

Azo Dye Degradation By Actinobacteria

Project Report

*Submitted in partial fulfillment of the requirements for the award of Bachelor of Science
in Biotechnology*

By

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade “A++” by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Sriman Karunakaran Raveendran (41770034) who carried out the project entitled "Azo Dye Degradation By Actinobacteria" under our supervision from 20th September 2023 to 28th March 2024.

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Abstract

Azo dyes are widely prevalent in commercial coloring due to their strong retention provided by their chemical properties. Their environmental impacts include genetic defects, hormonal imbalance, and growth distortion. This impact is further bolstered by the equally impactful chemical removal process, due to which a natural mostly microbial removal method is required. In this study, actinobacteria are chosen due to their known environmental resilience, diverse enzymatic repertoire, and cost-effectiveness. Six Soil isolates were obtained from the historical textile production hubs Chromepet and Kanchipuram regions using the serial dilution method and inoculation onto different media plates AIA (Actinobacteria Isolated Agar) and SCA (Starch Casein Agar) media. Six potential strains were streaked onto ISP-2 media plates for preservation, and the strains were treated with specific azo dyes (Turquoise Blue, and Reactive Yellow) at 1000 ppm. Four strains showed high tolerance in azo dye presence. Further study would be conducted using ISP-2 media broth with varying azo dye concentrations (50 ppm, 100 ppm, 500 ppm, and 1000 ppm) with emphasis on specific azo dye-bacterial relationships. The degree of azo dye decolorization was conducted through 9-day spectrophotometer analysis. Optimization strategies will be implemented for enhanced yield, degradative efficacy, and growth time by media modification and physical and chemical factors configuration such as pH and temperature.

Key words: Actinobacteria, Azo dye, Turquoise Blue, LCMS, Reactive Yellow

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List of Abbreviation

Abbreviation	Full Form
CO	Chromepet Outskirts Strain
PPM	Parts Per Million
ISP2	International Streptomyces Project 2
AIA	Actinobacteria Isolation Agar
SCA	Starch Casein Agar
L	Liter (used with a typo as "10 ml")
rpm	Revolutions Per Minute
TB	Turquoise Blue
RY	Reactive Yellow
Ao	Initial Absorbance
At	Absorbance After Time t
ISP	International Streptomyces Project (used with numbers to specify different media)
KNO ₃	Potassium Nitrate
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
HCL	Hydrochloric Acid
HPLC	High-Performance Liquid Chromatography
AMC	Arial Mycelium Pigment
RSP	Reverse Substrate Pigment
NA	Nutrient Agar
PCR	Polymerase Chain Reaction
BSA	Bovine Serum Albumin
BLAST	Basic Local Alignment Search Tool
MEGA	Molecular Evolutionary Genetics Analysis

Chapter - 1 Introduction

Throughout human history, humans have been extensively portraying the artistic tendencies of the human mind, through ever-expanding artistic pieces including but not limited to architecture, engineering, science, art etc. These artistic tendencies also included intricate textiles and painting which required dyes of quality that resisted biological degradation and could resist constant sunlight. These worries were put to bed when the first azo dyes were manufactured by C. Mene in 1861, it was a yellow dye we refer to as Aniline Yellow present as an orange powder it was produced by a coupling reaction between benzene diazonium chloride and aniline in an acidic medium, which had no real industrial significance due to it being an acid sensitive dye.

The method of creating azo dyes is intricate and starts with the diazotization of an aromatic primary amine, which is an essential stage in producing diazonium salt. Azo dyes are created when this salt is combined with a substrate that is typically aromatic. It is possible to produce symmetrical and asymmetric azo dyes using this approach, which gives designers of molecules more creative freedom. Furthermore, during the synthesis process, several chemical groups, such as hydrazone, chromene, and derivatives of 2-aminothiophene and 2-aminothiazoles, can be added to the dyes. These varied chemical functions provide azo dyes with their vast spectrum of hues and characteristics, which makes them adaptable substances for use in paints, textiles, and plastics, among other uses. (Benkhaya et al., 2020)

Azo dyes are industrially used as colouring agents mostly in leather, paints, photographs, cosmetics, and most commonly textiles where it currently dominates nearly 70% of the dye market (Nikfar & Jaberidoost, 2014),(Benkhaya et al., 2020). This domination and ever presence in human life is dangerous medically and environmentally speaking, as its production is fraught with numerous problems such as during the dye production approximately 10 to 15% of the total dye used by the industries are lost during the dyeing process and, thus, are released into the environment (Nam & Renganathan, 2000).

The industrial favorites, azo dyes are overused due to their low biodegradability, stability under light and long history of production for textile production, but medically speaking, its recalcitrant nature along with its perceived toxic, carcinogenic and mutagenic nature has pushed research to produce efficient manners of dye removal from the environment (de Campos Ventura-Camargo & Aparecida Marin-Morales, 2013).

Examples of azo dye-caused disorders are hepatocarcinogenicity in male mice when given as little as 0.027 to 0.15 $\mu\text{mole/g}$ body weight [8] and the frequent presence of bladder tumour in azo dye workers compared to the general society (Sudiana et al., 2022). From the environmental perspective, it has been demonstrated that the xenobiotic and recalcitrant properties of azo dyes have a negative effect on animal and human health, leading to serious diseases. As a matter of fact, azo dyes are regarded as organic compounds that can cause cancer and mutation.

The health of humans and animals is not considerably affected by short-term exposure to azo dyes. On the other hand, prolonged exposure to harmful dyes causes these substances to bioaccumulate on the bodies of humans and other animals, which can result in several fatal diseases. Azo dyes usually enter the bodies of humans and animals through contact with contaminated surfaces. Azo dyes bioaccumulate over time in contaminated environments through inhalation or oral exposure. From an environmental standpoint, contaminated agricultural produce, aquatic biota, and the air all expose people to azo dyes (Ali et al., 2023).

It was also identified by the Central Pollution Control Board in 2012 that it is one of the 17 most polluting industries in India, categorized under the "red category" by the Ministry of Environment, Forest and Climate Change (MoEFCC). It has also been observed that the discharge of textile wastewater into natural water bodies can impact water clarity, the solubility of gases, and photosynthesis, and lead to the formation of carcinogenic and mutagenic aromatic amines (Bafana et al., 2011).

All these clear examples point to the underlying medical and environmental issue at hand which raises the need for an efficient and cheap method for the removal of these diazotized aromatic amines from the environment. Methods regarding the removal of azo dyes have evolved through the years, ranging from physicochemical methods such as using activated carbon, it has been observed to possess unique physicochemical properties that allow it to be a useful adsorbent for removing azo dye and not its advantages aren't limited to just absorption but also economically speaking, activated carbon requires less preparation than other adsorbents (Ramesh et al., 2017). In another case involving physiochemical methods a novel macro-architectures material Fe₃O₄-N-GO-sodium alginate (SA) gel film was produced, which when allowed to perform series azo dye removal from wastewater, achieved maxima removal efficiency of 74.22%, 45.72%, 37.75% for Congo Red, Acid Orange 7 and Amino Black 10B respectively (Chen et al., 2021). In this study azo dye removal was done through a highly reusable TiO₂ nanoparticle photocatalyst immobilized on steel mesh via PVDF coating, electro spraying, and thermal fixation with results showing total discolouration, total organic carbon removal at 22% with a pH of 6.8-7. (Ramasundaram et al., 2016)

Biological methods have included fungal such as in (Sudiana et al., 2022) where turquoise blue dye went through biodegradation using locally isolated fungi from a plantation area, the results showing. The study analyzed that at a concentration of 30 mg/L in an aqueous solution, the turquoise blue textile dye's maximum colour removal effectiveness was attained at 78.50% and 85.84% at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum*, while the colour removal efficiency was 82.17% at pH 5 for *Trametes hirsuta* with seven days of incubation time.

Bacterial methods where pure bacterial strains are used, for example *Thiosphaera pantotropha* ATCC 35512 and *Pseudomonas aeruginosa* (RS1) exposed to the nutritional broth at a static concentration where it successfully fully decolourized at an concentration 50 mg/L. The researchers evaluated the efficiency of these bacterial

strains in degrading and eliminating RY145 from the solution by methodical observation and analysis. (Garg et al., 2020)

Algal treatment has been also extensively researched, in a specific study the azo dye was treated with *C. vulgaris* or *O. tenuis* under sterile conditions, the aniline was quantified by the HPLC system showed that the Algal action caused 100% loss of aniline and no other organic compounds were detected indicating that the selected algae can degrade aniline effectively and turn it into some simple inorganic material. (Jinqi & Houtian, n.d.)

This study mainly focuses on bacterial azo dye degradation, the bacterial strain being used is an Actinobacteria, and the dyes being used are Turquoise Blue and Reactive Yellow. Among the dyes, turquoise blue dye, a well-known reactive dye, is often used in the dyeing process and produces a dark blue color in wastewater effluent (Sudiana et al., 2022). Using the monochlorotriazine group as the reactive site and copper-phthalocyanine as the chromophore, one of the most popular commercial reactive dyes produces a turquoise-blue colour (Osugi et al., 2003).

According to the study, at a concentration of 30 mg/L in an aqueous solution, the best colour removal effectiveness for turquoise blue textile dye was obtained at 78.50% and 85.84% at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum*, respectively. Furthermore, at pH 5, *Trametes hirsuta* demonstrated an 82.17% colour removal effectiveness. These results imply that these wood-degrading fungi may be involved in environmentally benign processes of textile colour degradation. (Sudiana et al., 2022)

Like other reactive dyes, reactive yellow reacts with fibers to create covalent links that create a permanent bond that cannot be broken down by repeatedly treating the fibers with boiling water in a neutral environment. Chemically, they are α -bromoacrylamido derivatives based on azo, desulphonated bases. (Naebe et al., 2010) In another study dealing with reactive yellow 145 biodegradation *Pseudomonas aeruginosa* (RS1) and

Thiosphaera pantotropha ATCC 35512 completed the decolorization of 50 mg/L RY145 in 96 and 72 hours, respectively, when grown in static circumstances. Without an external carbon supply, the acclimatized cultures demonstrated 54% decolourization in mineral media. (Garg et al., 2020)

Actinobacteria are Gram-positive bacteria with a GC-rich linear genome and the substantial metabolic capacity to create secondary metabolites with a wide range of structural variety and commercial significance. Most of this phylum's members have a complex mycelial lifecycle and generate aerial and substrate mycelium with distinctive colouring and sporulation. Actinobacteria have been extensively studied because of their biotechnological significance, and this research has demonstrated that they can be discovered throughout both terrestrial and aquatic environments, with a range of sources. Actinobacteria can be discovered in an astonishing array of ecosystems, comprising freshwater and marine environments, soils, plants, and animals. (Jose et al., 2021)

People's health and well-being have greatly benefited from microbes all throughout the world. They can produce a wide range of secondary metabolites, which make up half of the medications sold today and supply agriculture with numerous necessary goods, in addition to the numerous primary metabolites they produce, such as amino acids, vitamins, and nucleotides. Over a million natural chemicals have been found thanks to technological advancements in screening programs, separation, and isolation processes. About 20-25% of all natural products that have been identified exhibit biological action, and of these, 10% come from microorganisms. Moreover, actinomycetes produce 45% of the 22,500 physiologically active chemicals that have been isolated from microbes thus far, followed by fungi (38%), and unicellular bacteria (17%). (Demain & Sanchez, 2009)

The study looks at the potential microbial removal of commercial colouring azo dyes by actinobacteria as well as the environmental impact of these colours. Four of the six soil isolates from textile industry centers showed great tolerance, with CO-18 in particular successfully absorbing the azo dyes. The other six isolates were evaluated

for tolerance to two specific azo dyes, Reactive Yellow and Turquoise Blue. Through the use of different dye concentrations, spectrophotometer measurement, and optimization techniques for improved degradation and growth, future studies will examine particular azo dye-bacterial connections. Advanced methods such as 16s rRNA, FTIR, and LCMS will also be used to identify strains and post-degradation metabolites.

Chapter - 2 Review of Literature

2.1 Introduction

Azo dyes comprise the majority of textile dyes used in the industry, often accompanied by gallons of discharge onto portable water bodies. The structure of these synthetic compounds is based on the cyclic ring with an - N = N - bond, with the desired colours and properties associated through slight variations in the structure such as different azo groups. These recalcitrant synthetic azo compounds have a considerable negative effect on the water bodies of our world, therefore many methods. Physical methods range from absorption, coagulation, flocculation, and filtration to ion exchange. Physical methods are commonly considered the most efficient dye removal method due to their impressive removal percentages, having around 86.8 to 90%. Chemical methods have included electrochemical treatment, oxidation and photochemical treatments. The greatest percentage of dye removal was found in chemical dye removal procedures, which ranged from 88.8 to 99%. Nevertheless, chemical dye removal techniques are frequently plagued by intolerable drawbacks such as particular machinery and calls for a lot of electrical power. Reactors or additional machinery used for chemical dye removal require a lot of electricity to run. In addition, users of chemical colour removal procedures frequently identify large-scale chemical and reagent use as a problem. The creation of harmful secondary pollution after the chemical colour removal process, which presents an extra disposal issue, is another undesirable characteristic of this procedure. The main reasons biological approaches are preferred for dye removal are their low cost and ease of usage. Biological dye removal techniques involve the use of live organisms in their operations. Engineering ethics should be upheld and this method applied with caution. With removal rates ranging from 76 to 90.1%, biological dye removal techniques offer a respectable removal rate. When combined with their associated low costs, biological removal techniques are a strong competitor. (Katheresan et al., 2018).

