Faculty of Biotechnology

Biodegradation of Azo-Dyes

Research Project 1

RS-400

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Receipt of Research Ethics Application

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	Project Title: biodegradation of AZO-Dyes Applicant Signature: Youssef Mohamed	
University Research Ethics Committee	Documents to be submitted by	( ) Application form ( ) Annex I ( ) Annex II ( ) Annex III ( ) Annex IV ( ) Annex V ( )Annex VI

	University Research Ethics Committee Coordinator's _____ FACULTY OF BIOTECHNOLOGY ETHICS COMMITTEE M S A _____
DATE	21/3/2024

I undertake to carry out research in accordance with the University's Research Ethics Policy. In the case of Research degree, I confirm that approval has been given by UREC.

Youssef Mohamed

Signature of Applicant
Date21/3/2024

I have discussed the project with the applicant, I confirm that all participants are suitably trained and qualified to undertake this research and I approve it.

Signature of External Supervisor ***Date 21/3/2021***

Sadia Saad

I have discussed the project with the applicant, I confirm that all participants are suitably trained and qualified to undertake this research and I approve it.

Signature of Internal Supervisor ***Date***

I have discussed the project with the applicant, I confirm that all participants are suitably trained and qualified to undertake this research and I approve it.

Signature of Head of Research committee ***Date***

Approved

FACULTY OF BIOTECHNOLOGY ETHICS COMMITTEE M S A

Provisional

Rejected

Annex I: Recruitment of participants by Host Institute / consent

I hereby agree and acknowledge that the student: Youssef Mohamed Ali Mahran

From the Faculty of Biotechnology at MSA University will be working under my direct supervision and following all the biological safety measures

Recommendations if {Provisional} and Justifications if {Rejected}:

documented written consents provided and stamped from the institution and the students are not responsible or involved in this procedure.

- Participants blood, placental, bone marrow (and/or others) human samples are provided by the institution and the students are not allowed to be in physical contact with any of the participants.
- The provided samples are disease-free and safe for the students and coworkers.
- The students are following the Environmental Rule and Regulations that are addressed in the institution according to the Egyptian Policies.
- Students must follow the lab safety regulations and precautions exhibited by the institution.

Institution: Supervisor:

Agri-lab Microorganism Department

Date: 21/3/2024

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List of abbreviations:

• AZO-dyes (or Azo dyes):	A class of synthetic dyes containing the functional group -N=N-
• CI:	Color Index (used for classifying dyes based on hue)
• DO1:	Disperse Orange 1 (a specific azo dye)
• HCC	Hepatocellular carcinoma (a type of liver cancer)
• IARC:	International Agency for Research on Cancer
• LED:	Light Emitting Diode (not mentioned in the text, but likely a typo for something else)
• mg/kg:	Milligrams per kilogram
• NADH:	Nicotinamide Adenine Dinucleotide (reduced form)
• ROS:	Reactive Oxygen Species
• spp.:	refers to multiple species of a genus (not mentioned in the text, but likely a typo for something else)
• AZOR (or Azor)	Likely a typo for AZO (Azo dye)
• COD	Chemical Oxygen Demand (a water quality parameter)
• CRP	refers to Cleavage Products (resulting from the breakdown of a molecule)
• DCIP	Dichlorophenolindophenol (a chemical compound)
• E. coli - Escherichia coli (	(a type of bacteria)
• HPLC	High Performance Liquid Chromatography (an analytical technique)
• NMR	Nuclear Magnetic Resonance (an analytical technique)

• OD	Optical Density (a measurement of light absorbance)
• PCR	Likely a typo for spectrophotometer (an instrument for measuring light absorbance)
• rRNA	Ribosomal RNA (a type of RNA molecule)
• rpm	Revolutions Per Minute (a unit of rotational speed)
• SDS	Sodium Dodecyl Sulfate (a detergent)
• TE	Tris-EDTA buffer (a commonly used buffer solution)
• TSA	Tryptic Soy Agar (a growth medium for bacteria)
• TSS	Total Suspended Solids (a water quality parameter)

Abstract:

Azo-dyes, made through a simple diazotization and coupling process, are the most widely used type, accounting for over 60% of all dyes and 70% of all dyes in industry. Their unique functional group distinguishes them from other dyes. They are widely used in many industries including food industry, paper printing, textile cosmetics, and manufacturing. the effect exist on the environment, and the human health and well-being is very important, because AZO colorants have been found to increase the mutagenic activity of surface and groundwater, when polluted with textile process shows very dangerous impact to the soil fertility, natural resources, aquatic live, and overall ecosystem. The removal of textile into the water can alter the pH and increase the biological oxygen demand and chemical oxygen demand which affect the water quality and shows about approximately 280,000 tons of textile colorant that are released into the atmosphere, which in the end affect the aquatic environment. Therefore, the current research aimed to investigate the biodegradation of azo dyes in manufacture waste water utilizing bacteria. To reduce the environment pollutants and carcinogenic effects of methyl red and methylene blue synthetic azo dyes The biodegradation occurred through the preparation of mineral media supplemented with the dye (methyl red & methylene blue) then the bacteria used (*Escherichia coli& Staphylococcus*) is added to the media containing the dyes in which the bacteria were incubated at 37 °C for 5-10 days. the wavelengths was then measured at 450 nm using spectrophotometer along 5-10 days to evaluate the bacteria's ability to degrade dyes, Results showed that after 6 days of incubation the dye was completely degraded in both tested samples The present study approach showed a significant improvement in degrading those dyes comparing to the current used methods

Keywords: Azo-dyes, Diazotization, Dispersibility, Alkyl, Aryl radicals

2. Introduction:

2.1 Background on azo dye history

AZO-dyes are made using a straightforward diazotization and coupling process. To achieve the required colour qualities, dye production, and particle size for better dispersibility, various approaches and adjustments are taken. More than 60% of all dyes are azo dyes, which are the most widely used type. Azole dyes make up about 70% of all dyes used in industry. The functional group (-N=N-) that unites two symmetrical, asymmetrical, identical, or non-azo alkyl or aryl radicals is what distinguishes these compounds Benkhaya; *et al.*,(2020) .The history of AZO dye goes back to 19 century when it was first commercialized by synthetic colorants dye that was discovered by W.H.perkin in 1856. Well, AZO dyes Named for their biological and chemical composition they contain colorant (N=N) AZO nitrogen group. they have become the most used dye in the world. They are widely used in industries like food industry, paper printing, textile cosmetics, and manufacturing. the effect exist on the environment, and the human health and well-being is very important, because AZO colorants have been found to increase the mutagenic activity of surface and groundwater, when polluted with textile process shows very dangerous impact to the soil fertility, natural resources, aquatic live, and overall ecosystem. the removal of textile into the water can alter the pH and increase the biological oxygen demand and chemical oxygen demand which affect the water quality and shows about approximately 280,000 tons of textile colorant that are released into the atmosphere, which in the end affect the aquatic environment as well the existence of dye in the environment leads to damage, and which caused the scientist to extend the researches, and how to get rid and eliminate these dyes, various physical and chemical methods and mechanisms and techniques have been used for the removal of them from the sewage, however, two ways have been studied with promising results, which they are

microbial degradation, and decolorization, which may have a potential method for addressing the environmental impact of these colors Rehman; A. *et al.*, (2020).

2.2 Classification By the number of azo groups

the chemical composition and the structure of AZO dyes is different from each type of AZO dyes since there is a lot of types of them but the most know one and the simplest of them is Azo reactive dye which shown in figure [1].

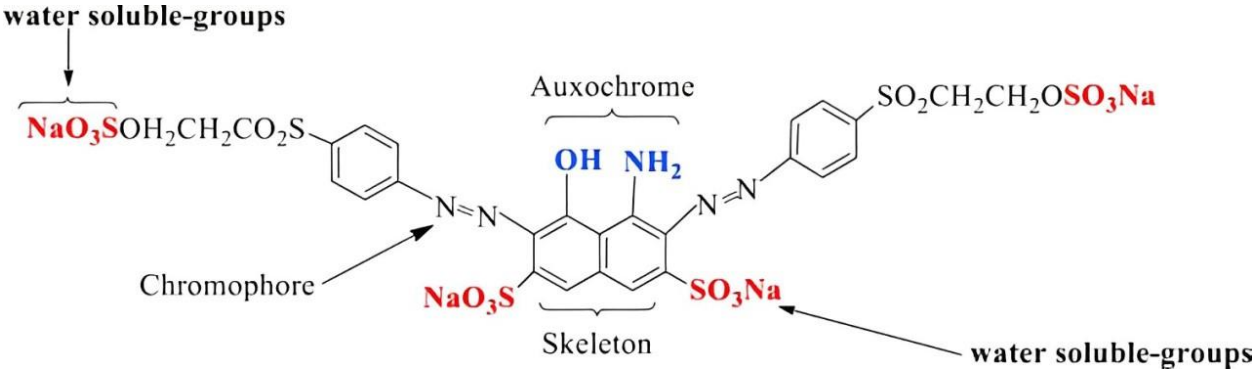

Figure 1: Structure of the azo reactive dye. Cited from Said Benkhaya; *et al.*,(2020)

However, AZO dyes are classified according to their color index (CI) "Astronomers utilize a numerical system called the color index to characterize the hue of stars and other celestial objects. It is a measurement of the brightness difference between two distinct colors or light wavelengths that an object emits. The difference in the magnitudes (brightness) of an item measured through two different filters or passbands is specifically described as the colour index"

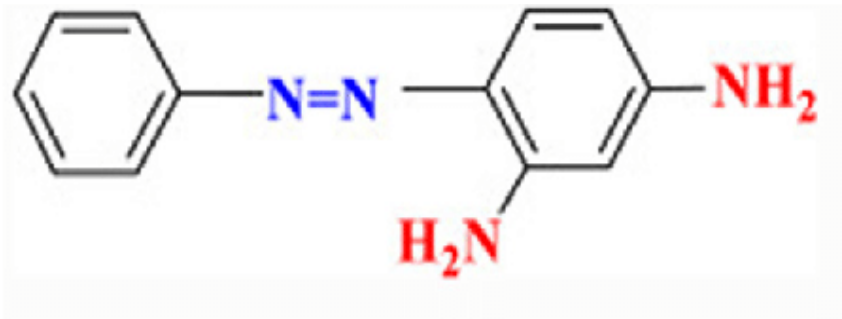

Figure (2) shows the structure of monoazo dye cited from Rehman; A. *et al.*, (2020).

