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Microbial System for Pollution Control, Carbon Capture and Environmental Monitoring Using Algae, Bacteria and Biofilms

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ABSTRACT

Environmental pollution caused by industrialisation and urbanization has increased the need for sustainable and eco-friendly technologies for pollution control and environmental management. The present study aimed to investigate the potential of microorganisms for environmental biotechnology applications, with emphasis on bioremediation, carbon capture, wastewater treatment, and environmental monitoring. Soil samples collected from polluted sites were used for the isolation and characterisation of bacteria with desirable environmental attributes. Morphological, microscopic, and biochemical analyses tentatively identified the selected isolates as probable Pseudomonas species, Bacillus species, and Morganella morganii. These isolates exhibited resistance to heavy metals, the ability to utilize hydrocarbons, potential plastic degradation, and good adaptability to pollutant-stressed conditions, indicating their suitability for bioremediation. Microalgae were cultivated under bicarbonate-supplemented conditions to evaluate their carbon sequestration potential. Enhanced biomass production and positive lipid accumulation demonstrated their applicability for atmospheric CO_2 capture and renewable biofuel production. Microbial biofilms were also developed and assessed for wastewater treatment, where stable biofilm formation and a noticeable reduction in wastewater turbidity confirmed their effectiveness in pollutant removal. The study successfully achieved all the proposed objectives and demonstrated that bacteria, microalgae, and microbial biofilms can be integrated into a sustainable environmental biotechnology system. The findings highlight the potential of microbial resources as cost-effective and environmentally friendly alternatives for pollution remediation, wastewater treatment, carbon management, and environmental monitoring. This work provides a scientific foundation for future molecular characterisation, process optimisation, and large-scale implementation of microbial technologies for sustainable environmental management.

Keywords: *Environmental biotechnology, Bioremediation, Heavy metal resistance, Hydrocarbon degradation, Microalgae, Carbon sequestration, Biofilm, Wastewater treatment, Environmental monitoring.*

1. Introduction

Environmental pollution has become one of the most significant global challenges of the twenty-first century, threatening ecosystem stability, biodiversity, public health, and sustainable development [1]. Rapid industrialization, urbanization, population growth, and expanding agricultural and manufacturing activities have considerably increased the release of hazardous contaminants into the environment [2]. Industrial effluents, municipal wastewater, agricultural runoff, petroleum products, plastics, pharmaceutical residues, and greenhouse gases continuously contaminate soil, water, and the atmosphere, reducing environmental quality and disrupting natural ecological processes [3].

The persistence of these pollutants has exceeded the natural self-purification capacity of ecosystems, making the development of sustainable pollution control technologies an urgent global priority.

Among various environmental contaminants, heavy metals are considered particularly hazardous because they are non-biodegradable, persistent, and toxic even at low concentrations [4]. Metals such as lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), nickel (Ni), copper (Cu), and zinc (Zn) are released through mining, electroplating, battery manufacturing, textile processing, metal finishing, and fertiliser industries. Once discharged into the environment, these metals accumulate in soil and aquatic ecosystems, enter food chains through plants and aquatic organisms, and undergo bioaccumulation and biomagnification.

Their presence adversely affects microbial diversity, soil fertility, enzyme activities, and aquatic life while causing neurological disorders, kidney damage, respiratory diseases, reproductive abnormalities, and carcinogenic effects in humans and animals.

Petroleum hydrocarbons constitute another major class of environmental pollutants resulting from oil exploration, refining, transportation, accidental spills, and industrial discharge [5]. Among these contaminants, polycyclic aromatic hydrocarbons (PAHs) are of particular concern because of their chemical stability, hydrophobic nature, persistence, and carcinogenic potential. Hydrocarbon contamination alters soil structure, suppresses indigenous microbial communities, reduces nutrient availability, and inhibits plant growth. In aquatic environments, oil films reduce oxygen transfer and light penetration, negatively affecting photosynthetic organisms, fish populations, and overall ecosystem productivity.

Agricultural intensification has substantially increased the application of synthetic fertilizers, pesticides, herbicides, and fungicides to improve crop productivity; however, their excessive and indiscriminate use has become a major source of environmental contamination [6]. These agrochemicals enter surrounding ecosystems through runoff, leaching, and irrigation, contaminating soil and water resources. Persistent pesticide residues suppress beneficial soil microorganisms, interfere with nutrient cycling, reduce biodiversity, and affect non-target organisms including insects, birds, aquatic fauna, and mammals. Furthermore, many pesticide compounds accumulate in food chains and are associated with endocrine disruption, neurotoxicity, reproductive disorders, and chronic health complications.

Plastic pollution has emerged as another critical environmental issue due to the exponential increase in the production and consumption of synthetic polymers for domestic, industrial, agricultural, and medical applications [7]. Conventional plastics such as polyethene, polypropylene, polyethylene terephthalate, polystyrene, and polyvinyl chloride exhibit remarkable durability but resist natural microbial degradation, allowing them to persist in the environment for decades. Environmental weathering gradually fragments these materials into microplastics and nanoplastics, which have been detected in soil, freshwater, marine ecosystems, atmospheric dust, food products, and even human tissues. These particles adsorb heavy metals and organic pollutants, facilitate contaminant transport through food webs, and adversely affect both terrestrial and aquatic organisms.

In addition to solid and liquid pollutants, the continuous increase in atmospheric carbon dioxide (CO_2) concentration has intensified concerns regarding global climate change [8]. Anthropogenic activities such as fossil fuel combustion, industrial production, transportation, cement manufacturing, and deforestation release enormous quantities of greenhouse gases, leading to global warming, sea-level rise, glacier melting, ocean acidification, altered rainfall patterns, and an increased frequency of extreme weather events. These climatic changes negatively affect agricultural productivity, freshwater availability, biodiversity, and ecosystem resilience, emphasising the need for effective carbon capture technologies that complement pollution control strategies. Conventional physicochemical methods, including chemical precipitation, adsorption, membrane filtration, ion exchange, coagulation-flocculation, solvent extraction, and incineration, have been extensively employed for environmental remediation [9].

Although these technologies can effectively remove specific contaminants, they often require high capital investment, substantial energy input, and continuous chemical consumption. Moreover, many conventional treatment methods generate secondary pollutants or toxic sludge requiring additional processing, thereby increasing operational costs and environmental burden. Their efficiency is also limited when treating complex pollutant mixtures or contaminants present at low concentrations.

These limitations have accelerated the search for environmentally sustainable alternatives capable of achieving efficient pollutant removal with minimal ecological impact [10]. Biological remediation technologies based on microorganisms have emerged as promising approaches because of their remarkable metabolic diversity, adaptability, and ability to degrade, transform, immobilize, or detoxify a wide range of environmental contaminants under natural conditions. Such eco-friendly systems not only reduce pollution but also contribute to ecosystem restoration while supporting the principles of sustainable environmental management.

