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Assessment of Bioefficacy of *Achromobacter xylosoxidans* KUESCCHK-6 Isolated from Textile Contaminated Soil in Treating Textile Effluent and its Impact on *Vigna mungo*

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ABSTRACT

Textile effluents are major pollutants with varied contaminants. Traditional treatment methods are costly and produce sludge, necessitating alternative, eco-friendly solutions. Biological treatment methods are receiving attention as it is proven to be cheap, environment friendly and highly efficient treatment method for dye effluent in industrial scale as compared to the other available treatment methods. The present work evaluates the bioremediation of textile effluent using a pure culture of a bacterium isolated from the soil samples contaminated with textile wastewater. The strain was identified as *Achromobacter xylosoxidans* KUESCCHK-6 (GenBank Accession Number: OM475749) through 16S rRNA molecular analysis. This bacterial strain was used to treat textile effluent under specific conditions: glucose as the carbon source, urea as the nitrogen source, a C/N ratio of 6:1, a temperature of 35°C, a pH of 8.5, and a static incubation period of 5 days. The results indicated that the strain effectively reduced various physiochemical parameters of the raw textile wastewater: color by 87.94%, BOD by 80.61%, COD by 80.96%, EC by 73.11%, fluoride by 81.15%, phosphate by 79.57%, sodium by 76.88%, and turbidity by 81.02%. Additionally, metal ions including iron were removed by 84.83%, while other metals such as zinc, nickel, manganese, copper, lead, cadmium, total chromium, arsenic, barium, cobalt, and boron were reduced to below detectable limits. Phytotoxicity tests confirmed the non-toxic nature of the treated effluent. Overall, the study concludes that *Achromobacter xylosoxidans* KUESCCHK-6 is a promising candidate for the bioremediation of textile industrial effluents, with potential for commercial application.

Key Words	Bioremediation, Textile effluent, Bacteria, *Achromobacter xylosoxidans*, Physico-chemical parameter, Phytotoxicity study, *Vigna mungo*
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INTRODUCTION

Textiles rank second after food among the substantial desires of human life. The textile industry converts fibers into yarn and fabrics, applying dyes and finishes through various processes. Numerous dyes and chemicals used in wet processing generate wastewater that poses environmental risks. Therefore, it is crucial to thoroughly treat this wastewater before release. Textile effluents are highly heterogeneous, containing substantial amounts of toxic and hard-to-treat substances from dyeing and finishing operations (Table 1).

Table 1: Total water consumed during wet process

Process	Water Consumption (L/1000 kg of products)
Sizing	500-8200
Desizing	2500-21000
Scouring	20000-45000
Bleaching	2500-25000
Mercerizing	17000-32000
Dyeing	10000-300000
Printing	8000-16000

The primary issue with textile industry wastewater is the use of dyes to impart color. Effluents contain various contaminants, including surfactants, salts, heavy metals, enzymes, and oxidizing and reducing agents. Over time, the release of these polluted effluents degrades the quality of nearby surface and groundwater. Untreated effluents from harmful and persistent chemicals in wet processing are especially detrimental to the environment (Madhav et al. 2018).

Textile effluents contain trace metals like Cr, As, Cu, and Zn, which can harm the environment. Heavy metals pose significant environmental and health risks due to their ability to disrupt cellular structures and biomolecules. They bind strongly to essential metal ion sites, destabilizing cellular components like enzymes, DNA, and RNA, leading to replication errors, mutations, and potentially cancer. These metals are toxic, persistent, and accumulate in living organisms. Toxic heavy metals, such as chromium, nickel, zinc, cadmium, lead, and others, can hinder plant growth by adversely affecting photosynthesis, enzymatic activities, and mineral nutrition (Kuanar et al. 2022). The eradication of toxic textile dyes through wastewater treatment is essential to mitigate their harmful environmental and biological impacts. Synthetic dyes, prevalent in industries like fabric printing and leather coloring, are major pollutants, contributing to significant environmental damage. Textile effluents, rich in organic and inorganic pollutants, heavy metals, and colorants, present serious ecological risks due to their high pH, color, and elevated COD and BOD levels. In aquatic environments, these dyes can form non-biodegradable, hazardous by-products. Given their

mutagenic and carcinogenic properties, decolorization of these effluents is crucial before discharge (Verma et al. 2022).

Pollutants from the textile industry alter the physicochemical parameters of receiving water bodies. Parameters such as color, odor, temperature, chemical oxygen demand (COD), biological oxygen demand (BOD), pH, fats, oil, nitrogen, phosphorus, total suspended solids (TSS), total solids (TS), total dissolved solids (TDS), sulfate, calcium, magnesium, and chloride can reach their highest levels according to Bureau of Indian Standard limits (Kousar et al. 2020).

Different physicochemical methods, such as coagulation, adsorption, filtration, and ion exchange, have been developed for treating textile effluents. However, membrane filtration faces limitations due to cost and clogging issues, making adsorption a more preferable method. Despite this, biological treatment methods are superior to chemical processes. They generate fewer by-products like solid wastes, have lower operational costs, and achieve complete mineralization of dyes, making them more effective for dye removal compared to physicochemical treatments (Adane et al. 2021).