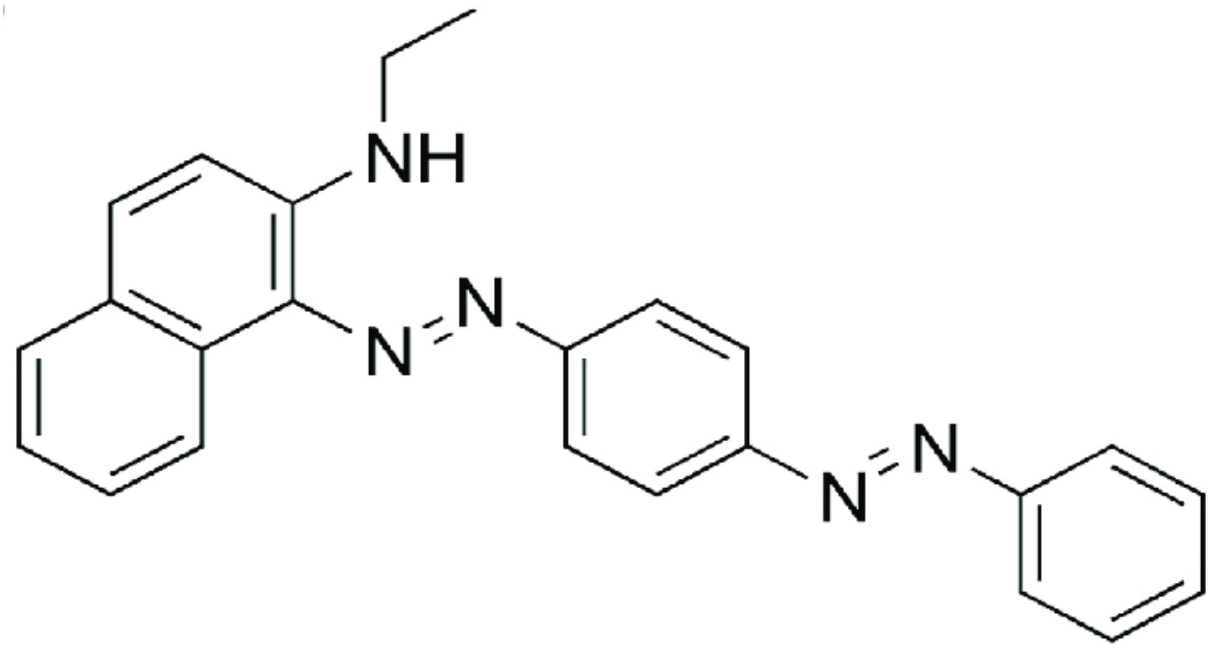

Figure (3) shows the chemical structure of disazo dye M. Daszykowski; *et al.,* (2020)

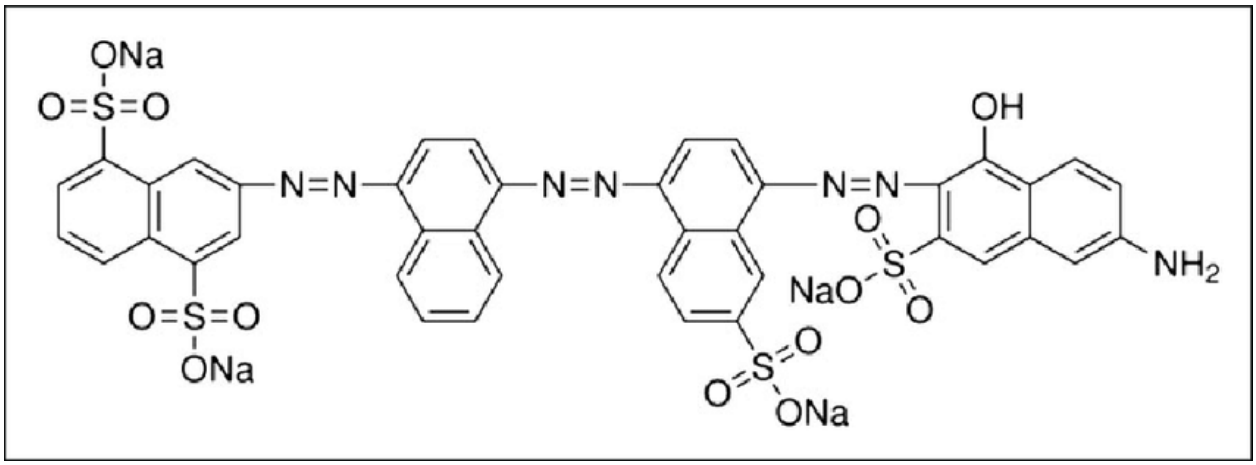

Figure (4) shows the chemical structure of trisazo dye J, López-Cervantes, et al (2018)

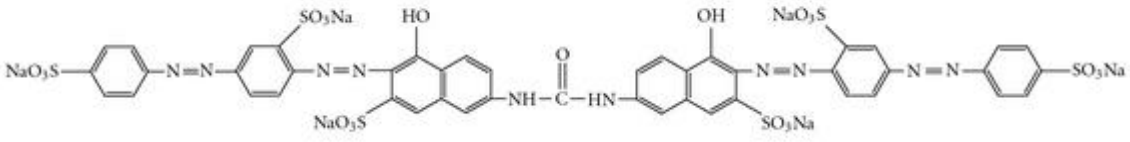

Figure (5) shows the chemical structure of polyazo dye Neifar, M., et al (2011)

Table (1) shows the Classification of azo dyes in Color Index Benkhaya, et al (2020)

Chemical class	Color index number
Monozo	11000–19999
Disazo	20000–29999
Trisazo	30000–34999
Polyazo	35000–36999
Azoic	37000–39999

Each type of these dyes are different form each other by the number of (-N=N-) group in their chemical structure and thus the function of each is different.

2.3 Classification of AZO dyes by their application:

The classification of azo dyes by application goes by different types. At first the acid is obtained which is it are used in dying nylon or silk or wool and they are synthetic fiber. Also, they have the functionality and the ability to be water soluble and also require acidic environment to be dyed Sarwar, A. et al (2023) Secondly, the basic dyes that's obtained which is it are used for dyeing actylic and polyester compound they are cationic which are having positive charge on their surface they are also water soluble and required basic environment for dyeing Islam Kiron, M. (2013) There's also direct dyes which are used for cotton and rayon and other cellulosic fiber are applied to the fiber with no need to be fixed Karthikeyan, J. (2002). There is disperse dyes which are used to color synthetic fiber like polyester and nylon and acetate they are to be soluble in the water and require very high temperature and the very high pressure to be fixed Bahramifar, N. (2004). Also, there is a type of dyes called reactive dyes which form equivalent bond with the fiber at the end resulting to very good colorfastness they are used widely for coloring fibers like cotton Johannes, H. H. (2004) There is also a solvent dyes are soluble in organic solvent, but insoluble in the water. Also, they have the functionality to be used for coloring plastic and waxes and hydrocarbon material. Littlejohn, D. (2020). There is also vat dyes which which is

they are grouped by fabulous colorfastness and ability to resist the light and being washed. However, they are commonly used for coloring cotton and cellulose fiber. El Harfi, S., & El Harfi, A. (2017).

2.4 Harmful impact of azo-dyes

2.4.1 Azo dyes (4-ABP) aminobiphenyl

Azo dyes (4-ABP) aminobiphenyl was shown to be linked with the occurrence of liver cancer in which this compound is located in tobacco and industrial waste and kitchen oil and food coloring dyes, studies have shown that at low levels of exposure to this compound might have the potential to increase the probability of liver carcinogenesis in the human cell. It also lead to (HCC) hepatocellular carcinoma. The activation of signaling pathways (Ras/MEK/ERK) by (4-ABP) have relation with cell growth and movement throughout development of liver cancer, the mechanism works by introducing azo dyes that eventually causes cancer by transforming 4-ABP to N-hydroxyl derivatives in which they react with DNA and lead to oxidative damage, 4-ABP also inhibits the production of repairing mechanisms of DNA by altering the miRNAs that by turn alter the repair proteins, the low concentration of 4-ABP stimulate the cell proliferation and migration by producing ROS in the (Ras/MEK/ERK) pathways.

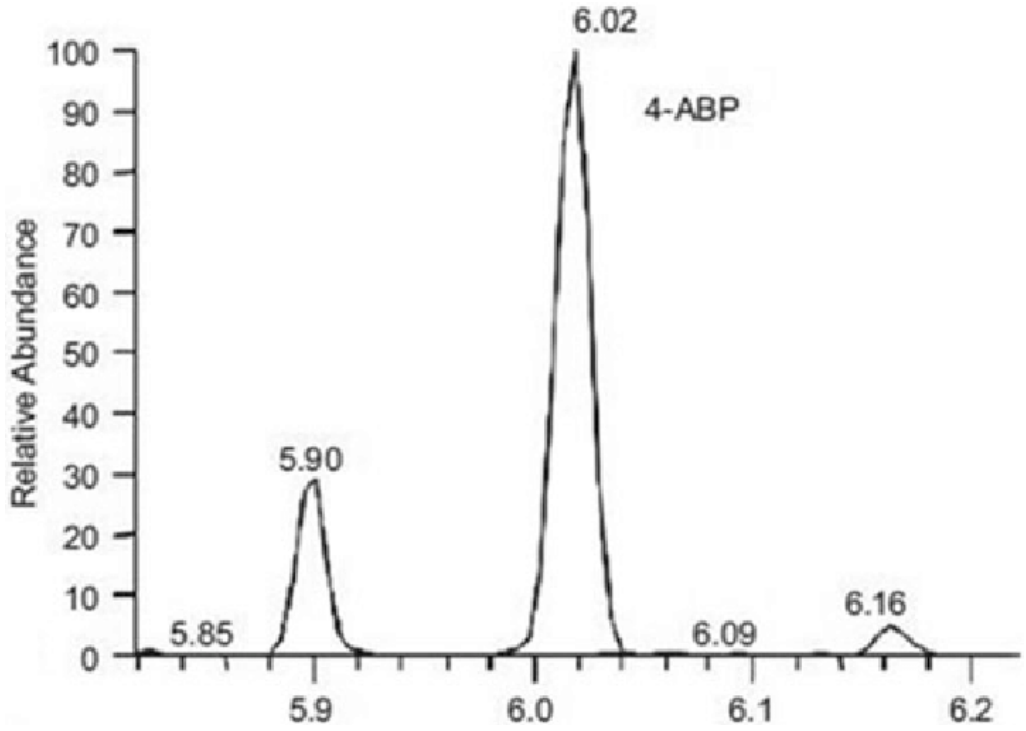

Figure (6) shows Chromatogram of 4-aminobiphenyl in a urine sample from a smoker Hui, Boon (2019)

2.4.2 Sudan I

Sudan I is a hydrophobic azo dye that is predominantly utilised in several industries such as plastics, printing inks, waxes, leather, fabrics, and floor polish. According to the International Agency for Research on Cancer (IARC), it is categorised as a level 3 carcinogen and is prohibited in most countries for use in food items because of its cancer-causing qualities. Potentiality of Sudan I as a carcinogenic substance in food products: Sudan I, other Sudan dyes, is used in food products despite the legal prohibition for colour retention due to its cheap and its ability to stain vibrant colours. In May 2003 rectification about Sudan I contamination was identified in chilli powder and other food items in Europe and Asia. China reported cases of Sudan I contamination in several food items amongst these being chilli sauce, beef, and eggs. Sudan dyes have been identified as a rising problem in food where precise identification is necessary for regulatory reasons. The analytical techniques used for Sudan dyes detection including LC, ELISA and GC-MS are applied frequently. Sudan dyes

when first analysed, the main technique employed is liquid chromatography, normally employing a C18 column, and appropriate mobile phase. The ELISA assay is known to be very sensitive and effective in the detection of Sudan dyes especially in foods. Sudan dyes in particular in chilli have been positively identified through use of gas chromatography-mass spectrometry (GC-MS). powder. Chen, H. (2012).

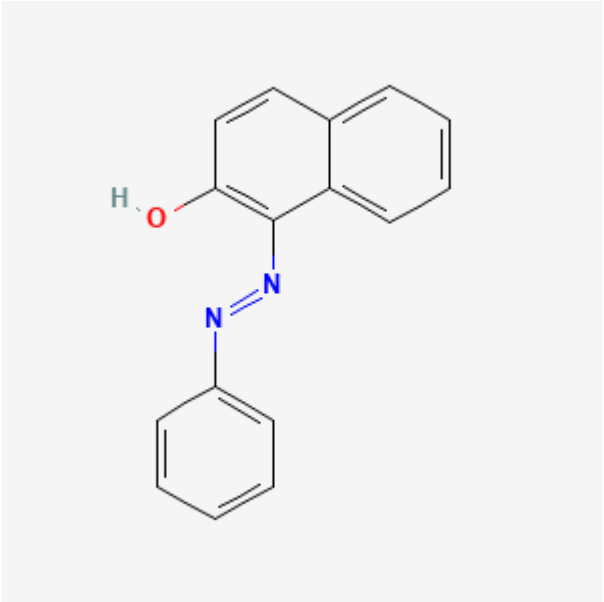

Fig (7) shows the structure of Sudan I dye molecule National Center for Biotechnology Information (2024).

