Evaluation of Bioremediation Competence of Indigenous Bacterial Strain *Brevibacillus panacihumi KUESCCHK-5* isolated from Textile Effluent and its Effects on *Vigna mungo*

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Abstract
The textile industry generates highly contaminated wastewater containing harmful substances, posing significant environmental hazards. Bioremediation using natural organisms offers an effective treatment solution. This study involved the characterization of physico-chemical parameters of textile wastewater, followed by treatment using indigenous bacterial strain isolated from the effluent. The bacterial strain isolated from textile effluent identified as Brevibacillus panacihumi KUESCCHK-5 via 16S rRNA gene sequencing, was evaluated for its bioremediation potential under static conditions over five days. The strain achieved a decolorization rate of 99.38%-100% under optimal conditions (40°C, pH 9.5, peptone as a nitrogen source, glucose as a carbon source with a 4:1 carbon-to-nitrogen ratio). The treatment significantly reduced pollution parameters: BOD by 94.16%-99.11%, COD by 89.24%-98.83%, electrical conductivity by 79.06%-88.97%, fluoride by 89.08%-97.81%, phosphate by 91.38%-97.94%, turbidity by 89.76%-97.44% and sodium by 89.88%-97.65% at different effluent concentrations (Raw, 75%, 50% and 25% concentrations).

ANOVA analysis indicated significant differences in pollutant levels across different effluent concentrations (p = 0.0436) and t-tests showed that the removal efficiency of physico-chemical parameters was significant at the 5% level but not at the 1% level. Additionally, heavy metals were reduced to below detectable limits and the treated effluent was non-toxic to Vigna mungo. These results indicate that Brevibacillus panacihumi is a promising, eco-friendly and cost-effective agent for treating textile effluent.

Keywords: *Brevibacillus panacihumi*, Textile Effluent Treatment, Biomass Production, Pollutant Removal, Phytotoxicity, ANOVA.

Introduction

The textile industry is one of the largest in the world, using over 10,000 dyes and producing over 700,000 tons of synthetic dyes annually. It plays a vital role in the economic growth of South Asia, particularly in India, Bangladesh, Pakistan and Sri Lanka, providing significant employment without requiring special skills. The industry generates substantial effluents, dirt slurry and solid waste daily. Textile products are made from three types of fibers: cellulose, protein and synthetic. Each fiber type uses different dyes: cellulose fibers use reactive, direct, naphthol and indigo dyes; protein fibers use acid and lanaset dyes; synthetic fibers use disperse, basic and direct dyes. The industry consumes large amounts of chemicals and water, using about 200 liters of water to produce 1 kilogram of textile.

Textile dyeing effluents contain trace amounts of heavy metals like iron, lead, nickel, copper, zinc and chromium. Synthetic azo dyes in these effluents are carcinogenic and toxic, posing severe health risks. These effluents alter the physical, chemical and biological nature of aquatic environments, impacting community health, livestock, wildlife, fish and biodiversity by changing turbidity, odor, noise, temperature and pH. Textile printing and dyeing processes: pretreatment, dyeing, printing and finishing, produce large amounts of effluents with pollutants such as starch, waxes, carboxyl methyl cellulose (CMC), polyvinyl alcohol, wetting agents, sodium hypochlorite, chlorine, sodium hydroxide, hydrogen peroxide, acids, surfactants, sodium silicate, sodium phosphate and short cotton fibers. These effluents contain dissolved solids (DS) of starch, strong colors, high BOD_5, low suspended solids (SS), heavy metals, oily residues and are slightly alkaline.

Textile dyeing effluents are very toxic, primarily due to sulfur, naphthol, vat dyes, heavy metals and various auxiliary chemicals. Gujarat's textile industries discharge the most effluents in India. The water is further contaminated by unsafe substances including formaldehyde-based dye fixatives, hydrocarbon-based softeners and non-biodegradable dyeing chemicals. Up to 200,000 tons of dyes are lost annually due to inefficient dyeing processes, increasing turbidity and causing unpleasant odors and appearance from coloring and oily scums[10].

