Investigating the Potential Additive Effects of UV and Heat Pretreatment in the Bioremediation of Low-density Polyethylene (LDPE) Lab Waste by *Bacillus cereus* and *Pseudomonas aeruginosa*

Final Thesis

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Declaration of authorship

We, **Tim Hannah** and **Natacha Jean**, confirm that this work submitted for assessment is our own and is expressed in our own words. Any uses made within it of the works of any other author, in any form (ideas, equations, figures, texts, tables, programs), are properly acknowledged at the point of use. A list of the references used is included.

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Abstract

Plastic pollution, of which wet laboratory research is a major contributor to, has a major, well documented impact on the environment. Currently, biodegradable plastics and recycling are two ways used to curb plastic waste, however, the latter isn't done at a scale high enough to cope with demand; consequently these plastics end up in landfill, where they remain to degrade slowly for decades. One potential solution proposed is the introduction of plastic degrading organisms via bioremediation. This study looks into investigating the effectiveness of *Bacillus cereus* and *Pseudomonas aeruginosa* in degrading low-density polyethylene (LDPE) that has been pre-treated by UV, heat, and/or as a combination of both in order to examine its efficiency and to determine if these effects are worth exploring in the future. The results of this study revealed that *B. cereus* and *P. aeruginosa* do efficiently degrade the pre-treated LDPE, though these outcomes depend on the method of pre-treatment and length of the remediation.

Key words: pre-treated LDPE, bioremediation, *B. cereus*, *P. aeruginosa*

1. Introduction

1.1. Background Information

1.1.1. Plastic Bioremediation

The impacts of plastic pollution and the UV oxidative formation of microplastics (MPs) on the environment have been a subject of focus across a broad range of scientific areas since their first recorded observations by Thompson *et al.* (2004). As plastic consumption continues to grow, so too do the instances in which MPs have had a detrimental impact on organisms in the environment; nearly 700 recorded species have been negatively affected by the internalisation of MPs (Lokesh *et al.*, 2023), including many freshwater and marine species (Alak *et al.*, 2022), terrestrial plant and animal species (Machado *et al.,* 2017), and most recently, microplastics have been reportedly observed during human cardiac surgery (Yang *et al.*, 2023).

The general public has become more conscientious of their plastic consumption; despite efforts to use alternatives such as biodegradable plastics (Ghosh & Jones, 2021), or implement plastic recycling (through both government and NGO schemes) at the personal and industrial level (Ertz *et al.*, 2023, Labcycle, 2022), the quantity of recycled plastic varies by geographical location, generally as a net outcome, not enough is being recycled to impact the rate in which plastics are used so more of it ends up in landfill which, in turn, contributes to the issues of microplastic pollution (Hopewell *et al.*, 2009). In searching for a solution, many instances of plastic degrading microorganisms were reported, and thus the idea of bioremediation (the use of organisms to consume pollutants) became a popular avenue of research for tackling plastic pollution (Sivan, 2011).

Though most plastics are semi to fully synthetically manufactured, structurally, these materials are formed of natural hydrocarbons and are therefore organic chemicals that can act as a source of carbon to any organisms that have the appropriate biochemical pathways to process them (Sharma, 2018). Many of the organisms that have been determined to be appropriate for the bioremediation of plastic/microplastics are bacteria and fungal species; because of this, candidates for research have predominantly been within these kingdoms (Shahnawaz *et al.*, 2019), and generally,

existing species found in the polluted environment are targeted for use (Malachova *et al.,* 2020).

1.1.2. Applications of UV and Heat as a Pre-Treatment for Plastic Degradation

Though degradation of plastics by bacterial species is possible, the process is slow and inefficient (Kale *et al.,* 2015) due to the lack of hydrolytic or functional groups for enzymatic breakdown (Taghavi *et al.,* 2021). There have been many solutions proposed to solve this issue such as the use of specialised enzymes like PETase and METase (Yoshida *et al.*, 2016), encouraging biofilm attachment by use of surfactants (Vimala & Mathew, 2016), as well as artificially ageing the material by treatment with radiation, heat, and chemical solvents (Taghavi *et al.,* 2021).

Of all of the proposed solutions, the most simple and cost effective means of increasing degradation efficiency is through the use of UV radiation, and by the use of heat, (Chaudry *et al.*, 2021). Both UV and heat pre-treatment work by damaging the molecular structure of plastics by introducing oxygen free radicals that can be readily used and further processed by bacteria that possess the adequate biochemical pathways, (Gewert *et al*., 2015).

1.1.3. Low Density Polyethylene (LDPE) and Wet Lab Research Plastic Waste

Two forms of polyethylene (low density - LDPE and high density - HDPE) combined represent about 69% of all plastic waste (De la Rosa, *et al.*, 2019), and as such, represents a bulk of the volume of global plastic pollutants (Alali *et al.*, 2023).

Wet lab research as an industry represents one of the largest contributors of plastic waste to landfill, accounting for around 5.5 million tonnes per year of mostly single use plastics, (Urbina *et al.*, 2015). The majority of these single use products are produced from LDPE, and whilst headway has been made to address the issues of lab plastic waste (Alvez *et al.* 2021), there still exists a need for a cheap, efficient, and scalable method for bioremediation of this form of plastic waste at the industrial scale.

The first major hurdle to achieving an effective solution is the identification of species that are able to biodegrade LDPE; many species have been shown to be successful candidates, (Shah *et al.*, 2008). Low degradation efficiency however, means that steps must be made to optimise the conditions for bioremediation (such as temperature, supplemental chemicals, pretreatments, pH, remediation time, etc.) to make the process efficient and viable in upscaling to an industrial level to deal with the scale of the plastic pollution problem.

1.1.4. Bacillus cereus and Pseudomonas aeruginosa as Organisms of Interest for LDPE Degradation

Bioremediation can be achieved by either the use of pre-existing species within the polluted environment, or through the introduction of external species assuming that the ecosystem can handle the introduction of a new species (Megharaj *et al*., 2011).

Whilst more convenient, the use of native species for bioremediation is limited to the assumption that any of the species available are able to biochemically degrade the target pollutant. In the instances where the native microflora are unable to process plastics such as LDPE, new species have to be introduced to the ecosystem that have proven success at LDPE biodegradation; two such bacteria that have shown potential as a bioremediation species for both aquatic and terrestrial environments are *Bacillus cereus* (Auta *et al.*, 2017, Suresh *et al.*, 2011), and *Pseudomonas aeruginosa* (Tamnou *et al.*, 2021, Kyaw, 2012).

Both of these bacteria are ubiquitous species (Moradeli *et al.*, 2017, Carlin, *et al.*, 2010) making them easy to incorporate into any given environment, and they are also common wet lab research strains, making them well understood targets for potential candidate species in LDPE bioremediation.

1.2. Rationale

The rationale for the following research is based on the idea that *B. cereus* and *P. aeruginosa* are well researched, easy to incorporate species that have shown the ability to biodegrade LDPE, making them worth exploring in terms of bioremediation candidates for addressing plastic pollution by wet lab organisations. Though the

scientific literature has not shown these species to have significant efficiency as LDPE bioremediators, there are steps that can be taken to increase their degradation efficacy.

Many studies have shown that UV, heat, as well as a combination of the two used as a pre-treatment on LDPE increases the effectiveness of a bacterial species' ability to degrade it; the effects of these pre-treatments, however, have not been tested specifically using *B*. *cereus* or *P. aeruginosa* degradation ability. The purpose of this study aims to observe any potential additive effects of LDPE pre-treatment that may lead to these bacterial species becoming more viable candidates in LDPE bioremediation at an industrial scale.

1.3. Objectives and Hypothesis

1.3.1. Objectives

- To determine if glucose is required to maintain *B. cereus* and *P. aeriginosa*'s viability in the media.
- To evaluate any enhanced effects of LDPE degradation when pre-treated by UV, heat, or as a combination by utilising *B. cereus* and *P. aeruginosa*.
- To determine if any observed enhancements to bioremediation efficiency of *B. cereus* and *P. aeruginosa* is significant enough to continue exploring.

1.3.2. Hypothesis

- There is a minimal amount of glucose that must be present for B. cereus and P. aeruginosa to survive in minimal salt media.
- Exposure of LDPE to UV, heat, and/or a combination of both will lead to degradation of the plastic, validating its use as a pre-treatment for bioremediation.
- Pre-treatment of LDPE will result in a more efficient degradation of plastics by *B. cereus* and *P. aeruginosa*.

2. Materials and Methods

2.1. List of Materials

2.1.1. Culture

Bacillus cereus (ATCC 11778) and *Pseudomonas aeruginosa* (ATCC 27853) are the two cultures used as the organisms of interest for plastic biodegradation of the LDPE samples.

2.1.2. Media and Chemicals

The main media used is Minimal Salt Media (MSM), which provides nutrients for the bacteria in order for it to survive. A carbon-source such as glucose is added separately to the media, which will allow the bacteria to proliferate and start consuming the LDPE samples once they're submerged. The recipe to prepare the MSM was taken from Sekar *et al.,* 2011 and is summarised in Table 1. The media was autoclaved at 121ºC for 15 minutes before proceeding to the next steps of the experiment.

Table 1. Recipe to prepare MSM per 1 L of distilled water as tabulated from Sekar *et al.,* 2011, autoclaved at 121ºC for 15 minutes.

Chemical Compound (solid form)	Amount (g/L)
Dipotassium hydrogen phosphate, K_2HPO_4	1.73
Potassium dihydrogen phosphate, KH_2PO_4	0.68
Magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$	0.1
Iron (II) sulfate heptahydrate, $FeSO_4 \cdot 7H_2O$	0.03
Sodium chloride, NaCl	4.0
Ammonium nitrate, NH_4NO_3	1.0
Calcium chloride dihydrate, $CaCl_2 \cdot 2H_2O$	0.02

In addition to MSM, Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA), and Phosphate Buffer Saline (PBS) are the other media used; they were prepared according

to the manufacturers' instructions, and autoclaved at 121ºC for 15 minutes. Finally, D-glucose was prepared to determine the glucose concentration necessary to aid in the bacterial growth once added in MSM. D-glucose was autoclave at 121ºC for 15 minutes.

2.1.3. Equipment

The shaking incubator is utilised for the biodegradation of the LDPE samples as it provides constant agitation, preventing the plastic and bacterial cells from settling at the bottom. The constant agitation also supports continuous mixing of the solution, allowing the bacteria to make contact and adhere to the plastic. A UV lamp and an oven (heat source) are used to pre-treat the LDPE samples before placing them in the MSM and *B. cereus*/*P. aeruginosa* media. After the incubation period, a gravimetric apparatus was used to perform a gravimetric assay to collect the degraded LDPE samples to quantify the lost mass. IR spectroscopy was used to analyse the LDPE samples before and after UV and heat treatment, as well as after the bioremediation.