Microorganisms have emerged as highly effective biological agents for environmental remediation because of their remarkable metabolic diversity, rapid adaptability, and ability to survive under adverse environmental conditions [11]. Microbial communities inhabiting contaminated environments develop specialized physiological and biochemical mechanisms that enable them to tolerate, transform, and detoxify a broad spectrum of pollutants. Through processes such as biosorption, bioaccumulation, biodegradation, biomineralisation, biotransformation, and enzymatic detoxification, microorganisms can remove or convert hazardous contaminants into less toxic forms. Compared with conventional physicochemical approaches, microbial remediation is environmentally friendly, energy-efficient, and capable of restoring contaminated ecosystems without generating significant secondary pollutants. These advantages have established microorganisms as indispensable components of sustainable environmental biotechnology.

Among different microbial groups, bacteria have demonstrated exceptional potential for pollution control due to their rapid growth, metabolic versatility, and ability to utilise diverse organic and inorganic compounds as sources of carbon and energy [12]. Numerous bacterial species produce extracellular enzymes, oxygenases, dehydrogenases, hydrolases, and oxidoreductases that facilitate the degradation of petroleum hydrocarbons, pesticides, phenolic compounds, dyes, and other recalcitrant pollutants. Heavy-metal-resistant bacteria further contribute to environmental remediation through mechanisms including biosorption, intracellular accumulation, extracellular precipitation, active efflux systems, and enzymatic transformation, thereby reducing metal toxicity and bioavailability. The diverse metabolic capabilities of bacteria make them valuable resources for wastewater treatment, soil remediation, and restoration of contaminated environments.

In recent years, microbial biofilms have received considerable attention as highly efficient biological systems for environmental remediation [13]. Biofilms consist of structured microbial communities embedded within a self-produced extracellular polymeric substance (EPS) matrix composed primarily of polysaccharides, proteins, lipids, and extracellular DNA.

This matrix provides structural stability, enhances microbial adhesion to different surfaces, and protects microbial cells against environmental stress, toxic chemicals, desiccation, and fluctuations in pH, temperature, and nutrient availability. The close association of microorganisms within biofilms also promotes metabolic cooperation, quorum sensing, and efficient degradation of complex pollutant mixtures. Consequently, biofilm-based technologies have been successfully investigated for wastewater treatment, heavy metal removal, hydrocarbon degradation, and bioremediation of contaminated aquatic and terrestrial ecosystems.

Microalgae have also gained considerable importance in environmental biotechnology because they simultaneously contribute to pollution remediation and atmospheric carbon capture [14]. Through photosynthesis, microalgae efficiently assimilate carbon dioxide while producing oxygen and generating biomass rich in lipids, carbohydrates, proteins, pigments, and other valuable biomolecules. Their high photosynthetic efficiency and rapid growth make them attractive biological systems for reducing greenhouse gas emissions and mitigating climate change. In addition, microalgae effectively remove excess nitrogen, phosphorus, heavy metals, and organic pollutants from municipal and industrial wastewater, thereby improving water quality while producing biomass suitable for biofuel production, biofertilizers, animal feed, pharmaceuticals, and other value-added products [15]. The integration of wastewater treatment with carbon sequestration and biomass generation highlights the multifunctional role of microalgae in sustainable environmental management.

Recent advances in environmental biotechnology have demonstrated that integrating bacteria, microbial biofilms, and microalgae can significantly improve remediation efficiency compared with individual biological systems [16]. In these integrated systems, bacteria degrade complex organic pollutants, biofilms enhance microbial stability and resistance under stressful environmental conditions, and microalgae assimilate carbon dioxide while removing nutrients from wastewater. The synergistic interactions among these microorganisms improve pollutant removal, enhance treatment efficiency, increase system stability, and support resource recovery. Such integrated microbial consortia therefore represent a promising approach for addressing multiple environmental challenges simultaneously, including wastewater treatment, carbon capture, pollutant degradation, and biomass production.

Besides remediation, microorganisms can also serve as sensitive biological indicators for environmental monitoring [17]. Changes in microbial diversity, biofilm architecture, enzymatic activities, and physiological responses provide valuable information regarding pollutant toxicity, ecosystem health, and remediation efficiency. Heavy-metal-resistant bacteria, hydrocarbon-degrading microorganisms, and algal communities have been widely investigated as bioindicators because their abundance and metabolic responses reflect environmental contamination. Integrating microbial monitoring with biological treatment technologies therefore offers a comprehensive strategy for pollution assessment and sustainable ecosystem management.

Considering the increasing complexity of environmental pollution and the limitations of conventional remediation technologies, there is a growing need to develop integrated biological systems capable of simultaneously controlling pollution, capturing atmospheric carbon, and monitoring environmental quality [18]. The present study focuses on the application of bacteria, microbial biofilms, and microalgae as complementary biological components for sustainable environmental management. By integrating the pollutant-degrading capabilities of bacteria, the structural stability of biofilms, and the carbon-fixing potential of microalgae, this study aims to provide an eco-friendly and cost-effective strategy for pollution control, carbon sequestration, wastewater treatment, and environmental monitoring. The findings are expected to contribute to the advancement of microbial biotechnology and support the development of sustainable solutions for addressing contemporary environmental challenges.

2. Materials and Methods

2.1 Study Design and Sample Collection

The study was designed to evaluate the potential of microbial systems for pollution control, carbon capture, and environmental monitoring using bacteria, microalgae, and microbial biofilms. Polluted soil samples were collected aseptically from selected contaminated sites using sterile sampling tools and transferred into sterile polyethene bags. The samples were transported to the laboratory under refrigerated conditions and processed within 24 h for microbiological analysis.

2.2 Isolation and Purification of Bacterial Isolates

One gram of each soil sample was suspended in 9 mL of sterile distilled water and serially diluted up to 10^{-6}. Aliquots (100 µL) from appropriate dilutions were spread onto Nutrient Agar (NA) and Luria–Bertani (LB) agar plates. The plates were incubated at 37°C for 24 h. Morphologically distinct colonies were selected based on colony colour, size, elevation, texture, and margin characteristics. Pure cultures were obtained by repeated streak plating and maintained on Nutrient Agar slants at 4°C until further use [19].

2.3 Morphological and Biochemical Characterization

Purified bacterial isolates were characterized by colony morphology, Gram staining, and microscopic examination. Preliminary identification was performed using standard biochemical tests, including catalase, oxidase, citrate utilisation, indole production, methyl red, Voges–Proskauer, urease, triple sugar iron (TSI), and carbohydrate fermentation tests according to standard microbiological procedures.

2.4 Screening for Heavy Metal Resistance

The resistance of bacterial isolates to heavy metals was evaluated on Nutrient Agar supplemented separately with copper sulphate ($CuSO_4$), lead nitrate [$Pb(NO_3)_2$], and potassium dichromate ($K_2Cr_2O_7$). Fresh bacterial cultures were spot inoculated onto the metal-containing media and incubated at 37°C for 24 h. Growth on the supplemented media was considered indicative of heavy metal tolerance [20].

