

EFFECTIVE APPROACH OF BACTERIAL DEGRADATION USING POLYMERIC ORGANOGEL FOR MUNICIPAL WASTE WATER TREATMENT

PROJECT WORK

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A++" by NAAC I12B Status by UGC Approved by AICTE

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APRIL-2024



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of N.THREISSHA (41770036), A.KAVIYA DHARISHINI (41770011) who carried out the project entitled "AN EFFECTIVE APPROACH BACTERIAL DEGRADATION USING POLYMERIC ORGANOGEL FOR MUNICIPAL WASTE WATER TREATMENT" under our supervision from January 2024 to April 2024


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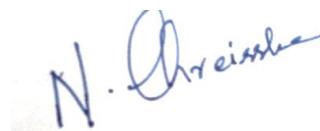

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DECLARATION

We N.THREISSHA (41770036) , A. KAVIYA DHARISHINI (41770011) here by declare that the Project Report entitled **“EFFECTIVE APPROACH OF BACTERIAL DEGRADATION USING POLYMERIC ORGANOGEL FOR MUNICIPAL WASTEWATER TREATMENT”** done by us under the guidance of a Dr. M. BAVANI LATHA (Internal) and Dr. SUDHA UTHAMAN (External) and Dr. SANJEEVI PRASATH (External) at International Research Centre at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ACKNOWLEDGEMENT

We are pleased to acknowledge my sincere thanks to The Board of Management of **Sathyabama Institute of Science and Technology** for their encouragement in doing this project and for completing it successfully. We are grateful to them.

We convey thanks to **Dr. REKHA CHAKRAVARTHI, Dean, School of Science and Humanities** and **Dr. V. RAMESH KUMAR, M. Phil, Ph. d., Head Of the Department, Department of Biotechnology**, for providing me necessary support during the progressive reviews.

We would like to express my sincere and deep sense of gratitude to our internal guide **Dr. M. BAVANI LATHA (Assistant Professor)** and external guide **Dr. SUDHA UTHAMAN (Assistant Professor, Research)** and **Dr. SANJEEVI PRASATH (Assistant Professor, Research)** for their valuable guidance, suggestions and constant encouragement paved the way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of their view work.

ABSTRACT

Water scarcity and pollution have emerged as critical global challenges, emphasizing the urgent need for effective wastewater treatment methods. This research addresses this pressing issue by introducing a novel approach utilizing organogel adsorbents for wastewater treatment. Despite water covering a significant portion of the Earth's surface and being essential for human activities and ecosystem health, its contamination due to various anthropogenic sources poses significant threats to both its quality and availability. The proposed method harnesses the unique properties of organogels, synthesized from organic solvents, to efficiently absorb pollutants and microorganisms from wastewater, rendering it safe for reuse. Through rigorous experimentation and analysis, this study demonstrates the efficacy and viability of organogel-based wastewater treatment, offering a promising solution to mitigate water pollution and enhance water resource sustainability. By integrating innovative technologies with environmental stewardship principles, this research contributes to advancing sustainable water management practices and safeguarding human health and the environment.

Keywords: Wastewater treatment, organogel, Antimicrobial sensitivity, sewage water.

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LIST OF ABBREVIATION

CTA	Chain Transfer Agent
DMF	Dimethylformamide
AIBN	Azobisisobutyronitrile
H ₂ SO ₄	Sulphuric acid
NaNO ₃	Sodium Nitrate
KMnO ₄	Potassium Permanganate
HCl	Hydro chloric acid
FeCl ₃	ferric chloride hexahydrate
FeSO ₄	ferrous sulphate
NH ₄	Ammonia
P ₄ VP/ GO-Fe ₂ O ₃	Poly 4-vinylpyridine Graphene oxide/ferric oxide
P ₄ VP	Poly 4- vinyl pyridine
P ₄ VP/ GO	Poly 4-vinyl pyridine graphene oxide
DBB	Dibromobutane
DBD	Dibromodecane
C-C	Carbon-Carbon
C-H	Carbon-Hydrogen
N+-H	Nitrogen-Carbon
N-O	Nitrogen-Oxygen
Staph.sp.	<i>Staphylococcus</i> species
e.coli sp.	<i>Escherichia Coli</i> species
Pseudomonas sp.	<i>Pseudomonas</i> species
FTIR	Fourier Transform Infrared Spectroscopy

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CHAPTER 1

INTRODUCTION

1.1 GELS

Gels are characterized as polymers and their swollen forms with three-dimensional network structures, remaining insoluble in any solvents. The interplay between the cross linked structure of gels and the properties of swelling matter is significant. Linear and branched polymers absorb, swell, and eventually disperse as individual molecules in suitable solvents. In contrast, the swelling of cross linked polymers is limited due to their three-dimensional network structure, although they can still swell through solvent interaction.

Gels occupy a unique space not encountered in solids, liquids, or gases. Swollen gels saturated with solvent exist in transitional states between solid and liquid phases. Their properties can range from viscous liquids to firm solids, influenced by factors such as chemical composition and external conditions. Gelation predominantly occurs via cross linking, a process not exclusively reliant on covalent bond formation but also achievable through secondary forces like hydrogen bonding.

To sum up, gels are cross linked three-dimensional networks capable of absorbing solvents and swelling to a limited extent without dissolution. Their presence in intermediate states between solid and liquid underscores their distinctive characteristics in material science. Aizo yamauchi *et al.*,(2001)

1.1.1 Organogel

A gel can be defined as a soft material that exhibits properties of both solids and liquids, comprising a network of solid components, known as the gelator, intertwined with a liquid component. This network of aggregates immobilizes the liquid phase, preventing it from flowing primarily through surface tension effects. Depending on the nature of the liquid component, a gel can be categorized as a hydrogel when water is involved or an organogel when an organic solvent is present.

Organogels, specifically, are non-crystalline, non-glassy thermo reversible (thermoplastic) solid materials with viscoelastic properties. They can be considered semi-solid preparations where an external a polar phase is immobilized within the spaces of a three-dimensional networked structure. This network arises from physical interactions among self-assembled structures of compounds acting as gelators. Often, these systems rely on the self-assembly of structurant molecules to form the gel network.

1.1.2 Advantage of organogel;

Ease of preparation.

- More stable than other types of gel.
- Avoid first pass metabolism.
- Organogels are moisture insensitive.
- Cost reduction due to less number of ingredients.
- Thermodynamically stable.
- They are less greasy and can be easily removed from the water.

(Mujawar, N. K., Ghatage, S. L., & Yeligar, V. C. (2014))

1.2 ANTIMICROBIAL SENSITIVITY

The aim of susceptibility testing is to anticipate the probable efficacy of treating a patient's infection with a specific antimicrobial agent. Empirical therapies remain effective for certain bacterial pathogens because resistance mechanisms may not have been acquired or are still infrequent. Susceptibility testing proves valuable and indispensable for prevalent bacterial species that are not reliably responsive to drugs of choice due to acquired resistance mechanisms. Two crucial functions of susceptibility testing include detecting overt resistance and qualitatively assessing susceptibility to antimicrobial agents, particularly with species that hold direct therapeutic significance. **(James H. Jorgensen *et al.*,1998)**

1.3 WASTEWATER TREATMENT

Clean water is a vital resource, covering a significant portion of the Earth's surface and serving as a crucial element for various human activities and ecosystems. However, the pollution of water sources, primarily caused by human activities such as domestic and industrial use, poses a serious threat to its quality and availability.