Textile wastewater can cause serious health issues such as hemorrhage, skin ulceration, nausea, irritation and dermatitis. Chemicals in the wastewater block sunlight, increasing biological oxygen demand and inhibiting photosynthesis and reoxygenation. It contains high organic content, dyes, toxic chemicals and inorganic compounds like sodium hydroxide, hydrochloric acid, sodium chloride and detergents. While cotton is eco-friendly, over 50% of its

products are dyed with reactive dyes, resulting in effluents that are colored, saline and have high BOD/COD, which are ecologically harmful[3,9].

The treatment of dyeing wastewater is crucial. Physical and chemical methods are often economically unfeasible due to high power and chemical requirements and they produce significant sludge, causing secondary pollution. Bioremediation, using microorganisms to degrade pollutants into less harmful forms, is preferred. Bacteria, fungi and algae can remove pollutants from wastewater. Microorganisms used can be indigenous or isolated. Molecular techniques, like PCR amplification of the 16S rRNA gene, enhance the detection and identification of specific microorganisms in effluent. BLAST comparisons with the GenBank database help to identify phylogenetic relatives. Utilizing microbial communities from textile effluents for bioremediation is promising due to their genetic and biochemical adaptation to toxic compounds.

Effective *in situ* bioremediation requires sufficient indigenous microbial populations and suitable environmental conditions[7,8]. As a result, this study aims to isolate, identify and describe indigenous bacteria found in textile industry effluent. It further investigates the influence of untreated and treated textile effluents on the growth of *Vigna mungo*.

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 effluent: Bacteria from the effluent were identified by serially diluting the sample from 10^{-1} to 10^{-10} and plating it on nutrient agar media (NAM). From each concentration, 100µl of the effluent suspension was added to Petri plates containing 20ml of sterile NAM. The sample was spread on the agar plate using the L-Rod (spread plate technique) and incubated at 37°C for 24 hours. After incubation, bacterial growth was observed on the agar plates and sub-cultured to obtain pure culture. Pure bacterial isolate was achieved using the quadrant streaking method[2].

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[4]. 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[6].

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.

Physicochemical characterization and colour analysis: The effluent's physico-chemical properties were analyzed in the laboratory according to APHA[1] standards (Table 1). 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) and C_e is the colour concentration after treatment (mg/L)[12].

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 nutrient agar medium. This isolate displayed distinct morphological and colonial features (Figure 1). 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[4], it was determined that the isolate belonged to the genus *Brevibacillus*.

Table 1
Standard methods adopted for physico-chemical characterization of textile industry effluent

S.N.	Parameter	Methods APHA (2017)	Instrument Used	Units
1.	Color	Spectrophotometric Method	Spectrophotometer	--
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	Turbidometrc 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

Several biochemical tests were performed on the isolate including starch hydrolysis, gelatin hydrolysis, citrate utilization, nitrate reduction, urease test, methyl red test, indole production test, catalase test, oxidase test and hydrogen sulfide production test. The results revealed unique morphological and biochemical characteristics including colony traits, suggesting distinct structure and functions (Table 2 and table 3).

Gram staining tests indicated that the isolate exhibited a Gram-negative reaction with rough, bacilli-shaped morphology. The 16S rRNA sequence of the bacterial isolate was deposited in GenBank, identifying it as *Brevibacillus panacihumi* KUESCCHK-8 (Accession number- OM475766) 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.

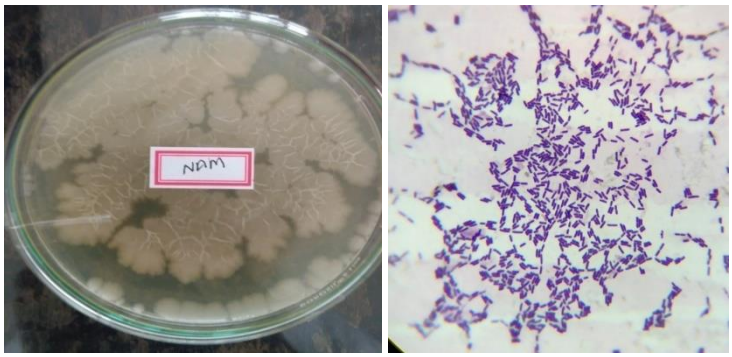

Figure 1: Macroscopic and microscopic observation of isolated strain

Table 2
Biochemical characteristics of the isolated strain

S.N.	Biochemical Test	Bacterial isolate
1.	Starch hydrolysis test	-
2.	Gelatin hydrolysis test	-
3.	Citrate utilization test	+
4.	Nitrate reduction test	-
5.	Urease test	-
6.	Methyl red test	-
7.	Indole production test	-
8.	Catalase test	+
9.	Oxidase test	-
10.	Hydrogen Sulphide production test	-

