MICRO AND NANOPLASTICS AS CARRIERS FOR THE SPREAD OF ANTIBIOTIC RESISTANCE IN THE AQUATIC ENVIRONMENT

Report

to the Water Research Commission

by

Amoah ID[1], Rajcoomar S[1], Kumari S[1], Bux, F[1]

[1]Institute for Water and Wastewater Technology,
Durban University of Technology

WRC report no. 2952/1/26
ISBN 978-0-6392-0802-2

August 2026

WATER
RESEARCH
COMMISSION

DISCLAIMER

This report has been reviewed by the Water Research Commission (WRC) and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the WRC, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

EXECUTIVE SUMMARY

BACKGROUND

The occurrence of micro- and nanoplastics in wastewater has been considered a major environmental hazard. This is mainly due to the increased use and subsequent discharge of plastics into the aquatic environment. The occurrence of these microplastics in wastewater has been reported as one of the main pathways through which microplastics are released into the aquatic environment. The ecological impact of the occurrence of microplastics in the water environment has been reported extensively globally and in South Africa. Furthermore, these microplastics could have an impact beyond their direct role as contaminants. These microplastics could adsorb harmful agents, such as pharmaceuticals and pathogenic organisms onto their surface. Microplastics in the aquatic environment are widely reported as a potential substrate for biofilm formation, creating an environment for phylogenetically and functionally diverse communities of bacteria, algae, protozoans, and fungi. Biofilm formation creates several advantages for the attached microorganisms, such as competition and survival strategies, including possibilities for forming stable consortia, horizontal gene exchange, accumulation of nutrients, and protection against toxic substances and desiccation. It is therefore important to understand the extent to which microplastics are released into wastewater in South Africa, the role of these microplastics as carriers of microorganisms, and the assessment of the removal of these microplastics during wastewater treatment.

AIMS

The following were the aims of the project:

1. To identify and characterise the major types of MNPs in different wastewater sources in South Africa
2. To evaluate UWTP removal of MNPs under various wastewater treatment configurations
3. To evaluate on a laboratory scale, DNA sorption to MNPs and factors influencing the sorption.
4. To evaluate the impact of chlorination and UV exposure/treatment on MNP-sorbed DNA and MNP-bound microorganisms

METHODOLOGY

The study was conducted in Durban, focusing on two wastewater treatment plants. The project's aims were achieved by first developing a revised methodology for detecting and quantifying microplastics in wastewater. This revised method involves removing organic matter through hydrogen peroxide digestion and filtering the sample through a series of sieves with different pore sizes. Microplastics were then recovered from the sieves and classified either by shape (microfragments, beads, film, or fibre) or by size. The size categories used were >5000 µm, 500-5000 µm, 180-500 µm, 100-180 µm, and 25-100 µm, achieved using sieves with varying pore sizes. Additionally, chemical characterization of the recovered microplastics was performed by identifying the plastic polymers using pyrolysis-gas chromatograph mass spectrometry (pyro-GC/MS). To evaluate the effectiveness of wastewater treatment in removing these microplastics, samples of raw (untreated) and treated wastewater were

collected, and the concentration of various microplastics was determined. DNA sorption to microplastics was tested by exposing commercially purchased microplastics to *Escherichia coli* genomic DNA for different durations (5-60 minutes at 10-minute intervals). The attached DNA was then washed, concentrated, and amplified using qPCR. However, no attachment was observed. Therefore, the potential role of microplastics as carriers for biofilms was examined by exposing the commercial microplastics to untreated wastewater for 5 weeks. The attachment of microorganisms (biofilm) to the microplastics was assessed weekly, and the biofilm was quantified. The effects of exposure time, temperature, light, and oxygen on biofilm formation were studied. The impact of ultraviolet and chlorine treatment on microplastic-bound microorganisms was tested by exposing the microplastics with attached biofilms to UV and chlorine for varying durations (10, 20, 30, and 60 minutes). The effects were then evaluated through culturing using heterotrophic plate count.

RESULTS AND DISCUSSION

Microfragments, beads, fibres, and films were the most common microplastics identified based on their shape. However, the most abundant were the microfragments, with mean concentrations in the untreated wastewater of 5712 (±3064) particles/L. The fibres were the least abundant microplastic identified, with mean concentrations of 113.2 (±39.2) particles/L of wastewater. These concentrations are known to correspond to the different types of plastics in use in the connected populations and fragmentation of macroplastics. The most common size categories were the 500-5000 µm size category among all the different shapes detected, except for the film, where the most abundant was the 25- 100 µm size range.

Removal of the microplastics differed depending on the size and the type of microplastic (microfragments). Microfragments in the > 5000 µm size category did not show any reduction in the effluents. However, for the smaller sizes like the 500-5000 µm size range, 91% removal was observed. In the 25-100 µm size range, an approximate 42% removal was recorded. The variation in the removal of the microplastic particles shows that this could be related to the type and size of the microplastics.

The Pyro-GC/MS data showed that the common plastic polymers were polyvinyl chloride (PVC), Polyethylene or polythene (PE), Polypropylene (PP), Polystyrene (PS) and Polyamide 6 (PA6). Among these polymers, the most common were PVC (5.6 µg/mg), PE (3.6 µg/mg) and PA6 (3.3 µg/mg) in the untreated wastewater. In the effluents, these concentrations were significantly reduced for all the different polymers, except for PS, which recorded an increase to 0.86 µg/mg in the effluents from 0.07 µg/mg in the raw wastewater. These findings give an indication of the type of polymers commonly used in plastics within the catchment and the removal of microplastics irrespective of the polymer.

Highest biofilm formation was observed on week 3, with an OD of 1.77. Thereafter, a decline in OD value was observed, reaching an OD of 1.1 by week 5. This change in biofilm concentration, represented by changes in the OD, corresponded to changes in the nutrient concentration in the wastewater. A positive correlation ($r = 0.824$) and ($r = 0.1$) was observed between changes in biofilm concentration and nitrite ($NO_{2)}$ and ammonia (NH_4), respectively. Meanwhile, changes in the biofilm concentration had a negative correlation ($r=-0.673$) with nitrate ($NO_{3)}$. Dark conditions, 25°C, and aerobic conditions presented the highest median biofilm concentrations of 1.6, 1.7 and 1.6, respectively. It was also observed that PE microplastics had higher biofilm concentrations compared to PP. These results suggest that

biofilm formation on microplastics is influenced by the surrounding environmental conditions. Therefore, similar conditions at wastewater treatment plants could facilitate biofilm formation on microplastics present in wastewater.

Despite these biofilms being able to protect microbial cells from tertiary treatment, UV and chlorine treatment were able to inactivate the attached biofilms. It was observed that biofilms were inactivated after 30 mins of UV treatment, whereas 10 min was sufficient to inactivate biofilm by chlorine treatment.

CONCLUSIONS

The study established that wastewater in Durban, South Africa, could be a common source of microplastics in the aquatic environment. Microfragments, beads, film and fibre were the common microplastics identified, with microfragments the most abundant. This is potentially due to the weathering of macroplastics. Furthermore, the study showed that most microplastics fall within the 500-5000 µm size range. The common polymers identified in these microplastics were polyvinyl chloride (PVC), Polyethylene or polythene (PE), Polypropylene (PP), Polystyrene (PS) and Polyamide 6 (PA6). PVC dominated based on the weight of microplastics recovered. The efficiency of the wastewater treatment plants in removing these microplastics differed significantly between the shapes and sizes of the microplastics. For instance, up to 91% of microfragments in the size range of 500-5000 µm were removed during treatment.

This study did not demonstrate DNA sorption to microplastics. However, it found that dark conditions, a temperature of 25 °C, and aerobic environments were optimal for biofilm attachment on microplastics. Tertiary treatment showed significant potential for completely inactivating these microorganisms, achieving full inactivation after 10 minutes of chlorination and 30 minutes of UV exposure. Overall, the study successfully met its objectives, except for demonstrating DNA sorption to microplastics.

RECOMMENDATIONS

The study makes the following recommendations:

1. Development or improvement of methods for the detection and quantification of these microplastics, including nanoplastics.
2. There is a need for capacity development to reduce the reliance on external organisations for plastic (in this case, microplastic) analysis.
3. It is recommended that further studies could add an assessment of microplastic removal by different wastewater treatment technologies or stages.
4. Microbial community profiling is also recommended to help determine if the microbial community is influenced by the type of microplastics, the polymers, and the environmental conditions.

ACKNOWLEDGEMENTS

The project team gratefully acknowledges the funding provided by the Water Research Commission of South Africa. We also acknowledge the support of members of the Joint Programme Initiative on Water (JPIWater) project titled “Nanocarriers”, with special thanks to the Norwegian Institute for Water Research (NIVA) for assistance with the pyrolysis–gas chromatography–mass spectrometry (pyro-GC/MS) analysis. Appreciation is further extended to the managers of the wastewater treatment plant where sampling was conducted, as well as to the WRC Research Manager, Dr Eunice Ubomba-Jaswa, and the Project Coordinator, Ms Penny Jaca.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

PE	Polyethylene
PP	Polypropylene
HDPE	High density polyethylene
LDPE	Low density polyethylene
PP	Polypropylene
EPS	Extra polymeric substances
MNPs	Micro- and nanoplastics
UWTP	Urban wastewater treatment plant
DNA	Deoxyribonucleic acid
WWTP	Wastewater treatment plant
OM	Organic material
MPs	Microplastics
pyro-GC/MS	Pyrolysis-gas chromatography mass spectrometry
NIVA	Norwegian Institute for Water Research
OD	Optical density
SEM	Scanning electron microscopy
HPC	Heterotrophic plate count
PVC	Polyvinyl chloride
PS	Polystyrene
PA6	Polyamide 6

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Plastic debris in aquatic environments has emerged as a major global pollution issue (Rummel et al., 2017). Plastics are synthetic polymers produced through the polymerisation of monomers derived from fossil fuels such as oil and gas (Cole et al., 2011). Their lightweight nature, durability, and low cost make them widely used in everyday products. However, these same properties render plastics highly resistant to degradation, complicating waste management and disposal (Sivan, 2011). The rapid increase in plastic production and use has resulted in elevated inputs of plastic waste into aquatic environments, including oceans, rivers, and lakes (Carr et al., 2016).

Larger plastic debris found in aquatic environments is referred to as macroplastics and poses aesthetic, economic, and ecological challenges, particularly through negative impacts on aquatic organisms. More recently, microplastics have emerged as a significant environmental concern (Cole et al., 2011). These emerging contaminants are widespread and ubiquitous in aquatic environments and pose a potential threat to aquatic biota (Harrison et al., 2018).

Microplastics are defined as plastic particles ≤5 mm in diameter (Shen et al., 2019) and are classified as either primary or secondary. Primary microplastics are intentionally manufactured for use in products such as personal care and cleaning items, whereas secondary microplastics originate from the fragmentation and degradation of larger plastic materials (Talvite et al., 2017). They are commonly composed of polymers such as polyethylene (PE), polypropylene (PP), polyester, and others (Carr et al., 2016). Microplastics originate from both land-based sources, including urban runoff, and aquatic sources, such as the degradation of macroplastics within water bodies (Hammer et al., 2012).

Wastewater is recognised as a major land-based source and pathway for microplastics entering aquatic environments. Microplastics detected in marine systems closely resemble those found in wastewater effluents (Ziajahromi et al., 2016). Of particular concern is the ability of microplastics in wastewater to adsorb harmful substances, including pharmaceuticals and pathogenic microorganisms, onto their surfaces (Ziajahromi et al., 2017). Additionally, microplastics can adsorb nutrients from wastewater, facilitating biofilm formation and subsequent colonisation by bacteria and other microorganisms (Joo et al., 2021).

Biofilm formation occurs through the secretion of extracellular polymeric substances (EPS) by microorganisms (Rummel et al., 2017). EPS, composed primarily of proteins and glycolipids, provides structural stability and enables microbial attachment to surfaces (Flemming et al., 2007; O'Toole et al., 2000). These biofilms may consist of phylogenetically and functionally diverse communities, including bacteria, algae, protozoa, and fungi, and confer advantages such as enhanced survival, competition, stable consortia formation, and horizontal gene transfer (Flemming et al., 2016).

The development of biofilms can alter the buoyancy and sinking behaviour of micro- and nanoplastics (MNPs), influencing their transport, removal efficiency in wastewater treatment processes, and distribution in aquatic environments (Rummel et al., 2017). Biofilms may also enhance the weathering and biodegradation of plastics, potentially affecting their persistence and ecological impacts. Consequently, biofilm formation may increase the adverse environmental effects associated with MNPs.

Several factors influence biofilm development and composition on microplastic surfaces, including environmental conditions, geographic location, particle size, surface properties, and polymer type (Carreres Calabuig et al., 2019). In wastewater treatment plants, variables such as flow velocity, temperature, and nutrient concentrations can affect microbial attachment.

Rougher surfaces and larger particles, which provide greater surface area, tend to promote increased biofilm formation (Donlan, 2002). As a result, microplastics can act as vectors for microorganisms and associated pollutants, facilitating their transport into aquatic environments and posing risks to aquatic organisms and human health (Talvite et al., 2017). Furthermore, microplastics may enhance the dissemination of pathogenic microorganisms and promote gene exchange, including the spread of antibiotic resistance.

This study aimed to determine the occurrence, concentration, and characterisation of microplastics in wastewater in South Africa, with a focus on Durban, and to assess potential risks associated with their interactions with microorganisms. This research formed part of the NANOCARRIERS project under the Joint Programme Initiative on Water (JPIWATER), a collaborative effort between European Union member states and South Africa.

1.2 PROJECT AIMS

Based on the background information and motivation described above, the following were the aims of the project;

1. To identify and characterise the major types of MNPs in different wastewater sources in South Africa.
2. To evaluate UWTP removal of MNPs under various wastewater treatment configurations.
3. To evaluate, on a laboratory scale, DNA sorption to MNPs and factors influencing the sorption.
4. To evaluate the impact of chlorination and UV exposure/treatment on MNP-sorbed DNA and MNP-bound microorganisms.

1.3 SCOPE AND LIMITATIONS

The study focused on the detection, characterisation, and concentration of microplastics in wastewater in Durban, South Africa. This involved the optimisation of methodologies for the detection and characterisation based on shape, size and polymers of the plastics. The study also looked at the efficiency of wastewater treatment plants in removing these microplastics. To further elucidate the potential risks associated with the occurrence of microplastics in wastewater. Although the title of the study included nanoplastics, the optimised method failed to capture nanoplastics due to their small sizes.

