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Decolorization of structurally dissimilar dyes by a termite derived bacterial consortium BUTC17 in a cost effective nutrient medium

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ABSTRACT

The present study aimed at isolating bacterial strains from termites and to check their efficiency as individual strains and as a consortium in decolorizing 14 structurally different dyes at static and shaking conditions. Twelve morphologically different colonies were identified during the initial phase of this experiment which are further subjected for their dye decolorizing capability. Initial screening established that decolorization of dyes resulted better when individual strains in static incubation (79.5%) condition was applied. But further experiments on employing various consortia made clear that consortia comprising 4 individual isolates displayed a better decolorization efficiency of Reactive blue 220 which was calculated to be about 94.3% When various media was used to make out a cost effective media for decolorization, it was observed that decolorizing medium in static condition favored better decolorization of 95.3% and 94.4% of Reactive blue 220 and Reactive red 195 and growth of the consortium was also well supported under this condition. The overall results suggested that these bacterial strains as a consortium holds a great novelty and will render a higher performance of decolorization in a economical nutrient medium which in turn has a huge application when applied in a large scale.

Keywords: Biodecolorization, Termite, Microorganisms, Reactive dyes, Consortium

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INTRODUCTION

Textile industries play a vital role in the economic increases in India and water is one of the major products of nature used enormously by human beings and it is natural that any growing community generates enormous waste water or sewage [1]. Textile industries dispose the waste water during the process of dyeing and also generate the effluent system [2] and such effluent containing dyes are organic colors having less water solubility [3]. Such generated dyes are toxic and carcinogenic in nature and contamination by these toxic chemicals is emerging as a serious global problem. Dyes from various industrial effluents may also cause skin cancer due to photosensitization and photodynamic damage [4] and employing wastewater treatment for removal of these toxics and colour will make the effluent usable for industrial or domestic use [5].

Environmental biotechnology relies upon the pollutant degrading capacities of naturally occurring microbial consortium in which bacteria play central role [6]. Microbial consortia are used as black boxes without analyzing the constituent microbial populations for environmental remediation. The complexity of microbial consortium enables them to act on a variety of pollutants [7]. Bacterial consortium generally has several advantages. Firstly, they do not

require sterile conditions, thus greatly reducing costs. Secondly, they are in general more stable towards changes in pH, temperature and feed composition, when compared with pure cultures [8]. Finally, there is a higher possibility of a complete mineralization of the dye since few strains have been found that it can metabolize wide range of compounds [9]. The advantage of bacterial application in textile industrial wastewater is normally faster [10]. Fungus-cultivating termites, which feed on wood, contain wide variety of intestinal microbial symbionts which are able to mineralize monoaromatic compounds. Such microorganisms are a potential resource in biotechnological processes for alleviating pollution from synthetic compounds [11, 12].

Termite gut harbors 10^6–10^8 microorganisms comprising >300 species of protists, bacteria, and archaea of symbiotic microorganisms which play key physiological functions such as cellulose and hemicelluloses digestion, acetogenesis, hydrogenesis, methanogenesis, sulfate reduction, and nitrogen fixation [13, 14]. Very few studies have been performed using sequential microaerophilic/ aerobic conditions with the same microorganism, preferring the use of consortia or different microorganisms, used separately under anaerobic, microaerophilic and aerobic conditions [15]. Symbiotic relationship between termites and their intestinal microbes was demonstrated and concluded that both termite soil and termite gut bacteria play an important role in polymer depolymerization [16] and application of such potent microorganisms from termites in environmental or industrial processes have a greater potential in treating dye related waste waters.

In this regard, the present study was designed which will serve as a platform to unravel the potential of a novel bacterial consortium capable of decolorizing structurally different dye stuffs. This was assessed with the following objectives: a) Isolation of bacterial strains from termite homogenate and checking their decolorization efficiency against 14 different dyes under static and shaking condition; b) Development of different consortium from indigenous strains and their decolorization pattern against 14 dyes; c) Identification of potent bacterial strains in BUTC17 consortium by morphological and biochemical characters and d) Selection of effective nutrient medium for better decolorization of selected dyes in optimized conditions.

MATERIALS AND METHODS

2.1. Description of sampling site

The environment of Bharathiar University, Coimbatore abutting the foothills of Western Ghats is unique one, is also a part of Nilgiri Biosphere Reserve (NBR) buffer zone [17] and the University environment is a flourishing ground for a rich diversity of flora and fauna and it is an ideal for a copious variety of insects and other organisms.

2.2. Collection of termites

Wood-eating termites (*Macrotermes* sp), one of the most interesting examples of a complex symbiotic community of prokaryotic and eukaryotic microorganisms and these microorganisms are responsible for digestion of lignocellulosic substances. Hence termites were collected from Bharathiar university campus with the help of sterilized spatula and separated using forceps from soil and kept in sterile polyethylene bags. The termite containing polybags were transported to laboratory immediately.

2.3. Preparation of termite homogenate

Termites were washed with sterile distilled water and surface sterilized with diluted ethanol (70%). Ten grams of termites were homogenized in a sterile mortar with sterile distilled water and the homogenate was transferred to flasks containing 50 ml of sterile saline blank. The flask was kept in a shaker at 120 rpm for 15 mins for uniform distribution and this homogenate was used for isolation of bacterial strains.

