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Decolorization Study of Reactive Red-11 by using Dye Degrading Bacterial Strain *Lysinibacillus boronitolerans* CMGS-2

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A B S T R A C T

Many bacterial isolates showed decolorisation of reactive red -11 as a sole source of carbon, among the strains, a potential thermophillic newly isolated and first time reporting dye degrading bacterial strain, *Lysinibacillus boronitolerans* CMGS-2 has been isolated from the textile dye treatment unit solapur, Maharashtra and identified by 16s rRNA. It was Motile and spore forming bacteria Showed decolorisation of 88.78% of reactive red -11 dyes (200mg/liter) within 48 hours of incubation under static condition with dye as carbon source. Abiotic and biotic parameters were framed to enhance the capacity of isolate, finally 96.8% of decolourisation was achieved with 400mg/L RR-11, at pH-9, at temperature 45ºC, Inoculum concentration was 5ml, and 1gm/L yeast extract as nutritional source, within 12 hours of incubation. The novel characteristics of CMGS-2 was found, it showed decolorisation of 95.9% even at 50ºC temperature. The complete decolorisation of reactive red-11(RR-11) dye confirmed by using UV-VIS Spectrophotometer. It is deposited in the IMTECH, MTCC Chandigarh with MTCC number-12531.

Introduction

Nature itself colorful and synthetic dyes are available in two forms natural and manmade synthetic dyes. Nowadays various chemically synthesized dyes which became essential part of life (Manohar *et al.*, 2001). Synthetic dyes creating a lot of problems to nature. Dyes are being widely used in many industries, especially the textile industries. These industries release, untreated dye contaminated water in to the environment, which leads complications on the earth and water bodies. One of the survey raveled textiles industries are the major source of polluters of India (Kumar *et al.*, 2012). These industries are greatest generators of liquid, it is estimated that daily nearly 280000 tons of contaminated dyes released in water.

In Textile industries 93% of the outgoing water comes out as colored wastewater and also contains high concentration of organic compounds and heavy metals (Gupta *et al.*, 2014). Strict Legislations has to be made to control especially azo based dyes. These azo dyes widely used in the industries majorly reactive azo dyes are largest synthetic dyes

constituting 60-70% of all organic colorants (Saratale *et al.,* 2013). In a waste water dye concentration may ranges from 5-1500 mg/L (Jadhav *et al.,* 2010). Due to heavy discharge of synthetic dyes leads accumulation, which effects on aquatic life, and humans, and presence of aromatic compounds, makes dyes to xenobiotic, carcinogenic and mutagenic (Lu *et al.,* 2010). Structurally these dyes contains N=N bonds, attached to a benzene ring, various other compounds like SO_3H, SO_2NH_2, attached to a aromatic nucleus which makes into recalcitrant (Jain *et al.,* 2012). Even a little amount of dye discharges in water leads ecological instability to aqualives, reduction in photosynthesis, toxicity level increases, alternations BOD, COD, TOC,PH, ranges etc (Lade *et al.,*2012).

Various microorganisms like fungi, actinomycetes, bacteria are used, out of all bacteria are used widely. Bacterial degradation is fastest compare to fungal decolarisation (Tapia and Tusell, 2011). Bacteria having ability to degrade dye in a single or mixed cultures (Chang *et al.,* 2010). Presently work carried out to achieve effective decolorisation, *Lysinibacillus boronitolarans* CMGS-2 given excellent results in the decolorisation RR-11.

Materials and Methods

Source of sample-samples brought from in and surroundings of dying industries, soil samples, dye contaminated soil, industrial discharged water sample were collected from solapur, Maharashtra.

Dyes and chemicals

A total of six dyes were used in this study, Purchased from sigma aldirich (U.S.A), Heena and Colorise dying industries (Gujarat, India). They were Reactive blue 4, Reactive yellow 86, Reactive navy blue 59, Reactive orange 16, and Reactive violet 1. and Reactive red 11. Out of these dyes, isolate shown good decolorisation in reactive red 11(RR-11) and it is carried further for the further experiments. For the preparation synthetic media and broth, HI-media, SD-fine chemicals were used.

