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PHYSICOCHEMICAL CHARACTERISTICS, ISOLATION AND SCREENING OF BACTERIA FOR DEGRADATION OF DYES FROM TANNERY EFFLUENTS

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ABSTRACT: Tannery industries uses versatile chemicals during tanning process. This tannery effluent when discharged into water bodies alter the physical, chemical and biological characteristics of water. The present study was an attempt for the assessment of different physicochemicalparameters, isolation and identification of bacteria through 16srRNAbased molecular techniques and evaluation of their ability to decolorize the dyes. The optimal dosage of coagulant was found to be 8%. The results of the parameters showed that the effluent was blackish colour with unpleasant odour, acidic in $P^H$with high organic and inorganic load of Total suspended solids(TSS), Total dissolved solid (TDS), Biological oxygen demand (BOD), Chemical oxygen demand (COD), Total Chromium, Copper, Chloride and Sodium. Twelve bacterial isolates were isolated and among these three bacterial isolates were screened which shows maximum decolourization of dyes and identified by 16srRNA sequencing. They were identified as *Bacillus cereus*, *Enterobacter cloacae and Enterobacteriaceae bacterium.* Optimization of different parameters was done by the potential strain *Bacillus cereus*. The results from the present study shows the potential of the bacteria to degrade the dye in tannery effluents that can help to solve the pollution problem.

KEYWORDS: Untreated tannery effluent, Polyaluminium Chloride (PAC), Physicochemical Parameters, Isolation and Identification, Decolourization.

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1.INTRODUCTION

Tannery is one of the important industries causing waterPollution. Tannery waste water are highly complex and are characterized by high contents of organic, inorganic and nitrogenous compounds, chromium sulfides, suspended solids and dissolved solids. The tanning process is almost completely a wetprocess that consumes significant amount of water and generates about 90% of the used water as effluent. Coagulation or flocculation is a frequently applied process in the primary purification of industrial wastewater. Alum and PAC are widely used as coagulants in water and wastewater treatment for the removal of impurities from effluent including colloidal particles and dissolved organic substances. There are many factors that affect the Process of coagulation such as P^H, alkalinity and dose of coagulant [1].Microbes in the environment play an important role in cycling and destroying them through biodegradation [2]. Coagulation and flocculation of tannery waste water by using various inorganic coagulants is to reduce the total organic load, total solids and to remove the toxic metals such as chromium before the biological treatment of tannery effluent [3].The major public concern over tanneries areabout odours and water pollution from untreated discharges. Other problems have arisen more recently from the increasing use of synthetic chemicals such as pesticides, solvents,dyes, finishing agents and chemicals which cause problems of toxicity and persistence [4]. Degradation is defined as the process of degrading hazardous organic contaminants into environmental safe levels in soil and water. The enzymes involved in degradation uses environmental contaminants as source of food and make them ideal for degradation [5]. Based on different researchers on different countries and different environmental samples like soil, water and vegetable in all Cr(VI) is above the limit. Cr(VI) has different health effects and cause toxicity, mutagenic, carcinogenic and high blood pressure for human and untreated water discharged from the tannery industry also affect seed germination of the plants[6]. Chromium can also cause a temporary effects such as dizziness, headache, irritation of eyes, skin or lungs, allergic reactions, poisoning of liver , Kidney , nervous system due to lack of oxygen. Tannery effluent with high TDS can also cause gastrointestinal irritations, chrome ulcers, acute irritations dermatitis and allergic eczematous dermatitis [7].The treated waste water can also be used for various non-potable purposes including agriculture, aquacultural purposes and also during leather tanning [8]. The main objectives of the study is to determine the effect of PAC dosage for coagulation, analysing the physicochemical parameters of untreated tannery effluents, isolation and identification of bacteria from the tannery effluent through 16srRNA based molecular technique, ability of the bacteria to degrade the dye present in the tannery effluent and to optimize the environmental factors for degradation by using the potential strain at different p^H, temperature, carbon and nitrogen source.

2. MATERIALS AND METHODS

Sample collection

The tannery effluent was collected from common Effluent Treatment Plant (CETP) Pallavaram, Chennai during (Oct 2017- March 2018). The effluent were brought to the laboratory and stored at 20^0C .

Treatment of tannery effluent by the process of Coagulation

500ml of tannery effluents was taken in five conical flask. To each conical flask various concentrations ranging from (6-10%) of polyaluminium chloride (PAC) was added. It was vortexed with magnetic stirrer for10 minutes and kept undisturbed for 24hrs. Coagulation formed was measured in cm and optimum concentration dosage of PAC for coagulation was selected. The supernantantwas filtered after coagulationandUV scanning was done. After scanning, highest wavelength (nm) absorbance was selected for further studies.

