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Potential applications of indigenous bacterial consortium for the treatment of textile effluents and condition optimization by RSM technique

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ABSTRACT

In the present investigation, dyes effluent samples were randomly selected and subjected to biological treatment. The physicochemical characterization such as pH, turbidity, chemical oxygen demand (COD) biological oxygen demand (BOD), conductivity, color and total dissolved solids (TDS) were studied before and after treatment. The biological treatment was employed on selected dye effluent samples. These isolates were depicted as B1 to B17. High reduction in values of COD, BOD and TDS were observed for B7 in all the effluent samples, while B11, B4 and B15 showed moderate responses. The other isolates did not show any significant response regarding improvement in water quality parameters. For optimal medium and culture conditions response surface methodology was used and up to 50% color and COD removal was observed when B7 was applied on the samples in accordance with analysis of variance (ANOVA). Furthermore, B7 isolate revealed an efficient performance and was identified as *Bacillus subtilis* by using morphological, biochemical and genomic sequencing.

Capsule Summary: The textile dyes effluent samples were subjected to biological treatment using various isolates and based on improvement in water quality parameters, the *B. subtilis* found to be highly efficient for the treatment of textile effluent.

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INTRODUCTION

Polluted water contains organic and inorganic dye residue, acidic and basic residues, suspended solid particles, polymeric hydrocarbons (PAHs), remains of pesticides, fats, oils, lubricants and heavy metals.(Oller et al., 2011). It has a negative impact on human health and aquatic life when this water is consumed. Today, Clothing and textiles are one of the world's oldest and largest industries. Textile processing plants accounts for 17 to 20% of the total industrial effluent (Holkar et al., 2016). Wastewater generated by industrial processes is highly dependent on the kind of fibers, chemicals, and dyes used. Most of the environmental pollutants come from tanning and pre-tanning processes, which account for about 90% of them. The paper industry is one of the most water-intensive in the economy, coming in third number for the amount of water it uses (Sridhar et al., 2011). Food preservers and colors are also widely used in the food industry to keep food-products safe and appealing (Boguniewicz-Zablocka et al., 2020)

Carcinogenic amines and other chemical pollutants such as pentachlorophenol, chlorine bleaching, softeners, hazardous metals, biocides, free formaldehyde, fire retardants and halogen carriers are found in various manufacturing effluents. The discharge of untreated textile effluents into water resources causes pH shifts, gas solubility, eutrophication and an increase in levels of TOC, COD and BOD, all of which have serious consequences. It also has a negative impact on terrestrial ecology as it lowers soil fertility, plant growth, plant production and seed germination. This is a direct threat to the environment. Dye-tainted water may cause cancer, dermatitis, allergic reactions, gene mutations, and more in humans. (Mustafa et al., 2021).

There are several ways to reduce the amount of dye in textile effluent. It is possible to remove colors via a process known as flocculation, which uses both physical and chemical methods to form loosely clumped masses of dye molecules known as flocs. The affinity and capacity to adsorb the adsorbate are the primary considerations in selecting the adsorbent for the adsorption process. Approaches include physical, chemical, and thermal treatment for the modification of carbon surfaces. Thermal treatment with water flow at 700 °C is the most effective approach (Pereira et al., 2003).

In the process of removing salts from water, reverse osmosis is primarily used. Dye removal can benefit from ultrafiltration and nanofiltration methods. Copper, cadmium, nickel, and chromium are among the heavy metals and hazardous chemicals formed as a result of the treatment process.

Phytoremediation is cheap, safe, and environmentally friendly. It is completely a solar powered process (Khandare and Govindwar, 2015). Polysaccharides like carrageenan and alginate make up the majority of algal biomass. The microorganisms in the soil and the roots of the plants perform an important function (de-Bashan et al., 2012). Enzymatic degradation, Biosorption or a mixture of both can be used to reduce the amount of dye in the effluent. To remove dye residues from the aqueous medium, biosorption is used, which involves using an inactive or dead microbial culture.

Acids, alkalis, reactive and non-reactive dyes may all be removed by the live microbial culture. Due to the particular structure of mycelia in fungi culture, dye reduction is more effective (Kaushik and Malik, 2009). Extracellular enzymes secreted by these fungi make it easier to dissolve the insoluble compounds. Because of this, fungi are considered as a viable alternative to conventional approaches. In anaerobic conditions, the dye reduction process can also take place.

The anaerobic bacterial culture is suitable for all dyes and can withstand high concentrations of dye. Microbial Fuel Cells (MFCs) have recently been employed to clean dye wastewater and generate electricity (Solís et al., 2012). Hence, the present investigation was undertaken to treat dyes, effluent samples biologically. The physicochemical characterization such as pH, turbidity, chemical oxygen demand (COD) biological oxygen demand (BOD), conductivity, color and total dissolved solids (TDS) were studied before and after treatment. The conditions were optimized by employing RSM technique.

MATERIAL AND METHODS

Experimental design and sample collection

This research was conducted at the center of environmental protection studies (CEPS), Pakistan Council of Scientific and Industrial Research (PCSIR), and Government College University Lahore, Pakistan. This research involves the characterization of industrial dye effluents (IDE) and its treatment by using isolated bacterial population and by using consortium of this microbial population. The chemicals used in this research were all of analytical grade. The culture media and other chemical reagents were prepared in deionized water.

Physicochemical characterization of dye effluent

All the physicochemical characteristics of dye effluent were carried out in the Institute of Industrial Biotechnology, Government College University, Lahore and Centre for Environmental Protection Studies (CEPS), Pakistan Centre of Scientific and Industrial Research (PCSIR) laboratories complex, Lahore. The physicochemical evaluation of IDE samples includes certain parameters such as: total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), color, turbidity, Ph, conductivity. All these characteristics were determined by using standard analytical methods (APHA, 2005; APHA, 2012).

Identification of isolated bacterial strain

Identification of one bacterial strain that was found to be effective in producing good results such as reduction in values of COD, BOD TDS and profound decolorization was performed. Identification was established through morphological features, biochemical characteristics, genome analysis and plating on differential media. For the identification of non-fastidious, gram negative bacteria and hemolytic, fastidious pathogenic bacteria, Blood agar and MacConkey agar were utilized following 2 hrs. incubation respectively. (Baharuddin et al., 2020).