The list below presents the rest of the materials/supplies utilised:

- 1 mL, 5 mL, and 10 mL serological pipettes
- P100 and P1000 Micropipettes
- Micropipettes tips
- Sterile syringe
- Inoculating loop
- Sterile spreaders
- McFarland Standards
- Gravimetric assay apparatus:
  → Buchner funnel
  → Vacuum flask
  → Filter paper
- Analytical balance

2.2. Methods

2.2.1. Glucose assay

Prior to inoculating the pre-treated LDPE samples in MSM, the minimal concentration of glucose had to be determined in order to add the amount that will maintain the bacteria's survival, but also force its starvation so that it uses the LDPE as a carbon-source for nutrients. To start, liquid cultures of *B. cereus* and *P. aeruginosa* were prepared by inoculating 1 colony from their respective purity plate in TSB and incubating at 35ºC for 24 hours. After the incubation period, the concentration of the liquid cultures were determined by using McFarland standards. A serial dilution was prepared from a diluted stock solution of the *B. cereus*/*P. aeruginosa* culture to achieve $1x10^3$ dilution, from which 0.1 mL was inoculated in MSM-filled tubes. Starting the glucose assay, 1 M of sterilised glucose was prepared and placed in a sterile tube. Duplicate tubes of MSM filled with 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, and 20 % (v/v) glucose were prepared for *B. cereus* and *P. aeruginosa*. The tubes were incubated at room temperature for 7 days. After the incubation period, the tubes were observed for turbidity. Using TSA plates, spread plating was performed on the lowest concentration that exhibited turbidity, as well as the concentration below and above to test for cell viability. The plates were incubated at 35ºC for 24 hours.

2.2.2. Plastic Sample Preparation

As the point of focus for this investigative paper is centered on plastic waste in laboratories, micropipette tips were chosen as the LDPE samples. The method of shredding the tips was selected to ensure a flat surface for the bacteria to adhere on to the plastic to facilitate the biodegradation. The process involved cutting 50 micropipette tips using scissors sanitised with 1.6 % citrus disinfectant and cutting them in small pieces. These pieces were then placed in a blender and set to 'shred' mode to effectively shred the plastic. These pieces were portioned accordingly to treat them under UV, heat, and UV + heat combined. IR spectroscopy was used to analyse a piece of the plastic to establish a base spectra and to be used as a point of comparison for IR spectra of the plastic samples post-UV/heat treatment and post-remediation.

2.2.3. UV/Heat Treatment of Plastic Sample

The shredded plastic pieces were pre-treated under three conditions:
Condition (1): the plastic pieces were placed under a UV lamp (UVP - Compact UV Lamp, 4 W, 254 nm), where they were left for 6 days. On day 7, the pieces were subjected to a higher intensity UV lamp to further ensure degradation.
Condition (2): the plastic pieces were placed in an oven as a heat source, with the temperature set at 60ºC for up to 24 hours.
Condition (3): the plastic pieces underwent combined treatment of both UV and heat as described in condition 1 and 2.

Once the plastic pieces had undergone the treatments, they were analysed by IR spectroscopy to identify LDPE degradation species and proceeded towards bioremediation.

2.2.4. Bioremediation of LDPE

Falcon tubes were assembled in duplicates for *B. cereus* and *P. aeruginosa* bioremediation media. For *B. cereus* media, 2.0 % glucose (0.8 mL) was pipetted in its set of Falcon tubes and MSM was added to make up a total volume of 40 mL. Similarly, for *P. aeruginosa*, 3.0 % glucose (1.2 mL) was pipetted in its set of Falcon tubes and MSM was added to make up a total of 40 mL. 1.0 g of the pre-treated shredded LDPE pieces (UV, heat, and UV + heat) were aseptically added to each tube, followed by 0.1 mL of 1.0 % SDS and 0.1 mL of each bacteria in their respective sets. Moreover, several controls were prepared in the following manner: a negative control consisting of MSM and untreated LDPE; and two types of positive controls, the first consisting of MSM, untreated LDPE and *B.cereus*/*P. aeruginosa* (without glucose), and the second consisting of TSB., untreated LDPE, and *B. cereus*/*P. aeruginosa*. The tubes were incubated in an incubator shaker at 200 rpm at room temperature. The LDPE samples were evaluated and analysed at 2, 3, and 4 weeks by IR spectroscopy to observe any changes in the presence of the pre-treated degradation species and the introduction of new species from bioremediation; microbial counts to examine the bacterial growth reflective of the ability for the bacteria to use LDPE as a carbon-source; and gravimetric assay to quantify the amount of degraded LDPE.

3. Results

3.1 Qualitative Results

3.1.1 IR spectra

Figure 1a) and 1b) display the IR spectra of pre-treated LDPE before bioremediation, as obtained from IR spectroscopy.

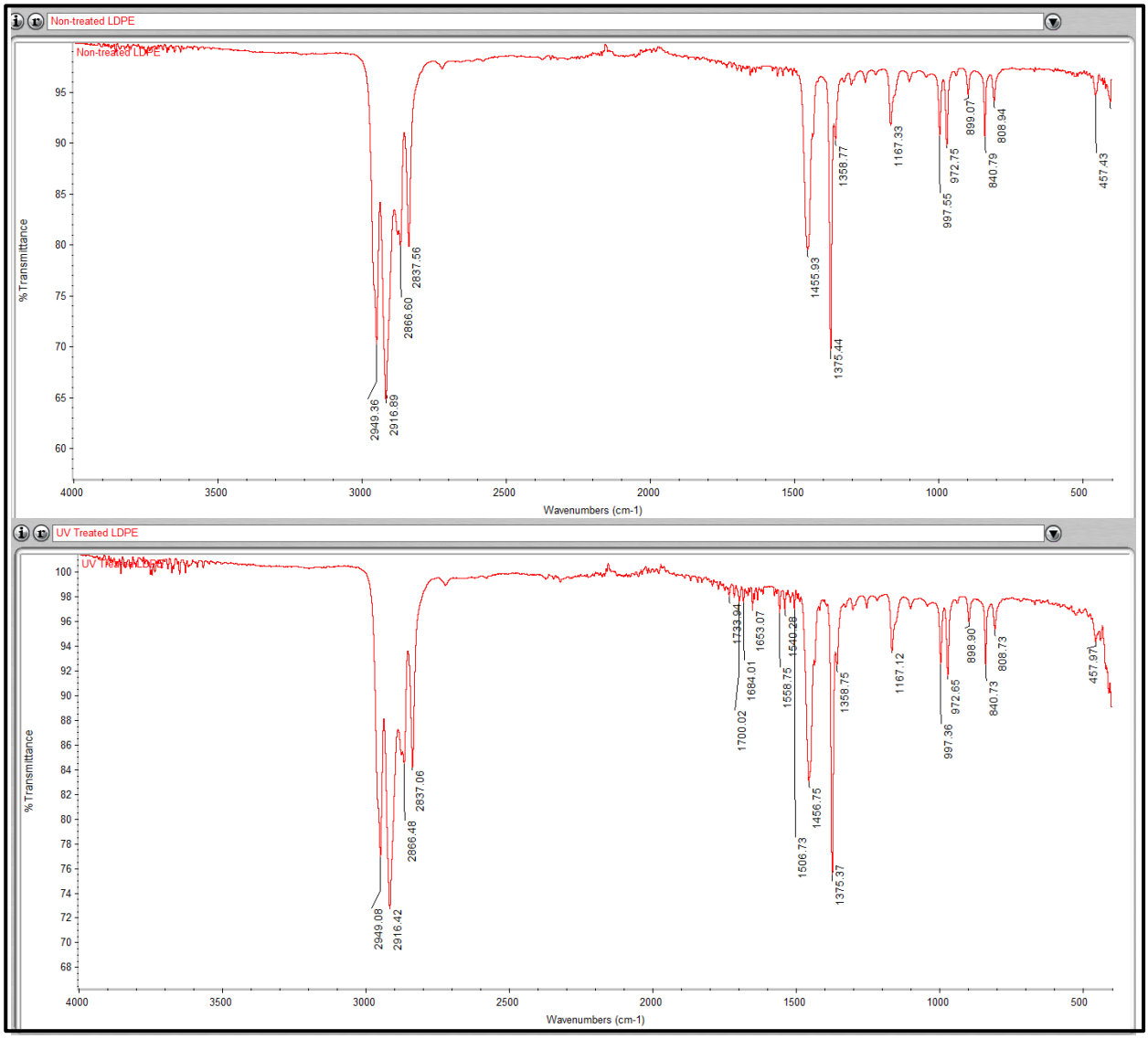

Figure 1a). IR spectra of non-treated (top) and UV-treated LDPE (bottom) pre-remediation.