The inefficiency or lack of wastewater treatment facilities is the main cause of water pollution. To achieve zero pollution, adopting alternative technologies is essential. Current focus is on sustainable wastewater treatment techniques. Bioremediation is a promising option due to its ability to detoxify effluents and its environmentally friendly approach. This process uses naturally occurring microorganisms and plants to break down hazardous substances into less toxic or non-toxic forms. It is typically more cost-effective than other remediation methods and avoids issues like atmospheric emissions and waste generation. Numerous studies have shown the effectiveness of microremediation techniques, using various microorganisms (bacteria and fungi) to remove a wide range of pollutants from industrial wastewater (Chaithra et al. 2022).

Bioremediation is a pollution remediation technique that employs naturally occurring or genetically modified organisms to degrade or transform toxic chemicals into less toxic or non-toxic substances. This method leverages bacteria, fungi, and other biological agents to remove pollutants from contaminated sites by stimulating microorganisms to use the pollutants as a source of food and energy (Chaithra & Kousar 2022).

Microorganisms like *Shewanella oneidensis, Pseudomonas delafieldii, Dehalococcoides, Lysinibacillus*, and *species of Sphingomonas* are effectively used in the removal of hexavalent chromium, dibenzothiophene, trichloroethane, nickel, and carbazole, respectively, in the presence of nanoparticles (Hemalatha et al. 2022)

Molecular-based techniques offer new possibilities for detecting specific microorganisms in soil or effluent and quantifying target gene expression. Recent advances in PCR amplification of the 16S rRNA gene and sequence analysis have enhanced the identification of unknown bacterial isolates. The 16S rRNA gene contains conserved sequences across all bacteria, aiding in identification. Bioremediation leverages the catalytic abilities of microorganisms to accelerate

pollutant degradation, using compounds in influent waste as nutrients. The Basic Local Alignment Search Tool (BLAST) compares partial sequences to the GenBank database to identify phylogenetic relatives. Utilizing microbial communities from textile effluents for bioremediation is promising due to their genetic and biochemical adaptations to toxic compounds. Effective *in-situ* bioremediation requires sufficient indigenous microbial populations with strong degradation capacities and suitable environmental conditions (Chaithra et al. 2023).

The current research aims to isolate, identify, and describe indigenous bacteria from textile industry effluent-contaminated soil using 16S rRNA sequencing. These identified bacteria will then be utilized for the treatment of textile effluent under optimal conditions. The effectiveness of the treatment process will be evaluated through a phytotoxicity study on *Vigna mungo* using the treated effluent.

MATERIAL AND METHODS

Effluent sample collection

The effluent for this study was collected from a textile mill in Banglore, Karnataka using the grab sampling technique at the inlet of an effluent treatment plant. The samples were immediately transported to the laboratory of the Department of P.G. Studies and Research in Environmental Science at Kuvempu University for further analysis. To preserve the samples, they were stored in a refrigerator at 4°C.

Isolation of bacterial isolate from soil

1g soil sample was suspended in 10ml of sterile distilled water and further diluted. From each dilution, 100µl was spread on petri plates containing 20ml of sterile Nutrient Agar Media (NAM) using the L-Rod (spread plate technique) and incubated at 37°C for 24 hours. Bacterial growth was observed and sub-cultured for pure culture, achieved through the quadrant streaking method (Aneja 2018).

Identification, morphological characterization and biochemical characterization of isolated bacterial strain

The study focused on examining various characteristics of a bacterial colony, including color, shape, margin, elevation, surface, and arrangement. Standard gram staining procedures were used for the morphological characterization of the isolate. Once a pure culture was obtained, biochemical methods were employed to identify the bacterial culture. The isolated strain was preserved on a nutrient agar slant and stored in a refrigerator at 4°C. Both macroscopic and microscopic analysis, along with biochemical tests and molecular methods, were used to further characterize the isolated bacterial strain. The results were compared with those in **Bergey's Manual of Determinative Bacteriology, 9th edition**. Biochemical tests conducted included starch hydrolysis, gelatin hydrolysis, citrate utilization, nitrate reduction, the urease test, the methyl red test, the indole production test, the catalase test, the oxidase test, and hydrogen sulfide production. These tests followed the methodologies described in **"Microbiology: A Laboratory Manual" Cappuccino**.

Isolation, identification, and phylogenetic analysis using 16S rRNA gene sequencing

The National Center for Biotechnology Information (NCBI) database and MEGA version 5 verified the identity of ITS sequence fragments using the Basic Local Alignment Search Tool (BLAST) in GenBank. BLASTn in NCBI was used for searches. Additionally, 16S ribosomal RNA (rRNA) gene sequencing, a common method for bacterial identification and phylogenetic analysis, was performed. Purified 16S rRNA gene sequences were obtained and aligned with homologous sequences using NCBI BLAST. Clustal W software aligned the sequences based on maximum identity scores. MEGA 7 was then used to generate a distance matrix and construct a phylogenetic tree for accurate classification.

