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DEPARTMENT OF BIOLOGICAL AND CHEMICAL ENGINEERING

Screening and Identification of Potential Dye-Degrading Bacteria from Maa Garment Effluent

BY

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A Thesis Submitted To the Department Of Biological and Chemical Engineering in Partial Fulfillment of the Requirements for the Master of Science in Industrial Biotechnology

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DECLARATION

I, Berihu Zenawi, hereby present for consideration by the Department of Biological and Chemical Engineering with in the Mekelle Institute of Technology at Mekelle University, my thesis entitled Screening and Identification of Potential Dye Degrading Bacteria Isolated from Maa Garment Effluents in partial fulfillment of the requirement for the degree of Masters in Industrial Biotechnology. I sincerely declare that this thesis is the product of my own efforts. No other person has published a similar study which I might have copied, and at no stage will this thesis be published without my consent and that of the Department of Biological and Chemical Engineering

Name of the students Berihu Zenawi G/zher Signature _____ Date _____

Approval Sheet

S/N	Advisors, Examiners, Dep't Head and PG Coordinator name	Signature	Date
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LIST OF ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DO	Dissolved oxygen
E	East
E. coli	*Escherichia coli*
g/l	Gram per litter
K. pneumonia	*Klebsiella pneumonia*
KOH	Potassium hydroxide
mg/l	Milligram per litter
N	North
Nm	Nanometer
P. argunosa	*Pseudomonas aeruginosae*
Rpm	Revolution per minute
rRNA	Ribosomal ribonucleic acid
Sp.	Species
TDS	Total dissolved solid
TSI	Triple sugar iron test
TSS	Total suspended solid

Abstract

The textile industry is a major contributor to water pollution, releasing effluents containing 10% - 15% unused dyes. These, dyes are resistant to biodegradation because their complex aromatic structures pose significant threats to aquatic ecosystems and human health. This study aimed to isolate, screen, and identify potential dye-degrading bacteria from the effluents of the Maa Garment and Textile Factory. Physicochemical parameters, including pH, temperature, total suspended solids (TSS), total dissolved solids (TDS), biological oxygen demand (BOD), and chemical oxygen demand (COD), were analyzed. Bacterial isolates were cultivated in dye-containing media, and their decolorization efficiency was evaluated using spectrophotometry under varying conditions: temperatures (25°C, 30°C, 37°C, and 40°C), pH levels (5, 7, and 9), and dye concentrations (50, 100, and 150 mg/L). The collected samples exhibited pH levels ranging from 7.2 to 7.5 and the temperature varied significantly, with one sample reaching 38°C. A total of 16 bacterial isolates were screened for their decolorization capabilities under varying conditions of temperature, pH, and dye concentration. The results indicated that optimal decolorization occurred at 37°C and pH 7, particularly at a dye concentration of 50 mg/L. Under these conditions, the *Pseudomonas aeruginosae* isolates H5P, C2P, and C4P achieved 90% decolorization of reactive dyes. There were statistically significant differences ($p < 0.001$) among all environmental factors tested. These findings suggest that the isolated bacterial strains have considerable potential for the bioremediation of textile wastewater. This biological approach represents an environmentally sustainable and cost-effective alternative to conventional treatment methods. Further field trials and studies involving a broader range of dyes are recommended to validate their application in real-world wastewater treatment systems.

***Keywords*:** Biodegradation, Decolorization, Dye, Environmental pollution, Microorganisms, Screening, Textile effluent

1. INTRODUCTION

1.1. Background

Rapid industrial development resulting in environmental pollution is a challenge facing the modern world (Sakpal & Tarfe, 2021). The textile industry is a major source of environmental pollution, primarily due to the discharge of wastewater containing reactive dyes. The synthetic dye's nature and complex aromatic molecular structure make it more difficult and unstable for biodegradation (de Vasconcelos *et al*., 2024**).** These dyes contain colors that hinder light diffusion in water and harm artistic integrity, leading to a decrease in dissolved oxygen levels and affecting the photosynthesis rate of aquatic life, not only causing significant esthetic issues in water bodies but also posing serious health risks due to their toxicity and persistence in the environment (Sreedharan *et al*., 2021).

In Ethiopia, the textile sector has seen rapid growth, with many factories producing substantial volumes of dye-laden effluents in Africa and a top-ranked source of water pollution (Gizaw *et al*., 2024). One such example is Maa Garment and Textile Factory, located in Quiha, Tigray region, Ethiopia. This factory is an essential part of the local textile industry and plays a significant role in the economy by providing jobs and contributing to the export sector. However, like many textile facilities worldwide, Maa Garment discharges untreated or poorly treated wastewater into the environment, posing potential ecological and health risks. The effluents from this factory are particularly concerning due to their high dye content, which is challenging to treat through conventional methods. The existing wastewater treatment facilities in Tigray often do not handle the volume of wastewater generated, leading to the discharge of untreated or poorly treated wastewater into the environment. Many areas lack proper treatment plants, and those that exist are frequently non-functional or poorly maintained (Asgedom *et al*., 2023).

Traditional wastewater treatment methods, including chemical coagulation and oxidation, are commonly used. However, such approaches often generate harmful byproducts and incur high operational costs (Tanaya *et al*., 2024). In recent years, biological treatments using specific bacterial strains have emerged as promising alternatives. Bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have demonstrated remarkable capabilities in degrading

azo dyes, achieving decolorization rates of up to 90% under optimal conditions (Moyo *et al.*, 2022). Despite these advancements, there are critical gaps in the research regarding the long-term effectiveness and adaptability of these microbial treatments in real-world industrial settings.

1.2. Statement of the Problem

The rapid expansion of industrial activity has increased the discharge of hazardous effluents containing heavy metals, dyes, and organic contaminants. These pollutants pose serious risks to environmental and human health, including respiratory illnesses, skin disorders, and chronic conditions. Regardless of the current treatment methods and regulatory frameworks, many industries have failed to remove these dangerous chemicals. In Tigray, the textile industry continues to grow, but the existing wastewater treatment facilities are insufficient or non-functional. Large volumes of untreated wastewater, including high concentrations of reactive dyes, are being discharged into the water bodies. The harmful impact of these dyes on aquatic ecosystems, as well as their potential to contaminate drinking water and soil, requires the urgent development of effective and sustainable treatment methods. Traditional chemical treatment methods are not only costly but also often produce harmful byproducts, highlighting the need for alternative, eco-friendly solutions. As a result, there is a critical need to explore alternative treatment strategies, particularly biological methods that use naturally occurring dye-degrading agents, which offer a more sustainable and cost-effective solution to this issue. There is a lack of comprehensive data on effective bacterial strains, particularly in the Tigray context, and a limited understanding of how environmental conditions affect their performance. To fill these gaps, this study isolates, screens, and identifies bacterial strains that can break down textile colors, optimizes the degrading conditions, and assesses the suitability of the strains for application in environmentally friendly wastewater treatment systems.

1.3. Objective

1.3.1. General Objective

The major objective of this study is screening, and identifying potential dye-degrading bacteria isolated from Maa Garment textile effluents.

1.3.2. Specific Objectives

- To determine the physicochemical characteristics of textile effluents
- To screen bacterial isolates for dye-degrading activity under laboratory conditions
- To assess and optimize environmental factors (such as temperature, pH, and dye concentration) for the best decolorization performance of the bacterial isolates
- To assess the decolorization efficiency of promising bacteria isolates using spectrophotometric methods
- To identify bacterial isolates using morphological, physiological, and biochemical techniques

1.4. Significance of the Study

The release of dye-laden wastewater from the textile industry poses serious ecological risks, including reduced soil fertility, eutrophication, and harm to aquatic ecosystems. This study focuses on identifying dye-degrading bacteria from MAA Garment Textile effluents, offering eco-friendly bioremediation strategies to restore water quality, protect biodiversity, and improve public health. Biological treatments using bacteria provide sustainable and cost-effective alternatives to traditional methods, reducing environmental toxicity while supporting a circular economy. This research aims to uncover novel enzymatic pathways for industrial applications and explore genetic enhancements for improved degradation efficiency. The findings also promote cleaner production practices in the textile industry, contribute to scientific knowledge, and align with global sustainability goals. Overall, this study seeks to isolate bacterial strains capable of degrading reactive dyes and to optimize the biodegradation process by adjusting key environmental factors. To address these challenges, this study isolates and identifies bacterial strains from textile effluents, optimizing key environmental factors such as pH, temperature, and dye concentration to enhance biodegradation, supports environmental protection, economic efficiency, and sustainable industrial practices, addressing key challenges in pollution control and resource management.

2. LITERATURE REVIEW

2.1. Characteristics of the textile effluent

2.1.1. Color

Textile effluent is highly colored due to dyes and pigments used in production, with even low concentrations visibly impacting water quality. Most dyes are unstable, resistant to biodegradation, and difficult to remove, leading to artistic damage, reduced light diffusion, and lower dissolved oxygen levels, which disrupt photosynthesis in aquatic ecosystems. Additionally, synthetic dyes pose carcinogenic and mutagenic risks to humans (Anjali *et al.*, 2022). Their persistence makes color removal crucial, as concentrations below 1 ppm can hinder aquatic plant growth, affect gas solubility, and harm microbes (Mohamad Hanapi *et al.*, 2022). Studies show that after 24 hours of incubation, decolorization exceeds 60%, but the process slows significantly beyond this point, reaching only 1.3% removal at 120 and 168 hours. Effective removal remains a major challenge, prompting the exploration of methods such as adsorption, biological treatment, and advanced oxidation processes for textile wastewater treatment (Verma, 2021).

2.1.2. pH level and temperature

The dye and certain chemicals employed in the production process could affect the pH of the textile effluents. While some effluents are alkaline, others can be acidic. In dyeing processing using caustic soda, the pH of the effluent is typically alkaline, often exceeding the neutral range of 7. The pH of the textile effluent can affect the stability and solubility of the dyes and the action of the microorganisms involved in the degradation process (Abu Bakar *et al*., 2020). The pH of the textile effluent ranges from 6 to 10; therefore, maintaining the appropriate pH level of the textile wastewater is important for effective treatment. The high pH levels can be attributed to the use of alkaline substances in the dyeing and finishing processes. An elevated pH can adversely affect aquatic life and disrupt the natural balance of ecosystems. Maintaining the pH within permissible limits is critical for effective biological treatment processes (Chockalingam *et al*., 2019).

The temperature of the textile effluent also varies depending on the location and the season. The temperature of the textile effluent can influence the rate of microbial breakdown and chemical reactions generally higher than that of the receiving water bodies because of the heat produced during various processes, such as dyeing and washing. The high temperatures can have a negative impact on aquatic life by making oxygen less soluble in water (de Vasconcelos *et al*., 2024).

2.1.3. Total suspended solids

Total suspended solids are a critical parameter in water quality assessment, because they can affect aquatic life and the overall health of ecosystems. Monitoring these levels helps in evaluating pollution sources and implementing effective management practices. The total suspended solids (TSS) in the textile effluent consist of various substances that are suspended in water. In addition, it contains organic matter, salt, carbonates, chlorides, phosphates, and nitrates of calcium, magnesium, sodium, and potassium (Younas *et al.*, 2017). The discharge of untreated suspended particles into the aquatic environment can lead to anaerobic conditions and sludge formation. The textile effluent contains high levels of suspended solids, such as fibers, particles, and other debris (Adjovu *et al*., 2023). It can be challenging to effectively discharge wastewater when these solids clog pipes and other infrastructure (Wang *et al*., 2022).

2.1.4. Total dissolved solids

TDS is an additional crucial metric that indicates the amount of dissolved materials present in the water. The chemicals employed and the soluble organic and inorganic materials present in the processing units combine to generate them. Textile effluents often have elevated TDS levels owing to the presence of dyes, salts, and other chemicals used in the production process. The amount of all organic and inorganic materials present in the molecular, ionized, or suspended water is known as TDS (Islam & Mostafa, 2019). This includes the presence of soluble salts, such as carbonate, bicarbonate, calcium, chloride, magnesium, nitrate, phosphate, sodium, and sulfate that yield ions. Dissolved salts in water cause skin dehydration in animals and have a purgative effect and an unpleasant mineral taste. The increase in the osmotic pressure of the soil water can lead to higher respiration rates in plants, which may ultimately result in reduced

growth and yield for most species. Water with high TDS is unpalatable and potentially harmful to health and the environment (Chockalingam *et al*., 2019). These hazardous and harmful substances in the TDS, especially heavy metals (e.g., zinc, copper, chromium, nickel, cadmium, etc.), have strong biological toxicities, causing serious environmental problems and harm to humans. For the final discharge, the amount of materials dissolved into the effluent ranged from 1250 to 3610 mg/L. These higher amounts of total dissolved solids may be due to the release of chemical agents used during various processes in the textile industry (Abu Bakar *et al*., 2020).

2.1.5. Biological oxygen demand and chemical oxygen demand

Biological oxygen demand (BOD) and chemical oxygen demand (COD) are essential indicators of the organic content in the textile effluents. High BOD values indicate a significant presence of biodegradable organic matter and pollution in wastewater; however, COD reveals both biodegradable and non-biodegradable materials. The BOD and COD of the textile effluent are typically high because of the presence of organic compounds, such as dyes and other processing aids. These values indicate the amount of oxygen required for the biodegradation of the organic matter in the wastewater (Dhameliya & Ambasana, 2023). The amount of dissolved oxygen used by aerobic microorganisms during the decomposition of organic matter in water is influenced by the biochemical oxygen demand (BOD) (Hossain *et al*., 2018). It is an important water quality parameter; subsequently, it delivers a biological index to assess the effect of discharge water on the environment. Higher BOD values indicate a reduction in dissolved oxygen in aquatic life (Anjali & Singh, 2022).

On the other hand, the chemical oxygen demand is the ability of wastewater to consume oxygen during the decomposition of organic matter and the oxidation of inorganic chemicals, such as ammonia and nitrite. It is also a measure of water and wastewater quality, with BOD levels ranging from 200 to 600 mg/L and COD levels from 800 to 3000 mg/L in various textile industries, highlighting the need for advanced treatment techniques to reduce these parameters before discharge. The BOD5/COD ratio of wastewater is an essential indicator of its biodegradability (Panhwar *et al*., 2024).

2.1.6. General description and chemical composition of the dyes used in the textile industry

Dyes are organic compounds used in textiles and other materials to impart color by absorbing specific wavelengths of light. The textile industry employs a wide range of dyes, with synthetic dye production reaching approximately 700,000 tons annually, covering over 100,000 types (Bogale *et al*., 2024). Dyes are classified based on fiber compatibility, application method, and chemical structure, but a significant portion remains unfixed in textile effluents, posing environmental risks if untreated (Garcha *et al*., 2016). Their chemical composition includes chromophores, responsible for color via conjugated double bonds, and auxochromes, which enhance fiber binding and modify shades. Common chromophores include azo (-N=N-), anthraquinone, carbonyl (>C=O), and ethylenic (>C=C<), while auxochromes consist of hydroxyl (-OH), amino (-NH2), and sulfonic acid (-SO3H) groups (Patel Jay *et al*., 2023; Alzain *et al*., 2023b). The interaction between these components determines dye performance and fiber affinity. The rising demand for synthetic dyes has intensified wastewater pollution, making dye effluent a significant environmental concern (Islam & Mostafa, 2019).

2.1.7. Types of dyes

Dyes are classified into several types based on their chemical structure and fiber compatibility. Reactive dyes form covalent bonds with fiber groups such as *-OH*, *-NH*, and *-SH*, enabling strong adhesion, wide color range, and durability. They are primarily used for silk, wool, cotton, and cellulose fibers but exhibit significant hydrolysis, leading to environmental concerns (Patel *et al.*, 2022). Disperse dyes, mostly from the azo or anthraquinone categories, are used for synthetic fibers like polyester and nylon, requiring high temperatures for dyeing due to low water solubility (Sarkar *et al.*, 2017).

Acidic dyes, the largest class, are anionic and soluble in water, applied to wool, silk, and nylon under acidic conditions, offering good wash and light fastness (Afrin *et al*., 2021). Basic dyes contain cationic groups and are used for materials like nylon, wool, and leather, known for vibrant colors but lower fastness (Tanaya *et al*., 2024). Azo dyes, accounting for 70% of synthetic dyes, are highly adaptable but pose environmental and health risks due to their mutagenic and carcinogenic properties (Benkhaya *et al*., 2020). Sulfur dyes are used mainly for

cotton, providing dark shades but producing harmful dye bath effluents, prompting efforts to replace sulfide-reducing chemicals with sustainable alternatives (Verma, 2021). Vat dyes, insoluble in water, require reduction into a soluble form for dyeing cellulose fibers, offering excellent fastness in washing and light but are unsuitable for wool and silk due to alkaline sensitivity (Adane *et al.*, 2021). Each dye type has unique applications, properties, and environmental considerations, influencing their industrial use and sustainability (Parthasarathy *et al*., 2022).

