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RESEARCH ARTICLE

Biodegradation of textile dye effluent through Indigenous bacteria

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ABSTRACT

The textile industry is considered as one of the major generators of toxic chemical wastewater in India. Dyes released by the textile industries pose a threat to environmental safety. Dye decolorization through biological means has gained momentum as these are cheap and can be applied to a wide range of dyes. The present study concentrates in the isolation, identification of indigenous bacteria namely D1, D2, D3 and D4 from textile dye effluent collected from the local textile dyeing shop located at Gurahakuan, Banda district, Uttar Pradesh, India, and evaluation of their ability to decolorize dyes sample. The isolated bacteria were identified through morphological and biochemical characteristics. Scanning electron microscope (SEM) analysis of isolated bacteria showed that all the bacteria appeared rod-shaped with size ranging from 1.33 to 2.84 µm. The physico-chemical analysis of dye effluent indicated the bluish-black color of the effluent having pH of about 8. The Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) value of the raw sample was estimated to be 470 mg/l and 800 mg/l, respectively, for dye effluent sample. The value of Total Dissolved Solids (TDS) and total Suspended Solids (TSS) was estimated to be 1760 mg/l and 560 mg/l, respectively, in our dye effluent sample. The study aims to isolate and optimize four bacterial isolates having the ability to degrade and decolorize azo dyes produced in the final dying effluent. The optimization results revealed that all the bacteria showed maximum growth at pH 8, temperature 35°C and declines further. All the isolated bacterial species showed significant potential for dye decolorization and degradation at varying wavelengths such as 420, 480, 506, 520, 620 and 668 nm but maximum removal of color (about 88%) was obtained at 668 nm after 48h by bacterial isolate D3. Thus, these selected native bacteria can be employed as a vital biological tool for developing decentralized wastewater treatment systems for decolorization of dye effluents through biosorption or biodegradation which is a cost-effective process.

***Key words*:** Azo dyes; Bacteria; Biosorption; Decolorization; Scanning Electron Microscope; Textile Effluent

1) INTRODUCTION

Rapid industrialization and urbanization lead to continuous deterioration of the eco system. Textile, printing, food and cosmetics industries are the largest source of dye containing effluent that on discharge generates serious environmental threats [1]. Significant problems arise from the high levels of production and low levels of recycling of these pollutants from textile industries [2]. The colored effluent discharged by these industries contain toxic organic residues including mixture of chemically versatile dyes leads to the serious pollution of surface water, ground waters and soil [3]. Dyes are usually aromatic and heterocyclic compounds and are often recalcitrant, some of them being toxic and even carcinogenic. These dyes include several structural varieties such as acid, reactive, basic, disperse, azo, diazo, anthraquinone based and metal complex dyes. Azo dyes comprise a diverse group of synthetic chemicals that are widely used by the leather, textile, cosmetics and paper product industries [4]. Their pollution potential seems from their possible toxicity and carcinogenicity which is mainly due to components such as benzidine and other aromatic compounds, however dyes are more difficult to treat because of their synthetic origin and mainly complex aromatic molecular structures [5]. Such structures are often constructed to resist fading on exposure to sweat, soap, water, light or oxidizing agents and this renders them more stable and less amenable to biodegradation [6, 7]. Inefficiency of the dyeing processes, poor handling of spent effluent and insufficient treatment of wastes of the dyestuff industries lead to dye contamination of the environment such as soil and natural water bodies [8]. Many physical and chemical methods have been employed for decolorization and degradation of dyes but these methods have limitations such as high running cost and disposal of large amount of sludge produced during these processes [9]. On the contrary, biological methods are extensively been used which offers several advantages such as cheap, simple, produce smaller volumes of sludge and high flexibility [10]. Many bacteria belonging to genera

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Bacillus, Micrococcus, Proteus, Pseudomonas, Sphingomonas and Staphylococcus [11-16], fungi and algae [17, 18] are employed for biotreatment of textile dyes under aerobic and anaerobic conditions.