2.5 Screening for Hydrocarbon Degradation

Hydrocarbon-degrading ability was assessed using Bushnell–Haas agar supplemented with naphthalene as the sole carbon source. The bacterial isolates were inoculated onto the medium and incubated at 30°C for 7 days. Visible bacterial growth indicated the ability of the isolates to utilise hydrocarbons as a carbon source [21].

2.6 Screening for Plastic-Degrading Activity

Plastic degradation potential was evaluated indirectly by determining extracellular lipase production on Tween 80 agar. The isolates were inoculated onto the medium and incubated at 37°C for 48 h. Formation of a clear hydrolysis zone surrounding the colonies was recorded as positive lipase activity, suggesting the potential for plastic degradation [22].

2.7 Cultivation of Microalgae for Carbon Capture

Microalgae were cultivated in a sterile nutrient medium containing sodium bicarbonate as the inorganic carbon source together with magnesium sulphate ($MgSO_4$), calcium chloride ($CaCl_2$), phosphate buffer, and urea. The cultures were maintained under a 12 h light/12 h dark photoperiod at room temperature with continuous aeration where required. Algal growth was monitored by observing biomass accumulation and green pigmentation throughout the cultivation period [23].

2.8 Biomass Harvesting and Lipid Extraction

After sufficient growth, the algal biomass was harvested by centrifugation at 5000 rpm for 10 min or by sedimentation. The biomass was washed with distilled water and dried at 60°C until a constant weight. Lipids were extracted from the dried biomass using a chloroform:methanol solvent system (2:1, v/v), and the extracted lipid fraction was quantified gravimetrically to evaluate its biofuel potential [24].

2.9 Development of Microbial Biofilms

Selected bacterial isolates demonstrating promising bioremediation characteristics were cultivated in nutrient broth under static conditions to promote biofilm formation. Following incubation, the developed biofilms were exposed to wastewater samples containing organic and inorganic pollutants. The stability of the biofilms and their treatment efficiency were assessed by monitoring visible reductions in turbidity, colour, and suspended pollutants [25].

2.10 Evaluation of Environmental Monitoring Potential

The environmental monitoring potential of the selected bacterial isolates was evaluated by comparing their growth under heavy metal stress, hydrocarbon-containing media, and wastewater conditions. The growth pattern, survival ability, and biofilm-forming capacity of each isolate were recorded. Isolates exhibiting consistent growth under different environmental stress conditions were considered potential microbial indicators of environmental pollution.

3. Result and Observation

Polluted soil samples were processed for the isolation of bacteria using serial dilution and spread plate techniques on Nutrient Agar and Luria–Bertani (LB) agar. Following incubation, morphologically distinct colonies were purified by repeated streaking to obtain pure cultures. Based on colony morphology and growth characteristics, three representative bacterial isolates were selected for further characterisation and evaluation of their bioremediation potential against heavy metals, hydrocarbons, and plastics, as well as for subsequent biofilm development and environmental monitoring studies.

Isolation of Bacterial Strains from Polluted Soil Samples

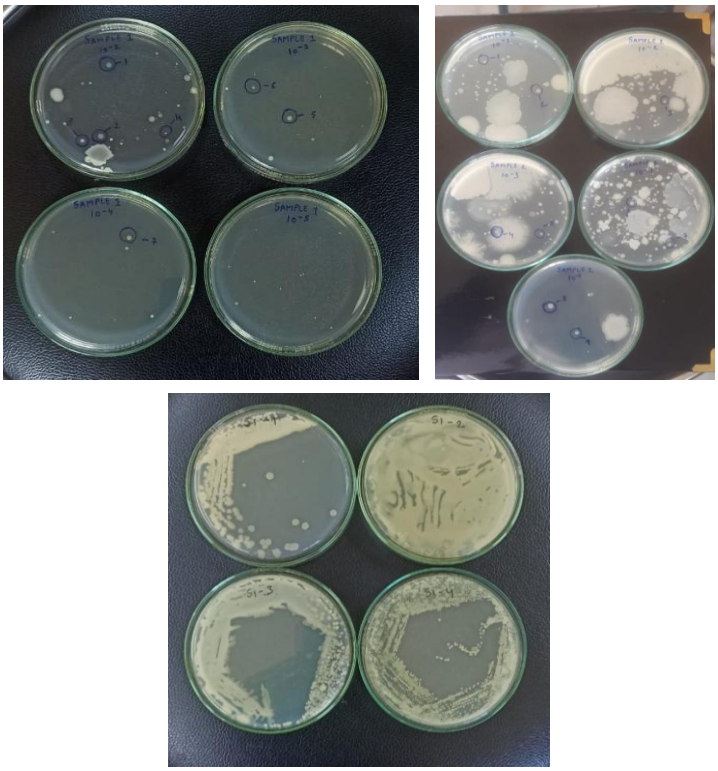

Figure 1. Isolation of morphologically distinct bacterial colonies from polluted soil samples following serial dilution and spread plate technique

Observation

Following incubation, numerous bacterial colonies with distinct morphological characteristics, including variations in colour, size, texture, elevation, and margin, were observed on the culture plates. The serial dilution method produced well-isolated colonies, facilitating the purification of individual bacterial isolates. Based on morphological diversity and consistent growth upon subculturing, three representative isolates were selected for further analysis. The purified isolates were preserved on Nutrient Agar slants and subsequently used for screening of heavy metal resistance, hydrocarbon degradation, plastic degradation potential, and biochemical characterisation. The successful isolation of these bacteria demonstrated that polluted soils harbour metabolically diverse microorganisms with potential applications in environmental bioremediation and biotechnology.

Screening of Bacterial Isolates for Heavy Metal Resistance

The purified bacterial isolates were evaluated for their ability to tolerate heavy metals using Nutrient Agar supplemented with copper sulphate, lead nitrate, and potassium dichromate. Fresh bacterial cultures were spot inoculated onto heavy metal-containing media and incubated for 24 hours under laboratory conditions. The presence or absence of bacterial growth was recorded to determine heavy metal resistance.

