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Removal Of Congo Red Dye Using Surface Modified Activated Carbon And Bacteria

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ABSTRACT: Surface modified activated carbon and hydrocarbon degrading bacteria can be used effectively as adsorbent to eliminate Congo red (CR) from aqueous solutions. Carbon adsorbents, also known as activated carbons, are adsorbed on carbonaceous materials that have high carbon, low ash, and high volatiles mass by physical, chemical, or a mixture of both. Activated carbon (AC) is the most widely used adsorbent due to its high porous surface area, adjustable pore structure, thermos-compatibility and low acidic/base reactivity. Hydrocarbon-degrading bacteria was isolated from the contaminated soil from automobile workshops. CR is used in textile industry due to their properties such as large variety shades, resistance to decoloring and lower energy consumption dyes are aromatic compounds with one to many –N=N– groups. They are widely used in various industries such as textile, paper, cosmetic, rubber, pesticides, and leather. Azo dyes, released in the environment through textile effluents, have hazardous effects on the aquatic as well as human life. Continuous use and discharge into the environment are becoming a global problem. CR has a carcinogenic structure due to its aromatic amine. Its structural stability makes it difficult for it to be biodegradable. Hence there has been a lot of focus on the treatment of these contaminants.

KEYWORDS: Activated carbon, Hydrocarbon degrading bacteria, Congo red

I. INTRODUCTION

Water pollution is considered to be a growing concern due to the swift expansion of the chemical, pharmaceutical, and agricultural sectors. Opportunity to approach of fresh drinking water is be- coming a significant hurdle and a current issue in many regions globally. People are facing problems the supply of drinking water, alongside issues regarding its purity. This is largely due to increased demands of the industry, agriculture and overpopulation [1]. According to WHO, nearly half of the populations in developing nations experiences health problems, which are related with microbiology or chemically contaminated drinking water. Many chemical compounds, including pesticides, steroids,antibiotics, and dyes, enter the aquatic environment. Among the chemical pollutants, organic dyes, in particular, are highlighted as significant in nature due to its toxic nature [2]. Dyes are consumed ranging from small-scale to large-scale industries inculcating tanneries, food,cosmetic, textile, medicinal sectors with the production exceeding 1,000,000 tons all over the world. The textile industry is a significant contributor to dye emissions into the ecosystem with dying operations alone releasing about 7.5 metric tons annually [3].

Dyes are hazardous, carcinogenic, toxic, and has bad effects on human health,environment and wat ecosystem [3]. They are classified like basic dyes, acidic dyes, azo dyes, disperse dyes, anthraquinone based and metal complex dyes [4-8]. The principal consumer of dyes is the textile industries. A large amount of dyes are lost due to the waste stream during the coloring process in textile and paint industries [9-16].About 15 percent of the total dyes are lost among the total dyes produced worldwide during the dyeing processes of the textile runoff [1]. The colored effluents from these textile and color industries not only contribute to water discoloration, but also serve as the pollutant for living organisms by decreasing or even stopping the light and water re-oxygenation capacity,thus, disrupting aquatic life. This leads to serious threat to human life due to carcinogenic nature of some dyes [7, 17-22]. Azo dyes deliver more than 50 % of textile dyes and are characterized by their nitrogen -bonds. Each year, textile industries produce more than 700,000 tons [23,24].Synthetic dyes (SODs) are underestimated as an environmental contaminant; currently, SODs are considered a micropollutant because of their low concentration in aquatic ecosystems (ng/l- µg/l). SODs are ubiquitous due to industrial production and numerous areas. The production and use of large quantities of dyes and their wide range of applications generate a large amount of coloured wastewater and various types of post-product waste. The textile sector is a major source of dyes in aquatic environments: dye wastage is estimated to be at least 5 % during various dyeing processes and can be as high as 50 % depending on the type of fabric and dye. This amounts to nearly 200 billion litres of coloured effluents per year. Synthetic organic dyes are non-biodegradable,making them more difficult to clear up using commercially available sewage treatment plant (STP) methods.

Available data confirms the toxic properties of synthetic organic dyes, such as cancer causing, allergent and dermatic effects, making their production and use potentially unsafe [25]. So far, there are several certified feasible methods for dyes removal.These methods include physical methods such as adsorption and membrane separation and coagulation, chemical methods such as ion change, oxidation and catalytic degradation, and biological methods such as microbial degradation. Adsorption is the most attractive and efficient of these methods. Not only is it easy to operate, but it is also green, cheap, and sensitive to various types of toxic substances, with the highest satisfactory performance.There are various adsorbents that have studied to remove the toxic organic color pigments,including nano-materials, the polymer, agri-industrial waste. Activated carbons are made from a wide variety of natural and synthetic compounds and have been used in adsorbent technology for a long time. It has a high surface area (more than 1000 m2g/l), highly porous structure, chemical stability and mechanical stability. These characteristics make activated carbons useful in many applications such as medical treatment, catalytic converter support and decontamination [26].

Various treatment methods have been employed to eliminate dyes from wastewater, which can be divided into physical, chemical, and biological methods [27]. A lot of literature has been written on the good effects of active carbon on adsorption to organic dyes. The adsorption of biosorbs of great interest to many environmental researchers, as it is able to bind to both inorganic and organic molecules due to various chemical or physical mechanisms on the surface of the microorganisms. The adsorption capacity of bacteria,algae, fungi, yeast and seaweed have all been studied. Certain aerobic bacterial strains (e.g.*Geobacillus sp, Pepsinophilus,Coccus sp.,Coccus rosea,Sphinhomonas pyrimobilus* and *Bacillus badius*) are able to break azo bond through oxygen-insensitive (oxygen-sensitive) or aerobic (anaerobic) Azoreductases. Researchers have investigated the adsorption of cationic and anionic dyes on activated carbon as well as on several natural adsorbents [28-30]. In this study, we focused on increasing the adsorption capacity using nitric acid(acidified activated carbon) entrapped bacterium as an adsorbent to remove the Congo Red(CR). The bacterium used in this study was able to metabolize the hydrocarbon.Structural modifications in the bacteria surface modified activated carbon were characterized using SEM. The adsorption of the CR from the aqueous solution were studied using the bacteria surface-modified activated carbon as adsorbents. [28-30].