2.2. Health and Environmental Impacts of Dyes

2.2.1. Impact of dyes on the environment

Dyes negatively impact soil microbial populations, degrade water bodies, and harm flora and fauna due to their persistence, accumulation in aquatic sediments, and decomposition into carcinogenic or mutagenic compounds with low aerobic biodegradability (Hassaan & Nemr, 2017). Their toxicity varies based on exposure time and concentration, leading to growth reduction, neurosensory damage, metabolic stress, and death in fish, limiting human activities such as drinking, fishing, and irrigation. Additionally, dyes disrupt soil microorganisms, affecting plant germination and shoot/root elongation (Lellis *et al*., 2019). Even minimal dye concentrations in wastewater reduce transparency, interfere with gas solubility, block light transmission, lower dissolved oxygen levels, and hinder aquatic photosynthesis (Ajaz *et al*., 2020). The direct release of dye effluents and their breakdown products—containing carcinogens like benzoin and nitric oxide—pose significant health risks to plants, animals, and humans, further straining ecosystems (Al-Tohamy *et al*., 2022).

The dissolved oxygen concentration primarily affects the growth of plants, as plants play a major role in ecology by providing organic matter that contributes to soil fertility, protecting the soil from erosion, and serving as a habitat for organisms. Hence, the presence of dyes negatively affects the chlorophyll content of plants (Ngo & Tischler, 2022). The greater environmental concern with dyes is their absorption and reflection of sunlight entering the water since the presence of very small amounts of days in the water is highly visible, which affects the quality and transparency of water bodies, leading to damage to the aquatic environment by stopping the

reoxygenation capacity of the receiving water and interrupting sunlight that can go through the photosynthesis process (Gita *et a*l., 2017).

2.2.2. Impact of dyes on Human Health

Many dyes are water-soluble and easily absorbed through the skin, ingestion, or inhalation, posing carcinogenic and allergic risks. Upon absorption, dyes undergo biotransformation through intestinal microorganisms, skin enzymes, or liver reactions, distributing throughout the body and affecting the immune system, metabolism, growth, and fertility while causing damage to vital organs such as the liver, spleen, kidneys, and heart (Benkhaya *et al*., 2020). Exposure to synthetic dyes can weaken immune responses by inducing lymphocyte cell death, disrupting cytokine production, and generating reactive oxygen species (ROS) that impair immune cell function (Jadhav *et al*., 2016). Additionally, dyes can negatively impact the gastrointestinal system, causing nausea, vomiting, and reduced food intake, while liver toxicity further hampers metabolism and growth. Reproductive health is also at risk, as certain dyes act as endocrine disruptors, interfering with hormone signaling and leading to fertility issues due to damage to the ovaries or testes (Barciela *et al.*, 2023).

The accumulation of dyes and their metabolites can lead to multi-organ toxicity. Liver Damage: The liver metabolizes many dyes, and their metabolites can be hepatotoxic, leading to conditions such as fatty liver disease or hepatitis. The kidneys are responsible for excreting waste products, including dye metabolites (Bharagava & Chowdhary, 2018). Accumulation can lead to nephrotoxicity, affecting kidney function. Some dyes can induce oxidative stress or inflammation in cardiac tissues, leading to heart damage.

Direct contact with dyes can lead to skin sensitization and allergies. Dyes can bind to proteins, forming hapten-protein complexes that trigger allergic reactions in sensitized individuals. The immune response to these allergens results in inflammation, which can manifest as rashes or dermatitis (Al-Tohamy *et al*., 2022). Certain dyes, particularly those containing aromatic amines, have been shown to cause DNA damage. Upon reduction by intestinal micro flora, dyes can release aromatic amines, which can intercalate into DNA strands, leading to mutations. The resulting DNA damage can lead to errors during cell division, which may result in malignant

transformations and tumor formation. Contact with dyes can lead to irritation of the eyes. Many dyes can cause direct chemical burns or irritation to the conjunctiva and cornea, leading to redness, swelling, and discomfort (Bogale *et al*., 2024).

2.2.3. Impact of dyes on Animal Health

Dyes in the water ecosystem have an impact on aquatic biota. Following uptake or consumption of these dyes, the mammalian intestinal anaerobic bacteria cleave the dyes into toxic colorless aromatic amines and will be absorbed by the intestine, causing significant physiological disorders including intermittent fever, hypertension, renal damage, and cramps through the food chains reaching humans (Ngo & Tischler, 2022). Aquatic vertebrates like fish and tadpoles contact the aquatic systems through gills, the primary organs for respiration, osmoregulation, and acid and base balance, making them highly susceptible to dye pollution and causing physiological malfunctions in these organs leading to homeostatic disorder (Alzain *et al*., 2023a).

Hydra attenuata, used as a model for studying the ecotoxicological impact of dyes, exhibits antioxidant defense mechanisms but is highly affected by Disperse Red 1 in freshwater. High concentrations of this dye interfere with metabolic processes, reducing energy production and overall efficiency (Gita *et al*., 2017). The dye overwhelms antioxidant defenses, increasing oxidative stress and cellular damage, leading to morphological changes such as alterations in body shape and size, impairing survival (Gulcin, 2020). Histological examinations show structural damage affecting health and function, while neurotransmitter distribution is disrupted, altering behavior and responsiveness. These effects impact locomotion and survival behaviors, while decreased feeding rates—likely due to sensory impairment—negatively affect growth and reproductive success, contributing to population declines in affected environments (Varjani *et al*., 2020a).

2.3. Biodegradation of dyes by microorganisms

Biodegradation is an essential environmental process in which microorganisms, particularly bacteria, break down organic materials into simpler, non-toxic compounds through enzymatic activity. The duration of this process varies depending on the material and environmental

conditions. Biodegradation occurs in three main stages: biodeterioration, biofragmentation, and bioassimilation (Nawanir, 2022).

Biodeterioration, the initial stage, involves microorganisms colonizing the material's surface, initiating physiochemical alterations that weaken its structure. Enzymatic and metabolic interactions degrade its composition, influenced by moisture, temperature, pH, and nutrient availability. Despite often being seen as destructive, biodeterioration plays a crucial role in natural recycling by breaking down both organic and synthetic substances for ecosystem reintegration (Han *et al*., 2024).

Biofragmentation is the second stage of biodegradation and is the lytic process that encompasses the splitting or breaking of the bonds of a polymer into oligomers and monomers as shown in Figure 1. This process is facilitated by the action of extracellular enzymes produced by the microorganism. Biofrgametation is essential as it increases the surface area of the material, allowing for more efficient microbial attack and further degradation. The resulting smaller fragments can be more easily assimilated by microorganisms. When biofragmentation happens in the presence of oxygen, it is called aerobic digestion and anaerobic digestion when it happens in the absence of oxygen. Resultant biomass, residual material, water, and carbon dioxide are produced by both reactions. However, anaerobic digestion yields additional products like methane gas or hydrogen sulfide (Coppola *et al*., 2021).

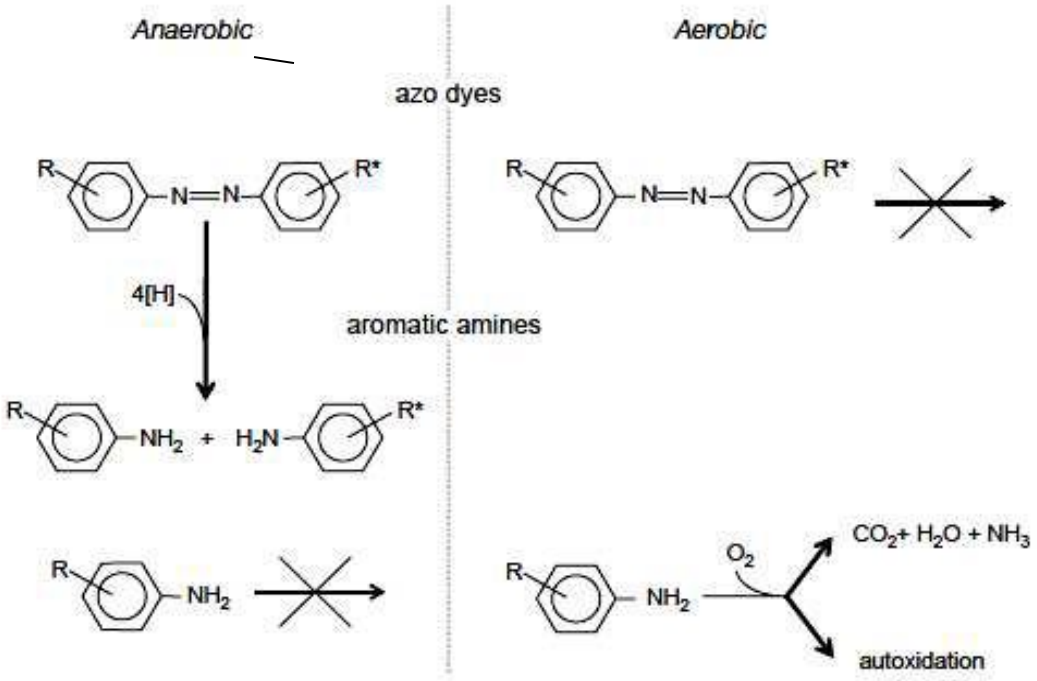

Figure 1: The lytic process of dyes during anaerobic and aerobic treatment (Selvaraj *et al*., 2021)

2.3.1. Aerobic biodegradation

The most interesting organic pollutant oxidase has been identified in microorganisms that live under aerobic conditions (Mirbolooki *et al*., 2017; Melese, 2020). Aerobic biodegradation, also known as aerobic respiration, is the breakdown of organic pollutants by microorganisms when oxygen is present. The major byproducts of aerobic respiration are carbon dioxide, water, and an increased population of microorganisms (Bremer, 2022). The aerobic treatment potentially has superior performance on organic pollutant degradation than the anaerobic treatment because all sludge generated in the aerobic biological treatment returns into the anaerobic biological stage through a sedimentation tank. Because the sludge in the anaerobic biochemical stage has sufficient hydraulic retention time to carry out anaerobic digestion thoroughly (Fernandes *et al*., 2022).

2.3.2. Anaerobic biodegradation

Anaerobic biodegradation is the breaking down of organic material by microorganisms in the absence of oxygen, also known as anaerobic respiration. The anaerobic systems could decrease

the color intensity more adequately than the aerobic processes (Guadie *et al*., 2017). The presence of organic substances like glucose, yeast extract, hydrolyzed starch, propionate, butyrate, and acetate are used as electron donors for azo reduction instead of oxygen. It is effectively used to treat wastewater sludge and biodegradable waste because it provides volume and mass reduction of the input material (Sutkar *et al*., 2022). Anaerobic digestion is a renewable energy source since the process produces methane and carbon dioxide-rich biogas suitable for energy production, serving to replace fossil energy. Also, the nutrient-rich solids left after digestion can be used as toxins. The anaerobic process includes hydrolysis, which is an enzyme-mediated transformation of complex organic compounds into simple compounds; acid genesis is the bacterial conversion of simple compounds into the substrate; and methanogenesis is the bacterial conversion of methanogenic substrates into methane and carbon dioxide (del Rosario Salazar Sánchez *et al*., 2023).

The final stage of biodegradation is bioassimilation, where the smaller fragments produced during biofragmentation is taken up by microorganisms. In this stage, the fragments are metabolized, leading to the conversion of these materials into biomass or energy. The end products of bioassimilation are typically carbon dioxide, water, and other simple inorganic compounds, which are less harmful to the environment. This stage is dynamic for the recycling of nutrients back into the ecosystem. Bioassimilation is the process of the products of the accomplished former biofragmentation being absorbed by the surrounding medium at the microbial cell level. Most of the products of the biodeterioration and biofragmentation that have just been completed are transported throughout the cells by means of biological mechanisms that regulate the movement of solutes like small molecules and ions through biological membranes (Sutkar *et al*., 2022). Other derivative products of biodeterioration and biofragmentation that have not been sufficiently decomposed and conditioned to an easily assimilatable degree will have to undergo further decomposition and bio-conditioning to break down and simplify them enough to be diffused through the microbial cell membranes (Moshood *et al*., 2022).

2.3.3. Bacterial biodegradation mechanism of dyes

Traditional wastewater treatment in the textile industry includes chemical coagulation, adsorption, advanced oxidation processes (AOPs), and biological methods. Chemical coagulation

and flocculation effectively remove suspended particles and color but generate harmful byproducts, increase sludge production, and require large chemical inputs (Verma, 2021). Adsorption using activated carbon is efficient but costly, necessitating frequent adsorbent regeneration. AOPs, including ozonation, UV treatment, and Fenton's reagent, are energy-intensive and unsuitable for large-scale applications (Moyo *et al*., 2022). Biological treatments, utilizing microorganisms for organic pollutant degradation, offer a more sustainable and cost-effective alternative. Microbial biodegradation of dyes occurs through biodeterioration, biofragmentation, and bioassimilation, contributing to environmental detoxification. Bacterial species such as *Klebsiella, Salmonella, Escherichia coli,* and *Pseudomonas* exhibit strong dye decolorization and detoxification capabilities (Upadhyay *et al*., 2023). These microorganisms are widely used due to their ease of cultivation, rapid growth under diverse conditions, and ability to withstand extreme environmental factors like temperature, pH, and salinity fluctuations. Their expression of azoreductase enzymes plays a crucial role in dye decolorization (Akansha *et al*., 2023).

The bacterial oxide reductive enzymes are significant in the degradation of dyes, and the dynamic metabolism of bacteria assists them in using complex xenobiotic compounds of the dyestuff as a substrate. The mechanism of bacteria for the biodegradation of dyes is divided into two stages. The initial phase is the reductive breakage of azo bonds (-N=N-) in the chromophore group, which entails the two-stage transfer of four electrons. In each step, two electrons are transferred from the dye to its final electron acceptor by an enzymatic biotransformation reaction, which leads to the formation of colorless aromatic amines (Pinheiro *et al*., 2022). Further degradation of the resulting toxic aromatic amines into simpler, nontoxic forms under anaerobic and aerobic conditions is the second step (Shah, 2014).

A variety of enzymes produced by bacteria are crucial to dye breakdown. The key enzymes include azoreductase. These enzymes reduce azo bonds (N=N) in azo dyes, converting them into colorless amines, which are less toxic and easier to further degrade (Ajaz *et al*., 2020). The optimum pH for azoreductase activity usually drops within the range of 6–8 although some enzymes may exhibit optimal activity at slightly acidic or alkaline pH values. The optimal temperature for azoreductase activity typically range between 25 and 40℃ (Goud *et al*., 2020). Laccase enzymes are multicopper oxidases that can oxidize various phenolic compounds and

effectively decolorize various dyes. Ligninolytic enzymes produced by certain fungi and bacteria are known for their ability to degrade complex aromatic compounds, including dyes *(Patel Jay et al*., 2023). The optimum pH for laccase activity, depending on the specific laccase and dye targeted, commonly ranges from 4 to 7. Nonetheless, it has been noted that some laccases perform best at greater pH levels, up to pH 9. The optimal temperature for laccase activity typically falls within the range of 25–60 ℃ (Sridharan *et al*., 2021).

Bacterial cells adsorb dye molecules via ionic bonds, hydrogen bonds, and van der Waals forces, concentrating them for enzymatic degradation. Some bacteria uptake dye molecules and metabolites, accumulating them within cells, aiding detoxification and transformation into less toxic forms (Bhatia *et al*., 2017). Bacteria utilize dyes as carbon and energy sources, degrading them into carbon dioxide and water through metabolic pathways, completing biodegradation. Effective bacterial strains enhance wastewater treatment and reduce dye effluent impact, supporting eco-friendly bioremediation (Mishra *et al*., 2022).

Species such as *Bacillus, Citrobacter, Pseudomonas, Clostridium, Staphylococcus, Proteus, Shigella, E. coli*, and *Micrococcus* play a crucial role in anaerobic dye decolorization, ensuring high mineralization, cost-efficiency, and minimal sludge production (Singh *et al*., 2017). *Escherichia coli* reactive blue and red from 75 to 98.5 % (Rane & Joshi, 2021) and (M-Ridha *et al*., 2020). *Klebsiella sp*. 94-97% (Desai, 2017). *P. aeruginosa* Reactive Red 91% (Sheth & Dave, 2009; Ajaz *et al*., 2020) and Reactive Yellow 96% (Moyo *et al*., 2022). From industrial effluent, *Klebsiella pneumoniae* was isolated in 24 hours, resulting in 90 % decolonization of DB-284 under 37℃ and a pH of about 7 (Das *et al*., 2023). Additionally, reactive blue-19 at a concentration of 100 mg L−1 in the incubation of 24 h at pH 7 and 37℃ temperature degrades 90 % by *Klebsiella sp*., as also reported by (Holkar *et al*., 2018). *Pseudomonas aeruginosa* RS1 for Reactive Yellow 145 at a concentration of 50 mg L−1 and an incubation time of 96 h at a pH of 7.5–8 and at 37 ℃ biodegraded 100 %, also reported by (Bharathi *et al*., 2022).