The most promising microorganisms for dye wastewater treatment are those isolated from sites contaminated with dyes (indigenous) because they have adapted to survive in adverse conditions [11, 19]. A potential low-cost alternative method used for color removal from textile effluent is biosorption [20]. This process utilizes the recalcitrance of dyes and affinity to adhere to surfaces as means of removing them through adsorption on biomass [21, 22]. Strong biosorption behavior of certain types of microbial biomass toward metallic ions and other pollutants, such as textile dyes, is a function of the chemical makeup of the microbial cells of which the biomass consists. Several workers [23, 24] described that chemical groups like acetamide group of chitin, phosphate groups in nucleic acids, amino, amido, sulfhydryl, carboxyl groups in proteins and hydroxyl in polysaccharides in biomass could attract and sequester charged pollutants. Bacteria and fungi along with their enzymes such as lignin- degrading enzyme and exopolymeric substances are used for decolorization of effluents under controlled conditions. The present study deals with the isolation, morphological and biochemical identification of indigenous bacteria and evaluation of ability of selected bacteria to decolorize dyes.

2) MATERIALS AND METHODS

2.1) Reagents and Chemicals

All chemicals used were of analytical reagent grade. All aqueous solutions were prepared in double distilled water.

2.2) Sample Collection

The textile dye effluent sample was collected from the local textile dyeing shop located at Gurahakuan, Banda district, Uttar Pradesh, India. The sample was collected in sterile air-tight translucent bottles. Standard procedures (spot and grab) were followed during sampling and samples were transported to the laboratory and stored at 4°C.

2.3) Analysis of physicochemical parameters of the dye effluent

The physicochemical characterization of the effluent was made by analyzing various parameters, viz., color, temperature, pH, total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) by the standard methods for examination of water and sewage as described in APHA [25]. The pH was determined by electronic digital pH meter (Cyberscan).

2.4) Isolation and characterization of bacteria from the textile dye effluent

Nutrient agar medium (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used for the isolation of bacteria from the dye effluent. The bacteria were isolated through serial dilution technique and incubated at 32°C for 24 h. After incubation, numerous bacterial colonies were observed. All colonies were isolated, sub-cultured and identified by several morphological and biochemical methods (Gram's Staining, Motility Test, Indole Test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test, Starch Hydrolases, Gelatin Hydrolases, Casein Test, Urea Test) according to Bergey's Manual of Determinative Bacteriology [26].

The shape and size of bacteria was determined by electronic digital Scanning Electron Microscope (JEOL-JSM -6490LV) by following standard method.

2.5) Growth Kinetics of Microbes

The growth pattern of selected bacteria was studied by measuring the bacterial growth in nutrient broth spectrophotometrically at 600 nm by UV- VIS spectrophotometer. The microbial growth was measured at different environmental factors such as pH and temperature.

2.6) Decolorization of dye effluent

The ability of bacterial isolates to decolorize textile dyes was carried out in nutrient broth amended with 50% of the dye effluent. The isolated bacteria were incubated in nutrient broth supplemented with 50% of dye sample at 32° C for two days. Similarly, a control set was also prepared without bacterial culture. Aliquot (3 mL) of culture media was withdrawn at different time intervals (24 and 48 h) then centrifuged at 8,000 rpm for 15 min so that all the cell deposited at the bottom and supernatant was collected to observe decolorization ability. Decolorization potential of isolates was monitored by measuring the absorbance of the cell free supernatant by using the spectrophotometer at different wavelength (420, 480, 506, 520, 620 and 668 nm). All the experiment was conducted in triplicates. The decolorization activity was expressed in terms of percentage decolorization using following formula:

Decolorization % = $(At_0 - At_f)/At_f \times 100$

Where,

At_0 = initial absorbance

At_f = absorbance after incubation time

3) RESULTS AND DISCUSSION

3.1) Analysis of physico-chemical parameters of the dye effluent

The dye effluent was collected from clothes dyeing shop situated at Gurahakuan, Banda, U.P., India. The physico-chemical analysis of dye effluent was performed. The dye effluent appeared bluish-black in color. The temperature of the sample was estimated about 37°C and pH about 8. The BOD of the raw sample after 3 days indicates the mean value of 470 mg/l. A high BOD values show that the effluent have high oxygen demanding waste which causes the depletion of dissolved oxygen (DO), which is a fundamental requirement for aquatic life. The COD value was estimated 800 mg/l of dye effluent sample. The highest COD gives valuable information about the pollution potential of textile industrial effluent. The value of TDS and TSS was estimated 1760 mg/l and 560 mg/l respectively, in the tested dye effluent sample.

3.2) Isolation and characterization of bacteria from dye effluent

Four bacteria (D1, D2, D3 and D4) were isolated from dye effluent through serial dilution technique and further studied for identification.

