Comparative analysis of isolated microplastics in select food samples and its effect on the growth and lactose fermentation activity of lactic acid bacteria

Final Thesis

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Abstract

Microplastics (MPs) have become a prevalent and concerning presence in our environment, proliferating even in the products we consume. However, limited studies are concerned with the extraction of MPs from food and their direct impact on microorganisms, especially those in the gut, specifically lactic acid bacteria (LAB). This study aims to effectively extract MPs from select food samples: apple, salmon, and bottled water, and assess their impact on the growth and lactose fermentation activity of *L. fermentum*, and the potential implications on overall health. MPs were extracted through a synergistic approach of digestion, density separation, and filtration techniques. Growth of *L. fermentum* was assessed by conditioning the MRS culture broths with the extracted MPs and measured through OD value readings and pour plate counts. Lactose fermentation activity was measured via absorbance readings on the color change (degree of fermentation) of enriched media. Results showed that MPs were present in all samples, with MPs mostly made of PET and some PPG, and salmon having the highest percentage among the samples. Furthermore, the bottled water generally had the highest reduction in both the growth and lactose fermentation activity, and was significantly lower than the positive control and other samples. Hence, this shows that MPs are truly present in the food we consume and can impact the growth and activity of LAB, providing preliminary data implicating potential effects on our gut and consequently, overall health.

Declaration of Authorship

We, Andrea Collado and Elyssa Jane Gargallano, 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.

Signatures

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- Andrea and Elyssa

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1.0 Introduction

1.1 Background of the Study

1.1.1 Microplastics and Its Impact on the Environment

Microplastics (MPs) are plastic particles that are smaller than 5 mm and can stem from both primary sources such as microbeads and fibers from synthetic clothing, as well as secondary sources, such as the degradation of larger plastic debris (Haque & Fan, 2023). These particles have become widespread, with annual global production at 390.7 million metric tons (Jaganmohan, 2024), contaminating water, soil and even air. The bioaccumulation of MPs make them a serious environmental concern and have impacts that are extensive and complex, such as disrupting the ecosystems by changing the microbial community structures, fostering the growth of harmful biofilms, and potentially affecting the health and behavior of various species. Studies have also shown that many organisms, including marine life and land animals, ingest MPs which leads to their accumulation across the food chain. MPs can also attract and carry toxic chemicals via surface adsorption, which increases the potential harm when consumed by organisms and poses health risks to humans via contaminated food and water sources (Salamone et al., 2024). Although the effects on human health over time are still being investigated, the presence of MPs in food and drinking water has sparked serious concern (Haque & Fan, 2023).

1.1.2 Microplastics Impact and Implications on Health

Microplastics have been increasingly accumulating into the environment, raising concerns about the potential health impact on humans. These particles are able to enter the human body through ingestion, inhalation, and skin contact from food, water, air and

many more (Yee et al, 2021). As MPs start to degrade, residual monomers are left behind and released, which is known to be a hazard for human health. Researchers have identified and classified the effects of ingesting MPs into three stages: blockage and damage of the digestive system, release of toxic chemicals into the body, and the assimilation of these substances by organs and tissues. As humans increase their exposure to MPs, they are absorbed into the body through different pathways and accumulate in various organs, including the lungs, liver, kidney and intestine. Studies have found MPs as a cause for inflammatory responses within these tissues, but it is not confirmed whether they will develop into serious illnesses (Ziani et al., 2023).

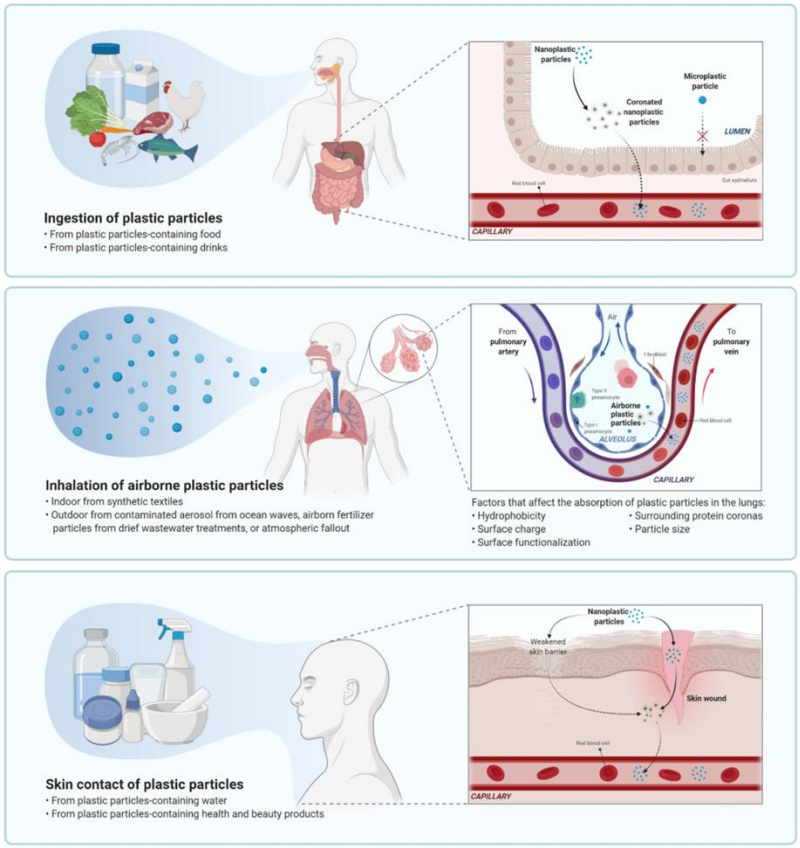

Figure 1. Three routes that microplastics can enter the human body (Yee et al., 2021)

1.1.3 Current Techniques in Microplastic Separation and Extraction

Current techniques take advantage of the physical and chemical properties of MPs such as density and resistance to degradation for separation and extraction from samples. Commonly employed ones include sieving, density separation, filtration, and chemical digestion, and while effective, often results in ~5-10% loss (Rani et al., 2023). Other techniques include the use of magnets, oil, and enzymes, but they require more resources and are generally more time-consuming in terms of the process and sample preparation (Rani et al., 2023). However, these techniques are often applied to environmental samples (e.g. soil, water, plants) in the majority of the literature, while those from food and drinks are limited and are difficult to navigate due to the complex matrices of the sample and are often processed via chemical digestion and filtration (Bai, Liu, Guo, Zeng, & Guo, 2022).

1.1.4 Synergistic Method for Microplastic Extraction

A synergistic approach using different techniques have been found to increase the recovery of MPs in order to maximize the amount extracted and reduce potential loss. In this experiment, it was crucial to recover most of the MPs in the suspected food samples in an attempt to observe whether they were present and ascertain the prominent material type while also separating organic matter. A chemical digestion using H_2O_2 was found to effectively separate organic matter and have a higher recovery of various types of MP at 53±4.4%. Digestion must occur at a maximum of 50°C to remain within the heat deflection limits of the MPs. Moreover, the addition of H_2O_2 effectively breaks down solid organic matter, leaving little to no residue without having to degrade the MPs themselves. When coupled with density separation using $ZnCl_2$, an

increase in recovery efficiency can be observed by ~5% (Radford et al., 2021). Density separation also allows better separation of MPs from any remaining organic matter or sample in solution and allows for a broader range of MPs collected (Table 1). Alternatively, oil can be used to separate MPs by density as it was found to be statistically comparable with $ZnCl_2$ in MP recovery, but can be difficult to filter. Filtration then ensures complete separation from the sample and chemicals, allowing for complete MP recovery.

Table 1. Types of microplastics and corresponding density literature values (Radford et al., 2021)

Type of MP	Density (g cm^{-3})
Polyethylene Terephthalate (PET)	1.37-1.45
High Density Polyethylene (HDPE)	0.93-0.97
Polyvinyl Chloride (PVC)	1.16-1.58
Low Density Polyethylene (LDPE)	0.91-0.92
Polypropylene (PP)	0.9-0.91
Polystyrene (PS)	0.015-0.03

1.1.5 Analytical Methods for Identification of Microplastics

Identification of MPs has always been a challenge among researchers due to the size of the particles and the varying types of plastics with different properties. The most common method for identification is through visual inspection, and although cost-effective and simple, can be labor-intensive and time-consuming. Moreover, visual identification is dependent on visual characteristics of MPs such as color and size, which can be difficult to discern when transparent or broken into smaller fragments (Kwon et al., 2020). The use of stains such as Rose Bengal on the sample and viewing it under UV has been a common technique, allowing for easier distinction as stains can either bind to the MPs or to the background (Ribeiro, Duarte, & Da Costa, 2024).

However, this method does not completely confirm that the material is actually plastic as it only provides distinct visualization and not the chemical properties of the MPs, and hence was not opted for this study. On the other hand, analyzing the sample through IR spectroscopy has been popular among studies investigating microplastics in different environmental samples. Other than being relatively cost-effective and less time-consuming than other methods (i.e. Raman spectroscopy, fluorescence microscopy, SEM), it provides a more specific insight into the chemical properties of the material present in the sample, allowing for confirmation that what is present is actually plastic (Kwon et al., 2020). This study then intends to identify MPs through visual identification as a preliminary assessment and confirming the material or type through IR spectroscopy.

1.1.6 Lactic Acid Bacteria (LAB) and Its Influence on Overall Health

In the human gut, LAB was seen as a subdominant but numerically relevant presence that contributed to and maintained stasis in the microbiota which in turn, influences one's overall health. Changes in the population of LAB have been correlated negatively with diseases, such as a reduction in *Lactobacillus* resulting in a variety of illnesses (e.g. type 1 diabetes, IBS, multiple sclerosis, and HIV), while an enrichment was correlated with Crohn's disease and rheumatoid arthritis (De Filippis & Pasolli, 2020), hence maintaining the appropriate number of certain bacteria in the gut can be crucial. Because of this, consumption of probiotic-rich and lactose-fermented products have been widely recommended as major contributors to gut health (Mathur, Beresford, & Cotter, 2020). Studies have shown that the consumption of LAB-rich products results in a diverse range of health-promoting effects including better lactose digestion, weight maintenance, exopolysaccharide production with a number of benefits such as

cholesterol-lowering, antidiabetic, antioxidant, and immunomodulatory properties, and increase vitamin levels and produce neuroactive molecules, among other things (De Filippis & Pasolli, 2020). Such benefits are often the by-products of metabolic activities and processes undergone by LAB, one of which being the fermentation of lactose, where lactose from consumed food or drinks is fermented by the bacteria in the gastrointestinal tract and split into short chain fatty acids (SCFAs) that contribute to the aforementioned benefits (Figure 2) (Mann, Lam, & Uhlig, 2024).

