Biodecolourisation of the Azo Dye, Congo Red Using the Bacterium, *Lactobacillus Delbrueckii*

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Abstract

Background: Waste water released from the use of synthetic dyes in different textile and other dye stuff industries require treatment before it is discharged into the environment to prevent groundwater contamination. Considerable interest has been on decolourisation and degradation of dyes by microorganisms due to their efficiency and duration of treatment.

Objective: The present study has been designed to isolate a bacterial strain capable of decolourising Congo Red and to test its efficiency of decolourisation.

Materials and Methods: In the present study the natural isolate, *Lactobacillus delbrueckii* was isolated from the contaminated soil and used in the decolourisation of the dye, Congo Red. Decolourisation was tested in concentration ranging from 10 to 1000ppm for 15 days. Activities of α-amylase and protease were determined during decolourisation.

Results: Decolourisation was achieved within 15 days at lower concentration. The enzymes α-amylase and protease produced by this bacteriumexhibited increased activity with the increase in treatment period.

Conclusion: The results showed practical application potential of this bacterial strain in decolouration of dye effluents that can help to solve the pollution problems caused by textile and dyeing industries.

Key words: *Biodecolourisation, Lactobacillus delbrueckii, Congo Red, Azo dyes.*

Introduction

"Water", the elixir of life is being polluted by various means which should be prevented to avoid serious environmental hazards. Pollutants include chemical substances, organisms, products or physical properties that are released intentionally or inadvertently by man into the environment with actual or potential adverse, harmful, unpleasant and inconvenient effects. Such undesirable effects may be direct or indirect being mediated through resource organisms or climate change. Water pollutantsare anything put into water which were not there in natural state. Regular and plentiful supply of clean water is necessary for the survival and health of most living organisms. As a consequence of rapidly expanding industrialization and excessive population growth, most of our rivers, lakes, streams and other water bodies are being increasingly polluted. Therefore, treatment of waste water has to be done through the stateofart technique employing microorganisms.Tamil Nadu is dominated by textile industries that use varieties of dyes and chemicals. Their effluents are let out into the rivers without any pretreatment, causing environmental pollution. One of the very pressing environmental problems of textile industry is the removal of colour from effluent prior to discharge to local sewage treatment.[1]

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But the dyes that are normally found in waste water treatment plants are highly resistant to microbial degradation. This is because dyestuffs are designed to resist chemical fading and light induced oxidative fading. Other factors involved in reducing biological degradation of dyes include properties such as high-water solubility, high molecular weight and fused aromatic ring structures. Textile dyes are classified as azo, diazo, cationic and basic based on the nature of their chemical structure.[2]

Amongst all, reactive azo dyes are most problematic due to their excess consumption and high-water solubility. Furthermore, the sulfonic acid and the azo groups are rare amongst the natural products and thus both confer xenobiotic character on this class of compounds. Therefore, bacterial isolates from dye effluent amended soils were used for decolourisation process. Some of the dyes and their degradation products have proved to be toxic, mutagenic and carcinogenic in nature. Thus, removal of dyes from effluents has been given utmost importance.Numerous physical and chemical techniques based on coagulation–flocculation, precipitation, membrane filtration, ion exchange, electrochemical destruction, photochemical degradation, ozonation and adsorption are available for the removal of colour in textile wastewater. The majority of colourremoval techniques work either by concentrating the colour into the sludge, or by partial to complete breakdown of the coloured molecules. Although some of the physico–chemical processes have been shown to be effective, their application is limited due to excess usage of chemicals, sludge generation with subsequent disposal problems, high installation as well as operating cost and sensitivity to variable wastewater input. Biodecolourisation constitutes an attractive alternative to physico-chemical methods, mainly due to its reputation as low cost, eco-friendly and publicly acceptable treatment technology. Thus, a microbial capacity of the degradation of recalcitrant textile dye is developing that may be harnessed for dye removal by biotechnological processes.The objective of the present investigation was to isolate a bacterial strain that is capable of decolourisation of the reactive azo dye, Congo Red. The rate of decolourisation as well as protease and amylase activity of the selected strain was also studied.

