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Effects of Ultrasound on the Growth and Cell Mass of Potential Pathogenic and Food-Spoilage Microorganisms Isolated from the Soil Environment

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Abstract

Soil microorganisms play essential roles in nutrient cycling and soil fertility; however, certain pathogenic and spoilage microorganisms inhabiting soil can contaminate agricultural produce and constitute significant risks to human and animal health. This study investigated the effects of ultrasonic exposure on selected potential pathogenic and spoilage microorganisms isolated from the soil environment. Soil samples collected from Crawford University, Nigeria, were subjected to microbiological analyses using standard isolation and identification techniques. Microbial growth and biomass accumulation were monitored spectrophotometrically at 500 nm. A custom-built sono-bioreactor equipped with two 27-W speakers was used to expose the isolated microorganisms to ultrasonic frequencies of 1 kHz, 4 kHz, and 9 kHz for 24 h. The isolates were identified as *Proteus vulgaris*, *Bacillus subtilis*, *Candida albicans*, and *Candida tropicalis*. The results revealed a statistically significant ($p < 0.05$) frequency-dependent response across all tested microorganisms. Exposure to low-frequency ultrasound (1 kHz) stimulated microbial proliferation and biomass production, indicating a bio-stimulatory effect. Among the isolates, *Bacillus subtilis* and *Candida tropicalis* exhibited the highest growth rates, attaining cell densities of 1.02×10^9 CFU mL^{-1} and 6.90×10^7 CFU mL^{-1}, respectively, with corresponding cell masses of 1.029 g and 0.690 g. In contrast, increasing ultrasonic frequency to 4 kHz and 9 kHz resulted in progressive inhibition of microbial growth and viability. The strongest antimicrobial effect was observed at 9 kHz, where the population of *Bacillus subtilis* decreased to 2×10^7 CFU mL^{-1}, while the cell mass of *Candida tropicalis* declined to 0.227 g. These findings demonstrate the potential of ultrasound technology as an environmentally friendly strategy for controlling pathogenic and food-spoilage microorganisms, with promising applications in medical, agricultural, and food-processing industries.

Keywords

Cell mass;
Microbial growth;
Non-chemical microbial control;
Ultrasound; Soil microorganisms.

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INTRODUCTION

Soil is a highly dynamic and complex ecosystem that harbors an enormous diversity of microorganisms, including bacteria, fungi, yeasts, actinomycetes, protozoa, and algae, which collectively play crucial roles in maintaining soil structure, fertility, and ecological balance (Chen *et al.*, 2024; Akbar *et al.*, 2025). Soil bacteria are involved in essential processes such as organic matter decomposition, nitrogen fixation, nitrification, denitrification, and the production of growth-promoting substances, while some species function as plant or human pathogens (Wang *et al.*, 2024).

Common bacterial genera found in soil include *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Clostridium*, and *Nitrosomonas*, many of which are involved in organic matter decomposition, nitrogen fixation, nitrification, and the production of plant growth-promoting substances, while others may act as opportunistic pathogens (Wang *et al.*, 2024). The growth and cell mass of microorganisms in the soil environment are key indicators of microbial activity and soil functional status, as microbial biomass constitutes the living component responsible for most biochemical transformations in soil (Semenov *et al.*, 2025). Overgrowth of specific microbial groups, including pathogenic bacteria and fungi, may further disrupt soil microbial balance and increase the incidence of soil-borne diseases, ultimately impairing soil productivity and plant health (Akbar *et al.*, 2025).

Various strategies have been employed to reduce or inhibit the growth and cell mass of microorganisms in the soil, particularly where excessive microbial activity results in nutrient depletion, disease incidence, or soil quality deterioration. Conventional approaches include the application of chemical fumigants and disinfectants, crop rotation, soil solarization, and the use of antimicrobial amendments, all of which have demonstrated varying degrees of effectiveness in suppressing microbial populations (Li *et al.*, 2026). However, these methods are often associated with significant limitations, including non-specific toxicity, disruption of beneficial microbial communities, persistence of chemical residues, environmental contamination, and the development of microbial resistance (Akbar *et al.*, 2025).

In addition, biological control methods, though environmentally friendly, may exhibit inconsistent performance under fluctuating soil conditions and complex microbial interactions (Sharma *et al.*, 2025). These challenges underscore the imperative need to explore alternative, non-chemical, and more targeted approaches capable of modulating microbial growth and biomass without compromising soil ecological balance.

