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RESEARCH ARTICLE

Decolourisation of azo dyes by marine bacterial strains

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Abstract

Azo dyes are widely used in the textile dyeing process due to the superior fastness for the fabric, high stability in light and washing, cost effectiveness of their synthesis and variety of colours are available in comparison to natural dyes and resistant to microbial attack. The discharge of these industrial effluent containing azo dyes results in creating undesirable conditions that are lethal to resident organisms. Various physical and chemical methods are employed for the remediation of the hazardous effluent but they may have disadvantages and limitations. Bioremediation is an effective technique, which is ecofriendly, cost effective, simple structural set up, less sludge producing properties. Realizing the importance of marine microbes in the degradation of azodyes, the present study focussed on the azo dye degradation potential of marine bacterial strains *in vitro*. A total of 12 bacterial strains were isolated from marine samples collected from three sampling site, Sanghumukham, Veli and Vizhinjam coast along the Arabian Sea. The isolated strains were screened for their potential to tolerate Congo red and Methyl red. The strains which showed maximum tolerance were selected for the decolourisation assay. Effects of dye concentration and incubation period on decolourisation were studied. Corresponding growth of the bacterial strains were measured in terms of absorbance at 600 nm. The results revealed that most of the selected strains shown >50% decolourisation with slight variation and the growth and decolourisation are inter-related. The present study proved that marine bacterial strains are very effective in degrading azo dyes in an eco-friendly way.

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INTRODUCTION

Water pollution causes some damage to an ocean, river, lake or other water resource. Effluent discharged from the dye-based industries plays a major role in water pollution. Zollinger et al., 1987 reported that among the various industries, the textile dyeing industries discharge large volume of waste water after dyeing process. Azo dyes are water soluble synthetic organic compounds, (Bell et al., 2000) which have wide applications in the textile dyeing, paper printing etc. Synthetic azo dyes are also found to produce carcinogenic compounds in the environment. To prevent contamination of vulnerable water and soil resources, removal of these dye pollutants is of great importance. Various physical and chemical methods viz., coagulation precipitation adsorption by activated carbon, charcoal, oxidation by ozone, ionizing radiation and ultra filtration are employed for the remediation of the hazardous effluent but they may have disadvantages and limitations, (Chen *et al*., 1999). They are proven to be costly and produce large amounts of sludge as secondary pollutants. All these techniques are minimizing the toxicity level not to neutralize the toxicity (Cooper, 1993; Maier *et al*., 2004). So more studies are now focused on methods involving biological treatments which use microorganisms to degrade azodyes. Bioremediation is found to be an effective technique for removal of azo dyes. A number of research groups investigated the capability of bacteria in the degradation of azo dyes. Marine microorganisms are reported to have much potential (Lekshmi et al., 2014). With a view to the

significance of marine bacterial strains for dye degradation, the present study was conducted to find the capacity of marine microbes for azo dye degradation and the degradation potential was optimized in terms of concentration of dye and period of incubation and the results are presented.

Materials and Methods

Sample collection

Marine water samples for the isolation of bacterial strains were collected from Sangumugham, Vizhinjam and Veli coast, Thiruvananthapuram, Kerala. The samples were collected in sterile bottles and were brought to laboratory maintaining a cold chain and refrigerated.

The dyes selected were Congo red and Methyl red.

Enrichment, Isolation and Characterization of bacterial strains

Enrichment of bacteria was done by the serial dilution and pour plate technique in marine Zobell broth. Biochemical characterization of the bacterial cultures was carried out based on Bergeys Manual of Determinative Bacteriology (1994) and Cappucino and Sherman, (1999).

Screening of bacterial isolates for selected dyes

For primary screening all the isolated strains were tested for tolerance to selected dye (congo red and methyl red) first in Marine Zobell Broth by taking the absorbance of culture broth at 600 nm in Elico Spectrophotometer. This was confirmed by plate assay in plates added with 1 % of selected dye and incubated for 48 hrs and the clear zone around the bacterial colonies indicated the ability of the organism to tolerate azo dyes. Unspotted plate was used as the control. The bacterial strains which showed maximum tolerance to the selected dyes were selected for further decolourisation study.

