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RESEARCH ARTICLE

DEGRADATION POTENTIAL OF *NOCARDIA SPP. AND E. COLI* AGAINST SOME COMMONLY USED DYES

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ABSTRACT

Different native bacterial strains were isolated from the dye effluent and were acclimatized and screened for the degradation experiments against five commonly used dyes viz. Methyl Red, Methyl Orange, Erichrome Black, Crystal Violet and Malachite Green. RGL and MRL values for these bacterial strains against the dyes were evaluated by keeping them on solid culture media or Dye Modified Media (DMM). The relative decolorization potential of bacterial strains was determined by growing them in liquid media and its modifications. From the screening experiments several bacterial isolate strains emerged as 'Degrader strains' that possess a good deal as they displayed good values of MRL and RGL against various dyes in DMM. It was found that these degrader strains were *Nocardia spp. and E. coli* that displayed a good deal of decolorization against all the dyes tested. Best results were obtained for *Nocardia spp.* followed by *E. coli* against all the tested dyes. Most accessible dyes for decolorization by these bacterial strains were Erichrome Black and Methyl Orange. *Nocardia spp.* displayed a maximum decolorization percentage of 54% against Methyl Orange and 51% against Methyl Red dye.

INTRODUCTION

The expansion of worldwide textile industry has led to an equivalent expansion in the use of such synthetic dyestuffs, resulting in a rise in environmental pollution due to the contamination of wastewater with these dyestuffs (Pandey *et al*., 2007; Saratele *et al*., 2011). Characteristics of the waste water from textile industries vary depending on the process employed (Mohan *et al*., 2002). Accordingly wastewater generated from of the operations in wet processing such as desiring, scouring, bleaching, mercerizing, dyeing, printing and finishing differ considerably (Gupta *et al*., 2003; Ponraj *et al*., 2011). The concentration of dye contained in the effluent varies between 10-200 mg/ml depending on the dyeing process (Kumar *et al*., 2006). Dyes used in the textile industry are difficult to remove by conventional waste water treatment methods since they are stable to light and oxidizing agents and are resistant to aerobic digestion. Presence of carcinogens has also been reported in combined waste water of dyeing and printing units (Robinson *et al*., 2001). The slow rate of decomposition of dyes present in waste water necessitates treatment methods to accelerate the process (Vandevivere *et al*., 1998). The methods employed for alleviating the environmental problems caused by the textile dye effluent include physical, chemical and biological treatment processes. Biological methods of removal involve use of microorganisms such as bacteria and fungi to convert the pollutants into nontoxic harmless substances. Biological processes convert organic compounds to water and carbon dioxide, have low cost sustainable and are easy to use (Chang and Kuo, 2000). Microbial degradation and decolorization of dyes have received much attention from the viewpoint of treating industrial wastewater containing dyes (Salar *et al*., 2012). The effect of various concentrations of dye on decolourization performance by using the isolated bacterial strains was studied (Humnabadkar *et al*., 2008, Mohan *et al*., 1999).

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Study area

The study of screening of native bacterial stains and their evaluation for dye degradation potential was conducted at Tonk district, which is located in north-eastern part of the Rajasthan state between 75°07' to 76°19' east longitude and 25°41' to 26°34' north latitude.

MATERIALS AND METHODS

Decolorization by bacterial strains

Screening of various indigenous bacterial isolates for the decolorization of dyes under different experimental conditions

Different bacterial strains were isolated from the dye effluent and were initially serially diluted and sub cultured. These unidentified strains were acclimatized and screened for the

higher concentration of dyes by the adaptation experiments and by the degradation experiments respectively by keeping them on solid culture media of increasing carpet dye concentrations. Then the relative decolorization potential of bacterial strains was determined by growing them in liquid media and its modifications at 37^0C for 8 days.

Assessment of dye biodegradation potential by bacterial species Isolation and identification of bacteria from the dye waste water and soil

Various samples were collected from the selected sites of study area A and B. These samples were then serially diluted in sterile distilled water and plated onto nutrient agar plates and incubated for 48 hours at 30^0C. Discrete bacterial strains were initially grouped on the basis of gram staining and morphological characteristics such as motility and colony forms. Bacterial colonies were then picked and sub cultured on agar plates and subjected to further biochemical tests for identification according to Bergey's manual of determinative bacteriology (9^{th} edition). The different biochemical tests performed include, catalase test, oxidase test, spore staining, starch hydrolysis, citrate utilization test, methyl red vogas prokaver test (MR-VP), nitrate reduction test, gelatin liquefaction test, triple sugar iron test for lactose/glucose fermentation, H_2S production, indole production test and urease test.

liquid media was also supplemented with 0.5% yeast extract in all the DMM preparations to enhance the rate of decolorization. The dyes were added to the liquid DMM supplemented with 0.5% yeast extract and all the culture preparations in triplicate were kept at 37^0C for 8 days under static conditions

Observation and Results

Selection of degrader-strains is based upon the concept that a strain was considered better if it decolorized the dyes. The decolorization was monitored by the Zone of decolorization on solid media, and confirmed by the spectrophotometric analysis done in the liquid nutrient media. Two bacterial stains or species stamped their presence by creating a good zone of decolorization among the screened bacterial strains and these were- *Nocardia spp. and E. coli.* These bacterial species displayed a good deal of decolorization against all five tested dyes (Table 2). Best results were obtained for *Nocardia spp.* followed by *E. coli.* Most accessible dyes for decolorization by these bacterial strains were Erichrome Black and Methyl Orange. *Nocardia spp.*displayed a decolorization percentage of 54% for Methyl Orange and 51% against Methyl Red and Erichrome Black dyes as well as 49% and 39% for Crystal Violet and Malachite Green dyes respectively. While the second tested bacterial degrader strain *E.coli* displayed a decolorization percentage of 59% against Erichrome Black and 46% against Methyl Red dyes.

