International Journal of
Life Science
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Science, 2019; 7 (2):316-320
ISSN:2320-7817(p) | 2320-964X(o)

Original Article Open Access

Biodecolorisation of Reactive orange-16 by *Lysinibacillus Boronitolerans* CMGS-2

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Manuscript details:

Received: 23.05.2019
Accepted: 12.06.2019
Published: 25.06.2019

Editor: Dr. Arvind Chavhan

Cite this article as:
Madhuri R Basutkar and Channappa T Shivannavar (2019) Biodecolorisation of Reactive orange-16 by *Lysinibacillus Boronitolerans* CMGS-2, *Int. J. of. Life Science*, Volume 7(2): 316-320.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Colors are the part of life, as the nature is itself color full gives pleasant to eyes and feel good, fresh, polite and respectful. But in present days it becomes reverse due to excessive use of synthetic colors leading to the environment pollution especially the hydrosphere. These pollutants are released into air, water and soil and have detrimental effects on the health of humans, plants, animals and microbes. In this direction isolate *Lysinibacillus boronitolerans* CMGS-2 (bp-1404) NCBI accession number-KT602857 and MTCC number-12531 was showed a maximum decolorisation of Reactive orange-16, under optimized parameters, the final decolorisation confirmed by UV-VIS Spectrometer and reveals isolate is helpful in the treatment of synthetic dyes.

Keywords: *Lysinibacillus boronitolerans,* accession number, Reactive orange-16(RO-16), UV-VIS Spectrometer.

INTRODUCTION

Human endeavors for the production and improvement of goods and services cannot be absolutely stopped because these are needed by humans for their survival on earth. Alternatively, we must look for green processes - that lead to the production of eco-friendly products. We must also focus our attention on ways for the eradication and reduction of the existing environmental pollution. Thus, for a sustainable human society, we need green chemistry and environmental remediation. A tremendous increase raised in the awareness of the toxic and carcinogenic effects of many polluting chemicals, which were earlier not considered hazardous substances (King *et al.,* 1997). Most of the industries, especially textile industries are posing a threat to the water bodies as these discharge effluents with various harmful and toxic components, including dyes. The mill effluent is also often of a high temperature and pH, both of which are extremely damaging. The colloidal matter present along with colors and oily scum increases the turbidity and gives the water a bad appearance and foul smell. Dye-house effluent typically contains only 0.6–0.8 g/l dye, but the

pollution it causes is mainly due to durability of the dyes in the wastewater (Jadhav *et al*., 2008). Therefore, it is necessary to search for and develop effective treatments and technologies for the decolorization of dyes in such effluents.

Biological technique includes the decolorisation using microorganisms, enzymes etc. Microbial communities are of primary importance in degradation of dye contaminated soils and water as microorganisms alter to dye chemistry and mobility through reduction, accumulation, mobilization and immobilization (Kumar and Bhatt, 2011). In recent years, biodégradation has become a viable alternative and proven to be a promising technology. Microorganisms have been successfully employed as sources for bioremediation (Khan and Husain, 2007). Present study deals with the decolorisation of Reactive orange-16, by the isolate and decolorisation was confirmed through UV-VIS Spectrophotometer.

MATERIALS AND METHODS

Dye- reactive orange-16 purchased by sigma Aldrich details of dye is given below- Reactive Orange-16 (RO-16) is bright yellow orange powder also called with other synonyms as Reactive Orange 3R, Reactive Orange KN-5R, Reactive Brilliant Orange KN-5R and it is a sulfonated nonvolatile, polycyclic aromatic compound.

Structurally it has one benzene ring with sulfonyl ethyl hydrogen sulfate attached to the naphthalene through azo bonding and naphthalene having one amino methyl, one OH and one sulfonate $NaSO_3$ groups makes it more recalcitrant in nature and also structurally it is called as mono azo reactive dye. This is most widely used in cotton or viscose fiber dyeing and also used for cotton and viscose fiber and in printing. It is soluble in water and methanol.

Its color index number is C.I.17757, and CAS Registry Number: 12225,

Molecular Formula: $C_{20}H_{17}N_3Na_2O_{11}S_3$,

Molecular Weight: 617.54, λ_{max} of 500 nm and chemicals required for the decolorisation study purchased from Hi- media.

