Research Square

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Growth Optimization and Spectroscopic Assessment of Low-Density Polyethylene Deterioration by Bacillus paranthracis and Pseudomonas aeruginosa

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Abstract

Low-density polyethylene (LDPE) is persistent in the environment, and microbial degradation offers a potential laboratory-scale approach to reducing its environmental burden. This study assessed how temperature, pH, supplementary carbon and nitrogen sources, and trace elements affect the growth of two LDPE-associated bacterial isolates from municipal dumpsite soil. We identified the isolates by 16S rRNA gene sequencing and whole-genome analysis as Bacillus paranthracis and Pseudomonas aeruginosa, respectively; we resolved the taxonomic assignment of the Bacillus isolate using whole-genome data because 16S rRNA provides limited resolution within the *Bacillus cereus* group. Under the tested conditions, *B. paranthracis* showed maximum growth at 30 °C and pH 7.0, whereas *P. aeruginosa* showed maximum growth at 35 °C and pH 7.0. Glucose, yeast extract, and the combined trace-element treatment $FeSO_4$, $MnSO_4$, and $ZnSO_4$ supported higher growth in the respective screening experiments. In a 90-day LDPE assay, apparent mass loss was 3.59 ± 0.01% for *B. paranthracis* and 4.85 ± 0.24% for *P. aeruginosa*. ATR-FTIR revealed treatment-associated changes in oxygen-containing functional groups. Because complete mineralization, carbon dioxide evolution, and polymer molecular-weight reduction were not measured, the results are interpreted as evidence of LDPE deterioration rather than complete biodegradation. Further work should include abiotic controls, carbon mineralization, molecular-weight analysis and statistically designed optimization.

Introduction

Plastics are man-made, long-chain polymers. The word ‘plastic’ derives from the Greek ‘plastikos’, meaning “to mold into anything” (Lutcavage et al., 1997; Kumar et al., 2017; Sussman et al., 1998). Plastics are mostly organic polymers, often with heteroatoms, that are resistant to biodegradation by enzymes and microorganisms. (Zaeko *et al*., 2015). Plastic is more affordable, thermal, flexible, robust, and electromagnetic insulator material (Mahitha et al., 2022). In 100 years, synthetic plastics are used widely in daily life including packaging, manufacturing, toys, automobiles, industries, buildings, health sectors, electronics, agriculture, transportation (Demirkan et al., 2020; Bodor et al., 2024). Wood, metal and glass materials are replaced by plastics because of their flexible, durable, cheap, high strength, light weight nature (Yao et al., 2022; Abatenh et al., 2017; Bodor et al., 2024).

Residential waste, industrial waste, agricultural waste, electronic waste, medical waste disposal are the main sources of plastic. Domestic wastes may be in form of plastic carrier bags, packaging wastes from different forms of food, papers, diapers, vegetable wastes, animal waste, and others, which can be hazardous or non-hazardous. Plastic waste occurs in industrial activities in the construction, electronics, automotive, and packaging industries, having many applications in the agricultural industry, including irrigation, protecting crops, and packaging. Various types of plastic PPE have played an important role in saving human life during the COVID-19 pandemic. (Kibria, et al., 2023; Surendarnath, et al., 2025; Vanapalli, et al., 2021). Plastic waste, especially carry bags, causes severe environmental and health hazards through methane emissions, leachate contamination, disease spread, and toxic air pollution from open burning (Kumar, et al., 2017).

The MSWM (Municipal Solid Waste Management) system in India is failing to cope up with rapid urbanization, and there are some areas where improvement is needed in order to make waste management sustainable (Joshi & Ahmed, 2016). Despite the advancements in sustainable waste management, improper waste management practices owing to lack of financial resources and operational efficiency still lead to biodiversity loss, ecosystem destruction, resource exhaustion, pollution by microplastics, and production of municipal waste (Singh & Sharma, 2016). Plastics are robust and cheap materials that persist in nature and pose great threats to the ecosystem, hence the need for microbial degradation through the actions of bacteria and fungi (Doshi & Chhaya, 2024).

