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ORIGINAL ARTICLE **OPEN ACCESS**

Molecular Identification of Bacterial Strain Using 16s rRNA Sequencing and Assessment of its Efficiency on Reduction of Sulphate in Textile Industry Effluent

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ABSTRACT
Textile industry effluents are among the most difficult to treat, due to considerable amount of recalcitrant and toxic substances. Many treatment technologies are already in use, but due to their drawbacks, standard biological treatment methods are acceptable. The present study aimed to isolate and molecularly identify a bacterial strain for assessing its efficacy in sulphate reduction. Employing the pour plate technique, a microbial strain was isolated from textile industry effluent. Molecular analysis identified the strain as Brevibacillus panacihumi, deposited at the National Center for Biotechnology Information (NCBI) under accession number OM475766. The strain's ability to reduce sulphate from textile industry wastewater was tested under specified conditions: pH 9.5, temperature 40°C, fructose as the carbon source, beef extract as the nitrogen source, and a carbon/nitrogen ratio of 6:1, with treatment duration of 5 days under static conditions. Results indicated that Brevibacillus panacihumi effectively reduced sulphate levels in textile industry effluents, with reduction percentages ranging from 85.38% to 92.16%. These findings underscore the potential of the isolated bacteria as promising candidates for sulphate concentration reduction in textile industry effluents.
***Keywords**: Textile industry effluent, bacteria, Brevibacillus panacihumi, sulphate, NCBI, sequencing.*

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INTRODUCTION

In India, water contamination is a serious environmental concern (1). The textile industry is considered one of the major worldwide industries which produce vast amount of effluent (2 and 3). The direct dumping of this waste water into the environment impacts the region's ecological status by causing a number of adverse effects (4 and 5). Consequently, wastewater is generated, having a very diverse range of contaminants that must be treated prior to disposal (6 and 7). Sulfates, particularly sodium sulfate (Na_2SO_4) and ammonium sulfate ($(NH_4)2SO_4$), are used in the textile industry for various purposes like dyeing, printing, textile finishing, desizing, scouring, bleaching, water treatment, cleaning and catalysts in textile processes. High levels of sulfates in effluents can contribute to environmental pollution. Discharging high levels of sulfate into water bodies can have several significant environmental and ecological effects like salinity and osmotic stress, impact on aquatic life, harmful algal blooms, corrosion and infrastructure damage, sulfide formation (sulfates can be converted into hydrogen sulfide (H_2S) under certain anaerobic conditions, hydrogen sulfide is highly toxic to aquatic life, even at low concentrations, and exposure to it can cause harm or mortality to fish and other aquatic organisms), eutrophication and environmental imbalance. Managing sulfate levels in textile industry effluents is crucial to ensure compliance with environmental regulations and minimize environmental impact. Execution of physical/ chemical strategies has the innate drawbacks of being economically unfeasible as they require greater power and chemical substances and are not able to take away the recalcitrant azo dyes and/or their natural metabolites completely, generating a vast quantity of sludge which can lead to secondary pollutants problems, and concerning complex procedures (8). Bioremediation is getting prominence because of its low cost, effectiveness, and environmental stewardship, and the metabolites formed during biodegradation are typically nontoxic or comparatively less hazardous in nature (9 and 10). Bacteria, algae, and fungi are among the microorganisms used in biological degradation approaches. Microorganisms used in the treatment process may be either indigenous or isolated from elsewhere (11 and 12). In recent years, PCR

amplification of the 16S rRNA gene and subsequent sequence analysis has improved the identification of unknown bacterial isolates (13). Using microbial communities recovered from textile industry effluents as bioremediation in the environment is a promising strategy. This is because of the metabolic arsenal of these microorganisms, which could be used for bioremediation due to the genetic and biochemical adaptation of this community to the toxic compounds (14). As a result, the current research aims to isolate, identify and describe indigenous bacteria found in textile industry effluent. So, the purpose of this study was to isolate and characterize bacteria from textile industry effluent for use in reduction of sulpahte in the textile industry.

MATERIAL AND METHODS

Effluent sample collection

The effluent was collected and transported in sterile, dry polypropylene bottles. During transportation the samples were kept cold. After keeping the sample in the refrigerator (at 4^0 C), the bacteria was isolated. Sulphate was measured using standard method of APHA (15).