Synthetic mineral salt medium for the decolorisation experiments (Brilon *et al.,* 1981) with little modifications

$Na_2HPO_4.2H_2O$ -12.00g, KH_2PO_4 -2.00g, NH_4NO_3 -0.50g, $MgCl_2.6H_2O$ -0.10g
$Ca(NO_3)_2.4H_2O$ - 50.00mg,$FeCl_2.4H_2O$ - 7.50mg,
Distilled water -1000.00ml, pH -7.00
Trace elements solution (mg/l)
$FeSO_4.7H_2O$ -0.10, $ZnSO_4.7H_2O$- 0.05, $CuSO_4.5H_2O$- 0.02, CaCl2.6H2O - 0.005, MnSO4.H2O -0.017.

Preparation of stock solution

Isolation, screening, and identification, biochemical tests for dye degrading bacteria:- Ten grams of soil sample and 10 ml of water effluents were added in 100 ml of normal saline (0.9%) in 250 ml conical flasks and kept on rotary shaker at 150 rpm for 1 hour and stopped it left for the settling of soil. After settling 10ml of supernatant was used for the inoculating the MSM broth containing 50 ppm Reactive red -11 dye as sole of carbon and incubated at 35 ^{0} C and kept for observations more than 50% decolorised flasks were selected visually observed daily till there is complete or more than 50 % change in the visual color. The degradation was confirmed by UV-vis spectrophotometer Optical density checked at 540nm. Maximum degradation showing flasks were selected, further processed for isolation purpose, loopful of decolrised sample streaked on the, MSM agar medium containing reactive red -11 dye. Microbial colonies showing clear

zone, were picked up and again cultured on the NA agar plate and observed for the colony characteristics and performed the gram staining motility. Different types of colonies were selected and subculture on MSM agar slants.

Decolorization process

Isolates grown in 10ml nutrient broth and incubated for 24hours of duration and transferred it to a 100 ml MSM medium containing 50 ppm of dye and incubated at 35 ^{0}C maximum decolorized flasks were selected and isolated the bacterial strains pure culture of dye degrading bacterial strains were selected and sub cultured on Nutrient Agar slants. Potential isolates were preserved at 4.c in 30% glycerol and further used for biochemical property analysis (holt *et al.*, 1994).

Decolorisation assay

For investigating the RR-11 degradation, isolated bacterial cultures used. For the decolorization 10 % of bacterial inoculums were added to the 100 ml of MSM broth with 50ppm of RR-11 dye (Decolorization medium). The decolorization medium without culture served as control. The flasks were incubated at 35 ^{0}C. Every 4 hour 1 ml of the sample was drawn from each flask and analyzed for the dye concentration broth taken in the 2ml centrifuged tubes centrifuged at 10000rpm for the 10mins, supernatant of culture drawn and optical density checked at 540 nm by using in a UV – Vis spectrophotometer. A decrease in the optical density with incubation time period is taken as an indicator of decolorization. To confirm the decolorization is due to degradation of dye not due to change in the pH of the medium and adsorption or absorption by change in the pH of the culture filtrate with addition HCl or NaOH. The adsorption was tested by dissolving the culture pellet in the solvent. Similarly absorption was performing by analyzing the dye in the cell lysates. The percentage of degradation was calculated.by using formula. Calculation of % decolorization:- % Decolorization = Initial absorbance-Observed absorbance/Initial absorbance x 100. Maximum degradation shown isolate selected for the identification.

Results and Discussion

Optimization study of RR-11 decolorisation by isolate CMGS -2

Effect of pH on the decolorisation of Reactive Red-11

For effective degradation pH is an important factor. Experiment carried out different pH ranges from 4to 14, isolate CMGS-2 shown an excellent degradation in alkaline condition. At pH 8,- 96% and pH-8 is optimum for our isolate and with other pH at 9-it was 95.9%, in at pH -10 it was showed 95.8 maximum decolorisation, it reveals isolate CMGS-2 uses alkaline pH for the better decolorisation. For industrial usage alkaline condition is optimum, to maintain neutral pH needs lots of efforts. Various reports shown on degradation in the alkaline pH range. (Bhatt *et al.,* 2012) shown bacterial consortia SpNb1, shown degradation in the range from 6-9pH. at pH 7.5 with maximum dye decolorization 94.95 ± 0.09 observed. *Shiwanella* sps IFN4 shown degradation in mixed azo dyes pH ranging from 5-9(Imran *et al.,* 2014).

Effect of temperature on the decolorisation Of Reactive Red-11

Decolorisation study carried out in different temperature ranges, from 20ºC to 50 ºC. Isolate CMGS-2 shown maximum decolorisation 40ºC 96.06%. At 45$^{.c}$ it was 96.23%. At-50ºC -95.6% even it showed decolorisation in 20ºC -66.78% in 12 hours of duration. From these it reveals that isolate

degrades dye in range of temperature. Decolorization of Acid Orange dye by *Staphylococcus hominis* RMLRT03 strain was found to be 35°C with 92.38% decolorization (Singh *et al.,* 2014).