2.4. Isolation of bacterial strains from termites

One ml of termite homogenate was serially diluted up to 10^{-5} dilution using sterile saline blanks. The nutrient agar medium (Peptone 5.0 g/L; NaCl 5.0 g/L; Beef extract 3.0 g/L; Yeast extract 3.0 g/L; Agar 2.0 g/L; pH 7.0 ± 0.2) was prepared and sterilized at 15 lbs at 121^{o}C for 15 mins in an autoclave. After thorough mixing, one ml of diluted samples from 10^{-4} and 10^{-5} was pipetted out into sterilized petridishes and about 15 ml of sterile nutrient agar medium (40 ± 2 ^{o}C) was dispersed and the petridishes were gently rotated clock wise and anti- clock wise direction for uniform distribution of the samples. The solidified plates were incubated at 30±2 ^{o}C for 24 - 48 hrs [18]. After incubation, the colonies of bacteria were enumerated and expressed as cfu/ml and twelve morphologically different bacterial colonies were isolated and purified by repeated streaking on nutrient agar plates. The purified colonies were tested for dye decolorization.

2.5. Development of inoculum of individual strains

Twelve morphologically different colonies isolated from pour plate technique were individually inoculated in 100 ml sterile nutrient broth in a 250 ml flask and incubated at 30±2 ^{o}C for 16 – 18 hrs.

2.6. Preparation of dye solution

Commercial grade dyes (Table 1) were obtained from local textile industry situated in Coimbatore, Tamilnadu, India and laboratory based dyes were purchased from Hi Media, Mumbai and were used without further purification. An initial stock solution of 1000 mg/L of the dye solution was dissolved by dissolving 1.0 gm of the dye in sterile distilled water and made up to 1000 ml. From this stock solution, required concentrations of dye solutions were prepared and filter sterilized using membrane filter (0.45µ) and used for further study.

Table 1: Dyes used in the study

S.No	Dyes	λ_{max} (nm)	S.No	Dyes	λ_{max} (nm)
1.	Congo red	500	8.	Reactive red 195	545
2.	Methyl orange	460	9.	Reactive yellow 84	354
3.	Methylene blue	600	10.	Reactive orange 72	388
4.	Brilliant green	613	11.	Reactive brown 18	468
5.	Reactive blue 222	610	12.	Reactive yellow 17	351
6.	Reactive blue 140	660	13.	Reactive brown 36	473
7.	Reactive blue 220	612	14.	Reactive blue 4	495

2.7. Dye decolorization by individual bacterial strains under static - shaking conditions

Hundred ml of nutrient broth medium was prepared in 250 ml Erlenmeyer flasks and sterilized at 15 lbs at 121^{o}C for 15 mins in an autoclave. All the 14 dyes were filter sterilized and added to nutrient broth medium individually at 50 mg/L concentration. To this, 1.0 ml inoculum of individual bacterial strains prepared was inoculated in separate flasks. One set of the flasks were incubated in a thermostat controlled shaker at 120 rpm at 30 °C for 48 hrs and another set of flasks were incubated at static condition in an incubator at 30 ^{o}C for 48 hrs. Appropriate control flasks containing dye without inoculum were also maintained. The experiment was conducted in triplicate for concurrent results. After incubation, the samples were analyzed for percent decolorization. The bacterial strains which exhibited maximum decolorization of the dyes tested were selected for further studies.

2.8. Development of consortium of strains

The potent dye decolorizers among the 12 isolated strains were selected and grown in nutrient broth for 16 – 18 hrs. Ten ml of culture was taken from each flask and mixed well in different combinations listed in Table. The OD was adjusted to 1.0 at 600 nm with sterile medium and used as inoculum of consortia.

2.9. Decolorization of dyes by consortia of strains

Decolorization was assessed by consortia of strains adopting the method mentioned earlier. After incubation, the samples were analyzed for percent decolorization. The consortium which recorded maximum decolorization of the dyes tested were selected and used for further decolorization experiments.

2.10. Decolorization measurement

The samples after incubation were centrifuged at 10,000 rpm for 15 mins and the suspended biomass was separated. The absorption spectra of the clear supernatant were recorded at λ_{max} of the dyes using a spectrophotometer (UV-Vis 3210, Hitachi, Japan). Medium containing dyes without the inoculum was taken as control. The initial and final absorbance values obtained were then used to calculate percentage decolorization of the dye.

$$\% \text{ decolorization} = \frac{\text{Initial absorbance value - Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

2.11. Selection of dyes and dye decolorizers

Among all the dyes examined, Reactive blue 220 and Reactive red 195 were decolorized at higher percentage and hence these two dyes were selected for further studies. Consortium comprising all the four strains which showed maximum decolorization of the above two dyes was designated as BUTC17 and was selected for further experiments.

2.12. Preparation of inoculum of bacterial consortium BUTC17

One ml of grown culture of potent dye decolorizers (designated as T3, T6, T10 and T11) was taken from each flask and inoculated in 50 ml sterile nutrient broth and incubated for 16-18 hrs. After 18 hrs of incubation, 10 ml of the broth was drawn from each flask, mixed thoroughly and adjusted to 1.0 OD at 600 nm using sterile medium in a spectrophotometer (UV-Vis Hitachi, 3210). One ml of the above bacterial consortium BUTC17 was used as inoculum for all the experiments.

