ISOLATION, IDENTIFICATION AND EFFICACY ASSESSMENT OF BACTERIA DEGRADING AZO DYE & REACTIVE DYE FROM RIVER SLUDGE SAMPLE

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A thesis submitted to the Department of Mathematics & Natural Science in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology.

Department of Mathematics & Natural Science

Brac University

November, 2022

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

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Ethics Statement

Hereby, we consciously assure that for the study the following is fulfilled:

1) This material is the authors' own original work, which has not been previously published elsewhere.

2) The paper is not currently being considered for publication elsewhere.

3) The paper reflects the authors' own research and analysis in a truthful and complete manner.

4) The paper properly credits the meaningful contributions of co-authors and co-researchers.

5) The results are appropriately placed in the context of prior and existing research.

6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.

The violation of the Ethical Statement rules may result in severe consequences.

I agree with the above statements and declare that this submission follows the policies of Solid State Ionics as outlined in the Guide for Authors and in the Ethical Statement.

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Abstract:

Textile and dyeing industry's effluents and wastes are considered one of the top water pollutants in Bangladesh which ultimately leads to public health hazard. This study intends to explore the capacity of local bacteria to degrade dye and the roles of pH concentration and temperature in facilitating Azo dye and Reactive Dye degradation. Samples from river sludge were taken from the Dhaleshwari and Turag rivers and serially diluted. Then bacterial isolation was randomly selected based on morphology and each individual colony of focus was inoculated in dye-mixed nutritional broth. Colonies were cultured for seven days at various temperatures and had different pH concentrations to study biodegradability. To determine the actual decolourizationn (%) amount, optical density values were measured for each sample in the meanwhile. All microorganisms demonstrated successful biodegradability for selected dyes. Biochemical tests were carried out for the identification of the bacterial strains. This study found that *Bacillus spp.* degraded azo dye at a rate of 69.26% at pH 6 & 37 ˚c. Furthermore, *Bacillus spp.* Attained 52.70 % degradation at pH 6 and 45 °c. The results disclosed that *Bacillus spp.* were the most efficient in degrading reactive and azo dyes, followed by *Bacillus subtilis* and *Staphylococcus hominis*. This study can further instigate the scope of the biodegradability of dye to be ready for commercial usage for effluent treatment.

This thesis is dedicated to Akash Ahmed, our loving supervisor, mentor & teacher, who taught us to never give up.

Table of contents:

Declaration……(ii)

Approval……...……...(iii)

Ethics statement……..……..……...….(iv)

Abstract…….(v)

Dedication……...…….(vi)

Table of contents…….(vii)

List of tables……...……..(ix)

List of figures……(x)

Chapter

1. Introduction & Background…….…….(xi)

1.1 Basic information…….…….(xi)

1.2 Azo dye…….…….(xi)

1.3 Reactive dye…….…….(xii)

1.4 Effects on environment & ecosystem…….….(xiii)

1.5 Effects on human health…….…….(xiii)

1.6 Remarkable benefits of Biodegradation……..….(xiv)

1.7 Objective & aim of the study……....(xiv)

2. Materials & Methodology……....(xv)

2.1 Sample collection ……....(xv)

2.2 Bacteria isolation ……(xv)

2.3 Media & dye……....(xvi)

2.4. Decolorization assessment…….…....(xvii)

2.5 Quantitative assessment ……(xviii)

2.6 Biochemist test…….(xix)

3. Result & observation ……..…(xxii)

3.1 CFU count……..…..(xxii)

3.2 Optical density result…….…..…(xxiii)

3.3 Decolorization (%) result…….……..(xxvi)

3.4 Identification of bacteria……(xxxi)

3.5 Effect of pH on dye decolorization …….…….…….(xxxi)

3.6 Effect of temperature variation on dye decolorization ……..…..(xxxii)

4. Discussion…….……(xxxiii)

5. Conclusion…….……(xxxv)

6. References ……(xxxvi)

List of Tables:

1. Colony forming unit count of Dhaleshwari river……(xxii)
2. Colony forming unit count of Turag river……...……(xxii)

List of figures:

1. Chemical structure of azo dye…….....…(xi)
2. Chemical structure of reactive dye……...…..(xii)
3. 5% w/v azo dye & reactive dye added nutrient broth ……..…..(xvi)
4. Decolorization of 5% w/v azo dye…….……..…..(xvii)
5. Decolorization of 5% w/v reactive dye…….…....(xvii)
6. Decolonization (%) formula for calculating decolorization percentage of dye by organisms……..……(xviii)
7. OD value of azo dye from pH 4 & 37°c…….…..(xxiv)
8. OD value of azo dye from pH 4 & 45°c……..…….(xxiv)
9. OD value of azo dye from pH 6 & 37°c……..……..…...(xxiv)
10. OD value of azo dye from pH 6 & 45°c……..……..…..(xxiv)
11. OD value of azo dye from pH 8 & 37°c……..……..(xxv)
12. OD value of azo dye from pH 8 & 45°c……..……..…...(xxv)
13. OD value of reactive dye from pH 4 & 37°c……..……..(xxv)
14. OD value of reactive dye from pH 4 & 45°c……..……..…..(xxv)
15. OD value of reactive dye from pH 6 & 37°c……..……..(xxvi)
16. OD value of reactive dye from pH 6 & 45°c……..……....(xxvi)
17. OD value of reactive dye from pH 8 & 37°c……..……..(xxvi)
18. OD value of reactive dye from pH 8 & 45°c……..……..(xxvi)
19. Decolorization (%) of azo dye…….(xxvii)
20. Decolorization (%) of reactive dye……...……(xxviii)
21. Decolorization (%) of azo dye at pH 4, pH 6 & pH 8 by each organism……(xxxi)
22. Decolorization (%) of reactive dye at pH 4, pH 6 & pH 8 by each organism…..…(xxxii)
23. Decolorization (%) of azo dye at 37°C and 45°C by each organism……..…(xxxiii)
24. Decolorization (%) of reactive dye at 37°C and 45°C by each organism……..(xxxiv)

Chapter 1

Introduction & Background:

1.1 Basic Information:

Out of 5 major basic needs of human life, cloth is one of them. In recent decades, the need for garment products has risen sky high. Bangladesh is the 3rd largest garment products exporter in the world also becoming a significant part of the economy. According to BGMEA, this garments sector maintains a 6% annual average GDP growth rate. But the textile industry has been considered a major water-wasting industry. In China, approximately more than 2.5-billion-liters of water is being wasted every year (Cook, 2021). Over 2, 80,000 tons of textile dyes are thought to be released into industrial wastewater each year, globally. Various types of dye are used in the garments sector for dyeing & conditioning throughout the production period. The most common types of dye used in Bangladesh are Azo dye, Acid dye, Reactive Dye, Direct Dye, All-Purpose dye etc.

1.2 Azo dye:

The first synthetic dye, was named Mauveine in 1856 by William Henry Perkin. Afterwards, there have been thousands of new synthetic dyes were developed. Among most of the significant synthetic dyes, azo dyes have been used extensively in the production of textiles, printing, and paper, among other things. (Benkhaya et al., 2020). In actuality, around 50% of the dyes used in industry today are azo dyes (Otutu, 2013).