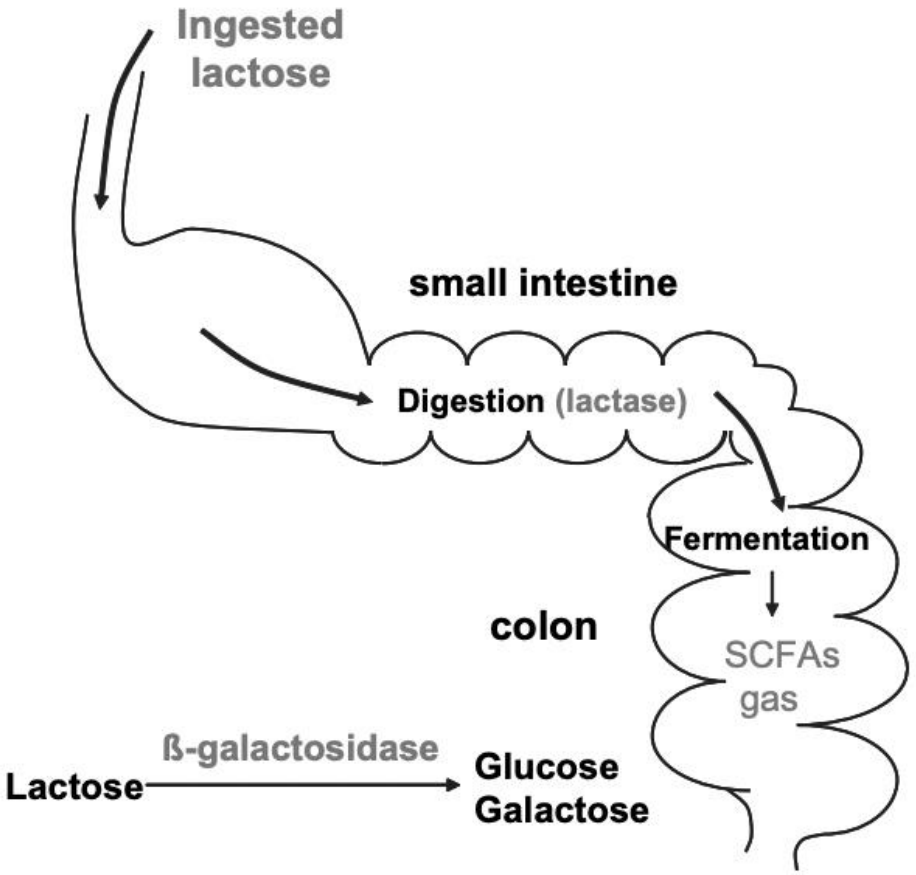

Figure 2. Lactose pathway in the gastrointestinal tract (Venema, 2011)

In this study, *Lactobacillus fermentum* was chosen as the culture of interest and served as the representative organism for lactic acid bacteria to obtain preliminary information on how MPs can impact LAB species and their metabolic activity. *L.*

fermentum, like other LAB, play a crucial role in gut health and have exhibited diverse regulatory mechanisms as well as preventive and therapeutic effects (Zhao, Zhong, Liu, Wang, & Gao, 2021). While a mixed culture containing various species of LAB is desirable to mimic gut microbiota and relationships, various factors had to be considered such as how the growth of each species would impact each other in the presence or absence of MPs, identification difficulties, and time constraints. Moreover, lactose fermentation was used to generally assess the metabolic activity of LAB as it is a major process that generates ATP essential for LAB's growth and other metabolic activities (Axelsson, 2004) and allow a survival advantage in acidic environments. Furthermore, as stated above, the fermentation of lactose is crucial for LAB to produce lactic acid which improves gut health by inhibiting harmful bacteria as well as producing fermented dairy products with probiotic benefits (Holzapfel & Wood, 2014).

1.2 Rationale

The demand for plastic products has increased worldwide, resulting in the rapid accumulation of plastic wastes that are broken down into unnoticeable fragments called microplastics, that circulate the environment for thousands of years (Chamas et al., 2020). Various studies have investigated its impact on environmental health and developed techniques to effectively extract them from different sources, e.g. soil, water, air, other living matter. However, these studies are often focused on common environmental sources such as soil and water, while there is less research on the extraction of MPs from food sources despite its prevalence (Abbasi, Turner, Hoseini, & Amiri, 2021). Moreover, despite evidence on the implications of microplastics on human health (Leslie et al., 2022; Koongolla et al., 2020), there are limited studies that

investigate the direct effects of MP consumption on humans and other living organisms (Parsaeimehr, Miller, & Ozbay, 2023). Hence, this study aims to isolate microplastics by utilizing and modifying existing techniques to extract microplastics from select food samples: apple, salmon, and bottled water. This study will also investigate the effect of microplastics on the growth and lactose fermentation activity of *L. fermentum* as a representative organism for lactic acid bacteria to ascertain potential implications for probiotic gut and overall health.

1.3 Objectives

1. To isolate, identify, and quantify (in %) microplastics from select food samples of varying sources (land, water, processed) by modifying existing extraction techniques and IR spectroscopy
2. To assess the rate of growth inhibition of lactic acid bacteria in the presence of microplastics determined by viable plate count and OD value measurements
3. To evaluate the impact on metabolic activity of lactic acid bacteria in the presence of microplastics by assessing the degree of lactose fermentation activity in enriched media

1.4 Hypotheses

1. Microplastics will be extracted from the food samples, with bottled water containing the highest amount (by weight and %) of microplastics. PET and fibers will be the most likely material found.

2. The presence of microplastics will inhibit the growth rate of *L. fermentum*, reducing its OD value and viable cell count readings.
3. A reduction in the lactose fermentation activity with respect to growth will be observed.

2.0 Materials and Methods

2.1 List of Materials

2.1.1 Cultures

The primary culture used for testing was *Lactobacillus fermentum* (ATCC 9338) provided by the Centennial College Prep Room (Morningside). The culture was received on an MRS plate that was sub-cultured on a new plate and incubated anaerobically for 72 hours at 37°C.

2.1.2 Media and Chemicals

The media and chemicals used in the project are listed in Table 2. All chemicals and media were provided by the Centennial College Prep Room (Morningside).

Table 2. Media or chemicals used in the study with lot numbers/storage, and manufacturer/location

Chemical or Media	Manufacturer/ Location	Lot Number/ Storage
Lactobacillus MRS Broth	Criterion	627112
Agar	VWR Life Sciences	24C2956243
Hydrogen Peroxide (30%)	H-13B	Fridge: Door
Zinc Chloride	Sigma- Aldrich	MKCC7426
Polypeptone (bovine/porcine origin)		
Yeast Extract		
Tween 80		
Dipotassium Phosphate		
Sodium Acetate		

Diammonium Citrate
Magnesium Sulfate
Manganese Sulfate
Bromocresol Purple
Concentrated Phosphate Buffer

2.1.3 Equipment

All equipment necessary for the experiment were provided by the Centennial College Morningside Campus Microbiology Prep Room. The *L. fermentum* required incubation in a candle jar to ensure a microaerophilic environment optimal for growth. The Genesys™ 30 Visible Spectrophotometer was used to measure the absorbance of the culture broths at 600 nm for both the assessment of growth and lactose fermentation activity. The dried filter papers containing the MPs were visually analyzed for any microplastics using a dissecting microscope. The filter papers containing the MPs from the samples were also analyzed using the IR spectrometer using ATR provided by the Centennial College 436 Chemistry Room (Morningside) to confirm that the materials observed from the microscope were plastic.

2.1.4 Other Supplies

- 100 mL and 250 mL media bottles
- Inoculating loop
- P8 grade filter paper (20-25 μm)
- Erlenmeyer flasks
- Glass petri dishes
- Ultrapure water (Millipore)
- Sterile petri dishes
- Slide cap tubes
- Durham tubes
- Shaking Incubator
- Filtering flasks
- Sterile buchner funnels

- BD GasPak EZ Anaerobe
- Food samples

2.2 Methods

2.2.1 Sub-culturing of Bacteria on Media

The *L. fermentum* bacteria was sub-cultured onto two MRS agar plates using a streak plate method, and incubated at 35°C for 72 hours (3 days) inside a candle jar. The liquid culture used for testing was prepared by inoculation of *Lactobacillus* MRS broth with a single colony from the *L. fermentum* bacteria using an inoculation loop and incubated in the same conditions as the MRS agar plates inside the same candle jar. The *L. fermentum* was regularly sub-cultured to ensure robustness for subsequent tests.

2.2.2 Extraction of Microplastics from Food Samples

(a) Sample Collection and Preparation

The apple was produced by Gala Foodland Ontario (4132) and was obtained from a local grocery stre. It was washed and cut into smaller pieces. The salmon (farmed) was obtained fresh from a local grocery store and was also cut into smaller pieces. The water bottle (Kirkland) was obtained from Costco Wholesale and kept in the fridge until used for filtration. 20 g of the samples were then weighed and placed into sterile 250 mL media bottles.

(b) Chemical Digestion of Solid Samples

Fifty (50) mL of 30% hydrogen peroxide (H_2O_2) was added to each of the media bottles containing the 20 g of food samples (apple and salmon) for digestion

and to remove organic matter. After which, the media bottles were placed into a shaking incubator at 50°C 100 RPM for 24-48 hours (Radford et al., 2021).

(c) Density Separation and Filtration

1.5 M of Zinc Chloride ($ZnCl_2$) solution was aseptically prepared in Erlenmeyer flasks for density separation of microplastics from the sample. 220 mL of this solution was added to the media bottles containing the digested food samples with 30% hydrogen peroxide. After adding the zinc chloride, the media bottles were then mixed by shaking the bottles vigorously for 30 seconds to allow the $ZnCl_2$ to interact with the entire sample solution. After the contents were mixed, the media bottles were then placed into the locker, making sure to not move the media bottles to allow the contents to settle properly (Radford et al., 2021).

After 72 hours, the supernatant of the solution was filtered over a P8 grade filter paper using a filtration system with a filtering flask and a Buchner funnel to effectively separate the MPs from the solution. Separate filtering setups were used for each sample to avoid cross-contamination, and ultrapure water was used to wash off any oil or residue from the samples on the filter paper (Bai, Liu, Guo, Zeng, & Guo, 2022).

(d) Visual Identification and IR Spectroscopy of Extracted Microplastics

Dried filter papers containing the MPs from the samples were visually inspected for any material resembling microplastics with the use of a dissecting microscope. Common visual identifiers are bright-colored fragments, fiber-like material, and transparent-like fragments (Mariano et al., 2021).

The filter papers were then analyzed via ATR-IR spectroscopy in order to confirm that the material observed under the microscope were plastic. Diagnostic peaks and their wavenumbers (cm^{-1}) in the analyzed samples were compared with reference spectra of different plastic polymer types (Table 1). Prior to this, a blank filter paper was read before the samples in order to rule out any interfering or overlapping peaks from the filter paper in the spectra.

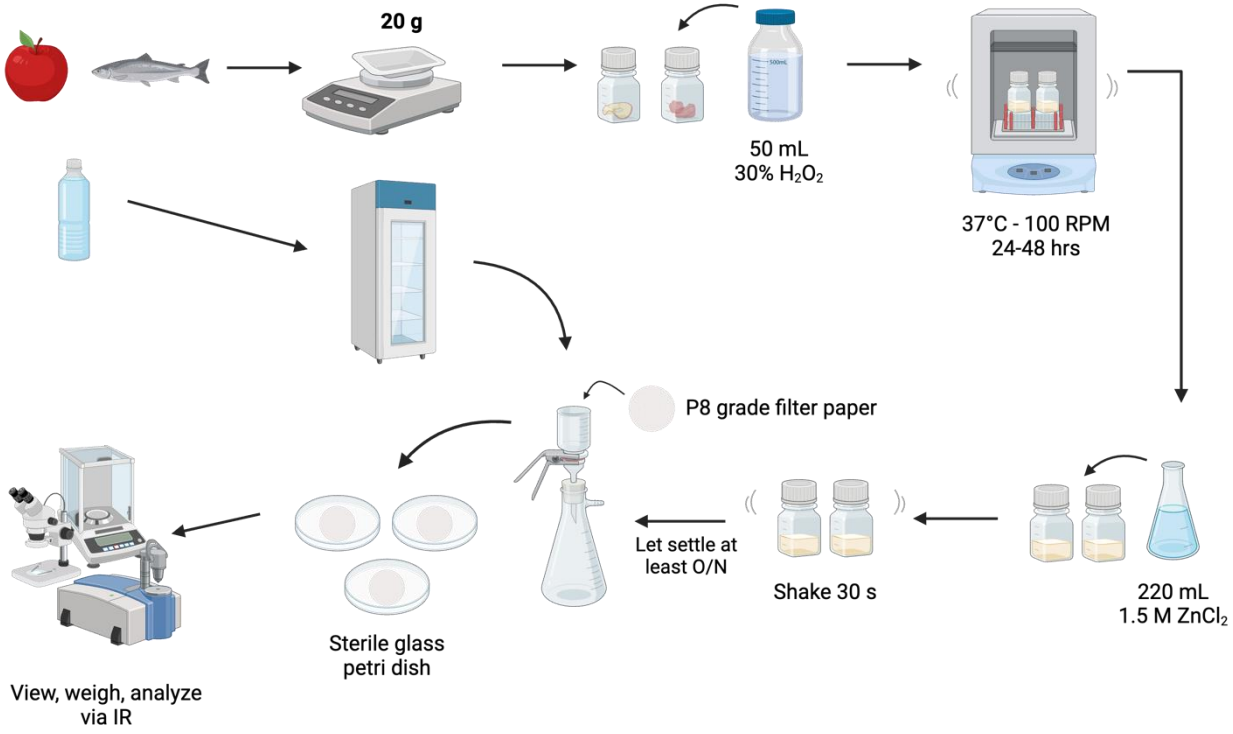

Figure 3. Procedural flowchart on the extraction of microplastics from food samples

2.2.3 Assessing the Growth of L. fermentum in Microplastic Presence

(a) Preparation of Culture Broths with Microplastics

Culture broths were prepared in 100 mL media bottles containing 50 mL of MRS broth each. This was done in bottles to allow for easier addition of filter papers containing MPs from the samples. Controls were set up in the same manner.