2.13. Selection of broth medium

Various media such as Nutrient broth, Basal minimal medium, Mineral salts medium, Basal medium and Decolorizing medium were used to study their effect on decolorization of Reactive blue 220 and Reactive red 195 at a concentration of 50 mg/L in similar method mentioned earlier. Apart from measuring decolorization, growth in terms of biomass was also were measured.

2.14. Bacterial growth assay

Bacterial growth was monitored after the termination of the experiment by measuring the turbidity at 600 nm in a spectrophotometer (UV-Vis 3210, Hitachi, Japan). Un-inoculated culture medium was used as blank.

2.15. Identification of bacterial strains in bacterial consortium BUTC17

The four bacterial strains present in the consortium BUTC17 were identified based on their morphological and biochemical characteristics employing standard protocols and identified to various genera [19].

RESULTS

3.1. Bacterial population in termite homogenate

The termite samples subjected for the enumeration of total heterotrophic bacterial population revealed that the bacterial count in it was found to be $54.4x10^4$ cfu/ml. Morphologically 12 different bacterial strains (T1-12) were isolated.

3.2. Decolorization of dyes by individual bacterial strains under static and shaking incubation

3.2.1. In shaking condition

Individual bacterial strains when screened for their decolorization efficiency against 14 different dyes established that the Strain T6 exhibited better decolorization of Reactive red 195 (47.3 %) and Reactive blue 220 (44.9%) followed by Reactive blue 222 (41.9%) and Reactive brown 18 (41.3%). Other dyes tested with the strain T6 showed a comparatively lesser decolorization which was in the range of 27.7% – 38.7%. The decolorization pattern of the dyes by remaining bacterial strains was in the range of 8.6% - 38.7% (Table 2).

Table 2: Decolorization of dyes by individual bacterial strains in shaking condition

Bacterial culture	Decolorization (%) of different dyes													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
T1	12.7	16.3	15.4	11.3	16.7	15.3	26.7	33.2	18.3	14.7	9.3	20.6	23.4	11.0
T2	11.5	17.4	9.1	13.4	21.7	11.5	20.3	30.5	15.2	11.5	18.7	18.6	19.6	14.3
T3	13.9	11.5	8.6	7.5	19.5	20.3	17.5	28.6	11.1	17.6	11.5	21.3	21.4	10.8
T4	18.6	13.2	11.3	13.2	11.3	18.1	11.8	19.3	15.7	28.1	20.3	19.4	17.6	11.6
T5	25.4	17.9	13.3	9.5	18.5	15.5	17.8	28.5	17.5	33.4	19.6	21.8	23.3	15.7
T6	32.5	31.7	28.5	28.4	41.9	32.8	44.9	47.3	38.7	32.8	41.3	35.3	32.7	27.7
T7	27.8	19.6	15.6	23.2	17.6	20.2	21.3	28.5	21.5	19.8	29.5	28.7	21.3	23.6
T8	22.3	15.4	18.4	19.4	18.9	19.5	19.5	33.8	25.8	29.7	32.8	24.6	27.4	21.0
T9	19.6	27.5	15.4	21.1	22.6	24.7	23.4	37.4	25.2	38.7	27.6	32.3	31.7	19.5
T10	21.0	24.3	12.8	27.5	25.0	21.3	27.7	27.8	27.0	31.5	33.2	30.0	28.5	23.4
T11	28.7	28.6	19.1	25.0	21.4	38.5	32.0	26.7	29.7	33.4	31.5	27.5	27.5	21.2
T12	36.3	21.7	17.6	23.3	21.5	30.3	37.6	30.8	30.1	31.5	31.8	31.6	33.3	20.4

3.2.2. In static condition

Similarly, the decolorization efficiency of all the 12 bacterial strains in nutrient broth in static condition showed that isolates T3, T6, T10 and T11 exhibited higher percentage of decolorization when compared to the other bacterial strains tested (Table 3).

Table 3: Decolorization of dyes by individual bacterial strains in static condition

Bacterial culture	Decolorization (%)of different dyes													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
T1	14.5	46.7	29.6	34.5	45.6	53.2	60.3	61.2	64.6	64.3	58.3	55.4	45.3	23.4
T2	21.6	53.0	30.1	39.7	36.2	55.4	68.7	63.5	61.5	56.8	52.6	58.7	51.6	23.3
T3	43.2	62.5	45.6	52.6	45.7	66.4	71.1	75.4	68.5	66.3	65.4	69.4	62.5	43.5
T4	24.2	46.2	38.5	18.4	36.4	49.6	57.6	66.8	60.2	58.7	58.7	50.0	40.2	32.6
T5	32.5	53.8	31.0	41.1	29.3	31.5	66.8	68.2	58.7	61.4	65.4	41.8	56.4	21.7
T6	50.3	64.5	55.3	65.3	59.2	70.4	79.5	77.5	73.5	77.5	74.7	69.4	61.4	41.0
T7	29.7	49.7	32.1	43.2	40.1	53.2	62.8	57.3	53.4	61.4	59.4	55.3	56.6	35.2
T8	31.4	41.6	29.3	45.7	51.2	50.1	66.5	65.4	64.5	60.2	56.6	60.3	53.4	41.0
T9	41.0	41.5	35.4	44.3	51.7	50.5	62.3	69.2	62.3	61.5	54.3	62.2	60.3	39.8
T10	44.2	63.1	48.2	65.0	57.7	64.3	75.7	71.7	70.4	71.0	68.6	67.8	73.5	41.5
T11	42.1	60.3	40.0	53.5	54.2	68.0	79.1	75.4	69.3	69.0	66.3	68.1	60.4	44.2
T12	40.2	32.7	33.5	54.3	48.5	51.3	67.3	63.0	67.0	64.0	56.3	61.5	57.0	41.2