Materials and Methods

Isolation of the bacterial strain

For the isolation of bacteria, 1g of soil sample was serially diluted up to 10^{-6}dilution.The isolation was carried out in nutrient agar medium by streaking a loopful of 10^{-6}dilution sample using streak plate technique.[3]

Selection of dye

Effluents which contain azo reactive dyes are very difficult to treat in environmental systems, due to the sulphonic acid groups, which makes the dyes verywater-soluble and polar. Therefore, such a reactive azo dye, Congo Red was selected. The molecular formula of Congo Red is $C_{32}\ H_{22}\ NO\ Na_2\ O_6\ S_2$ and the molecular weight is696.68.[4]

Preparation of dye stock solution

0.1 g of Congo Red was dissolved in 100mlof distilled water. This was kept as stock solution.

Absorption maxima for the dye

Using a dye concentration, absorption maxima was determined as 470nm from the absorption spectrum.

Minimal broth for decolourisation experiment

The composition of the minimal broth used is as follows.$K_2HPO_4$7.0g; $KH_2PO_4$2.0g; $(NH_4)_2\ SO_4$1.0g; Glucose1.0g; Sodium citrate0.5g; $MgSO_4$. $7H_200.1$g; at pH 7.0 +0.2 and 25°C.[5]

Preparation of the inoculum

The isolated colony was taken separately and inoculated on to Nutrient brothand kept in the shaker for overnight incubation.

Inoculation of the isolated strain

To the autoclaved broth, the pure dye Congo Redwas added in different concentrations like 10, 20, 30, 40, 50, 100, 200, 500 and 1000ppm separately. To each of this 0.1ml of culture was added and kept in the roomtemperature for incubation. OD readings were taken for 1st, 2nd, 3rd, 4th, 5th, 6th, and 7thhour using colorimetry. The readings were also taken for 15days using colorimeter after every 24 hours.

Identification of the isolated strain

The isolated bacterial strain was tentatively identified as *Lactobacillus delbrueckii* based on biochemical tests.

Decolourisation assay

Decolourisation activity expressed in terms of percent decolourisation wasdetermined by the following formula.

$D(\%) = [(A_{ini} - A_{fin}) / A_{ini}] \times 100$

Where, D= Decolourisation activity

A_{ini}= Initial absorbance

A_{fin}= Final absorbance after incubation time.

Two types of controls were used as, Uninoculated sterile control with dye and Inoculated control without dye.

Enzyme Assay

2ml of samples were taken from the concentrations, 10, 20, 30, 40 and 50 ppm ofthe dye. The samples were centrifuged at 10,000 rpm for 15 minutes. Thesupernatant obtained was stored in a refrigerator by adding 1% sodium azide which inhibits the enzyme activity. This was done up to 5 days after inoculation of bacterialstrain. The activity ofα- amylase and protease produced by bacteriawasdetermined using different methods.[6]

Findings

Results and Discussion

Soil samples that were analysed qualitatively had various bacterial colonies.From which, one of the strains was identified and used for the decolourisation of thedye. Most species of this nonspore forming bacterium ferment glucose into lactose, hence the name *Lactobacillus*.[7] In Gram staining, it was viewed as violet in colour which indicates the Gram positive bacilli. It did not form spores in spore formation test, and so it may be *Corynebacterium*or *Lactobacillus*. In catalase test, there was no bubbleformation and so it was confirmed as *Lactobacillus sp*. By glucose test, it could be *Lactobacillus casei* or *Lactobacillus delbrueckii* because of the absence of colourchange which indicates the absence of carbohydrate fermentation. In mannitol test.the organism did not ferment mannitol with the absence of yellow colourformation. So the organism was tentatively identified as *Lactobacillus delbrueckii*. *L.delbrueckii* are Gram positive, facultatively anaerobic,nonmotile andnonspore forming, rod shaped members of the industrially important lactic acidbacteria. Like other lactic acid bacteria, *L.delbrueckii* is acid tolerant, cannotsynthesize porphyrins and possess a strictly fermentative metabolism with lactic acid as the major metabolic end product.[8]

Wastewater from textile dye units is one of the major environmentallyundesirable pollutants.[9]The main causes ofpollution in textile industry are desizing, bleaching, dyeing and printing.[10] So, large amounts of dye stuff are being directly lost to thewastewater and find their way to the environment. Therefore, to solve thisproblem microbial degradation was carried out in the present study.

The major environmental problem of colourants is the removal of dyes fromthe effluent. The untreated effluents of these industries may be highly colouredand thus particularly dangerous when discharged into open water bodies. The dyeconcentration may be much less than 1ppm, but the dye is visible even at lowconcentration.[8]Dyes are carcinogenic, mutagenic, allergenic and toxic in nature. So microbes are employed for dyedecolourisation. In the present study, Congo Red was degraded by employing *L.delbrueckii*. The decolourisation has been observed and it is due to the enzymeproduction and utilization of dye by the organism. Based on the absorption maxima the wavelength was chosen as470 nm for Congo Red.