1. Physical

1.1 Absorption

The goal of the study was to remove methylene blue (MB) from water by employing a modified gum arabic, polyacrylamide, and polyacrylate superabsorbent hydrogel (SH). The SH performed exceptionally well in MB absorption, removing 98% of the dye per gram of SH, or 48 mg of dye, which was the maximum absorption capacity recorded. Initial MB concentration was 50 mg L⁻¹, hydrogel mass was 50 mg, and pH was 8 during the experiment. But when the hydrogel came into contact with a single orange II solution, an interesting thing happened: the hydrogel absorbed water, which made the solution richer in orange II, rather than the dye. This unexpected outcome suggested distinct absorption pathways by demonstrating the hydrogel's opposing effect on orange II in comparison to MB. Subsequent testing showed that the SH could only absorb MB in an aqueous solution when orange II was also present. Orange II, on the other hand, behaved differently. It used an inverted mechanism in which the SH absorbed water from the dye selectively, increasing the concentration of orange II in the solution by over 50 times above its starting concentration. The research suggested that the ionic combination formed between the imine groups of MB and the ionized carboxylic groups of SH was responsible for this unusual behavior, underscoring the intricate and specific absorption mechanisms involved in dye removal procedures utilizing superabsorbent hydrogels. (Paulino et al., 2006)

Using corn-cob activated carbon, the study explored the complex dynamics of pH influence and mass dose effects on dye adsorption, providing subtle insights into the underlying mechanisms. A distinct pattern surfaced over a pH range of 2 to 10 at a constant temperature of 30°C: higher pH levels were associated with increased dye absorption efficiency and process overall efficiency, as indicated by greater dye absorption rates and improved efficiency percentages. Interestingly, a pH neutral of 7 was shown to have a peak in adsorption capacity, highlighting the significance of pH neutrality for the best possible adsorption results. Conversely, a phenomenon of reduced adsorption was seen at lower pH values, especially in the acidic range. It was

proposed that this decrease resulted from increased H^+ ion competition for binding sites on the adsorbent surface, which in turn limited the number of sites available for dye molecules to attach and hence decreased total adsorption rates.

An interaction between dose and response wherein increasing adsorbent mass led to higher dye removal rates due to increased surface area and accessible adsorption sites. the effect of different dosages of corn-cob adsorbent on dye adsorption. Under continuous dye concentration settings, a saturation effect was noted at higher dosages, indicating a complex interaction of components such as aggregation. The utilization of H_2SO_4 -activated corn cob activated carbon, which was examined using a variety of isotherm models to comprehend multi-layered dye adsorption mechanisms, was a key component of the research. Thermodynamic studies demonstrated how the adsorption mechanism absorbs heat. This thorough investigation not only adds to our knowledge of adsorption processes but also provides useful information for improving wastewater treatment methods. (Aljeboree and Alkaim, 2019)

1.2 Filtration

The goal of the current work is to investigate the unique characteristics of nanofibrous filtration membranes made of keratin and polyamide blends for the treatment of dye-containing wastewater. Goat hair, a tannery waste, was used to extract keratin protein using the sulphitolysis technique. Using the electrospinning method, the retrieved keratin was combined with polyamide to create a nanofibrous membrane. The characteristics of the synthesized pristine polyamide and keratin-modified polyamide membranes were assessed and contrasted.

The effects of dye concentration, membrane flow, membrane capacity, and solution pH have all been studied. The membrane achieves greater filtration efficiencies and enhanced flux due to the presence of very fine nanofibers and increased porosity. The blend membranes' dye removal efficacy at pH 2 was 100, 99, 98, 90, and 83% for dye concentrations of 100, 200, 250, 300, and 400 ppm, respectively. In every way, the keratin-polyamide blend membrane demonstrated superior qualities. The current

study's findings suggest that keratin in filter membranes holds promise for eliminating colouring from wastewater.(David et al., 2020)

One of the biggest textile cottage industries in Southeast Asia is thought to be the batik sector. Wax is used in batik production as a physical barrier, and the dying, fixing, and washing processes use a lot of water. This results in the production of hazardous wastewater that contains wax, dye, and other auxiliaries. To achieve regulatory emission levels, an effective wastewater treatment process is considered essential. Reactive Blue 15, reactive red 194, reactive yellow 145, reactive black 5, and reactive orange 16 are the five distinct fibre reactive dye wastewater samples for which the use of polyamide nano-membrane dye removal was assessed in this work. The dyes under discussion were tested for 60 minutes of filtration at a low concentration of 16 mg/l. Based on analytical studies conducted both before and after the process, the filtering efficiency was computed. Every sample had a flux that varied from 7.8 to 9.2 ml/cm² s. It was found that the samples' permeate pH values increased somewhat, falling between 6.4 and 7.1. When comparing the sample dye solution from the feed to the permeate sample during the filtration cycles, conductivity measurements for the samples showed a sharp decline. The permeate samples' chemical oxygen demand value was lowered to zero, indicating a dye removal effectiveness of more than 90% and successfully fulfilling the environmental legal requirement.(Rashidi et al., 2015)

Improved water flux, high hydrophilicity, and anti-biofouling behaviour are just a few of the beneficial characteristics of polymeric nanocomposite membranes, which take the whole package to improve membrane performance, flexibility, affordability, and separation efficiency. With a focus on textile dye removal, phase-inversion technology was used in this study to produce PVDF membranes containing aminated graphene oxide (NH₂-GO). These membranes showed remarkable water flux, up to about 170.2 (J/L.h-1.m-2), and a remarkable BSA rejection rate of 98.2%. They also demonstrated significant dye removal capability, removing approximately 88.5% of methyl orange and 96.6% of methylene blue. This demonstrated the effectiveness of aminated graphene oxide-based PVDF membranes in improving membrane

performance for dye removal applications. Using aminated graphene oxide in polyvinylidene fluoride (PVDF) membranes is a potentially effective way to create membranes with better performance attributes. These membranes provide notable increases in water flux, dye removal efficiency, and protein rejection rates by taking advantage of the special qualities of NH₂-GO, such as its improved adsorption capabilities and surface chemistry. This highlights the possibility of improving membrane performance and resolving issues in dye removal procedures in diverse industrial and environmental situations using aminated graphene oxide-based PVDF membranes as a material choice.(Ahmad et al., 2022)

1.3 Coagulation and Flocculation

To decolorize Crystal Ponceau 6R (AR 44) in wastewater, this study investigates the potential of two natural organic polymers (NOPs): *Brachystegia eurycoma* coagulant (BEC) and *Vigna subterranean* coagulant (VSC). The study conducted precursor materials characterization studies and employed a thorough kinetics analysis. The decolorization processes were assessed by measuring the decline in particle concentration over time while varying the independent parameters. The proximate analysis revealed that BEC and VSC had protein levels of 19.77% and 18.15%, respectively. The functional test detected -OH, N-H, and C=H functional groups. Surface morphology analysis revealed various compact-net architectures, varying pore widths, and rough surfaces. VSC exhibited a higher removal efficiency than BEC, with optimal values of 88.8% and 73.3%, respectively. The coagulation order (α) and coagulation rate constant (K) for BEC and VSC were determined to be $6.38 \times 10^{-4} \text{ L mg}^{-1} \text{ min}^{-1}$, 1.8 and $4.03 \times 10^{-3} \text{ L mg}^{-1} \text{ min}^{-1}$, 1.9, respectively. The coagulation times, T_{ag} , were found to be 31.35 and 26.96 min for BEC and VSC, respectively, indicating rapid coagulation. The coagulation-flocculation kinetics, with $R^2 > 0.997$, showed that the process fits the pseudo-second-order model, suggesting that chemisorption governs the rate-controlling mechanism. The cross-validation test accurately predicted the experimental data in the mass transfer investigation, with a percentage mean relative deviation modulus (M%) of 2.54 for VSC and 3.26 for BEC.

These coagulants demonstrated notable adsorption characteristics and contributed significantly to advancing wastewater treatment via coagulation-flocculation. Similar modeling and kinetics research on particle behavior are essential in water treatment procedures.(Obiora-Okafo et al., 2022)

In order to remove color from a disperse dye solution, the potential of aluminum-based water treatment residuals (WTR) discharged from water treatment plants as a coagulant was assessed. We looked at how color removal was affected by the WTR dose, initial dye concentration, and initial pH. According to the findings, color loss increased at lower pH levels. At pH 3.0 and a WTR dose of 3,000 mg/L, maximum color reductions of 88, 87, and 76% were achieved for starting dye concentrations of 25, 50, and 75 mg/L, respectively. Various artificial neural networks (ANNs) were created in order to forecast the loss of color. The models demonstrated excellent performance, with correlation coefficient (R^2) values over 0.90. The outcomes demonstrated that the non-linear behavior of the system is incorporated into the simulation using ANN, and there was a strong correlation between the observed and anticipated values of color removals. According to the study, it would be desirable to reuse water treatment sludge as a coagulant for color removal.(Gadekar & Ahammed, 2016)

Two groups of dye solutions prepared in a laboratory were tested for their color removal efficiency using coagulation; one group included dyes with identical chromophores and auxochromes, while the other group included dyes with either different auxochromes and chromophores. In order to obtain enough data to predict and model the color removal efficiency, a number of coagulants, including polyaluminum chloride (PAC), cationic polyacrylamide (CPAM), anionic polyacrylamide (APAM), ferric chloride (FeCl_3), aluminum chloride (AlCl_3), and magnesium chloride (MgCl_2), were chosen to cover a wide range of experimental variations. The best-performing coagulants for each dye group—disperse, acid, reactive, and basic—were used to further explore the dye removal mechanism by coagulation and determine the impact of coagulant dosage on color removal efficiency.

The results demonstrated that, for each dye group with the same structures and functional groups, the best dye-coagulant combinations were, in descending order, disperse dye-MgCl₂ at a pH of 12, acid dye-MgCl₂ at a pH of 12, reactive dye-PAC at a pH of 7.5, and basic dye-APAM at a pH of 8.3. Dye types with the same auxochromes and charges showed extremely comparable color removal efficiency when treated with the same coagulant. It was demonstrated that the coagulation impact was significantly influenced by the condition of the dye molecules in the water. The experimental findings showed that the dye structures had an effect on the coagulation's efficacy in removing dye color.(Mcyotto et al., 2021)

2. Chemical Methods

2.1 Nanoparticles

TiO₂ nanoparticles were used to create a highly reusable photocatalyst that breaks down organic contaminants in water. A three-step process was used to immobilize the particles on steel mesh (SM): dip-coating the SM (2.5 cm × 5.0 cm) to form a poly(vinylidene fluoride) (PVDF) binder interface; electrospraying TiO₂ nanoparticles dispersed in methanol (Degussa P25, 1 mg/mL); and thermally fixing them at 100 MPa for increased mechanical stability. As the electrospray volumes varied from 10 to 60 mL, the TiO₂ loading on both sides of the PVDF-coated SM rose from 0.20 to 1.60 mg. Under UV light, the SM sample loaded with 1.10 mg TiO₂ (SM-TiO₂) had the best photocatalytic oxidation of methylene blue (MB), demonstrating consistent performance over 20 tests. It's interesting to note that SM-TiO₂ thermally fixed at 160°C exhibited greater photocatalytic efficacy than those fixed at 180°C and 200°C. This is because, at higher temperatures, the melted PVDF resin layer entraps and inactivates TiO₂ nanoparticles. With rate constants ranging from 0.0164 to 0.0725 min⁻¹, the improved SM-TiO₂ showed good performance on a variety of organic pollutants, including MB, methyl orange, reactive blue 4, sulfamethoxazole, and microcystin-LR. The importance of careful fabrication techniques and thermal treatment in boosting the photocatalytic activity of TiO₂-based nanocomposites for

water purification applications is highlighted in this work. The results of this study aid in the creation of effective and long-lasting photocatalysts to tackle environmental issues related to the pollution of water systems by organic pollutants.(Ramasundaram et al., 2016)

The study examined the adsorption of turquoise blue dye onto activated carbon from an aqueous solution using microwave assistance and zinc chloride. *Delonix regia* pods were used to make activated carbons. The carbon that was created was known as microwave-assisted zinc chloride-activated carbon, or MWZAC. BET analysis, XRD, FTIR, and pHZPC were used to determine the properties of the MWZAC. Adsorption tests in batch mode were conducted. Adsorption was investigated in many parameters, including temperature, pH of the solution, agitation time, initial dye concentration, and dose of the adsorbent. The system's kinetics were examined using linearized versions of the Webber-Morris, Ho, and Lagergren models. Langmuir, Freundlich, T2mpkin, and Dubinin-Raduskevich isotherms were used to fit equilibrium data. Based on the R² value, the order of best describing isotherms was determined. Using Vant Hoff plots, thermodynamic parameters including ΔH° , ΔS° , and ΔG° were found. 0.2 M HCl, 0.2 M NaOH, and distilled water were used in desorption procedures to replenish the exhausted MWZAC.(Ramesh et al., 2017)

Wastewater containing series azo dyes was successfully removed by a novel macro-architectures material Fe₃O₄-N-GO-sodium alginate (SA) gel film which was successfully created. As long as the mass ratio of Fe₃O₄-N-GO to sodium alginate was 0.11, the ideal adsorption rates were reached, leading to the maximum removal efficiency of 74.22%, 45.72%, and 37.75% for Congo Red, Acid Orange 7, and Amino Black 10B, respectively. For three dyes, the ideal adsorption temperature was 30 °C, and the adsorption equilibrium was attained in 150 minutes. For the three azo dyes, the adsorption kinetic model of Fe₃O₄-N-GO-SA matched the quasi-second-order reaction model, and the adsorption isotherm more closely resembled the Freundlich adsorption. Chemisorption was the primary mechanism for modulating the speed in the multi-layer heterogeneous adsorption process, which was mediated by both

physical and chemical adsorption. The research would offer a theoretical foundation for the environmental application of materials related to macro-architectures.(Chen et al., 2021)