Despite the sustainability and cost-effectiveness of biological treatments, significant challenges remain in textile wastewater biodegradation. Reactive dyes, due to their complex chemical structures, are particularly resistant to traditional microbial degradation. Existing research has largely focused on a narrow range of dyes or laboratory settings that fail to reflect real industrial

effluent conditions (Mishra *et al*., 2022). Additionally, key environmental factors such as pH, temperature, and dye concentration critically impact microbial activity, yet their optimization for practical applications is often overlooked (Sreedharan *et al*., 2021). While progress has been made, many studies primarily examine azo dyes, neglecting the diversity of reactive dyes used in the textile industry, which are more resistant to degradation (Akansha *et al*., 2023). Furthermore, inadequate exploration of environmental factor optimization limits the effectiveness of microbial treatments, as many studies fail to account for the variability in industrial effluents (Gita *et al*., 2017).

Understanding the effects of these factors on biodegradation is crucial for real-world applications. Microbial Strain Identification: Although bacterial species such as *Pseudomonas aeruginosae* and *Klebsiella pneumoniae* have been reported to degrade reactive dyes, research on the long-term stability and adaptability of these strains under diverse environmental conditions is limited. More studies are needed to characterize and identify novel microbial strains with superior dye-degrading capabilities (Tanaya *et al*., 2024). Scalability and Industrial Application: Existing studies generally fail to address the scalability of microbial treatments for large-scale industrial applications. Many biological treatments that have been success in the laboratory do not efficiently translate to industrial-scale wastewater treatment (Dixit & Garg, 2021). This study aims to address these gaps by isolating and screening bacterial strains capable of degrading a broader spectrum of reactive dyes commonly used in textile industries. The study also explores the optimization of environmental conditions, including temperature, pH, and dye concentration, to determine the optimal conditions for biodegradation. Additionally, this study focuses on evaluating the long-term adaptability of the microbial strains under real-world effluent conditions, aiming to develop more effective, sustainable, and scalable biological treatments for textile wastewater.

2.4. Factors Affecting Biodegradation of Dyes

2.4.1. Availability of Nutrients

The existence and abundance of microorganisms in an environment are determined not only by available carbon but also by various physical and chemical factors. These include oxygen

availability, nutrient availability, pH, temperature, salinity, and water activity, which are parameters that influence the degradation process of contaminant. These parameters impact the structure and physiology of microbial communities and change the physical and chemical properties of the pollutants (Krishnan *et al.*, 2017).

As a result, the effectiveness of these treatment systems depends upon the survival and adaptability of the microorganisms during the treatment processes. Dyes are deficient in carbon and nitrogen sources since most microorganisms generally cannot utilize dyes as carbon or nitrogen sources for growth, and the biodegradation of dyes without any supplement of these sources is very difficult. Such bacterial cultures require carbohydrate sources or complex organic sources such as yeast extract, peptone, or a combination of both for the high and quick dye decomposition rate. The rate and efficiency of biodegradation are affected by the addition of nutrients, which also modifies the vital nutritional balance for microbial growth and reproduction. To survive and continue the microbial activities of microorganisms, they need some nutrients such as carbon, nitrogen, and phosphorus in the ratio of **2:1:1**, respectively (Chittal *et al.*, 2019). Although the dye degradation efficiency can be increased by the addition of glucose because it is the most effective and easily available carbon source for microbial metabolism of dyes or color intermediates, and phosphorus has been identified as a critical component for microbial development (Varjani & Upasani, 2019).

2.4.2. Temperature

Temperature influences microbial growth and metabolic activity because it highly influences microbial physiological properties to facilitate or slow down the biodegradation process (Arunprasath *et al*., 2019). Biological enzymes that have participated in the degradation pathway have an optimum temperature and will not have the same metabolic turnover for every temperature. Besides, the degradation process for specific compounds requires a specific temperature. High temperatures can cause azo-reductase to become denatured. It is observed that increasing the temperature in a certain range increases the decolorization rate, and further increasing the temperature drastically decreases the degradation rate because the activity of microorganisms decreases (Alzain *et al*., 2023a).

The most microbes, the average optimal temperature for their enzymatic activity is around 25–40 ℃ (Shi *et al*., 2021). It has been demonstrated that the rate of color removal increases with temperature up to a certain point, after which the decolonization activity somewhat decreases. Extreme temperature harms most microorganisms by affecting growth, losing cell viability, or denaturing related enzymes (Guo *et al*., 2020). *Enterobacter sp.* decolorization of Reactive Black 5 increased in temperature from 22°*C* to 37°*C*, and a further increase in temperature to 42°*C* drastically affected the decolorization activity of the bacteria. The rate of temperature required for the removal of color corresponds with the optimum cell culture growth temperature of 35-45 ^{0}C**,** and the decline of color deletion activity at a high temperature may cause the azo-reductase enzyme to become denatured or cause cells to become less viable. Nonetheless, it has been demonstrated that the azo reductase enzyme is reasonably thermostable and may continue to function at temperatures as high as 60 ℃ when used with specific whole bacterial cell preparations (Ajaz *et a*l., 2020).

2.4.3. pH

The pH is an important factor for the growth of bacteria and also an essential characteristic for effluent treatment. The measurement of pH in degradation could indicate the potential for microbial growth. Also, the acidity, basicity, and alkalinity nature of the compound has an impact on microbial metabolic activity and the removal process (Wang *et al*., 2022). The decolorization rate is higher at optimal pH and decreases at a more acidic or alkaline pH. Textile industrial processes take place mostly under alkaline conditions; thus, tolerance to high pH is important in particular. The optimal often being between 6.0 and 10.0 (Jamee & Siddique, 2019). However the higher or lower pH values showed substandard results; even slight changes are highly susceptible to metabolic processes. The decolorization rate decreases at a more acidic or alkaline pH nonetheless is higher at optimal pH. The effectiveness of dye decolorization is highly affected by pH, and the optimum pH of bacterial biodegradation is commonly optimized from 6.0 to 10.0. Adjusting the pH of the solution might solve the problems of deciding on the microbial strain performance well at effluent pH and enhance the ability of dye degrade (Yaseen & Scholz, 2019). The pH of the effluent can affect the rate of dye degradation in dye-containing wastewater; therefore the issue can be resolved by modifying the pH to support the growth of

dye-degrading bacteria or by selecting the microbial species. That can grow at the effluent pH (Varjani *et al*., 2020b).

2.4.4. Dissolved oxygen

Different organisms want oxygen; others also do not want oxygen based on their requirement to enable the biodegradation rate in a superior way. Since the majority of living things require oxygen as a gas, biodegradation occurs in both anaerobic and aerobic settings. The biodegradation metabolism in most situations can improve with the presence of oxygen (Macaulay, 2015). Diverse groups of bacteria decolorize dyes under anaerobic, facultative anaerobic and aerobic conditions. The structure of the synthetic dyes is broken down by reductive enzymes, which are more active in anoxic environments for those working in anaerobic environments. Dissolved oxygen is considered an inhibitor of the azo dye reduction process, since both molecules act as electron acceptors and oxygen is a much stronger oxidant (Jamee & Siddique, 2019). Then the decolorization is significantly affected by the high-redox-potential electron acceptors and dissolved oxygen. In the dye reduction process, dissolved oxygen is considered an inhibitor, and oxygen is a great deal stronger oxidant given that both molecules act as electron acceptors. The hang-up of dye reduction has a tendency to only be a temporary effect rather than an irretrievable effect under aerobic conditions. Environmental conditions can affect the dye degradation and decolorization process directly depending on the reductive or oxidative status of the environment and indirectly influencing microbial metabolism (Lee *et al*., 2018).

2.4.5. Effect of dye concentration and structure

The dye structure and concentration have a significant influence on the rate of dye decolorization. Low dye concentration may not be recognized by enzymes that are secreted by dye-degrading bacteria. On the other hand, high dye concentration is toxic to bacteria and also affects the degradation of dye by blocking enzyme active sites (Zhuang *et al*., 2020). Dyes are preeminent to decolorize in the situation of low molecular weight and simple structure. Although high molecular weight and complex structure-holding dyes have low decolorization rates (Li *et al*., 2019).

Dye concentration gradually decreases the decolorization rate. This may be because of the toxic effect of dyes on the microorganisms (Jamee & Siddique, 2019). In addition to dye molecules with various structures obstructing the azoreductase active sites, there are a number of additional potential causes, such as an insufficient cell-to-dye ratio. By preventing the azo (-N=N-) link from cleaving, complex dye structure also affects the decolorization process. The microorganism and azo dye concentration affect the degradation activity. The increased dye concentration decreases dye decolorization and degradation rate (Li *et al*., 2019). The dye concentration in textile wastewater has also been observed to vary widely, with some studies reporting amounts between 10 and 50 mg/L and others reporting values between 10 and 250 mg/L (R Ananthashankar, 2013). Most findings showed that the maximum degradation efficiency of 97% was achieved at a concentration of 50 mg/L. It is therefore important that any potential organisms should be able to effectively degrade or decolorize the target dyes without any inhibition at these dye concentrations for the bacteria to be effective in industrial applications (Moyo *et al*., 2022).

Low concentrations of dyes have little impact on biodegradation efficiency since they may not be identified by enzymes secreted from microorganisms. High levels of some pollutants' poisonous properties, on the other hand, can slow down decontamination and have harmful consequences for microbes (Khan & Mathur, 2015). *Lead* and *cadmium,* which are major contaminants, found in the environment, are extremely poisonous to humans, animals, plants, and microbes, which can damage cell membranes, alter the particularity of enzymes, and destroy the structure of DNA. The displacement of essential metals from their native binding sites or ligand interaction toxicity is created (Shi *et al*., 2021).

Color removal of dyes demonstrated with simpler structure and low molecular weight is important. Biodegradation of dyes depends on not only the breakage of bonds but also the molecular structure of dyes and the substituent group types on the aromatic rings (Chen *et al*., 2020). An impact on oxidation has been shown through the nature of substituents on the aromatic ring. Studies have verified that electron-donating methyl and methyl substituents improve the enzymatic degradation of azo phenols, while electron-withdrawing nitro, chloro, and fluoro substituents inhibit oxidation**.** Usually dyes with low molecular weights and simple chemical structures exhibit higher decolorization rates (Zhuang *et al*., 2020).

2.5. Isolation and screening of dye-degrading bacteria

2.5.1. Isolation of dye-degrading bacteria

Dye-contaminated environments like textile effluent treatment plants, soil samples near dye manufacturing, and industrial wastewater discharge serve as rich sources for isolating dye-degrading bacteria. Enrichment culture techniques employing a specific dye as the sole carbon source are commonly used to selectively cultivate bacteria capable of degrading the target level. Isolation of dye-degrading bacteria typically involves the enrichment culture technique, where samples from contaminated sites are incubated in media containing specific dyes as the sole carbon source (Sakpal & Tarfe, 2021).

This method has been widely adopted due to its effectiveness in selecting bacteria capable of degrading complex organic compounds (Singh *et al*., 2017). The successful isolation of *Pseudomonas putida* from textile effluents exhibited significant decolorization of azo dyes. Another promising approach is the use of selective media that incorporate dyes along with other nutrients. This method not only facilitates the growth of dye-degrading bacteria but also suppresses non-degrading species (Mehzabin & Ahsan, 2024). Recent advancements in molecular techniques, such as metagenomics, have also enhanced the isolation process by allowing researchers to identify and cultivate previously uncultivable bacteria (Li *et al*., 2023).

2.5.2. Screening of dye-degrading bacteria

Visual color change in dye-containing medium after bacterial incubation serves as a simple screening method for dye degradation, with clear zones around colonies indicating degradation. Various assays assess bacterial dye-degrading abilities, with spectrophotometry being the most common. This technique quantifies decolorization by measuring the absorbance decrease at the dye's maximum wavelength post-incubation (Rahayu *et al*., 2023). And the decolorization of the dye percentage that was degraded by the isolates was calculated quantitatively. The spectrophotometry approach allows for the assessment of the degradation efficiency of isolated bacteria by measuring the absorbance of its wavelength at its maximum level (Afena *et al*., 2021). Screening methods are crucial for identifying bacteria with the ability to degrade dyes effectively. Common techniques include colorimetric assays, where the reduction in dye

concentration is measured by spectrophotometry. Assessing decolorization efficiency is a best method of screening process that involves incubating isolated bacteria in dye-containing media by measuring the absorbance at specific wavelengths (Roopa *et al*., 2024).

Absorbance measurements are crucial for tracking dye biodegradation in microbiological and industrial biotechnology studies. The Beer-Lambert Law establishes a direct correlation between absorbance and the concentration of an absorbing species in solution. Researchers monitor absorbance variations at specific wavelengths to quantify dye decolorization, serving as an indicator of microbial degradation (Montesinos-Cruz & Somerville, 2024). Every dye has a unique maximum absorbance wavelength (λ max) that indicates how much light it can absorb at its highest level in the visible spectrum. The formula Decolorization (%) = (A0−At/A0) ×100 is frequently used to determine the percentage of decolorization. Absorbance-based spectrophotometry is still a quick, inexpensive, and non-destructive method for screening a lot of isolates and determining how well they degrade dyes (Bachmann & Miller, 2020).

2.5.4. Characterization of dye-degrading bacteria

Characterization of these bacteria typically involves morphological, biochemical, and molecular techniques. Morphological characterization often includes microscopic examination and colony morphology assessment. Biochemical tests, such as catalase and oxidase tests, help in preliminary identification (Afena *et al*., 2021). Molecular techniques, particularly 16S rRNA gene sequencing, have become essential for accurate identification. Molecular techniques such as PCR and sequencing can be employed to identify specific genes associated with dye degradation pathways. This genetic screening provides insights into the metabolic capabilities of the isolated strains. Likewise, the use of high-throughput screening methods has accelerated the identification of effective dye-degrading bacteria, allowing for the simultaneous evaluation of multiple strains (Roopa *et al*., 2024).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Quiha Maa garment textile factory, located 10 kilometers southeast of Mekelle, the capital and special zone of Ethiopia's Tigray region. It is sited around 780 kilometers (480 miles) north of Addis Ababa, the capital of Ethiopia, it is 2,254 meters (7,395 feet) above sea level, with latitude 13^{o} 32 N and longitude 39^{o} 33 E. The mean annual temperature lied is between 16° and 20° Celsius (Hagos, 2023).

3.2. Sample Collection

Fifteen effluent samples of 30 ml were collected from different chambers of the Maa garment textile factory. The samples were collected in sterile plastic bottles and stored in an icebox container. Then, it was transported immediately to the Veterinary Medicine of Microbiology Laboratory and stored at a 4℃ refrigerator to avoid any physicochemical change in the wastewater.

3.3. Selection of dyes

Three types of dyes were selected namely reactive blue, reactive yellow, and reactive red dye, based on their chemical composition and most widely used in textile industries. The presence of reactive dyes can increase BOD and COD levels, indicating high organic pollution and potential toxicity to aquatic life.

3.4. Physicochemical Analysis of Effluents

Wastewater samples were analyzed for physicochemical parameters using APHA standard methods (Federation & Association, 2005). Color, odor, temperature, pH, chemical oxygen demand (COD), and biological oxygen demand (BOD) were measured using established techniques. Color intensity was determined by measuring absorbance at 450 nm in a colorimeter calibrated with distilled water. Temperature was recorded using a thermometer or probe after stabilization. pH was measured with a calibrated pH meter, ensuring accurate readings. COD

analysis involved digesting 50 mL of wastewater with potassium dichromate, sulfuric acid, and silver sulfate at 150°C for 2 hours, followed by spectrophotometric measurement at 600 nm. BOD was assessed by diluting 10 mL of textile wastewater into 90 mL of distilled water, measuring initial dissolved oxygen (DO1), incubating the sample at 20°C for five days, and recording final DO5 for calculation (Younas *et al*., 2017).

3.5. Isolation of Bacteria from Industrial Effluents

The collected samples were analyzed for the presence of microorganisms. The first 1 mL of each effluent sample was transferred into 9 mL of sterile saline solution in a test tube and shaken vigorously. The solutions were serially diluted, and 1 mL of bacterial culture mixed with the nutrient medium was plated using the pour plate technique on petri dishes. The bacteria were inoculated on nutrient agar medium containing (Peptone 5 g/l; yeast extract 1.5 g/l; beef extract 1.5 g; NaCl 5 g/l; agar 15 g/l; and pH to 7.00) and incubated at 37°C for 24 hours. Then pure isolates were obtained by the sub-culturing method, preserved in slant agar, and stored at 4°C for further tests (Sriram & Reetha, 2015).