Bacterial strains which recorded better decolorization of all the tested dyes were in the order of T6 > T10 > T11 > T3. Among the four strains, minimum decolorization of 40.0% was recorded in the strain T11 for Methylene blue dye whereas maximum decolorization of 79.5% was recorded by the strain T6 when Reactive blue 220 was amended in the medium. It was observed that decolorization of the dyes in general was established better by individual strains in static incubation condition than shaking incubation condition. Based on this observation, selected four strains in various combinations (consortia) were assessed for their decolorization efficiency under static condition in the presence of all the fourteen dyes.

3.3. Decolorization of dyes by consortia of strains

Decolorization efficiency of four potent dye decolorizing bacterial strains in different combinations (consortia) was monitored with fourteen different dyes in nutrient broth medium in static condition which supported that the consortia comprising all the four strains (T3, T6, T10 and T11) found to record higher decolorization percentage for all the dyes tested when compared to other combination of consortium having 2 and 3 mixed cultures. Among this, the consortium exhibited maximum decolorization of Reactive blue 220 (94.3%) followed by Reactive red 195 (93.7%). The decolorization performance of the remaining dyes by the consortia (T3, T6, T10 and T11) were in the order of Reactive blue 222 > Reactive orange 72 > Reactive yellow 17 > Reactive blue 140 > Reactive yellow 84> Reactive brown 36 > Reactive brown 18> Methyl orange > Brilliant green > Congo red > Methylene blue > Reactive blue 4 (Table 4).

Table 4: Decolorization of dyes by consortia developed from individual strains

Composition of Bacterial consortia	Decolorization (%) of different dyes (Static mode)													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Two isolates combination														
T3 + T6	54.2	68.1	58.2	66.0	61.7	73.3	81.7	71.7	70.4	80.0	74.6	69.8	71.5	46.5
T3 + T10	50.2	62.8	58.4	62.8	56.6	67.5	82.4	78.6	66.3	70.3	69.5	67.2	69.1	41.2
T3 + T11	51.4	61.5	66.5	59.6	66.4	61.2	72.2	72.3	65.4	69.8	71.2	70.2	66.6	50.1
T6 + T10	55.5	60.4	60.4	63.3	75.7	70.3	73.5	77.6	61.1	63.2	60.5	63.4	67.8	61.5
T6 + T11	56.3	69.6	56.4	60.5	69.5	60.4	81.3	76.5	59.8	71.2	67.5	71.2	66.3	49.5
T10 + T11	52.3	58.7	61.2	62.1	72.1	65.2	79.3	80.1	68.7	78.3	57.2	70.2	65.3	45.3
Three isolates combination														
T3 + T6 + T10	57.4	71.0	63.8	66.8	84.2	76.4	89.5	87.1	73.0	77.2	78.6	73.0	80.8	48.6
T3 + T6 + T11	66.3	63.0	71.0	68.5	82.2	69.5	83.3	77.3	71.3	81.7	81.3	77.2	71.5	50.0
T6 + T10 + T11	62.7	63.4	69.6	68.4	75.5	68.2	87. 4	81.4	75.2	83.3	74.4	79.4	70.5	51.1
T3 + T10 + T11	72.2	71.8	70.7	74.2	70.5	75.1	85.1	84.1	78.2	77.3	81.1	76.6	75.6	51.4
Four isolates combination (BUTC 17)														
T3 + T6 + T10 + T11	75.2	83.1	71.2	78.4	91.4	89.0	94.3	93.7	87.2	90.3	85.5	89.2	86.5	64.2

Further experiments were carried out with Reactive blue 220 and Reactive red 195 under static condition and the consortium (T3, T6, T10 and T11) which exhibited better decolorization potential was designated as BUTC17 and used thereafter for further studies.

3.4. Selection of broth medium for decolorization of Reactive blue 220 and Reactive red 195 by bacterial consortium BUTC17

Various broth media such as Nutrient broth, Basal minimal medium, Mineral salts medium, Basal medium and Decolorizing medium used to investigate the influence of broth medium on decolorization of Reactive blue 220 and Reactive red 195 and growth of the bacterial consortium BUTC17 revealed that decolorization of Reactive blue 220 was found to be higher (95.3%) in Decolorizing medium followed by Nutrient broth medium (94.2%). Similarly growth of the consortium was 2.53 OD and 2.52 OD in decolorizing medium and nutrient broth medium respectively (Figure 1).