Decolourisation Assay

Decolourisation assay was done in Mineral Salt Medium (NaCl 1g, $CaCl_2.2H_2O$ 0.1g, $MgSO_4$ 0.5g, KH_2PO_4 1g, Na_2HPO_4 1g, yeast extract 4g). The bacterial cultures were transferred to fresh mineral salt medium containing 100 ppm of selected dyes at 37 ^{0}C, under static condition for 48 hrs. After 48 hrs 5ml aliquot of the culture media were withdrawn and centrifuged at 5,000 rpm for 10 minutes. The supernatants were used to assay azo dye reduction by measuring residual absorption at the appropriate wavelength for each azo dye (λ max =506nm 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)

$$\% \text{ Decolourization} = \frac{\text{Initial absorbance value-final absorbance value}}{\text{Initial absorbance value}} \times 100$$

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

Effect of concentration and incubation period on dye decolourisation

In order to find out optimum factors for dye decolorization the selected strains were inoculated in basal salt media with congo red and methyl red and decolorization rate was checked by above said method at varying parameters, viz, different dye concentrations (10, 50, 100 and 150 ppm)and incubation period(24,48,72 and 96hr).

Results and discussion

Isolation and Screening of bacterial strains

A total of 12 bacterial strains were isolated from the selected marine samples. 4(Ab1, Ab2, Ab3 and Ab4) strains were isolated from Sangumugham, 4 (Bb1, Bb2, Bb3 and Bb4) from Vizhinjam and 4 (Tb1, Tb2, Tb3 and Tb4) from veli coast. Biochemical characterisation was based on standard procedures. Screening was done to find the efficient bacterial strains capable of decolourising the selected dyes. The tolerance test at 0.1% of congo red and methyl red was confirmed by plate assay at 1% congo red and methyl red. In the case of congo red, strains Ab1, Ab3, Bb1, Bb3, Tb2 and Tb3 and in the case of methyl red, strains Ab1, Ab3, Bb2, Bb3, Tb2 and Tb3 showed maximum growth than other strains. All the isolated strains showed maximum growth at 48 hrs of incubation.

Decolourization assay for Congo red and Methyl red

The strains which showed maximum tolerance were selected for the decolourisation assay. The percentage decolourisation was derived from initial and observed absorption at the appropriate wavelength for each azo dye and corresponding growth of the bacterial strains were measured in terms of cell OD at 600 nm. The results are shown in Table: 1. With regard to congo red; the percentage decolourisation of almost all the selected strains showed excellent decolourisation with irrelevant variation. Over all decolourisation ranged from 79.2% to 90.6%. Among the strains Tb2 showed maximum decolourisation and the strain Bb1 showed comparatively low decolourisation with the other strains (90.6% and 79.2% respectively). All the other strains showed almost equal range of decolourisation. Regarding growth, majority of the strains showed remarkable growth when added with congo red . Strain Tb2 showed maximum growth and minimum growth was showed by strain Bb1 (0.399 and 0.221 respectively). In the case of methyl red, out of the 6 strains Tb2 showed maximum potential where as the least efficiency was shown by Bb2 .All the strains showed a decolourisation rate <60%. With regard to the growth Ab1 showed maximum growth and Bb2, minimum growth, (0.225 and 0.046) respectively.

Effect of dye concentration on Congo red decolourisation

The dye concentrations significantly affected the decolourization potential of bacteria. Corresponding growth rate of the bacterial strains were also measured for congo red and is shown in the Fig: 1 and Fig: 2.