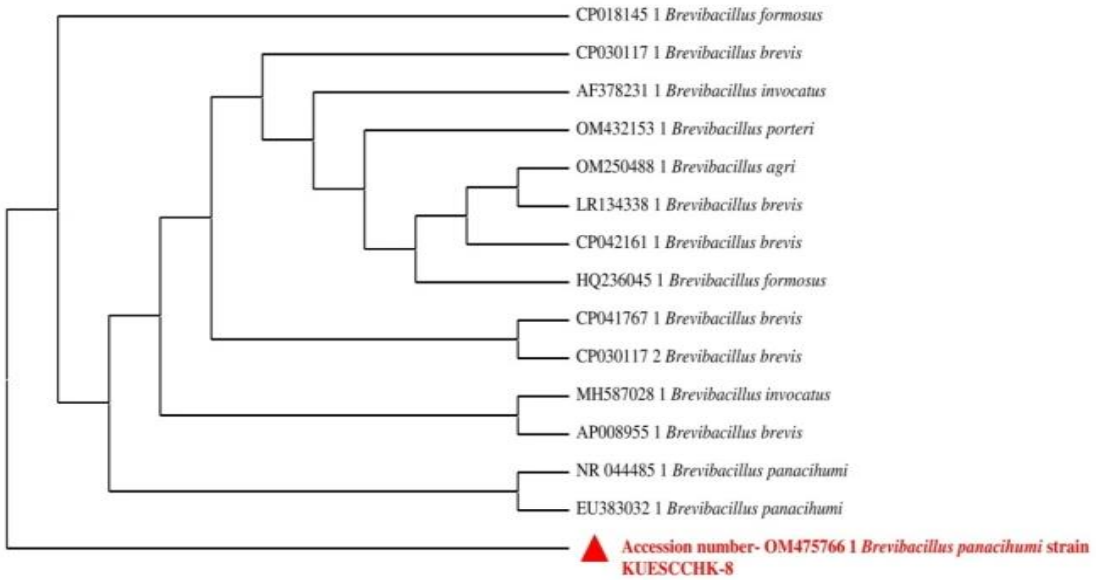

Figure 2: Phylogenetic tree of 16S rRNA gene sequences of isolate *Brevibacillus panacihumi*

The impact of varying temperatures, pH levels, carbon sources, nitrogen sources and carbon-to-nitrogen ratios (C/N ratio) on *Brevibacillus panacihumi*: The optimal conditions for biomass production by *Brevibacillus panacihumi* 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 40°C and a pH of 9.5, with peptone serving as the nitrogen source and glucose as the carbon source, with a C/N ratio of 4:1. These conditions yielded the highest biomass output, which is essential for the efficient treatment of textile industry effluent as shown in table 4 and figure 3.

Table 3
Morphological features of isolated bacterial strain

Morphology features		Bacterial isolate
Colony Morphology	Shape	Round
	Texture	Smooth
	Colour	Creamy
Microscopic characters	Cell shape	Rods
	Motility	Motile
	Spore Formation	+ve

Table 4
Biomass production by bacterial strain *Brevibacillus panacihumi* at optimized parameter