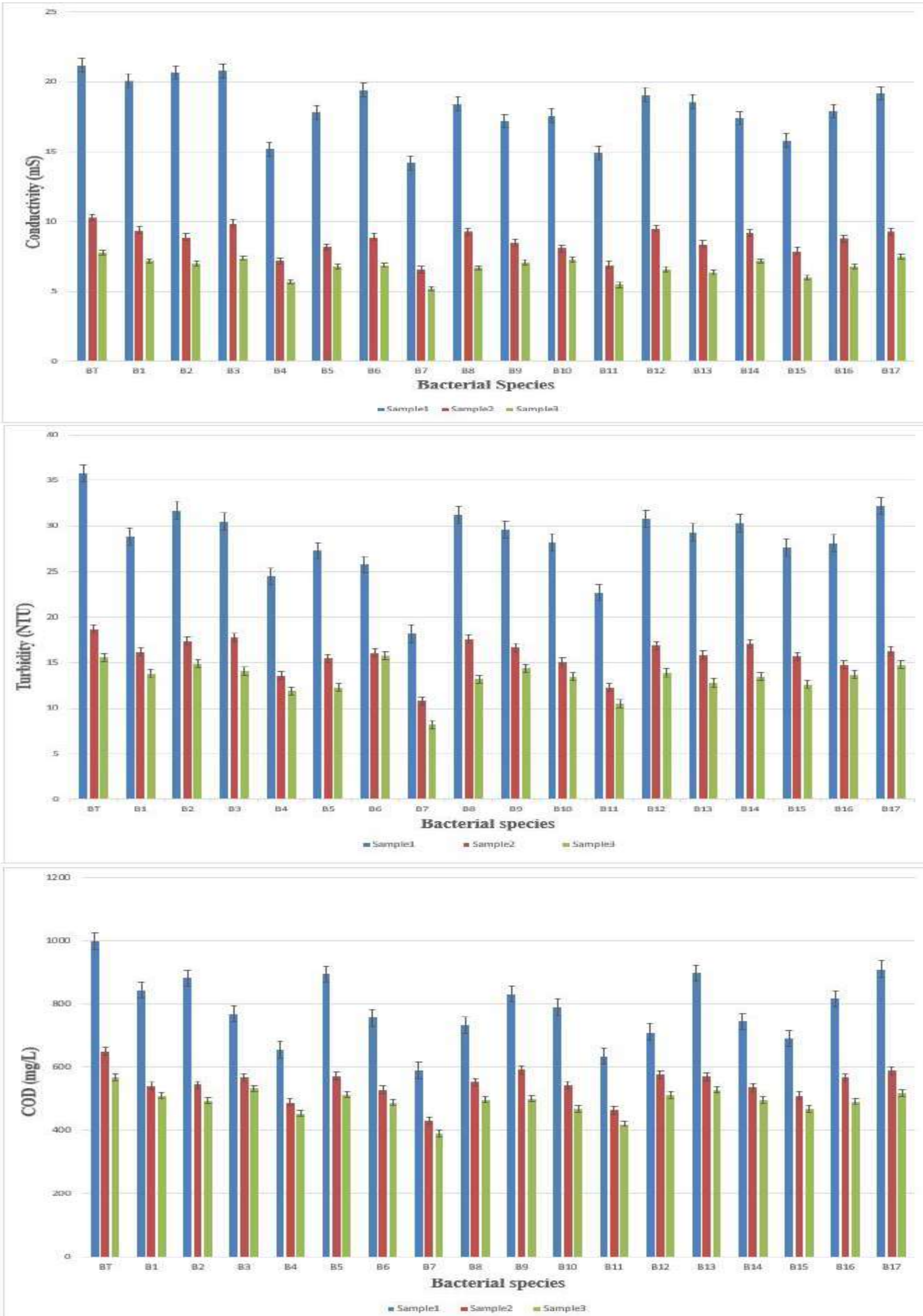


Fig. 1: Water quality parameters values after treatment, conductivity, Turbidity and COD

Morphological characterization

Morphological characterization (size, shape and color) of the bacterial isolate was done by staining the cultured bacteria on a glass slide and examined under 100X microscope. (Afrin et al., 2021).

Biochemical characterization

Oxidase test, indole test, citrate test and catalase test were performed to check the biochemical characteristics for the identification of the bacterial strain according to the methods of (Hussain et al., 2013), (Hadi and Dewi, 2019) and (Begum et al., 2017) respectively.

DNA sequencing of bacterial strain

The most potent bacterial strain was sent to Korea for the sequencing of their DNA.

Optimization process for decolorization of dye

For condition optimization, Design-Expert 6.0.8 software using response surface approach was employed. The optimization approach was used to boost the effects of just those strains of bacteria that had the highest decolorization efficiency. Response Surface Methodology (RSM) was used to find the culture conditions and best medium. Optimal medium parameters include pH, temperature, inoculum size, and glucose concentration that have been optimized. Table 1 shows the levels of these four parameters. Using 30 sets of independent experimentation, the central composite design was applied to optimize the culture conditions as indicated in Table 2. The following equation was used for expressing the relationship between coded and actual values for statistical analysis (Eq. 1).

$$X_i = \frac{(U_i - U_o)}{\Delta U} \quad (1)$$

Where, X_i is the coded value, U_i is the actual value, U_o is the real value centre point and ΔU is the step change in real values. For the coefficient analysis, ANOVA was used. If the *P*-value (probability > *F*) was less than 0.05, the model was considered significant. The experiment was carried out in accordance with the designed level and factors. Centrifugation was used to separate the treated effluent. The scanning spectrophotometer was used to determine the dye concentration. All the tests were repeated three times, and the average results were utilized in computations. The decolorization rate was calculated using Eq. 2.

$$Decolourization\ (\%) = \frac{(OD_i - OD_f)}{OD_i} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Physiochemical characterization

Industrial dye effluent physiochemical characterization for certain parameters i.e., turbidity, PH, conductivity, color, Total dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) were analyzed by using standard methods of water and wastewater. (Rice, 2017). Results for the above-mentioned parameters of collected samples have been summarized in Table 1. The result of pH is in range while all the other parameters having higher values as compared to NEQS as shown.

Isolation of bacteria

For the purpose of biological treatment all three collected samples were subjected to isolation of indigenous bacterial species present within the effluent. Ten isolates were obtained from sample one and designated as B1, B2, B3,…. B10. Similarly, four and three bacterial species were isolated from sample 2 and 3 respectively and were designated as B 11, B12, B17 respectively.

Treatment of samples with isolated bacterial strains

All the collected samples were treated with seventeen (B1 to B17) isolated bacterial strains by pouring 250mL of each sample into all the seventeen-flasks containing bacterial strains (2mL of freshly prepared inoculum of bacterial strains). A total of 51 flasks were used in each trial and three such trials were conducted to produce results on the basis of triplicate analysis. Dye degradation efficacy and other physiochemical parameters were checked.

Water quality analysis

Conductivity

The conductivity of all the samples after treatment with isolated bacterial species was measured. Bacterial species B7 gave the best reduction in conductivity in all three samples (Sample 1 42%, Sample 2 39% and Sample 3 40%) and is more effective in conductivity reduction than B4, B11 and B15. While the other remaining species were least effective on all samples. Results are shown in Fig. 1. A study was done on isolation of bacterial species from dairy sludge in which one bacterial specie DSI_3 showed better reduction in conductivity as compared to other bacterial specie. (Porwal et al., 2015)

Turbidity

Turbidity of all three samples was measured after treatment with isolated bacterial species. Among all the bacterial species B7 showed the maximum reduction in turbidity (48% in Sample 1, 60% in Sample 2 and 47% in Sample 3) while B4, B11, B15 were less effective. Rest of the bacterial species were least effective on all samples.