Isolation of bacterial isolates from an effluent sample

Bacteria from effluent were determined by serial dilution of 10^{-1} to 10^{-10} and plating in Nutrient Agar Media (NAM). The culture plates were incubated in a bacteriological incubator at 37^0 C for 24 hours. On the Agar Plate, mixed bacterial growth was observed after 24 hours. Each developed colony was sub-cultured to obtain pure cultures.

Isolation of bacterial strain

Based on the size, shape, color, elevation, transparency and other characteristics of their colony morphology, one bacterial isolate was selected from the mother plate. Biochemical methods were used to identify the bacterial culture after they were isolated as pure culture. Biochemical tests such as starch hydrolysis, gelatin hydrolysis, citrate utilization, nitrate reduction, urease test, methyl red test, indole production test, catalase test, oxidase test, and hydrogen sulphide production test were performed to characterize the isolated bacterial strain using the methods described in "Microbiology: A Laboratory Manual". The isolate was morphologically characterized using the standard Gram staining procedure (16).

Optimization condition for bacterial isolate

Effect of different temperatures, pH, carbon sources, nitrogen sources and carbon to nitrogen ratio (C/N ratio) on isolated bacterial strain

To optimize the different cultural conditions viz. temperature range from 25°C, 30°C, 35°C, 40°C, 45°C and 50°C and pH was adjusted to 5.5, 6.5, 7.5, 8.5, 9.5 and 10.5. Different carbon sources such as fructose, maltose, glucose, lactose and sucrose and different nitrogen sources such as urea, yeast extract, peptone and Beef Extract were analyzed. Different Carbon to Nitrogen ratio (C/N ratio) such as 1:1, 2:1, 4:1, 6:1, 8:1 and 10:1 was optimized (17).

Isolation and Identification of Isolated Bacterial Isolates using 16S rRNA gene sequencing

The National Center for Biotechnology Information (NCBI, https://blast.ncbi.nlm.nih.gov/) database and MEGA version 5 were used to verify ITS sequence fragment identity using the Basic Local Alignment Search Tool (BLAST) in GenBank. A phylogenetic tree was created to required to conduct a better classification.

RESULT AND DISCUSSION

Isolation of bacterial strains, morphological and biochemical characterization

One bacterial strain was isolated from effluent based on unique colonial characteristics on nutrient agar medium. Biochemical tests are used to distinguish different bacteria based on their biochemical responses to various biochemical compounds. One bacterium were subjected to standard biochemical tests. Bergey's Manual of Systematic Bacteriology was used to identify the isolate which belonged to *Brevibacillus.*

Starch hydrolysis, gelatin hydrolysis, citrate utilization, nitrate reduction, urease test, methyl red test, indole production test, catalase test, oxidase test, and hydrogen sulphide production test were among the biochemical tests performed on the isolate (Table 1). According to Gram staining tests, the bacterial colony had gram-positive **(Figure 1)** reaction with rough and bacilli in shape.

Extraction of DNA, PCR amplification and 16SrRNA gene sequencing for bacterial isolate

The 27F and 1492R primers were used to amplify a fragment of the 16S rRNA gene, and the 16S rRNA gene sequence was used to perform BLAST against the NCBI Genbank database. BLAST search is usually employed using nucleotide collection (BLASTn). The 16s rRNA sequences of bacterial isolate was deposited in GenBank like *Brevibacillus panacihumi KUESCCHK-8 (*Accession number-OM475766) (Table 2).

Phylogenetic analysis

The 16S rRNA gene sequence of isolated bacteria was compared using the BLAST online tool in the NCBI Genbank database. The first ten sequences were chosen and aligned using the multiple alignment software programs MEGA 7. Phylogenetic analysis was performed based on maximum identity scores. The

evolutionary relationship of the bacterial strains with the other relevant bacteria was presented in Figure 2 and their similarities are found in the GenBank database.

