128 Microbial Degradation of Polyethylene Plastic Under Varying NaCl Concentrations

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Introduction

Polyethylene Terephthalate (PET) is a commonly used polymer found in bottles, fabrics, and other plastic products. Its lightweight, durable nature prevents it from breaking down in the environment, contributing to pollution that can persist for over 450 years. This experiment aims to determine whether exposing the PETase enzyme in an *E. coli* K12 chassis to varying NaCl concentrations affects both PET breakdown and K12 growth.

Prior research shows that K-12 grows best between 0.2 M and 0.5 M NaCl, a range that supports osmotic balance, membrane integrity, and metabolism. Below this range, low osmotic pressure reduces growth; above it, cells face osmotic stress, dehydration, and metabolic slowdown. Within this sweet spot, K-12 maintains ion transport and turgor for reproduction. This study will explore whether pushing NaCl levels toward the upper limit changes PETase enzyme efficacy.

Experimental Design

Seven vials containing varying molar concentrations of NaCl and 1 gram of UV-degraded PET (excluding the negative control) were prepared. Over a two-week period, experimental runs were conducted in pairs. For each run, two sterile flasks were prepared, each containing 98 mL of minimal media, 2 mL of liquid bacterial culture, and the corresponding vial from the sample set.

Following preparation, an initial OD600 reading was recorded to establish a baseline for bacterial growth. Sterile magnetic stir bars were placed inside each flask to ensure that each solution was homogeneous. Each flask was then sealed with a sterile CO2 sensor, secured using parafilm to maintain an airtight environment.

To accommodate the CO2 sensors, a custom incubator was designed with wiring access ports. The incubator maintained a temperature range of 35–40 degrees Celsius. Upon placement of the flasks, the stir column was initiated, and real-time CO2 levels were recorded using the LabQuest CO2 sensor.

After 24 hours of incubation, the flasks were removed for final OD600 measurement to assess bacterial growth over the period. All glassware and materials were then cleaned and autoclaved in preparation for the next experimental set.

Methodology

1. Media Preparation
•All *E. coli* K12 cultures grown in M9 minimal media to control nutrient variables and focus on PET hydrolysis.
•M9 media recipe (per 1L):
- 200 mL M9 Salts (5X)
- 4 mL Glucose (20% w/v; final 0.4%)
- 2 mL $MgSO_4$ (1 M; final 2 mM)
- 0.1 mL $CaCl_2$ (0.1 M; final 100 µM)
- Sterile DI water to 1L

•Media autoclaved at 121°C, despite glucose caramelization risks.

2. NaCl Experimental Conditions
•NaCl added to flasks at: 0 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M, 1.0 M
•Each flask inoculated with *E. coli* K12 at 2% inoculum

3. CO_2 Assays
•PETase degrades PET into TPA and EG, which enter respiration → CO_2 production
•CO_2 output measured with Vernier Go Direct CO_2 Sensors
- Connected to LabQuest, reading every 10 mins for 24 hrs
- Data exported as .txt files

•Flasks sealed with parafilm to prevent gas exchange

Fig 1. Experimental Flasks with CO2 sensor and stir column initiated

4. OD600 Spectrophotometry
•OD600 measures bacterial cell density via light scattering
•Readings taken at start and end of culture period
•Blank: 1 mL M9 minimal media
•Samples drawn from top to avoid PET chunks affecting clarity

5. PET Preparation
•PET plastic pre-treated under UV light for 200 hours
•Ground into powder using a spice grinder for uniform degradation

Results

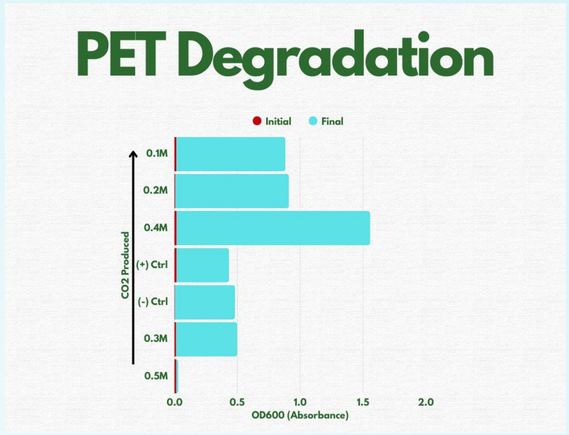

Fig 2. results of experimentation. Experimental groups are in descending order of Co2 output; while being graphed with respect to their OD600. This allows us to see which condition was optimal for microbial degradation.

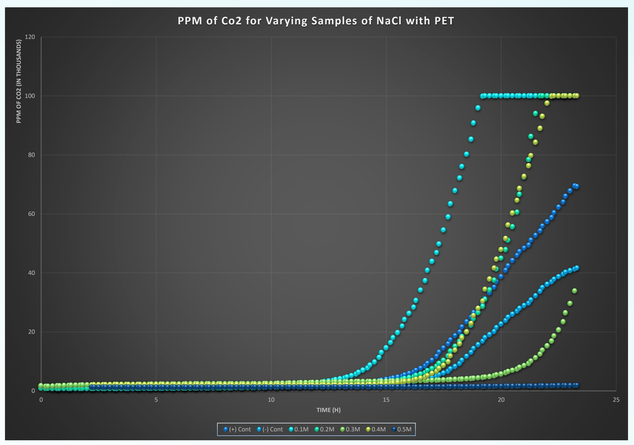

Fig 3. Co2 production of varying NaCl samples

Conclusion/Setbacks

Conclusion

•**0.1 M NaCl** produced the highest CO_2 output, indicating this concentration led to the most effective PET degradation.

•**0.4 M NaCl** had the highest OD600, meaning K12 thrived at this salt level. However, the lower CO_2 output highlights that more bacterial growth doesn't necessarily lead to better plastic breakdown.

•**UV pretreatment (200 hours)** was critical. It oxidized the PET surface, increasing hydrophilicity and allowing PETase to better bind and hydrolyze the polymer.

•**Why measure both OD600 and CO_2?** OD600 captured cell growth, while CO_2 output reflected actual PET metabolism.

•**Results:** more trials are needed to confirm the ideal balance of bacterial growth and enzyme activity across salt concentrations.

Setbacks and How We Solved Them

•**Setback 1: Pioreactor System Limitations**
We originally planned to use a Pioreactor for real-time OD600 and CO_2 data, but it failed to connect via Bluetooth. Its sensor also only detected ambient CO_2—not gas produced inside the culture flask.
Solution: We built a custom setup with an incubation.

• **Setback 2: Insufficient Initial Growth**
Our early cultures (positive and negative controls) barely grew because we used too few CFUs from plated bacteria.
Solution: We switched to using standardized liquid cultures and inoculated each flask with a consistent 2% volume. We also treated the OD600 blank under the same growth conditions to ensure accurate comparison.