Available online at www.ewijst.org

ISSN: 0975-7112 (Print)
ISSN: 0975-7120 (Online)

Environ. We Int. J. Sci. Tech. 11 (2016) 09-16

Environment & We
An International
Journal of Science
& Technology

Effects of Co-substrates on Biodegradation of Azo Dyes: A case study of Marine Bacteria

V. Sreelekshmi, M. Lekshmi and Ayona Jayadev*
Department of Environmental Sciences, All Saints' College, Thiruvananthapuram, Kerala, India
*Email: sureshayona@gmail.com

Article history:
Received 13 March 2016
Received in revised form
28 March 2016
Accepted 28 March 2016
Available online 31 March
2016

Keywords:
Azo dyes,
Bioremediation,
Co-substrates,
Percentage decolourization,
Growth rate,

Abstract

Presence of colour in water will make it unsuitable for human consumption. Textile industries have the major role in imparting colour in natural water bodies as their outlets are opened to them. These industries rely upon azo dyes as it impart brilliant colour to fabrics. But its presence in water bodies creates serious problems. Structure of an azo compound is a very important factor to be considered during the treatment of colored wastewater as it seriously affects the decolorization performance of microbial cultures. Since azo compounds have less carbon content, co-substrates can make good the deficiency. This study was carried out to find the efficacy of marine bacterial strains to degrade two selected azo dyes (congo red and methyl red) on giving three co-substrates (starch, glucose and mannitol). For decolourization of congo red, starch was found to be the best co-substrate (79.2% to 93% decolourization) and of methyl red, mannitol (50% strains shows >90% decolourization) was found to achieve best results. The decolourisation potential and growth of majority of the strains in methyl red was found to get reduced in the presence of starch. Next to mannitol strains utilise glucose (>50 % decolorisation).

Introduction

Among the pollution categories, water pollution has been identified as a major problem in the modern world. The increasing demand for drinkable water, and its dwindling supply, has made the treatment and reuse of industrial effluents an utmost requirement. One of the major industries, which contribute to the physical pollution of water, is textile as well as coir dyeing industry, which impart colour to the receiving water bodies, making it unaesthetic as well as objectionable for drinking purpose. Zolinger *et al.,* 1987 reported textile industry is a major contributor of wastewater discharged to surface water bodies. Substantial quantities of dyes have been deposited in the environment, particularly in streams and rivers. These colourful dyes persist in the

environment, because of their structural complexity. Previous studies reported that chemical structure and complexity of the dyes significantly influence the decolorisation rate (Pasti-Grisgby *et al*., 1992). There are physico-chemical treatments available for dye decolourization. But there are reports showing the formation of more problematic intermediate products such as primary amines which are carcinogenic resulting from such treatments. They have the added disadvantage that these are water soluble and hence can be easily absorbed through skin contact and inhalation (Nikulina *et al*., 1995)

Azo dyes come under the category of zenobiotic compounds, which are very resistant to biodegradation, (Stolz, 2001; Perkowski and Ledakowicz, 2002). Many of earlier workers (Verma and Madamwar, 2003; Moosvi *et al*., 2005; Pandey *et a*l., 2007; Khalid *et al*., 2008) reported that microorganisms could reduce azo dyes by secreting various enzymes. The products thus formed are further mineralized into simpler compounds, thereby utilizing for their energy requirement (Stolz, 2001). Bacterial degradation of azo dyes is found to be relatively faster. Lin and Leu, 2008, reported that there are a wide range of both aerobic and anaerobic bacteria which brings about degradation of azo dyes. Of these organisms, some uses the dyes as carbon source where as others as nitrogen source (Coughlin *et al.,* 2002); others may simply reduce the azo group by special oxygen tolerant azo reductases.

