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Decolorization of Textile Dying Mill Effluent by using a Bacterial Strain *Lysinibacillus boronitolerans* CMGS-2

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A B S T R A C T

Water is a essential source, nowadays days the resources of the water and its availability is lacking. Industrial pollutions like textiles, leather, food industries, pharmaceuticals are the major part of pollutants and creating a major problems. Especially textile areas, uses high colorant synthetic chemical dyes, and also recalcitrant, mutagenic in nature. Without treatment these chemicals were leaving to water bodies, the whole ecosystem disturbs, in the form of alteration in the BOD, COD, DO, PH, TDS, and accumulation of heavy metals, and toxic compounds in the water may results in the death of flora and fauna. And also causes effects on humans and became necessity to treat waste water and reuse it and textile dyes were part of it. By these concept we isolated a novel dye degrading bacterial strain, *Lysinibacillus boronitolerans* CMGS-2 has been isolated from the textile dye treatment unit solapur, Maharashtra and identified by 16s rRNA and. It is deposited in the IMTECH, MTCC Chandigarh with MTCC number-12531 showed a promising result in the decolorisation of textile effluents in raw samples and as well in poly urethane foams (PUF).

Introduction

Industrialization is considered to be the key to development in economic terms. However, it is also recognized to be the root problems from environmental perspective. The recognition that environmental pollution is a worldwide threat to public health has given rise to new initiatives for environmental restoration for both economic and ecological reasons. The industrial effluents contain toxic and hazardous pollutants. Earlier for coloring natural colours were using, best example for the natural color is turmeric, a naturally occurring yellow color dye, it is powerful antiseptic and indigo another natural dye gives cooling effect (Siva, 2007). When these natural dyes were replaced by the synthetic dyes, the actual problem of water pollution was started. The discovery of synthetic dye Mauveine by Perkins in 1856 has provided a tool for the production of wide range of dyes that are color fast and come in a wider color range and brighter shades (Zollinger, 1987). It is estimated that approximately dye discharged by textile processing, primarily belongs around class of Reactive Azo dyes (36%), Acid (25%), Direct (15%) dyes and

followed by remaining other classes of dyes (Pandey *et al.,* 2007). Most of the azo dyes are water soluble and readily to absorb through skin contact and inhalation leading to the risk of cancer and allergic reactions, an irritant for the eyes and highly toxic, if inhaled or consumed (Nikulina *et al.,* 1995). Mechanisms of microbial color removal Azo compounds are susceptible to biological degradation under both aerobic and anaerobic conditions (Khehra *et al.,* 2005). In general microbial degradation of azo dyes involves the reductive cleavage of azo bonds (–N=N–) with the help of an azoreductase enzyme under anaerobic conditions, and this involves a transfer of four-electrons (reducing equivalents). This then proceeds through two stages at the azo linkage, and in each stage two electrons are transferred to the azo dye, which acts as a final electron acceptor, resulting in dye decolorization and the formation of colorless solutions (Chang *et al.,* 2000).

The resulting intermediate metabolites (e.g., aromatic amines) are then further degraded aerobically or anaerobically (Chang *et al.,* 2001; Pandey *et al.,* 2007). A major mechanism behind biodegradation of synthetic dyes in microbial systems is because of the biotransformation enzymes (Raghukumar *et al.,* 1996).

It is generally recognized that azo reductases play an important role in bacterial dye decolorization (Kuhad *et al.,* 2004). Among synthetic dyes, azo dyes constitute the largest and most versatile class with the greatest variety of colors (Bafana *et al.,* 2009).

The efficiency of dye degradation of microbes is one of the major criteria in selecting the microorganisms for the bioremediation process therefore more and more worker are interested in isolation of microorganisms from the environment which having capacity to degrade efficiently more number of dyes individually and in mixture at higher concentrations. The use of such microorganisms for the removal of recalcitrant dyes from industrial effluents offers considerable advantages; the process is relatively inexpensive, the running costs are low, and the end products of complete mineralization are not toxic).

Thus, biodegradation is a promising approach for the remediation of recalcitrant synthetic dyes present in the wastewater. With the aim of detoxification we tested our desire organism on textile effluents and results studied by using UV-VIS Spectrophotometer (Table 1 and 2).

Decolorisation study of textile effluents from dying industry unit sample

Sampling of effluent sample

Cloth dying unit effluent (Bright red colored) before dumping was collected and used for the treatment using *L. boronitolerans* CMGS-2.

Treatment process of effluents

Freshly grown isolate *L. boronitolerans* CMGS-2 in the nutrient broth and PUF immobilized CMGS-2 strain selected for the treatment of textile effluents.

Protocol for the treatment of effluent from the dying industry with isolated CMGS-2 strain

Step-1

The effluent collected from cloth dyeing unit was taken distributed 100 mL each in the conical flasks. Out of those first flask kept as control without adding isolated bacterial culture of CMGS-2 and routine observation was done.

Immobilization of the bacterial isolate on poly urethane foam (PUF)

For the treatment of cloth dying unit effluent the bacterial cells of *L. boronitolerans* CMGS-2 immobilized on the polyurethane foam matrix (PUF). In brief the polyurethane foam was cut into approximately 5 mm cubes, washed twice with distilled water and autoclaved at 15 lbs pressure for 20 min and dried.

