Computational Modeling of Bacteria Formation and Dynamics Under Varying Radiation Doses

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Computational Modeling of Bacteria Formation and Dynamics Under Varying Radiation Doses

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ABSTRACT

Most bacteria naturally live in biofilms, complex communities bound by extracellular polymers and attached to surfaces. Biofilms are pervasive in healthcare, affecting 80% of microbial infections and costing billions annually. Pseudomonas aeruginosa and Staphylococcus aureus, the focus of this study, are significant contributors to biofilm-associated infections. A novel approach using low-energy beta-emitting radioisotopes is proposed to create self-sterilizing surfaces that mitigate biofilm formation through reactive oxygen species (ROS) generation. This computational modeling study explores the dynamics of biofilm inhibition under varying radiation doses, with implications for improving infection control on medical devices and instruments.

Keywords: biofilms — colony formation units — reactive oxygen species

KEYWORDS

The key terms relevant to this study are defined as follows:

- **Biofilm**: A complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix.
- **Radioactive Material Packet (RAM)**: Radioactive material packet encased by thermoplastic disk and plastic wrap.
- **Reactive Oxygen Species (ROS)**: Chemically reactive molecules containing oxygen, which can damage cell structures and play a role in the response to radiation exposure.
- **Colony Forming Units (CFU)**: A unit used to estimate the number of viable bacteria or fungal cells in a sample, based on the ability of each unit to form a colony.
- **Optical Density (OD)**: a logarithmic measurement of how much light is absorbed by a substance when light passes through it.

INTRODUCTION

Most bacteria naturally exist in biofilms, which are complex communities bound by extracellular polymers and often attached to surfaces (1-3). Biofilms can form on a wide range of materials, and once mature, they are extremely difficult to eliminate, evading both antibiotics and the immune system (4, 5). Consequently, biofilms represent a significant and growing issue in healthcare, implicated in an estimated 80% of all microbial infections (6-9). Each year, biofilm infections affect over 17 million Americans, result in at least 550,000 deaths, and cost the US healthcare system billions of dollars (10-13).

Approximately 60-70% of hospital-acquired infections (more than 106,000 annually in the US) are associated with biofilms formed on biomaterials or in-dwelling medical devices (14-16). Infection rates for commonly used devices,

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such as cardiac implants, hemodialyzers, central venous catheters, urinary catheters, and ventilators, range from 20% to 40% (17). With advancements in medical technology, the incidence of biofilm infections on these devices is also increasing. Patients with risk factors such as high BMI, diabetes, dependence on hemodialysis, or those taking immunosuppressants are at elevated risk of developing biofilm-associated infections, and these risk factors are becoming more common (18). The model organisms for this study, Pseudomonas aeruginosa and Staphylococcus aureus, are major contributors to biofilm infections on medical devices (17, 19, 20). A mature biofilm's outer layer acts as a highly effective shield against external agents (4, 5, 21). Once a biofilm is established, eradicating it can be so challenging that often the only viable response is to remove the contaminated device, leading to increased costs and risks for the patient (22).

Even surgical instruments and reusable tools, such as endoscopes, are vulnerable to biofilm contamination (23-25). These biofilms can survive conventional disinfection methods and create reservoirs of bacteria that can be inadvertently transferred to other patients, resulting in post-operative or post-procedure infections (26-31). Staphylococcus aureus and Pseudomonas aeruginosa biofilms are frequently responsible for such contaminations (27-29, 32, 33). Instruments with complex geometries, such as hinges or intricate topographies, are especially susceptible since their design provides crevices that are difficult to disinfect effectively (24, 33, 34).

The most common method for preventing biofilm infections is to maintain good sterile techniques during surgeries or device deployments and to administer prophylactic antibiotics (35-38). However, these methods still allow infection rates as high as those discussed above (39). Moreover, prophylactic antibiotic use comes with the risk of accelerating antibiotic resistance. These challenges have prompted the development of antimicrobial and antifouling surfaces to resist biofilm formation. Existing efforts primarily involve modifying the surface texture and chemical properties to inhibit bacterial adhesion or creating materials that release antimicrobial agents (22, 40-47). However, such approaches have shown limited clinical success (39). Bacteria possess numerous, redundant mechanisms for attachment to surfaces. Over time, antimicrobial coatings lose their effectiveness due to diffusion or chemical degradation, and adsorption of conditioning materials further reduces antifouling properties. The widespread use of antibiotic-containing materials may also contribute to the increasing problem of antibiotic resistance (48).