3.6. Screening of the Potential Dye-Degrading Bacterial Isolates

A liquid enrichment medium supplemented with a specific dye that contains (peptone 5 g/L, sodium chloride, 5 g/l, yeast extract and beef extract 1.5 g/l, and reactive dyes of 50 mg/l at a pH 7) then incubated at 37°C for 24 hours. Following incubation of the medium isolated of bacteria were inoculated on the enrichment media and incubated at 37℃ for 24 hours later the results were assessed for biodegradation of reactive dyes by observing any clear color changes and the bacteria were identified as potential of reactive dye degraders. Then pure culture stocks of isolate were taken and stored at 4°C for further study (Jamee & Siddique, 2019).

3.7. Optimization of environmental factors

The optimization of environmental factors for the highest degradation rate of three reactive dyes by selected bacterial isolates involved studying the effects of temperature, pH, and dye concentration. For temperature optimization, the decolorization of reactive dyes was assessed at 25, 30, 37, and 40℃, using a medium enriched with dyes at a concentration of 50 mg/L, with pH

maintained at 7. The effect of pH was examined at levels of 5, 7, and 9 on the decolorization efficiency of the isolates, utilizing nutrient broth with reactive dyes at the same concentration and 37°C for incubation, with 0.01 M KOH and HCL used to regulate pH. Additionally, the impact of varying dye concentrations was evaluated through the broth culture method, with degradation assessed at concentrations of 50, 100, and 150 mg/L (Desai, 2017).

3.8. Dye decolorization assay

The bacterial isolate was enriched by co-incubating in nutrient broth containing reactive dyes. And inoculated test tubes were incubated for 24 hours. Following incubation, dye decolorization was observed, then samples were centrifuged at 10,000 rpm for 30 minutes, and the supernatants were confirmed to decolorize dyes by selected isolates, which were determined at their specific maximum wavelength using a UV spectrophotometer, and absorbance was recorded (Roopa *et al.*, 2024). Decolorization potential was measured in terms of percentage decolorization using a UV-spectrophotometer via the following formula, and dye-containing media without microbial inoculation served as controls.

$$\% \text{ Dye decolorization} = \frac{\text{Initial absorbance} - \text{final absorbance}}{\text{Initial absorbance}} \times 100$$

3.9. Identification of Dye-Degrading Bacterial Isolates

All bacterial isolates were characterized and identified culturally and morphologically based on their form, color, elevation, and margin, the surface of colonies on a nutrient agar plate, size, shape, cell arrangement, and staining properties. The biochemical tests were performed according to the laboratory manual and workbook in microbiology to checking the presence of a particular substance or enzyme produced by the bacterial isolate, and Bergey's Manual of Systematic Bacteriology was used to identify the isolates' species (Karim *et al*., 2018).

3.9.1. Gram staining test

All isolated colonies were subjected to Gram staining in accordance with standard protocol. A mild heat fixation was used to apply a smear of bacterial cells to a sterile glass slide. For one minute, a crystal violet solution was poured over the heat-fixed smear. Water was used to wash

the smear. Gram's iodine was used as a mordant, and then 95% ethyl alcohol was used to decolorize the smear before it was rinsed with water. Gram staining was performed for all isolated colonies according to the standard procedure. Finally, safranin was used as a counter stain for 60-80 seconds and washed with water. Cells were then examined under a microscope (National Institute of Open Schooling, 2012).

3.9.2. Motility test

Bacterial isolates were inoculated into the peptone water and incubated at 37^{o}C for 24 hrs. A drop of isolates was placed on the test using a sterile loop. Then the cover slip was carefully inverted and placed over the concave portion of the hanging drop. Finally, the result was observed for motility under a microscope.

3.9.3. Catalase test

2 ml of the H_2O_2 solution was poured into a sterile glass slide using a sterile wooden stick, and colonies of the test organisms were immersed in the hydrogen peroxide solution. The formation of bubbles was then observed (Sriram & Reetha, 2015).

3.9.4. KOH test

2 ml of potassium hydrogen oxide solution was poured into a sterile glass slide using a wooden stick, and colonies of the test organisms were immersed in the KOH solution. The formation of gelatin was then observed.

3.9.5. Indole Test

The isolates were inoculated into the peptone water tubes, and the tubes were kept for incubation at 37℃ for 24 hrs. After the incubation period, 2-3 drops of KOVAC 'S reagent containing P-dimethyl aminobenzaldehyde-5g, Amylalcohol-75, and Hcl-25ml were added, and the results were observed.

3.9.6. Citrate Test

In citrate agar containing (g/L) agar (15.0), NaCl (5.0), sodium citrate dipotassium (2.0), ammonium dihydrogen phosphate (1.0), phosphate (1.0), magnesium sulfate (0.20), and

bromothymol blue (0.08) dissolved in 1000 mL of distilled water, the isolates were streaked on agar slants and incubated at 37°C for 24 hours. After the incubation period, the results were observed.

3.9.7. Triple Sugar Iron (TSI) Test

The test was performed to detect the ability of organisms to ferment glucose, lactose, sucrose, and H2S. The isolates were stabbed in the TSI medium. The test medium contains 3 sugars: glucose (0.1%), lactose, and sucrose (1% each). Phenol red serves as an indicator. The medium contains a butt and a slant. Ferrous sulfate serves as an indicator of H_2S production. The medium is inoculated with a stab method on the butt and a stroke method on the slant at 37℃ for 24 hrs. After the incubation period, the results were being observed (Paul *et al*., 2020).

3.10. Data Analysis

All experiments were repeated in triplicates, and results were presented as Mean ± Standard deviation. The data were analyzed using mean and variance techniques, specifically Two-way ANOVA and Fisher's Least Significant Difference (LSD) test, to assess differences among treatments. Statistical significance was determined at $p < 0.05$ using Minitab 19 software.

4. RESULTS AND DISCUSSION

4.1. Physicochemical Analysis

The physicochemical analysis of wastewater samples from the Maa Garment and Textile Factory showed variations in key parameters, influencing treatment strategies and environmental impact as shown in Table 1. The pH ranged from 7.2 to 7.5, indicating a neutral to slightly alkaline condition, which is favorable for microbial activity. Sample 1 had a notably higher temperature (38°C), suggesting potential thermal pollution, whereas Samples 2 and 3 recorded lower temperatures with a value of 26.3°C and 26°C, respectively. COD values remained consistent (58.3–59 mg/L), reflecting similar organic pollutant levels, while BOD ranged from 26 to 27.6 mg/L, indicating moderate oxygen demand for degradation. TDS concentrations were highest in Sample 2 (2866 mg/L), followed by Sample 1 (2700 mg/L) and Sample 3 (2633 mg/L), revealing high dissolved substance levels impacting aquatic ecosystems. TSS was relatively low (28–30 mg/L), indicating minimal particulate presence. Odor intensity varied, with Samples 1 and 2 categorized as unpleasant, while Sample 3 had a milder odor. Color differences among samples gray (Sample 1), amber (Sample 2), and black (Sample 3) suggest contamination or anaerobic decomposition. These findings emphasize the need for proper wastewater treatment, as previous studies (Sisay *et al*., 2017; Holkar *et al*., 2016; Abu Bakar *et al*., 2020) have reported similar physicochemical characteristics in textile effluents, reinforcing the necessity of continuous monitoring to mitigate pollution risks.

Table 1: Physicochemical Characteristics of Samples

Parameters	Sample 1	Samples 2	Sample 3
pH	7.5	7.2	7.4
Temperature	38	26.3	26
Chemical oxygen demand	58.3	59	58
Biological oxygen demand	27.3	27.6	26
Total dissolved solid	2700	2866	2633
Total suspended solid	30	29	28
Odor	Unpleasant	Unpleasant	less odor
Color	Gray	Amber	Black

4.2 Isolation of bacteria

The cultural characteristics of 30 bacterial isolates from textile wastewater were assessed using selective media, including Nutrient Agar (NA), MacConkey Agar (MAC), Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMB), and Salmonella Shigella Agar (SSA) as shown in Table 2. All isolates showed positive growth on NA, confirming viability. Most were lactose fermenters (LF) on MAC, producing acid that turned the medium pink, while non-lactose fermenters (NLF), such as B5, C2, C3, C4, C6, C7, C8, C9, and C10, did not utilize lactose. None of the isolates fermented mannitol on MSA. On EMB, isolates H1–H10 exhibited green sheen and pink mucoid colonies, indicating strong lactose fermentation, while B5 and C3 appeared colorless or blue. SSA results showed that most isolates were negative for hydrogen sulfide (H2S) production, except H4 and H5, which displayed black-centered colonies.

Identifying bacterial strains from textile effluent is essential for bioremediation, as selective media aid in enriching for dye-degrading bacteria. Previous studies by Sakpal & Tarfe (2021) and Singh *et al*. (2017), highlight the significance of selective enrichment in isolating strains with high decolorization efficiency. Mishra *et al*. (2022) and Afena *et al*. (2021) further demonstrated the role of microbial diversity in textile effluent bioremediation. Given their metabolic versatility, lactose-fermenting isolates are particularly suited for dye wastewater treatment, offering potential applications in biotechnology, environmental management, and industrial waste treatment.

Table 2: Cultural characteristics of isolated bacteria

No	**Isolated bacteria**	**Cultural characteristics**				
		NA	**MAC**	**MSA**	**EMB**	**SSA**
1	H1	+VE	LF	-VE	Green sheen, pink mucoid, colorless	-VE
2	H2	+VE	LF	-VE	Green sheen, pink mucoid, colorless	Colorless
3	H3	+VE	LF	-VE	Green sheen, pink mucoid, colorless	-VE
4	H4	+VE	LF	-VE	Green sheen, pink mucoid, colorless	Colorless with a black
5	H5	+VE	LF	-VE	Pink mucoid, colorless	Colorless with black center
6	H6	+VE	LF	-VE	Pink mucoid, colorless	-VE

7	H7	+VE	LF	-VE	Pink mucoid, colorless	-VE
8	H8	+VE	LF	-VE	Pink mucoid, colorless	-VE
9	H9	+VE	LF	-VE	Pink mucoid, colorless	-VE
10	H10	+VE	LF	-VE	Pink mucoid, colorless	-VE
11	B1	+VE	LF	-VE	Colorless	-VE
12	B2	+VE	LF	-VE	Colorless	-VE
13	B3	+VE	LF	-VE	Colorless	-VE
14	B4	+VE	LF	-VE	-VE	-VE
15	B5	+VE	NLF	-VE	Blue colonies	-VE
16	B6	+VE	LF	-VE	Colorless	Colorless
17	B7	+VE	LF	-VE	Colorless	-VE
18	B8	+VE	LF	-VE	Colorless	-VE
19	B9	+VE	LF	-VE	Colorless	-VE
20	B10	+VE	LF	-VE	Colorless	-VE
21	C1	+VE	LF	-VE	-VE	-VE
22	C2	+VE	NLF	-VE	Colorless	Colorless
23	C3	+VE	NLF	-VE	-VE	-VE
24	C4	+VE	NLF	-VE	Colorless	-VE
25	C5	+VE	LF	-VE	-VE	-VE
26	C6	+VE	NLF	-VE	Colorless	-VE
27	C7	+VE	NLF	-VE	Colorless	-VE
28	C8	+VE	NLF	-VE	Colorless	-VE
29	C9	+VE	NLF	-VE	Colorless	-VE
30	C10	+VE	NLF	-VE	Colorless	-VE

N.B: H stands for Homogenization, B for Biological wastewater, C stands for Clarification, NA for Nutrient Agar, MAC for MacConkey Agar ,MSA for Mannitol Salt Agar, EMB for Eosin Methylene Blue Agar, SSA for Salmonella Shigella agar, LF for Lactose fermentor, NLF for Non Lactose Fermentor, -VE for Negative and +VE for Positive.

4.3. Screening of potential bacteria for biodegradation of selected dyes

Of the 30 bacterial isolates screened, 16 showed positive decolorization, while 14 failed to degrade the dyes as Table 3 indicates. Isolates H1–H10 and selected group C members (C2, C4, C6, C8, and C10) exhibited strong dye degradation, suggesting enzymatic pathways essential for breakdown. Conversely, isolates B1–B10 and certain group C members (C1, C3, C5, and C9) did not degrade the dyes, indicating limitations in reactive dye degradation. Effective isolates

utilized dyes as their sole energy source, making them promising for textile dye removal (Sakpal & Tarfe, 2021). Bacteria causing dye color change in nutrient broth as Figure 2 were identified as efficient decolorizers, which is consistent with previous study (Rahayu *et al*., 2023). The screening of bacterial isolates for dye degradation is essential for bioremediation, with positive results highlighting their potential in wastewater treatment. The finding of the current study aligns with previous research done on azo dye degradation (Pinheiro *et al*., 2022).

Some isolates can decolorize up to 90% of specific dyes within 24 hours, demonstrating the effectiveness of microbial techniques in wastewater treatment (Roopa *et al*., 2024). These bacteria likely produce azoreductase, an enzyme responsible for cleaving azo bonds, leading to dye decolorization. Understanding these enzymatic mechanisms is essential for optimizing bioremediation strategies. According to Akansha *et al*. (2023), azoreductases play a significant role in enhancing azo dye degradation. The successful screening of bacterial isolates strengthens their potential for textile wastewater treatment, and their high decolorization rates suggest that integrating them into bioremediation efforts can mitigate the environmental impact of textile effluents.

Table 3: Screening of potential dye degradation of isolated bacteria

No	**Isolated bacteria**	**Decolorization of reactive dyes based on color change**		
		Red	**Blue**	**Yellow**
1	H1	+VE	+VE	+VE
2	H2	+VE	+VE	+VE
3	H3	+VE	+VE	+VE
4	H4	+VE	+VE	+VE
5	H5	+VE	+VE	+VE
6	H6	+VE	+VE	+VE
7	H7	+VE	+VE	+VE

8	H8	+VE	+VE	+VE
9	H9	+VE	+VE	+VE
10	H10	+VE	+VE	+VE
11	B1	-VE	-VE	-VE
12	B2	-VE	-VE	-VE
13	B3	-VE	-VE	-VE
14	B4	-VE	-VE	-VE
15	B5	-VE	-VE	-VE
16	B6	-VE	-VE	-VE
17	B7	-VE	-VE	-VE
18	B8	-VE	-VE	-VE
19	B9	-VE	-VE	-VE
20	B10	-VE	-VE	-VE
21	C1	-VE	-VE	-VE
22	C2	+VE	+VE	+VE
23	C3	-VE	-VE	-VE
24	C4	+VE	+VE	+VE
25	C5	-VE	-VE	-VE
26	C6	+VE	+VE	+VE
27	C7	+VE	+VE	+VE
28	C8	+VE	+VE	+VE

29	C9	-VE	-VE	-VE
30	C10	+VE	+VE	+VE

N.B: H stands for homogenization, B for biological treatment, and C for clarification; +VE stand for color change, and –VE stands for unchanged color.

Figure 2 : Screening of isolated bacteria based on their color change of the given dyes

NB: Figure A is reactive red, figure B is reactive yellow and figure C is reactive blue while the clear solution indicates successful degradation, likely due to bacterial isolates having enzymatic activity.

4.3.1. Zone of decolorization of screened bacteria

The results in Table 4 highlight variations in the zone of inhibition among bacterial isolates, reflecting differences in dye degradation efficiency. H5SAL exhibited the largest decolorization zones (37 mm for red, 35 mm for blue, and 33 mm for yellow), indicating strong biodegradation capacity, while C2SH showed similarly high effectiveness (36 mm for red, 34 mm for blue, and 32 mm for yellow). Moderate decolorization zones were recorded for H1E, H2E, H3E, and H5E (27–32 mm), suggesting stable but slightly lower biodegradation potential, while C2P and H5P demonstrated adaptability (29–33 mm). Lower decolorization zones observed in H1K, H2K, H3K, and H4K (15–23 mm) indicate reduced efficiency; with H3K showing the smallest

decolorization zone for yellow dye (15 mm). The effectiveness of H5SAL, C2SH, and H4SAL suggests their potential for wastewater treatment.

Variations in decolorization zones likely result from enzymatic differences, with high-performing isolates producing azoreductases, known for breaking down azo bonds (Ajaz *et al*., 2020). Larger decolorization zones signify greater biodegradation potential, supporting their use in wastewater treatment (Mehzabin & Ahsan, 2024) and (Bharathi *et al*., 2022). Identifying effective microbial strains is essential, as dye chemical structures influence microbial activity by affecting solubility and functional group interactions.