Therefore, the present study aimed to optimize temperature, pH, carbon and nitrogen sources, and trace elements for the growth of *B. paranthracis* and *P. aeruginosa* on LDPE, using an OFAT design, and to identify the conditions that maximize LDPE biodegradation potential.

Material

LDPE-degrading bacterial isolates

Two LDPE-degrading bacterial isolates have been isolated from municipal dump site soil and selected based on a weight loss study and FTIR assay of a polyethylene bag piece (Doshi, et al., 2026). The isolates were provisionally identified as *Bacillus paramycoides* and *Pseudomonas aeruginosa*, having accession no. PZ134689 and PZ134699, respectively, by 16s rRNA gene sequencing on NCBI portal; https://submit.ncbi.nlm.nih.gov/. Whole-genome analysis subsequently assigned the isolate to *Bacillus paranthracis* and *Pseudomonas aeruginosa* based on genome-wide taxonomic comparisons. Because 16s rRNA resolution within the *Bacillus cereus* group can be limited, whole-genome identification was used for the final taxonomic assignment.

Pretreatment of LDPE piece

Low-density polyethylene (LDPE), bags used in this study were obtained from local market plastic bags having 25 × 35 cm dimensions and approximately 20–30 µm thickness, Ahmedabad, India. LDPE was cut into small pieces of about (2 cm×2 cm) and put into a sterile beaker with distilled water, where it was kept under static conditions for an hour. Additionally, they were aseptically exposed for 30 minutes to a 70% (v/v) ethanol solution once. The LDPE pieces were then put onto a clean Petri dish. The LDPE pieces were air-dried and weighed 10.0 mg (Hussein, et al., 2015, Katru, et al., 2023).

Optimization of polyethylene biodegradation

The selected conditions represented the best-performing levels among the tested individual factors and should not be interpreted as a statistically optimized global condition. The following factors are used for this purpose: pH, temperature, carbon source, nitrogen source, and trace elements (Hussein, et al., 2015).

Effect of Temperature

50 ml of liquid MSM along with one piece of LDPE bag (2 cm X 2 cm) was prepared and put in 100 ml Erlenmeyer flasks containing (pH 7) at different temperatures (25 ℃, 30 ℃, 35 ℃, 40 ℃) in triplicate (n = 3) for both isolates (5% v/v) in separate flasks. Bacterial growth was quantified spectrophotometrically to check out the effect of temperature on *B. paranthracis* and *P. aeruginosa* individually under static conditions (Hussein, et al., 2015; Dhaka, et al., 2024; Katru, et al., 2023; Seong, et al., 2024).

Effect of pH

To evaluate the impact of pH on the degradation efficiency of LDPE by both isolates, 50 ml of liquid MSM along with one piece of LDPE bag (2 cm X 2 cm) was prepared and put in 100 ml Erlenmeyer flasks containing various pH levels (5.5, 6.5, 7.0, 8.0, and 9.0) in triplicate (n = 3) for both isolates in separate flasks. The cultures (5% v/v) were then incubated under static conditions, and after incubation, bacterial growth was quantified spectrophotometrically (Hussein, et al., 2015; Katru, et al., 2023; Seong, et al., 2024).

Effect of Carbon Source

To determine the influence of carbon sources on their capacity to degrade LDPE, 50 ml of liquid MSM along with one piece of LDPE bag (2 cm X 2 cm) was prepared and put in 100 ml Erlenmeyer flasks containing different carbon sources (only LDPE, Glucose, Sucrose, and Glycerol) were added individually at an equal concentration of 1.0 g/L to ensure comparison on an equivalent weight basis in triplicate (n = 3) for both isolates (5% v/v) separate flask. Both isolates were then allowed to grow in an incubator at 37 ℃ and pH 7.0 under static conditions. Bacterial growth was quantified spectrophotometrically (Seong, et al., 2024; Yao, et al., 2022).

Effect of Nitrogen Source

For determining the influence of nitrogen sources on degradation of the two isolates, 50 ml of liquid MSM along with one piece of LDPE bag (2 cm X 2 cm) was prepared and put in 100 ml Erlenmeyer flasks containing various nitrogen sources (Ammonium Chloride; NH_4Cl, Urea, Yeast extract and Peptone) were made in triplicate (n = 3) for both isolates (5% v/v) separate flask. Incubation was done at 37 ℃ and at pH of 7.0 under static conditions. Bacterial growth was quantified spectrophotometrically (Seong, et al., 2024).