The decolorization of RB-172 by *Shewanella haliotis* DW01 was found to be significant at 30 ºC and 35 ºC with maximum decolorization 78% at 35^{0C} in 12h (Radhika *et al.,* 2014).

Effect of salt on the degradation Of Reactive Red-11

Reactive dye contain high amount of sodium salts. To check the salt tolerance of our isolate salt concentration is ranging from 1% to 5%. For 1%- it showed 95.4%,. At 5%- it was 86%. The concentration of salt increases the decolorisation capacity shown decreasing order.

It reveals that the high concentration of salt decreases the decolorisation. (De Baere *et al.,* 1987) demonstrated that increase in the sodium concentration degradation rate decreases. Panswad *et al.,* I1999) revealed that these may due plamolysis causes, which make decolorisation slower. (Bheemaraddi *et al.,* 2014) reported *Paracoccus* sps shown decolorisation up to 6% salt concentration.

Effect of inoculum on the degradation Of Reactive Red-11

Inoculum concentration helps in the better decolorization of dye, isolate CMGS-2. 95.5%. as per (Sharma *et al.,* 2015) 5ml inoculums size was the optimum for the decolorization (Sahastrabuhe *et al.,* 2014) revealed *Enterococcus faecalis* YZ66 shown 10% of inoculums size as optimum. Bheemaraddi *et al.,* (2014) studied 5 to 20% of inoculums size for the decolorisation and decolourization activity of *Paracoccus* has shown high in 20% of inoculums size.

Effect of dye on the Decolorisation of Reactive Red-11

Decolorisation capacity of CMGS-2 was checked with the increasing concentration of dye for the duration of 12 hours of duration, dye concentration was checked from 100ppm to 1000ppm. Optimum dye concentration was 200mg/-it was 98%, and at 400 ppm it was 98.1%, was optimum for the better degradation and further experiment carried out by the same concentration of the dye. Further at, for 1000ppm isolate was showing 43% decolorisation. The results CMGS-2 was higher compare to other reports, *Enterocooccus fecalis* YZ66 shown decolorisation up to 700rpm (Sahastrabudhe *et al.,* 2014). The decolorization of textile dye reactive golden yellow 84 by the isolated bacterium *Rhizobium* sp. F5 showed upto 300mg/L decolorisation within 24 hours of duration (Narsinge *et al.,* 2013). Various Reports Showed increase the capacity of dye there were decrease in the degradation that may be due to reactive azo dye inhibits nucleic acid synthesis in microbial cell growth (Kalyani *et al.,* 2009).

Effect of Static and Shaking Conditions on the decolorisation of Reactive Red-11

To study the effect of aeration on the degradation of reactive red -11, flasks were kept on shaker at 180 rpm. For 12hour of duration. Isolate CMGS-2 shown better decolorisation in the static condition. (sahasrabudhe *et al.,* 2014) Showed static condition only favorable for the decolorisation.

Optimization of additional nutrients for maximum decolorization of isolate CMGS

Effect of corbon and nitrogen sources on the decolorization of Reactive Red-11

Different carbon sources were taken to

increase the efficiency of decolorisation, but isolate shown no significant increase in the decolorisation of RR-11 dye, in glucose 1gm/L It Showed 90.5%. Sucrose it was 84.3%, starch 73.4% reveals isolate CMGS-2 used only dye as a carbon soul source of energy, in other reports showed, Oturkar *et al.,* (2010) showed additional carbon sources is essential for increase in dye degradation efficiency of *Bacillus lentu* B1377. (Shah *et al.,* 2014) studied *Pseudomonas spp* showed moderate decolorization in the presence of sucrose (50%) lactose (45%) and starch (55%) and decolorization in the presence of glucose was reported (62%).

Effect of nitrogen sources on the degradation Of Reactive Red-11

Various nitrogen sources are used, to check the efficiency of isolate CMGS-2.in yeast extract it shown 97.4%, beef extract it shown 93.2%, peptone it was 88.1%, potassium nitrate it shown 71.5%. Ammonium nitrate shown degradation 70.1%, and these study results yeast extract was the excellent substrate for the better degradation. Similarly *Pseudomonas aeruginosa* GSM3 showed complete decolorization of Reactive Violet 5 within 16 h in the presence of yeast extract (Bheemaraddi *et al.,* 2014).

