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Biodegradation of Textile Wastewater by Naturally Attenuated *Enterobacter* sp.

N. Sharma*†, H. Bhagwani*, N.Yadav* and D. Chahar**

*Environmental Microbiology and Toxicology Lab, Department of Zoology, Poddar International College, Sector-7, Shipra Path, Mansarovar, Jaipur-302020, India

**Department of Zoology, Shri R. K. Post Graduate College, Bissau, Jhunjhunu-331027, Rajasthan, India

†Corresponding Author: N. Sharma; nehamicrobiologist@gmail.com

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ABSTRACT

The exponential increase in anthropogenic activities has led to the accumulation of xenobiotics into the environment, synthetic dyes being one of the culprits. Noteworthy is the fact that the textile industry utilizes enormous volumes of water for dyeing and printing unit operations thereby generating wastewater proportionately. Taking into consideration, implications of toxic textile effluents, a pilot study was planned to screen for naturally attenuated bacterial isolates capable of degrading textile effluents. Requisite effluent samples were collected from Kelki Printers Co-operative Society Limited, Sanganer, Jaipur and bacterial screening was carried out by bioaccumulation of Remazol Brilliant Blue R (RBRR) (formation of halo around colonies). Of the 19 bacterial isolates obtained, the most promiscuous isolate was biochemically characterized as *Enterobacter* sp. For biodegradative investigations, it was inoculated in sterilized textile effluent and incubated at 37°C for 7 days under agitating conditions. Pre and post bacterial inoculation (1% v/v), Physico-chemical parameters were analysed following standard procedures. A significant ($p<0.05$) lowering of pollution indicators was monitored when contrasted with abiotic control. The present study was aimed to explore the role of naturally attenuated and effluent adapted *Enterobacter* sp. screened from untreated textile effluent based on its colour (RBRR) removal efficacy under *in vitro* conditions. Furthermore, it was also explored for its biodegradative properties to minimize the level of potential pollution indicators through the microcosm approach. This pilot study based on a three-tier approach encompassing bioprospecting, bio enrichment and bioaugmentation plausibly provided insights for enhanced degradation of real dye wastewaters by unlocking the biochemical pathways of adapted microbes.

INTRODUCTION

Attainment of Sustainable Development Goals (SDGs) has become questionable in the current scenario, taking into account the ever-increasing concerns of environmental pollution. Industrial practices have contributed to socio-economic development but lack of sustained industrial practices aggravates bioaccumulation and persistence of toxicants. Synthetic dyes are extensively used by textile industries leading to the generation of enormous volumes of coloured effluents post dyeing and printing process (Sharma et al. 2019). Besides synthetic dyes, effluents are composed of heavy metals, bleaching agents, surfactants and other recalcitrant compounds (Mondal et al. 2017). Mainstream investigations have focused upon evaluation of physico-chemical profile of textile effluents and their potential toxicological implications (Bhatia et al. 2018).

Catering to address toxicity hazards generated by textile effluents, biological interventions utilizing the role of indigenous microbes for biodegradation have been investigated in recent past (Chanwala et al. 2019, Vikrant et al. 2018). Majorly, bacteria have been explored for their metabolic potential to degrade synthetic dyes, heavy metals and chemical surfactants from dye house effluents. Different mechanisms have been explored for microbial mediated dye removal like biosorption (Solis et al. 2012), biodegradation by catalytic enzymes like azoreductase (Ehlfarash et al. 2017), laccase (Mirzadeh et al. 2014), peroxidase (Saroj et al. 2014).

Considering the above-cited facts, we proposed an *in situ* bioremediation study wherein, the role of microbes native to dye house effluent was explored to biodegrade textile effluent based on its dye removal efficacy in synthetic medium.

MATERIALS AND METHODS

Study Area: Sanganer town with co-ordinates (26°49′ to 26°51′N latitude and 75°46′ to 75°51′ longitude) is located within outskirts of Jaipur, famous for its ethnic hues, hand-

made paper and block prints. It houses around 700 small and medium textile units which release toxic effluents into adjoining drains (Tambi 2013).

Sampling: Kelko Printers Co-operative Society Limited, Sanganer, Jaipur (Fig. 1a) was chosen for the collection of requisite samples (Fig. 1b) which were transported and stored following standard procedures (APHA 2000).