A novel alternative, unexplored so far, involves the use of radioactivity, distinct from chemical antimicrobials, to create materials with self-sterilizing surfaces. This approach is expected to be less susceptible to evolutionary evasion by bacteria compared to conventional antibiotics, and it could potentially be combined with antifouling or antimicrobial material properties for a multi-faceted defense against biofilm formation. Self-sterilizing surfaces could be effective even on complex or hinged instruments, as they do not rely on the ability of an external agent to reach the biofilm. Even if this radiation-based approach cannot entirely prevent biofilm formation, it could significantly slow biofilm development, providing a critical time window during which a tool or implant remains safe while the risk of biofilm development is highest (22). By delaying biofilm formation and the resulting resistance to antimicrobials and the immune system, this method would extend the timeframe during which bacteria can be effectively eliminated.

SAFETY CONSIDERATIONS

Radioactive isotopes can emit three types of radiation: alpha particles (helium nuclei), beta particles (electrons), and gamma radiation (electromagnetic radiation, or photons). Gamma radiation is often used to kill bacteria and prevent biofilm formation, particularly in food safety applications (60, 61). Similarly, electrons emitted by radioisotopes or generated by accelerators can penetrate materials based on their energy, and electron beams have been proven effective for food sterilization (62, 63). Beta-minus particles (electrons) and beta-plus particles (positrons) are widely used in medical procedures, such as Positron Emission Tomography (PET), and many beta-emitting radioisotopes are also employed in cancer treatments (64). The maximum energies of these beta emissions, used in cancer therapy, typically range from 0.6 to 2 MeV (64). However, for our application, we propose using low-energy beta emissions with energies far below these levels, comparable to those found safe for treating conditions like eczema, psoriasis, and mycosis fungoides (65, 66).

In our approach, we will use pure beta emitters that do not emit gamma radiation, thereby eliminating the associated safety risks. For surgical tools and instruments used temporarily (such as catheters or endoscopes), the risk of mutagenic effects is minimized due to both the low-energy nature of the beta particles and the brief exposure time. Additionally,

these beta particles are easily blocked by standard surgical gloves or by the thin aqueous layer separating devices from tissues. For devices implanted for longer periods, selecting an isotope with an appropriate half-life allows for an optimized dose duration—one that minimizes radiation exposure while ensuring the surface remains sterile during the critical initial period of susceptibility to infection. The thin aqueous layer surrounding the implant further shields host tissue from the beta emissions during this time (22, 67).

APPROACH

The experimental data includes CFU counts derived from biofilm growth under beta-emitting radioisotope surfaces. Simulation parameters will reflect laboratory findings to ensure model accuracy.

Introduction

The objective of this study is to measure the effect of radiation dose on biofilm biomass. Our working hypothesis is that radiation will have a dose-dependent impact on reducing biofilm biomass and antibiotic tolerance by inhibiting bacterial metabolism within the biofilm. To test this, we will grow biofilms under proof-of-concept self-sterilizing surfaces and utilize standard microbiological assays to quantify the effects of varying radiation doses.

The rationale behind this approach is that self-sterilizing surfaces, which do not depend solely on conventional cleaning methods or prophylactic antibiotics, could provide an innovative way to prevent biofilm formation. Such surfaces are less likely to contribute to the development of antibiotic resistance and are not easily circumvented through evolutionary adaptation.

Bacteria, Biofilms and Radiation

The primary model organism for this study is Pseudomonas aeruginosa, an important pathogen in cystic fibrosis, diabetes, and chronic pulmonary disease. P. aeruginosa is responsible for many hospital-acquired infections, particularly those involving implanted medical devices, and is well-established as a model organism for biofilm studies (75). It is a rod-shaped, Gram-negative bacterium, with an envelope consisting of a thin peptidoglycan layer between inner and outer lipid bilayer membranes (76). By contrast, Staphylococcus aureus, which will also be studied, is a spherical, Gram-positive bacterium. Unlike Gram-negative bacteria, Gram-positive bacteria lack an outer membrane, but they have a significantly thicker peptidoglycan layer surrounding their inner membrane. Despite this structural difference, we do not expect the peptidoglycan layer to significantly impact the generation of reactive oxygen species (ROS) inside the bacterium, even though the peptidoglycan may act as an attenuating material.