Filter papers with MPs extracted from each sample: apple, salmon, and bottled water were aseptically added into their respective bottles with 50 mL MRS broth. One mL of the 72-hour starting culture of *L. fermentum* was then inoculated to each bottle, swirled, and incubated at 37°C for 72 hours in microaerophilic conditions.

After 72 hours, the OD readings and plate counts were taken respectively for each culture broth. Then, 1 mL of the MP-conditioned culture from the first set of sample broths were each inoculated into another set of culture broths with the second set of filter papers with MPs of each sample, respectively. They were then sent for incubation at 37°C for 72 hours in microaerophilic conditions before OD readings and plate counts were taken.

(b) Preparation of Bacterial and MP control broths

Controls for the culture and MPs were set up the same way as the sample culture broths at 50 mL MRS in 100 mL media bottles. A 50 mL MRS broth with 1 mL of the starting *L. fermentum* culture was used as a positive control for growth. The addition of filter papers containing MPs from each sample were also inoculated into their own 50 mL MRS broth without the culture as a control and blank for the

absorbance readings. A sterile 50 mL MRS broth was also used as a negative control for the entire set-up.

(c) Assessing Growth via Optical Density Readings

After 72 hours of incubation under microaerophilic conditions at 37°C, optical density readings at 600 nm were taken for each culture broth to assess growth of *L. fermentum* in the presence of MPs. Absorbance at 600 nm was read using the Genesys™ 30 Visible Spectrophotometer, and readings were recorded accordingly. All readings were taken in duplicates to avoid biases and errors.

(d) Assessing Growth via Viable Plate Counts

Viable plate counts were then conducted via pour plate after the 72 hour incubation period. The culture broths were diluted using phosphate buffer tubes within a countable range and plated on sterile petri dishes. Tempered MRS agar was then poured into each, swirled, and set before incubating at 35°C for 72 hours in microaerophilic conditions. Plates were done in duplicates in order to avoid biases and errors.

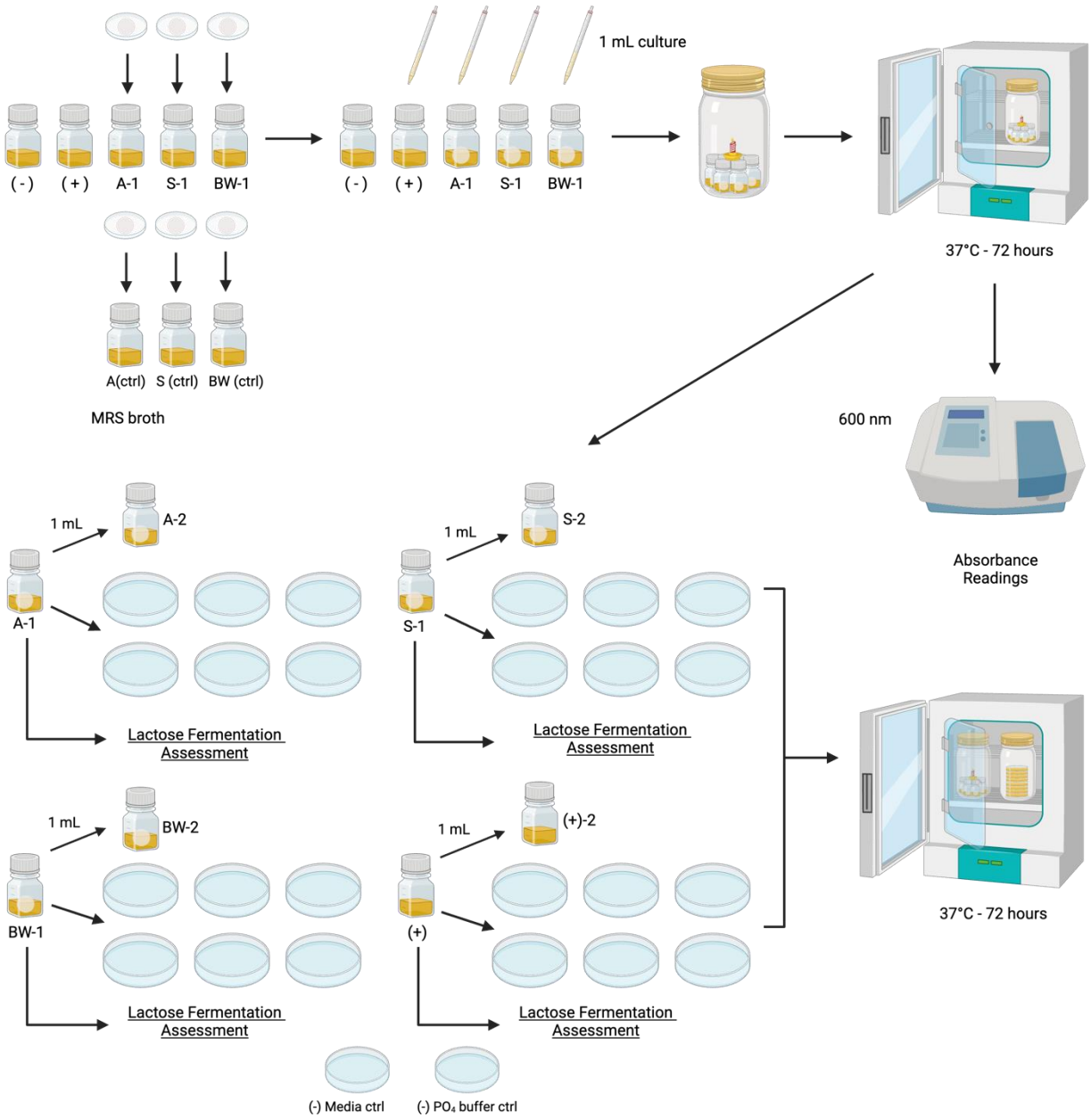

Figure 4. Procedural flowchart of the growth assay in the presence of MPs

2.2.4 Assessing the Lactose Fermentation Activity through Enriched Media

(a) Preparation of Media

Media for assessing the lactose fermentation activity was derived from Biomerieux API CHL 50 inoculation fluid, specifically the test media for lactose fermentation of *Lactobacilli* species. It was prepared following the formulation below (Table 3) in slide caps tubes, containing 5 mL of the media and 5 mL MRS broth supplement to support growth, bringing each tube to a total of 10 mL each. Durham tubs were also added to capture any production of gas during fermentation.

Table 3. Media formulation for lactose fermentation activity (Biomérieux)

Ingredient	**Amount**
Polypeptone (bovine/porcine origin)	10 g
Yeast extract	5g
Tween 80	1 mL
Dipotassium phosphate	2 g
Sodium acetate	5 g
Diammonium citrate	2 g
Magnesium sulfate	0.20 g
Manganese sulfate	0.05 g
Bromocresol purple	0.17 g
Demineralized water	1000 mL
pH: 6.7 - 7.1	

(b) Assessing Lactose Fermentation Activity of MP-conditioned cultures

0.1 mL of culture conditioned with MPs from each sample broth after 72 hours of incubation was inoculated into the test tubes containing the lactose media and Durham tubes. The tubes were then set up anaerobically and incubated for 72 hours at 37°C. Tubes were done in duplicates to avoid biases and errors. *L.*

fermentum cultured without MPs in the broth was used as the positive control while a sterile tube of the lactose media was used as the negative control.

After incubation, any color changes and gas production were noted. Absorbance readings at 600 nm were also taken to roughly quantify the amount of lactose fermented based on the degree of color change or fermentation.

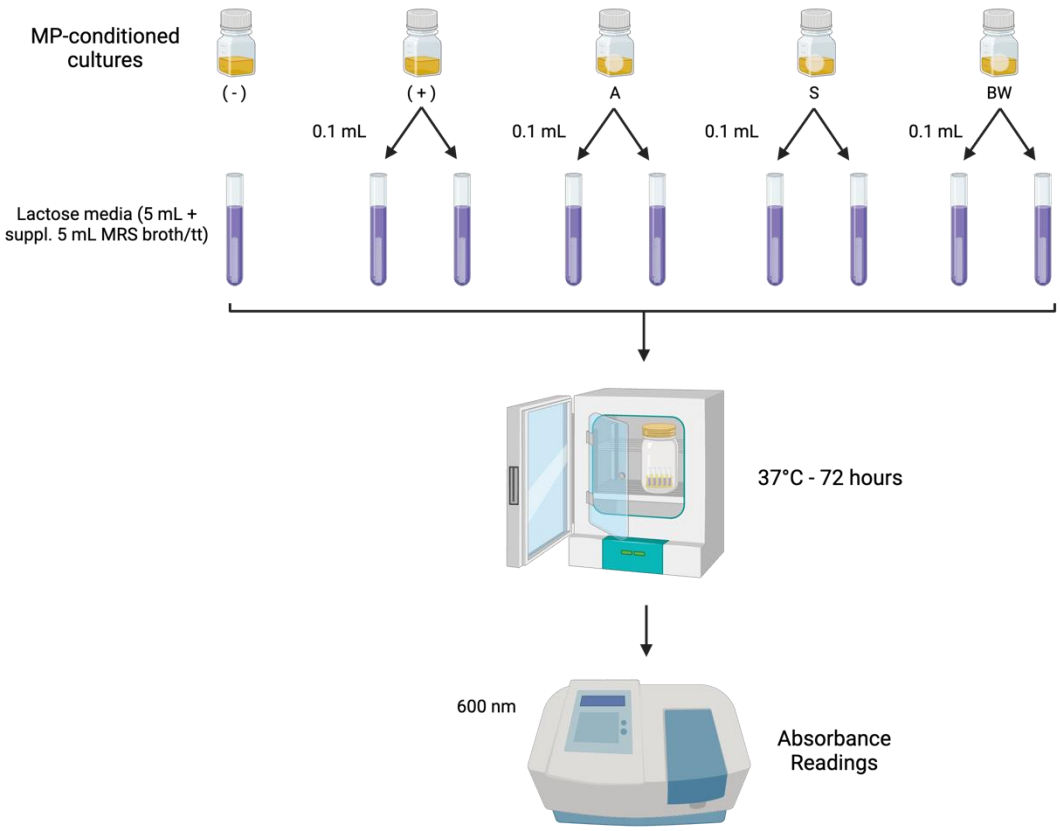

Figure 5. Procedural flowchart of the lactose fermentation activity assay

3.0 Results

3.1 Microplastic Extraction

Extracted microplastics from the food samples were identified through visual assessment via dissecting microscope and confirmed by IR analysis and compared with reference spectra provided by the College Chemistry lab and an online spectral database from the National Institute of Advanced Industrial Science and Technology (AIST), as demonstrated in the figures below.