Screening and characterisation of indigenous bacteria: Naturally attenuated bacterial strains were isolated from textile effluent and qualitatively screened by plate assay by supplementing Remazol Brilliant Blue R (RBRR) dye (1% w/v) in Nutrient Agar with following composition (Table 1) (Shah 2014). Based on the formation of halo, the most promising strain was biochemically characterized (Cappucino & Shermann 2009).

Table 1: Composition of Nutrient Agar.

S. No	Component	Quantity (g)
1	Beef extract	3
2	NaCl	5
3	Peptone	5
4	Agar	15
5	Remazol Brilliant Blue R	1
6	Distilled Water	1000 mL

Acclimatisation assay: The screened isolate was grown in Nutrient Broth (NB) (composition similar to Nutrient Agar with the exclusion of agar) comprising two sets. In the first set, RBRR was amended (1% w/v) (NBD), while another set was devoid of RBRR and was referred to as negative control (NBND). Both the sets were incubated at 37°C for 24 to 48 hours under agitated conditions (120 rpm) till a desirable ($O.D._{600}$= 0.6) was attained (Sharma et al. 2019).

Biodegradation assay: Heat killed effluent (500 mL) was inoculated with acclimatized bacterial cells (1%w/v) (TEXE). Similarly, 500 mL heat-killed effluent devoid of inoculum (TEXC) was used as abiotic control to obliterate the role of pre-existing micro-flora in a biodegradation study (Bennet et al. 2002) (Fig. 2).

Pre-sterilized textile effluent samples were analysed for the reduction in pollution indicators viz. colour, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), chloride, nitrate and phosphate (APHA 2000). Degradation study was carried out following the protocol devised by Rajeswari et al. (2013). Briefly, aliquots of samples (50 mL) were withdrawn and centrifuged at 4,000

Fig. 1a: Sampling site.

Fig. 1b: Raw textile effluent.

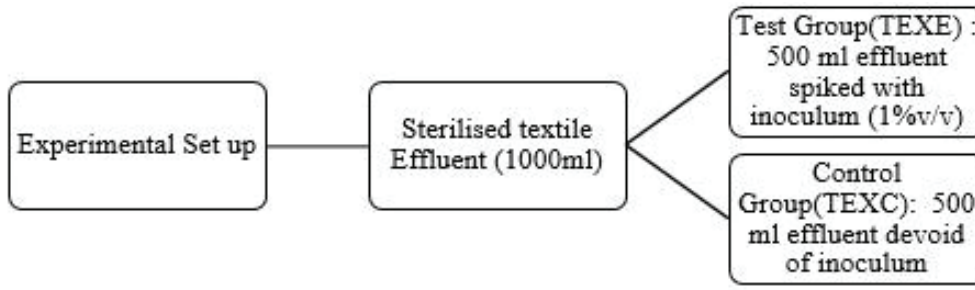

Fig. 2: Experimental set-up for biodegradation study.

rpm (REMI CM-8 Plus) for 15 minutes to remove biomass. Bio-efficacy of bacterial strains to reduce pollution indicators was analysed in Cell-Free Extract (CFE) and expressed as % pollutant removal (Sharma et al. 2014) as shown in the following equation:

$$\%\ Pollutant\ Removal = \frac{TEXC\left(\frac{mg}{L}\right) - TEXE\left(\frac{mg}{L}\right)}{TEXC\left(\frac{mg}{L}\right)} \times 100 \quad \ldots(1)$$

RESULTS AND DISCUSSION

Screening and characterisation of indigenous bacteria: Based on the halo formation, the most potential bacterial isolate was biochemically characterized as *Enterobacter* sp. (Fig. 3a). Formation of halo around bacterial colonies (Fig. 3b) is the phenomenon attributed to uptake of dye by cell membranes and/or cell walls through physical adsorption, electrostatic interaction, ion exchange, chelation and chemical precipitation and the structure remains intact (Ali 2010) concomitant with accumulation of redox-active enzymes or biochemical substances being released into medium during the growth of bacterial cells (Khalid et al. 2008). Screening of micro-organisms from dye contaminated sites has gained momentum in the recent past, the strategy being explored for biodegradation of textile effluents (Lalnunhlimi & Krishnaswamy 2016). Chaube et al. (2010) analysed soil samples collected from Nag Nadi (River) for isolation of dye degrading bacteria and found the isolates to be chromogenic mixture used for differentiation of organism found to be *Proteus* sp., *Pseudomonas* sp. and *Enterococcus* sp.