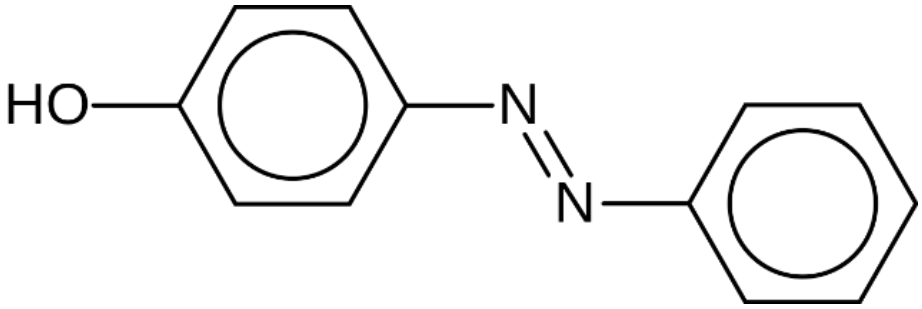

Figure: Chemical Structure of Azo dye [Source: https://en.wikipedia.org/wiki/File:4-hydroxyphenylazobenzene.png]

Azo dyes are common dyes, and their fundamental formula is R-N=N-R, where the azo group (-N=N) and the Rs are aromatic functional groups to which they are attached. They produce vivid colors like red and yellow, which are frequently used to color commercial items including meals, leather, cosmetics, and most textiles. These colors, which are made of aromatic hydrocarbons from benzene, toluene, aniline, phenol, and naphthalene, are persistent substances that are challenging to degrade naturally or chemically (Puvaneswari,N., Muthukrishnan, and J., Gunasekaran, P., 2006).

1.3 Reactive Dye:

The reactive dye does have a reactive group but it may chemically react with a substrate such as cotton, wool, polyamide, etc. during the correct conditions to generate covalent dye-substrate linkages. Here, the dye has a reactive group that forms a covalent link with the polymer of the fiber and acts as an essential part of the fiber. The terminal -OH (hydroxyl) group of cellulose fibers or the terminal -NH2 (amino) group of polyamide or wool fibers act as the receptors for the forming of this covalent bond between the dye molecule and the fibers.

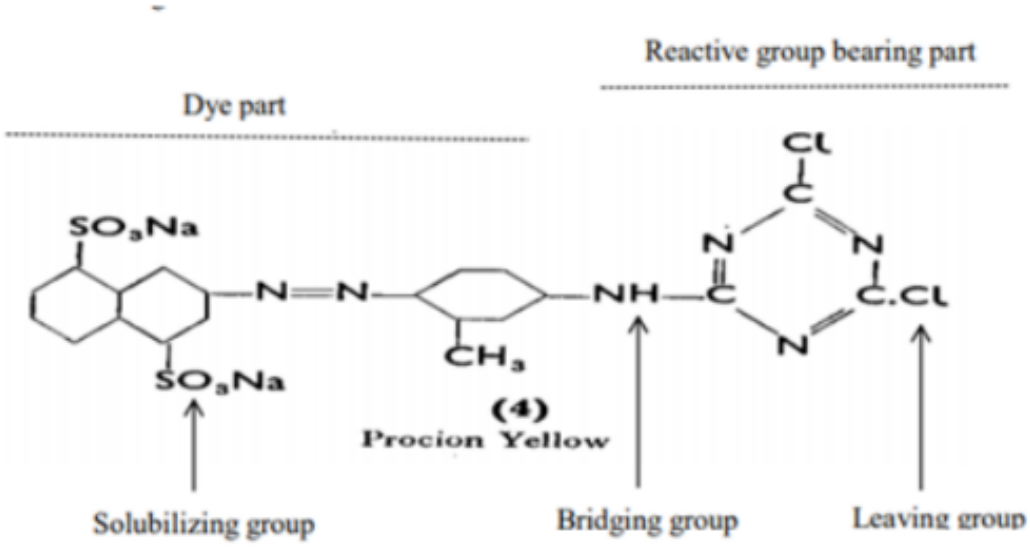

Figure: Chemical Structure of Reactive dye [Source: https://diutestudents.blogspot.com/2019/05/reactive-dyes-and-its-mechanism.html]

This is the only kind of dye that has reactive groups, which interact chemically with the molecules of the fiber polymer to establish covalent bonds. This covalent bond is created between the reactive group and the terminal -NH2 (amino) group of wool fiber, the terminal -OH (hydroxyl) group of polyamide, and the cellulose fiber polymer. A common characteristic of reactive dye molecules is, Dyes have an anionic functional group, an acidic chemical structure, and a reactive direct or mordant dye that forms a covalent bond with fiber (Safwan, 2022).

1.4 Effects on environment & ecosystem:

Despite being a huge financial support and generating workplace for millions of people every year, the garment industry causes devastating natural pollution. Textile and dyeing industry's effluents and wastes are considered one of the top water pollutants in Bangladesh. A few rivers beside Dhaka and Gazipur city have been declared biologically dead by the government due to the effluents and dyeing waste from the nearby garment industries, as the amount of dissolved oxygen is nearly zero in the water (Bangladesh's Polluted Waters: Rivers Dying Due to Dyeing, 2019). Every day large amounts of waste materials and severe polluting effluents are dumped into direct river water via pipelines, drains or sewers without any waste treatment activity.

Along with other toxic materials, the industrial dyes from the effluents pollute the river water every day. Thus, it contaminates nearby agricultural fields, and other surface water sources and destroys marine life and biodiversity. These textiles and dyeing effluents are directly involved in altering the chemical, physical and marine biological assets of river waters by causing the temperature and pH change, foul smell and turbidity change.

1.5 Effects on human health:

Concerning point is, every day these river water building up major issues like a public health risk, contaminating livestock, marine environment, fish and other aquatic animals, crop and agricultural fields. The presence of the dyes in the water not only makes it impotent to drink but also raises many possibilities of causing water-borne diseases like nausea, skin ulcer and damage, hemorrhage, severe bronchial and respiratory irritation and diseases (Islam et al., 2011).

1.6 Remarkable benefits of Biodegradation:

Multiple physical and chemical processes, including ozonation, photochemical reaction, sodium hypochlorite, electrochemical destruction, activated carbon, silica gel, membrane filtration etc. can be used to treat textile wastewater to eliminate dyes. Even though these methods are used to treat effluent, they have many drawbacks, including the production of sludge, extra by-products, the necessity of lengthy retention time. Additionally, the requirement for high concentrations of dissolved oxygen, which is nearly expensive and this is only possible to apply on a small quantity (Robinson et al.,2002). All those techniques have been found to be less effective due to either heavy cost, low efficiency, poor adaptability, interference from other wastewater elements, and the treatment of the trash they produce in process makes them poor choice (Kaushik et al, 2009).

Currently, it has been studied that a variety of species of gram-negative bacteria from various genus, including *Aeromonas, Escherichia, Citrobacter, Pseudomonas, and Sphingomonas*, are capable to degrade dye solutions or simulated effluents. (Forgacs E et al.,2004). Additionally, it has been found certain gram-positive bacteria, including *Bacillus, Clostridium, Nocardia, Paenibacillus and Streptomyces, Micrococcus*, etc., decompose synthetic dyes. It has been found that while bacterial strains grew well in aerobic or shaking incubators, color removal was accomplished with a high degree of efficiency. Bacterial biodegradation provides a more practical, affordable, and ecofriendly way to solve the issue without the burden of a lengthy procedure and a lot of hazardous waste.

1.7 Objective & aim of the study:

The objective of the study is to isolate a native bacteria strain from sample of river sludge that has the potential to degrade reactive and azo dyes effectively. Additionally, the isolates identification using several biochemical tests.

The purpose was also to determine how different temperature and pH levels impacted the decolorization process.

Chapter 2

Materials & Methodology:

2.1 Sample collection:

The Dhaleshwari River at Savar and the Turag River at Tongi were the two locations from where samples of river sludge were taken. Sludge was collected from both rivers & immediately transported also preserved at 4°C for use as the source of microbes in the later.

2.2 Bacteria Isolation:

Among each sample, sludge was diluted in order to prevent a high bacterial load by 10-fold Serial Dilution method. By using the spread plate technique, diluted sample was obtained and inoculated in LB Agar & Bacillus Cereus Agar. The plates were incubated for 24 hours at 37 °C. Each plate's CFU was counted following incubation.