The phenomenon of biofilm association with microplastics was explored. This looked at the determination of the conditions that aid biofilm formation. This study focused only on the exposure time (maximum 5 weeks), temperature (20°C, 25°C and 35°C), light and oxygen on the biofilm formation. Furthermore, the tertiary treatment options considered were UV and chlorination; no other treatment approaches were considered.

CHAPTER 2: METHODOLOGY

2.0 METHODOLOGICAL OUTLINE

The methodology applied in this study is presented in three phases:

1. The first describes the method development and application in identifying and characterising MNPs in wastewater. This aspect covers Objectives 1 and 2. It must be noted that the recovery of nanoplastics in the study was unsuccessful mainly due to the small size of these plastics. Therefore, the methods mainly focus on microplastic detection and characterisation. This characterisation focused on both the physical and chemical nature of the recovered microplastics. Further details for these methods are presented below.
2. The second method aims at describing the association of nucleic acid with microplastics, therefore covering Objective 3. However, it must be stated that due to the negative results obtained, that is, the non-detection of nucleic acid attachment to microplastics in the wastewater, this was expanded to study the association of microorganisms with these microplastics in the form of biofilms.
3. The third and final method focused on Objective 4, which is to evaluate the impact of tertiary treatment methods, such as chlorination and UV treatment, on the attached microorganisms.

2.1 IDENTIFICATION AND CHARACTERISATION OF MICROPLASTICS MNPS IN WASTEWATER IN SOUTH AFRICA

2.1.1 Study location and wastewater sampling

The Shallcross and Isipingo WWTPs in Durban, South Africa were selected for this study. The Isipingo WWTP largely treats domestic wastewater; however, the Shallcross WWTP treats both domestic and industrial wastewater. The configurations of these two WWTPs are presented in Figures 1 and 2 below. Wastewater samples with a volume of 5 L were collected from the influent and effluent, with a 10-L stainless steel bucket attached to a metal wire and poured through sieves with different pore sizes. These were 5000µm, 500 µm, 180 µm, 100 µm, and 25 µm pore sizes. Materials collected on the 25 µm sieve were washed thoroughly to ensure complete collection of all MNPs in the filtrate. A revised methodology was used to isolate these microplastics from the wastewater (Figure 3).

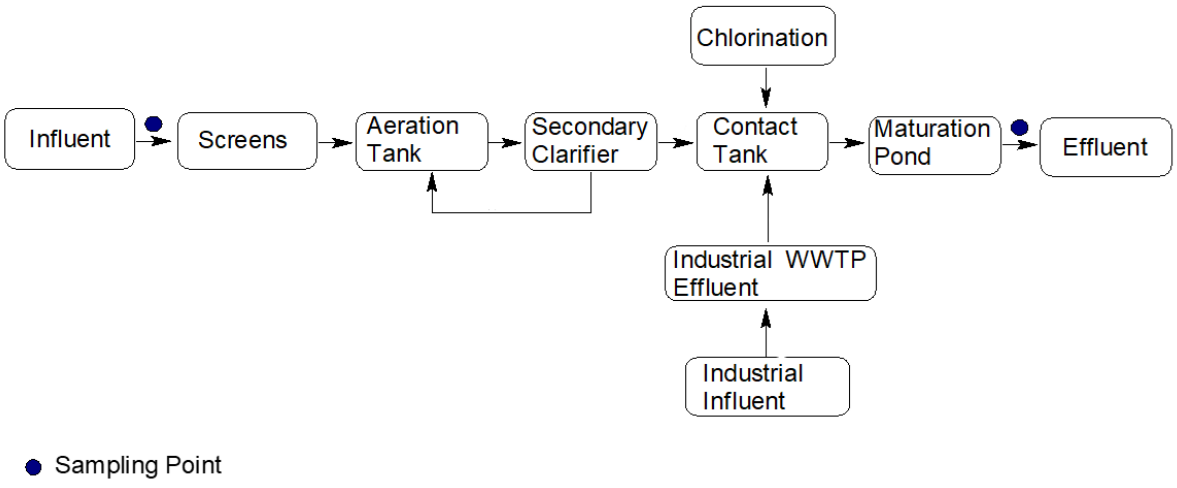


Figure 1: Shallcross WWTP sampling points

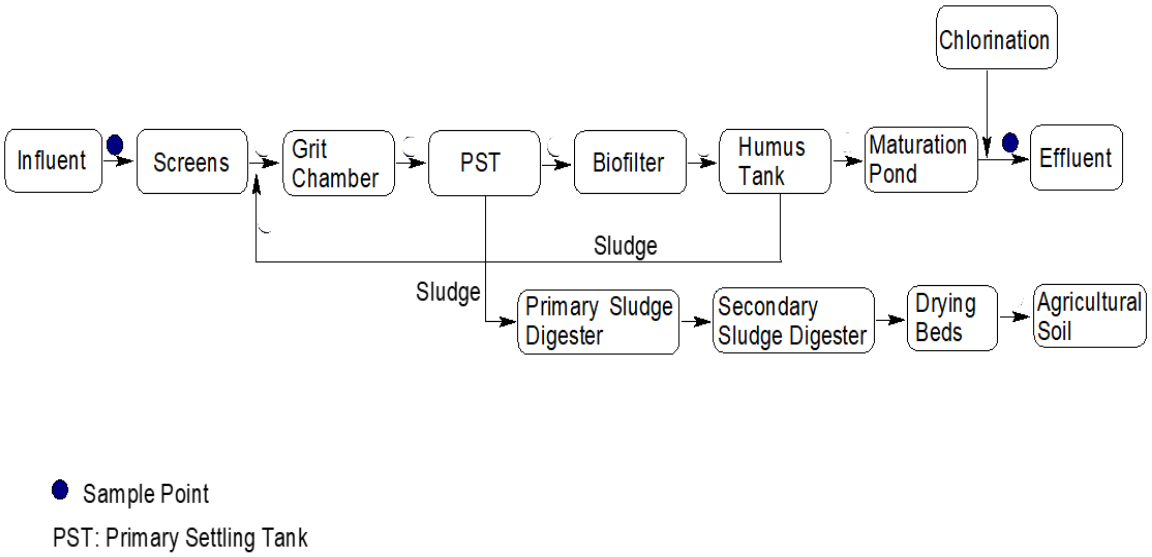


Figure 2: Isipingo WWTP sampling points

2.1.2 Identification and characterisation of microplastics

A new modified method was developed based on reports in the literature and laboratory experience to characterize MPs based on physical properties such as size and colour. This method is described below.

Samples were run through 4.75-mm and 330-µm stacked sieves. The 0.330–4.75 mm fraction was stored in glass beakers in a drying oven at 75°C. Organic material (OM) was then degraded through wet peroxide oxidation (0.05 mol/L Fe (II) and 30% hydrogen peroxide) at ~75°C. Plastic resists wet peroxide oxidation, while OM is degraded (Eriksen et al. 2013, Tagg et al. 2015). Sodium chloride (final concentration = 6 mol/L) was then added for a salinity-based density separation. MPs are expected to float at the surface, and heavier material drained from the sample (Baker et al. 2011). Microplastics were filtered (Whatman glass fibre filters, 0.7 µm nominal pore size, filtered area = 5.23 cm^2; Whatman, Piscataway, New Jersey, USA) and counted under a dissecting microscope. Figure 3 presents the sample process flow used. Using the physical characteristics of microplastic particles, we recorded the classification type (i.e., fiber, film, fragment, pellet, foam, pellet, or fragment) for each item (Eriksen

et al. 2013). The samples were dried and sent for chemical characterisation using pyrolysis-gas chromatography mass spectrometry (pyro-GC/MS) at the Norwegian Institute for Water Research (NIVA).

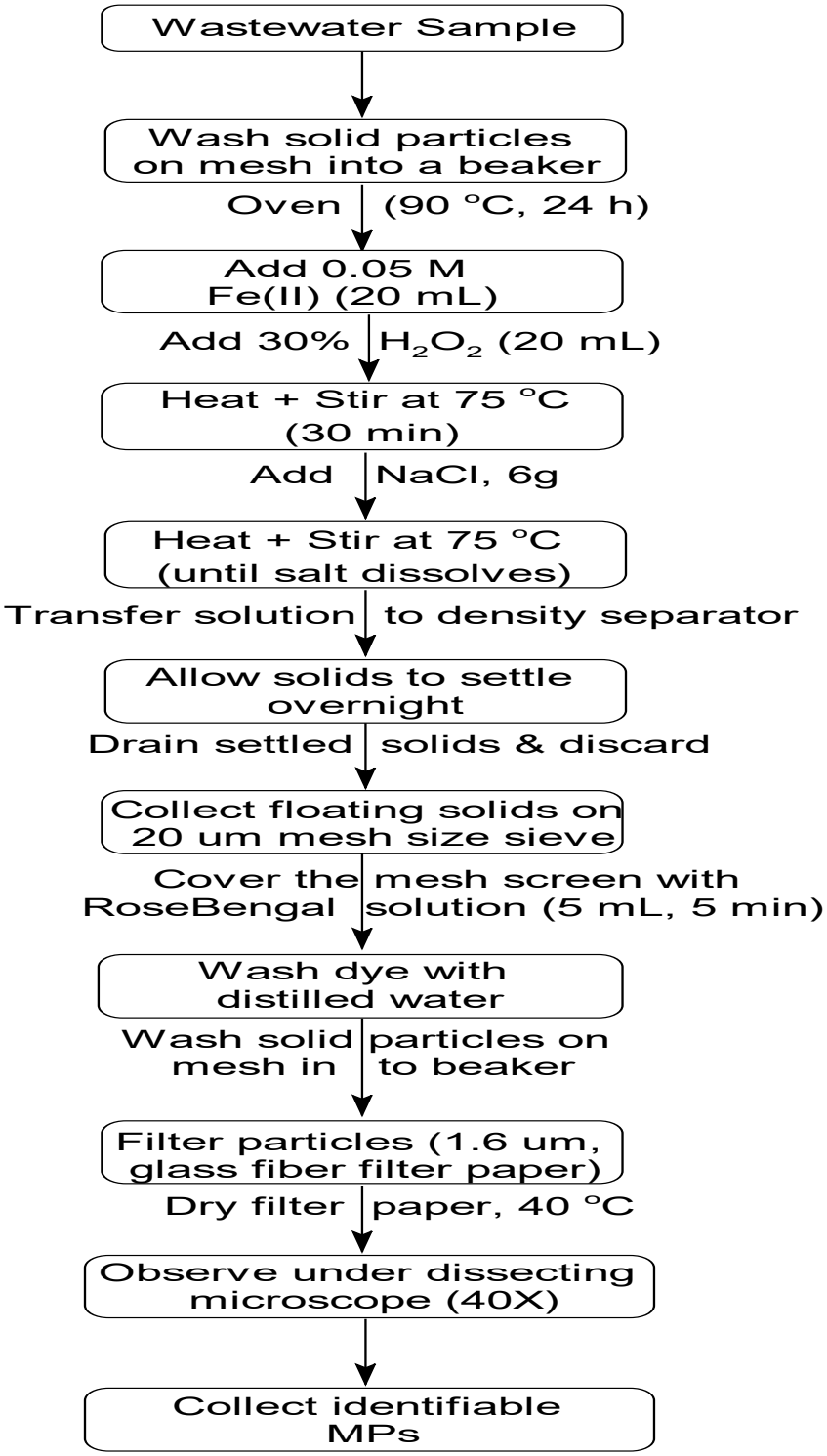


Figure 3: Methodology overview for MPs quantification and characterisation in wastewater samples (Adapted from Masura *et al.*, 2015 and Ziajahromi *et al.*, 2017).

2.2 FACTORS INFLUENCING BIOFILM FORMATION ON MICROPLASTICS

2.2.1 Experimental design

The factors chosen for the experiment to determine the conditions affecting biofilm formation on the selected types of microplastics were exposure time, temperature, light and oxygen. Sterile high-density polyethylene (HDPE), low-density polyethylene (LDPE) and polypropylene (PP) microplastic particles of varying sizes were purchased (Merck (PTY) LTD, South Africa) for this experiment.

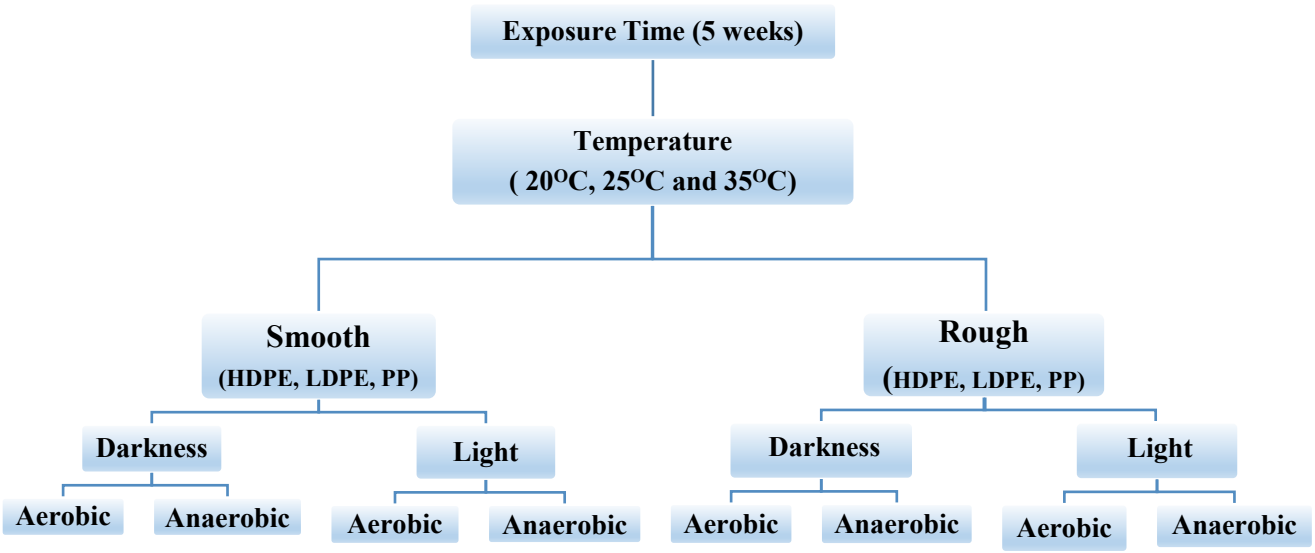


Figure 4: Graphical representation of the experimental conditions used to determine optimum environmental conditions for bacterial attachment.