Media for the decolorization (DM) :

Mmineral salt media prepared as per (Brion *et al*.,1981) with some modifications. MS medium was prepared by adding 10 mL of solution-2 to 100 mL of solution-1and adjusted pH-7.0. The solution-1 was prepared by adding gms/L of $Na_2HPO_4.2H_2O$ (12.00), KH_2PO_4 (2.00), NH_4NO_3 (0.50), $MgCl_2.6H_2O$ (0.10), $Ca(NO_3)_2.4H_2O$ (50.00 mg), $FeCl_2.4H_2O$ (7.50 mg) to 1000 mL distilled water. The solution-2 (trace element solution) was prepared by adding mg/L of $FeSO_4.7H_2O$ (0.10), $MnCl_2.4H_2O$ (3.0), $ZnSO_4.7H_2O$ (10.0), $CuSO_4.5H_2O$ (1.0), $MnSO_4.H_2O$(0.017), $NiCl_2.6H_2O$ (2.0), $Na_2MoO_4.2H_2O$ (3.0), H_3BO_3 (30.0), $CuCl_2.2H_2O$ (1.0) into 1000 mL of distill water. MSM blended with 50mg/L reactive orange16 dye used as dye as sole source of energy for the isolates, un inoculated media serves as control for the decolorisation study. Isolation of isolate was done as per Anjaneya *et al.* (2011). Ten grams of soil sample or 10 mL of water sample (turbid) were added to 100 mL normal saline (0.9%) containing in 500 mL conical flasks containing 100 mL of normal saline and kept on rotary shaker at 120 rpm for one hour and left at room temperature without shaking until all suspended particles were settle down. The supernatant was used for the screening of RO-16 decolorizing microorganisms. Twenty ml of supernatant was inoculated to 100 mL Mineral Salt Medium (MSM) containing 50 mg/L RO-16 as sole source of carbon and incubated at 35^0 C till visible color changed in the flask. The flasks showing more than 50% reduction in the color intensity were selected and decolorisation was confirmed by UV-Vis spectrophotometer taking optical density at 540 nm. Again 20 mL of declorized culture was inoculated into fresh 100 mL DM (Decolorizing medium) containing flasks and were incubated once again and observed for the more than 50% of reduction in the initially added dye. Again, the flasks showing maximum decolorization were selected for the isolation of RR-11 decolorizing microorganism. A 0.25 mL of culture from decolorized those flasks was taken out and inoculated on the MS agar medium containing 50 mg/L of RO-16 by pour/spread plate method. The plates were incubated at 35^0 C till visible growth appeared on the plates. The colonies showing clear zones around them were picked up and streak on the nutrient agar plates and study the cultural and morphological characteristics. Further physiological and biochemical tests were performed to identify isolate up to genus level. The characterized cultures were subculture on MS agar containing 0.1% yeast extract and 100 mg/L RO-16 slants and after growth two slants were stored at 4 ^{0}C after adding 25% of sterile glycerol on the culture surface and one slant used for the further study.

Conformation of decolorisation

For the decolorisation study UV-VIS spectrophotometric study was done ,the decolorized samples optical density checked at 500 nm corresponding to λ_{max} of the dye and With increase in incubation time the peak height at 500 nm goes on decreased and disappeared after 16 hrs of incubation indicates complete decolorisation of added RO-16 Dave and Dave, (2009).

RESULTS

Optimization of various biotic and abiotic parameters

Effect of static and shaking (aeration) conditions

The influence of static and shaking conditions on the decolorization performance of RO-16, by isolate CMGS-2 was studied. The results showed that decolorization of RO-16 by CMGS-2 within 12 hrs under static condition. In contrast under shaking condition organism showed only, 75.2%, of RO-16 decolorization respectively. Therefore, further optimization studies were performed only under static condition.

Optimization of temperature

The decolorization of RO-16 was tested over wide range temperatures from 20 to 50^0 C with an interval of 5^0 C. It was observed that all three isolates showed more than 86% of decolorization between 30^0 C to 45^0 C. CMGS-2 showed maximum of 97.6% of decolorization at 45^0 C and more than 65% of decolorization was observed from 20 to 50^0 C.

Optimization of pH

The optimization of pH for maximum decolorization of RO-16 by isolate was determined using over a wide range of pH from 4 to 14 with the interval of 1. isolates showed maximum of around 90% of RO-16 decolorization at pH-7 to 9. Bacterial isolate CMGS-2 showed consistent decolorisation above 50% in all ranges of pH tested and showed maximum decolorization of 97.4% at pH-9 and was optimum pH for the isolate to decolorize maximum amount of dye.

Optimization of Inoculum size

The optimization of inoculum size for RO-16 decolorization by three isolates was determined with different volumes of initial bacterial inoculums (bacterial concentration was 10^6/mL). CMGS-2 showed more than 96% of decolorization at 5% of inoculum size under static incubation with optimum conditions with pH-8 and temperature 40^0 C.