Analysis of Physicochemical parameters of tannery effluents

The supernatant formed after coagulation was used forphysicochemical parameter analysis. The parameters such as P^H ,Total suspended solids (TSS), Total dissolved solid (TDS), Biological oxygen Demand (BOD), Chemical oxygenDemand (COD), Total Chromium,Copper, Chloride, Electrical conductivity (EC), Total hardness, Calcium and Sodium wasestimated by using standard methods[9] and the results werecompared with pollution control acts[10]. The results were statisticallyanalysed using standard deviation to examine the significance difference.

Isolation of dye degrading bacterial isolates from tannery effluents

The sludge formed after coagulation was taken and the bacterial isolation was carried out by spread plate method, following the procedures [11].After incubation, the colonies were isolated and purified by streaking in nutrient agar plates. The well grown bacterial cultures was used for screening techniques.

Screening of dye degrading bacterial isolates

The culture was screened out by inoculating them on 100ml of nutrient broth with 10ml dye effluent. The flask was incubated at 37^{o}C for 24hrs. After incubation, the OD value was measured

at selected wavelength maxima (λm) . The isolates which showed more than 70% degradation was selected for further studies.

Identification of selected isolates

Based on the dye degrading ability the selected isolates were identified by 16srRNA sequencing. The genomic DNA were amplified with universal bacterial primers. For species level identification, the sequences obtained were compared with the Genebank database using the BLAST program facility of NCBI. The sequences were analysed using the check chimera and the similarity rank programs of the Ribosomal Database project is also analysed by using BLAST program [12].

Effect of dye decolourization

100ml of nutrient broth with 15ml of tannery effluent was taken. The P^H was adjusted to7. It was autoclaved at121°C for 15minutes at 15 lbs. After autoclaving, 5ml of the culture was inoculated in the broth. The initial absorbance was taken and the flask was kept in the shaker incubator at 37ºC for 24hrs. After incubation, 10ml of the broth was taken, filtered and centrifuged at 5000rpm for 20 minutes. Decolourization was measured with the supernantant by measuring the absorbanceat 480nm. Among these Organisms, only one potential strain which shows more decolourization was used for optimization studies.

Decolourization assay

Decolourization was measured in percentage by using spectrophotometer. The Percentage of decolourization was calculated from the following formula,

$$\% \text{ of Decolourization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} X\ 100$$

Optimization of environmental factors for efficient decolourization

Effect of p^H

To 15ml of nutrient broth, 3.5ml of effluent was added in each flask. Various P^H(6,6.5, 7, 7.5 and 8)was adjusted and it was autoclaved. After autoclaving, each flask was inoculated with 5ml of culture.The initial absorbance was taken and the flask was kept in the shaker incubator at 37ºC for 24hrs. After incubation, final OD was measured.

Effect of temperature

To 15ml of nutrient broth, 3.5ml of effluent was added in each flask. After autoclaving, each flask was inoculated with 5ml of culture. The initial absorbance was taken and the flask was incubated at various temperature (20ºC, 25ºC, 30ºC, 35ºC, 40ºC) for 24hrs. After incubation,final OD was measured.

Effect of carbon source

To 15ml of nutrient broth, different carbon sources (glucose, rice, sucrose, wheat) was added. 3.5ml of effluent was added in each flask andit was autoclaved. After autoclaving, 5ml of the

culture was added in each flask and the initial absorbance was measured. Then the flask was kept in the shaker incubator at 37°C for 24hrs. After incubation,final ODvalue was measured.

Effect of nitrogen source

To 15ml of nutrient broth,different nitrogen sources (ammonium sulfate, ammonium chloride, urea, yeast, and peptone) was added. 3.5ml of effluent was added in each flask and it was autoclaved. After autoclaving, 5ml of culture was added in each flask and the initial absorbance was measured. Then the flask was kept in the shaker incubator at 37°C for 24hrs. Afterincubation,final OD value was measured.

3. RESULTS AND DISCUSSION

Tannery effluent not only affects the quality of drinking water but also has deleterious impact on the soil microflora and aquatic ecosystem. Dye present in the tannery effluent is also one of the major source of environmental toxicity.

Treatment of tannery effluent by the process of coagulation

Coagulation is a chemical water treatment technique prior to sedimentation and filtration to enhance the ability of a treatment process to remove particles. The optimal dosage of coagulant used for coagulation was found to be 8%.

Table 1: Physicochemical parameters of tannery effluent for a period of six months from Oct 2017 – Mar 2018.