Effect of yeast extract on the degradation Of Reactive Red-11

Out of all nitrogen sources yeast extract played a main role in the degradation of reactive dyes. For the optimization of 0.5%, 1.0%, 1.5%, 2.0%, yeast extract was taken. CMGS-2 showed Optimum degradation in 0.1% of yeast extract.

Fig.1 Optimization of pH on the decolorisation of Reactive Red-11

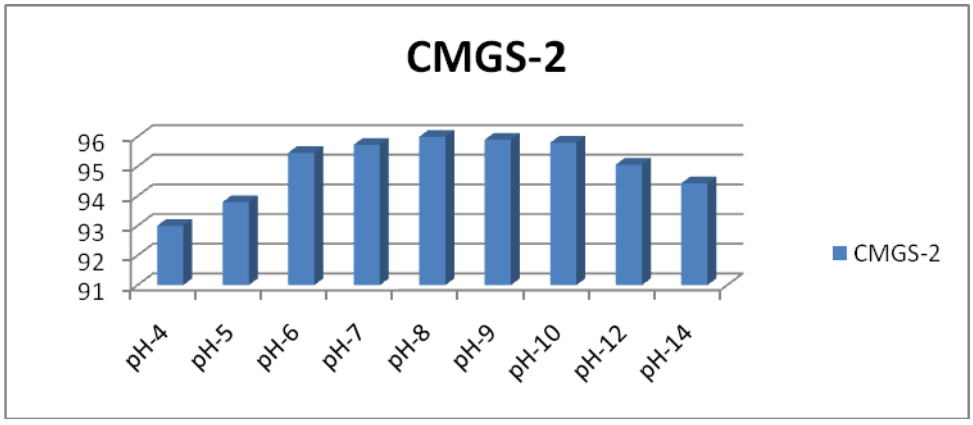


Fig.2 Optimization of temperature on the decolorisation of Reactive Red-11

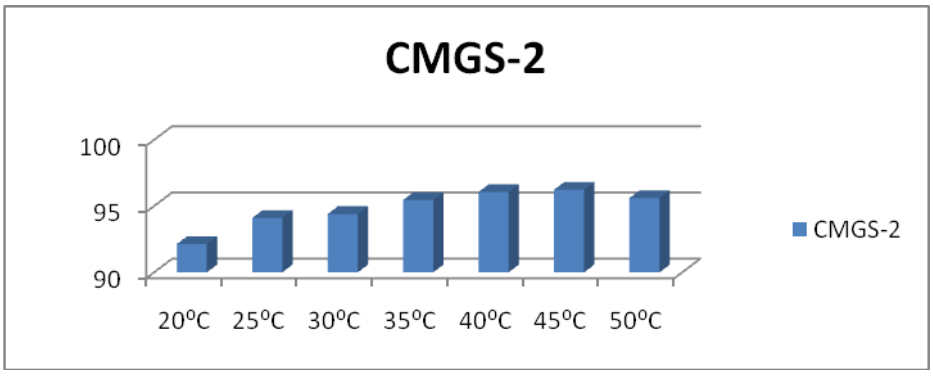


Fig.3 Effect of salt concentration on the decolorisation of Reactive Red-11

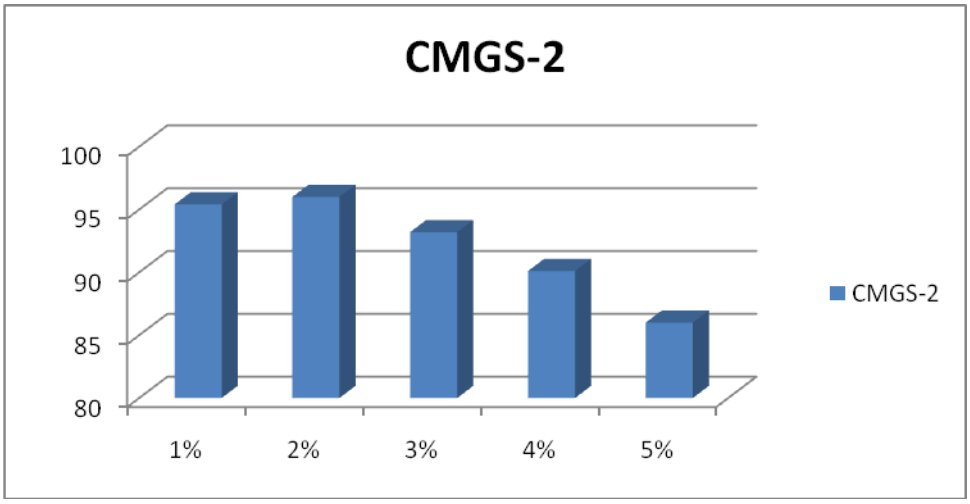


Fig.4 Optimization of inoculum concentration on the decolorisation of Reactive Red-11