2.2 Electrochemical Treatment

This study examined the effectiveness of the electro-coagulation-flotation (EC-F) procedure for removing the double azo bond dye Acid Red 66 (AR66) by employing waste metal scraps of Al and Fe that were collected from the construction and demolition trash of the Indian Institute of Technology Madras (IIT M) campus. At pH 7, the primary working parameters, namely current density and electrical conductivity, were individually tuned, starting with a dye concentration of 50 mg/L. Various electrode configurations and connection types (series (MP-S, BP-S), parallel MP-P)) were examined under pre-optimized settings to maximize AR66 dye removal while minimizing energy usage. A better COD reduction was seen with a series connection in bipolar electrode mode (BP-S), going from 164 mg/L to 26.2 mg/L with full decolourization (BDL). The hybrid electrode system of Fe-Al-Fe-Al-Fe-Al demonstrated full decolourization, 86.3% TSS reduction, and a maximum decrease of COD from 164 mg/L to 11.3 mg/L. The degradation of AR66 dye during electrolysis resulted in the creation of intermediates with m/z 195, m/z 210.6, and m/z 159.3, according to LC-MS analysis. When comparing the hybrid electrode system to the Al ($\varphi = 30.1\%$) and Fe ($\varphi = 98.3\%$) electrode systems under comparable operating conditions, the hybrid electrode system showed the highest current efficiency (CE $\varphi = 107\%$). By using the right hybrid electrode combination, removal efficiency can be increased and energy consumption can be decreased as compared to using the same electrode material for the cathode and anode. It also investigated how aeration affected the system's performance. Aeration greatly increased both full decolourization (100%) and COD removal efficiency (98.3%). By using waste metal scrap as electrodes, the treatment process's overall cost was lowered from 1.6 \$/m³ to 0.06 \$/m³. In addition to lowering environmental metal accumulation, using discarded metal scrap as electrodes lowers the cost of the EC-F process.(Nippatla & Philip, 2020)

The research was done using an aluminium electrode to investigate the electrochemical (EC) breakdown mechanism of basic green 4 (BG), also referred to as malachite green, a triphenylmethane dye. Over 85% of BG degradation was seen within 50 minutes of treatment at the ideal conditions (current density= 117.64 A m^{-2} , starting dye concentration= 125 mg L^{-1} , pH=6.5, electrode gap=1 cm, and NaCl concentration= 1.5 g L^{-1}). Analysis using high-performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GCMS), Fourier transform infrared (FTIR), UV-visible, and high-resolution mass spectroscopy (HRMS) revealed that the degradation happened via the cleavage of conjugated structure and N-de-methylation. The intermediate products found included N, N, N', N'-tetramethyl-4,4'-diaminobenzophenone; 4, 4'-bis-aminobenzophenone; N-methyl-para-aminophenol following breakage of the triphenylmethane ring; and hydroxymethylated intermediates during the N-de-methylation of the dye. Zeta potential analysis revealed a hard acid-base interaction between the hydroxide produced in situ during the EC treatment process and the $-\text{N}(\text{CH}_3)_2$ group of the dye molecule, as well as aluminium ions. During the EC treatment process, it was seen that active species such as hydrogen peroxide, ozone, and chlorinated oxidizing compounds were generated; additionally, it was observed that the BG degradation was caused by an attack by the $\bullet\text{OH}$ radical. (Singh et al., 2013)

The transport of organic compounds in the soil from the cathode to the anode reservoir was due to the electromigration phenomenon. Toxicity tests were performed to evaluate the reuse of the soil after remediation, then, the germination of sunflower seeds was carried out, achieving a significant percentage of germination in central soil positions (65% and 92%). Finally, the effluent generated by ER was treated with BDD-electrolysis, obtaining complete discolouration after 80 min and a quasi-complete elimination of organic matter (more than 95%) after 120 min due to the contribution of persulfate ($\text{S}_2\text{O}_8^{2-}$) electrochemically generated at BDD anode. The process of electromigration was responsible for the movement of organic compounds in the soil from the cathode to the anode reservoir. After conducting toxicity tests to assess the soil's suitability for reuse following remediation, sunflower seed germination was

conducted, with notable percentages of germination (65% and 92%) in the centre soil sites. Ultimately, BDD-electrolysis was used to treat the ER effluent, resulting in total discolouration after 80 minutes and a near-complete removal of organic matter (more than 95%) after 120 minutes. This was attributed to the production of persulfate ($S_2O_8^{2-}$), which was produced electrochemically at the BDD anode. (de Paiva et al., 2018)

2.3 Photochemical Treatment

The following circumstances were used for the primary analysis: (i) dye solution self-photolysis with UV light; (ii) dye solution with H_2O_2 in the dark; and (iii) dye solution with H_2O_2 and UV light. Since the energy of 365 nm was insufficient to break down the dye molecule, the dye turned out to be resistant to direct UV-photolysis. In the dark, 8.55% decolourization and 3.0% degradation were noted for the dye solution containing H_2O_2 . Nevertheless, in just 150 minutes, the combined effects of UV and H_2O_2 resulted in 88.68% decolourization and 59.85% deterioration. In the UV- H_2O_2 process, the hydroxyl radicals produced by photolysis of hydrogen peroxide react to degrade the dye [Eqs. (1)-(3)]: $h\nu \rightarrow :OH + :OH$ (1) $:OH + \text{dye} \rightarrow \text{dye intermediate}$ (2) $:OH + \text{dye intermediate} \rightarrow CO_2 + H_2O + \text{mineralization products}$ (3). The dye molecule breaks down due to the potent oxidizing effect of the hydroxyl radical. (Muruganandham, 2004)

UV irradiation has been used to study the photochemical and photocatalytic degradation of aqueous solutions of Solophenyl Green (SG) BLE 155%, an azo dye preparation that is highly persistent in heavily coloured textile fluids. For modest initial dye concentrations, the pure photochemical technique proved to be quite effective. Commercial titanium dioxide was used for photocatalytic breakdown at higher concentrations, and titanium dioxide mixes with various activated carbons (AC) suspended in the solution were also used. It was discovered that a first-order rate rule described the degradation kinetics of photocatalytic dyestuffs. It was shown that the activated carbon's presence improved the titanium dioxide catalyst's photo efficiency.

The apparent first-order rate constant of the process increased differently in response to different activated carbon compounds. The adsorption equilibrium constants (KC) and KC values for the organic molecules and reaction rate constants, respectively, were $1.58 \text{ mg l}^{-1} \text{ min}^{-1}$ and 0.0923 l mg^{-1} for the TiO_2/UV process and $2.64 \text{ mg l}^{-1} \text{ min}^{-1}$ and 0.0928 l mg^{-1} for the $\text{TiO}_2 + \text{AC}/\text{UV}$ system with the highest synergy factor. (da Silva & Faria, 2003)

Using the $\text{H}_2\text{O}_2/\text{UV-C}$ and $\text{TiO}_2/\text{UV-A}$ advanced oxidation systems, respectively, aqueous solutions of hydrolyzed Reactive Black 5 (RB5) dye, a well-known stand-in for non-biodegradable azo dyes, were photochemically and photocatalytically treated. The observed kinetics of $\text{OH}^\bullet$ -induced colour loss were empirically explained by taking into account the reaction pH, oxidant dose, and initial dyestuff concentration. As a result, after 120 minutes of advanced oxidation at the designated optimized reaction conditions, the waste dyestuff solutions could be fully decoloured ($k_d, \text{max} = 0.155 \text{ min}^{-1}$; $\text{H}_2\text{O}_2/\text{UV-C}$ system) and effectively mineralized ($k_{\text{TOC}}, \text{max} = 0.01 \text{ min}^{-1}$; $\text{H}_2\text{O}_2/\text{UV-C}$ system), with an average overall TOC removal of 78%. Rather than being controlled by direct UV-C or UV-A photolysis, the $\text{H}_2\text{O}_2/\text{UV-C}$ treatment was primarily controlled by $\text{OH}^\bullet$ type oxidation. (Alaton & Balcioglu, 2001)

3. Biological Methods

3.1 Bacterial Treatment

UV-vis analyses and colourless bacterial cells suggested that *Citrobacter* sp. CK3 exhibited decolorizing activity through biodegradation, rather than inactive surface adsorption. It is the first time that a bacterial strain of *Citrobacter* sp. has been reported with decolorizing ability against both azo and anthraquinone dyes. High decolourization extent and facile conditions show the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents. In contrast to inert surface adsorption, *Citrobacter* sp. CK3 appeared to demonstrate decolorizing activity through

biodegradation, according to UV-vis studies and colorless bacterial cells. This is the first time that a *Citrobacter* sp. bacterial strain that can decolorize both azo and anthraquinone dyes has been documented. This bacterial strain has the potential to be employed in the biological treatment of dyeing mill effluents due to its high decolorization extent and easy conditions.(Wang et al., 2009)

In the resting cell experiment, the soil bacteria isolated in this study were three actinobacteria strains and one *Paraburkholderia* sp, which exhibited the decolourization activity of azo dyes and demonstrated the usage of methyl red as their exclusive carbon source for proliferation. Thus, utilizing brilliant black as a substrate, their capacity to bioabsorb, degrade, or a combination of both methods was examined. After a full day of incubation, the strains DP-A9 and DP-L11 showed total biodegradation of 173.54 mg/L brilliant black, whereas the strains DP-D10 and DP-P12 displayed partial decolourization of 83.3 mg/L and 36.4 mg/L, respectively, by a combination of biosorption and biodegradation. Furthermore, a highly diverse set of genes encoding potential dye-degrading enzymes was revealed in the shotgun-assembled genome of these strains, opening up new research opportunities to examine azo dye metabolism in greater depth. This thorough investigation emphasizes the significance that these soil bacterial strains can play in sustainable environmental management techniques by highlighting their potential for bioremediation of azo dye-contaminated environments.(Kumaran et al., 2022)

The goal of the current work is to assess the capacity of the established microbial consortium SDS to degrade Red HE3B using two bacterial cultures, *Pseudomonas aeruginosa* strain BCH (PA) and *Providencia* sp. SDS (PS), which were initially isolated from soil contaminated with dye. When it came to the decolorization and degradation of Red HE3B, the consortium outperformed the individual bacterial strains significantly. In just one-hour, Red HE3B (50 mg l⁻¹) underwent 100% decolourization due to the high metabolic activity of these bacteria. Significant increases in the levels of several dye-decolorizing enzymes, including DCIP reductase, laccase, azoreductase, and veratryl alcohol oxidase, as compared to the control, indicate that

these enzymes are involved in the decolourization and degradation process as a whole. GC-MS, FTIR, and HPLC analyses were performed to examine the biodegradation process in detail. Utilizing *Allium cepa* root cells, toxicological investigations were conducted both before and after microbial treatment about cytotoxicity, genotoxicity, oxidative stress, antioxidant enzyme status, protein oxidation, and lipid peroxidation study. The results of the *A. cepa* toxicity analysis show that the dye Red HE3B causes oxidative stress, which in turn has harmful effects on the root cells. In contrast, the dye's breakdown products are comparatively less toxic. Additionally, investigations on phytotoxicity showed that microbial treatment promotes Red HE3B detoxification.(Phugare et al., 2011)

3.2 Fungal Treatment

The agaric white-rot fungus *Clitocybula dusenii* partially decoloured raw mixed-dye wastewater from a textile dye mill. When the fungus was cultivated with dye effluent, its Mn peroxidase (MnP) and laccase activity were higher than those of control cultures. Using a four-fold diluted dye-containing effluent on a five-day pre-grown mycelium, maximum decolourization rates were attained over 20 days at 28°C, with the MnP activity increasing correspondingly with the concentration of raw dye wastewater in the medium. As demonstrated by notable changes in absorbance values across the spectrum, the application of *C. dusenii* led to the decolourization of an actual effluent that contained a complex mixture of azo and anthraquinonic dyes, indicating dye breakdown as opposed to a physical adsorption phenomenon. In ideal circumstances, after 20 days of growth, up to 87% of the dyes in the effluent were decolourized, according to measured absorbance obtained from integrated spectrum scans (400-700 nm). Other fungi have also been shown in earlier studies to successfully degrade dyes. For instance, an artificial dye-containing effluent was broken down by *B. adusta* and *P. tremellosa* to the extent of 85% and 79%, respectively. The presence of spots in the treated effluent indicated decreased

residual colour and the absence of further by-products with benzene ring structures, which was further validated by thin-layer chromatography of culture supernatants. These findings imply that *C. dusenii* can decolourize and degrade actual industrial effluents that contain textile dyes, hence lowering the toxicity and pollutant charge connected to these colours.(Wesenberg et al., 2002)

With an emphasis on colours, heavy metals, and aromatic amines, the study examined the capacity of the ascomycete fungus *N. pironii* to proliferate and maintain metabolic activity in the presence of waste from the dye industry. At tested concentrations, the fungus effectively removed o-toluidine and other colours, with an approximately 90% decolourization rate for azo dyes, according to the research. Furthermore, the decolourization process was marginally affected by the presence of metals, although the fungus maintained significant levels of decolourization. Given its high tolerance to dangerous substances and effective removal skills, the study demonstrated the potential of using *N. pironii* as a tool for removing toxic contaminants from the textile sector. The study also looked at *N. pironii* growth in dye industry waste leachate, and the results showed that the presence of leachate significantly increased fungal biomass. With 10% leachate added, the biomass content almost doubled, although the biosynthetic processes were slowed down by greater leachate concentrations. The fungus was shown to be capable of growing in all tested concentrations and removing o-tolidine from the growth medium at different speeds. This allowed the investigation to also look at the elimination of o-tolidine. These results highlight *N. pironii*'s potential to be an important part of wastewater treatment systems that remove dangerous chemicals from the textile industry, aiding in efforts to clean up the environment.(Góralczyk-Bińkowska et al., 2021)

Using a strong fungal consortium consisting of *Aspergillus niger* SAR-6, *Penicillium oxalicum* SAR-3, and *Aspergillus flavus* SAB-3, the study investigated the degradation of azo dyes. The group demonstrated remarkable effectiveness in the degradation of different azo dyes, such as Acid Red 183, Direct Blue 15, and Direct Red 75, attaining almost total decolourization at reduced starting concentrations between 200 and 400

mg/L. After 168 hours, SAR-3 had the highest manganese peroxidase activity (367.4 UL-1), followed by SAR-6 (240.6 UL-1) and SAB-3 (211.5 UL-1) in the enzymatic activity analysis. The strains' identities and compatibility within the consortium were confirmed by morphological and molecular identification, which also showed consistent growth patterns over seven days—a prerequisite for effective and prolonged dye degradation processes. Additionally, the efficacy of the consortium was demonstrated in simulated textile wastewater, exhibiting a noteworthy decolorization from 100 mg/L (92%) to 900 mg/L (36%). The toxicity investigation demonstrated a significant reduction in the metabolites resulting from dye degradation, which was especially noticeable at lower dye concentrations (200–400 mg/L). The consortium's superiority in terms of dye decolourization and toxicity reduction was highlighted by a comparative comparison with other research, establishing it as a potential and environmentally benign alternative for treating wastewater contaminated with azo dyes from the textile sector.(Saroj et al., 2015)