Biomass production by bacterial strain *Brevibacillus panacihumi*									
Different Temperature (ºC)	**Biomass Production in g/L**	**Different pH**	**Biomass Production in g/L**	**Different Nitrogen Sources**	**Biomass Production in g/L**	**Different Carbon Sources**	**Biomass Production in g/L**	**Different Carbon to Nitrogen Ratio**	**Biomass Production in g/L**
25ºC	0.7	5.5	0.1	Urea	1.3	Fructose	4.1	01:01	2.7
30ºC	2.9	6.5	1.1	Yeast Extract	3.2	Maltose	6.3	02:01	5.9
35ºC	4.1	7.5	3.9	Peptone	4.5	Glucose	10.2	04:01	8.7
40ºC	5.3	8.5	4.7	Beef Extract	3.1	Lactose	3.3	06:01	6.3
45ºC	1.8	9.5	6.1			Sucrose	7.1	08:01	3.3
50ºC	0.3	10.5	4.3						

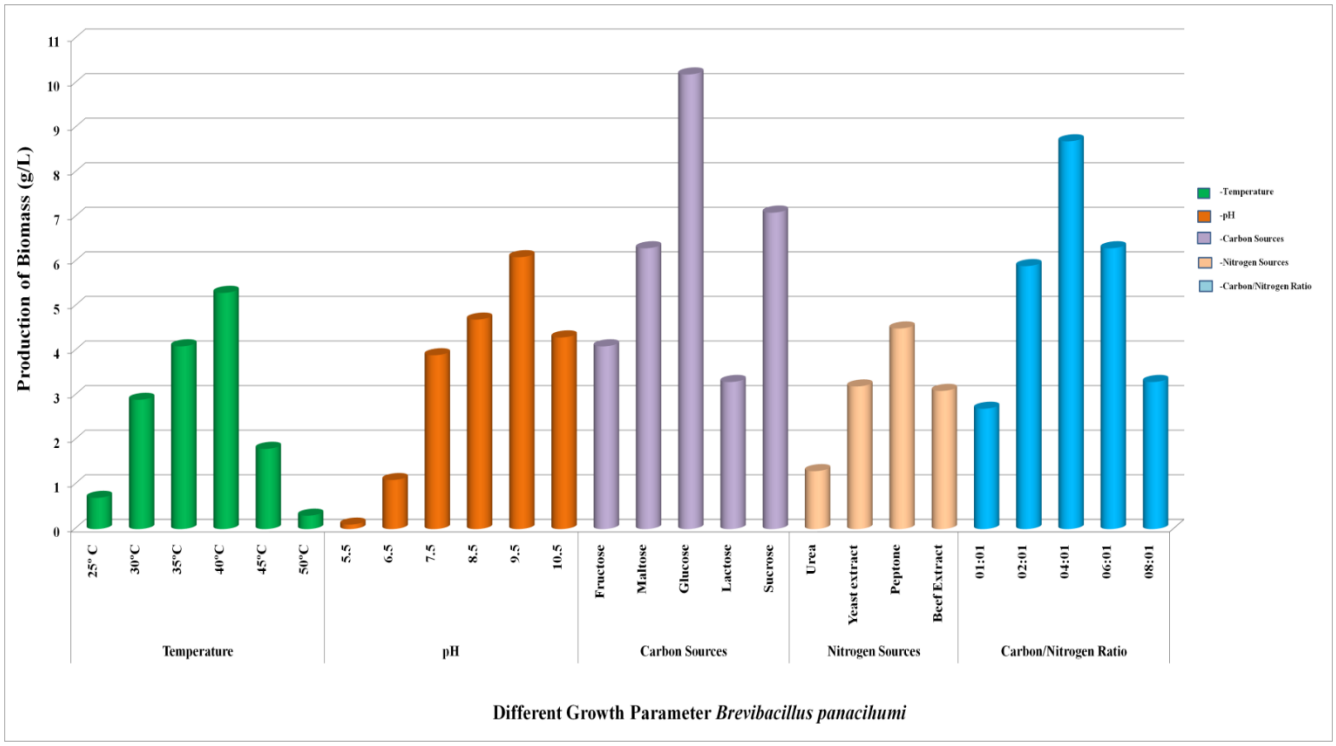

Figure 3: Biomass production by *Brevibacillus panacihumi* in different growth parameters

