INTERNATIONAL JOURNAL OF LATEST TECHNOLOGY IN ENGINEERING, MANAGEMENT & APPLIED SCIENCE (IJLTEMAS)

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XV, Issue VII, July 2026

Comparative Effects of Selected Food Preservatives from Expired Food Products on the Growth and Biodegradation Potential of Mesophilic Saprophytic Bacteria

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DOI: https://doi.org/10.51583/IJLTEMAS.2026.150700027

Received: 21 July 2026; Accepted: 26 July 2026; Published: 06 August 2026

ABSTRACT

The growing number of expired processed food products has become a serious environmental concern since food preservatives used to prolong product shelf life since it can also inhibit the microbes responsible for organic waste decomposition. This study examined the impact of Class II food preservatives found in expired food products. The class II preservatives, such as sulphur dioxide, nitrates and nitrites, benzoic acid, and sorbic acid, were tested on the growth and biodegradation capability of mesophilic saprophytic bacteria involved in solid organic waste degradation. The four bacterial species, such as Pseudomonas spp., Bacillus subtilis, Staphylococcus spp., and Cellulomonas spp., were studied by comparing the breakdown period of preservative-treated food waste to untreated controls. The significance of preservation effects was determined using statistical methods such as independent sample t-tests, one-way and two-way ANOVA, and linear regression. All of preservatives considerably extended the degradation duration in comparison to the control ($p < 0.05$). Independent t-tests revealed significant differences in sulphur dioxide ($t = -8.45$, $p = 0.00017$), nitrates and nitrites ($t = -11.90$, $p = 0.00006$), benzoic acid ($t = -10.06$, $p = 0.00014$), and sorbic acid ($t = -6.26$, $p = 0.00133$). Two-way ANOVA revealed significant effects of preservative treatments and microbial species on degradation behavior ($p < 0.001$). Regression analysis demonstrated a strong positive connection between natural degradation time and preservative-treated degradation time ($r = 0.925$), demonstrating that preservatives have a significant negative effect on microbial activity.

Keywords: Expired food products; Food preservatives; Mesophilic saprophytic bacteria; Organic waste biodegradation; Statistical analysis; ANOVA; Regression analysis; Environmental microbiology.

INTRODUCTIONS

Food waste has emerged as one of the most significant environmental challenges worldwide, with approximately one-third of food produced for human consumption being lost or wasted throughout the food supply chain. The disposal of food waste not only results in economic losses but also contributes substantially to greenhouse gas emissions, environmental pollution, and inefficient utilization of natural resources. Organic food waste constitutes the largest biodegradable fraction of municipal solid waste and is primarily decomposed through microbial activity, making efficient biodegradation essential for sustainable waste management (Urugo et al., 2024; Xue, 2024). Food manufacturing industries frequently use chemical preservatives such as sulfur dioxide, benzoic acid, sorbic acid, nitrates, and nitrites to increase shelf life and guarantee the microbiological safety of processed goods. By interfering with microbial metabolism, disrupting cell membrane permeability, and inhibiting enzymes, these preservatives prevent the growth of harmful and spoiling bacteria. Even while these substances successfully maintain food quality while it is being stored, leftover preservatives can still have antimicrobial effects on the environment and stay in expired food products after they are disposed (Snyder et al., 2024)

Mesophilic saprophytic bacteria are responsible for the breakdown of complex organic substrates, such as carbon and energy sources. The breakdown of carbohydrates, proteins, lipids, and cellulose is influenced by

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bacterial species such as Pseudomonas, Bacillus, Cellulomonas, and Staphylococcus, which promote nutrient recycling and reduce waste accumulation (Rastogi et al., 2020; Duan et al., 2024). The extrinsic factors influence the growth of bacterial species. These factors include temperature, moisture content, oxygen availability, pH, and the presence of inhibitory substances (Duan et al., 2024; Gao et al., 2024). Therefore, antimicrobial compounds in food waste may have a detrimental effect on microbial proliferation, enzyme activity, and microbial community structure, delaying the organic waste's decomposition.(Gao et al., 2024; Snyder et al., 2024).