Effect of different temperatures, pH, carbon sources, nitrogen sources and carbon to nitrogen ratio (C/N ratio) on production of biomass yield

Understanding how temperature influences the growth of this bacterium is crucial for optimizing its cultivation conditions and potentially exploiting its biomass production for various applications. The biomass production notably increased to 2.9 g/L at 30°C, indicating the bacterium's preference for moderately warm conditions. The peak biomass production of 5.3 g/L occurred at 40°C, suggesting an optimal temperature for *Brevibacillus panacihumi's* growth and metabolism. However, beyond 40°C, there was a decline in biomass production and further to 0.3 g/L at 50°C, indicating the bacterium's sensitivity to temperatures above its optimal range (Table 3 and Figure 3). A temperature optimum at 30°C has been shown (18) ,(19) in *Brevibacillus borstelensis and Brevibacillus panacihumi*. (20) have shown that at 35°C, the highest oil degradation rate i.e., 31.15% was attained by *Brevibacillus laterosporus*. This indicates that temperature around 30°C–40°C proves to be optimum for biomass production. In the present study, the effect of pH on the biomass production of *Brevibacillus panacihumi* was observed. The results revealed a trend of increasing biomass production with higher pH levels, peaking at pH 9.5 with 6.1 g/L. At lower pH levels, biomass production was significantly lower, with only 0.1 g/L observed at pH 5.5. These findings suggest that *Brevibacillus panacihumi* thrives in slightly alkaline conditions, with optimal biomass production achieved at pH 9.5 (Table 3 and Figure 3). Liu *et al.,* (21) revealed that *Brevibacillus laterosporus* shows highest production of algicidal substances at pH value 4-8. Furthermore, *Bacillus species* has its highest enzyme activity at pH 5-9 studies by Kim *et al.,* (22). This reflects that both acidic and an alkaline pH could inhibit biomass production of *Brevibacillus panacihumi*. The biomass production of *Brevibacillus panacihumi* varied with different nitrogen sources, with peptone resulting in the highest yield of 4.5 g/L. Yeast extract also supported significant biomass production at 3.2 g/L, while urea and beef extract resulted in lower biomass yields of 1.3 g/L and 3.1 g/L, respectively (Table 3 and Figure 3). Yüksekdağ *et al.,* (23) explored the effects of different nitrogen sources on the production of PHB yield. The highest level of PHB accumulation was observed in the media with proteaz peptone in *B. subtilis* (78.69%) and in *B. megaterium* (77.00%). Various carbon sources impact the biomass production of *Brevibacillus panacihumi.* Our findings indicate that glucose exhibited the highest efficacy, resulting in a biomass yield of 10.2 g/L, while fructose and lactose resulted in lower yields of 4.1 g/L and 3.3 g/L, respectively (Table 3 and Figure 3). Demirkan *et al.,* (24), reported that highest production of antibacterial substances was achieved by glucose as carbon source. When examining different carbon to nitrogen ratios' effects on biomass production in *Brevibacillus panacihumi,* it was noted that the 04:01 ratio resulted in the highest biomass yield, reaching 8.7 g/L, while the ratios of 01:01 and 08:01 resulted in lower biomass yields of 2.7 g/L and 3.3 g/L, respectively (Table 3 and Figure 3). Reddy *et al.,* (25) reported that optimum levels of biosurfactant were produced by *Achromobacter xyloxidans*, when grown in C/N ratio of 16:1 at pH 8. A similar study was reported by Vigneshwaran *et al.,* (26) while studying the effect of nitrogen sources in the production of biosurfactant by a strain *Bacillus subtilis.*

Reduction of Sulphate

Sulfates are abundant in nature, found in minerals, rocks, soils, water bodies, and organisms. They are vital in geological and biological processes. Industrially, sulfates are utilized in various sectors from chemical manufacturing to pharmaceuticals. The CPCB's guidelines mandate sulfate levels in treated effluents at 250 mg/L for discharge into inland surface waters. The raw effluent sample exhibited a substantial decrease in sulphate concentration from 1454.75 mg/L before treatment to 212.65 mg/L after treatment, resulting in an 85.38% reduction. Similarly, in diluted effluent samples (75%, 50%, and 25%concentrations), there was significant reductions in sulphate concentration post-treatment, with percentages of 87.13%, 90.36%, and 92.16%, respectively, compared to their respective initial concentration (Table 4 and Figure 4). At a 25% concentration, bacterial strain exhibited their highest reduction rate. These findings are in agreement with Chatterjee *et al.,* (27), who worked on bioreduction of toxic hexavalent chromium using *Brevibacillus species isolated from tannery effluent. Brevibacillus species* showed maximum reduction of about 85% for 100 mg/l of hexavalent chromium at temperature 37 °C and pH 7.0 in 48 h. *Brevibacillus panacihumi* is a bacterium known for its versatility in environmental remediation, including sulfate reduction. It possesses enzymes like sulfate reductase that catalyze the reduction of sulfate to sulfide. This reduction process often occurs under anaerobic (low oxygen) conditions. The sulfide produced can be beneficial for certain environmental applications, such as the bioremediation of sulfate-containing wastewater or soils.