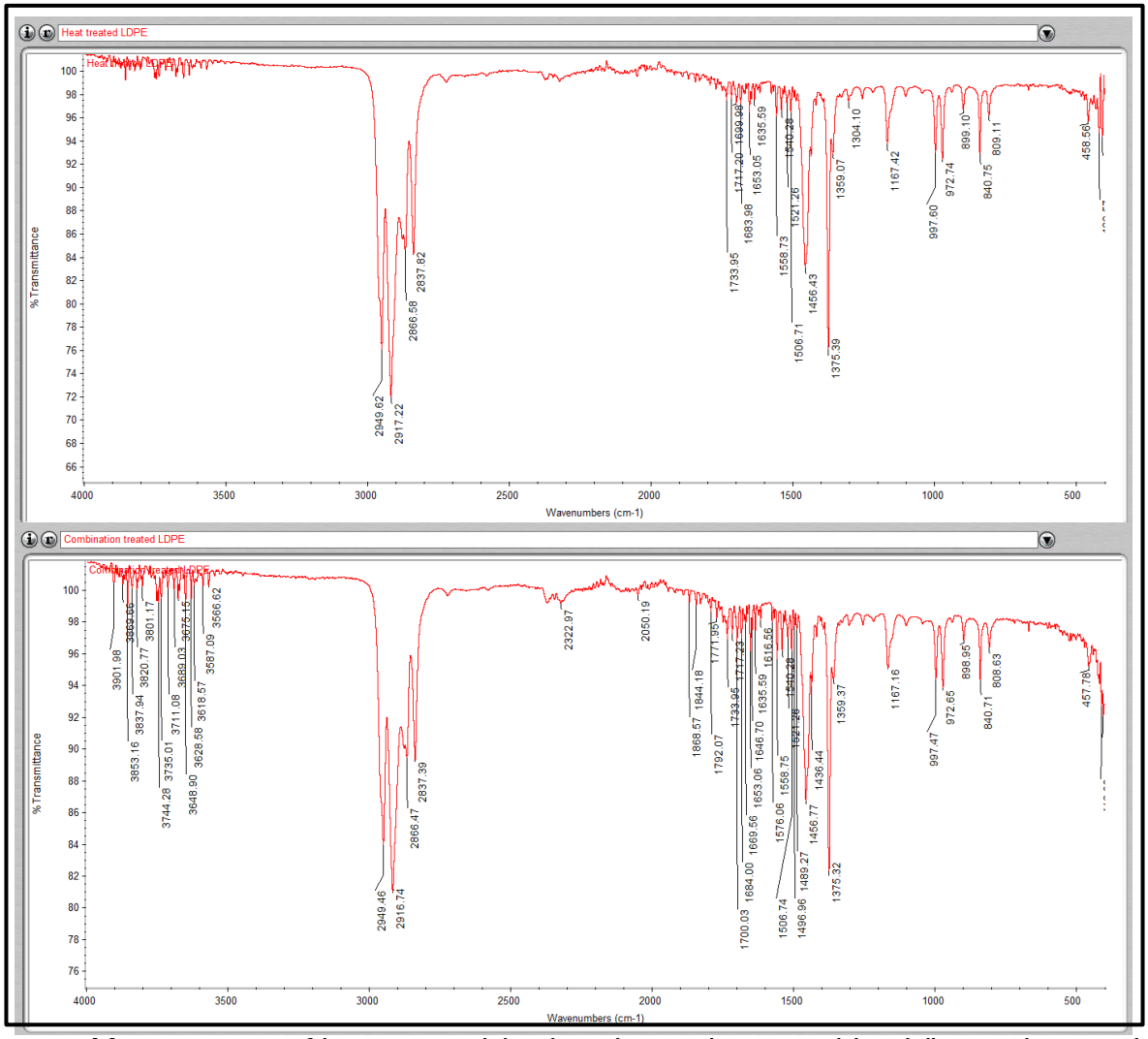

Figure 1b). IR spectra of heat-treated (top) and UV + heat combined (bottom) treated LDPE pre-remediation.

Figure 2a) and 2b) show the IR spectra of pre-treated LDPE bioremediated by *B. cereus* after 4 weeks obtained from IR spectroscopy. Similarly, figure 3a) and 3b) show the IR spectra of pre-treated LDPE bioremediated by *P. aeruginosa* after 4 weeks from IR spectroscopy.

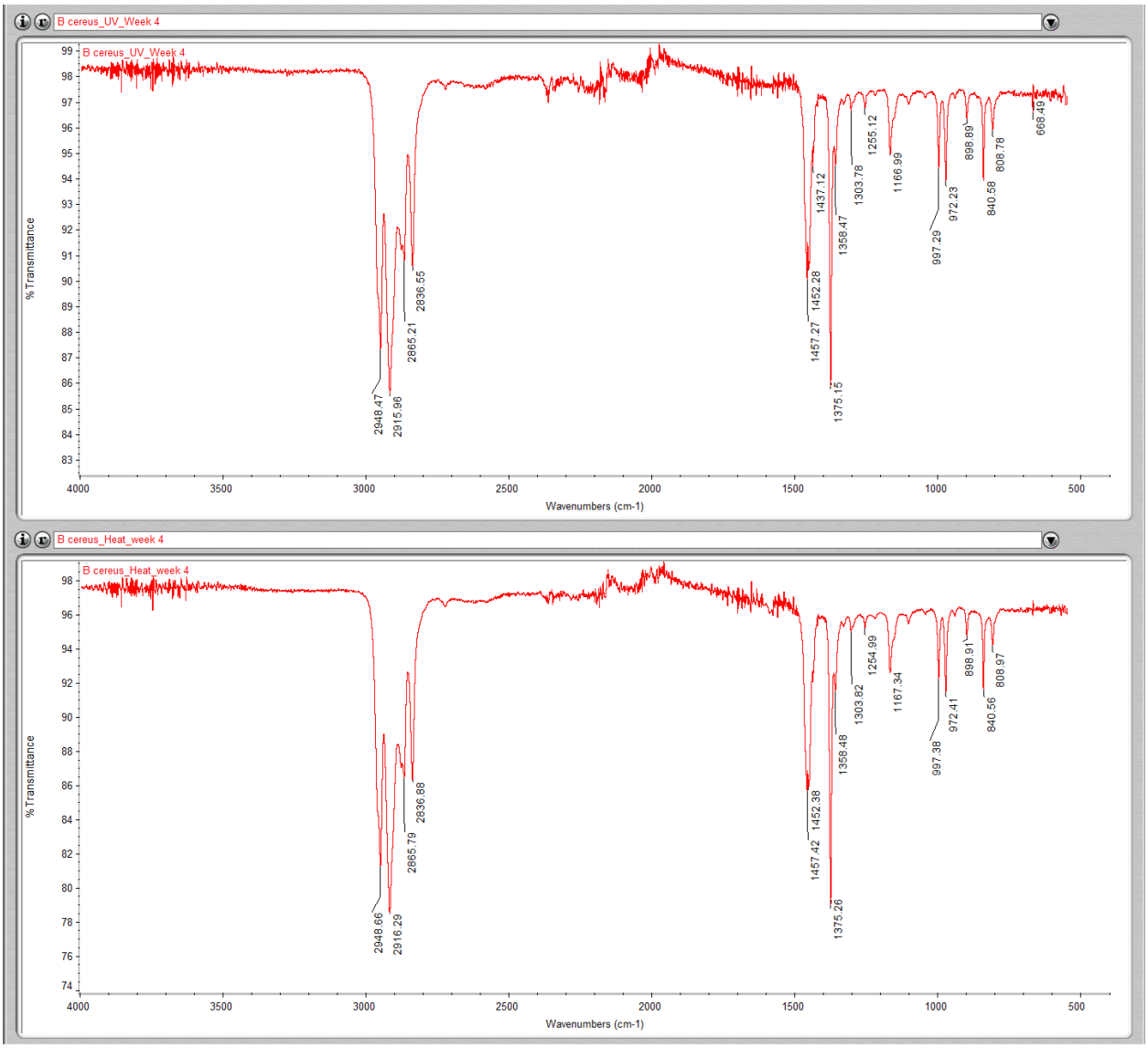

Figure 2a). IR spectra of UV-treated LDPE (top) and heat-treated LDPE (bottom) remediated by *B. cereus* after 4 weeks.

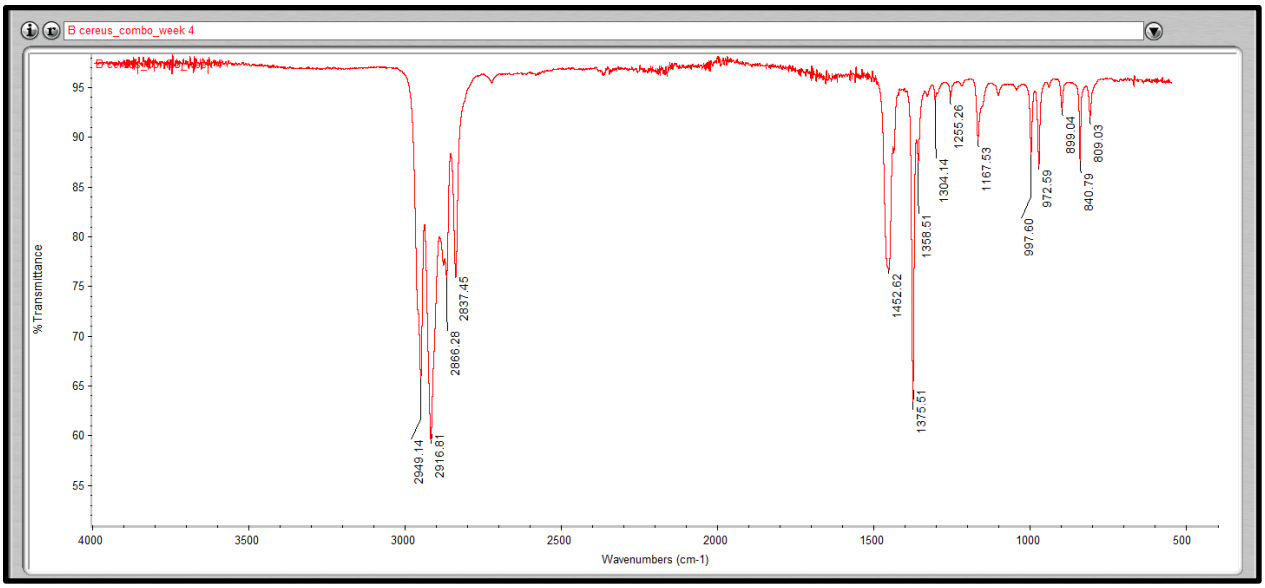

Figure 2b). IR spectra of UV and heat-treated LDPE remediated by P. aeruginosa after 4 weeks.