Untreated wastewater was collected from the Isipingo Wastewater treatment plant (Umlazi, KwaZulu- Natal, South Africa) and then added to 100 mL Erlenmeyer flasks containing 2g of microplastic particles. There were separate setups for each type of microplastic particle. Both rough and smooth setups for each type of microplastic were then subjected to the selected factors. For the first variable, which was exposure time, the experiment was run over a period

of five weeks to determine the optimum time for biofilm formation on the microplastics. These flasks were incubated in shaking incubators (100 rpm) at three different temperatures (20°C, 25°C and 35°C) (Figure 4). The flasks were also exposed to light and dark conditions to determine the potential impact of light exposure on biofilm formation using separate experiments. The dark conditions were achieved by covering the flasks with foil, preventing light from entering, keeping all other parameters like light conditions. In addition, Furthermore, biofilm formation under aerobic and anaerobic conditions was determined. For aerobic conditions, the flasks were covered with perforated gauze. Anaerobic conditions were created by sealed reactors initially sparged with nitrogen gas.

2.2.2 Analysis of biofilm formation

Microplastics from the experimental setups described above were sampled weekly to determine the concentration of biofilm on the plastic particles based on optical density (OD) measurements. This was done by rinsing the microplastics with sterile water and placing them onto nutrient broth. The nutrient broth containing the microplastic particles was vigorously shaken both manually and using a vortex for ~1 min to remove the attached biofilms. The nutrient broth was then incubated for 24 h at 37 °C. The absorbance of the nutrient broth cultures was measured at 660 nm wavelength using the Spectroquant Pharo 300 Spectrophotometer. Nutrient (nitrate, nitrite, and ammonia) concentration in the wastewater used in the flasks was measured at week 1, week 3 and week 5 using the Thermo Scientific Discrete Gallery Analyser.

2.3 CONFIRMATION OF BIOFILM FORMATION VIA SCANNING ELECTRON MICROSCOPY

Before the studies on factors that could potentially facilitate microbial association with MPs in wastewater, in the form of biofilms, the actual association was confirmed both in-situ and in

the laboratory. To confirm the attachment of bacteria to microplastics, scanning electron microscopy (SEM) analysis was performed. The in-situ analysis involved recovery of MPs from WWTPs using a revised methodology (Appendix I). This involved filtering the sampled wastewater through a 0.18mm sieve, density separation with NaCl and a final filtration with 0.45 μm filter paper. The recovered MP particles were washed with sterile distilled water and fixed in 2.5% glutaraldehyde for approximately 8 hours in a fridge. The fixed samples were then rinsed with 0.1 M sodium phosphate buffer (pH 7.2) and dehydrated using an alcohol series consisting of 30%, 50%, 70%, 90%, 95% and 100% alcohol (10 min each). To minimise the distortion of the microplastics before the SEM analysis, critical point drying (CPD) was used. Plastic particles were then mounted on a stub covered with carbon glue and coated with gold palladium. The stub was then placed in the SEM, and images were recorded and stored. Confirmation of microbial association with MPs under laboratory settings followed a similar approach.

2.4 EVALUATION OF THE IMPACT OF CHLORINATION AND UV EXPOSURE/TREATMENT ON MICROPLASTIC-BOUND MICROORGANISMS

Two tertiary treatment options were tested in this study: chlorination and ultraviolet treatment.

2.4.1 Chlorination

The flasks containing the microplastic particles and wastewater were exposed to a sodium hypochlorite concentration of 5 mg/L for 10-, 20-, 30- and 60-min contact time at a CT value of 150 mg min/L (WWTP conditions) (USEPA, 1999). Chlorination was terminated by the addition of a sodium thiosulfate solution (1.5%) (Zheng et al, 2017). Microplastic particles and wastewater samples were collected for subsequent analysis.

2.4.2 UV treatment

The microplastic particles and wastewater from the flasks were transferred into Petri dishes. UVC light emitting 254 nm was applied at a 10cm distance between the light and the petri dishes. The exposure was done for 10, 15, 30 and 60 mins (Lin et al, 2020). Microplastic particles and wastewater samples were collected at each time interval.

2.4.3 Heterotrophic plate count (HPC)

HPC was done in accordance with standard methods of water and wastewater tests (APHA, 2011) to determine the effect of UV and chlorine on biofilms. Ten MPs after biofilm formation were collected and added to an Eppendorf tube with 2 mL of PBS. The tubes were shaken vigorously and then vortexed (~1 min) to ensure maximum removal of the biofilms attached to the microplastics. Approximately 0.2 mL of biofilm suspension was spread onto m-HPC agar and incubated at 35°C for 48 ± 3 h. Colonies were counted, and CFU/MP was calculated using the following equation:

$$\frac{CFU}{MP} = \frac{number\ of\ colonies\ \times 2\ ml\ (volume\ of\ PBS)}{10} \quad [1]$$

2.6 STATISTICAL ANALYSIS

All data were captured in Excel (Microsoft Corp, USA) and diamond plots were used to present the distribution of the data obtained for biofilm concentrations under various conditions. The t-test was applied to demonstrate the significance between two data categories, such as MP concentrations in effluents and influents, Light and Dark conditions, anaerobic and aerobic conditions; Rough and Smooth microplastics during the biofilm formation assessment.

ANOVA was used to demonstrate the significance between the different types of MPs detected, the three different temperatures (20°C, 25°C and 35°C) for biofilm formation assessment; and between the three types of microplastics (HDPE, LDPE and PP). The Pearson correlation coefficient (r) was used to determine the relationship between biofilm concentration and nutrient concentration.

CHAPTER 3: RESULTS AND DISCUSSION

3.0 RESULTS AND DISCUSSION STRUCTURE

This chapter is also presented in a similar format to the methodology section. The results and discussion are presented in three stages.

1. Characterisation of microplastics: Presents results of both the physical (shape) and chemical characterisation (Pyro-GC/MS results).
2. Concentration of microplastics in wastewater and removal efficiency during wastewater treatment.
3. Factors affecting biofilm association with microplastics in wastewater
4. Impact of chlorination and UV treatment on MNP-bound microorganisms/biofilms.

These results are presented and discussed in detail below.

3.1 CLASSIFICATION BASED ON SHAPE

Microplastics (MPs) based on shape varied greatly in the wastewater analysed; this indicates the difference in sources of these MPs. Based on the classification of MPs used in this study, Figures 5-8 present some of the common MPs detected. Figure 5 presents examples of microfibers; these are mainly found in fabrics and could therefore be the main source of these in the wastewater samples (Browne, 2015; Napper and Thompson, 2016). Microfragments were also found; these could be from bottles or containers made of plastics. Their presence in the wastewater samples could be because of degradation of these bottles or containers. In addition to the above-mentioned MPs, microfilms were also found in large quantities in the wastewater samples (Figure 7). These microfilms are usually found in packaging materials; therefore, the influence of these potential sources on the MP concentration in the wastewater

is confirmed. Furthermore, microbeads were also found (See Figure 8), although these were in very low concentrations. The presence of these different types of microplastics could be due to their use for different purposes by the connected communities. For instance, microbeads have been reported to originate from cosmetic products (Horton et al., 2017). These types of microplastics represent the most common classification of MPs based on the shape (Feldberg, 2018).