Next, single colonies were chosen at random after being visually observed for colony morphology,

2.3 Media and Dye:

LB Agar:

LB Agar which is consists of 10 g/liter Tryptone, 5g/liter Yeast Extract, 10g/liter NaCl and 1.5% of final concentration Agar powder. All the ingredients were dissolved in distilled or deionized water before the mixture was heated to boiling. Sterilization was accomplished by autoclaving for 15 minutes between 121 and 124°C at 15lbs pressure.

Bacillus Cereus Agar:

Bacillus Cereus Agar Base which is consists of 1g/liter Peptone, 10g/liter Mannitol, 2g/liter Sodium chloride, 0.1g/liter Magnesium sulphate, 2.51g/liter Disodium hydrogen phosphate, 0.250g/liter Potassium dihydrogen phosphate, 10 g/liter Sodium pyruvate, 0.120 g/liter Bromo thymol blue & 15g/liter Agar powder. All the ingredients were dissolved in distilled or deionized

water before the mixture was heated to boiling. Sterilization was conducted by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Nutrient Broth:

Nutrient Broth is consisting of 1g/liter beef extract, 2g/liter yeast extract, 5g/liter peptone and 5g/liter NaCl (Sodium chloride). Each component was dissolved in distilled or deionized water before the mixture was heated to boiling. Afterwards Sterilization was accomplished by autoclaving for 15 minutes between 121 and 124°C at 15lbs pressure.

Dye selection:

Reactive Red 3BX which is azo dye & Reactobond Blue H-RSPL which is reactive dye was used for the study. Each dye was added to nutrient broth to evaluate if sample isolated bacteria degraded them.

2.4 Decolorization Assessment:

A combination of 5% w/v azo dye and reactive dye was added to 100ml of nutrient broth to assess the degradation of the dyes. Afterwards, selected isolates were inoculated in the nutrient broth which containing dyes. The broth was then incubated for 24 hours at 37 °C in incubator.

Figure: 5% w/v azo dye & reactive dye added nutrient broth

Figure: Decolorization of 5% w/v Azo dye

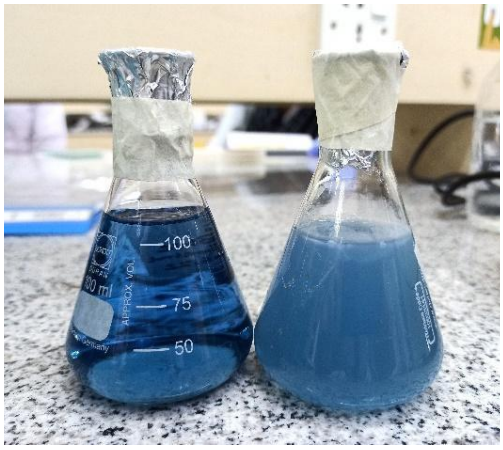

Figure: Decolorization of 5% w/v Reactive dye (Control in the left side)

pH:

For both red and blue dye, three different pH; pH 4, pH 6, and pH 8 were tried to see which was the most efficient pH for dye degradation. Thus, nutrient broth was prepared at these three

distinct pH levels. Afterwards, the isolates were inoculated and incubated and values of optical density were obtained.

Temperature:

Two different temperature 37 °C & 45 °C were adjusted while incubation to find out the effective temperature for dye degradation.

2.5 Quantitative Assessment:

The term "optical density" means how quickly a light wave passes through an object. The logarithmic ratio between radiation imposed on a substance and radiation it transmits is used to quantify optical density. Therefore, optical density determines how fast light travels through an object. The optical density is mostly influenced by the light wave's wavelength (Madhu 2018). The property is measured using spectroscopy, particularly for quantitative analysis (Helmenstine, 2022). Dyes are elements that absorb light with wavelength in the visible spectrum, between 400 and 700 nm. (Van der Zee et al., 2003).

Freshly cultured isolates were once again inoculated in the nutrient broth & incubated for 24 hours at 37 °C and 120 rpm in a shaking incubator for 7 days. Following that, Optical density measurements were carried out using a spectrophotometer to assess the dye degradation. To determine the initial absorbance of the control, the OD of the control solution was measured at 500nm using dye-free nutritional broth as the reference. each Sample was collected in Eppendorf every 24 hours & spectrophotometer was used to determine the optical density of the supernatant for the red dye at 500 nm. This was done again over the course of seven days to get optical density values every 24 hours. Similarly, for blue dye the OD of the control was measured at 550nm and also the optical density for seven days was measured at 550nm and the values were collected.

$$\text{Decolorization (\%)} = \left[\frac{(A_0 - A_t)}{A_0} \right] \times 100,$$

Figure: Decolorization (%) formula for calculating decolorization percentage of dye by organisms

The decolorization method was then figured out using the above-mentioned formula. Here, A_0 is initial absorbance of media with dye before inoculation & A_t is absorbance of media with dye after bacterial decolonization process. Later the Decolorization values (%) were noted.

2.6 Biochemical test:

Several biochemical tests were carried out to identify bacteria strains.

Gram Staining test:

Based on the characteristics of their cell walls, the Gram stain is a differentiating staining technique used to classify bacteria into the gram-positive or gram-negative categories. It is often referred to as Gram's procedure or Gram staining. The process is dependent on the reaction between peptidoglycan in the cell walls of bacteria. For the Gram stain, bacteria are stained, the color is fixed using a mordant, the cells are decolorized and then a counterstain is applied (Helmenstine, 2019). A loopful of sample was used to generate a smear of suspension on a clean, glass slide. Next, glass slide was air dried and heat fixed. Following that, Crystal Violet was poured, held for about 60 seconds, and then rinsed with distilled water. The gram's iodine was then added, left on for 1 minute, and then removed using distilled water. Following that, washed with 95% alcohol for around 10 seconds & then rinsed with distilled water. Safranin was applied for around a minute before being rinsed with water once more. The glass slide was then air dried and examined under a microscope after that.

Catalase test:

The catalase test aids in the identification of the catalase enzyme in bacteria. The catalase enzyme works to neutralize hydrogen peroxide's bactericidal effects (Reiner, 2016). Ethanol was

used to clean and disinfect a glass slide. On the slide, bacterial culture were placed &v 2-3 drops of 3% hydrogen peroxide was added. It was then watched for thirty seconds. Depending on the glass Slide created bubbles that could be positive or negative.

Oxidase test:

An enzyme termed indophenols oxidase is produced as part of the oxidase test. The reagent's redox dye is oxidized by this enzyme, changing its color from yellow to dark purple. This test was carried out by filter paper method (Shields & Cathcart, 2010). On a piece of filter paper, few drops of the oxidase test reagent were added. In the reagent-soaked paper a loopful of bacteria was smeared onto it using sterile wooden stick & waited for 30 seconds. Later the results for each bacteria was observed.

TSI test:

The measurement of carbohydrate fermentation and H2S generation uses the tubed differential medium known as triple sugar iron (TSI) agar. Additionally, gas produced by the metabolism of carbohydrates can be found. Bacteria may process carbohydrates either fermentatively or aerobically. The TSI classifies bacteria according to how they ferment lactose, glucose, and sucrose as well as how they produce hydrogen sulfide (Lehman, 2005). All isolates were inoculated on TSI agar media & was incubated for 24hours and after incubation was results from each bacteria were checked & noted.

Motility test:

The motility of microorganisms is assessed using a motility test medium. Motility has long been acknowledged as a crucial taxonomic tool and biological characteristic of microorganisms. An easy way to assess motility is with a motility test medium containing triphenyltetrazolium chloride (Shields & Cathcart, 2011). A well-isolated colony of each isolate was used to stab the medium to within 1 cm of the tube's bottom in order to test for motility. Growth was observed after 24 hours of incubation at 35°C.