In this study, we concentrated on increasing the adsorption capacity using nitric acid(acidified activated carbon) entrapped bacterium as an adsorbent to remove the Congo Red(CR). The bacterium used in this study was able to metabolize the hydrocarbon. Structural modifications in the bacteria surface modified activated carbon were characterized using SEM. The adsorption of the CR from the aqueous solution were studied using the bacteria surface modified activated carbon as adsorbents. Adsorption data were subject to kinetics analysis. Equilibrium data were subject to isotherm model analysis. The structural properties of the absorbent and its adsorption performance were elucidated[31].

Congo Red and its Harmful Effects : Dyes and pigments are widely used in different industries and a large amount of these coloring compounds goes into the effluent water during the dyeing process. The carcinogenic and potentially toxic nature of these compounds represents an increasing hazard to aquatic life. Therefore, various physicochemical methods such as coagulation, adsorption,filtration, precipitation and oxidation have been used for dye removal [32].Reactive dyes are an important class of commercially available synthetic dyes. Reactive dyes are mainly characterized due to their excellent binding capability initiated due to the formation of a covalent bond between the reactive groups of dyes and the surface groups present on the textile fibres. Reactive dyes are extensively used in textile industries, and their discharge in the ecosystem represents rising environmental pollutions,nowadays, because of their non-biodegradability, carcinogenicity, toxicity, and mutagenicity. Reactive dyes are the most problematic dyes among others, as they tend to pass unaffected through conventional treatment systems [33].

CR is among the most frequently used azo dyes worldwide. CR is a diazo benzidine based anionic dye containing double azo (-N=N-) linkage. CR is highly toxic and carcinogenic and is metabolized well by known human carcinogen benzidine in many organisms. Due to health risks, several countries had banned the use of CR dye. The intricate aromatic molecular structures of CR dye provide them optical, thermal and physicochemical stability, thus making them difficult to biodegrade. As a result, the existence of CR dye in water, even in dilute concentrations is highly undesirable [34]. Due to CR's ability to cause cancer, mutagenesis, and other toxicities to flora, wildlife, and humans, it is now necessary to remove CR from industrial CR-laden (waste)water. A total of 300 research and review-based papers are chosen for this review, examined, and subjected to additional evaluation. The consequences of CR dye's simulated toxicity on aquatic fauna and flora are explored. It is discovered that CR is phytotoxic to aquatic flora and has the potential to interfere with aquatic fauna's reproductive activity. A full examination and discussion of CR removal options was also conducted, with adsorption technology emerging as the most effective method for CR decontamination [35]. When CR

concentrations are high, it can have a devastating effect on the human body by being cytotoxic (genotoxic, hemotoxic, and neurotoxic), carcinogenic, and mutagenic. It has an impact on various organs, including the reproductive, respiratory, ocular, and skin systems. A carcinogenic substance and its poisonous metabolite, benzidine, produces allergic reactions. Because it forms a covalent bond with cellular macromolecules, the bladder cancer-causing agent benzidine inhibits cellular function. There are limitations on the use of azo dyes in a number of nations. Although azo dyes enhance the material's aesthetic appeal, its toxicity is a significant issue. These colours produce water pollution and have a negative impact on various living things, which the affects our ecosystem. This is because they are released into the water. Thus, their toxicity is a serious problem.Numerous different creatures, including humans, plants, animals, bacteria, algae, protozoa, and protozoa are poisoned by CR. In addition to being carcinogenic, it is also mutagenic and teratogenic. It should be noted that while CR is not harmful on its own, toxic amines result from its decrease. We take precautions to protect diverse living things in order to reduce the negative effects and fatalities brought on by various azo dyes[36].

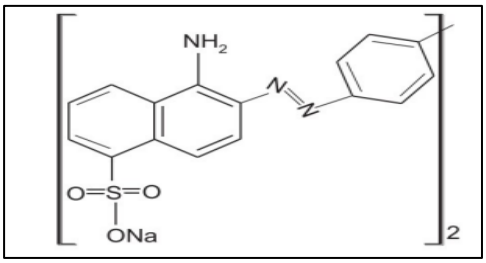

Figure 1:Structure of CR dye

Bioremediation of Dyes : During synthesis and processing of dyes, about 15% of the total world productions are lost with wastewater [37]. Various bioremediation methods are used for dye removal.

Enzymes : Enzymes are typically used in an immobilized form because free enzymes are less robust, making them vulnerable to higher temperatures (70-80 ºC), sudden changes in pH, and high substrate concentrations. Additionally, free enzymes tend to deactivate relatively quickly. Immobilization renders enzymes resistant to harsh environments and allows for easy recovery and recycling.Peroxidases are commonly utilized as versatile catalysts in various industrial processes. For instance, Horseradish peroxidase (HRP) enzymes have been employed in the degradation or remediation of synthetic dyes and toxic compounds of industrial origin. However, due to the high cost of HRP, researchers have explored alternative iron porphyrins [38]. For instance, Horseradish peroxidase (HRP) enzymes have been employed in the degradation or remediation of synthetic dyes and toxic compounds of industrial origin. However, due to the high cost of HRP, researchers have explored alternative iron porphyrins [38]. Both HRP and soybean peroxidase (SBP) from Glycine max are typical oxidoreductases. In the presence of H_2O_2, peroxidases catalyze the oxidation of phenols, anilines, benzidines, biphenyls, and other aromatic compounds [39].