Determination of optimal growth conditions for isolated bacterial strain

The growth characteristics of the bacterial isolate were analyzed by measuring biomass in g/L. The analysis was conducted at pH levels of 6.0 to 10.0 and temperatures of 25°C to 45°C, using nutrient broth for growth. Various carbon sources (glucose, fructose, sucrose, maltose, lactose) and nitrogen sources (peptone, beef extract, urea, yeast extract) were tested. The carbon to nitrogen (C/N) ratio was optimized at concentrations of 1:1, 2:1, 4:1, 8:1, and 16:1. After a 5-day incubation period under these varying conditions, biomass was measured. The optimal conditions for the highest biomass production were identified as the best for removing color and various physico-chemical characteristics from textile industry effluent.

Experimental setup for degradation studies

Bioremediation of textile industry effluent was undertaken at the laboratory scale. The effluent was diluted to three dilutions: 25%, 50%, and 75%, using either distilled water or deionized water. The purpose of dilution was to investigate the degradation efficiency of organisms at varying concentrations. Under aseptic conditions, 5mL of spore suspension from each organism was introduced into the effluent treatment systems (5mL/ L). Treatment was then carried out for duration of 5 days under static conditions in accordance with optimal conditions for the organisms. Static conditions were preferred to facilitate biofilm formation and enhance organism efficiency in pollution remediation. Following treatment, the effluent was analyzed for physico-chemical parameters.

The formula used to calculate the percentage reduction in physico-chemical parameters after treatment with the study microorganisms is as follows:

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

Physicochemical characterization and colour analysis

The effluent's physico-chemical properties were analyzed in the laboratory according to APHA (2017) standards (Table 2). Decolorization was assessed spectrophotometrically by measuring the absorbance peak of untreated effluent at 470 nm, expressed in mg/L. Post-treatment, samples were centrifuged at 8000 rpm, and the supernatant's absorbance was measured using a UV-Visible spectrophotometer at 470 nm. The percentage of decolorization was then calculated using a specific formula.

$$\% \text{ Decolourization} = \frac{(C_0 - C_e)}{C_0} \times 100$$

Where, C_0 is the initial concentration of colour (mg/L)

C_e is the colour concentration after treatment (mg/L) **(Sharma *et al.*, 2010)**

Table 2. Standard methods adopted for physico-chemical characterization of textile industry effluent

Sl. No.	Parameter	Methods APHA (2017)	Instrument Used	Units
1.	Color	Spectrophotometric Method	Spectrophotometer	mg/L
2.	Electrical Conductivity	Electrometric Method	Conductivity Meter	µmhos/cm
3.	BOD	Winkler's Method	Titration	mg/L
4.	COD	Potassium dichromate Method	COD reflux	mg/L
5.	Turbidity	Turbidometric Method	Turbidity meter	NTU
6.	Phosphate	Spectrophotometric Method	Spectrophotometer	mg/L
7.	Fluoride	SPANDA method	Spectrophotometer	mg/L
8.	Iron	Spectrophotometric Method	Spectrophotometer	mg/L
9.	Sodium	Flame Photometry	Flame atomic emission Spectrometry	mg/L
10.	Potassium	Flame Photometry	Flame atomic emission spectrometry	mg/L
11.	Manganese	Atomic Absorption Method	Atomic Absorption Spectrometry	mg/L
12.	Zinc			
13.	Nickel			
14.	Cadmium			
15.	Arsenic			
16.	Lead			
17.	Total Chromium			
18.	Barium			
19.	Cobalt			
20.	Copper	Atomic Absorption Method	Spectrophotometer	mg/L
21.	Boron	Curcumin method	Spectrophotometer	mg/L

22.	Spore Count	Haemocytometer Chamber	Haemocytometer	Spores/ml

Bacterial cell counting using dilution technique

Serial dilutions of a bacterial suspension were made across 10 dilution blanks. Samples from each dilution were plated on nutrient agar petri dishes. After incubating for 24-48 hours, colonies were counted using a colony counter. The number of organisms per plate was calculated by multiplying the colony count by the dilution factor, and the cell concentration per milliliter in the spore suspension was determined using the provided formula.

$$\text{Number of cells/ mL} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Phytotoxicity studies of treated effluent

To assess the toxicity of treated textile industry effluent, phytotoxicity studies were conducted using *Vigna mungo* seeds at room temperature. The experiments were primarily focused on studying seed germination and root elongation. Healthy, uniform seeds were washed, sterilized at 24°C for 30 minutes, and 10 mature seeds were used per test. Daily, 10mL of treated effluent, untreated effluent, and distilled water (control) were administered to observe effects on seed germination and growth over a week. The number of germinated seeds, root, and shoot lengths were recorded. Results were presented using established methods to assess seed germination index (GI), relative seed germination (RSG), and relative root elongation (RRE).

$$\text{RSG}(\%) = \frac{\text{Number of seeds germinated in the sample extracted}}{\text{Number of seeds germinated in the control}} \times 100$$

$$\text{RRE}\ (\%) = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \times 100$$

$$\text{GI}\ (\%) = \frac{(\%\ \text{Seed germination}) \times (\%\ \text{Root elongation})}{100}$$

RESULTS AND DISCUSSION

Isolation of bacteria

A bacterial colony was isolated from the effluent based on its unique colonial characteristics observed on a NAM. Gram staining tests indicated that the isolate exhibited a gram-negative reaction with smooth, bacilli-shaped morphology (Figure 1). This isolate displayed distinct morphological and colonial features. To identify the bacteria, various biochemical tests were conducted, and the responses to different biochemical compounds were assessed. By consulting Bergey's Manual of Systematic Bacteriology, it was determined that the isolate belonged to the genus *Achromobacter*.