3.3 Algal Treatment

This study aims to investigate how the decolourization capacities of green algae, cyanobacteria, and diatoms are impacted by the differences in molecular structures and functional groups found in our models, monoazo dye (Tartrazine) and diazo dye (Ponceau). The findings showed that the azo dyes were removed quickly during the first three days of the trial and more slowly over the next six days. After six days of incubation, the highest decolourization was seen at 5 ppm tartrazine with *S. bijugatus* (68%) and *N. muscourm*. The molecular makeup of the dyes and the type of algae utilized seem to have an impact on how much colour loss is reduced. After two days of incubation, the diatom culture *Nitzschia perminuta* entirely died. It was estimated how much azo reductase from algae breaks down the azo linkage to convert azo dyes into aromatic amines. The infrared spectra showed a decrease in the azo band at 1642-1631 cm⁻¹ and a new peak at 3300 cm⁻¹. To examine the sorption behaviour of algae, the Langmuir equilibrium model was put to the test. (Hafez Omar, 2008)

Textile dye wastewater leakage into untreated waterways is a serious health and environmental hazard. The abundance of linked research findings suggests that color removal in particular has recently attracted significant scientific attention. Several physicochemical decolourization processes have been described during the past 20 years; the textile industry has accepted just a small number of these techniques. The main reasons for their non-implementation have been their high cost, low efficiency, and limited applicability to a broad range of dyes. There has been a lot of discussion over microbes' capacity to perform dye decolourization. Considered to be a significant source for decolorizing dye and textile effluent are green and blue-green algae. The green algae *Haematococcus* sp., *Chlorella* sp., *Chlorella vulgaris*, *Scenedesmus obliquus*, *S. Officinalis*, and *S. quadricauda*, as well as the blue-green algae *Arthrospira maxima*, were utilized for the decolourization process. The dye Congo red and textile dye effluent were selected for this experiment. This organism's protein and chlorophyll contents were measured both before and after the treatment. Of the seven microalgae, *Haematococcus* sp. demonstrated the greatest degradation, which was discovered at 10 ppm, or 98%, and which quickly and effectively decolorizes textile effluent. (Mahalakshmi et al., 2015)

Using calcium alginate, *Chlorella* (*pyrenoidosa*) was rendered immobile. Following immobilization, algal growth and physiological activity both increased. Cell development and physiological activity are largely independent of algal size and starting density. The support's ability to divide algal cells was limited in the absence of bubbling air that contained 2% CO₂. The number of individual algae cells grew and the distribution of algae within the support was non-uniform, suggesting that the immobilized algae's growth and cell division were restricted by the support's resistance to mass transfer of CO₂. Following a 2% CO₂ algal growth bubble, there was a significant increase in cell division and a normal restoration of individual cell size. Study of the degradation of dye (direct brown NM) by immobilized algae was better than that of free algae. Bubbling air containing 2% CO₂ in the culture solution was more favorable for increasing decolorization rate. Preliminary study of co-immobilization of algae plus bacterium illustrated that the addition of bacterium

increased the anabolic activity of algae, thus increased the decolorization capacity if immobilized algae for dye.(Guolan et al., 2000)

Chapter - 3 Aim And Objectives

1. AIM:

Investigate and develop a natural microbial-based method utilizing actinobacteria for the effective removal and degradation of azo dyes from soil.

2. Objectives

- In vitro screening of *Streptomyces* from dye-contaminated soil for the degradation of Turquoise Blue and Reactive Yellow dyes under standard laboratory conditions.
- Degradation of Turquoise Blue and Reactive Yellow dyes by the potential *Streptomyces* sp. using the shake flask method.
- Confirming the degradative metabolites produced during degradation using HPLC. Characterization and taxonomic identification of the potential *Streptomyces* sp. using DNA 16sRNA.

Chapter - 4 Methods and Methodology

Methods and Methodology

4.1 ACTINOBACTERIA'S ISOLATION AND CHARACTERIZATION FROM DYE-CONTAMINATED SOIL

4.1.1 Collection of Sample

An exact sample of the Chromepet Outskirts Strain (CO) was taken from the Tiruneermalai High Way in Tiruneermalai, Chennai, Tamil Nadu 600044, at coordinates 12°57'56.5"N 80°06'38.5"E, in order to isolate *Streptomyces* that degrade dyes. Three separate samples were obtained, and each was identified by the precise distance it was from the extraction site and nearest water source. Sample 2 was placed 5 cm away from the water source, Sample 3 was placed 2 cm away, and Sample 1 was exactly positioned 10 cm away. The approaches described in the methodology of Radhakrishnan et al. (2016) were closely followed during the gathering process. To preserve the integrity of the specimens that were gathered throughout transportation, these samples were meticulously placed in sterile plastic zip-lock containers.

4.1.2 Isolation of actinobacteria from a soil sample

The soil sample was put in a flask and mixed with 10 ml of water using a spinning shaker for thirty minutes. After the material was heat-treated (i.e., dried out by placing it in a hot air oven on a glass petri plate), 10 millilitres of distilled water and one gram of CO were combined. The suspension was diluted up to a 10⁻⁵ ratio. Using a sterile tool, 100µl of the solution from the 10⁻³, 10⁻⁴, and 10⁻⁵ dilutions was spread out onto Actinobacteria Isolation Agar (AIA) and Starch Casein agar medium (SCA) using the spread plate method using L Shaped glass rods. Colonies showing suspected actinobacterial morphology were picked up from the isolation agar plates using a sterile L-shaped loop and inoculated on yeast extract-malt extract agar (ISP2 medium) to achieve improved growth. The plates were incubated at 28°C for 7-14 days.

Morphologically different actinobacterial cultures were selected and preserved using ISP2 agar slants as well as in 30% glycerol broth. The isolation methods regarding the heat-treatment method was followed in accordance with (Radhakrishnan et al., 2016).

4.1.3 Characterization of actinobacteria

After being injected into the ISP2 agar medium and incubated for 10 days at 28°C, actinobacterial colonies from a soil sample were culturally characterized. The growth, consistency, colour of the aerial mass, presence of reverse side pigment, and generation of soluble pigments of the colonies were all observed. Moreover, during the incubation phase, the morphological traits of the colonies—which include their size, color, and texture—were examined. The Characterization of actinobacteria steps regarding the specificities was followed in accordance with (Radhakrishnan et al., 2016).

4.2 SCREENING OF DYE DEGRADING ACTINOBACTERIA

4.2.1 Screening of Dye Degrading Actinobacteria.

A dot plot assay was used to measure each bacterial strain's degradation of Turquoise Blue dye in ISP2 medium. To monitor degradation, 6 distinct bacterial strains were spotted onto ISP2 agar plates at a concentration of 100 PPM and incubated at 28°C for 7 days. The culture was checked for dye degradation by testing for transparency after seven days of incubation. For additional testing, the culture with the degradative ability is subcultured again onto ISP2 agar plates.

4.2.2 Decolorization of Turquoise Blue (TB) and Reactive Yellow (RY) by selected strain under different concentrations.

Actinobacterial strain CO demonstrated significant degradation efficacy against Turquoise Blue (TB) and Reactive Yellow (RY) dyes at concentrations ranging from 50 to 500 ppm. Following the selection of strain CO via the plate method, degradation experiments were conducted using the shake flask method in ISP2 broth.

The degradation process involved inoculating strain CO into different concentrations of TB and RY dyes and incubating them in a shaker at 120 rpm. Samples were drawn

aseptically on the 5th, 6th, and 7th days of incubation and then centrifuged at 10000 rpm for 10 minutes. UV-Vis Spectroscopy was utilized to estimate dye decolourization, with TB measured at 630 nm and RY at 410 nm. The method regarding the use of multiple dye concentrations for assessment of the degradative capability regarding the strain CO18 albeit modified in terms concentration values due the dyes in use was followed in accordance with (Hanis et al., 2020)

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t . Graphical representations were constructed based on concentration and absorbance data. Control experiments with ISP2 broth containing dye but no inoculum were conducted in parallel to ensure biological mediation of decolorization. The percentage decolorization was calculated using the provided formula, confirming the efficacy of strain CO in degrading Turquoise Blue and Reactive Yellow dyes. The method regarding the use of UV Spectrophotometer for quantifying the degradative capability of the strain CO18 albeit modified in terms of UV Spectrophotometer values regarding the dyes was followed in accordance with (Hanis et al., 2020). The formula at hand for calculating the degradative efficacy has been taken from (Guadie et al., 2017).

4.3 EFFECT OF DIFFERENT SOURCES ON DECOLORIZATION OF TURQUOISE BLUE (TB)

4.3.1 Effect of Carbon Sources on the degradation of Turquoise Blue by strain CO18. To maximize the carbon sources in the ISP2 broth, a range of carbon sources were utilized, including lactose, sucrose, xylose, inositol, mannitol, starch, glucose, and no sugar (lack of carbon source). Glucose, the typical carbon source, was replaced with each carbon source at a concentration of 1% in the ISP2 medium. Turquoise blue dye at a concentration of 100 PPM were simultaneously added to each broth, and the mixture was autoclaved. Initial UV-Vis Spectroscopy readings were obtained at corresponding wavelengths (630 nm for Turquoise Blue) for calculating Degradative Efficacy.

After the modified media-dye broths were added to a 96-well plate, the actinobacterial strain CO18 was added to each filled well, and the plate was shaken at 120 revolutions per minute. There were no variations in colour and carbonation in the broths. Samples were incubated for 7 days before being aseptically extracted using sterile micropipettes and centrifuged for 10 minutes at 10,000 rpm. UV-Vis Spectroscopy was used to test the degradative efficacy by looking at 100µl of sample supernatant in a 96-well microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t . Graphical representations were constructed based on concentration and absorbance data.

For comparative research, bar graphs were created using concentration and absorbance data. Parallel control tests were carried out using ISP2 broth that included dye but no inoculum to verify the biological mediation of decolorization. Using the

supplied formula, the percentage of decolorization was computed, verifying that strain CO was effective in breaking down Turquoise Blue. The use of multiple carbon sources in regards to optimization producing an comparative study was taken from (Khani et al., 2016)

4.3.2 Effect of Nitrogen Sources on the degradation of Turquoise Blue by strain CO18.

To maximize the nitrogen sources in the ISP2 broth, a range of nitrogen sources were utilized, including yeast, peptone, malt, soyabean meal, KNO₃, and no sugar (lack of nitrogen source). Yeast, the typical nitrogen source, was replaced with each nitrogen source at a concentration of 1% in the ISP2 medium. Turquoise blue dye at a concentration of 100 PPM were simultaneously added to each broth, and the mixture was autoclaved. Initial UV-Vis Spectroscopy readings were obtained at corresponding wavelengths (630 nm for Turquoise Blue) for calculating Degradative Efficacy.

After the modified media-dye broths were added to a 96-well plate, the actinobacterial strain CO18 was added to each filled well, and the plate was shaken at 120 revolutions per minute. There were no variations in colour and carbonation in the broths. Samples were incubated for 7 days before being aseptically extracted using sterile micropipettes and centrifuged for 10 minutes at 10,000 rpm. UV-Vis Spectroscopy was used to test the degradative efficacy by looking at 100µl of sample supernatant in a 96-well microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t . Graphical representations were constructed based on concentration and absorbance data.

For comparative research, bar graphs were created using concentration and absorbance data. Parallel control tests were carried out using ISP2 broth that included dye but no inoculum to verify the biological mediation of decolorization. Using the

supplied formula, the percentage of decolorization was computed, verifying that strain CO was effective in breaking down the dyes Reactive Yellow and Turquoise Blue. The use of multiple nitrogen sources in regards to optimization producing an comparative study was taken from de(Guadie et al., 2017)

4.3.3 Effect of Mineral Sources on the degradation of Turquoise Blue by strain CO18.

To maximize the mineral sources in the ISP2 broth, a range of mineral sources were utilized, including CaCl₃, MnCl₂, FeSO₄, MgSO₄ and No Source (lack of mineral source). NaCl, the typical mineral source, was replaced with each mineral source at a concentration of 1% in the ISP2 medium. Turquoise blue dye at a concentration of 100 PPM were simultaneously added to each broth, and the mixture was autoclaved. Initial UV-Vis Spectroscopy readings were obtained at corresponding wavelengths (630 nm for Turquoise Blue) for calculating Degradative Efficacy.

After the modified media-dye broths were added to a 96-well plate, the actinobacterial strain CO18 was added to each filled well, and the plate was shaken at 120 revolutions per minute. There were no variations in colour and carbonation in the broths. Samples were incubated for 7 days before being aseptically extracted using sterile micropipettes and centrifuged for 10 minutes at 10,000 rpm. UV-Vis Spectroscopy was used to test the degradative efficacy by looking at 100µl of sample supernatant in a 96-well microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t . Graphical representations were constructed based on concentration and absorbance data.

For comparative research, bar graphs were created using concentration and absorbance data. Parallel control tests were carried out using ISP2 broth that included dye but no inoculum to verify the biological mediation of decolorization. Using the

supplied formula, the percentage of decolorization was computed, verifying that strain CO was effective in breaking down the dyes Reactive Yellow and Turquoise Blue. The use of multiple mineral sources in regards to optimization producing a comparative study is a modified method present in (Khani et al., 2016).

4.3.4 Effect of Ph on the degradation of Turquoise Blue by strain CO18.

To maximize the Ph presence in the ISP2 broth, a range of Ph levels were utilized, including 4, 5, 6, 7, 8 and 9. The typical Ph level 7 post dye addition, was modified using 5M HCL and 10% NaOH for acidity and alkalinity respectively in the ISP2 medium. Turquoise blue dye at a concentration of 100 PPM were simultaneously added to each broth, and the mixture was autoclaved. Initial UV-Vis Spectroscopy readings were obtained at corresponding wavelengths (630 nm for Turquoise Blue) for calculating Degradative Efficacy.