Urease test:

The urease test reveals the presence of microbes that can hydrolyze urea to generate ammonia and carbon dioxide. It is mainly used for distinguishing *Enterobacteriaceae* from urease-positive *Proteeae.* 2% urea and phenol red, a pH indicator, present in the urease test media. The hue changes from yellow (pH 6.8) to bright pink (pH 7) as a result of a rise in pH put on by the synthesis of ammonia (pH 8.2) (Brink, 2010). A single colony of each isolate was inoculated after making the urease test medium and was then incubated. Results were noticed and documented following incubation.

Indole test:

The indole test evaluates an organism's capacity to break down the amino acid tryptophan and generate indole (P. MacWilliams, 2009). Tryptophan Broth containing 5 g of NaCl and 10 g of Tryptone per 1000ml of distilled water was prepared and were filled in test tubes, autoclaved thereafter. After that, the bacterial culture was introduced and kept at 37°C for 24 hours for incubation.

A few drops of Kovac's reagent were added after incubation, and the mixture was then monitored for 10 to 15 minutes. A red or pink ring structure formation on top is considered to be positive, else negative.

MR-VP test:

The Voges-Proskauer and methyl red tests are a part of the IMViC test of biochemical assessments. To differentiate between members of the *Enterobacteriaceae* family, MR-VP tests were performed (McDevitt, 2009). For conduct this test, MR VP test medium was prepared, and a single colony of each isolate was inoculated and incubated. Results were observed and recorded after incubation.

Citrate test:

The citrate test determines if a bacterial isolate can use citrate as a source of carbon and energy. The production of alkaline waste products from citrate metabolism is the basis for a positive testing. The shade of a pH indicator changes to show the following rise in the medium's pH. The

citrate test is used to identify environmental isolates and gram-negative pathogens (P. MacWilliams, 2009). A single colony of each isolate was put into the citrate test medium and incubated. Results were evaluated and noted following incubation.

Nitrate Reduction test:

A useful part of biochemical test batteries for identifying bacteria is the nitrate reduction test. It can be used to distinguish between different gram-negative bacilli, identify species of Neisseria and distinguish them from *Moraxella* and *Kingella* species, and make it easier to identify *Corynebacterium* and other *Asporogenous* gram-positive bacilli by species. Each isolate was incubated as a single colony in the nitrate reduction test medium. Results were assessed and recorded after incubation.

Chapter 3

Result & Observation:

3.1 CFU count

CFU count of both samples are mentioned below:

Media	CFU per ml
LB	TNTC
LB	2.46×10^6
LB	4.2×10^7
LB	TFTC
BC	1.9×10^6
BC	9×10^6
BC	6.4×10^7

BC	TFTC

Table: Colony Forming Unit count of Dhaleshwari River Sample

Media	CFU per ml
LB	TNTC
LB	1.2×10^{7}
LB	TFTC
LB	TFTC
BC	2.1×10^{6}
BC	6.4×10^{6}
BC	TFTC
BC	TFTC

Table: Colony Forming Unit count from Turag River Sample

3.2 Optical Density result:

Optical Density of Azo dye is visualized through graph below:

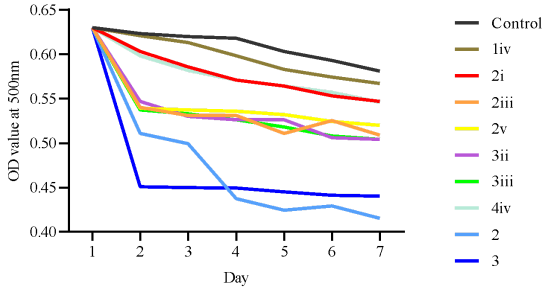

Figure: OD value from pH 4 & 37°C batch

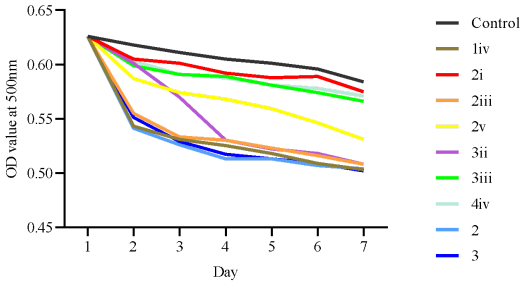

Figure: OD value from pH 4 & 45°C batch

Here in the left figure, it visualizes Optical Density value at 500nm wavelength of each isolate from pH 4 azo dye medium & 37°C incubation for 7 days. On the right-side figure, Optical Density value at 500nm wavelength of each isolate from pH 4 azo dye medium & 45°C incubation for 7 days.

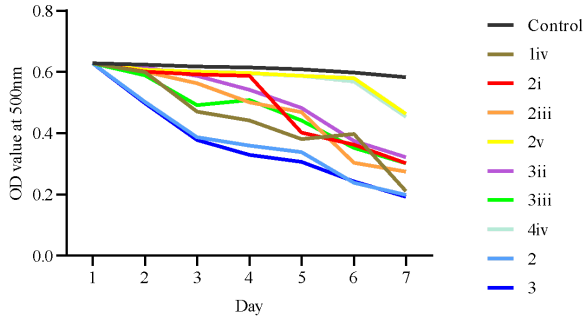

Figure: OD value from pH 6 & 37°C batch

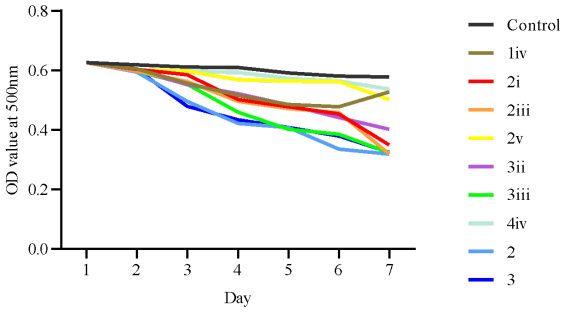

Figure: OD value from pH 6 & 45°C batch

Here in the left side figure, it visualizes Optical Density value at 500nm wavelength of each isolate from pH 6 azo dye medium & 37°C incubation for 7 days. On the other side figure, Optical Density value at 500nm wavelength of each isolate from pH 6 azo dye medium & 37°C incubation for 7 days.

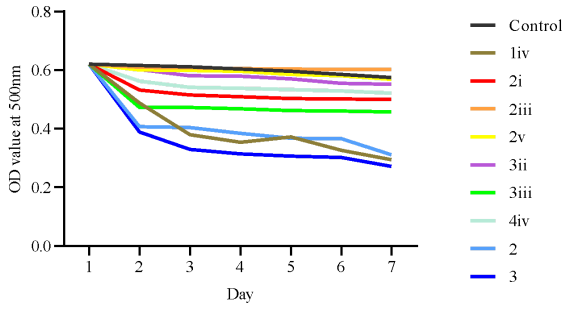

Figure: OD value from pH 8 & 37°C batch

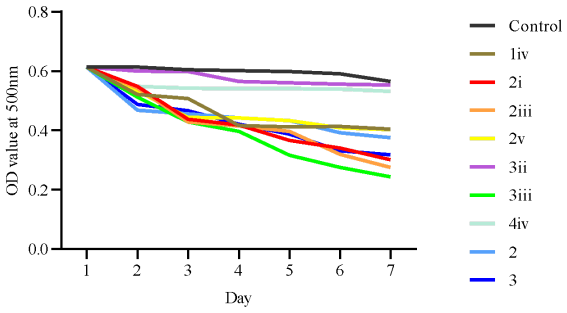

Figure: OD value from pH 8 & 45°C batch

On the left side figure, it visualizes Optical Density value at 500nm wavelength of each isolate from pH 8 azo dye medium & 37°C incubation for 7 days. On the other side figure, Optical Density value at 500nm wavelength of each isolate from pH 8 azo dye medium & 37°C incubation for 7 days.