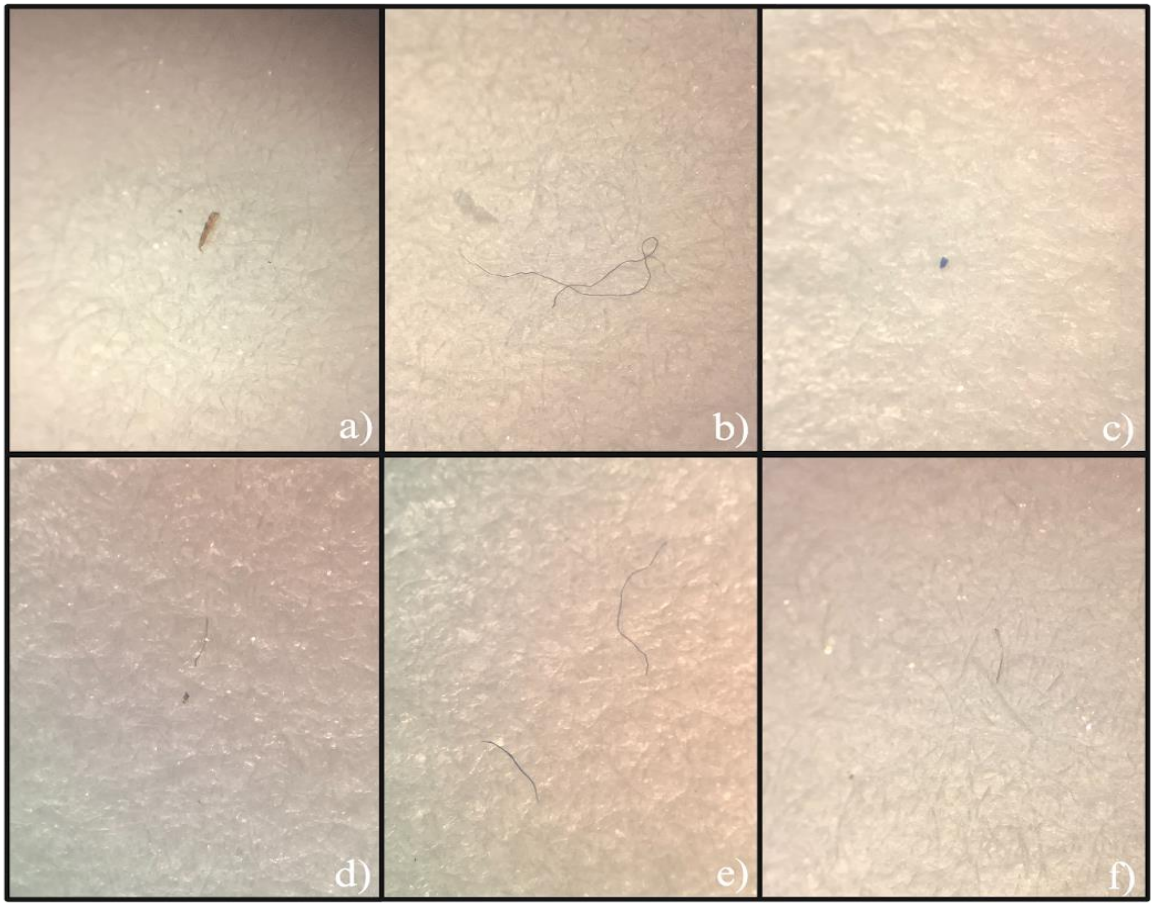

Figure 6. Microplastics extracted from the apple sample of two sets under 40X magnification

In Figure 6, the sample shows microplastics in the form of small brown fragments, thin transparent fibres, black fibres and a blue bead-like particle, indicating a mix of synthetic fragments and fibres. These findings suggest that the apple could have been exposed to environmental contamination during growth, harvesting or packaging and contain potential microplastics.

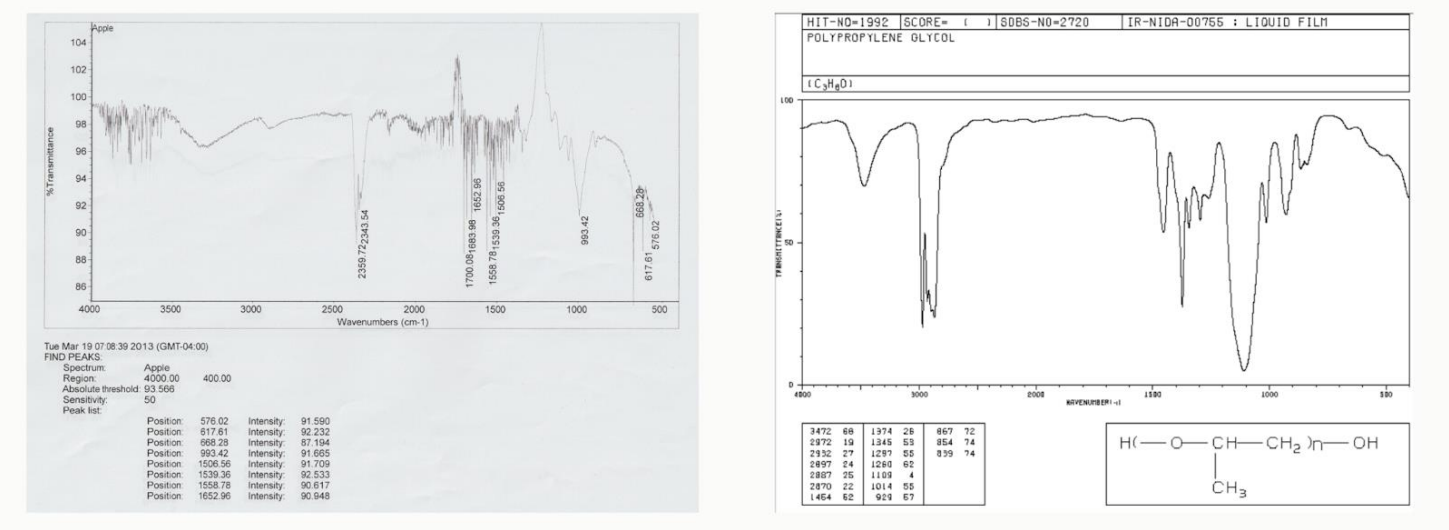

Figure 7. IR spectrum of the apple sample (left) and polypropylene glycol (PPG) (right; AIST, https://sdbs.db.aist.go.jp/Disclaimer.aspx)

Figure 7 reveals that the sample shows prominent peaks around 3300-3500 cm^{-1} (O-H stretching), ~2300 cm^{-1} (C-H stretching) and ~1000 cm^{-1} (C-O stretching). These peaks are consistent with that of the polypropylene glycol (PPG) and its chemical structure. This suggests that microplastics, specifically PPG, in the apple may have been introduced during handling, packaging or preservation as it is often used in food packaging and coatings on fruits (Agency for Toxic Substances and Disease Registry, 1997). Other peaks observed in the sample spectrum were ignored as these overlapped with the peaks seen in the filter paper without the sample (See Appendix, Figure 17).

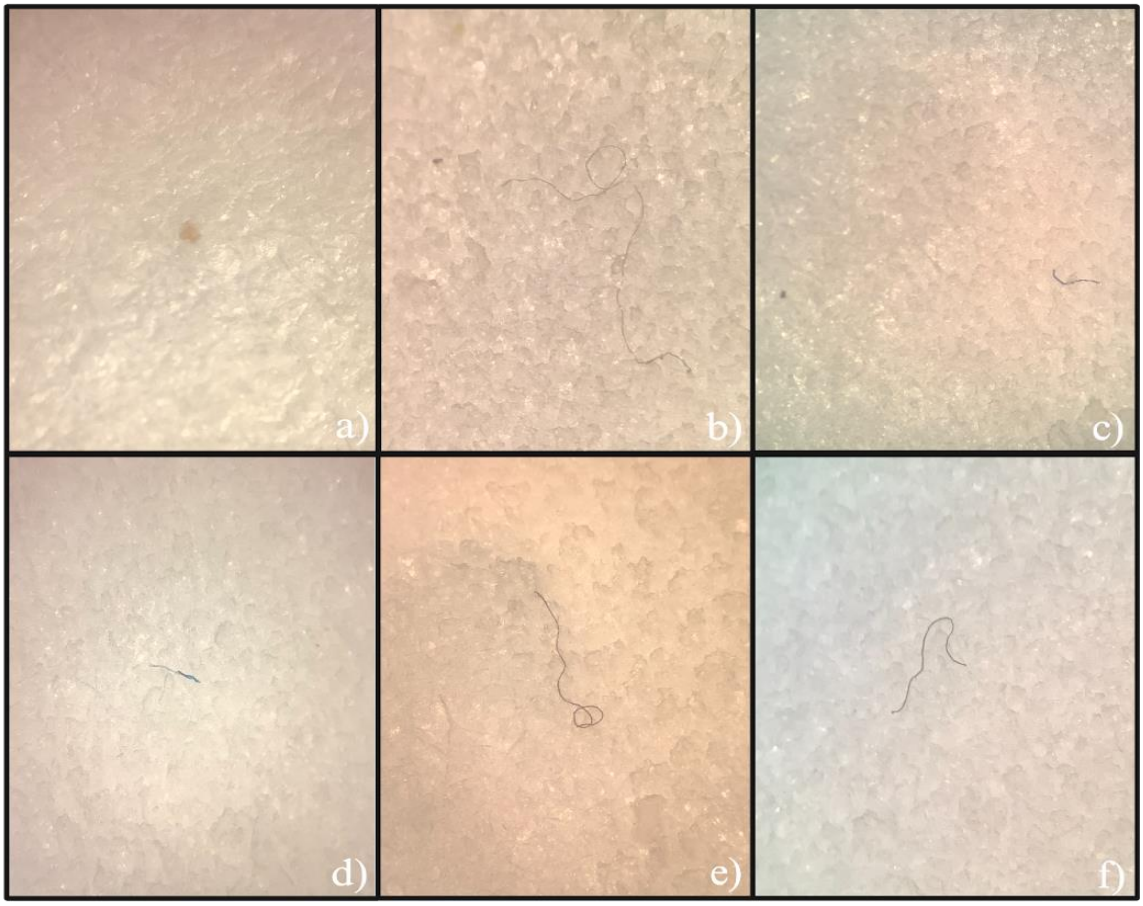

Figure 8. Microplastics extracted from the salmon sample of two sets under 40X magnification

In Figure 8, the sample shows microplastics in the form of brown fragments, transparent fibres and distinct blue and black fibres. The twisted and looped structures are characteristics of synthetic microplastics, possibly from food packaging, handling processes, or the exposure and consumption of microplastics in its habitat.

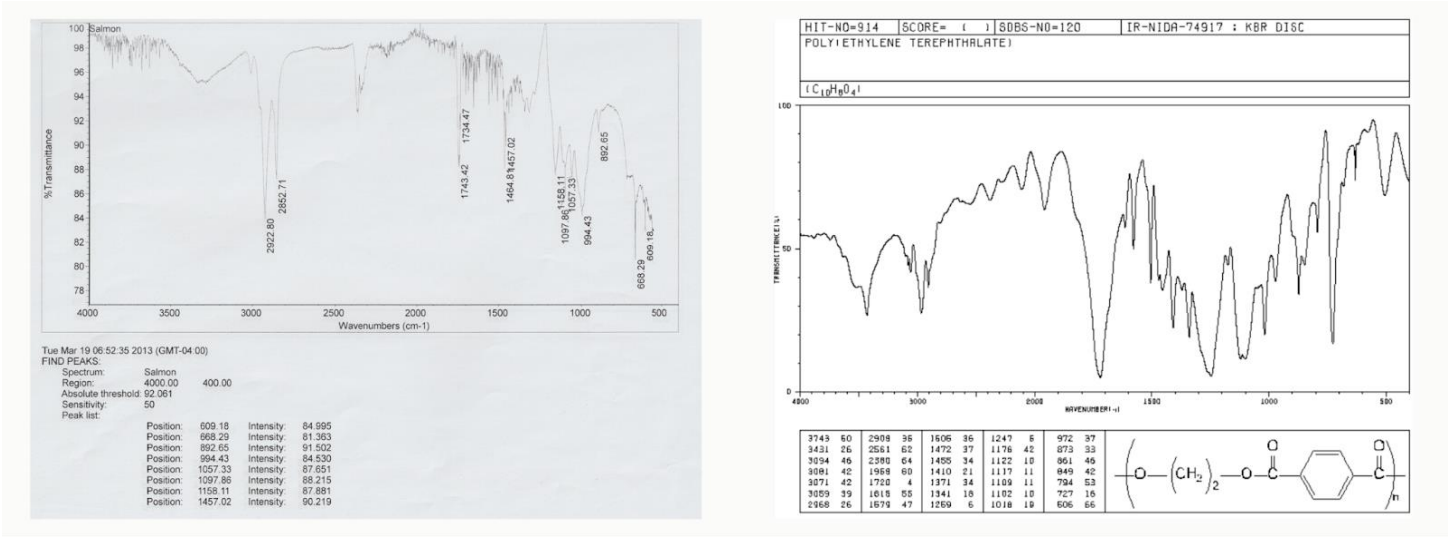

Figure 9. IR spectrum of the salmon sample (left) and poly-(ethylene terephthalate) (PET) (right)

In Figure 9, the sample spectrum displays strong peaks at 3300-3500 cm^{-1} (O-H/N-H stretching), ~1740 cm^{-1} (C=O stretching) and 1000-1200 cm^{-1} (C-O stretching). This spectrum matches with that of the polyethylene terephthalate (PET), as these wavenumbers indicate the presence of relevant functional groups found in the PET chemical structure. This suggests possible microplastic exposure from food packaging or environmental exposure. Other peaks observed in the sample spectrum were ignored as these overlapped with the peaks seen in the filter paper without the sample (See Appendix, Figure 17).

