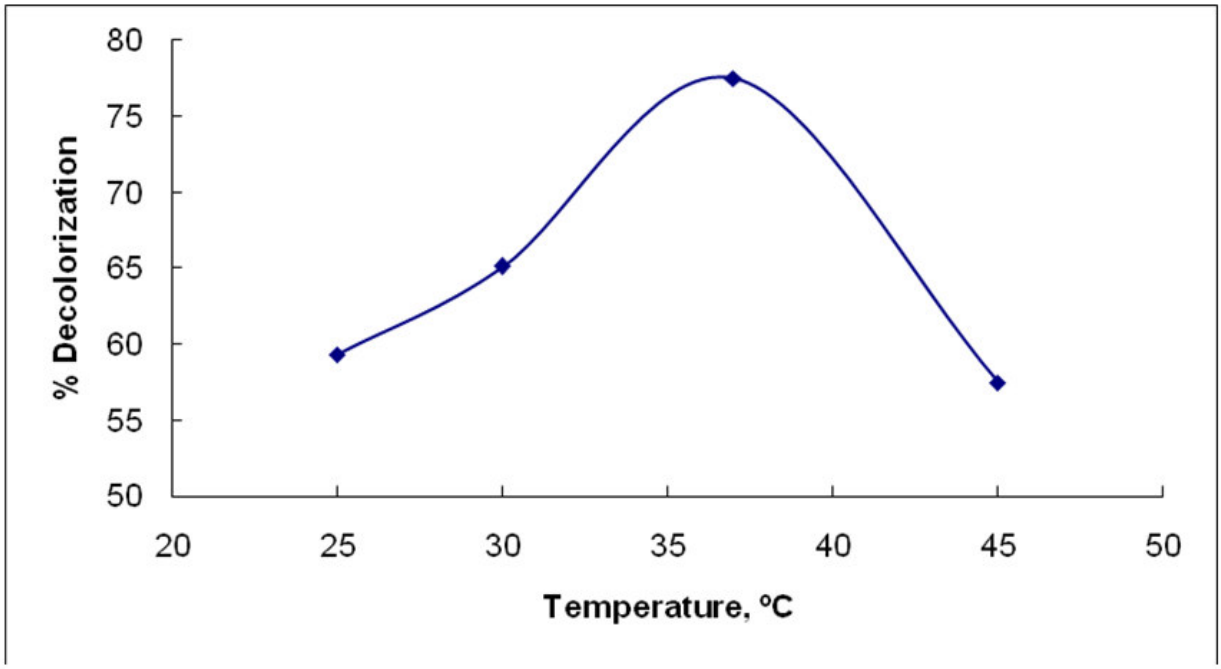

Figure 5
Effect of temperature on %decolorization

Effect of inoculum volume

The percentage decolorization increased from 71.42 to 92.9% with an increase in inoculum volume from 2 to 8% respectively due to the presence of young and activating cells in inoculums (Figure-6 & Table-4). The highest decolorization was achieved with the highest inoculum volume. This observation was similar to the reports given by Hazrat Ali et.al[20] and Lin-Na Du et.al[21], where dye removal capacity increased significantly with an increase in initial inoculum volume.

