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Investigation of Microplastic Removal Using Foam Produced from Rhamnolipids

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ABSTRACT

Microplastics generated from the breakdown of larger plastic pieces are the emerging contaminant in aquatic ecosystems at high levels, due to inappropriate disposal or mismanagement. With a particle size within 2-5 μm, it has been reported that microplastics are extensive pollutants in water bodies. However, with a wide variety of scientific articles recording the abundance of plastics in the aquatic environment, there is a lack of studies examining microplastics removal. Although multiple studies have used sedimentation and filtration as separation techniques to remove microplastic particles, no study has been able to remove these pollutants without secondary water pollution.

The focus of this study was to investigate the potential use of foam generated from rhamnolipids biosurfactant to remove polyethylene and car tire residue microplastics. Rhamnolipids are a type of biosurfactant that can mimic its chemical counterparts and display less toxicity, higher biodegradability and higher frothing ability.

The research was divided in four stages. In the first stage, the ability of rhamnolipids for the removal of polyethylene microplastic particles was assessed using a microplastic removal system. Then, a two-level factorial Placket Burman design was used for the identification of key parameters for the PE removal efficiency. The effects of rhamnolipids concentration, PE concentration, time, air flow rate, NaCl concentration and system configuration on PE recovery were investigated. The analysis carried out demonstrated the rhamnolipids' potential as a foaming agent and its ability to remove 92.42% of PE particles when the system operated for 40 min with a rhamnolipid concentration of 5 g/L, a PE concentration of 0.5 g/L, air flow rate of 2 Lpm and NaCl concentration of 0.5M as the medium. The ANOVA analysis identified the variables rhamnolipid concentration and operating time, as critical parameters that influence the PE removal efficiency.

In the second phase of the study, response surface methodology was used to investigate the effect of the variables rhamnolipid concentration (X_1), operating time (X_2), PE microplastic size (X_3) and PE concentration (X_4) and their impact on PE removal. This evaluation found that the variables operating time and its interactions with the rhamnolipid concentration and PE size parameters, can significantly impact the removal of polyethylene powder at the 5% level. A least squares quadratic fit that correlates the predictors and the response variable was created with an R^2 of 0.82.

Finally in the third stage of the study, the efficiency of rhamnolipids foam for the removal of car tire residue was assessed. Response surface methodology was used to investigate the effect of the

variables rhamnolipid concentration (X_1), operating time (X_2,), Ctr size (X_3) and Ctr concentration (X_4) and their impact on Ctr removal (Y). The analysis found that the predictors X_1, X_2 and X_3 have a significant effect on the response variable with pvalues of less than 0.05. The interactions X_1X_2, X_1X_3, X_2X_3, and the squared terms X_1^2, X_2^2 and X_3^2 were also found to be significant. The factor X_1, had the strongest effect on Y with an estimated coefficient of 39.811 and a low p-value of 1.5761e-06. A multiple regression that correlates the parameters and the response value was developed with an R2 of 0.89.

Keywords: Microplastics, biosurfactants, foam, rhamnolipids

SUMMARY FOR LAY AUDIENCE

Microplastic particles are contaminants of emerging concern due to their recalcitrant nature and their abundance in various environments. Although multiple research projects have focused on reporting their occurrence in water bodies, to the best of our knowledge, no study has investigated the potential use of biosurfactants to capture and remove microplastics.

The focus of this study was to investigate the potential use of foam-based rhamnolipids for the removal of polyethylene and car tire waste microplastics. Rhamnolipids are a type of biosurfactant that can mimic their counterparts in terms of biodegradability, low toxicity, and high foaming ability. Moreover, previous research indicated the ability of rhamnolipids to remove several contaminants from water. Since the ability of rhamnolipids to remove heavy compounds was confirmed through several investigations, this study studied the microplastics removal efficiency of rhamnolipids foam. This investigation is divided into six chapters. Chapter 1 presents an introduction to the research and thesis structure; chapter 2 provides a literature review on microplastics in the environment and biosurfactant production. Chapter 3 presents the results on the first phase of experiments regarding the design of the microplastics removal unit and assessment of the system for the removal of PE microplastics particles using rhamnolipids. Chapter 4 provides the results of the investigation of the PE removal efficiency using different PE sizes and concentrations of rhamnolipids. Chapter 5 reports the results of the investigation of the Ctr removal efficiency using rhamnolipids, and chapter 6 summarizes the conclusions of the study and provides some recommendations for future work.