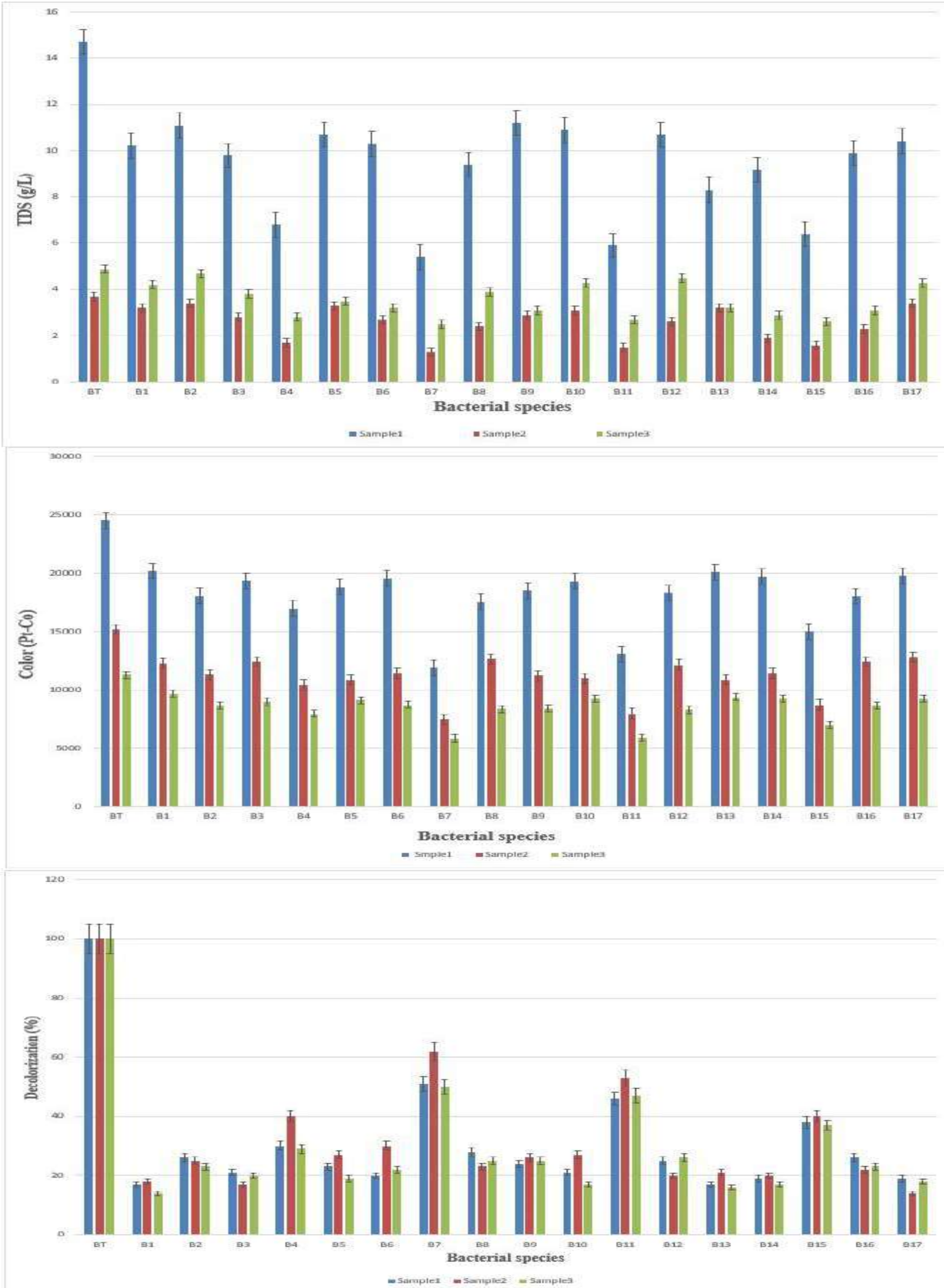


Fig. 2: Water quality parameters values after treatment, TDS, color and decolorization percentage

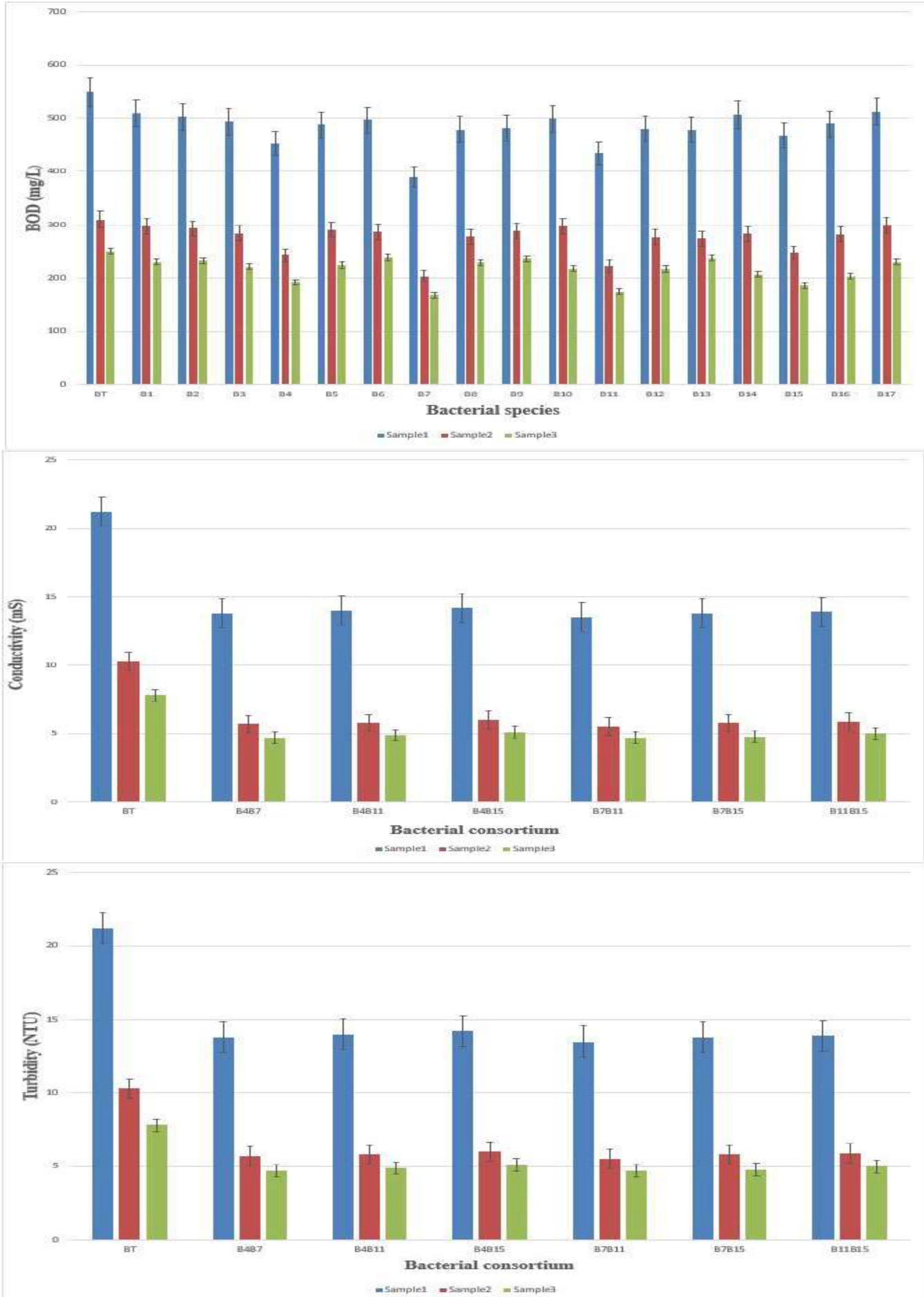


Fig. 3: Water quality parameters values after treatment, BOD, conductivity and turbidity