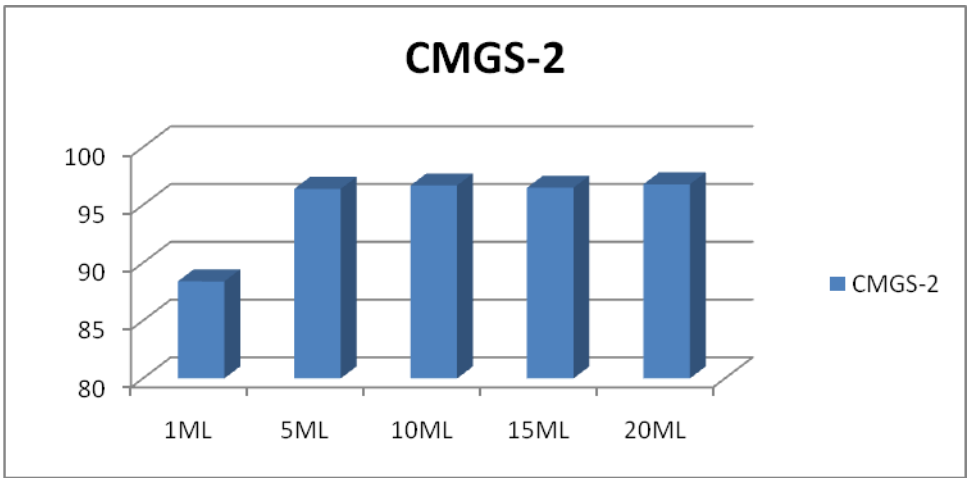


Fig.5 Effect of dye concentration on the decolorisation of Reactive Red-11

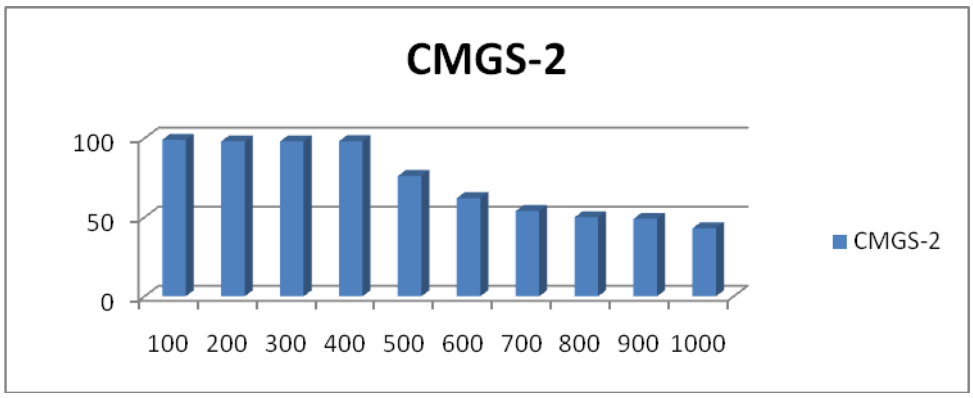


Fig.6 Effect of aeration on the decolorisation of Reactive Red-11

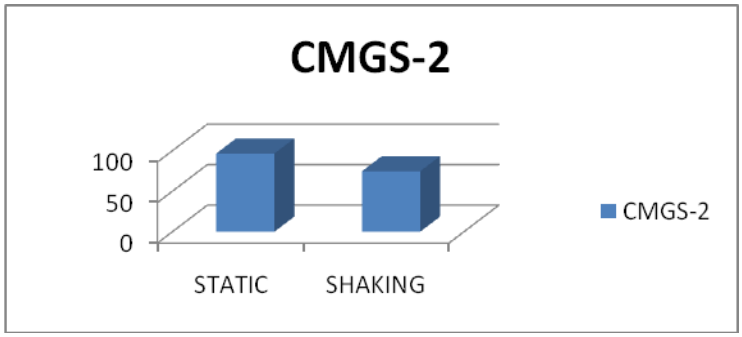


Fig.7 Optimization of yeast concentration on the decolorisation of Reactive Red-11

