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Study On: "Bacterial Decolourization of Textile Grade Coravat Red Dye by *Rhizobium Mayense*"

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Abstract: *Rhizobium isolate obtained from nodules of stem and root of Aeschynomene indica plant has ability to decolourize of textile grade Coravat Red dye. The Rhizobium isolate was identified by 16S rRNA sequencing as Rhizobium mayense. The ability of the Rhizobium mayense for the effective decolourization Textile grade Coravat Red Dye was evaluated. The effects of various physicochemical parameters as pH, temperature, Carbon, Nitrogen, Incubation condition and Incubation period were analysed for maximum decolourization. The Rhizobium mayense efficiently decolourized 50mcg / ml concentration dye after 48 hours of incubation period at optimum pH 7.4 and at 27°C under aerobic shaking conditions as 85.82% and 90.92% respectively. The dye decolourization efficiency of this strain was maximum at 0.3%(w/v) Sucrose as carbon source and was 83.93% and at 0.1% (w/v) Ammonium sulphate as a nitrogen source and was 84.68%.The bio-decolourization of Textile grade Coravat Red Dye was confirmed by UV- Spectrophotometer.*

Keywords: *16S rRNA sequencing, Rhizobium mayense, Textile grade Coravat Red Dye, Decolourization, UV-Spectrophotometer.*

1. INTRODUCTION

Synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetics and other industries (Ren et al, 2006). Major classes of the synthetic dyes include azo, anthroquinone and triarylmethane dyes (Khadijah, et al, 2009). Among these azo dyes represent the largest and most versatile class of synthetic dyes. There are more than 1, 00,000 commercially available dyes with over 7 X 10^5 tons of dyestuff produced annually (Zollinger H., 1987). Generally colours are visible at a dye concentration of more than1 mg / l is undesirable and an average concentration of 300 mg / l has been reported from textile manufacturing processes (Goncalves,et al, 2000). They are toxic and xenobiotic in nature. Some azo dyes are carcinogenic in nature to the human being. Most of the time the textile effluents are discharged into the water bodies without any treatment. The difficulties encountered in the waste water treatment resulting from dyeing operations lies in the wide variability of the dyes used and excessive colour to the effluent. Colour present in the effluent affect the photosynthetic activity in aquatic life due to reduced light penetration and may be toxic to some aquatic organisms due to presence of metals and chlorides (Van der Zee, 2006).

For the decolourization of coloured textile effluent, several physico-chemical methods are developed over the last 25 years. These include filtration, coagulation, flocculation, carbon activated, flocculation and chemical flocculation. All these methods are effective but are expensive, low efficiency and inapplicability to a wide variety of dyes. Since no single process is able to decolorize all textile effluents, a solution is to use the combination of different methods (Banat et al, 1996). The success of a biological process for colour removal from textile effluent mainly depends on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures. Many bacteria, actinomycetes, yeast and molds are able to remove dyes by adsorption from the effluent (Zhou,W.;Zimmermann, W.,1993).Microbial decolourization is the eco-friendly and cost effective method and alternative to physical and chemical methods. (Verma, P. and Madamwar, D., 2003)

The objectives of the present study were:

- To identify the stem and root nodulating bacterium of the *Aeschynomene indica* plant
- To study the potential of *Rhizobium mayense* in decolourization of Textile grade Carovat Red Dye
- To study the effect of different physico-chemical parameters on dye decolourization

2. MATERIALS AND METHODS

➢ Dyes and Chemicals

The azo dye Textile grade Carovat Red was obtained Central Institute for Research on Cotton Technology, Mumbai. This is extensively used in dyeing of cotton fibres. Absorption maxima for the dye was obtained by scanning dye solution over visible range of 450-750nm. The stock solution was prepared in Mineral Salt Medium with the concentration of 50 mcg / ml. All other chemicals were of analytical grade and were obtained from Merck and Hi Media.

➢ Isolate used for decolourization

Rhizobium mayense is the root and stem nodulating bacterium isolated from *Aeschynomene indica* plant using Yeast Extract Mannitol Agar and was identified by 16S rRNA sequencing.