Trace Elements

To establish the influence of the trace elements on degradation capacity of both isolates, 50 ml of liquid MSM along with one piece of LDPE bag (2 cm X 2 cm) was prepared and put in 100 ml Erlenmeyer flasks containing various trace elements (Ferrous sulfate; $FeSO_4$, Manganese (II) Sulfate; $MnSO_4$, Zinc Sulfate; $ZnSO_4$, $FeSO_4$ + $MnSO_4$ + $ZnSO_4$) were prepared and subsequently incubated at 37 ℃ and pH 7.0 under static condition in triplicate (n = 3) for both isolates (5% v/v) separate flask. Upon completion of the incubation period, bacterial growth was quantified spectrophotometrically. (Yao, et al., 2022).

Biodegradation Assay: Weight loss study and ATR - FTIR

Based on the previous experimental study, Pure bacterial isolates were inoculated into 100 mL of MSM containing 5% (v/v) actively growing culture and a 2 × 2 cm LDPE film as the sole carbon source. Cultures were incubated statically at 37 ℃ for 90 days in triplicate (n = 3), and polyethylene films were collected every 15-day interval to determine biodegradation by percentage weight loss after washing and drying (Doshi, et al., 2026). The percentage weight loss was calculated by the following equation (Nadeem et al. 2021).

$$\varvec{W}\varvec{e}\varvec{i}\varvec{g}\varvec{h}\varvec{t}\ \varvec{l}\varvec{o}\varvec{s}\varvec{s}\ (\varvec{\%}) = \frac{\varvec{I}\varvec{n}\varvec{i}\varvec{}}{}$$

Modification of polymer bonds as a result of biodegradation was performed through Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy. After being subjected to bacterial action for 90 days, the LDPE films were collected, cleaned using 2% SDS solution, air-dried under vacuum, and examined from 4000–500 cm^{-1} wavelength. The degree of biodegradation of the polymers was evaluated depending on changes in functional groups and polymer bonds (Doshi, et al., 2026).

Results and Discussion

Biodegradation serves as an economic means for recycling of wastes and performs faster than what would have been done naturally if conditions were optimized (Tokiwa et al., 2009).

Effect of Temperature

The effect on growth of *B. paranthracis* and *P. aeruginosa* was determined at 25 ℃, 30 ℃, 35 ℃ and 40 ℃ for 96 h using OD_{600}. Both isolates were mesophilic in nature, with an increasing OD value from 24 to 72 h followed by a drop at 96 h, signifying the entry into the stationary phase. *B. paranthracis* grew optimally at 30°C (OD_{600} 1.60 ± 0.02 at 72 h) while *P. aeruginosa* grew optimally at 35°C (OD_{600} 1.80 ± 0.03 at 72 h). Growth at 25 ℃ and 40 ℃ was much lower in both isolates, indicating that suboptimal temperatures had adverse effects on bacterial growth. The higher growth observed for *P. aeruginosa* under these conditions was consistent with its higher mass loss in the separate LDPE assay and FTIR spectral changes. However, growth alone does not establish LDPE biodegradation (Doshi, et al., 2026).

The LDPE-degrading ability was observed for *Pseudomonas fluorescens, Pseudomonas aeruginosa*, and *Acinetobacter ursingii* when these bacteria were incubated in MSM at pH 7.0 and 30°C for 15 days. The isolates have optical density values of 0.351, 0.272, and 0.241 with the production of dissolved CO_2 of 1.18, 1.05, and 0.95 g/L, indicating their efficient LDPE-degrading activity (Hussein, et al., 2015). Under optimum conditions, the bacterial strain *SARR1* exhibited 38.3 ± 1.27% biodegradation efficiency of the pre-treated LDPE films at 37°C and pH 7.0 within 30 days of incubation (Rani, et al., 2021).

Optimization study for polyester degrading enzyme production by *Actinomadura sp. S14, Actinomadura sp. TF1, Streptomyces sp. APL3, and Laceyella sp. TP4* showed the maximum activity of enzyme was produced at 40–60 ℃ temperature (Sriyapaia *et al*., 2018).