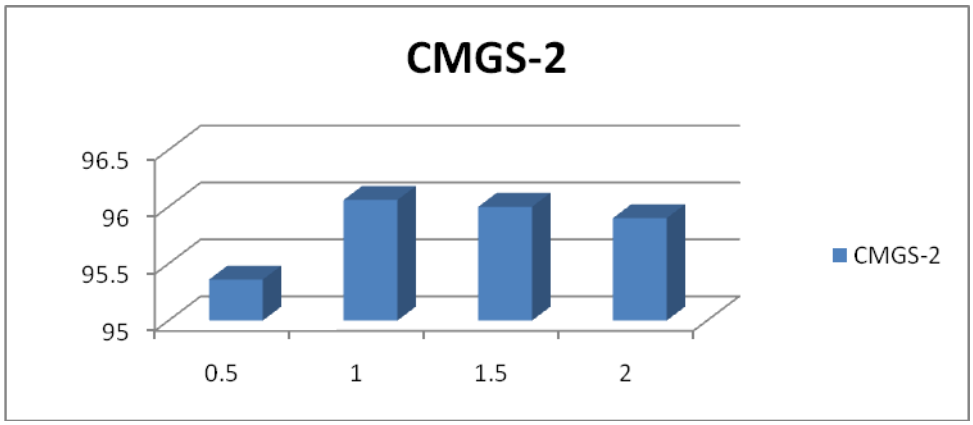
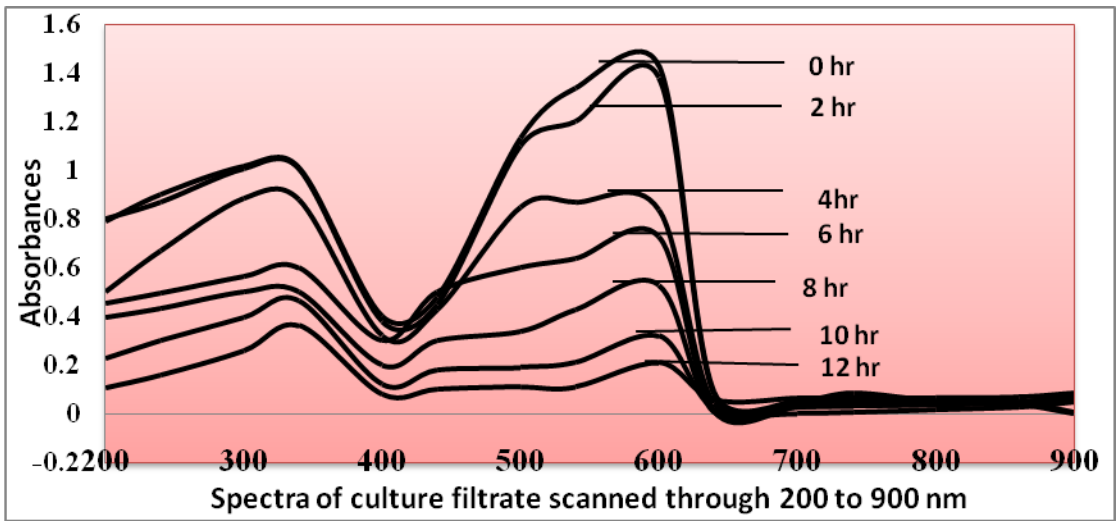


Fig.8 Decolorization of RR-11 by *L.boronitolerans* at 0hr and 12 hr of incubation



With Increase Concentration Of yeast extract it does not effected on decolorisation Dawkar *et al.*,(2009) reported decolorisation of the *bacillus sp. vus* Yeast extract was the best medium for faster decolorization.

UV-Vis Spectroscopy

The supernatants of different intervals of incubated DM with RR-11 and CMGS-2 were subjected to scan between 190 to 900 nm UV-Vis spectrophotometer results are shown in Fig. 4.24. A single peak at 540 nm corresponding to λ_{max} of the dye and two intense peaks at 250 nm and 325 nm corresponding to phenyl and naphthol rings of RR-11 respectively. With increase in incubation time the peak height at 540 nm goes on decreased and disappeared after 12 hrs of incubation indicates complete decolorisation of added RR-11 similarly many researchers studied decolorisation using UV-Spectrophoometer, Reactive red -195 at 542 nm by *Micrococcus glutamicus* by Sahasrabudhe *et al.*, (2014). Metanil yellow at 430 nm by *Lysinibacillus* sp. AK2 (Anjeneya *et al.*, 2011).

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