Optimum incubation time for maximum RO-16 decolorization by isolates under optimum condition.

To know the minimum incubation time required for the maximum decolorisation of RO-16 by each isolates by incubating isolate separately in 100 mL of DM with different concentrations of RO-16 (100 to 1000 mg/L) under static conditions with optimized parameters. Results showed that all three isolates were decolorized the initially added RO-16 maximum of more than 95% within 16 hrs of incubation. CMGS-2 showed maximum of 98.8% decolorization up to 400 mg/L within 12 hrs. Further increased in the dye concentration decreasing of decolorisation was noticed in isolate, percent decolorisation of RO-16 with dye concentrations above 500 mg to 1000 mg/L was observed up to 24 hrs of incubation with each isolate.

Effect of salt concentration

Effect of salt concentration on the RO-16 decolorization efficiency of all three was checked by incubating DM with different concentration of NaCl (1 to 5%). Isolate CMGS-2 not affected more at higher salt concentrations even up to 5% of NaCl and was more than 86% of decolorization.

Effect of additional nutritional sources

To check the effect of additional nutritional source on the decolorization capacity of the organism was performed with various carbon and nitrogen sources. Carbon sources selected like glucose, sucrose and starch were not shown any additional increase in the decolorization activity by bacterial isolate tested. Among nitrogen sources selected only yeast extract alone showed increase in the RO-16 decolorization efficiency of isolate by reducing the incubation time from 72 hrs to 16 hrs. CMGS-2 98.7% within 12 hrs of incubation. However, ammonium nitrate and potassium nitrate showed reduction in the decolorization capacity of the isolates.

Optimization of Yeast extract concentration for maximum decolorization RO-16

Further to determine the optimum concentration of yeast extract for maximum decolorization of RO-16 by isolate, the DM was incubated with different concentrations of yeast extract ranges from 0.05% to 0.2%. The results showed that maximum decolorization was with 0.1% of yeast extract for isolate, further increased in concentration of yeast extract was not shown any effect on the efficiency of RO-16 decolorization.

DISCUSSION

The textile industries are consumed yearly 34 million tons of more than 10,000 varieties of synthetic dyes (Amoozegar *et al*., 2011). When natural water bodies received such effluents alters the pH, turbidity, biological oxygen demand (BOD), chemical oxygen demand (COD), water quality and affects the flora and fauna (Wu *et al*., 2011).

To fulfill the demands of increased world's population, various industries were raised. Industries needs huge amount of water, especially in textile industries, for the production and application of dyes on clothes and these industries leave dye contaminated effluents in to the natural water bodies. One of the major problems that humans are facing is the restoration of the contaminated environment. Textile dye contributes as the most important environment polluting agents, textile industries are the largest consumers of dyes and pigments accounting 80% of total production (Jyothi Kumar Thakur *et al.,* 2014). The present study carried out by isolating a potential isolate from samples collected from different sources like textile dye effluents area inside textile industries samples and treatment unit soil at MIDC, Solapur. The bacterial isolate CMGS-2 isolated from the dye contaminated soil of textile dye waste treatment unit by biochemical and molecular methods it was identified as *Lysinibacillus Boronitolerans* CMGS-2.

As per the literature on the degradation of azo reactive dyes the optimum pH for the maximum decolorization of the dye by bacterial isolates in the range of 6-10 (Aksu, 2003; Guo *et al.,* 2007; Singh *et al.,* 2014). However, our results are revealed isolate have a capacity to decolorize more than 70% at pH-5 though pH-12 and maximum at pH 8 and 9. Our results are in accordance with the report of Imran *et al.* (2014) where they showed that isolate *Shewanella* sp. IFN4 decolorized mixed azo dyes in the pH range of 5-9. CMGS-2 showed maximum decolorisation of RO-16 at 40^0 C. Our results showed CMGS-2 in thermophilic in nature. Among the many reports Ali *et al.* (2010) have reported that the mesophilic range is 25^0 – 45^0C suits for waste water treatment and dye effluents in tropical conditions, our results were according to that.