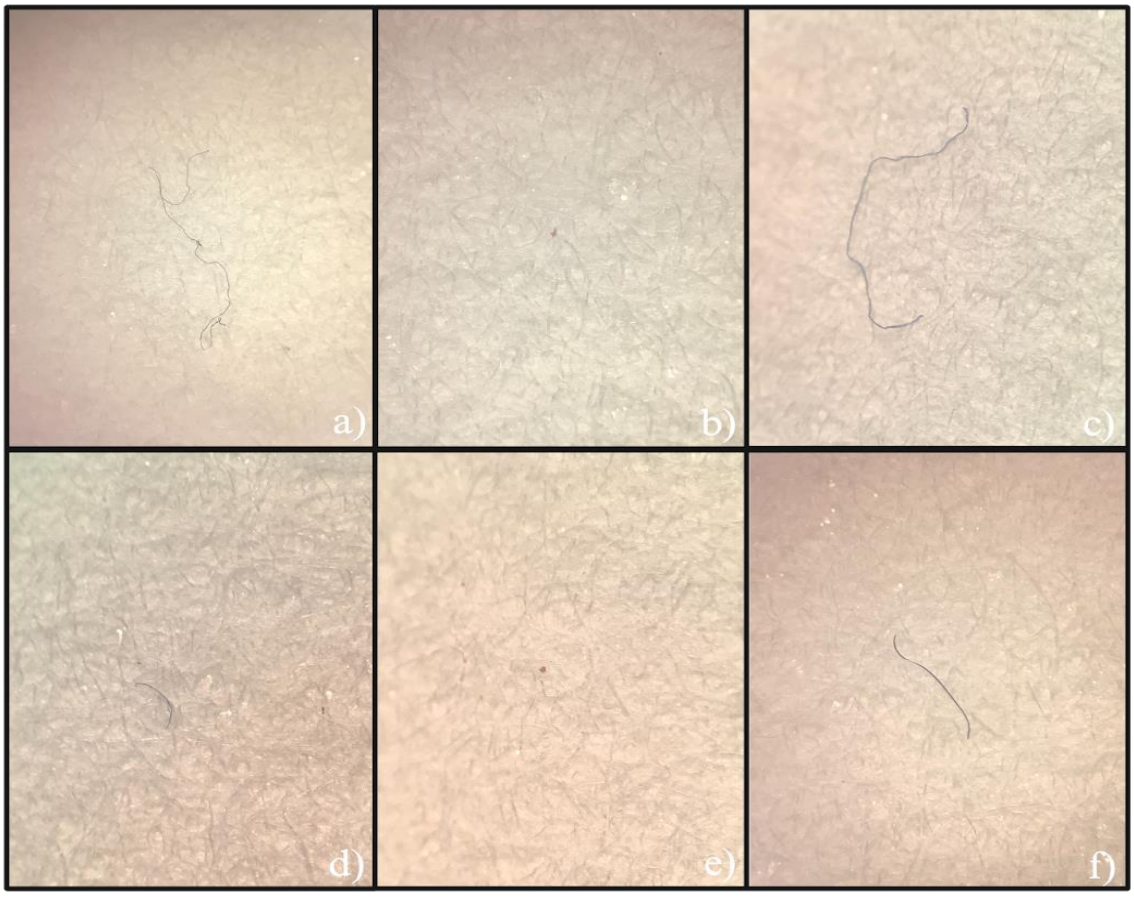

Figure 10. Microplastics extracted from the bottled water sample of two sets under 40X magnification

In Figure 10, the sample contains a variety of microplastics, mostly thin fibres, with transparent, blue and black being the most common colours. The presence of looped and curled fibres suggests that contamination could be from synthetic materials used in bottle manufacturing, bottle caps or during storage.

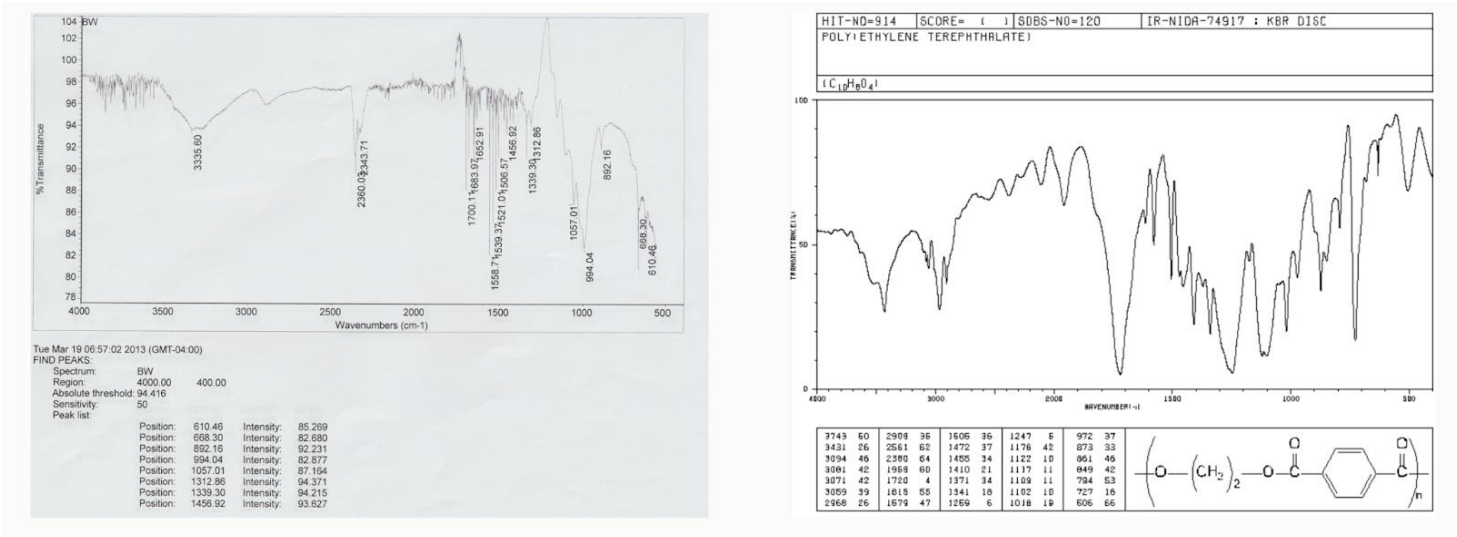

Figure 11. IR spectrum of the bottled water sample (left) and poly-(ethylene terephthalate) (PET) (right)

In Figure 11, the sample features broad O-H stretching (~3300-3500 cm^{-1}), C=O stretching (~1500-1700 cm^{-1}) and C-O stretching (~1000 cm^{-1}). These peaks are consistent with the spectrum of Polyethylene Terephthalate (PET), which is a common plastic used in water bottle manufacturing and the possible source for microplastic exposure and contamination.

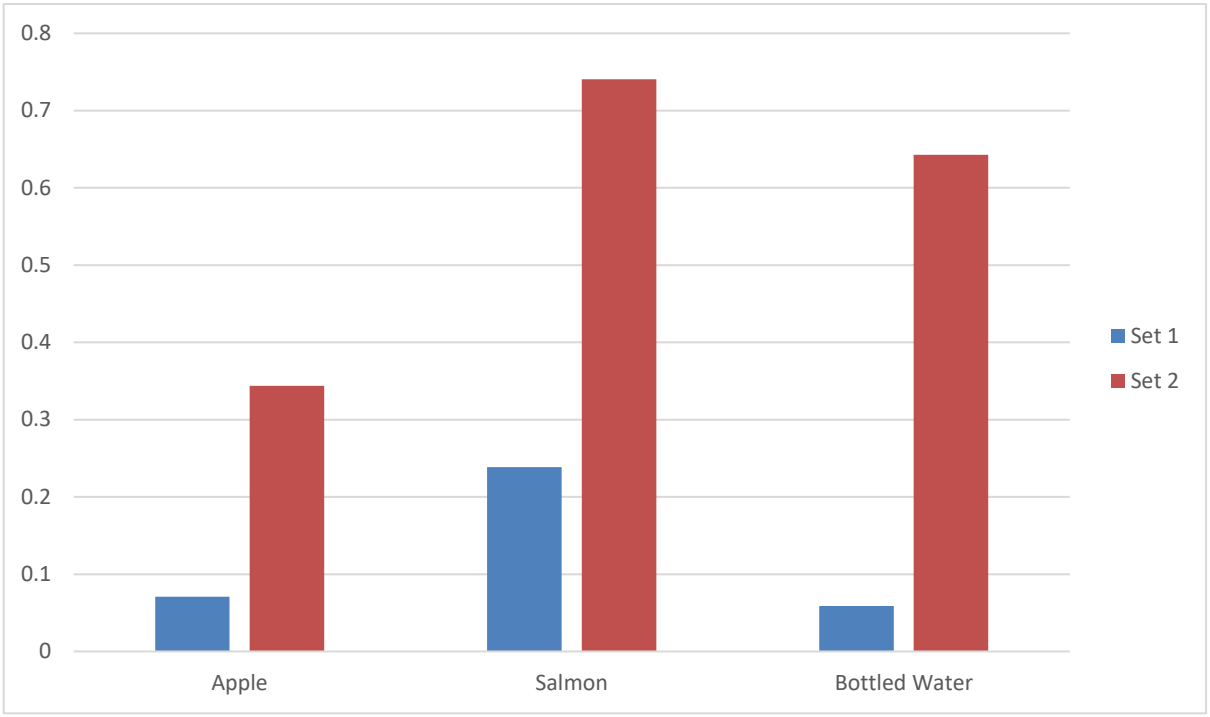

Figure 12. Weight of microplastics extracted from the food samples in two sets

Figure 12 shows the weight of the microplastics extracted from each food sample in two sets. As seen above, salmon had the highest weight in both sets 1 and 2 at 0.2387 g and 0.7407 g, respectively (See Appendix, Table 5). On the other hand, bottled water followed salmon in the 2nd set at 0.6427 g, but was lowest during the 1st set at 0.0587 g. The apple samples were similar in that it followed salmon during the 1st set at 0.0707 g, but was lowest in the 2nd set at 0.3437. It can be noted, however, that the average weight obtained from the second set of samples were higher than that of the first set, which could be attributed to the distribution of MPs in the sample itself.