2.4.3 DO1

DO1 focuses on organic chemicals which are not soluble in water and that is used in the textile industry. These compounds pose risks to the lives of water dwelling animals or aquatic plants as well as human individuals due to their toxicity and persistence within the environment. Azo dyes might penetrate human body via ingestion, dermal contact or by inhalation of particles containing azo dyes. The organisms within the intestinal microbiota play a role in the metabolism of azo dyes with azo reduction being a function that is significant in terms of toxicity and mutagenicity. In several studies evidences showed that DO1 is capable of mutagens and DNA damage. Impact of Azo Colors on the Environment and Health Being toxic substances, showing stability in the environment, and classified as

environmental pollutants, azo pigments, for example, Disperse Orange 1 (DO1) may have severe consequences on human health and the environment. Annually, almost 4,500 kilogrammes of dye are discharged into rivers, with the disperse class making a substantial contribution. Azo dyes have the potential to build up in sediments or soils, leading to the contamination of drinking water. Conventional techniques of treating wastewater have difficulties in eliminating these dyes because of their chemical persistence. Exposure and Metabolism of Azo Dyes in Humans. Humans can be exposed to azo dyes such as DO1 through eating, absorption through the skin, or inhalation. The human intestinal microbiota is involved in the metabolic process of azo dyes, notably by carrying out azo reduction. DO1 has been demonstrated to cause mutations, DNA harm, and cellular demise in numerous investigations. Electrochemical Characteristics and Reduction of DO1 exhibits solubility in an aqueous solution when combined with the Fongranal FB dispersant, demonstrating a minimum stability period of 90 days. The presence of micellar habitats in water can influence the channel of electron transport during the electrochemical reduction of DO1. Products resulting from the decrease of DO1 can present significant risks to human health and the environment. Final thoughts DO1 exhibits solubility in a particular aqueous solution when combined with a dispersant and maintains stability for a prolonged duration. Decreasing the dissolved oxygen level (DO1) can result in the creation of substances that may be detrimental to human health and the environment. D,Rawat,. *Etal*,.(2016)

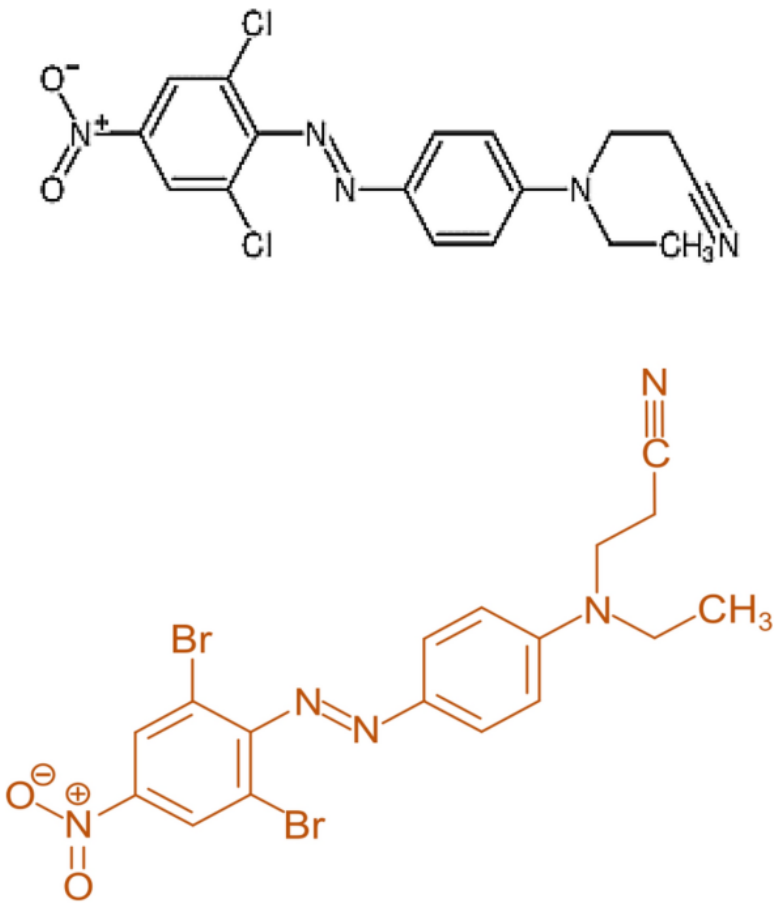

Figure (8) Concurrent cleanup of Disperse orange-2RL Azo dye and fatty acids through bioremediation D,Rawat,. *Etal*,.(2016)

2.4.4 Brilliant Blue FCF (E133)

Detrimental Effects of Brilliant Blue Dye on Human Health and the Environment Effects on Health the food business utilises brilliant blue dye, which can pose significant dangers to human health if not adequately controlled. The breakdown of brilliant blue can result in the creation of sulfonate groups, which can have detrimental effects. The presence of chloride ions in the dye can impede the breakdown process, which may have an effect on health. Ecological Consequences Brilliant blue dye is a prominent contaminant found in industrial effluents, particularly in the agri-food industry. Its tenacity renders it difficult to eliminate by conventional techniques such as coagulation or filtering. The presence of dye in water bodies can have detrimental effects on aquatic creatures and cause disturbances in ecosystems. Drhimer, F., *et al*, (2023)

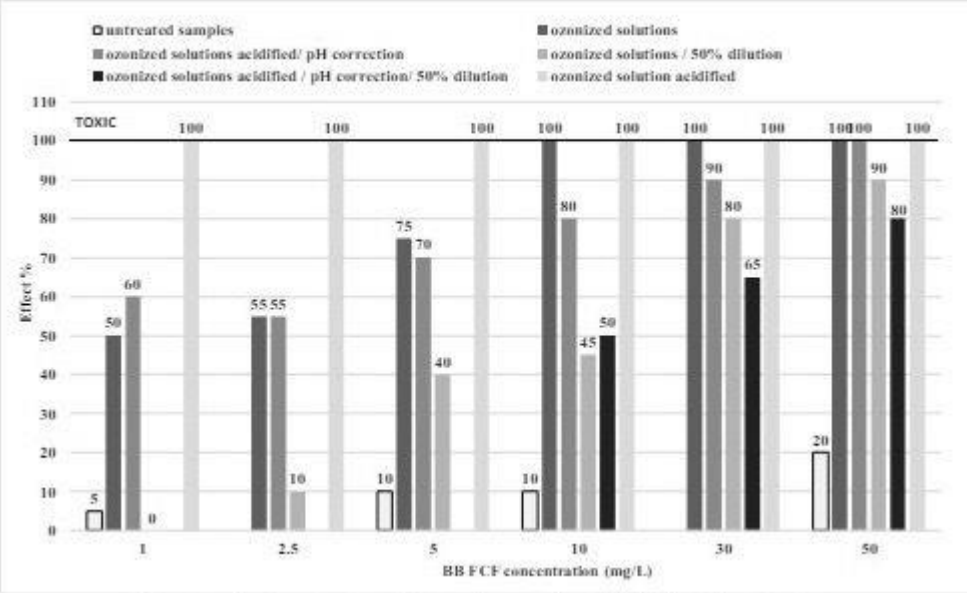

Figure (9). Shows the acute toxicity of ozonized BB FCF solutions on planktonic crustaceans

Maria, T,.*et al* ,.(2022)

2.4.5 Fast Green FCF" (E143) or "Green S" (E142)

Fast Green FCF (E143) is a man-made food dye that has been discovered to have negative impacts on human health, such as causing hives, skin inflammation, swelling, and worsening of asthma symptoms. Fast Green FCF and certain other food colours are believed to have carcinogenic properties. The mutagenicity of a substance is dependent on the amount ingested. A study revealed that Fast Green FCF and other colourants can have genotoxic effects, even when present at quantities within the allowed limits. The toxicity of combinations of colours was greater than that of individual components. The toxicity levels exhibited a direct correlation with the concentration of the colourants. Furthermore, a reduction in concentrations by 50% below the allowed limits resulted in a considerable decrease in toxicity. The acceptable daily intake (ADI) of approved colours ranges from 0.1 to 25 milligrammes per kilogramme of body weight**.** Swaroop, V. R., Roy, D. D., & Vijayakumar, T. (2011).

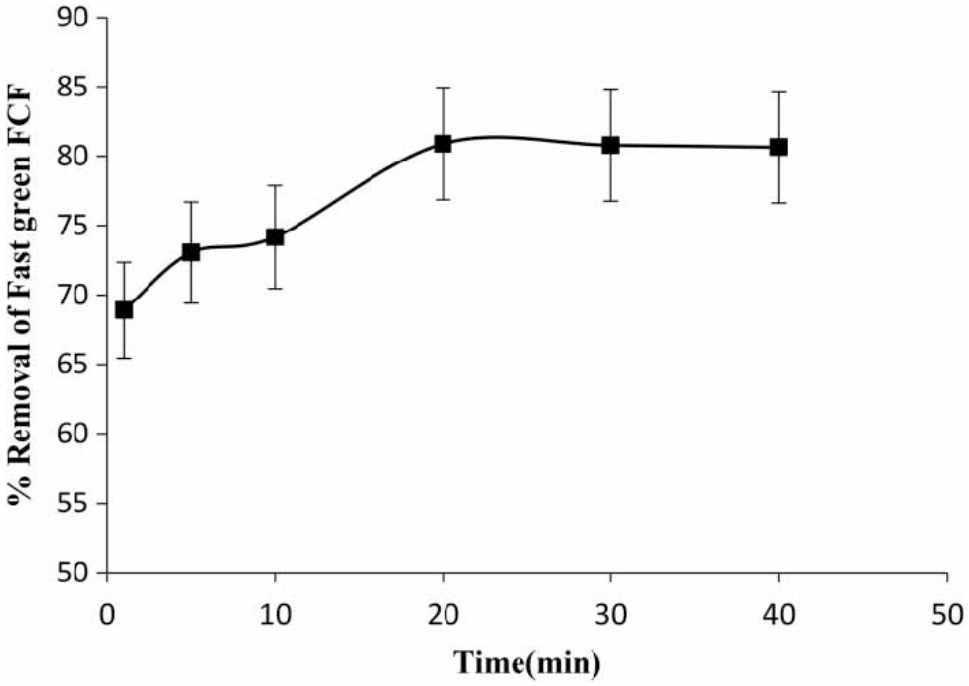

Figure (10) shows Effect of contact time on the removal percentage of Fast Green FCF dye

Abdi, Sara & Nasiri, Masoud. (2017).

2.4.6 azo acid dyes

Azo acid dyes present substantial environmental and human health hazards as a result of their persistent existence in the environment. Plants undergo toxicity tests to evaluate the impact of industrial wastes. Adsorption methods, employing substances such as sawdust and microbes, can effectively eliminate colour molecules from wastewater prior to its release. Nevertheless, the utilisation of these colours might result in water sources becoming contaminated, hence impacting ecosystems and rendering species unfit for human consumption. Research has demonstrated that employing pine sawdust as a biosorbent and conducting toxicity testing using Lactuca sativa seeds can successfully remove the colour from Acid Blue 29 dye in water-based solutions, hence reducing the associated risks. The application of biosorption techniques has demonstrated potential in mitigating the environmental consequences of dye effluents. However, additional investigation is required to enhance the effectiveness of dye removal and minimise pollution discharge into the environment.