Figure 1: Influence of various broth media on decolorization of Reactive blue 220 and growth of the consortia

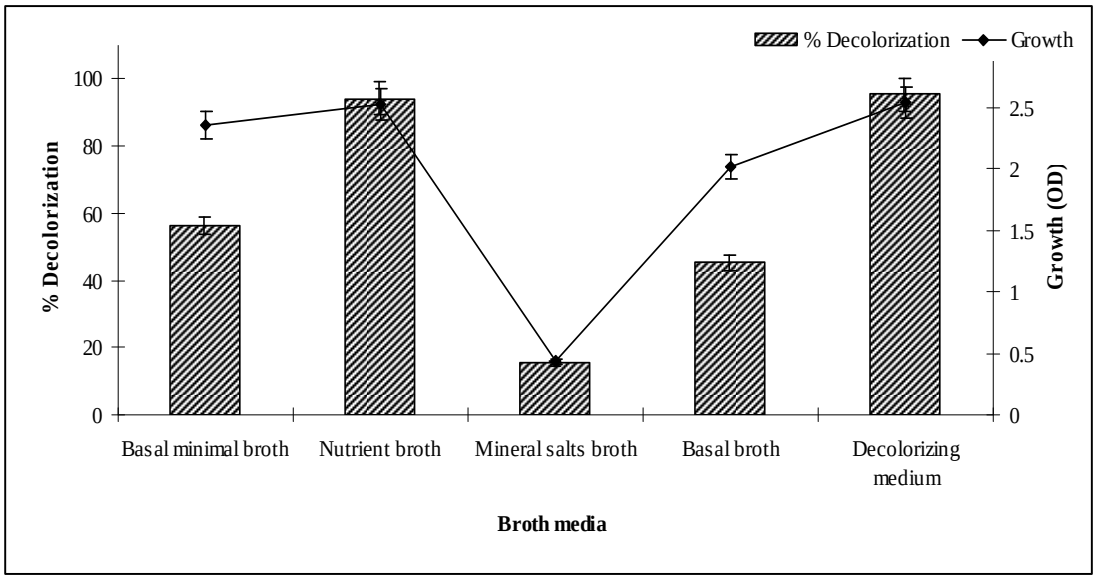
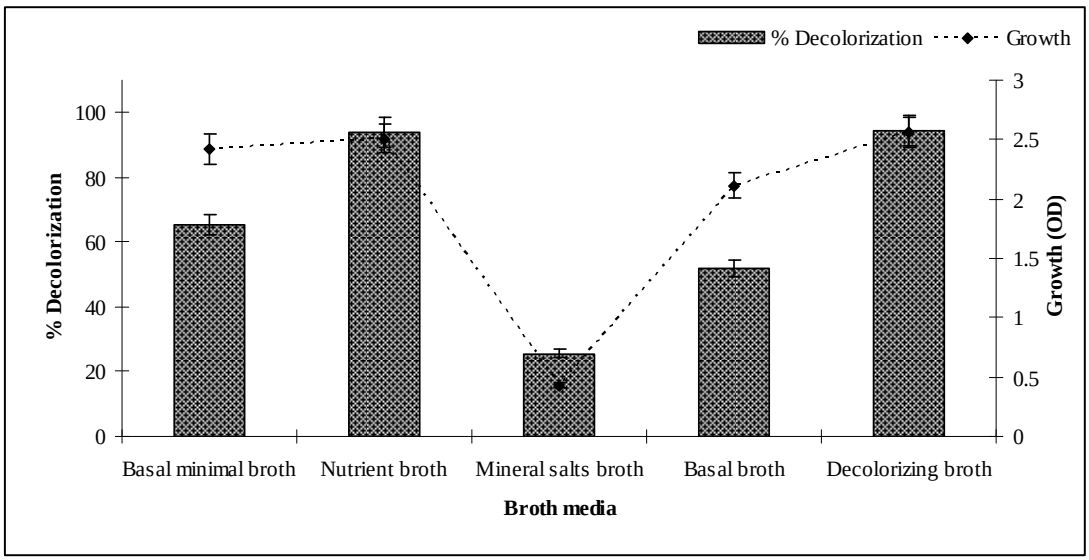


Figure 2: Influence of various broth media on and decolorization of Reactive red 195 and growth of the consortia



Another dye, Reactive red 195 was decolorized to the maximum (94.4%) in Decolorizing medium (Figure 2) followed by Nutrient broth medium (93.7%). The growth was recorded maximum in Decolorizing medium (2.56%) followed by nutrient broth medium (2.51%). In general, it was observed that decolorizing medium had significant effect on decolorization of Reactive red 195 and growth of the bacterial consortium when compared to other media

tested during the study. From the experiment, it was observed that decolorizing medium was found to be the ideal medium which resulted in better decolorization of both the dyes as well as resulted in maximum growth of the bacterial consortium in static condition and hence employing such cost-effective nutrient medium will serve as an ideal resource for large scale applications.

3.5. Identification of bacterial strains present in the bacterial consortium BUTC17

Bacterial consortium BUTC17 containing T3, T6, T10 and T11 bacterial strains were identified as *Bacillus* sp3, *Bacillus* sp6, *Bacillus* sp10 and *Enterobacter* sp 11, employing morphological and biochemical characters and the results are presented in table 5.