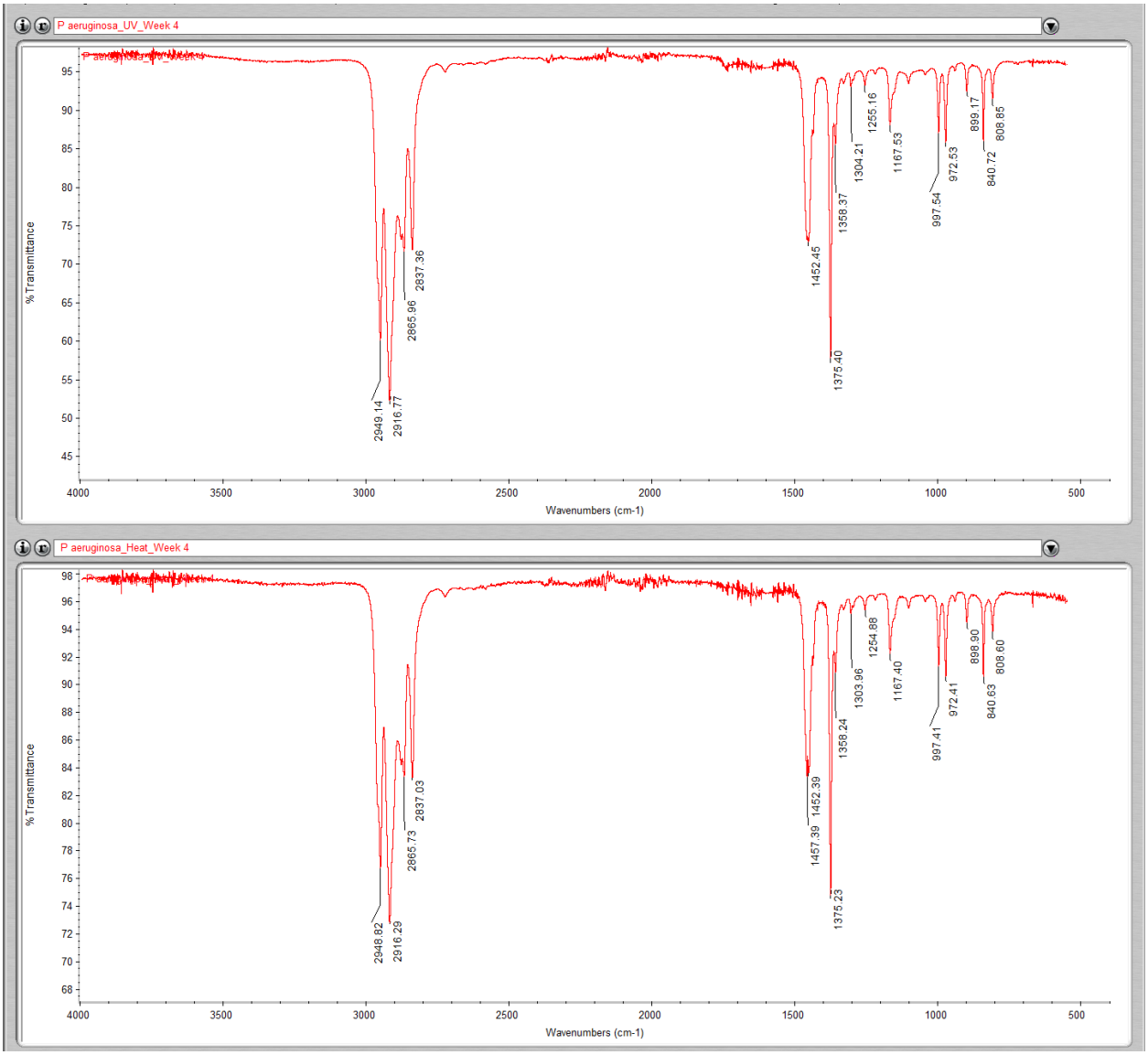

Figure 3a). IR spectra of UV-treated LDPE (top) and heat-treated LDPE (bottom) remediated by *P. aeruginosa* after 4 weeks.

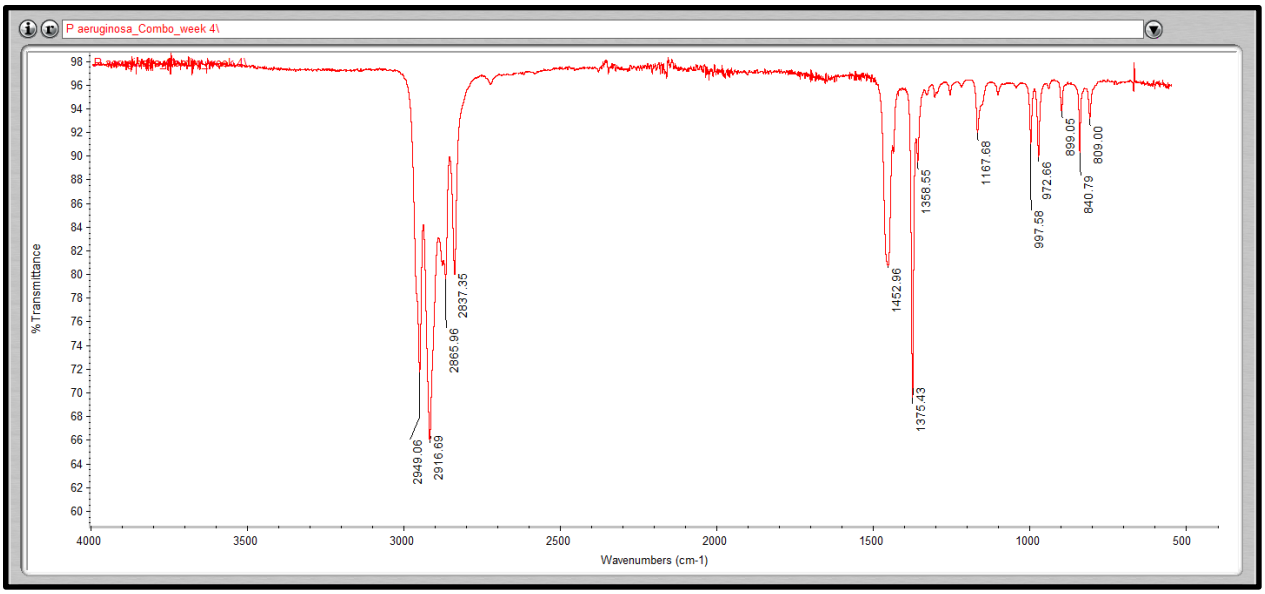

Figure 3b). IR spectra of UV and heat treated LDPE remediated by *P. aeruginosa* after 4 weeks.

3.1.2 Viability Plates

The figures below show the results of the viability plates of the remediated LDPE samples by *B. cereus* and *P. aeruginosa* after 4 weeks to confirm the growth of bacteria. Similarly for the controls, they were plated after 4 weeks to confirm sterility of media and viability of bacteria in media without glucose.

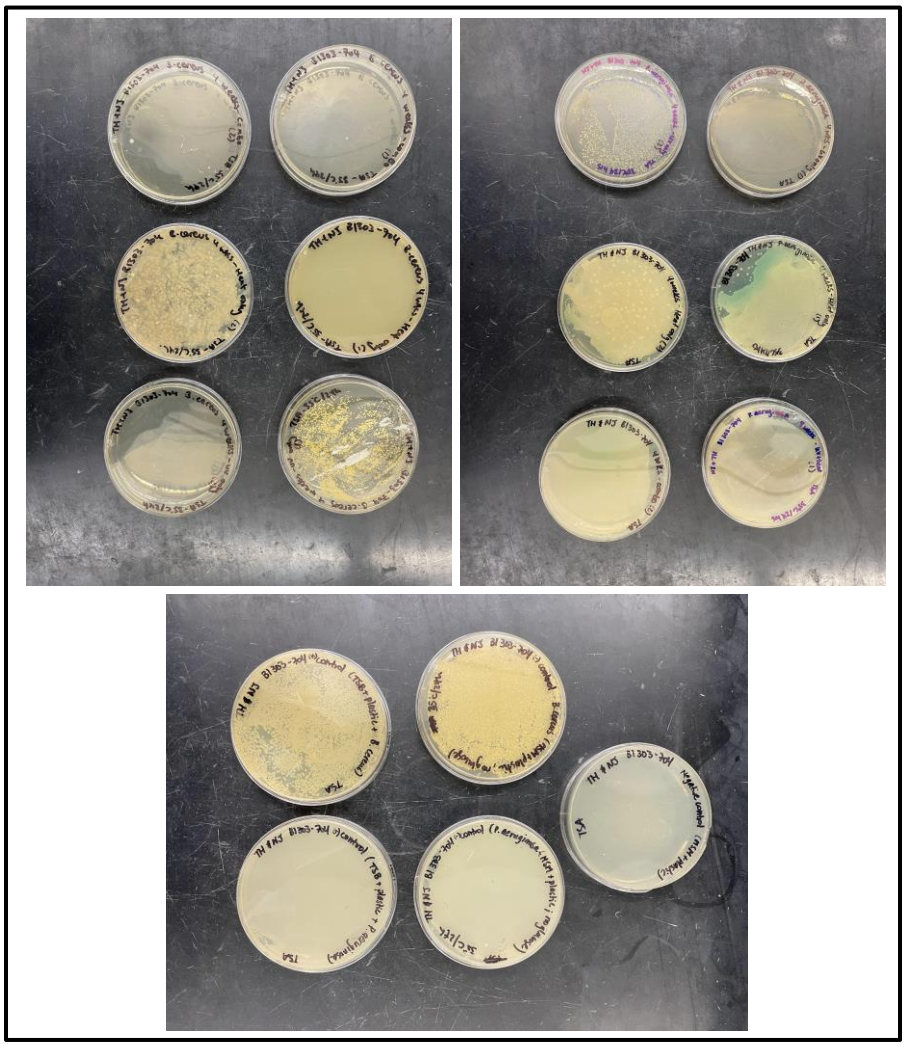

Figure 4. Viability plates of remediated pre-treated LDPE by *B. cereus* (left picture) and *P. aeruginosa* (right picture) and controls (bottom center) after 4 weeks, incubated at 35ºC for 24 hours.

3.2 Quantitative Results

3.2.1 Gravimetric assay - Percent mass loss

The following tables present the results of the remediated pre-treated LDPE samples after 2, 3, and 4 weeks. An initial mass of 1.0 g was placed in the tubes pre-remediation and the final mass for each trial was collected post-remediation via gravimetric assay;their mass was measured by weight by difference. The average of the mass loss was calculated to obtain an average % weight loss. Similarly for the controls, 1.0 g of untreated LDPE was placed pre-remediation and its final mass was measured post-remediation after 4 weeks to calculate the % weight loss.

Week 2 Results:

Table 2. Gravimetric analysis of 1.0 g of bioremediated LDPE samples in duplicates by *B. cereus* in MSM spiked with 2.0 % glucose, incubated in an incubator shaker for 2 weeks at 200 rpm at room temperature.

B. cereus							
Pre-treatment method	Initial mass (g)	Final mass - Trial 1 (g)	Final mass - Trial 2 (g)	Difference of mass (g)		Average mass (g)	Avg % weight loss
				#1	#2		
UV	1.0000	0.9377	0.9260	0.0623	0.0740	0.06815	6.9
Heat	1.0000	0.8838	0.7522	0.1162	0.2478	0.1820	18.2
Combo	1.0000	0.8811	0.8956	0.1189	0.1044	0.11165	11.2

Table 3. Gravimetric analysis of 1.0 g of bioremediated LDPE samples in duplicates by *P. aeruginosa* in MSM spiked with 3.0 % glucose, incubated in an incubator shaker for 2 weeks at 200 rpm at room temperature.