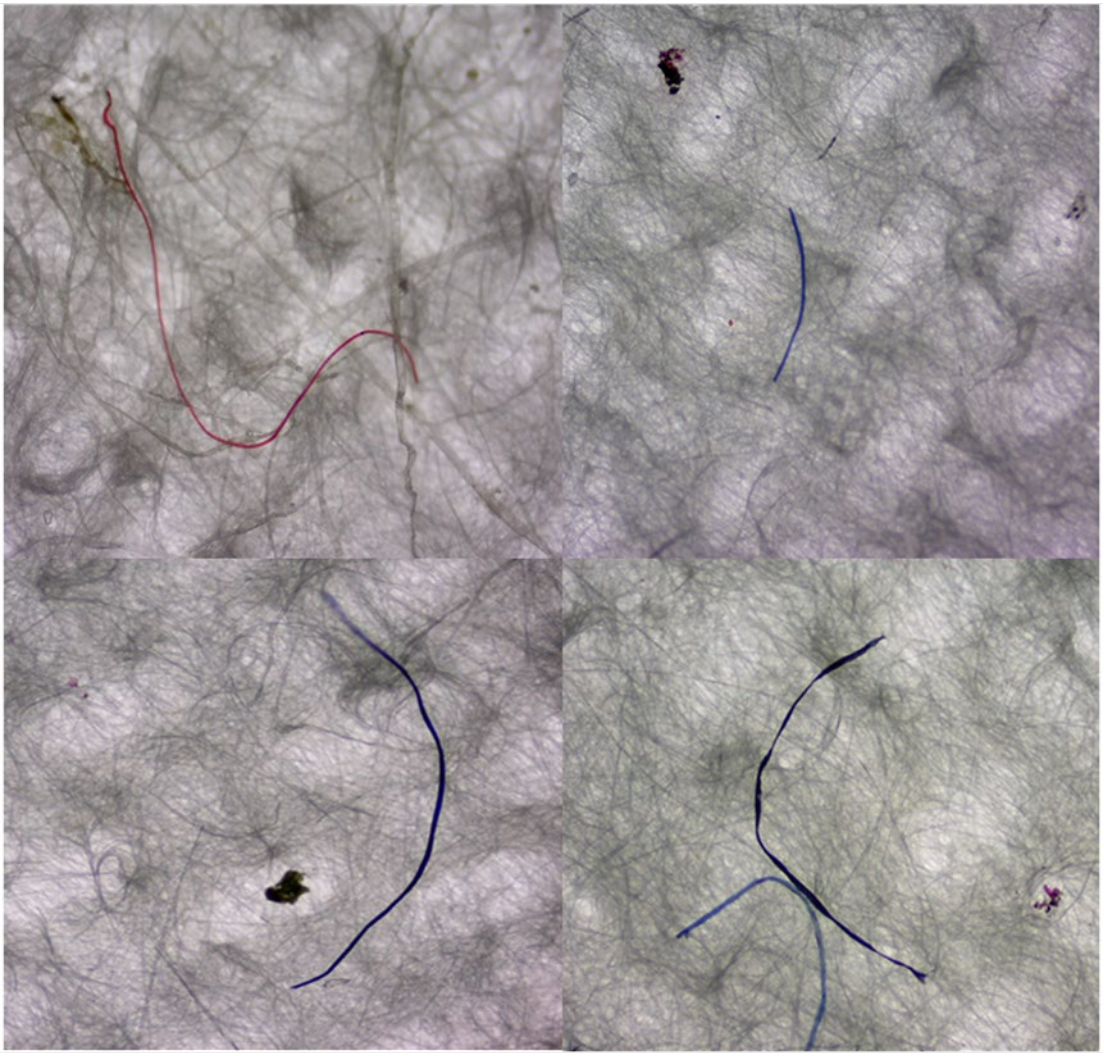


Figure 5: Examples of microfibers found in the wastewater samples

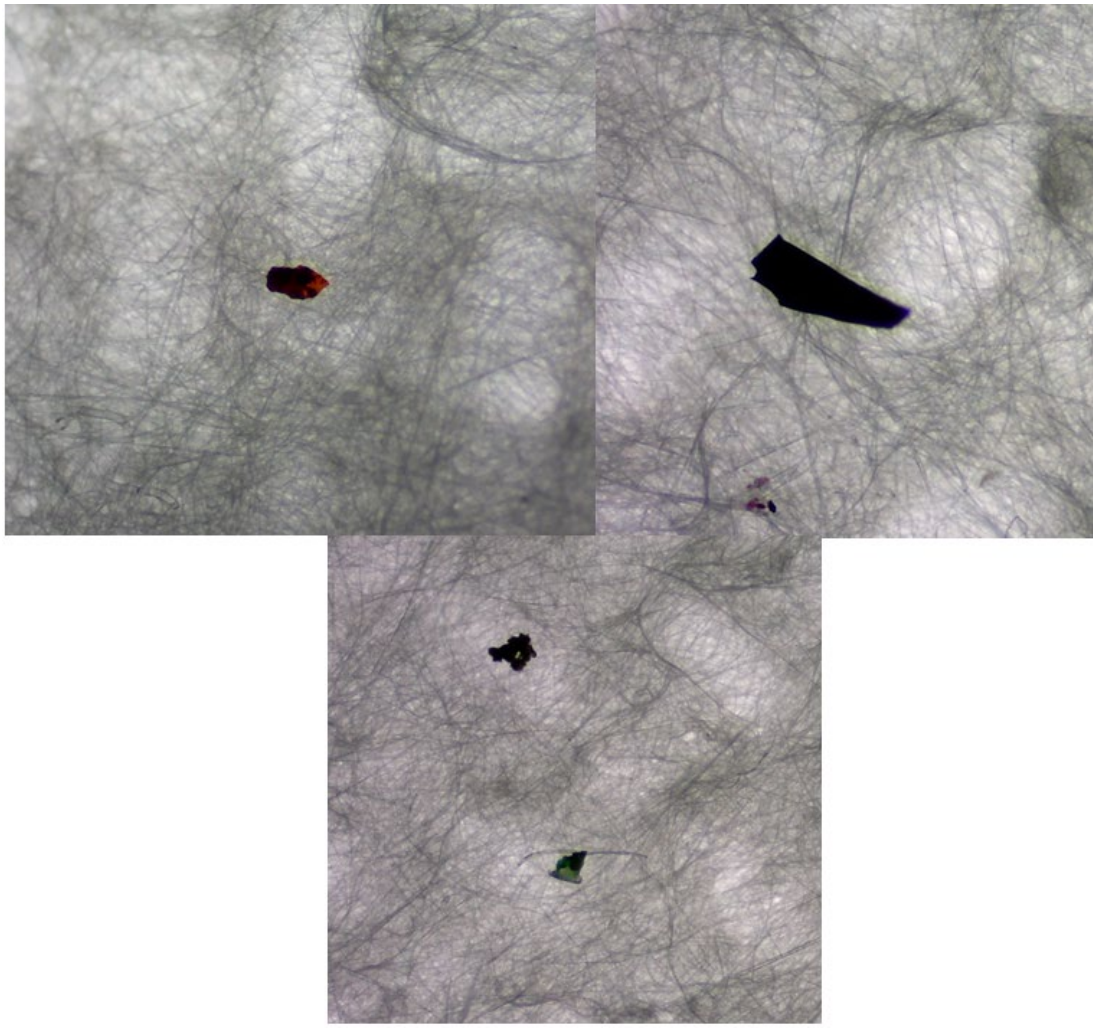


Figure 6: Examples of microfragments detected in the wastewater samples

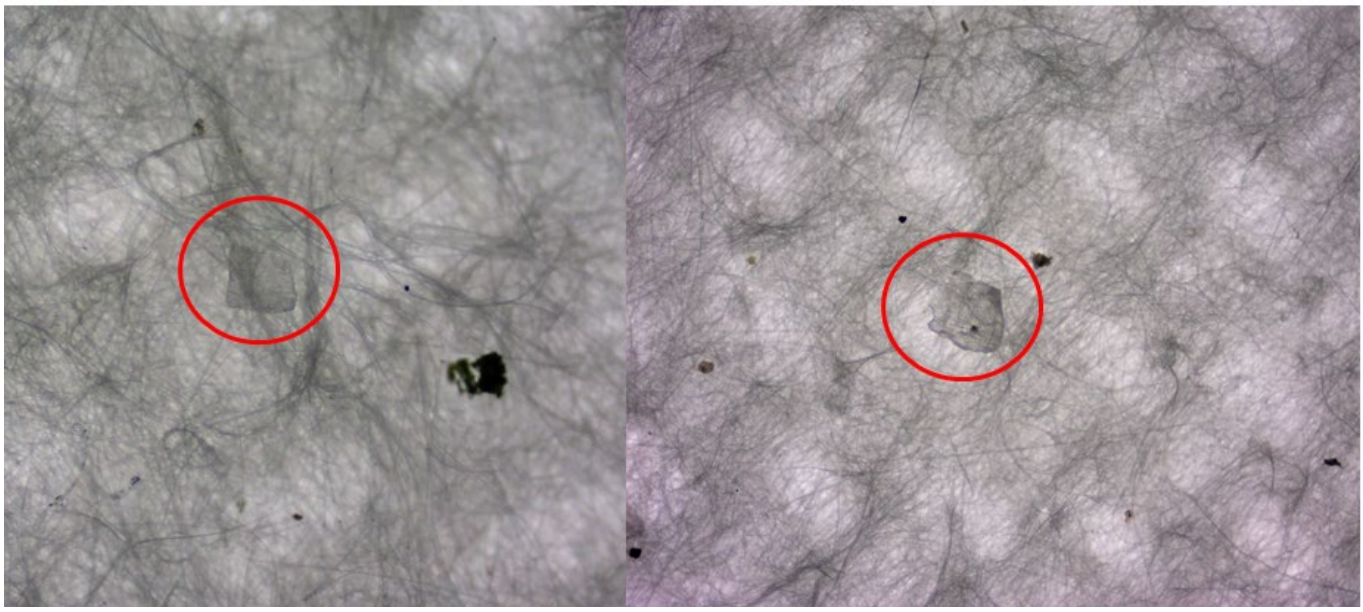


Figure 7: Examples of microfilms (circled in red) found in the wastewater samples

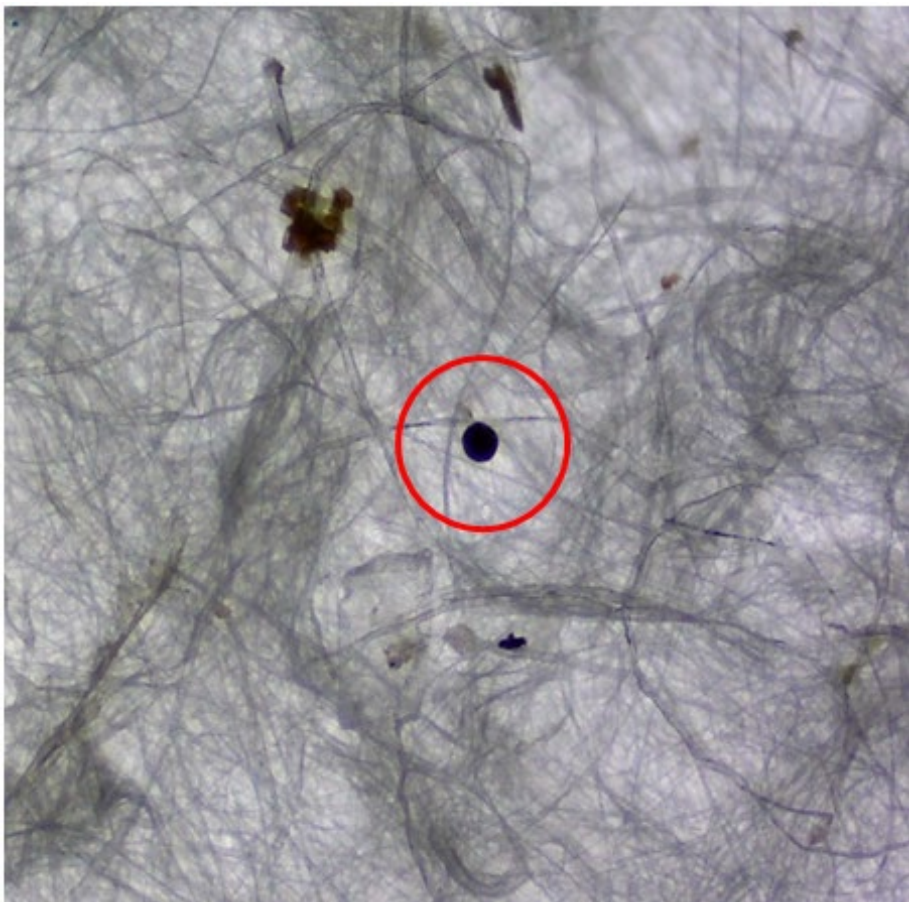


Figure 8: Examples of a microbead detected in the wastewater samples.

3.2 CHEMICAL IDENTIFICATION OF THE MICROPLASTICS

The Pyro-GC/MS analysis identified five main types of polymers in the microplastics recovered in the wastewater. These were polyvinyl chloride (PVC), polyethylene or polythene (PE), polypropylene (PP), polystyrene (PS) and polyamide 6 (PA6), also referred to as Nylon 6 or polycaprolactam.

The most common among polymers were PVC, PE and PA6, with concentrations in the untreated wastewater of 5.6 µg/mg, 3.6 µg/mg and 3.3 µg/mg, respectively. However, in the treated wastewater, the concentrations were reduced significantly for all types of polymers. However, the concentration of PS in the treated wastewater was significantly higher (0.86 µg/mg) compared to the untreated wastewater (0.07 µg/mg) (Figure 9). These results indicate a reduction in the various polymer types except for PS.

Lares et al. (2018) reported polyester and polyethylene as the most common plastic polymers in wastewater, making up to 79.1% of all polymers detected. The common occurrence of polyamide (nylon) fibres was also reported, accounting for 3.7% of all MPs recovered. Comparatively, this current study reported the presence of PVC in the wastewater, in contrast to the data obtained by Lares et al. (2018). This could be attributed to the difference in the study location and potential difference in the extent of use of these MPs or plastics that degrade into these MPs. It is, therefore, that studies on the common MPs detected in wastewater within South Africa could provide further insight into this. It is also worth mentioning that the Lares et al. (2018) study used FITR as the analytical tool to identify the polymer. However, this study used pyro-GC/MS. For instance, Primpke et al. (2020) observed that using FTIR failed to detect PVC from various samples such as wastewater, marine surface water and sediments.

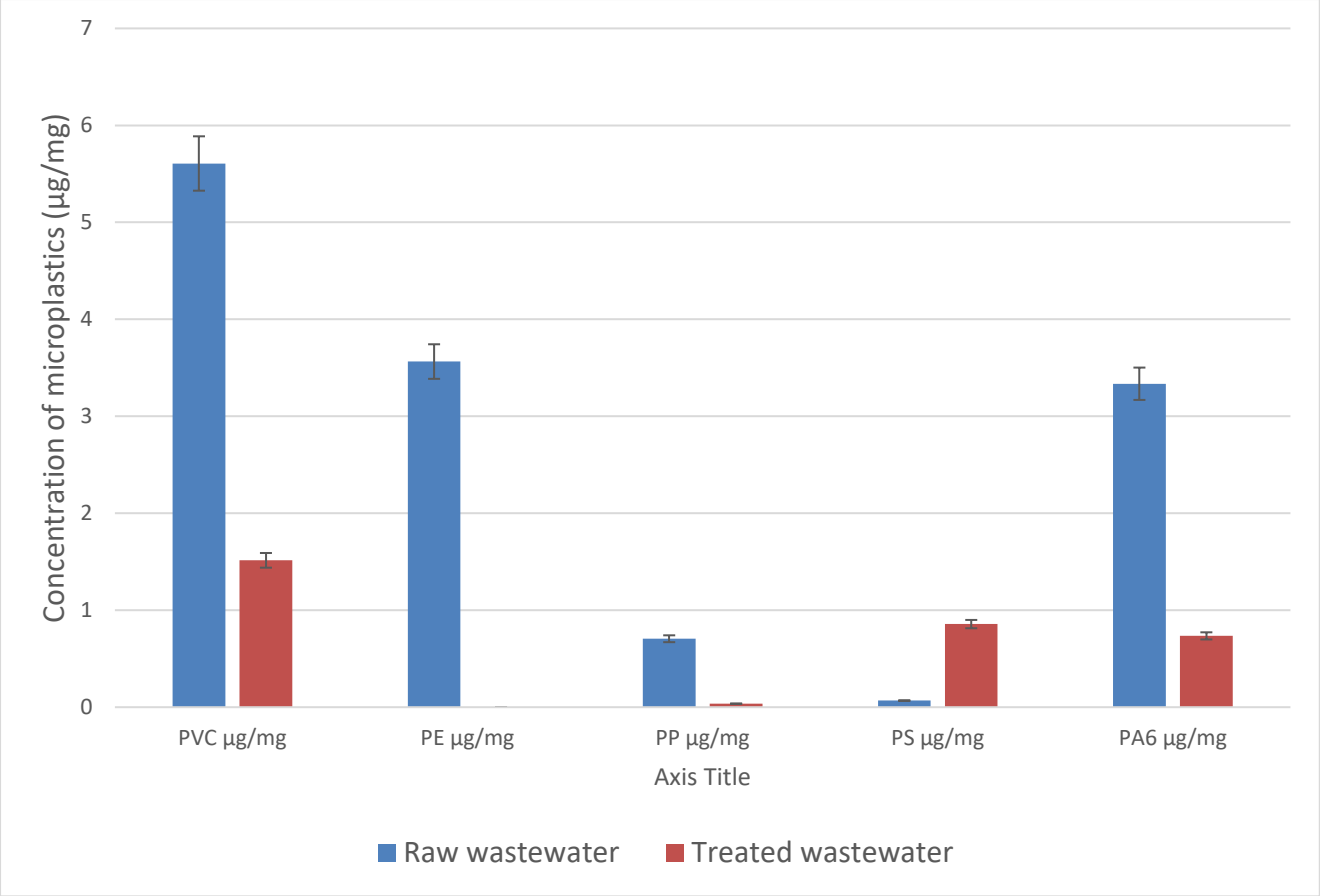


Figure 9: Concentration of major plastic polymers in the recovered microplastics

3.3 CONCENTRATION AND REMOVAL OF MICROPLASTICS IN WASTEWATER

3.3.1 Microplastic occurrence and concentration in untreated wastewater

Varying concentrations of MPs were recorded in the wastewater, either treated or untreated. Irrespective of the type of wastewater (treated or untreated), the most abundant MP type, based on the physical characteristic (shape), was microfragments. For instance, in the untreated wastewater, the mean concentration of microfragments was 5712 (±3064) particles/L of wastewater. The least abundant was the fibre microplastics, with a mean concentration of 113.2 (±39.2) particles/L of wastewater.

Horton et al. (2017) reported in their studies plastic flakes and fibres were the two most abundant microplastic types (67.3% and 18.5% respectively), with microbeads only

contributing to 3% of total particles. The difference in the concentration or proportion of the different types of MPs in the referenced study and this study could be attributed to the different sources of wastewater and the possible different plastics in use. The predominance of microfragments in the aquatic environment has been reported in other studies (Wagner et al., 2019; Tagg et al., 2020). This could be attributed to the fragmentation of macroplastics into smaller microplastics.

Furthermore, the size of the various MPs detected in the untreated wastewater had varying concentrations. As shown in Table 1, the most predominant size was the 500-5000 size range among all the shapes of MPs except the film. Among the film type of MPs, the most abundant size category was the 25-100 (8647 MPs/L). There is no evidence in the literature to support the dominance of a particular size category; different sizes of MPs have been reported in the literature, as shown in the literature reviews by Elkhatib and Oyanedel-Craver (2020) and Zhang and Chen (2020).

3.3.2 Removal of MPs during wastewater treatment

In the treated wastewater, although the microfragments were still the most abundant, the least abundant in this case were the bead MPs, with mean concentrations of 32.8 (±12.1) particles/L of wastewater.

In relation to the efficiency of the WWTPs in removing these MPs, varying removal percentages were observed. For instance, for microfragments in the > 5000 size category an increase in concentrations in the final effluents was observed (Figure 10). However, in the smaller sizes up to 91% removal was observed for the 500-5000 size range and a low of approximately 42% in the 25-100 size range.

It must be noted that irrespective of the size range, the concentrations of fibre in the treated wastewater were observed to be consistently higher than the untreated wastewater (Figure 10).

However, as the sizes reduced, this increase in concentrations was lower. The only type of MPs that recorded a consistent increase in removal from the big sizes to the small sizes was the films. For instance, in the > 5000 size category a four-fold increase in the effluent was observed (-233 % removal), but in the 25-100 size range approximately 98% removal was observed. This indicates differential removal of these MPs during wastewater treatment. This differential removal could be attributed to the type of MP. For instance, Carr et al. (2016) reported complete removal of MPs in the size range of 45–400 μm. Murphy et al. (2016) similarly found that microplastics were significantly reduced in effluent following a secondary treatment process. In the current study, removal of the MPs was categorized by type (microfragments, microbeads, etc.) and size; this could be the reason why this study observed the differential removal. For instance, the highest removal was observed for the microbeads, film and to some extent the microfragments (Figure 10). However, there was no removal observed for the fibres irrespective of the size range.

Table 1: Application of the modified method in determining the concentration of microplastics in the wastewater categorised by size and colour.