Effect of pH

The impact of pH on the growth of *B. paranthracis* and *P. aeruginosa* was determined at pH values of 5.5, 6.5, 7.0, 8.0, and 9.0 over a period of 96 h using OD_{600} measurements. Both isolates showed the highest growth rates under neutral pH conditions, with a progressive increase in OD values from 24 to 72 h followed by a slight decrease or stabilization at 96 h. This means that the stationary phase of growth started for *B. paranthracis* and *P. aeruginosa*. The highest growth rate was reached for pH 7.0 (OD_{600} 1.45 ± 0.02 for *B. paranthracis* and OD_{600} 1.78 ± 0.03 for *P. aeruginosa* at 72 h), whereas lower growth was detected under acidic (pH 5.5–6.5) and alkaline (pH 9.0) pH levels. Despite the fact that both isolates showed rather good growth at pH 8.0, optimal bacterial proliferation was observed for neutral pH conditions. Higher growth rate of *P. aeruginosa* at pH 7.0 correlates well with the efficiency of LDPE biodegradation and changes in the FTIR spectrum of the bacteria at neutral pH observed earlier (Doshi, et al., 2026).

Optimization study for polyester-degrading enzyme production by *Actinomadura sp. S14, Actinomadura sp. TF1, Streptomyces sp. APL3, and Laceyella sp. TP4* showed the maximum activity of the enzyme was produced at 6.0–8.0 pH (Sriyapaia *et al*., 2018). S-6 (*Paenibacillus sp.*) and S-2 (*Serratia sp.*): The optimum conditions for the LDPE biodegradation are contact time 72 h, temperature 37 ℃, pH 7.1, volume of inoculum 4% v/v, weight of LDPE films 0.030 g (Mallisetty, et al., 2023). The biodegradation test was done at different pH and temperatures, and was determined through microbial growth, optical density, and weight loss of polyethylene in 28 days. *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Pseudomonas putida*, and *Arthrobacter citreus* showed good potential to degrade polyethylene. The weight loss was from 10–50% and occurred maximally at pH 7.0 and 37 ℃ (Bashir, et al., 2025).

Effect of Carbon Source

The impact of various carbon sources on the growth rate of *B. paranthracis* and *P. aeruginosa* was studied through the optical density (OD_{600}) determination within 96 h of incubation when isolated bacteria were grown in the medium containing LDPE, glucose, sucrose, and glycerol. These experiments assessed growth responses to supplementary carbon sources; they were not used as direct measures of LDPE mineralization. As seen, both strains demonstrated slow growth within 24–72 h; then the growth rate was stabilized or decreased after 96 h. In regard to different carbon sources, it should be noted that *P. aeruginosa* grew best in the medium containing glucose (OD_{600} 0.80 ± 0.05 at 96 h), while for *B. paranthracis*, growth rates in glucose and glycerol were almost similar (OD_{600} 0.61 ± 0.02 at 72 h). Also, sucrose stimulated bacterial growth, and the lowest optical density value was revealed for bacteria grown in the medium containing only LDPE. It should be mentioned that the increased growth rate of *P. aeruginosa* in the optimized carbon source correlates with its higher efficiency of LDPE biodegradation and changes of FTIR spectra obtained previously (Doshi, et al., 2026).

Optimization study for polyester-degrading enzyme production by *Actinomadura sp. S14, Actinomadura sp. TF1, Streptomyces sp. APL3, and Laceyella sp. TP4* showed that the maximum activity of the enzyme was produced at the incubation with 1% (w/v) PCL (18 U/mL), 0.5% (w/v) PLA (22.3 U/mL), 1% (w/v) PBS (19.4 U/mL), and 0.5% (w/v) PBSA (6.3 U/mL) as carbon source, respectively (Sriyapaia *et al*., 2018).