Table 4
Effect of inoculum volume on %decolorization

S.No	Inoculum volume, ml	% Decolorization
1	2	71.42
2	4	78.61
3	6	85.13
4	8	92.9

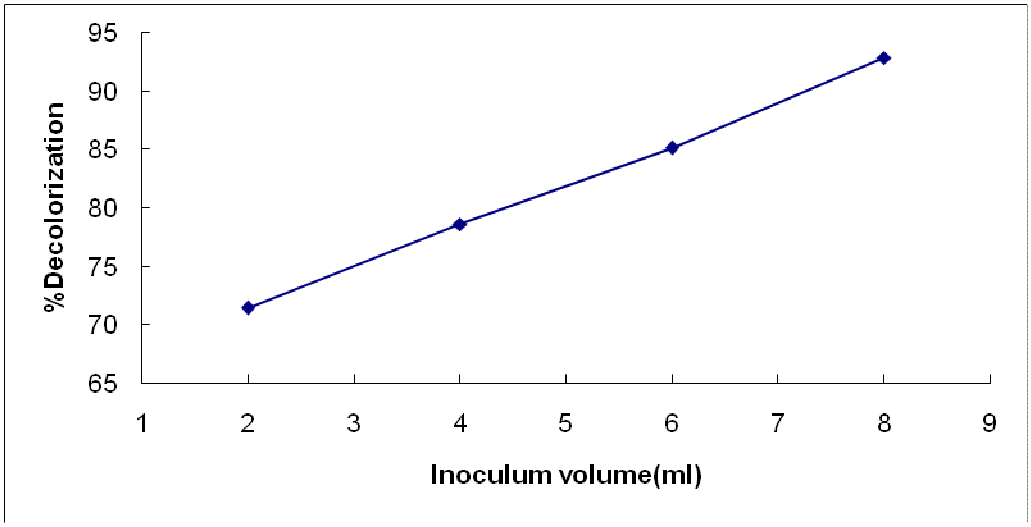

Figure 6
Effect of inoculum volume on %decolorization

Effect of carbon source

To explore the carbon effect on decolorization of dyes, experiments were performed with different carbon sources (glucose, sucrose, lactose and starch) at a concentration of 1% (w/v) and keeping other conditions constant (pH 7, dye conc. 30ppm, temperature 37 ^{O}C, inoculum volume 5%). Dye degradation up to 83.25% with glucose as a co-substrate was achieved (Figure-7 & Table-5). Other sources of carbon showed minimum degradation when compared to glucose. Glucose seemed to be effective to promote the decolorization over other carbon sources, probably due to the preference of the cells in assimilating the added carbon source. Similar results were observed by Bhoomi Joshi et.al[23], they proposed lactose as an optimum carbon source during their study on biodegradation and decolorization of food azo dye.

Table 5
Effect of carbon source on %decolorization

S.NO	Carbon Source	%decolorization
1	Lactose	82.13
2	Glucose	83.25
3	Sucrose	79.39
4	Starch	80.27