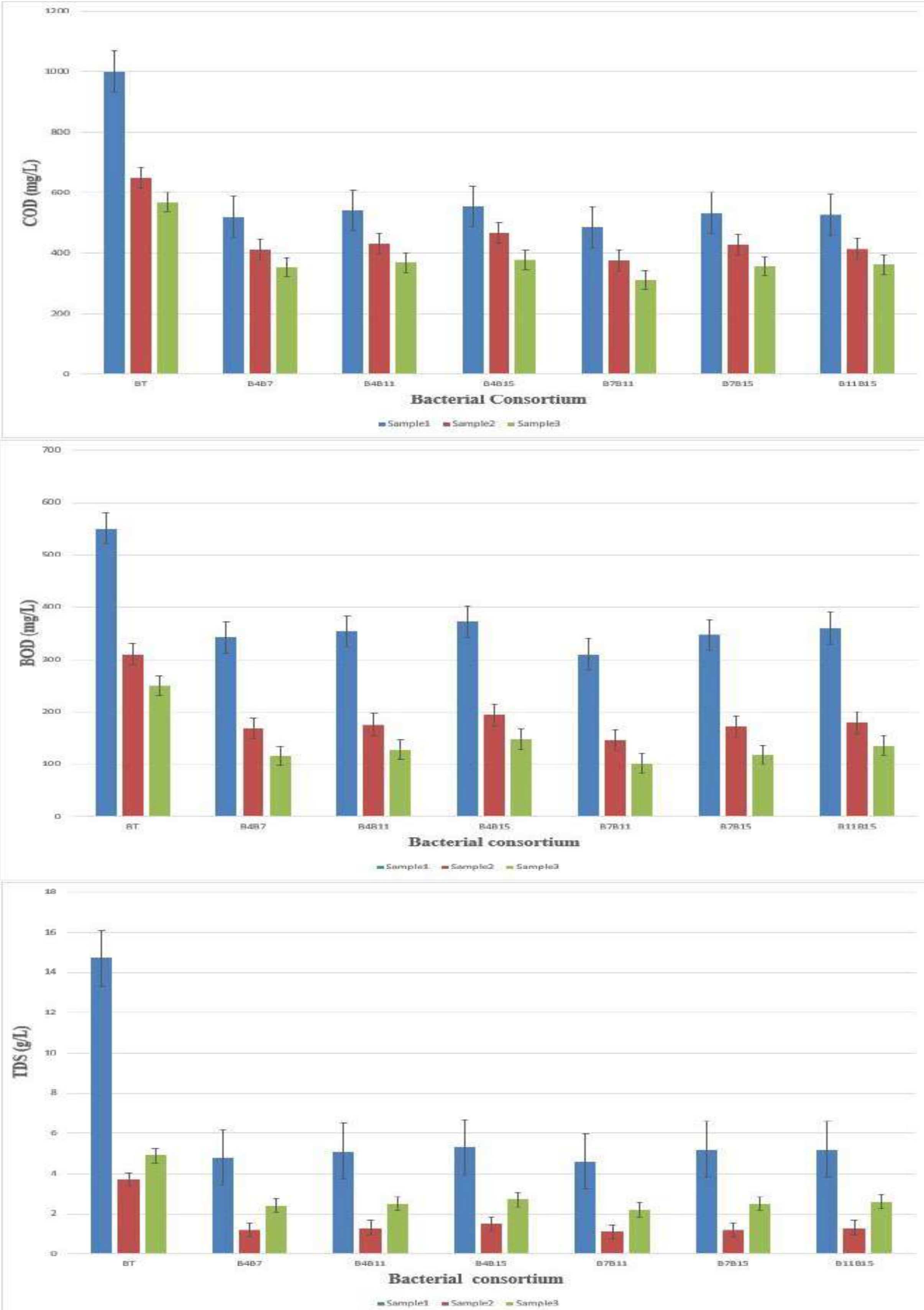


Fig. 4: Water quality parameters values after treatment, COD, BOD and TDS

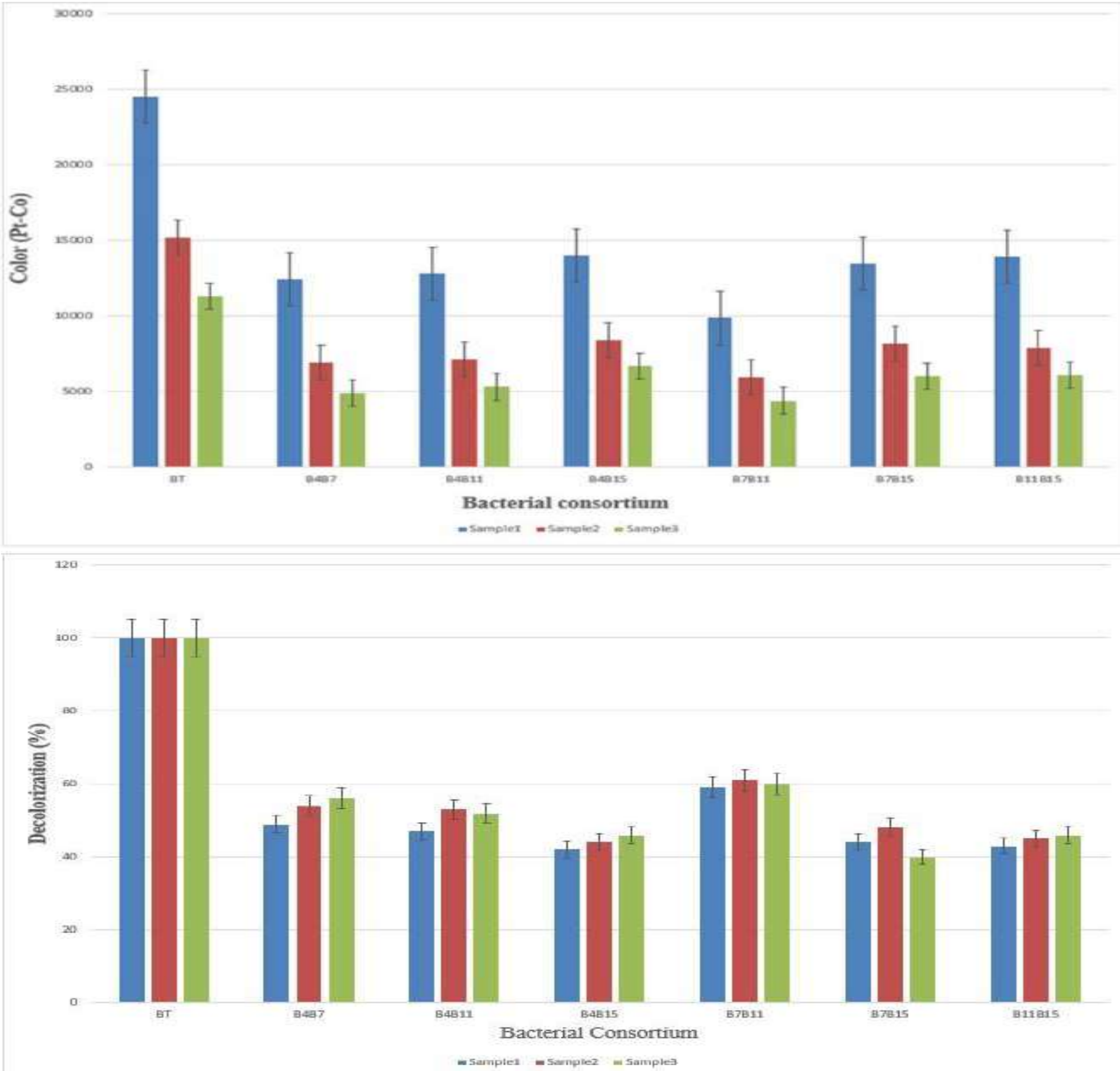
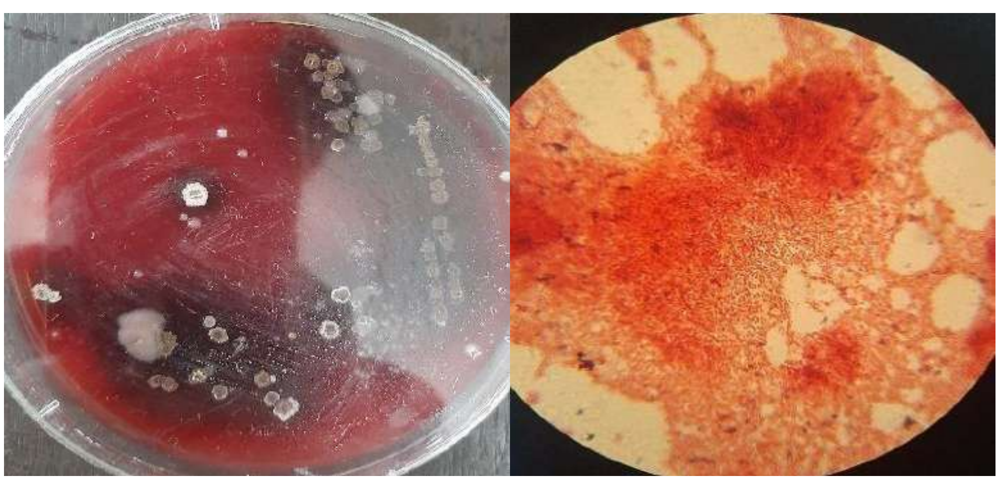
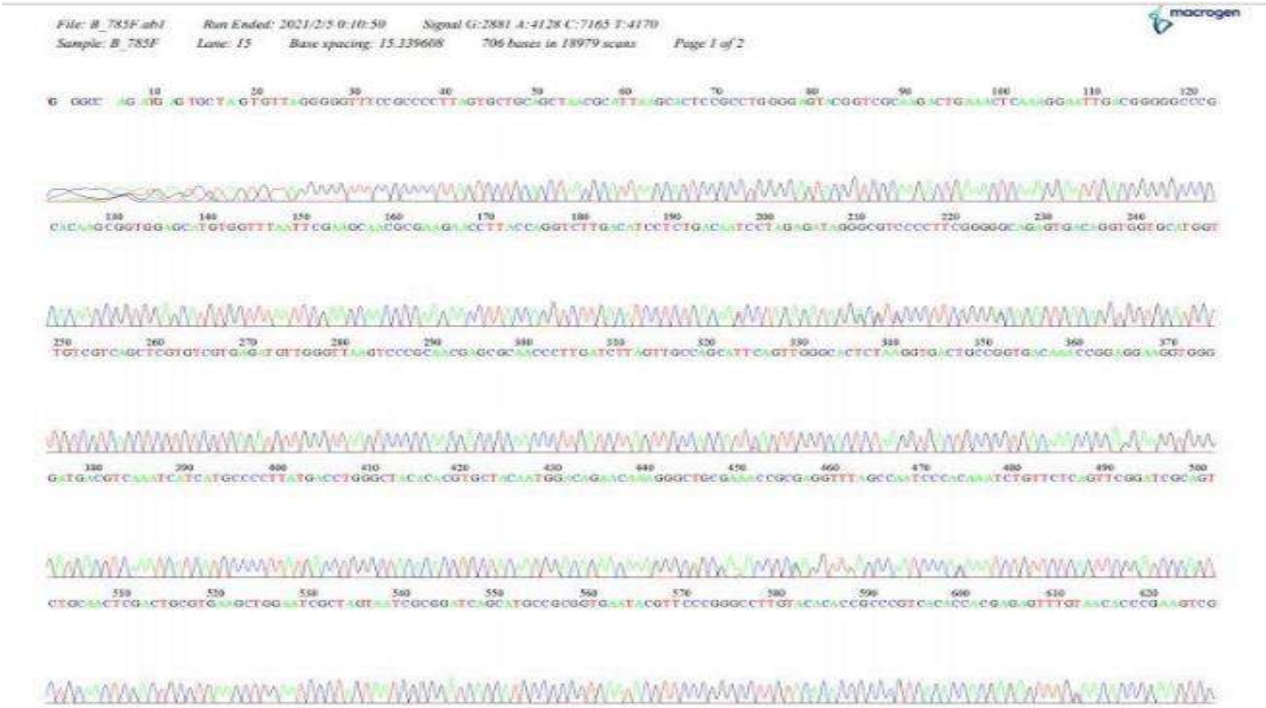