Optical Density of Reactive dye is visualized through graph below:

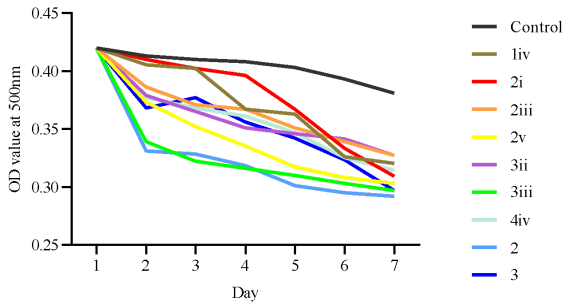

Figure: OD value from pH 4 & 37°C batch

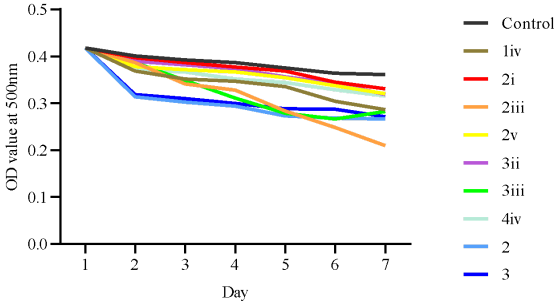

Figure: OD value from pH 4 & 45°C batch

Here in the left figure, it visualizes Optical Density value at 550nm wavelength of each isolate from pH 4 reactive dye medium & 37°C incubation for 7 days. On the right-side figure, Optical Density value at 550nm wavelength of each isolate from pH 4 reactive dye medium & 45°C incubation for 7 days.

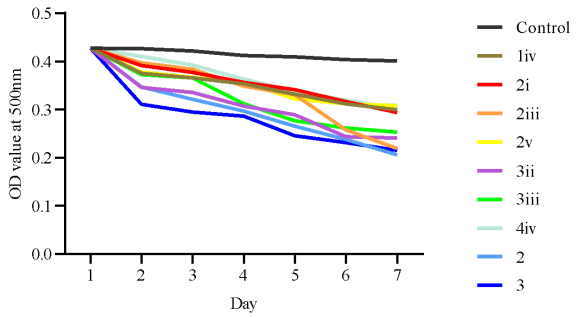

Figure: OD value from pH 6 & 37°C batch

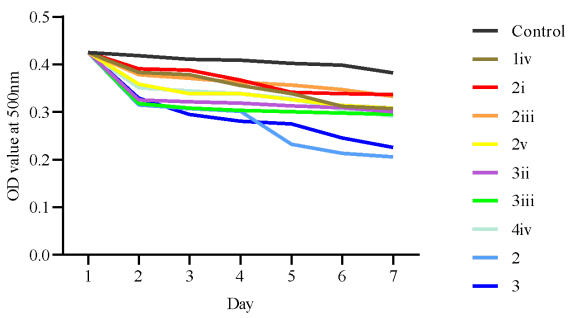

Figure: OD value from pH 6 & 45°C batch

On the left figure, it visualizes Optical Density value at 550nm wavelength of each isolate from pH 6 reactive dye medium & 37°C incubation for 7 days. On the right-side figure, Optical Density value at 550nm wavelength of each isolate from pH 6 reactive dye medium & 45°C incubation for 7 days.

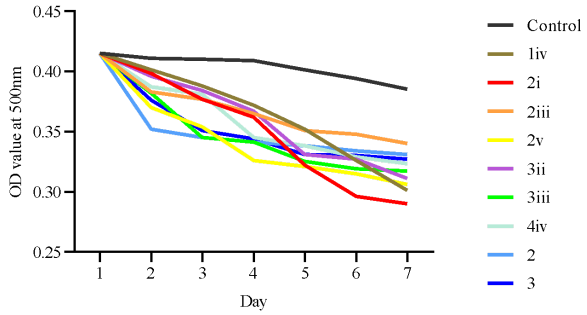

Figure: OD value from pH 8 & 37°C batch

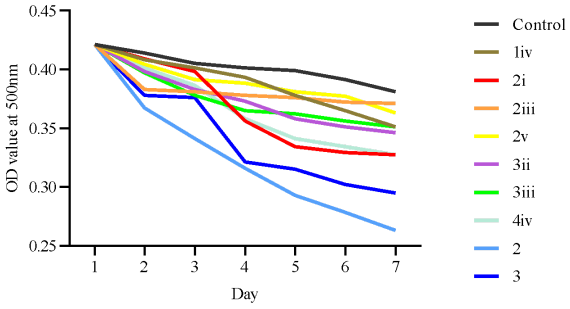

Figure: OD value from pH 8 & 45°C batch

The left figure, which visualizes Optical Density value at 550nm wavelength of each isolate from pH 8 reactive dye medium & 37°C incubation for 7 days. On the right-side figure, Optical Density value at 550nm wavelength of each isolate from pH 8 reactive dye medium & 45°C incubation for 7 days.

3.3: Decolorization (%) result

Decolorization (%) of Azo dye is visualized through bar chart below:

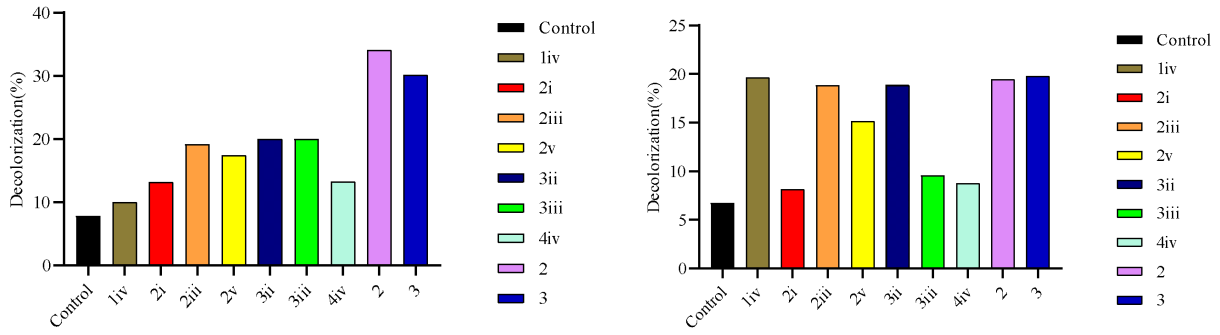

Figure: Decolorization (%) value of each bacterium from pH 4 azo dye medium at 37 & 45°C incubation for 7 days

In the left figure Decolorization (%) value of each bacterium from pH 4 azo dye medium & 37°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 10%, (Organism 2i) decolorized 13.17%, (organism 2ii) decolorized 19.20%, (organism 2v) decolorized 17.46%, (organism 3ii) decolorized 20% also (organism 3iii) decolorized 20%, (organism 4iv) decolorized 13.33%, (organism 2) decolorized 34.12% & (organism 3) decolorized 30.15%. In contrast, in the right figure, Decolorization (%) value of each bacterium from pH 4 azo dye medium & 45°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 19.64%, (Organism 2i) decolorized 8.14%, (organism 2ii) decolorized 18.84%, (organism 2v) decolorized 15.17%, (organism 3ii) decolorized 18.85%, (organism 3iii) decolorized 9.58%, (organism 4iv) decolorized 8.78%, (organism 2) decolorized 19.48% & (organism 3) decolorized 19.81%.

