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Bioremediation By *Bacillus Subtilis*: Isolation, Molecular Characterization And Process Optimization

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Abstract
Bioremediation is a green and viable practice that involves the use of microorganisms to break down pollutants in the environment instead of the use of chemicals as practiced in the conventional method. In the current research, a bacterial strain was isolated and the sewage water of Ezhakulam, Neyyattinkara and it was identified as *Bacillus subtilis* by morphological, biochemical, and molecular characterization. The isolate was highly proteolytic and had high potential of biodegrading methylene blue dye, a model pollutant. The efficiency of degradation peaked at 48 hours, then decreased, which could be explained by the fact that nutrients were used up, or by toxic intermediates that were formed.Optimization experiments proved that environmental conditions had a considerable effect on the efficiency of biodegradation. The highest percentage of dye removal occurred at 37°C with alkaline pH and 2 percent NaCl solution which increased the activity of the enzyme and dissolution of the pollutant. At optimized conditions the isolate had a maximum removal efficiency of which is compared to 32.76 when the conditions were normal. These results indicate the metabolic and environmental plasticity of Bacillus subtilis, such as the resistance to the extreme environment and the ability to generate various degradative enzymes.
In general, this paper shows that the Bacillus subtilis can be used as an effective bioremediation agent to treat wastewater. The findings also indicate the significance of maximization of physicochemical conditions to increase microbial degradation capacity and enable the use of bacterial systems in sustainable environmental management.

Keywords: Bioremediation; *Bacillus subtilis*; Environmental optimization; Methylene blue; Wastewater treatment

1) Introduction

The bioremediation technique, a biological process in which the living organisms (primarily bacteria) are used to clean up the pollutants instead of using chemicals as a sustainable approach (Atlas *et al*., 1995). The ubiquitous soil and water bacteria create enzymes (e.g. azoreductases, laccases, peroxidases, dioxygenases) which decompose dyes, hydrocarbons and other organics (Ikram *et al*., 2022; de Souza *et al*., 2021). They can survive pH extremes, temperature extremes as well as nutrient deprivation due to their resistant endospores. The most recent developments are enzyme engineering, genomic or omics strategies, engineered microbial consortia and pilot-scale bioreactors. Bacillus biofilms immobilized and recombinant strains have demonstrated greater degradative abilities (de Souza *et al*., 2021; Almamoori*et al*., 2024).
The idea of microbes consuming pollutants has long been known since the early 20th century, however it was formally worked out into the sphere of bioremediation in the late 20th century (Cerniglia *et al*., 1992). A groundbreaking experiment proved that oil and fuel residues could be degraded by natural bacterial consortia, and this is one of the first recorded applications of microbes to clean up (Sayler *et al*., 2003). In 1970s and 1980s, microbial cleanup became of interest as a reaction to environmental crises (e.g. oil spills, industrial contamination). During the 1950s1980s researchers defined certain pathways of aromatic hydrocarbons and phenols (e.g. oxygenase mediated oxidation) and put down the biochemical basis of biodegradation (Cerniglia *et al*.,1992).
Genetic and ecological studies had identified enzymes (oxygenases, reductases) and mobile genetic components (plasmids) that facilitated the mineralization of xenobiotic compounds by bacteria (Atlas *et al*., 1995; Van Ham and Renneckar, 2000). This was also around the time when the first genetically modified microbes that would degrade pollutants emerged (Sayler *et al*., 1993). High-throughput sequencing and the so called omics technologies were developed in the 2000s and illuminated whole degradation processes and microflora of polluted locations. At the same time, Bacillus species became potential bioremediators: the strains obtained on the soil and wastewater could effectively degrade dyes, oils, and phenolic wastes (Ikram, 2022; de Souza *et al*., 2021).
Bioremediation takes advantage of the metabolic flexibility of microbes to transform pollutants into end-products that are harmless (CO 2, H 2 O, biomass) (Foght *et al*., 2008). Some of the processes involve bioaugmentation (the addition of degrader microbes) and biostimulation (the increase of native populations). Bacteria tend to be a favorite as it grows

quickly and is flexible. Another interesting genus of bacteria is Bacillus: Gram-positive, rod-shaped, facultatively aerobic bacteria typical of soils, sediments and wastewater. To the environment, many species of Bacillus release an assortment of degradative enzymes, such as laccases, peroxidases, azoreductases, dehydrogenases and dioxygenases (Ikram *et al*., 2022; de Souza *et al*., 2021). As an illustration, the synthetic azo dyes are cut into colorless amino acids by azoreductases, and the phenolic and aromatic pollutes are oxidised by laccases and peroxidases.

The fearsome ability of Bacillus spp. also contributes to bioremediation. They create endospores that survive dryness, heat, acid and base extremes and toxins. B. subtilis in particular can survive at pH of about 4 -10 as well as 15 -50 C (Tang *et al*., 2019). The biosurfactants (e.g. surfactin, fengycin) are also produced by Bacillus and increase the solubility of hydrophobic contaminants (de Souza et al., 2021). Secreted enzymes, stress tolerance coupled with generally recognised-as-safe (GRAS) status are combined to make Bacillus strains ideal agents in contaminated wastewater and soil treatments. In fact, several articles have identified the Bacillus in the textile effluent, oil sludge and dye contaminated locations, revealing the ability to decolorize dyes and break down oil, without genetic engineering (Ikram *et al*., 2022).