Wastewater, comprising domestic and industrial waste along with infiltration/inflow and stormwater, is a major contributor to water pollution. The discharge of untreated wastewater into rivers and streams leads to the contamination of water bodies, impacting both human health and aquatic ecosystems.

To address this issue, wastewater treatment processes have been developed. These processes, including primary, secondary, and tertiary treatments, aim to remove contaminants such as suspended solids, pathogens, and organic matter from wastewater before its release back into the environment.

In conclusion, addressing water pollution, especially from wastewater, is crucial for safeguarding water resources and ensuring the well-being of both human populations and the environment. Effective management strategies, along with advancements in wastewater treatment technologies, are essential for mitigating

the harmful effects of pollution and preserving clean water for future generations.(**Sonune, A et al., (2004)**)

There are 8 stages of wastewater treatment process

1.3.1 Preliminary treatment

Preliminary treatment plays a crucial role in wastewater management by targeting the removal of coarse solids and other sizable materials commonly found in untreated wastewater. These materials encompass a broad range, including pieces of wood, cloth, paper, plastics, and general debris, often mixed with fecal matter. Additionally, this process addresses heavy inorganic solids such as sand and gravel, alongside metallic or glass objects, collectively known as grit. Moreover, preliminary treatment targets the reduction of excessive oils and greases present in the wastewater stream.

1.3.2 Primary treatment

Primary treatment is specifically engineered to eliminate both organic and inorganic solids through physical processes like sedimentation and flotation. This stage typically removes around 25-50% of the incoming biochemical oxygen demand (BODs), 50-70% of total suspended solids (SS), and approximately 65% of oil and grease present in the wastewater. While primary sedimentation also removes some organic nitrogen, organic phosphorus, and heavy metals associated with solids, it doesn't affect colloidal and dissolved constituents. The output from primary sedimentation units is termed primary effluent.

1.3.3 Secondary treatment

Secondary treatment builds upon primary treatment by further purifying effluent to remove remaining organics and suspended solids. Typically, suspended solids constitute around 30%, colloidal solids 6%, and dissolved solids 65%. While primary treatment targets suspended solids, secondary treatment tackles colloidal and dissolved organic and inorganic solids. To meet strict standards, secondary treatment is crucial for thorough organic removal. It involves biological processes

utilizing diverse microorganisms in controlled settings. Various aerobic biological methods are employed, differing in oxygen supply and organic matter metabolism rates

1.3.4 Tertiary treatment

Tertiary treatment involves adding unit operations to the flow scheme after conventional secondary treatment. These additions can range from simple, like adding a filter for suspended solids removal, to complex, incorporating multiple unit processes for organic, suspended solids, nitrogen, and phosphorous removal. Physicochemical treatment blends biological and physical-chemical processes to achieve the desired effluent quality. Combined biological-physical-chemical treatment differs from tertiary treatment in that it mixes biological and physicochemical treatments rather than adding unit processes after conventional biological treatment. **(Sonune, A et al.,2004)**

1.4 SIGNIFICANCE

1.4.1 Prevent waterborne pollution

Wastewater treatment is essential for preventing waterborne pollution. Key processes include water clarification to remove solids and scum, while heavy metals are efficiently eliminated through methods like adsorption. Silt management quickly recycles wash water for irrigation. Dust suppression ingeniously uses treated oil and gas wastewater. Truck wheel wash systems conserve water by segregating and treating wheel wash wastewater. This approach emphasizes responsible water management, ensuring both efficiency and environmental stewardship.

1.4.2 Improve efficiency

Enhance industrial manufacturing efficiency by implementing liquid-solids separation technology for wastewater treatment. This system removes particles, including inorganic substances and debris, from stormwater runoff, leading to increased operational efficiency and reduced costs.

1.4.2 Increase by-product recovery

In general, any industrial process using a lot of water has a significant amount of by-product washed and wasted into wastewater streams. Having a wastewater treatment system set in place can help you find treasure, such as scraps from ingredients, fines left from steel, and other residual materials that would otherwise be put to waste.

1.4.3 Minimise waste

Minimize waste generated by your facility through the adoption of an industrial wastewater treatment system. This approach not only results in cost savings but also guarantees the safe and eco-friendly removal of chemically-treated water.

1.4.4 Clean and safe processed water

Water scarcity is a global challenge, and recycling wastewater provides a sustainable solution. Wastewater treatment removes toxins, turning it into clean, safe water for reuse. This process accelerates purification, offering an efficient alternative to conventional water sources. Wastewater reuse not only saves costs but also aligns with responsible environmental stewardship, addressing the pressing need for sustainable water management.

1.5 ORGANISM

Bacteria in wastewater, originating mainly from the human intestine, are diverse and can pose health risks. Among the notable pathogens are *Salmonella* spp., *Escherichia* spp., *Shigella* spp., *Yersinia* spp., *Klebsiella* spp., *Leptospira* spp., *Vibrio cholerae*, *Aeromonas hydrophila*, *Legionella pneumophila*, *Mycobacterium* spp., and *Pseudomonas*.

These bacteria cause various health issues, such as salmonellosis, infections, shigellosis, and respiratory infections. *Klebsiella* spp. can lead to respiratory and urinary tract infections, while *Leptospira* spp. causes leptospirosis. Proper wastewater management is crucial to prevent the spread of these pathogens and protect public health. Sanitation measures and water quality monitoring are

essential for avoiding the risks associated with bacterial contaminants in wastewater.

The most predominant organism present in the wastewater are E.coli, Pseudomonas and Staphylococcus.(Yesilay, G *et al.*, 2023)

1.5.1.E.coli

Escherichia coli is a type of gram-negative bacterium. It possesses a rod-shaped structure, is facultative anaerobic, and belongs to the coliform group within the Escherichia genus. This bacterium is frequently located in the lower intestine of warm-blooded organisms. Escherichia coli (E. coli) is a common constituent of the gut micro biota in humans and warm-blooded animals, contributing to a healthy digestive environment. This bacterium is released into the natural environment through two main pathways: direct expulsion with faeces and indirect transmission via treated wastewater. Whether through natural waste or treated water, E. coli disperses into ecosystems, emphasizing the need for effective wastewater management and environmental monitoring to mitigate potential health and ecological risks.

1.5.2. Staphylococcus

Staphylococcus is a Gram-positive bacterium. These bacteria, appearing spherical under a microscope, cluster in grape-like formations. Staphylococcus species are facultative anaerobes, capable of thriving in both aerobic and anaerobic conditions, contributing to their widespread presence. The genus encompasses various species, each with unique characteristics, influencing their significance in microbiology and health sciences. Drug-resistant and pathogenic Staphylococcus spp. strains can enter surface waters and air through wastewater discharge. This increases the environmental pool of genetic elements for antibiotic resistance and virulence, posing a health risk to wastewater treatment plant employees exposed to bio aerosols daily. Preventative measures are crucial to mitigate these threats.

1.5.3. *Pseudomonas*

Pseudomonas is a Gram-negative bacterial genus in the family Pseudomonadaceae, classified under Gammaproteobacteria. They are known for diverse metabolic capabilities and inhabit various environments, with some species having pathogenic or beneficial roles.

