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Identification of Novel Bacterial Strains for Enhanced Decolourization of Synthetic Dyes

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Abstract

Synthetic dyes present in textile effluents are significant pollutants of aquatic environments, where they accumulate and pose severe ecological and health risks by entering the food chain. Bacterial bioremediation offers an effective approach to the degradation and management of these contaminants. This study deals with the identification and characterization of three novel bacterial strains capable of decolorizing Methylene Blue, Direct Red 80, and Direct Green 2B dyes. Various physicochemical parameters were optimized to enhance the efficiency of the decolorization process. Among the strains tested, Pseudomonas aeruginosa immobilized using different alginate support matrices, resulted in an 97.7% dye degradation overnight. The findings highlight the potential of these bacterial strains in bioremediation, providing a sustainable approach to treating industrial wastewater contaminated with dyes.

Keywords: Bacterial bioremediation, Direct Green 2B, Direct Red 80, entrapment, gelatin alginate, Methylene Blue, optimization, starch alginate, synthetic dyes.

Introduction

The widespread use of dyes across industries has enhanced product appearance and quality. Unlike pigments, dyes dissolve in their medium for lasting coloration. Natural dyes, derived from plants, animals, and minerals, are valued for their environmental friendliness but face challenges in industrial-scale use due to inconsistent concentrations and quick fading. This has led to the dominance of synthetic dyes, which are man-made from chemical compounds like petroleum or coal tar. Synthetic dyes offer vibrant colours, better colourfastness, and consistency, making them the preferred choice for mass production[1].

Synthetic dyes, essential to industries like textiles, account for 75% of global dye usage, with annual production reaching 800,000 tons. Developing countries like India, Bangladesh, and Vietnam play key roles in this industry[2]. However, synthetic dyes contain hazardous heavy metals, such as lead and chromium, which disrupt aquatic ecosystems by reducing light penetration and impairing photosynthesis. These toxins also pose health risks to humans, including allergic reactions and cancer[3].

Given the limitations and harmful effects of synthetic dyes, there is an increasing need for sustainable solutions to mitigate their environmental impact. Traditional chemical and physical wastewater treatment methods, such as oxidation, reduction, and membrane filtration, often fall short due to their high costs, energy requirements, and secondary pollution[4,5]. In contrast, biological treatment methods, particularly those involving bacteria, offer a promising alternative. Bacteria possess diverse enzymatic activities that enable the breakdown of complex dye structures, leading to more environmentally friendly remediation of dye wastewater[6].

Bacterial degradation of synthetic dyes is aided by enzymes like oxidoreductase, which break down complex dye structures into less harmful compounds[7]. Bacteria from genera such as Bacillus, Pseudomonas, and Shewanella are effective in treating dye-contaminated environments[8]. Additionally, bacterial consortia groups of different bacterial species offer superior dye decolorization and degradation by adapting to environmental changes and utilizing various metabolic activities[9].

Isolating bacterial strains from polluted sites enhances bioremediation, as these strains are adapted to synthetic dyes and can be used alone or in consortia for efficient degradation. Using mineral salt medium (MSM) in biodegradation studies supports the growth of degradative bacteria like *Bacillus subtilis* and *Pseudomonas aeruginosa*, optimizing pollutant breakdown[10,11].

Factors like pH, temperature, and heavy metals affect dye degradation, making their understanding vital for optimizing bioremediation[12]. Immobilizing bacterial cells on a solid matrix improves stability, extends operational lifespan, and reduces costs. Immobilized cells are more resilient to environmental changes and offer sustained, efficient dye degradation over time[9].

Materials and Methods

Design of experiment: This research involved identifying dye degrading bacteria from the effluent sample collected from a dyeing unit in Cubbonpet Bengaluru, India, measuring

decolourization potential of Bacterial isolates, optimizing growth conditions and entrapment of bacterial cells in variant alginate supports.

Dye Chemical and culture media: Commercial-grade textiles dyes - Direct Green 2B, Direct Red-80 procured from Rathi Chemicals and Dyes. A common synthetic dye Methylene Blue of laboratory grade was used in the experiment. Other chemicals and solvents used for the experiment were of analytical grade. These were procured from HiMedia Laboratories Private Limited, Mumbai, Maharashtra, India.

Table-1: Composition of mineral salts medium.

Ingredients	Quantity (g/l)
KH_2PO_4	0.1%
K_2HPO_4	0.1%
$MgSO_4$	0.02%
$CaCl_2$	0.002%
NH_4Cl	0.1%
NH_4NO_3	0.1%
NaCl	0.1%
$FeCl_3$	0.005%
Glucose	0.4%
Yeast extract	0.4%
Dye	1mg/mL
agar	25g
Distilled water	100mL
pH	6.8

Collection of samples: Waste water Effluent from the textile dyeing unit (Cubbonpet, Bengaluru) was collected in a small container. The effluent sample was refrigerated at 4°C until use.

Isolation, screening and identification of dye degrading bacteria: 500 µl of Effluent was added to 25 ml of mineral salts medium with 1% of Glucose as a sole source of carbon along with the respective dyes were dispensed. Tubes were incubated for a period of 2 days at 37°C under static conditions. Centrifuged at 6000rpm for 6-8 mins and the cell pellet obtained was streaked on the mineral salts agar medium with the respective dye along with 10^{-6} and 10^{-7} serial dilutions, the distinct bacterial colonies from each pellet plate were selected streaked on fresh agar plates. A single colony was picked from the pure cultures of dyes and were maintained further on respective agar media with 1% dye. The isolates were identified by gram staining, biochemical tests according to Bergey's manual and molecular identification using 16s rRNA sequencing (Sanger Sequencing).

DNA extraction and PCR amplification of dye degrading bacteria: DNA was extracted using the Nucleospin Microbial DNA Kit, and its quality was assessed through 1% agarose gel electrophoresis. The gel was visualized with a UV Transilluminator (Himedia). The 16S rRNA gene fragment was amplified using 27_F and 1492_R primers, resulting in a single discrete PCR amplicon band when resolved on 1.2% agarose gel. This PCR amplicon was then purified to remove any contaminants. DNA sequencing reactions for both forward and reverse primers were conducted using the BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. The consensus sequence of the 16S rRNA gene was generated from the forward and reverse sequences using BioEdit software. The 16S rRNA gene sequence was subsequently used to perform a BLAST search against the NCBI GenBank database. Based on the highest identity scores and alignments using the Clustal W multiple alignment program, a distance matrix was generated, and a phylogenetic tree was constructed using MEGA 11 software.

Amplification of 16S rRNA: The 16S rRNA region was amplified by using primers (Forward and Reverse as detailed in Table-3). Amplified PCR products were visualized on 1.2% agarose gel.

Table-2: Details of polymerase chain reaction composition.

Component	Component Volume
GoTaq Green Master mix	25 µL
F primer	3 µL
R Primer	3 µL
Template DNA	6 µL
Nuclease-Free Water	13 µL
Total reaction volume	50 µL

Table-3: Primers used for 16S rRNA gene amplification and its sequencing.

Marker	Primer Name	Primer Sequences	T_m
16S rRNA	27_F	5' AGAGTTTGATCMTG GCTCAG 3'	56.28°C
	1492_R	5' GGTTACCTTGTTACG ACTT 3'	52.35°C

Sequencing: PCR products were processed for cleanup to remove unincorporated nucleotide and residual primers using Exonuclease-I and Shrimp Alkaline phosphatase enzyme followed by cycle sequencing reaction using BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.). For Cycle sequencing the same PCR primers were used. The thermal cycler conditions were an initial denaturation of 2 min at 960C and 35 cycles of 30 sec at 960C, 15 sec at 550C, and 4 min at 600C. Cycle sequencing is followed by sequencing cleanup by ethanol precipitation followed by dissolving template in HiDi formamide and bidirectionally sequenced in ABI 3730 Genetic analyzer.