Table 3 summarizes the biochemical characteristics of the bacterial strain isolated from textile effluent-contaminated soil. The strain tested negative for starch hydrolysis, gelatin hydrolysis, methyl red, and

indole production, indicating it does not break down starch or gelatin, perform mixed-acid fermentation, or produce indole. Conversely, it tested positive for citrate utilization, nitrate reduction, urease activity, catalase, and oxidase, showing its ability to use citrate as a carbon source, reduce nitrate, hydrolyze urea, and produce enzymes involved in hydrogen peroxide breakdown and cytochrome c oxidation. The strain was also negative for hydrogen sulfide production, indicating it does not produce this compound from sulfur-containing sources. These results reflect the strain's specific metabolic capabilities and potential for bioremediation applications.

Table 4 summarizes the morphological features of the bacterial strain isolated from textile effluent-contaminated soil. On solid media, the colonies of the strain appear round in shape, with a smooth texture and a creamy color. Microscopically, the cells are rod-shaped and exhibit motility, which indicates that the strain is capable of moving. Additionally, the strain does not form spores, as indicated by a negative result for spore formation. These morphological characteristics provide a basic identification framework for the bacterial strain and offer insight into its growth patterns and structural features. The results revealed unique morphological and biochemical characteristics, including colony traits, suggesting distinct structure and functions.

The 16S rRNA sequence of the bacterial isolate was deposited in GenBank, identifying it as *Achromobacter xylosoxidans* KUESCCHK-6 (Accession number- OM475749) with 100% similarity in partial gene sequencing. The evolutionary relationship of this bacterial strain with other relevant bacteria can be found in Figure 2 within the GenBank database.

Figure 1: Macroscopic and microscopic observation of isolated strain

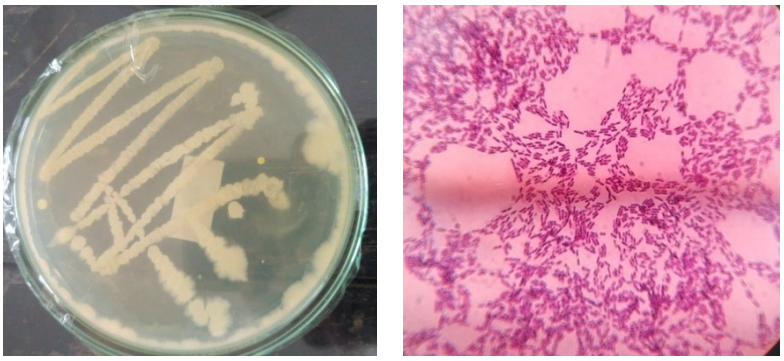


Table 3: Biochemical characteristics of the isolated strain

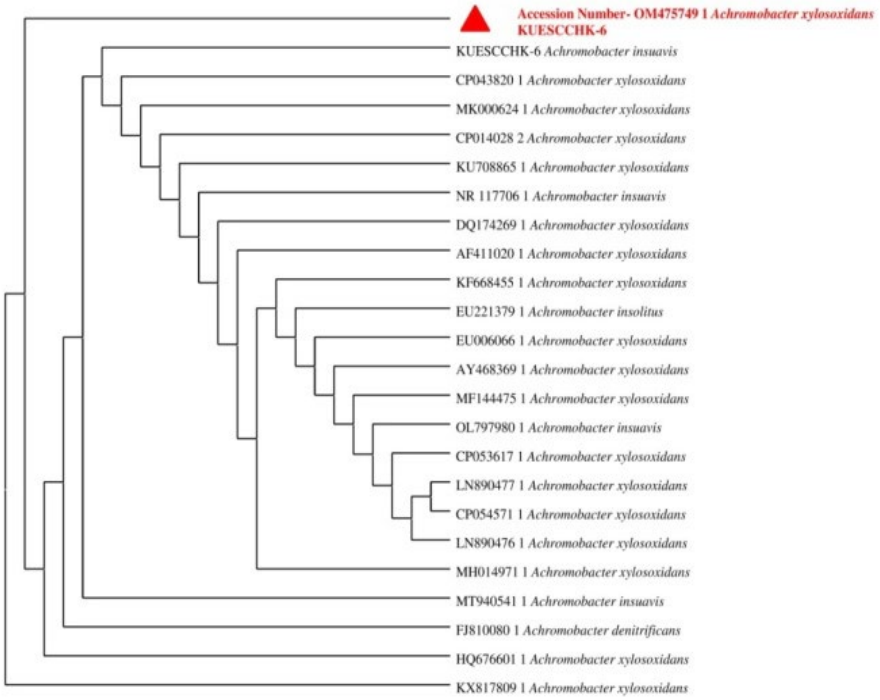
SL. No.	Biochemical Test	Bacterial Isolate
1.	**Starch hydrolysis**	-
2.	**Gelatin hydrolysis**	-
3.	**Citrate utilization**	+
4.	**Nitrate reduction**	+
5.	**Urease**	+
6.	**Methyl red**	-
7.	**Indole production**	-
8.	**Catalase**	+

