International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 5 -2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.05.012

"Decolorization of reactive Orange 84 Azo dye by *Bacillus cereus* CMGS-5"

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Abstract

Advances in the technology and living standards, increase in the production unwanted wastes and synthetic dyes are the part of it. Dyes are the artificially synthesized colored chemical compounds. Many of them polycyclic hydrocarbons, recalcitrant, and toxic in nature. These chemicals were in nature not degraded by easily and alter the stability of ecosystems. Therefore it is necessary to treat these dyes with proper methods. By taking the task of decolorization , we isolated the a *Bacillus cereus* CMGS-5 bacterial strain, isolated by the textile dye effluent showed decolorization of reactive orange-84 dye, the optimum pH-8 and temp range-40^0 C and optimum dye concentration was 100mg/L, having capacity to decolorize nine structurally different kinds of dyes, and the decolorization confirmed by UV-VIS Spectrophotometer.

Keywords: Decolorization, UV-VIS Spectrophotometer, Optimization.

Introduction

Dyes are the chemical substances with an affinity towards substrate to which it is applied. They are widely used in paper, food, leather, textiles, cosmetics, printing, photography and pharmaceutical industries (Jirasripongam, *et al*., 2007). This dye became essential to humans, but the actual problem comes in degradation of synthetic dyes. Especially azo based dyes have a complex aromatic molecular structure which makes them more stable and difficult to be degraded. (Aksu, 2005). Azo dyes produce some metabolites which are highly toxic, carcinogenic and mutagenic in nature. (Saratale, *et al*., 2011; Gottileb, 2003). Therefore, there is a need of proper treatment to avoid these harmful effects on water bodies (Supaka, *et al*., 2004). Hence, various methods are employed for treating these industrial effluents. The physical and chemical methods are not effective as compared to biological treatment, because they are of high cost consuming and difficult to cleave azo bonds completely (Chacko and Subramaniam, 2011). The biological treatment is very effective. Many organisms are capable of degrading the industrial dyes such as; bacteria, fungi, actinomycetes, yeast and algae. Out of these organisms, the bacteria show maximum degradation within a short period of time when compared to the other organisms (Govindwar, *et al*., 2011). In this direction we selected the novel bacterial dye degrading strain *bacillus cereus* CMGS-5 and the reactive orange-84, widely used in the textiles areas specially on silks, cotton , viscous clothes and in

printings. The purpose of selecting this dye is a polycyclic aromatic, double bond –azo class containing dye. It contains two chlorine groups, eight sulphonic groups, and 12 benzene rings, through these complex structure makes dye into more recalcitrant and toxigenic in nature, easy to fade, water soluble in nature. By these properties of dye we considered for the decolorization studies.

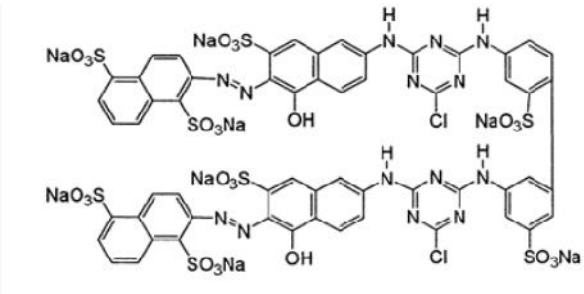
Materials and Methods

Materials: Dyes:

The dyes used in this study are azo reactive dyes namely Reactive orange -84, Reactive blue-4, Reactive yellow – 84, Reactive red -11, Reactive orange-16 and were procured from Colorise and Heena Textiles Industries, Ahmadabad (Gujarat) and sigma Aldrich U.S.A. Out of these reactive orange -84, is a polycyclic aromatic, double bond –azo class containing dye. It contains two chlorine groups, eight sulphonic acid groups, and 12 benzene rings, through these complex structure makes dye into more recalcitrant and toxigenic in nature, water soluble in nature. By these properties of dye we considered for the decolorization studies.