Microbiological decolorization : Microbiological degradation and decolorization offer cost-effective and environmentally friendly alternatives to chemical decomposition and enzymatic processes. Bacteria and fungi are primarily focused on for the biodegradation of azo dyes. Literature reports indicate that a mutant strain of Escherichia coli can decolorize azo dye Reactive Red 22.Research has shown that *Rhodobacter sphaeroides* is capable of decolorizing Methyl Orange, while *Rhodopseudomonas palustris* has demonstrated effectiveness in decolorizing an azo dye called Acid Red B [40]. Microorganisms utilized for decolorization or degradation of triphenyl methane dyes include bacteria such as *Bacillus subtillus, Pseudomonas pseudomallei, E. coli,* and *Citrobacter sp.*, as well as yeasts like *Rhodotorula rubra* and *Rhodotorula sp*., and fungi like *Cunninghamella elegans* and *Phanerochaete chrysosporium*. Actinomycetes like *Norcardia coralline* are also employed.studies on crystal violet decolorization have also been conducted [41]. Reports suggest that certain species of ascomycetous yeast, including *Candida tropicalis, C.zeylanoides, Issatchenkia occidentalis,* and *Debaryomyces polymorphus*, are capable of enzymatic dependent decolorization and biodegradation of mutagenic Methyl Red and various azo dyes [42].

Other Methods for Degrading Dye *:* Till date, a number of approaches, including physical (adsorption, membrane separation, and coagulation), chemical (ion change, oxidation, and catalytic degradation),as well as biological methods (microbial degradation), have been validated as feasible for eliminating dyes.Adsorption is thought to be the most alluring and effective techniqueamong these. Along with being simple to use, it is also affordable, en vironmentally friendly,and impervious to various harmful substances while providing satisfactory performance.

To remove the harmful organic colourants, a variety of adsorbents have been studied, including metal- organic frameworks, polymers, agro-industrial waste, and nanomaterials. With its enormous porous surface area, tunable pore structure, thermo-stability, and low acid/base reactivity, activated carbon (AC) has been the most widely used adsorbent. The Society for Applied Microbiology Journal of Applied Microbiology creates a carbon-based electropositive absorbent. They discovered that the new adsorbent was favourable for Congo red (CR) and had a monolayer adsorption capability.Adsorption is regarded as the most effective approach among these and is considered the most appealing and effective plan [27] .

When compared to other physical and chemical methods, biological therapy has proven to be the most cost-effective choice. For the treatment of industrial effluents, biodegradation methods such as fungal decolorization (e.g.,*Phanerochaete chrysosporium, Trametes sp.,* and *Aspergillus sp.*), microbial degradation,by (alive or dead) microbial biomass, or bioremediation systems have been often used. By cleaving the azo link and producing aromatic amines as a byproduct, bacteria like *Bacteroides sp., Eubacterium sp., and Clostridium sp.* are used in anaerobic biological treatment procedures to decolorize azo dye solutions. Numerous colours have been documented as being oxidatively decolored by aerobic microorganisms.Azo dyes consistently emerged asthe most resistant chemicals from a variety of classes.For the decolorization of azo dyes in bioreactors, dye-degrading fungi are used. The xenobiotic nature of azo dyes prevents complete degradation. The bioremediation of textile effluents predominantly involves the extracellular enzymes laccase and azo- reductase [43].Numerous microorganisms, including fungi, actinomycetes, hydrophila, and Bacillus Cereus, have been reported to have dye decolorizing ability.

Mechanism behind Decolorization of Azo Dyes : Decolourization of azo dyes typically occurs under aerobic and anaerobic conditions as well as in anaerobic conditions due to diverse bacteria. Elimination of colour by an anaerobic microbiological approach could be regarded as successful, mainly due to the activity of an anaerobic reductase slicing N = N resulting in amines. These amines are not only toxic but also oncogenic, mutagenic and above all repel further decolourment under anaerobic conditions However, pure in in addition to mixed bacterial culture has been exposed to anaerobic degradation It has also been suggested that a fortuitous process of decolorization of anaerobic azo dye is an azo-dye-dependent process, where the anaerobic dyes provide an electron acceptor to the carriers of the electron transport chain. Other researchers suggest that decolorization may be contributed to non-specific extracellular reactions between reduced compounds produced by an anaerobicproduct.

Aerobic azo reductases were shown to be able to co-factor with both NAD (NADH) and NADH and to reductively cleave not only the carbonylated growth substrate of the bacteria but also sulfonate structural analogues. This type of azo-reductase activity was observed in Pseudomonas strain K22, KF46 after purification and characterization. This enzyme system was flavin-free. These bacteria do not use azo dye for growth and require additional organic carbon sources. There are few bacteria that can grow on an azo compound as their sole carbon source. These bacteria also cleave -N=N-bonds reductively, and use amino acids as carbon and energy sources for growth. These organisms are substrate-specific [44] of dyes capable of detoxifying aromatic amines formed by anaerobic decolourization of azo colourants. Decolouring an azo dye using pure cultures of bacteria is successful; however, different isolates often cannot completely decolorise an azo dye and are also responsible for producing carcinogenic aromatic amines as intermediate products that require further degradation [45]. A low redox potential (50 mV) has been shown to be responsible for the effective decolorization of a range of synthetic dyes in anaerobic applications. Some researchers suggest that oxygen inhibits azo bond reduction activity, as aerobic respiration may be dominant in the utilization of NADH, thereby inhibiting the electron transfer from NADH to an azo bond.

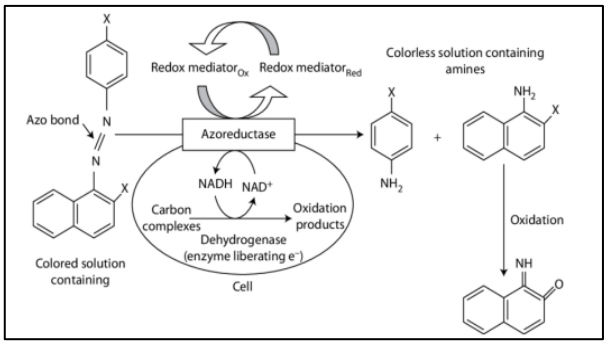

Figure 2:Mechanism of bacterial decolorization of Azo dye

Surface Modification of Activated Carbon : Adsorption properties can be improved by several research studies. The main focus of the research is on modification process such as raw material, carbonization process, activation conditions, etc. Another approach is to modify the surface structure of the AC. This is done by changing the functional groups of the surface. Surface modifications are a way to alter the structure of AC. These modifications include adding new atoms, formation of functional groups and alteration of the textural properties of the surface. Surface modifications have a significant impact on adsorption capability. Adsorptive capacity can be determined by various factors such as surface chemistry, functional groups, porous structure, adhesiveness, and adsorbate /adsorbent interactions. The majority of surface studies involve the addition of oxygen or other atoms to the AC structure, resulting in changes in the surface of the AC. These surface modifications involve chemical and physical treatments. Modifications are caused by several substances, such as acids,bases, and oxidizing agents, which produce waste that must be treated and properly disposed of after the process. The structure of the AC is non-polar, so the ACs are organophilic. The surface structure of this material have a structure that indicates the polarity of the molecules on the surface. The polarity can be increased by modification of surface, mainly surface oxidation. Another type of modification is gas plasma under some conditions. Therefore, new surface modification methods must be perform to better utilize activated carbons [46].