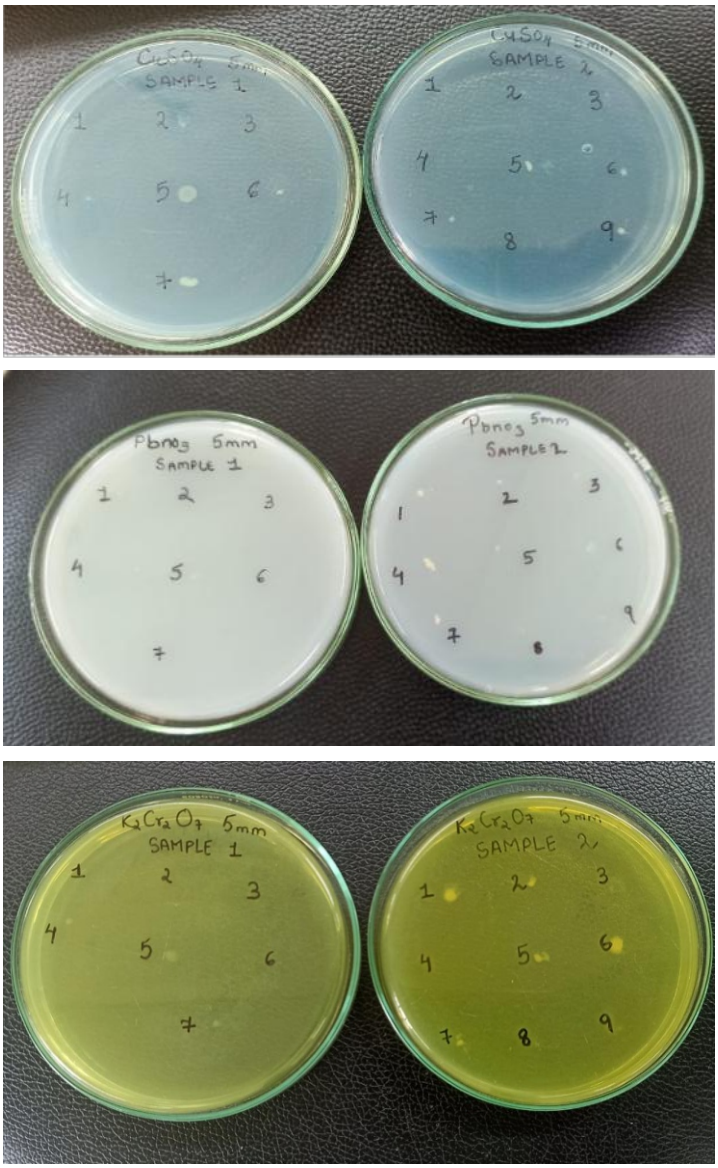

Figure 2. Growth of bacterial isolates on Nutrient Agar supplemented with heavy metal salts for evaluation of heavy metal resistance

Observation

All three bacterial isolates (S18, S21, and S24) demonstrated visible growth on media containing heavy metal salts, indicating their ability to tolerate elevated concentrations of toxic metals. Growth was characterised by the formation of healthy bacterial colonies comparable to those observed on control media, suggesting that the isolates possess adaptive mechanisms enabling survival under heavy metal stress.

The ability of these isolates to grow in the presence of copper sulphate, lead nitrate, and potassium dichromate indicates the presence of physiological and biochemical mechanisms associated with heavy metal resistance. Such characteristics are considered important for microorganisms intended for environmental bioremediation, particularly in the treatment of heavy metal-contaminated soils and industrial effluents.

Screening of Bacterial Isolates for Hydrocarbon Degradation

The bacterial isolates obtained from polluted soil samples were further evaluated for their hydrocarbon degradation potential using Bushnell–Haas Agar supplemented with naphthalene as the sole carbon source.

Bushnell–Haas medium lacks an alternative carbon source and therefore supports the growth of only those microorganisms capable of utilising hydrocarbons for their metabolic activities. The purified isolates were spot inoculated onto the prepared medium and incubated at 30–37°C for 3–5 days under laboratory conditions.

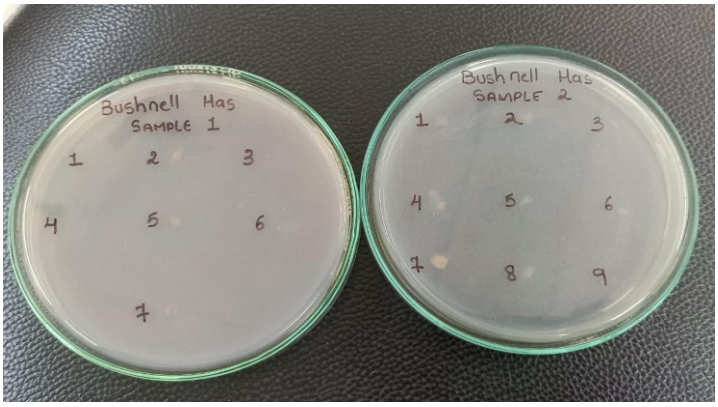

Figure 3. Growth of bacterial isolates on Bushnell–Haas Agar indicating hydrocarbon utilisation

Observation

Growth of all three bacterial isolates was observed on Bushnell–Haas Agar supplemented with naphthalene, indicating their ability to utilize hydrocarbons as the sole carbon source. Isolates S18 and S21 exhibited comparatively higher growth, whereas S24 showed moderate growth. These findings suggest that the selected isolates possess hydrocarbon-degrading capabilities and demonstrate adaptation to hydrocarbon-contaminated environments, highlighting their potential application in the bioremediation of petroleum-contaminated soil and wastewater.

Screening of Bacterial Isolates for Plastic Degradation

To evaluate their potential for plastic degradation, the purified bacterial isolates were screened on Tween 80 Agar, which is commonly employed for the preliminary detection of extracellular lipase activity. Lipase-producing microorganisms are capable of hydrolyzing lipid-like substrates and are often associated with the biodegradation of synthetic polymeric materials. The isolates were spot inoculated onto Tween 80 Agar plates and incubated under suitable laboratory conditions.

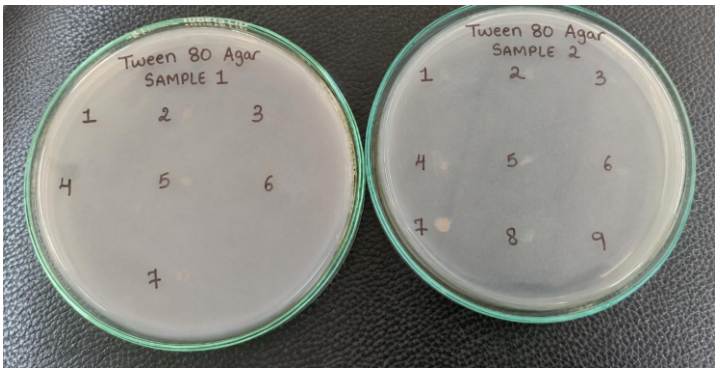

Figure 4. Lipase activity of bacterial isolates on Tween 80 agar indicates potential plastic degradation capability

Observation

Clear hydrolysis zones were observed around the bacterial colonies on Tween 80 Agar, indicating extracellular lipase production. Among the isolates, S18 exhibited the largest hydrolysis zone, followed by S21 and S24, demonstrating positive lipase activity.

These results suggest that the selected isolates possess lipolytic enzymes associated with the degradation of ester-containing compounds and indicate their potential application in the biodegradation of plastic waste and environmental bioremediation.

Characterisation of Selected Bacterial Isolates

The bacterial isolates exhibiting positive results during pollutant screening were subjected to detailed morphological, microscopic, physiological, and biochemical characterization for preliminary identification. Colony morphology, Gram staining, motility, and a series of biochemical tests were performed using standard microbiological procedures. These analyses were carried out to evaluate the physiological characteristics of the isolates and determine their probable taxonomic identity.

Morphological Characterization

The selected bacterial isolates exhibited distinct colony characteristics with respect to colour, size, elevation, surface texture, and colony margins. Isolate S18 produced smooth, circular colonies with creamy pigmentation, whereas isolate S21 formed relatively larger colonies with rough surfaces characteristic of spore-forming bacteria. Isolate S24 developed small, circular, translucent colonies with smooth margins. These morphological differences indicated the presence of taxonomically distinct bacterial populations within the polluted soil samples.