Physico-chemical treatment for dye degradation is found to be less effective. Microbial decolorisation and degradation (bioremediation) of dyes is seen as cost effective method for removing the pollutants, (Banat *et al*., 1996). Bacteria are the organisms with unique potential to be present in almost any kind of environment because of their genetic plasticity. Previous works have shown that marine bacteria are much more efficient than their freshwater and terrestrial counter parts in many aspects. This is because of their presence in the most adverse environments owing to varying nature of temperature, pH, salinity, sea surface temperature, currents, precipitation regimes and wind patterns (Dash *et al*., 2012). Marine organisms have to adjust frequently to these changing conditions and hence they can be used for biodegradation of various recalcitrant compounds. Reports are there to show compared to other microbes; harsh conditions tolerant marine bacteria have an extensive capacity to degrade variety of organic compounds (Manogari *et al.,* 2008).

Several reports, (Lekshmi *et al*., 2015; Jayadev *et al.,* 2015; Navami *et al.,* 2015; Lekshmi and Jayadev, 2015) show that marine bacteria are veterans in enzyme production. Quite a lot of microbial enzymes find their application in various industries. The effect of concentration on azo dye degradation, another part of this work is also published, (Sreelekshmi *et al*., 2015). Biodegradation of azo dyes by marine bacteria is the result of the production of an enzyme. Reductive cleavage of the chromophoric group of recalcitrant synthetic dyes requires redox equivalents (electron donors), (Kudlich *et al.,* 1997; Zee *et al.,* 2000). This reduction (decolorization) is carried out mainly by azo reductases, which is a key enzyme involved in the azo dye reduction. This enzyme catalyzes the reductive cleavage of azo bond by utilizing reducing equivalents (NADH or NADPH) generated from the metabolism of organic compounds. Source of these reducing equivalents are azo dyes and/or co-substrate. Co-substrates can generate redox

equivalents for the reductive cleavage. In the present study we try to find out the co-substrate having the better ability to donate electrons for reductive cleavage.

Meterials and Methods

Marine water samples were collected from Sanghumukham, Vizhinjam and Veli Beach, Trivandrum, Kerala, India. Azo dyes selected for the study were Congo red and Methyl red because of their easy commercial availability and their wide application in research field. The co-substrates selected for the study are glucose, starch and mannitol.

Isolation of bacteria: Water samples were collected in sterile bottles following standard procedures and brought to the laboratory and refrigerated till isolation. Bacterial strains were isolated by serial dilution and pour plate method in zobell marine broth. Biochemical characterisation of the bacterial strains was carried out based on Bergeys Manual of Determinative Bacteriology (1994) and Cappucino and Sherman, (1999). Bacterial strains streaked on marine zobell agar plates were maintained at static condition for further studies.

Screening for efficient decolorising bacteria: Screening was done to identify efficient bacterial strains capable of decolorizing azo dyes, congo red and methyl red. This was done by inoculating Zobell marine broth having 0.1 % of respective dyes and the measuring the absorbance of supernatant of culture broth at 600nm in Elico Spectrophotometer after 24hr for 2 successive days. It was confirmed by plate assay in the Basal salt media (NaCl 1.0 g, $CaCl_2$. $2H_2O$ 0.1 gm, $MgSO_4$. $7H_2O$ 0.5 g, KH_2PO_4 1.0 g, Na_2HPO_4 1.0 g, Yeast extract 4.0 g and Agar 5.0 g). Plates with 1% dyes incubated at 30^0C for 48 hrs shows clear zone around bacterial colonies. Un-inoculated test tubes and plates containing azo dyes were also set to check the abiotic decolourization of dye. Bacterial strains showing tolerance to both screening tests selected for further decolourisation studies.

Decolourisation assay: Decolourisation assay was done in Mineral Salt Medium (MSM) (NaCl 1.0 g, $CaCl_2$. $2H_2O$ 0.1 g, $MgSO_4$. $7H_2O$ 0.5 g, KH_2PO_4 1.0 g, Na_2HPO_4 1.0 g, Yeast extract 4.0 g). The bacterial cultures were inoculated to fresh Mineral Salt Media containing 100 ppm concentration of selected dyes at 37°C, under static condition for 5 days. After every 24 hours, 3 mL aliquot were withdrawn from both un-inoculated and inoculated culture media and centrifuged at 5000 rpm for 10 minutes. The culture supernatants were used to assay azo dye reduction by measuring residual absorption at the appropriate wavelength for each dye (λ max =506 nm for congo red and 502 nm for methyl red respectively). The percentage of decolourization was calculated from the difference between initial and final values using the following formula (Phugare *et al*., 2011).