The methods of modification can be classified into chemical and physical. The chemical method allows you to increases the number of functional surface groups by the action of oxidizers or reductives. This modification have to be done in the liquid or in the gas phase. The oxidizers used in the liquid state are usually inorganic acids (such as nitric acid or sulphuric acid), hydrogen peroxide and organic acids. Gas related modification can be done by using oxygen derived from air, water vapor, carbon dioxide, ozone and nitric oxide.The most efficient method is to oxidize liquids, as ease to control the process and to get a large amount of oxidation of the surface. However, one of the drawbacks of thismethod is that the surface becomes contaminated with reduced forms of oxidants, which necessitates material purification and results in excess amounts of waste. The acidic and alkaline properties can be improved by gas oxidation, but this method is more less efficient than oxidation by liquid substances.

The number and types of oxygen groups formed are largely determined by the oxidant used and by the temperature at which the modification is made. In the liquid phase, large amounts of acidic oxygen group tend to be formed. Heat-induced modifications of activated carbon were made using HNO_3 and HCl. HNO_3 enabled the formation of more acidic oxygen groups on the surface of the activated carbon, while HCl also improved the adsorption properties of modified coal with respect to lead ions. Alkaline agents may also be used to modify activated carbon. Potassium hydroxy hydroxide sig-nificantly enhances the physicochemical characteristics of activated carbon. Material with a well defined pore structure, high adsorption capacity in comparison with methanol, and enriched with surface oxygen groups may be obtained [47].

Advantages of surface modification are high acidic and functional groups on activated carbon surface, enhanced chelation ability with metal species surface, increases uptake of organics, enhances in built catalytic oxidation capability. And the disadvantages are may reduce Brunauer-Emmett-Teller (BET) surface area and pore volume has more adverse effect on uptake of organics may give off undesired SO_2, (treatment with H_2SO_4) or NO_2, (treatment with HNO_3) gases. May, in some cases, decrease the adsorption of metal ions [48].

II. MATERIALS AND METHODS

Collection of soil sample

50g of soil sample is collected from an automobile shop in Chiyaram. The sample was placed in a refrigerator at 4°C

Isolation and Screening of Hydrocarbon Degrading Bacteria

In the process of serial dilution, the procedure begins by drawing 1 ml of a thoroughly mixed sample into a pipette. This 1 ml is then added to the first tube, which already contains 9 ml of solvent, resulting in a total volume of 10 ml. This initial step establishes a dilution factor of 10^{-1}. The pipette is then emptied and refilled with the mixture from the 10^{-1} tube, transferring 1 ml of this solution into the second tube. This action creates a dilution factor of 10^{-2} for the contents of the second tube. This process is repeated with each subsequent tube. This repetition results in a final dilution factor of 10^{-8} for bacteria or cells in the eighth tube.

In the spread plate method, 0.1 ml of the desired dilution series is carefully pipetted into the centre of the agar plate's surface. To ensure even distribution, an L-shaped glass spreader is dipped in alcohol and heated on a Bunsen burner. The sample is then spread evenly over the agar surface using the sterile glass spreader, while rotating the petri dish simultaneously for uniform coverage.

Figure 3:Serial dilution of diluted soil sample solution

To sterilize the wire loop, it is touched to the outside of the agar plate and then dipped into the broth culture containing the bacteria. With the plate lid slightly lifted, the wire loop is inserted and dragged over the top third of the plate's surface in a 'zig-zag-like' pattern. Care is taken not to touch any of the previously streaked areas. Finally, the remaining plate is filled using the loop in a 'zig-zag-like' manner. The completed plate is then incubated at 37°C for 24 hours to allow for bacterial growth and colony formation.

Confirmatory Test for Dye Decolorizing Bacteria

The detection of laccase using the guaiacol substrate involves several steps. 3 ml of guaiacol is added to 50 ml of Nutrient broth, which is then poured into three Petri dishes. Bacteria from agar slants are spread onto these Petri dishes, and they are then incubated at 37°C for 48 hours to allow for laccase activity. For the preparation of the master culture, a loopful of bacteria is taken from agar slants labeled SS1(A), SS2(B), and SS3(C). These bacteria are transferred into 150 ml of nutrient broth and incubated at 37°C for 24 hours to promote growth. CR broth was prepared with a concentration of 40 mg/l of nutrient broth and 150 ml of the mixture was transferred to 7 conical flasks. In the decolorization assay, 600µl of bacterial suspension is added to 150 ml of CR broth in each of the seven conical flasks. The absorbance of the decolorized media solution is then measured at 490 nm using a UV-Vis spectrophotometer against Nutrient broth without dye as a reference. The decolorization assay is calculated using the equation [49]. This assay helps quantify the effectiveness of the bacteria in decolorizing the CR broth, providing insight into laccase activity.

$$\%\ \text{Decolorization} = \frac{\text{Initial O.D} - \text{Final O.D}}{\text{Initial O.D}} \times 100$$

Identification of Unknown Bacteria using 16S rRNA Sequencing

Following the decolorization assay, it was observed that certain unknown bacteria exhibited a higher capacity for degrading Congo Red dye compared to the other two microorganisms. To further characterize and identify these potent dye-degrading bacteria, the next step involves 16S rRNA sequencing. This sequencing was conducted at VETA Genomics,Thrissur provided a detailed genetic profile to elucidate the identity of these bacteria