After the modified media-dye broths were added to a 96-well plate, the actinobacterial strain CO18 was added to each filled well, and the plate was shaken at 120 revolutions per minute. There were no variations in colour and carbonation in the broths. Samples were incubated for 7 days before being aseptically extracted using sterile micropipettes and centrifuged for 10 minutes at 10,000 rpm. UV-Vis Spectroscopy was used to test the degradative efficacy by looking at 100µl of sample supernatant in a 96-well microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t . Graphical representations were constructed based on concentration and absorbance data.

For comparative research, bar graphs were created using concentration and absorbance data. Parallel control tests were carried out using ISP2 broth that included dye but no inoculum to verify the biological mediation of decolorization. Using the supplied formula, the percentage of decolorization was computed, verifying that strain CO was effective in breaking down the dyes Reactive Yellow and Turquoise Blue. The use of multiple Ph levels in regards to optimization producing a comparative study was taken from (Barathi et al., 2020)

4.3.5 Effect of Salt Concentration on the degradation of Turquoise Blue by strain CO18.

To maximize the salt presence in the ISP2 broth, a range of salt concentrations were utilized, including 0.1, 0.5, 1, 3, 4, 5 and No Source (no salt presence). The typical salt concentration 1% post dye addition, was replaced with each salt concentration in the ISP2 medium. Turquoise blue dye at a concentration of 100 PPM were simultaneously added to each broth, and the mixture was autoclaved. Initial UV-Vis Spectroscopy readings were obtained at corresponding wavelengths (630 nm for Turquoise Blue) for calculating Degradative Efficacy.

After the modified media-dye broths were added to a 96-well plate, the actinobacterial strain CO18 was added to each filled well, and the plate was shaken at 120 revolutions per minute. There were no variations in colour and carbonation in the broths. Samples were incubated for 7 days before being aseptically extracted using sterile micropipettes and centrifuged for 10 minutes at 10,000 rpm. UV-Vis Spectroscopy was used to test the degradative efficacy by looking at 100µl of sample supernatant in a 96-well microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_0 is the initial absorbance and A_t is the absorbance after time t . Graphical representations were constructed based on concentration and absorbance data.

For comparative research, bar graphs were created using concentration and absorbance data. Parallel control tests were carried out using ISP2 broth that included dye but no inoculum to verify the biological mediation of decolorization. Using the supplied formula, the percentage of decolorization was computed, verifying that strain CO was effective in breaking down the dyes Reactive Yellow and Turquoise Blue. The use of multiple salt concentrations in regards to optimization producing a comparative study was taken from (Wang et al., 2020)

4.3.6 Effect of Sea Water Concentration on the degradation of Turquoise Blue by strain CO18.

To maximize the sea water presence in the ISP2 broth, a range of sea water concentrations were utilized, including 50, 100 and No Source (no sea water presence). The typical sea water concentration 0% post dye addition, was replaced with each sea water concentration in the ISP2 medium with distil water dilution. Turquoise blue dye at a concentration of 100 PPM were simultaneously added to each broth, and the mixture was autoclaved. Initial UV-Vis Spectroscopy readings were obtained at corresponding wavelengths (630 nm for Turquoise Blue) for calculating Degradative Efficacy.

After the modified media-dye broths were added to a 96-well plate, the actinobacterial strain CO18 was added to each filled well, and the plate was shaken at 120 revolutions per minute. There were no variations in colour and carbonation in the broths. Samples were incubated for 7 days before being aseptically extracted using sterile micropipettes and centrifuged for 10 minutes at 10,000 rpm. UV-Vis Spectroscopy was used to test the degradative efficacy by looking at 100 μ l of sample supernatant in a 96-well microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t . Graphical representations were constructed based on concentration and absorbance data.

For comparative research, bar graphs were created using concentration and absorbance data. Parallel control tests were carried out using ISP2 broth that included dye but no inoculum to verify the biological mediation of decolorization. Using the supplied formula, the percentage of decolorization was computed, verifying that strain CO was effective in breaking down the dyes Reactive Yellow and Turquoise Blue. The use of multiple sea water concentrations in regards to optimization producing a comparative study is a modified version of a method present in (Wang et al., 2020).

4.4 Bacterial Growth Optimization on Solid Agar

4.4.1 Solid Media Modifications Relating to Carbon, Nitrogen and Mineral Sources.

To maximize the bacterial growth under ISP2 agar plates, a range of carbon sources were utilized, including lactose, sucrose, xylose, inositol, mannitol, starch, glucose, and no sugar (lack of carbon source). Glucose, the typical carbon source, was replaced with each carbon source at a concentration of 1% in the ISP2 medium. For nitrogen source optimization a range of nitrogen sources were utilized, including yeast, peptone, malt, soyabean meal, KNO_3 , and no sugar (lack of nitrogen source). Yeast, the typical nitrogen source, was replaced with each nitrogen source at a concentration of 1% in the ISP2 medium. For mineral source optimization a range of mineral sources were utilized, including CaCl_3 , MnCl_2 , FeSO_4 , MgSO_4 and No Source (lack of mineral source). NaCl , the typical mineral source, was replaced with each mineral source at a concentration of 1% in the ISP2 medium. Media was prepared in a 12-well microtiter plate after which they were incubated for 7 days before being aseptically observed for Consistency, Growth, Arial Mycelium Pigment (AMC), Reverse Substrate Pigment

(RSP), Arial Mycelium presence and Substrate Mycelium presence. The use of different media in regards to optimization producing a comparative study is a modified version of a method present in (Wang et al., 2020). The characterization protocol presents in followed in accordance to (Radhakrishnan et al., 2016).

4.5 TAXONOMY STUDY AND IDENTIFICATION OF POTENTIAL STRAIN

4.5.1 Micromorphology

Actinobacterial strain CO18 was observed under bright field microscopy at 40X magnification to visualize the micromorphology

4.5.2 Cultural characterization

For cultural characterization, the actinobacterial strain CO18 was inoculated ontp different media compositions including Actinomycetes Isolation Agar (AIA), Czapek Dox Agar (CDA), Oatmeal Agar (ISP3), Glycerol Asparagine Agar Base (ISP5), Peptone Yeast Extract Iron Agar (ISP6), Inorganic Salt Starch Agar (ISP4), Nutrient Agar (NA) and Starch Casein Agar (SCA). Media was prepared in a 12-well microtiter plate after which they were incubated for 7 days before being aseptically observed for Consistency, Growth, Arial Mycelium Pigment (AMC), Reverse Substrate Pigment (RSP), Arial Mycelium presence and Substrate Mycelium presence. (Radhakrishnan et al., 2016)

Chapter – 5 Results and Discussions

5.1 ACTINOBACTERIA'S ISOLATION AND CHARACTERIZATION FROM DYE-CONTAMINATED SOIL

5.1.1 Collection and Isolation of actinobacteria from a soil sample

An exact sample of the Chromepet Outskirts Strain (CO) was taken from the Tiruneermalai High Way in Tiruneermalai, Chennai, Tamil Nadu 600044, at coordinates 12°57'56.5"N 80°06'38.5"E, in order to isolate *Streptomyces* that degrade dyes. Three separate samples were obtained, and each was identified by the precise distance it was from the extraction site and nearest water source. Sample 2 was placed 5 cm away from the water source, Sample 3 was placed 2 cm away, and Sample 1 was exactly positioned 10 cm away.. To preserve the integrity of the specimens that were gathered throughout transportation, these samples were meticulously placed in sterile plastic zip-lock containers.

After that, the soil sample was put into a flask and shaken for thirty minutes with 10 milliliters of water. Following heat treatment, which involved drying the material on a glass petri plate in a hot air oven, 10 milliliters of distilled water and one gram of CO were added. A 10⁻⁶ ratio was achieved by diluting the suspension. Using sterile instruments, 100µl of the solution from the 10⁻⁴, 10⁻⁵, and 10⁻⁶ dilutions was disseminated using the spread plate method with L-shaped glass rods onto Actinobacteria Isolation Agar (AIA) and Starch Casein agar medium (SCA).

Using a sterile L-shaped loop, colonies with putative actinobacterial morphology were removed from the isolation agar plates and inoculated on yeast extract-malt extract agar (ISP2 medium) to promote growth. After that, the plates were cultured for 7-14 days at 28°C. Actinobacterial cultures with unique morphologies were chosen and kept in 30% glycerol broth and ISP2 agar slants.

The approaches described in the methodology of Radhakrishnan et al. (2016) were closely followed during the gathering process. The location of the sample collection is shown in Fig. 1, and a flask holding the collected sand samples is shown in Fig. 2 and the Fig. 3 depicts the serial dilution being done for all 3 samples from 10⁻¹ to 10⁻⁶. The Fig. 4 depicts the initial cultures on SCA and AIA plates.



Fig. 5.1 Location



Fig. 5.2 Sand samples in flask S1, S2 and S3.



Fig. 5.3 Serial Dilution



Fig. 5.4 SCA and AIA cultures / 1st isolation

5.1.2 Morphological Characterization of Serial Diluted Actinobacteria

Actinobacterial colonies from a soil sample were obtained and cultured for ten days at 28°C after being injected into the ISP2 agar medium. We observed the colonies' development, homogeneity, color, presence of reverse side pigment, and production of soluble pigments. In addition, the colonies' morphological characteristics—such as their size, color, and texture—were studied during the incubation stage. The Characterization of actinobacteria steps regarding the specificities was followed in accordance with (Radhakrishnan et al., 2016). The cultures grew at a steady pace with

visible growth. The morphological characteristics of the observed cultures are shown in Table 5.1

Table 5.1 Morphological Characteristics of isolated Cultures

Sno	Strain	Texture	Elevation	Growth	AMC	RSP	Arial Mycelium	Substrate Mycelium
1	CO1	Powder	Umbonate	Medium	Black	Black	+	+
2	CO2	Powder	Umbonate	Medium	Black	Black	+	+
3	CO3	Powder	Umbonate	Good	White	White	+	+
4	CO4	Powder	Umbonate	Medium	Red	Red	+	+
5	CO5	Powder	Umbonate	Good	Red	Red	+	+
6	CO6	Powder	Umbonate	Low	Black	Black	+	+
7	CO7	Powder	Umbonate	High	White	White	+	+
8	CO8	Powder	Umbonate	Medium	Black	-	+	-
9	CO9	Powder	Umbonate	Medium	Black	-	+	-
10	CO10	Smooth	Umbonate	Low	White	-	+	-
11	CO12	Powder	Umbonate	Low	Black	Black	+	+
12	CO13	Powder	Umbonate	Good	Orange	-	+	-
13	CO14	Powder	Umbonate	Good	Green	Black	+	+
14	CO15	Powder	Umbonate	Low	White	-	+	-
15	CO18	Powder	Umbonate	Medium	Black	Black	+	+
16	CO19	Powder	Umbonate	Low	White	-	+	-
17	KT01	Powder	Umbonate	Good	Black	Black	+	+

5.2 SCREENING OF DYE DEGRADING ACTINOBACTERIA

5.2.1 Screening of Dye Degrading Actinobacteria.

The amount of Turquoise Blue dye that each bacterial strain degraded in ISP2 media was measured using a dot plot technique. Six different bacterial strains were spotted onto ISP2 agar plates at a concentration of 100 PPM and cultured at 28°C for seven days in order to track deterioration. After incubating the culture for seven days, transparency was tested to see if the dye had degraded. The culture possessing the degradative ability is recultured onto ISP2 agar plates for supplementary examination.

The Fig. 5.5 shows the post incubation screening plate. The plates were prepared using 100 PPM Turquoise Blue dye concentration in ISP2 plates.

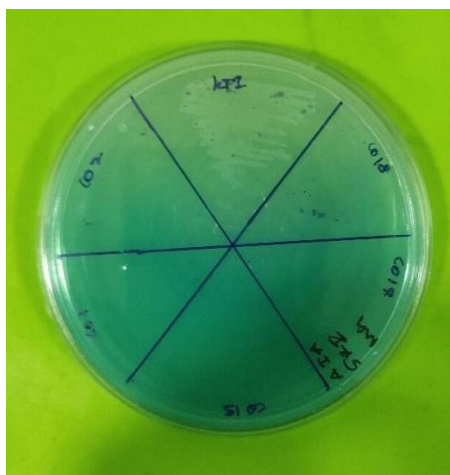


Fig. 5.5 Screening plate post incubation

5.2.2 Decolorization of Turquoise Blue (TB) by selected strain under different concentrations.

The Actinobacterial strain CO18 demonstrated significant degradation efficacy against Turquoise Blue (TB) dye at concentrations ranging from 100, 250 and 500 ppm. Following strain CO18 selection via the plate method, degradation experiments were conducted in ISP2 broth using the shake flask method. The degradation process involved inoculating strain CO18 into various TB and RY dye concentrations and incubating them in a shaker at 120 rpm. Samples were drawn aseptically on days 1-

9, centrifuged, and analyzed using UV-Vis Spectroscopy at 630 nm for TB and 410 nm for RY.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t . Graphical representations were constructed based on concentration and absorbance data. Control experiments were conducted to confirm biological mediation of decolorization. The graphical representation of the data is presented in Fig 5.7.

The individual concentration degradation data is given in the following graphs for Fig. 5.8, Fig. 5.9 and Fig. 5.10.