% Decolourization = (Initial absorbance value-final absorbance value/ Initial absorbance value) 100

Corresponding growth of the bacterial strains were measured by taking absorbance of the culture broth at 600 nm. Un-inoculated MSM set as blank.

Effect of dye degradation with different co-substrates: In order to assess the effects of different carbon sources on bacterial decolorization, carbon sources namely glucose, starch and mannitol were used at the rate of 4 gL^{-1}. Decolorization was determined by using the protocol as described above.

Results and Discussion

Isolation and Screening of bacterial strains: From the collected water samples 12 bacterial strains were isolated. Four bacterial strains from (Ab1, Ab2, Ab3 and Ab4) Sanghumukham, four strains (Bb1, Bb2, Bb3, Bb4) from Vizhinjam and four strains (Tb1, Tb2, Tb3, Tb4) from Veli coast. All the bacterial strains were subjected to standard biochemical tests. In the case of congo red, bacterial strains Ab1, Ab3, Bb1, Bb3, Tb2 , Tb3 and in methyl red, strains Ab1, Ab3, Bb2, Bb3, Tb2 , Tb3 showed efficiency compared with other strains for decolourization ability.

Decolourization assay: Bacterial strains showing tolerance to selected dyes were selected for the assay. Decolourization assay was performed to calculate percentage decolourization by taking initial and final absorbance values using UV-Visible spectrophotometer. Un-inoculated medium was set as blank. Growth rate was also measured simultaneously in terms of their cell density (OD of the medium) at 600 nm.

Effect of co- substrates on Congo red decolourisation: The influence of co-substrates on Congo red decolourization and its corresponding growth rate were shown in figure 1 and figure 2. The results revealed that all the strains showed significant decolourisation rate and growth when added with different co-substrates. All the strains showed their maximum decolourisation potential with starch when compared to glucose and mannitol with slight variation which is insignificant. Padmavathy *et al.,* (2002) and Bhoosreddy (2014) also reported that starch acts as the efficient co-substrate. Over all decolourisation ranged from 73.7% to 90.6 % for glucose, 79.2% to 93 % for starch and 67.3% to 85.3% for mannitol respectively. Strain Tb2 showed maximum decolourisation and growth when starch was used as co-substrate (93% and 0.674 respectively). Of the remaining two co-substrates, strains can utilise glucose compare to mannitol. For decolourization of congo red, starch was found to be the best co-substrate (79.2% to 93% decolourization).

Effect of co-substrates on methyl red decolourisation

The effect of different co-substrate on methyl red decolourisation and corresponding bacterial growth was checked and the results are shown in Figure 3 and figure 4. Greater visible fluctuation in the utilisation of carbon sources can be seen here (figure 3). Except Ab1 all the other strains showed maximum decolourisation and growth when mannitol was used as co-substrate. Ab1 showed maximum reduction potential with glucose. Bb2, Bb3 and Tb3 showed > 90 % decolourisation in the presence of glucose. Maximum decolourisation and growth was shown by Tb3 (99.4 % and 0.698 respectively). It was observed that with starch as co-substrate, Tb3 showed no decolourising potential but it showed a slight growth (0.043). The decolourisation

potential and growth of majority of the strains was found to get reduced in the presence of starch. Next to mannitol strains utilise glucose (>50 % decolorisation).