Sl.no:	Parameters	CPCB 1995	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18
1	Colour	Colourless	Blackish	Blackish	Blackish	Blackish	Blackish	Blackish
2	Odour	Odourless	Unpleasant	Unpleasant	Unpleasant	Unpleasant	Unpleasant	Unpleasant
3	Total Suspended Solid (mg/l)	100	125.3 ±1.5275	124.3 ±1.5275	126.1 ±1.2583	128.5 ±1.3229	122.1 ±1.7652	122.8 ±1.9809
4	PH	5.5-9.0	5.87 ±0.0306	5.96 ±0.0321	5.89 ±0.0252	5.84 ±0.0351	5.85 ±0.03	8.57 ±0.02
5	Total dissolved solid (mg/l)	2100	8841 ±1.6215	8780 ±1.58	8843 ±1.7214	8781 ±1.6073	8760 ±1.7559	8830 ±1.5
6	BOD (mg/l)	30	320.5 ±1.8028	349.9 ±1.3528	400.5 ±1.8028	310.1 ±1.7559	360.9 ±1.6523	384.8 ±1.2583
7	COD (mg/l)	250	1039.7 ±1.464	1489.8 ±1.2583	1300.6 ±1.5275	1599.8 ±1.2583	1100.3 ±1.5275	1699.6 ±1.5275
8	Total Chromium	2	0.02 ±0.0173	2.30 ±0.4359	2.74 ±0.5666	4.38 ±0.5918	4.03 ±0.9851	7.03 ±0.0975
9	Copper (mg/l)	3	0.06 ±0.0306	5.42 ±0.5687	5.78 ±0.586	5.72 ±0.5333	3.65 ±0.5329	5.39 ±0.6038
10	Chloride (mg/l)	1000	1284.9 ±1.7782	1473.5 ±1.5604	1310.5 ±1.5	1101.9 ±1.6825	1696.6 ±1.8965	1505.3 ±1.4552
11	Electrical conductivity (µmhos / cm)	400	13600 ±1.8305	13200 ±1.3614	13400 ±1.8028	13320 ±1.6823	13451 ±1.8028	13510 ±1.7559
12	Total hardness (mg/l)	1000	471.4 ±1.5069	520.8 ±1.893	580.5 ±1.3299	496.3 ±1.5275	501.0 ±1.7321	470.3 ±1.5275
13	Calcium (mg/l)	100	82.5 ±1.5	96.3 ±1.5275	179.1 ±1.0408	87.9 ±1.7755	130.3 ±1.5275	144.3 ±1.5275
14	Sodium (mg/l)	600	616.8 ±1.6073	636.3 ±1.5275	622.8 ±1.6073	690.1 ±1.0504	640.9 ±1.675	685.4 ±1.5698

± Standard Deviation

Analysis of Physicochemical parameters of tannery effluents

The Physicochemical parameters of untreated tannery effluent was analysedfor a period of 6 months (Oct 2017- Mar 2018). The results of the study shows that the effluent was blackish with unpleasantodour. TSS ranges from 122.1 mg/l ± 1.7652 to 128.5 mg/l ± 1.3229 than the standard. High amount of suspended solids in effluent will deplete the oxygen and reduce the aquatic life. The pH of the tannery effluent was highly acidic ranges from 5.84 ± 0.0351to 5.96 ± 0.0351 and low pH will affect the physiology of fishes [13]. The Maximum values of total dissolved solid ranges from 8760 mg/l ±1.7559 to 8843 mg/l ±1.7214. High concentration of dissolved solids affects the density of water and osmoregulation of fresh water organisms.BOD has a minimum range of 310.1mg/l ±1.7559 to maximum 400.5 mg/l ±1.8028. High levels of biological oxygen demand is due to high organic load. Increase in BOD leads to depletion of dissolved oxygen [14]. COD ranges from 1039.7 mg/l ±1.4640 to 1699.6 mg/l ±1.5275. COD is used for the determination of total oxygen demand by organic material present in the effluent. This effluent is unsuitable for aquatic organisms due to reduction in DO content [15]. Total chromium ranges from 0.02mg/l ±0.0173 to 7.03 mg/l ±0.9750. Continuous discharge of chromium in low concentration has been reported to be toxic to aquatic life and it disrupt the aquatic food chain [16]. Copper ranges from 0.06mg/l ±0.0306 to 5.78 mg / l ±0.5860. Copper is an essential element in mammalian nutrition. High levels of copper leads to adverse health effects [17]. Chloride ranges from 1101.9mg /l ±1.6825 to 1696.6 mg/l ± 1.8965. Chloride is used in hide ofskinpreservation. High level of Chloride leads to the breakdown in cell structure. Theelectrical conductivity was found to be high from 13200 μ mhos/Cm ±1.3614 to 13600 μ mhos/Cm ± 1.8305.This is due to the presence of inorganic substances and salts. High electrical conductivity level may be due to higher concentration of acid-base and salt in water. Total hardness was found to be highfrom 470.3 mg/l ±1.5275 to 580.5 mg/l ±1.3229. Calcium has maximum range of 179.1mg/l ± 1.0408 and sodium has maximum range of 690.1 mg/ l ± 1.0504. High colour intensity, high value of physicochemical parameters such as COD, TDS, TSS, Chloride, Sodium and nitrate of untreated tannery effluent was also studied [18].