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List of Abbreviations

Symbol/Acronym	Description	Unit
C_{PE}	Concentration of rhamnolipids	g/L
C_{RMLP}	Concentration of rhamnolipids	g/L
CMC	Critical micelle concentration	
Ctr	Car tire residue	
E_{24}	Emulsification index	
ESAW	Enriched seawater, artificial water medium	
FTIR	Fourier transform infrared	
MP	Microplastic	
PB	Plackett Burman	
PE	Polyethylene	
PP	Polypropylene	
PET	Polyethylene terephthalate	
PVC	Polyvinyl chloride	
PS	Polystyrene	
PC	Polycarbonate	
R^2	Regression coefficient	
RMSE	Root mean squared error	
ST	Surface tension	mN/m

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CHAPTER 1

1. Introduction

1.1. Background

In 2018, the global production of plastics reached a total of 359 million tonnes with less than half of it recycled or consigned to landfill [1]. Of the remaining 205 million tonnes, a portion of plastic material is considered to still be in use, while the rest is thrown into the oceans. Due to the non-biodegradable nature of plastics, this synthetic debris can affect the marine environment. Not only plastic waste physically harms wildlife but it carries other toxic pollutants that contaminate water bodies [2].

Microplastics generated from the breakdown of larger plastic pieces, are the emerging contaminant in the aquatic ecosystems at high levels. With a particle size within 2-5 μm, it has been reported that microplastics are bioavailable throughout the food web [3]. Numerous researchers have found that ingested and inhaled microplastics cause tissue and cell damage in animals [4]. On the other hand, in patients with plastic implants disruption in the cellular processes has been reported, still with a lack of studies in this field [5]. Even though, there is no evidence of plastic consumption, with humans as top predators, there is high probability rate of human plastic ingestion [6].

In 2016, a study carried out by the scientist Dirk Zeller reported that by the year 2050, the number of plastics in the ocean will be more than the number of fish. In other words, if the plastic production keeps increasing and its poor disposal remains, plastics in the ocean will outweigh fish in 2050. However, with a wide variety of articles recording the abundance of plastic in the aquatic environment, there is no establish system capable of removing microplastics in water.

The purpose of this study is to investigate strategies to remove microplastics in water using biosurfactant based foam. These strategies can be applied to be part of a water treatment plant for human consumption. Foam production will be studied using a microbial surfactant that is less toxic than its chemical counterparts and that can be recovered after the extraction process [7]. Hence, during microplastics separation no other pollutant will be added to water bodies, which constitutes an environmentally friendlier alternative [8].

Biosurfactants are compounds that exhibit surface and interfacial properties and they are synthesized from various microorganisms such as yeast, fungi and bacteria [9]. Several studies have been reported on their ability to mimic their chemical counterparts by displaying unique

properties including emulsification, solubilization, foam formation, low toxicity, high biodegradability and higher environmental compatibility, among others [8], [10]. Their ability to remove heavy metals have been used with a high efficiency [11]. Since biosurfactants can remove heavy particles and have affinity for both hydrophilic and hydrophobic phases, it can be said they are capable of potentially removing microplastic particles. The addition of a biosurfactant to water, acts as a surface-active compound. Therefore, microbial surfactants change the surface layer properties of water, creating the perfect environment for air bubbles to trap microplastic particles and take them successfully to the surface.