Sequence alignment and assembly: PCR products were then processed for direct bi-directionally sequencing using ABI PRISM 3730 × 1 Genetic Analyzer (Applied Biosystems, USA). The resulting DNA sequences were aligned using CLUSTALW in MEGA 11, manually trimmed and edited to obtain complete sequences. The confirmation of species depends on the sequence similarity score. Homology searches were carried out using the BLASTn program against the NCBI GenBank database. ML tree was constructed using MEGA 11 with all positions containing gaps and missing data included for analysis. Clade supports were calculated based on 1,000 bootstrap resembling.

Decolourization activities: A loopful of bacterial inoculum from a pure culture plate was inoculated into 10 ml of mineral salts medium containing 1% dye and incubated at 37°C under static aerobic conditions, with pH maintained at 7.0 throughout. A control was prepared using mineral salts medium with the respective dyes but without bacterial culture. The culture suspensions were centrifuged at 8,000 rpm for 10 minutes at 4°C to remove biomass. The level of decolorization was assessed by measuring the absorbance of the culture supernatant using a UV-visible spectrophotometer. Percentage of degradation of Dye by the cultures was calculated using the formula:

Percent degradation =[Initial OD – Final OD)/Initial OD x 100]

Optimization of the Decolourization Process: To optimize the decolourization process, various factors, including carbon and nitrogen sources, pH, temperature, inorganic metal ions, surfactants, and dye concentration, were systematically tested.

Effect of Carbon Source: The impact of additional carbon sources on dye degradation was studied by adding glucose, sucrose, maltose, glycerol, and cellulose at a concentration of 1 mg/L to the mineral salts medium. Other parameters, such as pH (7.0), temperature (30°C), dye concentration (1%), culture inoculation, and static incubation conditions, were kept constant.

Effect of Nitrogen Source: The effect of various nitrogen sources on dye degradation was examined by adding yeast extract, urea, ammonium sulphate, peptone, and potassium nitrate at a concentration of 1 mg/L. All other conditions were maintained as mentioned earlier.

Effect of pH and Temperature: Degradation studies were conducted at different pH levels, ranging from 3 to 11 in intervals of 2. The tubes were incubated at varying temperatures, from 0°C to 40°C, under static conditions.

Effect of Inorganic Metal Ions: To investigate the effect of inorganic metal ions on dye degradation, salts of Fe, K, Na, Zn, and Co were added to the mineral salts medium at a concentration of 1 mg/L.

Effect of Surfactants: The impact of surfactants on dye degradation was studied by adding Tween-20, Tween-80, Cetyl Pyridinium Chloride, Triton X-100, and Sodium Dodecyl Sulphate at a concentration of 1 mg/L.

Effect of Dye Concentration: Degradation studies were also carried out by adding different dye concentrations, ranging from 50 µL to 250 µL in intervals of 50 µL, to the mineral salts medium.

Batch Degradation of Dyes Using Immobilized Cells: Ca-alginate entrapment of bacterial strains was performed following Bettman and Rehm (1984). A 3% w/v sodium alginate solution was sterilized by autoclaving at 121°C for 15 minutes. Fresh bacterial pellets (3% w/v) were mixed with the sterilized alginate solution and extruded into a cold, sterile 0.2 M calcium chloride solution, forming 2 mm diameter gel beads. The beads were hardened by agitation in fresh 0.2 M calcium chloride for 2 hours, washed with sterile distilled water, and stored in 0.2 M calcium chloride at 4°C. For gelatin and starch alginate beads, 4% sodium alginate was prepared. Gelatin 1 g /100mL and starch 1 g dissolved in 10 mL water (1g/100mL) was added to the alginate solution, followed by the bacterial culture (4%). The mixture was extruded, hardened, washed, and stored similarly, as mentioned above the procedure was modified[13].

Results and Discussion

Morphological features of isolated bacteria: The isolates MB3, DR1, and G1, which demonstrated enhanced decolourization potential, were cultured on mineral salts media. Isolate MB3 formed circular colonies with a smooth surface, DR1 exhibited mucoidal colonies with a round shape, and G1 produced flat colonies with irregular and slightly ruffled edges. All three isolates were confirmed to be Gram-negative.

Identification of Bacteria Using Sanger Sequencing: All strains were identified correctly up to the species level with Sanger Sequencing. MB3, DR1, and G1 isolates were identified as *Serratia marcescens* strainSCQ1, *Klebsiella pneumoniae* strain INF206-sc-2280074, *Pseudomonas aeruginosa* strain VITVLC-6.

Table-4: Results of Biochemical Test.

Biochemical Test	MB3	DR1	G1
Catalase	Positive	Positive	Positive
Oxidase	Negative	Negative	Positive
Indole Production	Negative	Negative	Negative
Methyl Red	Variable	Negative	Negative
Voges-Proskauer	Positive	Positive	Negative
Citrate Utilization	Positive	Positive	Positive
Lactose Fermentation	Negative	Positive	Negative
Glucose Fermentation	Positive	Positive	Positive
Gelatin Hydrolysis	Positive	Negative	Positive
Motility	Positive	Negative	Positive
Hydrogen Sulfide Production	Negative	Negative	Negative
Pigment Production	Red	None	Green