Fig. 5: Water quality parameters values after treatment, color and decolorization percentage



(a) (b)

Fig. 6: (a) Identification through differential media and (b) Identification through gram staining method



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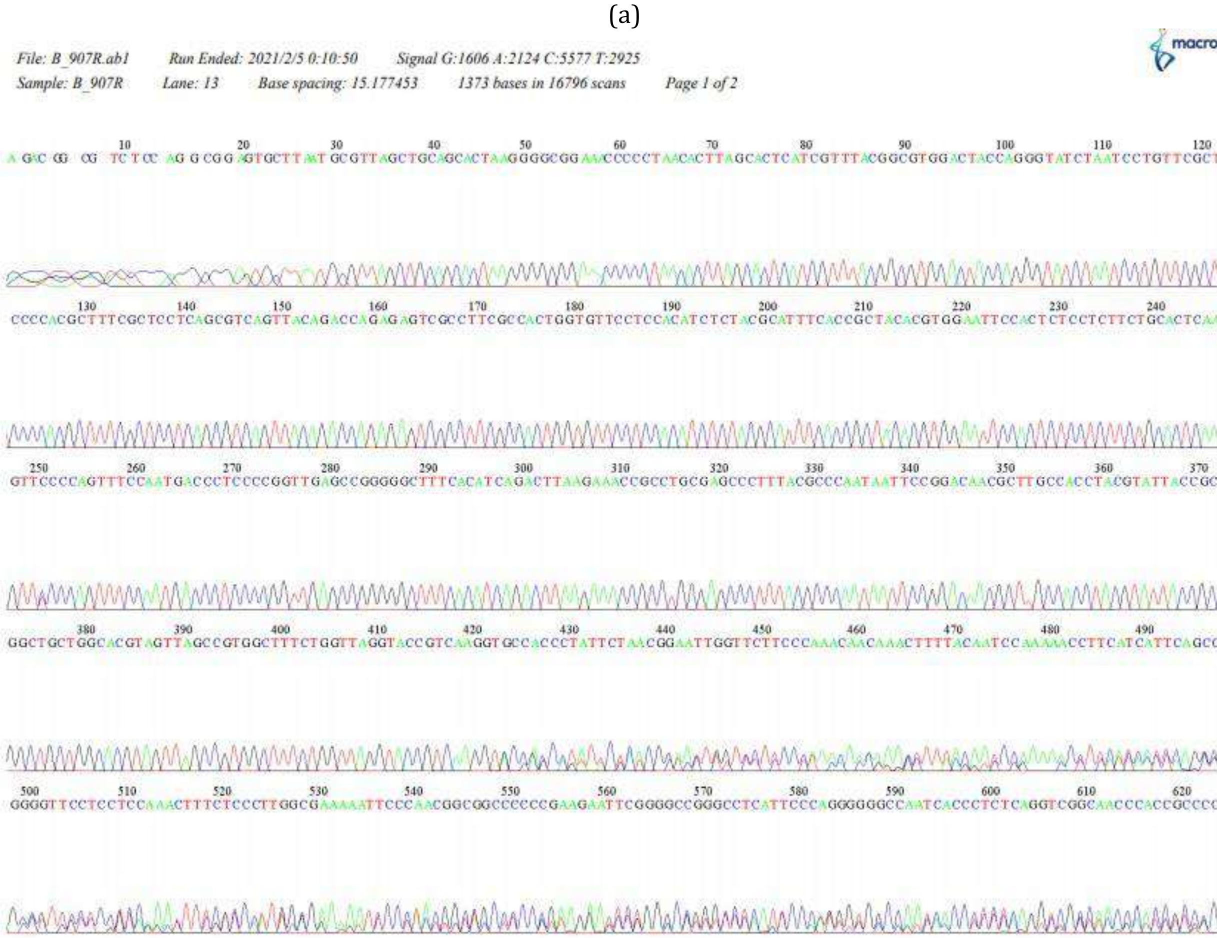
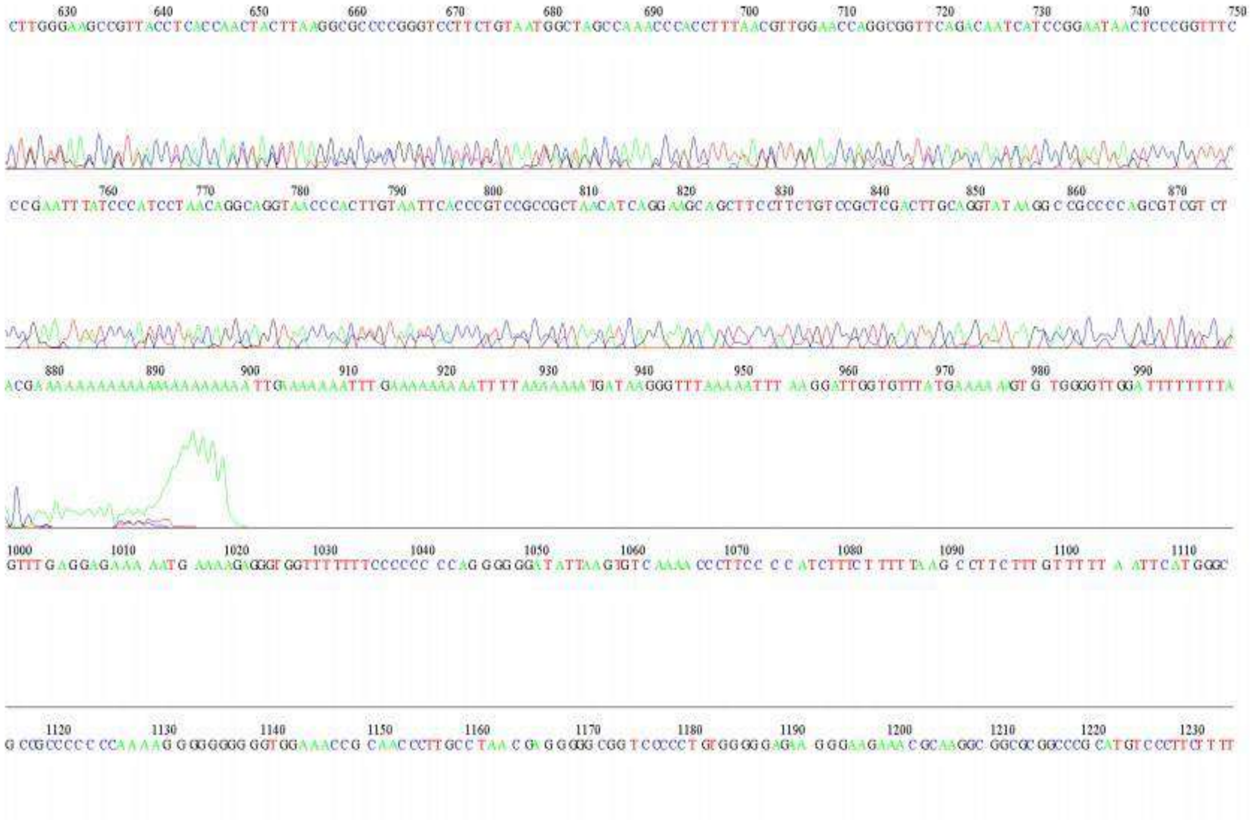
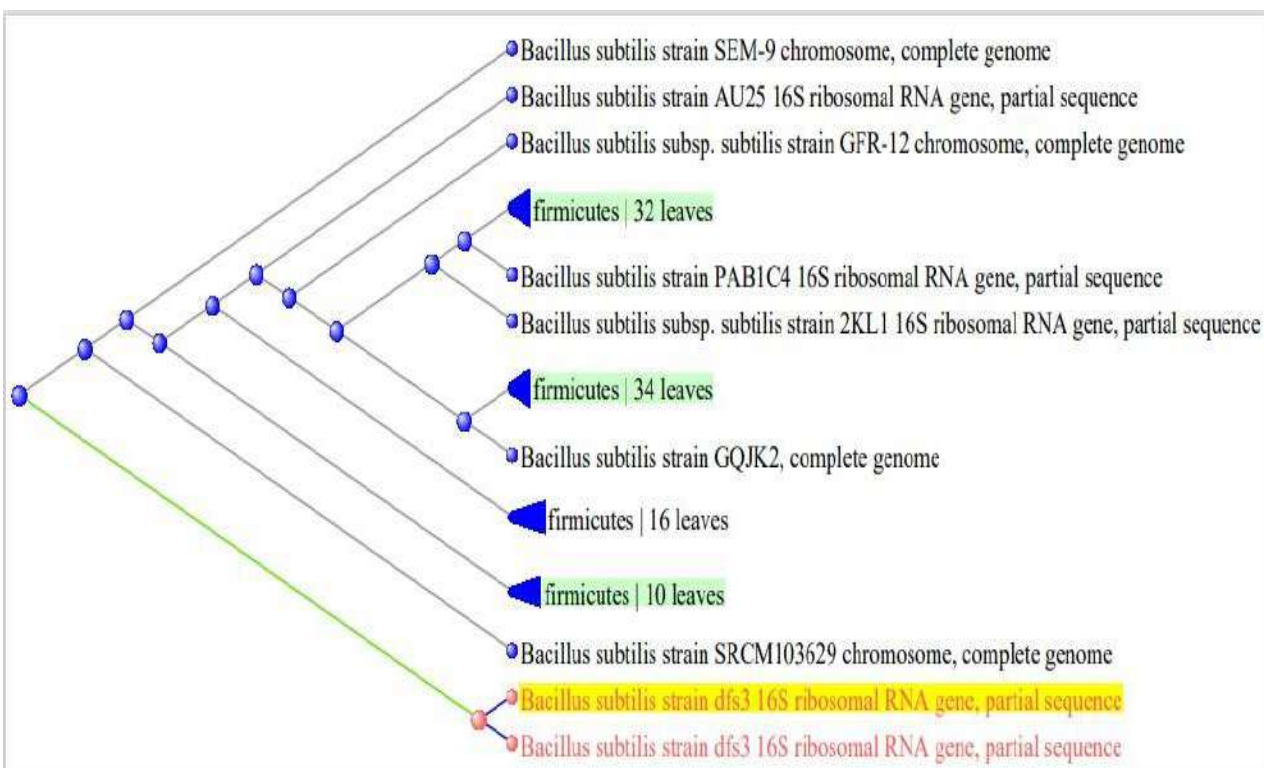