L.delbrueckii was found to decolourise the dye when grown in minimal medium along with Congo Red in different concentrations like 10, 20, 30, 40, 50, 100, 200, 500 and 1000 ppm. After the inoculation of bacterial culture into the medium containing dye, OD readings were measured after every one hour uptoseven hours.

Short duration treatment of Congo Red employing *L.delbrueckii* in hours shows slight variation in all the concentrations after one hourof treatment. As the time duration increased, there was a decrease in optical densityreadings in 10, 20 and 30 ppm. But, when the time duration increased, no change was observed in 40, 50, 100, 200, 500 and 1000 ppm. For instance, from 1st hour to 4th hourthe OD reading was 0.54 and in 5th hour it was 0.53. The decolourisation activity of the strain is shown in Fig.1.

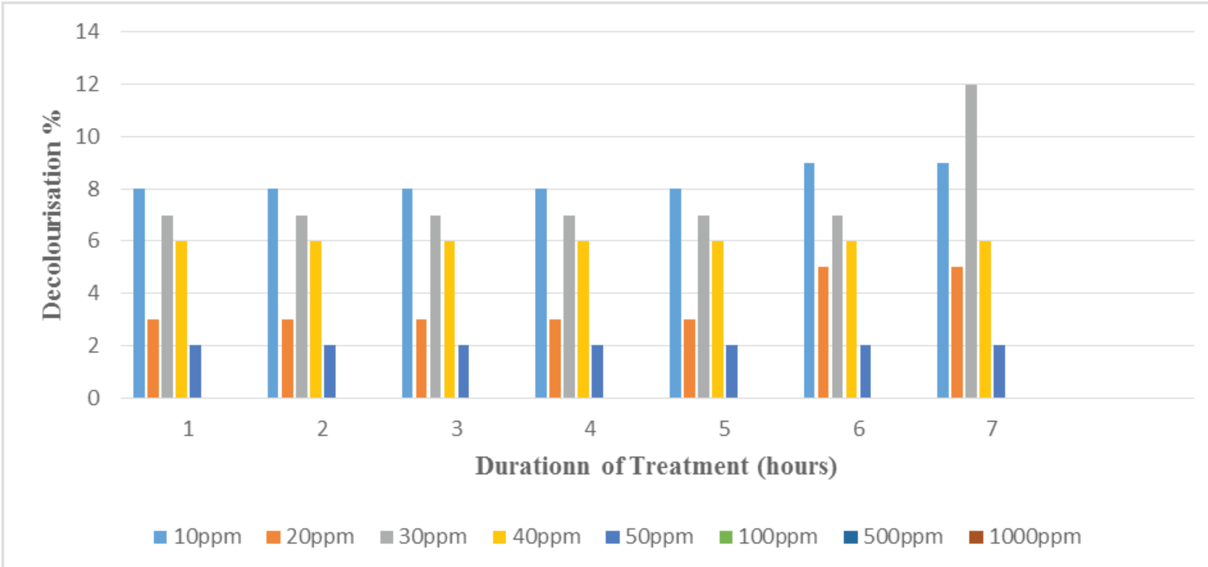

Fig.1. Decolourisation activity of *Lactobacillus delbrueckii* on Congo Red in short duration

Long duration treatment with *L.delbrueckii* showed 100% decolourisation oftheCongoRed in 10 and 20 ppm with maximum of 15 days of incubation. Thepercentage of decolourisation in higher concentrations was from 61.4 to 78.3% whichare shown in Fig.2. Dye decolourisation may take place through dye removal bysimple adsorption of the dye at the cell surface or degradation by the cells. Cellswould become deeply coloured if the dyes were removed by adsorption, but cellsremain colourless if they degrade the dye.[11]In thepresent study, the bacterial strain absorbed the dye and the cells present inthe bottom of the flask had become deeply coloured which indicates thedecolourisation.