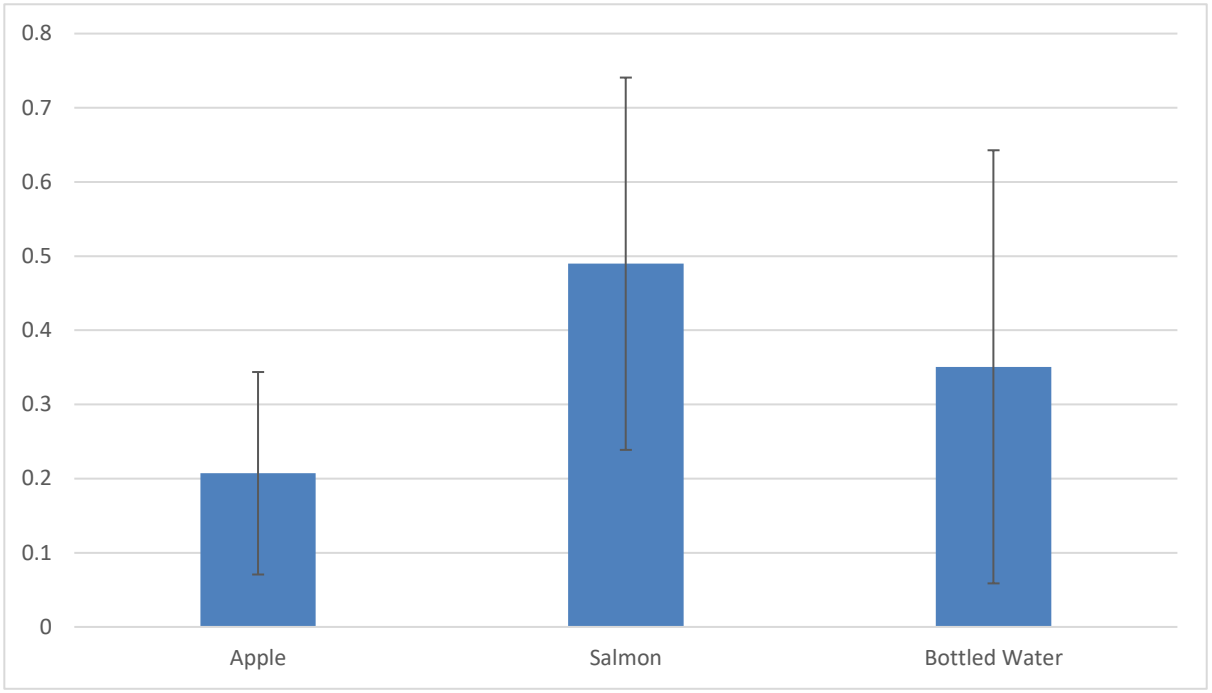

Figure 13. Average weight of microplastics extracted from the food samples

In figure 13, it can be noted that the salmon had the highest average weight among the microplastics extracted from each sample. That being said, residues observed in the filter papers could have contributed to its weight. Furthermore, the apple sample had the lowest average weight extracted from both sets. However, given the overlapping error bars, there was not much of a significant difference among the samples' weight.

3.2 Growth of *L. fermentum* in Microplastic Presence

The growth of *L. fermentum* in the presence of microplastics were assessed by measuring the optical density readings and performing a pour plate experiment in duplicates with two sets of samples, as demonstrated in Figures 14 and 15 below.

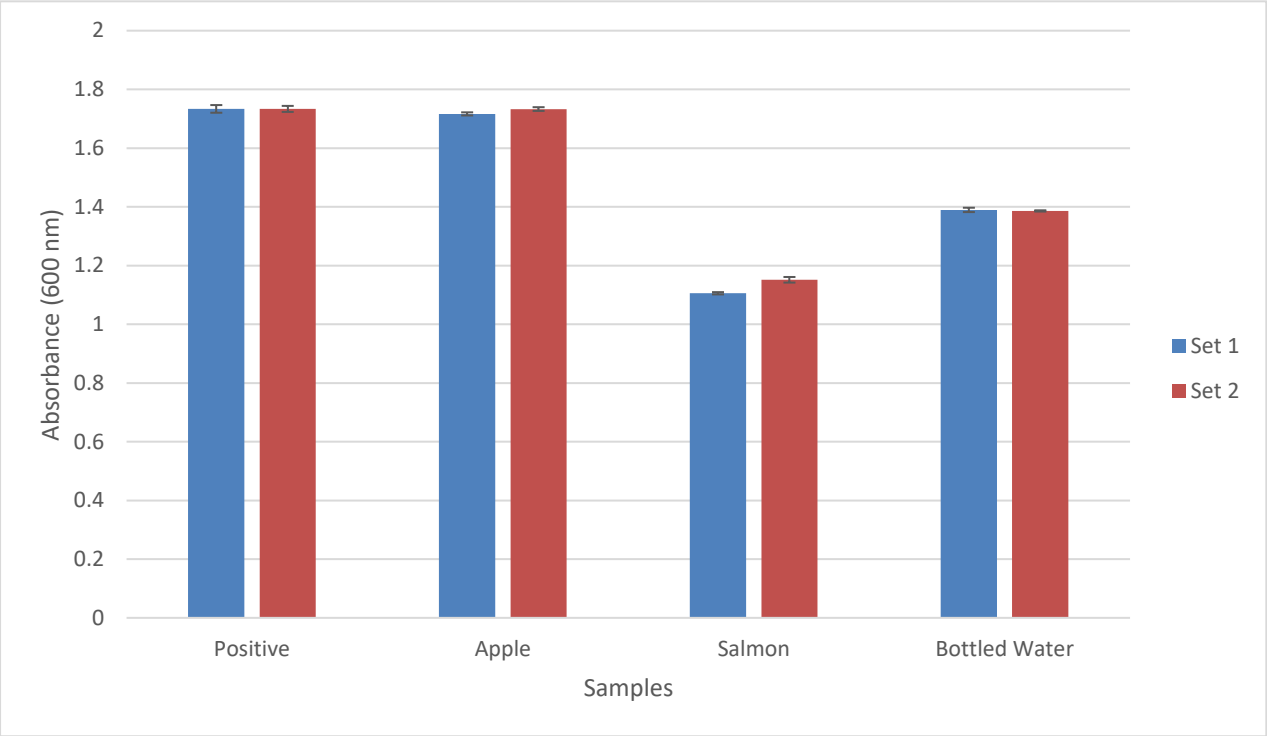

Figure 14. OD readings of microplastic-conditioned *L. fermentum* after 72 hours of incubation at 37°C

Figure 14 shows the OD readings of the *L. fermentum* culture conditioned with MPs from select food samples. As seen above, both sets of samples had the same trend in absorbance readings, wherein the culture broths inoculated with MPs from apple had the highest average readings and were comparable to that of the positive control (See Appendix, Table 6). However, they were significantly greater than the culture broths conditioned in MPs from salmon and bottled water. On the other hand, salmon was found to have the lowest average absorbance for both sets. All samples were also significantly different from each other in both sets.

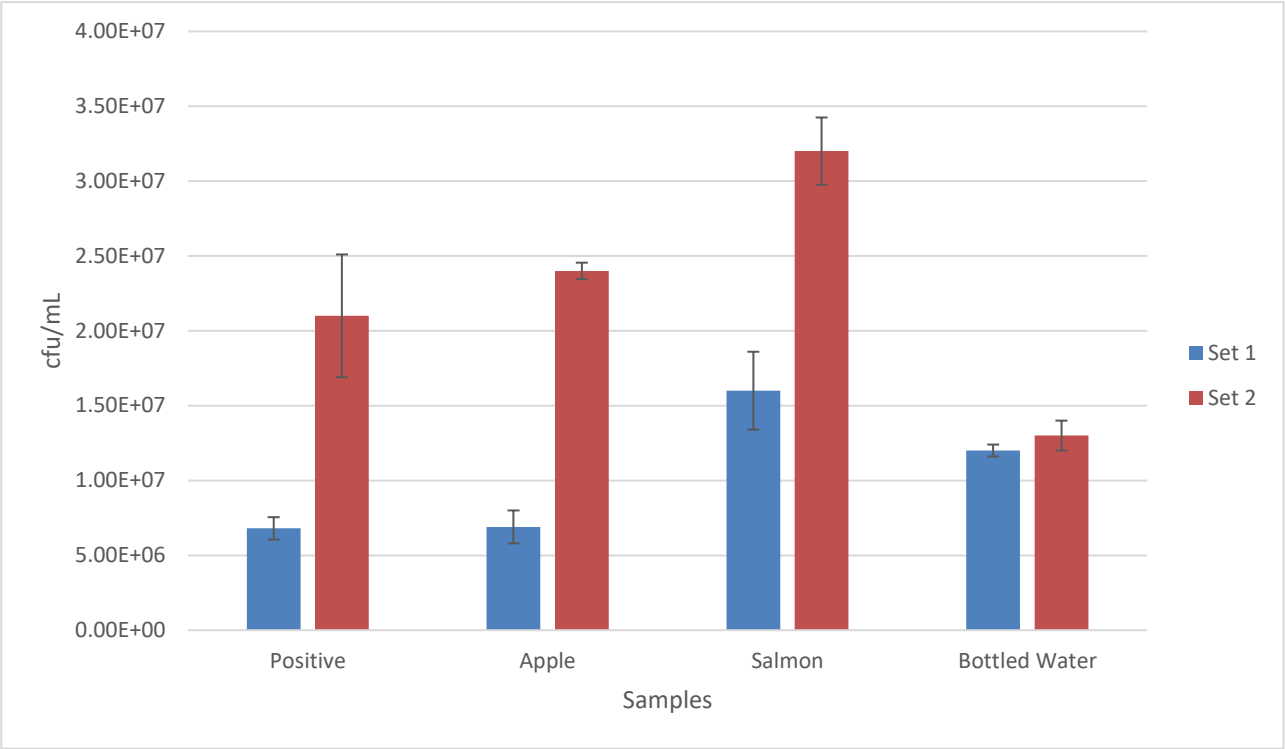

Figure 15. Plate counts of microplastic-conditioned *L. fermentum* after 72 hours of incubation at 37°C

Viable plate count through the pour plate method was conducted to measure the growth of viable bacteria after exposure to MPs. Figure 15 above reveals that the first set of culture broths with samples had a generally lower average concentration (cfu/mL) than those of set 2 which may be due to a sudden change in the environment causing the decrease or slow growth. However, the trends for both sets were fairly similar in that the culture broths for apple were statistically comparable to that of the positive control while the broths for salmon were significantly higher. On the other hand, the broths containing MPs from bottled water revealed a higher concentration during the first set of conditioning then was significantly lower during the second set. All samples were significantly differently from each other (See Appendix, Table 7).

3.3 Lactose Fermentation Activity of MP-conditioned *L. fermentum*

The lactose fermentation activity of the MP-conditioned culture was also assessed to determine its impact and implications on the cell's metabolic processes by quantifying the change in color through absorbance readings. Figure 16 below shows the absorbance results taken in duplicates for two sets of samples.