The maximum electron energy of Sulphur-35 ($\sim$167 keV) results in a penetration depth of approximately 700 µm, while the average energy of emitted beta particles ($\sim$49 keV) corresponds to a penetration depth of 205 µm. Both values are much greater than the size of a bacterium ($\sim$1 µm), suggesting that the number of radicals formed within each bacterial type should be similar. However, significant physiological differences exist between Gram-negative and Gram-positive bacteria, as well as variations within each group. Comparing the effects of radiation on S. aureus and P. aeruginosa will help us understand the relative effectiveness of different biochemical mechanisms for DNA repair.

Although radiation has not yet been explored as a method for biofilm prevention, previous research has examined its effect on established biofilms. Alpha radiation (helium nuclei) has been shown to reduce the metabolic activity of fungal biofilms, while radioactive thermal water from hot springs has been found to reduce the metabolic activity of bacterial biofilms, including those formed by P. aeruginosa and S. aureus (77, 78). In both cases, metabolic activity was assessed using the XTT assay. Furthermore, gamma radiation has been shown to reduce the number of viable cells in pathogenic biofilms (79, 80). Although radiation's effects on biofilms specifically have not been widely studied, ionizing radiation in general can effectively kill a broad range of pathogenic bacteria and fungi, including those capable of forming biofilms (81-86).

Proof-of-Concept

Our study investigates self-sterilizing surfaces using Sulphur-35-labeled NaCl (S-35-M), a commercially available radioisotope from Perkin-Elmer. S-35-M has a half-life of approximately three months and emits electrons with a maximum energy of around 167 keV. Our lab is authorized to handle this isotope, and we have initiated preliminary studies supported by seed funding from the University of Texas at Austin.

These initial studies aim to develop a method to culture biofilms in close proximity (approximately 10 µm) to the radioisotope without direct contact between the bacteria and the radioactive material. This restriction is based on our expectation that any practical application would involve embedding radioisotopes at a controlled depth beneath a surface. Such a design is more suitable for creating self-sterilizing surfaces for real-world applications, compared to non-contained radioactive materials, which carry greater risks of exposure to people.

To conduct our experiments, we use petri dishes containing LB nutrient agar gels, with bacteria placed on the gel in each well. To simulate radiation-functionalized surfaces, we place a sealed packet consisting of a thin plastic film and a plastic disk—sized precisely to fit each well—on top of the agar. The packet contains either the radioactive material (RAM) or a non-radioactive control.

We confirmed that these packets do not leak for the 24-hour biofilm growth period by using food coloring as a test material. Since the plastic packet reduces the bacteria's access to oxygen from the air, we supplement the LB nutrient agar with nitrate (in the form of potassium nitrate) to provide an alternative electron acceptor. For comparison, each experimental setup is paired with a non-radioactive control sample, which starts with the same overnight bacterial culture and uses the same batch of nutrient agar.

METHODOLOGY

Summary

Protocol for performing a growth assay on bacterial biofilms subjected to radioactivity, to examine the effects of radioactivity on the growth of bacterial biofilms. Biofilms are grown in the vicinity of beta emitters and their growth is estimated with CFU measurements.

Materials

Radioactive material (RAM), plastic coverslip, plastic film, glue, PAO1 WT, LB, LB agar, filter disk (pore size = 0.2 μm), 96-well plate

Fabrication of RAM Packets

Day 1

1. Pipette desired amount of RAM (we have used 5 μL of fluid) (Perkin Elmer, NEG709A001MC) on the center of a plastic coverslip (Thermo Scientific, 174950). Leave the coverslip with RAM inside a lidded petri dish. Use one petri dish for each coverslip to prevent static electricity between coverslips from causing movement and spilling the RAM. Let the RAM air dry on coverslips for one day.