Isolation of dye degrading bacterial isolates from tannery effluents

The present study was focused on biodegradation of tannery dye effluent by using bacteria isolated from tannery effluent. Therefore, totally twelve bacterial single colonies (TEI- TE12) were isolated and streaked in nutrient agarmedium based on their morphological characters.

Screening of dye degrading bacterial isolates

These bacterial isolates were screened for the decolourization ofdye effluents by measuring using spectrophotometer. Bacterial isolates TE3, TE10 and TE12 were screened out which shows more than 70% decolourization and characterized using various biochemical test and confirmed through molecular approach. Decolourization of dye solution take place in two ways, either adsorption on

the microbial biomass or biodegradation of the dye molecules by the bacterial cells [19]. The percentage of decolourization was shown in Fig1

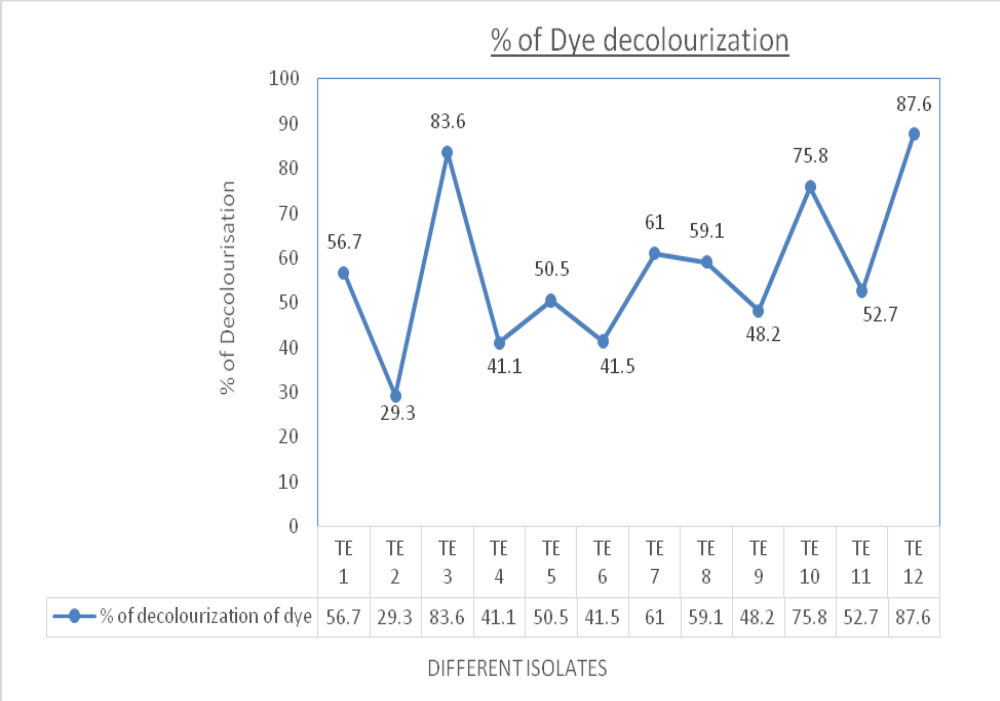
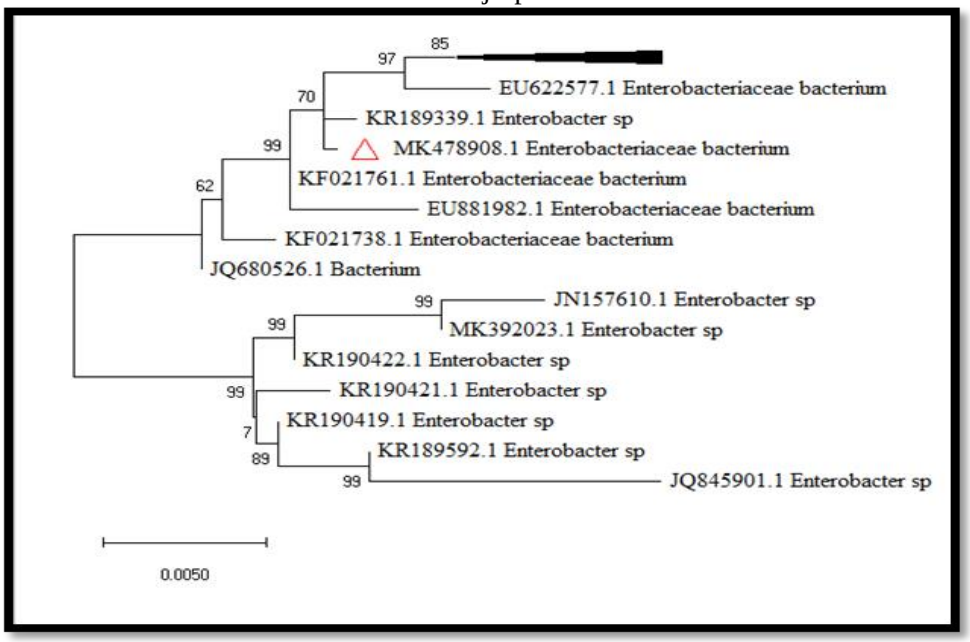
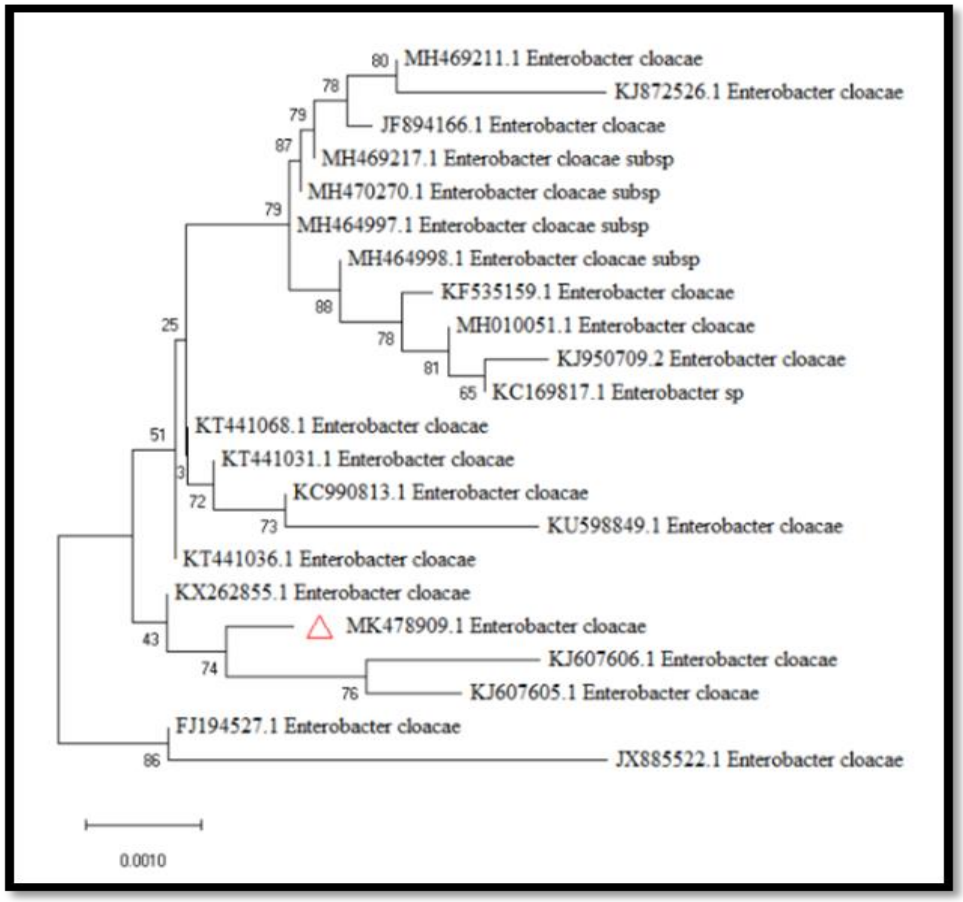