➢ Efficiency of the isolate for Textile grade Carovat Red dye decolourization (Wang, H., et al,2009)

Experiments with *Rhizobium mayense* were performed in Mineral Salt Medium with composition: K_2HPO_4: 0.16 gm, KH_2PO_4: 0.02 gm, $(NH_4)\ SO_4$:0.1gm, $MgSO_4.7H_2O$: 0.02 gm, $FeSO_4.7H_2O$: 0.001 gm, NaCl: 0.01gm, $CaCl_2.2H_2O$: 0.002gm, Glucose :0.3 gm, Yeast extract: 0.1 gm, Distilled Water: 100ml, pH: 7.5. 250 ml flasks containing 100 ml medium each were autoclaved for 20 minutes at 121°C and 15 lb pressure. Inoculum (2% v/v) was added aseptically to the experimental flasks with initial dye concentration of 50 mcg / ml and incubated at room temperature at 150 rpm. Suitable control without any inoculum was also run along with experimental flasks. Samples were withdrawn at 24 hours interval for 72 hours. The withdrawn samples were centrifuged at 10,000 rpm for 20 minutes at 5°C (Temperature controlled BioEra refrigerated Centrifuge). The absorbance of the supernatant of the withdrawn samples was measured spectrophotometrically at 540 nm (Labindia UV 3000, UV/Vis Spectrophotometer).

➢ Influence of pH on the decolourization of Textile grade Carovat Red dye

2.0 ml of the fresh culture was inoculated in sterile Mineral Salt Medium adjusted with different pH values as 7.0, 7.4, 8.0. All the flasks were incubated at 27°C on a shaker with speed of 150 rpm and in the incubator at a static condition. The experiment was carried out in duplicate. Non-inoculated culture medium was used as control.

➢ Influence of temperature on the decolourization of Textile grade Carovat Red dye

2.0 ml of the fresh culture was inoculated in sterile Mineral Salt Medium .The flasks were incubated at 27°C, 37°C and 45°C on a shaker with speed of 150 rpm and in the incubators in a static condition. The experiment was carried out in duplicate. Un-inoculated culture medium was used as control.

➢ Influence of nitrogen on the decolourization of Textile grade Carovat Red dye

2.0 ml of the fresh culture was inoculated in sterile Mineral Salt Medium with 0.1 gm % of $(NH_4)\ SO_4$, $NaNO_2$, $NaNO_3$, Urea and Beef extract as a sole source of nitrogen. All the flasks were incubated at 27°C on a shaker with the speed of 150 rpm and at a static condition in the incubator. The experiment was carried out in duplicate. Un-inoculated culture medium was used as control.

➢ Effect of carbon on the decolourization of Textile grade Carovat Red dye

2.0 ml of the fresh culture was inoculated in sterile Mineral Salt Medium with 0.3 gm % of Glucose, Mannitol, Sucrose, Lactose and Starch as a sole source of carbon. All the flasks were incubated at 27°C on a shaker with the speed of 150 rpm and in the incubator at a static condition. The experiment was carried out in duplicate. Non-inoculated culture medium was used as control.

The decolourization was expressed in terms of percentage of dye decolourization and was calculated by using following formula:

$$\% \text{ Dye Decolourization} = \frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial Absorbance}} \times 100$$

3. OBSERVATIONS AND RESULTS

The stem nodulating isolate obtained from *Aeschynomene indica* plant is identified by 16S rRNA sequencing technique. The DNA sequence of AIRS-I isolate was compared with Gene Bank Data Base using BLAST algorithm available from NCBI (www.ncbi.nlm.nih.gov). It showed 98.64% query coverage as ***Rhizobium mayense*** by 16S rRNA with as Accession number of JX855172 in Gene Bank Data Base. The isolate was named ***Rhizobium mayense.*** The Sequences of the primer pair used for amplification -