P. aeruginosa							
Pre-treatment method	Initial mass (g)	Final mass - Trial 1 (g)	Final mass - Trial 2 (g)	Difference of mass (g)		Average mass (g)	Avg % weight loss
				#1	#2		
UV	1.0000	0.8407	0.9553	0.1573	0.0447	0.1020	10.2

Heat	1.0000	0.9882	0.9082	0.0118	0.0918	00518	5.2
Combo	1.0000	0.9237	0.8117	0.0763	0.1883	0.1323	13.2

Week 3 Results:

Table 4. Gravimetric analysis of 1.0 g of bioremediated pre-treated LDPE samples in duplicates by *B. cereus* in MSM spiked with 2.0 % glucose, incubated in an incubator shaker for 3 weeks at 200 rpm at room temperature.

B. cereus							
Pre-treatment method	Initial mass (g)	Final mass - Trial 1 (g)	Final mass - Trial 2 (g)	Difference of mass (g)		Average mass (g)	Avg % weight loss
				#1	#2		
UV	1.0000	0.9260	0.8888	0.0794	0.1112	0.0953	9.5
Heat	1.0000	0.9543	0.9464	0.0457	0.0563	0.04965	5.0
Combo	1.0000	0.9108	0.9325	0.0892	0.0675	0.07835	7.8

Table 5. Gravimetric analysis of 1.0 g of bioremediated pre-treated LDPE samples in duplicates by *P. aeruginosa* in MSM spiked with 3.0 % glucose, incubated in an incubator shaker for 3 weeks at 200 rpm at room temperature.

P. aeruginosa							
Pre-treatment method	Initial mass (g)	Final mass - Trial 1 (g)	Final mass - Trial 2 (g)	Difference of mass (g)		Average mass (g)	Avg % weight loss
				#1	#2		
UV	1.0000	0.8985	0.8700	0.1015	0.1300	0.11575	11.6
Heat	1.0000	0.9292	0.9722	0.0708	0.0278	0.0493	4.9
Combo	1.0000	0.8819	0.8663	0.1181	0.1337	0.1259	12.6

Week 4 Results + Controls:

Table 6. Gravimetric analysis of 1.0 g of bioremediated pre-treated LDPE samples in duplicates by *B. cereus* in MSM spiked with 2.0 % glucose, incubated in an incubator shaker for 4 weeks at 200 rpm at room temperature.

B. cereus							
Pre-treatment method	Initial mass (g)	Final mass - Trial 1 (g)	Final mass - Trial 2 (g)	Difference of mass (g)		Average mass (g)	Avg % weight loss
				#1	#2		
UV	1.0000	0. 8754	0.9048	0.1246	0.0952	0.1099	11.0
Heat	1.0000	0.8077	0.8953	0.1923	0.1047	0.1485	14.9
Combo	1.0000	0.8569	0.8379	0.1431	0.1621	0.1526	15.3

Table 7. Gravimetric analysis of 1.0 g of bioremediated pre-treated LDPE samples in duplicates by *P. aeruginosa* in MSM spiked with 3.0 % glucose, incubated in an incubator shaker for 4 weeks at 200 rpm at room temperature.

P. aeruginosa							
Pre-treatment method	Initial mass (g)	Final mass - Trial 1 (g)	Final mass - Trial 2 (g)	Difference of mass (g)		Average mass (g)	Avg % weight loss
				#1	#2		
UV	1.0000	0.9419	0.8613	0.0581	0.1387	0.0984	9.8
Heat	1.0000	0.9577	0.9337	0.0423	0.0663	0.0543	5.4
Combo	1.0000	0.9131	0.9196	0.0869	0.0804	0.08365	8.4

Table 8. Gravimetric analysis of 1.0 g bioremediated untreated LDPE samples set-up as controls, incubated in an incubator shaker for 4 weeks at 200 rpm at room temperature.

Controls	Initial mass (g)	Final mass (g)	% weight loss
MSM + plastic	1.0000	0.9963	0.37
*MSM + plastic + *B. cereus*	1.0000	1.0000	0
*MSM + plastic + *P. aeruginosa*	1.0000	1.0000	0
TSB + plastic + *B. cereus*	1.0000	0.9820	1.80
TSB + plastic + *P. aeruginosa*	1.0000	0.9655	3.45

**Note: no glucose was added to the negative controls of MSM and plastic with the test organism to evaluate if they would consume the plastic as a carbon-source on its own.*

Figure 5 presents charts of the average % weight loss from the bioremediated LDPE samples for each week after adjusting for any anomalous data points. The charts generalise the trends that show which pre-treated LDPE was most effectively degraded by the bacteria.

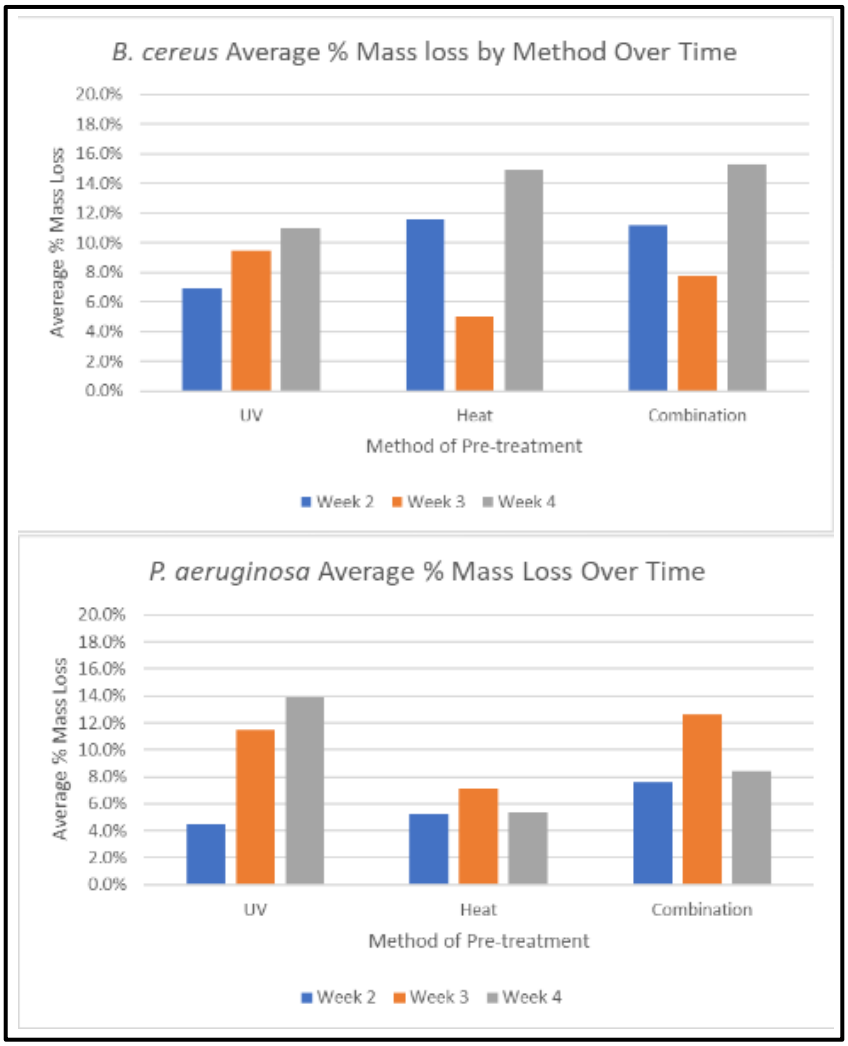

Figure 5. Visual representation presented of the average % weight loss post-remediation of the pre-treated LDPE experienced after 2, 3, and 4 weeks by *B. cereus* (top) and *P. aeruginosa* (bottom).

Figure 6 presents charts comparing the % weight loss of the controls to the pre-treated LDPE sample that experienced the highest mass loss by each organism.

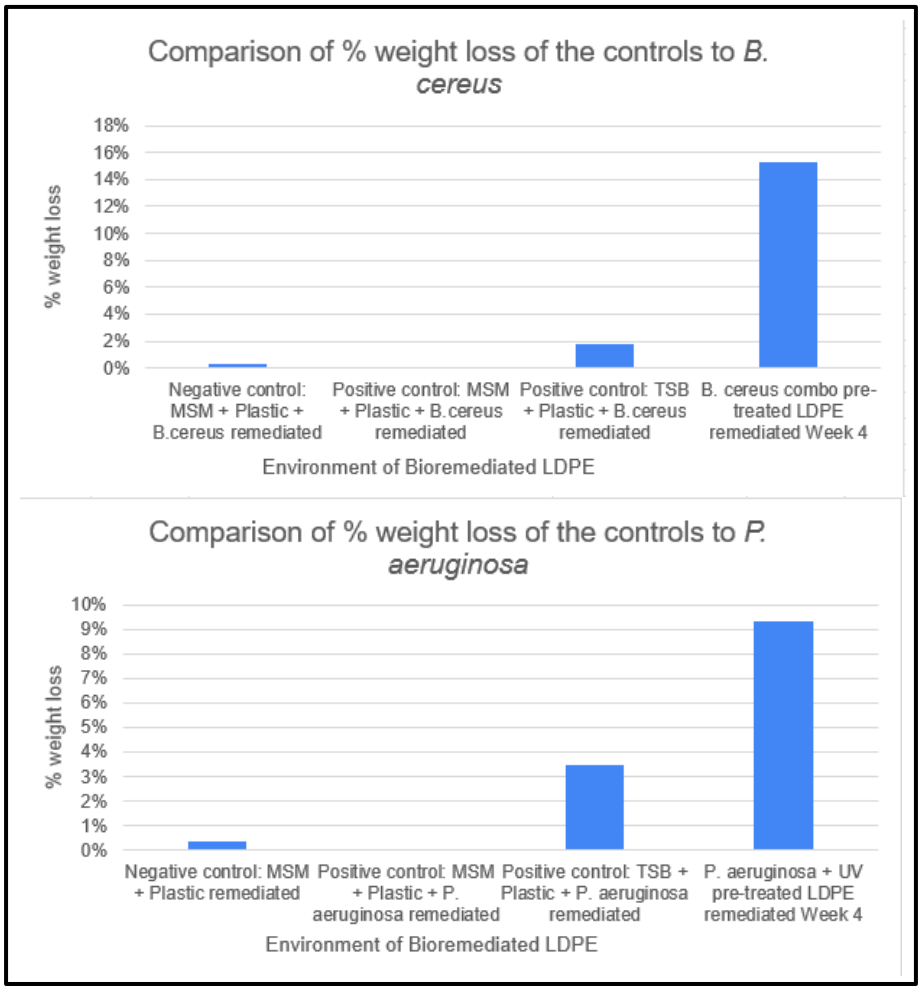

Figure 6. Visual representation comparing the % weight loss of the remediated LDPE samples in the controls to the highest % weight loss of remediated UV + heat pre-treated LDPE with *B. cereus* (top) and to the highest % weight loss of remediated UV pre-treated LDPE with *P. aeruginosa.*

4. Discussion and Conclusion

4.1 Qualitative Analysis

4.1.1 IR analysis

LDPE samples were analysed using attenuated total reflectance infra-red spectroscopy (ATR-IR) at three key experimental stages; before plastic treatment, after treatment (UV, heat, and combination), and after remediation (at the 2, 3, and 4 week marks). All expected identifier peaks were resolved for the non-treated LDPE sample (as shown in Figure 1a) including the CH_2 asymmetric stretch, symmetrical stretch, and bending deformation at 2815, 2837, and 1455 cm^{-1} respectively (Chaudhary et al., 2021).