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 *Brevibacillus panacihumi* KUESCCHK-5: Effluent samples were analyzed before and after treatment at different concentrations (raw effluent, 75%, 50%, 25%) and compared to a control (Figures 5 and 6). Data in tables 5 and 6 show various effluent parameters before and after treatment with *Brevibacillus panacihumi*. Initially, parameters such as color, BOD, COD, EC, fluoride, phosphate, sodium and turbidity were high in the raw effluent. After treatment, these parameters significantly decreased across all concentrations. For example, the color dropped from 1.33 mg/L in raw effluent to 0 mg/L at 25% concentration. BOD decreased from 40.49 mg/L to 1.46 mg/L, COD reduced from 286.47 mg/L to 7.71 mg/L and electrical conductivity (EC) fell from 1485.37 µmhos/cm to 292.92 µmhos/cm.

Fluoride levels dropped from 0.42 mg/L to 0.03 mg/L, phosphate decreased to 0.20 mg/L and sodium reduced to 18.72 mg/L. Turbidity significantly dropped from 18.49 mg/L to 1.53 mg/L, while iron concentration decreased from 0.74 mg/L to 0 mg/L. Additionally, metals such as zinc, nickel, manganese, copper, lead, cadmium, total chromium, arsenic, barium, cobalt and boron were below detectable limits after treatment with bacterial strain. This demonstrates the high efficacy of *Brevibacillus panacihumi* in significantly reducing pollutants (Figure 6).

A similar study conducted by Bhatia et al[5] investigated the biodegradation and decolorization of disperse red 167 dye using the bacterium *Brevibacillus panacihumi*, known for its production of the azo-reductase enzyme. The bacterium's effectiveness in degrading the dye was assessed at concentrations of 50, 100 and 150 mg/L in a laboratory environment. After 96 hours, the dye concentration decreased by 58.83 ± 0.76% at 50 mg/L, 52.40 ± 0.45% at 100 mg/L and 47.10 ± 0.10% at 150 mg/L.

Statistical analysis ANOVA followed by Tukey's Test: The ANOVA analysis tested the hypothesis that there is no significant difference in the mean concentrations of parameters across different effluent dilutions (raw, 75%, 50%, 25%, control). The calculated F-statistic was 2.5579 and the p-value was 0.0436. Since the p-value is less than the 0.05 significance level and the F-statistic exceeds the F critical value of 2.4675, we reject the null hypothesis (Table 7). This indicates that there are statistically significant differences in the concentrations of the parameters among the different effluent dilutions, demonstrating that the treatment process significantly affects the measured parameters. These results are important for evaluating the effectiveness of the treatment process.

The ANOVA followed by Tukey's test indicated that the results are statistically significant, as shown in tables 5, 6 and 7. The t-test results in tables 8, 9, 10 and 11 demonstrate that the p-values are less than 5% but greater than 1%.

Table 5
Concentration of parameters before treatment at different effluent concentrations

Parameters	Units	Raw effluent	75% concentration	50% concentration	25% concentration	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 6
Concentration of parameters after treatment with *Brevibacillus panacihumi*

Parameters	Units	Raw effluent	75% concentration	50% concentration	25% concentration	Control
Color concentration	mg/L	1.33±0.02*	0*	0*	0*	210.26±0.08*
BOD	mg/L	40.49±0.25*	16.57±0.20*	7.49±0.21*	1.46±0.12*	660.30±0.09*
COD	mg/L	286.47±0.15*	169.46±0.20*	76.45±0.20*	7.71±0.15*	2568.54±0.15*
EC	µmhos/cm	1485.37±0.09*	1059.20±0.08*	656.83±0.03*	292.92±0.05*	6796.63±0.12*
Fluoride	mg/L	0.42±0.07*	0.25±0.05*	0.14±0.03*	0.03±0.01*	3.65±0.06*
Phosphate	mg/L	1.42±0.04*	0.92±0.03*	0.50±0.06*	0.20±0.04*	15.76±0.10*
Sodium	mg/L	231.4±0.10*	145.25±0.11*	65.60±0.11*	18.72±0.2*	2121.27±0.04*
Turbidity	mg/L	18.49±0.09*	11.48±0.04*	5.28±0.08*	1.53±0.11*	660.30±0.09*
Iron	mg/L	0.74±0.06*	0.51±0.04*	0.19±0.09*	0±0*	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±0.01*

Key: mg/L = milligram per liter, BDL: Below detectable limit, Values are expressed as mean ± SEM (n=3),* $p<0.05$; **$p>0.01$, denotes significance with respect initial values (before treatment) using one way ANOVA followed by Tukey's test.