2.5 replacements of AZO dyes

2.5.1 Reactive dyes

Reactive dyes are frequently employed in the dyeing process of cotton garments because of their capacity to generate vibrant hues and maintain their colour intensity even after repeated washing. Nevertheless, these dyes possess substantial environmental repercussions, such as elevated levels of dissolved solids and oxygen demand in the wastewater, pollution caused by the wastewater, and a high demand for oxygen and colour load. Some initiatives to reduce the negative impact include utilising different dyeing methods, treating wastewater, employing substances that can break down naturally, modifying cotton with biodegradable polymers, and investigating new technologies such as ultrasonic energy and supercritical carbon dioxide. However, the textile industry faces obstacles in widely adopting environmentally sustainable choices due to their high costs. Concurrent remediation of Disperse orange-2RL Azo dye and fatty acids using bioremediation. Khatri, A.,*etal*,(2015)

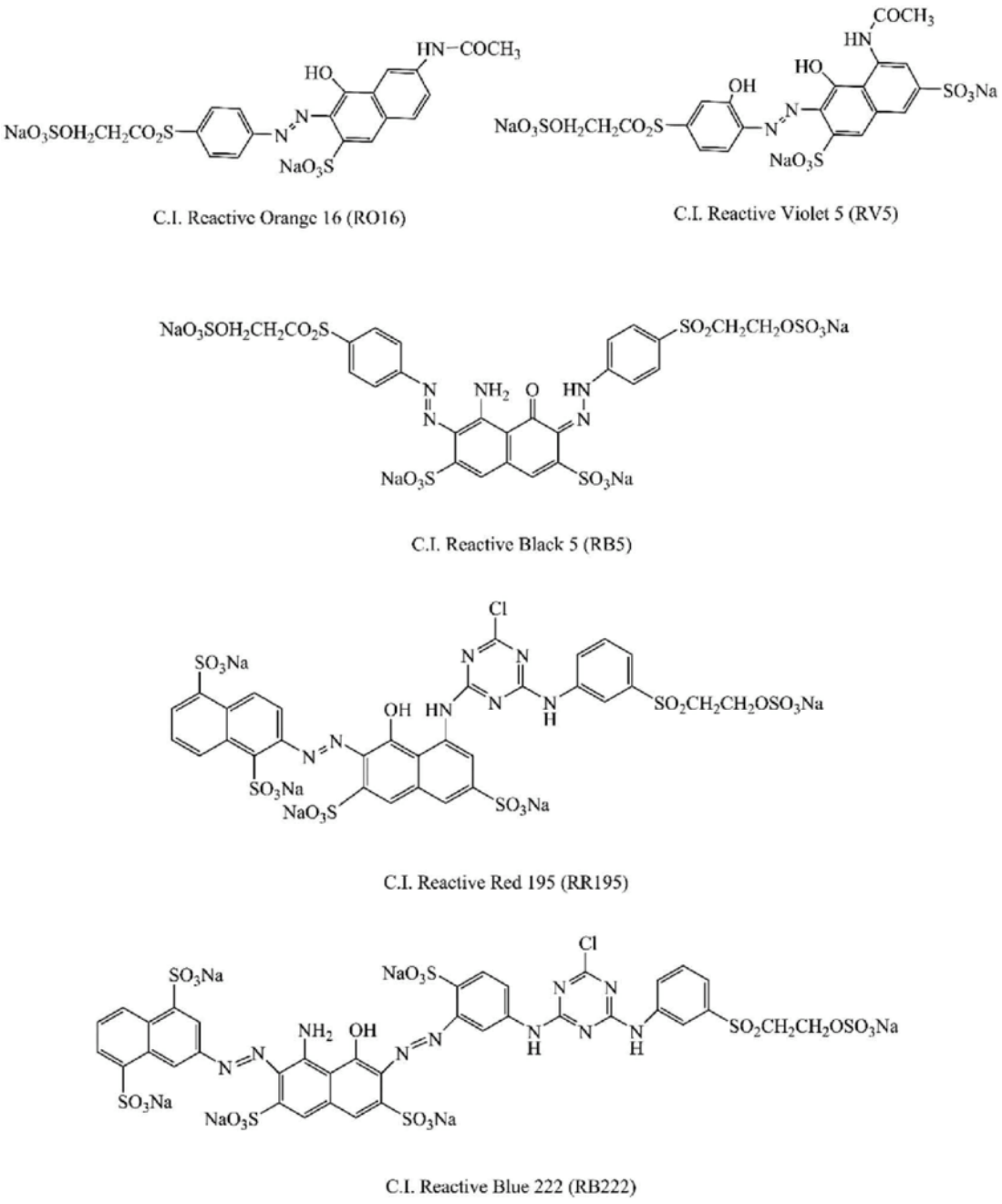

Figure (11). Molecular structures of reactive dye. Khatri, A.,*etal*,(2015)

2.5.2 Natural dyes

Plant-based colours, such as henna, turmeric, and Acacia catechu, are renowned for their cosmetic and antimicrobial attributes. Furthermore, they are used in the pharmaceutical industry to act as pigments for products and are also known extenders that have therapeutic properties. These dyes do not cause any harm to the environment and have health benefits such as being antiseptic and antibacterial in nature. Natural products that have been found to

contain dye pigment include turmeric, indigo and Punica granatum L. These plants are known to have no toxic effects, few side reactions and hold curative values to a higher degree. Natural dye is encouraged for its use since it is harmless; it has a low impact and offer medicinal benefits. The natural dyes that are vital in medicine are antibacterial in nature and rating high in the presence of different pathogens. These dyes are used in dyeing of textile and fabrics besides finding uses in several pharmaceutical industries due to their natural nature, being a good substitute to the synthetic dyes. Some natural dyes such as Acacia catechu, Kerria lacca, Rubia cordifolia, Rumex maritimus have found to have antibacterial properties, which might serve medicinal purpose. Bellews has reported that turmeric and other plant colours contain antibiotic properties; appropriately, they are seen as a natural, functional replacement for medicines. Some of these natural colours containing naphthoquinones include lawsone extracted from henna, juglone from walnut and lapachol from alkanet; all these colourants have antibacterial and antifungal properties thereby enhancing their therapeutic effects. Henna is known for its functions of anticancer, anti-inflammatory, analgesic effects and fever reductions. The natural dye can be used in ways that make them safer to be used in therapeutic solutions and be a better alternative to synthetic colourants. This is due to their efficiency, no side effects and representative of therapeutic properties which reflect the world on shift to natural products in the field of medicine. There are increasing calls to incorporate these natural dyes in drug products to ensure their appropriate use given the now well-established health benefits of natural products. The natural pigments sourced from the plant, insects, animals and minerals are known to possess the curative properties. It is important to note that these pigments are made of chemicals which have therapeutic qualities for example the antimicrobial properties of the agent used in preparation of turmeric. Punica granatum L. or pomegranate is known for its bacteriostatic properties due to action of tannins. The plant dyes which have been observed to

contain higher amounts of naphthoquinoides are antibacterial as well as antifungal. The chemical composition of these pigments goes beyond their ability to colour, since substances such as tannin and gallic acid play a role in producing their effects. The growing recognition and application of natural dyes in several industries underscore their non-toxic properties and therapeutic advantages. Chengaiah, B.,*et al* (2010)

2.5.3 Digital printing

Digital printing is a technology that enables the precise and controlled application of materials through a digital process. Digital Materials Deposition (DMD) is a precise digital printing technique employed for the production of highly effective dye-sensitized solar cells (DSSCs) within the framework of the document. DMD technology streamlines the manufacturing process of DSSCs by minimising material usage and enhancing efficiency in comparison to traditional techniques such as screen printing. Digital printing provides numerous benefits compared to traditional processes, such as improved productivity, decreased use of materials, and enhanced functionality. The technology enables faster production of solar cells, streamlines the process, and enhances the 3D structuring of TiO2 photoelectrodes, resulting in enhanced electron collecting efficiency and overall performance of solar cells. Raïssi, M.,*et al*,(2020)

2.5.4 Sustainable dyeing technologies

Sustainable dyeing methods strive to improve the sustainability of textiles through the creation of synthetic dyes, the utilisation of ecologically friendly chemicals in dyeing processes, and the chemical modification of fibres prior to dyeing. These technologies provide a decreased environmental footprint, increased dyeing efficiency, wider spectrum of colours, and improved resistance to fading from light and washing. Their motivation stems from the desire to reduce costs, comply with more stringent environmental rules, and meet consumer demand for sustainable products. Khatri, A., & White, M. (2015).

Figure: (12). Shows the methods of sustainable dyeing Arunraj,. A, *et al*, (2024)

2.6 Current used approaches to degrade azo dyes

2.6.1 Nanoparticles Based Microbial Enzyme Conjugates

Microbial enzyme conjugated nanoparticles are a type of bioconjugates in which microbial enzymes are combined with nanoparticles and there are several advantages of using bioconjugate enzymes over using enzymes independently. They also afford enhanced stability and efficiency through the enzymes being encased in a protective formation that prevents the breakdown of substrate, the increased amortization of substrate load by the enzymes, and convenient recovery and reuse. Further, they can also be functionalized with specific ligands or molecules for targeting certain regions of a biological system in an organism. Nanoparticles can be engineered to also have other properties, like magnetic property, or surface chemistry, or a certain function; this gives the nanoparticles multiple jobs to accomplish. However, there are several drawbacks linked with this strategy They include the following; Limited output boost, Possible decrease in enzyme performance, Possible toxic outcome, complex evaluation, Increased costs, and immune system stimulation. These factors

are crucial and make it essential to critically evaluate and analyze these problems for each specific application. However, there are challenges when it comes to the use of nanoparticles that are blended with microbial enzymes, with notable areas that appear to hold promising future applications in biotechnology, medicine, and environmental remediation.Selvaraj, V.,*etal.,* (2021).

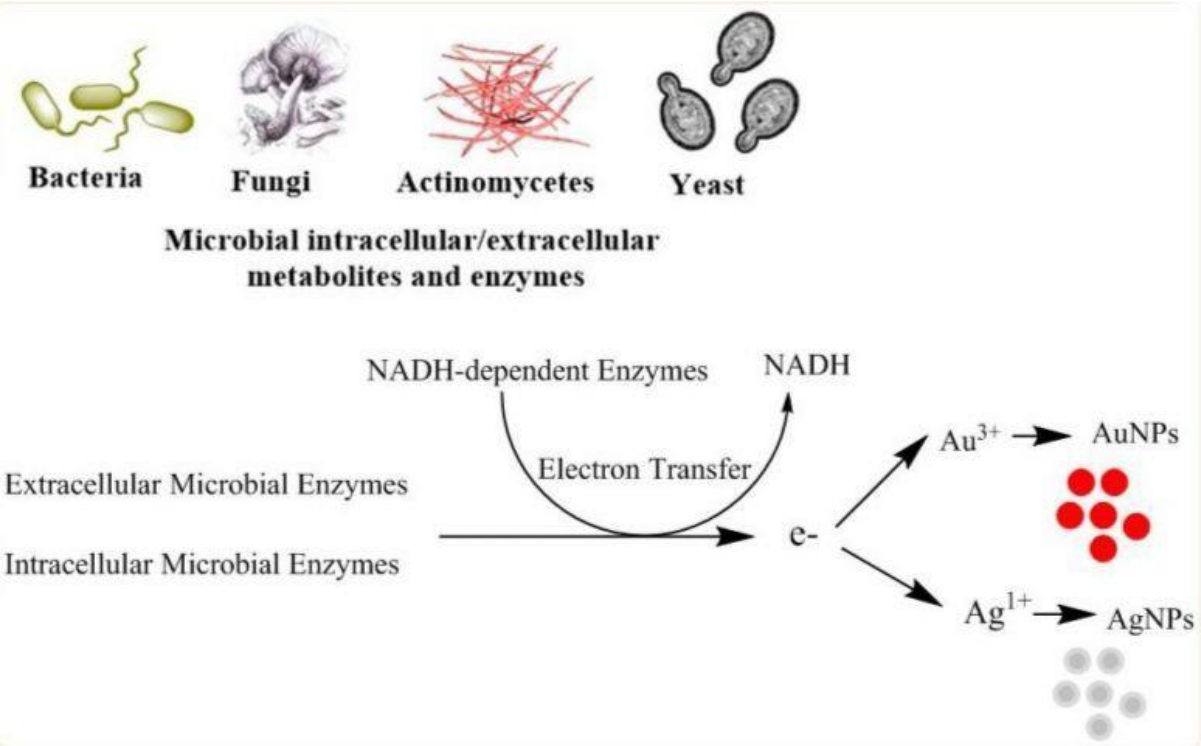

Figure (13) shows Role of NADH and NADH-dependent microbial enzymes in the synthesis of metal nanoparticles Ovais, M., *et al*,(2018)

2.6.2 Aerobic Treatment with Azo-Reductase Enzyme

Azo-reductase enzyme is employed in aerobic treatment as a means to degrade azo dyes, which are artificial dyes that contain azo linkages. Microorganism-derived enzymes are introduced into wastewater or contaminated settings that contain azo dyes. Under situations when oxygen is present, these enzymes facilitate the process of reducing azo bonds, leading to the creation of aromatic amines and other secondary substances. Nevertheless, this procedure has inherent disadvantages, such as restricted substrate selectivity, compromised enzyme stability and activity, reduced degradation rates, possible secondary contamination,

and elevated enzyme availability and cost. Effective management and treatment can reduce these dangers and ensure the efficient breakdown of azo dyes. Selvaraj, V.,*etal.,* (2021).