According to recent research, chemical additives and antimicrobial substances found in food waste can change the composition of microbial communities, inhibit microbial metabolism, and lower the effectiveness of biodegradation during the composting and waste treatment processes. Such inhibition may lengthen the time it takes for decomposition to occur, reduce the effectiveness of composting, and raise the environmental cost of disposing of organic waste. The direct impact of leftover food preservatives from manufactured foods that have expired on the biodegradation capacity of naturally occurring mesophilic saprophytic bacteria is not well understood, despite substantial study on compost microbiology and food preservation (Siddique F et al., 2024).

MATERIALS AND METHODS

Study Design

The present study was designed to investigate the effects of selected food preservatives commonly present in expired processed food products on the biodegradation potential of mesophilic saprophytic bacteria involved in the decomposition of solid organic waste. A comparative experimental design was employed by evaluating the degradation time of preservative-treated food waste against untreated control samples. Statistical analyses were performed to determine the significance of preservative-induced changes in microbial degradation.

Microorganisms

Four mesophilic saprophytic bacterial species commonly associated with organic waste decomposition were selected for the study:

- *Pseudomonas* spp.
- *Bacillus subtilis*
- *Staphylococcus* spp.
- *Cellulomonas* spp.

These microorganisms were selected because of their ability to degrade carbohydrates, proteins, lipids, and cellulose during aerobic decomposition of organic waste.

Selection of Food Preservatives

Four commonly used Class II food preservatives were selected based on their widespread application in processed foods.

Food Preservative	**Food Product**
Sulphur dioxide (SO_2)	Fruit-based beverages
Nitrates and nitrites	Processed meat products
Benzoic acid and sodium benzoate	Spice-based processed products
Sorbic acid and potassium sorbate	Tomato ketchup and vegetable products

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Expired commercially available food products containing these preservatives were used as representative sources of preservative-treated organic waste.

Experimental Procedure

Equal quantities of solid organic food waste were prepared from expired processed food products containing the selected preservatives. Untreated organic waste without preservatives served as the control. Each bacterial culture was inoculated separately into both control and preservative-treated organic waste samples under mesophilic aerobic conditions. The degradation process was monitored until complete decomposition of the organic substrate was observed. The degradation period was recorded in days for each microorganism under both control and preservative-treated conditions.

Experimental Variables

Independent variable

- Type of food preservative

Dependent variable

- Time required for biodegradation of solid organic waste (days)

Controlled variables

- Temperature
- Moisture content
- Aeration
- Initial quantity of waste
- Inoculum concentration
- Incubation period
- pH

Statistical Analysis

Experimental data were analysed using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA).

Independent Sample *t*-Test

Independent sample *t*-tests were conducted to compare degradation time between control and preservative-treated samples for each food preservative. Statistical significance was considered at $p < 0.05$.

One-Way Analysis of Variance (ANOVA)

One-way ANOVA was performed to evaluate the effect of different preservative treatments on degradation time.

Two-Way Analysis of Variance

Two-way ANOVA was used to determine the interaction between bacterial species and preservative treatments on the degradation behaviour of solid organic waste.

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Regression Analysis

Simple linear regression analysis was performed to examine the relationship between natural degradation time and degradation time in the presence of food preservatives. The regression equation and Pearson correlation coefficient (*r*) were calculated to evaluate the strength of the association.

A significance level of **α = 0.05** was used for all statistical analyses.

Data Presentation

Results were expressed as degradation time (days) and presented using tables and statistical parameters including mean values, *t*-values, F-values, regression equations, correlation coefficients (*r*), and corresponding *p*-values. Statistical significance was accepted when $p < 0.05$.