ATR-IR analysis after degradation treatments with UV, heat, and combination were able to resolve peaks corresponding to degradation species as described in prior literature; specifically the 1558 cm^{-1} peak related to formation of carboxylate containing species by UV oxidation (Weiland *et al.*, 1995), as well as the 1733 cm^{-1} peak related to formation of carbonyl containing species from heat degradation, and the 1771 cm^{-1} peak related to thermal degradation of UV treated plastics (Awasthi *et al.*, 2017).

Interestingly, from as early as the 2-week remediation point and consistently through to the 4th week, significant decreases in the degradation peaks were observed when post-remediation samples were analysed; this indicates that the degradation species were being removed. As the LDPE sample represents the only carbon source within the MSM media, it is likely that these degradation species were internalised by the bacterial culture for use as a nutrient carbon source; this was confirmed by plating of the culture from the remediation media onto TSA plates to determine bacterial viability.

4.1.2 Viability Plates

To test for bacterial viability within the MSM media after each remediation trial, aliquots of culture were plated onto TSA plates to check for cell growth after incubation. Lawn growth was observed in the majority of cases, showing that both *B. cereus* and *P. aeruginosa* were able to utilise degradation species from the pre-treated LDPE as a source of carbon nutrients to stay viable.

There were several instances where cultures of *P. aeruginosa* in UV treated LDPE showed no growth on viability testing plates, which is in direct contradiction to the

gravimetric assay data that showed degradation of UV treated LDPE to be the most successful for *P. aeruginosa* (as discussed below). One possible explanation is the majority of *P. aeruginosa* could be found as biofilms on the surface of the LDPE sample which would not only account for the high level of degradation, but also for the anomalous viability plating results as aliquots were only taken from the MSM media.

4.2 Quantitative Analysis

4.2.1 Mass loss

Analysis from the gravimetric assay data shows varying results for each bacterial species in terms of efficiency and general degradation trends.

In the case of *B. cereus*, the most effective method of pre-treatment for increasing remediation efficiency changed over time, with heat treatments being the most effective in the short term, and a combination treatment of both UV and heat being the most effective in the long term. As discussed in the IR analysis section, spectra of heat treated LDPE have a defined carbonyl peak at the ~1720 cm^{-1} mark, representing the main degradation species formed by this type of treatment. Carbonyl groups are common substituents for a range of biomolecules (Schaller, 2023); many of which feed both directly and indirectly into the cellular respiration pathway (i.e. saccharides, fatty acids, and ketones). Because of this, *B. cereus* contains an array of enzymes that are capable of efficiently utilising carbonyl species for nutrients and conversion to cellular energy, and thus would account for why the heat treated LDPE samples were remediated so efficiently at the start. In a study by Gilan *et al.* (2004), it was found that the presence of carbonyl residues from polyethylene was the main initiator for biofilm formation and biodegradation to begin, which is commensurate with the results outlined above.

We saw a general trend of better degradation (in terms of % mass loss) over time, with the most significant degradation occurring at week 4 for all pre-treatment types, but especially for the combination treatment. This general trend made logical sense in that the longer the bacteria was given to degrade the LDPE, the more % mass loss would be observed. From our results, we can see that *B. cereus* is able to use degradation species from both UV and heat treatment as sources of carbon nutrients; this factor is important in understanding why the most significant degradation occurred

through the combination treatment, as the bacteria had access to multiple degradation species for nutrients as opposed to singular species as seen in the UV or heat treated samples; the more degradation species available for use as nutrients, the more LDPE degradation was observed.

For *P. aeruginosa*, the most effective pre-treatment in the short term was shown to be the combination treatment, however the UV treatment was found to be the most effective by the 4th week. Interestingly, heat treatments individually showed low degradation efficiency, but when used in tandem with UV, we saw much greater overall degradation; looking back to the IR specs for the combination treatment, it was observed that higher instances of hydroperoxide species were formed when compared to the individuals. These hydroperoxides have been shown in literature to eventually interact with other free radicals and lead to the formation of more carbonyl and carboxylate species (Albertsson *et al.,* 1987), and therefore it could be that the combination treatment was initially the most effective due to the availability of a higher concentration of nutrients formed from the abundance of hydroperoxides.

The overall effectiveness of UV degradation for *P. aeruginosa* is likely what caused the efficiency of the combination treatment to carry over across week 2 and 3, however the dip in the fourth week is likely due to a lack of bioavailable carboxylate species for nutrients, which is not an issue seen in week 4 for the stand alone UV treatment.

4.3 Limitations

Several limitations exist in the design of the above research that, if rectified, would give a clearer picture and greater confidence in the results obtained.

Though IR spectroscopy was useful in confirming the formation and removal of degradation species by pre-treatments, the data given does not elucidate further about what specific degradation species were formed, which, if known, would be useful in determining the means by which *B. cereus* and *P. aeruginosa* were able to metabolise them. Often, IR spectroscopy is partnered with NMR analysis to determine the molecules being formed (Gundlach *et al.*, 2017), and in this instance, both ^{1}H or ^{13}C spectroscopy could be used to determine the identities of the carboxylate and carbonyl species.

Another limitation in the above experimental design is that, although the LDPE samples were analysed by IR, the media was not tested to determine if the degradation species had been released into the solution. In the next iterations of this experiment, it is suggested that the MSM media from bioremediation samples should also be tested to determine if the degradation species were being internalised by the bacteria, or if they had simply been washed into the media. Alternatively, the use of radiolabeled carbon (^{14}C) in the production of the LDPE sample (as shown by Albertsson, 1978) could be employed to track internalisation of degradation species and quantify the amounts in the cell and in the media.

Lastly, issues with fungal contamination were seen in MSM bottles throughout the length of the project, this means that it is possible that contamination may have occurred within our remediation samples, leading to competition for resources (in an already nutrient scarce environment) and a skewing in the analysis of our bacteria's ability to degrade LDPE. In future iterations, it is recommended that some form of antifungal compound be included as part of the remediation recipe to decrease the potential for fungal contaminations.

4.4 Further Studies

Aside from the suggested changes to the experimental designs outlined in the limitations section, there are several avenues worth exploring as future studies for this research.

Firstly, as much of the purpose of this research was to determine if pre-treatments of LDPE could lead to more efficient degradation by *B. cereus* and *P. aeruginosa,* the next logical step would be to scale up the operation from a proof-of-concept size to one that is more befitting of a standard research lab. If effective degradation was still observed at this scale, then further scaling up to an industrial size may also be a viable next step.

Next, due to constraints of time, many of the important environmental factors that affect the metabolic efficiency of *B. cereus* and *P. aeruginosa* were ignored such as optimal temperature, pH, etc. In future studies, optimisation of these factors would be worth exploring as they could only improve on the remediation efficiency for each bacterial species.

Another interesting area of research would be determine the ways in which the two bacterial species are able to degrade LDPE in the first place; this information could be then used to further enhance or optimise pathways by understanding important cofactors, coenzymes or substrates that may need to be part of the media to further optimise the remediation process. One such method for determining the biochemistry of LDPE degradation would be the construction of a cDNA library for both *B. cereus* and *P. aeruginosa* at different stages of the remediation process; this would highlight what enzymes were transcriptionally active (Soares *et al.*, 1994) and give insight into metabolic pathways required for LDPE degradation.

Lastly, further optimisations to the actual bacteria could also be made in the form of recombinant genetic engineering to introduce enzymes such as PETase and METase (as described by Yoshida *et al.,* 2016) that have been shown to increase plastic degradation up to 60% without pre-treatment; by combining all of the tools at our disposal, it may be possible to further exceed the current best efforts.

4.5 Conclusion

To conclude, *B. cereus* and *P. aeruginosa* were shown to be efficient LDPE bioremediators based on their ability to metabolise the degradation of carbonyl and carboxylate species that were introduced from the pre-treated LDPE, as well as the ability to efficiently degrade it based on high % weight loss ranging from 5-18 %. The results of the bioremediated pre-treated LDPE varied by treatment method and also by the length of the remediation; *B. cereus* was shown to effectively degrade LDPE pre-treated by UV and heat combined after 4 weeks, while *P. aeruginosa* effectively degraded LDPE pre-treated by UV only after 3 weeks. The methods utilised for pre-treatment clearly helped in the efficiency of the bioremediation by the test organisms, however, their effect when internalised by the organisms differs depending on the treatment itself. Furthermore, the addition of glucose in the MSM is required to sustain the bacteria in the media to drive it to consume the LDPE once depleted. Though the objectives of the investigative study were met, there were some limitations to the experimental design that may have affected the outcome of the bioremediation, such as determining the identity of the carboxylate/carbonyl species formed and if any of it was released in the media. To add, fungal contamination may have occurred, thus possibly

competing with *B. cereus* and *P. aeruginosa* to consume LDPE as a carbon-source. It is recommended that IR spectroscopy be paired with NMR spectroscopy to identify the carboxylate/carbonyl species that formed from the pre-treatment and/or released in the media, as well as adding an antifungal compound to prevent fungal growth in the media. For future studies, expanding the research to include other environmental parameters such as temperature and pH to optimise the degradation, as well as engineering enzymes that would specifically aid and accelerate the bioremediation of LDPE.