The general trend of the observation was that the growth increased as the concentration increased from 10 to 100 ppm and then there was a sharp decrease in growth as dye concentration increased to 150 and further to 200 ppm. At 100 ppm of congo red Bb1 showed maximum decolourisation. But a variation to this general trend was observed in case of the bacterial strain Tb2. Tb2 showed maximum decolourisation and at 150 ppm. The decolourization rate was lower at lower concentration of dye. This may be because of low growth as observed in those concentrations. Except the strains Bb3, Ab3 and Bb1 all the other strains followed the similar trend. Strain Bb3 showed 62.9 % decolourization at 10 ppm, where as Ab3 and Bb1 showed 55.6 and 58.2 % decolourisation respectively at 50 ppm. Strain Bb1 was found to tolerate all the tested concentrations. It showed > 50 % decolourisation.

Effect of dye concentration on methyl red decolourisation

The influence of specific dye concentration on the decolourisation and corresponding growth of the bacterial strains in Methyl red are shown in Fig: 3 and Fig: 4. As the concentration increased from 10 to 100 ppm, there occurred an increase in growth and decolourisation and there after a sudden drop was found as the concentration increased from 100 to 200 ppm. Except Ab3 and Bb2 all the other strains showed maximum growth and decolourisation at 100 ppm. Among the strains, Tb3 showed maximum decolourisation percentage and minimum was shown by Tb2 (58.4 and 8 % respectively).

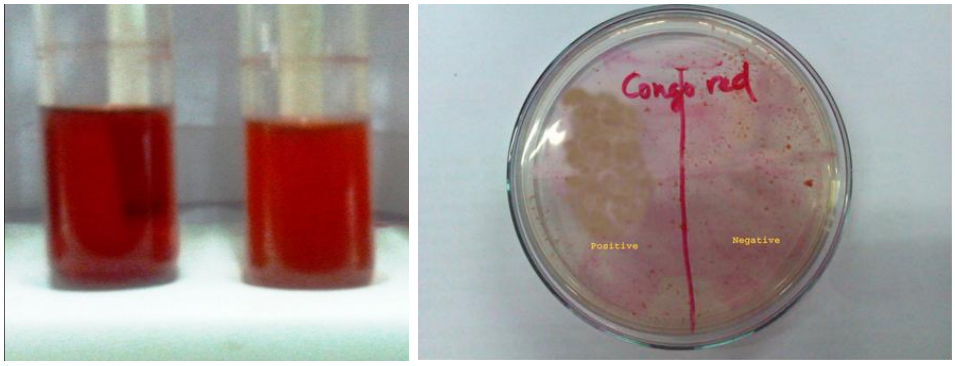
Effect of incubation period on Congo red decolourisation

The influence of incubation time for the rate of dye decolourisation activity of bacterial strains and corresponding growth is shown in Fig: 5 and 6. It was observed that the decolourisation and growth increases with the time period. For almost all the strains 48 hours of incubation was found to be the optimum time period for growth and incubation period except the strains Bb1 and Bb3 which showed maximum growth and decolourisation at 72 hrs of incubation (Fig: 5). At 48 hrs of incubation strain Tb2 showed maximum decolourisation followed by Ab3 (90. 6 and 89.5 % respectively). The highest growth rate was also shown by the strain Tb2 (0.612). Here the growth and decolourisation followed a similar pattern i.e. as the growth increases decolourisation increases and vice-versa.