Ultrasound which is defined as acoustic waves with frequencies exceeding the upper limit of human hearing (>20 kHz), represents a rapidly evolving technology in the realm of microbial biotechnology, environmental remediation, and food processing. The ability of ultrasound to influence microbial cells arises from the convergence of mechanical, thermal, and chemical effects.

Mechanically, the collapse of cavitation bubbles produces shock waves and microstreaming that can disrupt cell walls and membranes, alter permeability, and shear cellular appendages such as flagella and pili (Mondal *et al.*, 2021). Ultrasound's dualistic nature allows it to serve both as a stimulant and as an antimicrobial agent, depending on key

operational parameters such as frequency, intensity (power density), duty cycle, and exposure duration. Low-frequency ultrasound (20-100 kHz) with moderate power densities often produces violent cavitation events, leading to pronounced mechanical disruption and microbial inactivation (Lauteri, *et al.*, 2023; Tiwari *et al.*, 2009). In contrast, high-frequency ultrasound (>100 kHz) generates smaller, more numerous cavitation bubbles that collapse less violently, favoring enhanced radical formation and milder mechanical effects (Ashokkumar, 2015). The dual nature of ultrasound makes it a powerful yet delicate tool in microbial physiology and process engineering, determining the optimal parameters for desired biological outcomes requires a deep understanding of microbial cell structure, ultrasound physics, and the specific objectives of treatment.

Conventional methods used to control microbial growth in soil are often limited by high cost, environmental persistence, non-specific toxicity, and disruption of beneficial microbial communities, making safer and more targeted alternatives necessary. Ultrasound represents a novel, non-chemical, and controllable physical approach with the potential to regulate microbial growth and cell mass through its ability to alter cellular structure and metabolic activity without leaving harmful residues. Investigating the effects of ultrasound on potential pathogenic and food spoilage microorganisms isolated from the soil environment is therefore the aim of this study.

MATERIALS AND METHODS

Microorganisms and Culture Media

Soil microorganisms used in this study were obtained from different locations within Crawford University, Nigeria. The bacterial isolates were cultivated on Nutrient Agar (BDH Chemicals, UK), while yeast isolates were cultivated on Potato Dextrose Agar (BDH Chemicals, UK).

Collection and Preparation of Soil Samples

Soil samples were collected using sterile techniques from various sites within the university campus. The samples were transported immediately to the laboratory and stored under ambient conditions prior to microbiological analysis.

Construction of the Sono-bioreactor

A sono-bioreactor was constructed using two 27-watt speakers supported by two 12-volt batteries. The system operated for 12 hours at moderate frequencies of 1, 4, and 9 kHz, and could be extended up to 20 kHz, though with reduced battery life of approximately 6 hours. This system was designed to evaluate the effects of ultrasonic waves on microbial growth and cell mass.