Appendices

Appendix A) - Calculations

Sample calculation - Average % weight loss of bioremediated LDPE

Initial mass of UV-treated LDPE: 1.0 g

Final mass of remediated UV-treated LDPE with B. cereus after 2 weeks:

Trial 1 = 0.9377 g Trial 2 = 0.9260 g:

Final average mass from trial 1 and 2 = 0.06815 g

The average percent (%) weight loss of the remediated LDPE can be calculated as followed in equation (1):

$$\% \text{ weight loss} = \frac{Initial\ mass - average\ final\ mass}{Initial\ mass} \times 100 \quad (1)$$

The average % weight loss of remediated UV-treated LDPE after 2 weeks is:

$$\% \text{ weight loss} = \frac{1.0\ g - 0.06815\ g}{1.0\ g} \times 100 = 6.815\ \% \approx 6.8\ \%$$

∴ **6.8 %** of UV-treated LDPE was degraded by *B. cereus* after 2 weeks.

Appendix B) - Additional Figures

This appendix is reserved for extra figures that showcase the process of the experimental protocol.

Figure I. Glucose assay of *B. cereus* (left) and *P. aeruginosa* (right). Glucose concentrations ranging from 0.5-20 % were prepared from adding 1 M glucose to MSM media and inoculating 0.1 mL of bacteria.

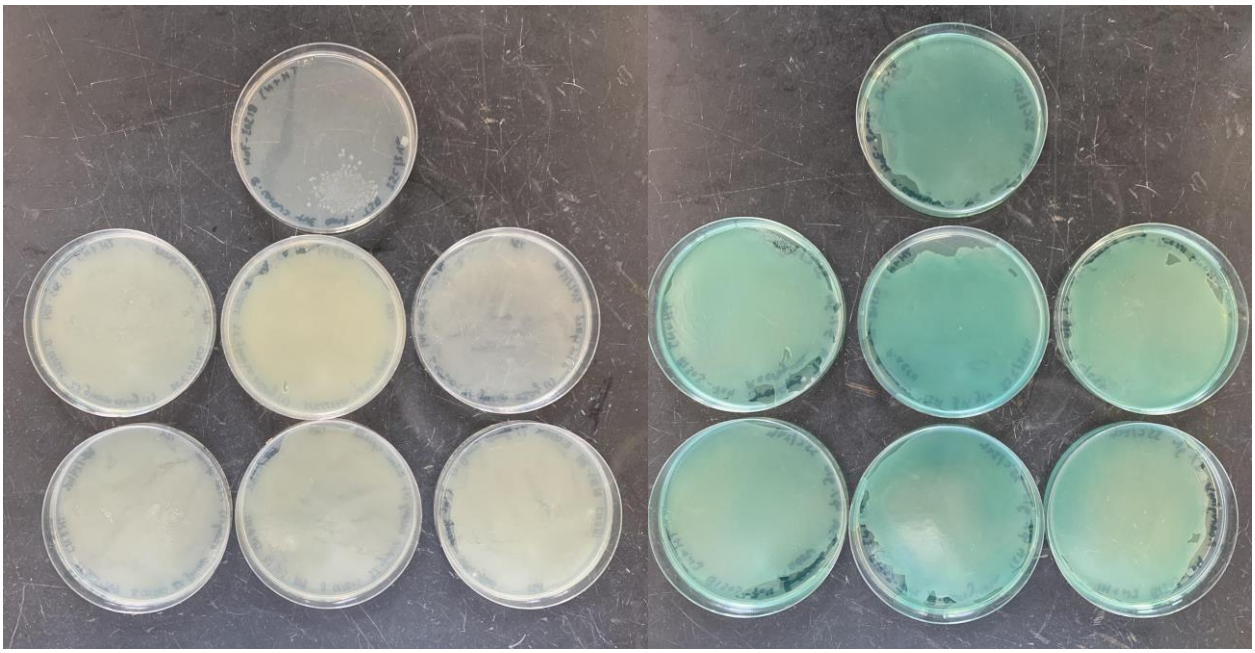

Figure II. Results of the viability plates of the glucose assay to determine minimal amount of glucose to add to MSM for bioremediation. In the left picture, *B. cereus* was viable in glucose concentration starting with 2.0, 3.0, and 4.0 %, respectively. In the right picture, *P. aeruginosa* was viable in glucose concentration starting with 3.0, 4.0, and 5.0 %. Subsequently, 2.0 % and 3.0 % glucose was used in the bioremediation of LDPE for *B. cereus* and *P. aeruginosa*, respectively.

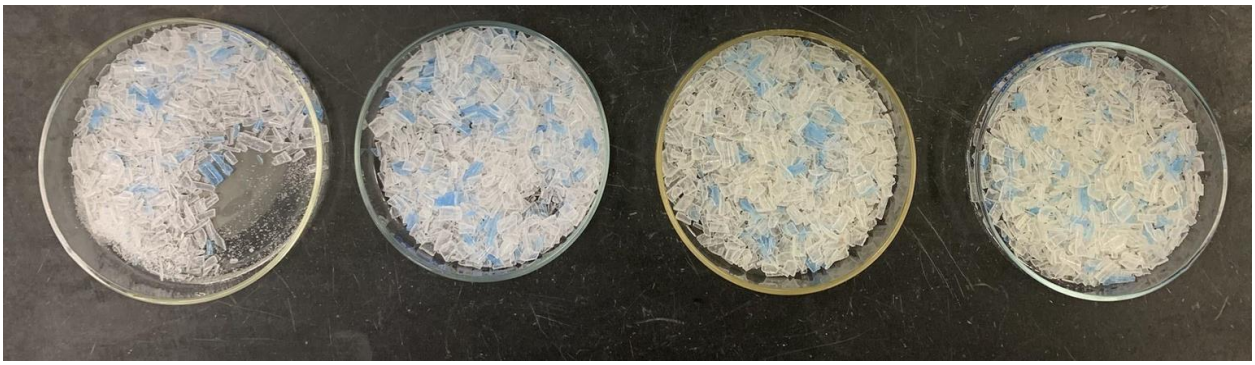

Figure III. *From left to right*: untreated LDPE; heat-treated LDPE, UV-treated LDPE, and UV + heat treated LDPE.

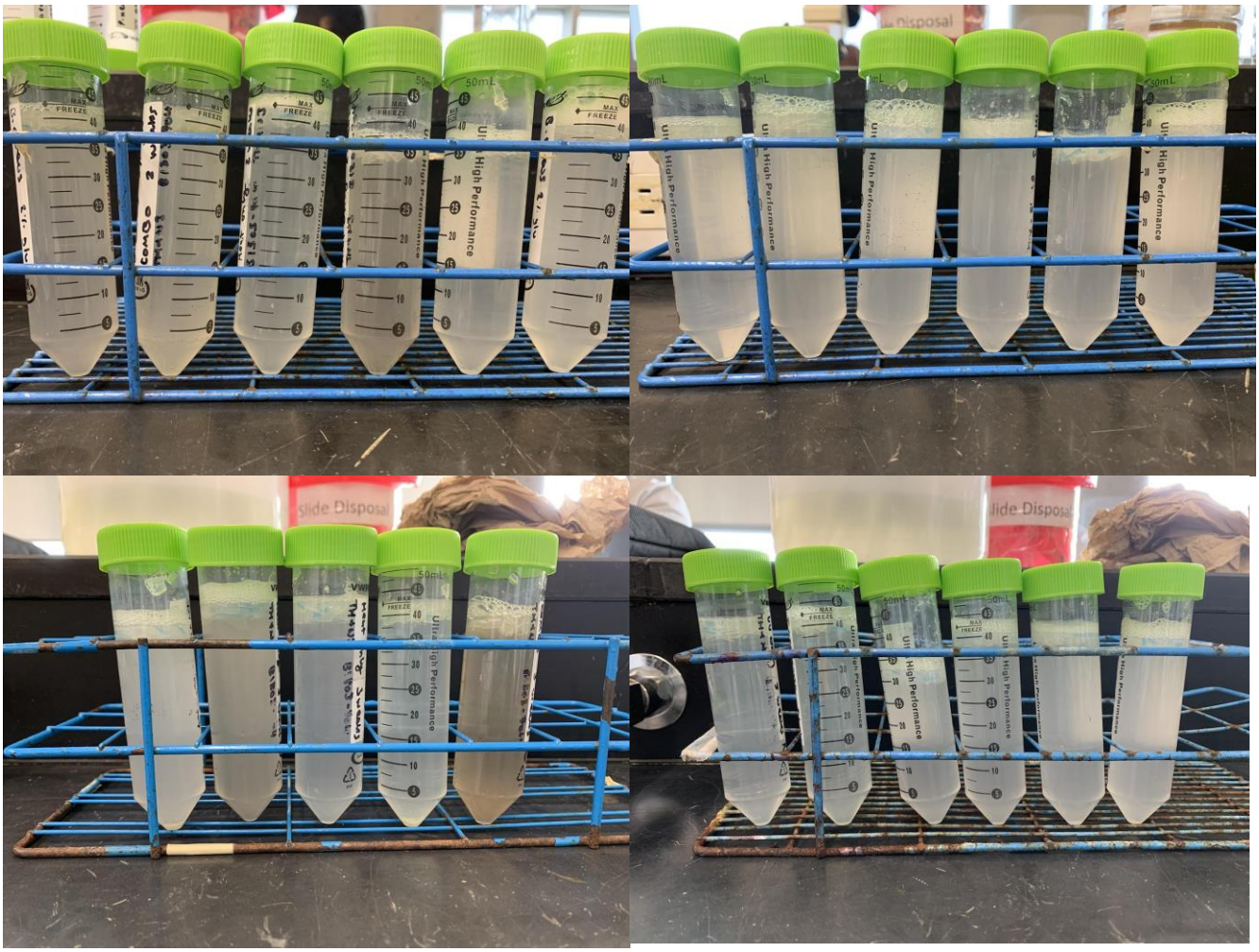

Figure IV. Top two pictures: on the left, bioremediated LDPE (UV, heat, and UV + heat) by *B. cereus* after 2 weeks ; on the right, bioremediated LDPE (UV, heat, and UV + heat) by *P. aeruginosa* after 2 weeks. Bottom two pictures: on the left, bioremediated LDPE (UV, heat, and UV + heat) by *B. cereus* after 3 weeks ; on the right, bioremediated LDPE (UV, heat, and UV + heat) by *P. aeruginosa* after 3 weeks .