Table 1: **Biochemical Characteristics of the Bacterial Strain Isolated from Textile Effluent**

Sl. No.	Biochemical Test	Bacterial Isolate
		Brevibacillus panacihumi
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	-

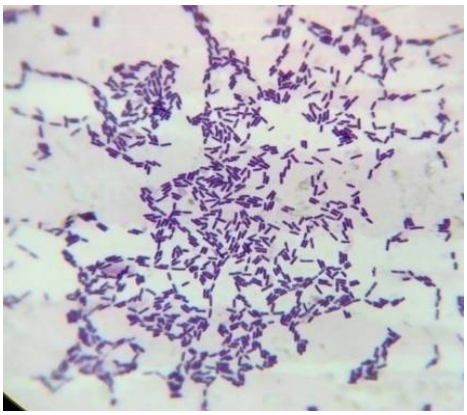

Figure 1: Microscopic observation of representative isolate *Brevibacillus panacihumi* (Gram +ve)

Table 2. The NCBI database BLASTn result

Accession number	Description	Maximum Identity
OM475766	*Brevibacillus panacihumi*	100%

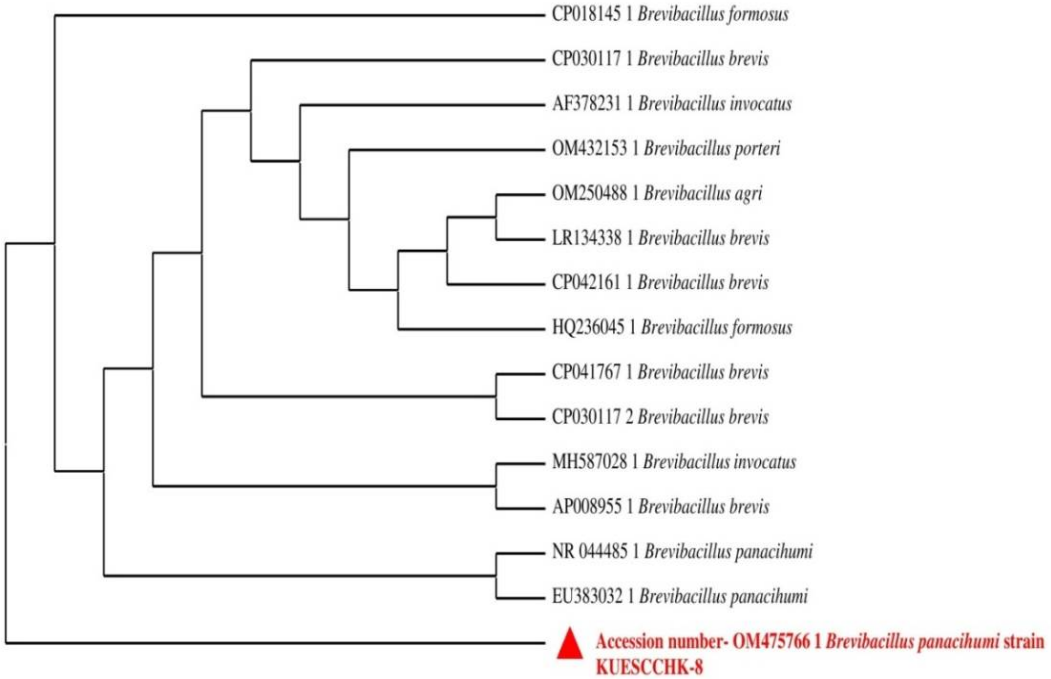

Figure 2: Phylogenetic tree of 16S rRNA gene sequences of isolate *Brevibacillus panacihumi*

Table 3: Biomass production by bacterial strain *Brevibacillus panacihumi* at different pH, temperature, carbon sources, nitrogen sources and carbon to nitrogen ratio