Table 7
ANOVA Table for Analysis of Variance between and Within Groups

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	5749567.161	4	1437391.79	2.557882861	0.043606525	2.467494
Within Groups	53384860.64	95	561945.9014			
Total	59134427.8	99				

Note: SS-Sum of Squares, Df- Degrees of Freedom, MS-Mean Square, F-Statistic, F Crit-Critical Value of F.

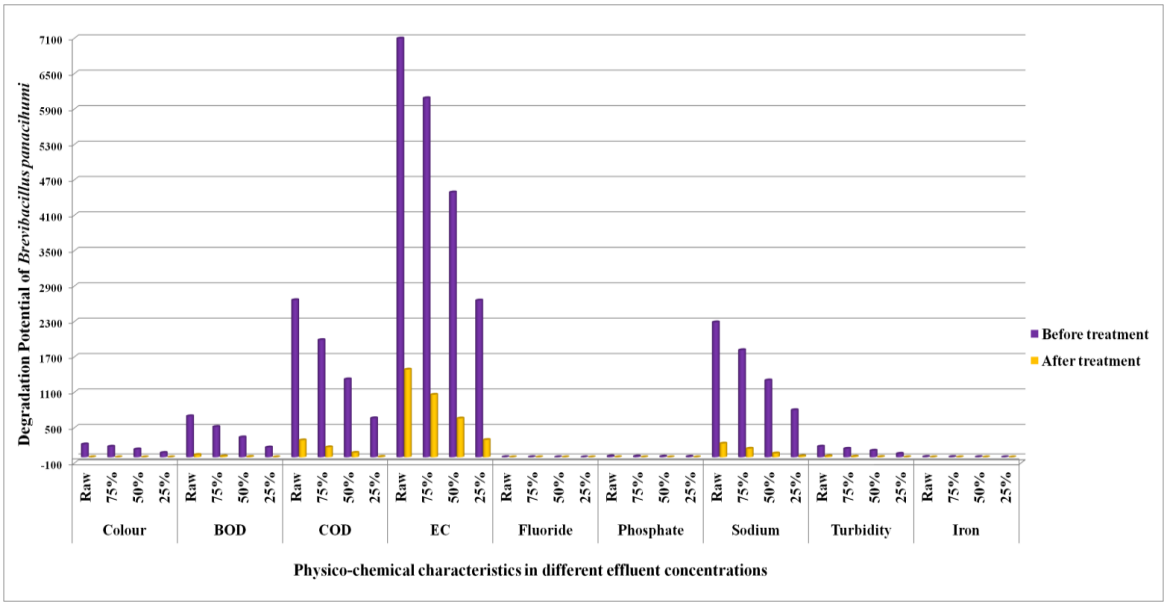

Figure 4: Physico-chemical parameters reduction by *Brevibacillus panacihumi* at different effluent concentration

Figure 5: Effluent concentrations before treatment with study organism

Figure 6: Effluent treated with *Brevibacillus panacihumi*

Table 8
t-Test: Paired Two Sample for Means for raw effluent

	Before treatment	After treatment
Mean	658.7388	103.3065
Variance	2871207.73	112130.3278
Observations	20	20
Pearson Correlation	0.97418311	
Hypothesized Mean Difference	0	
Df	19	
t Stat	1.81266997	
Sig	5%	1%
P(T<=t) one-tail	0.04285757	
t Critical one-tail	1.72913279	2.539
P(T<=t) two-tail	0.08571514	
t Critical two-tail	2.09302405	2.861

Table 9
t-Test: Paired Two Sample for Means for 75% concentration

	Before treatment	**After treatment**
Mean	537.6204	70.182
Variance	2041345	56506.11
Observations	20	20
Pearson Correlation	0.976214	
Hypothesized Mean Difference	0	
Df	19	
t Stat	1.745225	
Sig	5%	1%
P(T<=t) one-tail	0.048549	
t Critical one-tail	1.729133	2.539
P(T<=t) two-tail	0.097098	
t Critical two-tail	2.093024	2.861