1.6.TOTAL VIABLE COUNT

Quantitative results may lack reproducibility, particularly when based on a small sample size. The accuracy of counting methods hinges on the medium employed, its pH, and the necessary incubation conditions. Consequently, the precision of quantitative determinations may vary, contingent upon the materials and technical procedures utilized. Relying solely on a single determination typically proves unreliable; instead, averaging multiple determinations enhances accuracy, particularly in instances where the counting method lacks standardization.

Agar plate methodology involves inoculating a predetermined volume of sample onto Petri dishes containing a liquid medium that solidifies after thorough mixing. Upon solidification, microorganisms within the gel proliferate until they form discernible colonies that can be counted using a magnifying lens or electronic device. Typically, the sample is diluted, and various dilutions are prepared. Colonies are counted when their numbers range between 20 and 200. This approach operates on the principle that each viable microorganism will reproduce, resulting in a visible colony. It assumes that bacteria exist as isolated single cells, are uniformly dispersed within the sample, and do not aggregate. Although plate counting is labor-intensive, it boasts high sensitivity. Theoretically, if only one bacterium per millilitre is present in a sample, a visible colony should emerge after seeding one millilitre of the specimen.

CHAPTER 2

LITERATURE SURVEY

2.1 ORGANOGELE

Sanjeevi Prasath Sridhar *et al.*, (2021) states that the synthesis of an organogel from poly(styrene-*block*-4-vinylpyridine) (PS-*b*-P4VP) block copolymer cross-linked with dibromododecane. Characterization confirms its gel formation and ability to adsorb anionic dyes selectively, driven by electrostatic interactions. The organogel offers cost-effective, recyclable, and efficient removal of anionic dyes, outperforming other gel-based absorbents with just 2 wt % usage. Its durability suggests suitability for long-term water treatment. Future research aims to explore its potential as a filtration membrane for removing anions from saline water.

Biplab Mondal *et al.*, (2020) States that a dipeptide-based synthetic amphiphile, featuring a myristyl chain, forms hydrogels in pH 6.9–8.5 and organogels in various solvents like petroleum ether, diesel, kerosene, and petrol. Characterization techniques include transmission electron microscopy, X-ray diffraction, Fourier-transform infrared spectroscopy, and rheology. The resulting xerogel efficiently traps toxic organic dyes from wastewater, while the hydrogel removes heavy metal ions Pb²⁺ and Cd²⁺. Dye adsorption kinetics follow the Freundlich isotherm equation. Notably, the gelator promptly gels fuel oil, kerosene, diesel, and petrol in saltwater-oil mixtures, showing potential for oil spill recovery and wastewater treatment. It can be recycled multiple times without significant loss of activity, promising sustainability in environmental remediation with peptide-based gelators.

Jason Y.C.Lim *et al.*, (2019) States that explores the potential of molecular gels for treating polluted water by offering a large surface area for pollutant sorption and ease of chemical modification. Exceptional performance has been reported in removing various pollutants such as oil, toxic cations and anions, dyes, and pharmaceutical products. Key advances in hydrogel development, including small

molecule supramolecular gelators, polymers, and composites, are highlighted. The study emphasizes the importance of considering practical aspects such as gel mechanical properties and recyclability for real-life applications. It aims to stimulate further research into practical gel remediators that can complement existing water treatment technologies.

Peter Adeniyi Alaba *et al.*, (2018) States that various polymeric adsorbents as alternatives to activated carbon for wastewater treatment. Nano-magnetic polymers (NMPs) offer fast separation due to their magnetic properties, while polysaccharides like chitosan show high reactivity and selectivity. Covalent organic polymers (COPs) exhibit comparable removal efficiency to activated carbon for heavy metals. Additionally, extracellular polymeric substances (EPS) play crucial roles in biological wastewater treatments. Further research is needed to optimize these materials for industrial use, addressing durability and back pressure limitations, and exploring composite polymeric materials.

Mohammad m ghobashy *et al.*, (2021) states that a novel chalcone derivative (referred to as G1) has been developed and synthesized, demonstrating impressive gelation properties across a range of solvents, including organic solvents, fuel, and edible oils. Characterization through UV-Vis, FTIR, and XRD techniques has revealed that the formation of three-dimensional networks in G1 gels is primarily driven by hydrogen bonding, van der Waals forces, and π - π interactions. This comprehensive understanding of the molecular interactions governing gel formation sheds light on the versatile applications of G1 in various solvent systems. Additionally, scanning electron microscopy analysis of xerogel G1-n-butanol further corroborates these findings, highlighting the high porosity and confirming the mechanisms underlying gel network formation. Such insights pave the way for the utilization of G1-based gels in diverse fields ranging from pharmaceuticals to materials science.

Ran Wang *et al.*, (2021) States that the design and synthesis of diverse hydrogel types, such as organogels, supramolecular gels, and composite hydrogels, with applications in wastewater treatment and biomedicine. A notable focus is on a

collagen-protein-based hydrogel, enabling controlled drug delivery via electrostatic interactions. Additionally, organogels show promise in regulating drug release rates. Composite hydrogels designed for wastewater treatment exhibit effective dye adsorption through host-guest and electrostatic interactions. These findings advance self-assembly systems and nanomaterials design, with implications for physics, biosensors, catalysis, and environmental remediation.

Abishek pan *et al.*, (2019) States that the synthesis of carbohydrate-based organogels through reversible addition-fragmentation chain transfer (RAFT) polymerization of a β -D-glucose pentaacetate containing methacrylate monomer (Ac-glu-HEMA), with a di-vinyl cross-linker. These organogels can be transformed into hydrogels by treatment with sodium methoxide (NaOMe). Characterization techniques such as solid-state ^{13}C cross-polarization/magic-angle spinning (CP/MAS) NMR, Fourier transform infrared (FTIR) spectroscopy, and field emission scanning electron microscopy (FE-SEM) were employed to analyze the materials. The swelling behaviour of the gels in both organic solvents and water was investigated, along with their ability to absorb model bioactive molecules such as methylene blue (MB) and rhodamine B (RhB), and to absorb/release silver nitrate. The results indicate the potential of these gels for environmental and biomedical applications, leveraging their 3D cross-linked polymeric structures and capability to encapsulate or release substances in response to environmental cues.

Haiying guo *et al.*, (2015) States that the facile preparation and dye adsorption capacity of GO/PEI hydrogels. PEI was selected for its abundant amine groups capable of forming hydrogen bonds with GO. SEM and XRD analyses confirm successful cross-linking of GO sheets within the PEI network, while Raman spectra indicate minimal structural changes in GO sheets pre- and post-gelation. The resulting GO/PEI hydrogels demonstrate efficient removal of both methylene blue (MB) and rhodamine B (RhB), following the pseudo-second-order model. This research enhances our understanding of GO-based polymer-containing hydrogels as effective dye adsorbents for wastewater treatment applications.