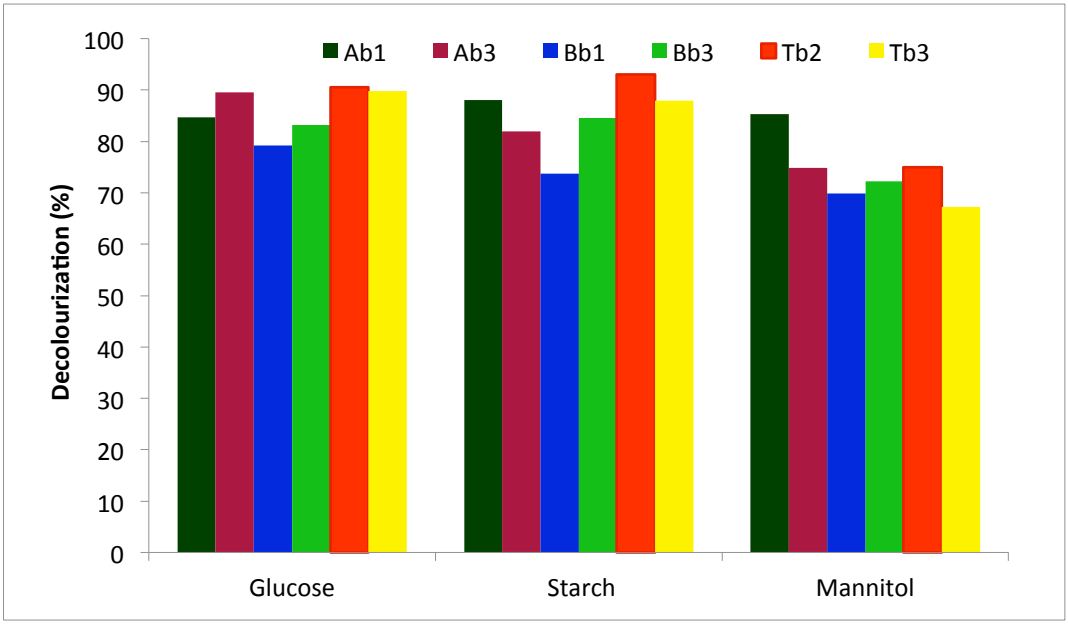

Figure 1 Decolourization of Congo red by bacterial strains with different co-substrates

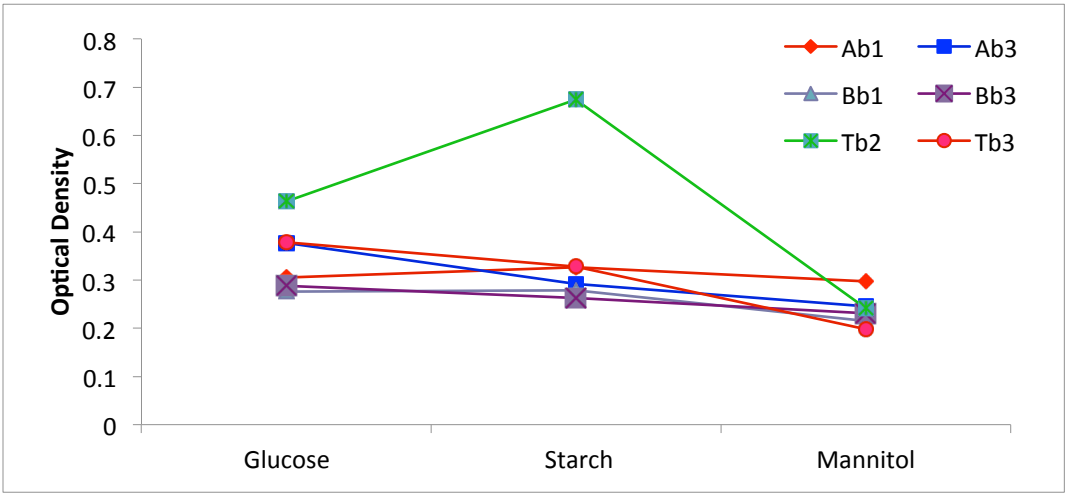

Figure 2 Growth of bacterial strains (in terms of cell density) in Congo red with different co-substrates