(b)

Fig. 7: The figure part a shows the distribution and part b shows the expression of potent bacterium, i.e., *B. subtilis*



(a)



(b)

Fig. 8: (a) The figure part (a) shows the expression of most potent bacterium, i.e., *B. subtilis* and part (b) shows phylogenetic tree of most potent bacterium, i.e., *B. subtilis*

Results are shown in Fig. 1. According to a study, three bacterial specie were isolated from dairy effluents. It was observed that after aeration reduction turbidity was 45.3% and after filtration further reduction was observed. The overall reduction was 99.3% (Al-Wasify et al., 2017).

Chemical oxygen demand (COD)

COD of all sample 1,2 and 3 were measured after treatment with isolated bacterial species. B7 bacterial specie showed the maximum reduction in COD in all of the three samples (58% in Sample 1, 70% in Sample 2 and 65% in Sample 3) while B4, B11, B15 were less effective. Rest of the bacterial species were least effective on all samples. Results are shown in Fig. 1. In a study, 67.1% COD reduction was observed for bacterial specie 1 that was isolated from dairy effluent with 1% inoculum and 48.3% was observed for S3 with 5% inoculum.(Sreemoyee and Priti, 2013)

Biological oxygen demand (BOD)

BOD of all three samples was calculated after treatment with isolated bacterial strains. B7 bacterial species exhibited the maximum reduction of BOD (67% in Sample 1, 64% in Sample 2 and 70% in Sample 3) as compared to B4, B11, and B15. While rest of the bacterial species were least effective when treated with all three samples. Results are shown in Fig. 1. 78.7% overall reduction in BOD was observed after aeration and filtration after treatment of isolated bacterial specie with effluents in a study. (Porwal et al., 2015)

Total dissolved solids (TDS)

The treatment results of TDS with isolated bacterial species, on collected samples were analyzed and are depicted in Fig. 2. Bacterial specie B7 exhibited maximum reduction in TDS with all samples (37% in Sample 1, 40% in Sample 2 and 42% in Sample 3) as compared to other three B4, B11 and B15 bacterial species. While rest of the bacterial species were less effective. Results are shown in Fig. 2. A bacterial specie *Pseudomonas. sp* was isolated from rubber processing industry. It was used for remediation of this industry effluents and it showed 68.8% reduction in total dissolved solid (TDS). (Shruthi et al., 2012)

Color

Color reduction after treatment of isolated bacterial species with all three samples was measured. Bacterial specie B7 showed maximum color reduction in all samples (48% in Sample 1, 49% in Sample 2 and 60% in Sample 3) as compared to B4, B11 and B14 bacterial species. While rest of the bacterial species exhibited least reduction in color. Results are shown in Fig. 2.

Decolorization efficiency

Decolorization efficiency of isolated bacterial species with all the samples was calculated. It was seen that bacterial specie B7 exhibited maximum decolorization efficiency with all samples (50% in Sample 1, 60% in Sample 2 and 49% in Sample 3) as compared to B4, B11 and B14. While other bacterial species such as (B1, B2, B3, B5, B6, B8, B9, B10, B12, B13, B14, B16 and B17) showed least effect. Results are shown in Fig. 2. *Bacillus cereus* was isolated from tannery industry and was used for decolorization of orange II dye. It showed 68.5% dye decolorization with in the incubation period of 96 hrs (Garg et al., 2013).

Treatment of samples with bacterial consortium effect on water quality

Consortium of those isolated bacterial species which show maximum results against collected samples was prepared. Total six combinations of bacterial consortium were applied on effluent samples. 250 ml of each sample was poured in conical flask and 2ml of freshly prepared inoculum was added into each flask. Dye degradation efficiency and physical parameters were checked as described above.