Because of its popularity as a study bacterium, B. subtilis is illustrative of bioremediation properties. It has a wide range of enzymatic armament: azoreductases (to break azo dyes), peroxidases and laccases (to break aromatic oxidizers), and dioxygenases (to break rings) (Ikram *et al*., 2022). Under aerobic conditions, complex dyes can be broken down by B. subtilis; the enzyme Orange II, which is an azo dye, was broken down into simple aromatic amines by the enzyme, resulting in decolorization of the compound (>85 percent) (Ikram *et al*., 2022). All these amines are then further oxidized by downstream enzymes into non-toxic end-products. In line with this, *B. subtilis* secretes dye-decolorizing peroxidases which operate within expansive temperature scopes (de Souza *et al*., 2021).Hydrolases and oxygenases have also been reported to degrade crude oil n-alkanes (C13 30) using B. subtilis (Tang *et al.,* 2019). One example is strain BL-27 that mineralized 3080 percent of long-chain alkanes in days and could contemplate 410 pH and salinity of up to 5 percent (Tang *et al*., 2019).

B. subtilis pollutant degradation ability in adverse environments was supported by its ecological ability to exist as biofilms and spores. The spores also guarantee the stability of the genome and can be made to express degradative enzymes on the surface (de Souza *et al*., 2021). Its fast development and genetic malleability have transformed B. subtilis into an enzyme engineering model, with mutated laccases and further-stabilized peroxidases (de Souza *et al*., 2021). Synthetic dyes (methylene blue, malachite green, Congo red), aromatic phenols, and petroleum compounds are among the examples of pollutants that the B. subtilis was capable of depleting (Ikram *et al*.,2022). The metabolic plasticity and stress resilience of the species (through enzymes that survive heat and form spores) are the reasons behind choosing it as an agent to treat wastewater.

Recent bioremediation combines molecular biology and engineering and oldfashioned microbiology. The latest developments are the genome sequencing of Bacillus strains to determine catabolic genes, transcriptomics/proteomics to characterize the pollutant-activated pathways, and metagenomics to study mixed consortia (Wang et al., 2020; Ilma *et al*., 2025). As a case in point, Bacillus, by omics analyses have revealed new azoreductase and dioxygenase variants that can be used to engineer targeted enzymes. Synthetic biology strategies (e.g. CRISPR editing) are also under investigation in order to facilitate pollutant-specific pathways in B. subtilis, but the field application is not yet feasible because of regulation.

The application of the bacillus with other degraders (e.g. Pseudomonas, Rhizobium) to pollutant mixtures to increase stability is now becoming the dominant approach to bioaugmentation strategies (Ilma *et al.,* 2025; Wang *et al*., 2020). Certain pilot studies have been used in industrial effluents and have been found to be superior in the removal of hydrocarbons and dyes when Bacillus consortia are used in bioreactors instead of single strains. Moreover, biosurfactants of Bacillus scale is enhanced to expand bioavailability of hydrophobic pollutants (de Souza *et al*., 2021).

Immobilized-cell and engineered bioreactors are currently being used. As an example, fluidized-bed and packed-bed reactors using Bacillus biofilms have been experimented on textile effluents and the high dye breakdown under controlled conditions was achieved (Wang*et al*., 2020). Spore-immobilized enzymes allow using reusable biocatalysts when using continuous flow systems (de Souza *et al*., 2021).

Optimization and Environmental Factors.

Environmental conditions are important in bioremediation. The most important are the pH, the temperature, the oxygen level, and the availability of nutrients. Bacillus strains are mostly neutral to slightly alkaline (6–8) and mesophilic (2537 o C) to ensure the maximum activity of enzymes (Tang *et al*., 2019; Ikram *et al*., 2022). Beyond these ranges, there is retarded growth and catalysis. As an illustration, B. subtilis BL-27 exhibited the best oil-degradation at pH 7 and 45 o C with a drastic decrease beyond this range (Tang *et al*., 2019).

The other control point is oxygen: Bacillus usually degrade dyes under aerobic conditions through oxidative enzymes, but other reductive reactions (e.g. azo cleavage) could be optimal under microaerophilic conditions. Reductases can be provoked with the help of redox mediators (e.g. vitamin C) (Ikram *et al*., 2022). Nutrient supplements (carbon sources such as glucose, nitrogen) can be used to stimulate degradation due to the energy available to the growth. But, too much material can result in excess biomass or by-products.

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Others include concentration of the pollutant (toxicity by excess dye), and ionic strength (salinity). Certain Bacillus strains can endure moderate salinity (less than 5%), although presence of high salt or co-contaminants of heavy metals may cause activity to be impaired (Tang *et al*., 2019). In the case of hydrophobic pollutants (PAHs, oils), the addition or production of bioavailability-enhancing biosurfactants is essential (de Souza et al., 2021). Practically, to optimize these parameters (e.g. by use of design of experiments techniques) to maximize the rate of removal and avoid the formation of toxic intermediates is used. It is necessary to understand the impact of these factors on metabolic pathways to predictively scale up bioremediation (Ikram *et al*., 2022; Ilma *et al*., 2025). The focused application of B. subtilis under optimized conditions can be a better ecofriendly approach to remove the accumulated pollutants from ecosystem.