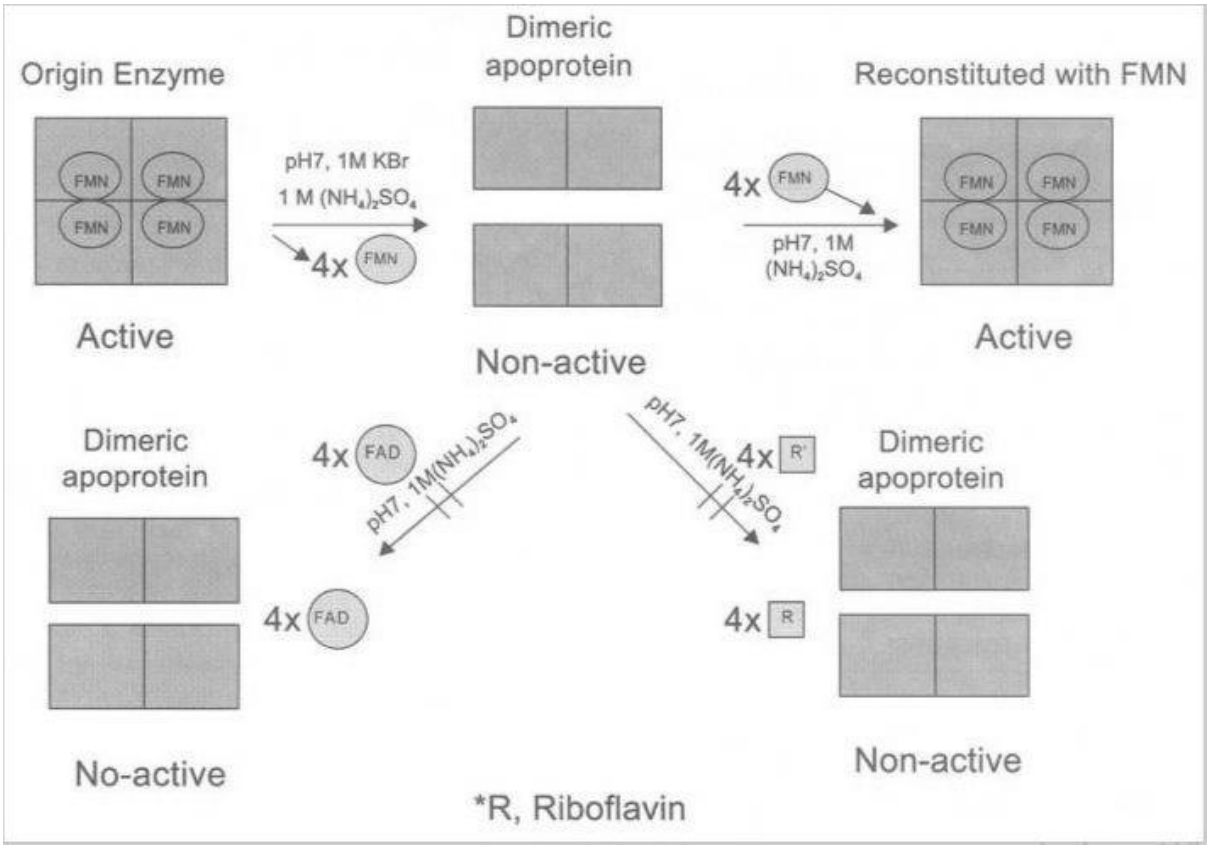

Figure (14) shows Mechanisms of the dissociation and re-association of azoreductase of *Staphylococcus aureus* expressed in *Escherichia coli* Chen H. (2006).

2.6.3 Conventional Activated Sludge Treatment:

Conventional activated sludge treatment is a technique employed to break down azo dyes in wastewater by combining the wastewater with a diverse microbial community in an oxygen-rich environment. This method involves the breaking up of colours by enzyme actions and biochemical processes which involve bacteria and fungus. However, there are some drawbacks that are linked with this process such as: sludge thickening, limited biodegradation of the complex azo dyes, formation of highly stable metabolites, nutrient loading and control, possible formation of the sludge and biosolids,and; high energy and operational costs. These disadvantages they lead to poor sedimentation; reduction in the efficiency of treatment,

increased operation challenges. Since they are hazardous components, it is important that such waste products be managed efficiently to prevent potential pollution of the environment or threat to human life. Selvaraj, V.,*etal.*, (2021).

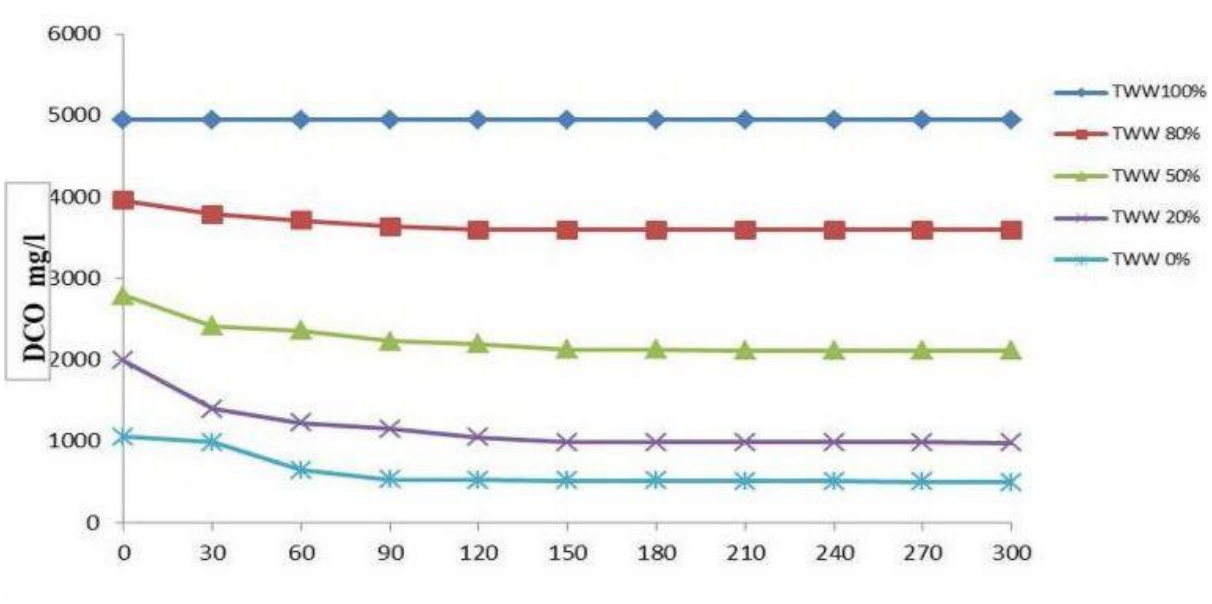

Figure (15) shows Kinetics of substrate removal (COD) as a function of time and concentration of textile waste water Gebrati, L.,*et al*,(2019)

2.6.4 Photo-catalytic Degradation of Azo Dyes

Photo-catalytic degradation of azo dyes is a process that employs light energy and a catalyst to disintegrate azo dyes into less complex and less harmful substances. The procedure entails utilising semiconductor materials such as titanium dioxide (TiO2) to trigger the deterioration process. Nevertheless, this approach is constrained by its restricted scope, dependence on UV or visible light, difficulties in retrieving and reutilizing the photo-catalyst, generation of secondary products, and possible concerns regarding cost and stability. Furthermore, the task of optimising factors such as catalyst loading, pH, temperature, and light intensity might pose challenges, potentially necessitating the use of sophisticated monitoring and control systems. In general, the process necessitates meticulous optimisation and regulation to guarantee its efficacy and safety. Selvaraj, V.,*etal.*, (2021).

3. Methodology:

Methods:

3.1 Collection of Water Samples:

3.1.1The initial stage:

Gathering water samples from the intended source. The collection methodology will vary based on the unique ecosystem under investigation, such as rivers, lakes, or wastewater treatment plants. Sterile containers are commonly employed to prevent the introduction of undesired microorganisms and maintain a contamination-free environment.

3.1.2 Cultivation and Separation of Bacteria Capable of Degrading Dye Enhancement:

The technique of co-incubation is utilised. The water sample is introduced into a nutrient broth that contains a certain dye (the specific type of dye is not mentioned, however it could potentially be Azo dyes based on subsequent procedures). The combination is subjected to incubation for a duration of 24 hours at a temperature of 37°C, which is conducive to the proliferation of bacteria. The objective of this phase is to enhance the environment for bacteria that have the ability to consume the dye as a source of nourishment, hence facilitating their rapid reproduction. Solitude: The "pour plate method" is employed to separate individual bacterial colonies from the enriched culture. A minute quantity of the enriched culture is evenly distributed over a solidified nutrient agar medium that is additionally supplemented with the corresponding dye employed in the previous enrichment process. The plate is thereafter placed in an incubator and kept at a temperature of 25°C for a period of 3-5 days. This temperature is conducive to the proliferation of many types of bacteria found in the environment. Following the incubation period, the plates are inspected for colonies that exhibit distinct clear zones around them. The presence of these transparent areas indicates that the bacteria present in those regions are breaking down the dye in the

media. Colonies exhibiting ideal properties, such as optimal size, shape, and clear zone size, are selected for further research.

3.1.3 Bacterial Degradation Identification (Optional):

The methodology does not extensively explore the process of precise identification, although it does reference the utilisation of a Pasteur Device Manual. This implies the possibility of an extra procedure in which the separated bacteria could undergo a swift examination using these strips to obtain an initial identification based on features such as Gramme staining (which differentiates between Gram-positive and Gram-negative bacteria) and the presence of specific bacteria like Staphylococcus aureus.

3.1.4 Assay for the degradation of dye:

The purpose of this stage is to measure the capacity of the bacteria that have been separated to break down the dye. The isolated bacteria culture is placed in an incubator along with a fresh decolorization solution that contains 5% (v/v) of the specific dye being targeted. The incubation takes place at a temperature of 37°C while being shaken at a speed of 500 rpm (rotations per minute) in order to guarantee effective mixing and aeration for the growth of bacteria. Culture samples are collected periodically (on days 1, 2, 3, 4, 5, and 10) and subjected to centrifugation to separate the bacterial cells (pellet) from the liquid portion (supernatant). The liquid portion, which contains the dye that has been broken down or is still present, is subsequently examined using a spectrophotometer (while not explicitly stated, it may be inferred from the optical density data). The spectrophotometer quantifies the degree to which a solution absorbs light at a particular wavelength (480 nm in the case of methylene blue dye, which is presented as an illustrative example). A greater absorbance value implies a higher concentration of dye that has not been degraded, whereas a lower absorbance value indicates more effective degradation by the bacteria. There are two control flasks provided: one contains the dye and medium without bacteria, which is used to measure any degradation

of the dye that may occur naturally. Another sample consisting of a medium containing bacteria but without any colour (to compensate for any cloudiness induced by the microorganisms themselves). The percentage of dye degradation is determined by applying a formula that considers the beginning and final optical density (OD) measurements of the bacterial culture supernatant.