Table 4: Zone of decolorization of biodegradation of reactive dyes

No	**Isolates**	**Zone of decolorization of biodegradation in mm**		
		Red	**Blue**	**Yellow**
1	H1E	32	31	30
2	H2E	31	29	27
3	H3E	30	28	27
4	H5E	27	26	26
5	H1K	23	20	19
6	H2K	22	21	20
7	H3K	19	17	15
8	H4K	21	20	18
9	H5K	25	24	22
10	C2SH	36	34	32
11	H2SH	33	31	30
12	H4SAL	34	32	31
13	H5SAL	37	35	33
14	C2P	33	31	29
15	C4P	28	27	25
16	H5P	32	31	30

N.B: H1E, H2E, H3E, and H5E stand for *Escherichia coli*; H1K, H2K, H3K, H4K, and H5K stand for *Klebseilla pneumonia*; C2H, and H2SH stand for *Shigella sp*.; H4SAL, and H5SAL stand for *Salmonella typhimurium*; and C2P, C4P and H5P stand for *Pseudomonas aerguinosae*.

4.4. Determination percent of decolorization on environmental factors

4.4.1. Effect of Temperature on Biodegradation

The results show the performance of bacterial isolates in decolorizing three reactive dyes, red, blue, and yellow, at four different temperatures of 25°C, 30°C, 37°C, and 40°C by 16 bacterial isolates, as shown in Table 5, which indicates that temperature significantly impacts the decolorization efficiency of reactive dyes. The analysis revealed a significant effect of temperature on biodegradation performance ($p = 0.000$). The highest mean biodegradation rate was observed at 37℃ with a mean of 0.894176. The isolated bacterial strains show an increase in biodegradation when the temperature increases from 25 to 37℃. The temperature with a maximum bacterial isolate reaches its peak at 37°C. This recommends that this temperature was optimal for microbial activity, promoting enzyme production and metabolic processes involved in dye degradation. As the temperature increased from 37 to 40℃, the biodegradation decreased in all isolates. Isolates like H5P, C2P, C4P, and H1E, with a temperature mean of 0.71, 0.70, 0.68, and 0.62, respectively, performed extremely well at higher temperatures, signifying they are suitable for warmer environments, encouraging bioremediation. In contrast, H4K shows the lowest mean of 0.49, indicating its potential susceptibility to temperature fluctuations.

In the decolorization of industrial effluent samples *pseudomonas sp*., ranges from 87 to 95% at 37℃ similar to the current study (Pinheiro *et al.,* 2022). At 40°C, all isolates show a decrease in performance, which may indicate thermal stress or denaturation of enzymes, reducing the bacterial ability to degrade dyes by affecting their metabolic rates (Alzain *et al*., 2023a). Also, lower decolorization rates, particularly at 25°C suggest, they are less effective in dye degradation compared to the top performers. The decrease in performance at 25℃ indicates that lower temperatures may hinder the metabolic activity of the bacterial isolates. A decline in performance could be a sign of heat stress or enzyme denaturation, which would impact on the metabolic rates of the bacteria and limit their capacity to break down dyes (Ajaz *et al*., 2020). For microbial activity, temperatures between 30 and 37°C are ideal. Previous research validated the conclusions of this investigation (Shi *et al*., 2021). When the temperature was raised from

22°C to 37°C, Enterobacter sp. decolorized Reactive Black 5, and when the temperature was raised to 42°C, the decolorization activity of the bacteria was significantly impacted, as reported by (Guo *et al*., 2020) which is comparable to the current work. The results show that the temperature significantly impacts reactive dye biodegradation, with 37℃ being optimal. Variability in performance among bacterial isolates suggests specific strains may be more suitable for bioremediation applications.

Table 5: Percent of Biodegradation at Variable Temperature

No	**Isolated bacteria**	**Color of reactive dye**	**Percentage of decolorization of dyes at variable temperature**			
			25℃	**30℃**	**37℃**	**40℃**
1	H1 E	Red	36% ± 0.64%	63% ± 2.60%	96% ± 0.56%	57.78% ± 1.99%
		Blue	36% ± 1.14%	63% ± 1.61%	93% ± 1.23%	57.33% ± 1.17%
		Yellow	35% ± 0.50%	62% ± 0.89%	96% ± 0.51%	54.55% ± 1.40%
2	H2 E	Red	34% ± 1.07%	60% ± 1.05%	86% ± 1.48%	57.32% ± 1.16%
		Blue	35% ± 1.11%	60% ± 0.21%	86% ± 2.33%	55.64% ± 2.09%
		Yellow	34% ± 0.76%	59% ± 1.40%	87% ± 1.73%	55.27% ± 1.66%
3	H3 E	Red	35% ± 0.74%	58% ± 1.18%	89% ± 1.80%	55.05% ± 1.98%
		Blue	34% ± 0.00%	59% ± 0.41%	87% ± 1.46%	56.20% ± 2.56%
		Yellow	32% ± 1.60%	60% ± 0.47%	90% ± 1.47%	54.13% ± 1.85%
4	H5 E	Red	33% ± 0.97%	57% ± 1.52%	87% ± 1.53%	55.62% ± 1.72%
		Blue	33% ± 2.49%	59% ± 1.75%	86% ± 1.88%	56.44% ± 2.14%
		Yellow	32% ± 0.40%	55% ± 0.75%	88% ± 1.44%	53.87% ± 1.78%
5	H1 K	Red	25% ± 1.13%	51% ± 1.28%	88% ± 0.86%	47.09% ± 1.14%
		Blue	26% ± 0.82%	49% ± 1.62%	88% ± 0.25%	46.31% ± 1.13%
		Yellow	25% ± 1.43%	50% ± 1.15%	87% ± 1.38%	44.22% ± 1.07%
6	H2 K	Red	28% ± 1.08%	48% ± 0.58%	86% ± 1.41%	45.51% ± 2.39%
		Blue	29% ± 0.74%	47% ± 0.00%	88% ± 1.56%	44.52% ± 1.68%
		Yellow	25% ± 0.84%	50% ± 1.03%	88% ± 1.47%	42.99% ± 1.17%

7	H3 K	Red	29% ± 1.87%	53% ± 2.06%	93% ± 1.62%	47.14% ± 1.68%
		Blue	30% ± 1.19%	51% ± 2.83%	91% ± 0.70%	46.54% ± 0.67%
		Yellow	28% ± 2.60%	52% ± 0.70%	92% ± 0.39%	44.72% ± 2.02%
8	H4 K	Red	25% ± 1.36%	50% ± 1.00%	82% ± 2.28%	43.41% ± 1.02%
		Blue	28% ± 1.72%	46% ± 1.39%	83% ± 1.62%	43.19% ± 1.99%
		Yellow	25% ± 0.05%	49% ± 1.64%	83% ± 1.15%	41.38% ± 0.71%
9	H5 K	Red	27% ± 0.00%	49% ± 1.96%	87% ± 1.32%	45.64% ± 1.85%
		Blue	29% ± 0.62%	48% ± 1.15%	87% ± 0.91%	45.07% ± 0.39%
		Yellow	25% ± 1.56%	47% ± 1.29%	87% ± 1.02%	43.16% ± 0.77%
10	C2 SH	Red	20% ± 0.37%	46% ± 1.48%	98% ± 0.47%	30.72% ± 0.99%
		Blue	21% ± 0.91%	44% ± 1.84%	97% ± 0.00%	29.85% ± 0.87%
		Yellow	21% ± 0.84%	43% ± 2.46%	97% ± 0.50%	27.10% ± 0.85%
11	H2 SH	Red	20% ± 0.75%	44% ± 0.64%	91% ± 0.94%	29.86% ± 2.40%
		Blue	19% ± 0.65%	42% ± 1.12%	89% ± 1.42%	29.00% ± 0.00%
		Yellow	18% ± 1.01%	43% ± 0.28%	90% ± 1.39%	26.76% ± 1.97%
12	H4 SAL	Red	22% ± 0.00%	49% ± 1.11%	92% ± 1.55%	32.13% ± 1.26%
		Blue	23% ± 0.65%	51% ± 1.72%	91% ± 1.30%	31.18% ± 2.15%
		Yellow	22% ± 0.94%	48% ± 1.61%	91% ± 0.98%	30.54% ± 1.51%
13	H5 SAL	Red	24% ± 1.06%	52% ± 1.27%	98% ± 0.66%	33.67% ± 1.89%
		Blue	24% ± 1.49%	50% ± 0.26%	98% ± 1.02%	33.05% ± 1.00%
		Yellow	23% ± 1.22%	50% ± 1.97%	98% ± 0.51%	31.11% ± 1.02%
14	C2 P	Red	52% ± 1.45%	72% ± 1.43%	87% ± 1.64%	70.21% ± 1.42%
		Blue	53% ± 0.00%	73% ± 1.11%	86% ± 1.42%	68.62% ± 0.57%
		Yellow	51% ± 0.56%	72% ± 0.16%	89% ± 1.45%	65.82% ± 1.50%
15	C4 P	Red	51% ± 0.00%	70% ± 1.74%	85% ± 2.02%	67.63% ± 1.19%
		Blue	52% ± 0.64%	70% ± 1.22%	84% ± 1.99%	66.95% ± 1.65%

		Yellow	51% ± 0.76%	70% ± 3.08%	86% ± 1.41%	63.05% ± 0.09%
16	H5 P	Red	54% ± 1.22%	74% ± 1.51%	90% ± 1.62%	71.52% ± 1.46%
		Blue	54% ± 0.89%	74% ± 0.65%	87% ± 1.35%	70.00% ± 0.00%
		Yellow	53% ± 1.11%	72%±0.77%	90%±1.47%	68.11%±1.84%

N.B. : H1E, H2E, H3E, and H5E stand for *Escherichia coli*; H1K, H2K, H3K, H4K, and H5K stand for *Klebseilla pneumoniae*; C2H, and H2SH stand for *Shigella sp.*; H4SAL, and H5SAL stand for *Salmonella typhimurium*; and C2P, C4P and H5P stand for *Pseudomonas aerguinosa*.

The statistical analysis Table 6 highlights significant effects of bacterial isolates, dye color, and temperature on decolorization efficiency. Isolate (DF = 15, MS = 0.25301, P = 0.000) exhibited a highly significant influence, confirming that different bacterial strains vary in their dye degradation potential. Color (DF = 2, MS = 0.00649, P = 0.000) also showed significance, suggesting that dye composition affects microbial degradation rates. Temperature (DF = 3, MS = 8.46353, P = 0.000) had the strongest effect, emphasizing its critical role in optimizing enzymatic activity for efficient dye removal.

The interaction between Isolate and Temperature (DF = 45, MS = 0.04760, P = 0.000) was highly significant, showing that bacterial strains respond differently to temperature variations. Likewise, Color and Temperature (DF = 6, MS = 0.00357, P = 0.000) exhibited significance, indicating that dye degradation efficiency depends on temperature conditions. However, Isolate and Color (DF = 30, MS = 0.00014, P = 0.826) was not significant, meaning microbial effectiveness remains consistent across dye types. The three-way interaction (Isolate, Color, and Temperature) (DF = 90, MS = 0.00025, P = 0.034) showed a minor effect, suggesting that while temperature and isolates significantly impact dye degradation, color plays a relatively smaller role. These findings highlight the need for temperature optimization and strain selection for effective dye bioremediation.

Table 6: Analysis of Variance at Temperature

Sources of Variation	**DF**	**Mean Square**	**P-Value**
Isolate	15	0.25301	0.000
Color	2	0.00649	0.000
Temperature	3	8.46353	0.000

Isolate*Color	30	0.00014	0.826
Isolate*Temperature	45	0.04760	0.000
Color*Temperature	6	0.00357	0.000
Isolate*Color*Temperature	90	0.00025	0.034
Error	384	0.00018	
Total	575		

The results in Table 7 indicate variations in bacterial dye decolorization efficiency across different temperatures, with H5P exhibiting the highest rate (71.36%), followed by C2P (70.03%) and C4P (68.05%), suggesting strong enzymatic activity and adaptability to temperature fluctuations. Moderate decolorization efficiencies were observed in H1E, H2E, H3E, and H5E, ranging from 58.09% to 62.37%, indicating stable but lower degradation potential. H3K recorded 54.72%, while H1K, H2K, and H5K showed slightly reduced efficiency (51.65%–52.18%), demonstrating a decline in decolorization at varying temperatures. The least efficient isolates, H5SAL, H4K, C2SH, H2SH, and H4SAL, exhibited decolorization rates below 51.18%, suggesting limited ability to function effectively under specific temperature conditions. These findings highlight the significance of temperature optimization in microbial biodegradation, with H5P, C2P, and C4P emerging as promising candidates for dye removal in wastewater treatment applications.

Table 7: Fisher pairwise comparisons: isolate Grouping information using Fisher LSD Method and 95% Confidence

No	**Isolates**	**Mean % of decolorization on Temperature**
1	H5P	0.713575^{a}
2	C2P	0.700347^{b}
3	C4P	0.680465^{c}
4	H1E	0.623692^{d}
5	H2E	0.590529^{e}
6	H3E	0.589387^{e}
7	H5E	0.580867^{f}

8	H3K	0.547237[g]
9	H1K	0.521785[h]
10	H2K	0.516886[h]
11	H5K	0.516512[h]
12	H5SAL	0.5118[i]
13	H4K	0.499414[i]
14	C2SH	0.478032[j]
15	H2SH	0.452147[k]
16	H4SAL	0.452147[l]

The Table 8 results indicate slight variations in the mean percentage of decolorization among reactive dyes, with red dye exhibiting the highest efficiency at 56.64%, followed by blue dye at 56.15% and yellow dye at 55.49%. The differences suggest that microbial degradation varies slightly based on dye color, though all dyes undergo comparable levels of decolorization. These findings highlight the general effectiveness of bacterial isolates in breaking down reactive dyes, providing valuable insights for optimizing bioremediation strategies in wastewater treatment.

Table 8: Fisher Pairwise Comparisons: Color

No	**Color**	**Mean % decolorization of reactive dyes**
1	Red	0.566443[a]
2	Blue	0.561484[b]
3	Yellow	0.554853[c]

The results indicate significant variations in dye decolorization efficiency across different temperatures, with 37°C showing the highest rate at 89.42%, confirming that this temperature provides optimal conditions for microbial activity as shown in Table 9. Decolorization efficiency declined at 30°C (55.00%) and 40°C (48.10%), suggesting that deviations from the optimal temperature reduce bacterial effectiveness. The lowest efficiency was recorded at 25°C (31.85%), indicating that lower temperatures significantly hinder biodegradation. These findings emphasize the importance of temperature optimization in microbial dye degradation, with 37°C being the most favorable for enzymatic activity and effective dye removal.

Table 9: Fisher Pairwise Comparisons: Temperature

No	Temperature	Percentage of decolorization on Different Temperatures
1	37 ℃	0.894176[a]
2	30 ℃	0.550021[b]
3	40 ℃	0.480984[c]
4	25 ℃	0.318525[d]

4.4.2. Effect of pH on Biodegradation

The results in Table 10 highlight variations in bacterial dye decolorization efficiency across different pH levels, with pH 7 showing the highest activity, where decolorization ranged from 82% to 98%, confirming that neutral conditions favor microbial dye degradation. H1E, H2E, and H5P exhibited particularly strong decolorization at pH 7, with efficiencies exceeding 90% across all dye colors. In contrast, at pH 5, decolorization rates were notably lower, ranging between 20% and 50%, suggesting that acidic conditions hinder bacterial dye breakdown. C2SH, H2SH, and HS SAL had the weakest decolorization at pH 5, indicating reduced biodegradation capacity under acidic conditions. At pH 9, decolorization varied among isolates but was generally lower than at pH 7. C2P, C4P, and H5P displayed the highest efficiency at pH 9, exceeding 55%, demonstrating adaptability to alkaline environments, while C2SH, H2SH, and HS SAL showed significantly lower efficiency, with decolorization dropping below 30%, highlighting their reduced activity under alkaline conditions.

Research supports the importance of neutral pH in enhancing microbial enzymatic activity crucial for dye degradation (Upadhyay *et al*., 2023). Studies by (Wang *et al.*, 2022), confirm that pH influences microbial metabolic activity and dye removal efficiency, with optimal degradation observed at pH 7 and 37°C, where *Pseudomonas aeruginosa* achieved 90% decolorization. Similar findings by (Das *et al*., 2023) demonstrate that pH 7 supports high biodegradation rates. (Jamee & Siddique, 2019) noted that enzyme activity declines outside the optimal range, affecting degradation at pH 5 and 9, where lower biodegradation rates which highlights superior decolorization at neutral pH. Research by (Goud *et al*., 2020) indicates that enzymatic activity thrives between pH 6 and 8, with extreme pH values negatively impacting degradation rates.

(Yaseen & Scholz, 2019) emphasize that optimizing pH conditions significantly enhances biodegradation efficiency. These findings confirm that reactive dye biodegradation is strongly pH-dependent, with neutral pH being the most effective. Additionally, variations in isolate performance suggest that certain strains may be better suited for bioremediation, reinforcing the importance of selecting the most effective bacterial species for wastewater treatment applications.