Gram Staining and Microscopic Examination

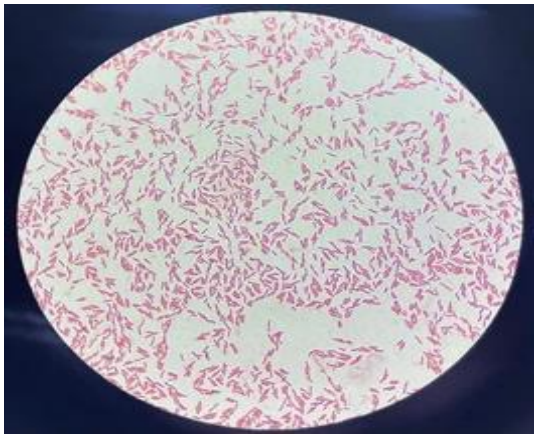

Figure 5. Gram staining of bacterial isolate S18 showing Gram-negative rod-shaped cells

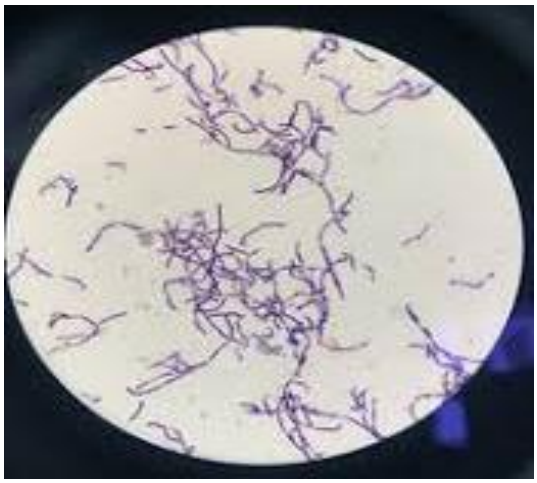

Figure 6. Gram staining of bacterial isolate S21 showing Gram-positive rod-shaped cells

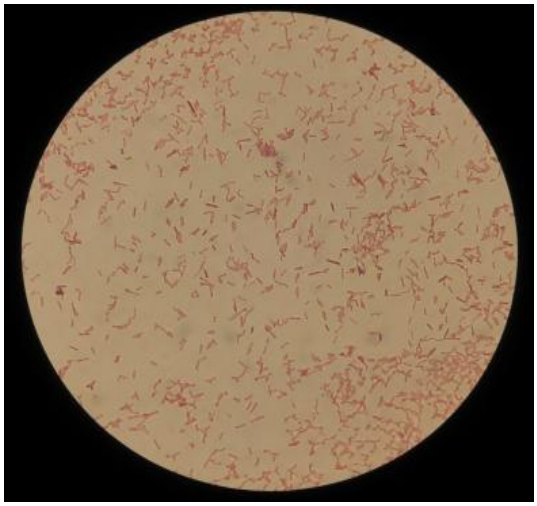

Figure 7. Gram staining of bacterial isolate S24 showing Gram-negative rod-shaped cells

Observation

Microscopic examination following Gram staining revealed distinct cellular morphologies among the isolates. Isolate S18 appeared as Gram-negative rods, whereas isolate S24 also exhibited Gram-negative rod-shaped cells typical of enteric bacteria. In contrast, isolate S21 appeared as Gram-positive rod-shaped cells with characteristics consistent with spore-forming bacteria. These microscopic observations provided important preliminary information for bacterial identification and supported the subsequent biochemical characterization.

Biochemical Characterisation of Isolate S18

The purified bacterial isolate S18 was subjected to a series of standard biochemical tests to determine its physiological and metabolic characteristics. The tests included Gram staining, motility, catalase, oxidase, citrate utilization, nitrate reduction, Triple Sugar Iron (TSI), urease, indole, methyl red (MR), Voges–Proskauer (VP), glucose fermentation, hydrocarbon utilization, heavy metal resistance, and biofilm formation. The results obtained are presented in Table 1.

Table 1. Biochemical characteristics of isolate S18 (Probable Pseudomonas sp.).

Test	Result	Interpretation
Gram staining	Gram-negative rods	Characteristics of Pseudomonas spp.
Motility test	Positive	Indicates presence of polar flagella
Catalase test	Positive	Bubble formation observed after addition of H_2O_2
Oxidase test	Positive	Development of dark purple colour
Citrate utilization	Positive	Growth with blue colour on Simmons citrate agar
Nitrate reduction	Positive	Reduction of nitrate to nitrite
TSI test	K/K	Indicates non-fermentative metabolism
Urease test	Negative	No urea hydrolysis observed
Indole test	Negative	No red ring formation
Methyl Red	Negative	No stable acid production
Voges–Proskauer	Negative	No acetoin production
Glucose fermentation	Negative/Oxidative	Characteristic of non-fermentative bacteria
Growth on Bushnell–Haas medium	Positive	Hydrocarbon utilization
Heavy metal resistance	Positive	Growth on metal-supplemented media
Biofilm formation	Positive	Ability to develop biofilm

Observation

The biochemical characterization of isolate S18 revealed features consistent with a probable *Pseudomonas* sp. The isolate was identified as a Gram-negative, motile rod showing positive reactions for catalase, oxidase, citrate utilization, nitrate reduction, hydrocarbon utilization, heavy metal resistance, and biofilm formation, while exhibiting negative reactions for urease, indole, methyl red, and Voges–Proskauer tests. A K/K reaction on Triple Sugar Iron (TSI) agar indicated a non-fermentative metabolism. Based on its morphological, physiological, and biochemical characteristics, isolate S18 was tentatively identified as a probable *Pseudomonas* species with promising potential for environmental bioremediation and wastewater treatment.

Biochemical Characterization of Isolate S21

The selected isolate S21 was subjected to biochemical characterization using conventional microbiological methods to determine its physiological and metabolic properties. The results obtained are summarized in Table 2.

Table 2. Biochemical characteristics of isolate S21 (Probable Bacillus sp.).