	Untreated wastewater				Treated wastewater (effluent)			
Size range	**Fragment**	**Fibre**	**Bead**	**Film**	**Fragment**	**Fibre**	**Bead**	**Film**
> 5000	55	16	13	12	316	384	20	40
500 – 5000	16520	243	482	3899	1440	1392	80	208
180 – 500	1810	54	62	435	368	484	24	20
100 – 180	1697	109	35	262	372	372	12	8
25 - 100	8480	144	176	8647	4920	568	28	208

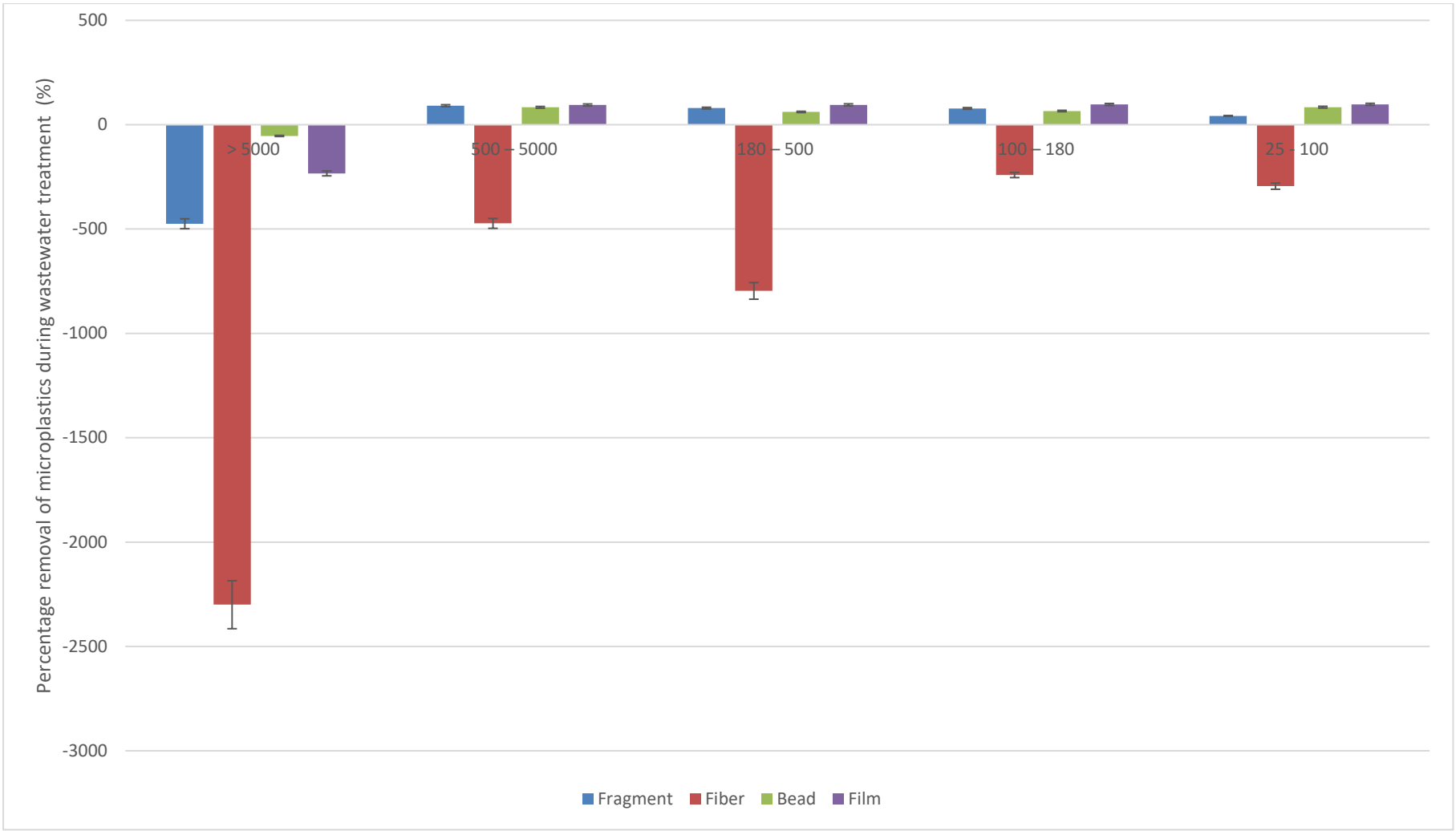


Figure 10: Removal of microplastics during wastewater treatment (%).

3.4 FACTORS AFFECTING BIOFILM ASSOCIATION WITH MICROPLASTICS IN WASTEWATER

SEM analysis confirmed the association of microorganisms with MPs, both in-situ and in laboratory experiments. As shown in Figure S2 (Appendix), SEM analysis showed attachment of extracellular polymeric substances (EPS) on the MP surface isolated from both the treated and untreated wastewater. However, in the MPs exposed to wastewater in the laboratory, the SEM analysis showed clear bacterial attachment to the MPs (Figure S4 and S5). The observed microbial attachment to the MPs in the laboratory experiments, compared to the MPs isolated from the WWTPs, could be due to the fact that the exposure time in the laboratory experiments was long enough for biofilm formation.

3.4.1 Biofilm formation and the usage of nutrients in the wastewater

Biofilm formation was observed in all experiments irrespective of the conditions; however, a difference in the amount of biofilm was evident. An increase in biofilm formation on both microplastics was observed from week one to week five, with the highest optical density of 1.77 observed in 3 weeks. Thereafter, a decrease in optical density to 1.1 in week 5 was observed, indicating a decline in biofilm attachment.

Nutrient concentration in the wastewater showed a decrease in the concentration of ammonia (NH_4) from 33,45 mg/L in week 1 to 7,95 mg/L in week 5, while nitrate (NO_3) concentrations increased from 0 mg/L in week 1 to 40,65 mg/l in week 5. The concentration of nitrite (NO_2) increased from 0.01 mg/L in week 1 to 0,74 mg/L in week 3 and thereafter decreased to 0 mg/L in week 5 (Figure 11). When compared statistically, the correlation analysis showed a positive linear relationship between biofilm concentration and NO_2 ($r = 0.824$) and NH_4 ($r = 0.1$). Whereas for biofilm concentration and NO_3, the correlation analysis showed a negative linear relationship ($r = -0.673$). Biofilm formation is a highly regulated process and occurs over a period, as shown in this study and

each step is influenced by various environmental factors (Huang et al, 2019). Nutrient availability is one such important factor. Biofilms are more abundant in nutrient-rich environments. Biofilm growth is influenced by inorganic nutrients such as nitrogen as well as organic matter present in wastewater (Liu et al., 2016). These nutrient-rich environments promote the transition of bacterial cells from planktonic form to biofilm state, while the decrease or depletion in nutrient concentrations results in the detachment of biofilms from surfaces (Sehar and Naz, 2016). The nutrients required for biofilm formation include carbon, nitrogen sources and phosphorus sources (He et al., 2021). Nitrogen is a key nutrient in biofilm development; it is a building block for proteins and other genetic materials (DNA and RNA). Autotrophic nitrifying bacteria or nitrifiers are a major class of biofilm-forming microorganisms (Liu et al., 2016). Wastewater contains large amounts of organic waste; therefore contains a high ammonia concentration that can be toxic to aquatic life. Hence, nitrogen removal from wastewater is required. The nitrification process, during which ammonia (NH_4) is converted to nitrite (NO_2) then to nitrate (NO_3) by nitrifying bacteria, is an essential process in wastewater treatment (Muhammad et al., 2020).

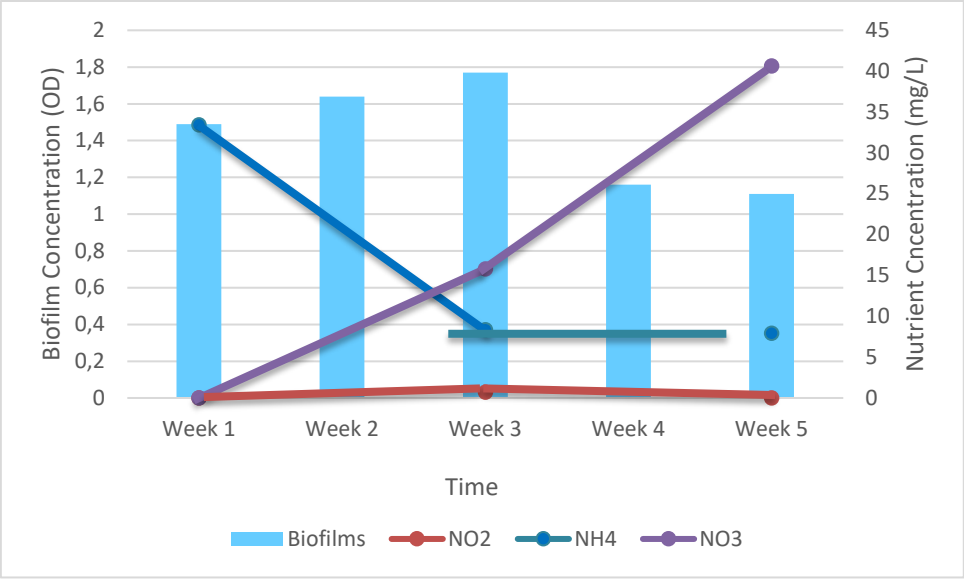


Figure 11: The amount of biofilm formed on microplastic particles and nutrient usage during a period of 5 weeks

3.4.2 The difference in biofilm formation based on the different types of microplastics

The median biofilm values on HDPE and PP remain in a similar range from week 1 to week 5. In contrast, the median biofilm values for LDPE varied during each week compared to HDPE and PP. As presented in Figure 12 (a), the highest median biofilm concentration of 1.8 was observed for LDPE and HDPE in Week 3, and a slightly lower concentration for PP (median of 1.75). There was an observed difference in the biofilm concentration for the three temperatures studied (20, 25 and 35 °C). At 20°C, the highest median biofilm concentration of 1.65 was observed for HDPE. However, at 25°C and 35°C, the highest median biofilm concentrations were observed for the LDPE microplastics, with values of 1.7 and 1.65, respectively. The difference in biofilm concentration on the different types of microplastics at different temperatures was not statistically significant (p-value ≥ 0.05). This shows that the temperature ranges studied had no significant influence on the biofilm formation of these microplastics. The metabolism of bacteria is closely associated with the presence of enzymes (Achinas et al., 2019). Biofilm formation depends on the enzyme reaction rate, which is directly affected by temperature. Studies have also shown that bacteria have a greater surface area at low temperatures than at higher temperatures (Govaert et al., 2018). A study done by Townsley and Yildiz, (2015) reported that biofilm formation by *Vibrio cholerae* at 15 °C and 25 °C showed greater thickness and better structure when compared to biofilms formed at 37 °C. This is in relation to the number of bacterial appendages. The number of flagella from bacteria is temperature-dependent. When the number of flagella increases, the surface area of bacteria increases and the chance of bacterial adhesion (Townsley and Yildiz., 2015). These reports corroborate the findings in this study, because the highest median biofilm value of 1.7 was observed for 25 °C (Figure 12(d)). The temperature with the highest biofilm formation indicates a close relation with environmental conditions, which is mostly 25 °C.

Similar observations were made when assessing the biofilm concentrations on microplastics subjected to light and dark conditions. For instance, as shown in Figure 12 (c), the highest median biofilm concentrations of 1.7 and 1.6 were recorded on the LDPE microplastic subjected to light and

dark conditions, respectively. Light is regarded as an important variable that directly drives the composition of a microbial community (Piwosz et al., 2020). The overall biofilm system is shaped by the complex interactions between autotrophic and heterotrophic microbes (Schmidt et al., 2018). This was also observed for aerobic and anaerobic conditions (Figure 12 (d)) and for smooth and rough microplastics (Figure 12 (e)). Interestingly, polyethylene (HDPE and LDPE) microplastics showed a higher biofilm concentration formation than polypropylene (PP) microplastics under various conditions considered in this study.

Anaerobic and aerobic conditions are known to affect the morphological changes of biofilms, which may alter the diffusibility of substances and enable metabolic adaptation by maximising the exchange of nutrients and waste products (Toyofuku et al., 2016). Figure 12 (c) shows that the highest median value of 1.6 was observed under aerobic conditions. This indicated a higher biofilm formation under aerobic conditions than under anaerobic conditions. During the transport of microplastic particles through the environment, the attached biofilm-forming organism must survive various stressful environmental conditions, particularly high oxygen levels (Reuter et al, 2010). Low oxygen conditions increase biofilm formation, whereas normal oxygen levels may decrease biofilm formation (Totani et al., 2017). From this study, we observed better biofilm formation under aerobic conditions, suggesting that anaerobic conditions may reduce the number of biofilms that formed on the microplastic particles. However, the influence of oxygen varies among different species of biofilm-forming microorganisms (Alvarez-Ordóñez et al., 2019); therefore, further studies on the microbial community attached to these microplastics are needed. The microbial community profiling will provide critical answers for further understanding of the role of aerobic and anaerobic conditions on biofilm formation.

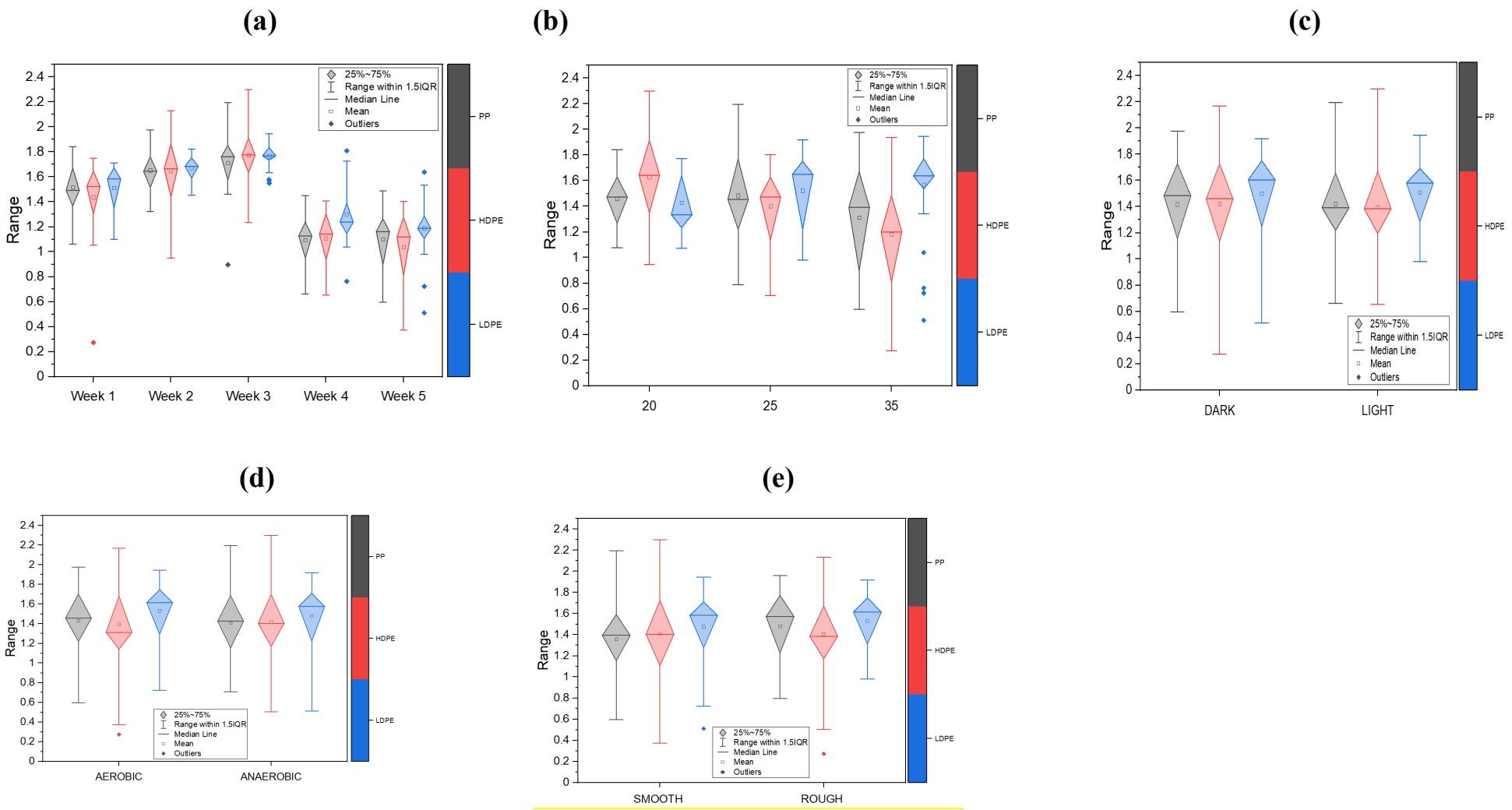


Figure 12: Diamond box plots showing the difference in biofilm formation on the different microplastics at the different experimental conditions. (a) Impact of duration of exposure on biofilm formation (b) Impact of temperature on biofilm formation (c) Impact of dark or light conditions on biofilm formation (d) Impact of aerobic and anaerobic conditions on biofilm formation (e) The difference in biofilm formation on smooth and rough microplastic particles.