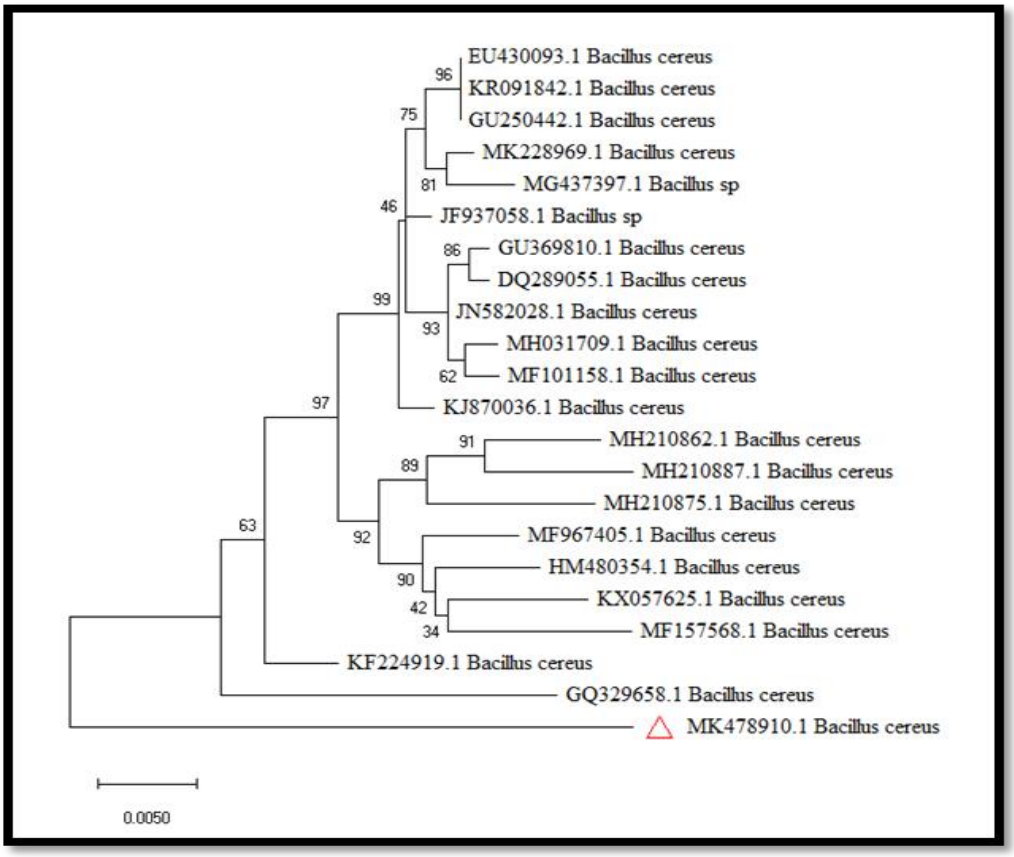
Fig 1: Shows the percentage of dye degradation by using bacterial isolates.

Identification of selected isolates

Bacterial genomic DNA was isolated as per the standard protocol [20]. The DNA extracted was used as the template for the amplification of16srRNA gene. The universal primers (Forward primer 5'AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-GGTTACCTTGTTACGACT-3')were used for the amplification of the 16srRNA gene fragment. The amplified PCR product were electrophoresed on 1 % agarose gel. The gel was stained in ethidium bromide and photographed with gel documentation system. For species level identification, sequences were compared with the Genebank databaseusing the BLAST program (http://blast.ncbi.nlm.nih.gov)[21]. The nucleotide sequence data reported in this study has been deposited in the NCBI nucleotide sequence database under the accession number of MK478908, MK478909, and MK478910 and confirmed as *Enterobacteriaceaebacterium, Enterobacter cloacae and Bacillus cereus*. Chromium resistant bacterial strain *Bacillus cereus*was also isolated from tannery effluent [22]. *Enterobacter cloacae* strain reduces hexavalent chromium was also identified [23].





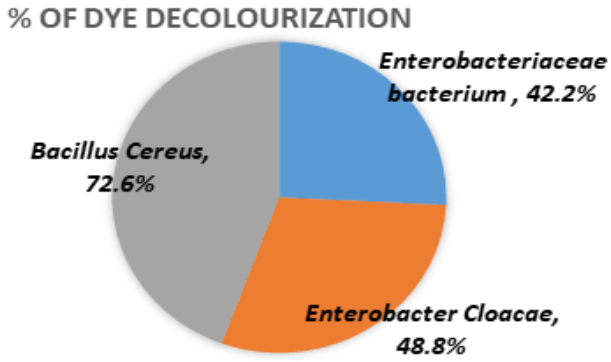


Effect of dye decolourization

The decolourization pattern was measured by inoculating with *Enterobacteriaceae bacterium, Enterobacter cloacae and Bacillus cereus* .The decolourization was expressed as percentage (%) and estimated by the formula

$$\% \text{ of Decolourization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} X\ 100$$

Among these organisms, only one potential strain *Bacillus Cereus* which shows more decolourization was used foroptimizationeffect. Bacterial isolates, *Bacillus* sp. and *Pseudomonas* sp. have potential to decolourize the dye effluent [24]. He found that *Bacillus* sp., has higher decolourization ability than *Pseudomonas* species. This is due to the faster decolourization of effluent by bacteria with the metabolic activities. The percentage of decolourization was shown in fig 2.



Organism	% of dye decolourization
Enterobacteriaceae bacterium	42.2
Enterobacter Cloacae	48.8
Bacillus Cereus	72.6

Fig 2: Shows the effect of dye decolourization

Effect of pH

The optimal densities (OD) was measured and the percentageof decolourization at different p^H was shown in Fig 3. Initially thedye concentration was high and gradually dye concentration decreases depending on time interval.p^H 7 was optimum during bioremediation by different bacterial strains[25][26]. The optimum P^H for the decolourization of dye was found to be p^H 7.