Conductivity

Conductivity was measured after treatment of four most effective bacterial species in six different possible combinations with all three samples. Maximum reduction in conductivity was observed by bacterial specie B7B11 consortium (60% in Sample 1, 51% in Sample 2 and 65% in Sample 3). B4B7 and B4B11 were less effective while rest of the combinations (B4B15, B7B15 and B11B15) were least effective. Results are shown in Fig. 3. About 88.7% reduction in conductivity was observed by use of consortium of five bacterial species isolated from dairy waste water in a study. (Al-Wasify et al., 2017)

Turbidity

Turbidity was measured after treatment of four most effective bacterial species in six different possible combinations with collected samples. Maximum reduction in turbidity was observed by B7B11 consortium (50% in Sample 1, 49% in Sample 2 and 52% in Sample 3). B4B7 and B4B11 were less effective while rest of the combinations (B4B15, B7B15 and B11B15) were least effective. Results are shown in Fig. 3. 79% reduction in turbidity was observed in sample by using microbial consortium in a study (Costa et al., 2018) and these results revealed the promising efficiency for turbidity value improvement.

Chemical oxygen demand (COD)

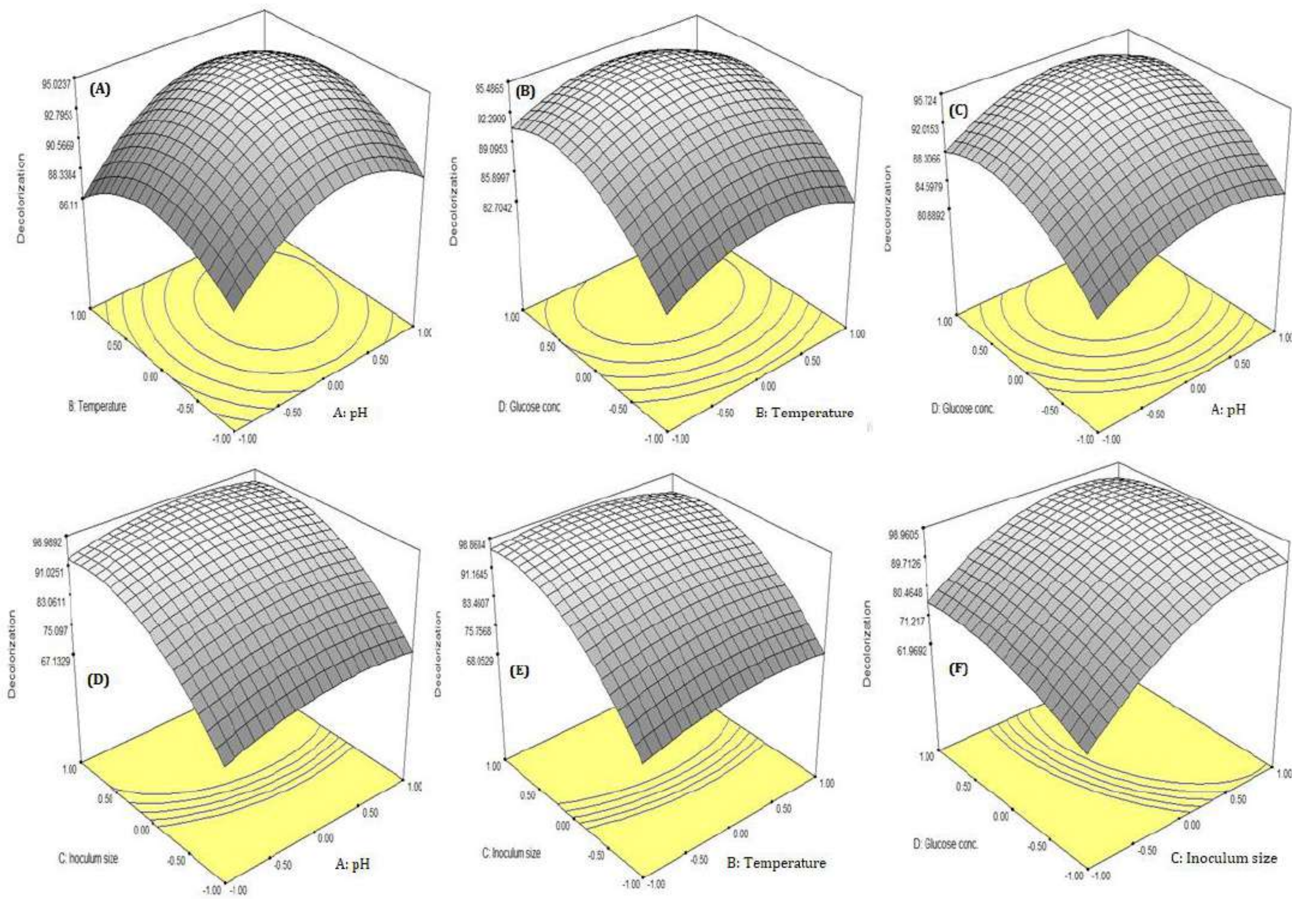


Fig. 9: Interactive effect of input variable on the decolorization, (A) pH versus temperature, (B) temperature versus glucose conc., (C) pH versus glucose conc., (D) pH versus inoculum size, (E) temperature versus inoculum size (F) inoculum size vers glucose conc.

COD of investigated samples were measured after treatment of four most effective bacterial species in six different possible combinations. Maximum reduction in COD was observed by B7B11 consortium (48% in Sample 1, 58% in Sample 2 and 54% in Sample 3). B4B7 and B4B11 were less effective while rest of the combinations (B4B15, B7B15 and B11B15) were least effective. Results are shown in Fig. 4. BMP1 that was the consortium of six isolated bacterial species showed maximum COD reduction by 61.35%. (Mahmood et al., 2013)

Biological oxygen demand (BOD)

BOD was measured after treatment of four most effective bacterial species in six different possible combinations of collected samples. Maximum reduction in BOD was observed by B7B11 consortium (56% in Sample 1, 48% in Sample 2 and 42% in Sample 3). B4B7 and B4B11 were less effective while rest of the combinations (B4B15, B7B15 and B11B15) were least effective. Results are shown in Fig. 4. BMP1 that was the consortium of six isolated bacterial species showed maximum BOD reduction by 59.49%. (Mahmood et al., 2013).

Total dissolved solid (TDS)

TDS was measured after treatment of four most effective bacterial species in six different possible combinations with all three samples. Maximum reduction in TDS was observed by B7B11 consortium (40% in Sample 1, 43% in Sample 2 and 42% in Sample 3). B4B7 and B4B11 were less effective while rest of the combinations (B4B15, B7B15 and B11B15) were least effective. Results are shown in Fig. 4 BMP1 that was the consortium of six isolated bacterial species showed maximum TDS reduction by 44.93% (Mahmood et al., 2013).

Color

Color was measured after treatment of four most effective bacterial species in six different possible combinations with all three samples. Maximum reduction in color was observed by B7B11 consortium (40% in Sample 1, 44% in Sample 2 and 43% in Sample 3). B4B7 and B4B11 were less effective while rest of the combinations (B4B15, B7B15 and B11B15) were least effective. Results are shown in Fig. 5. 94% color reduction was exhibited by a consortium within 37 hrs. with the pH 6.5-8.5 in a study (Moosvi et al., 2005).

Decolorization efficiency

Decolorization efficiency was measured after treatment of four most effective bacterial species in six different possible combinations with all three samples. Maximum decolorization efficiency was observed by B7B11 consortium (59% in Sample 1, 60% in Sample 2 and 60% in Sample 3). B4B7 and B4B11 were less effective while rest of the combinations (B4B15, B7B15 and B11B15) were least effective. Results are shown in Fig. 5. 90 % decolorization efficiency was achieved through bacterial consortium in a study. (Costa et al., 2018).