Information and molecular Structure of Reactive orange 84:

Name: C.I Reactive orange 84, C.I.292810
Other names:- Reactive orange HE4R, Reactive orange KE-R.
Molecular Formula: $C_{58}H_{30}Cl_2N_{14}Na_8O_{26}S_8$
Molecular weight: 1850.29
CAS Registry Number: 91261-29-9.
Molecular structure: Double azo class dye



Chemicals:-The chemicals, reagents, media and other requirements used in the study were of analytical grade and procured from Hi-media Pvt. Ltd. Mumbai.

Bacterial culture:

The locally isolated dye degrading bacterial strain CMGS-5 present in this laboratory was used in this study. The bacterial culture collected was first sub cultured on the nutrient agar (NA) plate. The isolated colonies were used for the identification and characterization of isolate by conventional and molecular biological methods. Using conventional methods like cultural, morphological and biochemical methods isolate was tentatively identified as *Bacillus* sps and was confirmed by the 16S rRNA sequence homology. The pure culture of this characterized bacterium was maintained on the NA slants with 25% glycerol in duplicates at 4^o C for future use.

Preparation of pre-inoculum:

Single isolated colony of isolate CMGS-5 on the mineral salt agar medium containing dye (50 mg/L) was aseptically added to 10 ml of nutrient broth and incubated at 35^o C for overnight. After checking the purity of the culture transfer all 10ml to MSB containing 50 mg/L of Reactive Orange -84 and 0.01% of yeast extract and incubated under static conditions. The complete decolorized dye culture was once again checked for its purity and was used as pre-inoculums for further studies.

Preparation of Media and Reagents:

The required media, reagents and solutions were prepared using analytic grade chemicals and solvents by following the standard methods in the microbiological lab manuals and in the concerned literature of decolorization of reactive dyes.

Mineral Salt Medium (MSM): The mineral salt medium (MSM) broth was prepared by adding $Na_2HPO_4.2H_2O$ -12.00 g, KH2PO4 -2.00 g, NH_4NO_3 - 0.50 g, $MgCl_2$. $6H_2O$ -0.10 g, $Ca(NO_3)_2$. $4H_2O$ - 50.00 mg, $FeCl_2.4H_2O$ - 7.50 mg to 1000 ml of distilled water and to this 10 ml of trace elements solution was added before adjusted the pH 7.0. Trace elements solution was prepared by adding $FeSO_4.7H_2O$ -10 mg, $ZnSO_4$. $7H_2O$- 10 mg, $CuSO_4.5H_2O$- 1 mg, $CaCl_2.6H_2O$ - 1 mg, $MnSO_4.H_2O$ -1.7 mg to 1000 ml of distilled water. MS agar medium was prepared by adding 1.8 % agar to MSM broth. All media were sterilized at 121^o C for 15min before use.

Preparation of decolorizing medium (DM): The MSM blended with required amount of testing dyes was used as decolorizing medium (DM) for isolation of dye decolorizing bacteria, optimization of various parameters and determining the decolorizing efficiency of isolated bacteria. DM without inoculants was served as control.

The media required for culturing, maintaining, purity testing, biochemical testing were prepared according to the methods mentioned in the standard microbiology laboratory manuals. The composition of the each media is presented in the Appendix.

Preparation of stains and reagents: The required stains for grams and spores- staining and reagents for biochemical tests were prepared using standard microbiology laboratory manuals the composition of each item is given in the appendix.

Methods:

All the methods used in this study were performed by following the procedures described in the standard microbiology laboratory manuals and in the subject concerned literatures from periodicals.

Characterization and identification of dye degrading bacterial isolate CMGS-5:

The isolated potent RO-84 decolorizing bacterial strain CMGS-5 was characterized on the basis of their cultural, morphological, staining, biochemical and physiological properties. Based on these properties the isolated bacteria tentatively identified up to genus level. Details are described as follows:

1. Cultural characters on different media.

A loop full of freshly culture was streaked by quadrant streak method on the testing agar media and was incubated at 35^{o} C for 24 hrs. After incubation the culture observed for cultural characters like colony size, shape, color, texture, margin etc., on:

a. MSM agar media with RO-84
b. Nutrient agar

2. Morphological characters:

Gram Staining, Shape, size, arrangement of cell, motility, and spore staining were performed.