Preparation of Surface Modified Activated Carbon and Bacteria

The preparation of surface-modified activated carbon begins with 6 grams of activated carbon (AC), which is ground under 100 mesh and then subjected to acid washing. It undergoes a treatment with 100 ml of nitric acid (0.72 mol/l) once, followed by two washes with distilled water. The mixture is then centrifuged at 10,000 rpm for 15 minutes to remove the supernatant water, and the resulting pellet is dried under a hot air oven for 30 minutes [6]. For the preparation of the mixture of bacterial suspension in 0.9% NaCl solution and surface-modified activated carbon, a 10 ml bacterial culture is first centrifuged at 10,000 rpm for 10 minutes. To the pellet, 5 ml of distilled water is added, and this mixture is centrifuged twice more at 10,000 rpm for 10 minutes each time. The resulting pellet is collected and transferred to a boiling tube, where 10 ml of 0.9% NaCl solution is added. The choice of 0.9% NaCl solution is crucial as it is isotonic with cell protoplasm, minimizing the chances of cell death. This isotonicity prevents the swelling or shrinking of cells. The use of saline solution in serial dilution or culture suspension is preferred due to its isotonic nature. In contrast, water is hypotonic, which means its solute concentration is lower than inside the cell.This difference causes water to enter the bacterial cell, potentially disrupting it [50].The final step involves adding 5 grams of surface-modified activated carbon to the bacterial saline suspension and vortexing the mixture for 30 minutes to ensure thorough mixing and interaction between the bacteria and the modified carbon surface.

Characterization of Surface Modified Activated Carbon by FTIR Analysis

FTIR analysis is a sophisticated technique used to describe molecular structure and chemical composition based

FTIR analysis provides valuable information about molecular structure and chemical composition, enabling the characterization of surface modifications of activated carbon induced by the presence of *Bacillus cereus*.
The FTIR analysis was done at Inter Instrumentation Centre (IUIC), MG University, Kottayam using a Fourier transform spectrophotometer model SHIMADZU IR Prestige-21 for analyzing surface modification of Sample 2 (activated carbon with *Bacillus cereus* in 0.9% NaCl solution) and Sample 1 (activated carbon in 0.9% NaCl saline solution)

Adsorption Experiment
Prepare sample containing 5ml of 40mg/l Congo Red dye solution and 2ml of the mixture of activated carbon and *Bacillus cereus* in saline solution, and the control containing 5ml of 40mg/l Congo Red dye solution and 2ml of saline solution. At the beginning of the incubation period, measure the absorbance of the sample and the control using a spectrophotometer. Put the control and the sample into a shaker incubator. Preheat the shaker incubator to 37 degrees Celsius for five days. Take the samples out of the shaker incubator after they have been incubated for three days.Using the spectrophotometer, determine the absorbance of the sample and the control at 490nm.Determine the absorbance change from the first to the last reading for the sample and the control. To evaluate how well the activated carbon and *Bacillus cereus* mixture removes Congo Red dye, compare the absorbance change between the sample and the control.

III. RESULT AND DISCUSSIONS

Isolation and Screening of Hydrocarbon degrading Bacteria
In the spread plate method, colonies are observed to form in the dilutions of 10^{-4}, 10^{-5}, and 10^{-8}. These dilutions represent different concentrations of the original bacterial sample that were plated and allowed to grow on agar plates. The presence of visible colonies in these dilutions indicates the viability and growth of the bacteria at these specific dilution factors. In the streak plate method, three distinct organisms are individually streaked onto separate nutrient agar plates.

Figure 4:Colony formation

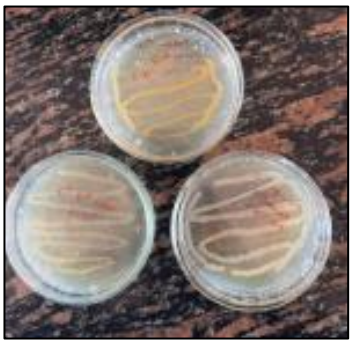

Figure 5:Three different pure bacterial isolates by streak plate method

The purpose of streaking is to obtain isolated colonies. Each organism is streaked in its own plate to prevent cross-contamination and to facilitate the growth of pure cultures.After incubation, these plates will exhibit distinct colonies characteristic of each organism.

To establish pure cultures, the preparation of agar slants involves streaking three different organisms onto the surface of the slants. The streaking technique creates isolated lines of growth, promoting the development of distinct colonies.

Figure 6:Inoculated agar slants after incubation

Confirmatory Test for Dye Decolorizing Bacteria

In the detection of laccase using the guaiacol substrate, laccase, an enzyme responsible for oxidizing guaiacol into a brown-colored compound, was tested. However, it was observed that laccase did not play a role in decolorization. The negative result from the guaiacol test indicates the absence of laccase activity. Therefore, it can be concluded that laccase enzyme is not involved in the process of dye decolorization. Instead, there are other enzymes, such as azo-reductase, peroxidases, and others, that are responsible for the decolorization of dyes. These enzymes may be the key players in the breakdown and removal of color from dye molecules, highlighting the diverse mechanisms employed by microorganisms in environmental.For the preparation of the master culture, three distinct bacterial suspensions were meticulously prepared. The process involved cultivating these bacteria in nutrient rich broth media under optimal conditions to promote robust growth and ensure a healthy culture.

Figure 7:Inoculated different bacterial strains in seven dye media containing flasks before incubation

Figure 8:Bacterial growth at the 7th day of incubation

Figure 9: Bacterial growth at the 14th day of incubation

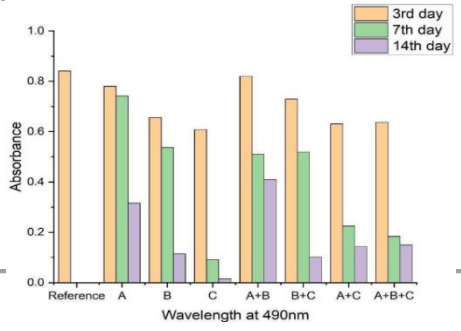

Figure 10:Decolorization of 7 CR samples after
3rd,7th and 14th day

After the 14th day, the flask containing bacterial strain 'C' exhibits a noticeable fading in the color of the CR dye, indicating a substantial degradation process. This strain 'C' demonstrates a remarkable ability to degrade the CR dye, surpassing other bacterial strains present in the flask by 71.46%. This means that the CR dye's color diminishes significantly more in the presence of strain 'C' compared to the other bacterial strains within the same time.