1.2. Objectives

Towards the completion of this study, one overall objective and several sub-objectives were proposed.

1.2.1. Overall objective

To investigate the potential of using foam generated from rhamnolipids' biosurfactant to capture and remove polyethylene and car tire residue microplastics.

1.2.2. Specific objectives

1. To investigate the use of foam generated from rhamnolipids to remove PE microplastic particles in fresh and saltwater systems using foam-based rhamnolipids.
2. To study the removal of polyethylene of various sizes using rhamnolipids.
3. To evaluate the potential use of rhamnolipids to remove car tire residue microplastics.

1.3. Thesis Structure

The major findings of this study are organized into 6 chapters:

- Chapter 1: Presents an introduction to the research and thesis structure.

- Chapter 2: Provides a literature review on microplastics in the environment and biosurfactant production.

- Chapter 3: Presents the results on the first phase of experiments regarding the design of the microplastics removal unit and assessment of the system for the removal of PE microplastics particles using rhamnolipids.

- Chapter 4: Provides the results of the investigation of the PE removal efficiency using different PE sizes and concentrations of rhamnolipids.

- Chapter 5: Reports the results of the investigation of the Ctr removal efficiency using rhamnolipids.

- Chapter 6: Summarizes the conclusions of the study and provides some recommendations for future work.

1.4. Mayor contributions

This study contributed to:

- Identify and describe challenging aspects of microplastics removal in water bodies and biosurfactant potential to remove plastic particles in aqueous systems.
- Modulate the microplastics removal system in different water bodies by changing the concentration of microplastics and biosurfactant in a water-polymer-biosurfactant system.
- Find optimal parameters that significantly affect the PE and Ctr removal efficiency when using foam-based rhamnolipids.
- Test the feasibility of the application of rhamnolipids to remove different microplastic particles in water.
- Conclude the efficiency of the process for microplastic extraction.

CHAPTER 2

2. Literature review

The objective of this chapter is to review the recent technical literature on microplastics removal in water. It is apparent that the effectiveness of the removal of microplastics is strongly influenced by the separation method and the plastic size. Thus, this literature review chapter considers the effect of these variables.

2.1. Introduction

Plastics are materials made of semisynthetic or synthetic compounds which can be molded to form very rigid or slightly elastic objects [12]. Plastics are typically organic polymers of high molecular mass that often contain other substances [13]. Their chemical and physical properties of liquid polymers are modified with additives and shaped to convert them into solids with dimensionally stable forms [14].

Their chemical and physical properties include high versatility to be tailored to specific technical needs, lower density than competing materials, excellent thermal and electrical insulation properties, low cost and imperviousness to water and chemicals, temperature, and light resistance [15]. These characteristics have led plastics to replace and displace a wide variety of materials such as wood, paper, stone, leather, metal, glass and ceramic.

Currently, plastics are present in all aspect of daily life involving clothing, packaging materials, transportation and telecommunications [13]. Hence, considering this versatility, it is not surprising that the last detailed report on the annual global production of plastics, for 2022, showed it to exceed 450 million tonnes [16].

Even though the benefits of plastics are undeniable, unsustainable use and disposal of plastics, mainly in discardable form, is causing persistent environmental contamination, becoming a major concern over the last few decades. [17]

Due to the rapid increase in the production and disposal of plastic, it is estimated that plastic waste constitutes approximately 10% of the total municipal waste worldwide [1], [18]. Although a fraction of plastic waste is recycled, end of life plastics accumulate in landfills, and natural habitats where they may take a few hundred years to decompose [19]. Once released, it may be subjected to degradation [20], which leads to fragmentation of larger materials into microplastics, defined as plastic particles less than 5mm [21].

However, of special concern are plastics that enter the marine environment, which have been

calculated to be 10% of the total plastics produced worldwide [20]. This floating debris is continuously mixed by the actions of wind and waves and becomes widely dispersed over huge surface areas. The environmental impacts of these particles include entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasion, as these floating debris can constitute new routes for invasive species [22].