Table 5: Morphological and biochemical characteristics of the potent strains of bacteria

S. No	Characteristics	Strain – T3	Strain – T6	Strain – T10	Strain – T11
1.	Gram's staining test	Gram + ve	Gram + ve	Gram + ve	Gram - ve
2.	Morphology	Oval	Rods	Rods	Rods
3.	Motility	Motile	Non motile	Non motile	Motile
4.	Spore staining	+	+	+	-
5.	Cultural characteristics	White waxy growth	White waxy growth	Translucent	White
6.	Indole production	-	-	-	-
7.	MR reaction	-	-	-	-
8.	VP Reaction	+	+	+	+
9.	Citrate utilization	-	-	-	+
10.	Starch hydrolysis	-	+	+	-
11.	Urease activity	-	-	-	+
12.	NO_3 production	+	+	+	-
13.	H_2S production	-	-	-	-
14.	Catalase activity	+	+	+	+
15.	Oxidation Fermentation	-	-	-	-
16.	Gelatin liquefaction	+	+	+	-
17.	Arginine hydrolase	+	+	+	+
18.	Lysine decarboxylase	-	-	-	-
19.	Casein hydrolysis	+	+	+	-
20.	Lactose	-	-	-	+
21.	Dextrose	+	+	-	+
23.	Sucrose	-	-	-	-
24.	Adonitol	+	-	-	-
25.	Arabinose	-	-	+	-
26.	Cellobiose	-	-	-	+
27.	Xylose	+	+	+	-
28.	Dulcitol	-	-	-	+
29.	Fructose	-	+	+	-
30.	Inositol	-	+	-	-
31.	Maltose	-	-	-	+
32.	Mannitol	-	-	+	-
33.	Raffinose	-	-	-	+
Identified as		***Bacillus* sp**	***Bacillus* sp**	***Bacillus* sp**	***Enterobacter* sp**

DISCUSSION

Among various contaminants, dyes are common pollutants in industrial wastewaters and many of them are known to be toxic and carcinogenic [20]. In most developed and developing countries, by introducing stringent environmental regulations, these industries are forced to develop on-site or in-plant treatment facilities and minimize the concentration of contaminant to acceptable limits prior to their discharge [21]. However, before selecting a wastewater treatment facility, a considerable amount of laboratory and engineering work must be completed prior to system design [22].

4.1. Termite gut flora

In this study bacterial strains were isolated from termite homogenate sample prepared from *Macrotermes* sp and the bacterial count was found to be 54.4 x 10^4 cfu/ml and a similar report on isolation of bacterial strains from *Macrotermes michaelseni* harboring 2.2 × 10^6 bacterial cells per ml of gut homogenate was reported [12]. Another report reveals that free living aerobic bacteria (*Azotobacter* sp and *Beijerinckia* sp) and facultative nitrogen fixing anaerobe are identified as *Klebsiella* sp and *Clostridium* sp from *Macrotermes* sp [23].

4.2. Dye decolorization performance of individual strains

Interesting reports on degradation of complex substances by termite gut flora have given a belief that the termite bacteria may posses the dye degrading efficiency. In the present study, higher percentage of decolorization was observed for Reactive blue 220 (94.3%), followed by Reactive red 195 (93.7%), Reactive orange 84, Reactive brown 18, Reactive blue 222, Reactive brown 9 and Congo red. The effectiveness of all the selected isolates in decolorizing these dyes may depend on the structure and complexity of the dyes, particularly on the nature and position of substituent in the aromatic rings and the resulting interactions with the azo bond [24, 25].

In line with our studies, [26] studied the metabolism of monoaromatic compounds such as benzoic acid, cinnamic acid, ferrulic acid and phenylpropanoic acid by guts homogenates of the wood-feeding *Nasutitermes lujae* termites and demonstrated the occurrence of oxygen-mediated ring cleavage.

Benzoic acid and celluloses degrading termite gut bacteria was studied by [12] and the isolated strains were identified to be *Stenotrophomonas maltophila*, *Stenotrophomonas acidaminophila and Xanthomonas campestris*. [27] suggested that highly substituted tri azo DB71 dye showed longer decolorization times (168 hrs). It has been reported that the azo compounds with hydroxyl or amino groups are more likely to be degraded than those with methyl, methoxy, sulfo or nitro groups and usually, the presence of sulfonates in reactive dye structures results in low levels of color removal.

Decolorization pattern was found to be very unique since dyes used were of chemically different. This was supported by the studies performed by [28] where decolorization of Acid red B, Reactive blue GL, Acid red G and RBR X-3B by *Rhodopseudomonas palustris* found that dye with less molecular mass (Acid red B) demonstrated the best degradability. An additional support in this aspect is stated by [9] that dyes with simple structures and low molecular weights usually exhibit higher rates of color removal, whereas color removal is more difficult with highly substituted, high molecular weight dyes. Contrarily certain microorganisms are found to reduce Amaranth more quickly than Acid Orange 7 in spite of the presence of three sulphonic groups in the structure of Amaranth [29].

4.3. Effects of aeration on decolorization

In the present investigation, it was observed that, the culture under agitated conditions demonstrated very less color removal (47.2 % for Reactive blue 220 and 45.3% for Reactive red 195), while the static culture decolorized higher (94.3% of Reactive blue 220 and 93.7% of Reactive red195) of 50 mg/L of the dyes at 24 hrs of incubation. From this, it was clear that near micro-aerophilic conditions supported for a better performance of the bacterial consortia for decolorization, though the cell growth was poorer than that under aerobic conditions. This kind of aeration dependant performance in the present study may be due to several factors in which a supportive reference is reduction of azo bond mediated by facultative as well as obligate anaerobic or under microaerophilic conditions [30, 31]. Similarly, [32] reported decolorization of azo dyes by *Proteus mirabilis* to be 20% in shake cultures and more than 95% dye removal was estimated in static anoxic culture, even when associated with low level of cell growth than shaking condition. Contrarily, several aerobic azo dye degrading strains capable of using dye as sole source of carbon and nitrogen [33], degradation by oxidative reaction [34], dye reducing enzymes under aerobic conditions [35, 36] were also reported earlier.