Effect of incubation period on methyl red decolourisation

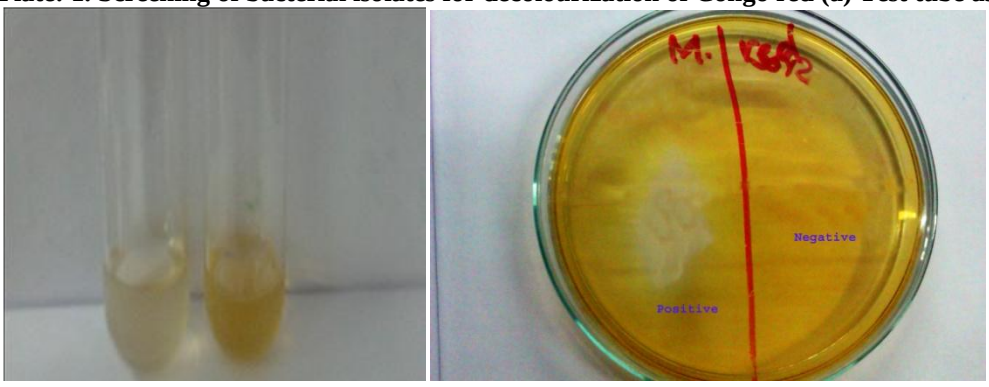
The influence of incubation period on dye decolourisation and corresponding growth was shown in Fig: 7 and Fig 8. The results revealed that the growth showed a gradual increase from 24 to 48 hrs and from 48 to 72 hrs a slight variation was exhibited by the strains. For the strains Ab1 and Bb2 the growth remains stable. Almost all the strains showed maximum decolourisation at 48 hrs of incubation after that decolourisation range seems to get stable. Among the strains Tb3 showed maximum decolourisation followed by Tb2 and Ab1 (56.8, 55.8 and 53.7 respectively). Least decolourisation was shown by Bb2 (13.1%).

Biodegradation of azodyes by microorganisms has gained much attention owing to the disadvantages of conventional treatment method. Tannery and textile effluents are characterized to have high salinity (Ahmed et al., 2011). Hence the marine microbes may have potential applications in the bioremediation of textile and tannery effluents they can survive the harsh saline conditions of the textile dye effluent. In the present study among the twelve strains six strains (Ab1, Ab3, Bb1, Bb3, Tb2 Tb3) showed maximum tolerance to congo red and six strains (Ab1, Ab3, Bb2, Bb3, Tb2 Tb3) showed maximum tolerance to methyl red. In the case of congo red 90 % of the strains showed more than 80 % decolourisation but in the case of methyl red only 30 % of the strains showed more than 50 % degradation. The highly variable potential of different bacterial strains to decolorize different azo dyes is highlighted in earlier reports (Maier *et al*., 2004, Khalid *et al*., 2008). Previous studies reported that chemical structure and complexity of the dyes significantly influence the decolourization rate (Pasti-Grigsby *et al*., 1992). The bacterial growth and decolourisation followed a direct relation in decolourisation assay i.e. as the growth increases decolourisation increases and vice versa. In the present study, decolourisation decreases with increase in concentration from 150ppm to 200 ppm of both dyes. Lower decolourization rate at high dye stuff concentration was due to the inhibitory effects of high dye stuff in bacterial growth. Similar results are also observed by Khataee et al., (2009). The incubation period plays an important role in decolourisation. In the present study 48 hrs of incubation was found to be the optimum for the decolourization of 100 ppm of dye. The bacterial growth gets saturated at 48 hrs of incubation and there after the growth gets declined affects decolourisation.



(a) (b)

Plate: 1. Screening of bacterial isolates for decolourization of Congo red (a) Test tube asssay, (b) Plate assay



a) b)

Plate: 2. Screening of bacterial isolates for decolourization of Methyl red (a) Test tube asssay, (b) Plate assay

Table: 1 Percentage degradation and corresponding growth of bacterial strains added with Congo red and methyl red

Bacterial strains	Percentage decolourisation of congo red	Growth (cell OD at 600 nm) of bacterial strains added with congo red	Percentage decolourisation of methyl red	Growth (cell OD at 600 nm) of bacterial strains added with methylred
Ab1	84.7	0.262	53.6	0.225
Ab3	89.5	0.371	24.7	0.131
Bb1	79.2	0.221	-	-
Bb2	-	-	13.1	0.046
Bb3	83.2	0.378	30	0.140
Tb2	90.6	0.399	55.2	0.196
Tb3	89.8	0.308	58.4	0.175

