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Ecofriendly Bioremediation of Acid Orange – An Electron Deficient Xenobiotic Chromogen by Haloalkaliphilic Bacterial Consortium TVU- AO 64

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Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. Halophilic bacterial isolates, TVU-AO6 and TVU-AO4 were found to be the efficient strains exhibiting maximum decolorization efficiency towards Acid Orange and they were selected for the development of consortium TVU-AO 64. On optimization studies, the halophilic bacterial consortium exhibited maximum decolorization efficiency at 35°C, Slightly alkaline pH 8 and static condition after 24 h of incubation in the presence of sucrose and yeast extract as suitable carbon and nitrogen sources respectively.

Introduction

The first synthetic dye, Mauevin was manufactured in the year 1856 since then more than 100,000 new synthetic dyes have been generated (Nigam *et al.*, 2001). Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade (Fu and Viraraghavan, 2002). The textile industry accounts for two-thirds of the total dye stuff market. Azo dyes, the largest chemical class of dyes with the greatest variety of colors, have been used extensively for textile, dyeing and paper painting (Fang *et al.*, 2004; Vijayanand and Hemapriya, 2013). Besides the unpleasant appearance of the dye-polluted wastewater, the disposal of these

waters into receiving waters causes damage to the environment as they significantly affect the photosynthetic activity in aquatic life due to reduced light penetration and may also be toxic to some aquatic organisms due to the presence of metals, chlorides etc. and the breakdown products of dyes in them (Cetin and Donmez, 2006; Fan *et al.*, 2009).

Dye pollutants from the textile industry are a major source of environmental contamination. Considerable amount of these pollutants draw off during the textile dyeing and finishing operations. Now-a-days, colored water is unattractive and generates more and more complaints. Dyes have obtained notoriety as

hazardous substances, because most of them are toxic and persistent in the environment (Schrack *et al.*, 2007). Remediation of dye waste by several physical and chemical methods including precipitation, flocculation, coagulation, adsorption and wet oxidation has been suggested but none of them has been widely used due to their certain inherent limitations (Bechtold *et al.*, 2006; Vijayanand and Hemapriya, 2014). All these methods have different color removal capabilities, capital costs and operating speed. So the need of efficient and economic processes to treat these effluents increases (Khelifi *et al.*, 2009). Despite the existence of a variety of chemical and physical treatment processes, bioremediation of textile industry effluent is still seen as an attractive solution due to its reputation as a low cost, eco-friendly and public-acceptable treatment technology (Lucas *et al.*, 2008).

Thus, the main objective of this study was to observe microbial decolorization and biodegradation of Acid Orange-10 by bacterial consortium TVU-357. The optimal cultural conditions for maximizing bacterial biomass and decolorization were investigated.

Materials and Methods

Dye stuff used

Synthetic textile azo dye, Acid Orange used for this study was procured from a local textile dyeing unit. Stock solution was prepared by dissolving 1 g of Acid Orange in 100 ml distilled water. All chemicals used were of the highest purity available and of an analytical grade.

Isolation and screening of bacterial strains decolorizing acid orange

The effluent samples were serially diluted and spread over Halophilic agar medium

containing 50 ppm of Acid Orange. pH was adjusted to 7.0 before autoclaving and incubated at 37 °C for 5 days. Colonies surrounded by halo (decolorized) zones were picked and streaked on halophilic agar plates containing Acid Orange. The plates were re-incubated at 37°C for 3 days to confirm their abilities to decolorize Acid Orange. Different colonies of dye decolorizing bacteria were picked and re-streaked several times to obtain pure cultures.

Decolorization assay

A loopful of bacterial culture was inoculated in Erlenmeyer flask containing 100 ml of nutrient broth and incubated at 150 rpm at 37°C for 24 h. Then, 1 ml of 24 h old culture of the bacterial strain was inoculated in 100 ml of nutrient broth containing 50 ppm of Acid Orange and re-incubated at 37°C till complete decolorization occurs. Suitable control without any inoculum was also run along with experimental flasks. 1.0 ml of sample was withdrawn every 12 h and centrifuged at 10,000 rpm for 15 min. Decolorization extent was determined by measuring the absorbance of the culture supernatant at 510 nm using UV-visible spectrophotometer, according to Shyamala *et al.*, (2014).