Effect of Nitrogen Source

Influence of various types of nitrogen sources on the growth of *B. paranthracis* and *P. aeruginosa* was estimated using NH_4Cl, yeast extract, urea, and peptone in the time period up to 96 h using optical density (OD_{600}). Both strains showed an increase in their growth gradually up to 72 h, after which there was a small decrease or stabilization in growth until 96 h. Among the nitrogen sources tested, yeast extract supported the highest optical density for both isolates, since the maximum OD_{600} of *B. paranthracis* was 1.49 ± 0.02 and for *P. aeruginosa* it was 1.58 ± 0.03. The growth on peptone was also considerable, but growth on NH_4Cl and urea gave the lowest optical density values during the incubation process. The increased growth of *P. aeruginosa* in yeast extract corresponds to its previously observed increased efficiency in LDPE degradation and FTIR changes (Doshi, et al., 2026).

Trace Elements

The influence of various trace elements on the development of *B. paranthracis* and *P. aeruginosa* was studied using $FeSO_4$, $MnSO_4$, $ZnSO_4$, and their combination $FeSO_4$ + $MnSO_4$ + $ZnSO_4$ within 96 h according to the results of the optical density (OD_{600}). It was found out that both strains demonstrated an increase in growth from 24 to 72 h, then a slight reduction or stability at 96 h. Among individual trace elements, $FeSO_4$ had the most favorable effect on bacterial growth; $MnSO_4$ and $ZnSO_4$ also improved the growth of microorganisms compared to the control (no trace elements). Most importantly, the simultaneous use of $FeSO_4$, $MnSO_4$, and $ZnSO_4$ increased the growth of both isolates up to the maximal level – OD_{600} values were 1.67 ± 0.06 for *B. paranthracis* and 1.79 ± 0.09 for *P. aeruginosa* after 48 h. In addition, it was established that the higher growth of *P. aeruginosa* compared to the control strain was connected with the greater efficiency of LDPE biodegradation by the same strain and the significant changes in the FTIR spectrum as per previous studies (Doshi, et al., 2026).

Weight loss study and FTIR

From previous experimental studies, biodegradation assays were carried out in triplicate by incubating 10 mg LDPE strips in MSM medium with each strain of bacteria for 90 days. It was observed that *P. aeruginosa* had better biodegradation potential (4.85 ± 0.24% weight loss) as compared to *B. paranthracis* (3.59 ± 0.01%). Also, FTIR spectra (4000 – 500 cm^{-1}) demonstrated substantial chemical changes in LDPE films after bacterial exposure. *P. aeruginosa* caused higher production of O-H and C = O functional groups with a considerable decrease in peaks typical for polyethylene, showing intensive oxidation of carbon chains and decreased crystallinity, while *B. paranthracis* demonstrated less pronounced spectrum changes (Doshi, et al., 2026).

Conclusion

The present study identified pH 7.0 and mesophilic temperatures as favorable conditions for the growth of the two LDPE-associated isolates under the tested conditions. Among the supplementary nutrients examined, glucose, yeast extract, and the combined trace-element treatment supported higher bacterial growth in the screening experiments. After 90 days, LDPE films incubated with *P. aeruginosa* exhibited greater apparent mass loss than those incubated with *B. paranthracis*. ATR-FTIR revealed treatment-associated changes in oxygen-containing functional groups, consistent with oxidative modification of the polymer surface. Because abiotic controls, CO_2 evolution, and polymer molecular-weight analysis were not fully established, these results should be interpreted as evidence of LDPE deterioration rather than complete biodegradation. Future work should employ statistically designed optimization, include appropriate abiotic controls, quantify CO_2 evolution, and incorporate SEM and molecular-weight analyses to more conclusively establish biodegradation mechanisms and extent.

Declarations

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Ethics and Consent to Participate declarations: Ethical approval was not required for this study, as it did not involve human participants, animals, or human-derived materials. The study involved environmental soil samples and bacterial isolates used for laboratory-based biodegradation experiments.

Author Contributions: Kruti Doshi contributed to the conceptualization, methodology, investigation, data collection, experimental work, data analysis, and writing of the original manuscript. Ripal Fadiya contributed to reviewing the manuscript. Dhruvil Brahmbhatt contributed to supervision, research guidance, interpretation of results, and critical review and editing of the manuscript. All authors read and approved the final version of the manuscript.