3. Biochemical tests:

Indole, Methyl red, Voges Proskaur, citrate utilization, gelatin hydrolysis, urease test, utilization test in this was performed for the characteristic identification of CMGS-5.

4. 16S rRNA sequence analysis of isolate CMGS-5:

Further identification and confirmation of isolated bacterium CMGS-5 up to species level by 16S rRNA sequence analysis the pure culture was sent to Royal life sciences, Hyderabad.

Decolorisation assay:

For investigating the ability of the bacterial cultures for decolorization of reactive orange-84, 10 % of bacterial inoculum was added to the 100 ml of MSM with 100 mg/L of reactive orange-84 (Decolorization medium). The decolorization medium without culture served as control. The flasks were incubated at 35^{0} C. To determine rate of decolorization every 4 hour 3 ml of the sample was drawn from each flask and centrifuged at 10,000 rpm for 10 min then supernatant used for taking optical density at 500 nm in a UV–Vis spectrophotometer. A decrease in the optical density compared to control was taken as an indication of decolorization. To confirm the decolorization is due to degradation of dye or due to change in the pH of the medium, adsorption or absorption. To know decolorization is due to change in the pH, the culture filtrate was checked for alteration in the initial pH, if so; again check the color change in supernatant by adding HCl or NaOH. The decolorization is due to adsorption was tested by dissolving the culture pellet in the solvent. Similarly absorption was performed by analyzing the dye in the cell lysate.

Decolorization procedure: Dye decolorisation in MS broth supplemented with yeast extract (0.1% w/v) and reactive orange-84 (100mg/L) complete decolorisation of dye occurred within 24 hours of duration. Dye decolorization was confirmed by the checking optical density at 500 nm for different intervals of time. The percentage of decolorisation was calculated by following equation.

Calculation of % decolorization:-

Percentage of decolorization =initial O.D-final-O.D X 100/initial O.D.

Determination of reactive orange-84 decolorization efficiency of isolate CMGS-5:

To determine the reactive orange-84 decolorization efficiency of isolate CMGS-5 was performed adding 100 mg/L reactive orange-84 to 100 ml of decolorization medium with all optimized conditions then added 10 % of bacterial inoculums and incubated at 35^0 C. To determine rate of % RO-84 decolorization at different time intervals of incubation every 4 hour of interval 3 ml of the sample was drawn from flask aseptically and centrifuged at 10,000 rpm for 10 min then supernatant was used for scanning from 190 to 900 nm in a UV–Vis spectrophotometer. A decrease in the optical density at λ_{max} 500 nm and no peaks at different wave length during the scanning and compared to control indicates decolorization of dye is due to degradation.

Results and Discussion

Characterisation and identification of bacterial isolate CMGS-5:

The results of cultural, morphological, and biochemical characteristics are given in the table1 & figures 1 and 2. The colonies of isolate CMGS-5 on nutrient agar medium observed to be round medium size, with regular margin, smooth and opaque in appearance. The Grams staining reveals that isolate is rod shaped arranged in chain with endospores in the centre of the cells and are found to be non-motile. Results of biochemical tests are listed in the table-2. More than 25 sugars utilization test was performed for this isolate using Hi-carbo Kit procured from Hi-media, the results shown in table -3 Based on all these tests, the isolate CHGS5 was tentatively identified as *Bacillus* sps. This was confirmed and further identified up to species level by 16S r DNA sequence homology with available data base. The 16S rDNA sequence of this isolate and the dendrogram showing the position of our isolate among the Bacillus species presented in figure 3(A) and (B).