2) Materials and Methods

2.1)Sample Collection

Sewage samples were collected from wastewater source, Ezhakulam, Neyyattinkara(Figure1) in sterile, screw-capped containers. Care was taken to avoid contamination during sampling. The samples were transported to the laboratory immediately and processed within a few hours of collection to preserve microbial viability.

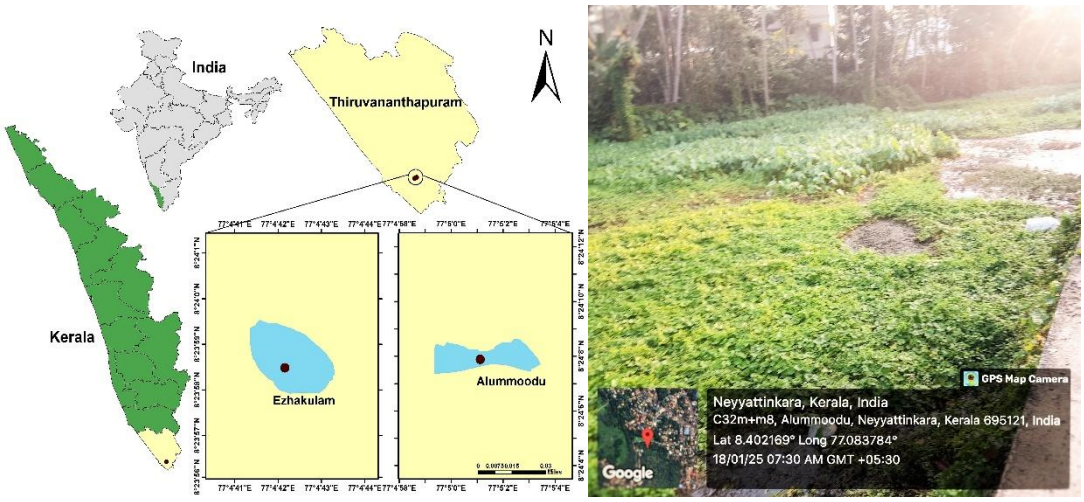

Figure 1: Sample collection site, Ezhakulam pond

2.2) Isolation of Bacteria

Bacterial isolates were obtained using the serial dilution and spread plate technique. One milliliter of sewage sample was serially diluted in sterile distilled water up to appropriate dilution levels. Aliquots (0.1 mL) from selected dilutions were spread evenly onto nutrient agar plates using a sterile spreader. The plates were incubated at 37°C for 24 hours. After incubation, distinct colonies differing in size, shape, color, and texture were selected for further analysis.

2.3) Purification of Bacterial Isolates

Selected colonies were purified by repeated streaking on fresh nutrient agar plates using the streak plate method. The plates were incubated at 37°C for 24 hours. Pure cultures were confirmed based on uniform colony morphology and were maintained on nutrient agar slants at 4°C for further studies.

2.4) Identification of Selected Bacterial Isolate

The bacterial isolate was identified based on morphological and biochemical characteristics. Colony morphology was observed with respect to shape, size, color, elevation, and margin. Gram staining was performed to determine the Gram reaction and cell morphology. Biochemical tests were carried out following standard microbiological procedures.

2.5) Screening of microbial isolates

The microbial colonies isolated from the sewage water were subjected to screening their ability to degrade the compounds.

The isolated bacterial strains were screened for specific enzymatic activities essential for breaking down sewage components. Organic Matter Degradation: Test for Protease (using Skim Milk Agar) to identify strains that break down proteins.

2.6) Screening of Bacterial Isolates for Bioremediation Potential

The biodegradation potential of bacterial isolates was assessed using the methylene blue reduction assay. 50 ml nutrient broth was prepared and was inoculated with the 24 hrold bacterial culture and sewage sample (Sample) aseptically. A

control was maintained which contains only sewage sample and both samples were incubated at 4 hours initially at 37°C. After 4 hours 1ml of methylene blue, 1 ml of phosphate buffer, 5 ml of cholorform solution was added to 10 ml Sample with inoculum and control was centrifuged at 10,000 rpm for 10 minutes. After centrifugation the bottom layer was carefully removed and the amount of pollutant degraded was measured by observing the optical density using a UV–Visible spectrophotometer at 625 nmThe degradation of methylene blue was monitored at time intervals of 4, 24, 48, 72, and 96 hours. Isolates showing maximum percentage removal were selected for further studies.

Percentage removal was calculated using the formula:

$$\text{Percentage Removal} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control}} \times 100$$

2.7)Molecular characterization of Selected bacterial Strain

After screening the selected bacterial strain was subjected for molecular characterization. The bacterial DNA was isolated by following standardized protocol (Rachana mol *et al* ., 2017) and the DNA were amplified. The amplified DNA were sequenced and using Mega 5.0 the alignment was done. The maximum similar sequence were compared and the phylogenetic tree were constructed.

2.8)Optimization of Environmental Parameters

To determine the optimum conditions for biodegradation, the effect of temperature, pH, and incubation time was evaluated.

2.9) Effect of Temperature

Biodegradation experiments were conducted at different temperatures, including 4°C, room temperature, and 37°C. Dye degradation was monitored by measuring optical density after incubation, and percentage removal was calculated.

2.10) Effect of pH

The effect of pH on biodegradation was studied by adjusting the pH of the medium to different levels (pH 5, pH 6, pH 7, pH 8 , pH 9 and pH 10) using appropriate buffer solutions. Bacterial cultures were inoculated into each medium and incubated under identical conditions. Optical density readings were recorded to determine degradation efficiency.