Decolorization efficiency (%) = $\frac{\text{Dye (i)} - \text{Dye (r)}}{\text{Dye (i)}} \times 100$

Where, Dye (i) refers to the initial dye concentration, Dye (r) refers to the residual dye concentration. Decolorization experiments were performed in triplicates.

Development of halophilic bacterial consortium TVU-AO 64

Bacterial strains TVU-6 and TVU-4 that showed maximum decolorization percentage on Acid Orange were aerobically co-cultured

in halophilic broth to develop an efficient Halophilic consortium. The pH was adjusted to 7.0. For frequent use, the culture was maintained by transfer to a fresh medium at 24 h intervals.

Optimization of culture conditions for decolorization of acid orange by TVU-AO 64

Effect of temperature, pH, agitation rates and dye concentration

The effect of temperature, pH, agitation rates and dye concentration on dye decolorizing ability of Halophilic Bacterial Consortium TVU-AO 64 was studied. This was carried out by incubating the bacterial strain at different temperatures (20-50°C), different pH values of the medium (pH 4.0-10.0), different agitation speeds (0-200 rpm) and various dye concentrations (200 -1000 ppm).

Effect of carbon and nitrogen sources

The effect of various soluble carbon sources (1% w/v) (glucose, sucrose, lactose, maltose, starch) and nitrogen sources (tryptone, beef extract, peptone, yeast extract and meat extract) on dye decolorization extent of Acid Orange by halophilic bacterial consortium TVU-AO 64, was investigated.

Results and Discussion

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. Textile effluents are of global concern because they color the drains and ultimately the receiving water bodies (Olukanni *et al.*, 2006; Hemapriya and Vijayanand, 2013). An important element in guiding the direction and development of

decolorization technology should logically depend upon a sound scientific knowledge, which undoubtedly warrants for further research. In view of the need for a technically and economically satisfying treatment technology, a flurry of emerging technologies are being proposed and tested at different stages of commercialization. Broader validation of these new technologies and integration of different methods in the current treatment schemes will most likely in the near future, render these both efficient and economically viable (Goncalves *et al.*, 2000).

Isolation and screening of bacterial strains decolorizing acid orange

Ten different bacterial isolates, designated as TVU-AO1 to TVU-AO10 that was capable of decolorizing Acid Orange were isolated from different effluent samples. Among the above mentioned isolates, TVU-AO6 and TVU-AO4 isolates were found to be the efficient bacterial strains exhibiting maximum decolorization efficiency and were used for the development of consortium TVU-AO 64.

Optimization of culture conditions for maximizing decolorizing ability of TVU-AO 64

Effect of incubation time

Color removal capability of the consortium was found to be dependent on the growth of the bacteria. Decolorization process started after 4 h of incubation, gradually increased and reached their highest levels after 24 h of incubation (Fig 1). Hence, the optimal culture conditions for decolorizing ability of the consortium TVU-AO 64 were carried out at 24 h of incubation. In contrast, *Pseudomonas* sp. and *Bacillus* sp. showed maximum dye decolorization efficiency of 89% on Orange 3R at the end of 144 h incubation under optimum conditions (Ponraj *et al.*, 2011).

Effect of temperature

The results shown in Fig 2 has revealed that color removal efficiency of the bacterial consortium TVU-AO 64 reached highest levels between 30-40°C, with optimum being 35°C after 24 h of incubation, indicating that the decolorization process was directly proportional to the increase in incubation temperature. Decolorization activity was significantly suppressed at temperatures more than 40°C, which might be due to the loss of cell viability or denaturation of the enzymes responsible for the decolorization at elevated temperatures (Cetin and Donmez, 2006; Carvalho *et al.*, 2008).