4.4. Efficiency of bacterial consortia in dye decolorization

Large quantities of reactive dyes are now used in textile and dyestuff industries in India. Thus reactive group of dyes were selected, screened and further studied on degradation by potent dye decolorizing bacterial consortium BUTC17. In the present study, maximum decolorization of the all the dyes was found to be better in the medium inoculated with total bacterial consortium rather than the inoculum of individual strains. Amongst them, bacterial consortium BUTC17 exhibited higher percentage of decolorization of Reactive blue 220 (94.3%) Reactive red 195 (93.7%) under static condition. This may be due to the uniqueness of the bacterial consortium BUTC17 which as a whole in a consortium might have played a role in better decolorization performance. A consortium comprises of three organisms to degrade a mixture of dyes by co-metabolism was developed and observed that the consortium could decolorize efficiently all the three dyes tested [37]. Similar observations were made by [38] when a consortium HM-4 based on combinations of four selected isolates showed decolorization efficiency of 20 mg/L of AR-88 in 20 hrs whereas the individual isolate took 60 hrs to achieve complete decolorization. Similar results were observed by [39] who studied the role of non decolorizers *Escherichia coli* DH5α species in decolorization of Reactive red 2 in association with decolorizer, *Pseudomonas luteola.* It was observed that the presence of E. coli

DH5α increased the decolorization efficiency of *P. luteola*. Thus, addition of DH5α into a mixed culture containing *P. luteoa* as a major decolorizer may lead to a bioaugmentation effect on decolorization activity.

Earlier report on decolorization of dyes by [39] revealed unique observations where six bacterial strains (*Shewanella* sp) isolated from activated sludge showed greatest ability to decolorize azo dyes (AR-88, RB-5, and DR-81) in liquid medium up to a concentration of 300 mg/L. It also showed that AR-88 required 8 hrs of incubation and 500 mg/L of RB-5 and DR-81 within 20 hrs and were found to carry efficient enzymatic system for the cleavage of azo bonds which caused rapid decolorization of higher concentrations under reduced (static) conditions. They also suggested these strains may have potential application for treatment of wastewaters contaminated with azo dyes.

4.5. Effect of medium composition

It has been found that medium compositions are critical to the efficiency of microbial decolorization [40] and the reduction of azo dyes depends on the presence and availability of a co-substrate because it acts as an electron donor for the azo dye reduction [27]). Many different co-substrates were found to suite as electron donor, like glucose [41] and yeast extract [27].

In the present study, both the dyes (Reactive blue 220 and Reactive red 195) were decolorized to the maximum in Decolorizing medium (greater than 90%) followed by Nutrient broth medium and similarly the growth of the bacterial consortium BUTC17 was recorded to the maximum in the Decolorizing medium than the latter one and glucose was identified as the ideal carbon source.

The effect of medium compositions on azo dye decolorization under microaerophilic conditions has been found that medium compositions are critical to the efficiency of microbial decolorization [41, 42]. Previous studies conducted by [43], showed that Fast acid red GR decolorization by *Shewanella decolorationis* S12 exhibited different azo reducing efficiencies with different electron donors under microaerophilic conditions. They identified lactate (20 mM) as the best optimal carbon source than formate, glucose and sucrose. It was observed that color removal took place in all the media tested whereas lower decolorizing rates were observed in the presence of substrates other than lactate and suggested that this phenomenon may be due to (1) with resting cells but no carbon source, and (2) with each carbon source but no cells.

CONCLUSION

From the present investigation, it is very obvious that the termite derived bacterial consortium BUTC17 can be utilized for treatment of dye bearing waste waters since the present study confirms the ability of this consortium to decolorize 14 different dyes with decolorization efficiency of more than 60% in static experimental mode. Studies on complete physiology of such novel microbes and their enzyme production for better performance is of utmost importance before they could be opted as a better choice for treating textile dye containing wastewater in an industrial scale. Further standardization of the treatment process for bioremediation of dye contaminated soil employing this consortium is in progress.

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REFERENCES

[1] Deviram GVNS, Pradeep KV, Gyana Prasuna R, *Eur. J. Exp. Biol*, **2011**, 1 (3): 216 – 222.
[2] Tariq J, Ashraf M, Jaffer M, Afzal M, *Water Res*, **1996**, 30: 1337 - 1344.
[3] Malik G M, Zadafiya S K, *Der Chemica Sinica*, **2010**, 1 (3): 15-21.
[4] Gandhi J, Dangi R, Sharma J C, Verma N, Bhardwaj S, *Der Chemica Sinica*, **2010**, 1 (3), 77.
[5] Mehta M R, Goyal N, Prajapati V P, *Adv. Appl. Sci. Res*, **2011**, 2 (2): 315.
[6] Liu S, Suflita J M, *Trends Biotechnol*, **1993**, 11: 344 - 352.
[7] Watanabe K, Baker P W, *J. Biosci. Bioeng*, **2000**, 89: 1 - 11.
[8] Forgacs E, Cserhati T, Oros G, *Environ. Int*, **2004**, 30*:* 953 - 971.
[9] Pearce C I, Lloyd J R, Guthrie J T, *Dyes Pigments*, **2003**, 58: 179 - 196.