Table 5.2 Table Representing Degradative Efficacy of CO18 regarding Turquoise Blue and Reactive Yellow (RY)

Sno	Day	Concentration (ppm)	UV Spec Results (Turquoise Blue and Reactive Yellow)	Degradative Efficacy (%)
1	Day 1	100	0.324	0
2			1.455	0
3		250	0.723	0
4			1.522	0
5		500	1.746	0
6			2.268	0
7	Day 3	100	0.32	1.234568
8			0.796	45.2921
9		250	0.722	0.138313
10			1.343	11.76084

11		500	1.734	0.687285
12			1.869	17.59259
13	Day 5	100	0.257	20.67901
14			0.72	50.51546
15		250	0.617	14.66113
16			1.127	25.95269
17		500	1.589	8.991982
18			1.882	17.0194
19	Day 7	100	0.212	34.5679
20			0.701	51.82131
21		250	0.47	34.99308
22			0.927	39.0933
23		500	1.48	15.23482
24			1.864	17.81305
25	Day 9	100	0.075	76.85185
26			0.631	56.6323
27		250	0.413	42.8769
28			0.464	69.5138
29		500	1.301	25.48683
30			1.796	20.81129

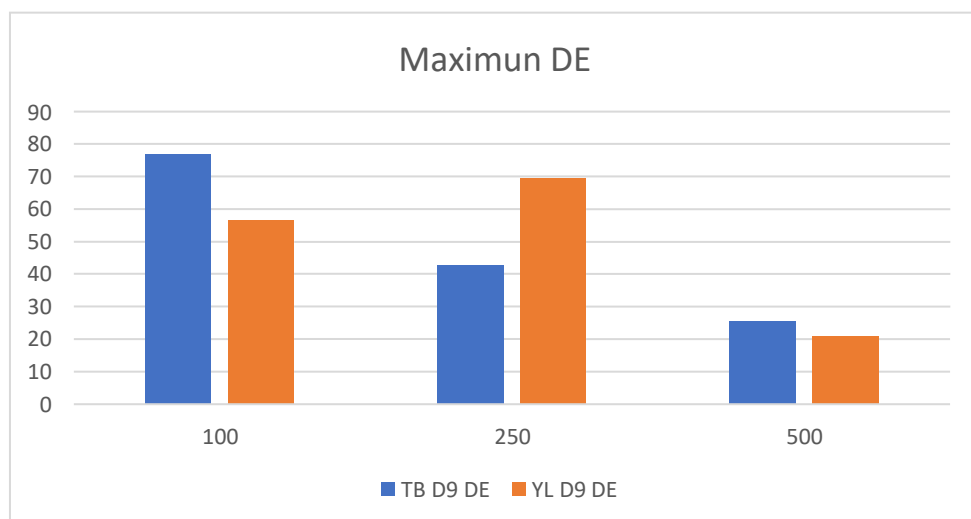


Fig. 5.6 Bar Graph regarding Concentration-Maximum Degradative Efficacy

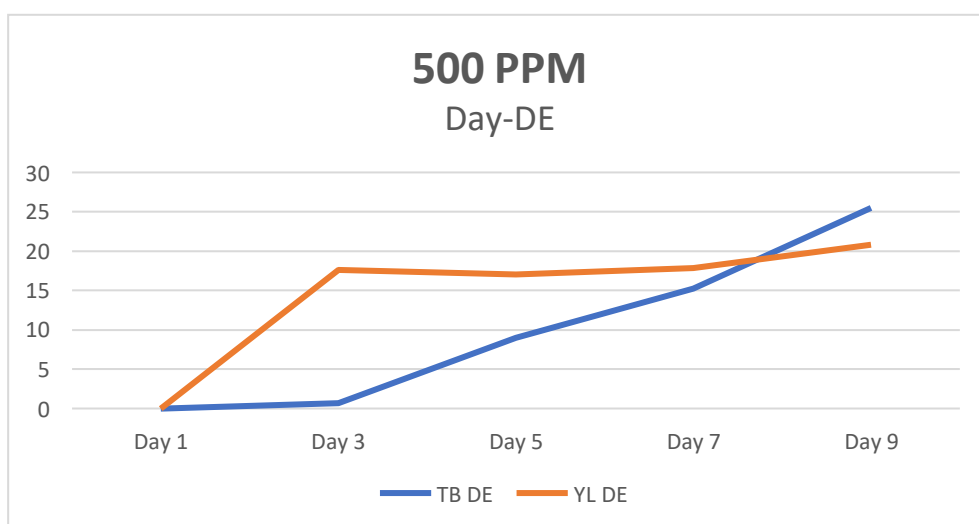


Fig. 5.7 Line Graph representing Day-Degradative Efficacy for 500 PPM

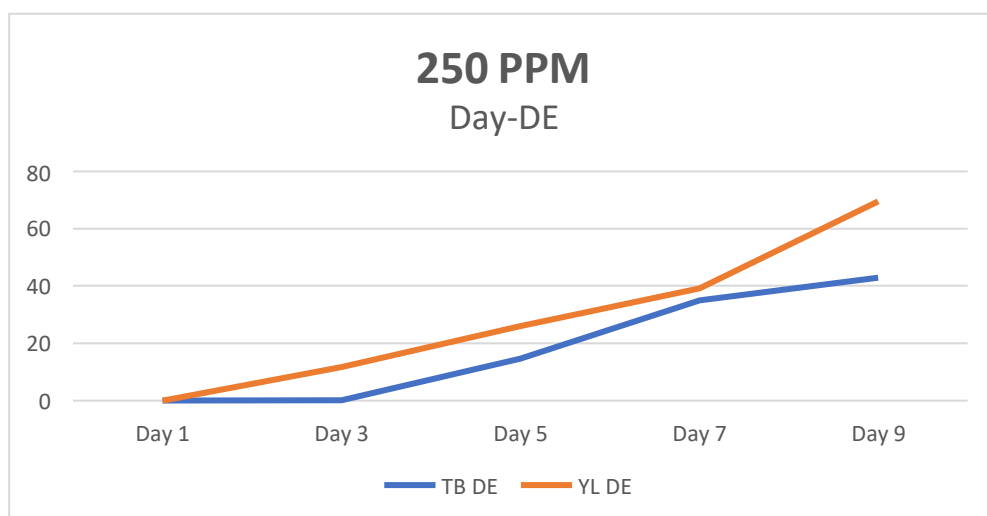


Fig. 5.8 Line Graph representing Day-Degradative Efficacy for 250 PPM

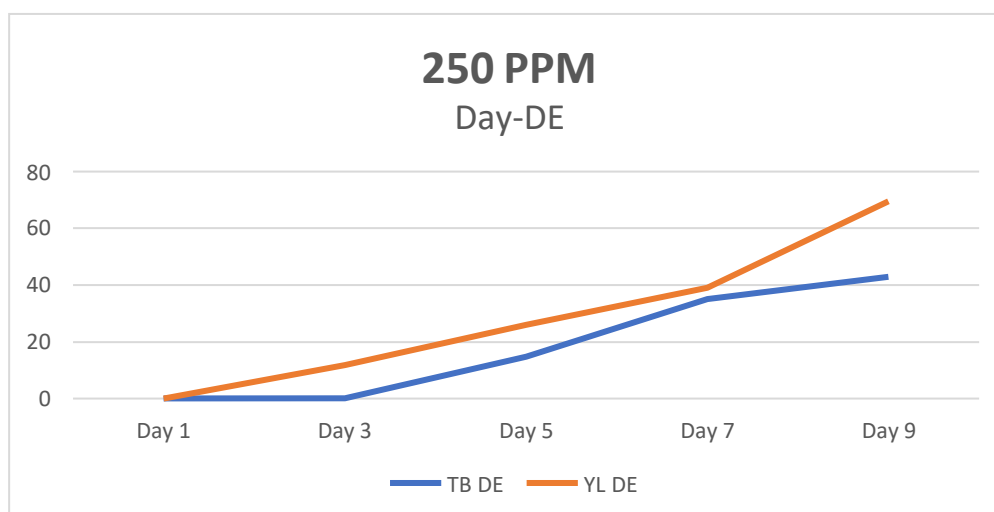


Fig. 5.9 Line Graph representing Day-Degradative Efficacy for 100 PPM

5.3 EFFECT OF DIFFERENT SOURCES ON DECOLORIZATION OF TURQUOISE BLUE (TB)

5.3.1 Effect of Carbon Sources on the degradation of Turquoise Blue by strain CO18.

The carbon sources employed in ISP2 broth at 1% concentration in place of glucose included lactose, sucrose, xylose, inositol, mannitol, starch, and no sugar. Each soup was mixed with 100 PPM of turquoise blue dye, and the dye's UV-Vis Spectroscopy values were obtained at 630 nm. In a 96-well plate, actinobacterial strain CO18 was added to the broths, agitated at 120 rpm, and incubated for 7 days without any discernible alterations. The sample supernatant was centrifuged, and the degradative efficacy was evaluated using UV-Vis Spectroscopy in a microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t .

The highest degradative efficacy among the carbon sources tested was observed with Starch (62.06%), while Mannitol showed the lowest efficacy at 33.82%. Complex carbohydrates like Starch, Fructose, and Lactose tended to exhibit higher degradative efficacy compared to simpler sugars such as Mannitol and no sugar, suggesting that the complexity of the carbon source influences degradation efficiency.

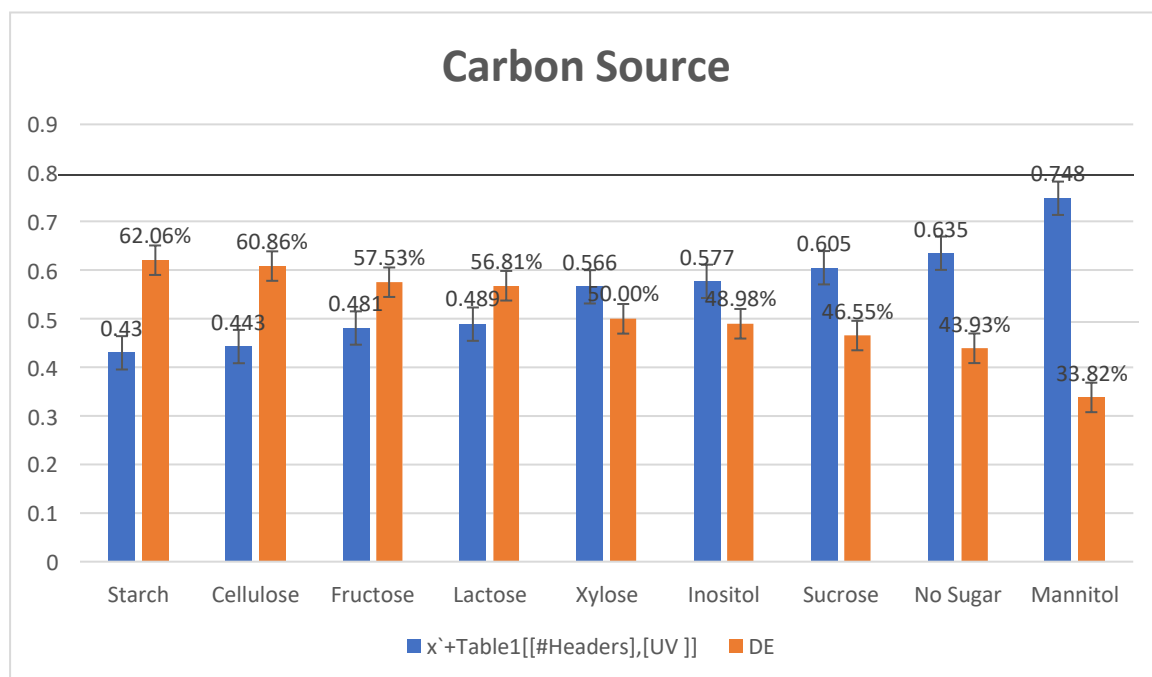


Fig. 5.10 Degradation Of Turquoise Blue By Strain CO18 Under Different Carbon Sources

Table 5.3 Degradation Of Turquoise Blue By Strain CO18 Under Different Sources Table

Sno	Component	UV Spectrophotometer Reading	Degradative efficacy
Carbon Source			
1	No Sugar	0.635	43.93%
2	Sucrose	0.605	46.55%
3	Xylose	0.566	50.00%
4	Inositol	0.577	48.98%
5	Mannitol	0.748	33.82%
6	Fructose	0.481	57.53%

7	Starch	0.43	62.06%
8	Cellulose	0.443	60.86%
9	Lactose	0.489	56.81%
Nitrogen Source			
10	Malt	0.726	35.91%
11	Yeast	0.678	40.16%
12	Peptone	0.946	15.67%
13	Soybean Meal	0.528	53.36%
14	KNO3	0.623	45.02%
15	No Source	0.782	30.91%
Mineral Source			
16	CaCl3	0.246	78.29%
17	MnCl2	0.365	67.78%
18	FeSO4	0.06	94.71%
19	MgSO4	0.432	61.85%
20	No Source	0.776	31.40%
Salt Concentration (%)			
21	0.1	0.499	55.93%
22	0.5	0.432	61.85%
23	1	0.425	62.47%
24	2	0.357	68.43%
25	3	0.333	70.55%
26	4	0.342	69.84%
27	5	0.302	73.28%
28	No Source	0.588	48.06%
Ph			
29	4	0.053	95.31%
29	5	0.084	92.61%
30	6	0.051	95.49%
31	7	0.431	61.93%
32	8	0.55	51.34%
33	9	0.625	44.79%
34	4	0.053	95.31%
Sea Water (%)			
35	No Source	0.399	64.70%
36	100	0.286	74.69%
37	50	0.303	73.21%

5.3.2 Effect of Nitrogen Sources on the degradation of Turquoise Blue by strain CO18.

In order to enhance nitrogen availability, a range of nitrogen sources were used at 1% concentration in ISP2 broth, including yeast, peptone, malt, soybean meal, KNO₃, and no sugar (lack of nitrogen source). Each broth received 100 PPM of turquoise blue dye, which was then added, autoclaved, and measured using UV-Vis Spectroscopy at 630 nm. A 96-well plate was filled with the actinobacterial strain CO18, shook, and left to incubate for seven days. The samples underwent aseptic extraction, centrifugation, and UV-Vis Spectroscopy analysis on the sample supernatant in a microtiter plate to determine the degradative efficacy.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t

Peptone had the lowest degradative efficacy at 15.67%, whereas FeSO₄ showed the maximum efficacy at 94.71%. When compared to organic nitrogen sources like peptone and malt, inorganic nitrogen sources like FeSO₄ and soybean meal shown more efficacy, suggesting that the type of nitrogen source has a major impact on the degradation process.