Different Temperature (°C)	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
25°C	0.7	5.5	0.1	Urea	1.3	Fructose	4.1	01:01	2.7
30°C	2.9	6.5	1.1	Yeast Extract	3.2	Maltose	6.3	02:01	5.9
35°C	4.1	7.5	3.9	Peptone	4.5	Glucose	10.2	04:01	8.7
40°C	5.3	8.5	4.7	Beef Extract	3.1	Lactose	3.3	06:01	6.3
45°C	1.8	9.5	6.1			Sucrose	7.1	08:01	3.3
50°C	0.3	10.5	4.3						

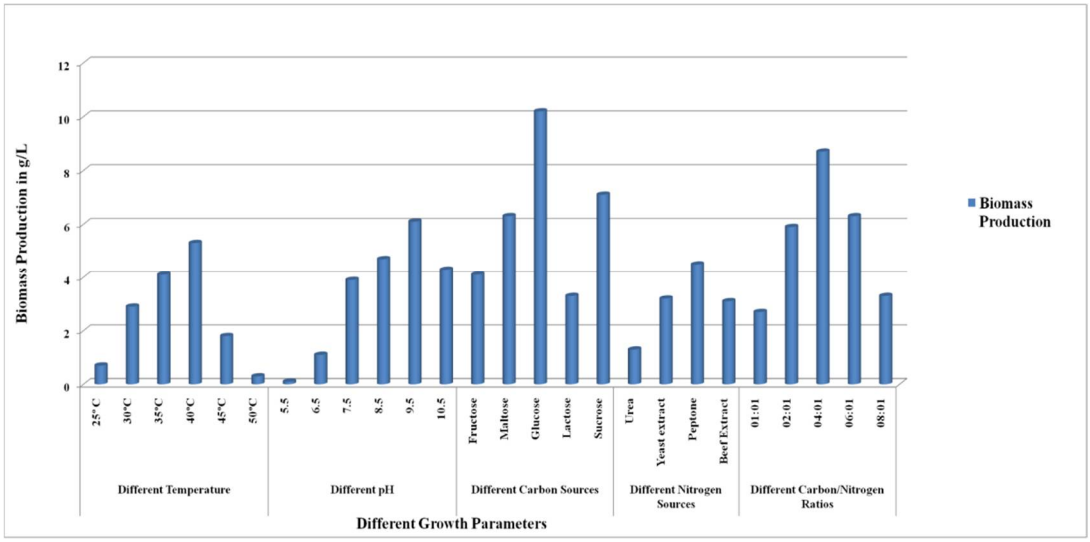

Figure 3: Production of biomass by *Brevibacillus panacihumi* at different pH, temperature, carbon sources, nitrogen sources and carbon/nitrogen ratio

Table 4: Concentration of sulphate before and after treatment

Effluent Concentrations	Sulphate (mg/L) removal by *Brevibacillus panacihumi*		
	Before treatment	After treatment	Percentage reduction (%)
Control	1454.75±0.05	1389.30±0.05	4.49
Raw	1454.75±0.21	212.65±0.15	85.38
75%	1107.57±0.15	142.53±0.12	87.13
50%	802.76±0.15	77.36±0.21	90.36
25%	515.71±0.05	40.41±0.15	92.16

Note: ^{1}= mgL^{-} milligram per liter. Values are expressed as mean±SD (n=3)