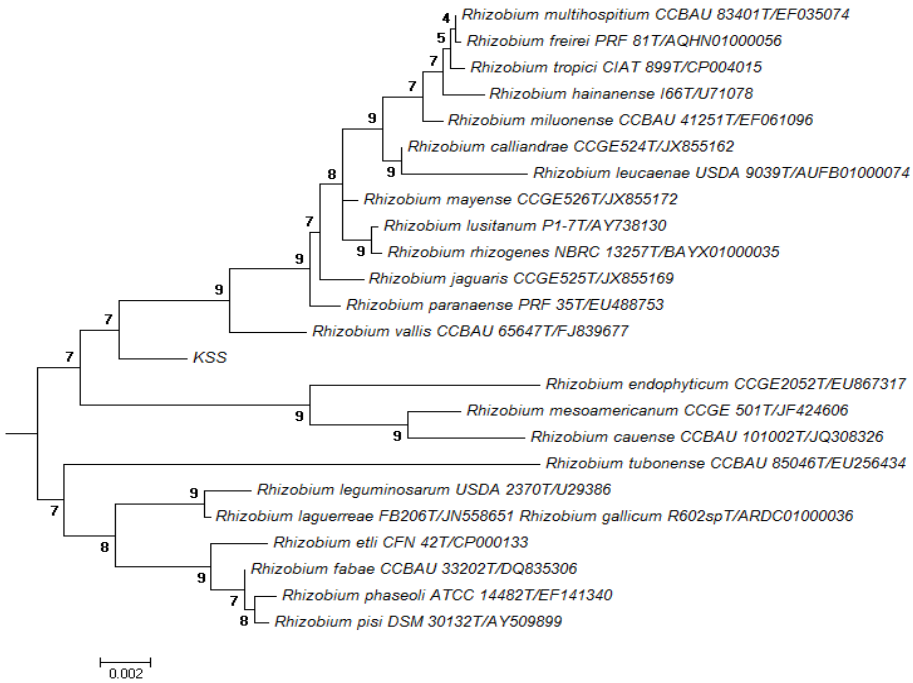
RPP2 – CCAAGCTTCTAGACGGITACCTTGTTACGACTT

FDD2 – CCGGATCCGTCGACAGAGTTTGATCITGGCTCAG

3.1. The Obtained DNA Sequence

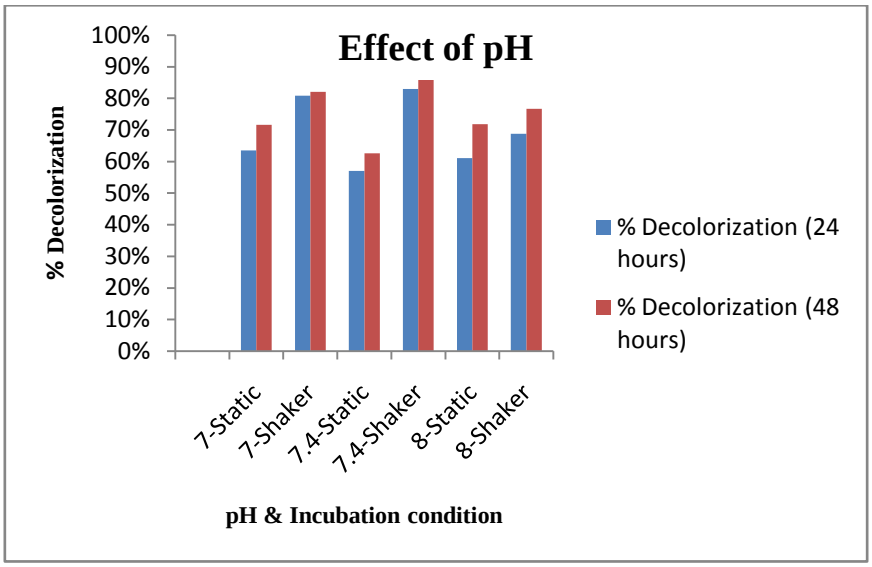
CGTGGGTATTTGGCAATGGGCGCTAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGG
CCCTAGGGTTGTAAAGCTCTTTCACCGGAGAAGATAATGACGGTATCCGGAGAAGAAGC
CCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAA
TTACTGGGCGTAAAGCGCACGTAGGCGGATCGATCAGTCAGGGGTGAAATCCCAGGGCT
CAACCCTGGAACTGCCTTTGATACTGTCGATCTGGAGTATGGAAGAGGTGAGTGGAATTC
CGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCA
CTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCC
TGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGTCGGGCAGTATACTGTTCGGTGGCG
CAGCTAACGCATTAAACATTCCGCCTGGGGAGTACGGTC