2. Repeat Step 1 but without RAM as the negative control of this experiment.

Day 2

3. Cut plastic film into a 4 x 4 cm square.

4. Apply glue along the periphery of a coverslip, encircling the RAM in the center.

5. Place the coverslip with RAM and glue upside down on the center of a plastic film square (see Figure 1). Then leave this in a petri dish and let the glue air dry for one day.

* Use exactly enough glue so that as the coverslip is placed on plastic film, the glue does not smear into the RAM and forms a barrier between the RAM and the plastic film.

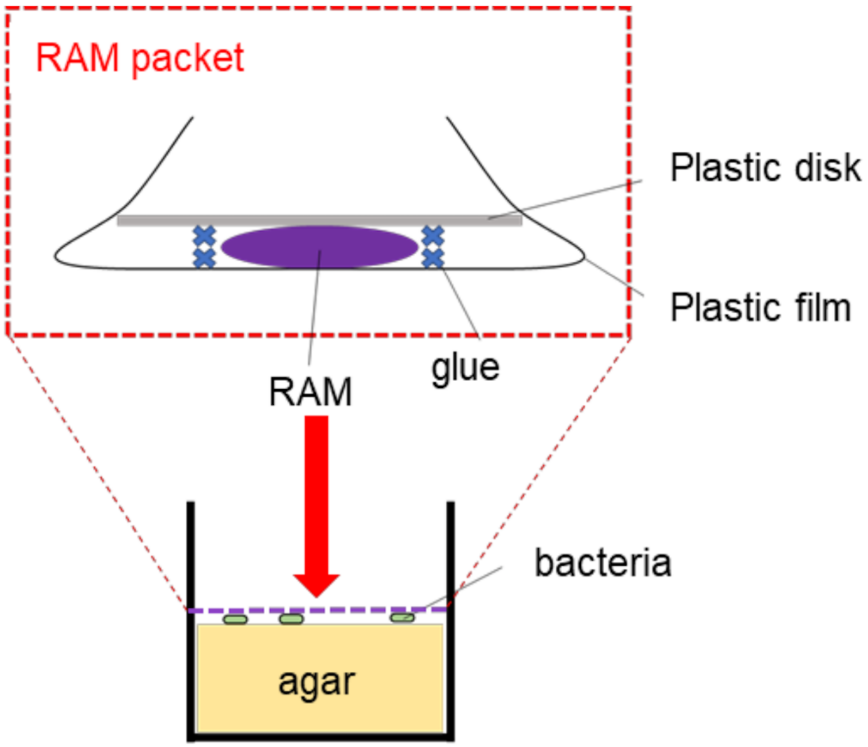


Figure 1: Experimental Schematic. A sealed packet of radioactive material (RAM) is placed atop nutrient agar on which bacteria have been deposited.

Apparatus Preparation

Day 1

1. Cover the separation ring with plastic film and glue it together at contact points to maintain tautness of film (reduce slack to a minimum).
2. Start by putting glue on the top of the ring, carefully without extra glue spilling or having too little to adhere.
3. Hold the film firmly (without ripping it) and push it onto the side of the ring with the glue. Let it dry for a few hours before attempting to use it.

Biofilm Growth Assay with RAM Packets

Day 0

1. LB Agar plate: Mix 500 mL of MilliQ water and 20 g of agar powder. Autoclave for 45 minutes on liquid cycle, let cool slightly, and pour into petri plates until they solidify.

   (Note: this step is only necessary if you have not made LB Agar plates)

Day 1

2. Streak PAO1 WT or Staph (depending on what week it is) from the frozen stock onto an LB agar plate and incubate the plate in an incubator at 37°C overnight.

Day 2

3. Pick a single colony from the streak plate and transfer it to a glass tube containing 5 mL of LB media. Incubate the tube with shaking at 37°C overnight.

Day 3

4. Dilute the overnight liquid culture to an OD value of 10^{-3}.

* The following function calculates the required volume to dilute to 10 mL of LB media:

```
def odvalue(odvalue_1):
    odvalue_2 = odvalue_1 - 0.001
    ml = 0.010 / odvalue_2
    ul = ml * 1000
    return "Absorption - " + str(odvalue_1), "uL - " + str(ul)
```

5. Place a sterile filter disk (Fisher Scientific, 09-719-2A) on the surface of an LB agar plate. Let the moisture from the LB agar fully saturate the disk.