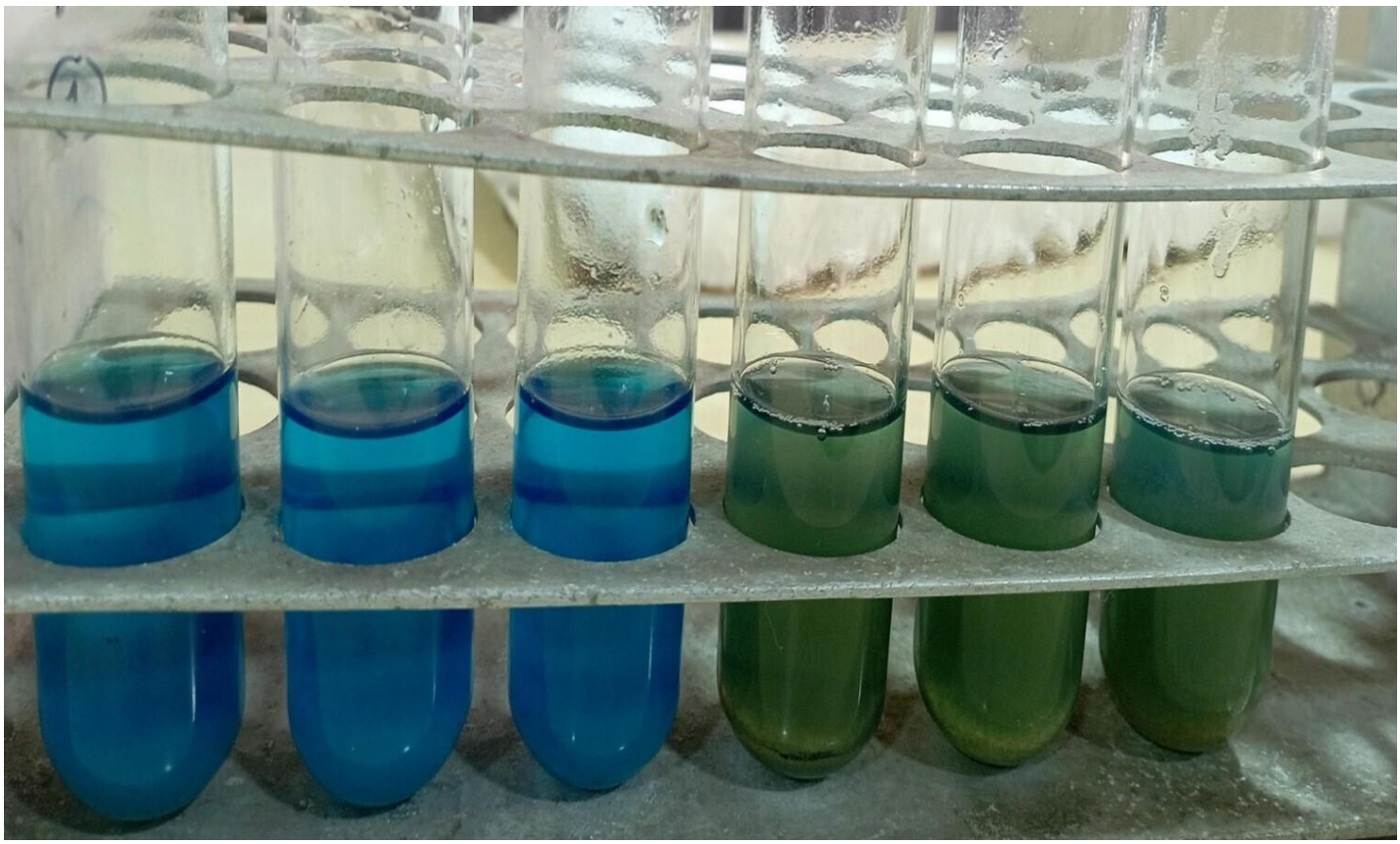

Figure-1: Screening of Bacterial Strains for Degradation of Methylene Blue and Direct Green 2B.

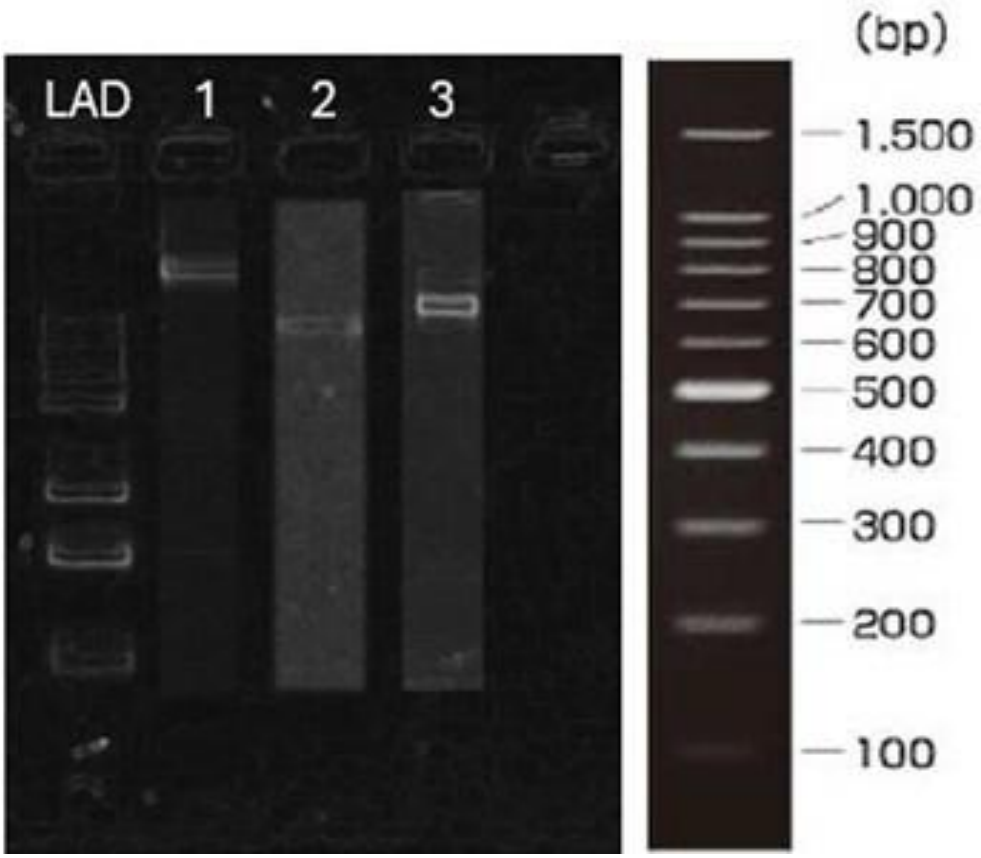

Figure-2: Gel Electrophoresis Using 1.2% agarose.

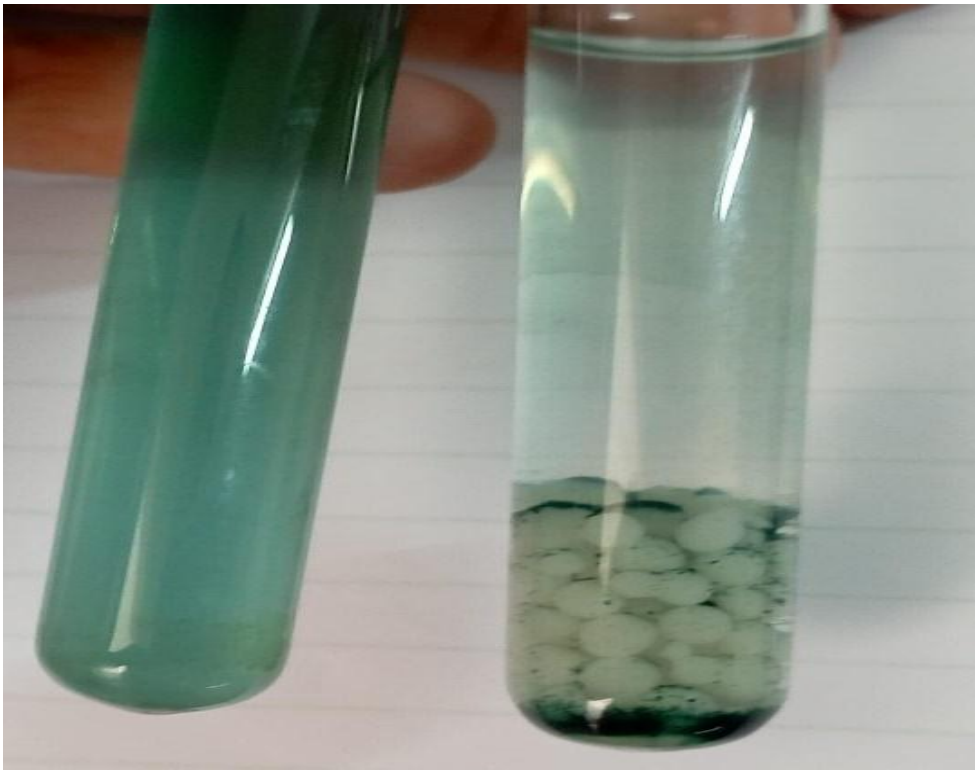

Figure-3: Degradation by G1 Immobilized in Starch Alginate.