Fig. 1: Growth on nutrient agar

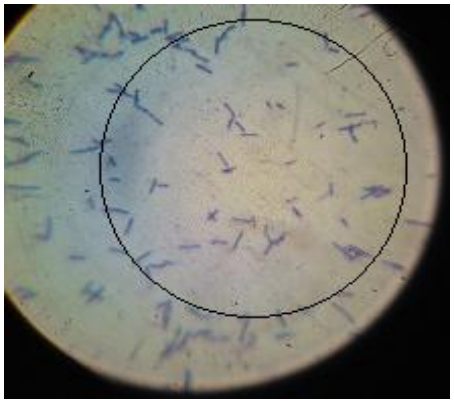


Fig. 2: Gram staining image

Table-1: Cultural and Morphological characteristics of CMGS-5

Tests	Observation
A. Colony character	
Size	Medium
Shape	Round with entire margin
Color	colorless to cream
Texture	Rough, fragile, opaque
B. Morphological Characteristics	
Grams staining	Blue, Small rods (Positive)
Motility	nonmotile
Cell shape and arrangement	Cells arranged in chain spores
Spore	are in centre
C. IMViC	
Indole	Positive
Methyl Red	Positive
Voges Proskaur	Positive
Citrate	Positive
D. Urease production	Negative
E. Gelatin hydrolysis	Positive
Tentatively identified as *Bacillus* species	

Table-2: Biochemical Tests

Test	Result
Indole	Positive
Methyl red	Positive
Voges Proskers	Positive
Citrate utilization	Positive
Gelatine liquifiction	Positive
Urease test	Negative

Table-3: Results of sugar utilization tests

Test	Result	Test	Result
Lactose	Negative	Sorbitol	Negative
Xylose	Negative	Adonitol	Negative
Maltose	Positive	Arabitol	Negative
Fructose	Positive	Erythritol	Negative
Dextrose	Positive	Alpha-methyl-D- glucoside	Negative
Galactose	Positive	Rhamnose	Positive
Raffinose	Negative	Cellobiose	Positive
Trehalose	Positive	Melezitose	Positive
Melibiose	Negative	Alpha-methyl-d - mannoside	Negative
L- Arabionose	Negative	Xylitol	
Mannose	Positive	ONPG	Negative
Inulin	Negative	Esculin hydrolysis	Positive
Sodium gluconate	Positive	d- arabinose	Positive
Glycerol	Negative	malonate utilization	Negative
Salicin	Positive	Sorbose	Negative
Dulcitol	Positive		Negative
Inositol	Negative		
	Negative		

A-

Forward_CMG5-5

GCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGA
GTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATA
GGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTC
TAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTAT
GACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCT
AATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCG
CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTTGGAGCCAGCCGCCTAAGGT
GG
GACAGATGATTGGGGTGAAGTCGT

>Reverse_CMG5-5

CTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTTAGAGCTTGGT
TTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAAC
TCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGC
TTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA
GGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGA
CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG
CGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAA
TAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAA
GTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCA
GAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTG
GCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGAT
TAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTA
GTGCTGAAGTTAACGCA

>Consensus_CMG5-5

GCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGA
GTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATA
GGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTC
TAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTAT
GACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCT
AATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCG
CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTTTGGAGCCAGCCGCCTAAGGT
GGGACAGATGATTGGGGTGAAGTCGTGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGG
CGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAG
TGCTGAAGTTAACGCA

Fig.3: A) 16S r RNA sequence of *Bacillus cereus* CMGS-5.

B-

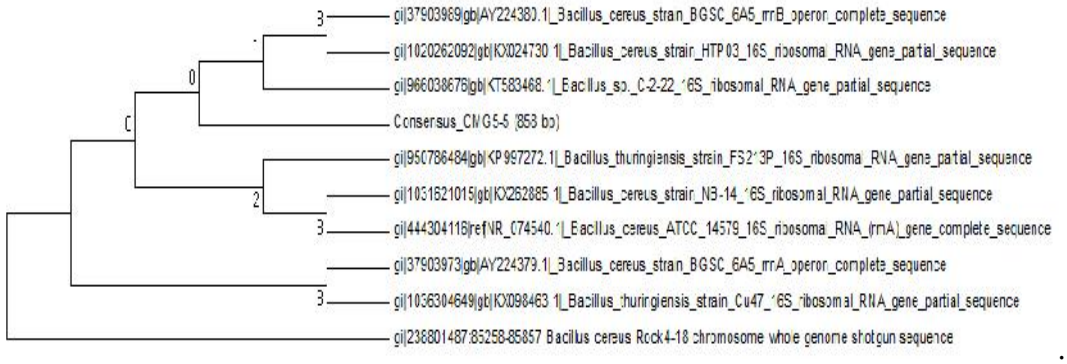


Fig.3: B) Phylogenetic tree of the genus *Bacillus cereus* CMGS-5

Optimization of biotic and abiotic factors:

The various biotic and abiotic factors were optimised for the maximum degradation by *Bacillus cereus* CMGS-5.