Table 10: Percentage of Biodegradation at Variable pH

No	Isolated bacteria	Color of reactive dyes	Percentage of decolorization at various pH		
			5	**7**	**9**
1	H1 E	Red	39.13% ± 1.73%	96% ± 0.56%	44.05% ± 1.35%
		Blue	38.07% ± 0.12%	93% ± 1.23%	42.74% ± 0.66%
		Yellow	37.14% ± 0.46%	96% ± 0.51%	42.58% ± 0.50%
2	H2 E	Red	35.52% ± 0.99%	86% ± 1.48%	41.41% ± 1.27%
		Blue	37.72% ± 1.65%	86% ± 2.33%	41.33% ± 0.67%
		Yellow	35.61% ± 0.58%	87% ± 1.73%	43.23% ± 0.40%
3	H3 E	Red	33.20% ± 0.72%	89% ± 1.80%	43.36% ± 0.71%
		Blue	37.81% ± 2.53%	87% ± 1.46%	42.35% ± 1.19%
		Yellow	35.57% ± 1.07%	90% ± 1.47%	43.91% ± 1.82%
4	H5 E	Red	33.35% ± 1.19%	87% ± 1.53%	43.53% ± 1.29%
		Blue	36.96% ± 2.69%	86% ± 1.88%	43.28% ± 1.11%
		Yellow	34.89% ± 1.65%	88% ± 1.44%	43.75% ± 1.80%
5	H1 K	Red	28.81% ± 1.37%	88% ± 0.86%	36.13% ± 1.80%
		Blue	27.87% ± 2.80%	88% ± 0.25%	36.03% ± 1.765

		Yellow	27.82% ± 2.86%	87% ± 1.38%	35.86% ± 1.80%
		Red	27.13% ± 1.00%	86% ± 1.41%	35.50% ± 1.32%
6	H2 K	Blue	26.47% ± 0.76%	88% ± 1.56%	34.82% ± 0.90%
		Yellow	28.56% ± 3.15%	88% ± 1.47%	35.41% ± 1.23%
		Red	29.26% ± 2.39%	93% ± 1.62%	37.53% ± 1.53%
7	H3 K	Blue	28.04% ± 0.07%	91% ± 0.70%	37.30% ± 0.60%
		Yellow	30.30% ± 2.87%	92% ± 0.39%	36.75% ± 1.14%
		Red	28.00% ± 1.73%	82% ± 2.28%	36.85% ± 1.78%
8	H4 K	Blue	25.42% ± 0.63%	83% ± 1.62%	37.64% ± 0.80%
		Yellow	30.70% ± 2.29%	83% ± 1.15%	36.94% ± 1.17%
		Red	29.77%±1.27%	87%±1.32%	37.41%±1.24%
9	H5 K	Blue	27.83% ± 2.44%	87% ± 0.91%	37.67% ± 0.53%
		Yellow	29.26% ± 1.63%	87% ± 1.02%	37.66% ± 1.59%
		Red	20.55% ± 1.48%	98% ± 0.47%	19.23% ± 1.33%
10	C2 SH	Blue	20.06% ± 0.10%	97% ± 0.00%	17.67% ± 0.585
		Yellow	22.25% ± 1.49%	97% ± 0.50%	18.28% ± 1.39%
		Red	20.53% ± 1.36%	91% ± 0.94%	17.84% ± 1.17%
11	H2 SH	Blue	21.57% ± 1.91%	89% ± 1.42%	18.10% ± 0.17%
		Yellow	19.36% ± 0.64%	90% ± 1.39%	18.23% ± 1.08%
		Red	21.67% ± 1.53%	92% ± 1.55%	24.03% ± 1.27%
12	H4 SAL	Blue	19.81% ± 1.67%	91% ± 1.30%	21.86% ± 1.085
		Yellow	18.65% ± 1.10%	91% ± 0.98%	21.75% ± 0.655

		Red	20.79% ± 1.66%	98% ± 0.66%	28.22% ± 1.15%
13	HS SAL	Blue	20.75% ± 0.00%	98% ± 1.02%	24.00% ± 1.37%
		Yellow	19.23% ± 1.75%	98% ± 0.51%	24.16% ± 0.36%
		Red	50.03% ± 1.57%	87% ± 1.64%	56.77% ± 2.34%
14	C2 P	Blue	49.09% ± 1.08%	86% ± 1.42%	54.95% ± 1.52%
		Yellow	45.83% ± 1.04%	89% ± 1.45%	51.15% ± 1.03%
		Red	47.62% ± 1.17%	85% ± 2.02%	57.13% ± 1.70%
15	C4 P	Blue	46.46% ± 1.00%	84% ± 1.99%	56.38% ± 1.24%
		Yellow	45.07% ± 0.92%	86% ± 1.41%	53.32% ± 0.40%
		Red	51.52% ± 0.56%	90% ± 1.62%	57.84% ± 1.60%
16	H5 P	Blue	49.23% ± 1.155	87% ± 1.35%	57.00% ± 0.00%
		Yellow	48.25% ± 1.73%	90% ± 1.47%	55.08% ± 1.38%

The statistical analysis of dye decolorization factors revealed significant effects for certain variables as Table 11 indicates. Isolate (DF = 15, MS = 0.1219, P = 0.000) had a highly significant influence on decolorization, indicating that bacterial isolates play a crucial role in dye degradation. pH (DF = 2, MS = 14.1358, P = 0.000) also showed a significant effect, suggesting that pH levels strongly impact microbial decolorization efficiency.

The interaction between Isolate and Color (DF = 30, MS = 0.0019, P = 0.046) was statistically significant, meaning that different bacterial isolates respond differently to various dye colors. Likewise, the interaction between Isolate and pH (DF = 30, MS = 0.0603, P = 0.000) was highly significant, further supporting the influence of pH on bacterial dye degradation. However, Color alone (DF = 2, MS = 0.0003, P = 0.795) and its interaction with pH (DF = 4, MS = 0.0025, P = 0.085) were not significant, suggesting that dye color does not independently affect decolorization rates.

The three-way interaction among Isolate, Color, and pH (DF = 60, MS = 0.0013, P = 0.426) was also not significant, indicating that their combined effect does not strongly influence decolorization beyond their individual contributions. The error term (DF = 288, MS = 0.0012) highlights variability within the data. These findings confirm that bacterial isolates and pH levels are the primary factors driving dye degradation efficiency, emphasizing the importance of pH optimization in bioremediation processes.

Table 11: Analysis of Variance at pH

Source	**DF**	MS	P- Value
Isolate	15	0.1219	0.000
Color	2	0.0003	0.795
PH	2	14.1358	0.000
Isolate*Color	30	0.0019	0.046
Isolate*PH	30	0.0603	0.000
Color*PH	4	0.0025	0.085
Isolate*Color*PH	60	0.0013	0.426
Error	288	0.0012	
Total	431		

The Table 12 results show notable variations in the mean percentage of dye decolorization among bacterial isolates at different pH levels. H5P exhibited the highest efficiency (65.14%), followed closely by C2P (63.34%) and C4P (62.40%), indicating strong adaptability to varying pH conditions. Moderate decolorization was observed in H1E, H3E, H2E, and H5E, with efficiencies ranging from 55.18% to 58.67%, reflecting stable but lower degradation potential. H3K and H5K recorded slightly lower values (52.78% and 51.24%), while H1K, H2K, and H4K showed further reduced performance (49.42%–50.58%), suggesting diminished effectiveness at varying pH levels.

Among the least efficient isolates, H5SAL achieved 47.83%, followed by C2SH (45.53%), H4SAL (44.68%), and H2SH (42.89%). These differences emphasize the significance of pH optimization in microbial dye biodegradation. High-performing strains such as H5P, C2P, and

C4P exhibit potential for wastewater treatment, particularly in environments where pH fluctuations influence dye degradation efficiency.

Table 12: Fisher Pairwise Comparisons: Isolate Grouping Information Using Fisher LSD Method and 95% Confidence

No	**Isolate**	**Mean % of decolorization of isolates on pH**
1	H5P	0.651385[a]
2	C2P	0.633367[a]
3	C4P	0.624019[b]
4	H1E	0.586700[c]
5	H3E	0.557044[d]
6	H2E	0.554367[d]
7	H5E	0.551804[d]
8	H3K	0.527826[e]
9	H5K	0.512444[e]
10	H1K	0.505878[f]
11	H2K	0.499189[f]
12	H4K	0.494237[f]
13	H5SAL	0.478337[g]
14	C2SH	0.455267[h]
15	H4SAL	0.446837[h]
16	H2SH	0.428930[i]

The results show in Table 13 slight variations in the mean percentage of decolorization among reactive dyes across 144 samples, with red dye exhibiting the highest efficiency at 53.30%, followed by blue (53.19%) and yellow (53.02%). The minimal differences suggest that bacterial isolates degrade different dye colors at similar rates, indicating that structural composition does not significantly impact microbial decolorization. These insights are valuable for optimizing bacterial strains in wastewater treatment to ensure effective dye removal regardless of color type.

Table 13: Grouping Information Using Fisher LSD Method and 95% Confidence

No	Color	Mean % of decolorization of reactive dyes
1	Red	0.533027[a]
2	Blue	0.531916[a]
3	Yellow	0.530237[a]

The results reveal significant variations in dye decolorization efficiency across different pH levels, with pH 7 showing the highest rate at 89.29%, indicating that neutral conditions optimize microbial activity for dye breakdown. In contrast, efficiency dropped to 37.86% at pH 9, suggesting reduced microbial effectiveness in alkaline environments, while pH 5 recorded the lowest efficiency at 32.37%, highlighting the inhibitory effects of acidic conditions on dye degradation as shown in Table 14. These findings emphasize the crucial role of pH optimization in enhancing microbial decolorization, with neutral conditions being the most favorable for enzymatic activity, reinforcing the need for pH control in wastewater treatment applications.

Table 14: Grouping Information Using Fisher LSD Method and 95% Confidence

No	pH	Mean % of decolorization of pH
1	7	0.892854[a]
2	9	0.378635[b]
3	5	0.323692[c]

4.4.3. Effect of Dye Concentration on Biodegradation

The results shown in Table 15 indicate that dye concentration significantly impacts biodegradation efficiency, with 50 mg/L facilitating optimal microbial activity. As concentration

increases to 100 mg/L and 150 mg/L, degradation rates decline, suggesting a saturation effect or toxicity. Isolates *H5SAL* and *C2SH* exhibited the highest decolorization efficiency (98.16% and 97.96%, respectively), while *H4K* showed lower performance (89.03%). The adaptability of *Pseudomonas* species reinforces their effectiveness in bioremediation, as enzymatic activity is most efficient at lower dye concentrations.

Consistent with previous research, *Pseudomonas aeruginosa RS1* achieved 100% biodegradation at 50 mg/L under optimal pH and temperature conditions (Rane & Joshi, 2021). Studies confirm that higher dye concentrations can inhibit bacterial activity, likely due to toxicity effects (Bharathi *et al*., 2022) and (R Ananthashankar, 2013). The decline in decolorization rates at 100 mg/L and 150 mg/L underscores the need for controlled dye concentrations to maximize microbial degradation efficiency. These findings provide valuable insights for designing effective bioremediation strategies tailored to dye-contaminated environments.

Table 15: Percent of Biodegradation at Variable Dye Concentration

No	**Isolated bacteria**	**Color of reactive dyes**	**Percentage decolorization at various dye concentration**		
			50 mg/l	**100 mg/l**	**150 mg/l**
1	H1 E	Red	96% ± 0.56%	65% ± 1.52%	43.75% ± 1.56%
		Blue	93% ± 1.23%	62% ± 1.955	43.47% ± 0.81%
		Yellow	96% ± 0.51%	62% ± 1.00%	42.62% ± 1.52%
2	H2 E	Red	86% ± 1.48%	60% ± 0.00%	41.97% ± 1.35%
		Blue	86% ± 2.33%	63% ± 3.16%	41.78% ± 1.72%
		Yellow	87% ± 1.73%	61% ± 1.10%	41.17% ± 1.61%
3	H3 E	Red	89% ± 1.80%	60% ± 1.29%	42.03% ± 1.77%
		Blue	87% ± 1.46%	61% ± 1.29%	41.02% ± 2.28%
		Yellow	90% ± 1.47%	61% ± 0.10%	39.93% ± 2.11%
4	H5 E	Red	87% ± 1.53%	62% ± 1.21%	40.06% ± 2.22%
		Blue	86% ± 1.88%	63% ± 2.42%	40.82% ± 2.97%
		Yellow	88% ± 1.44%	62% ± 1.55%	39.11% ± 2.45%

5	H1 K	Red	88% ± 0.86%	52% ± 2.16%	38.57% ± 1.91%
		Blue	88% ± 0.25%	54% ± 1.86%	37.97% ± 1.89%
		Yellow	87% ± 1.38%	51% ± 1.34%	35.63% ± 1.52%
6	H2 K	Red	86% ± 1.41%	52% ± 1.33%	38.79% ± 2.55%
		Blue	88% ± 1.56%	54% ± 2.29%	36.68% ± 1.86%
		Yellow	88% ± 1.47%	51% ± 1.29%	36.29% ± 1.46%
7	H3 K	Red	93% ± 1.62%	54% ± 2.41%	41.93% ± 1.10%
		Blue	91% ± 0.70%	56% ± 2.92%	38.92% ± 2.01%
		Yellow	92% ± 0.39%	54% ± 2.54%	36.67% ± 2.08%
8	H4 K	Red	82% ± 2.28%	53% ± 1.76%	39.59% ± 2.27%
		Blue	83% ± 1.62%	55% ± 2.81%	38.33% ± 1.15%
		Yellow	83% ± 1.15%	53% ± 1.53%	36.18% ± 1.28%
9	H5 K	Red	87% ± 1.32%	51% ± 1.46%	39.36% ± 1.39%
		Blue	87% ± 0.91%	54% ± 2.92%	37.92% ± 1.66%
		Yellow	87% ± 1.02%	50% ± 1.01%	36.16% ± 1.58%
10	C2 SH	Red	98% ± 0.47%	57% ± 1.05%	36.10% ± 2.71%
		Blue	97% ± 0.00%	56% ± 2.34%	33.75% ± 0.00%
		Yellow	97% ± 0.50%	57% ± 3.08%	31.57% ± 1.07%
11	H2 SH	Red	91% ± 0.94%	55% ± 2.65%	36.10% ± 1.15%
		Blue	89% ± 1.42%	57% ± 3.40%	31.92% ± 0.88%
		Yellow	90% ± 1.39%	59% ± 2.52%	30.59% ± 0.63%
12	H4 SAL	Red	92% ± 1.55%	59% ± 2.45%	35.47% ± 1.36%
		Blue	91% ± 1.30%	59% ± 2.63%	31.13% ± 1.31%
		Yellow	91% ± 0.98%	58% ± 2.60%	28.67% ± 2.08%
13	H5 SAL	Red	98% ± 0.66%	61% ± 2.23%	38.40% ± 1.42%
		Blue	98% ± 1.02%	61% ± 1.47%	35.42% ± 2.92%

		Yellow	98% ± 0.51%	60% ± 0.00%	34.36% ± 3.11%
14	C2 P	Red	87% ± 1.64%	73% ± 0.85%	56.67% ± 1.53%
		Blue	86% ± 1.42%	70% ± 1.95%	54.31% ± 1.75%
		Yellow	89% ± 1.45%	71% ± 2.00%	55.76% ± 3.295
15	C4 P	Red	85% ± 2.02%	70% ± 2.00%	55.45% ± 2.45%
		Blue	84% ± 1.99%	67% ± 2.02%	53.92% ± 1.02%
		Yellow	86% ± 1.41%	68% ± 2.08%	55.27% ± 3.10%
16	H5 P	Red	90% ± 1.62%	73% ± 1.86%	59.16% ± 1.35%
		Blue	87% ± 1.35%	71% ± 1.19%	55.92% ± 1.95%
		Yellow	90% ± 1.47%	70% ± 0.94%	53.99% ± 3.11%

The statistical analysis of dye decolorization factors shown in Table 16 indicates significant interactions among variables. Isolate (DF = 15, MS = 0.054, P = 0.000) exhibited a highly significant effect, suggesting that bacterial isolates strongly influence dye degradation. Color (DF = 2, MS = 0.005, P = 0.000) also showed a significant effect, indicating variation in decolorization efficiency among different dye colors. Dye concentration (DF = 2, MS = 8.596, P = 0.000) had the largest mean square value, demonstrating its dominant role in affecting decolorization.

Significant interactions were observed between Isolate and Color (DF = 30, MS = 0.0005266, P = 0.020) and Isolate and Dye Concentration (DF = 30, MS = 0.027, P = 0.000), indicating that bacterial degradation efficiency is influenced by both dye color and concentration. The interaction between Color and Dye Concentration (DF = 4, MS = 0.005, P = 0.000) was also significant, highlighting how dye concentration affects the breakdown rate of different colors.