Test	Result	Interpretation
Gram staining	Gram-positive rods	Characteristic morphology of Bacillus spp.
Motility	Positive	Indicates motile bacilli
Catalase	Positive	Presence of catalase enzyme
Oxidase	Positive/Variable	Aerobic metabolism
Citrate utilization	Negative	No citrate utilization
Urease	Positive	Urea hydrolysis observed
Indole	Positive/Variable	Strain-dependent reaction
Methyl Red	Positive	Stable acid production
Voges–Proskauer	Negative	No acetoin production
Nitrate reduction	Positive	Nitrate reduced
Glucose fermentation	Positive	Acid production observed
Maltose fermentation	Positive	Carbohydrate utilization
Xylose fermentation	Negative	No fermentation
TSI	A/A or K/A	Variable carbohydrate utilization
H_2S production	Negative	No black precipitate
Spore formation	Positive	Characteristics of Bacillus spp.
Heavy metal resistance	Positive	Growth observed
Bushnell–Haas medium	Positive	Hydrocarbon utilization

Observation

The biochemical characterization of isolate S21 revealed characteristics consistent with a probable Bacillus sp. The isolate was identified as a Gram-positive, motile, endospore-forming rod exhibiting positive reactions for catalase, nitrate reduction, glucose fermentation, maltose fermentation, heavy metal resistance, and hydrocarbon utilization. Growth on heavy-metal-supplemented media and Bushnell–Haas medium demonstrated its adaptability to pollutant-associated conditions. Based on its morphological, physiological, and biochemical characteristics, isolate S21 was tentatively identified as a probable Bacillus species with potential applications in environmental bioremediation

Biochemical Characterization of Isolate S24

The purified bacterial isolate S24 was characterized using morphological, microscopic, physiological, and biochemical tests. The characterisation included Gram staining, motility, catalase, oxidase, citrate utilization, urease, indole, methyl red (MR), Voges–Proskauer (VP), nitrate reduction, carbohydrate fermentation, Triple Sugar Iron (TSI), heavy metal resistance, and growth under pollutant-associated conditions.

Table 3. Biochemical characteristics of isolate S24 (Probable Morganella morganii).

Test	Result	Interpretation
Gram staining	Gram-negative rods	Characteristic morphology of enteric bacteria
Motility test	Positive	Indicates motile bacilli
Catalase test	Positive	Bubble formation observed after addition of H_2O_2
Oxidase test	Negative	No purple coloration observed
Citrate utilization test	Negative	No growth or blue coloration observed
Urease test	Positive	Urea hydrolysis observed
Indole test	Positive	Red ring formation after Kovac's reagent
Methyl Red (MR) test	Positive	Stable acid production
Voges–Proskauer (VP) test	Negative	No acetoin production
Nitrate reduction test	Variable	Requires further confirmation
Glucose fermentation	Positive	Acid production observed
Xylose fermentation	Negative	No carbohydrate utilization
Maltose fermentation	Positive	Carbohydrate fermentation observed
Triple Sugar Iron (TSI)	K/A	Glucose fermentation only
H_2S production	Negative	No black precipitate observed
Growth on heavy metal supplemented media	Positive	Heavy metal resistance
Growth on polluted environmental media	Positive	Environmental adaptability

Observation

The biochemical characterization of isolate S24 revealed characteristics consistent with a probable Morganella morganii. The isolate was identified as a Gram-negative, motile rod exhibiting positive reactions for catalase, urease, indole, methyl red, glucose fermentation, maltose fermentation, and heavy metal resistance, while showing negative reactions for oxidase, citrate utilization, Voges–Proskauer, xylose fermentation, and hydrogen sulphide production. A K/A reaction on Triple Sugar Iron (TSI) agar indicated glucose fermentation without lactose or sucrose fermentation. Based on its morphological, physiological, and biochemical characteristics, isolate S24 was tentatively identified as a probable Morganella morganii, demonstrating adaptability to polluted environments and potential for environmental bioremediation.

Comparative Evaluation of the Selected Bacterial Isolates

Comparative characterization of the three selected bacterial isolates (S18, S21, and S24) demonstrated distinct physiological and biochemical characteristics associated with environmental adaptation and pollutant resistance. Isolate S18 (probable *Pseudomonas* sp.) exhibited strong hydrocarbon utilization, heavy metal resistance, oxidase positivity, and robust biofilm-forming ability, indicating its suitability for hydrocarbon degradation and wastewater treatment. Isolate S21 (probable *Bacillus* sp.) showed endospore formation, catalase activity, nitrate reduction, carbohydrate utilization, and tolerance to pollutant-associated conditions, suggesting potential for long-term environmental remediation. Isolate S24 (probable *Morganella morganii*) demonstrated heavy metal resistance and adaptability to contaminated environments, indicating its potential role in environmental monitoring and bioremediation. Overall, the selected isolates exhibited complementary metabolic traits, highlighting their potential application in pollution control, biofilm-based remediation, and environmental biotechnology.

Cultivation of Microalgae for Atmospheric CO_2 Sequestration and Lipid-Based Biofuel Production

The second objective of the present study was to evaluate the potential of microalgae for atmospheric carbon dioxide (CO_2) sequestration and preliminary lipid production for biofuel applications. Microalgae are photosynthetic microorganisms capable of converting atmospheric CO_2 into biomass while producing valuable intracellular lipids that can serve as renewable feedstock for biofuel production. Therefore, laboratory cultivation of microalgae was carried out under controlled conditions to assess their growth performance, biomass production, and lipid accumulation.

A nutrient medium containing sodium bicarbonate as the inorganic carbon source, together with magnesium sulphate ($MgSO_4$), calcium chloride ($CaCl_2$), phosphate buffer, and urea, was prepared under sterile laboratory conditions.

The prepared medium was inoculated with an actively growing microalgal culture and incubated under a controlled photoperiod of 12 hours light and 12 hours of dark at room temperature. During the incubation period, algal growth was monitored by observing changes in green pigmentation, turbidity, and biomass accumulation.

Figure 8. Microalgal culture cultivated under laboratory conditions using bicarbonate-supplemented growth medium

Observation

The inoculated microalgal culture exhibited successful growth under controlled laboratory conditions. Progressive increase in green pigmentation and turbidity of the culture medium was observed throughout the incubation period, indicating active photosynthetic growth and biomass development. The culture remained healthy and stable under the 12-hour light and dark cycle, demonstrating its adaptability to the cultivation conditions.

Biomass Production

Visible biomass accumulation was observed during the cultivation period. The increase in suspended green biomass indicated efficient utilization of nutrients and inorganic carbon present in the growth medium. Following incubation, the algal biomass was successfully recovered by centrifugation or settling and subsequently dried for further analysis.

Harvesting of Microalgal Biomass

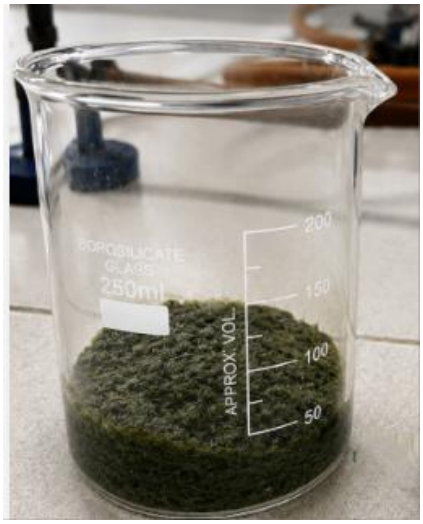

Figure 9. Harvested microalgal biomass recovered following cultivation

Observation

The harvested biomass appeared as a concentrated green pellet after centrifugation, indicating successful recovery of algal cells. The biomass was washed and dried under controlled conditions before being used for preliminary lipid extraction studies.