In the bioremediation of reactive azo dyes by microorganisms is mainly depends on the dye class as well as the types and number of substituent groups and dye concentration in the decolorising medium and effectiveness of the decolorisation mainly depends on dye decolorisation capacity of the isolate, a potential isolate decolorizes dye with maximum concentration and uses shorter incubation time. Isolate CMGS-2 showed maximum decolorisation of RO-16 up to 400 mg/L dye within 12 hrs of incubation, again with increase of dye concentration decolorisation activity lowered. Similar with results Jain *et al.* (2012) showed the complete decolorization of initially added 1500 mg/L of Reactive violet-5 within 42 hrs by a bacterial consortium SB4. Also, Chen *et al.* (2003); Kalyani *et al.* (2009) reported high concentration of dyes inhibits nucleic acid synthesis in microbial cell growth. The inoculums size of dye decolorizing organism depends on its tolerance to toxic substituent's, genetic makeup and adapting ability to toxic environment and nutrients availability. Our results on inoculum concentration of each isolate for the maximum decolorisation of RO-16 was different. In case isolate CMGS-2 showed maximum decolorisation in 5% inoculum concentration. Inoculum concentration varies from species to species in the report of Kumar and Bhatt, (2011) on decolorization of Red 3BN by *B. cereus* optimum inoculum size was 8% and *B. megaterium* inoculums concentration was 10%. In this study the selected bacterial isolate CMGS-2 showed effective decolorisation in all ranges of salts concentrations at 1% it was 98.2 and at 3% it was 94%, at 5% it showed 70% of decolorisation. Bheemaraddi *et al.* (2014) have reported *Paracoccus* sp. shown decolorisation up to 6% salt concentration. Khalid *et al.* (2008) reported salt tolerant bacterium may facilitate the development of biological treatment azo dye for the bioreactor because waste water from dyestuff manufacturing and textile processing industries shows presence of various acids, alkalis, metal ions and salt as impurities. Generally, azo dyes decolorization by mixed as well as pure culture requires additional carbon or nitrogen source because some dyes are deficient in nitrogen concentration. Carbon sources provide energy for the growth and survival of the bacteria and also transfer reducing equivalent to the dyes for azo bond cleavage (Ali *et al.,* 2010) but study in our various carbon and nitrogen sources were selected. Carbon source like glucose, sucrose and starch were used none of the isolate had shown any additional effect on decolorization efficiency in terms of either increase in percent decolorization or in reduction decolorisation time. These revels that microbe's uses dye as a sole source of carbon. However, among the nitrogen sources

tested only organic source that is yeast extract at 0.1% concentration enhanced the decolorisation capacity of organism. Our results are accordance with recent reports of Rajeshwari *et al.* (2011) where the bacterial isolate *Lysinibacillus* RSV-1 showed maximum 95% of decolorization with 100 mg/L initially added dye in the presence of yeast extract and not with addition of other inorganic sources and also with urea.

CONCLUSIONS

The main objectives of the present study were investigating the bacterial degradation of aromatic hydrocarbon derived Reactive dyes, which taken leading position in the production of Reactive dyes. We adapted various methodologies to optimize the environmental, nutritional and operational parameters to achieve optimum decolorisation of Reactive Orange-16 (RO-16) by selected isolate CMGS-2 and this study has contributed and explored the potential native bacterial isolate for the decolorisation of polycyclic reactive dyes under static condition.

REFERENCES

Anjaneya O, Yogesh Souche S, Santoshkumar M, Karegoudar TB (2011) Decolorization of Sulfonated Azo Dye Metanil Yellow by Newly Isolated Bacterial Strains: *Bacillus* sp. strain AK1 and *Lysinibacillus* sp. Strain AK2. *J. of Hazard Materials*, 190, 351–358.

Bheemaraddi M, Patil S, Shivannavar CT and Gaddad SM (2014) Isolation and characterization of *Paracoccus* sp. GSM2 capable of degrading textile azo dye Reactive Violet 5. *Scientific World J.* 2014:410704. doi:10.1155/2014/410704.

Brilon C, Beckmann W, Hellwig M and Knackmuss HJ (1981) Enrichment and isolation of naphthalenesulfonic acid-utilizing Pseudomonads. *Applied and Environmental Microbiology*, vol. 42, no. 1, pp. 39–43.

Dave SR, Dave RH (2009) Isolation and characterization of *Bacillus thuringiensis* for Acid red 119 dye decolorization. *Bioresource Technology*; 100(1): 249–253.

Jadhav SU, Kalme SD, Govindwar SP (2008) Biodegradation of Methyl red by *Galactomyces geotrichum* MTCC 1360. *Int Biodeterioration and Biodegrad*, 62:135–142.

Khan AA, Husain Q (2007) Potential of plant polyphenol oxidases in the decolorization and removal of textile and non-textile dyes. *J Environ Sei*, 19:396-402.

King BR, Long GM and Sheldon HK (1997) Practical environmental bioremediation: The field guide. Boca Raton: CRC.

Kumar Praveen GN and Sumangala K Bhatt (2011) Fungal Degradation of Azo dye- Red 3BN and Optimization of Physico-Chemical Parameters. *Inter J of Environ Sciences,* Volume 1, No 6.