9.	**Oxidase**	+
10.	**Hydrogen Sulphide production**	-

Table 4: Morphological features of isolated bacterial strain

Morphology features		**Bacterial isolate**
Colony Morphology	**Shape**	Round
	Texture	Smooth
	Colour	Creamy
Microscopic Characters	**Cell shape**	Rod
	Motility	Motile
	Spore Formation	-ve

Figure 2: Phylogenetic tree of 16S rRNA gene sequence of isolate *Achromobacter xylosoxidans*



The impact of varying pH levels, temperatures, carbon sources, nitrogen sources, and carbon-to-nitrogen ratios (C/N ratio) on *Achromobacter xylosoxidans*

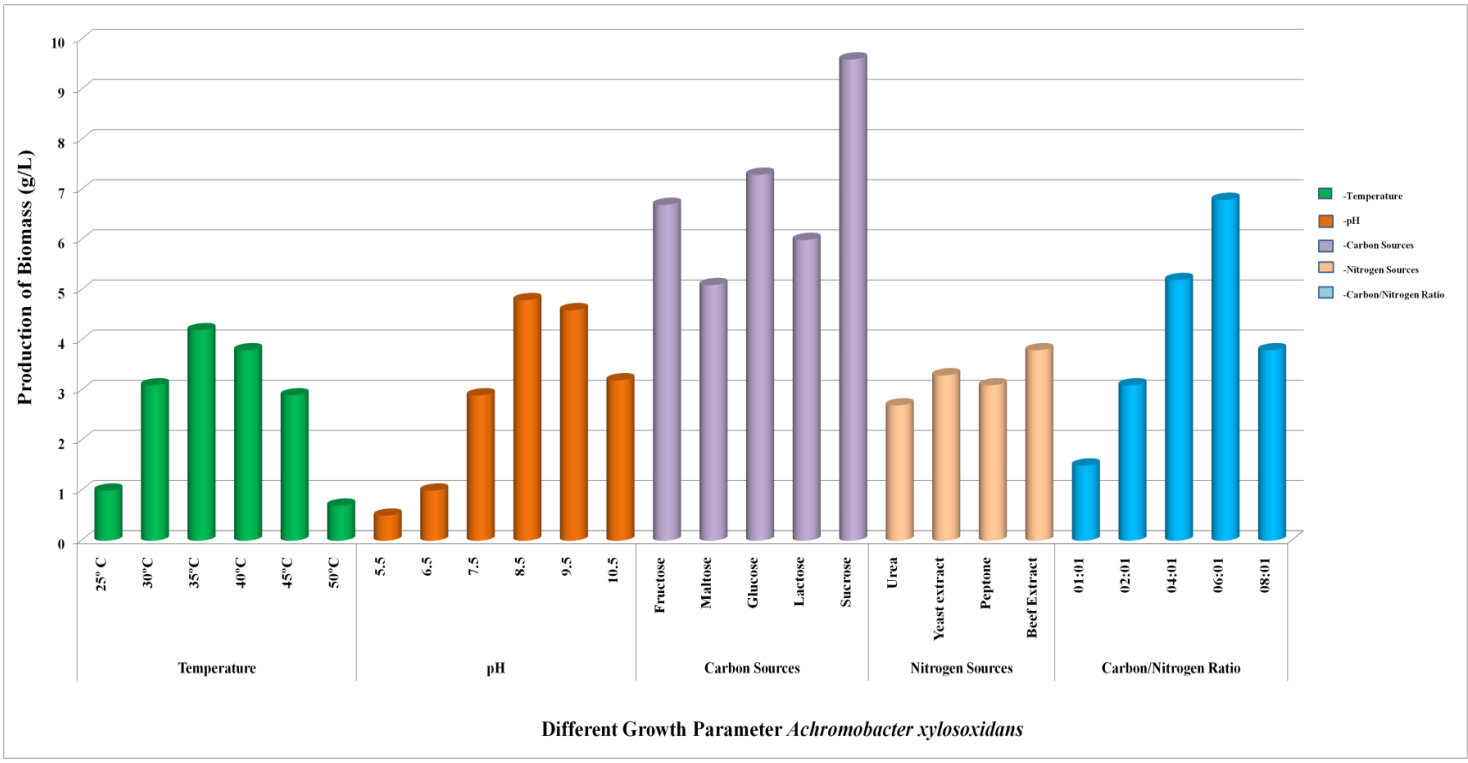
The optimal conditions for biomass production by *Achromobacter xylosoxidans* were identified through the variation of temperature, pH, nitrogen source, carbon source, and C/N ratio. Maximum biomass production was achieved at a temperature of 35°C and a pH of 8.5, with urea serving as the nitrogen source and glucose as the carbon source, and a C/N ratio of 06:01. These conditions yielded the highest biomass output, which is essential for the efficient treatment of textile industry effluent as shown in Table 5 and Figure 3.