Preliminary Lipid Extraction

Figure 10. Preliminary lipid extraction from harvested microalgal biomass

Observation

Organic solvent extraction of the dried algal biomass resulted in the formation of visible oily residues, indicating the presence of intracellular lipids. Although the extraction was carried out as a preliminary investigation, the positive lipid recovery demonstrated the potential of the cultivated microalgae as a renewable source of biofuel feedstock.

Parameter	Observation
Growth medium	Sodium bicarbonate-based algal medium
Light condition	12 h light / 12 h dark cycle
Growth observation	Positive
Green pigmentation	Increased gradually
Biomass accumulation	Moderate to high
Turbidity increase	Observed
Biomass recovery	Successful
Lipid extraction	Positive
Visible oily residue	Observed
CO_2 sequestration potential	Positive
Biofuel applicability	Potentially suitable

Table 4. Growth characteristics and biofuel potential of cultivated microalgae.

Component	Function
Sodium bicarbonate	Carbon source for CO_2 sequestration
Magnesium sulphate ($MgSO_4$)	Magnesium source for algal metabolism
Calcium chloride ($CaCl_2$	Calcium source for cellular activity
Phosphate buffer	Maintains pH stability
Urea	Nitrogen source for growth

Table 5. Composition of microalgal growth medium.

The successful cultivation of microalgae under bicarbonate-supplemented laboratory conditions demonstrated their ability to utilize inorganic carbon for photosynthetic growth and biomass production. Increased green pigmentation, biomass accumulation, and successful biomass recovery confirmed the effective growth of the algal culture under controlled environmental conditions. Preliminary lipid extraction further indicated the presence of intracellular lipids, suggesting the potential applicability of the cultivated microalgae for lipid-based biofuel production.

The findings demonstrate that microalgae can simultaneously contribute to atmospheric CO_2 sequestration and renewable bioresource generation, highlighting their importance as sustainable biological systems for carbon capture and future bioenergy applications. This objective successfully established the feasibility of integrating microalgal biotechnology into environmentally sustainable strategies for carbon management and renewable fuel production.

Development of Microbial Biofilms for Wastewater Treatment and Pollutant Reduction

The third objective of the present study was to develop microbial biofilms using the bacterial isolates obtained from polluted soil samples and to evaluate their potential for wastewater treatment. Microbial biofilms are structured communities of microorganisms attached to solid surfaces and embedded within a self-produced extracellular polymeric matrix. These biofilms exhibit enhanced resistance to environmental stress and play an important role in the biodegradation and removal of organic and inorganic pollutants from contaminated wastewater.

The three selected bacterial isolates obtained during Objective 1 were inoculated into suitable growth media and incubated under controlled laboratory conditions to promote biofilm formation. The cultures were maintained under static conditions, allowing the microorganisms to adhere to the surface and develop stable microbial biofilms.

Following biofilm formation, the developed biofilms were exposed to wastewater samples containing organic and inorganic pollutants. Treatment efficiency was evaluated by monitoring visible changes in wastewater characteristics, including turbidity, colour intensity, and pollutant load.

Development of Microbial Biofilms

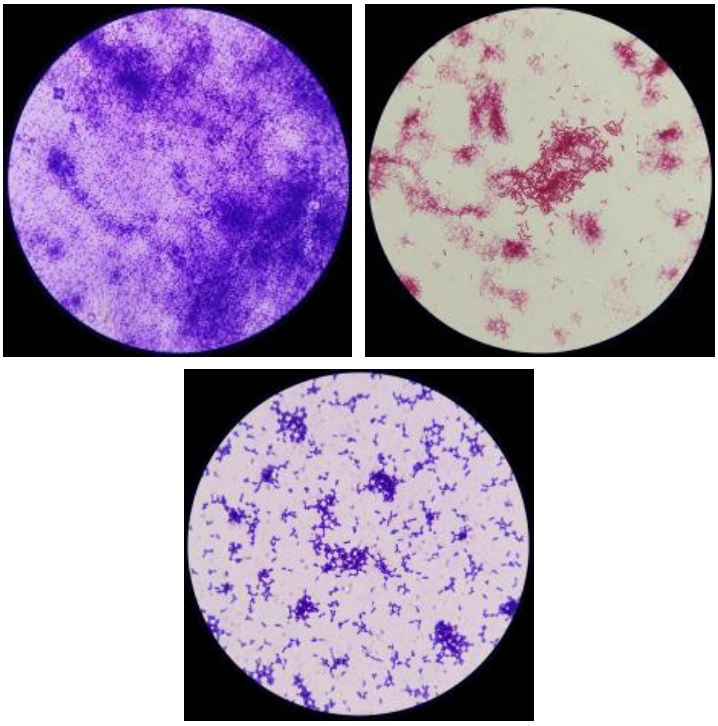

Figure 11. Microscopic observation of biofilm formation by isolates S18 (Pseudomonas sp.), S21 (Bacillus sp.), and S24 (Morganella morganii) following biofilm staining

Observation

Microscopic examination following biofilm staining revealed that all three bacterial isolates (S18, S21, and S24) successfully formed biofilms under static incubation conditions. Distinct clusters of bacterial cells attached to the surface were observed, indicating effective surface colonization and biofilm development. The stained biofilm structures appeared dense and well organised, demonstrating the ability of the isolates to establish stable microbial communities. These observations suggest that the selected isolates possess good biofilm-forming capability, which may enhance their persistence under environmental stress and support their potential application in wastewater treatment and environmental bioremediation.

Biofilm-Mediated Wastewater Treatment

Observation

Following exposure to wastewater samples, the developed biofilms remained viable and exhibited stable growth under contaminated conditions. A noticeable reduction in turbidity and colour intensity was observed after the treatment period, indicating preliminary removal of suspended and dissolved pollutants. These observations suggest that the microbial biofilms were capable of reducing the pollutant load present in the wastewater.

3. Adaptability of Biofilms Under Polluted Conditions

Observation

The microbial biofilms maintained their structural integrity and continued to grow under wastewater conditions, demonstrating high adaptability to pollutant-associated environments. The persistence of the biofilms throughout the treatment period indicates that the heavy-metal-resistant bacterial isolates possess characteristics favourable for long-term environmental remediation and wastewater treatment applications.

Table 6. Performance of developed microbial biofilms during wastewater treatment

Parameter	Observation
Biofilm formation	Positive
Surface attachment	Successful
Biofilm stability	Good
Growth under wastewater conditions	Positive
Reduction in turbidity	Observed
Reduction in colour intensity	Observed
Reduction in visible pollutant load	Observed
Adaptability under polluted conditions	High
Potential for wastewater treatment	Positive

Table 7. Characteristics of developed microbial biofilms.

Characteristic	Observation
Biofilm-producing isolates	S18, S21 and S24
Growth condition	Static incubation
Surface colonization	Successful
Biofilm persistence	Stable
Pollutant tolerance	Positive
Heavy metal resistance	Positive
Environmental adaptability	High
Application	Wastewater treatment and environmental bioremediation

Overall Findings

The present study successfully demonstrated the development of microbial biofilms using bacterial isolates obtained from polluted soil samples. The selected isolates effectively adhered to solid surfaces and formed stable biofilms capable of surviving under wastewater conditions. Preliminary observations revealed reductions in turbidity, colour intensity, and visible pollutant load following biofilm treatment, indicating the potential removal of organic and inorganic contaminants.