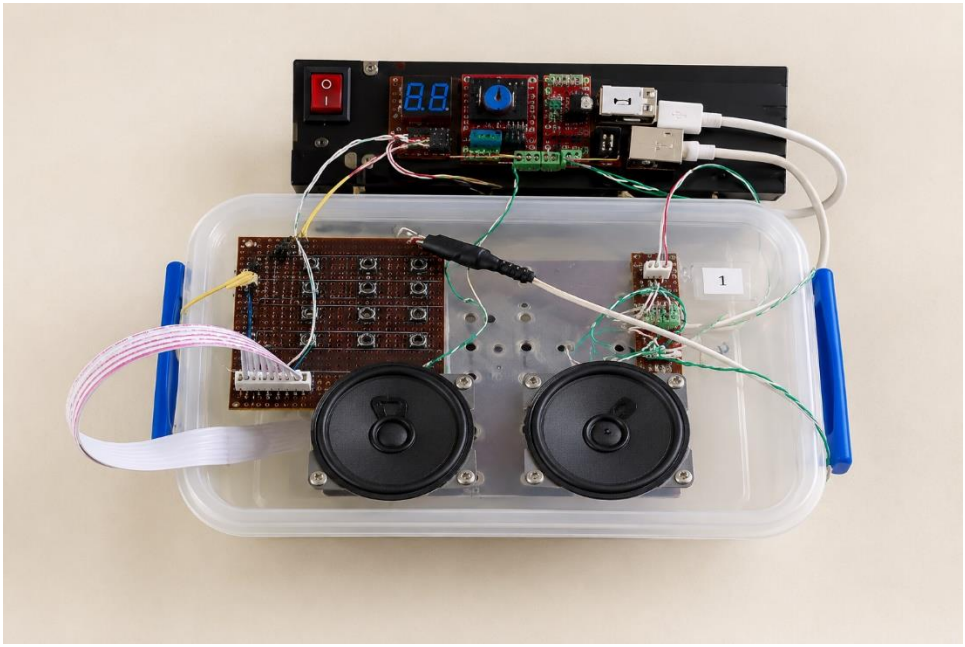

Figure1: Construction of a sono bioreactor

Preparation of Culture Media

Culture media used for this evaluation were potato dextrose agar (PDA) and Nutrient agar (NA) (BDH Chemicals, UK) for fungi and bacteria. Potato dextrose agar and Nutrient Agar of 39 g and 78 g were dissolved separately in 1 litre of distilled water and boiled to dissolve the medium completely before sterilizing with autoclave at 121 °C for 15 minutes. The pH of the sample of the PDA was adjusted to 3.5, after adding 10 ml of lactic acid. The media were thereafter cooled to 55 °C.

Isolation and Identification of Microorganisms

Isolation of microorganisms

Microorganisms were isolated from the soil samples. Using standard microbiological technique (serial dilution), 1 ml of the soil sample was pipetted and mixed in another 9 ml of sterile distilled water in a test-tube. The test-tube was shaken vigorously to homogenize. The exponential dilution continued to the fourth factor (10^{-4}). One millilitre (1 ml) of the fourth factor was aseptically transferred and plated in duplicate sets using sterile molten lukewarm potato dextrose agar and Nutrient agar for fungi and bacteria respectively. The poured plates were allowed to set and were incubated (Gallenkamp, England) at 35 °C (24 h) and 27 °C (48 h) for bacteria and fungi respectively. Sub culturing of distinct colonies were carried out to obtain pure cultures for further identification.

Identification of Microorganisms

Identification of bacteria

Bacterial isolates were identified based on colonial morphology including size, shape, color, elevation, margin, and consistency. Biochemical tests were also carried out (Mboto *et al.*, 2012; Cheesbrough, 2006)

1. **Catalase Test:** A loopful of culture was mixed with 3% hydrogen peroxide

on a clean glass slide; bubble formation indicated a positive result.

2. **Starch Hydrolysis Test:** Isolates were streaked on starch agar, incubated at 37 °C for 48 hours, and flooded with iodine solution to detect clear zones indicating amylase activity.
3. **Kligler Iron Agar (KIA) Test:** Differentiated bacteria based on glucose/lactose fermentation and H_2S production.
4. **Coagulase Test:** Determined the presence of coagulase enzyme by clot formation.
5. **Methyl Red/Voges–Proskauer (MR–VP) Tests:** Assessed mixed-acid fermentation and acetoin production (Tortora *et al.*, 2018).

Identification of Yeast cells

The yeast isolates were identified according to the method described by Cowan and Steel (1993) and Chessbrough (2006).

1. **Germ Tube Test:** A loopful of yeast was inoculated into 0.5 mL of serum and incubated at 37 °C for 48 hours. Germ tube formation was observed under ×40 magnification.
2. **Catalase Test:** Conducted similarly to bacteria, with bubble formation indicating positive catalase activity.
3. **Starch Hydrolysis Test:** Yeast was streaked on starch agar, incubated at 37 °C for 48 hours, and flooded with iodine to detect zones of clearance indicating amylase production.

Quantification of Microbial Growth

Bacterial and yeast growth was quantified using the McFarland turbidity standard. A 0.5 McFarland standard was prepared by mixing 1% barium chloride solution and 1% sulfuric acid in a 0.05:9.95 ratio, corresponding to approximately 5×10^6 CFU/mL. Optical density was measured at 500 nm using a spectrophotometer, and microbial concentrations were estimated by comparison with McFarland standards ranging from 0.5 to 10.0 for both bacteria and fungi.