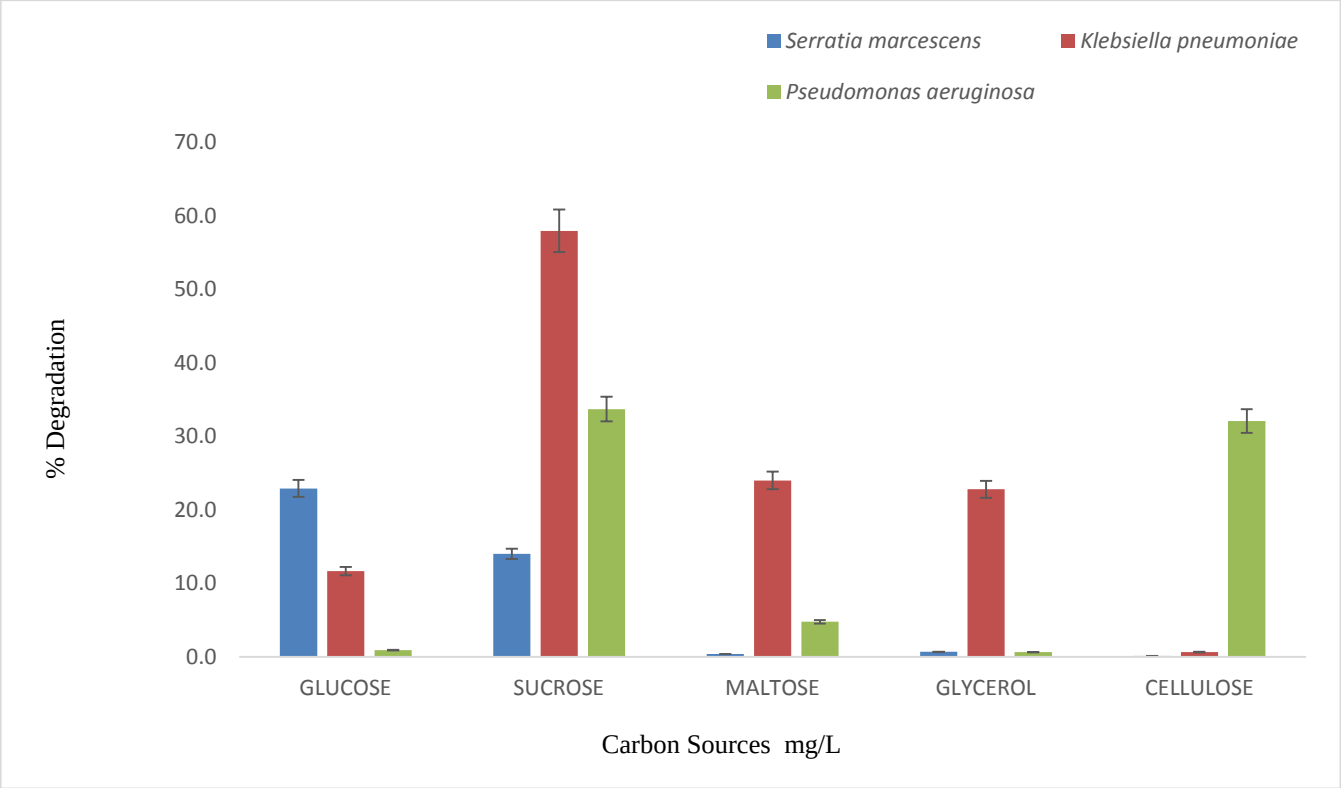

Figure-4: Effect of Different Carbon Sources.

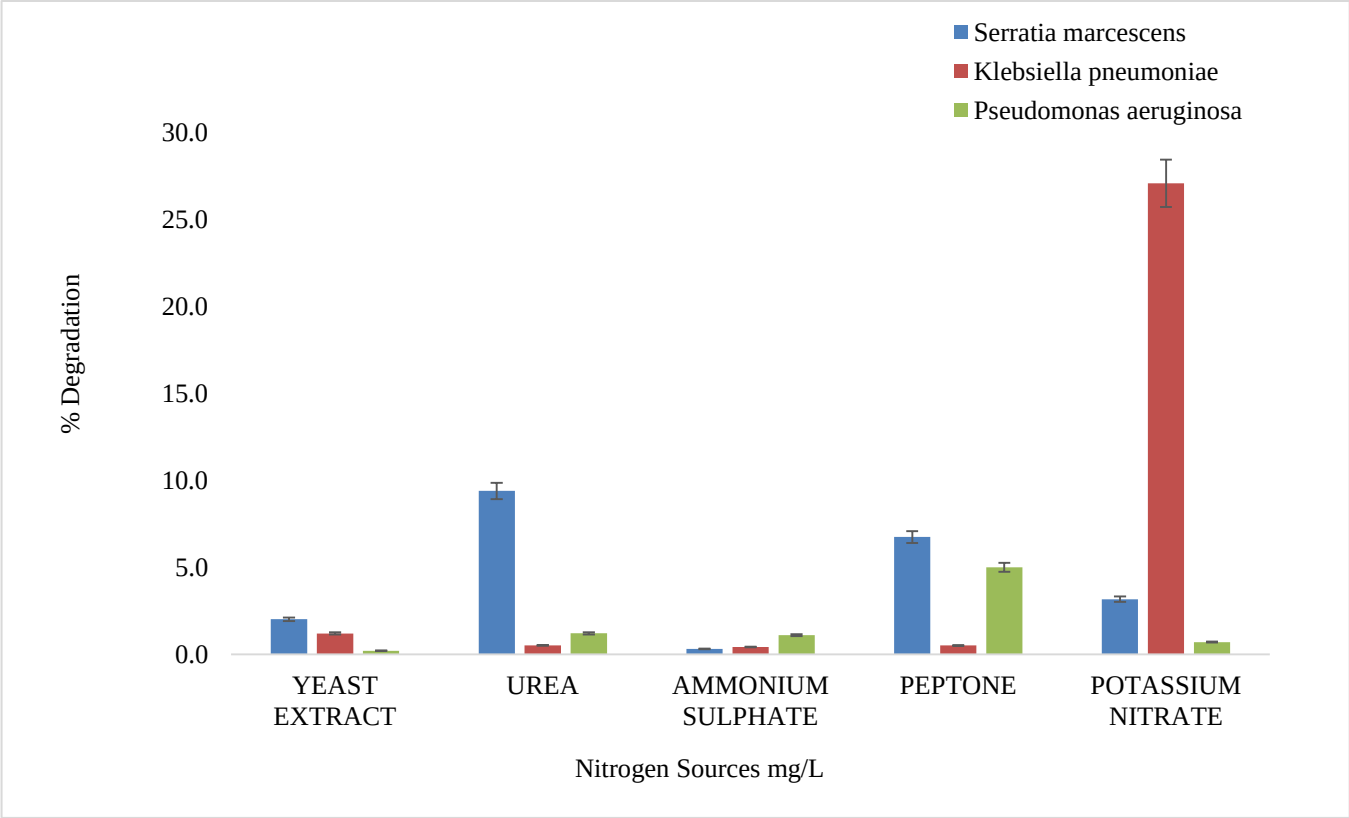

Figure-5: Effect of Different Nitrogen Sources.