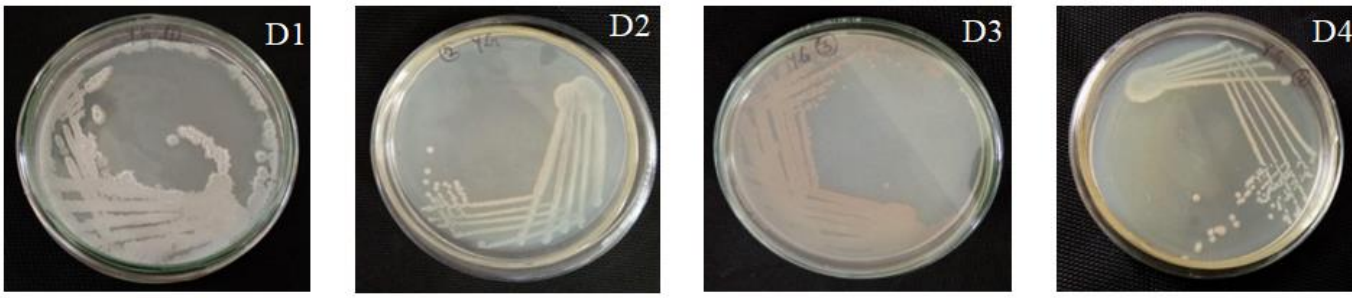


Figure 1: Photographs showing morphological characterization of selected bacteria

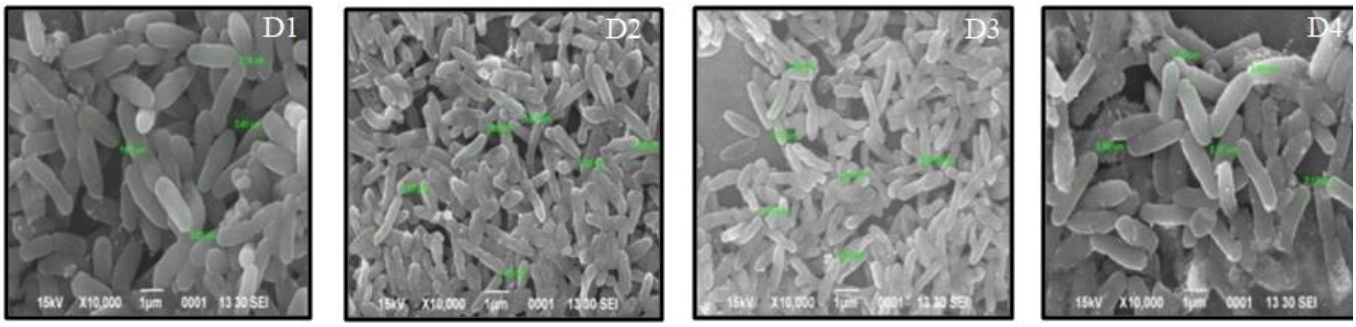


Figure 2: Photograph showing shapes of bacterial cell through SEM Analysis

Table 1: Morphological characteristics of bacterial colonies isolated from dye effluent

	D1	D2	D3	D4
Colony color	White	Off white	Orange	Off white
Cell morphology	Rod	Rod	Rod	Rod
Gram staining	Gram +ve	Gram –ve	Gram –ve	Gram –ve
Margin or edge	Irregular	Circular	Circular	Rounded
Surface structure	Rough, wrinkled	Smooth	Mucoid	Smooth
Elevation	Flat	Flat	Convex	Flat
Consistency	Stringy	Viscous	Viscous	Viscous
Optical feature	Opaque	Opaque	Opaque	Opaque

Table 2. Biochemical analysis of selected bacterial isolates

Test/ Enzymes	D1	D2	D3	D4
H_2S production	-ve	-ve	+ve	-ve
Casein hydrolysis	-ve	+ve	-ve	-ve
Urease test	-ve	-ve	+ve	-ve
Citrate utilization	+ve	-ve	-ve	-ve
Indole	-ve	-ve	-ve	-ve
Voges Proskauer	+ve	+ve	-ve	-ve
Methyl red	-ve	-ve	-ve	-ve
Catalase test	-ve	-ve	-ve	-ve
Amylase test	+ve	+ve	-ve	-ve
Cellulase test	-ve	-ve	-ve	-ve

Morphological characterization of bacteria: Selected bacteria were observed for their shape, size color, margin, arrangement and gram reaction along with the colony/cell morphology of individual isolates (Table 1 and Fig. 1). All the bacterial isolates used in the study had characteristic colony morphology. The bacterial isolates D2, D3 and D4 were found to be gram negative rods, but isolate D1 was found to be gram positive and rod shaped. The shape of all the four bacteria was also confirmed by Scanning electron microscope analysis. All the bacteria appeared to be rod shaped with size ranging from 1.33 to 2.84 µm as shown in figure 2.