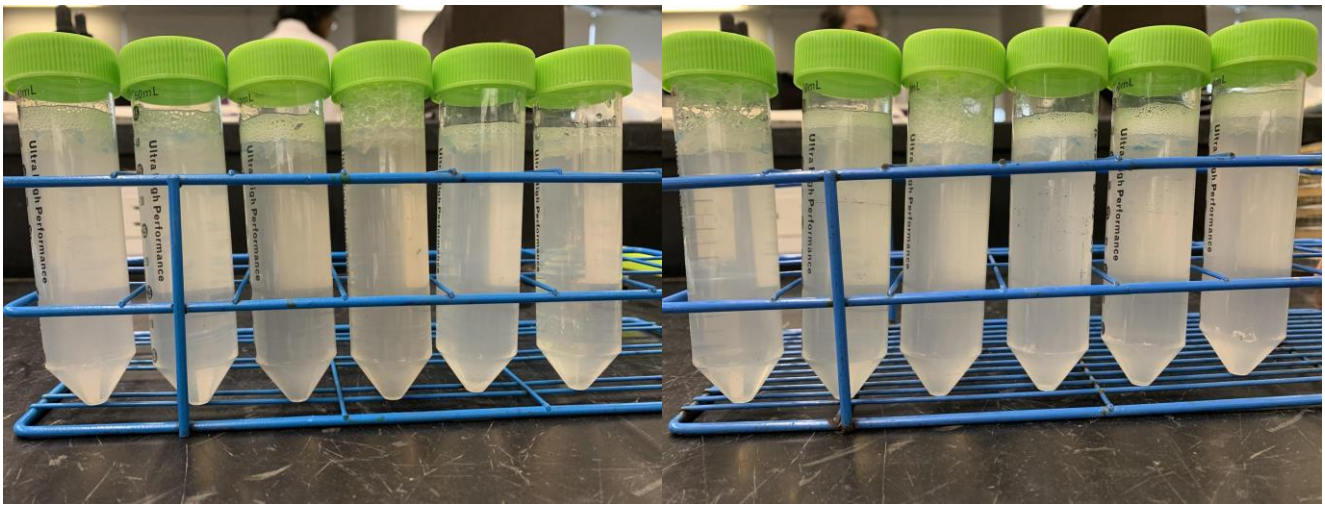

Figure V. On the left: bioremediated LDPE (UV, heat, and UV + heat) by *B. cereus* after 4 weeks ; on the right: bioremediated LDPE by *P. aeruginosa* after 4 weeks.

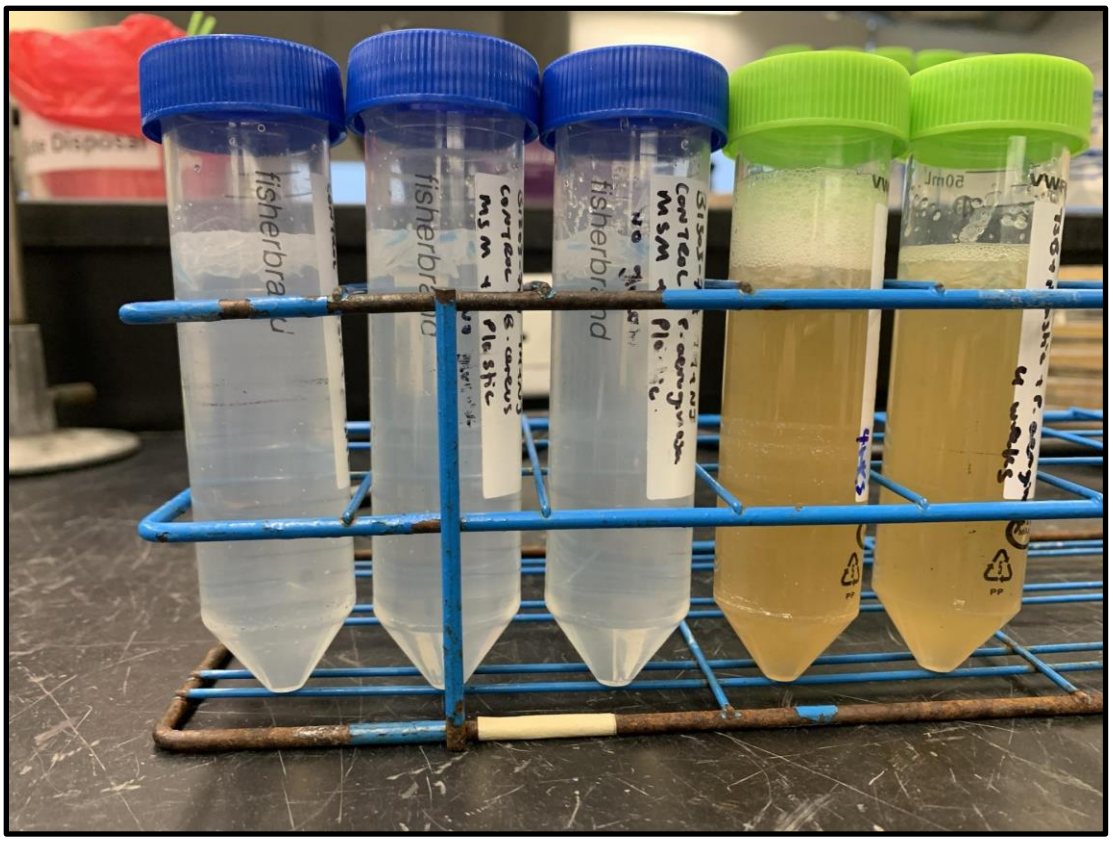

Figure VI. Controls bioremediated for 4 weeks, starting from left to right: MSM + plastic, MSM + plastic + *B. cereus*, MSM + plastic + *P. aeruginosa*, TSB + plastic + *B. cereus*, and TSB + plastic + *P. aeruginosa.*

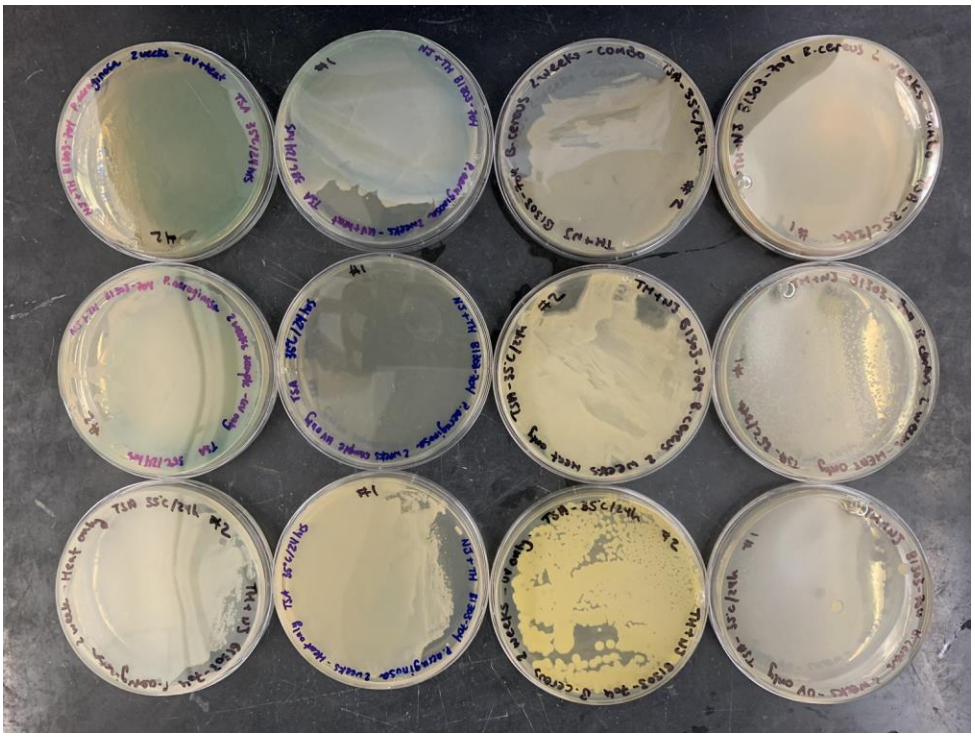

Figure VII. Results of viability plates from bioremediated LDPE by *B. cereus* and *P. aeruginosa* after 2 weeks.

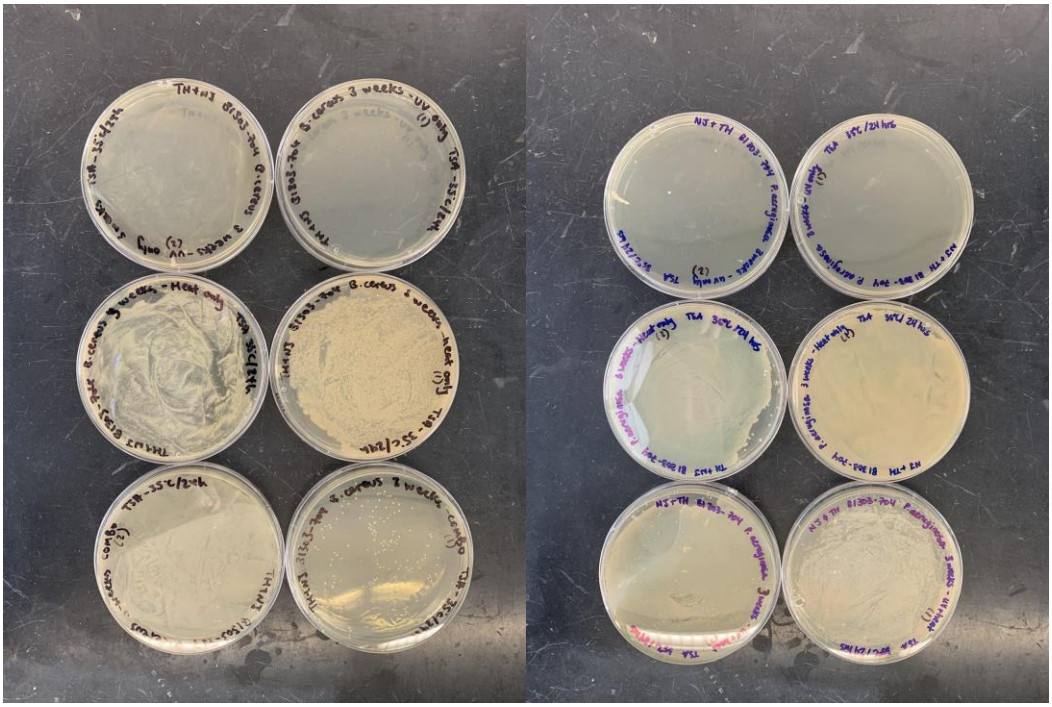

Figure VIII. Results of viability plates from bioremediated LDPE by *B. cereus* (left) and *P. aeruginosa* (right) after 3 weeks.

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