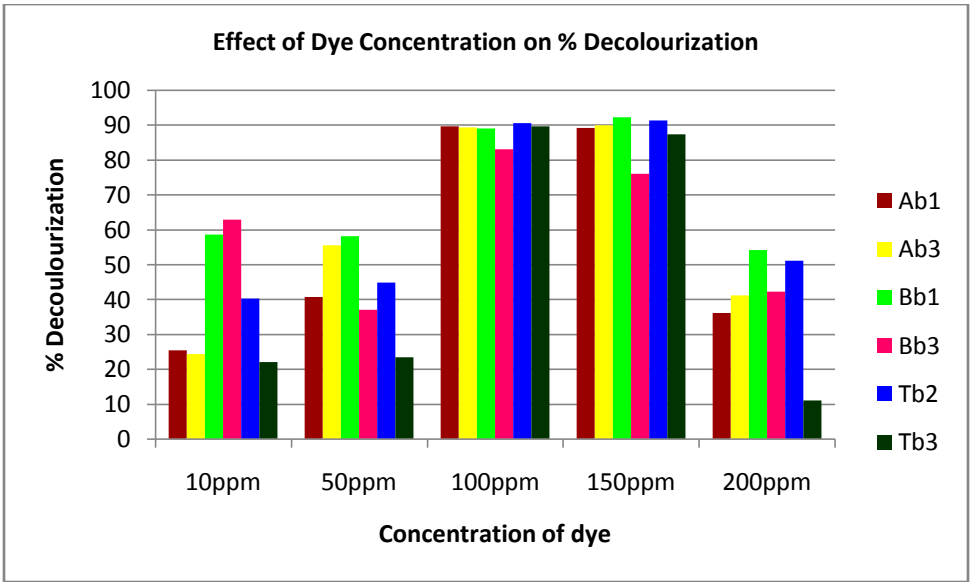


Fig: 1 Decolourization of Congo red at different concentrations

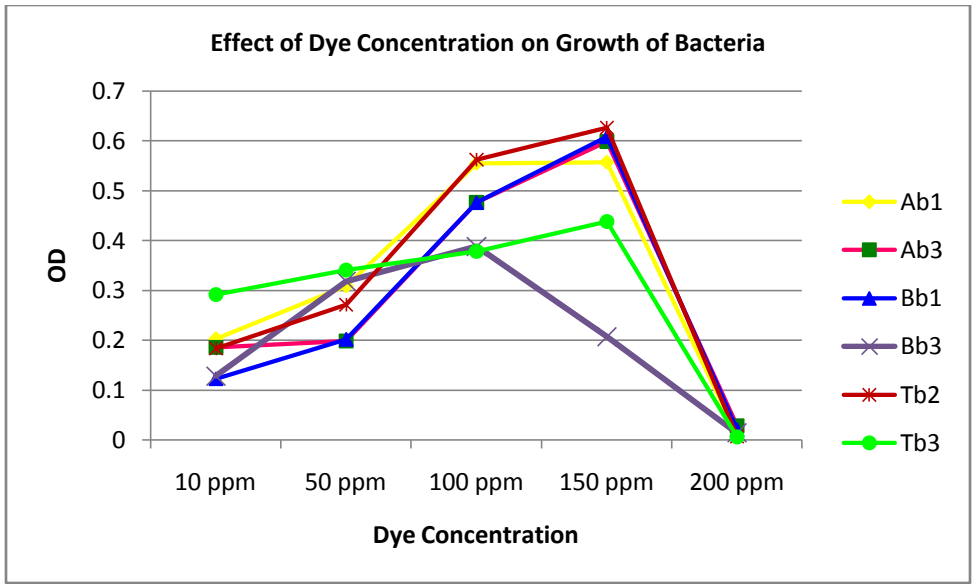


Fig: 2. Bacterial cell density in Congo red at different concentrations

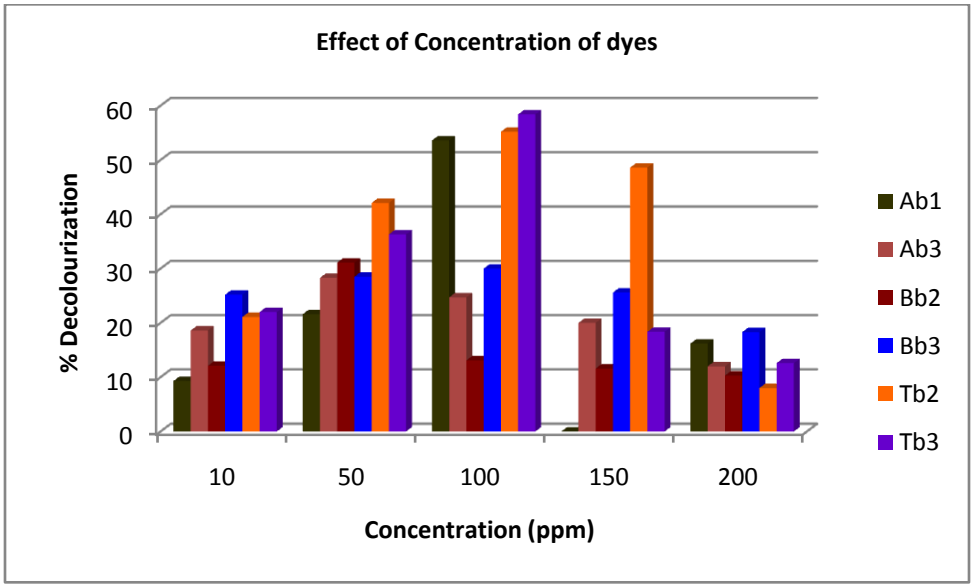