Biochemical characterization of selected bacterial isolates: Biochemical tests were also performed on the isolates showing their functional characteristics. The results of these tests are shown in table 2.

3.3 Optimization of growth conditions

Growth of isolated bacteria (D1, D2, D3 and D4) was observed by taking optical density at 600 nm at different time intervals i.e. 24h, 48h and 72h and 96h. In the initial hours growth of all the bacteria rapidly increases then become almost constant and later started declining (Fig. 3).

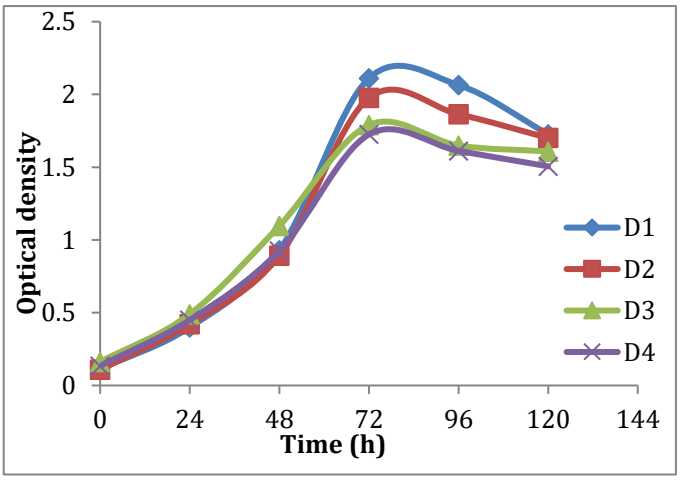
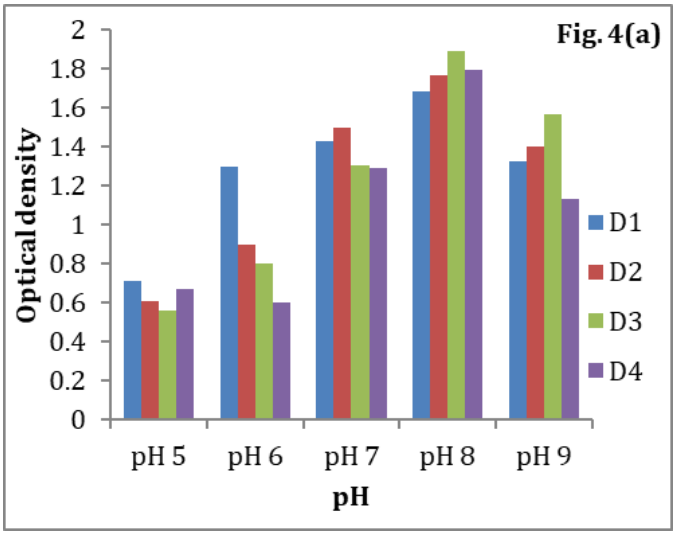


Figure 3: Growth pattern of different bacterial isolates

The optimization of pH and temperature for growth of all the bacteria was shown in fig. 4. All the bacteria showed

maximum growth at pH 8 and growth declines with further increase in pH. The growth of bacteria increases with increase in temperature. All the bacteria showed maximum growth at 35°C temperature and declined above this temperature.