Effect of static and shaking conditions:

The effect of aeration or oxygen is one of the critical factors to be considered in the decolourization of RO-84. The influence of static and shaking condition on the decolourization performance of RO-84 (100 mg/l) using *Bacillus cereus* CMGS-5.The 87.97% of decolourization of RO-84 within 24hours by CMGS-5 isolate under static condition while only 54.16% under shaking condition was observed. Thus static condition was adopted for all further decolourization experiments. (Mallikarjun, *et al.*, 2014). Showed static condition only favorable for the degradation. (S. Satheesh Babu, *et al* 2013) found that degradation rate decreased in shaking condition.. (Phugare, *et a*l., 2011) Revealed that many researchers found degradation is by bacterial cells nit through the abiotic factors. .

Optimization of pH

The pH is another important parameter of dye decolourization. Optimization of pH for maximum decolourization of RO -84 by *Bacillus cereus* CMGS-5 was determined by adjusting the decolorization medium containing flasks with increasing pH value 1 at a time starting from pH 4 to 14. At pH 8 the maximum decolorization of 86.65% was observed under static conditions. The organism retains its decolorization ability above 50% of initially added RO-84 through pH 5 to 14 and above 80% between 6-9 pH. We selected pH 8 as optimum for further RO-84 decolorization experiments. . Our results are in accordance with the earlier results on degradation of reactive Red-120 by *Bacillus lentus* BI-377 and (Joe, *et al*., 2008) reported that *Clostridium bifermentus* SL186 was more efficiently degraded the reactive Red 3B-A in alkaline pH of 10 . In contrast (Jain, *et al*., 2012) reported that mixed locally isolated different *Bacillus* sps called bacterial mixed culture SB4 decolorize more than 85% in a wide range of pH 5-8.5, maximum at pH-7 and decolorization was decreased drastically when pH was increased to 10.

Optimization of temperature

The decolorization of RO -84 by bacterial isolate *Bacillus cereus* CMGS-5 was performed over a wide range of temperatures, from 20-50˚C with intervals of 5˚C at a time. The maximum decolorization (87.56%) of initially added RO-84 within 24 hr at 40˚C. However, the percent decolorization was above 80 in a wide range of temperature from 30-50˚C and was decreased drastically when incubated at or below 25˚C. This shows that the isolate *Bacillus cereus* CMGS-5 is heat tolerant and we chose 40˚C as optimum temperature for our further experiments. Similarly isolate *Bacillus cereus* CMGS-5 decolorize the RO-84 maximum at 40^0 C and also retain its decolorizing activity above 80% even at 50^0 C. However, activity drastically decreased to 55% at 20^0 C. It retain its decolorizing activity above 70% between 25 to 55^0 C. Similar reports have been reported by (Oturkar, *et al*., 2010) of decolorization of reactive Red 120 by *Bacillus lentus* BI-377. In contrast, (Jain, *et al.*,2012) reported that decolorization of reactive violet-5R by bacterial mixed cultures-SB4 was maximum 37^0 C. Whereas, *Clostridium bifermentous* SL-186 showed decolorization maximum at 35^0 C (Joe, *et al*.,2008).

Effect of RO-84 dye concentrations on degrading efficiency of *B. cereus* CMGS-5:

In order to check the decolourization efficiency of isolate *B. cereus* CMGS-5 we exposed it for various initial concentrations of RO-84 (100-1000mg/lit). It was observed that with increase in the concentration of RO-84 the degradation efficiency of isolate was increased up to 200 mg/L thereafter reduced gradually and was about 60% at concentration of 1000 mg/L. *B. cereus* CMGS-5 was able to decolorize maximum of 87.57% initially added RO-84 within 24 hours of incubation up to 200 mg/L concentrations only. . The decolorization process by particular organisms for the removal of azo dyes depends on the class as well as varying substituent groups and dye concentration in the medium. The RO-84 is complex polycyclic with more sulfonic acid groups (SO_3H) which acts as detergents and surfactants and can inhibits the growth of the organism. The isolate *B.cereus* CMGS-5 more efficiently decolorizes RO-84 up to the concentration of 200mg/l within 24 hr of incubation further increase in the dye concentration observed the gradual reduction in the decolorizing efficiency and was 60% at 1000mg/l dye concentration and also increases the incubation period. Most of the earlier reports suggest 100% decolorization of initially added dye upto 200-400 mg/l within the 24 hr and it was reduced to 60-70% when increased the dye concentration to 1000mg/l. (Jain, *et al*.,2012; and Joe, *et al*.,2010) however, (Kalyani, *et al*., 2009) reported that *Pseudomomas* sps SUK1 could able to decolorize 80% of 5000 mg/l initially added reactive red 2 within 10 h.

Effect of salt concentration

To check the effect of salt concentration on the decolourization of RO -84 by *B. cereus* CMGS -5 by increasing 1% NaCl concentration at a time in the decolorization medium up to 10%. gradual decrease in the decolorization efficiency of *B. cereus* CMGS-5 with 1% of NaCl added. However, it is more than 60% decolorization even at 4% of salt and was only 37% at 5% of NaCl. This shows that this isolate is not tolerate higher salt concentrations.. Isolate *Bacillus cereus* CMGS-5 is moderate salt tolerant and decolorize the RO-84 more than 60% at 4% NaCl under the optimum decolorizing conditions. Our results are agree with (Oturkar, *et al*., 2010) in which maximum decolorization reactive red 120 by *Bacillus lentus* BI377 at 1% of salt concentration however (Jain, *et al*.,2012) reported less than 1% salt in the decolorizing medium favours the maximum degradation of reactive violet 5R by mixed bacterial culture SB4.

Optimization of size of inoculums:

The effect of inoculum size (1 -20%) on Ro-84 decolourization by *B. cereus* CMGS-5 under the optimized abiotic parameters was performed by increasing percent of inoculums in the flacks containing decolorizing medium from 1 to 20.The increase in inoculum size above 1% resulted in gradual increase in the percent decolourization up to 5% thereafter there is no considerable increase or decrease in the decolorization up to 20% of inoculums. In 5% inoculum size decolourization was maximum of 86.79% in 24 hours. Therefore the optimum inoculum size of 5% was fixed for further experiments.(Rajee, *et al*., 2011) used 5% inoculum of *Micrococcus* sps to decolorize azo dye Orange MR. During azo bond cleavage, the cells compete for electron donors which effect decolorization even at low percentage of inoculum size. In the degradation of red 3BN Bacillus cereus shown inoculums size 8% .(Praveen Kumar and sumangala.2012).

Effect of additional nutritional sources:

The effect of additional carbon and nitrogen nutritional sources on the RO-84 decolorizing efficiency of *B. cereus* CMGS-5 using different sugars, polysaccharides, organic and inorganic nitrogen sources was performed.

Effect of additional nitrogen sources:

The 1g/L of different nitrogen sources like yeast extract, sodium nitrate, ammonium nitrate was added to the decolorizing medium with all optimized conditions to know the effect on the RO-84 decolorization efficiency of isolate *B. cereus* CMGS-5. In yeast extract maximum decolourization of 89% were as in sodium nitrate and in ammonium nitrate found to be 83% and 82% respectively. The isolate *Bacillus cereus* CMGS-5 is capable of utilize 70% of RO-84 as a sole sources of carbon under static incubation within 72 hours. There was no 100% decolorization of this dye by isolate Bacillus *cereus* CMGS-5 even after addition of additional carbon and nitrogen sources but reduction of decolorization time period was observed. Many reports are support the addition of yeast extract as nitrogen source increases the decolorization process using bacteria (Oturkar, *et al*., 2010; Jain, *et al*.,2012). (Ola, *et al*., 2010) showed

that *Bacillus cereus* able to decolorize 81% of Cibacron red PUB in the presence of ammonium nitrate and sucrose while it decolorizes 75% Cibacron black PSG with addition of yeast extract and lactose.