Identification of Unknown Bacteria using 16s rRNA Sequencing : The identification of the unknown bacteria using 16S rRNA sequencing revealed that it belongs to the species *Bacillus cereus*. The successful identification of Bacillus cereus through 16S rRNA sequencing enhances our understanding of the microbial communities involved in dye decolorization.

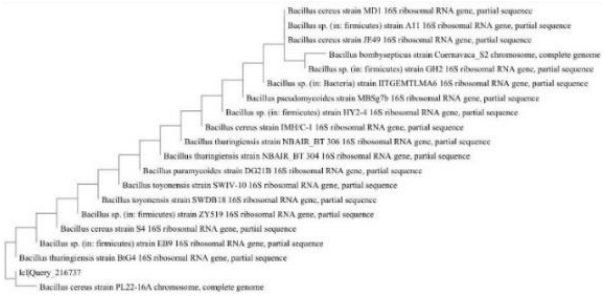

Figure 11:Phylogenetic tree of *Bacillus cereus*

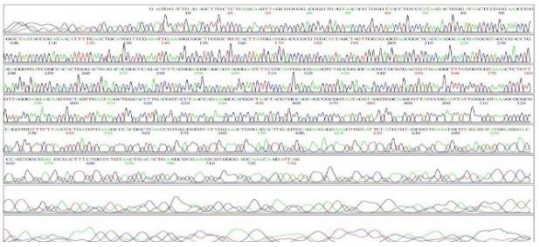

Figure 12:Forward chromatogram

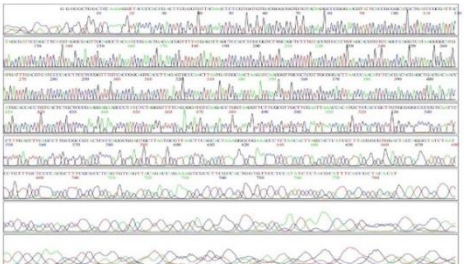

Figure 13: Reverse chromatogram

Preparation of Surface Modified Activated Carbon and Bacteria
A surface modified activated carbon is prepared with bacteria.

Figure 14:AC and Bacillus cereus in
0.9% saline solution

Figure 15:AC after surface modification

Characterization of Surface Modified Activated Carbon by FTIR Analysis

After surface modification of activated carbon and bacteria, the FTIR results show a reduction in the number of observed peaks compared to the unmodified samples. This suggests significant alterations in their chemical compositions or surface functional groups due to the modification process. The decrease in peaks post-modification implies that certain functional groups may have been removed or modified, resulting in a FTIR spectrum [51].

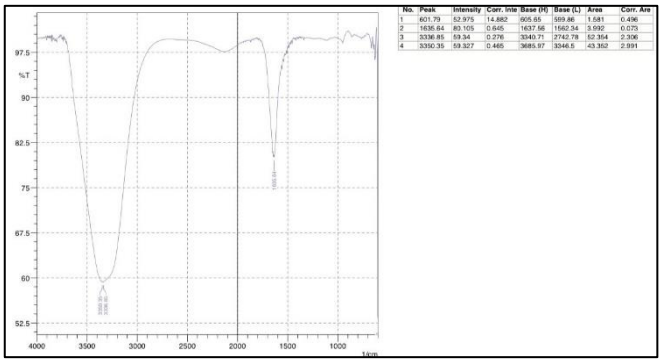

Figure 16:FTIR results of AC in 0.9 % saline solution

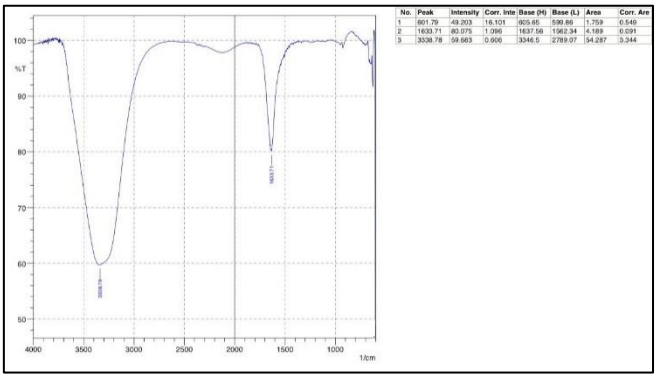

Figure 17 :FTIR results of AC and Bacillus cereus in 0.9% saline solution

Adsorption Experiment

After 3 days of incubation, absorbance of control was found to be 0.841 at 490nm and the absorbance of the sample was found to be 0.167. The percentage of decolorization of CR dye in presence of surface modified activated carbon is 80%. Without adding activated carbon, the percentage of decolorization was 28.65% after 3 days.

Figure 18:Sample and Control

IV. CONCLUSION

The removal of CR dye using surface-modified activated carbon and bacteria represents an innovative and effective approach towards addressing environmental concerns associated with dye pollution. This method combines the adsorption capabilities of surface-modified activated carbon with the biodegradation potential of bacteria, offering a synergistic and efficient solution for wastewater treatment. The surface modification of activated carbon enhances its adsorption capacity by creating active sites that attract and capture dye molecules. This modification also contributes to the selectivity and specificity of the adsorption process, making it particularly effective for the removal of Congo Red dye. The porous structure of activated carbon provides a large surface area, further optimizing its adsorption efficiency. The introduction of bacteria into the treatment process introduces a biological aspect to the dye removal. Bacteria, through their metabolic activities, can break down and degrade dye molecules into less harmful byproducts. This biodegradation complements the adsorption process, ensuring a more comprehensive and sustainable treatment of wastewater containing CR dye. Additionally, the use of bacteria in conjunction with surface-modified activated carbon offers the advantage of regeneration. Bacteria can be cultivated and sustained, providing a continuous and renewable mechanism for dye removal. This aspect contributes to the economic feasibility and long-term viability of the treatment approach. Overall, the combined use of surface-modified activated carbon and bacteria presents a promising solution for the removal of Congo Red dye from wastewater. This approach addresses both the physical and biological aspects of dye pollution, demonstrating efficiency, selectivity, and sustainability. Further research and optimization of this method could lead to its implementation on a larger scale, contributing to the mitigation of environmental impacts associated with dye contamination.