Effect of pH

Dye decolorization efficiency of the bacterial consortium TVU-AO 64 was detected over a broad range of pH (5.0-9.0), with optimum decolorization being exhibited at slightly alkaline pH (8.0). However, incubation at neutral pH (7.0) slightly reduced the dye decolorization efficiency of the bacterial

consortium TVU-AO 64 (Fig 3). Similarly, optimal pH values for the decolorization of Reactive Red RB by a microbial consortium was found to be 8.0 (Cetin and Donmez, 2006). In contrast, pure cultures of *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168 showed maximum decolorization efficiency at neutral pH (7.0) (Saratale *et al.*, 2009).

Effect of dye concentration

The influence of different dye concentrations (200 - 1000 ppm) were studied on decolorization ability of the bacterial consortium TVU-AO 64. The result shown in Fig 4 has revealed that the decolorization rate increased linearly with the increase in initial dye concentration upto 100 ppm. As the dye concentration increased in the culture medium, a decline in color removal was attained. At high concentration (1000 ppm), Acid Orange greatly suppressed decolorization ability. Similar result was reported by Hemapriya *et al.*, (2010).

Table.1 Effect of Various Carbon sources on decolorizing efficiency of TVU- AO 64

| Sl. No | Carbon Source (1% W/V) | Decolorization Efficiency |
|--------|------------------------|---------------------------|
| 1 | Glucose | 20.42 % |
| 2 | Sucrose | 93.76 % |
| 3 | Lactose | 62.76 % |
| 4 | Maltose | 54.24 % |
| 5 | Starch | 66.08 % |

Table.2 Effect of Various Nitrogen sources on decolorizing efficiency of TVU- AO 64

| Sl. No | Nitrogen Source (1% W/V) | Decolorization Efficiency |
|--------|--------------------------|---------------------------|
| 1 | Tryptone | 80.22 % |
| 2 | Beef Extract | 33.46 % |
| 3 | Peptone | 82.64 % |
| 4 | Yeast Extract | 94.64 % |
| 5 | Meat Extract | 36.24 % |

Fig.1 Effect of Incubation Time on decolorization efficiency of TVU- AO 64

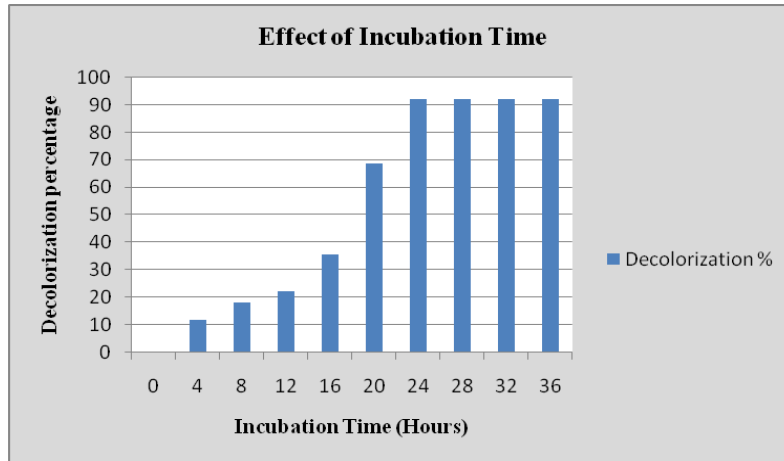


Fig.2 Effect of Incubation Temperature on decolorization efficiency of TVU- AO 64