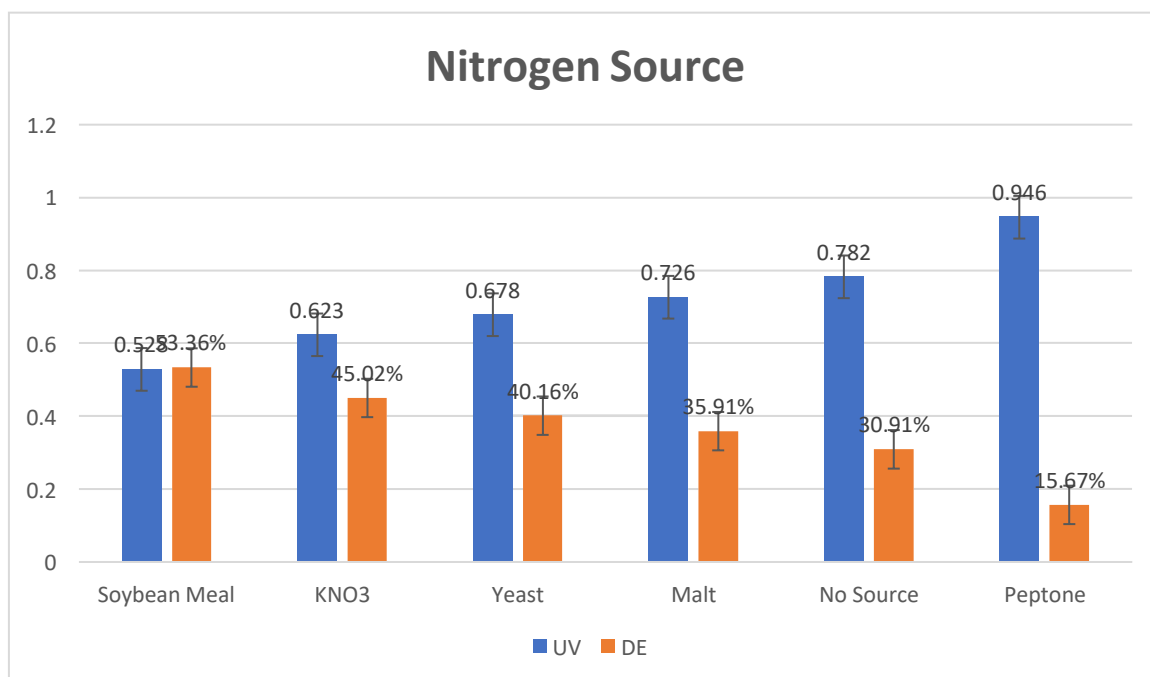


Fig. 5.11 Degradation Of Turquoise Blue By Strain CO18 Under Different Nitrogen Sources

5.3.3 Effect of Mineral Sources on the degradation of Turquoise Blue by strain CO18.

In place of NaCl, mineral sources such as CaCl₃, MnCl₂, FeSO₄, MgSO₄, and No Source were added to ISP2 broth at a concentration of 1%. Each soup was mixed with 100 PPM of turquoise blue dye, and results from UV-Vis Spectroscopy were obtained at 630 nm. A 96-well plate was filled with actinobacterial strain CO18, agitated at 120 rpm, and then allowed to incubate for seven days. There were no changes in carbonation or color. The samples were extracted aseptically, centrifuged, and their degradative efficacy was assessed using UV-Vis Spectroscopy on the sample supernatant in a microtiter plate.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t

FeSO₄ again demonstrated the highest degradative efficacy at 94.71%, while samples with no added mineral source had the lowest efficacy at 31.40%. Inorganic mineral sources such as FeSO₄ and CaCl₃

exhibited higher efficacy compared to samples without added minerals, highlighting the importance of mineral sources in enhancing degradation.

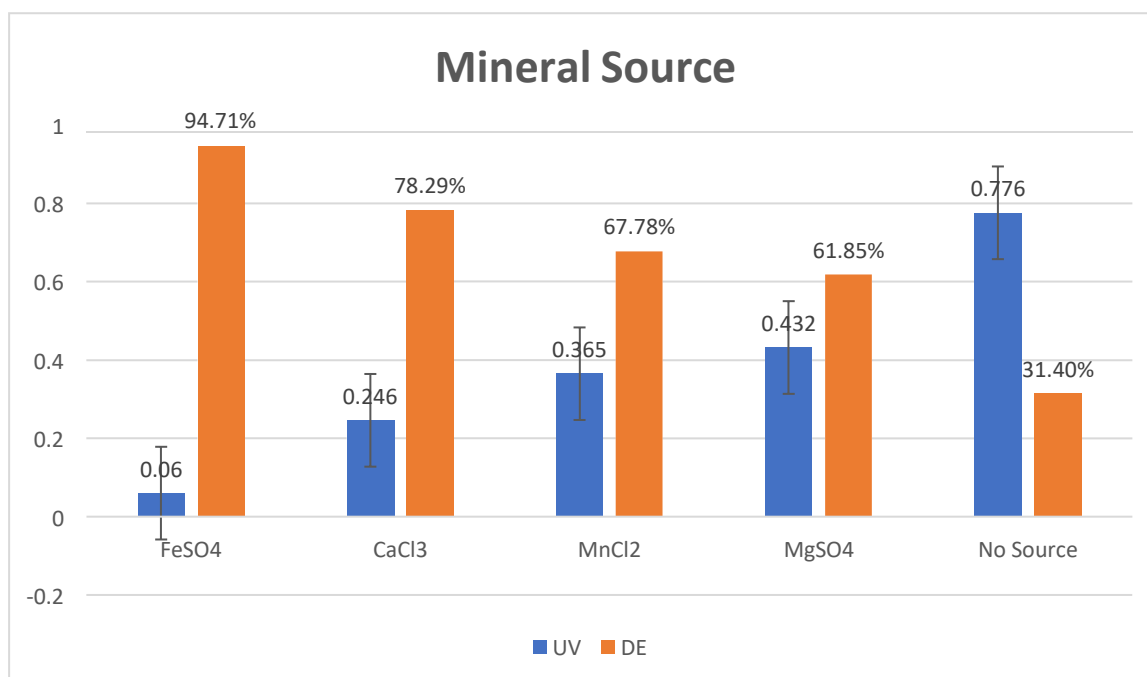


Fig. 5.12 Degradation Of Turquoise Blue By Strain CO18 Under Different Mineral Sources

5.3.4 Effect of Ph on the degradation of Turquoise Blue by strain CO18.

The ISP2 broth was prepared with a range of pH values (4, 5, 6, 7, 8, and 9). To change the normal pH 7 post-dye addition, 5M HCl and 10% NaOH were added. Each broth was treated with 100 PPM of turquoise blue dye, and 630 nm UV-Vis Spectroscopy values were obtained for preliminary analysis. Samples were cultured for 7 days after the modified broths were added to a 96-well plate along with the actinobacterial strain CO18. Aseptic extraction and centrifugation were then performed on the samples. After that, the degradative efficiency of the sample supernatant in a microtiter plate was evaluated using UV-Vis Spectroscopy.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_0 is the initial absorbance and A_t is the absorbance after time t

The degradative efficacy of optimal pH values 4 and 6 was found to be the highest at 95.31% and 95.49%, respectively, while pH 9 showed the lowest efficacy at 44.79%. The findings suggest that optimizing degradation efficiency requires keeping the pH within specific ranges.

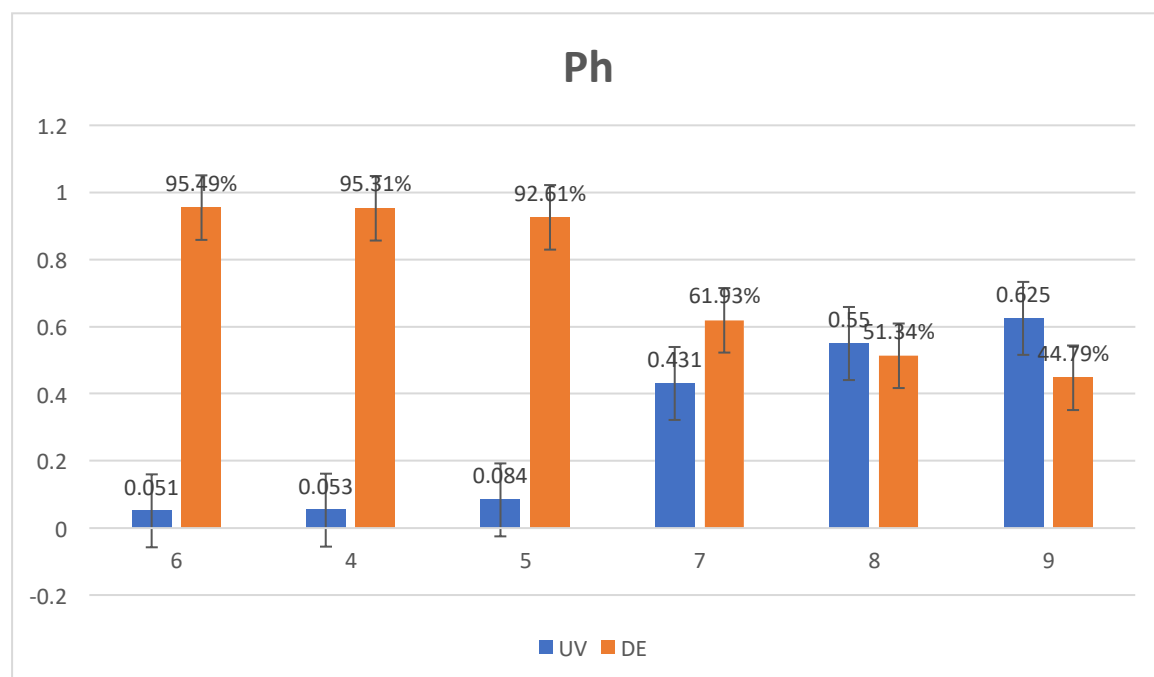


Fig. 5.13 Degradation Of Turquoise Blue By Strain CO18 Under Different Ph Levels

5.3.5 Effect of Salt Concentration on the degradation of Turquoise Blue by strain CO18.

To optimize the amount of salt present in ISP2 broth, a variety of salt concentrations were utilized, including 0.1%, 0.5%, 1%, 3%, 4%, 5%, and no salt at all. After adding 100 PPM of turquoise blue dye to each broth, it was autoclaved. Degradative Efficacy was computed using UV-Vis Spectroscopy measurements at 630 nm. A 96-well plate was filled with actinobacterial strain CO18, shook, and left to incubate for seven days.

After that, samples were removed, and their degradative activity was evaluated using UV-Vis Spectroscopy.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t .

The degradative efficacy of samples with additional salt was 48.06%, whereas samples with higher concentrations of salt, especially at 5%, demonstrated the maximum efficacy at 73.28%. This implies that higher concentrations of salt, with a 5% concentration demonstrating the greatest effect, can enhance degrading efficiency.

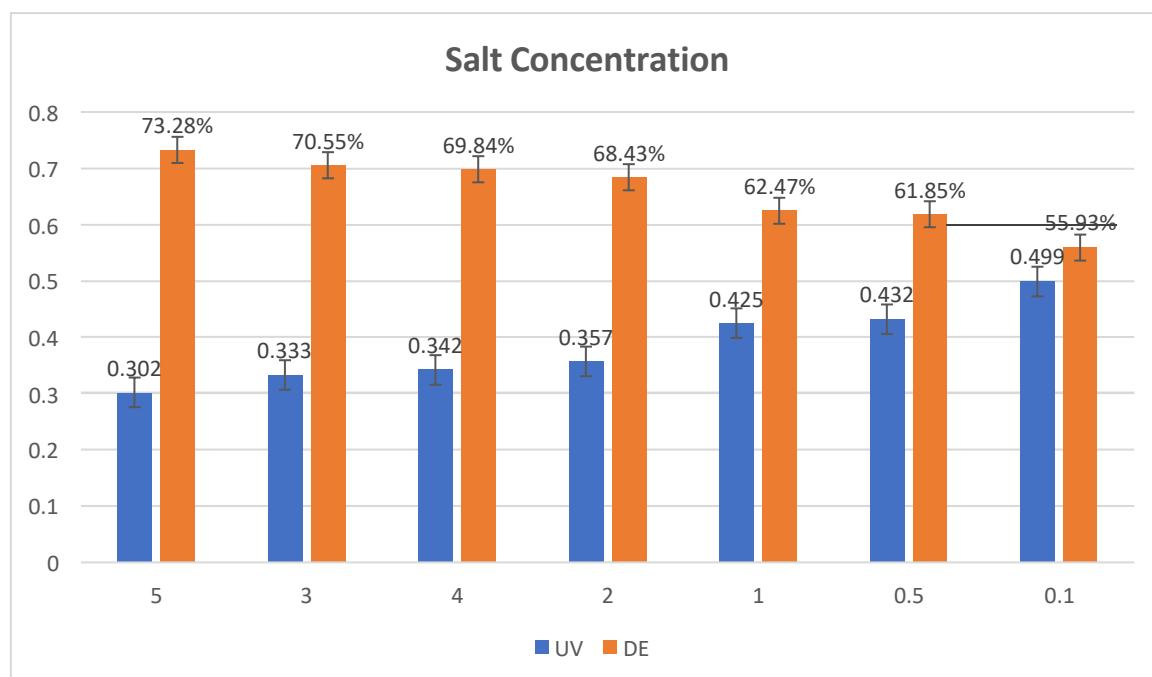


Fig. 5.14 Degradation Of Turquoise Blue By Strain CO18 Under Different Salt Concentrations

5.3.6 Effect of Sea Water Concentration on the degradation of Turquoise Blue by strain CO18.

To assess the impact of sea water concentration in ISP2 broth, varying concentrations of sea water (50%, 100%, and No Source) were utilized. Turquoise blue dye (100 PPM) was added to each broth, and UV-Vis Spectroscopy readings were taken at 630 nm to measure Degradative Efficacy. Actinobacterial strain CO18 was added to each well in a 96-well plate, which was then shaken and incubated for 7 days. Following incubation, samples were extracted and centrifuged for analysis using UV-Vis Spectroscopy, revealing insights into the degradative efficacy influenced by sea water concentration.