Then 5 g sterile foam cubes were placed in 100 mL of isolate *L. boronitolerans* CMGS-2 suspensions (9 x 10^9 cfu/mL) contained in 500 mL Erlenmeyer flasks, mixed for 2 hrs with the help of magnetic stirrer, and shaken for 1 hr at 150 rpm.

The conical flask left undistributed for 2 more hours. After removal of medium, a saline solution was used to wash the immobilized foam cubes for further studies (Veena *et al.*, 2015).

Step-2(a)

To the flask containing 100 mL of effluent and 10 mL of freshly grown culture CMGS-2 was added. (b) To the flask containing 100 mL of effluent 5 g of PUF cubes immobilized with CMGS-2 was added.

Step-3 (a)

To a flask containing 100 mL of effluent with 0.1% yeast extract and 10 mL of freshly grown culture isolate of CMGS-2 was added. b) To a 100 mL of effluent containing with 0.1% yeast extract 5 g of PUF cubes immobilized with CMGS-2 was added.

All these flasks were incubated for 48 hrs at 35 °C and reduction of color intensity of effluent was determined by UV-Vis spectrophotometer with respective optical density of dyes. Then calculated the percent decolorization and compared results (Lade *et al.*, 2012; Farhana *et al.*, 2015; Lone *et al.*, 2014).

Decolorisation assay

1 mL of the culture media was withdrawn at different time intervals and was centrifuged at 8000 rpm for 10 min to separate the bacterial cell mass. After that clear supernatant was used to measure the decolorization of dye at UV visible absorbance spectra. Controls without microorganism were always used. The percentage decolorization was calculated as follows: bacterial concentration was 10^6 per mL).

Decolorization (%) = [(Initial Absorbance– Final Absorbance)/Initial Absorbance] X 100

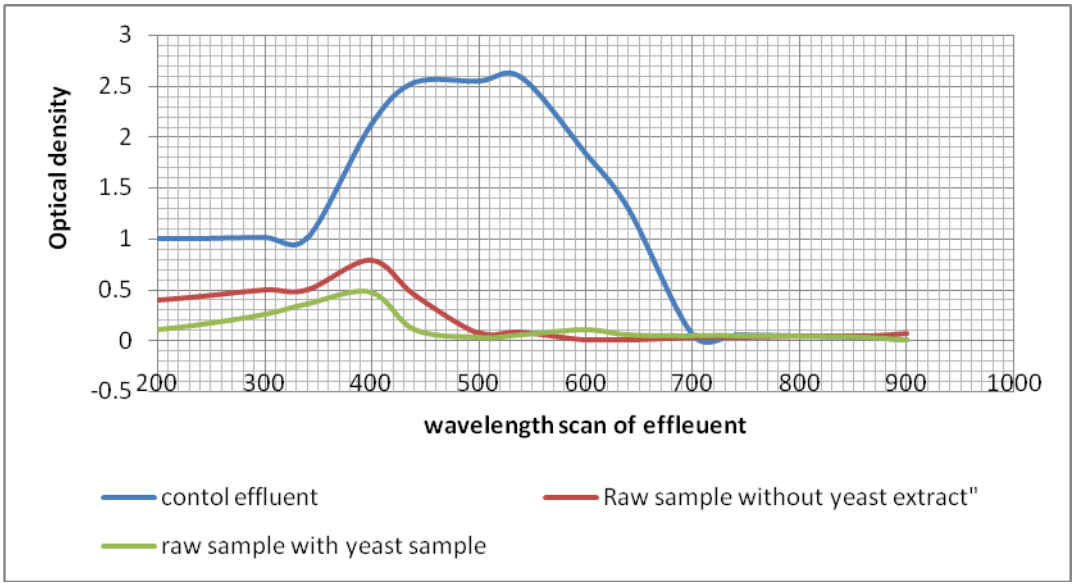
Table.1 Treatment of dying effluent by using grown culture of CMGS-2

Raw Sample (Control)	Raw Sample (Without Yeast Extract)	Raw Sample (With Yeast Extract)
0% (Decolorization)	79.17%	89.39%

Table.2 Decolorisation of dying effluent by using immobilized CMGS-2 on PUF

Raw Sample (Control)	Raw Sample (Without Yeast Extract)	Raw Sample (With Yeast Extract)
0% (Decolorisation)	86.32%	94.3%

Fig.1 Wavelength scan of untreated and treated dying effluents



Results of treatment of dying mill effluents

Effluent sample brought from local cloth dying mill was subjected to decolorization with immobilized and free cells of CMGS-2 following as per (Lone *et al.*, 2014). To the 100 mL of raw effluent flask added 10 mL pre grown inoculum of *Lysinibacillus boronitolerans* CMGS-2 to second flask of that one containing of 0.1% Y.E.(Yeast extract) (Fig. 1). Similarly to the flask containing 100 mL of raw effluent (one with 0.1% Y.E) were added 5 g of puf immobilized with CMGS-2 were incubated at 45° C for 48 hrs. Two flask containing raw effluent (with 0.1% Y.E) without inoculum served as control effluent treated with immobilized on PUF was shown 94.3% of decolorization as compared to free cells of CMGS-2 (89.39%) with yeast extract. However, without Y.E. it was 86.32% and 79.17% respectively.

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