2.11) Effect of Incubation Time

Biodegradation was monitored at different incubation periods (4, 24, 48, 72, and 96 hours) to determine the time required for maximum dye removal.

2.12) Effect of NaCl

The effect of NaCl on the bioremediation was evaluated by increasing the concentration of NaCl in media from 0.5% to 2.5%. The percentage of inhibition was calculated.

2.13) Measurement of Growth of Bacillus

The increase in the number of cells at a particular time period was studed by measuring the optical density at 600nm. Th time periods initiated from zero hour, 24h, 48h, 72h and 96h.

2.14) Comparison for Bioremediation activity of bacteria grown in enriched media and normal media

The bioremediation activity of *Bacillus subtilis* was compared by growing the culture in media which was optimized in conditions like p^H, temperature and media supplemented with NaCl and in media with normal growth conditions. After 48 hr of incubation the samples labelled as A1(normal media) and optimized media (A2) were performed for their bioremediation activity assay.

3) Results

3.1) Isolation and Purification of Bacteria from Sewage sample

The bacterial colonies isolated from the sewage sample were morphologically characterized and the most dominant white raised colonies were selected for further studies. Distinct, creamy-white, circular colonies with smooth margins are observed along the streak lines, indicating successful isolation and purification of the bacterial strain. (Fig 2). The identical colonies were purified and preserved for bioremediation screening and characterization (Fig 3).

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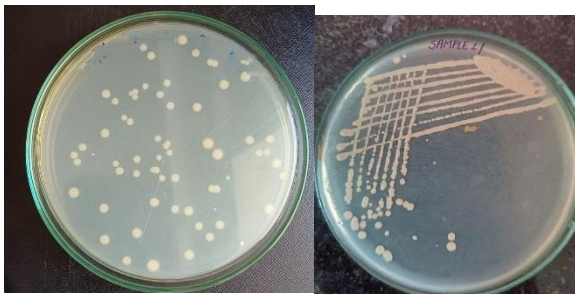

Figure 2 &3: *Isolation and Purification of Bacterial Colonies on Nutrient Agar Plate*.

3.2) Biochemical Characterization

The dominant colonies were identified as Gram-positive rod-shaped bacteria with catalase positive, citrate positive (Table 1). The biochemical characters inferred the strain as Bacillus sp.

Table 1: Morphological and Biochemical Identification of the Selected Bacterial Isolate

SI NO	BIOCHEMICAL TEST	OBSERVATION	RESULT
1	Gram staining	Purple colored Rod-shaped	Positive
2	Catalase Test	Immediate bubble formation	Positive
3	Indole Test	No red ring formation	Negative
4	Methyl Red Test	No red color formation	Negative
5	Voges-Proskauer Test	Red/Pink colour formation	Positive
6	Citrate Utilization Test	Blue colour change in media	Positive

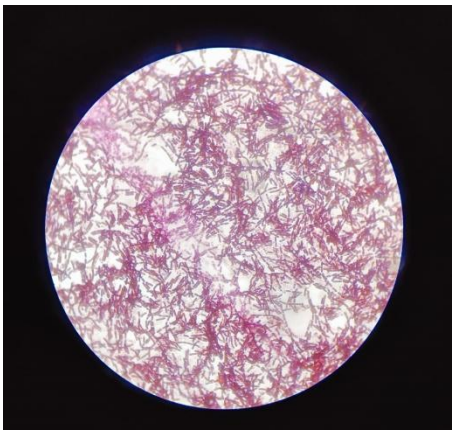

Figure 4:*Microscopic view of the bacterial isolate after Gram staining. The cells appear as purple colored, rod-shaped structures, indicating a Gram-positive reaction*.

3.3) Functional Screening Assays

Screen isolates for specific enzymatic activities essential for breaking down sewage components:

Organic Matter Degradation: Test for Protease (using Skim Milk Agar) and to identify strains that break down proteins.The bacterial strains inoculated in Skim milk agar media and isolate that degrade the casein in the skim milk agar media with clear zone of clearance is observed (Figure 5)

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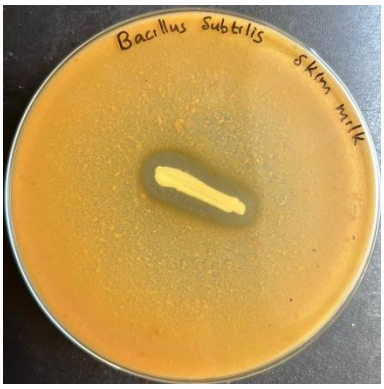

Figure 5:*Protease activity of Bacillus subtilis in skim milk Agar*

3.4) Screening of Bacterial isolate for Bioremediation

Bacterial isolates obtained from sewage samples were screened for their ability to degrade methylene blue by measuring the reduction in optical density over time. The results showed a gradual decrease in optical density of the sample compared to the control, indicating biodegradation activity. During the screening experiment, percentage removal increased from 17.15% at 4 hours to 22.31% at 48 hours. After 48 hours, the degradation efficiency decreased slightly, with 10.94% removal at 72 hours and 8.33% removal at 96 hours, suggesting that maximum biodegradation occurred at 48 hours.