The most common sources of microplastic contamination in the environment include microbeads from personal care products, fibers from textiles and clothing like polyester, acrylic and nylon, fragments from the breakdown of larger plastic products such as plastic packaging, bags and bottles (polyethylene, polyethylene terephthalate) pellets from plastic production, paint particles and fibers from artificial turf, and tire residue from vehicles [23] [24] [25] [26].

Polyethylene and car tire residue microplastics are widely considered to be good models for the study of microplastics in aquatic environments, given their prevalence in water and their similar physical and chemical properties to other types of microplastics. Polyethylene, for instance, is one of the most widely used plastics, found in an wide variety of products from cosmetics to plastic bags. Car tire residue, on the other hand, is a type of microplastic released into the environment through tire wear abrasion [27] [28]. Both microplastics have been identified in water bodies, including freshwater and marine environments, raising concern about their potential impact on aquatic life and human health [29] [30], [31].

Some of the most common conventional methods for capturing and removing these microplastics from water systems include filtration, sedimentation, and adsorption [32]. Filtration involves passing water through a physical barrier or membrane that can trap microplastic particles [33]. Sedimentation allows microplastics to settle at the bottom of a water system naturally or following the addition of coagulants. Adsorption entails using materials such as zeolites or activated carbon to attract and bind the microplastic particles in water, making it easy to extract them [34]. Despite their effectiveness, these methods have limitations. Filters may require frequent maintenance as they could become clogged over time, and the adsorbent materials need to be replaced periodically [35]. For this reason, there is a need to create new an environmentally friendly alternatives to remove these plastic contaminants. It is important to find effective techniques that mitigate and limit the presence of these microplastics in water systems.

2.2. Fate of microplastics in the environment

Microplastics, defined as plastic particles usually with a size < 5 mm, have become of increasing concern due to their threat to environmental quality preservation and related issues. Even though most studies agree on the previous size, other studies have considered microplastics as particles that fall within other size ranges, including <1 mm, < 2 mm [36], 2e6 mm [37] and < 10 mm [38]. Fig 2.1 shows the fate and transport of microplastic particles in the aquatic environment [39].

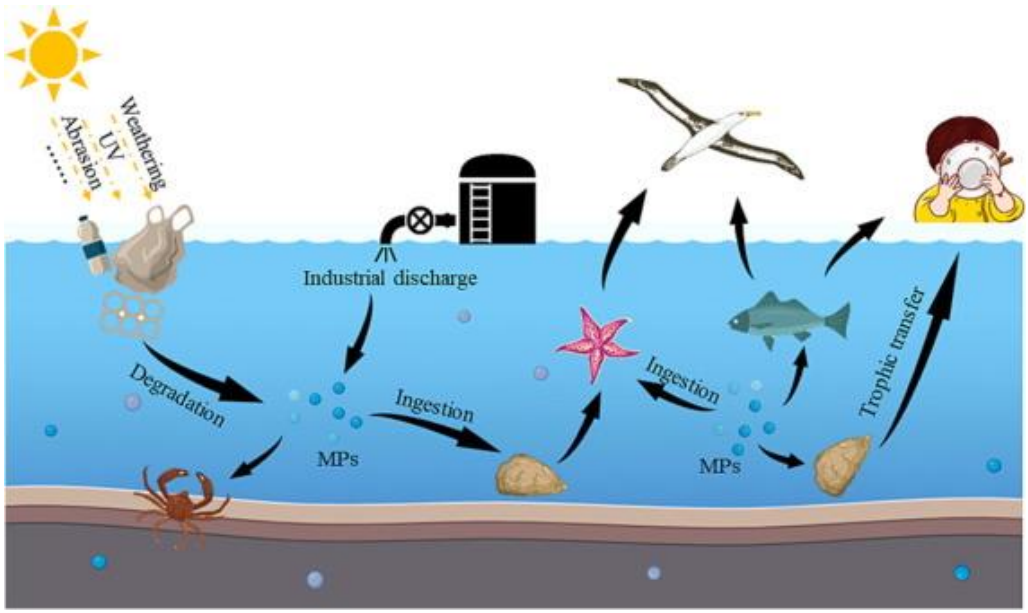

Figure 2.1. Fate and transport of microplastics in the aquatic environment [39]

2.3. Microplastics classification

According to multiple studies, microplastics can be classified depending on their actual source, as primary and secondary microplastics.