6. Pipette 3 μL of the liquid culture with OD = 10^{-3} onto the center of the disk. Use a sterile wooden stick to spread the liquid culture droplet around an area on the disk that matches the size of the plastic coverslip (diameter 1.3 cm).

7. Cut the excess RAM packet with a sterilized razor (see Figure 1). Place the RAM packet on the disk that covers the liquid culture.

8. Leave the LB agar plate with the disk and the RAM packet in the incubator at 37°C overnight.

Day 4

9. Transfer the filter disk with biofilms grown on top to a centrifuge tube filled with 15 mL of LB. Vortex the tube for 10 seconds, five times. Shake the tube laterally by hand for another 60 seconds.

10. Serially dilute the liquid culture from Step 13 to appropriate concentrations in a 96-well plate (200 μL total: 180 μL LB broth and 20 μL culture or another ratio of 1:10). Optimal dilution factors require trial and error and should be between 10^{-4} and 10^{-7}.

11. Pipette 50 μL of diluted liquid culture onto the surface of an LB agar plate. Spread the liquid culture evenly on the surface with an L-spreader.

12. Leave the plate in the incubator at 37°C overnight.

Day 5

13. Count the plate with a dilution factor that yields 3-300 colonies and calculate the corresponding CFU/mL.

RESULTS

The simulations predict that low-energy beta radiation significantly reduces biofilm biomass and enhances antibiotic susceptibility. Dose-response relationships highlight optimal radiation doses for biofilm control.

Constraints

This project has been ongoing for over a year and a half, and we have overcome many hurdles to finally gather usable data. Issues continue to arise with radiation leaking out of the packets due to the deterioration of plastic covers by ethanol; This stems from the need to mechanically and chemically clean the surface of these packets between tests to ensure they do not contribute bacteria to the next samples.

Because of the nature of this project and S-35's half-life of 90 days, there is only a month of data for P. Aeruginosa gathered currently. This means the modeling will be based on limited data.

Modeling

First, we model bacteria growth without any outside factors. We can do so by fitting our bacteria (P. Aeruginosa WT and S. Aereus) to a logistic growth model with our initial conditions and growth parameters (108, 109, 110, 111, 112) as stated below:

Calculating the Carrying Capacity $N_{\max}$ for Bacteria

To calculate the carrying capacity $N_{\max}$ for bacteria based on the amount of agar (in mg) in a Petri dish, we can use the following logic:

STEPS

1. **Assume a Bacterial Biomass Yield** (Y):

This is the amount of bacterial biomass (e.g., in mg) produced per unit of substrate consumed (e.g., mg agar). - Typical yields for bacteria range from **0.2 to 0.6 mg of biomass per mg of substrate**.

2. **Estimate the Biomass Capacity**:

Maximum Biomass (mg) = Y (Bacterial Biomass Yield (mg biomass/mg of substrate)) $\times$ S (Total Substrate (mg))

3. **Convert Biomass to CFU**: Use an approximate conversion factor based on bacterial cell size and dry weight. - **Dry weight per cell**: 10^{-12} g = 10^{-9} mg (varies slightly by species).

$$N_{\max} = \frac{\text{Maximum Biomass (mg)}}{\text{Dry weight per cell (mg)}}$$

KNOWN PARAMETERS

- **Agar amount in a Petri dish** (S): e.g., (S) mg – Around 0.8 mg agar per dish. - **Biomass yield** (Y): Estimated from literature or experiments. - **Dry weight per cell** (w): Varies by bacterial species.

FORMULA

$$N_{\max} = \frac{Y \cdot S}{w}$$

Adjustments for Specific Bacteria

YIELD FACTOR (Y)

- **Pseudomonas aeruginosa & Staphylococcus aureus**: 2.085 mg biomass/mg agar (aerobic conditions).

DRY WEIGHT PER CELL (W)

- **Pseudomonas aeruginosa**: $1.0 - 2.8 \times 10^{-10}$ mg/cell. - **Staphylococcus aureus**: 2.8×10^{-10} mg/cell.