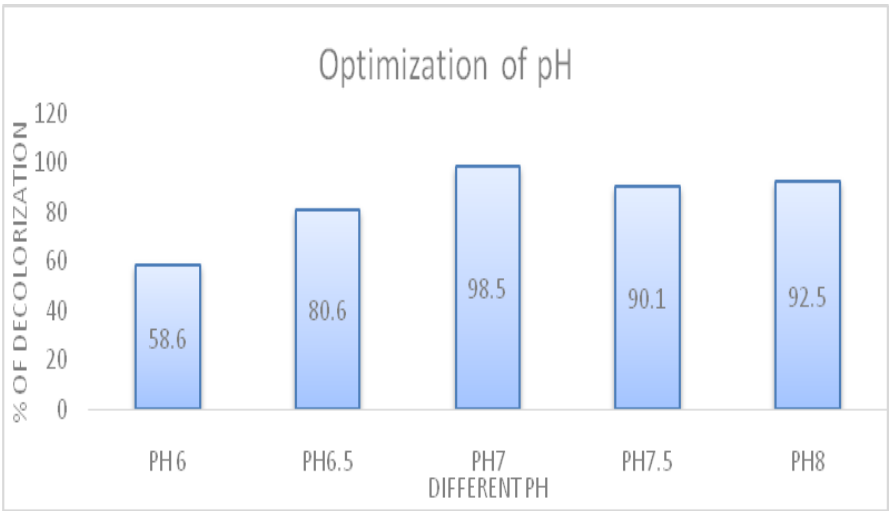


Fig 3: Shows the percentage of decolourization at different P^H by *Bacillus cereus*

Effect of temperature

Bacillus Cereus showed optimum temperature for degradation at 35°C. The temperature efficiency for dye decolourization vary between 30°C - 40°C[27][28][29] . The percentage ofdye decolourization at different temperature was shown in Fig4.

Effect of Carbon Source

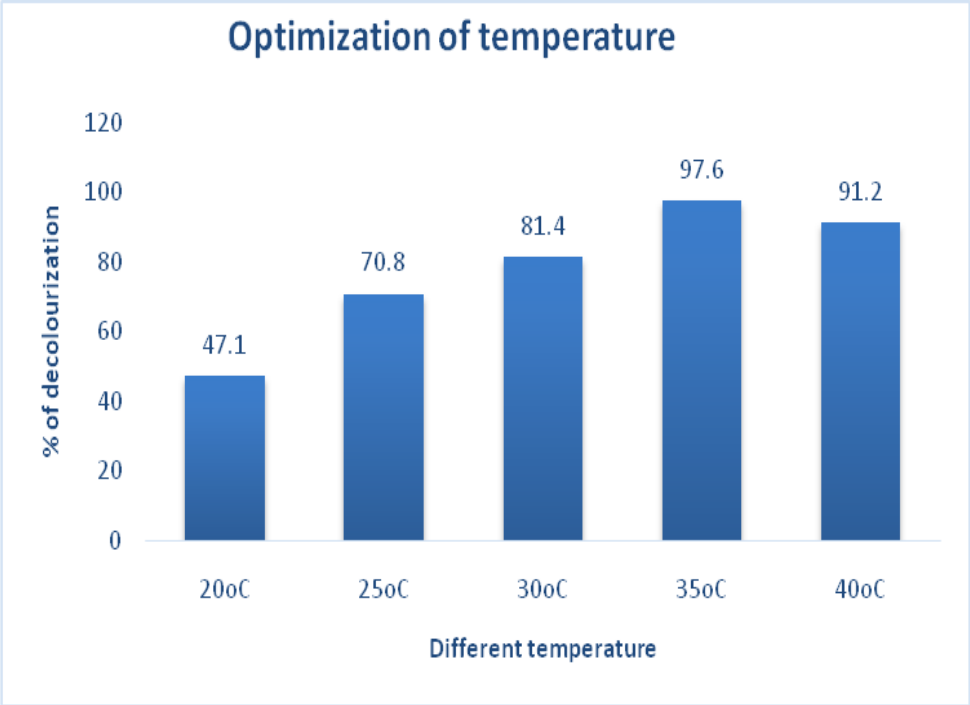


Fig 4: Shows the percentage of dye decolourization at different temperature by *Bacillus cereus*

The percentage of decolourization at different carbon source was shown in Fig 5. From the results the Optimum Carbon Source for decolourization was found to be glucose. Glucose is the better carbon source than sucrose [30]. Other researchers also reported that glucose is needed for azo dye decolourization by different microorganisms [31][32][33].