Acclimatisation assay: Bacterial growth curve was plotted as a measurement of $O.D_{600}$ versus incubation duration (in hours) (Fig. 4).

Enterobacter sp. was grown in nutrient broth amended with RBRR (1% w/v) and growth pattern was observed. Growth was found to be significantly increased ($p<0.05$) under dye induced conditions (NBD) (Sinha et al. 2009) attributing to the utilization of dye as a sole source of carbon and energy (Agarval & Singh 2012). Kuberan et al. (2011) reported a higher concentration of protein in *Listeria sp.* under (Black B) dye induced conditions. Contrary to the above-cited fact, Anjanyulu et al. (2005) have speculated the possibility that upon utilization of pollutants as a sole source of carbon and nitrogen, it is the toxicological manifestation which is apparent thus deteriorating the bacterial growth.

Biodegradation assay: A significant reduction in pollution indicators ($p<0.05$) of pre-sterilized textile effluent was adjudged as bioefficacy of *Enterobacter* sp. when contrasted with abiotic control and was expressed as per cent decrease. Table 2 elucidates the reduction in Physico-chemical properties in the effluent. The values are expressed at Mean ±S.D.

Colour: Our findings suggested, the dark blue colour of the effluent is attributed to excessive usage of anthraquinone dyes, Remazol Brilliant Blue R (RBRR) being commonly used for dyeing of cotton fabrics (Velayutham et al. 2018). Post bacterial treatment, 57.3% colour was removed owing to bacterial biosorption which is accumulation of chemicals and dyes by microbial mass (Bras et al. 2001).

pH: The pH of textile effluent was found to be 12 which is reportedly found to be associated with the use of bleaching agents and chemicals like sodium hypochlorite, sodium hydroxide, sodium phosphate and surfactants (Paul et al.

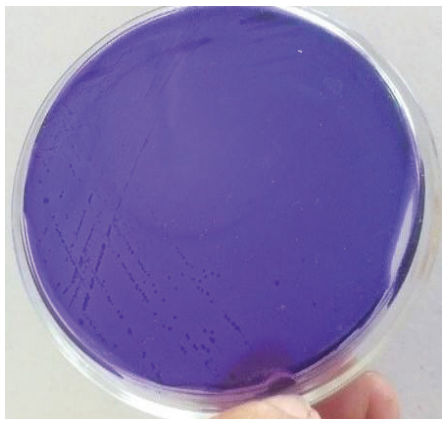

Fig. 3a: Pure culture of *Enterbacter* sp.

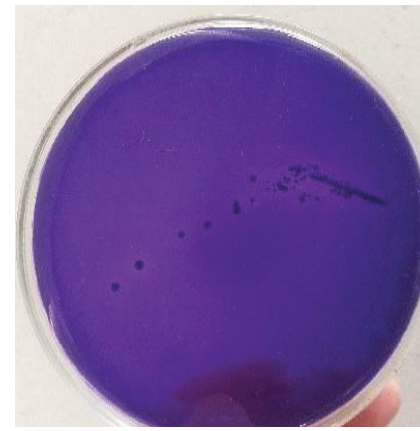

Fig. 3b: Halo around bacterial colonies.