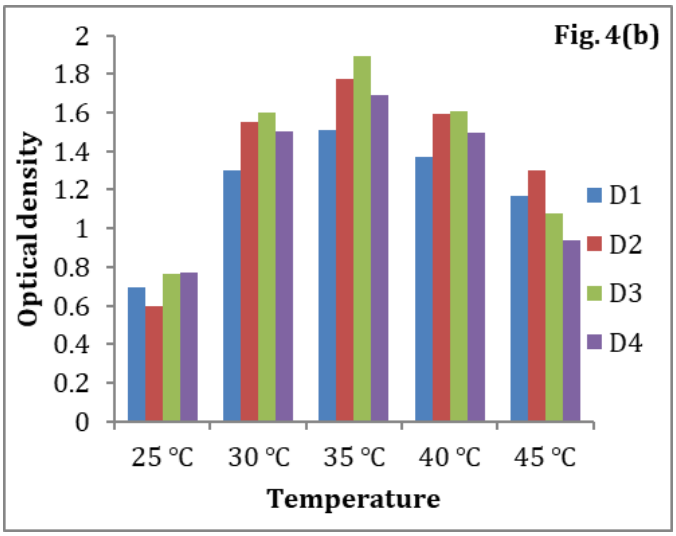
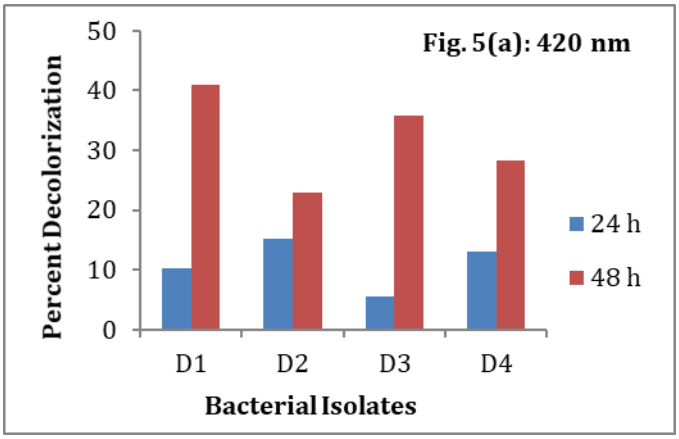
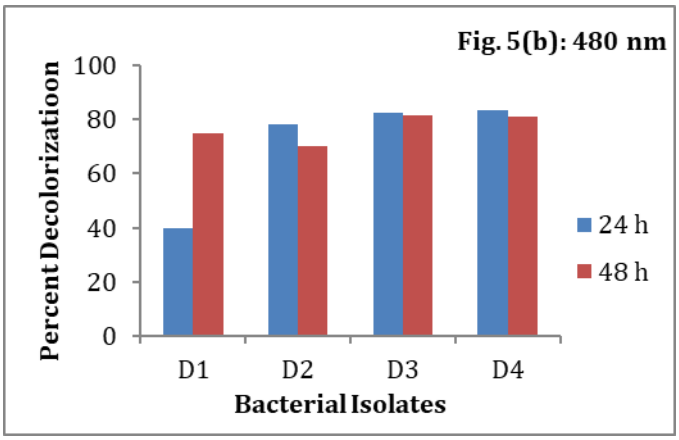
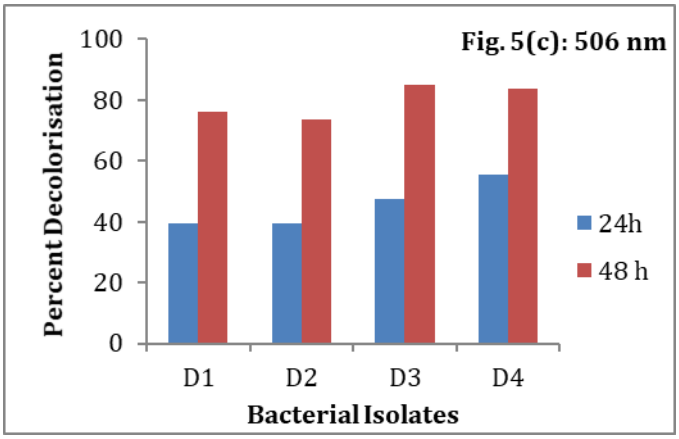
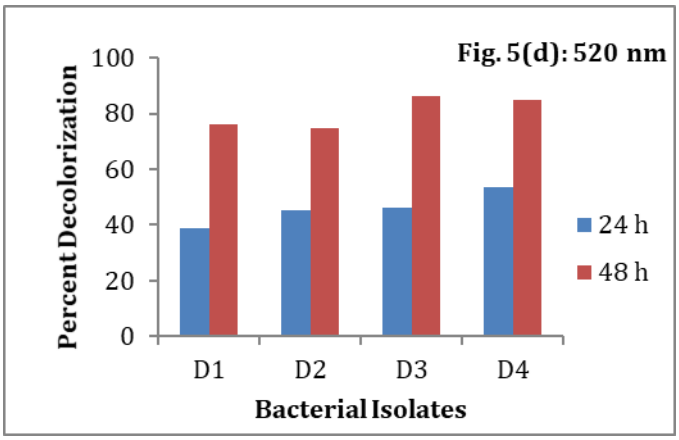