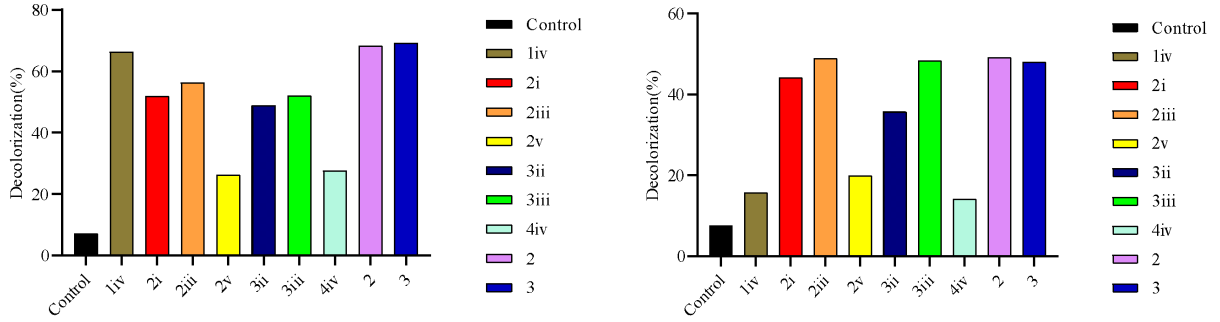

Figure: Decolorization (%) value of each bacterium from pH 6 azo dye medium at 37 & 45°C incubation for 7 days

On the left side figure Decolorization (%) value of each bacterium from pH 6 azo dye medium & 37°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 66.40%, (Organism 2i) decolorized 51.91%, (organism 2ii) decolorized 56.36%, (organism 2v) decolorized 26.27%, (organism 3ii) decolorized 48.88% also (organism 3iii) decolorized 52.07%, (organism 4iv) decolorized 27.71%, (organism 2) decolorized 68.31% & (organism 3) decolorized 69.26%. On the other side, in the right figure, Decolorization (%) value of each bacterium from pH 6 azo dye medium & 45°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 15.81%, (Organism 2i) decolorized 44.24%, (organism 2ii) decolorized 49.05%, (organism 2v) decolorized 19.97%, (organism 3ii) decolorized 37.78%, (organism 3iii) decolorized 48.21%, (organism 4iv) decolorized 14.22%, (organism 2) decolorized 49.25% & (organism 3) decolorized 48.08%.

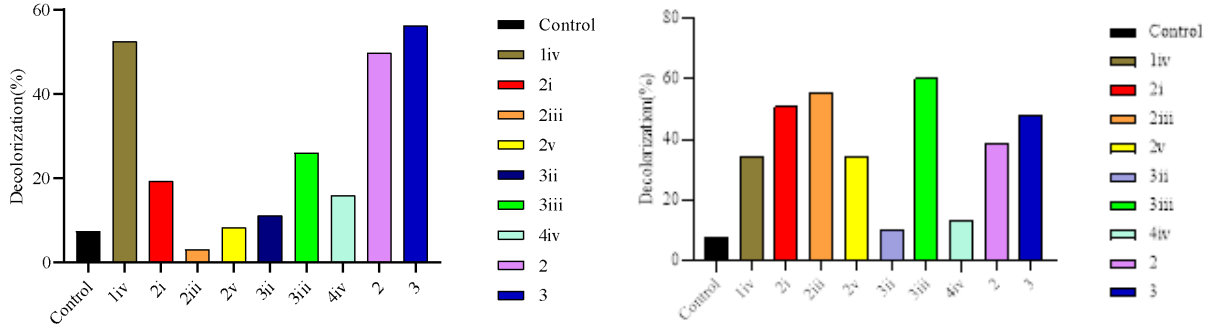

Figure: Decolorization (%) value of each bacterium from pH 8 azo dye medium at 37 & 45°C incubation for 7 days

Here, left side figure visualize Decolorization (%) value of each bacterium from pH 8 azo dye medium & 37°C incubation for 7 days. Here (Organism 1iv) decolorized 52.58%, (Organism 2i) decolorized 19.35%, (organism 2ii) decolorized 3.06%, (organism 2v) decolorized 8.89%, (organism 3ii) decolorized 11.13% also (organism 3iii) decolorized 26.13%, (organism 4iv) decolorized 15.96%, (organism 2) decolorized 49.85% & (organism 3) decolorized 56.29%. On opposite, in the right figure, Decolorization (%) value of each bacterium from pH 8 azo dye medium & 45°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 34.21%,

(Organism 2i) decolorized 50.97%, (organism 2ii) decolorized 55.21%, (organism 2v) decolorized 34.36%, (organism 3ii) decolorized 10.09%, (organism 3iii) decolorized 60.26%, (organism 4iv) decolorized 13.51%, (organism 2) decolorized 38.76% & (organism 3) decolorized 48.20%.

Decolorization (%) of Reactive dye is visualized through bar chart below:

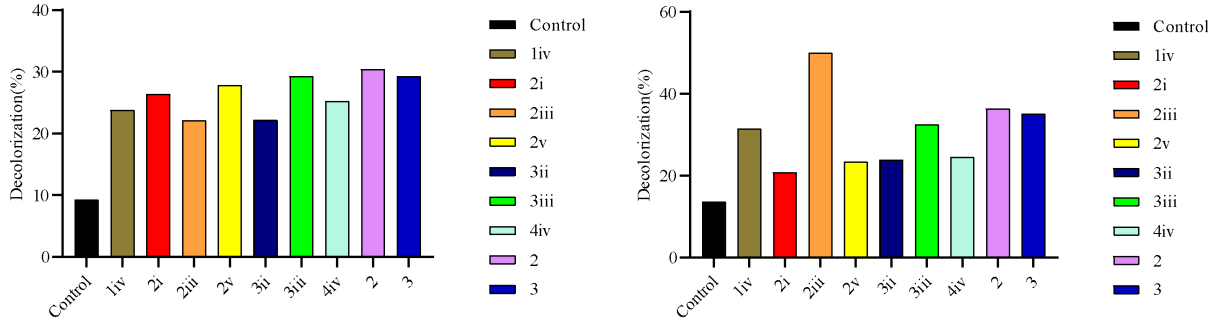

Figure: Decolorization (%) value of each bacterium from pH 4 reactive dye medium at 37 & 45°C incubation for 7 days

In left side figure Decolorization (%) value of each bacterium from pH 4 reactive dye medium & 37°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 23.80%, (Organism 2i) decolorized 26.43%, (organism 2ii) decolorized 22.14%, (organism 2v) decolorized 27.85%, (organism 3ii) decolorized 22.14% also (organism 3iii) decolorized 29.28%, (organism 4iv) decolorized 25.23%, (organism 2) decolorized 30.47% & (organism 3) decolorized 29.28%. On the other side, in the right figure, Decolorization (%) value of each bacterium from pH 4 reactive dye medium & 45°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 31.57%, (Organism 2i) decolorized 20.81%, (organism 2ii) decolorized 50%, (organism 2v) decolorized 23.45%, (organism 3ii) decolorized 23.92%, (organism 3iii) decolorized 32.53%, (organism 4iv) decolorized 24.64%, (organism 2) decolorized 36.36% & (organism 3) decolorized 35.16 %.