Table 5: Biomass production by bacterial strain *Achromobacter xylosoxidans* at optimized parameter

Biomass production by *Achromobacter xylosoxidans*

Temperature (ºC)	Biomass Production in g/L	pH	Biomass Production in g/L	Nitrogen Source	Biomass Production in g/L	Carbon Source	Biomass Production in g/L	Carbon to Nitrogen Ratio	Biomass Production in g/L
25ºC	1	5.5	0.5	Urea	2.7	Fructose	6.7	01:01	1.5
30ºC	3.1	6.5	1	Yeast Extract	3.3	Maltose	5.1	02:01	3.1
35ºC	4.2	7.5	2.9	Peptone	3.1	Glucose	7.3	04:01	5.2
40ºC	3.8	8.5	4.8	Beef Extract	3.8	Lactose	6	06:01	6.8
45ºC	2.9	9.5	4.6			Sucrose	9.6	08:01	3.8
50ºC	0.7	10.5	3.2						

Figure 3: Biomass production by *Achromobacter xylosoxidans* in different growth parameters



Bacterial counting using dilution technique

1mL of bacterial suspension containing 85,00,000 cells was used for textile industry effluent treatment.

Decolourization and physico-chemical parameters reduction by *Achromobacter xylosoxidans* KUESCCHK-5

Tables 6 and 7 illustrate the concentrations of various pollutants in textile effluent before and after treatment *with Achromobacter xylosoxidans*. Table 6 shows that the raw effluent had high pollutant levels, including a color concentration of 218.48 mg/L, BOD of 694.58 mg/L, COD of 2662.74 mg/L, and an EC of 7095.63 µmhos/cm. The concentrations of other contaminants such as fluoride, phosphate, sodium, turbidity, iron, and several heavy metals were also significantly high in the raw effluent (Figure 4).

Table 7 shows the effectiveness of *Achromobacter xylosoxidans* in treating the effluent. After treatment, there were significant reductions in pollutant concentrations across all effluent dilutions. For instance, color concentration dropped from 26.34 mg/L to 6.61 mg/L, BOD decreased from 134.63 mg/L to 19.61 mg/L, and COD was reduced from 506.55 mg/L to 97.56 mg/L. EC, fluoride, phosphate, sodium, and turbidity also saw marked reductions. At a 25% dilution level, the effluent treatment process achieved substantial reductions across key environmental parameters. Iron content was reduced by 91.56%, effectively removing this potentially toxic metal. The color of the effluent decreased by 91.07%, indicating the successful elimination of chromophoric substances. Reductions in BOD and COD by 88.16% and 85.23%, respectively, show a significant decrease in pollution levels. EC dropped by 81.07%, reflecting a reduction in dissolved salts. Fluoride and phosphate levels were lowered by 88.34% and 86.41%, respectively, minimizing risks of fluorosis and eutrophication. Turbidity was reduced by 89.10%, indicating clearer water, while sodium levels decreased by 83.01%, helping to prevent soil salinization. Additionally, heavy metals such as zinc, nickel, manganese, copper, lead, cadmium, total chromium, arsenic, barium, cobalt, and boron were either reduced to below detectable limits or significantly lowered. These results highlight *that Achromobacter xylosoxidans* is highly effective in reducing the concentration of pollutants in textile effluent (Figure 5 and 6).

Chaudhary et al. (2020) investigated the removal of Cr^{6+} using fungi isolated from tannery effluent. Their rDNA genes were subsequently identified as *Aspergillus niveus* MCC 1318, *Aspergillus flavus* MCC 1317 and *Aspergillus niger* MCC 1316. *A. niveus, A. niger* and *A. flavus* were capable of removing 80%, 82% and 88% Cr, respectively. The bioremediation efficiency of individual fungal strains for other heavy metals such as Zn, Pb, Cd, and Ni was observed to be 55%, 68%, 41%, and 69%, respectively for *A. niveus*, 60%, 59%, 59%, and 50%, respectively for *A. niger*, and 63%, 52%, 68%, and 58% for *A. flavus*. An exceptional removal of Zn, Pb, Cd, and Ni was observed in the case of fungal consortia, i.e., 80%, 70%, 80%, and 75%, respectively. Fourier transform infrared spectroscopy (FTIR) unveiled the adsorption phenomena by revealing the interactions of adsorbed Cr with methyl and methylene groups, secondary amides, and phosphate groups present in the fungal media.

Harish et al. (2024) noted similar findings in their investigations of dye decolorization utilizing immobilized *Achromobacter xylosoxidans* DDB6, which was isolated from textile effluent. Their study indicated that this strain effectively reduced the color of crystal violet, coomassie brilliant blue, and alizarin red by 67.20%, 28.58%, and 20.41%, respectively. Kundu et al. (2012) investigated the treatment of small-scale slaughterhouse wastewater using *Achromobacter xylosoxidans* S18. The results indicated a reduction in COD of the effluent by 88 ± 3% in the presence of glucose and ammonium chloride.

Achromobacter xylosoxidans is highly effective in reducing a wide range of pollutants, including color, BOD, COD, EC and heavy metals. Its performance is comparable or even superior to other bioremediation agents such as fungal strains like *Aspergillus species.* The broad spectrum of pollutant reductions achieved with *Achromobacter xylosoxidans* makes it a potent and versatile bioremediation agent for treating industrial effluents, particularly in sector like textiles.