REFERENCES

[1] Azizullah L, Khattak MNK, Richter P, Häder D-P. Water pollution in Pakistan and its impact on public health - A review. Environ Int 2013; 37: 479-97.

[2] Ismail, M., Akhtar, K., Khan, M.I., Kamal, T., Khan, M.A., M Asiri, A., Seo, J. and Khan, S.B., 2019. Pollution, toxicity and carcinogenicity of organic dyes and their catalytic bio-remediation. Current pharmaceutical design, 25(34), pp.3645-3663.

[3] Maheshwari, K., Agrawal, M. and Gupta, A.B., 2021. Dye pollution in water and wastewater. Novel materials for dye-containing wastewater treatment, pp.1-25.

[4] Khan SB, Faisal M, Rahman MM, Jamal A. Exploration of CeO_2 nanoparticles as a chemi-sensor and photo-catalyst for environmental applications. Sci Total Environ 2011; 409(15): 2987-92.

[5] Bhat IU, Anwar MNK, Appaturi JN. Polymer based palladium nanocatalyst for the degradation of nitrate and Congo red. J Polym Environ 2019; 27: 1475-87.

[6] Kodoth AK, Badalamoole V. Pectin based graft copolymer-ZnO hybrid nanocomposite for the adsorptive removal of crystal violet. J Polym Environ 2019; 27: 2040-53.

[7] Ramezani S, Zahedi P, Bahrami SH, Nemati Y. Microfluidic fabrication of nanoparticles based on ethyl acrylate-functionalized chitosan for adsorption of methylene blue from aqueous solutions. J Polym Environ 2019; 27: 1653-65.

[8] Wang YQ, Li YH, Li H, Zheng H, Du QJ. Equilibrium, kinetic and thermodynamic studies on methylene blue adsorption by konjac glucomannan/activated carbon aerogel. J Polym Environ 2019; 27: 1342-51.

[9] Arumugham T, Kaleekkal NJ, Rana D. Fabrication of novel aromatic amine functionalized nanofiltration (NF) membranes and testing its dye removal and desalting ability. Polym Test 2018; 72: 1-10.

[10] Barnabas CGS, Theerthagiri J, Santhanam A. Comparative photocatalytic degradation of organic dyes using silver nanoparticles synthesized from Padina tetrastromatica. Curr Nanosci 2018; 14: 71-5.

[11] Mazzoni M, Dagar J, Lai S, et al. Transformed double-capped gold nanorods in dye co-sensitized solar cells for semitransparent windows. Curr Nanosci 2019; 15: 309-18.

[12] Phukan S, Kakati D, Rashid MH. Use of invasive weed to synthesize shape-tunable gold nanoparticles and evaluation of their catalytic activities in dye reduction. Curr Nanosci 2018; 14: 511-9.

[13] Sekhar MC, Reddy BP, Mallikarjuna K, Krishna GG, Park SH. Biogenic fabrication of Au/Pd bimetallic quantum dots from mushroom extract and their application to organic dye pollutant reduction. Curr Nanosci 2018; 14: 313-8.

[14] Siong VLE, Lai CW, Juan JC, Lee KM, Leo BF, Khe CS. One-step solvothermal synthesis of rGO/TiO2 nano-composite for efficient solar photocatalytic degradation of methylene blue dye. Curr Nanosci 2019; 15: 157-62.

[15] Yang LL, Zhao Y, Li J, Zhou YW, Xiao X, Zhang WJ. Effects of calcination on sol-gel synthesis of hollow spherical 8%B-TiO2 for photocatalytic degradation of RBR X-3B-characterization and activity. Curr Nanosci 2019; 15: 289-95.

[16] Zhang WJ, Liu YX, Xin HL. Sol-gel preparation of hollow spherical x%B-TiO2 photocatalyst: the effect of boron content on RBR X-3B decoloration. Curr Nanosci 2018; 14: 209-15.

[17] Zhang WJ, Yang J, Du L. Sol-gel synthesis of a novel chi Sm2Ti2O7/HZSM-5 composite photocatalyst for the promoted activity on RBR X-3B degradation. Curr Nanosci 2018; 14: 17-25.

[18] Ali F, Khan SB, Kamal T, Anwar Y, Alamry KA, Asiri AM. Bactericidal and catalytic performance of green nanocomposite based-on chitosan/carbon black fiber supported monometallic and bimetallic nanoparticles. Chemosphere 2017; 188: 588-98.

[19] Haider A, Haider S, Kang IK, et al. A novel use of cellulose based filter paper containing silver nanoparticles for its potential application as wound dressing agent. Int J Biol Macromol 2018; 108: 455-61.

[20] Kamal T, Ahmad I, Khan SB, Asiri AM. Bacterial cellulose as support for biopolymer stabilized catalytic cobalt nanoparticles. Int J Biol Macromol 2019; 135: 1162-70.

[21] Khan MSJ, Kamal T, Ali F, Asiri AM, Khan SB. Chitosan-coated polyurethane sponge supported metal nanoparticles for catalytic reduction of organic pollutants. Int J Biol Macromol 2019; 132: 772- 83.

[22] Khan MSJ, Khan SB, Kamal T, Asiri AM. Agarose biopolymer coating on polyurethane sponge as host for catalytic silver metal nanoparticles. Polym Test 2019; 78: 105983.

[23] Ul-Islam M, Wajid Ullah M, Khan S, et al. Recent advancement in cellulose based nanocomposite for addressing environmental challenges. Recent Pat Nanotechnol 2016; 10(3): 169-80.

[24] Karimi L, Zohoori S. Superior photocatalytic degradation of azo dyes in aqueous solutions using TiO2/SrTiO3 nanocomposite. J Nanostructure Chem 2013; 3: 1-5.