However, the three-way interaction among Isolate, Color, and Dye Concentration (DF = 60, MS = 0.0002942, P = 0.635) was not statistically significant, suggesting that the combined effect of all three factors does not strongly influence dye decolorization beyond their individual contributions. The error term (DF = 288, MS = 0.0003183) indicates variability within the

dataset. Overall, the findings emphasize the importance of isolate selection and dye concentration in optimizing microbial decolorization processes.

Table 16: Analysis of Variance at Dye Concentration

Source	DF	MS	P-Value
Isolate	15	0.054	0.000
Color	2	0.005	0.000
Dye concentration	2	0.8.596	0.000
Isolate*Color	30	0.0005266	0.020
Isolate*Dye concentration	30	0.027	0.000
Color*Dye concentration	4	0.005	0.000
Isolate*Color*Dye concentration	60	0.0002942	0.635
Error	288	0.0003183	
Total	431		

The results in Table 17 indicate variations in the mean percentage of dye decolorization among bacterial isolates. H5P exhibited the highest decolorization efficiency (72.28%), followed closely by C2P (71.48%) and C4P (69.45%), suggesting their strong biodegradation potential. H1E and H5SAL demonstrated moderate decolorization rates, ranging from 66.95% to 64.76%. Several isolates, including H3E, H2E, H5E, and C2SH, showed efficiencies between 63.35% and 62.60%, indicating comparable degradation capability.

Lower decolorization rates were observed for H3K (61.89%), H4SAL (60.61%), and H2SH (60.02%). The least efficient isolates, H1K, H5K, H2K, and H4K, exhibited decolorization percentages between 59.24% and 58.30%. These differences suggest variations in enzymatic activity among isolates, influencing their effectiveness in breaking down dye compounds. The findings highlight the most promising bacterial strains for potential applications in wastewater bioremediation.

Table 17: Mean Percentage of Dye Decolorization by Bacterial Isolates

No	Isolate	Mean % of decolorization

1	H5P	0.722807[a]
2	C2P	0.714774[a]
3	C4P	0.694504[b]
4	H1E	0.669526[c]
5	H5SAL	0.647663[d]
6	H3E	0.633500[e]
7	H2E	0.631730[e]
8	H5E	0.630693[e]
9	C2SH	0.626037[e]
10	H3K	0.618885[f]
11	H4SAL	0.606152[g]
12	H2SH	0.600241[h]
13	H1K	0.592404[i]
14	H5K	0.589033[i]
15	H2K	0.587963[i]
16	H4K	0.583041[i]

The results show in Table 18 variations in the mean percentage of decolorization among reactive dyes across 144 samples, with red dye exhibiting the highest efficiency (64.09%), followed by yellow (63.25%) and blue (62.95%). The differences suggest varying microbial degradation efficiency, with red dye being more susceptible to biodegradation. The slightly lower decolorization of yellow and blue dyes may be due to differences in molecular structure, dye composition, or enzymatic activity influencing breakdown. These findings highlight the effectiveness of biodegradation and provide insights into optimizing bacterial strains for enhanced dye removal in wastewater treatment applications.

Table 18: Grouping Information Using Fisher LSD Method and 95% Confidence

No	Color	Mean % of decolorization of reactive dyes
1	Red	0.640867a
2	Yellow	0.632547[b]
3	Blue	0.629515[b]

At a 95% confidence level, Table 19 shows how well three colors—yellow, blue, and red—decolorize reactive dyes when applied using the Fisher LSD method. The highest mean percentage of decolorization is seen in red (0.640867), followed by yellow (0.632547) and blue (0.629515). Accordingly, among the three dyes, red dye decolorizes the most successfully. This implies that the chemical or biological methods used may be more effective for this color. The reactive dyes' structures may make them more susceptible to decolorization techniques. Yellows and blue's chemical makeups or conditions may also be similar.

Table 19: Grouping Information Using Fisher LSD Method and 95% Confidence

No	**Dye concentration**	**N**	**Mean % of decolorization of dye concentration**
1	50 mg/l	144	0.895006[a]
2	100 mg/l	144	0.597365[b]
3	150 mg/l	144	0.410558[c]

4.5. Identification of Isolated Bacteria

The biochemical characterization of bacterial isolates as shown in Table 20 was performed using Gram staining (GS), potassium hydroxide (KOH) solubility, catalase (CAT) activity, triple sugar iron (TSI) fermentation, sulfur-indole-motility (SIM) tests, and citrate utilization (CIT). All isolates tested negative for GS, confirming they are Gram-negative bacteria. They were positive for KOH, indicating cell wall fragility typical of Gram-negative organisms. CAT activity was consistently positive, confirming their ability to break down hydrogen peroxide. TSI results showed variations in sugar fermentation: *Escherichia coli* and *Klebsiella pneumoniae* presented A/A reactions, indicating fermentation of both glucose and lactose/sucrose with acid production,

while *Shigella spp.* and *Salmonella Typhimurium* exhibited K/A, fermenting glucose only. *Pseudomonas aeruginosa* displayed K/K, signifying no sugar fermentation.

Gas (GAS) production was observed in all A/A and K/A isolates, whereas hydrogen sulfide (H2S) production varied, with *Salmonella Typhimurium* producing H2S, while others did not. Motility (MOT) was positive in *Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella Typhimurium,* but negative in *Klebsiella pneumonia and Shigella spp.* Indole (IND) production was detected only in *Escherichia coli.* Citrate utilization (CIT) was positive in *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* indicating their ability to use citrate as a sole carbon source. These findings highlight metabolic diversity among the isolates, supporting their potential for environmental and wastewater bioremediation applications.

A study has successfully isolated dye-degrading bacteria from textile effluents, including *Pseudomonas aeruginosa* and *Shigella*, which showed significant dye decolorization (Sriram & Reetha, 2015). Similarly, *Klebsiella* was identified as an effective degrader (Dixit & Garg, 2021), and *Escherichia coli* was found capable of biodegrading reactive dyes (Rane & Joshi, 2021). These isolates exhibit diverse biochemical traits, with *E. coli* and *Klebsiella pneumoniae* as strong fermenters, while *Shigella* and *Salmonella* show varied fermentation patterns, indicating their biodegradation potential. *Pseudomonas aeruginosa* stands out for citrate utilization and non-fermentative metabolism, highlighting its adaptability in dye-contaminated environments. This data is essential for optimizing bacterial strains in bioremediation and wastewater treatment.

Table 20: Morphological and biochemical characteristics of screened isolated bacteria for biodegradation of reactive dye blue, red, and yellow dyes

Isolates code	GS	KOH	CAT	TSI			SIM				BACTERIA
				FER	GAS	H2S	MOT	H2S	IND	CIT	
H1E	-	+	+	A/A	+	-	+	-	+	-	*Escherichia coli*
H2E	-	+	+	A/A	+	-	+	-	+	-	*Escherichia coli*
H3E	-	+	+	A/A	+	-	+	-	+	-	*Escherichia coli*
H5E	-	+	+	A/A	+	-	+	-	+	-	*Escherichia coli*
H1K	-	+	+	A/A	+	-	-	-	-	+	*Klebsiella pneumonia*
H2K	-	+	+	A/A	+	-	-	-	-	+	*Klebsiella pneumonia*

H3K	-	+	+	A/A	+	-	-	-	-	+	*Klebsiella pneumonia*
H4K	-	+	+	A/A	+	-	-	-	-	+	*Klebsiella pneumonia*
H5K	-	+	+	A/A	+	-	-	-	-	+	*Klebsiella pneumonia*
C2SH	-	+	+	K/A	+	-	-	-	-	-	*Shigella spp*
H2SH	-	+	+	K/A	+	-	-	-	-	-	*Shigella spp*
H4SAL	-	+	+	K/A	+	+	+	+	-	-	*Salmonella Typhimurium*
H5SAL	-	+	+	K/A	+	+	+	+	-	-	*Salmonella Typhimurium*
C2P	-	+	+	K/K	-	-	+	-	-	+	*Pseudomonas aeruginosa*
C4P	-	+	+	K/K	-	-	+	-	-	+	*Pseudomonas aeruginosa*
H5P	-	+	+	K/K	-	-	+	-	-	+	*Pseudomonas aeruginosa*

NB : GS stands for gram staining test, KOH for potassium hydroxide test, CAT for catalase test, TSI for triple sugar iron test, SIM for sulfide indole motility test , FER for fermentation, GAS for gas production, H_2S for hydrogen sulfide production, MOT for motility test, IND for indole test, CIT for citrate test , - for negative result, + for positive result, A/A for yellow slant/ yellow butt, K/A for red slant/ yellow butt, and K/K for red slant/ red butt.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The physicochemical analysis confirmed the environmental burden of untreated wastewater, revealing high COD, BOD, and TDS levels that supported microbial communities capable of degrading complex dye compounds. Among 30 bacterial strains isolated, 16 exhibited significant decolorization of reactive red, blue, and yellow dyes, with Pseudomonas aeruginosa isolates H5P, C2P, and C4P achieving up to 90% degradation efficiency. Optimal decolorization occurred at 37°C, pH 7, and 50 mg/L dye concentration, while higher concentrations reduced efficiency due to substrate inhibition. ANOVA results confirmed that temperature, pH, and dye concentration significantly influence ($p < 0.001$) microbial degradation, making it a condition-dependent process. Identification through morphological, Gram-staining, and biochemical assays verified dominant strains such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Shigella spp*., known for their xenobiotic-degrading capabilities. Selecting high-performing bacterial isolates based on environmental conditions can enhance bioremediation strategies and mitigate pollution.

Effluent analysis revealed neutral to slightly alkaline pH levels (7.2–7.5), aligning with WHO guidelines and supporting microbial activity. Sample 1 exhibited thermal pollution (38°C), while high TDS levels (2633–2866 mg/L) posed potential risks to aquatic ecosystems. COD and BOD values remained consistent, reflecting organic pollution, while low TSS levels (28–30 mg/L) suggested minimal particulate matter presence. Unpleasant odors in Samples 1 and 2 indicated decomposing organic matter, while color variations (gray, amber, black) highlighted industrial discharge or anaerobic decomposition, emphasizing the need for effective effluent treatment. Screening of 30 bacterial isolates identified 16 effective strains capable of dye degradation, with H1–H10 and selected C-group isolates showing strong biodegradation potential due to enzymatic activity, particularly azoreductase production, which cleaves azo bonds. Temperature significantly affected decolorization, peaking at 37°C, though efficiency declined at 40°C due to enzyme denaturation. ANOVA confirmed the influence of bacterial strain, dye type, and temperature, identifying H5P, C2P, and C4P as the most effective isolates for textile wastewater bioremediation.

5.2. Recommendations

Based on the findings, the following recommendations are forwarded.

- ✓ The isolated bacterial species of *pseudomonas* (H5P, C2P, and C4P) can be utilized in developing bioremediation systems for treating industrial wastewater, providing a sustainable alternative to chemical treatment methods that often produce harmful byproducts.
- ✓ By effectively breaking down harmful dyes, these bacterial isolates have the potential to improve water quality in affected environments, protecting aquatic life and enhancing environmental health.
- ✓ These bacterial isolates can help textile producers include microbial treatments into their wastewater treatment procedures, encouraging eco-friendly business practices.
- ✓ This research shows the need for policies that encourage the adoption of biotechnological solutions in environmental management, potentially guiding regulations on effluent discharge standards.
- ✓ Future researchers should explore the genetic and enzymatic mechanisms behind dye degradation, which could lead to the development of genetically engineered strains with enhanced capabilities for environmental cleanup.

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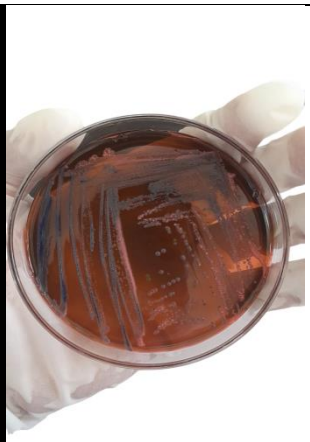
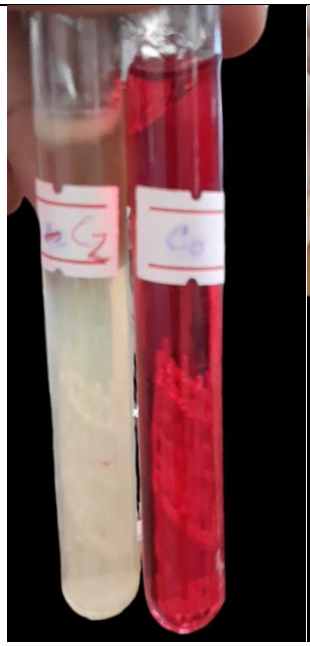
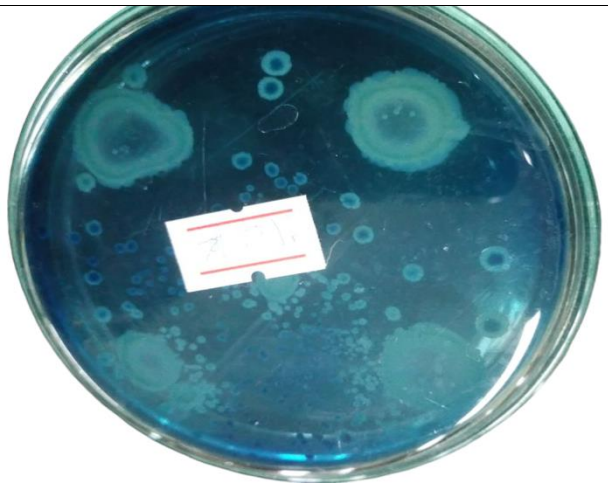
APPENDIX

1. LABORATORY IMAGES

No	Figure	Description
1		Textile effluent samples
2		Reactive dyes
3		Preparation of media

4		Bacteria culturing on nutrient agar media
5	 	Selective and differential media
6	  	Screened dye-biodegradation

7		Clear zone of inhibition
8	 	Biochemical test
9		UV spectrophotometry

2. Initial absorbance

CATAGORIES		Initial absorbance at their specific wave length		
		RED at 590nm	BLUE at 700nm	YELLOW at 430nm
TEMPERETURE	25	0.915	0.881	0.835
	30	0.854	0.86	0.812

	37	0.876	0.855	0.793
	40	0.755	0.733	0.714
PH	5	0.893	0.87	0.821
	7	0.876	0.855	0.793
	9	0.769	0.746	0.705
DYE CONCENTRATION	50	0.876	0.855	0.793
	100	1.741	1.69	1.437
	150	2.66	2.432	2.219

3. Final absorbance

3.1 Absorbance of reactive dyes at various temperatures

Isolate	REPLICATION	Absorbance of reactive dye at various Temperatures											
		at 25 ℃			at 30 ℃			at 37 ℃			at 40 ℃		
		Color			Color			Color			Color		
		RED	BLUE	YELLOW	RED	BLUE	YELLOW	RED	BLUE	YELLOW	RED	BLUE	YELLOW
		Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
H1E	R1	0.581	0.579	0.542	0.31	0.31	0.309	0.03	0.072	0.0325	0.3106	0.322	0.328
H1E	R2	0.588	0.565	0.537	0.342	0.336	0.318	0.04	0.063	0.0399	0.336	0.307	0.334
H1E	R3	0.576	0.559	0.545	0.3	0.316	0.303	0.035	0.05	0.0335	0.31	0.307	0.314
H2E	R1	0.615	0.583	0.561	0.34	0.344	0.346	0.138	0.137	0.118	0.332	0.33	0.332
H2E	R2	0.596	0.572	0.549	0.35	0.3416	0.329	0.117	0.1175	0.102	0.319	0.307	0.317
H2E	R3	0.611	0.563	0.551	0.333	0.34	0.324	0.114	0.097	0.0917	0.315	0.337	0.3086
H3E	R1	0.5925	0.581	0.5744	0.3697	0.3503	0.325	0.116	0.127	0.096	0.354	0.329	0.343
H3E	R2	0.6058	0.581	0.5566	0.3558	0.3546	0.3314	0.09	0.109	0.076	0.325	0.2995	0.3198
H3E	R3	0.5968	0.581	0.5829	0.35	0.3574	0.325	0.0876	0.103	0.076	0.338	0.3338	0.3198
H5E	R1	0.6021	0.6167	0.5673	0.3758	0.335	0.357	0.164	0.14	0.1063	0.35	0.3331	0.3435
H5E	R2	0.6131	0.5844	0.5645	0.3655	0.3579	0.3687	0.1	0.1175	0.0835	0.3275	0.3022	0.3257
H5E	R3	0.6197	0.5747	0.5607	0.35	0.3638	0.3593	0.139	0.11	0.096	0.3276	0.322	0.3188
H1K	R1	0.6976	0.66	0.643	0.418	0.4281	0.4087	0.11	0.103	0.1135	0.4093	0.403	0.406
H1K	R2	0.6894	0.6555	0.628	0.4289	0.455	0.415	0.099	0.1024	0.0955	0.3956	0.388	0.395
H1K	R3	0.677	0.6463	0.6194	0.4071	0.438	0.3965	0.096	0.099	0.0938	0.3935	0.389	0.3927
H2K	R1	0.6695	0.616	0.6379	0.452	0.455	0.416	0.137	0.119	0.111	0.4322	0.4166	0.416
H2K	R2	0.6679	0.6288	0.629	0.4436	0.455	0.4096	0.118	0.137	0.091	0.4	0.3928	0.405
H2K	R3	0.6517	0.6189	0.624	0.4445	0.455	0.399	0.113	0.094	0.091	0.4	0.41	0.399
H3K	R1	0.6415	0.6175	0.5946	0.3975	0.416	0.3897	0.071	0.078	0.069	0.3907	0.3884	0.3813