Table 6: Concentration of parameters before treatment at different effluent dilution

Parameters	Units	Raw effluent	75% dilution	50% dilution	25% dilution	Control
Color concentration	mg/L	218.48±0.04	179.51±0.04	133.74±0.03	74.07±0.07	218.48±0.04
BOD	mg/L	694.58±0.14	514.57±0.21	336.98±0.15	165.71±0.15	694.3±0.02
COD	mg/L	2662.74±0.12	1985.54±0.20	1320.49±0.15	660.57±0.17	2662.74±0.11
EC	µmhos/cm	7095.63±0.08	6083.60±0.11	4487.70±0.03	2656.30±0.06	7095.63±0.08
Fluoride	mg/L	3.91±0.03	2.96±0.02	2.15±0.07	1.37±0.11	3.91±0.0
Phosphate	mg/L	16.56±0.15	14.39±0.11	11.56±0.12	9.91±0.02	16.56±0.15
Sodium	mg/L	2287±0.02	1815.39±0.07	1302.49±0.12	798.66±0.11	2287.22±0.02
Turbidity	mg/L	180.66±0.09	144.25±0.10	111.76±0.12	59.91±0.02	180.66±0.09
Iron	mg/L	7.08±0.05	5.71±0.09	4.15±0.11	2.13±0.07	7.08±0.05
Zinc	mg/L	1.99±0.0003	1.59±0.001	1.09±0.0003	0.596±0.001	1.99±0.0003
Nickel	mg/L	2.34±0.0003	1.89±0.0003	1.24±0.0006	0.679±0.0006	2.34±0.0003
Manganese	mg/L	0.33±0.0003	0.26±0.001	0.18±0.0005	0.103±0.0006	0.33±0.0003
Copper	mg/L	0.66±0.0006	0.52±0.0006	0.35±0.0006	0.180±0.0008	0.66±0.0006
Lead	mg/L	1.004±0.001	0.79±0.001	0.53±0.001	0.275±0.002	1.004±0.001
Cadmium	mg/L	0.52±0.001	0.41±0.001	0.29±0.001	0.161±0.0006	0.52±0.001
Total Chromium	mg/L	0.73±0.001	0.61±0.001	0.42±0.001	0.243±0.001	0.73±0.001
Arsenic	mg/L	0.002±0.0003	0.0015±0	0.001±0	0.0006±0	0.002±0.0003
Barium	mg/L	0.04±0.003	0.036±0.001	0.027±0.0003	0.014±0	0.04±0.003
Cobalt	mg/L	0.20±0.003	0.15±0.0006	0.107±0.001	0.058±0.001	0.20±0.003
Boron	mg/L	0.32±0.01	0.23±0.002	0.164±0.002	0.080±0	0.32±0.01

Key: mg/L = milligram per liter. Values are expressed as mean ± SEM (n=3)

Table 7: Concentration of parameters after treatment with *Achromobacter xylosoxidans*

Parameters	Units	Raw effluent	75% dilution	50% dilution	25% dilution	Control
Color concentration	mg/L	26.34±0.02	19.07±0.03	13.15±0.03	6.61±0.01	210.26±0.08
BOD	mg/L	134.63±0.10	83.71±0.20	47.55±0.21	19.61±0.15	660.30±0.09
COD	mg/L	506.55±0.12	357.55±0.17	224.52±0.21	97.56±0.20	2568.54±0.15
EC	µmhos/cm	1907.37±0.12	1404.72±0.08	934.16±0.06	502.82±0.10	6796.63±0.12