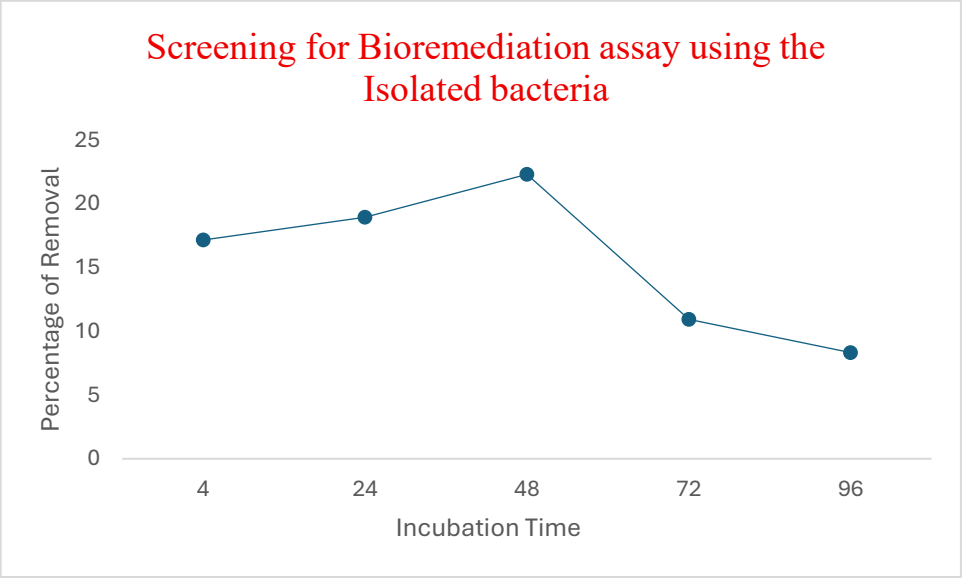

Figure 6: *Screening of isolated bacteria for bioremediation activity over a 96hr incubation period.*

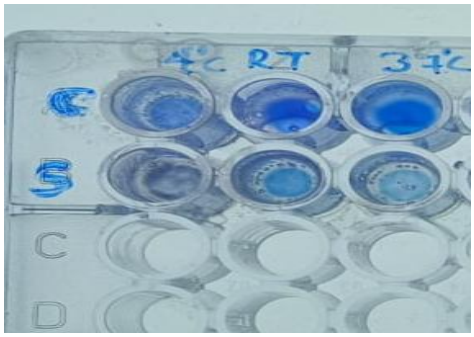

Figure6:*Microtitre plate showing the rate of bioremediation at different hours of incubation*
Molecular characterization of selected bacteria strain

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3.5) Phylogenetic tree

The query sequence exhibits a shared ancestry with the remaining species, which are all members of the genus Bacillus. These closely related species demonstrate a close genetic relationship to the query sequence. The query sequence branch is represented in the tree in a way that makes it very clear that it is in no way related to the other branches, which belong to the organism and have names for their generic categories that are either the same or different from one another. This makes it very clear that the query sequence branch is wholly separate from the other branches.

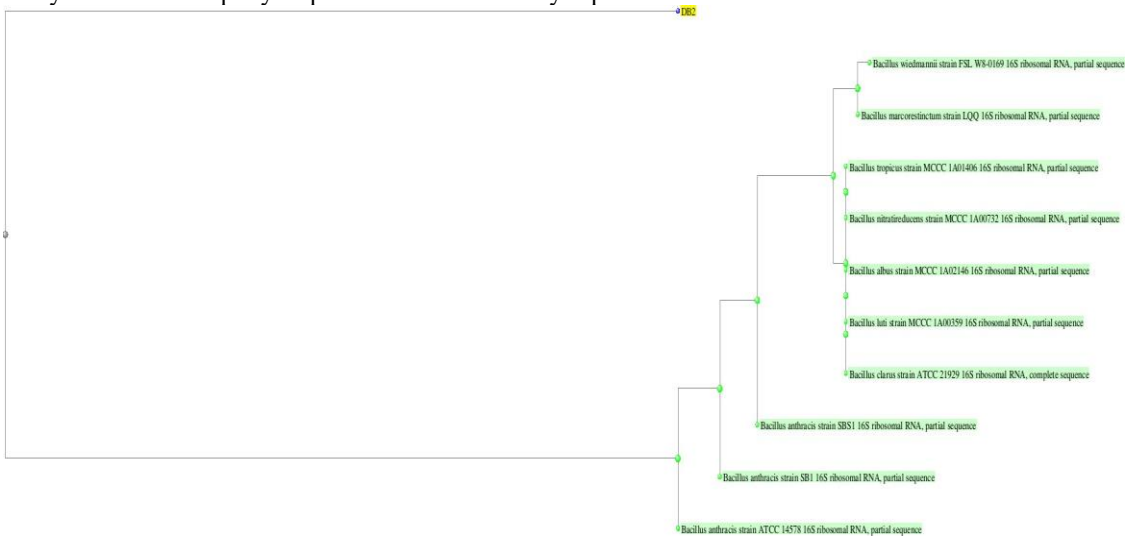

Figure 7:*Phylogenetic tree of bacteria 2 that depicts the relation of query sequence*

3.6) Measurement of Growth of Bacillus

The growth curve of the Bacillus subtilis was measured at 600nm from initial hour to 96 hr . The maximum growth was observed at 48h of incubation (Fig 8). After 48hr, decline in growth of bacteria was observed.