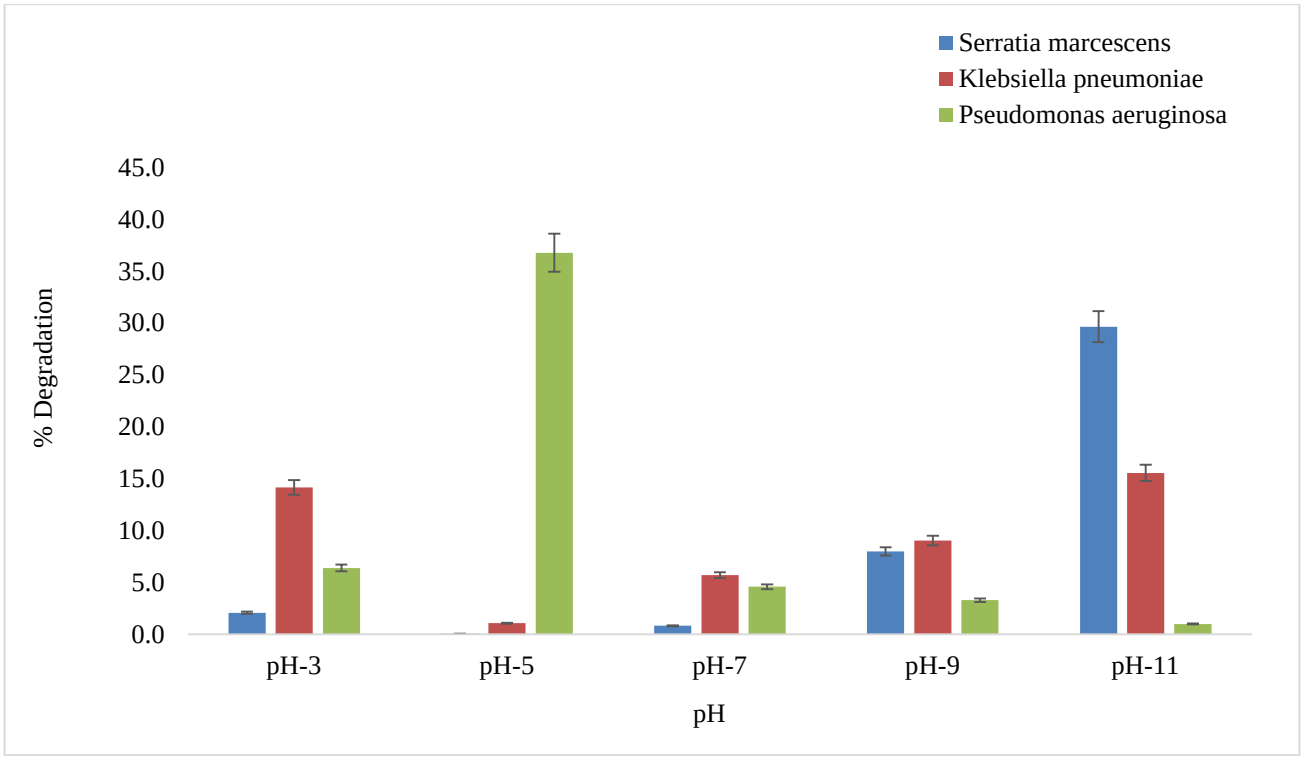

Figure-6: Effect of pH.

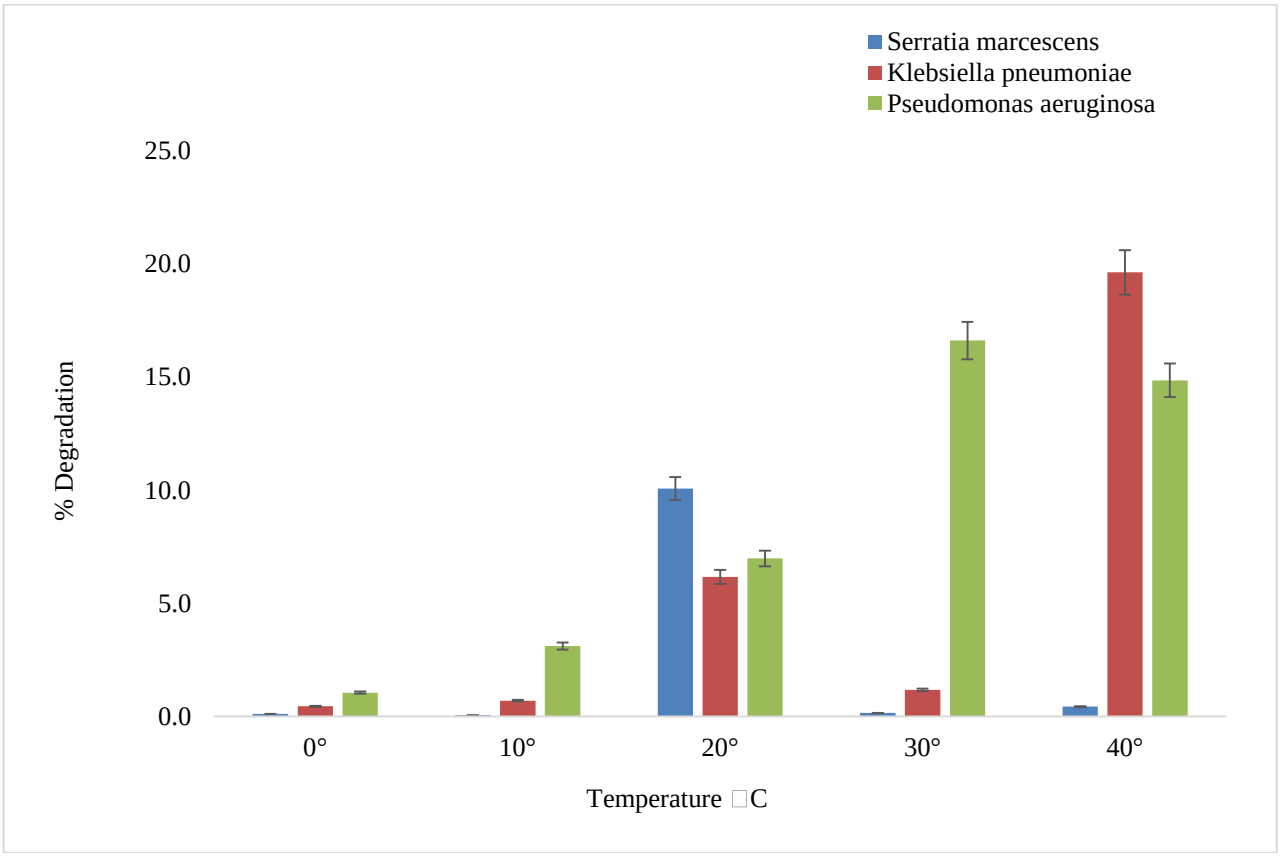

Figure-7: Effect of Temperature.