Fig: 3. Decolourization of methyl red by bacterial strains at different dye concentrations

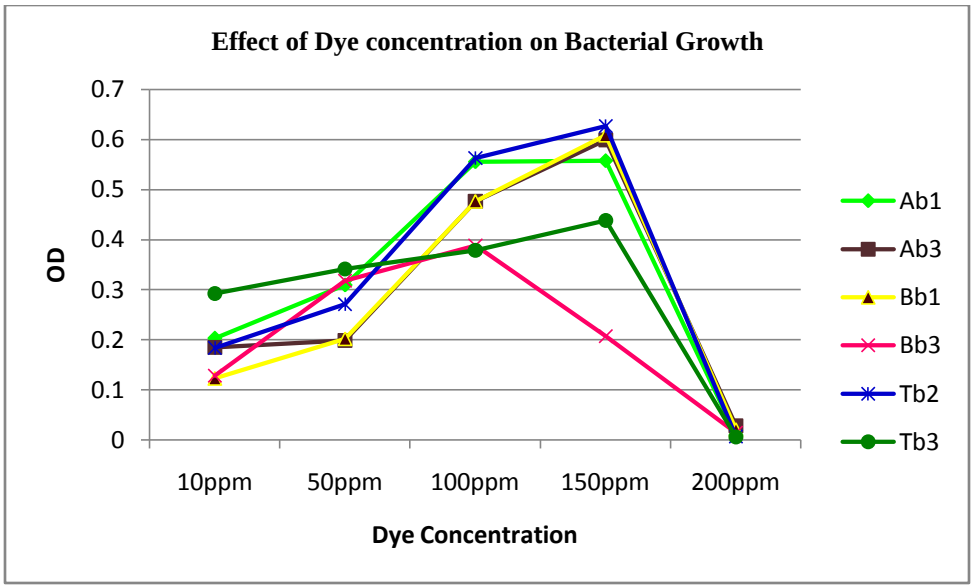


Fig: 4. Growth of bacterial strains (ODat 600 nm) in methyl red at different concentrations