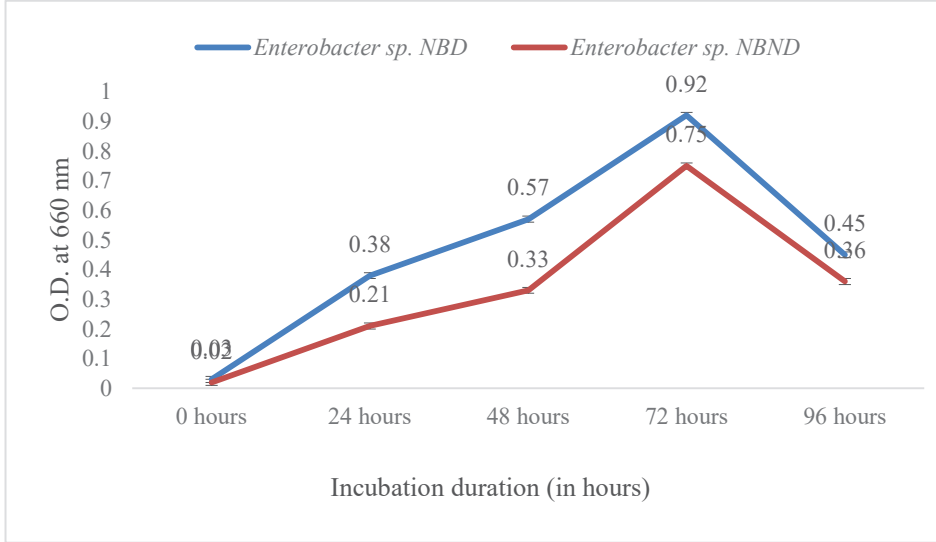

Fig. 4: Bacterial growth curve under dye induced and uninduced conditions.

2012). A similar trend (11.3±1.6) in pH of textile effluent was reported in our previous study (Sharma et al. 2013).

BOD: It is estimated as the amount of oxygen required for oxidation of organic matter by microbes. Significantly, the reduction of 54.8% (130±0.28 mg/L) was observed. High load of organic and inorganic constituents contributes to depletion in dissolved oxygen (DO) and an increase in BOD (Garg & Kaushik 2008).

COD: It determines the amount of chemically oxidizable organic matter. We observed a reduction of 61.1% (270±0.36 mg/L) in the bacterially treated effluent. Previously, we explored the effect of different inoculum concentration of monocultures and bacterial consortium for COD reduction in textile effluents (Sharma et al. 2014). Sur & Mukhopadhya (2017), have explored the potential of *Pseudomonas aureofaciens* and *Escherichia coli* using three-phase fluidized bioreactor for COD reduction in textile effluent.

Nitrate: Our findings reported a reduction of 16% (11.5±0.06 mg/L) owing to bio×efficacy of *Enterobacter* sp. Saharimoghaddam et al. (2018) explored the combinatorial effect of Gambusia fish and *Phragmites australis* in constructed wetlands for nitrate reduction in textile effluent. The biological denitrification is recommended for the removal of relatively low concentration of nitrogen components and it is operated by the so-called denitrifying bacteria in anoxic conditions, where they use nitrates as electron acceptors during their respiratory process in the place of the oxygen (Sharma & Dwivedi 2017).

Table 2: Reduction in Physico-chemical attributes of textile effluent post bacterial treatment.

Pollution indicators	Units	TEXC	TEXE	% Pollutant Removal $= \frac{TEXC\ \left(\frac{mg}{L}\right). - TEXE\left(\frac{mg}{L}\right)}{TEXC\left(\frac{mg}{L}\right)} \times 100$
Colour	-	Dark blue	Light blue	57.3
pH	-	12±0.1	8±0.07	33.3
BOD	mg/L	278±0.4	130±0.28	54.8
COD	mg/L	670±0.32	270±0.36	61.1
Nitrate	mg/L	13.7±0.06	11.5±0.06	16.0
Phosphate	mg/L	13.9±0.03	9.5±0.04	10.5

Phosphate: Different types of chemicals used in unit processes and unit operations contribute to high phosphate levels (Metcalf & Eddy 2003). Post bacterial inoculation, 10.5 % (9.5±0.04 mg/L) reduction was observed. Enhanced Biological Phosphate Removal (EPBR) has been investigated in industrial wastewaters, the fact based on extrapolation of bacterial metabolism leading to biomineralization of phosphate (Sharma 2018).

CONCLUSION

Textile effluents are characterized by the presence of synthetic colourants, heavy metals, bleaching agents, chlorinated compounds and other xenobiotic compounds significantly contributing to excessive water pollution. Microbes adapted to a harsh environment have been known to degrade and mineralize toxic components of effluents. The present study was aimed to explore the role of naturally attenuated and effluent adapted *Enterobacter* sp. screened from untreated textile effluent based on its colour (RBRR) removal efficacy under *in vitro* conditions. Furthermore, it was also explored for its biodegradative properties to minimize the level of potential pollution indicators through the microcosm approach. This pilot study based on a three-tier approach encompassing bioprospecting, bioenrichment and bioaugmentation plausibly provided insights for enhanced degradation of real dye wastewaters by unlocking the biochemical pathways of *Enterobacter* sp. which led to biodegradation. Further, our lab is focused on developing fungal-bacterial consortium for *in situ* bioremediation models.

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