H3K	R2	0.667	0.6288	0.6262	0.4192	0.4496	0.3998	0.068	0.078	0.064	0.4137	0.3975	0.41
H3K	R3	0.6359	0.6078	0.5845	0.3843	0.4024	0.39	0.045	0.068	0.0635	0.3926	0.3896	0.3927
H4K	R1	0.6954	0.6551	0.6262	0.4355	0.4816	0.4322	0.175	0.1557	0.137	0.436	0.4324	0.424
H4K	R2	0.6706	0.6344	0.627	0.428	0.4609	0.415	0.154	0.14	0.134	0.4228	0.4039	0.4175
H4K	R3	0.6808	0.6255	0.6265	0.4184	0.4609	0.406	0.135	0.128	0.1206	0.4228	0.4128	0.4141
H5K	R2	0.6679	0.6275	0.6429	0.4503	0.4303	0.4421	0.126	0.1168	0.111	0.4102	0.4046	0.4106
H5K	R3	0.6679	0.6221	0.624	0.4357	0.4303	0.4264	0.109	0.1045	0.098	0.3965	0.3993	0.406
H5K	R1	0.6679	0.6343	0.6179	0.4169	0.4141	0.4222	0.103	0.1026	0.096	0.4244	0.4031	0.399
C2SH	R2	0.7261	0.702	0.655	0.4785	0.498	0.4872	0.025	0.028	0.0249	0.5151	0.521	0.521
C2SH	R3	0.732	0.699	0.669	0.4631	0.4772	0.4559	0.018	0.028	0.0176	0.53	0.5084	0.526
C2SH	R1	0.732	0.687	0.6596	0.4535	0.468	0.4466	0.0175	0.028	0.024	0.5239	0.5131	0.514
H2SH	R2	0.724	0.7175	0.693	0.5225	0.5074	0.4628	0.092	0.075	0.087	0.509	0.52	0.5255
H2SH	R3	0.7363	0.7077	0.6826	0.4782	0.491	0.4606	0.1089	0.086	0.077	0.536	0.52	0.5355
H2SH	R1	0.7356	0.7077	0.6763	0.4782	0.49	0.4583	0.0855	0.07	0.065	0.54	0.52	0.5077
H4SAL	R2	0.7241	0.7175	0.693	0.4877	0.5074	0.4628	0.083	0.0866	0.0793	0.509	0.5204	0.5255
H4SAL	R3	0.7363	0.7077	0.6826	0.4782	0.4913	0.4606	0.067	0.077	0.0658	0.536	0.5204	0.5355
H4SAL	R1	0.7384	0.7077	0.6763	0.4782	0.49028	0.4583	0.055	0.064	0.0657	0.5436	0.5204	0.5077
H5SAL	R2	0.7046	0.67704	0.6484	0.3975	0.4338	0.3913	0.02	0.027	0.024	0.4945	0.498	0.499
H5SAL	R3	0.7008	0.67105	0.6436	0.4192	0.4338	0.4226	0.018	0.022	0.018	0.5171	0.489	0.49
H5SAL	R2	0.6862	0.6519	0.6288	0.4083	0.43	0.5081	0.009	0.0103	0.0158	0.4907	0.483	0.485
C2P	R3	0.448	0.41107	0.4101	0.2485	0.2414	0.2273	0.11	0.12	0.0793	0.2205	0.2346	0.2499
C2P	R1	0.4439	0.41107	0.4053	0.2437	0.2386	0.225	0.128	0.126	0.102	0.237	0.2264	0.2505
C2P	R2	0.4234	0.41107	0.4008	0.2254	0.2236	0.2273	0.1	0.1026	0.088	0.217	0.2288	0.2316
C4P	R3	0.448	0.4276	0.4175	0.2752	0.266	0.2712	0.14	0.145	0.116	0.249	0.2552	0.2712
C4P	R1	0.448	0.4189	0.4102	0.2584	0.2483	0.2279	0.135	0.152	0.11	0.2499	0.2403	0.2701
C4P	R2	0.448	0.4171	0.4048	0.2456	0.266	0.2279	0.108	0.12	0.095	0.234	0.2312	0.2712
H5P	R3	0.4328	0.414	0.4008	0.223	0.2231	0.235	0.07	0.1036	0.0793	0.2048	0.2199	0.2189
H5P	R1	0.4283	0.4055	0.3994	0.239	0.2332	0.2232	0.097	0.1207	0.0927	0.2267	0.2199	0.2427
H5P	R2	0.4117	0.3984	0.384	0.2135	0.2241	0.227	0.093	0.098	0.0695	0.2134	0.2199	0.2214

3.2. Final absorbance of reactive dyes at various pH

Isolate	REPLICATION	Absorbance of reactive dyes at various pH								
		at pH 5			at pH 7			at pH 9		
		Color			Color			Color		
		RED	BLUE	YELLOW	RED	BLUE	YELLOW	RED	BLUE	YELLOW
		Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
H1E	R1	0.5308	0.5394	0.5191	0.03	0.072	0.0325	0.423	0.4234	0.4027
H1E	R2	0.5608	0.5376	0.5118	0.04	0.063	0.0399	0.442	0.4327	0.4089
H1E	R3	0.5389	0.5394	0.517	0.035	0.05	0.0335	0.4256	0.4252	0.4027
H2E	R1	0.58	0.555	0.5336	0.138	0.137	0.118	0.6924	0.4334	0.401
H2E	R2	0.5813	0.5266	0.5281	0.117	0.1175	0.102	0.4477	0.4431	0.3969
H2E	R3	0.5655	0.5439	0.5241	0.114	0.097	0.0917	0.4425	0.4364	0.401
H3E	R1	0.598	0.55018	0.5344	0.116	0.127	0.096	0.4414	0.4401	0.4077
H3E	R2	0.6018	0.5159	0.5188	0.09	0.109	0.076	0.4306	0.4233	0.3821
H3E	R3	0.589	0.5569	0.53336	0.0876	0.103	0.076	0.4346	0.4257	0.3964
H5E	R1	0.607	0.5569	0.55	0.164	0.14	0.1063	0.438	0.4188	0.4089
H5E	R2	0.5911	0.522	0.5281	0.1	0.1175	0.0835	0.423	0.4177	0.3835
H5E	R3	0.5872	0.5664	0.5254	0.139	0.11	0.096	0.4414	0.4326	0.3972
H1K	R1	0.627	0.6264	0.599	0.11	0.103	0.1135	0.5044	0.4923	0.4667
H1K	R2	0.6626	0.6037	0.5664	0.099	0.1024	0.0955	0.492	0.4707	0.4448
H1K	R3	0.6443	0.6525	0.61205	0.096	0.099	0.0938	0.4767	0.4686	0.4448
H2K	R1	0.6608	0.6472	0.5978	0.137	0.119	0.111	0.499	0.4931	0.4653
H2K	R2	0.6474	0.6368	0.5569	0.118	0.137	0.091	0.5036	0.4798	0.4494
H2K	R3	0.6439	0.6351	0.6047	0.113	0.094	0.091	0.484	0.4856	0.45127
H3K	R1	0.6143	0.6264	0.5944	0.071	0.078	0.069	0.4787	0.469	0.454
H3K	R2	0.655	0.6253	0.5476	0.068	0.078	0.064	0.4929	0.4707	0.4455
H3K	R3	0.62518	0.6264	0.5747	0.045	0.068	0.0635	0.4695	0.4625	0.438
H4K	R1	0.6608	0.6546	0.5764	0.175	0.1557	0.137	0.499	0.4719	0.4535
H4K	R2	0.6319	0.6481	0.5476	0.154	0.14	0.134	0.484	0.461	0.4427
H4K	R3	0.6319	0.6438	0.5829	0.135	0.128	0.1206	0.4725	0.4625	0.4374
H5K	R2	0.6393	0.636	0.5829	0.126	0.1168	0.111	0.4921	0.4696	0.444
H5K	R3	0.617	0.6037	0.5664	0.109	0.1045	0.098	0.474	0.4625	0.4267
H5K	R1	0.6251	0.6438	0.593	0.103	0.1026	0.096	0.4777	0.4628	0.4476
C2SH	R2	0.6965	0.696	0.6485	0.025	0.028	0.0249	0.6191	0.6191	0.5857
C2SH	R3	0.7242	0.6945	0.6247	0.018	0.028	0.0176	0.6321	0.6117	0.5764

C2SH	R1	0.705	0.696	0.6416	0.0175	0.028	0.024	0.6119	0.6117	0.5661
H2SH	R2	0.723	0.6873	0.6677	0.092	0.075	0.087	0.6315	0.6117	0.5851
H2SH	R3	0.705	0.6638	0.6609	0.1089	0.086	0.077	0.2614	0.6095	0.5731
H2SH	R1	0.7002	0.696	0.6574	0.0855	0.07	0.065	0.622	0.6117	0.571
H4SAL	R2	0.6965	0.6914	0.6668	0.083	0.0866	0.0793	0.5952	0.5903	0.5556
H4SAL	R3	0.714	0.7142	0.6773	0.067	0.077	0.0658	0.5805	0.5838	0.5527
H4SAL	R1	0.6876	0.6873	0.6593	0.055	0.064	0.0657	0.5767	0.5744	0.5466
H5SAL	R2	0.7011	0.6894	0.6633	0.02	0.027	0.024	0.5479	0.5595	0.5305
H5SAL	R3	0.7242	0.6894	0.6773	0.018	0.022	0.018	0.5621	0.5785	0.5365
H5SAL	R2	0.7054	0.6894	0.6485	0.009	0.0103	0.0158	0.5459	0.5628	0.5358
C2P	R3	0.4484	0.4533	0.4515	0.11	0.12	0.0793	0.3306	0.3298	0.3525
C2P	R1	0.459	0.4402	0.4474	0.128	0.126	0.102	0.3512	0.3292	0.3422
C2P	R2	0.4286	0.4351	0.4351	0.1	0.1026	0.088	0.3153	0.3491	0.3384
C4P	R3	0.4594	0.4698	0.4597	0.14	0.145	0.116	0.3308	0.3357	0.3313
C4P	R1	0.4795	0.4718	0.4466	0.135	0.152	0.11	0.3422	0.3227	0.3299
C4P	R2	0.4643	0.4558	0.4433	0.108	0.12	0.095	0.316	0.3177	0.3259
H5P	R3	0.4315	49.78%	0.4198	0.07	0.1036	0.0793	0.318	0.3207	0.3102
H5P	R1	0.4384	47.90%	0.4408	0.097	0.1207	0.0927	0.3383	0.3207	0.3278
H5P	R2	0.4286	50%	0.4138	0.093	0.098	0.0695	0.3162	0.3207	0.3119

3.3. Final absorbance of reactive dyes at various dye concentration

Isolate	REPLICATION	Absorbance of reactive dyes on various dye concentration								
		at 50 mg/l			at 100 mg/l			at 150 mg/l		
		Color			Color			Color		
		RED	BLUE	YELLOW	RED	BLUE	YELLOW	RED	BLUE	YELLOW
		Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
H1E	R1	0.03	0.072	0.0325	0.611	0.676	0.546	1.463	1.386	1.267
H1E	R2	0.04	0.063	0.0399	0.644	0.65	0.531	1.542	1.352	1.243
H1E	R3	0.035	0.05	0.0335	0.59	0.6107	0.56	1.483	1.386	1.31
H2E	R1	0.138	0.137	0.118	0.696	0.642	0.575	1.58	1.435	1.338
H2E	R2	0.117	0.1175	0.102	0.696	0.559	0.546	1.508	1.368	1.31
H2E	R3	0.114	0.097	0.0917	0.696	0.659	0.549	1.543	1.445	1.268
H3E	R1	0.116	0.127	0.096	0.72	0.676	0.558	1.596	1.484	1.366

H3E	R2	0.09	0.109	0.076	0.679	0.634	0.56	1.518	1.267	1.279
H3E	R3	0.0876	0.103	0.076	0.714	0.645	0.56	1.51	1.333	1.35
H5E	R1	0.164	0.14	0.1063	0.686	0.642	0.751	1.652	1.478	1.399
H5E	R2	0.1	0.1175	0.0835	0.66	0.581	0.532	1.534	1.355	1.292
H5E	R3	0.139	0.11	0.096	0.644	0.659	0.533	1.596	1.484	1.362
H1K	R1	0.11	0.103	0.1135	0.811	0.811	0.704	1.675	1.55	1.465
H1K	R2	0.099	0.1024	0.0955	0.87	0.75	0.718	1.577	1.459	1.422
H1K	R3	0.096	0.099	0.0938	0.8	0.794	0.68	1.65	1.156	1.397
H2K	R1	0.137	0.119	0.111	0.864	0.791	0.724	1.702	1.592	1.442
H2K	R2	0.118	0.137	0.091	0.818	0.793	0.719	1.569	1.514	1.42
H2K	R3	0.113	0.094	0.091	0.8375	0.815	0.689	1.612	1.507	1.378
H3K	R1	0.071	0.078	0.069	0.835	0.76	0.657	1.542	1.532	1.442
H3K	R2	0.068	0.078	0.064	0.8	0.697	0.632	1.574	1.489	1.42
H3K	R3	0.045	0.068	0.0635	0.635	0.794	0.704	1.516	1.435	1.353
H4K	R1	0.175	0.1557	0.137	0.844	0.76	0.689	1.676	1.532	1.444
H4K	R2	0.154	0.14	0.134	0.783	0.718	0.646	1.569	1.483	1.415
H4K	R3	0.135	0.128	0.1206	0.814	0.812	0.675	1.574	1.483	1.387
H5K	R2	0.126	0.1168	0.111	0.87	0.783	0.721	1.622	1.556	1.449
H5K	R3	0.109	0.1045	0.098	0.853	0.728	0.704	1.572	1.489	1.42
H5K	R1	0.103	0.1026	0.096	0.82	0.827	0.732	1.644	1.483	1.379
C2SH	R2	0.025	0.028	0.0249	0.766	0.76	0.632	1.782	1.611	1.491
C2SH	R3	0.018	0.028	0.0176	0.753	0.693	0.564	1.667	1.611	1.532
C2SH	R1	0.0175	0.028	0.024	0.731	0.762	0.648	1.649	1.611	1.531
H2SH	R2	0.092	0.075	0.087	0.8	0.743	0.589	1.729	1.678	1.556
H2SH	R3	0.1089	0.086	0.077	0.731	0.665	0.546	1.667	1.635	1.533
H2SH	R1	0.0855	0.07	0.065	0.818	0.777	0.617	1.702	1.653	1.531
H4SAL	R2	0.083	0.0866	0.0793	0.683	0.696	0.618	1.744	1.678	1.597
H4SAL	R3	0.067	0.077	0.0658	0.766	0.654	0.553	1.675	1.641	1.531
H4SAL	R1	0.055	0.064	0.0657	0.706	0.743	0.617	1.729	1.729	1.619
H5SAL	R2	0.02	0.027	0.024	0.666	0.676	0.574	1.652	1.556	1.444
H5SAL	R3	0.018	0.022	0.018	0.731	0.633	0.574	1.667	1.647	1.531
H5SAL	R2	0.009	0.0103	0.0158	0.661	0.677	0.574	1.596	1.507	1.394
C2P	R3	0.11	0.12	0.0793	0.488	0.507	0.445	1.143	1.108	1.042
C2P	R1	0.128	0.126	0.102	0.47	0.528	0.387	1.197	1.155	0.9
C2P	R2	0.1	0.1026	0.088	0.452	0.463	0.416	1.117	1.07	1.042
C4P	R3	0.14	0.145	0.116	0.557	0.591	0.488	1.25	1.146	1.042
C4P	R1	0.135	0.152	0.11	0.487	0.549	0.431	1.119	1.118	0.91
C4P	R2	0.108	0.12	0.095	0.518	0.524	0.474	1.184	1.096	1.02
H5P	R3	0.07	0.1036	0.0793	0.458	0.509	0.431	1.09	1.118	1.043
H5P	R1	0.097	0.1207	0.0927	0.504	0.507	0.409	1.119	1.072	1.076
H5P	R2	0.093	0.098	0.0695	0.442	0.473	0.434	1.048	1.024	0.943