Table 1. MRL and RGL values for indigenous bacterial strains against all carpet dyes in solid DMM media

S. No.	Isolated bacterial strain	Carpet dye									
		Methyl Red		Methyl Orange		Crystal Violet		Erichrome Black		Malachite Green	
		MRL (mg/L)	RGL (mg/L)	MRL (mg/L)	RGL (mg/L)	MRL (mg/L)	RGL (mg/L)	MRL (mg/L)	RGL (mg/L)	MRL (mg/L)	RGL (mg/L)
1.	*Nocardia spp.*	310	200	300	190	280	150	270	140	220	100
2.	*E.coli*	280	190	280	170	250	120	290	180	200	90

Table 2. Decolorization percentage of bacterial strains against carpet dyes in liquid DMM media

Name of the dye	Decolorization percentage by Isolated Bacterial strains	
	Nocardia spp.	*E.coli*
Methyl Red	51	46
Methyl Orange	54	45
Crystal Violet	49	45
Erichrome Black	51	59
Malachite Green	39	32

We inoculate bacterial strains on solid culture media of DMM (Dye Modified Media) that contains 15% of agar (nutrient agar) in serially increasing concentration of all the carpet dyes tested and for this several concentrations of culture variations were prepared in the lab in which the dye concentration was kept between 10-1000 mg l^{-1}. The purpose of this variable concentration gradient was to just assess the maximum resistance level (MRL) and rich growth limit (RGL) of the respective strain tested against all the dyes. These values are actually the indication of the dye tolerance limit hence the degrader potential of given bacterial strain (Table 1). All the culture concentration variants were made in a set of 9 for each dye [8 for each bacterial strain + 1 control preparation]. Control preparation culture contains 15% agar + respective bacterial inoculation (without addition of dye) only. Now it is very crucial to examine the exact degradation potential of these carpet dyes in the liquid media of DMM. For this purpose the

A 45% decolorization percentage was noticed against Methyl Orange and Crystal Violet both, while a minimum decolorization percentage was shown against Malachite Green dyes, that was 32%.

DISCUSSION

As in the present study, the decolorization potential of two native strains of bacteria was revealed and these three degrader bacterial strains were- *Nocardia spp.* and *E.coli.* The bacterial strain *Nocardia spp*., got noticed in the reports, of Alexander (1994) and Azmi *et al.* (1998), suggesting it's decolorization role in the textile dyes, the results are similar with the reports of Yatome *et al*. (1993). In another study, it was revealed that *Nocardia atlantica* caused almost complete decolourization of Blue FNR and Red FNR, while at least 80% of other dyes tested. This study thus reveals that some bacteria inhabit in

textile effluent whereby utilize the dyes as their source of energy and nutrition, and imply their importance in treatment of industrial effluents. Similarly in the present study the decolorization potential of *Nocardia spp*., was reported in the decolorization of Methyl Red, Methyl Orange, Crystal Violet, Erichrome Black and Malachite Green. Decolorization capacity of *E.coli* has already been reported by Chang *et al.* (2000) Isik and Sponza (2003); Ghanem *et al.* (2011). The results of the present study also confirmed role of *E.coli* in the decolorization of carpet dyes.

As in the present study, the utilized mode of decolorization by bacterial species is primarily static, therefore anaerobic, In earlier studies also, the efficacy of various anaerobic treatment applications for the treatment and degradation of a wide variety of synthetic dyes has been demonstrated (Delee *et al.*, 1998). Many efforts have been devoted to the study of the influence of various technologies on the decomposition rate of dyes and the effect of the presence of other compounds in the media (Forgacs *et al.,* 2004). Bacterial decolorization is normally faster than fungal decolorization (Kalyani *et al.,* 2009). It is well known that bacteria degrade azo dyes reductively under anaerobic conditions into colorless aromatic amines (Ali *et al.* 2010). These aromatic amines formed during anaerobic cleavage of the azo dyes, could be further degraded in an aerobic treatment system (Kuhad *et al.,* 2004; Zee and Villaverde, 2005). The degradation rate of the bacterial strains and species depends on the types of bacterial species and the type of dyes used along with the set physicochemical parameters on which the degree of decolorization depends.

Therefore it is concluded from the present study that the drained dye waste water should be treated with the indigenous bacterial strains under optimum conditions so that the toxic recalcitrant dyes should be converted down into colourless, less harmful or harmless products that have little or no disastrous effect on the local microbial fauna and flora that are inhabitating the sinks or local water reservoirs of the area.

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