2.2 ADSORPTION CAPACITY OF ORGANOGE

Hem N. Naik *et al.*, (2023) States that a polymeric organogel was developed through the interaction of polyvinyl alcohol (PVA) with cellulose diacetate (CA), utilizing phthalic anhydride (PA) as a cross-linker. Notably, CA was synthesized from cellulose extracted from *Dalbergia sissoo* bark biomass. The resulting cross-linked organogel demonstrated enhanced stability and dye adsorption capacity in comparison to the pristine PVA organogel. Through thorough analysis, including evaluation of structure, morphology, and thermal stability, the researchers confirmed the efficacy of the organogel. Impressively, the organogel exhibited notable adsorption capabilities, achieving 78% for crystal violet and 69% for congo red, surpassing the performance of pristine PVA-based hydrogel. These findings underscore the potential of the developed organogel as an environmentally friendly, thermally stable, and efficient adsorbent for synthetic dye removal from wastewater.

Wafa Maatar *et al.*, (2013) States that the preparation of sponge-like cellulose organogels from nanofibrillated cellulose (NFC), enhanced through surface grafting of hexadecylamine. Chemical modification confirmed through spectroscopy improved adsorption of organic pollutants. Adsorption experiments showed capacities ranging from 50 to 400 mol g⁻¹, with rapid kinetics reaching equilibrium in 40 minutes. The proposed mechanism involves diffusion to the external surface followed by diffusion within hydrophobic chains. Despite the need for surface modification, the NFC organogel offers advantages like high surface area and sustainability. While modification increases costs, the material's reusability compensates. Overall, the study demonstrates the potential of this approach for efficient organic pollutant removal, balancing benefits with associated costs.

Sabrine Alila *et al.*, (2009) states that chemically modified cellulose fibers are effective for removing organic compounds and herbicides from water. By grafting hydrocarbon-bearing organic moieties onto the fibers, hydrophobic reservoirs are created for compound accumulation. Equilibrium and continuous adsorption

experiments demonstrated significantly increased adsorption capacity compared to unmodified fibers, ranging from 400 to 1000 mol g⁻¹ depending on solute structure and modification extent. Kinetic analysis identified internal diffusion as the rate-limiting step. Regeneration of exhausted columns via ethanol washing restored their adsorption capacity for multiple cycles, indicating reusability without degradation.

Kankana Baruah *et al.*, (2023) States that synthesizing an organogel to absorb pollutants like solvents and remove cationic dyes from water. Characterization involved various spectroscopic techniques and water contact angle determination. Swelling kinetics and adsorption studies revealed significant performance, with the organogel swelling up to 10 times its original weight in Acetic acid and achieving a 99.35% removal efficiency for dyes within 60 minutes. Additionally, the organogel showed excellent reusability for up to 10 cycles and effectively removed dyes from binary mixtures. Overall, the organogel demonstrates promising potential for environmental remediation, offering high swelling capacity and selectivity for cationic dyes.

Kankana Baruah *et al.*, (2023) States that a cost-effective polymeric adsorbent based on bio-degradable polymer polyvinyl alcohol to remove toxic heavy metal ions from underground water systems. Characterization using FT-IR, HNMR, and P-XRD, along with SEM morphological studies, was conducted. Metal ion detection and adsorption revealed removal rates of nearly 80% for arsenic ions, 70.5% and 70.7% for lead and chromium ions, and 60.7% for cadmium ions, respectively. Kinetics of adsorption and intraparticle diffusion studies were performed, with the highest adsorption capacity observed for arsenic at approximately 570.42 mg g⁻¹, highlighting the adsorbent's potential efficacy.

Reena kyariwkal *et al.*, (2023) states tha the potential of N₂,N₄,N₆-tri(1H-tetrazol-5-yl)-1,3,5-triazine-2,4,6-triamine (G8) combined with 3,3',3''-(benzenetricarbonyl)tris(azanediyl))tris(4-aminobenzoic acid) (GE) as an effective adsorbent for heavy metal removal in water remediation. G8GE organogel exhibits stability in aqueous environments and forms metallogels with Hg(II), Cd(II), and

Pb(II) salts. The hydrophobic nature of the gel enables prolonged retention of its structure in water. With a high adsorption capacity, G8GE efficiently removes toxic heavy metals from aqueous solutions and can be recycled for multiple cycles. Additionally, the study investigates the impact of gel formation methods on rheological and fluorescence properties, concluding that the adsorption method yields stronger metallogels without significant disruption to the G8GE network. DFT optimization supports the hypothesis of complementary hydrogen bond formation between G8 and GE. While further analysis is needed to understand adsorption capacities across different metal salts and the effects of various metal ions in contaminated water, the study highlights the potential of G8GE for water remediation and suggests new possibilities for enhancing gel materials for diverse applications.

Jiajun Chen *et al.*, (2023) States that the challenges posed by offshore oil leakage and industrial oily-wastewater by developing organogels with tunable crosslinking densities. These gels exhibit stable hydrophobic/lipophilic properties, high absorption capacity (1200%), and efficient adsorption of multiple emulsions (>99.0%), with excellent reusability. Additionally, they are assembled with 2D/3D substrates to achieve remarkable adsorption capacity (102.5 g/g) and recycling performance (50 cycles). Specifically, gelatinized non-woven fabric (Gel-NWF) demonstrates exceptional heavy oil/water mixture separation efficiency (>97.0%). Furthermore, the combination of organogels with carbon black on MS (GCB-MS) achieves outstanding photothermal conversion performance, heating the surface rapidly to 70.4 °C under sunlight radiation, with an ultra-high adsorption capacity of about 103 g/g for viscous crude oil. The integration of GCB-MS with a pump facilitates the development of continuous oil spill cleaning equipment, achieving a super-fast cleanup rate of 6.83 g/min. These hydrophobic organogels offer unprecedented capabilities for comprehensive treatment of oily-wastewater in complex environments, including layered oils, emulsions, and viscous crude oil spills, providing valuable insights for the expansion and application of organogels.

jiajun chen *et al.*,(2023) states that the successful construction of hydrophobic organogels with high sorption capacity for oils and selective sorption of oil/water emulsions (>99.0%). Additionally, a gel-coating strategy was developed to assemble these organogels with 2D and 3D substrates, inducing wettability changes and enhancing treatment capacity for oily wastewater. The resulting composite material exhibits excellent mechanical properties and environmental suitability.

Keziah E. Liebenberget *al.*,(2021) states that the successful fabrication and characterization of mesoporous neat PAN polymer monoliths and mesoporous composite polymer monoliths, analyzed using SEM, EDS, BET, and BJH methods. The materials demonstrated thermal stability and high adsorption capacity for Ru(III) and Pd(II) ions at a concentration of 1 mg·L⁻¹. Composite polymer monoliths exhibited smaller pore sizes and superior adsorption capacity for Rh(III), As(V), and Cr(VI) compared to neat PAN monoliths. Overall, these polymer materials show promise for wastewater treatment and precious metal remediation. However, further studies are recommended to explore adsorption mechanisms through adsorption-desorption experiments, kinetic and isotherm models, and characterization techniques like FTIR spectroscopy. Optimization of contact time is also suggested to determine adsorption equilibrium for each metal ion, providing deeper insights into the adsorption process.

2.3 P4VP

yichang ma *et al.*,(2019)states that novel blend membranes composed of poly(4-vinylpyridine)-b-polysulfone-b-poly(4-vinylpyridine) triblock copolymer and polysulfone for palladium (II) separation from electroplating rinsing wastewaters. The membranes, synthesized using RAFT polymerization, exhibit sponge-like structures with hydrophilic properties due to segregated 4-vinylpyridine (4-VP) segments. These membranes demonstrate high adsorption capacity for Pd (II) ions and effective separation performance in both simulated and actual electroplating rinsing wastewaters, making them promising for palladium recovery from low-concentration wastewaters.