3.2 Culture media:

3.2.1 Nutrient agar media

This medium is specifically formulated to selectively support the growth of bacteria that are capable of metabolising the target dye as a source of nutrients. Below is a comprehensive analysis of its constituent parts and intended function: Essential Medium: The recipe use a restricted selection of components, with the goal of supplying the necessary nutrients for the growth of most bacteria. This diminishes competition from bacteria that are unable to utilise the dye, hence facilitating the isolation of the targeted dye-degrading microorganisms. The precise elements comprise:

Agar (1g): Agar is a substance that acts as a gelling agent, causing the medium to solidify. This allows for the development of distinct colonies, which are visible clusters of bacteria that can be seen without the aid of a microscope. Potassium dihydrogen phosphate (0.15g) and sodium hydrogen phosphate (0.3g) function as a buffer system, which helps to maintain a constant pH level that is conducive to bacterial growth. Sodium Chloride (0.25g) supplies crucial electrolytes, which are charged ions necessary for multiple bacterial biological processes. Ammonium Chloride (0.1g) is used as a nitrogen source, which is an essential element for synthesising proteins and other important compounds in bacteria. Dye that matches. A crucial component to enhance this simplistic medium is a particular dye, the selection of which would be contingent upon the specific dye under investigation in the water samples. The inclusion of dye in the agar medium serves two functions:

Selective pressure is exerted on bacteria that lack the ability to break down or use the dye for their metabolic processes, resulting in their inefficient or nonexistent growth on this medium. This process aids in the eradication of undesirable bacteria and promotes the growth of bacteria with the required ability to break down dyes. Visualisation: The observation of a distinct area devoid of bacteria around a colony on the agar plate suggests that the bacteria are aggressively breaking down the dye in the surrounding substance. This facilitates the straightforward identification of prospective bacteria capable of degrading dyes during the process of selecting colonies.

3.2.2 Rose Bengal Agar:

This is a commercially manufactured, pre-made culture medium that is frequently used in microbiology laboratories for a variety of applications. Here is a concise summary of its attributes

Composition: The precise formulation of Rose Bengal Agar may exhibit subtle variations among different manufacturers, however it generally consists of:

Nutrient broth is a highly nutritious solution containing a variety of essential nutrients such as amino acids and vitamins. It is designed to facilitate the growth of a wide spectrum of bacteria.

Agar is a substance that is used to harden and support the growth of colonies, similar to low nutrition agar.

Rose Bengal dye possesses antifungal characteristics and can impede the growth of some fungi and yeasts that may contaminate the cultures.

Additional selecting factors: The composition of the medium may vary depending on the formulation by the manufacturer, which may include extra substances designed to specifically inhibit certain species of bacteria or enhance the development of others.

Deciding Between the Two Forms of Communication:

Within this specific approach, Rose Bengal Agar might be employed for the initial assessment or separation of bacteria from the water samples. The wide-ranging growth capabilities of this might enable the identification of diverse bacterial communities.

The minimal nutrient agar, when combined with the corresponding dye, fulfils a more precise function at a later stage of the process. By incorporating the specific dye into the medium, it serves as a discriminating agent to separate and enhance the growth of bacteria possessing the desired dye-degrading capability.

3.3 Enhancement of bacteria capable of degrading dyes:

The approach utilises a procedure known as co-incubation. The water sample is initially enhanced by incubating it with a nutrient broth. This broth has a diverse array of nutrients (such as amino acids, vitamins, etc.) that can facilitate the growth of a broad spectrum of bacteria found in the sample.

Crucially, the nutritional broth is further enriched with the specific dye(s) that are the focus of attention. The choice of dye(s) to be applied would be contingent upon the researcher's purpose. For instance, if the user is interested in isolating bacteria that break down Azo dyes, then Azo dyes would be employed in the enrichment process.

The co-incubation process takes place at a temperature of 37°C for a duration of 24 hours. This temperature is selected due to its ability to facilitate the proliferation of numerous environmental microorganisms. The colour in the broth exerts a selective pressure. Bacteria that lack the ability to metabolise the dye are unlikely to flourish or multiply as efficiently as those capable of breaking it down. This enrichment process facilitates the proliferation of bacteria capable of breaking down dyes within the entire bacterial community included in the water sample.

3.4 Isolation of Dye-Degrading Bacteria utilising the Pour Plate Method:

Following the process of enrichment, the pour plate technique is employed to separate and isolate individual bacterial colonies from the culture that has been enriched. This is the operational process:

A tiny amount of the enhanced culture is combined with molten nutrient agar medium. The medium also includes the identical dye(s) employed in the enrichment process. The inclusion of dye in the agar serves two purposes:

Selectivity: Bacteria incapable of degrading the dye are unlikely to exhibit robust growth or produce discernible colonies on this medium. This process aids in the eradication of undesirable bacteria and promotes the growth of bacteria with the required ability to degrade dyes.

Representation: A discernible region devoid of bacteria surrounding a colony on the agar plate signifies the active degradation of the dye in the surrounding medium by the bacteria. This facilitates the straightforward identification of prospective bacteria capable of degrading dyes during the process of selecting colonies.

Next, the combination of culture and liquefied agar is put onto a sterile petri plate and left to harden. This process results in the formation of a consolidated agar plate with the bacteria incorporated inside it.

The plate is subsequently placed in an incubator set at a lower temperature of 25°C for a period of 3-5 days. This facilitates the proliferation of colonies from viable bacteria already in the culture.

Following the incubation period, the plates are inspected for colonies that exhibit distinct clear zones around them. The presence of these transparent areas signifies that the bacteria present in those regions are breaking down the dye within the agar media.

3.5 Choosing colonies for additional analysis:

Not all colonies exhibiting clear zones may possess equivalent efficacy in dye degradation. The researchers may select multiple colonies for further examination, taking into consideration additional criteria:

Colony morphology: This pertains to the dimensions, form, hue, and consistency of the colony. Colony morphology can be utilised by skilled microbiologists to gather indications regarding the specific type of bacteria present.

Clear zone size: The dimensions of the clear area surrounding a colony can serve as a measure of the bacteria's effectiveness in breaking down the dye. Increased clear zones may indicate a greater capacity for decomposing dyes.

3.6 Removal of colour from dyes (stated but not included in the process of isolation):

The text also briefly alludes to a distinct procedure for assessing the reduction of dye colour, although it is not directly associated with the process of isolating. This phase entails cultivating a separate bacterial culture in a new medium that contains the desired dye, followed by monitoring the concentration of the dye over a period of time using a spectrophotometer. This enables a more accurate assessment of the efficacy of colour breakdown by the isolated bacteria.

3.7 The Pasteur Device:

Pasteur Devices are efficient diagnostic tools that can be utilised in clinical settings for a wide range of reasons. Within this particular framework, it appears to be a portable and versatile testing device that is specifically engineered to recognise distinct attributes of bacteria found in isolated colonies.

Categories of Strips: The 5-Slot Strip is specifically developed for the analysis of samples such as urine, swabs, and pus. The device probably contains distinct reagents (chemicals)

already filled in each slot, which can interact with bacterial components and generate a noticeable signal.

The 7-Slot Strip is designed for the analysis of blood, bodily fluids, and other internal samples. Like the 5-slot strip, it probably contains many pre-loaded reagents for different assays.

Acquiring a Blank: This step creates a reference point for the following sample measurements. The following is the procedure:

Programme Selection: The user selects a programme on the Pasteur Device that matches the type of sample being analysed, for as choosing the blood programme for a 5-slot strip. This programme is responsible for determining the meaning of the signals produced by the particular substances in the selected strip.

Strip insertion involves placing the suitable strip (either a 5-slot or 7-slot strip) into the Pasteur Device based on the chosen programme.

The "Blank Button" is pressed to start a process that determines a reference reading for each test on the strip. This takes into consideration any ambient signals or fluctuations within the strip itself.

3.8 Preparation of Samples:

This step entails preparing the isolated bacterial colony for examination using the selected strip:

Colony Suspension: To begin, a single bacterial colony is placed into a sterile saline solution to create a suspension. Saline solution creates a balanced and uniform mixture that supports the growth of microorganisms.

Procedure: Next, a minute quantity (20 µl) of the bacterial solution is introduced into each assigned slot on the strip utilising an insulin syringe. Alternatively, if the Pasteur Device permits, a small amount of the suspension might be introduced to each slot.

Incubation: The strip containing the sample is left to undergo incubation for a duration of 18 hours. The incubation period enables the bacteria to engage with the particular chemicals found in each slot of the strip.

3.9 Analysing the Example:

Following the incubation period, the findings are acquired by following these steps:

Programme Selection (Reiterated): In a manner akin to the previous stage, the user chooses the programme that corresponds to the type of sample and strip being utilised.

Strip insertion involves reinserting the same strip that was used for sample loading into the Pasteur Device.

Read Button: Depressing and maintaining pressure on the "Read Button" triggers the commencement of the analysis procedure. The device analyses the signals produced by the interactions between the bacteria and the substances in each slot.

Results Display: The Pasteur Device exhibits the outcomes of the analysis. The precise configuration of the outcomes may vary depending on the model, although it may encompass numerical values, symbols, or visual indicators displayed on the strip itself.

3.10Analysis and Possible Experiments:

The documentation is expected to contain instructions on how to interpret the findings shown by the gadget for each slot on the strip. These results may suggest the following:

Gramme Staining is a fundamental test used to categorise bacteria into two main groups: Gram-positive and Gram-negative. The 5-slot strip appears to include a specific slot (Slot 2) designed for recognising Gram-negative bacteria, whereas the 7-slot strip has distinct slots (Slot 2 and Slot 7) for both Gram-negative and Gram-positive bacteria.

Staphylococcus aureus is a distinct strain of bacterium known for its ability to cause a range of infections. Both the 5-slot and 7-slot strips appear to feature a specific slot (Slot 5) that is intended for detecting the presence of S. aureus.

Additional Possible Examinations: The remaining slots on the strips can be allocated for different testing based on the particular model and its functionalities. These may encompass examinations for antibiotic susceptibility, enzyme presence, or other attributes that can assist in initial bacterial identification.

4. Materials:

Table 2: Chemicals

□ Nutrient broth	Sigma-Aldrich, USA
□ Matching dyes for enrichment cultures (not specified)	Sigma-Aldrich, USA
□ Agar	Invitrogen, USA
□ Potassium dihydrogen phosphate	Sigma-Aldrich, USA
□ Sodium hydrogen phosphate	Gibco, USA
□ Sodium chloride	Biodiagnostic, Egypt
□ Ammonium chloride	Biodiagnostic, Egypt
□ Rose Bengal Agar (media)	Gibco, USA
□ Methylene blue dye	Sigma-Aldrich, USA
□ Saline solution	Sigma-Aldrich, USA

Table 3: List of tools used in the project.

□ Microscope	Thermofisher scientific, German
□ Centrifuge	Thermofisher scientific, German
□ Spectrophotometer (not directly mentioned but implied by OD measurements)	Thermofisher scientific, German
□ Insulin syringe	Sigma-Aldrich, USA
□ Pasteur Device Manual	Heidolph, Germany
5-slot strips	Sigma-Aldrich, USA
7-slot strips	Sigma-Aldrich, USA

5. Results:

The experiment involved using a plate with Methylene Blue to cultivate bacteria. Methylene Blue is a frequently employed dye in the field of microbiology, which aids in the visualisation and distinction of various bacterial species. The experiment yielded conclusive evidence of the proliferation of two distinct categories of microorganisms on the plate. This observation is noteworthy as it suggests the existence of numerous bacterial species in the sample under examination. Microscopic inspection was performed to enhance the characterization of the microorganisms in the sample. The objective of this study was to observe the structural characteristics of the bacteria and provide initial data regarding their classification. The examination detected the existence of two categories of bacteria: gram-positive cocci and gram-negative bacilli. Gram-positive cocci are bacteria with a spherical shape that maintain the crystal violet dye when subjected to the Gramme staining technique, resulting in a purple or blue colour when observed under a microscope. Gram-negative bacilli are bacteria with a rod-like structure that do not retain the crystal violet dye and instead

absorb the counterstain, causing them to look pink or red when viewed under a microscope. The distinction between gram-positive and gram-negative bacteria is a crucial stage in bacterial identification. Identification with Pasteur Device: To further determine the particular bacteria in the sample, a Pasteur device was employed. The Pasteur device, usually referred to as a biochemical test kit or system, is a commonly employed technique for the identification of microorganisms. The procedure comprises a sequence of examinations that evaluate diverse metabolic functions of bacteria, aiding in the process of identifying the specific species. The results obtained from the Pasteur apparatus indicated the presence of Staphylococcus and Escherichia coli (E. coli) bacteria. Staphylococcus is a group of bacteria that have a spherical shape and retain a purple stain when tested using a certain method. They are frequently present on the outer layer of the skin and the moist linings of the body in both humans and animals. It comprises multiple species, including Staphylococcus aureus and Staphylococcus epidermidis, which can exhibit different levels of pathogenicity.