Table 10
t-Test: Paired Two Sample for Means for 50% concentration

	Before treatment	**After treatment**
Mean	385.771	40.624
Variance	1091965	21507.98
Observations	20	20
Pearson Correlation	0.969766	
Hypothesized Mean Difference	0	
Df	19	
t Stat	1.708484	
Sig	5%	1%
P(T<=t) one-tail	0.051916	
t Critical one-tail	1.729133	2.539
P(T<=t) two-tail	0.103832	
t Critical two-tail	2.093024	2.861

Table 11
t-Test: Paired Two Sample for Means for 25% concentration

	Before treatment	**After treatment**
Mean	221.551	16.1285
Variance	378162.3	4263.892
Observations	20	20
Pearson Correlation	0.952877	
Hypothesized Mean Difference	0	
Df	19	
t Stat	1.661013	
Sig	5%	1%
P(T<=t) one-tail	0.056562	
t Critical one-tail	1.729133	2.539
P(T<=t) two-tail	0.113123	
t Critical two-tail	2.093024	2.861

Table 12
Effect of effluent treated with *Brevibacillus panacihumi* 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	21.9	17.5	95	92.87

This leads to the rejection of the null hypothesis (Ho) at 5% significance level and its acceptance at 1% significance level. Therefore, it can be concluded that the physico-chemical parameter removal efficiency of *Brevibacillus panacihumi* is significant at 5% significance level but not at 1% significance level.

Phytotoxicity study on treated effluent: A study on the phytotoxicity of treated industrial effluent focused on its impact on plant growth by evaluating how treated effluent affects seed germination and plant health. The research on effluent treated with *Brevibacillus panacihumi* yielded promising results. Seeds grown in the treated effluent exhibited a 95% germination rate, significantly only 40% observed with raw effluent. Additionally, seeds in the treated effluent demonstrated greater root and shoot growth compared to those in the raw effluent. The germination index was notably higher in the treated effluent at 92.87%, compared to just 6.7% in the raw effluent (Table 12). These findings underscore the effectiveness of *Brevibacillus panacihumi* treatment in reducing effluent phytotoxicity, indicating its potential for environmentally safe applications.

Kabra et al[11] investigated the phytotoxic effects of textile industry effluent on crops by comparing treated and untreated samples. Seeds of *P. mungo* and *S. vulgare* showed high germination rates in both distilled water and treated effluent. However, the plumule and radicle lengths were significantly shorter in seeds germinated in untreated effluent and dye mixture compared to those germinated in distilled water and treated effluent. This finding indicated the toxicity of the untreated effluent while the treated effluent was almost non-toxic and comparable to distilled water.

Conclusion

The study isolated *Brevibacillus panacihumi* KUESCCHK-8 from effluent and identified it using biochemical tests and 16S rRNA sequencing. Optimal biomass production was achieved at 40°C and pH 9.5, using peptone as the nitrogen source and glucose as the carbon source. These conditions are crucial for efficient textile effluent treatment. Effluent treatment with this strain significantly reduced pollutants such as color, BOD, COD, EC, fluoride, phosphate, sodium and turbidity and lowered metal concentrations to below detectable limits across all effluent concentrations, with the greatest reduction observed at 25% concentration.

ANOVA and t-tests confirmed the treatment's effectiveness, showing significant differences in pollutant levels. Phytotoxicity tests revealed a 95% seed germination rate in treated effluent, compared to 40% in raw effluent, with improved root and shoot growth. The germination index further validated the effectiveness of *Brevibacillus panacihumi* treatment in reducing effluent phytotoxicity. Overall, *Brevibacillus panacihumi* KUESCCHK-8 effectively treats textile effluent, reducing pollutants and phytotoxicity, showcasing its potential for sustainable wastewater management.

Control Treated effluent Raw

Figure 7: *Vigna mungo* grown in effluent treated with *Brevibacillus panacihumi*

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