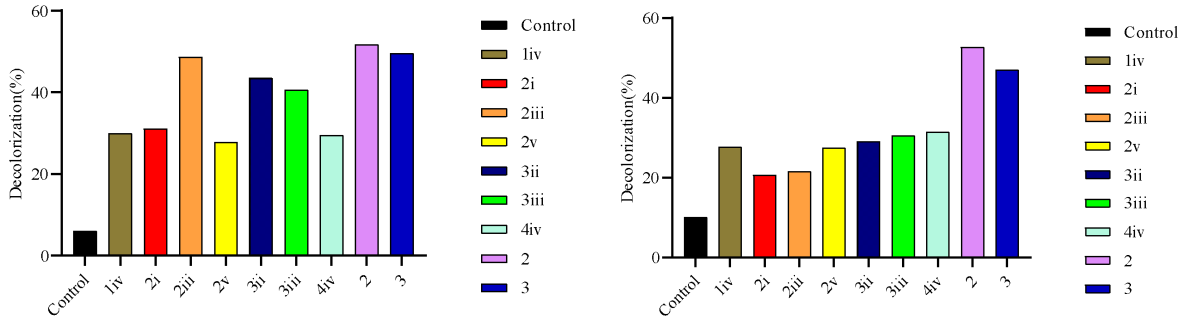

Figure: Decolorization (%) value of each bacterium from pH 6 reactive dye medium 37 & 45°C incubation for 7 days

Here in left side figure Decolorization (%) value of each bacterium from pH 6 reactive dye medium & 37°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 29.97%, (Organism 2i) decolorized 31.15%, (organism 2ii) decolorized 48.71%, (organism 2v) decolorized 27.86%, (organism 3ii) decolorized 43.55% also (organism 3iii) decolorized 40.75%, (organism 4iv) decolorized 29.50%, (organism 2) decolorized 51.75% & (organism 3) decolorized 49.64%.On opposite, in the right figure, Decolorization (%) value of each bacterium from pH 6 reactive dye medium & 45°C incubation for 7 days is visualized. Here (Organism 1iv) decolorized 27.76%, (Organism 2i) decolorized 20.70%, (organism 2ii) decolorized 21.64%, (organism 2v) decolorized 27.52%, (organism 3ii) decolorized 29.17%, (organism 3iii) decolorized 30.58%, (organism 4iv) decolorized 31.53%, (organism 2) decolorized 52.70% & (organism 3) decolorized 47.05%.

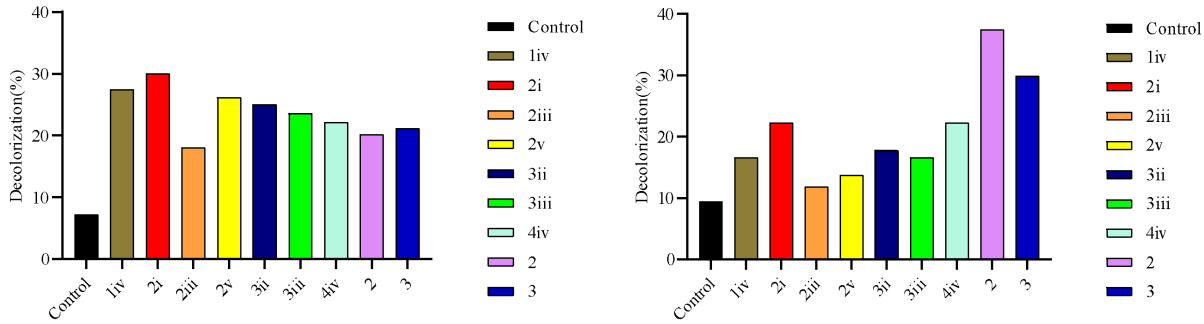

Figure: Decolorization (%) value of each bacterium from pH 8 reactive dye medium at 37 & 45°C incubation for 7 days

Organisms from the samples decolorized exceptionally well in this study. (Organism 1iv) decolorized 31.57% of reactive dye and up to 66.40% of azo dye. A maximum of 51.92% azo dye and 31.14% reactive dye were decolorized by (organism2i). Additionally, (organism2iii) decolorized 50% reactive dye and 56.36% azo dye. Furthermore, (organism2v) decolorized reactive dye by 27.85% and azo dye by a high of 34.36%, (organism 3ii) decolorized maximum 43.56% reactive dye and 48.88% azo dye, (organism 3iii) decolorized 40.75% reactive dye and a maximum of 60.26% azo dye. Moreover, (organism 4iv) decolorized 31.53% reactive dye and a maximum of 27.70% azo dye. A maximum of 68.31% azo dye and 51.76% reactive dye were also decolorized by (organism 2). Furthermore, (organism 3) decolorized maximal amounts of 49.64% reactive dye and 69.26% azo dye.

The most effective organism for decolorizing Azo dye, according to this study's findings, is (organism) 3. Additionally, (organism 2) is the organism with the most potential for decolorizing reactive dye.

3.4 Identification of bacteria:

Based on the biochemical tests following bacteria were found in this study:

- *Bacillus subtilis*. (1iv)
- *Bacillus subtilis.* (2i)
- *Bacillus spp.* (2iii)
- *Bacillus spp.* (2v)
- *Bacillus spp.* (3ii)
- *Bacillus spp.* (3iii)
- *Staphylococcus hominis.* (4iv)
- *Bacillus spp.* (2)
- *Bacillus spp.* (3)

3.5 Effect of pH on dye decolorization:

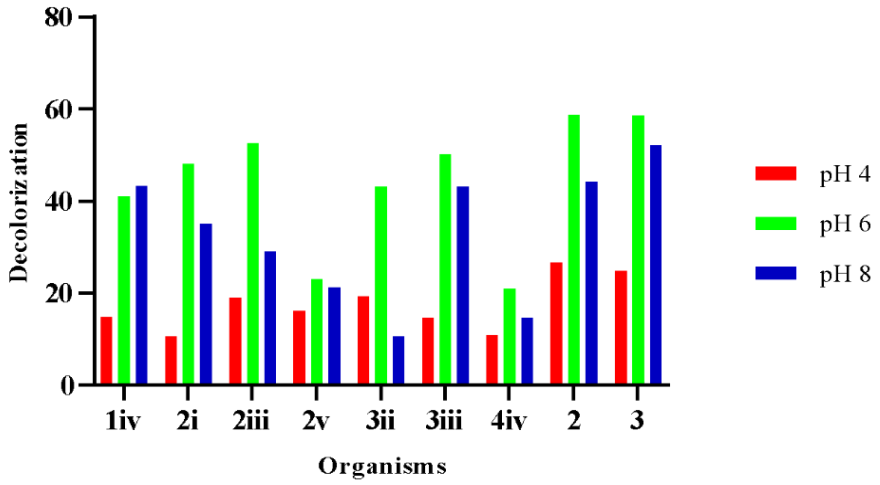

Figure: Decolorization (%) of azo dye at pH 4, pH 6 & pH 8 by each organism

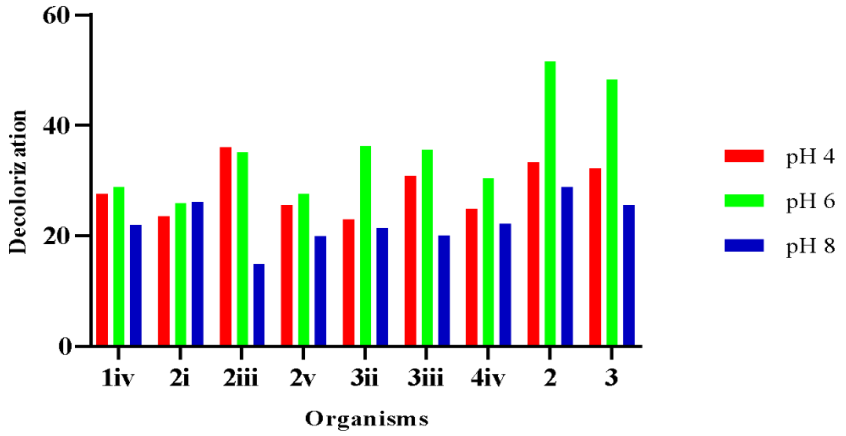

Figure: Decolorization (%) of reactive dye at pH 4, pH 6 & pH 8 by each organism

Effect of different pH for dye decolorization: Percentage of decolorization was observed at different pH concentration like pH 5, 6 & 8. Maximum 69.26% decolorization of the azo dye was observed in pH 6 by (Organism2), and maximum 34% decolorization of the dye was recorded in pH 4 by (Organism2) in this study (organism 3). Additionally, the highest 60.26% decolorization at pH 8 was found by (organism 3iii). Maximum 50% decolorization of reactive dye was seen in pH 4 by (Organism 2iii), while maximum 51.75% decolorization was observed in pH 6 by

(organism 2). Additionally, the highest 37.52% decolorization at pH 8 was recorded by (organism 2).