Data Availability Statement: The datasets generated and/or analyzed in this study have been deposited in the NCBI GenBank database and are publicly accessible under accession numbers PZ134689 (Bacillus paramycoides) and PZ134699 (Pseudomonas aeruginosa). The 16S rRNA gene sequences generated in this study are publicly available in the NCBI GenBank repository. The data can be accessed through the following permanent database link: https://www.ncbi.nlm.nih.gov/genbank/.

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Figures



Figure 1

Sources of Plastic Pollution (Kibria, *et al*., 2023; Surendarnath, *et al*., 2025; Vanapalli, *et al*., 2021)

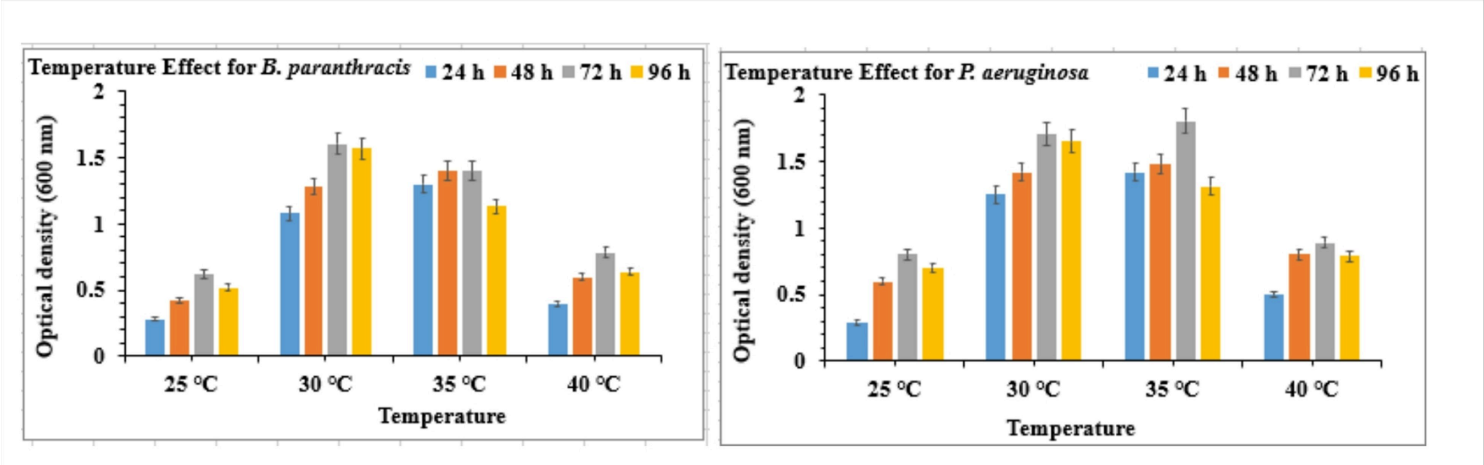

Figure 2

Effect of temperature on bacterial strains (*Bacillus paranthracis* and *Pseudomonas aeruginosa*)