3.2. Phylogenetic Tree of Rhizobium Mayense based on 16S rRNA Sequence



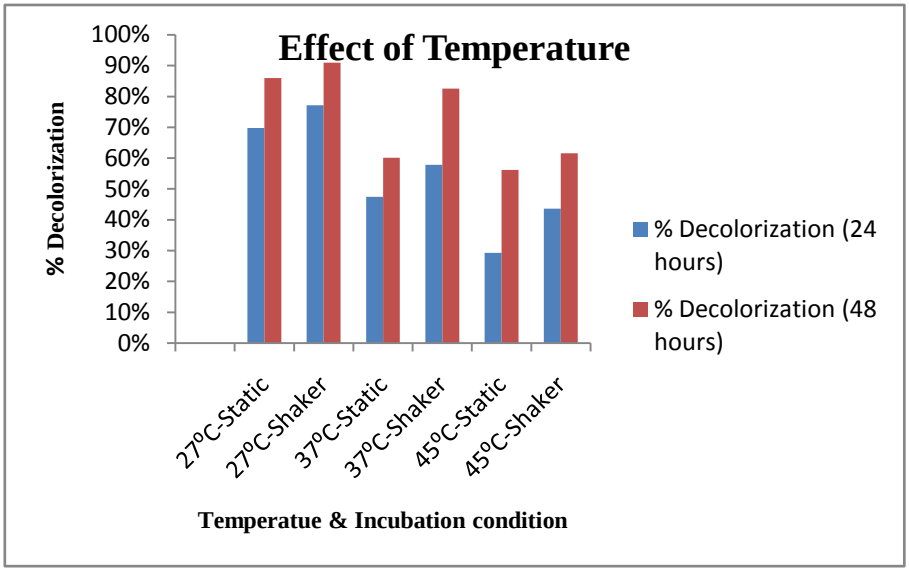
3.3. Effect of pH on Textile Grade Coravat Red Dye Decolourization

Sr.No.	pH value	Incubation condition	Percentage Dye Decolourization (24 hours)	Percentage Dye Decolourization (48 hours)
1	7.0	Static	63.51%	71.64%
		Shaker	80.90%	82.04%
2	7.4	Static	57.08%	62.57%
		Shaker	82.98%	85.82%
3	8.0	Static	61.05%	71.83%
		Shaker	68.80%	76.74%



3.4. Effect of Incubation Temperature and Condition on Textile Grade Coravat Red Dye Decolourization

Sr.No.	Incubation Temperature (°C)	Incubation Condition	Percentage Dye Decolourization (24 hours)	Percentage Dye Decolourization (48 hours)
1	Room Temperature	Static	69.75%	86.01%
		Shaker	77.12%	90.92%
2	37°C	Static	47.44 %	60.11%
		Shaker	57.84%	82.60%
3	45°C	Static	29.30%	56.21%
		Shaker	43.66%	61.58%



3.5. Effect of Nitrogen Source on Textile Grade Coravat Red Dye Decolourization

Sr. No.	Nitrogen Source	Incubation Condition	Percentage Dye Decolourization (24 hours)	Percentage Dye Decolourization (48 hours)
1	Urea	Static	3.21%	8.56%
		Shaker	7.56%	10.58%
2	Beef Extract	Static	10.12%	39.85%
		Shaker	20.26%	46.31%
3	Ammonium Sulphate	Static	51.20%	69.09%
		Shaker	67.29%	84.68%
4	Sodium Nitrate	Static	2.12%	8.00%
		Shaker	4.72%	26.46%
5	Sodium Nitrite	Static	45.74%	58.66%
		Shaker	61.05%	82.60%