The percentage of dye degradation was calculated using the formula:

$$\frac{(A_o - A_t)}{A_o} \times 100$$

Where A_o is the initial absorbance and A_t is the absorbance after time A_t

The degradative efficacy of samples containing 100% sea water was 74.69%, whereas samples containing no sea water had a lower efficacy of 64.70%. This implies that adding seawater may accelerate degradation, with the impact being more noticeable at larger concentrations.

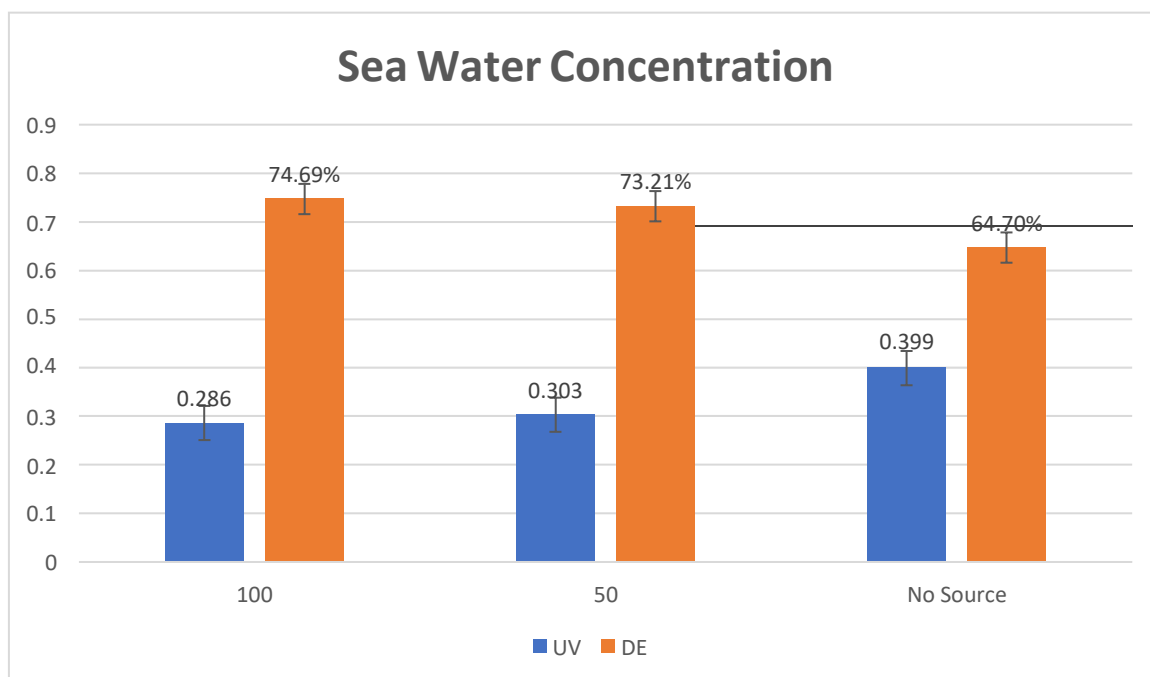


Fig. 5.15 Degradation Of Turquoise Blue By Strain CO18 Under Different Sea Water Concentrations

5.4 BACTERIAL GROWTH OPTIMIZATION ON SOLID AGAR

5.4.1 Solid Media Modifications Relating to Carbon, Nitrogen and Mineral Sources

A variety of sources were used in the optimization process, including carbon sources such as lactose, sucrose, xylose, inositol, mannitol, starch, glucose, and no sugar (lack of carbon source). Nitrogen sources included yeast, peptone, malt, soybean meal, KNO₃, and no sugar (lack of nitrogen source). Mineral sources comprised CaCl₂, MnCl₂, FeSO₄, MgSO₄, and No Source (lack of mineral source) were tested at 1% concentration in ISP2 agar plates to maximize bacterial growth. The media were incubated for 7 days and assessed for consistency, growth, aerial mycelium pigment (AMC), reverse substrate pigment (RSP), aerial mycelium presence, and substrate mycelium presence.

Table. 5.4 Solid Media Modifications Table Relating to Carbon, Nitrogen and Mineral Sources

Sn o	Compone nt	Consisten cy	Growt h	AMC	RSP	Arial Mycelium	Substrate Mycelium
Carbon Source							
1	No Sugar	Powder	Mediu m	Pale Green	Black	+	+
2	Sucrose	Powder	Mediu m	Green	Black	+	+
3	Xylose	Powder	High	Pale Green	Black	+	+
4	Inositol	Powder	Mediu m	Green	Black	+	+
5	Mannitol	Powder	Good	Green	Black	+	+
6	Starch	Powder	High	Green	Black	+	+
7	Glucose	Powder	High	Pale Green	Black	+	+
9	Lactose	Powder	Mediu m	Green	Black	+	+
Nitrogen Source							
10	Yeast	Powder	Low	Pale Green	Black	+	+
11	KNO ₃	Powder	Low	Pale Green	Black	+	+
12	No Source	Powder	Low	Pale Green	Black	+	+
Mineral Source							
13	CaCl ₂	Powder	Good	Pale Green	Black	+	+

14	MgSO ₄	Powder	Medium	Pale Green	Black	+	+
15	No Source	Powder	Good	Pale Green	Black	+	+

5.5 TAXONOMY STUDY AND IDENTIFICATION OF POTENTIAL STRAIN

5.5.1 Micromorphology

Actinobacterial strain CO18 was observed under bright field microscopy at 40X magnification to visualize the micromorphology

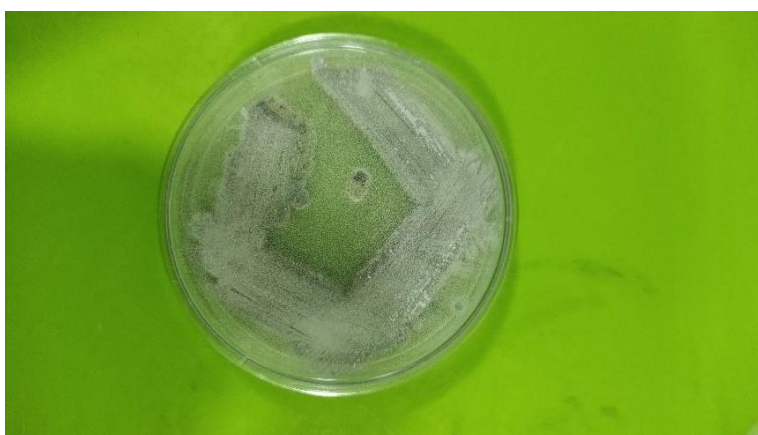


Fig. 5.16 Actinobacterial Strain CO18

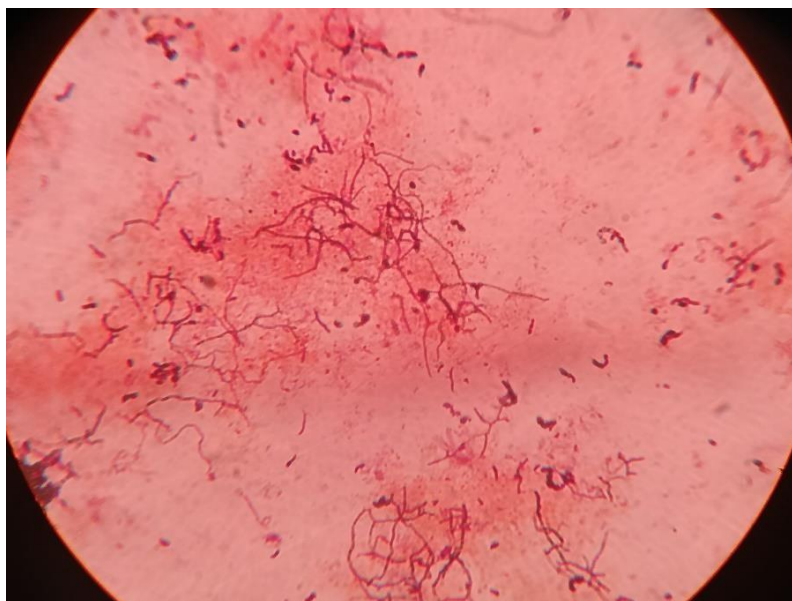


Fig. 5.17 Micromorphology of strain CO18

5.5.2 Cultural characterization

The actinobacterial strain CO18 was cultured on various media including AIA, CDA, ISP3, ISP5, ISP6, ISP4, NA, and SCA in a 12-well microtiter plate. After incubation for 7 days, cultures were examined for Consistency, Growth, Arial Mycelium Pigment (AMC), Reverse Substrate Pigment (RSP), Arial Mycelium presence, and Substrate Mycelium presence.

Table 5.5 Cultural characterization for Actinobacterial Strain CO18

	Component	Consistency	Growth	AMC	RSP	Arial Mycelium	Substrate Mycelium
Media							
16	AIA	Powder	Good	Green	Black	+	+
17	CDA	Powder	Good	Green	Black	+	+
18	ISP3	Powder	Medium	Green	Black	+	+
19	ISP5	Powder	Good	Green	Black	+	+

20	ISP6	Powder	Good	Green	Black	+	+
21	ISP4	Powder	Good	Green	Black	+	+
22	NA	Powder	Good	Green	Black	+	+
23	SCA	Powder	Good	Green	Black	+	+

CHAPTER - 6

CONCLUSION

Based on an extensive and detailed study, several significant conclusions emerge regarding the isolation, characterization, and dye-degrading capabilities of Actinobacterial Strain CO18. Initially, Actinobacteria were isolated from a soil sample using precise methods and characterized based on their distinct morphological features, with Strain CO18 displaying notable traits that made it a focus for further investigation into its potential for dye degradation. The subsequent experiments evaluating the degradation of Turquoise Blue (TB) dye by Strain CO18 unveiled its substantial efficacy across varying concentrations of the dye (100 ppm, 250 ppm, and 500 ppm) over a span of nine days. These degradation processes were intricately influenced by a myriad of factors, including the selection of carbon, nitrogen, and mineral sources, pH levels, salt concentrations, and sea water concentrations. Notably, Strain CO18 demonstrated heightened degradation efficiency when complex carbon sources like Starch were utilized, alongside nitrogen and mineral sources such as FeSO_4 and CaCl_2 . These findings underscore the importance of environmental conditions in optimizing degradation processes. Furthermore, Metabolite analysis employing High-Performance Liquid Chromatography (HPLC) offered valuable insights into the metabolic activities of Strain CO18, enhancing our understanding of its potential applications in bioremediation. Taxonomic studies and cultural characterizations provided additional confirmation of the unique characteristics and promising capabilities of Strain CO18, particularly in the realm of dye degradation, paving the way for future research and practical applications in environmental remediation efforts.

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APPENDICES

1. Starch Casein Agar (SCA)

REQUIREMENTS	AMOUNT (g/L)
Soluble starch	10
Casein (vitamin free)	0.3
Potassium nitrate	2
Magnesium sulphate	0.05
Ferrous sulphate	0.01
Dipotassium phosphate	2
Calcium carbonate	0.02
Sodium chloride	2
Agar Agar	20
pH	7

2. INTERNATIONAL STREPTOMYCES PROJECT – 2 (ISP-2)

REQUIREMENTS	AMOUNT (g/L)
Glucose	0.4
Yeast extract	0.4
Malt Extract	1
Sodium Chloride	1
Agar Agar	2.0
pH	7

3. INTERNATIONAL STREPTOMYCES PROJECT – 1 (ISP-1)

REQUIREMENTS	AMOUNT (g/L)
Casein enzymic hydrolysate	5

Yeast extract	3
Agar Agar	20
pH	8

4. INTERNATIONAL STREPTOMYCES PROJECT – 3 (ISP -3)

REQUIREMENTS	AMOUNT (g/L)
Oatmeal agar	30 g
Agar	15 g

5. INTERNATIONAL STREPTOMYCES PROJECT -5 (ISP -5)

REQUIREMENTS	AMOUNT (g/L)
L- Asparagine	1
Glycerol	10
K ₂ HPO ₄	1
Agar	20

6. INTERNATIONAL STREPTOMYCES PROJECT -7 (ISP -7)

REQUIREMENTS	AMOUNT (g/L)
L- ASPARAGINE	1.00
L- TYROSINE	0.50
DIPOTASSIUM PHOSPHATE	0.50
MAGNESIUM SULPHATE	0.50
SODIUM CHLORIDE	0.50
TRACE SALT SODIUM	1.00
AGAR	20

7. NUTRIENT AGAR

REQUIREMENTS	AMOUNT (g/L)
BEEF EXTRACT	1.00
YEAST EXTRACT	2.00
PEPTONE	5.00
SODIUM CHLORIDE	5.00
AGAR	15.0

8. CZAPEX DOX AGAR (CDA)

REQUIREMENTS	AMOUNT (g/L)
SUCROSE	30.0
SODIUM NITRITE	2.00
DIPOTASSIUM PHOSPHATE	1.00
MAGNESIUM SULPHATE	0.05
POTASSIUM CHLORIDE	0.05
FERROUS SULPHATE	0.01

7. GLUCOSE YEAST MALT AGAR (GYM)

REQUIREMENTS	AMOUNT (g/L)
PEPTIC DIGEST OF ANIMAL TISSUE	5.00
YEAST EXTRACT	5.00
GLUCOSE	2.00
MONOPOTASSIUM PHOSPHATE	0.50
DIPOTASSIUM PHOSPHATE	0.50
MAGNESIUM SULPHATE	0.30
SODIUM CHLORIDE	0.01
MANGANESE SULPHATE	0.01
ZINC SULPHATE	0.0016

COPPER SULFATE	0.0016
COBALT SULFATE	0.0016
AGAR	15.00

9. ACTINOMYCETE ISOLATION AGAR (AIA)

REQUIREMENTS	AMOUNT (g/L)
AIA	22.00
GLYCEROL	5.00
AGAR	15.00