3.6Effect of temperature variation on dye decolorization:

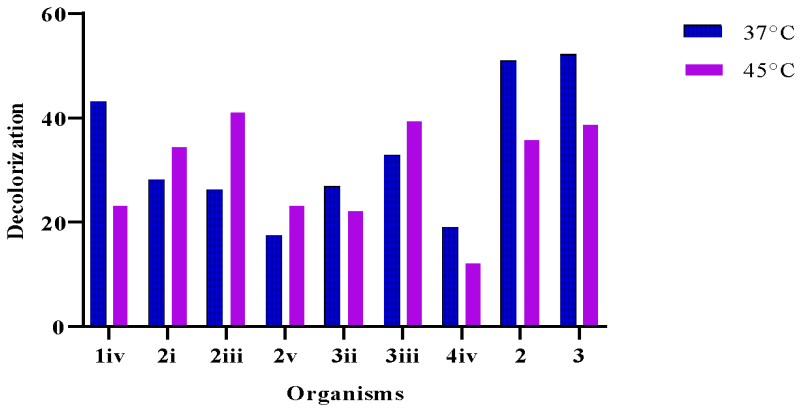

Figure: Decolorization (%) of azo dye at 37°C and 45°C by each organism

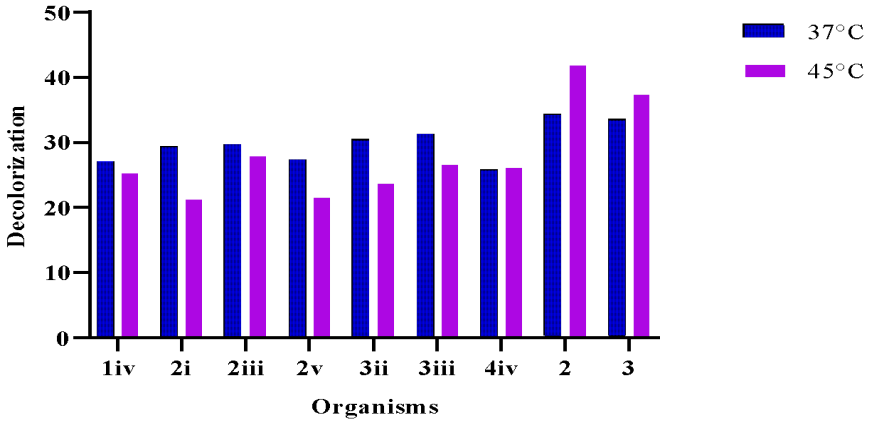

Figure: Decolorization (%) of reactive dye at 37°C and 45°C by each organism

Decolorization percentage was observed at two distinct temperatures, 37°C and 45°C. In this study, the highest decolorization of the Azo dye was noticed at 37°C by (Organism3). Besides that, 45°C was where the highest decolorization was seen (Organism3iii).

Maximum 51.75% decolorization of reactive dye was seen at 37°C by (Organism2). Additionally, maximum 51.52% decolorization was observed by (Organism3iii).

Chapter 4
Discussion

Azo & Reactive dye are mostly used in the textile industry. Dye contamination in major water resources like river has become a great threat for not only mankind, for the marine & animal life as well. According to estimates, Bangladesh's textile factories produced 1.80 million metric tons of garment in 2016, which produced 217 million cubic meters of wastewater that contains a variety of contaminants. If the textile industry continues to use traditional dyeing techniques, it is predicted that wastewater production would reach 349 million cubic meters by 2021. (Hossain et al., 2018).

Several studies have been done to find out the degradation of various dye used in the garments industry by using bacterial biodegradation method. In this study, three bacteria types were found which are *Bacillus subtilis, Bacillus spp.* & *Staphylococcus hominis* showed positive results from the decolourization of selected dyes.

Previous studies have shown that *Bacillus spp.* could decompose a wide range of dyes, including reactive and azo dyes. Additionally, it was discovered that, when compared to other studies, it has a very large ability to biodegrade dye effluent. (Venkata et al, 2013).

The majority of the studies were conducted using LB broth. However, this study was conducted following procedure of using Nutrient broth to figure out how bacteria degrade in normal growth media rather than rich media (Aqilah Hanis et al., 2020). According to Aqilah Hanis et al., 2020 the ideal temperature for *Bacillus* to decolorize azo was about 30°C. On contrary, according to this study, it was found that the ideal temperature for decolorizing azo dye is about 37°C. Additionally, the lowest degradation of azo dye was found pH 9 according to Aqilah Hanis et al., study which is completely different in this study as pH 4 was found least degradation concentration.

As stated by Singh et al., *Staphylococcus hominis*. decolorization capability of azo dye was observed. The novelty of this study is, no documentation was found that decolorize specifically reactive dye *Staphylococcus hominis.* in shaking condition.

According to a study mentioned that *Bacillus subtilis* bacteria had the highest decolorization rate of reactive dye at pH 8. In contrast, this study found that *Bacillus subtilis* best degradation was found in pH 6.

In the case of azo dye in this study, maximum degradation of 66.40% was achieved by *Bacillus subtilis* at pH 6 concentration & 37°C. On contrast, in terms of reactive dye maximum 31.57% degradation was achieved by *Bacillus subtilis* at pH 4 concentration & 45°C. Likewise, *Staphylococcus hominis* was able to degrade the azo dye in this study to its highest extent of 27.71% at pH 6 concentration and 37°C. In comparison, *Staphylococcus hominis* at pH 6 concentration and 45°C was able to degrade reactive dye at a maximum rate of 31.52%. Furthermore, a maximum degradation of 69.26% of the azo dye in this work was attained by *Bacillus spp.* at pH 6 concentration & 37°C. In comparison, *Bacillus spp*. at pH 6 concentration & 45°C obtained the highest 52.70% degradation of reactive dye.

Bacillus subtilis requires a higher temperature for getting significant degradation in terms of reactive dye than azo dye. Also, *Bacillus subtilis* needed more acidic concentration for degradation of reactive dye than azo dye. *Staphylococcus hominis* needed a high temperature to significantly degrade reactive dye as compared to azo dye. Likewise, *Bacillus Spp.* Also needed a high temperature to degrade reactive dye than azo dye.

According to the results of this study, pH 6 is the pH concentration that has the most potential for decolorizing azo dye. Additionally, pH 6 also most potential for decolorizing reactive dye. Also, 37°C is the most likely temperature and concentration for decolorizing reactive dye as well as azo dye.

Chapter 5

Conclusion:

The importance of the textile sector to the economy and way of life in our nation is indescribable. Biodegradation can play a crucial role in protecting us from many types of environmental pollution and health issues because of their connections to lots of issues with public health & natural pollution.

The textile sector frequently uses reactive and azo dyes. The biodegradation of these kinds of chemical dyes is difficult. In this study, reactive and azo dyes from the textile industry were chosen to study the feasibility of biodegradation by microorganisms. The goal of this study was also to determine the pH and temperature that would allow the organisms to decolorize the best. The sample's isolated organisms shown excellent effectiveness and decolorization capacity for these dyes. This study can be considered as a significant finding for the bioremediation sector.

It's an efficient, affordable, and straightforward technique to remediate numerous chemical contaminants. Further research & improvisation of method may increase the chance of using bacteria for dye effluents treatment in industrial level in near future.

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