Yuanshuai li *et al.*,(2020)States that CoFe₂O₄-P4VP@Ag nanoparticles were prepared and used as catalysts to remove Rhodamine B (RhB) and Ciprofloxacin (Cip) from wastewater. The nanoparticles showed excellent catalytic activity, with high removal efficiency and short reaction times. They were also reusable and stable, making them effective and environmentally-friendly catalysts for organic pollutant removal.

Li Zhang *et al.*,(2022)States that a graphene-enhanced supramolecular hydrogel composed of poly(acrylic acid) (PAA) and poly(4-vinylpyridine) (P4VP) for heavy metal removal from wastewater. The hydrogel, formed by integrating P4VP brushes grown from functionalized graphene oxide (GO) into a PAA matrix, exhibits high compressive strength and a porous 3D network. Under optimal conditions, the hydrogel demonstrates maximum adsorption capacities of 257.28 mg/g for Pb²⁺ and 175.79 mg/g for Cd²⁺. Adsorption kinetics follow a pseudo-second order model, and Langmuir isotherms are observed. Additionally, the hydrogel shows good reusability, with removal efficiency remaining above 80% after five cycles, underscoring its efficacy as a heavy metal adsorbent for wastewater treatment.

Abdel al *et al.*,(2010)States that Hydrogels containing pyridine and -OH groups show pH-dependent adsorption behavior. A composition of 50% pyridine groups and 50% -OH groups exhibits increased adsorption at lower pH but decreased adsorption at higher pH. This is due to the nature of pyridine groups as weak bases and the influence of pH on both dye and hydrogel structures. Despite pH variations, the hydrogels effectively remove acid dyes, even at low concentrations, and demonstrate reasonable uptake from wastewater. They also display good thermal stability and durability, suggesting potential for practical use in industrial wastewater treatment.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The aim of this project is to find polymer based organogel to remove the microbes from the municipal waste water.

3.2 SCOPE

- To find suitable organogel for the removal of microbes present in the municipal waste water.
- To determine the capacity of organogel in degrading the bacteria present in waste water
- To separate organogel after waste water treatment using ethanol wash

CHAPTER 4

MATERIALS AND METHODS

4.1 ORGANOGE

Materials

- ☐ 4-Vinylpyridine (Sigma-Aldrich, 98%) was purified by passing through an alumina column to remove the inhibitor.
- ☐ Chain transfer agent CTA (DMF, Sigma-Aldrich, 98%) and initiator 2,2'-azobis- (2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%) were recrystallized from methanol before use.
- ☐ DBD (Sigma-Aldrich, 98%) was used as the cross-linker
- ☐ H₂SO₄(Sulphuric acid)
- ☐ NaNO₃(Sodium Nitrate)
- ☐ KMnO₄(Potassium Permanganate)
- ☐ HCl (Hydro chloric acid)
- ☐ FeCl₃ (ferric chloride hexahydrate)
- ☐ FeSO₄ (ferrous sulphate)
- ☐ NH₄ (Ammonia)

4.1.2. Synthesis of Graphene Oxide(GO)

GO will be preparing through exfoliation of a chemically oxidized flake graphite powder by modified the Hummers method. In detail, commercial graphite powder is adding into concentrated in round-bottom flask under stirring in an ice bath. of NaNO₃ and of KMnO₄ are gradually put into the dispersion under intensive stirring for 30 min at an ice bath temperature until a purple-green mixture was obtained. This suspension is then transfer to a 35 °C water bath and magnetically stirred for 24 h to achieve a dark brown color paste. Subsequently, of DI water will be adding to the mixture and an oxidation reaction is allowing to proceed for another 96 h. The reaction will be terminate by addition of a good amount of DI water and 30%

H₂O solution. The mixture is subjected to centrifugation and suspension in 5% HCl solution and mechanical agitation to completely remove metal ions. The product is then washing with a copious amount of DI water to adjust the pH to around 6. The as- obtained graphite oxide is dispersing in DI water and exfoliated by a tip sonicator to prepare the GO dispersion.

4.1.3 Synthesis of Magnetite (Fe₃O₄)

Magnetite (Fe₃O₄) nanoparticles will be prepared by an aging stoichiometric mixture of ferric chloride hexahydrate (FeCl₃ • 6H₂O)(5.406g), and ferrous sulphate (FeSO₄)(2.702g), are dissolving in 200 mL distilled water in a three necked conical flask and heating the mixture at 60°C and 600 RPM under nitrogen atmosphere. After 1 h, 12 mL of ammonia is adding under constant stirring. The colour of the solution will changed to brown to black, indicating the forming of Fe₃O₄ nanoparticles. The black precipitate is washing repeatedly with DI water and keep drying for 24 h.

4.1.4 Synthesis of Poly 4 – vinylpyridine (P₄VP)

A two-step procedure was adopted for the synthesis of P₄VP by free radical polymerization. First, P₄VP-CTA was prepared. In a typical protocol, DDMAT/AIBN (6:1) (50mg) was dissolved in 20 ml of DMF, and the reactants were taken in a Schlenk flask fitted with a stir bar. The flask was hermetically sealed, and the mixture was degassed at 0°C for 30 min. Polymerization was carried out at 70°C for 24 h.

After polymerization, the reaction mixture was quenched by rapid cooling in an ice bath. Finally, the crude mixture was precipitated thrice into excess cold diethyl ether and dried under vacuum overnight. P₄VP-CTA was recovered as a yellow powder.

The ratio of 4-VP/AIBN was 300:1. After polymerization, the reaction mixture was quenched by rapid cooling in an ice bath. P₄VP-CTA was precipitated in cold hexane and dried under vacuum. The collected P₄VP-CTA powder had an orange color.

4.1.5 Preparation of P₄VP/ GO-Fe₂O₃Nanocomposite organogel.

Preparation of P₄VP/GO-Fe₂O₃ Nanocomposite gel is by adding P₄VP polymer GO-Fe₂O₃ and nanocomposites (Nanocomposite will be ball mixed for 6 h before adding to the polymer solution) and the mixture of crosslinkers such as dibromobutane (DBB) and dibromododecane (DBD) in DMF. The Solution is dispersed well using sonicator. The well mixed solution is placing for degassing by three freeze-pump-thaw cycles. The polymer solution is heating at 70°C for 4 hrs under an inert atmosphere. Finally, will obtain the strong & rigidP₄VP/GO-Fe₂O₃ Nanocomposite organogel.

4.2. ANTIMICROBIAL SENSITIVITY

Materials

- ☐ Eppendorf
- ☐ Pippette
- ☐ P₄VP
- ☐ P₄vp/GO-Fe₂O₃
- ☐ P₄VP/GO

4.2.2 Bacterial innoculum

The antibacterial activity of the Test (with exposure of gel) and control (without exposure of the gel) were examined by Gram positive bacteria (Staphylococcus) and Gram negative bacteria (E.coli and Pseudomonas).Bacterial strains were inoculated in the nutrient broth and incubated at 37°C for 24h in a shaker incubator. After incubation, the samples were immersed in the saline solution and serial dilution was performed. After the desired concentration was plated on the nutrient agar plates sand CFU was done. For the same set of samples acridine orange staining was performed to determine the viable and non viable cells under epifluorescence microscopy.