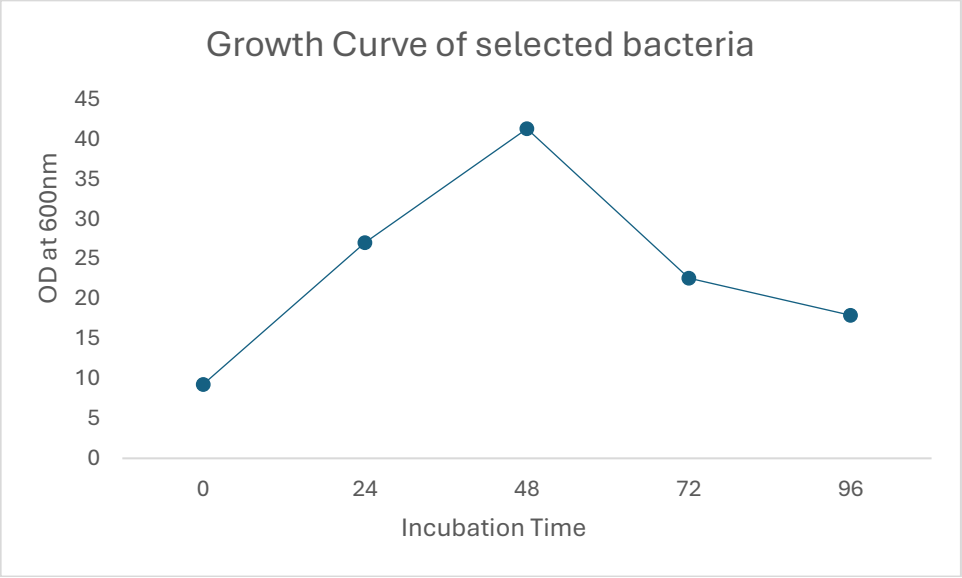

Figure 8: *Growth curve of Bacillus subtilis*

3.7)Optimization of parameters for Bioremediation

3.7.1)*Effect of Temperature on Bioremediation*

The effect of temperature on biodegradation efficiency was also evaluated. The results demonstrated that temperature significantly influenced dye degradation. At 4°C, percentage removal was 13.48%, indicating reduced microbial activity at low temperature. At room temperature, removal increased to 21.76%, showing moderate degradation. The highest degradation was observed at 37°C, where 43.77% removal was recorded, indicating that this temperature was optimal for bacterial activity and enzymatic reactions (fig 9).

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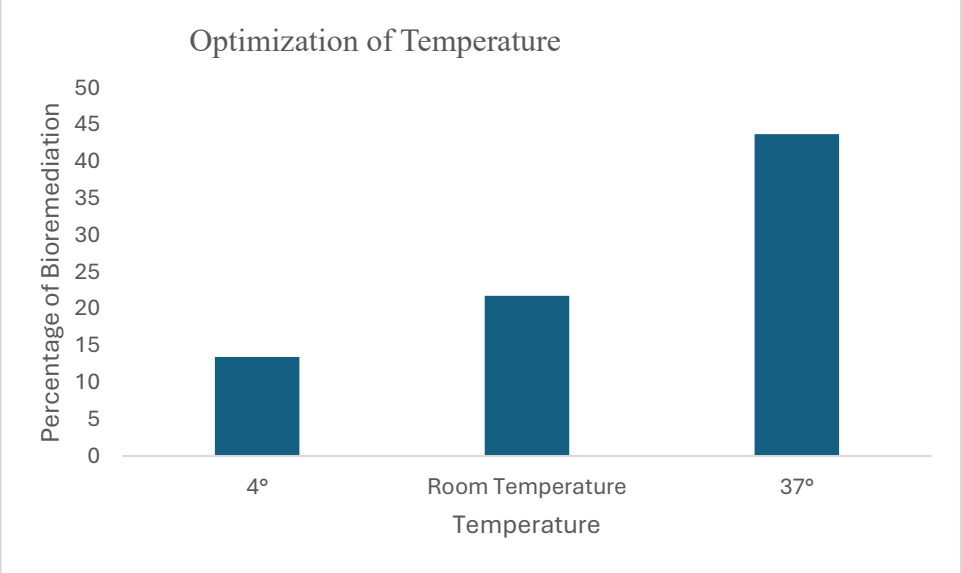

Figure 9:*Effect of Temperature on bioremediation by Bacillus subtills.*

3.7.2)Effect of pH on Bioremediation

The influence of pH on biodegradation efficiency was studied by conducting experiments at pH 5, pH 6, pH 7, pH 8, pH 9 and pH 10. The results showed that degradation varied with pH. At acidic pH lower while at alkaline pH, the percentage of removal was higher. The highest rate of removal was observed at pH 9, 44.98% (Fig 10). The highest degradation was observed at alkaline pH, indicating that alkaline conditions favored biodegradation by the bacterial isolate