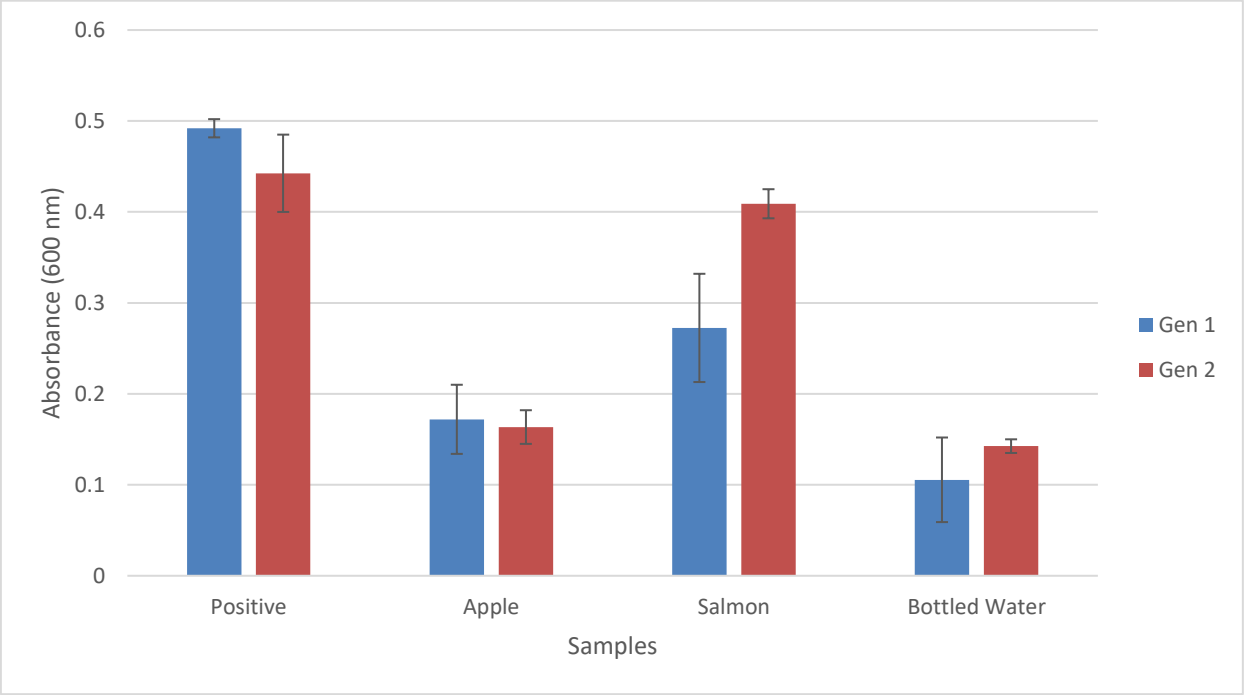

Figure 16. OD readings for the lactose fermentation activity of microplastic-conditioned *L. fermentum* after 72 hours of incubation at 37°C

According to these results, both sets followed the same trend in terms of data distribution. The first set of samples revealed a significant difference between each sample against the positive control, indicating a reduction in fermentation activity was observed. Set 2, on the other hand, had a similar trend with the exception of the salmon sample being comparable with the positive control (See Appendix, Table 8).

4.0 Discussion, Conclusion, and Recommendations

4.1 Discussion

Results obtained from this study provided information on the presence and amount of microplastics in select food samples representative of varying sources, as well as their impact on the growth and lactose fermentation activity of LAB, specifically, *L. fermentum*. In the extraction of microplastics from the food samples, it was revealed that the salmon had the highest yield in both sets, which contradicts the hypothesis where bottled water was the expected sample to have said results. However, it can be noted that some residues of the sample remained on the filter paper, which could have contributed to an increase in its weight. On the other hand, the bottled water and apple samples had the lowest yield in set 1 and 2, respectively (Figures 12 & 13) (See Appendix, Table 5). This difference in the amount from these samples may be attributed to the distribution of the MPs in the composition of the sample itself. That being said, the difference between these samples in the first set was not very significant, and was expected to have little impact in the succeeding experiments. Visual observations of the extracted microplastics showed mostly fiber-like materials of varying colors (red, blue, purple) and smaller pieces of transparent fragments, with the bottled water containing the most by visual estimation and comparison with other samples (Figures 6, 8, & 10). Confirmation of the material through IR analysis by comparing diagnostic peaks observed with a reference spectrum revealed that the samples mostly contained polyethylene terephthalate (PET), specifically from the salmon and bottled water (Figures 9 & 11). This confirmed the hypothesis in which PET plastics would be mostly observed due to their abundant use and presence in the community (Factors, 2024). However, in the apple samples, polypropylene glycol (PPG) was prominent based on

the similarity of their peaks, which may indicate the exposure to or use of PPG during processing as it is often used as an additive in food and food products as an emulsifier, thickener, stabilizer, and as an agent to extend shelf life and enhance their appearance as well as a packaging material (Agency for Toxic Substances and Disease Registry, 1997).

The study also showed that the *L. fermentum* conditioned with the MPs from bottled water had a significantly lower growth than that of the unconditioned culture, as well as the other samples. This information was significant as a noticeable impact is seen on their growth due to the presence of MPs. Although not much studies have seen their direct impact on microbes, various investigations on plastics and their implications for organism growth have noted that plastics can become physical interferences in an environment, in that their presence and composition disrupt the organisms' ability to uptake nutrients and molecules from their environment due to potential surface adsorption of said molecules (Balasubramanian, Muthuramalingam, Chen, & Chou, 2023) (Nicolescu et al., 2023). However, it can be observed that the growth in the first set of samples was higher than the positive control in the plate counts, while a significant reduction was seen in the second set (Figure 15). This could be due to the amount of MPs extracted during the first set which was significantly lower than that of the second set (See Appendix, Table 5). That being said, it further confirmed the researchers' speculations that the amount of growth would be inversely proportional with that of the amount of MPs in the sample. Moreover, analysis on the collated data between the samples' extracted MP amount, OD value readings, and plate counts revealed a consistent relationship in that a general reduction was observed with the bottled water. On the other hand, the two other samples revealed a similar consistency

in the OD value readings and plate counts with respect to the positive control, as well as the data obtained from the extraction process. For the salmon, the increase in viable growth was attributed to the residues on the filter paper which could have provided additional nourishment for the bacteria as well as masked or overpowered the presence of MPs, thus its proliferation in the plates. In the OD value readings, however, it was notably the lowest among the samples (Figure 14), in which the researchers speculate its slower growth rate, which may be due to a number of factors such as changes in the environment with the presence of residues or just another potential impact of MPs on their growth rate, which could also be another point of interest in future studies. The apple samples had the most consistent results, wherein it did not show any significant changes in the amount of growth in both OD value readings and plate counts which was directly attributed to the low amount of MPs extracted and potentially the type of MP (PPG), resulting in this little impact on their growth.

In terms of their lactose fermentation activity, a general reduction was observed among all the samples, with the bottled water having the highest reduction as it had the lowest absorbance readings in both sets (Figure 16). Conversely, the salmon sample had the highest absorbance readings with the second set being comparable to that of the positive control, indicating that there was some impact on its lactose fermentation activity but not by much. Interestingly, the apple samples were second to the bottled water in terms of reduction, which relatively disagreed with the consistency observed in the growth assay and the amount of MPs extracted. However, as previously mentioned, studies have revealed that MPs can adsorb certain molecules or nutrients from the environment affecting their ability to uptake the molecules needed for survival or to conduct their activities (Balasubramanian, Muthuramalingam, Chen, & Chou, 2023)

(Nicolescu et al., 2023). The lactose may have been adsorbed by the surfaces of the MPs, wherein larger fragments of MPs were reported in apple as compared to other fruits and vegetables, contributing to more surface area for molecules to be adsorbed (Aydın, Yozukmaz, Şener, Temiz, & Giannetto, 2023). Moreover, in a study by Kaseke, Lujic, & Velickovic (2023), although the interactions between carbohydrates and MPs are not completely understood yet, they also observed a reduction in the activity of to break down the starch in mussels in the presence of MPs, which could imply a potential impact on enzyme activity and/or gene expression. That being said, the hypothesis was confirmed in that the bottled water had the highest reduction in lactose fermentation activity relative to its growth. But, further investigation on the relationship between the growth, metabolic activity, size, and amount of MPs can be done to further elucidate the impact of MPs on bacterial growth and activity, providing implications for various microbiomes, specifically that of the gut.

4.2 Conclusion

This study showed that microplastics can be extracted from and were present in the selected food samples, with PET being the most prominent followed by PPG and salmon having the highest percentage in weight. In the growth and lactose fermentation activity assay, bottled water was revealed to have the highest general reduction among the samples and was significantly lower than that of the positive control, indicating the potential impact of MPs on LAB growth and activity. Further refinement on the extraction process from solid samples and investigations are needed to elucidate the relationship between the growth, metabolic activity, and amount of MPs and their impact on LAB. That being said, this undertaking has provided substantial preliminary data and

foundation for the direct impact of food-extracted microplastics on bacterial species, which could have implications on organism health, specifically that of microbial species and communities in the gut such as LAB, thus opening an entirely new discourse on the impacts of plastic on global and environmental health.

4.3 Recommendations

Due to the novel nature of this study, further modifications and refinement in certain aspects are necessary to enhance the robustness of the results. In the extraction process, the researchers suggest increasing the time for solid sample digestion to reduce residues on the filter papers containing MPs to obtain more accurate measurements in the MP amount. If available, measuring the amount of MPs via mass spectrometry would be more accurate than through analytical mass measurement. Also, the use of a density separator could improve the separation between the samples and MPs, reducing potential loss during the separation and filtration process. In terms of the growth and lactose fermentation activity, the addition of more sets of samples could strengthen the statistical aspect of the results as well as allow an analysis on the long-term impact of MPs on LAB growth and activity. Moreover, utilizing another bacterial species model in the gut with negative correlations such as *E. coli* or *Clostridium spp*. could allow for comparison on the MP impact and their potential implications in the gut microbiome balance and effect on overall health. Also, using a mixed culture instead of a single species could be done to mimic microbiome conditions as bacteria do not necessarily separate by species but rather interact with other species in a certain environment. Lastly, investigating the effect on other metabolic activities such as biomolecule or exopolysaccharide production, biofilm formation, and toxin production

could provide more insight on the potential effect on overall health, especially if assessed on a genetic or molecular level.

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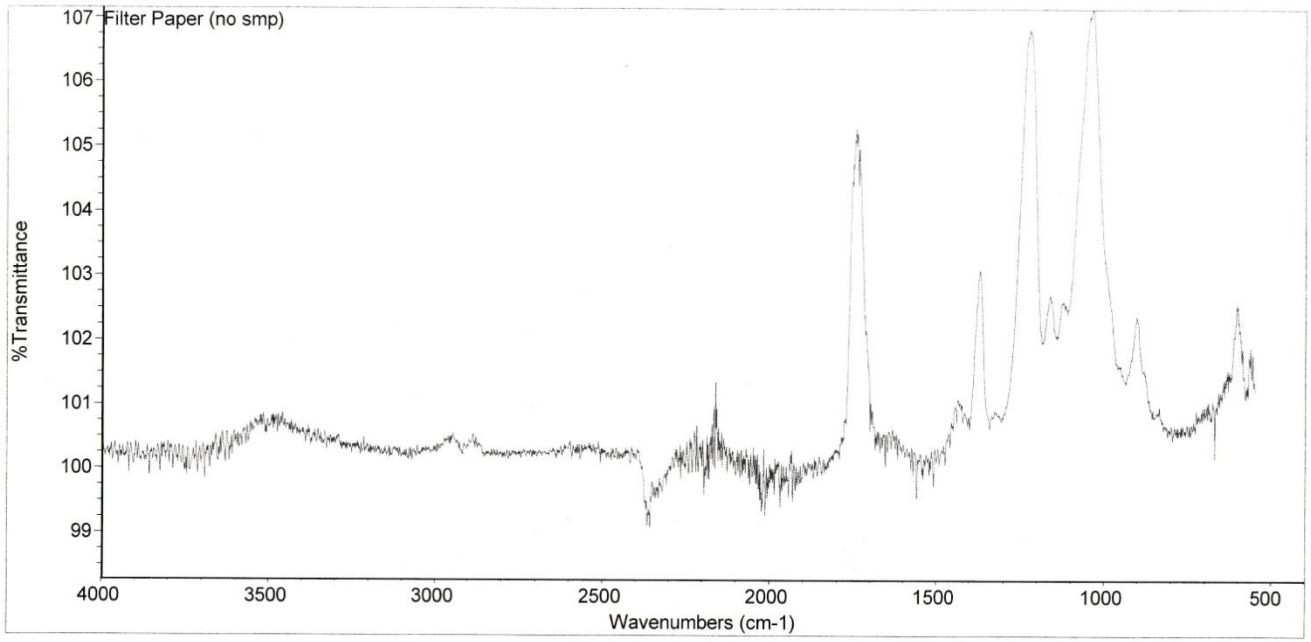
Appendix A. Blank Filter Paper Spectrum



Tue Mar 19 06:37:00 2013 (GMT-04:00)
FIND PEAKS:

Spectrum:	Filter Paper (no smp)	
Region:	4000.00	400.00
Absolute threshold:	97.512	
Sensitivity:	50	
Peak list:		

No peaks were found.