Escherichia coli (E. coli) is a type of bacteria that has a rod-like shape and is classified as gram-negative. It is frequently present in the intestines of animals that maintain a constant body temperature. Although the majority of E. coli strains are benign, several strains have the potential to cause serious gastrointestinal illnesses. The detection of Staphylococcus and E. coli species is significant as it offers crucial insights into the potential pathogenic microorganisms found in the sample of summary, the outcomes derived from the experiment utilising Methylene Blue, microscopic analysis, and the Pasteur apparatus enhance our thorough comprehension of the microbial makeup of the provided sample. These discoveries have great importance for future research, as they offer vital knowledge about the various microorganisms that exist and their potential effects on health and the environment.

5.1 Biodegradation Assay results

Table 4 shows the Biodegradation Assay results from spectrophotometer

DAY 1	**1.115**	**1.052**	**0.943498**	**94.34977578**	**5.650224**
DAY 2	**0.88**	**0.817**	**0.732735**	**73.2735426**	**26.72646**
DAY 3	**0.84**	**0.777**	**0.696861**	**69.68609865**	**30.3139**
DAY 4	**0.526**	**0.463**	**0.415247**	**41.52466368**	**58.47534**
DAY 5	**0.079**	**0.016**	**0.01435**	**1.434977578**	**98.56502**
DAY 6	**0.063**	**0**	**0**	**0**	**100**

The table displays the outcomes of a biodegradation experiment carried out for duration of six days. The table is composed of multiple columns, each representing a distinct day of the assay, and contains measurements pertaining to the biodegradation process. The initial column denotes the day of the test, spanning from Day 1 to Day 6. Each successive column represents distinct measurements recorded on a daily basis. The second column exhibits a numeric number denoting the initial concentration of the drug under examination. This value functions as a benchmark for the biodegradation process and enables the computation of degradation-percentages. The third column indicates the concentration of the chemical following the biodegradation process. This value represents the quantity of the material that remains on each particular day of the test. The fourth column displays the computed percentage of biodegradation attained on a daily basis. This percentage figure shows the degree of deterioration of the material, indicating the amount of the initial concentration that has been broken down or metabolised by the microbes or other degrading agents participating in the experiment. The fifth column offers an additional viewpoint on the biodegradation process by presenting the percentage of the substance that remains after degradation. This figure shows the remaining fraction of the material that has not undergone degradation and is still present in the sample at each specified day. The last column represents the complementary number to the preceding column, indicating the proportion of the substance

that has been effectively decomposed. This figure denotes the fraction of the chemical that has undergone metabolism or decomposition in the biodegradation process. The table illustrates the gradual breakdown of organic material over the six-day experiment. The values in each column facilitate the examination of degradation rates, patterns, and the overall efficiency of the biodegradation process. This information is crucial for analysing the potential environmental impact of the material under examination and evaluating the effectiveness of biodegradation processes or agents.

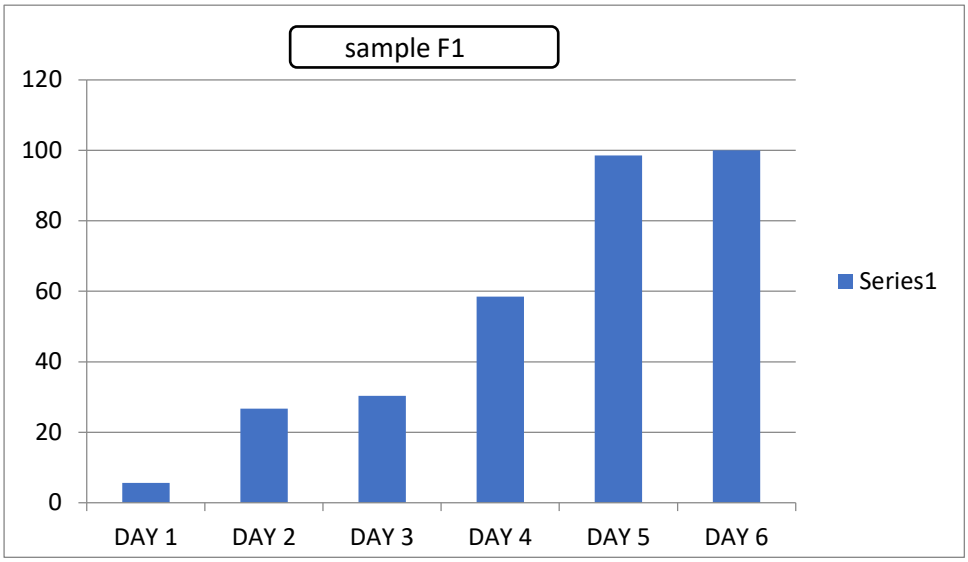

Figure (16) shows the effect of degradation of methylene blue using *Escherichia coli* (E. coli) bacteria

5.2 Observation of Curve 1 (F1 - E. coli Bacteria, Methylene Blue Azo Dye):

The graph depicting the findings of the biodegradation assay for F1 (E. coli bacteria) with methylene blue azo dye shows a progressive decrease in the concentration of the dye over the duration of the experiment. At the beginning, on the first day, the dye's concentration is relatively high, as shown by a higher value on the y-axis. During the course of the experiment, the concentration consistently declines, reaching its minimum level on Day 5.

The decrease in concentration indicates that the F1 (E. coli) bacteria are actively breaking down the methylene blue azo dye, leading to a substantial drop in its amount.

5.3 Table Description: Biodegradation Assay Results for Staphylococcus (K2)

SAMPLE K2	OD-480nm	Calculation		PERCENTAGE OF DYE IN MEDIS	PERCENTAGE OF DEGREDATION
DAY 1	1.46	0.626	0.428767	42.87671233	57.12329
DAY 2	1.43	0.596	0.408219	40.82191781	59.17808
DAY 3	1.37	0.536	0.367123	36.71232877	63.28767
DAY 4	1.205	0.371	0.25411	25.4109589	74.58904
DAY 5	0.93	0.096	0.065753	6.575342466	93.42466
DAY 6	0.834	0	0	0	100

The table shown offers comprehensive data regarding the outcomes of a biodegradation experiment specially carried out for Staphylococcus (K2) over a duration of six days. The table comprises multiple columns, each corresponding to a certain day of the test, and presents measurements pertaining to the biodegradation process. The initial column of the table indicates the day of the assay, spanning from Day 1 to Day 6. Each successive column represents distinct measurements recorded on a daily basis. The "OD-480nm" column indicates the optical density of the sample at a wavelength of 480 nanometers. This measurement acts as an indicator of the concentration or cloudiness of the sample, offering insights into the growth or metabolic activity of the Staphylococcus (K2) bacteria during the process of biodegradation. The "Calculation" column presents a computed value obtained from the optical density data. The computation is presumably derived from a particular formula or technique employed to transform the optical density observations into a

significant parameter for subsequent study. The "Percentage of Dye in Media" column represents the proportion of dye that remains in the media following the biodegradation process. This figure indicates the ratio of the dye that remains intact after being exposed to the Staphylococcus (K2) bacteria. It serves as a measure of the efficiency of the biodegradation process in breaking down the dye. The fifth column, titled "Percentage of Degradation," presents additional information that complements the preceding column. It indicates the percentage of the dye that has been effectively broken down. This result represents the proportion of the dye that has undergone metabolism or decomposition by the Staphylococcus (K2) bacteria during the process of biodegradation. The table illustrates the gradual breakdown of the dye by Staphylococcus (K2) over a period of six days. The data in each column facilitate the examination of degradation rates, patterns, and the overall efficiency of the biodegradation process for this particular bacterial strain. This information is vital for determining the possible utilisation of Staphylococcus (K2) in dye degradation procedures and evaluating its efficacy in decomposing the dye component.

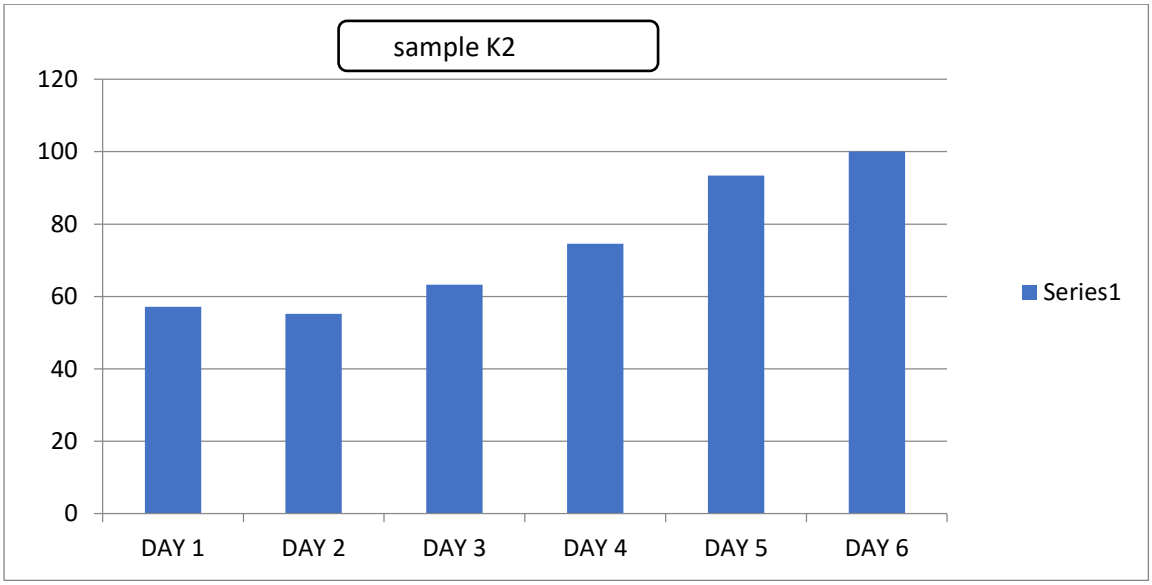

Figure (17) shows the effect of degradation of methylene blue using *Staphylococcus Bacteria*

5.4 Observation of Curve 2 (K2 - Staphylococcus Bacteria, Methylene Blue Azo Dye):

Unlike Curve 1, the curve depicting the biodegradation assay findings for K2 (Staphylococcus bacteria) with methylene blue azo dye exhibits a faster decrease in the dye's concentration. The concentration at the beginning of Day 1 is relatively high, comparable to Curve 1. Nevertheless, as the assay advances, the concentration diminishes at an accelerated pace, ultimately reaching its minimum value on Day 4. These findings suggest that the K2 (Staphylococcus) bacteria have a strong ability to break down the methylene blue azo dye, leading to a significant decrease in its concentration.

5.5 Comparative analysis of the two curves:

Upon comparing the two curves, it is apparent that the K2 (Staphylococcus) bacteria demonstrate a more effective and swift biodegradation process in contrast to F1 (E. coli) for the methylene blue azo dye. The dye concentration exhibits a more pronounced fall in the case of K2, reaching a lower level by Day 4. Conversely, F1 demonstrates a slower decline, reaching its minimum point on Day 5. This implies that the Staphylococcus bacteria have a higher level of efficiency in decomposing the methylene blue azo dye, maybe because of their distinct metabolic capabilities or enzymatic activity. Moreover, the general form of the curves suggests that both bacterial strains, F1 and K2, have the ability to break down the methylene blue azo dye as time progresses. Nevertheless, the speed and degree of deterioration vary between the two strains, emphasising the significance of taking into account the particular bacterial species engaged in the biodegradation of distinct compounds. These data highlight the possible differences in the efficacy of various bacterial strains in bioremediation applications or environmental clean-up efforts, particularly for methylene blue azo dye. To summarise, the comparison of the two curves demonstrates the divergent biodegradation capacities of F1 (E. coli) and K2 (Staphylococcus) bacteria for the methylene blue azo dye. The variations in degradation rates and concentrations achieved by each strain emphasise the need of comprehending the distinct microbial communities and their capacities

to break down diverse compounds, such as methylene blue azo dye. This information aids in the advancement of more precise and effective biodegradation techniques for the restoration of habitats contaminated with dyes.