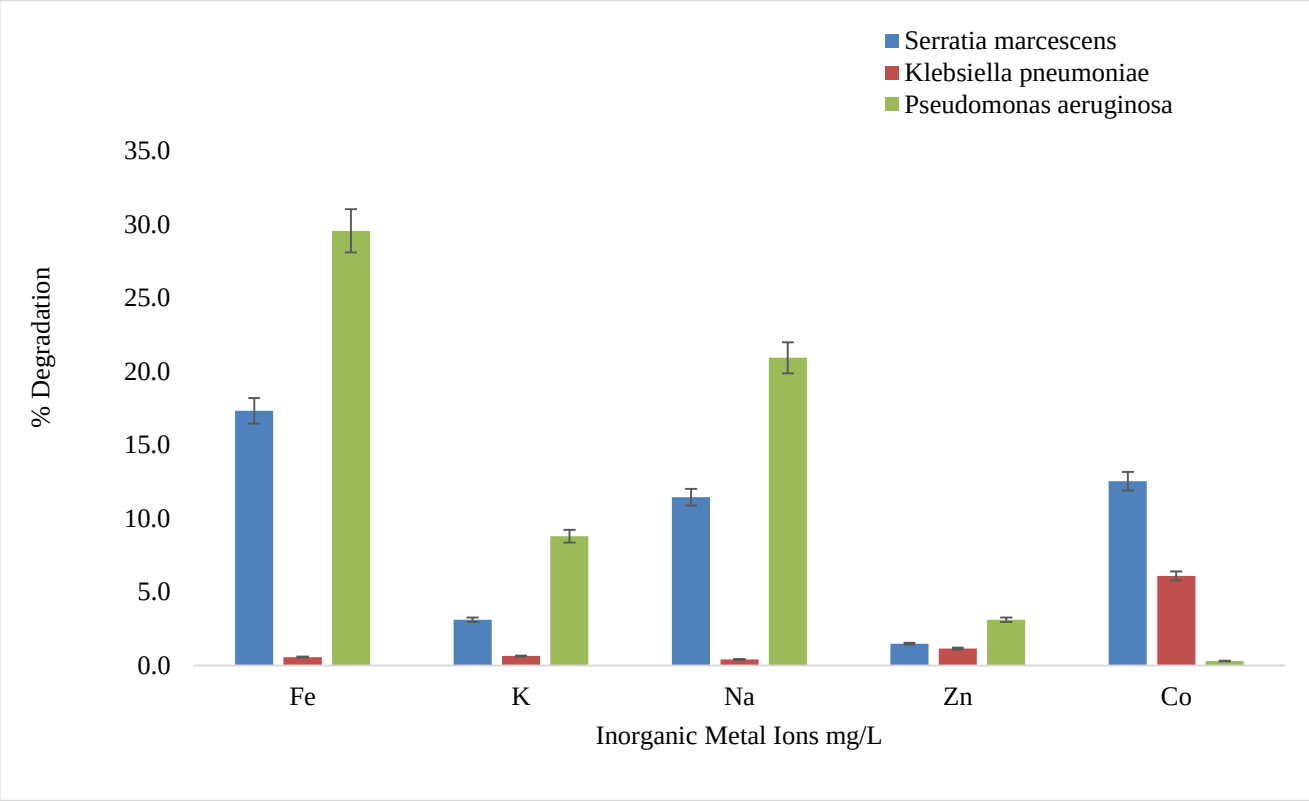

Figure-8: Effect of Inorganic Metal Ions.

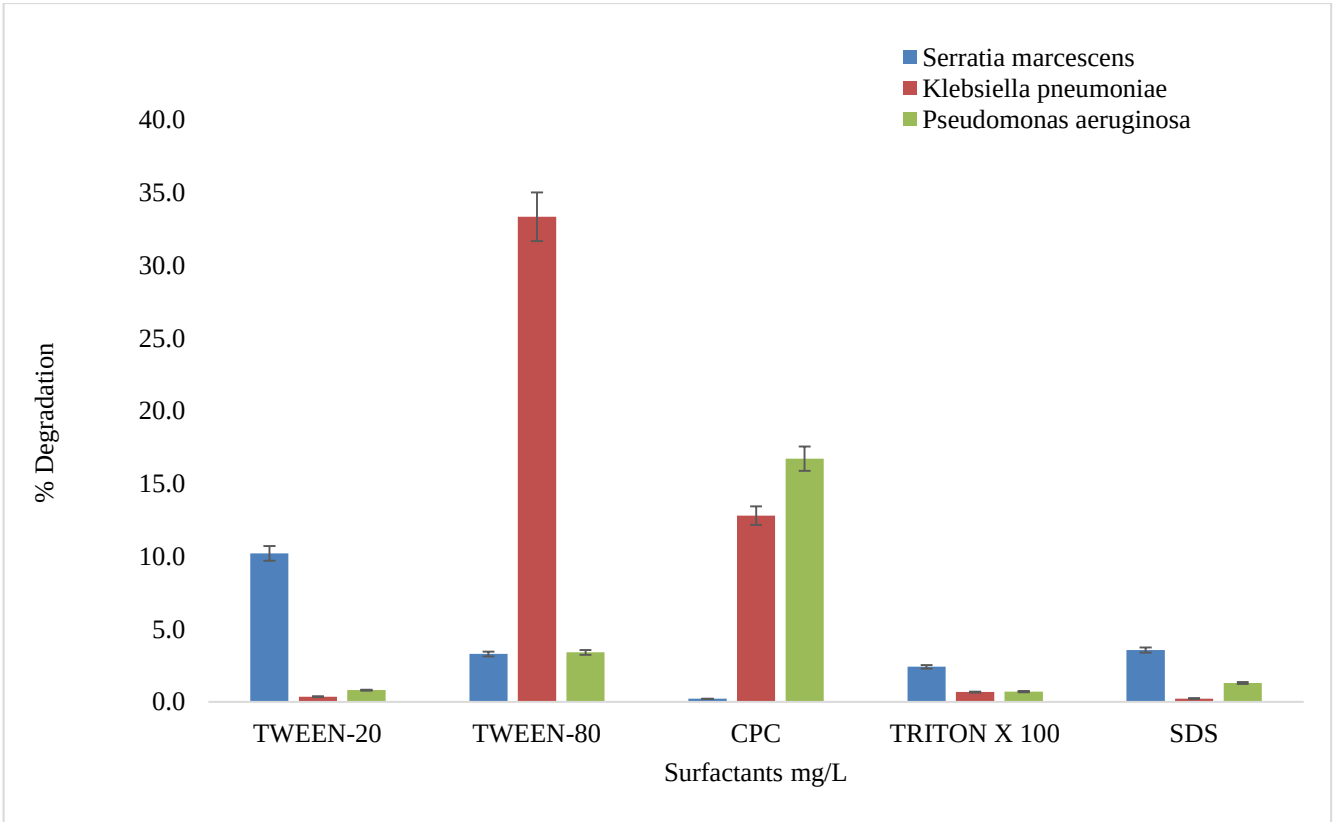

Figure-9: Effect of Surfactants.