Bacteria Growth

In the case of our study, the bacteria only ever grows in 20-30 hour periods, so this period is the most important area to look at. By modeling the expected growth of the bacteria, we can see that we will not have issue with carrying capacity of the LB agar plates.

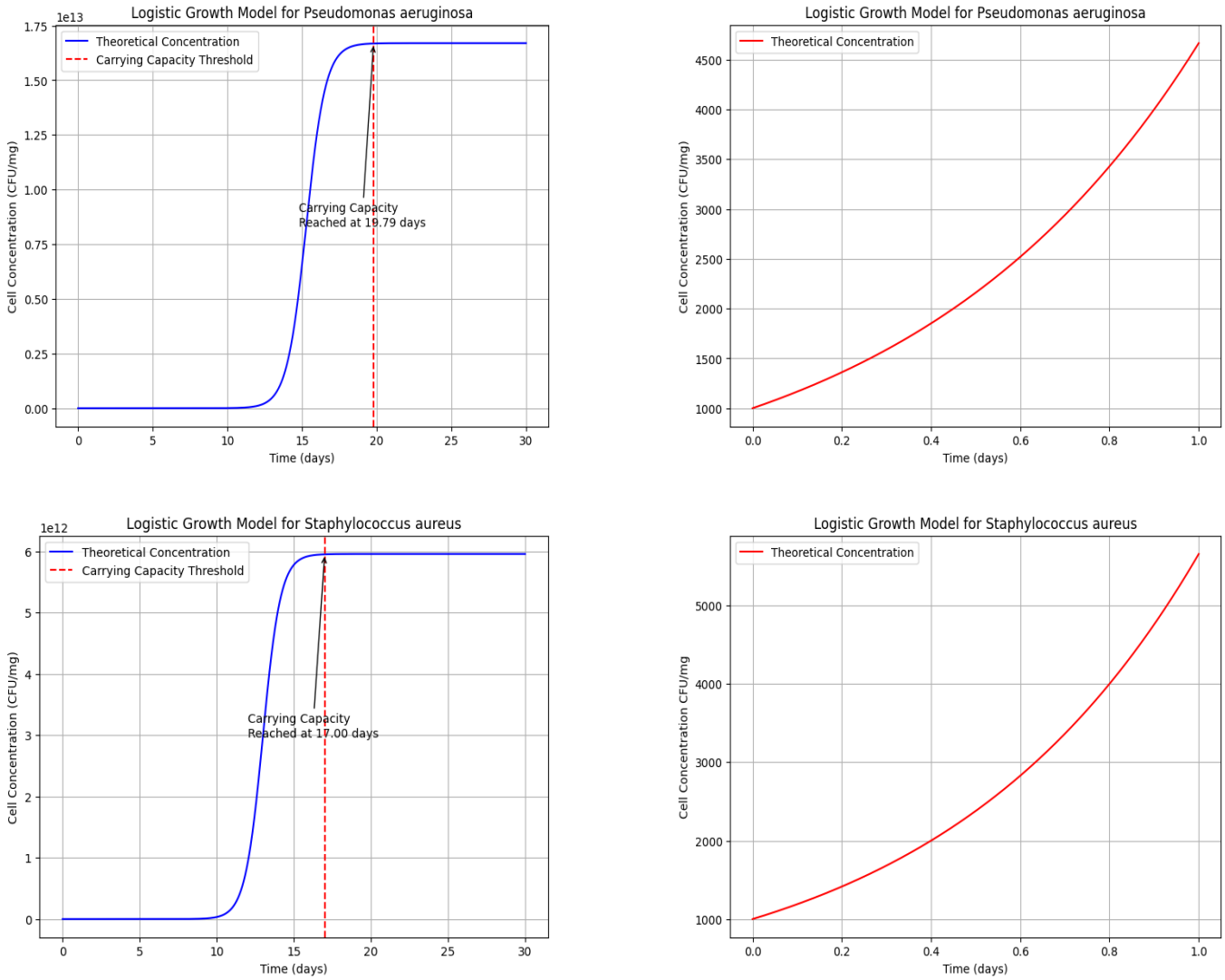


Figure 2: Modeling of P. Aeruginosa and S. Aereus over 1 and 20 day periods

Radioactive Decay

The half-life of a substance is the time required for its quantity to reduce to half of its initial amount. This concept is widely used in radioactive decay, pharmacokinetics, and various chemical processes (113). The mathematical equation for half-life decay is given as:

$$N(t) = N_0 \cdot \left(\frac{1}{2}\right)^{\frac{t}{T_{\text{half}}}}$$

where:

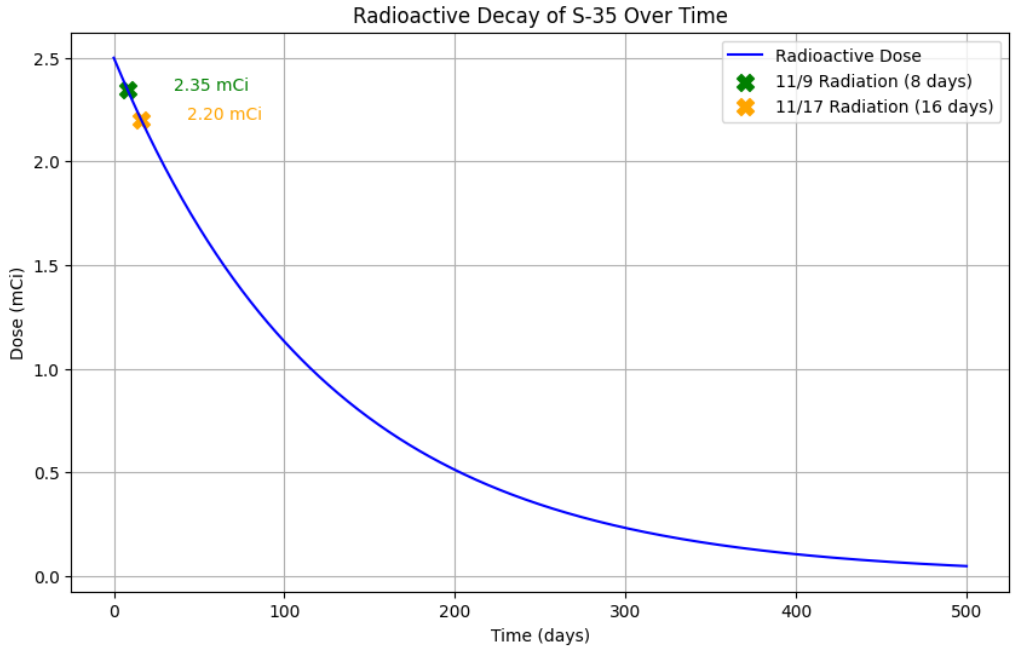
- $N(t)$ is the quantity of the substance remaining at time t.
- N_0 is the initial quantity of the substance.
- T_{half} is the half-life of the substance.
- t is the elapsed time.

This equation illustrates the exponential nature of decay, where the quantity decreases by half every T_{half} units of time. Both $N(t)$ and N_0 are in millicurie (mCi).

Knowing such, we can find the current dose for our RAM packets on the day the bacteria is exposed to them. where:

- The packet creation is 11-1-24
- $N_0 = 2.5\,\text{mCi}$ is the initial radioactive dose.
- $T_{\text{half}} = 87.4\,\text{days}$ is the half-life of S-35.

The decay is computed over a time range of $t \in [0, 500]$ days.



Radiation on Bacteria Growth

The relationship between radiation dose and CFU count is how we can quantify the effects of the beta-radiation on the growth of the bacteria. Using the limited data we have, the fit is based on an exponential growth/decay equation; our hypothesis, based on literature and past tests, indicates that the radiation will be ineffective until a threshold is reached, wherein as the radiation dose increases from there will cause exponential decreases in the CFU counts.

All data on specific days are collected from two replicates, one radioactive and one not, then diluted to 10^{-4} their original concentration before being spread on the growing plates. Multiple plates are use don the growing day, and the counts are averaged in order to reduce the variance present in biological samples.