[10] Ozdemir G, Pazarbasi B, Kocyigit A, Omeroglu E E, Yasa I, Karaboz I, *World J. Microbiol. Biotechnol*, **2008**, 24:1375 - 1381.
[11] Kato K, Kozaki S, Imamura T, Sakuranaga M, U. S. patent, Patent Number 6, **2000**.
[12] Ngugi D K, Tsanuo M K, Boga H I, *J. Basic Microbiol*, **2007**, 47: 87 - 92.
[13] Adams L, Boopathy R, *Bioresour. Technol*, **2005**, 96: 1592 - 1598.
[14] Nakajima H, Hongoh Y, Usami R, Kudo T, Ohkuma M, *FEMS Microbiol. Ecol*, **2005**, 54: 247 - 255.
[15] Sandhya S, Padmavathy S, Swaminathan K, Subrahmanyam Y V, Kaul S N, *Process Biochem*, **2005**, 40: 885 - 890.
[16] Varma A, Kolli B K, Pau J, Saxena S, Konig H, *FEMS Microbiol. Reviews*, **1994**, 15: 9- 28.
[17] Sekar T, Forest history of the Nilgiris, Tamil Nadu Forest Department, Chennai, **2004**.
[18] Azambuja P, Feder D, Garcia E S , *Exp. Parasitol*, **2004**, 107:89 - 96.
[19] Brenner D G, Krieg N R, Staley J T, (Ed.), Bergey's manual of systematic bacteriology, The Proteobacteria, II edition, volume II, Springer Verlog, New York, **2005**.
[20] Vijayaraghavan K, Yun Y S, *Biotechnol. Advances*, **2007**, 26: 266 - 291.
[21] Banat I M, Nigam P, Singh D, Marchant R, *Bioresour. Technol*, **1996**, 58: 217 - 227.
[22] Atkinson B, Daoud I S, *Adv. Biochem. Eng*, **1996**, 4: 41 -124.
[23] Gomathi V, Ramalakshmi A, Ramasamy K, *Entomol. Res*. **2007**, 35 (2): 75 - 78.
[24] Zimmermann T, Kulla H G, Leisinger T, *Eur. J. Biochem*, **1982**, 129: 197 - 203.
[25] Sani R, Banerjee U, *Enzyme Microbial Technol*, **1999**, 24: 433 - 437.
[26] Brune A, Miambi E, Breznak A, *Appl. Environ. Microbiol*, **1995**, 61, 2688–2695.
[27] Nigam P, Banat I M, Singh D, Marchant R, *Process Biochem*., **1996**, 31: 435 - 442.
[28] Liu G F, Zhou J, Wang J, Song Z, Qv Y, *World J. Microbiol. Biotechnol*, **2006**, 22 (10): 1069 - 1074.
[29] Bragger J L, Lloyd A W, Soozandehfar S H, Bloomheld S F, Marriott C, Martin G P e*t al*, *Int.J. Pharm*, **1997**, 157:61–71.
[30] Chung K T, Stevens S E, *Crit. Rev. Microbiol*, **1992**, 18:175–90.
[31] Hu T L, *Water Sci. Technol*, **1998**, 38: 299 - 306.
[32] Chen K C, Huang W T, Wu J Y, Houng J Y, *J. Ind. Microbiol. Biotechnol*, **1999**, 23: 686 - 690.
[33] Coughlin M F, Kinkk B K, Tepper A, Bishop P L, *Water Sci. Technol*, **1997**, 36 (1): 215 - 23.
[34] Field J A, Stams A J M, Kato M, Schraa G, *A Van Leeuwenhoek*, **1995**, 67: 47 - 77.
[35] Chung J S, Chou C, Lin Y C, Lin P J, Hu T L, *Water Res*, **2001**, 35(2): 2841 - 2852.
[36] Suzuki Y, Yodu T, Ruhul A, Sugiura W, *J. Biol. Chem*, **2000**, 276(12): 9059 - 9069.
[37] Senan R C, Abraham T E, *Biodegr*., **2004**, 15: 275 - 80.
[38] Khehra M S, Saini H S, Sharma D K, Chadha B S, Chimni S S, *Water Res*, **2005**, 39: 5135 - 5141.
[39] Chen B Y, Chen S Y, Lin M Y, Chang J S, *Process Biochem*, **2006**, 41: 1574 - 1581.
[40] Hu T L, *Bioresour. Technol*, **1994**, 49: 47 - 51.
[41] Carliell C M, Barclay S J, Naidoo N, Buckley C A, Mulholland D A, Senior E, *Water SA*, **1995**, 21: 61-69.
[42] Hu T, Wu S C, *Bioresour. Technol*., **2001**, 77: 93 -95.
[43] Hong Y, Chen X, Guo J, Xu Z, Xu M, Sun G, *Appl. Microbiol. Biotechnol*, **2007**, 74: 230 - 238.