The stability and persistence of the developed biofilms under contaminated conditions further confirmed the environmental adaptability of the selected bacterial isolates. These findings suggest that heavy-metal-resistant microorganisms possess significant potential for biofilm-mediated wastewater treatment and environmental bioremediation. The successful development of microbial biofilms in this study provides a promising foundation for the application of microbial systems in sustainable wastewater management and pollution control.

Assessment of Heavy-Metal-Resistant Microorganisms as Potential Indicators of Environmental Pollution

Heavy-metal-resistant microorganisms were evaluated to assess their potential as indicators of environmental pollution. The bacterial isolates (S18, S21, and S24) obtained from polluted soil samples were examined for their adaptability under heavy metal-containing media, hydrocarbon-supplemented Bushnell–Haas Agar, and wastewater conditions developed during the biofilm studies. Their growth, survival, biofilm-forming ability, and morphological stability were monitored under pollutant-stressed conditions.

Consistent growth and persistence across multiple contaminated environments demonstrated strong environmental adaptability, suggesting that these isolates can serve as potential microbial indicators for monitoring polluted ecosystems while also contributing to environmental bioremediation.

Growth of Bacterial Isolates Under Pollutant-Stressed Conditions

All three bacterial isolates (S18, S21, and S24) demonstrated consistent growth on heavy-metal-supplemented media, hydrocarbon-containing Bushnell–Haas Agar, and wastewater-associated environments. The isolates maintained active growth under these stressed conditions, indicating their ability to tolerate multiple environmental pollutants. Their persistence under contaminant exposure suggests the presence of adaptive physiological mechanisms associated with survival in polluted ecosystems.

Environmental Adaptability of the Selected Isolates

Observation

The selected bacterial isolates (S18, S21, and S24) exhibited stable morphological characteristics and maintained consistent growth throughout the experimental period. No significant reduction in growth was observed under heavy metal stress, hydrocarbon-associated conditions, or wastewater environments. The isolates also retained their biofilm-forming ability, indicating strong environmental adaptability and physiological stability under multiple pollutant-stressed conditions.

Evaluation of Microbial Indicators of Environmental Pollution

Observation

Repeated survival and growth of the isolates under contaminated environmental conditions indicated their ecological association with polluted habitats. Their ability to tolerate heavy metals, utilise hydrocarbons, survive in wastewater, and maintain biofilm formation demonstrates adaptive characteristics commonly associated with microorganisms inhabiting contaminated ecosystems. These findings suggest that the selected isolates have the potential to serve as biological indicators for environmental pollution monitoring.

Table 8. Adaptability of bacterial isolates under different pollutant-associated conditions

Parameter	Observation
Growth on heavy metal-containing media	Positive
Growth on Bushnell–Haas Agar	Positive
Hydrocarbon utilization	Positive
Growth in wastewater	Positive
Biofilm formation	Positive
Morphological stability	Maintained
Environmental adaptability	High
Survival under pollutant stress	Consistent
Potential as pollution indicator	Positive

Table 9. Environmental characteristics of the selected bacterial isolates

Characteristic	Observation
Heavy metal resistance	Positive
Hydrocarbon tolerance	Positive
Wastewater adaptability	Positive
Biofilm persistence	Stable
Growth under multiple stress conditions	Positive
Ecological association with polluted habitats	High
Suitability for environmental monitoring	Potentially suitable
Environmental application	Pollution monitoring and bioremediation

The present study demonstrated that the heavy-metal-resistant bacterial isolates obtained from polluted soil samples possess strong environmental adaptability under multiple pollutant-associated conditions. All three isolates (S18, S21, and S24) consistently survived and maintained growth in heavy metal-containing media, hydrocarbon-associated environments, and wastewater systems. Their ability to retain stable biofilm formation and physiological characteristics under stressed environmental conditions further highlights their resilience and ecological significance.

The repeated occurrence and persistence of these microorganisms in contaminated environments indicate their close association with pollutant-stressed ecosystems, suggesting their potential application as biological indicators of environmental pollution. In addition to their role in environmental monitoring, the isolates also demonstrated characteristics favorable for bioremediation, wastewater treatment, and pollutant degradation. These findings highlight the potential of the selected microorganisms for integrated environmental management, where they can simultaneously contribute to pollution monitoring and sustainable remediation strategies.

4. Conclusion

The present study demonstrated the potential of microorganisms as sustainable resources for environmental biotechnology. Bacterial isolates obtained from polluted soil showed resistance to heavy metals, the ability to utilise hydrocarbons, and potential for plastic degradation, indicating their adaptation to contaminated environments. Based on morphological, microscopic, and biochemical characteristics, the isolates were tentatively identified as probable *Pseudomonas* species, *Bacillus* species, and *Morganella morganii*.

The cultivated microalgae exhibited satisfactory growth under bicarbonate-supplemented conditions with enhanced biomass and lipid accumulation, suggesting their suitability for carbon dioxide sequestration and biofuel production. In addition, the developed microbial biofilms remained stable under wastewater conditions and effectively reduced turbidity, demonstrating their potential application in biological wastewater treatment.

The study achieved all the proposed objectives and highlights the complementary role of bacteria, microalgae, and biofilms in addressing environmental pollution. The findings support their future application in bioremediation, carbon capture, wastewater treatment, and environmental monitoring, providing a basis for further research and large-scale implementation of sustainable environmental technologies.

5. Recommendations

Based on the findings of the present study, molecular identification of the bacterial isolates using 16S rRNA gene sequencing is recommended to confirm their taxonomic identity. Future studies should quantitatively evaluate the efficiency of these microorganisms in degrading heavy metals, hydrocarbons, plastics, and other environmental pollutants under laboratory and field conditions. Optimisation of culture parameters, including pH, temperature, nutrient composition, and incubation time, is also suggested to improve microbial growth, biofilm formation, and pollutant removal efficiency.

Further research should focus on enhancing microalgal biomass production, carbon dioxide sequestration, and lipid accumulation to improve the feasibility of biofuel production. The developed microbial biofilms should be evaluated in pilot-scale wastewater treatment systems to assess their long-term stability and treatment performance under practical conditions. The pollutant-resistant bacterial isolates should also be investigated for their potential use as bioindicators of environmental pollution and their ability to degrade emerging contaminants such as pesticides, pharmaceutical residues, synthetic dyes, and microplastics. In addition, studies on the genetic and metabolic mechanisms underlying pollutant degradation and environmental adaptation would improve the development of efficient bioremediation technologies.

Pilot-scale validation and the integration of bacteria, microalgae, and microbial biofilms into a single bioremediation system are recommended to support the development of sustainable, cost-effective, and environmentally friendly technologies for pollution control, wastewater treatment, carbon capture, and ecosystem restoration.

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