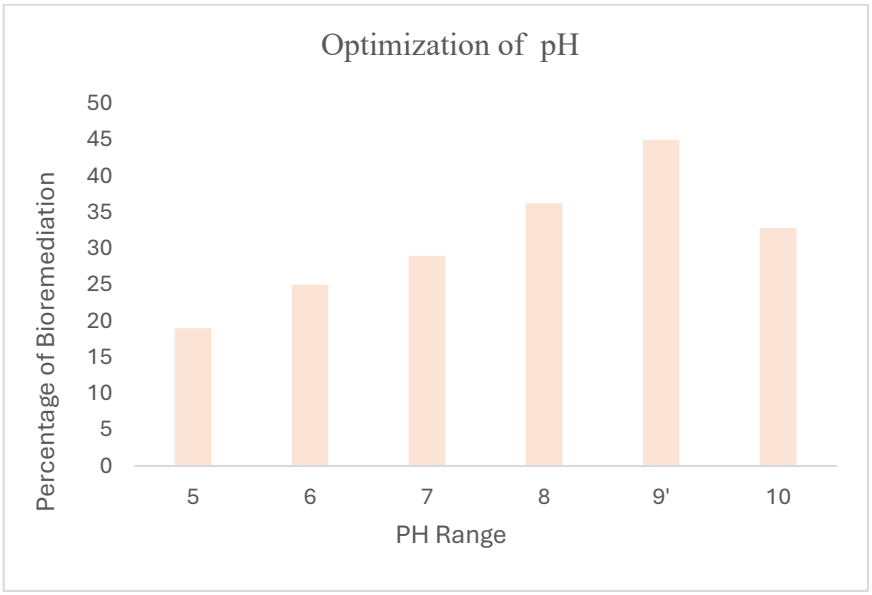

Figure 9:*Optimization of pH for the bioremediation assay*

3.7.3) Effect of NaCl on growth and bioremediation of sewage water

The minimal media was supplemented various concentrations of NaCl (0.5%, 1%, 1.5% and 2%). The salt concentration influenced the rate of removal of waste in sewage as type percentage of salt increased the inhibition rate also increased (Table2). At 2 % a maximum inhibition of 57.2 % was observed (fig10)

Sl.No	Concentration of NaCl	% Inhibition
1	0.5	33.5
2	1	45.19
3	1.5	48.1
4	2	57.20

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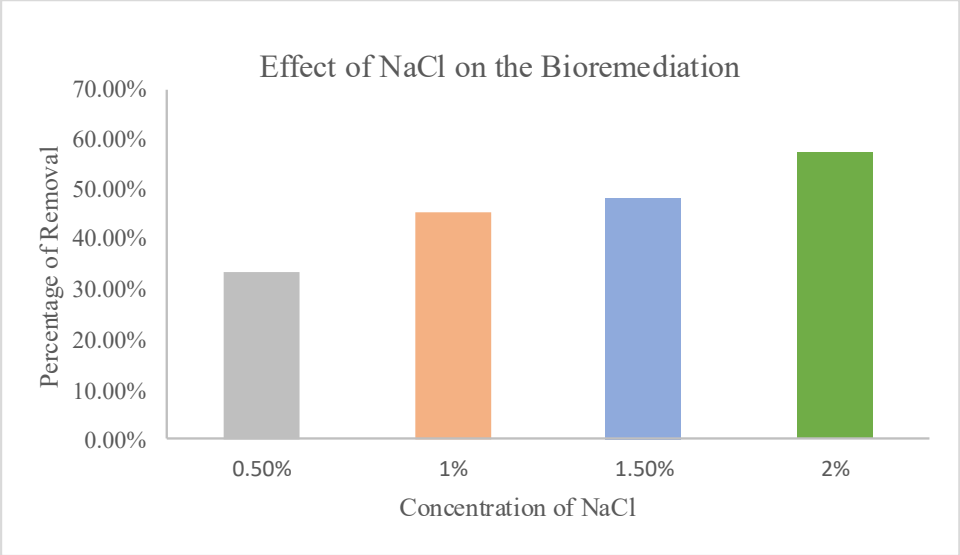

Figure 10:*Effect of NaCl on bioremediation by Bacillus subtilis*

3.7.4) Comparison for Bioremediation activity of bacteria grown in enriched media and normal media

On comparing the bioremediation activity of bacteria cultured in optimized media and normal media showed an increase in rate of bioremediation was observed in broth enriched with the parameters optimized. Under the optimized conditions with media supplemented with 2% NaCl incubated under 37 C with media p^H 9had an increased activity of 65% .The sample A1 containsthe bacillus subtills under normal growth conditions with maximum bioremediation of 32.76%. Mean while the bacillus subtilis at optimized media showed 68.78% bioremediation potential (fig 12).