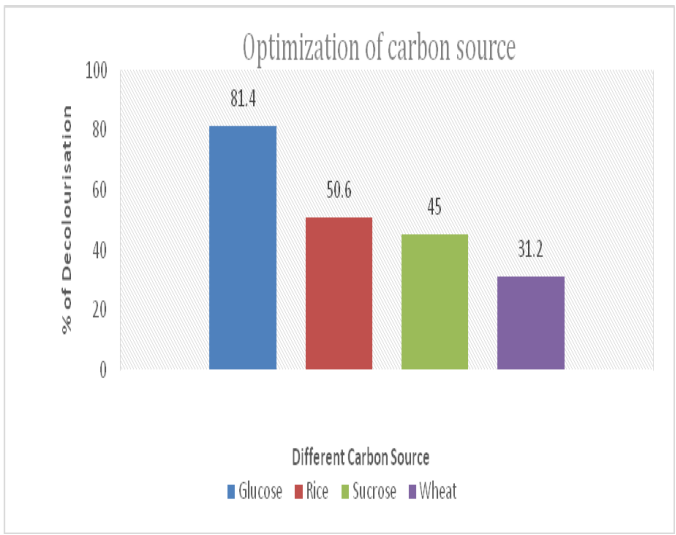


Fig 5: Shows the percentage of decolourization at different carbon source by *Bacillus cereus*

Effect of nitrogen Source

The percentage of decolourization at different nitrogen source was shown in Fig 6. From the result the Optimum nitrogen source fordecolourizationwas found to be ammonium chloride. Contrary to our results, peptone was found as a nitrogen source for dye decolourization under different culture conditions [34].

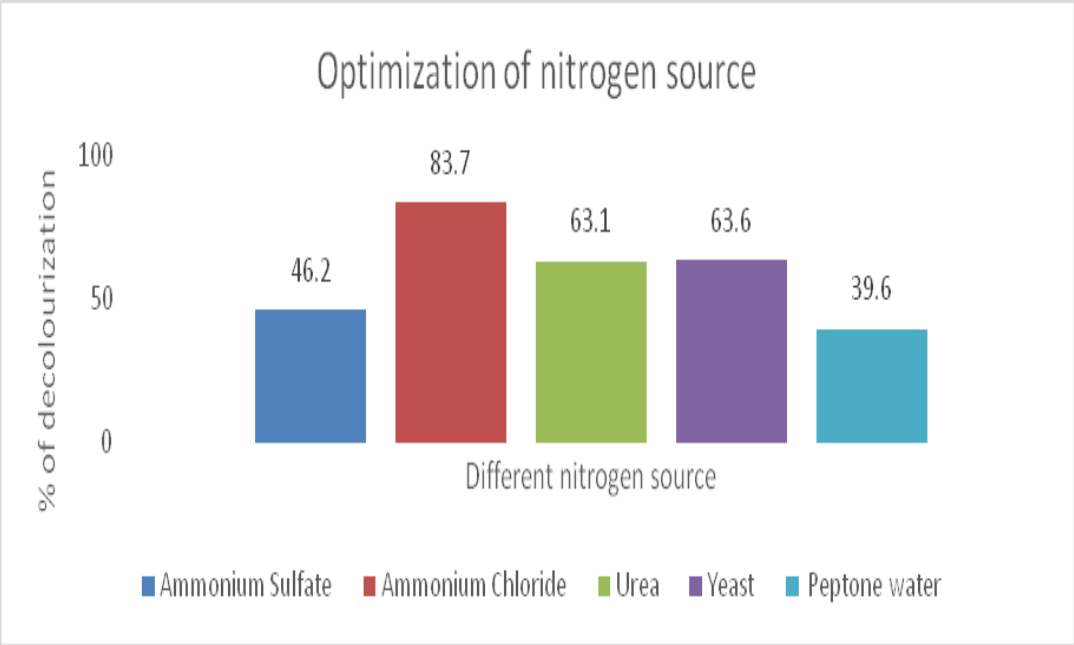


Fig 6: Shows the percentage of decolourization at different nitrogen source by *Bacillus cereus*

4. CONCLUSION

Synthetic dyes are used in many industries mainly in tanning and leather industry. The effluents from these industries are disposed withoutany treatment. Due to this, the water get polluted. To solve the pollution problem, microOrganism is used to degrade the dye present in the effluent. The results obtained shows that *Bacillus Cereus* isolated from the tannery effluent is the efficient strain to decolourization the dye. The Optimum p^H for the decolourization was found to be 7. The Optimum temperature was found to be 35^oC .The Optimum carbon source for decolourization was found to be glucose and the Optimum nitrogen source for decolourization wasfound to be ammonium Chloride.

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CONFLICT OF INTEREST

Author has no conflict of interest.

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