2.3.1. Primary Microplastics

Primary microplastics are manufactured to be of a microscopic size, and they can be typically found in cosmetics, household items, air-blasting media, personal hygiene products, such as, toothpaste and exfoliating creams, among others [40]. For example, it has been estimated that approximately 6% of the liquid skin-cleaning products sold in the European Union, Switzerland and Norway contain microplastics, of which over 93% consist of polyethylene (PE) [41]. After use, microplastics present in such products are frequently disposed of and can reach the environment through wastewater collection and treatment systems [42]. Another key source of primary microplastics is the raw materials used in the fabrication of plastic products. Accidental loss, inadequate handling, run-off from processing facilities, and residues from the manufacturing of plastic materials can also accumulate in the environment [36],[43].

2.3.2. Secondary Microplastics

Secondary microplastics, described as small plastic fragments, result from the breakdown of larger plastic particles. When exposed to the elements, the physical, biological, and chemical processes

to which these polymeric particles are subject to, can culminate in the reduction of the structural integrity of plastic debris, leading to their fragmentation. This fragmentation, however, can also occur before the plastics enter the environment, as is the case of synthetic fibers released during the washing of clothes [19]. The main sources of secondary microplastics are plastic material present in organic waste, fibers released from hygiene products, paints containing polymers and fibers released from synthetic textiles [44] [45].

2.3.3. Microplastics abundance

Determining the abundance of microplastics in the environment is a challenge due to their small size. Moreover, the vast array of ways through which these materials enter the environment, make their accurate quantification rather difficult. These constraints are further exacerbated by the lack of standardized methods in sampling, unit normalization and data expression, as well as a unified definition and characterization of microplastics [43].

As an artificial product, plastic sources are mostly inland. Although current wastewater treatment mechanical processes have shown considerable efficiency in the removal of microplastics, this greatly depends not only on the types of treatments used in wastewater treatment plants, but also on the nature of the materials present and their load [1]. It is generally assumed that, due to the low density of the majority of plastic materials, a significant proportion of these end up in the oceans. However, this may not necessarily be true, as, owing to phenomena such as hetero-aggregation with other detritus and suspended solids, these materials may be subject to settling in riverbeds, an assumption that has been substantiated by recent findings detailing such phenomena [47].

Driven by winds and ocean currents, plastic debris can be transported across vast distances and these materials can be found throughout the oceans, including the North and South Poles, remote islands and the deep ocean. Consequently, plastic litter is hence capable of permeating marine ecosystems worldwide. Circulation models suggest that all five subtropical gyres, constitute zones of accumulation for these debris [48]. These large-scale vortices act as ‘conveyor belts’, and, sustained by what are known as Ekman currents, collect the floating plastic debris released, which is subsequently accumulated into central convergence zones [49]. Despite the tremendous complexity of these distribution dynamics, direct measurements of microplastics in the surface have corroborated these models [50].

2.3.4. Degradation

Once in the environment, microplastics can undergo degradation through abiotic or biotic processes, which may act either simultaneously or sequentially. However, these mechanisms do

not depend on the environmental settings alone, but also on the physical and chemical characteristics of the polymeric materials [51]. This may also affect the degree to which microorganisms may attach and form biofilms over the surface of the microplastics, which, in turn, may influence the rate of the biotic degradation processes [51]. Both abiotic and biological degradation mechanisms may also be influenced by the complexity of the composition and polymeric structure of these materials. One study found that plastics with regular and short repeating units with high symmetries, such as Polypropylene (PP), Polyethylene (PE) and polyethylene terephthalate (PET), often limit the accessibility of enzymes and are therefore less susceptible to the action of these biomolecules. Hence, different blends of plastic materials have been described as exhibiting different sensitivities to ultraviolet (UV)-mediated degradation [52].