Observation Table

t-test Analysis

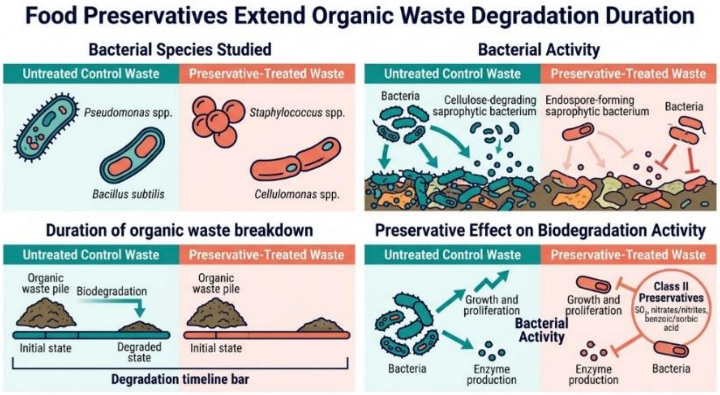
Independent sample t-tests showed significant differences between control and preservative-treated samples.

Preservative	t-value	p-value	Result
Sulphur dioxide	-8.45	0.00017	Significant
Nitrates & nitrites	-11.90	0.00006	Significant
Benzoic acid	-10.06	0.00014	Significant
Sorbic acid	-6.26	0.00133	Significant

Result: All calculated p-values were less than 0.05, indicating that food preservatives significantly increased degradation time.

ANOVA

Source of Variation	SS	df	MS	F	p-value	F crit
Preservative Treatment	39462.94	7	5637.56	87.43	1.60×10^{-15}	2.42
Microbial Observations (Error)	1548.00	24	64.50			
Total	41010.94	31				



RESULT

The ANOVA results indicate a highly significant difference among different preservative treatments because the calculated F-value is much greater than the critical F-value and $p < 0.05$.

Source of Variation	SS	df	MS	F	p-value	F crit
Microorganisms	1421.84	3	473.95	27.31	1.93×10^{-7}	3.07
Preservative Treatments	45551.2	7	6507.32	375	1.11×10^{-16}	2.49
Error	364.41	21	17.35			
Total	47337.5	31				

The two-way ANOVA demonstrates that: the calculated F-value is much greater than the critical F-value, and $p < 0.05$

1. Different microorganisms show significantly different degradation behaviour.
2. Food preservatives significantly increase the decomposition time of solid organic waste.

Regression Analysis: - Linear regression analysis showed a strong positive relationship between natural degradation time and degradation time in the presence of preservatives.

Regression Equation: $y = 1.176x + 38.87$

Correlation coefficient (r) = 0.925

FINAL CONCLUSION

The present investigation showed that food preservatives, which frequently appear in processed foods that have expired, possess a substantial impact on the biodegradation of solid organic waste by preventing mesophilic saprophytic bacteria from growing. When compared to untreated control samples, comparative analysis showed that all of the chosen preservatives—sorbic acid, benzoic acid, nitrates and nitrites, and sulfur dioxide—significantly lengthened the time it took for organic waste to decompose. Nitrates and nitrites showed the strongest inhibitory effect on microbial degradation among the preservatives tested, followed by sorbic acid, sulfur dioxide, and benzoic acid.

Regression analysis, one-way ANOVA, two-way ANOVA, independent sample t-tests, and other statistical

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analyses repeatedly verified that the observed differences were very significant ($p < 0.05$). The presence of food preservatives significantly slows down the organic waste's natural decomposition process, according to the strong positive association found by regression analysis. These results imply that even after food goods have expired, residual preservatives still retain antibacterial activity, which inhibits the growth and metabolic processes of microbes that break down organic matter.

The study draws attention to a significant environmental consequence of food waste containing preservatives: delayed biodegradation may prolong the buildup of organic waste in landfills and composting systems, which could lower the effectiveness of biological waste treatment procedures. Therefore, while building composting and biodegradation systems for expired processed foods, waste management techniques should take residual food preservatives into account.

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