Identification of isolated bacterial strain

Differential media

Table 1: Levels and factors of experimental design for dye reduction through bacterial and fungal consortium

Factors	Level 1 (-a)	Level 2 (-1)	Level 3 (0)	Level 4 (+1)	Level 5 (+a)
pH	5	6	7	8	9
Temperature	35	36	37	38	39
Inoculum Size	1.50	1.75	2	2.25	2.50
Glucose concentration	2.5	5.5	8.5	11.5	14.5

Table 2: Design for the experimental and predicted values of decolorization

S. No.	pH	Temperature (C°)	Inoculum size (%)	Glucose conc. (%)	Decolorization (%)
1	-1	-1	-1	-1	44.67
2	1	-1	-1	-1	49.43
3	-1	1	-1	-1	50.02
4	1	1	-1	-1	59.63
5	-1	-1	1	-1	80.02
6	1	-1	1	-1	81.23
7	-1	1	1	-1	78.54
8	1	1	1	-1	80.01
9	-1	-1	-1	1	64.65
10	1	-1	-1	1	67.13
11	-1	1	-1	1	68.02
12	1	1	-1	1	72.56
13	-1	-1	1	1	82.78
14	1	-1	1	1	85.94
15	-1	1	1	1	81.43
16	1	1	1	1	86.61
17	-2	0	0	0	84.05
18	2	0	0	0	91.37
19	0	-2	0	0	92.03
20	0	2	0	0	88.15
21	0	0	-2	0	37.28
22	0	0	2	0	94.12
23	0	0	0	-2	83.57
24	0	0	0	2	84.92
25	0	0	0	0	93.26
26	0	0	0	0	95.91
27	0	0	0	0	94.71
28	0	0	0	0	94.06
29	0	0	0	0	95.63
30	0	0	0	0	94.75

Table 3: Characterization of collected industrial dye samples

Parameters	Sample 1 (S-1)	Sample 2 (S-2)	Sample3 (S-3)	NEQS
pH	10.8	8.1	7.8	6-10
Conductivity (mS/cm)	21.5	10.1	7.3	--
Turbidity (NTU)	35.5	18.5	15.2	--
COD (mg/L)	1000	650	570	180 mg/L
TDS (mg/L)	14.7	3.7	4.9	3500 mg/L
Color (Pt-Co/Hazen)	24500	15200	11300	--
BOD (mg/L)	550	310	250	150 mg/L

Table 4: Identification of bacterial strain through gram staining

S. No	Basic Characteristics	Properties
1	Cell morphology	Rods
2	Gram nature	Positive
	Motility	Motile
3	Capsulated/ Non capsulated	Non capsulated
4	Filamentous/ Nonfilamenteous	Long filamentous
5	Size	1×3-4 µm
6	Spore position	Central spores
Identification of bacteria through different biochemical tests		
1	Catalase	Positive (+ve)
2	Citrate	Positive (+ve)

Most effective bacterial species were then chosen for further identification process. For this purpose, differential media method was used in which both MacConkey Agar and Blood Agar were used. Gray, opaque, granular, spreading flower like colonies with irregular perimeters grows on Blood Agar and no growth appears on the MacConkey Agar. It is clearly observed that isolated bacteria is gram positive as shown in blood agar. Gram staining method was used for further identification. Results are shown in Fig. 6(a, b).

Morphological characteristics

The results in Table 4 showed microscopic examination revealed that the isolated bacteria is gram positive because it has rod shape squared ends that might be in the form of single rod and short chains. It is observed as non-capsulated long filamentous bacterium. It has spores that are present at central position. The size of that bacterium is about 1×3^{-4} µm.

Biochemical tests

The results of Biochemical Tests are shown in Table 4. The overall results of morphological characteristics and biochemical tests showed that bacteria are gram positive and is *Bacillus subtilis.* The genomic sequencing technologies enable us the high-speed analysis of multiple genes simultaneously and also showed the distribution as well as phylogenetic tree of potent bacteria (Phillips et al., 2014). Distribution and expression of most potent bacterium i.e., *Bacillus subtilis* is shown in Fig. 7(a), 7(b) and 8(a) and its phylogenetic tree is shown in Fig. 8(b).

Optimization

The decolorization rate was calculated using response surface methodology and second-order polynomials. Various criteria were used to assess the model's quality. Within 24 hours, the decolorization efficiency ranges from 86% to 88% as the pH changed from -1.00 to 1.00 as shown in Fig. 9(a). It was claimed that pH was a significant parameter. Within 24 hours, the decolorization efficiency increased from 90% to 92% when the temperature was adjusted from -1.00 to 1.00. It was suggested that temperature was a key parameter as shown in Fig. 9(b). As shown in response surface plot of decolorization in Fig. 9(c) within 24 hours, the decolorization rate went from 88 to 90% as the pH climbed from -1.00 to 1.00. It was indicated that pH was an important parameter to consider. 91% to 98% change in decolorization rate was observed as pH increased from -1.00 to 1.00 within 24 hours as depicted in Fig. 9(d). So, pH was considered as important parameter. Similarly, 97% to 98% change in decolorization was calculated as the temperature thrived from -1.00 to 1.00 within 24 hours as shown in Fig. 9(a). Within 24 hours, the decolorization efficiency improved from 72% to 80% when the inoculum size was increased from -1.00 to 1.00. Results are shown in Fig. 9(b). In coding the values of the four independent variables, the Central Composite Design was used. According to the study of Mohana and his coworkers, dye reduction efficiency was increased from 75% to 98.5%. Glucose accelerated the decolorization process by inducing redox nucleotides, which accelerated the decolorization of azo dye. (Mohana et al., 2008). A study was based on the azo dyed reduction by adding glucose as an additional carbon source exhibited similar results (Carliell and Senior, 1995)

Our results are in close agreement with this study that the results of a randomized controlled trial, using a RSM and an intermediate design, bacterial consortiums grown in flask. The three levels of temperatures, salts, pH, and initial concentration of asphaltene as a substrate are considered to be the limits of the culture medium and incubation growing areas for 60 days. The highest rate of asphaltene biodegradation was 46.41% due to the consortium of crude oil including *Staphylococcus saprophyticus sp.* and *Bacillus cereus sp.* at 45 degrees C, salinity 160 gL^{-1}, pH 6.5, and 25 gL^{-1} initial asphaltene concentration (Shahebrahimi et al., 2020).

Similar results were found in a study based on degradation of bispyribac sodium (BS) by using bacterial consortium named as BDAM. Different cultural conditions (pH, temperature and inoculum size) were designed to degrade BS by consortium BDAM and the interactions of these structures were analyzed using 2 (3) complete factorial central composite design (CCD) based on Response Surface Methodology (RSM). Accurate values of temperature, pH and inoculum size were obtained at 40 oC, 8 and 0.4 g/L, respectively to achieve significant BS degradation (85.6%) (Ahmad et al., 2018; Solis et al., 2012).

Similar results were found in another study in which microbial consortium of two specie *Bacillus cereus* and *Streptomyces aureus* for the enhancement of degradation of insecticide cypermethrin. Response surface methodology determined the optimal conditions that were 28.2^{o} C and 7.5 pH (Chen et al., 2012).

Another study reported a triazo dye degradation using microbial co culture. The RSM was used to efficiently optimize the decolorization process. The experimental variables: dye concentration, yeast extract, and pH all have a substantial influence on the percentage of dye decolorization. Efficient decolorization by microbial co culture was depicted by results (Zin et al., 2020).

CONCLUSIONS

In this study, contaminants in food dyes were treated biologically by using different bacterial species. The main objectives of this study were to reduced color, along with other factors which includes turbidity, total dissolved solids (TDS), conductivity, chemical and biochemical oxygen demand. The effectiveness of color removal was 50% when treated with bacterial isolate and when treated with bacterial consortia the color was removed up to 60%. Overall, 95.5% color removal was achieved by treatment with the best bacterial isolate. Several parameters that include TDS, Turbidity, COD, BOD and conductivity were determined to evaluate the effectiveness of that biological treatment. The most effective treatment is performed by bacterial isolate *Bacillus subtilis*. This method of extracting food dye was relatively inexpensive and environment friendly. The microbes used were harmless, easily accessible and non-toxic sludge was produced.

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