4.3. Serial dilution of sample

Materials

- ☐ Conical flask
- ☐ Pipette
- ☐ Petri dish
- ☐ Control sample
- ☐ Test sample
- ☐ Sterile water
- ☐ Nutrient agar

4.3.2 Nutrient agar preparation

Table 4.1 Nutrient agar preparation

INGREDIENTS	Grams/Litres
Agar	15.00
Peptone	5.00
Sodium Chloride	5.00
Yeast Extract	2.00
Beef extract (ex. Buffalo)	1.00
Distilled water	1000

The above mentioned ingredient were weighed and mixed with distilled water and the PH was adjusted to requirement. Then the media was autoclaved at 121°C for 15 minutes and cooled to room temperature.

Procedure

1ml of control sample was taken and mixed with 9ml of distilled water and 1ml of the solution was taken and used for further dilutions and 9 test tubes were taken and marked to be 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 9ml of the distilled water was added to all the test tubes.

From the control sample 1ml of the solution was added to 10^{-1} test tube mixed well and 1ml of the sample from 10^{-1} was transferred to 10^{-2} . In the same way the samples were transferred till 10^{-9} tube and 1ml from 10^{-9} tube was discarded and From the serially diluted samples 10^{-1} , 10^{-3} , 10^{-5} , 10^{-7} , 10^{-9} , 100µl of the sample was added to agar plates and Then the plates were incubated at 37°C for about 24 hours.

The colonies obtained on the plate on the plate was stained using the appropriate method and The following procedures were repeated for the remaining Control and Test samples Total viable count was performed.

4.4 IDENTIFICATION OF MICROORGANISM

The organism was identified based on evaluation by observing morphological characteristics and biochemical test.

4.4.1 Gram staining

Materials

- ☐ Clean glass slide
- ☐ Inoculation loop
- ☐ Distilled water
- ☐ Ethanol
- ☐ Crystal violet
- ☐ Iodine
- ☐ Safranin

Procedure

The clean glass slide was taken. Take the culture with the sterilised loop and Slide was heat fixed for 2-3 minutes. Then drop of crystal violet was added to the slide and kept for 30 seconds The slide was washed with distilled water and adds 2 drops of Iodine and the slide was kept for 1 minute and then the slide was washed with 95% of ethanol.add 2-3 drops of Safranin to the glass slide finally the slide was washed with the distilled water.Then the slide was focused under the microscope.

CHAPTER 5

RESULT AND DISCUSSION

5.1 ORGANISM MORPHOLOGY

Microbes were subcultured from mother culture and stained using gram staining method to see their morphology.

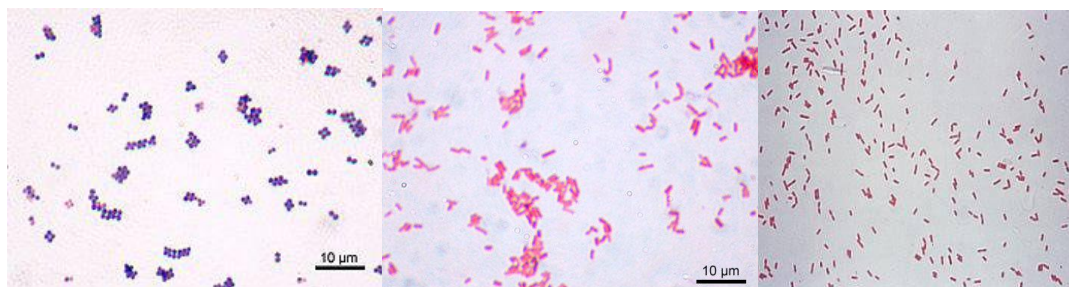


Fig 5.1 Staphylococcus sp.

Fig 5.2 E.Coli sp.

Fig 5.3 Pseudomonas sp.

5.1.1 E.coli sp.

Escherichia coli is a type of gram-negative bacterium. It possesses a rod-shaped structure, is facultative anaerobic, and belongs to the coliform group within the Escherichia genus (**Fig 5.2 E.Coli sp.**)

5.1.2 Staphylococcus sp.

Staphylococcus is a Gram-positive bacterium. These bacteria, appearing spherical under a microscope, cluster in grape-like formations. Staphylococcus species are facultative anaerobes, capable of thriving in both aerobic and anaerobic conditions, contributing to their widespread presence. (**Fig 5.1 Staphylococcus sp.**)

5.1.3 Pseudomonas sp.

Pseudomonas is a Gram-negative bacterial genus in the family Pseudomonadaceae, classified under Gammaproteobacteria. (**Fig 5.3 Pseudomonas sp.**)

For removing these organisms from sewage water. A polymeric based organogel is prepared.

5.2 CHARACTERIZATION OF GEL

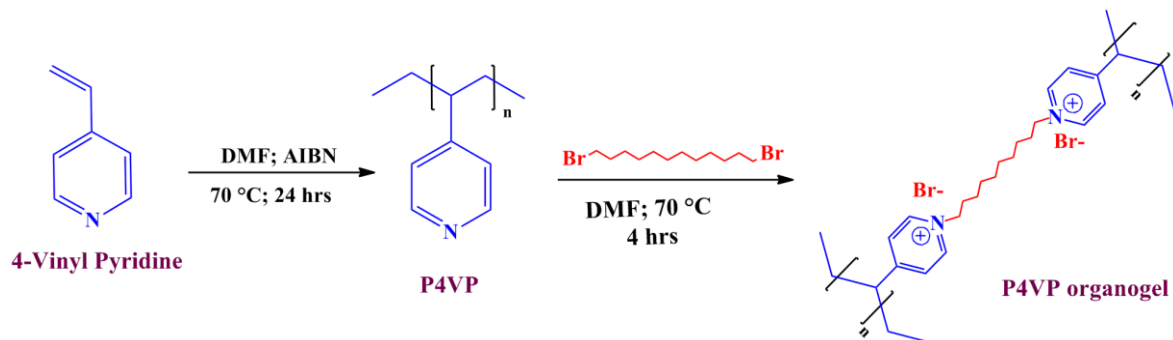


Fig 5.4 Characterization of gel

The above figure(**Fig 5.4**) explains that the first, P₄VP was prepared. In a typical protocol, DDMAT/AIBN (6:1) (50mg) was dissolved in 20 ml of DMF, and the mixture was degassed at 0°C for 30 min. Polymerization was carried out at 70°C for 24 h.

After polymerization, the reaction mixture was quenched by rapid cooling in an ice bath. P₄VP-CTA was recovered as a yellow powder.

The ratio of 4-VP/AIBN was 300:1. P₄VP was precipitated in cold hexane and dried under vacuum. The collected P₄VP powder had an orange color.