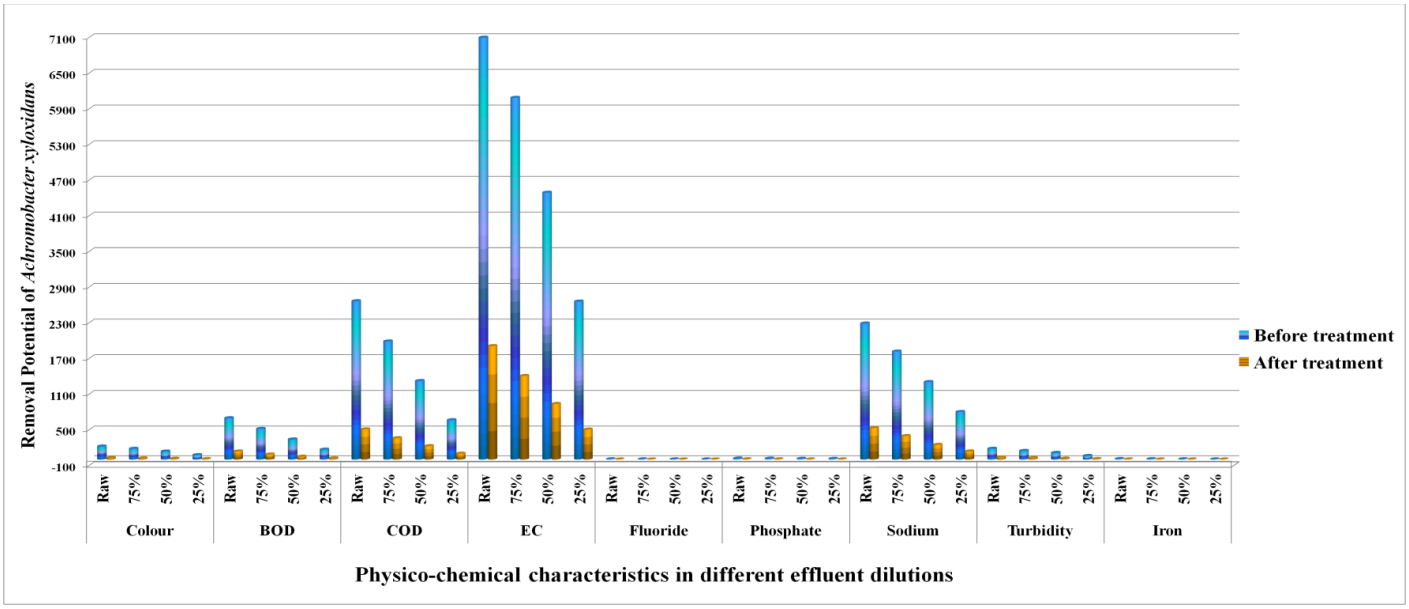
Fluoride	mg/L	0.73±0.06	0.48±0.07	0.31±0.05	0.16±0.05	3.65±0.06
Phosphate	mg/L	3.38±0.09	2.68±0.11	1.80±0.08	1.34±0.09	15.76±0.10
Sodium	mg/L	528.60±0.17	394.24±0.09	246.42±0.11	135.62±0.07	2121.27±0.04
Turbidity	mg/L	34.27±0.06	24.39±0.09	15.26±0.05	6.52±0.08	660.30±0.09
Iron	mg/L	1.07±0.04	0.74±0.05	0.49±0.11	0.18±0.08	6.42±0.21
Zinc	mg/L	***Below detectable limit***				1.99±0.0003
Nickel	mg/L					2.34±0.0003
Manganese	mg/L					0.33±0.0003
Copper	mg/L					0.66±0.0006
Lead	mg/L					1.004±0.001
Cadmium	mg/L					0.52±0.001
Total Chromium	mg/L					0.73±0.001
Arsenic	mg/L					0.002±0.0003
Barium	mg/L					0.04±0.003
Cobalt	mg/L					0.20±0.003
Boron	mg/L					0.32±0.01S

Key: mg/L = milligram per liter, BDL: Below detectable limit, Values are expressed as mean ± SEM (n=3)

Figure 4: Effluent dilutions before treatment with study organism

Figure 5: Effluent treated with *Achromobacter xylosoxidans*

Figure 6: Physico-chemical parameters reduction by *Achromobacter xylosoxidans* at different effluent dilution



Phytotoxicity study on effluent treated with *Achromobacter xylosoxidans*

The control group achieved a seed germination rate of 100%, while the treated effluent reached 90%, and the raw effluent had only a 40% germination rate. Additionally, seeds in the treated effluent exhibited more substantial root and shoot growth compared to those in the raw effluent. The germination index was notably lower for the raw effluent at 6.7%, whereas the effluent treated with *Achromobacter xylosoxidans* showed a germination index of 83.96% (Table 8 and Figure 7).

Rahman et al. (2018) investigated the phytotoxic effect of synthetic dye effluent on the seed germination of red amaranth. Results showed that germination experiments of *Amaranthus cruentus L.* (red amaranth) were conducted in sterilized petri dishes containing concentrations of 0%, 5%, 10%, 25%, 50%, 75%, and 100%. There was a gradual decrease in the percentage of seed germination and seedling growth with increased concentrations of effluents. Relative toxicity and phytotoxicity were extreme at 100% effluent concentration. Root and shoot lengths of seedlings exhibited the lowest values at raw effluent and peaked at 5%. Hence, it was concluded that diluted effluent favored the growth and germination of seeds, while concentrated effluent inhibited seed growth.

Table 8: Effect of effluent treated with *Achromobacter xylosoxidans* on the germination and growth of *Vigna mungo*

Sample	Shoot length (cm)	Root length (cm)	Germination (%)	GI (%)
Control	**22.4**	**17.9**	**100**	**100**
Raw	**4.9**	**3**	**40**	**6.7**

Treated	**17.6**	**16.7**	**90**	**83.96**

Figure 7: *Vigna mungo* grown in effluent treated with *Achromobacter xylosoxidans*

Control Treated effluent Raw

Conclusion

The study effectively demonstrates that *Achromobacter xylosoxidans* KUESCCHK-6, identified through 16S rRNA molecular analysis, is a highly efficient and eco-friendly solution for the bioremediation of textile effluents. Under optimal conditions, the strain significantly reduced key physiochemical parameters of the wastewater including color, BOD, COD, EC, fluoride, phosphate, sodium, and turbidity, as well as various metal ions to below detectable limits. The treated effluent was confirmed to be non-toxic through phytotoxicity tests. These findings suggest that *Achromobacter xylosoxidans* KUESCCHK-6 holds great promise for large-scale, sustainable bioremediation applications in the textile industry.

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