3.5 IMPACT OF TERTIARY TREATMENT ON MICROPLASTIC-BOUND MICROORGANISMS

The impact of UV and chlorination on the attached microorganisms (biofilms) varied depending on the treatment. Prior to the tertiary treatment, total heterotrophic concentration of up to 15000 cfu/MP was measured. However, after exposure to UV treatment, the colony counts reduced to 1 cfu/MP after 30 minutes (Figure 13). Further exposure beyond 30 minutes resulted in complete inactivation of all attached microorganisms. With respect to chlorination, no colony growth was observed from 10 to 60 min of treatment. These results show that bacterial inactivation was higher under chlorine treatment than under UV treatment. Some of the agar plates showing colony growth, etc., are shown in Figure S6 (Appendix)

Biofilms survive tertiary treatment mainly through the development of thick matrices, and UV radiation can only penetrate through the top layer of microbial cells (de Carvalho, 2017). The treatment efficiency using UV radiation is also influenced by the age of the biofilm, together with the increased thickness and EPS in biofilms (Argyraki et al., 2017; Luo et al., 2022). Nevertheless, there have been several studies that have reported the efficacy of UV radiation to inactivate microbial cells in biofilms. In this study, we focused on determining the impact of UV-C light and Chlorine on biofilms at different exposure times. Chlorination is one of the most common treatments used in wastewater treatment because of its persistent germicidal ability. The inactivation efficiency of chlorine for biofilms depends on its activation strength and diffusibility (Shen et al., 2017). Chlorine decreases biofilm hydrophobicity and adhesion by reacting with proteins and polysaccharides (Luo et al., 2022). A study done by Buse et al. (2019) reported that *L. pneumophila* biofilm was inactivated on PVC surfaces using free chlorine after 30 min of exposure (Buse et al., 2019). In this study, no bacterial growth was observed from biofilms and wastewater after 10 min of exposure to chlorine.

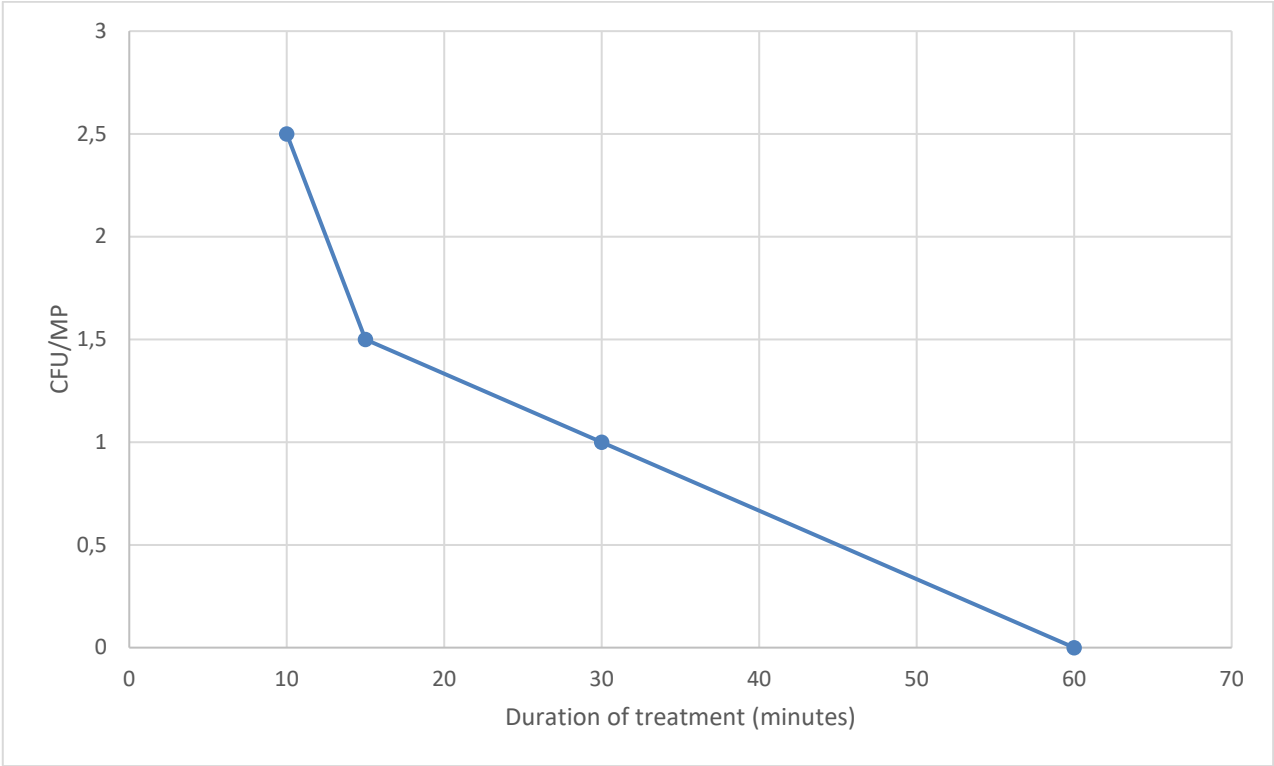


Figure 13: Colony forming units per microplastic particle before (0 minutes) and after UV treatment of biofilms formed on MPs

CHAPTER 4: CONCLUSIONS & RECOMMENDATIONS

4.1 CONCLUSIONS

Microplastics in wastewater could be from different sources, which can be deduced from the type of plastic. This study concluded that four main types of microplastics, based on shape, are present in wastewater within Durban: microfragments, beads, film, and fibre. The microfragments, usually formed from the degradation (fragmentation) of macroplastics, were the most common type of microplastics. Furthermore, the study highlighted the size differentiation in these microplastics, with the 500-5000 µm size range being the most common. Chemically, the most common polymer of plastic among these microplastics was polyvinyl chloride (PVC). This was found to be the most abundant, which could be attributed to the use of this material for several purposes, such as pipes. This also corroborates the dominance of the microfragments, since the degradation of PVC macroplastics results in the formation of microfragments.

It is worth noting that removal of these microplastics differed considerably depending on the shape of the microfragments (beads, film or fibre) and size (>5000 µm, 500-5000 µm, 180-500 µm, 100-180 µm, and 25-100 µm) of the microplastics. Microfragments in the >5000 µm did not show a reduction through wastewater treatment, attributable to potential reintroduction of these large particles into the maturation ponds of the wastewater treatment plants by wind. Removal of up 91% was observed for smaller sizes such as the 500-5000 µm size range.

This study also concluded that maximum biofilm formation occurs after three weeks; thereafter, a reduction in biofilm. The surrounding environmental conditions were found to play a major role in the growth and development of the biofilms. Conditions such as temperature (optimum of 25 °C), darkness, and aerobic conditions. This indicates that these could be the preferred conditions for biofilm formation in the wastewater treatment plants. Nutrient availability also affected biofilm development during the five weeks. The conversion of ammonia to nitrite and nitrate was observed

at the different stages of biofilm development, suggesting that nitrogen in the form of ammonia is one of the key components in biofilm development. This study also revealed that the physicochemical properties of the microplastic particles affect the concentration of biofilms that attach to the microplastic particles. The concentration of biofilm formed on both polyethylene (HDPE and LDPE) microplastics was higher than the biofilm concentration on polypropylene microplastics. Surface texture of the microplastic particles also affected biofilm formation. Rough microplastics had a higher concentration of biofilm attachment than smooth microplastics. Biofilms are known to shield microorganisms from tertiary treatment. In this study, it was observed that both UV and chlorine treatment were able to inactivate attached biofilms. Chlorine treatment required a 10 min contact time, whereas UV treatment was able to inactivate biofilms after 30 mins of exposure. Despite the insignificant difference between the various conditions and the biofilm concentration, it was observed that the conditions studied did have an influence on the biofilm formation on the microplastics. This suggests that, together with the conditions studied, there could be other contributing factors that may affect biofilm development that need to be studied further. In addition, the studied conditions may not only affect biofilm development but also the microbial composition of the biofilms.

4.2 RECOMMENDATIONS

Based on the results obtained, the following recommendations are made.

1. Further method development: Although the project initially included the detection of nanoplastics, recovering these plastics from the wastewater was a major challenge due to their smaller sizes. Therefore, to further describe in full the impact of micro- and nanoplastics in the environment, most especially water/wastewater, there is a need for simpler techniques to recover these nanoplastics.
2. Capacity development: Chemical characterisation of microplastics posed a serious challenge during the study due to two main reasons: lack of equipment and expertise. It is therefore

suggested that provision of the infrastructure for testing (analytical instruments) and training of personnel is very important for future work on this important area.

3. Determination of different wastewater treatment configurations on microplastic removal: The study established that removal of the microplastics during wastewater treatment plants differed between the different sizes of plastics, the shape, and by polymer type. This could be due to differences in efficiency based on these characteristics. However, this study did not focus on the removal achieved at each stage of wastewater, it is suggested that future studies in the area include an evaluation of each wastewater step to ascertain the removal of microplastics achieved at each stage.
4. Factors affecting biofilm formation: The factors considered in this study may not be the only factors with an effect on biofilm formation. Therefore, future studies should include the expansion of these factors.
5. Microbial community profiling: Biofilms are known to form a unique microbial community, most especially under different conditions and on different types of microplastics. Therefore, future studies can include a microbial community profile of the biofilms to provide information on the common types of microbes associated with the microplastics under these conditions.

REFERENCES

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E. and Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the total environment*, *586*, pp.127-141.

Feldberg, B., 2018. Macro implications of microplastics: a comparative study of microplastic distribution in Bahía Almirante, Bocas del Toro, Panama.

Primpke, S., Fischer, M., Lorenz, C., Gerdts, G. and Scholz-Böttcher, B.M., 2020. Comparison of pyrolysis gas chromatography/mass spectrometry and hyperspectral FTIR imaging spectroscopy for the analysis of microplastics. *Analytical and bioanalytical chemistry*, *412*(30), pp.8283-8298.

Wagner, S., Klöckner, P., Stier, B., Römer, M., Seiwert, B., Reemtsma, T. and Schmidt, C., 2019. Relationship between discharge and river plastic concentrations in a rural and an urban catchment. *Environmental science & technology*, *53*(17), pp.10082-10091.

Tagg, A.S., Sapp, M., Harrison, J.P., Sinclair, C.J., Bradley, E., Ju-Nam, Y. and Ojeda, J.J., 2020. Microplastic monitoring at different stages in a wastewater treatment plant using reflectance micro-FTIR imaging. *Frontiers in Environmental Science*, p.145.

Elkhatib, D. and Oyanedel-Craver, V., 2020. A critical review of extraction and identification methods of microplastics in wastewater and drinking water. *Environmental Science & Technology*, *54*(12), pp.7037-7049.

Browne, M. A., Bergmann, M., Gutow, L., & Klages, M. (2015). Marine anthropogenic litter. *Marine Anthropogenic Litter*, 229-244.

Napper, I.E. and Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Marine pollution bulletin*, *112*(1-2), pp.39-45.

Zhang, Z. and Chen, Y., 2020. Effects of microplastics on wastewater and sewage sludge treatment and their removal: A review. *Chemical Engineering Journal*, *382*, p.122955.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D. and Schmitt-Jansen, M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental science & technology letters*, *4*(7), pp.258-267.

Cole, M., Lindeque, P., Halsband, C. and Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin*, *62*(12), pp.2588-2597.

Sivan, A., 2011. New perspectives in plastic biodegradation. *Current opinion in biotechnology*, *22*(3), pp.422-426.

Carr, S.A., 2017. Sources and dispersive modes of micro-fibers in the environment. *Integrated environmental assessment and management*, *13*(3), pp.466-469.

Harrison, J.P., Boardman, C., O'Callaghan, K., Delort, A.M. and Song, J., 2018. Biodegradability standards for carrier bags and plastic films in aquatic environments: a critical review. *Royal Society open science*, *5*(5), p.171792.

Shen, M., Zhu, Y., Zhang, Y., Zeng, G., Wen, X., Yi, H., Ye, S., Ren, X. and Song, B., 2019. Micro (nano) plastics: unignorable vectors for organisms. *Marine pollution bulletin*, *139*, pp.328-331.

Talvitie, J., Mikola, A., Koistinen, A. and Setälä, O., 2017. Solutions to microplastic pollution–Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. *Water research*, *123*, pp.401-407.

Hammer, J., Kraak, M.H. and Parsons, J.R., 2012. Plastics in the marine environment: the dark side of a modern gift. *Reviews of environmental contamination and toxicology*, pp.1-44.

Ziajahromi, S., Neale, P.A. and Leusch, F.D., 2016. Wastewater treatment plant effluent as a source of microplastics: review of the fate, chemical interactions and potential risks to aquatic organisms. *Water science and technology*, *74*(10), pp.2253-2269.

Ziajahromi, S., Neale, P.A., Rintoul, L. and Leusch, F.D., 2017. Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. *Water research*, *112*, pp.93-99.