2.3.4.1. Abiotic Degradation

When exposed to the weathering elements, plastics undergo mechanical disintegration, and experience multiple cycles, pressure changes, water turbulence and damage caused by animals. This mechanical breakdown, nonetheless, differs from degradation, as the molecular bonds do not change, and the materials simply endure morphological modifications.

Photodegradation is generally considered to be the most efficient abiotic degradation route occurring in the environment. Plastics exposed to both visible (400e700nm) and high- energy UV radiation (290e400nm) can absorb such radiation, leading to a higher reactivity of their electrons, inducing oxidation and cleavage, degradation processes that are mediated, mostly, by chain scission and cross-linking reactions [52] [53].

Thermal degradation of plastics causes bond scissions of the main polymeric chain, leading to changes in the properties of the material, including alterations in tensile strength, molecular weight, crystallinity and even colour [54]. However, the high temperatures required for these modifications are hardly observed where microplastics occur in the environment [55]. Nonetheless, the thermal treatment of polymers may be environmentally relevant, as samples of PP subjected to thermal pretreatment have been demonstrated to be more susceptible to biodegradation when compared to none-pretreated samples by a factor of 25 [56].

Oxidation is also an important mechanism of plastic degradation in the environment. This process can be either thermal- or photoinduced and derives from the introduction of oxygen into the polymer matrix [57]. This, in turn, leads to the formation of carbonyl (CO) and hydroxyl (OH) functional groups, which contribute to subsequent biotic degradation routes [58]. The presence of ozone (O_3) in the atmosphere, even in small concentrations, accelerates the ageing process of plastics, as this powerful oxidant attacks covalent bonds, yielding cross- linking reactions and/or chain scissions leading to the production of free radicals [16].

Plastics can also undergo degradation through *hydrolytic degradation*. This process, however, is directly dependent of the presence of covalent bonds prone to hydrolysis, such as those found in ester and ether groups [52]. This results in the breaking of the length of the polymeric chain and, concomitantly, the molecular weight distribution, hence affecting properties such as material strength [52].

In the benthic zone, where the temperatures are low and the rates of sunlight penetration and oxidation-mediated mechanisms are minimal, abiotic degradation processes are negligible [59]. However, the continuous agitation and attrition with sediments constantly yields smaller particles that accumulate on the seabed, thus yielding an enduring source of environmental exposure [60]. Furthermore, a study highlighted that the existing high pressures found in these areas possibly contribute to the degradation of these materials, though such assertions require further confirmation [61].

2.3.4.2. Biodegradation

Biological degradation of plastics usually benefits from the abiotic degradation processes to which these materials are previously subject to, as the mechanical and structural changes resulting from such processes yield larger surface areas, more amenable to microbial colonization. Usually, the biological degradation of plastics initiates outside the cells, as secreted enzymes cleave the polymer chains by hydrolytic processes [51]. These mechanisms take place both in aquatic environment and in soils, and they are further enhanced by the formation of smaller, utilizable groups in the polymer chain. As the molecular weight of the polymers is gradually reduced, cross-linking reactions, enhanced by the increasing presence of water and oxygen, lead to the continuing loss of the polymeric structure and result in the presence of molecules that are increasingly subject to microbial action [61]. Ultimately, these reactions yield water-soluble oligomers and monomers, which then enter the mineralization process. These compounds can be transported through the semipermeable outer membrane of microorganisms and are assimilated as a carbon or nitrogen source through the appropriate metabolic pathway [62]. Although not complete, some materials can exhibit considerable rates of bioassimilation: PE, for example, has been demonstrated to be at least 60% assimilated after 180 days under composting conditions [63].