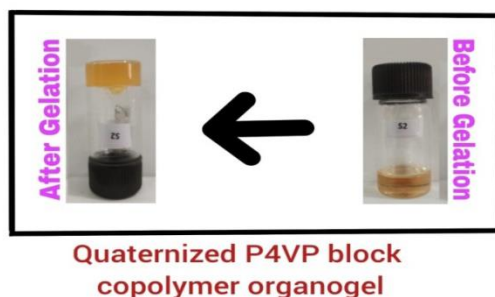
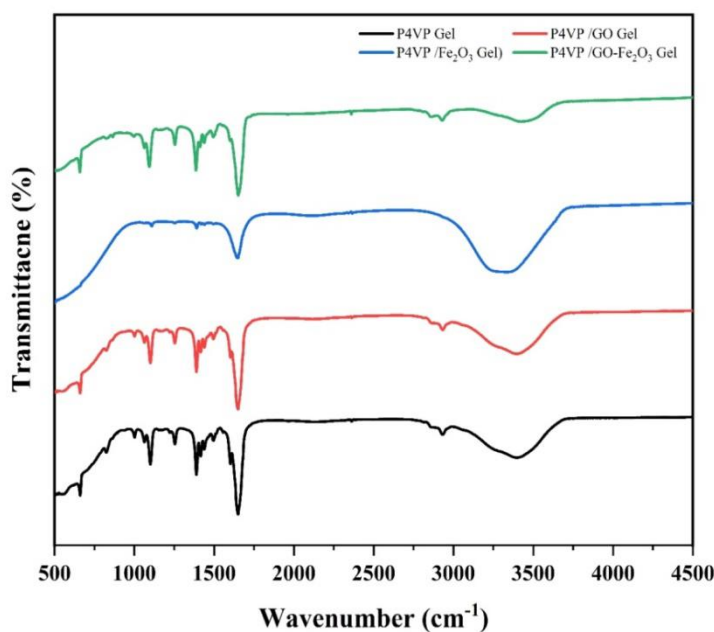


Fig 5.5 P₄VP organogel

5.3 FTIR ANALYSIS



Graph 1.1 FTIR

- C = C ; around 1600 - 2000 cm^{-1} .
- C – H Asymmetric aliphatic stretching; around 2950 – 3023 cm^{-1} .
- C – H Stretching 2923
- Aromatic symmetric – 1644 cm^{-1} .
- N+ - C Stretching 810 – 1145 cm^{-1} .
- N – O asymmetric stretching; around 1475 – 1550 cm^{-1} . this peak is related to N – O asymmetric stretching which indicating the presence of nitroalkane

Due to the presence of N⁺ ions the organogel acts as an adsorbent..

5.4 ANTIMICROBIAL SENSITIVITY

The colonies were treated with organogels and incubated for 24hrs and the serial dilution were done and plated and incubated for 24hrs.



Fig 5.6 Sample

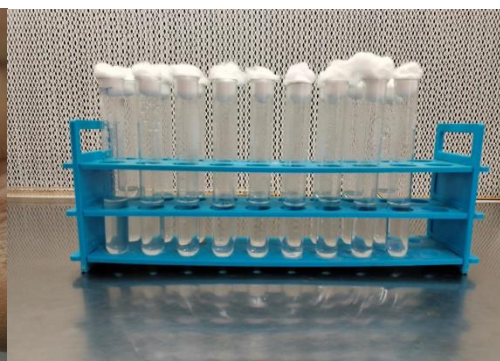


Fig 5.7 Serial Dilution

Table 5.1 Antimicrobial sensitivity test

<i>TYPE OF CULTURE</i>	STAPHYLOCOCCUS		E.COLI		PSEUDOMONAS	
TYPE OF GELS	CONTROL	TEST	CONTROL	TEST	CONTROL	TEST
P ₄ VP	350×10 ⁻⁹	125×10 ⁻⁹	32×10 ⁻⁹	9×10 ⁻⁹	94×10 ⁻⁹	16×10 ⁻⁹
P ₄ VP GO/Fe ₂ O ₃	144×10 ⁻⁹	5×10 ⁻⁹	76×10 ⁻⁹	19×10 ⁻⁹	170×10 ⁻⁹	116×10 ⁻⁹
P ₄ VP GO	315×10 ⁻⁹	30×10 ⁻⁹	154×10 ⁻⁹	24×10 ⁻⁹	626×10 ⁻⁹	14×10 ⁻⁹

5.4.1 P₄VP GEL

The colonies were treated with P₄VP gel for 0.05 gms and incubated for 24 hrs at 37°C and the gel was plated in the serial dilution method and incubated for 24 hrs and total viable counts were calculated.

***Staphylococcus* sp.**

By treating the *Staphylococcus* sp. with P₄VP gel the colonies were gradually decreased from the control plate from 350×10^{-9} to 125×10^{-9} .



Fig 5.8 Staph sp. (Control 350×10^{-9})



Fig 5.9 Staph sp. (Test 125×10^{-9})

***E. Coli* sp.**

By treating the *E. Coli* sp. With P₄VP gel the colonies experienced a gradual reduction from 32×10^{-9} to 9×10^{-9} .



Fig 5.10 *E.coli* sp

(Control 32×10^{-9})



Fig 5.11 *E.coli* sp.

(Test 19×10^{-9})

***Pseudomonas* sp.**

By treating the *Pseudomonas* sp. With P4VP gel Over the test, the colonies underwent a gradual decline, diminishing from 94×10^{-9} to 16×10^{-9}



Fig 5.12 *Pseudomonas* sp.

(Control 94×10^{-9})



Fig 5.13 *pseudomonas* sp.

(Test 16×10^{-9})

5.4.2 P₄VP GO/Fe₂O₃ Gel

The colonies were treated with P₄VP GO/Fe₂O₃ gel and incubated for 24 hrs and the gel was plated in the serial dilution method and incubated for 24 hrs and total viable counts were calculated.

***Staphylococcus* sp.**

By treating the *Staphylococcus* sp with P₄VP GO/Fe₂O₃ Gel The colonies gradually diminished in number from 144×10^{-9} to 5×10^{-9} .



Fig 5.14 Staph sp.

(Control 144×10^{-9})



Fig 5.15 staph sp.

(Test 15×10^{-9})

***Pseudomonas* sp.**

By treating the with *Pseudomonas* sp. P₄VP GO/Fe₂O₃ Gel The colonies underwent the gradual reduction from 170×10^{-3} to 116×10^{-3} in six degree reduction.



Fig 5.16 Pseudo. sp.

(Control 170×10^{-3})



Fig 5.17 Pseudo. sp.

(Test 116×10^{-3})

***E.Coli* sp.**

By treating the with *E.Coli* sp. P_4VP GO/Fe_2O_3 Gel The number of colonies gradually decreased from the control to test is 76×10^{-7} to 19×10^{-7} in 3 degree reduction.



Fig5.18 *E.coli* sp (Control 76×10^{-7})



Fig 5.19 *E.coli* sp (Test 15×10^{-7})

5.4.3. P_4VP GO Gel

The colonies were treated with P_4VP GOgel and incubated for 24 hrs and the gel was plated in the serial dilution method and incubated for 24 hrs and total viable counts were calculated.

***Staphylococcus* sp.**

By treating the *Staphylococcus* sp with P₄VP GOGelThe colonies were gradually decreased from the control to test from 315×10^{-9} to 30×10^{-7} .



Fig 5.20 Staph sp.

(Control 315×10^{-9})



Fig 5.21 Staphy sp

(Test 30×10^{-9})

***E.Coli* sp.**

By treating the with *E.Coli* sp. P₄VP GO Over the test ,the colonies underwent the gradual decline from control to test from 154×10^{-9} to 24×10^{-9} .