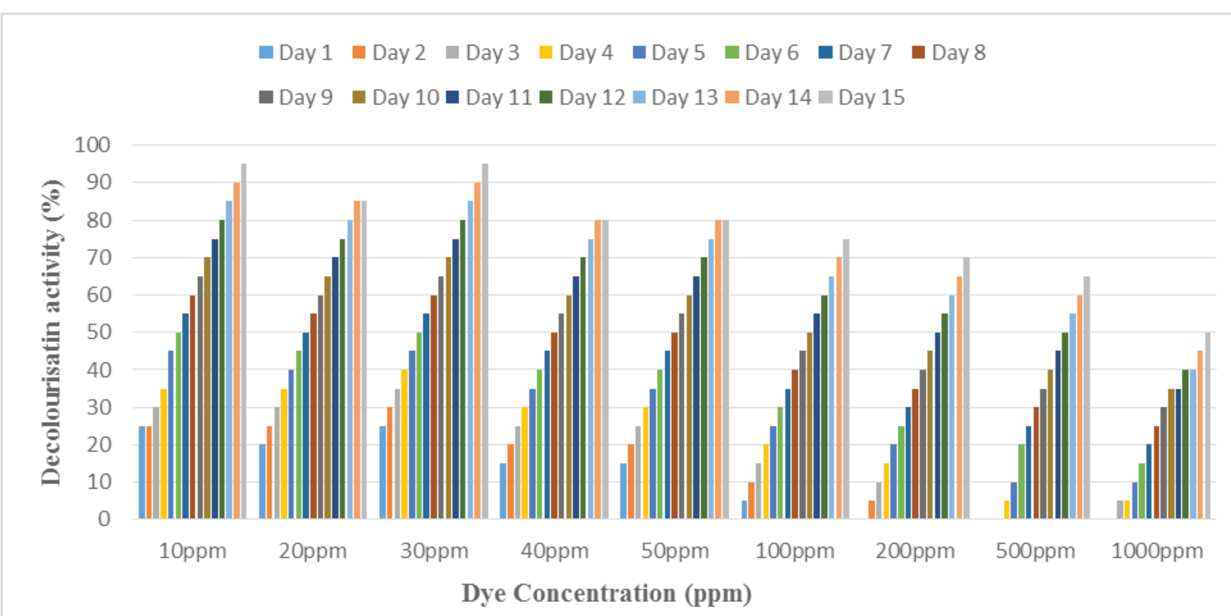

Fig.2. Decolourisation activity of *Lactobacillus delbrueckii*on Congo Red in long duration

Activities ofα-amylase and protease estimated during the decolourisationprocess by the bacterial isolate in dye amended broth shown in Fig.3 and Fig.4. The production of α -amylases in the decolourisation of Congo Redvaried from 0.7 to 1.6 mg/ml after five days of treatment (Fig.3). As the daysincreased, the amylase production decreased. In Congo Red, five days of treatmentshowed maximum protease production ranging from 25 to 250 µg/ml.