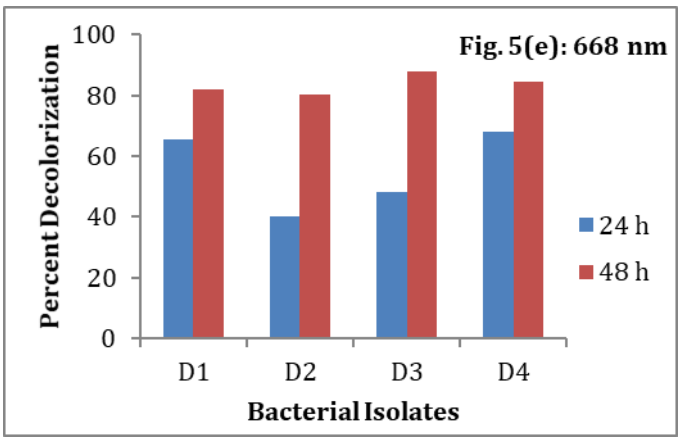
Figure 4: Effect of (a) pH and (b)temperature on the growth of different bacteria











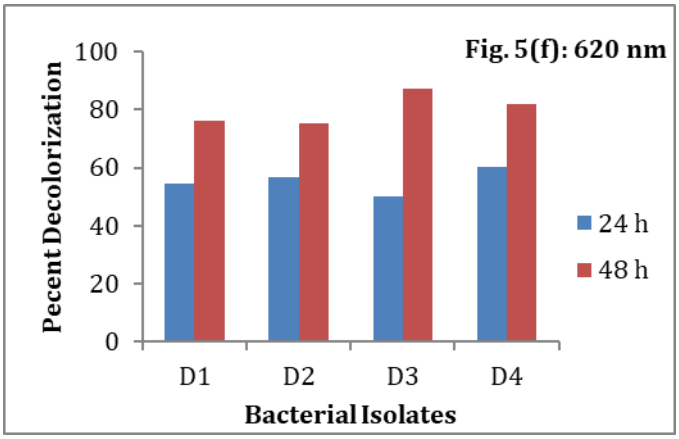


Figure 5 (a-f): Percent decolorization by bacterial isolates at different wavelengths during different time interval

3.4 Decolorization assay

The obtained dye effluent was mixture of many colors. The four bacterial isolates were observed for their color removal efficiencies in collected dye effluent sample at different time intervals. For studying the removal of color by bacterial isolates, the dye sample (50%) was treated with four isolated bacteria then color removal of dye sample was analyzed by spectrophotometer and further calculated by using the given formula. The absorbance was taken for obtained supernatant at different wavelengths such as 420, 450, 506, 520, 620, 668 nm (visible range for different colors) at different time interval. Percent dye removal was calculated and shown in **figure 5**. Maximum removal of color (about 88%) was obtained at 668 nm in 48h by bacterial isolate D3. Microbial decoloration can occur through two principal mechanisms: biosorption and enzymatic degradation or a combination of both [27-29]. Biomass from algae, yeast, filamentous fungi and bacteria has been used to remove dyes by biosorption [30]. The biosorption capacity of a microorganism is attributed to the heteropolysaccharide and lipid components of the cell wall, which contain different functional groups, including amino, carboxyl, hydroxyl, phosphate and other charged groups, causing strong attractive forces between the azo dye and the cell wall [31, 32]. Azo dyes are electron-deficient xenobiotic components because of their azo linkage (–NN), and in many cases, they have sulphonic (SO_3–) or other electron-withdrawing groups, which generate an electron deficiency [33-35] but under the appropriate conditions, they can be degraded by reductases [36, 37]. The anaerobic mechanism of microbial degradation of azo dyes to their corresponding amines is initiated by the cleavage of the azo linkage with the aid of an anaerobic azo-reductase and electron transfer by a redox mediator that acts as an electron shuttle between the extracellular dye and the intracellular reductase [36, 38].

4) CONCLUSION

It can be concluded from the present study that the indigenous microorganism has the ability to remediate the dye from the textile effluent. These observations has established that the bacteria are adaptive in nature and can degrade dye contaminants. The ability of the selected bacteria to tolerate and decolorize azo dyes present in dye effluent sample at high concentration gives an advantage for treatment of textile industry effluent. All the isolated bacterial species showed significant potential for dye decolorization and degradation. Further, it can be suggested that dye contaminated sites can potentially be recovered by a low cost bioremediation process with native bacterial species isolated from the textile effluent and dye disposal sites. Maximum removal of color (about 88%) was obtained at 668 nm in 48h by bacterial isolate D3. Thus, these selected bacteria can be employed as a vital biological tool for developing decentralized wastewater treatment systems for decolorization of dye effluents through biosorption or biodegradation which is a cost effective process.

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