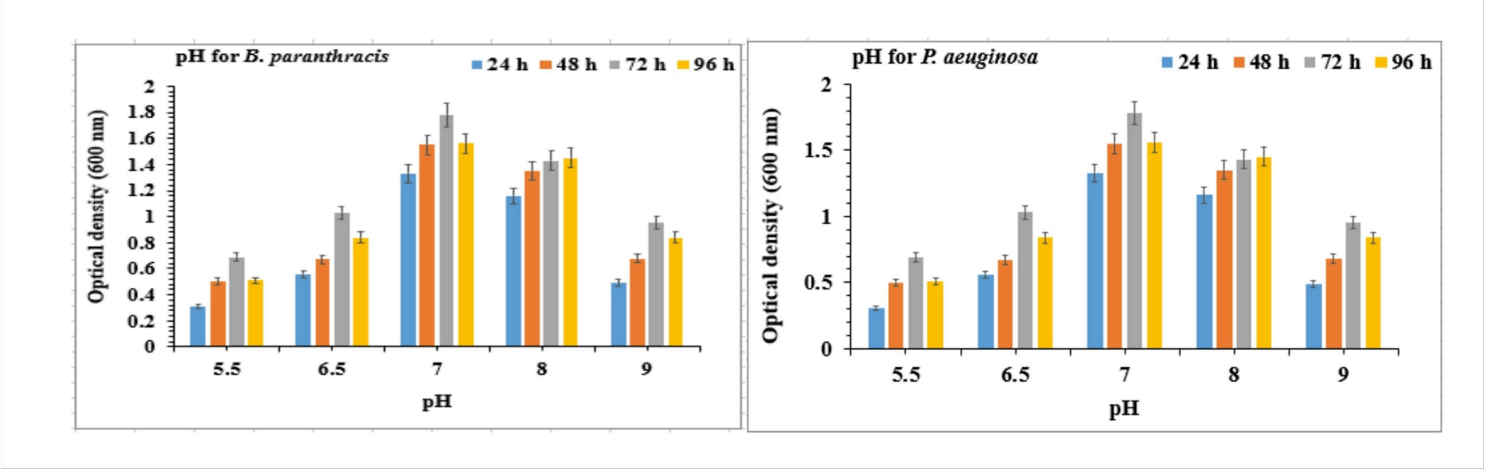

Figure 3

Effect of pH on bacterial strains (*Bacillus paranthracis* and *Pseudomonas aeruginosa*)

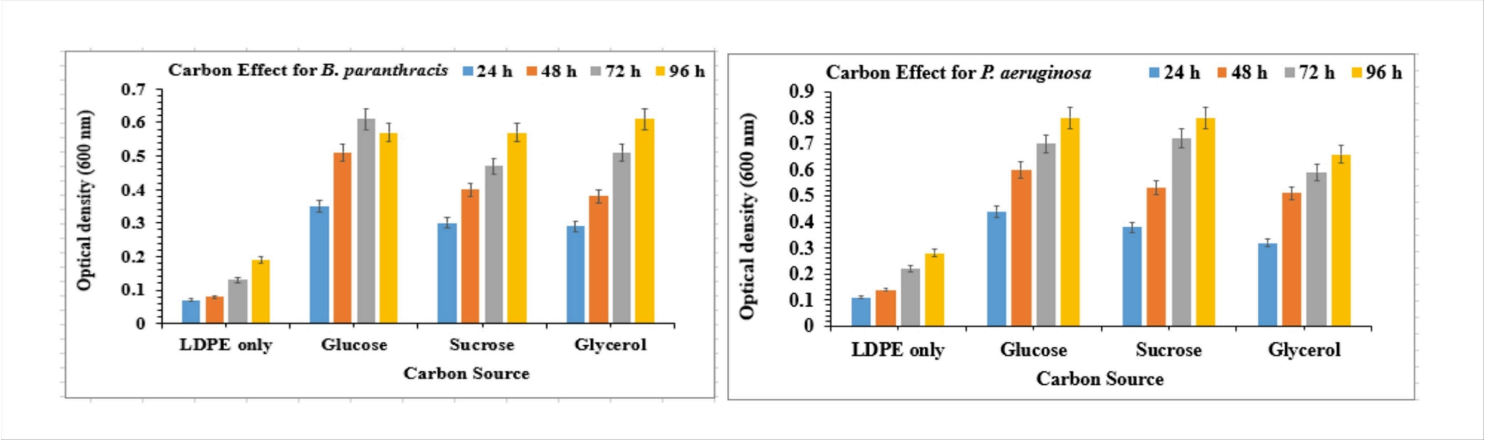

Figure 4

Effect of carbon sources on bacterial strains (*Bacillus paranthracis* and *Pseudomonas aeruginosa*)