Radiation on CFU Count	**Dilution From Original Replicate**	**CFU (Sample 1)**	**CFU (Sample 2)**
RAM	10^{-4}	13	14
CONTROL	10^{-4}	284	103
Percent Reduction		95.42%	86.41%
Dates		11-9-24	11-17-24
Dosage		2.35 mCi	2.20 mCi

For the sake of simplicity, no calculation of the attenuation by plastic wrap covering the radiation will be determined. The wrap is sufficiently thin, 20 microns (20e-6 m) thick, so that minimal energy is lost as electrons pass through the material. Propagation of error has not been calculated, so the plots are without statistical uncertainty.

DISCLAIMER: Because there are only 2 data points, it's unlikely that this model accurately reflects real trends between radiation dose and colony formation units.

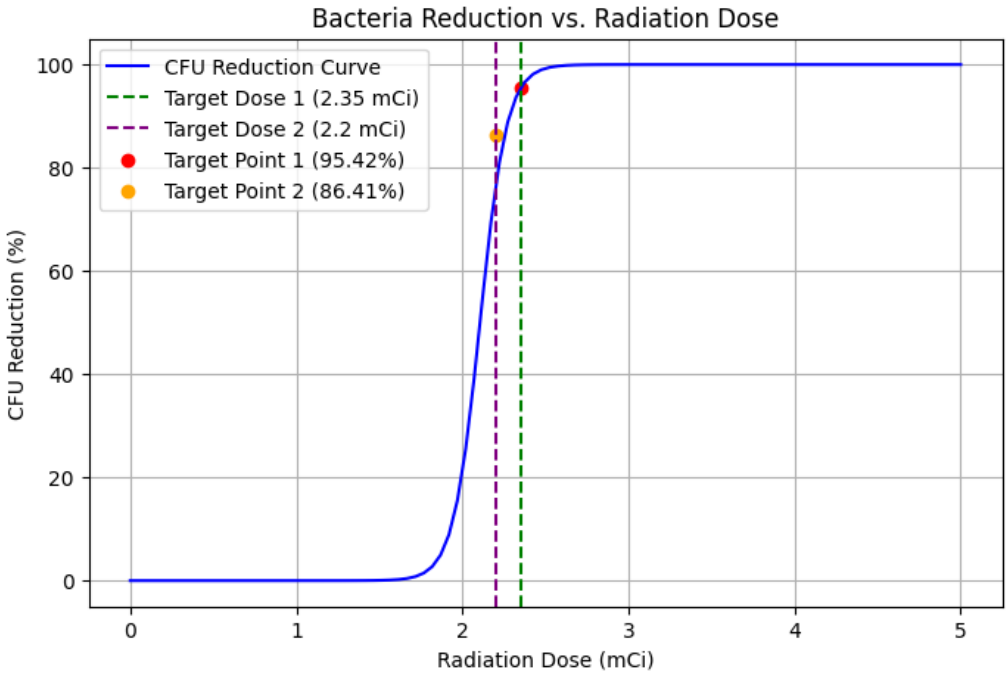


Figure 3: Predicted Reduction-Dose Curve Based on Current Data

SUMMARY

Our group has developed an innovative approach to combating bacterial biofilms using low-energy beta-emitting radioisotopes. We are specifically targeting problematic bacteria like Pseudomonas aeruginosa and Staphylococcus aureus, prominent strains that cause chronic illness through efficient bioifilm formation. By utilizing Sulphur-35-labeled NaCl, the study explores a novel method of generating reactive oxygen species that can effectively reduce biofilm biomass and enhance antibiotic susceptibility on surfaces. Biofilms, which are complex bacterial communities notoriously resistant to conventional treatments, pose a significant challenge in healthcare settings. The potential for use in medical technology bestows significant interest in its development.

The preliminary findings and computational modeling demonstrate the potential for creating self-sterilizing surfaces through radiation-based methods, offering a promising alternative to current disinfection techniques. While the results are encouraging, we acknowledge the need for further investigation to optimize radiation doses and comprehensively validate the long-term safety and efficacy of this approach under diverse clinical conditions. This groundbreaking research represents a critical step towards developing more effective strategies for preventing biofilm-related infections, potentially revolutionizing infection control in medical environments where traditional sterilization methods fall short.

All code used to generate plots can be found here: https://github.com/SpicedDill/Bacteria-Dynamics

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