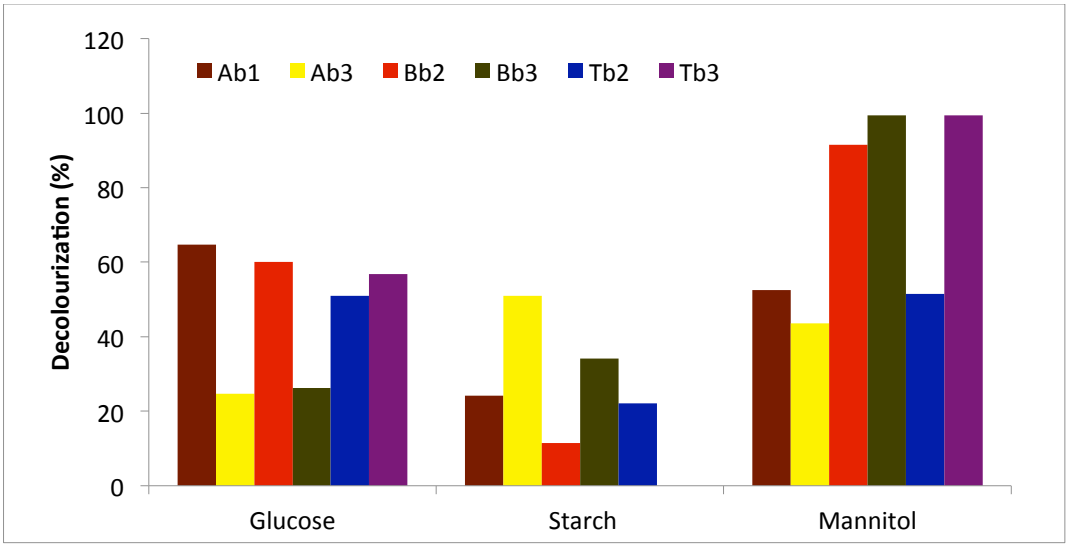

Figure 3 Decolourization of methyl red with different co-substrates

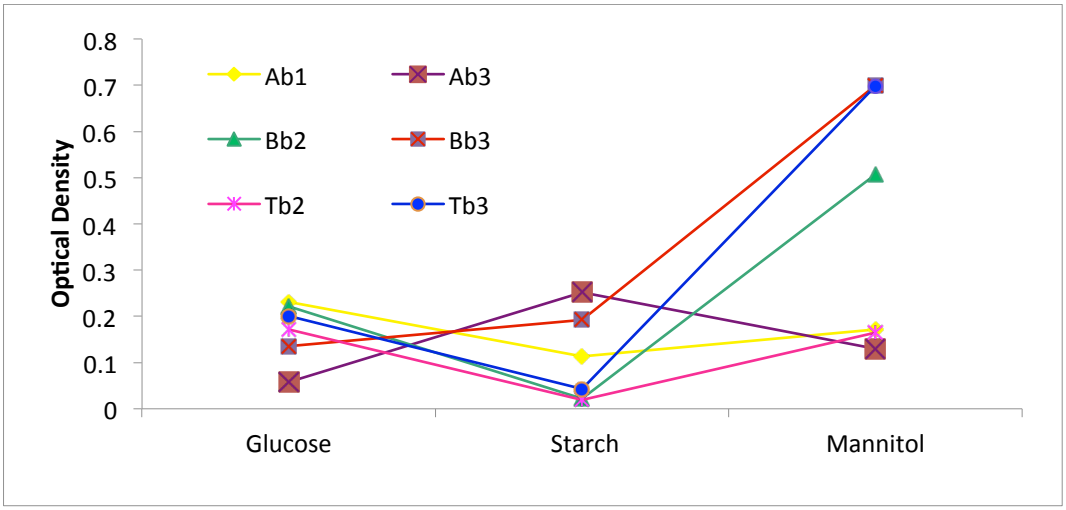

Figure 4 Growth of bacterial strains (OD) in methyl red with different co-substrates

The dyes are deficient in carbon content and decolourisation without any extra carbon source is very difficult. Co-substrates can acts as electron donors. Addition of carbon source seemed to be effective to promote the decolourization, probably due to the preference of the added carbon sources along with using the dye compound as the carbon source. Thus it can be concluded that this type of co-metabolic reduction will helps to remove dye content economically and in an ecofriendly manner.

Acknowledgement

The authors are grateful to University Grants Commission for providing the financial support to carry out this work.

Author's contribution: Dr. Ayona Jayadev (Assistant Professor and Principal Investigator) has final editing of menuscipt and also corresponding author; Dr. Lekshmi M. (Project fellow) has planned project

methodology and worte the menuscript and Ms. Sreelekshmi V. meticulously carried out the experiments under their leadership.

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