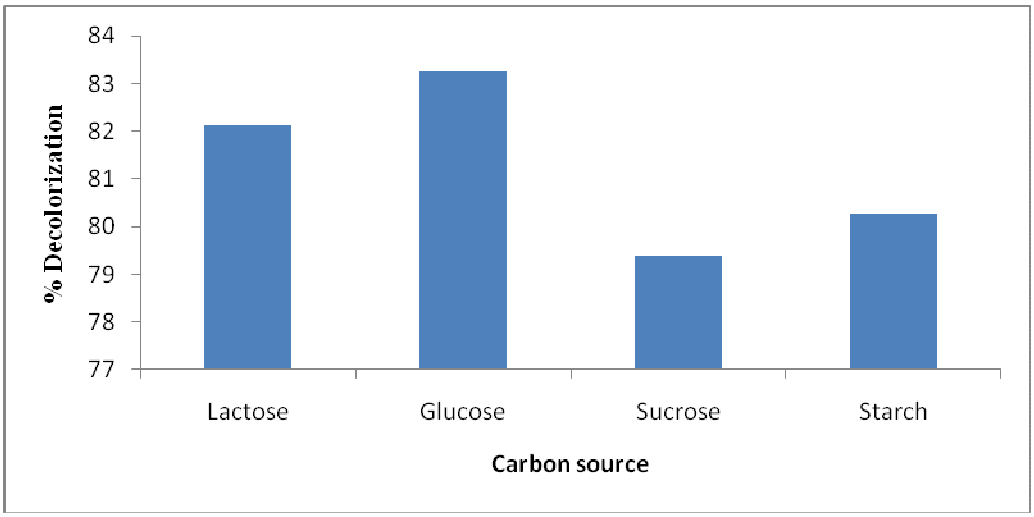

Figure 7
Effect of Carbon source on %decolorization

Effect of Nitrogen source

To study the impact of nitrogen source on decolorization of dyes, experiments were performed with different nitrogen sources (ammonium sulphate, ammonium nitrate, ammonium chloride and urea) at a concentration of 0.25% (w/v) and keeping other conditions constant (pH 7, dye conc. 30ppm/50ml, temperature 37^{O}C, inoculum volume 5%). Dye degradation up to 87.17% with ammonium sulphate as an additional source of nitrogen was observed for methylene blue (Figure-8 & Table-6). Maximum degradation was observed with 0.25% (w/v)

ammonium sulphate. Ammonium sulphate seemed to be effective to promote the decolorization. To decolorize more, the new cells needs to be generated and to generate new cell energy is required. Therefore, the use of carbon and nitrogen sources are required in order to balance the energy generated by bacteria on oxidation of substrate by respiration against the energy required to synthesize the new cells. Similar results were observed by Ponraj et.al[19].

Table 6
Effect Nitrogen source on %decolorization

S.No	Nitrogen Source	%decolorization
1	Ammonium sulphate	87.17
2	Ammonium nitrate	84.77
3	Urea	85.48
4	Ammonium chloride	82.35

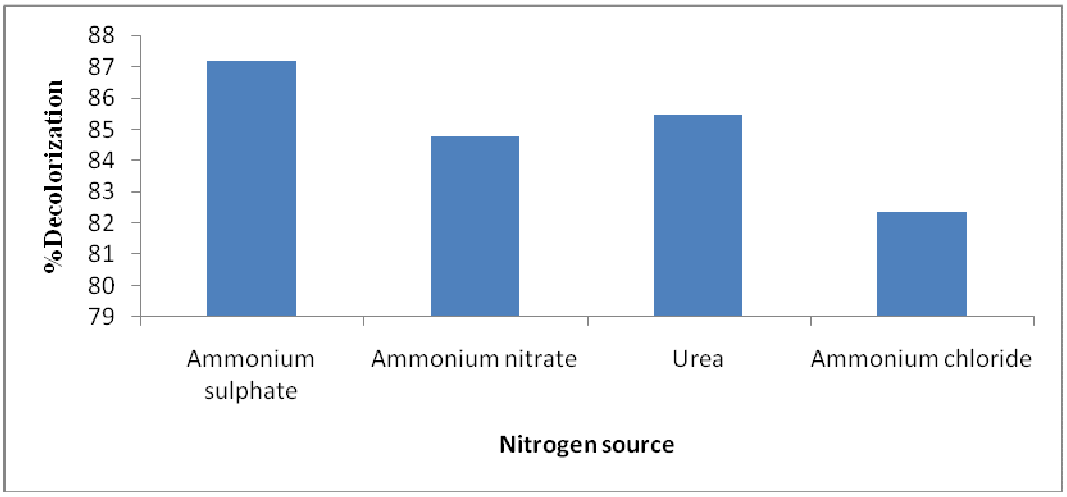

Figure 8
Effect of Nitrogen source on %decolorization

CONCLUSION

The present work helped in identifying a new source of bacteria for removal of dyes from effluent wastes containing low concentration of dyes. Analysis of results was made for about 100 experimental runs conducted. Based on the analysis the following conclusions were given below. The dye removal performances were strongly affected by parameters such as initial concentration, pH, inoculum volume, temperature, carbon source and nitrogen source. The effect of time of contact for maximum dye removal performance was found to be 4 hrs. The percentage of decolorization was decreased with an increase in the initial concentration of dyes and a significant biodegradation takes place at pH-7 .The percentage of decolorization increased with an increase in the inoculum volume and at 37^{o}C the biodegradation is maximum. Glucose seemed to be effective to promote the decolorization probably due to the preference of the cells in assimilating the added carbon source over using other carbon sources and the dye compound as the carbon source and ammonium sulphate, as a nitrogen source, seemed to be effective to promote the decolorization.

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