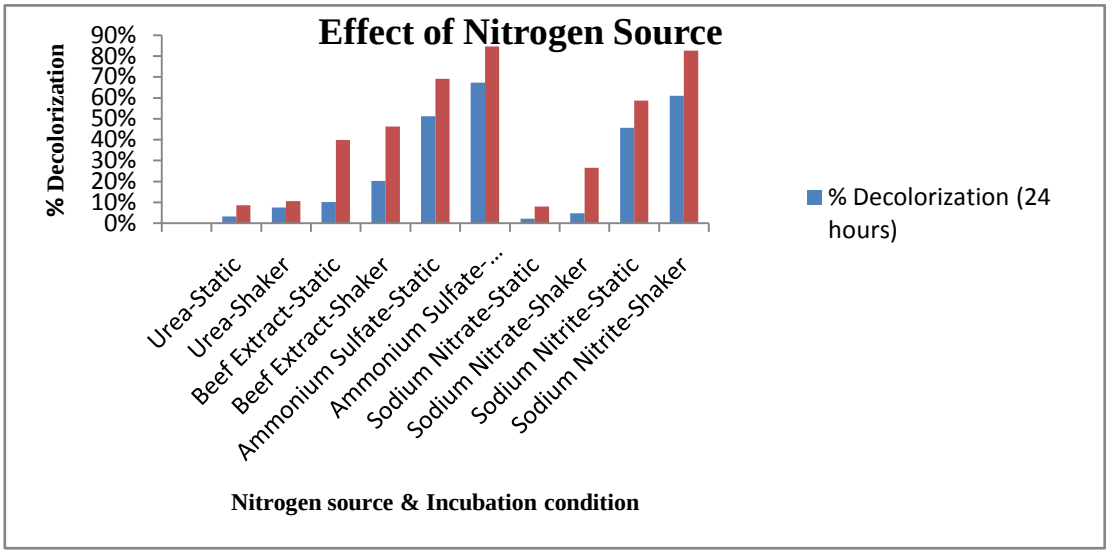
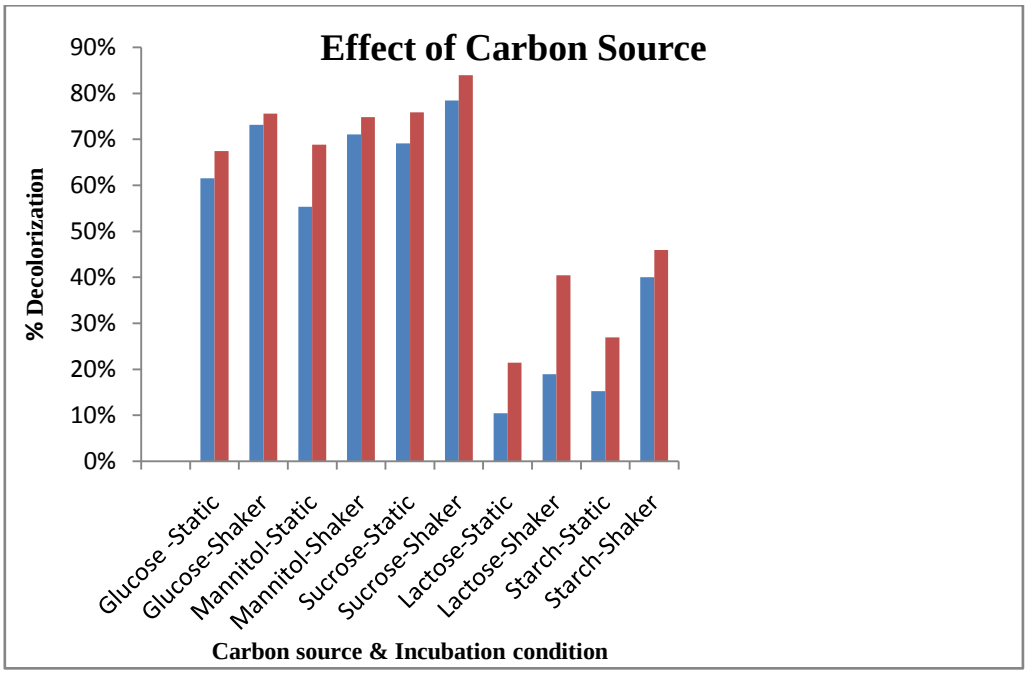


Fig3. *Effect of Nitrogen source on Textile Coravat Red Dye decolourization after 24 hours*

Fig4. *Effect of nitrogen source on Textile Coravat Red Dye decolourization after 48 hours*

3.6. Effect of Carbon Source on Textile Grade Coravat Red Dye Decolourization

Sr. No.	Carbon Source	Incubation Condition	Percentage Dye Decolourization (24 hours)	Percentage Dye Decolourization (48 hours)
1	Glucose	Static	61.53%	67.47%
		Shaker	73.15%	75.61%
2	Mannitol	Static	55.32%	68.87%
		Shaker	71.07%	74.85%
3	Sucrose	Static	69.12%	75.84%
		Shaker	78.44%	83.93%
4	Lactose	Static	10.45%	21.47%
		Shaker	18.90%	40.45%
5	Starch	Static	15.21%	26.95%
		Shaker	40.00%	45.93%