Joo, S.H., Liang, Y., Kim, M., Byun, J. and Choi, H., 2021. Microplastics with adsorbed contaminants: Mechanisms and Treatment. *Environmental Challenges*, *3*, p.100042.

Flemming, H.C., Neu, T.R. and Wozniak, D.J., 2007. The EPS matrix: the "house of biofilm cells". *Journal of bacteriology*, *189*(22), pp.7945-7947.

O'toole, G.A., 2003. To build a biofilm. *Journal of bacteriology*, *185*(9), pp.2687-2689.

Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A. and Kjelleberg, S., 2016. Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology*, *14*(9), pp.563-575.

Carreres-Calabuig, J.A., Rogers, K., Gorokhova, E. and Posth, N., 2019. Micro-by-micro interactions: how microorganisms influence the fate of marine microplastics.

Donlan, R.M., 2002. Biofilms: microbial life on surfaces. *Emerging infectious diseases*, *8*(9), p.881.

Masura, J., Baker, J., Foster, G. and Arthur, C., 2015. Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for quantifying synthetic particles in waters and sediments.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H. and Amato, S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Marine pollution bulletin*, *77*(1-2), pp.177-182.

Tagg, A.S., Sapp, M., Harrison, J.P. and Ojeda, J.J., 2015. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging. *Analytical chemistry*, *87*(12), pp.6032-6040.

Browne, M.A., 2015. Sources and pathways of microplastics to habitats. *Marine anthropogenic litter*, pp.229-244.

Napper, I.E. and Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Marine pollution bulletin*, *112*(1-2), pp.39-45.

Lares, M., Ncibi, M.C., Sillanpää, M. and Sillanpää, M., 2018. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water research*, *133*, pp.236-246.

Huang, Y., Zhao, Y., Wang, J., Zhang, M., Jia, W. and Qin, X., 2019. LDPE microplastic films alter microbial community composition and enzymatic activities in soil. *Environmental Pollution*, *254*, p.112983.

Liu, F.F., Liu, G.Z., Zhu, Z.L., Wang, S.C. and Zhao, F.F., 2019. Interactions between microplastics and phthalate esters as affected by microplastics characteristics and solution chemistry. *Chemosphere*, *214*, pp.688-694.

Sehar, S. and Naz, I., 2016. Role of the biofilms in wastewater treatment. *Microbial biofilms-importance and applications*, pp.121-144.

He, X., Zhang, S., Jiang, Y., Li, M., Yuan, J. and Wang, G., 2021. Influence mechanism of filling ratio on solid-phase denitrification with polycaprolactone as biofilm carrier. *Bioresource Technology*, *337*, p.125401.

Muhammad, M.H., Idris, A.L., Fan, X., Guo, Y., Yu, Y., Jin, X., Qiu, J., Guan, X. and Huang, T., 2020. Beyond risk: bacterial biofilms and their regulating approaches. *Frontiers in microbiology*, *11*, p.928.

Achinas, S., Charalampogiannis, N. and Euverink, G.J.W., 2019. A brief recap of microbial adhesion and biofilms. *Applied Sciences*, *9*(14), p.2801.

Govaert, M., Smet, C., Baka, M., Janssens, T. and Impe, J.V., 2018. Influence of incubation conditions on the formation of model biofilms by Listeria monocytogenes and Salmonella Typhimurium on abiotic surfaces. *Journal of Applied Microbiology*, *125*(6), pp.1890-1900.

Townsley, L. and Yildiz, F.H., 2015. Temperature affects c-di-GMP signalling and biofilm formation in V ibrio cholerae. *Environmental microbiology*, *17*(11), pp.4290-4305.

Piwosz, K., Shabarova, T., Pernthaler, J., Posch, T., Šimek, K., Porcal, P. and Salcher, M.M., 2020. Bacterial and eukaryotic small-subunit amplicon data do not provide a quantitative picture of microbial communities, but they are reliable in the context of ecological interpretations. *Msphere*, *5*(2), pp.e00052-20.

Toyofuku, M., Inaba, T., Kiyokawa, T., Obana, N., Yawata, Y. and Nomura, N., 2016. Environmental factors that shape biofilm formation. *Bioscience, biotechnology, and biochemistry*, *80*(1), pp.7-12.

Reuter, M., Mallett, A., Pearson, B.M. and van Vliet, A.H., 2010. Biofilm formation by Campylobacter jejuni is increased under aerobic conditions. *Applied and environmental microbiology*, *76*(7), pp.2122-2128.

Totani, T., Nishiuchi, Y., Tateishi, Y., Yoshida, Y., Kitanaka, H., Niki, M., Kaneko, Y. and Matsumoto, S., 2017. Effects of nutritional and ambient oxygen condition on biofilm formation in Mycobacterium avium subsp. hominissuis via altered glycolipid expression. *Scientific reports*, *7*(1), pp.1-12.

Alvarez-Ordóñez, A., Coughlan, L.M., Briandet, R. and Cotter, P.D., 2019. Biofilms in food processing environments: challenges and opportunities. *Annual Review of Food Science and Technology*, *10*, pp.173-195.

de Carvalho, C.C., 2017. Biofilms: Microbial strategies for surviving UV exposure. *Ultraviolet Light in Human Health, Diseases and Environment*, pp.233-239.

Argyraki, A., Markvart, M., Bjørndal, L., Bjarnsholt, T. and Petersen, P.M., 2017. Inactivation of Pseudomonas aeruginosa biofilm after ultraviolet light-emitting diode treatment: a comparative study between ultraviolet C and ultraviolet B. *Journal of Biomedical Optics*, *22*(6), p.065004.

Luo, X., Zhang, B., Lu, Y., Mei, Y. and Shen, L., 2022. Advances in application of ultraviolet irradiation for biofilm control in water and wastewater infrastructure. *Journal of Hazardous Materials*, *421*, p.126682.

Shen, Y., Huang, C., Lin, J., Wu, W., Ashbolt, N.J., Liu, W.T. and Nguyen, T.H., 2017. Effect of disinfectant exposure on Legionella pneumophila associated with simulated drinking water biofilms: release, inactivation, and infectivity. *Environmental Science & Technology*, *51*(4), pp.2087-2095.

Luo, L.W., Wu, Y.H., Chen, G.Q., Wang, H.B., Wang, Y.H., Tong, X., Bai, Y., Xu, Y.Q., Zhang, Z.W., Ikuno, N. and Hu, H.Y., 2022. Chlorine-resistant bacteria (CRB) in the reverse osmosis system for wastewater reclamation: Isolation, identification and membrane fouling mechanisms. *Water research*, *209*, p.117966.

Buse, H.Y., J. Morris, B., Struewing, I.T. and Szabo, J.G., 2019. Chlorine and monochloramine disinfection of Legionella pneumophila colonizing copper and polyvinyl chloride drinking water biofilms. *Applied and Environmental Microbiology*, *85*(7), pp.e02956-18.

APPENDICES

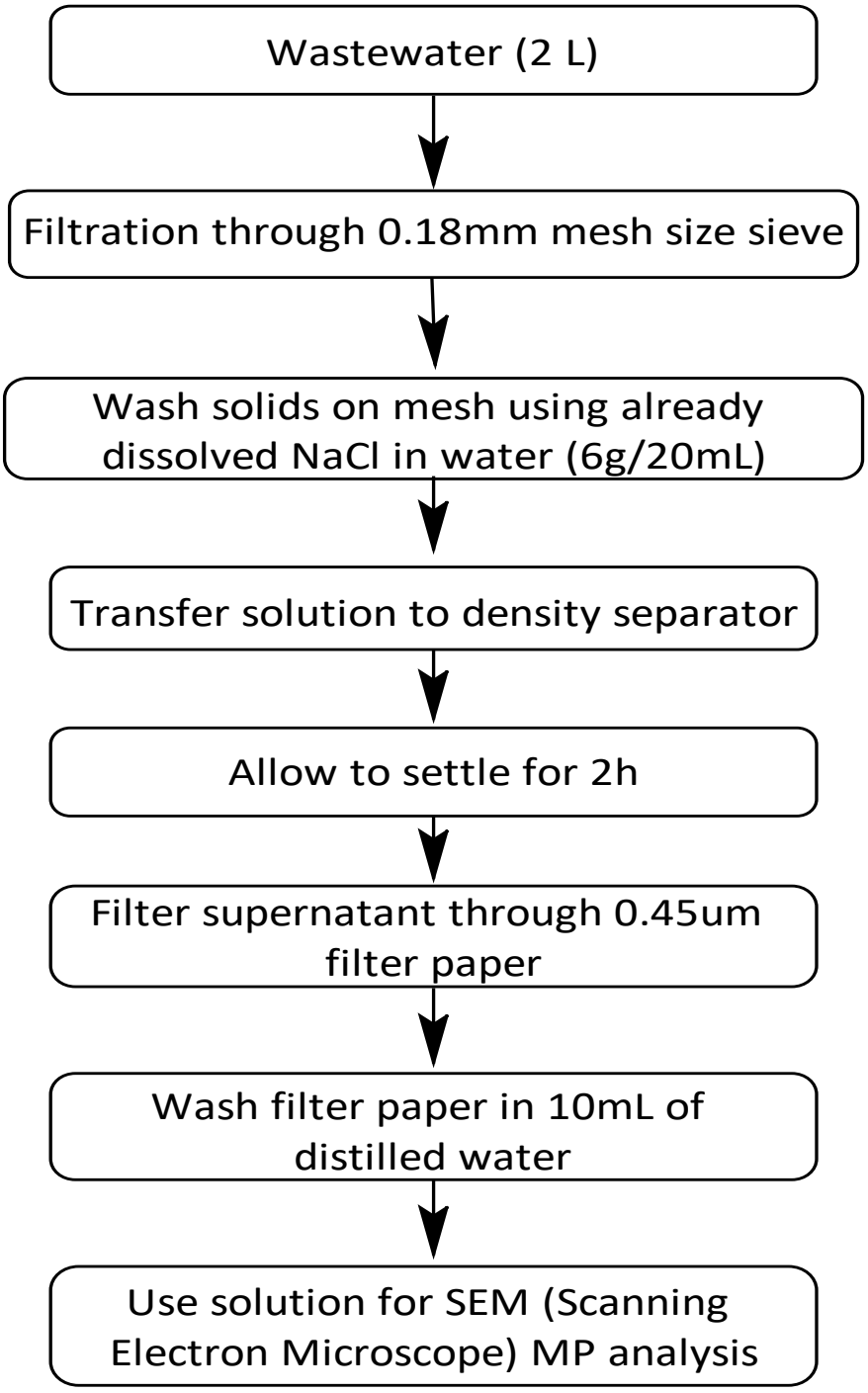


Figure S1: Microplastic isolation protocol for SEM

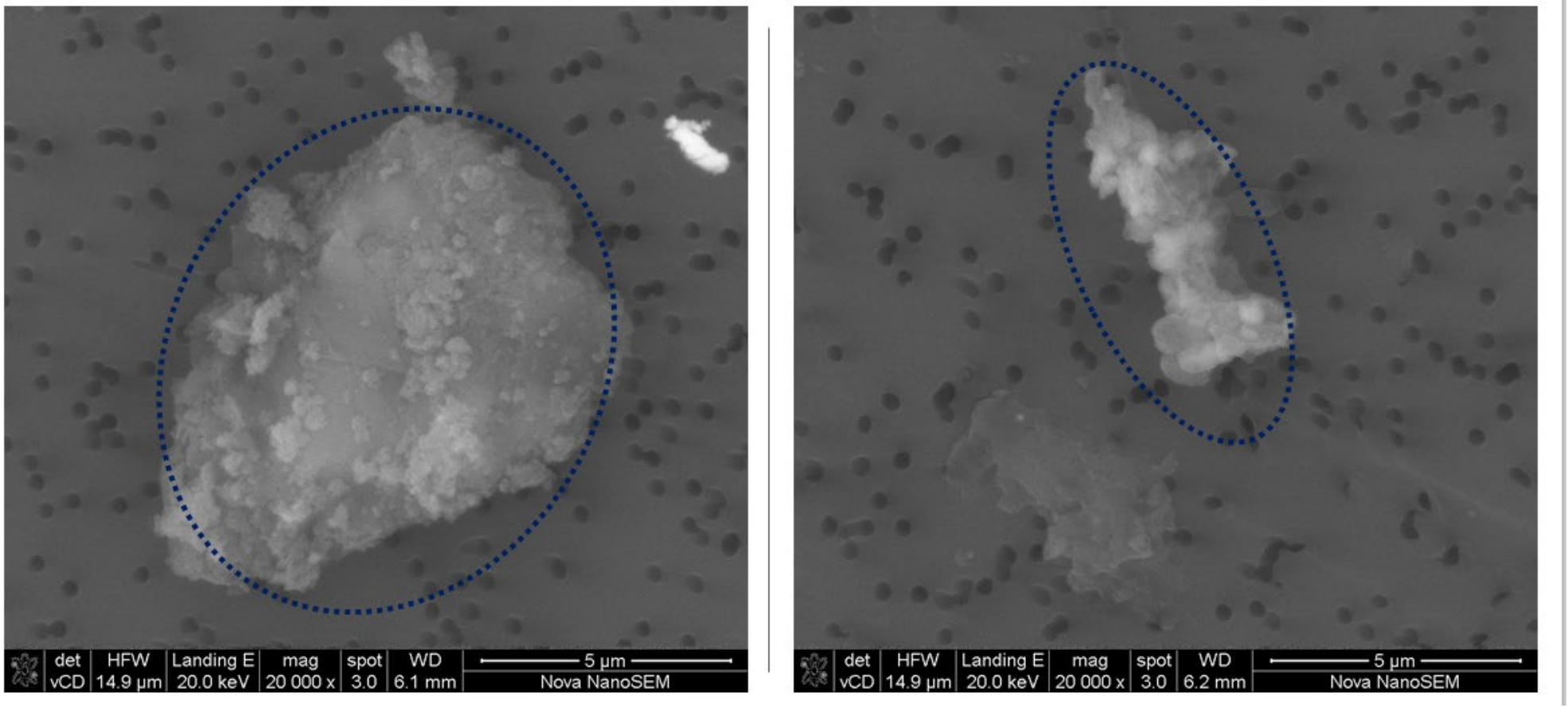


Figure S2: SEM images from microplastics isolated from raw wastewater, showing possible EPS on the microplastic surfaces