Determination of lowest concentration of yeast extract for maximum decolorization of RO-84.

Among the nitrogen sources tested, yeast extract was efficient in enhancing the decolourization of RO-84 by *B. cereus* CMGS-5. Now, to know the degree of influences of varying concentrations of yeast extract (0.5-2g/L) on decolourization efficiency of CMGS-5. The results are shown in table-12 and fig. 13. The decolourization efficiency of CMGS-5 was increased with increase in yeast extract concentration from 0.5-2 g/L. In 1.5 g/L of yeast extract and the maximum of 87.97% of decolourization was observed. Therefore 1.5 g/L of yeast extract concentration was used as optimal additional nitrogen source for all dye decolourization experiments in this study. (Jain, *et al*., 2012) reported that yeast extract was essential for regeneration of NADH which act as electron donor in azo bond reduction. So, yeast extract may increase the transports like NADH which are essential for the azo bond cleavage. (Mallikarjun, *et al*., 2014) shown 1g/L yeast extract optimum for 100mg /L RV-5 dye. (Rajeshwari, *et al*., 2012) shown yeast extract is a better substrate for degradation.

Effect of additional carbon source:

The effect of carbon sources like glucose, lactose, sucrose on the decolourization of reactive orange-84 by *B. cereus* CMGS-5 From the figure it is revealed that all sugars are not influenced on decolorization of RO-84 however the maximum of 88% decolourization was of both glucose and lactose and 86% with sucrose. However, in our study there is no much influence on decolorizing efficiency on addition of carbon sources similar reports are reported on RV-5 dye decolorization using bacterial strains (Joe *et al.,* 2008; Mallikarjun *et al.,* and 2014; Moosvi *et al.,* 2004).

Decolorization of structurally different dyes:-

To know the dye decolorizing efficiency of isolate *B. cereus* CMGS-5 for other structurally different dyes used in the textile industries we designed this experiment. The results are represented in table-4 and fig.4. We also observed that the organism has capacity to decolorize more than 50% initially added 8 dyes individually. More than 4 dyes degraded more than 90% remaining all are showed more than 50% except reactive blue-4 (48.56%). And figure represent the results of mixed dyes colorization by the potential strain bacillus cereus CMGS-5 at various incubation time. The percent decolorization increase up to 72 hours of incubation after that it slows down and become almost nil after 78 hours of incubation. The isolate *B. cereus*-CMGS5 is able to decolorize more than 9 structurally different reactive dyes tested in this study. This is might be due the consequences of natural adaptation as this isolated from the dye contaminated area at Solapur. Similar report have been reported by (Mallikarjun, *et al*., 2014; Ola, *et al*., 2010). Ola et al also reported that the ability of *B. cereus* to degrade more numbers of reactive dyes because they are naturally versatile and carries an efficient enzymatic system for the cleavage of azo bonds.

Table 4 dye decolorizing efficiency of isolate *B. cereus* CMGS-5

Different dyes	Percentage of decolourization
Reactive Orange 84	84.60%
Reactive Blue 59	92.10%
Reactive Yellow 84	51.50%
Reactive Blue 4	48.56%
Reactive Violet 1	90.51%
Reactive Red 11	93.21%
Reactive Orange 16	94.34%
Crystal Violet	63.68%
Mrthylene Blue	89.19%