[25] A. Tkaczyk, K. Mitrowska, and A. Posyniak, "Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: A review," Science of the total environment, vol. 717, p. 137222, 2020

[26] J. Andrade, K. Kunitomo, V. R. Wagenen, B. Kastigir, D. Gough, and W. Kolff, "Coated adsorbents for direct blood perfusion:: Hema/activated carbon." ASAIO Journal, vol. 17, no. 1, pp. 222–228, 197

[27] Vandevivere P, BianchiR, VerstraeteW. Review:treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies. Journal of Chemical Technology and Biotechnology 1998;72:289e302.

[28] Janosˇ P, Buchtova´ H, Milena Ry´znarova´ M. Sorption of dyes from aqueous solutions onto fly ash. Water Research 2003;37:4938e44.

[29] Faria P, O´ rf~ao J, Pereira M. Adsorption of anionic and cationic dyes on activated carbons with different surface chemistries. Water Research 2004;38:2043e52.

[30] Crini G. Non-conventional low-cost adsorbents for dye removal: a review. Bioresource Technology 2006;97:1061e85.

[31] A. Wang, C. Liu, X. Ge, W. Meng, Y. Pi, and C. Liu, "Enhanced removal of congo red dye from aqueous solution by surface modified activated carbon with bacteria," Journal of Applied Microbiology, vol. 131, no. 5, pp. 2270–2279, 2021.

[32] Muthirulan P, Nirmala Devi C, Meenakshi Sundaram M. Synchronous role of coupled adsorption and photocatalytic degradation on CAC-TiO2 composite generating excellent mineralization of alizarin cyanine green dye in aqueous solution. Arabian J Chem 2017; 10(Suppl. 1): S1477-83.

[33] Belessi V, Romanos G, Boukos N, Lambropoulou D, Trapalis C. Removal of reactive red 195 from aqueous solutions by adsorption on the surface of TiO2 nanoparticles. J Hazard Mater 2009; 170(2- 3): 836-44.

[34] Xia H, Chen L, Fang Y. Highly efficient removal of Congo red from wastewater by Nano-Cao. Sep Sci-Technology 2013; 48: 2681-7
[35] P. O. Oladoye, M. O. Bamigboye, O. D. Ogunbiyi, and M. T. Akano, "Toxicity and decontamination strategies of congo red dye," Groundwater for Sustainable Develop ment, vol. 19, p. 100844, 202
[36] S. I. Siddiqui, E. S. Allehyani, S. A. Al-Harbi, Z. Hasan, M. A. Abomuti, H. K. Rajor, and S. Oh, "Investigation of congo red toxicity towards different living organisms: A review," Processes, vol. 11, no. 3, p. 807, 2023
[37] Gupta AK, Pal A, Sahoo C. Photocatalytic degradation of a mixture of Crystal Violet (Basic Violet 3) and Methyl Red dye in aqueous suspensions using Ag+ doped TiO2. Dyes Pigments 2006; 69: 224- 32.
[38] Pirillo S, Rueda EH, Ferreira ML. Supported biocatalysts for Alizarin and Eriochrome Blue Black R degradation using hydrogen peroxide. Chem Eng J 2012; 204–206: 65-71.
[39] Pirillo S, Einschlag FSG, Ferreira ML, Rueda EH. Eriochrome Blue Black R and Fluorescein degradation by hydrogen peroxide oxidation with horseradish peroxidase and hematin as biocatalysts. J Mol Catal B Enzym 2010; 66: 63-71
[40] Chang JS, Kuo TS, Chao YP, Ho JY, Lin PJ. Azo dye decolorization with a mutant Escherichia coli strain. Biotechnol Lett 2000; 22: 807-12.
[41] Chen CH, Chang CF, Liu SM. Partial degradation mechanisms of malachite green and methyl violet B by Shewanella decolorationis NTOU1 under anaerobic conditions. J Hazard Mater 2010; 177(1- 3): 281-9.
[42] Hatvani N, Mécs I. Production of laccase and manganese peroxidase by Lentinus edodes on malt-containing by-product of the brewing process. Process Biochem 2001; 37: 491-6. [http://dx.doi.org/10.1016/S0032-9592(01)00236-9]
[43] K. Lokesh, R. Sivakiran et al., "Biological methods of dye removal from textile effluents-a review," Journal of Biochemical Technology, vol. 3, no. 5, pp. 177–180, 2014
[44] R. G. Saratale, G. D. Saratale, J.-S. Chang, and S. P. Govindwar, "Bacterial decol orization and degradation of azo dyes: a review," Journal of the Taiwan institute of Chemical Engineers, vol. 42, no. 1, pp. 138–157, 2011.
[45] M. Ajaz, S. Shakeel, and A. Rehman, "Microbial use for azo dye degradation—a strat egy for dye bioremediation," International Microbiology, vol. 23, pp. 149–159, 2020
[46] M. Pego, J. Carvalho, and D. Guedes, "Surface modifications of activated carbon and its impact on application," Surface Review and Letters, vol. 26, no. 01, p. 1830006, 2019.
[47] E. Wolak and A. Orzechowska-Zieba, "Change of the surface and structure of activated carbon as a result of hno3 modification," Adsorption, pp. 1–8, 2023. xii
[48] K. Foo and B. H. Hameed, "An overview of dye removal via activated carbon adsorption process," Desalination and Water Treatment, vol. 19, no. 1-3, pp. 255–274, 2010
[49] H. Shah, F. Yusof, and M. Z. Alam, "A new technique to estimate percentage decolorization of synthetic dyes on solid media by extracellular laccase from white-rot fungus," Bioremediation Journal, vol. 27, no. 1, pp. 66–74, 2023.
[50] R. G. Lobetti, K. E. Joubert, J. Picard, J. Carstens, and E. Pretorius, "Bacterial colonization of intravenous catheters in young dogs suspected to have parvoviral enteritis," Journal of the American Veterinary Medical Association, vol. 220, no. 9, pp. 1321– 1324, 2002.
[51] A. IkhtiarBakti and P. L. Gareso, "Characterization of active carbon prepared from coconuts shells using ftir, xrd and sem techniques," J. Ilm. Pendidik. Fis. Al-Biruni, vol. 7, pp. 33–39, 2018