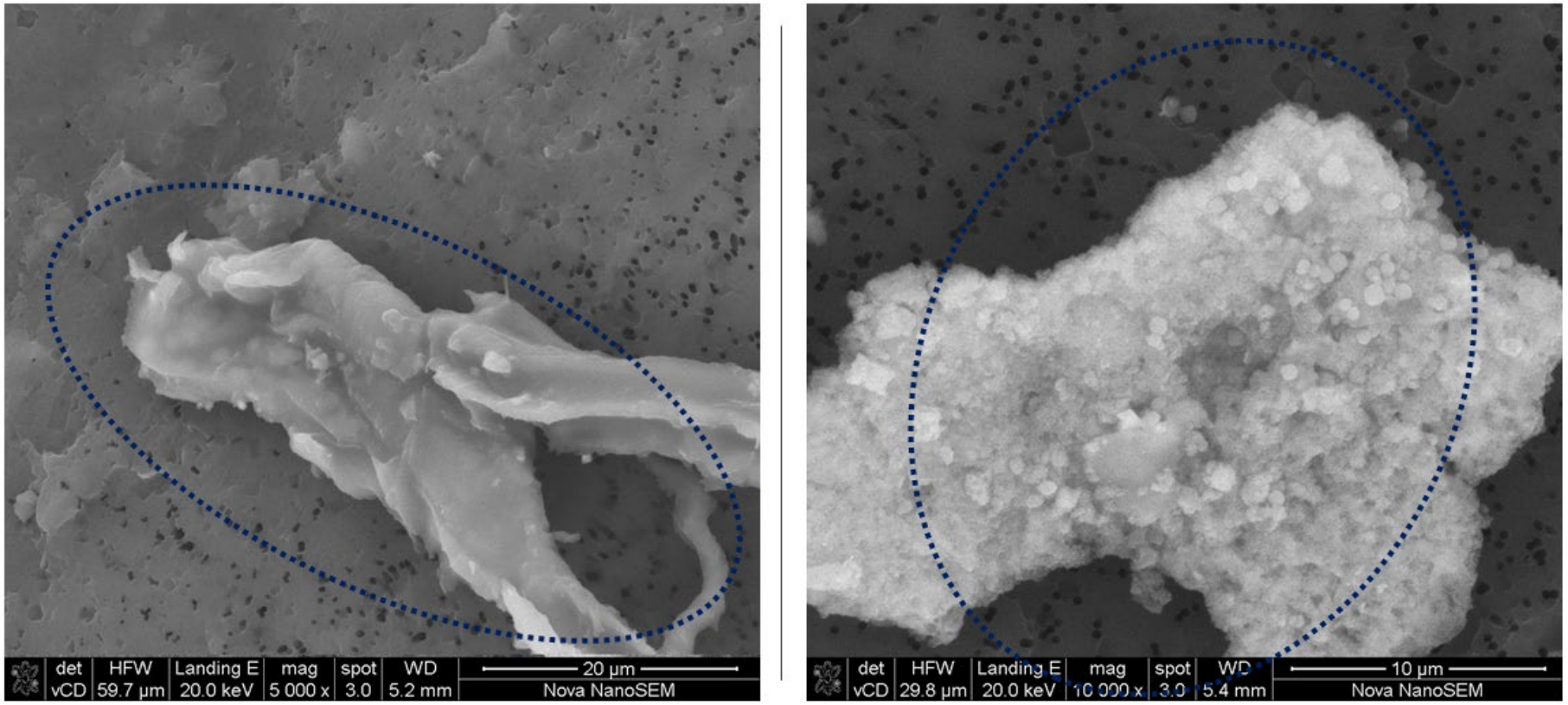


Figure S3: SEM images from microplastics isolated from treated wastewater, showing possible EPS on the microplastic surfaces

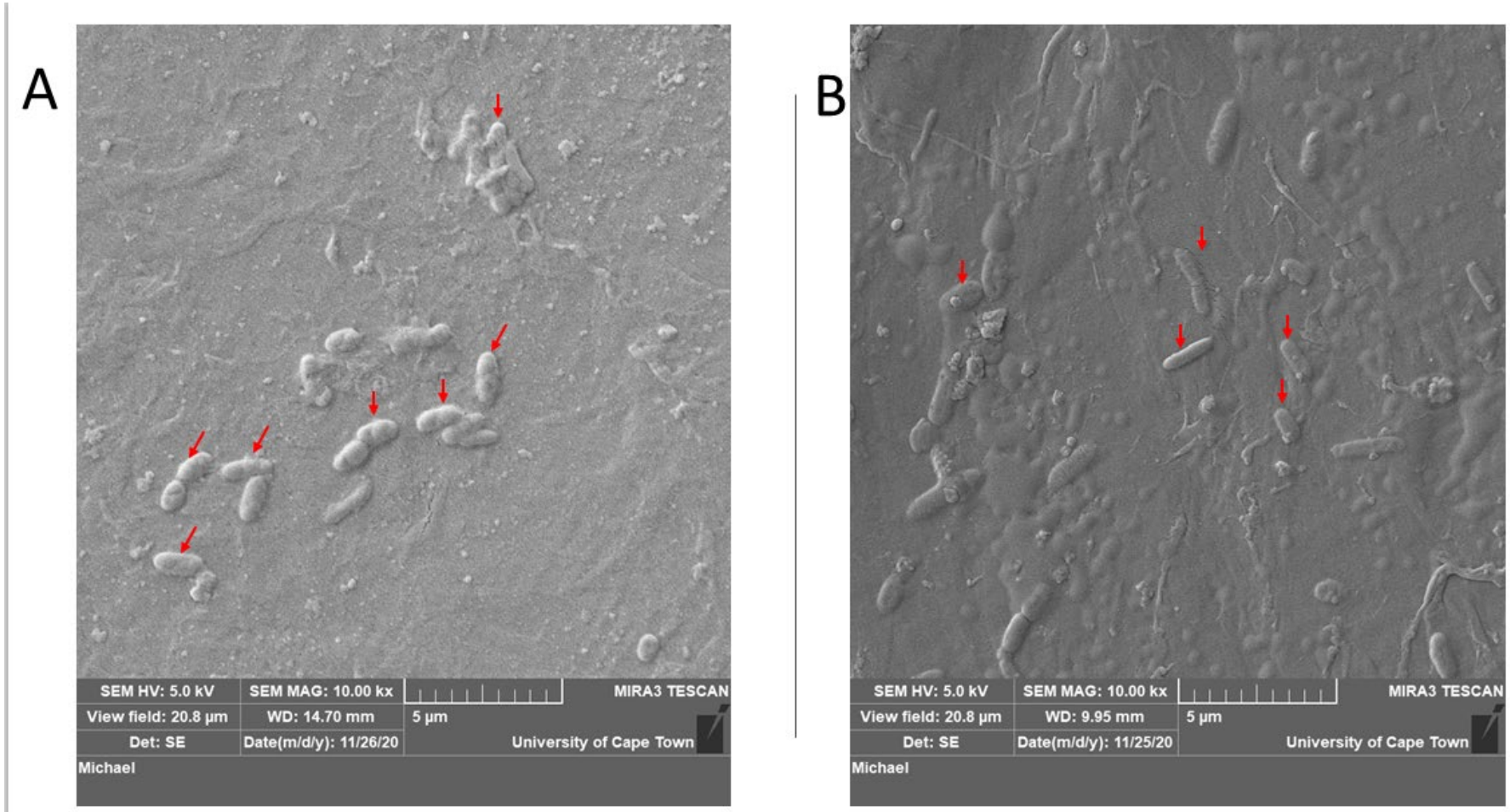


Figure S4: SEM images to show bacterial attachment to smooth (A) and rough (B) LDPE.

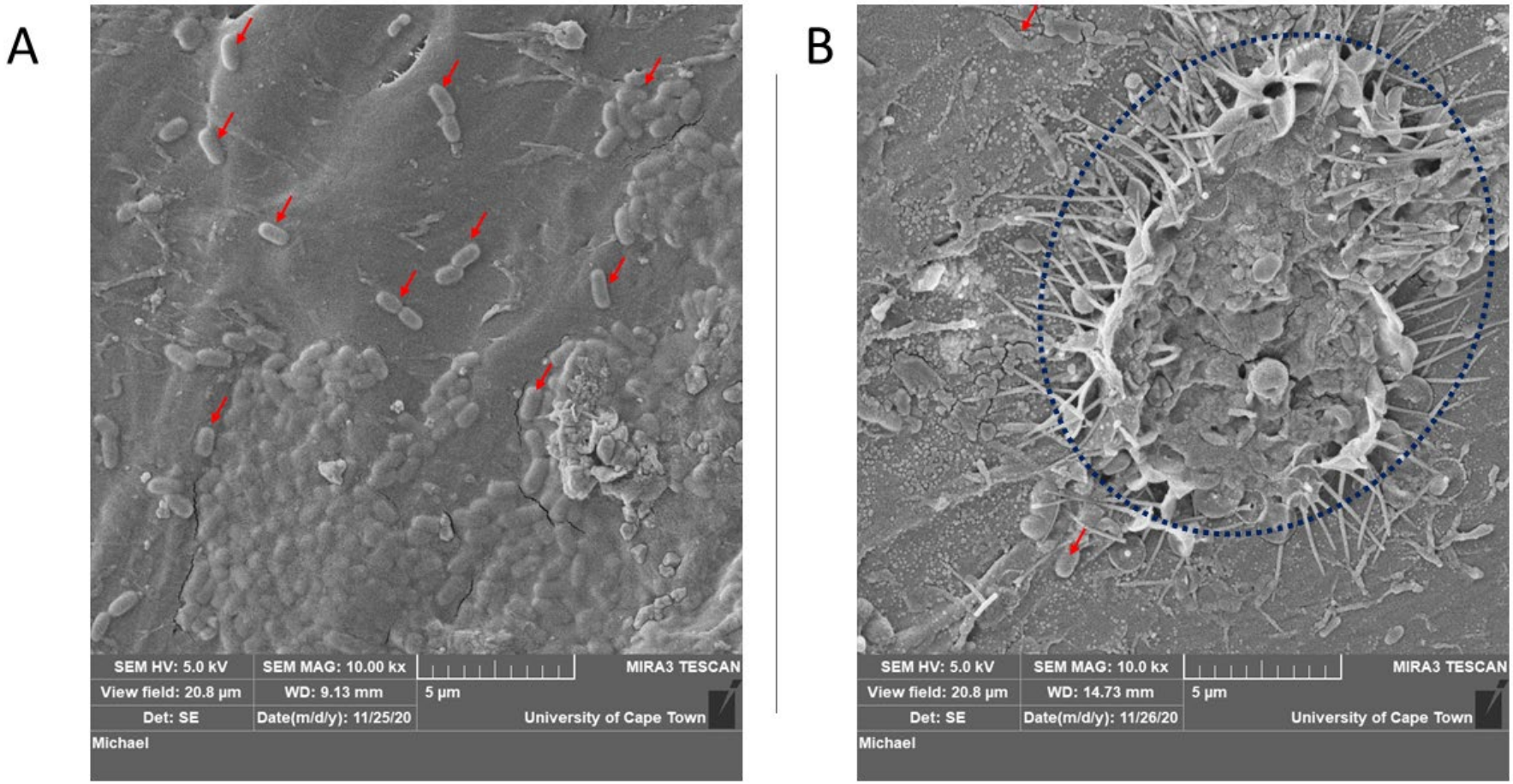
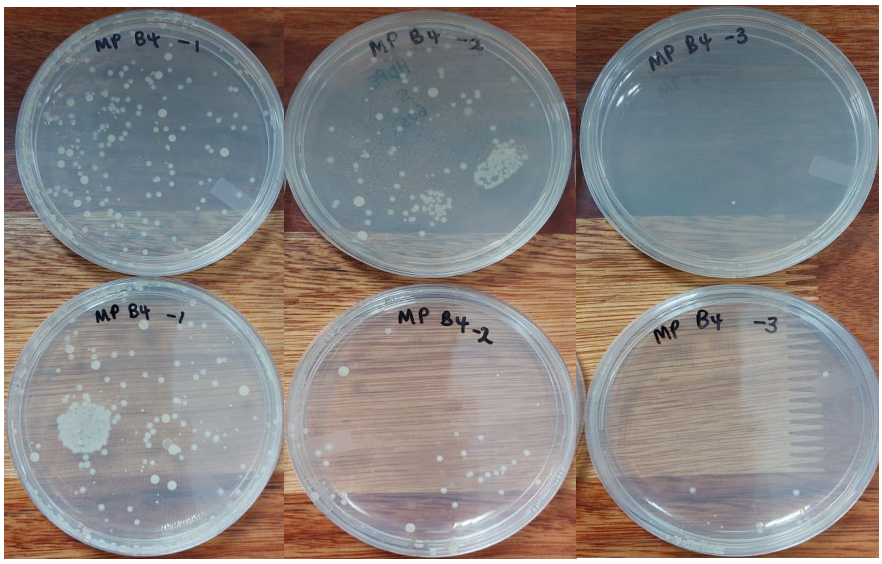
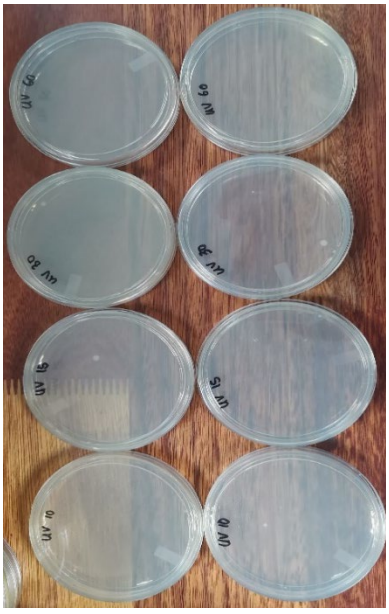


Figure S5: SEM images to show bacterial attachment to smooth (A) and rough (B) HDPE

(A)



(B)



(C)

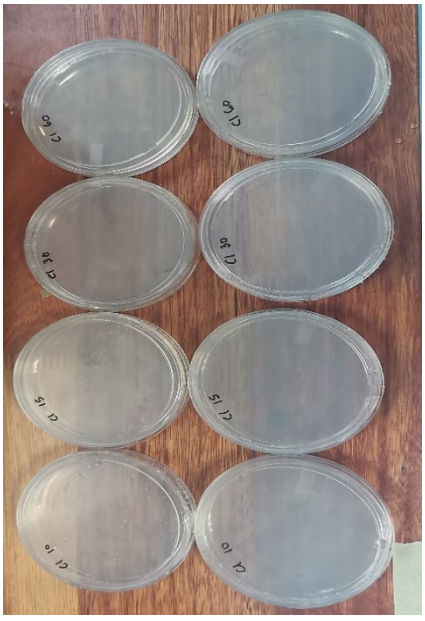


Figure S6: Colony growth on HPC agar from biofilm samples (A) before tertiary treatment and after (B) UV and (C) Chlorine tertiary treatment