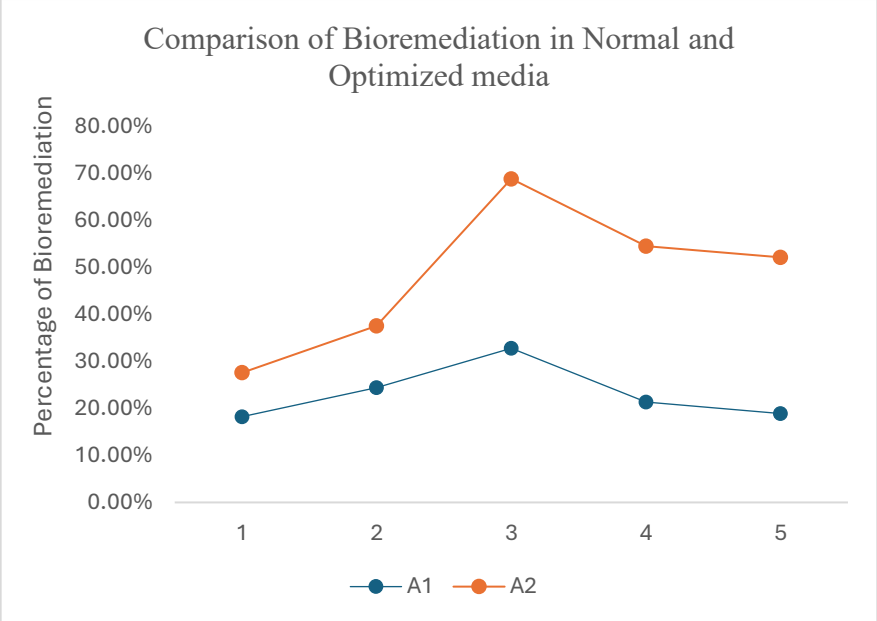

Fig 12: Comparison of bioremediation by Bacillus in normal and optimized media

4) Discussion

In the present study the isolation and characterization of creamy- white, smooth, circular colonies from waste water indicated the presence of well adapted heterotrophic bacteria which are capable of surviving in waste water. The morphological characterization by Gram staining and Biochemical characterization revealed the purified colonies as Gram-positive rod-shaped bacteria with catalase, Voges–Proskauer positive and utilizing citrate. The initial characterization study suggested that the bacterial strain belong to genus Bacillus. The spore forming and diverse metabolic activities of Bacillus species have been reported as their unique property for thriving in polluted environment (Pandey *et al*., 2016).The Bacillus species from waste water also showed catalase positive indicating its ability to withstand oxidative stress, which is common in sewage environment.

The Bacillus sp confirmed its protease activity by forming a clear zone of casein hydrolysis in Skim milk Agar media. The enzyme activity suggests that the isolate can degrade complex organic proteins present in sewage, contributing to organic matter reduction. Such enzymatic capabilities are crucial for effective bioremediation, as wastewater contains a variety of biodegradable macromolecules. (Gupta *et al*., 2002), reported a protease-producing *Bacillus* species have been widely reported for their role in organic matter degradation

Molecular characterization through phylogenetic analysis confirmed that the isolate shares close genetic similarity with other *Bacillus* species, reinforcing the biochemical identification. The distinct clustering of the query sequence within the *Bacillus* lineage highlights its evolutionary relationship and supports its classification.

Bioremediation potential was further validated through the degradation of methylene blue dye, a common model pollutant (ref). The gradual decrease in optical density over time indicates active metabolic degradation. The maximum removal efficiency observed at 48 hours suggests that the bacterial cells reached peak metabolic activity during this period. The subsequent decline in degradation efficiency after 48 hours may be attributed to factors such as nutrient depletion, accumulation of toxic intermediates, or reduced bacterial viability over prolonged incubation (ref). The study on dye degradation reported the decline in degradation efficiency after 48 hours may be due to nutrient depletion or accumulation of toxic intermediates, as reported in earlier studies (Forgacs *et al*., 2004).

Optimization studies demonstrated that temperature, pH, and salinity significantly influence bioremediation efficiency. The highest degradation at 37°C (43.77%) indicates that the isolate exhibits optimal enzymatic activity under mesophilic conditions, which aligns with the known growth range of *Bacillus* species and it is consistent with previous reports on *Bacillus* species (Madigan *et al*., 2018).

At lower temperatures (4°C), reduced activity reflects decreased enzymatic kinetics and microbial metabolism. The adaptability nature and physiological nature of Bacillus made this species as one of the important genus associated with biodegradation, which helps them grow and survive in an ample range of habitats, including hot springs, cold sea water, desert sands, etc. Diverse species of Bacillus was found to thrive in psychrophilic, thermophilic, acidophilic, alkaliphilic, or halotolerant conditions, making them well-suited to specific temperature ranges, pH levels, or salt concentration in the environment in which they live (Drobniewski 1993).

The pH optimization results revealed maximum degradation at alkaline pH 9 indicating that the isolate is alkaliphilic or alkali-tolerant. This is particularly advantageous for treating industrial effluents, which are often alkaline in nature. Reduced degradation at acidic pH further supports the enzyme sensitivity to pH variations.

The salt concentration of 2% favoured the bioremediation activity and may be due to increased enzyme production as there are reports on maximum enzyme production of Bacillus occurs at salt concentration of 1 to 5% (Joo & Chang, 2005).

A significant improvement in bioremediation efficiency (65% removal) was observed under optimized conditions compared to normal media. There are studies signifying the role of physicochemical parameters in improving biodegradation efficiency (Das and Chandran, 2011). This highlights the importance of environmental parameter optimization in enhancing microbial degradation efficiency. The enriched conditions might promoted enhanced microbial growth, improved substrate utilization and better enzyme expression.

Bacillus sp has a diversified adaptability nature, metabolic pathways enabling them to utilize hydrocarbons as carbon and energy sources. The application of Genetic engineering techniques to enhance the hydrocarbon-degrading capabilities of Bacillus spp. by introducing specific genes or pathways that improve their ability to break down hydrocarbons (Pan *et al*. 2023; Rafeeq *et al.* 2023). The sustainability of energy sources and conservation of ecosystem can be effectively improved using Bacillus sp, a promising approach for long -term environmental sustainability.

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