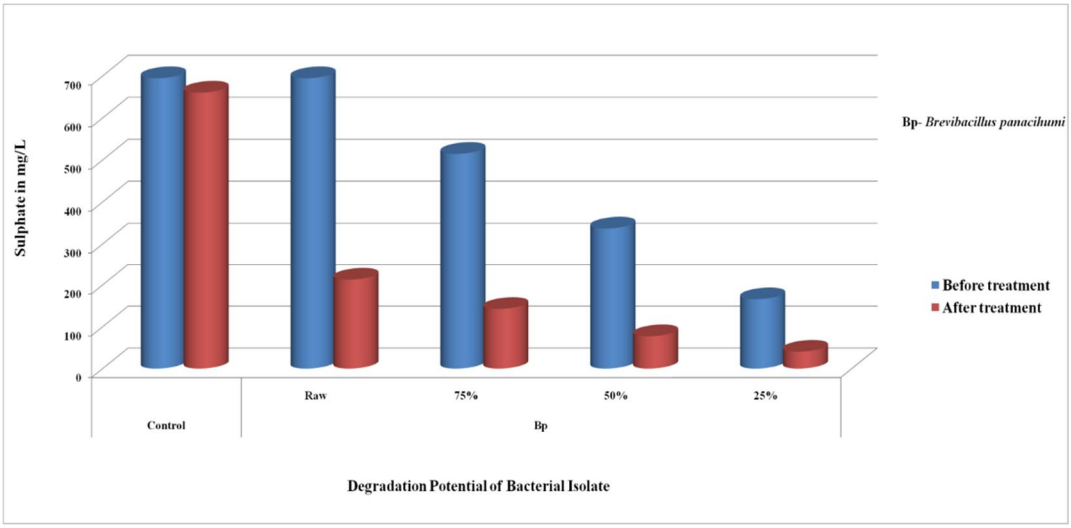

Figure 4: Sulphate reduction by study organism at different effluent concentrations

CONCLUSION

Textile industries are particularly problematic among industrial sectors because they generate large quantities of wastewater which when released into the environment without treatment, could have serious consequences. Environmental issues associated with textile activities are of great concern due to the extensive use of dyes. Various physical and chemical methods for removing contaminants in effluent are ineffective. The objective of the present study was to isolate and identify native bacteria from textile effluent and soil contaminated with effluent. The results indicated that bacterial strain in effluent possesses the ability to grow in polluted environment and survive in extreme conditions. Hence, isolated indigenous bacterial isolate could play a very important role in removal of contaminants in effluent especially sulphate reduction.

CONFLICT OF INTEREST

Author's declare that there is no conflict of interest.

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REFERENCES

1. Khatun, R. (2017). Water pollution: Causes, consequences, prevention method and role of WBPHED with special reference from Murshidabad District. International Journal of Scientific and Research Publications,7(8): 269-277.
2. Mondal P., Datta B. and Chaudhuri S. (2019). A highly heavy metal tolerant fusarium solani with efficient bioaccumulation potentiality from contaminated soil. Journal of Experimental Biology and Agricultural Sciences, 7 (6): 579 – 586.
3. Chandran, D. (2016). A review of the textile industries waste water treatment methodologies. Int. J. Sci. Eng. Res, 7(1): 392-403.
4. Mahmood, R. T., Asad, M. J., Asgher, M., Khan, F. S., Muzammil, K., Nasir, N. & Awais, M. (2022). First report on the bioremediation of textile industrial effluents by *Piptoporus betulinus* IEBL-3 by using response surface methodology. Applied Sciences, 12(3): 1090.
5. Yaseen, D. A., & Scholz, M. (2019). Textile dye wastewater characteristics and constituents of synthetic effluents: a critical review. International journal of environmental science and technology, 16: 1193-1226.
6. Kousar, H., Chaithra, C. & AkshathaK, U. (2021). Use of Aspergillus niger for the reduction of biological oxygen demand in textile industry effluent. Journal of Advanced Scientific Research, 12(2 Suppl 1): 351-354.
7. Hossain, K., Quaik, S., Ismail, N., Rafatullah, M., Avasan, M. & Shaik, R. (2016). Bioremediation and detoxification of the textile wastewater with membrane bioreactor using the white-rot fungus and reuse of wastewater. Iranian journal of biotechnology, 14(3): 154.
8. Adane T., Adugna A.T. and Alemayehu E. **(2021).** Textile industry effluent treatment techniques. Journal of Chemistry, 1-14.