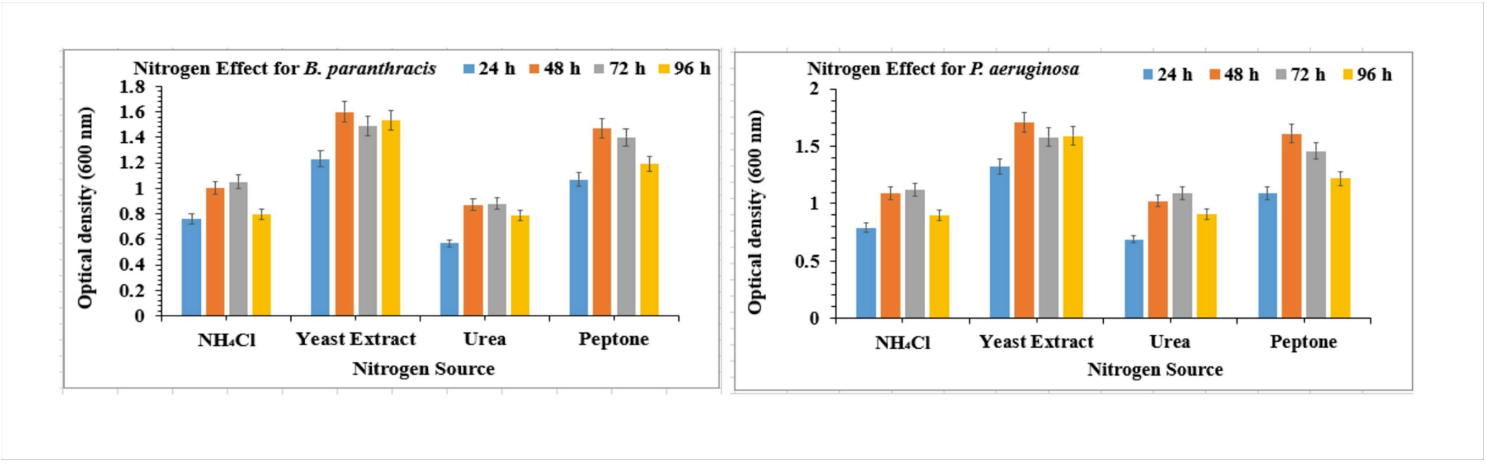

Figure 5

Effect of nitrogen sources on bacterial strains (*Bacillus paranthracis* and *Pseudomonas aeruginosa*)

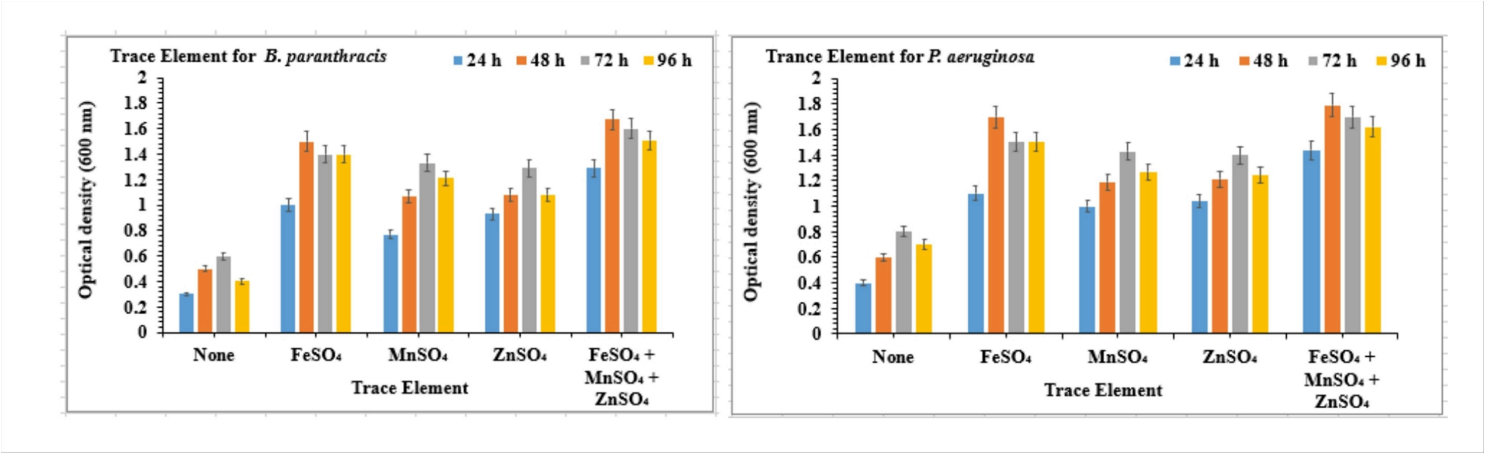

Figure 6

Effect of trace elements on bacterial strains (*Bacillus paranthracis* and *Pseudomonas aeruginosa*)