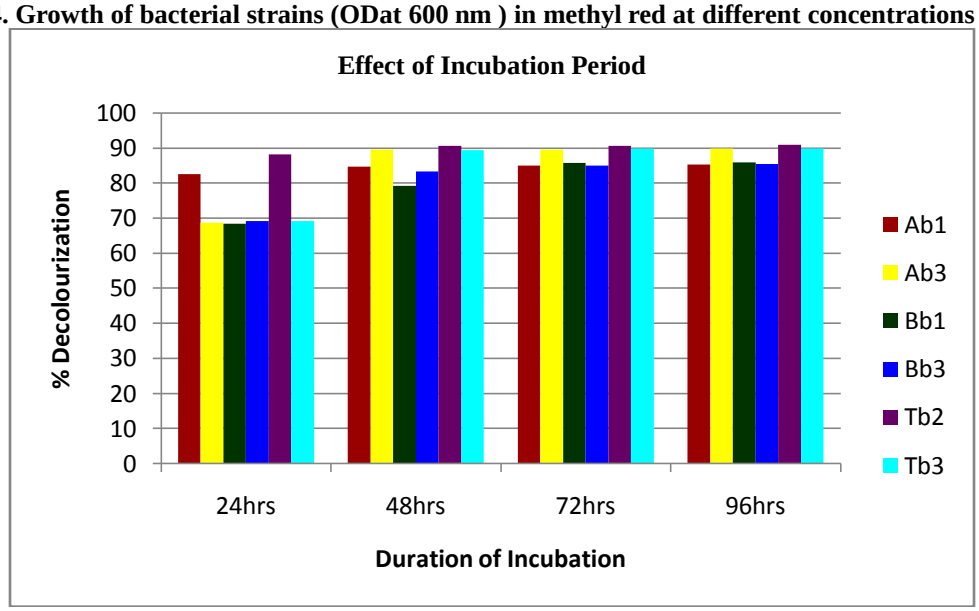


Fig: 5. Decolourization of Congo red at different time periods

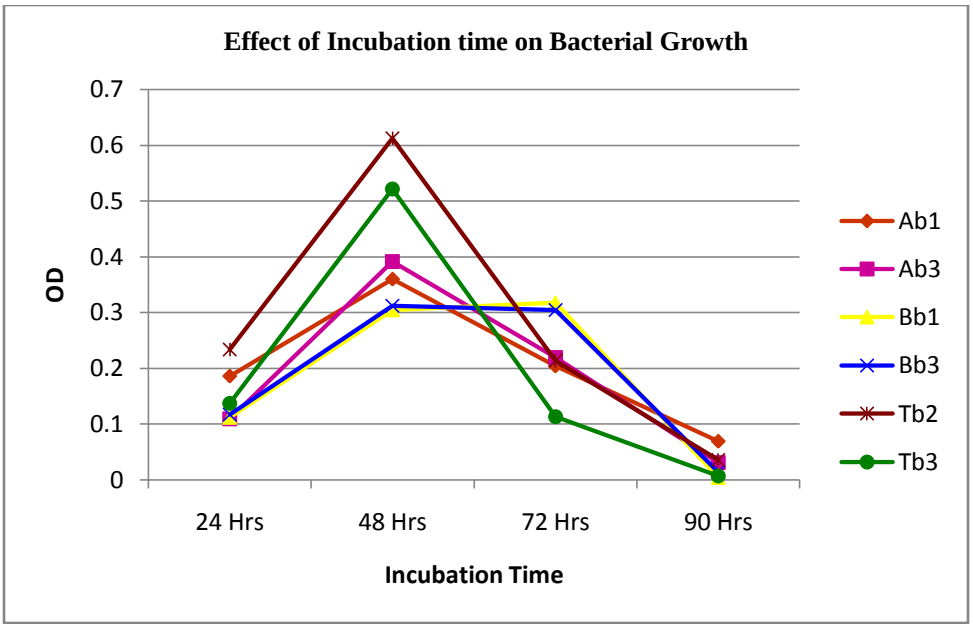


Fig: 6. Bacterial growth at different incubation time

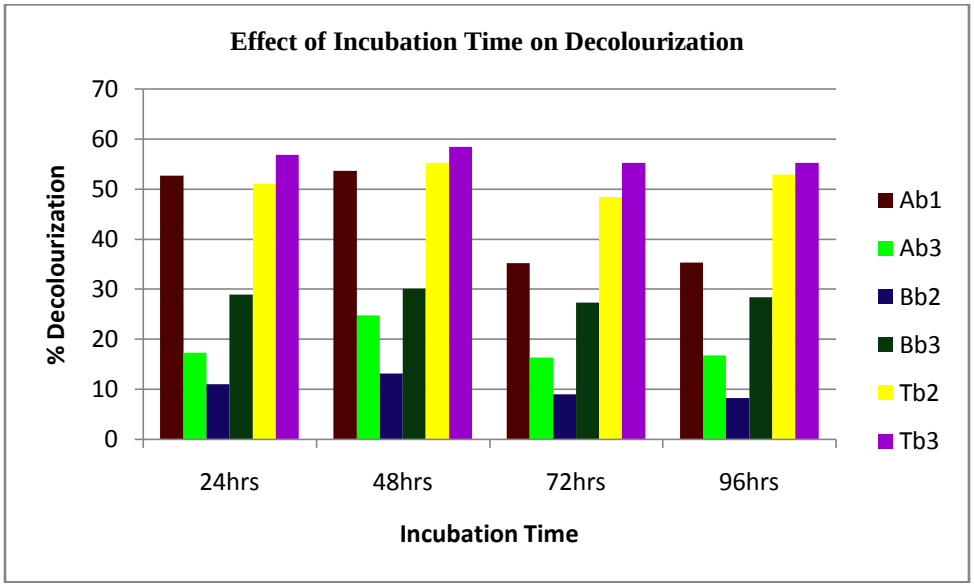


Fig: 7. Decolourization of methyl red at different time periods

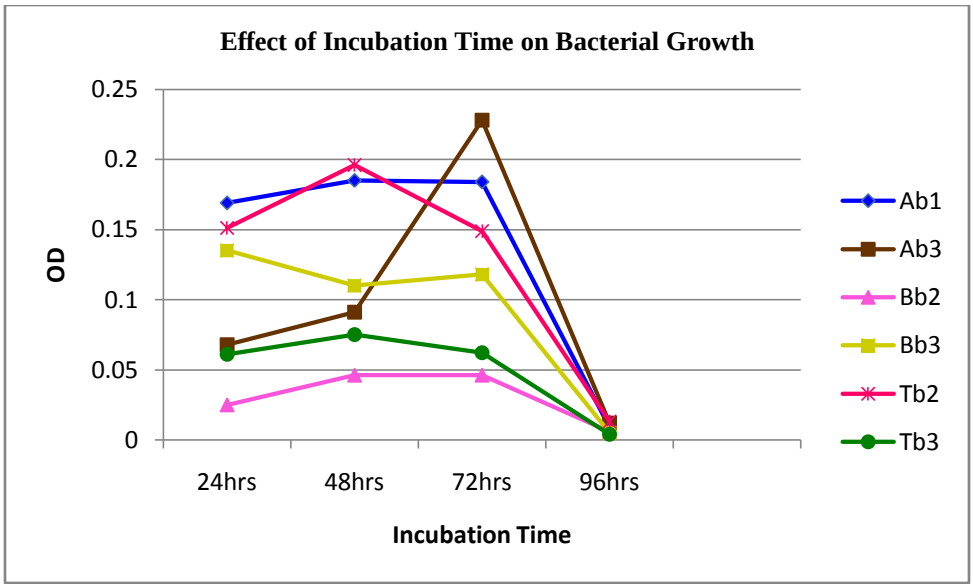


Fig: 8. Growth of bacterial strains (OD) in methyl red at different time periods

Conclusion

Based on the present study it may be concluded that biodegradation of azodyes by marine bacterial strains has proved to be very effective method in countering the textile dye pollution in an eco-friendly way. In the present study the selected bacterial strains isolated from the marine water samples have a high efficiency to tolerate congo red and methyl red. This has increased the scope for using these marine for the decolourisation harmful dyes. These strains can be positively used for further decolourisation studies. Further characterizations would help to commercially exploit these organisms for biodegradation of textile azodyes.

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