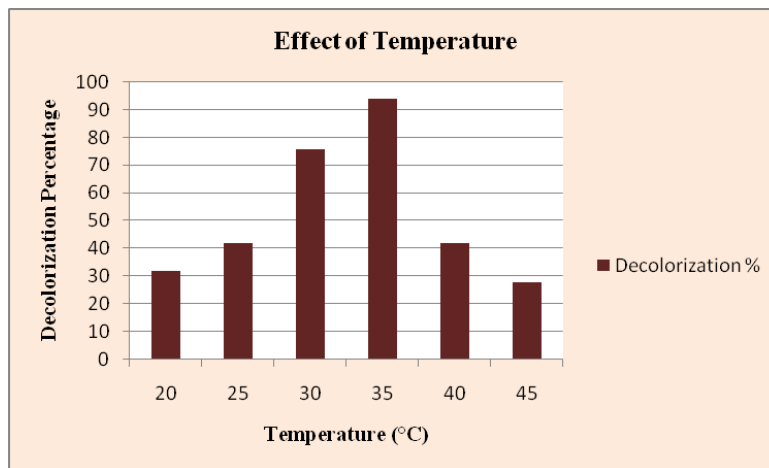


Fig.3 Effect of pH on decolorization efficiency of TVU- AO 64

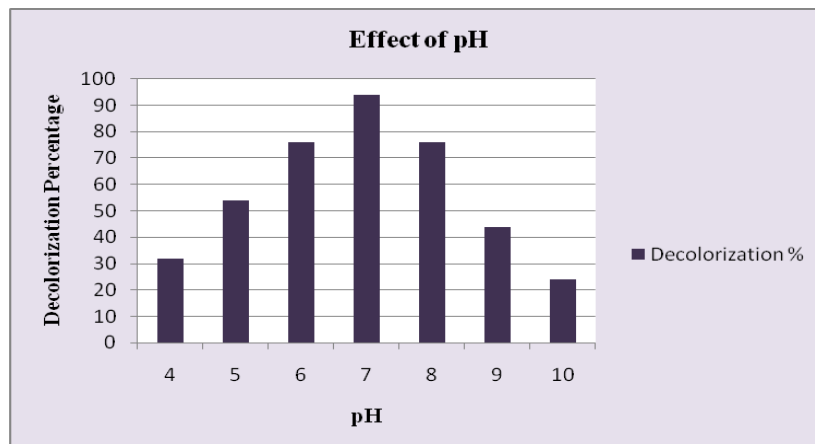


Fig.4 Effect of Dye concentration on decolorization efficiency of TVU- AO 64

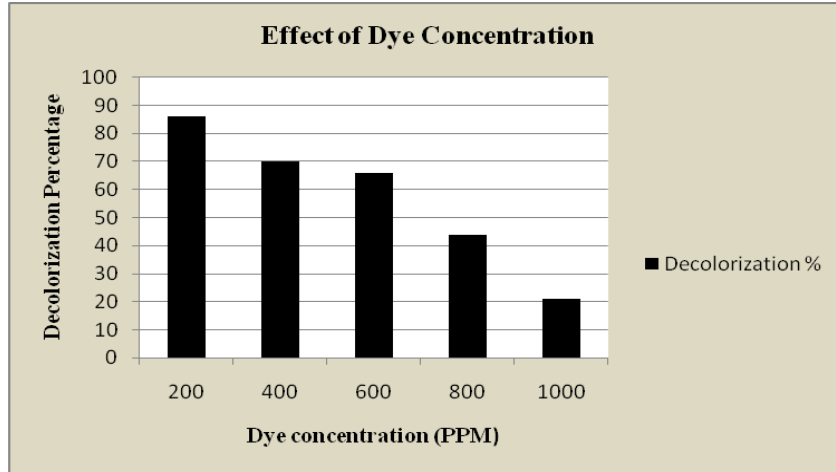


Fig.5 Effect of Agitation Speed on decolorization efficiency of TVU- AO 64

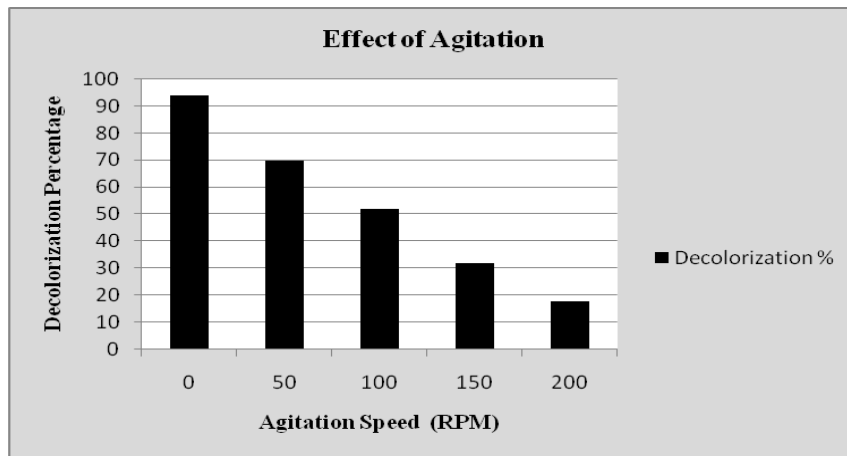
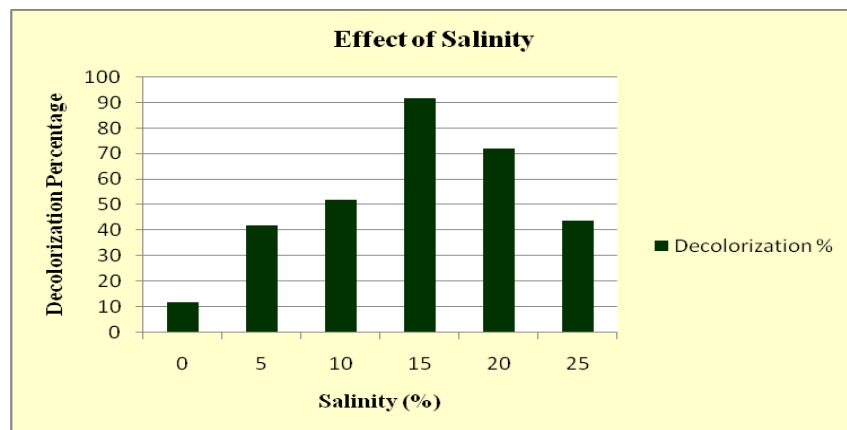


Fig.6 Effect of Salinity on decolorization efficiency of TVU- AO 64



Effect of agitation

The dye decolorization ability of the bacterial consortium TVU-AO 64 was found to be greatly decreased with increases in agitation speeds. At 200 rpm, the decolorization ability of the bacterial consortium was greatly inhibited (Fig 5). Static conditions proved to be effective in maximizing decolorization percentage of the consortium. Similarly, Oxygen inhibition on enzymatic reduction of azo dyes for bacterial species containing azoreductase system has also been reported (Chung and Stevans, 1993).

Effect of carbon sources

In a preliminary study, various soluble carbon sources were used to replace the original carbon source in the growth medium. The results obtained in Table 1 showed that the bacterial consortium was able to utilize most of the carbon sources tested, except Glucose, whereas sucrose instigated maximum decolorization efficiency after 24 h of incubation. Glucose repression often causes inhibition of the transcription of cyclic-AMP-dependent genes, which may include genes crucial to azo dye decolorization, such as azoreductase genes (White, 1995; Chang *et al.*, 2001).

Effect of nitrogen sources

The effect of various nitrogen sources organic nitrogen sources (tryptone, beef extract, peptone, yeast extract and meat extract) were investigated after 24 h of incubation. Among the various organic nitrogen sources tested, yeast extract was found to be the superior source in maximizing decolorizing ability of the consortium (Table 2). Similar result was reported by Deng *et al.*, (2008). The metabolism of yeast extract is considered to be essential for the regeneration of NADH that acts as electron donor for the reduction of azo bonds (Carliell *et al.*, 1995).

Effect of salinity

The effect of various NaCl concentrations (0-25 % W/V) on decolorization efficiency of TVU-AO 64 was investigated, after 24 h incubation. The consortium was able to decolorize Acid Orange in a broad range of NaCl concentrations (5-20 %) with optimum at 15 % (Fig 6). However, it required minimum 5 % NaCl for decolorization and below which no decolorization was observed. Similarly, Asad *et al.*, (2007) reported the decolorization of textile azo dyes in the presence of Halophilic bacterial isolate, *Halomonas* sp.

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