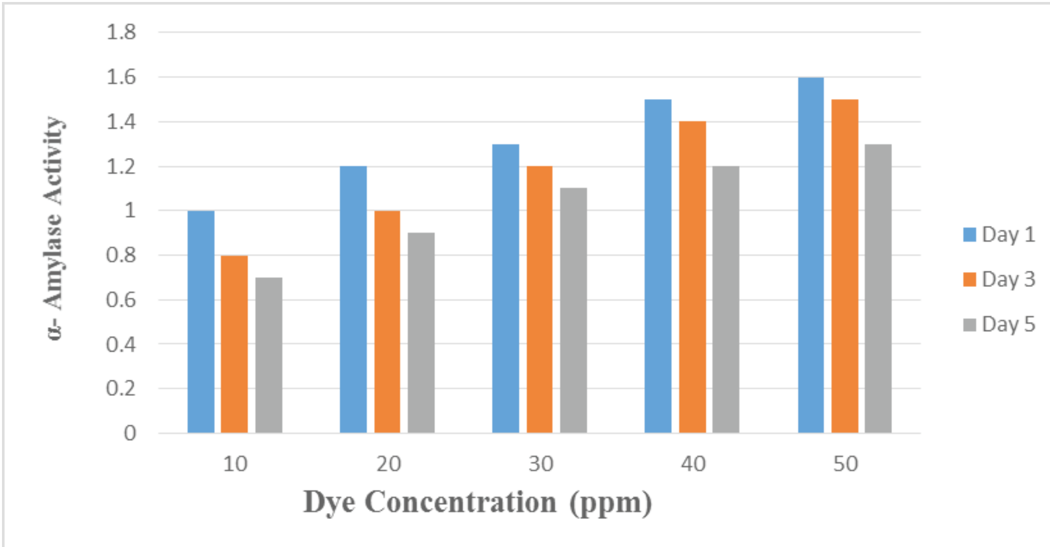

Fig.3α -Amylase activity during decolourisation of Congo Red by *L.delbrueckii*

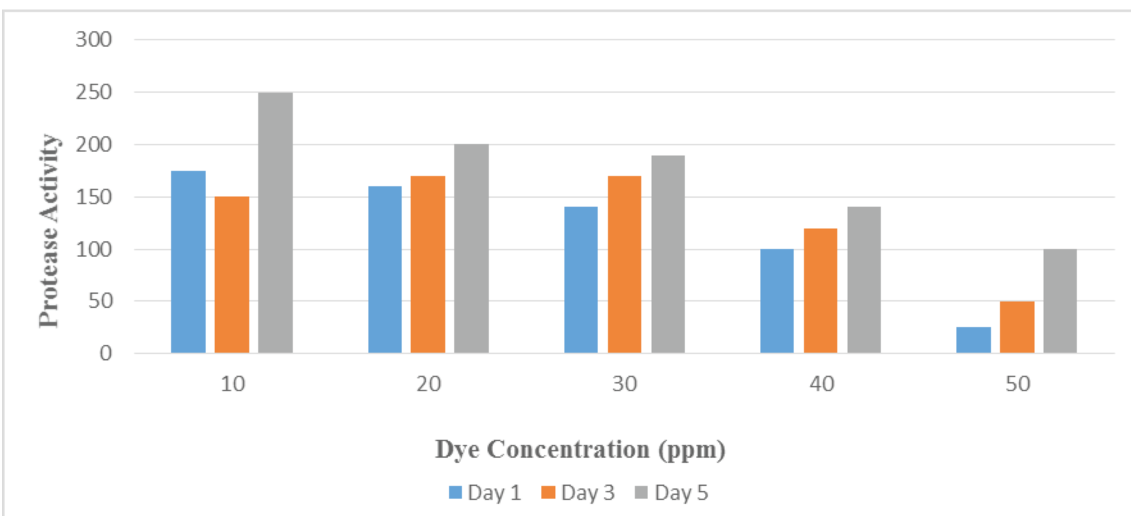

Fig.4. Protease activity during decolourisation of Congo Red by *Lactobacillus delbrueckii*

L.delbrueckii was more efficient in degrading CongoRed. Generally aromatic amines that are not mineralized accumulate in such environments andpose a major threat to human health and environment because of their welldocumented toxic, mutagenic and carcinogenic activity.[7]Although numerous physical and chemical techniques based on coagulationflocculation, precipitation, membrane filtration, ionexchange, electrochemical destruction, photochemical degradation, ozonation and adsorption are availablefor removal of colour from textile wastewater, they are not ideal.[2]

Table 1. Two way analysis of variance: Activity of *Lactobacillus delbrueckii* on the decolourisation activity of Congo Red in short duration and long duration

Duration	Source of Variation	SS	DF	MS	Calculated F value	Table F value at 5% level	Level of significance
Short	Treatment Period	0.030104	6	0.005017	0.941683217	2.294598289	NS
	Dye Concentration	17.2664	8	0.005017	411.1886172	2.138229149	S
Long	Treatment period	648.9853	15	43.26569	1.072265548	1.75049637	NS
	Dye concentration	312.3766	8	39.04707	0.96771439	2.01642714	NS

S- Significance; NS- Not Significant.

Table 1divulges the two way ANOVA results for the decolourisation efficiency of *L.delbrueckii* on Congo Red as a function of treatment period in hoursand dye concentration. In both short term and long term study, the variations due to treatment period and dye concentration are not significant, except for dye concentration during short term study which is significant.

Therefore, biological methods involve the use of microorganisms such as bacteria to turn pollutants into nontoxic and harmless substances to theenvironment. Biodegradation is the most environment friendly as it does notrequire large amounts of energy anddoes not generate toxicsubstances.[12]Decolourisation is a challenging process in the textile industry and a greatpotential for microbial decolourisation system exists for achieving total colourremoval within a few days.Bacteria from local dye environment are easily adapted to the environment as thebacteria originated from the dye contaminated soil of the local environment can develop enzymes for dye decolourisation. Therefore, treatment ofwastewater contaminated with dye using *L.delbrueckii* is an effective method.

Conclusion

Current investigation has confirmed the decolourisation of the Azo dye, Congo Red by the isolate, *L.delbrueckii* under *in vitro* conditions. Thus the study has confirmed the potential of *L.delbrueckii* in the decolourisation of the dye indicating its possible application in treatmentof textile dye effluents.

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