9. Dewi, R. S., Kasiamdari, R. S., Martani, E. & Purwestri, Y. A. (2018). Decolorization and detoxification of batik dye effluent containing Indigosol Blue-04B using fungi isolated from contaminated dye effluent. Indonesian Journal of Biotechnology, 23(2): 54-60.
10. Chaithra C. and Kousar H. (2022). Reduction of chemical oxygen demand in textile industry effluent using Aspergillus nige. Pollution Research, 41(1): 230-233
11. Adebajo S.O., Ojo A.E., Bankole P.O., Oladotun A.O., Akintokun P.O., Ogunbiyi E.O. and Bada A. (2022). Degradation of paint and textile industrial effluents by indigenous bacterial isolates. Bioremediation Journal, 2022: 1-10
12. Roy, D. C., Biswas, S. K., Sheam, M. M., Hasan, M. R., Saha, A. K., Roy, A. K. & Tang, S. S. (2020). Bioremediation of malachite green dye by two bacterial strains isolated from textile effluents. Current research in microbial sciences, 1: 37-43.
13. Pushpa M.V., Yogendra K., Mahadevan K.M. and Mahesh M. (2019). Isolation and identification of azo dye degrading microbes using 16s r RNA sequencing. Journal of Applied and Natural Science, 11(2): 245-249.
14. Bernal S.P., Lira M., Jean-Baptiste J., Garcia P.E., Batista E., Ottoni J.R. and Passarini M.R. (2021). Biotechnological potential of microorganisms from textile effluent: isolation, enzymatic activity and dye discoloration. Anais da Academia Brasileira de Ciências, 93(4): 1-12.
15. American Public Health Association (APHA) (2017). Standards Methods for the Examination of water and Wastewater, twenty–third ed. American Public Health Association, American Water Work Association, Water Work Federation, Washington DC.
16. Cappuccino, James. G. & Sherman, N. (2013). Microbiology: a laboratory manual. Pearson Higher Ed.
17. Chandani, N., Mazumder, P. B. and Bhattacharjee, A. (2018). Biosynthesis of biodegradable polymer by a potent soil bacterium from a stress-prone environment. Journal of Applied Biology and Biotechnology, 6(2): 54-60.
18. Mukherjee, R. & Bera, D. (2022). Statistical optimization of asparaginase production by a novel isolated bacterium *Brevibacillus borstelensis* ML12 using Plackett–Burman design and response surface methodology. Journal of Applied Biology and Biotechnology, 10(3): 12-21.
19. Wang, X. X., Li, C., Zhao, L. B., Wu, L., Zhou, P., An, W. & Chen, Y. (2014). Optimization of Fermentation Medium and Culture Conditions of Hydrocarbon-Degrading Bacterium *Brevibacillus panacihumi* W25. Advanced Materials Research, 1010: 69-72.
20. Zhan, Y.B., Tao, X.L., He, S.W., Song, S.F., Xing, J.J., Li F.M., Jiang, T. and Ma, L. (2017). Isolation, identification and degradation characteristics of oil degrading bacterial strain. Open Access Library Journal, 4(10): 1.
21. Liu, S., Yu, Z., Wu, Z., Cao, X., Cheng, R., & Song, X. (2023). Algicidal substances of *Brevibacillus laterosporus* and their effect on red tide organisms. Frontiers in Marine Science, 10: 1160585.
22. Kim, M. K., Sathiyaraj, S., Pulla, R. K. & Yang, D. C. (2009). *Brevibacillus panacihumi* sp. nov., a β-glucosidase-producing bacterium. International journal of systematic and evolutionary microbiology, 59(5): 1227-1231.
23. Yüksekdağ, Z. N., Aslim, B., Beyatli, Y. and Mercan, N. (2004). Effect of carbon and nitrogen sources and incubation times on poly-beta-hydroxybutyrate (PHB) synthesis by Bacillus subtilis 25 and Bacillus megaterium 12. African Journal of Biotechnology, 3(1): 63-66.
24. Demirkan, E., Aybey, A. and Alev, U. S. T. A. (2021). Optimization of culture conditions for antibacterial substance production from newly isolated *Brevibacillus laterosporus* EA62. The European Research Journal, 7(2): 152-158.
25. Reddy, G. S., Himavarshini, K., Prasanna, A. D., Harini, S., Nikitha, N. S., Sphoorthi, G. N. and Bhuvaneswari, M. (2021). Statistical optimization of production and surface tension of lipoprotein and lipopeptides using central composite design. Int. J. Life Sci. Pharma Res, 11(4): 104-112.
26. Vigneshwaran, C., Sivasubramanian, V., Vasantharaj, K., Krishnanand, N. and Jerold, M. (2018). Potential of *Brevibacillus* sp. AVN 13 isolated from crude oil contaminated soil for biosurfactant production and its optimization studies. Journal of environmental chemical engineering, 6(4): 4347-4356.
27. Chatterjee, S., Shekhawat, K. and Gupta, N. (2019). Bioreduction of toxic hexavalent chromium by novel indigenous microbe *Brevibacillus* agri isolated from tannery wastewater. International journal of environmental science and technology, 16: 3549-3556.

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