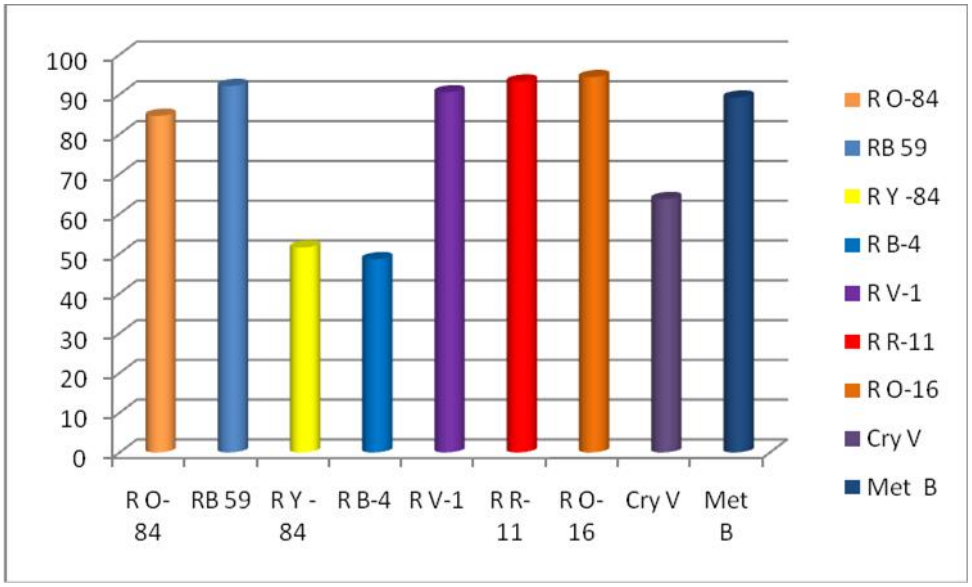


Fig-4: Decolorisation of different types of dye by *Bacillus cereus* CMGS-5.

Analysis of dye degradation through UV-VIS Spectrophotometer.

Decolourization assay of reactive orange 84 shows that results of optimization of reactive orange 84 dye decolourization by the bacterial strain bacillus cereus CMGS-5. CMGS-5 at various incubation time beginning with 4 hours of incubation time to 48 hrs with regular intervals of every 4 hrs of incubation time. The percent decolourization values were calculated by taking OD of culture supernatant of decolorizing culture at different incubation period and results are depicted in the figure maximum of 87.57 % dye degradation was achieved by the Bacillus CMGS-5 at an incubation time of 24hours. Further incubation does not shown increase in the decolourization even after an incubation time up to 48hrs so we observed that this organism can decolorize reactive 84 up to 87% but not completely and also confirms that decolourization of reactive orange 84 is due to degradation of dye (Fig 5).

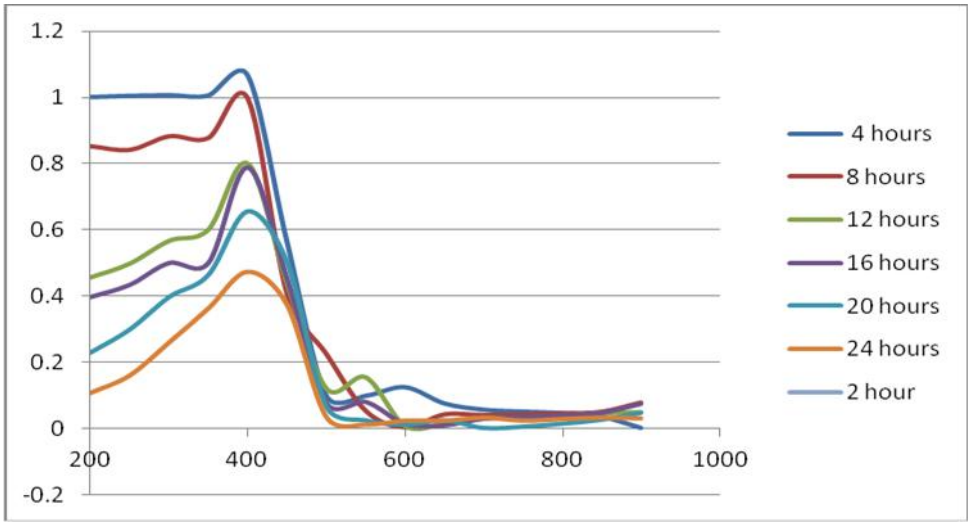


Fig.5: Percent decolorisation of RO-84 in different incubation by *Bacillus cereus* CMGS-5

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DOI:10.22192/ijarbs.2019.06.05.012	

How to cite this article:
Madhuri R. Basutkar, Mukesh Mala, Rashmi, Pruthvi, Subhaschandra M. Gaddad and C.T. Shivannavar. (2019). "Decolorization of reactive Orange 84 Azo dye by *Bacillus cereus* CMGS-5". Int. J. Adv. Res. Biol. Sci. 6(5): 98-109.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.05.012