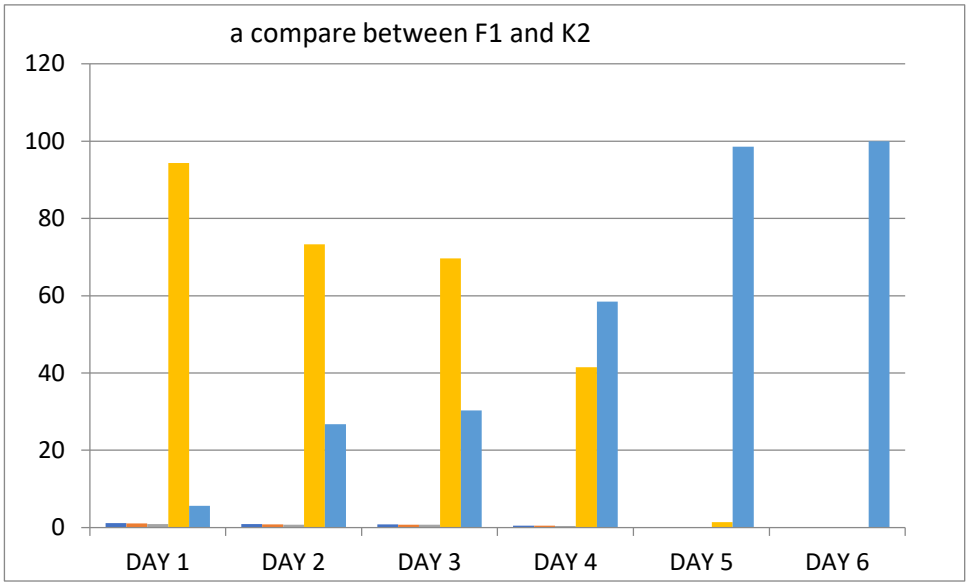

Figure (18) shows a comparison between the effects of degradation of methylene blue using *Escherichia coli* and *Staphylococcus*

This figure curve shows that *Staphylococcus* have more potential at degrading the color of methylene blue from the sample that was obtained

6. Discussion:

The first result shown is microbial identification; the bacterial strains that's were used in this approach were able to degrade the color of methylene blue only and the methyl red wasn't included in the spectrophotometer process due to the non-availability of any lead or prove that the bacterial strains used were able to perform degradation process at the first incubation so, This types of bacteria *Escherichia coli& Staphylococcus* has been then inoculated with the methyl red and methylene blue Azo dyes to examine its Degradation Efficiency on these dyes. It has been calculated through six days by the reading of the spectrophotometer. The percentage is calculated by using this equation: Degradation percentage = initial OD - Final

OD/initial OD *100. The supernatant containing Methylene blue and added to *Escherichia coli (K2)* underwent spectrophotometric measurement at 450 nm. On the first day, the deterioration percentage was 42.87%, indicating the highest level of efficiency in degradation. Subsequently, there was a decline of 40.82% on the second day, followed by a decrease of 36.71% on the third day, 25.41% on the fourth day, and 6.57% on the fifth day. And a 0.00% rate on the sixth day. The liquid portion containing Methylene blue was combined with Staphylococcus (F1) and subjected to spectrophotometric analysis at a wavelength of 450 nm. On the first day, the deterioration percentage was 94.34%, indicating the highest level of efficiency in degradation. Subsequently, there was a decline of 73.27% on the second day, followed by a decrease of 69.68% on the third day, 41.52% on the fourth day, and 1.43% on the fifth day. Finally, there was a complete absence of deterioration on the sixth day, with a value of 0.00%.

The purpose of this study is to degrade the azo dyes that found in manufacture waste water through decolorizing it by using bacterial strains that are capable of degrading these azo dyes with high efficiency and monitor the finals results through biodegradable sensors as an example. And the previous results are successfully achieved to ensure that decolorization of azo dyes reduce the carcinogenicity effect of these dyes in tektites and food as well as it is economically and environmentally friendly and help in maintaining the environment healthy. The results shows that the bacterial strains that is used has enormously effective as biodegradable for azo dyes as the percentage of the isolated bacteria (*Staphylococcus (F1)*) were efficient decolourizers for Methylene blue

Azo dyes possess a fused aromatic structure that contributes to their durability and long-lasting nature by preventing degradation. Wastewater treatment in dyeing and textile industries is difficult because it contains high levels of chemical oxygen demand (COD) and suspended particles, including surfactants, detergents, and dyestuff. Discharging this

substance into water bodies like rivers and lakes can result in ecological harm, including changes in pH levels, elevated chemical oxygen demand (COD), and noticeable discoloration. The discharge of effluent containing azo dye is not preferred since azo dye is very toxic, and results in formation of hazardous intermediates. Another issue that affects most of the azo dyes is that they are poisonous, and they chiefly affect the environment. Several investigations have played out confirmed that the waste released by dye processing and manufacturing businesses is cancerous and have a capacity to mutate various forms of life. The hazards resulting from problems such as the increase in toxicity of wastewater discharge have implications on both the environment and human health. Another method by which the activity of the reductive dye-degrading enzymes: azo reductase and NADH-DCIP reductase were evaluated was the spectrophotometric method, and these were on the cell-free extracts. There are standard rituals that were practiced in the interest of the purpose. The azo reductase assay involved reaction of methylene blue and enzyme solution For all methods azo reductase activity was measured and expressed as difference in absorbance. The measurements of NADH-DCIP reductase activity were done by altering a method that was given in the work of wet flesh and co-authors. Benkhaya; *et al.*,(2020)

Comparing the bacterial consortium with that of individual bacterial strains, the bacterial consortium had a lower efficiency in the biodegradation process of the two azo dyes. On the other hand, the ability to degrade one out of the tested azo dyes within 6 days of the treatment period. Specifically, they established that methylene blue is mainly biodegraded into CRP across all the bacterial strains used in the study. The release of azo dyes especially methylene blue into the water bearing environment is another issue of concern because amongst other evils that it brings it has the capacity to change the color in natural bodies of water. When assessing the potential risks of a substance, information on the substance's toxicity, its ability to cause mutations or else biotransformation products which may pose a threat. In this

context, to address the challenges that threaten the environment from the contamination of wastewater carrying dyes from various industries, Ikram et al (2022) conducted experiments to test the suitability of various bacterial strains in the degradation of these dyes. Their work revealed significant differences in the susceptibility of the two bacterial strains they studied to the azo dyes that they analysed. Staphylococcus (F1) proved to possess one hundred MN percent degradation capability, thus they are most proficient in degrading the methylene blue. E.coli, Staph, and Pseudomonas showed higher numbers in terms of quantity in the wastewater. For example, fryer contamination such as Salmonella enterica had the similar trend lower degradation efficiency as compared to Staphylococcus aureus, Escherichia coli have degradation potential 98%. 5%. In its convection, the results vindicate staphylococcus for the capacity to degrade azo dyes especially the ones found in textile industries' wastewater.

This speculation focuses on the extent to which Chlorella vulgaris and Aphanocapsa elachista can be used in biodegrading the dye. Now, for evaluation of colored pollutants like Disp orange 2RL and Reactive yellow 3 RN, Chlorella vulgaris and Aphanocapsa elachista proved to be effective in decolorizing pollutants. Mammalian azo reductase is crucial in the reduction of azo dyes so as to form aromatic amines. The presence of decomposition products upon decolorization was also confirmed using spectroscopic analysis. According to the study, the application of microalgae is recommended as an approch that can be used in textile and paper industries to alleviate the challenge of decolorizing dyes. As it Algae there are several limitations of using it for biodegradation of dye Some of the disadvantageous include; These include the fact that some algal species were found to be necessary, decolorization rates were inconsistent, and procedures took a long time, which could be a problem for industries. Further studies are needed to discover other products of degradation, understanding the impacts of intermediate degradation products on the final environment, and to ensure

complete removal of contaminants. Concerning the colours and algae species that the study has focused on, one can understand the necessity for further investigations in order to deal with a range of different contaminants and organisms. To sum up, these problems should be solved to reach the effective biomazae and dye biodegradation. El-Sheekh, M. M(2018)

The paper looks at the use of ionizing radiation where focus is given to its function in the treatment of Textile dye waste effluents; the focus here is in the ability of the radiation to augment the degradation of effluents within aqueous solutions. The main luminosities that come from water radiolysis are used to add the degradation progress. The text focuses on techniques of degradation that include UV-photolysis, fenton process, photo-fenton process, ozonation process, sono-lysis, photo-catalytic approach, biodegradation and radiation-induced degradation of dyes. However, there are few limitations that found in this study; for example, limited data of the effect of pH on degrading dye; also, there are few factors those are play role in degradation such as pH, dosage, availability of oxygen and temperature etc. This work also investigated the synthesis of low-level radicals in strong alkali solutions and how the relative solubility of species, oxygen concentrations, pH value, dosage, and temperature affect the degradation process. Other forms of analytic methods used in research are High Performance Liquid Chromatography (HPLC); Fourier Transformed Infra Red (FTIR); Gas Chromatography/Mass Spectrometry (GC/MS) and Nuclear Magnetic Resonance (NMR). Rauf, M. A., & Ashraf, S. S. (2009).

The biodegradation of azo dyes particularly methylene blue has more advantages than using algae, fungi or radiation for breaking the dyes as follows: Some members of the bacteria found are capable of having certain enzymes such as azo reductases, which can efficiently cleave azo bonds within dye molecules. These enzymes can increase the rate of degradation depending on the extent of richness which in turned can lead to quicker elimination of the dyes from the environment thus reducing impact on ecosystems and human health. It is for

this reason that there is an array of bacterial strains that possess the requisite ability to break down azo dyes. This makes it possible to identify and apply certain strains that can characterize high performance in the degradation of a particular dye or under certain environmental conditions. There are smaller micro-organisms called bacteria, which are quadratic and adaptable to several conditions in an environment down to the degradation process that could happen in a rather aggressive pH, temperature, and nutrient instability. The utilization of bacterial biodegradation is possible when treating industrial wastewater or when large scale bioremediation projects are envisaged especially if cost is an influencing factor in the process. In conclusion, bacterial biodegradation offers clear advantages over algae, fungi, and radiation methods as far as the degradation of azo dyes is concerned. It has given it enzymatic capacity, AE 461's flexibility in assorted circumstances and its affordable nature making it a functional solution in industrial wastewater treatment and large-scale treatment processes. Rehman; A. *et al.*, (2020).

Recommendations: I urge to conduct additional research to investigate the fundamental processes involved in the breakdown of azo dyes by E. coli and Staphylococcus bacteria. An in-depth comprehension of the biodegradation process and the possibility for more effective bioremediation solutions could be achieved by investigating aspects such as enzyme activity, metabolic pathways, and genetic drivers.

Conclusion: The study discovered that Staphylococcus (F1), a bacterial strain, is highly effective in breaking down azo dyes such as methylene blue that are present in industrial wastewater. The substance exhibited complete breakdown during a span of six days, whereas Escherichia coli had a lesser degree of degradation. Bacteria possess several advantages compared to algae or radiation, including enzymatic capacity, flexibility, and cost-effectiveness. They possess the ability to flourish in diverse environmental circumstances, rendering them well-suited for extensive bioremediation endeavors. Future study should

prioritize the identification of degradation products and their environmental consequences, investigate the degradation efficiency of Staphylococcus for different azo dyes, and enhance the biodegradation process for industrial use. This has the potential to enhance the efficiency and feasibility of utilizing microorganisms for the bioremediation of wastewater contaminated with azo dyes.

Limitations of your study: investigation about the use of *Escherichia coli& Staphylococcus* show significant hazard on the field of usage of my approach in human related area due to the potential toxicity that's exhibited from *Escherichia coli& Staphylococcus* so their use is limited in the non-human applications like degrading the azo dyes in waste water of factories

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