Figure 17. Blank filter paper IR Spectrum. Background Run and Reference

Appendix B. Microplastic Weight Calculations, Raw Data, and Analysis

Table 4. Microplastic weight calculations. Petri, filter paper, and raw weight data

	Petri + Filter Paper + MP (g)	Empty Petri (g)	Filter Paper (g)	Microplastic Weight
Set 1	94.07	93.714	0.2853	0.0707 g
	95.05	94.526	0.2853	0.2387 g
	101.27	100.926	0.2853	0.0587 g
Set 2	89.988	89.359	0.2853	0.3437 g
	94.526	93.500	0.2853	0.7407 g
	89.359	88.431	0.2853	0.6427 g

Table 5. Extracted microplastics amount data. Weight, averages, and standard deviations & error of mean values

Tube	S1 MP Weight (g)	S2 MP Weight (g)	Average	SD	SEM
Apple	0.0707	0.3437	0.2072	0.193040151	0.1365
Salmon	0.2387	0.7407	0.4897	0.354967604	0.251
Bottled Water	0.0587	0.6427	0.3507	0.41295036	0.292

Appendix C. Growth Assay Raw Data, Statistical Analysis, and Plate Counts

Table 6. Growth measurement: Optical density experiment data. Replicates, averages, and standard deviations & error of mean values

	Tube	R1	R2	Average	SD	SEM
Set 1	Positive	1.715	1.752	1.7335	0.0185	0.013081475
	Apple	1.709	1.724	1.7165	0.0075	0.005303301
	Salmon	1.111	1.1	1.1055	0.0055	0.003889087
	Bottled Water	1.4	1.379	1.3895	0.0105	0.007424621
Set 2	Positive	1.719	1.748	1.7335	0.0145	0.010253048
	Apple	1.724	1.742	1.733	0.009	0.006363961
	Salmon	1.165	1.138	1.1515	0.0135	0.009545942
	Bottled Water	1.389	1.383	1.386	0.003	0.00212132

Table 7. Growth measurement: Pour plate experiment data. Plate counts, averages, cfu/mL, and standard deviations & error of mean values

	Tube	Plate 1	Plate 2	cfu/mL	Average	SD	SEM
Set 1	**Positive**	6.00E+06	7.50E+06	6.80E+06	6.75E+06	1060660.172	750000
	Apple	5.80E+06	8.00E+06	6.90E+06	6.90E+06	1555634.919	1100000
	Salmon	1.82E+07	1.30E+07	1.60E+07	1.56E+07	3676955.262	2600000
	Bottled Water	1.18E+07	1.26E+07	1.20E+07	1.22E+07	565685.4249	400000
Set 2	**Positive**	2.83E+07	2.01E+07	2.10E+07	2.42E+07	5798275.606	4100000
	Apple	1.80E+07	1.69E+07	2.40E+07	1.75E+07	777817.4593	550000
	Salmon	2.57E+07	2.12E+07	3.20E+07	2.35E+07	3181980.515	2250000
	Bottled Water	1.23E+07	1.43E+07	1.30E+07	1.33E+07	1414213.562	1000000

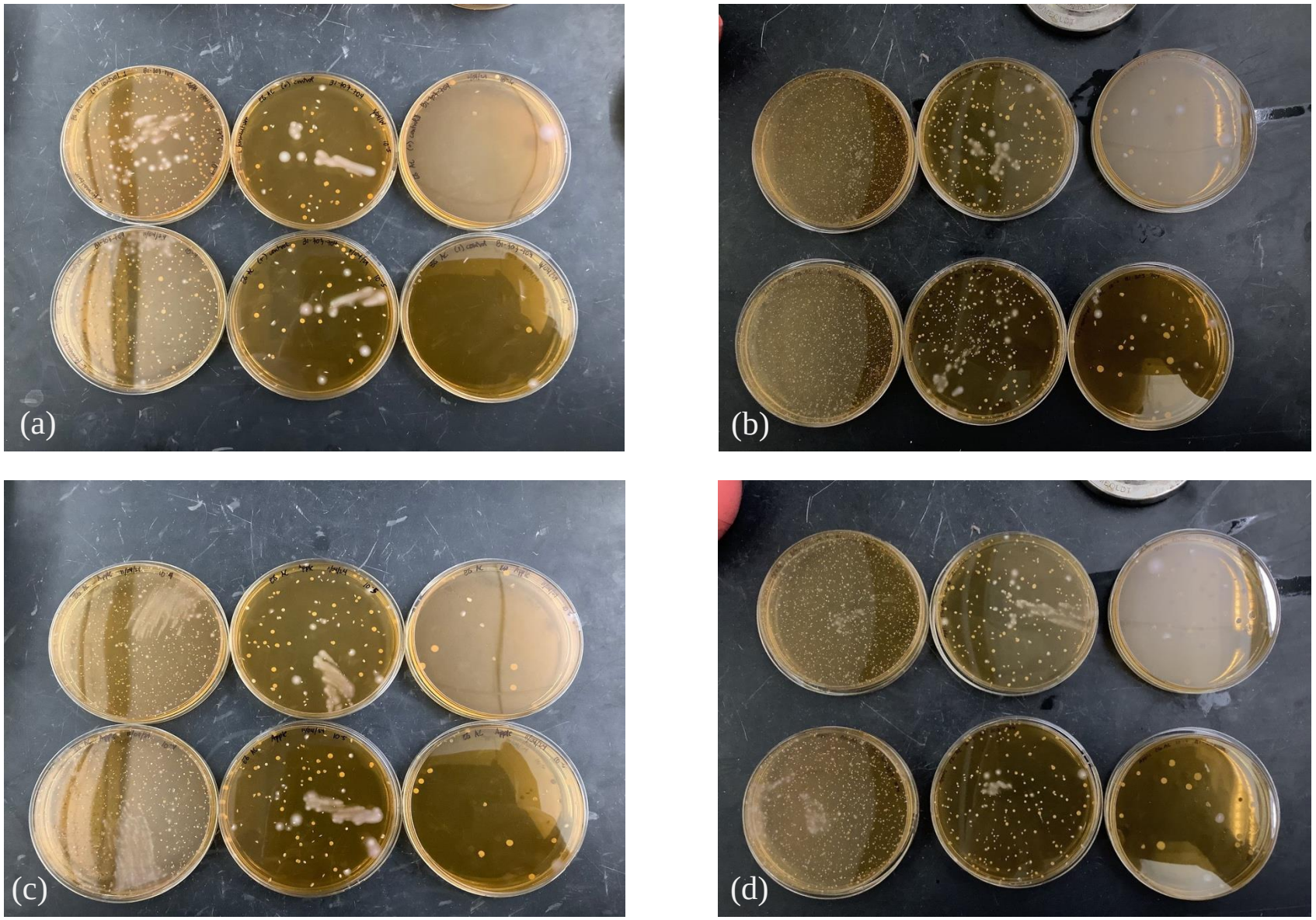


Figure 18. Pour plate experiment plates and colonies in positive control and apple samples. Positive Control (a) Set 1 and (b) Set 2; Apple (c) Set 1 and (d) Set 2

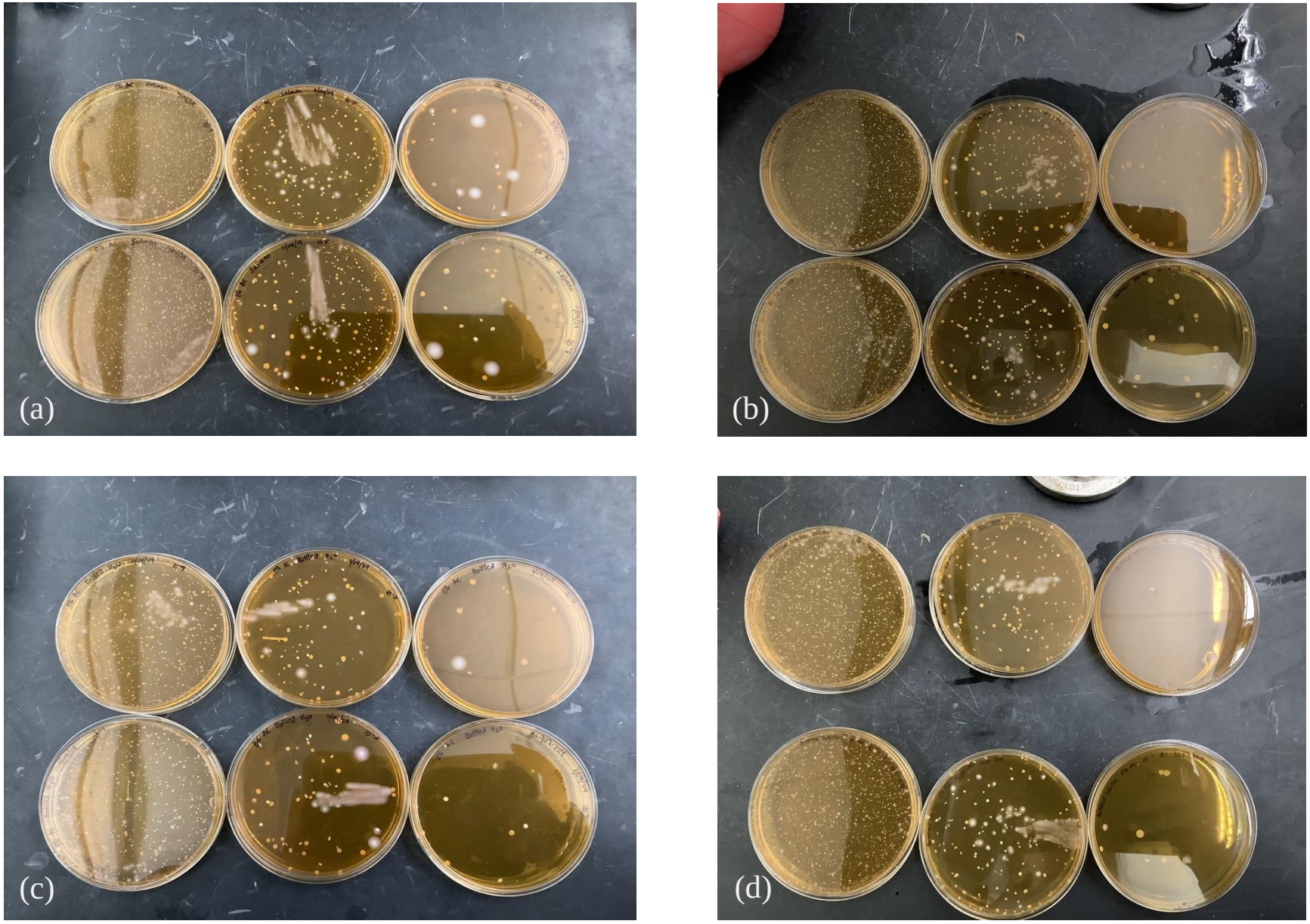


Figure 19. Pour plate experiment plates and colonies in salmon and bottled water samples. Salmon (a) Set 1 and (b) Set 2; Bottled Water (c) Set 1 and (d) Set 2

Appendix D. Lactose Fermentation Activity Raw Data and Statistical Analysis

Table 8. Lactose fermentation activity absorbance experiment data. Replicates, averages, and standard deviations & error of mean values

	Samples	R1	R2	Average	SD	SEM
Set 1	**Positive**	0.482	0.502	0.492	0.014142136	0.01
	Apple	0.134	0.21	0.172	0.053740115	0.038
	Salmon	0.213	0.332	0.2725	0.084145707	0.0595
	Bottled Water	0.152	0.059	0.1055	0.065760931	0.0465
Set 2	**Positive**	0.4	0.485	0.4425	0.060104076	0.0425
	Apple	0.145	0.182	0.1635	0.026162951	0.0185
	Salmon	0.393	0.425	0.409	0.022627417	0.016
	Bottled Water	0.135	0.15	0.1425	0.010606602	0.0075