Fig 5.22 E.coli sp.

(Control 154×10^{-9})



Fig 5.23 E.coli sp.

(Test 24×10^{-9})

***Pseudomonas* sp.**

By treating the with *Pseudomonas* sp. P₄VP GO The colonies diminished in number from control to test from 626×10^{-9} to 14×10^{-9} .



Fig 5.24 Pseudo sp. (Control 626×10^{-9})

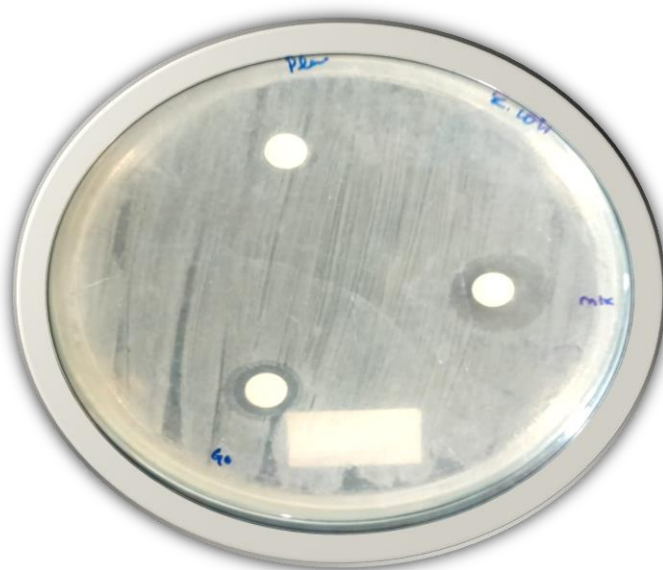


Fig 5.25 Pseudo sp. (Test 14×10^{-9})

5.5 ZONE INHIBITION

Table 5.2 *E.Coli.sp.* ZONE SIZE

<i>E.Coli sp.</i>	
TYPE OF GELS	ZONE SIZE
P ₄ VP	-
P ₄ VP GO	0.09 cm
P ₄ VP GO/Fe ₂ O ₃	1.5 cm

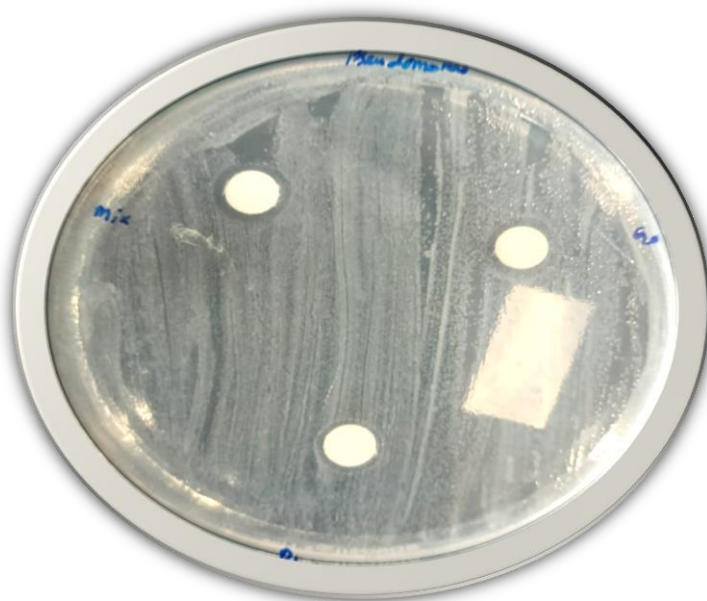


5.26 *E.coli* sp. (zone inhibition)

Among 3 gels(P₄VP) has given no zone and (P₄VP GO) has given 0.09 cm (P₄VP GO/Fe₂O₃) has given better results compared to all other gels by 1.5cm.

Table 5.3 *Pseudomonas* sp. (zone inhibition)

<i>Pseudomonas</i> sp.	
TYPE OF GELS	RESULT
P ₄ VP	-
P ₄ VP GO	0.05 cm
P ₄ VP GO/ Fe ₂ O ₃	1 cm



5.27 *Pseudomonas* sp. (Zone inhibition)

Among 3gels (P₄VP) has given no zone and (P₄VP GO) 0.5cm and (P₄VP GO/Fe₂O₃) has given better results compared to all other gels by the difference of 1cm.

Table 5.4 *Staphylococcus* sp.(Zone inhibition)

<i>Staphylococcus</i> sp.	
TYPE OF GELS	ZONE SIZE
P ₄ VP	-
P ₄ VP GO	1.3 cm
P ₄ VP GO/ Fe ₂ O ₃	1.6 cm



5.28 *Staphylococcus* sp. (Zone inhibition)

Among 3gels (P₄VP) gel has given no zone and (P₄VP GO) has given 1.3 cm and (P₄VP GO/Fe₂O₃) better results compared to all other gels by the difference of 1.6cm.

With the test performed and with the help of the results gathered (P₄VP GO/Fe₂O₃) has given better results among all other gels and this gel was treated with municipal waste water.

5.6 SEWAGE WATER

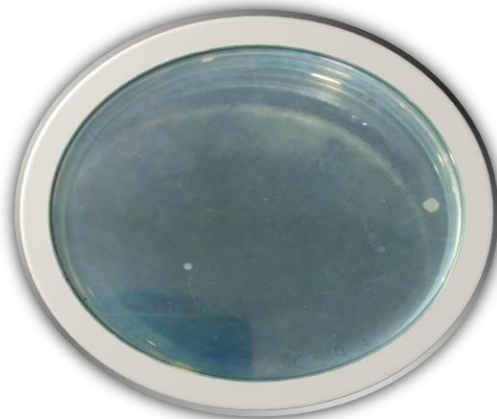
TABLE 5.5 SEWAGE WATER

SEWAGE WATER	CONTROL	TEST
1.	150×10^{-3}	2×10^{-3}

The above table indicates the result of sewage water which was exposed in P_4VP $GO Fe_2O_3$ for 24 Hrs and the sample were serially diluted and the diluted samples were poured on nutrient agar plate in pour plate method and the results were taken and the colonies were gradually decreased from control to test from 150×10^{-3} to 2×10^{-3} in six decrease order.



5.29 Sewage water (Control 150×10^{-3})



5.30 Sewage water (TEST 2×10^{-3})

CHAPTER 6

SUMMARY AND CONCLUSION:

Through our project, we have observed that there are certain microorganism present in the wastewater which can be reused if we treat it appropriately.

We have identified that organogel serves as a good adsorbent and actively removes the microorganism.

In antimicrobial sensitivity Test and zone inhibition test , we have analysed that P₄VP GO/Fe₂O₃ performs optimally compared to the other two gels when exposed to the microorganism.. With active engagement, it effectively eliminates microorganisms.

In conclusion, our findings highlight that the P₄VP GO/Fe₂O₃ organogel works as a good adsorbent in wastewater treatment and can eliminate the microorganism.

"Organogel was selected as the preferred material for its exceptional reusability, allowing for multiple cycles of use, and its effortless removal following the adsorption of microorganisms and impurities, thereby enhancing the efficiency and sustainability of the process."

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