Treatment of Microorganisms with Ultrasound

Bacterial and fungal isolates obtained from soil samples were subjected to ultrasonic treatment using the constructed sono-bioreactor. Microbial cultures were first suspended in sterile distilled water to a standardized turbidity equivalent to 0.5 McFarland (~5×10^6 CFU/mL). Aliquots of 5 mL of each suspension were transferred into sterile test tubes, covered with aluminum foil, and placed within the sono-bioreactor chamber. Ultrasonic waves were applied at frequencies of 1, 4, and 9 kHz for 24 hours.

Control samples were maintained under identical conditions without ultrasonic exposure. Effects of ultrasound on microbial growth and cell mass were monitored by measuring optical density at 500 nm at regular intervals. Following exposure, samples were streaked onto their respective media to assess viability, colony morphology, and growth reduction. Microbial biomass was additionally estimated gravimetrically by harvesting cells through centrifugation and measuring dry weight after oven-drying at 60 °C until constant weight was achieved.

Results

Identification of Bacteria and Yeasts Isolated from the Soil Environment

The results of the biochemical and microscopic tests used for the identification of soil microorganisms are presented in Table 1. Based on the morphological and biochemical tests, two bacterial species; *Proteus vulgaris* and *Bacillus subtilis* were identified among the isolates. In addition, fungal analysis revealed the presence of *Candida albicans* and *Candida tropicalis* as the predominant yeast species isolated from the soil environment as shown in Table 2.

Table 1: Identification of bacteria isolated from soil sample

Isolates	Catalase test	Kinger iron agar test	Coagulase test	Methyl red	Gram staining	Starch hydrolysis	Probable identity
A	+	H_2S+	+	+/-	-	+	*Proteus vulgaris*
B	+	Glucose fermenter	+	+/-	+	+	*Bacillus subtilis*
C	+	H_2S+	+	+/-	-	+	*Proteus vulgaris*

Table 2: Identification of yeasts from soil environment

Label	Macroscopy	Germ tube	Catalase	Starch hydrolysis	Yeast
Yeast A	Creamy color, elevated and mucoidal	+	+	+	*Candida albicans*
Yeast B	Creamy color,smooth and glaborous	+	-	+	*Candida tropicalis*

Frequency-Dependent Effects of Ultrasound on the Growth of Soil Microorganisms

The data presented in Tables 3 and 4 indicate that the variations in bacterial and fungal cell mass, as well as total viable counts (CFU/mL), across the different acoustic frequencies are statistically significant ($p < 0.05$). This low p-value indicates a less than 5% probability that the observed suppression of microbial growth occurred by random chance, confirming that the acoustic waves generated by the sono-bioreactor were the primary driver of microbial inhibition.

Among the bacterial isolates, Proteus vulgaris and Bacillus subtilis exhibited their highest CFU counts of 9.70×10^8 and 1.02×10^9 at 1 kHz, respectively, compared to lower counts at 4 kHz (3.67×10^8 and 4.03×10^8) and 9 kHz (1.03×10^7 and 2.0×10^7). Similarly, the yeast isolates, Candida albicans and Candida tropicalis, recorded CFU counts of 6.67×10^7 and 6.90×10^7 at 1 kHz, which declined to 4.47×10^7 and 5.75×10^7 at 4 kHz, and further to 3.11×10^7 and 2.27×10^7 at 9 kHz. These results indicate a clear frequency-dependent effect of ultrasound on microbial growth and viability (Tables 3 - 4). The mean growth response of microorganisms exposed to ultrasound frequencies of 1, 4 and 9 kHz were highest at 1 kHz and lowest at 9 kHz, indicating a frequency-dependent inhibitory effect on microbial growth.

Table 3: Effects of different ultrasound frequencies on the growth of bacterial isolates

Isolates	1000Hz		4000Hz		9000Hz	
	Bacterial count (Cfu/mL)	Cell mass	Bacterial count (Cfu/mL)	Cell mass	Bacterial count (Cfu/mL)	Cell mass
Proteus vulgaris	$9.70x10^8$	0.970	$3.67x10^8$	0.367	$1..03x10^7$	0.301
Bacillus subtilis	$1.02x10^9$	1.029	$4.03x10^8$	0.602	$2.0x10^7$	0.403

Table 4: Effects of Different Ultrasound Frequencies on the Growth of fungal isolates

Isolates	1000Hz		4000Hz		9000Hz	
	Fungal count (Cfu/mL)	Cell mass	Fungal count (Cfu/mL)	Cell mass	Fungal count (Cfu/mL)	Cell mass
Candida albicans	$6.67x10^7$	0.667	$4.47x10^7$	0.447	$3.11x10^7$	0.311
Candida tropicalis	$6.90x10^7$	0.690	$5.75x10^7$	0.575	$2.27x10^7$	0.227