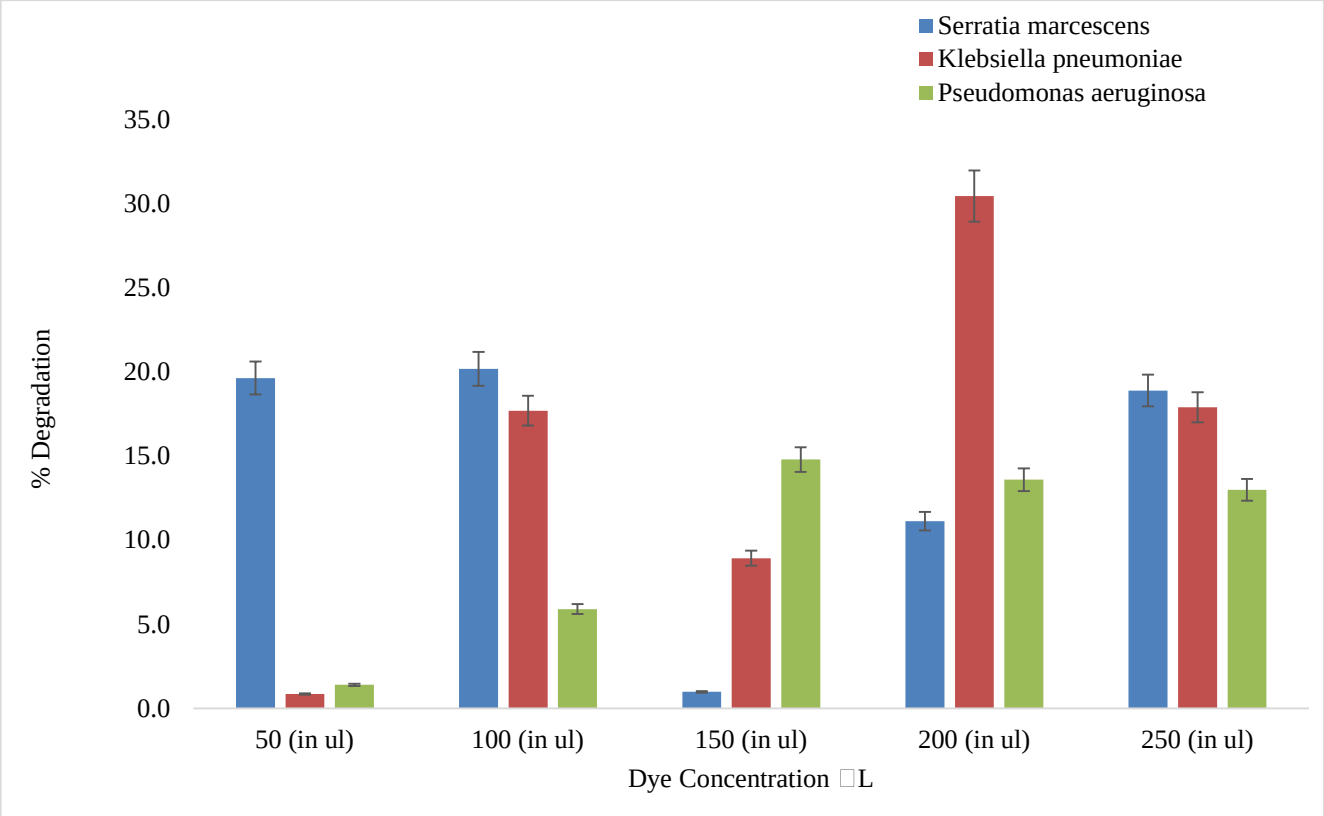

Figure-10: Effect of Dye Concentration.

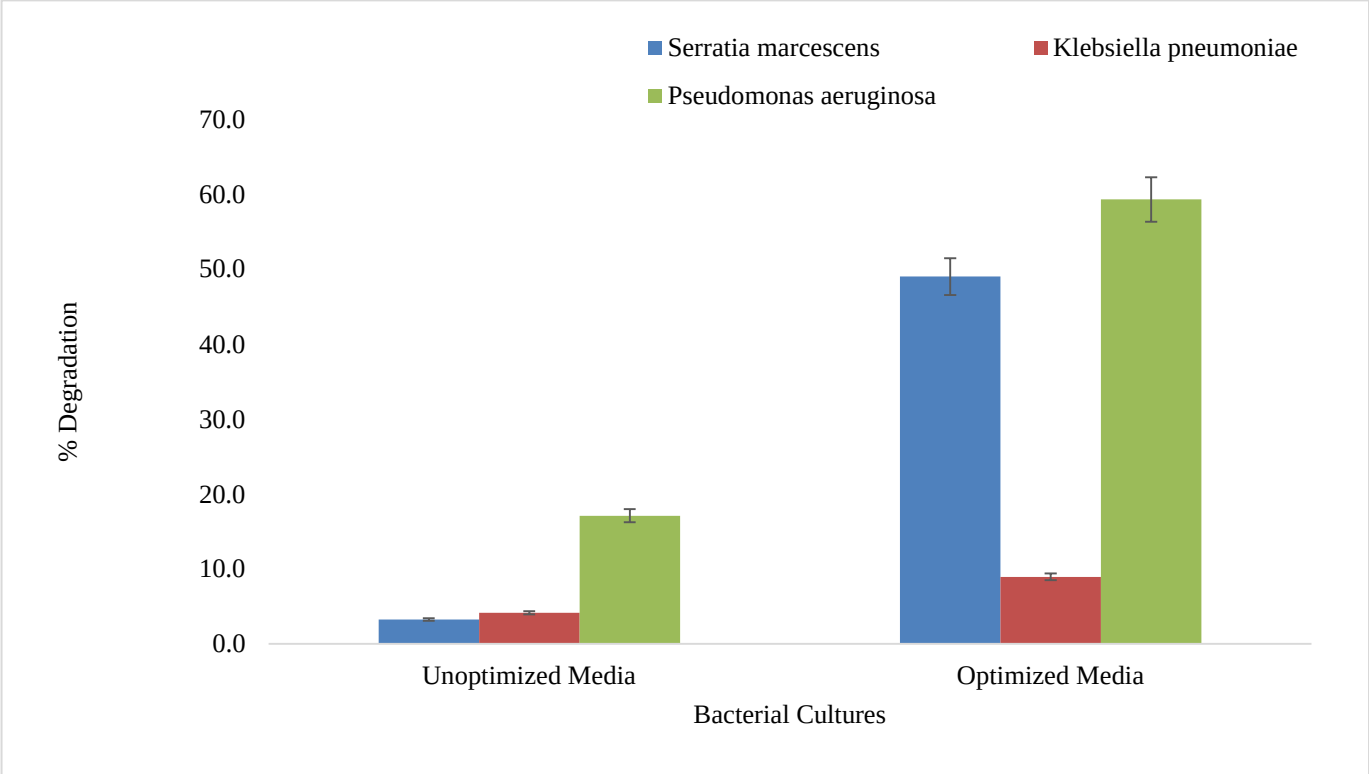

Figure-11: Effect of Unoptimized Media and Optimized Media.

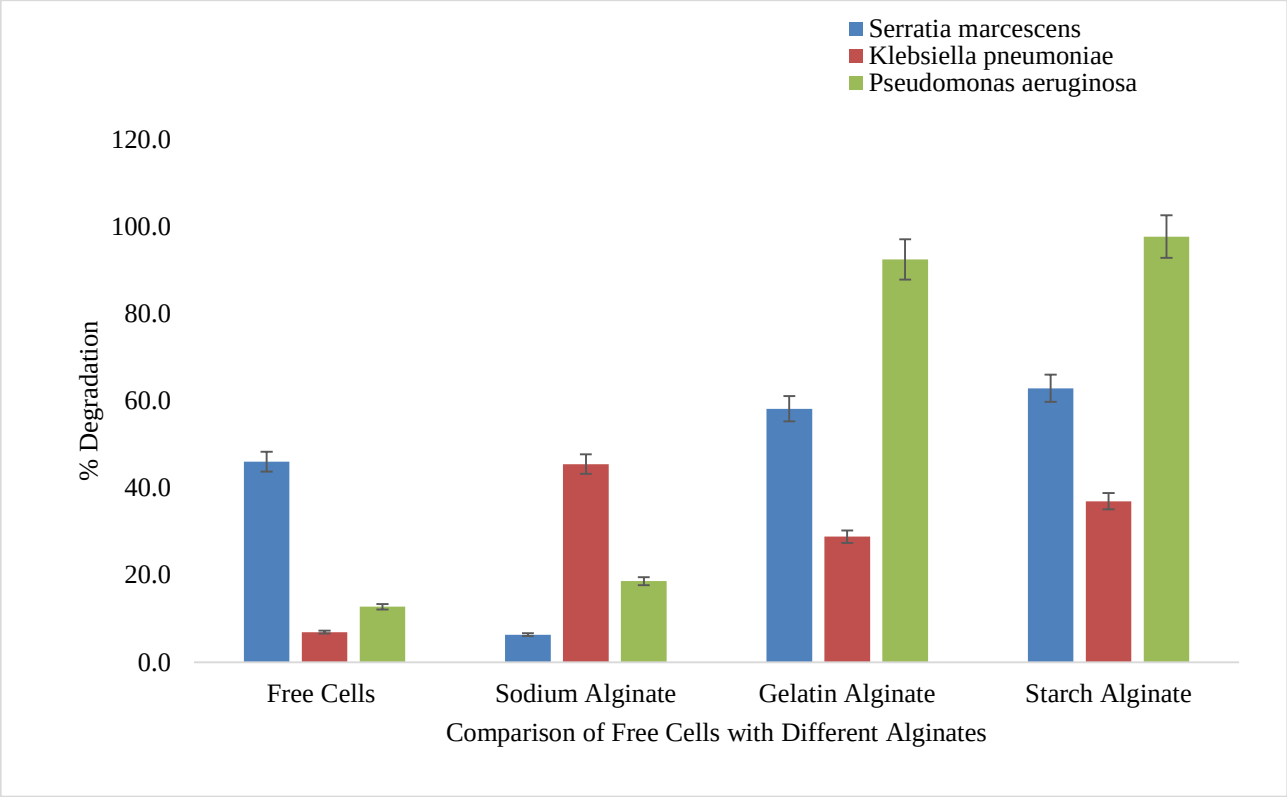

Figure-12: Comparison of Free Cells with Different Alginates.