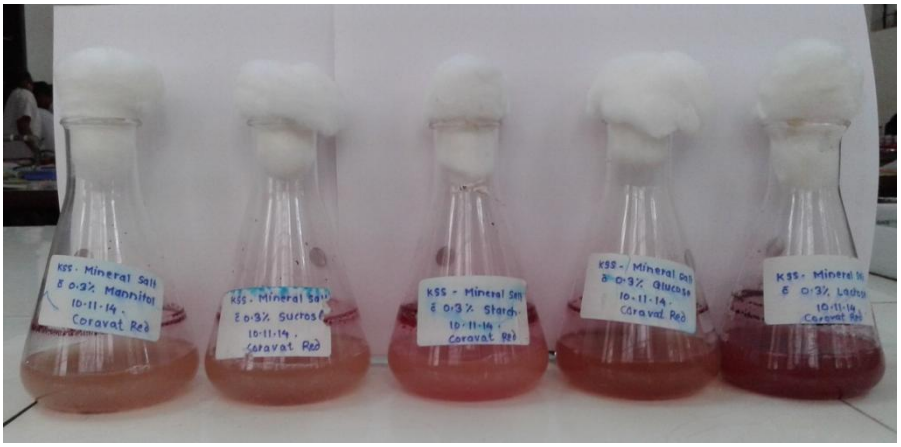


Fig1. *Effect of carbon source on Textile Coravat Red Dye decolourization after 24 hours*

Fig2. *Effect of carbon source on Textile Coravat Red Dye decolourization after 48 hours*

4. DISCUSSION

The stem and root nodulating bacterial strain *Rhizobium mayense* has shown 90.92% decolourization of Textile Grade Carovat Red Dye with 50 µg / ml initial concentration within 48 hours. Prolonged incubation resulted in decreased dye decolourization. This might be due to toxicity of the dye to the bacterial cells through the inhibition of metabolic activity, saturation of the dye and its products on the cell. The *Rhizobium mayense* showed maximum dye decolourization with 1.0gm% (w/v) Sucrose as a main carbon source. Other carbon sources tested were *viz.* Mannitol, Sucrose, Lactose and Starch and the organism also showed dye decolourization in their presence. Coughlin, M.F., et al, 1999 reported

that the dye decolourization of certain textile dyes like Orange II, A08 and AR-88 by *Sphingomonas* sp. strain ICX was observed only in the presence of carbohydrate. Addition of Ammonium sulphate at 0.1gm% (w/v) as nitrogen source resulted in maximum decolourization 84.68% by *Rhizobium mayense* . Nachiyar, C.V. and Rajkumar, G.S., 2003 reported that Ammonium salts were the best nitrogen sources for the decolourization of Navitan Fast Blue by *Pseudomonas aeruginosa.* Coughlin, M.F., et al, 1999 reported similar findings of Ammonium salts supporting decolourization of Orange II, A08 and AR-88 by *Sphingomonas* sp. strain ICX. Efficient decolourization of the dye was obtained at pH 7.4 by *Rhizobium mayense. Bacillus subtilis* HM exhibited colour removal capacity of Fast Red over a wide range of pH of 5 -9 with optimum pH at 7.0 (Mona, E.M. and Hoda, H.Y., 2008).The optimum temperature for *Rhizobium mayense* for maximum decolourization was 27°C with efficiency of 90.92%. Wang, H. e*t al*, 2009 studied decolourization of Reactive Black by bacterial strain Enterobacter sp. EC3 and found that with the increase in temperature from 22°C- 37°C, the decolourization rate was increased. Further increase in the temperature up to 42°C drastically affected decolourization.

5. CONCLUSION

The organism used in the present study is a root and stem nodulating Rhizobial strain obtained from *Aeschynomene indica* plant which was identified as *Rhizobium mayense* based on 16 S rRNA sequencing. The results showed that the dye decolourization mainly depends on carbon and nitrogen source in the medium, pH of the medium, incubation temperature and incubation condition. 90.92% decolourization of Textile Grade Coravat Red Dye was observed at 50 µg / ml initial concentration at pH 7.4 and at 27°C after 48 hours of incubation on a shaker. The results showed that the addition of Sucrose sugar at 1.0gm% (w/v) concentration and Ammonium sulphate at 0.1gm% (w/v) as nitrogen source resulted in maximum decolourization of Textile Grade Coravat Red Dye. The decolourization of Textile Grade Coravat Red Dye by *Rhizobium mayense* was confirmed by UV-Visible Spectrophotometer.

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