DISCUSSION

The isolation of *Bacillus subtilis*, *Proteus vulgaris*, *Candida albicans*, and *Candida tropicalis* aligns with well-documented baseline studies of soil ecology. *Bacillus subtilis* is a ubiquitous, spore-forming soil bacterium widely recognized for its metabolic versatility and production of plant growth-promoting substances (Chauhan *et al.*, 2023). Conversely, *Proteus vulgaris* and the *Candida* species (*C. albicans* and *C. tropicalis*) often represent opportunistic pathogens or transient components of the soil microbiota, thriving in organic-matter-rich niches where domestic or agricultural runoff introduces complex nutrient streams (Wang *et al.*, 2024; Akbar *et al.*, 2025). The dominance of these specific genera in the isolated samples highlights the structural diversity and potential pathogenic risks inherent in local soil environments.

The experimental data reveals a clear inverse relationship between acoustic frequency (from 1 kHz to 9 kHz) and microbial survival, with exposure to 9 kHz generating the most pronounced reductions in both colony-forming units (CFU/mL) and overall cell mass across all isolates. This trend can be interpreted through the fundamental physics of acoustic cavitation and microstreaming (Jalal & Leong, 2018)

The stimulation of growth at 1 kHz can be attributed to mild cavitation effects, which enhance mass transfer of nutrients and oxygen to microbial cells without causing structural damage (Tiwari *et al.*, 2009). This gentle stress may trigger adaptive responses in microbes, a phenomenon known as hormesis, where low-level stress enhances cellular metabolism and division (Calabrese *et al.*, 2007). In contrast, exposure to 4 kHz and 9 kHz produced more violent cavitation, generating microjets, shock waves, and reactive oxygen species (ROS) that likely disrupted cell walls, membranes, and enzymes, thereby reducing proliferation (Ashokkumar, 2015; Ojha *et al.*, 2017).

Species-specific differences were observed. *C. tropicalis* experienced a more pronounced reduction in growth at 9 kHz than *C. albicans*, suggesting slightly lower tolerance to oxidative and mechanical stress. Among the bacteria, *B. subtilis* was more sensitive to 4 kHz than *P. vulgaris*, which may be due to differences in cell wall architecture and stress-response mechanisms (Chen and Zhu, 2011; Gow *et al.*, 2012). These findings highlight the importance of microbial physiology and envelope structure in determining susceptibility to ultrasonic treatment. The ability of low-frequency ultrasound to enhance microbial growth, while higher frequencies inhibit it, presents practical opportunities for controlled microbial management. In biotechnology, low-frequency ultrasound could be used to boost growth of beneficial yeasts in fermentation processes, whereas higher frequencies could suppress unwanted microbial populations in food processing, environmental remediation, or soil treatment. This selective modulation mirrors the antimicrobial effects observed in physical treatments, such as shea butter coating of tomato fruits, which reduces microbial penetration and extends shelf life (Vignesh and Nair, 2019; Banjo and Malomo, 2024).

Overall, the study demonstrates that ultrasound is a frequency-dependent tool capable of both stimulating and suppressing microbial populations. Low-frequency treatment promoted proliferation via mild cavitation and enhanced nutrient availability,

whereas higher frequencies induced mechanical and oxidative stress that reduced viability (Bhat *et al.*, 2011).

CONCLUSION

The present study shows that ultrasound at low frequency (1 kHz) enhanced the growth of *Proteus vulgaris*, *Bacillus subtilis*, *Candida albicans*, and *Candida tropicalis*, while higher frequencies (4 kHz and 9 kHz) progressively suppressed their growth and viability. This suggests that ultrasound can stimulate microbial activity at mild frequencies while inhibiting growth at higher frequencies, likely through cavitation and oxidative stress mechanisms. Ultrasound is non-invasive, energy-efficient, and can be applied to modulate microbial populations without the use of chemical agents. This could be an alternative means of eliminating pathogenic and food spoilage microorganisms. Therefore, this study has established the potential of ultrasound in controlling microbial growth, offering applications in environmental microbiology, medicine, food safety, and industrial processes, while providing a sustainable and cost-effective alternative to chemical treatments.

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