Serratia marcescens was used to degrade Methylene Blue, Klebsiella pneumoniae for Direct Red-80, and Pseudomonas aeruginosafor Direct Green 2B.

Serratia marcescens showed the highest degradation with an additional carbon source, glucose, achieving 22.9%. Similarly, Klebsiella pneumoniae and Pseudomonas aeruginosa exhibited maximum degradation using sucrose as the carbon source, with 58% and 33.7%, respectively (Figure-4). In terms of additional nitrogen sources, the results were less significant. S. marcescens displayed 9.4% degradation with yeast extract, while K. pneumoniae achieved 27.1% degradation with potassium nitrate, and P. aeruginosa showed 5% degradation with peptone (Figure-5). S. marcescens and K. pneumoniae preferred a pH of 11, with degradations of 29.7% and 15.6%, respectively, while P. aeruginosa favored an acidic pH of 5, with a degradation rate of 36.8% (Figure-6). Temperature-wise, S. marcescens showed 10.1% degradation at 20°C, K. pneumoniae exhibited 19.6% degradation at 40°C, and P. aeruginosa displayed 16.6% degradation at 30°C (Figure-7). Among inorganic metal ions, iron (Fe) was preferred by both S. marcescens and P. aeruginosa, leading to degradations of 17.3% and 29.6%, respectively. K. pneumoniae, on the other hand, preferred cobalt (Co), resulting in a 6.1% degradation (Figure-8).

Surfactants also played a role in degradation, with Tween-20 influencing 10.2% degradation by S. marcescens, Tween-80 supporting 33.3% degradation by K. pneumoniae, and cetylpyridinium chloride (CPC) aiding in 16.7% degradation by P. aeruginosa (Figure-9). Optimal dye concentrations were found to be 100µl for S. marcescens, resulting in 20.2% degradation; 250µl for K. pneumoniae, with 17.9% degradation; and 150µl for P. aeruginosa, with 14.8% degradation (Figure-10).

When combining all the above-mentioned parameters to prepare an optimized media specific to each bacterial strain, an average increase of 35% in degradation was observed compared to unoptimized conditions (Figure-11). Immobilization further enhanced degradation, with S. marcescens achieving 62.9% degradation in starch-alginate beads, P. aeruginosa showing 97.7% degradation in the same starch-alginate beads, and K. pneumoniae recording 45.5% degradation in sodium alginate beads (Figure-12).

Conclusion

This study has successfully identified novel bacterial strains with exceptional dye degradation capabilities. The newly isolated Pseudomonas aeruginosa strain VITVLC-6 demonstrated maximum dye degradation rates, with 97.70% for Direct Green 2B in starch alginate beads and 92.49% in gelatin alginate beads, surpassing previously reported rates. Serratia marcescens strain SCQ1 also exhibited a 62.91% degradation of Methylene Blue, complementing previous findings on Serratia marcescens. Notably, the Klebsiella pneumonia strainINF206-

sc-2280074 demonstrated a 45.50% degradation of Direct Red, reinforcing the efficacy of this bacterium in dye decolorization as observed in other studies. The novel method of immobilization of bacterial cells, such as gelatin and starch alginate variants, was found to be effective in enhancing dye degradation as the results were recorded overnight it might be applicable in future applications of wastewater treatment. While the results are promising, further research is essential to adapt these techniques for industrial scale-up and to address the practical challenges associated with dye bioremediation. Future studies should focus on characterizing degradation metabolites using advanced techniques like HPLC and evaluating the reusability of immobilized cells through repeated batch degradation tests. These additional steps will help in ensuring the feasibility and safety of the bioremediation processes.

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References

1. Bishal, A., Ali, K., Ghosh, S., Parua, P., Bandyopadhyay, B., Mondal, S., Jana, M., Datta, A., Das, K. K., & Debnath, B. (2023). Natural dyes: Its origin, categories and application on textile fabrics in brief. *European Chemical Bulletin*, 12(8), 9780–9802.

2. Slama, H. B., Chenari Bouket, A., Pourhassan, Z., Alenezi, F. N., Silini, A., Cherif-Silini, H., ... & Belbahri, L. (2021). Diversity of synthetic dyes from textile industries, discharge impacts and treatment methods. *Applied Sciences*, 11(14), 6255.

3. Lin, J., Ye, W., Xie, M., Seo, D. H., Luo, J., Wan, Y., & Van der Bruggen, B. (2023). Environmental impacts and remediation of dye-containing wastewater. *Nature Reviews Earth & Environment*, 4(11), 785-803.

4. Katheresan, V., Kansedo, J., & Lau, S. Y. (2018). Efficiency of various recent wastewater dye removal methods: A review. *Journal of environmental chemical engineering*, 6(4), 4676-4697.

5. Crini, G., & Lichtfouse, E. (2019). Advantages and disadvantages of techniques used for wastewater treatment. *Environmental chemistry letters*, 17, 145-155.

6. Mohana, S., Acharya, B. K., & Madamwar, D. (2009). Distillery spent wash: treatment technologies and potential applications. *Journal of hazardous materials*, *163*(1), 12-25.

7. Jamee, R., & Siddique, R. (2019). Biodegradation of synthetic dyes of textile effluent by microorganisms: an environmentally and economically sustainable approach. *European journal of microbiology and immunology*, 9(4), 114-118.

8. Patel, Y., Chhaya, U., Rudakiya, D. M., & Joshi, S. (2021). Biological decolorization and degradation of synthetic dyes: a green step toward sustainable environment. *Microbial Rejuvenation of Polluted Environment*, 2, 77-110.

9. Kurade, M. B., Waghmode, T. R., Chakankar, M., Awasthi, M. K., Dawkar, V., & Jeon, B. H. (2023). Microbial consortium as an effective biocatalyst for the decolorization of textile dyes wastewater. In *Current Developments in Bioengineering and Biotechnology* (pp. 473-511). Elsevier.

10. Aline, S. D. B. (2018). Formulation of culture media using fish scale bioconversion. JESES, 4(1), 534-540.

11. Zhang, X., Zhang, D., Chu, S., Khalid, M., Wang, R., Chi, Y., ... & Zhou, P. (2023). Employing salt-tolerant bacteria Serratia marcescens subsp. SLS for biodegradation of oily kitchen waste. *Chemosphere*, 329, 138655.

12. Varjani, S., Rakholiya, P., Ng, H. Y., You, S., & Teixeira, J. A. (2020). Microbial degradation of dyes: An overview. *Bioresource Technology*, 314, 123728.

13. Kurade, M. B. (2023). Microbial consortium as an effective biocatalyst for the decolorization of textile dyes wastewater. In *Current Developments in Bioengineering and Biotechnology* (pp. 473-511). Elsevier. doi:https://doi.org/10.1016/B978-0-323-91235-8.00007-3

14. Susilowati, P. E., Rajiani, N. A., Hermawan, H., Zaeni, A., & Sudiana, I. N. (2019). The use Immobilized Bacteria-Alginate-Chitin for crack remediation. *IOP Conference Series: Earth and Environmental Science*, 299(1), 012010.