In oceans, at the benthic level, the rate of biodegradation is negligible, due to the reduced density of microbial communities in these environments. In less deep waters, though, the existing diverse microbial communities of autotrophs, heterotrophs and symbionts play an active role in the biodegradation of plastics, and, in fact, such communities have been found at the surface of plastic marine debris.

2.3.4.3. Bioassimilation

Bioassimilation is a complex process by which microorganisms utilize plastic materials as a carbon source for energy and growth [64]. This process involves the enzymatic breakdown of plastic polymers into smaller fragments or monomers, which can then be metabolized and assimilated by the microorganisms [65].

The microorganisms synthesise plastic-degrading enzymes that act on the plastic surface, breaking the polymer chains into smaller units, such as oligomers or monomers [66] . These enzymes can break down various types of plastics, including polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS), among others. The initial step of bioassimilation involves the attachment of microorganisms to the plastic surface. This can occur through physical adhesion or through the secretion of adhesive substances [67]. Once the plastic is degraded, the microorganisms can uptake the resulting breakdown products through various transport mechanisms and further metabolize them to produce energy, grow and carry out other metabolic activities [68].

2.4. Behavior and effects of microplastics

Laboratorial experiments have demonstrated that these polymers are ingested by cnidarians, rotifers, ciliates, annelids, copepods, mysids, cladocerans, amphipods, euphausiids, mussels, barnacles, tunicates, birds and fish [1] [37]. In fact, an interesting study in the early 1990s pointed to the preferential ingestion of yellow and tan particles by marine organisms, possibly because these particles were mistaken for prey items [69]. Direct effects of the ingestion of (micro) plastics include obstruction of the digestive tract and internal injury, causing reduced food consumption, decreased nutrition and, eventually, starvation and death [43]. Fig 2.2 shows a photograph of plastic debris found in the stomach of a fish in Portugal [70].

Figure 2.2. Photograph of plastic debris discovered in a fish's stomach. Photograph taken by Paulo Oliveira in Portugal [70]

Ingestion of microplastics in the terrestrial environment can occur, although works focusing on the fate and behaviour of microplastics in terrestrial settings are very limited and the direct and quantitative evidence of these materials in soils is, at best, scarce. Nevertheless, livestock have been described to ingest plastic particles, namely, sheep and goats [71], and, despite no specific effects were evaluated, the authors postulated that these animals could suffer from nutritional deficiencies.

Plastic materials, however, do not represent a threat to the environment solely in isolation, i.e., by themselves. Although plastics are, in essence, biochemically inert, most of these now include additives, usually of small molecular size, which are not chemically bound to the polymer. Polymerization reactions during plastic production are also incomplete, and unreacted residual monomers, solvents and additives can migrate away from the synthetic matrix. Therefore, they are able to leach from the plastic materials [72]. A vast majority of these frequently used additives are lipophilic, making them suitable for penetrating cell membranes and to subsequently participate in biochemical reactions, yielding not only severe behavioural effects, but also having reproductive consequences.

Polyvinyl chloride (PVC), polystyrene (PS) and polycarbonate (PC) have all been demonstrated to release toxic monomers associated with the development of reproductive abnormalities and cancer in invertebrates, but also in rodents and humans [46]. Additives used in the manufacture of plastics have also been proven to leach from plastics and ingested by marine organisms [36]. Moreover, plastics collected in aquatic environments have been shown to also contain other contaminants, including organic chemicals that were found to have been adsorbed from the ambient surroundings.

2.5. Sampling and detection

Due to their small size, there are intrinsic difficulties in the collection, handling, sampling, and identification from environmental samples. Microplastic research suffers from insufficient consistent data, which may be, in part, attributable to the current lack of standard operating protocols for sampling and detection [73].

Sampling and detection of microplastics in water samples, mainly involves net mesh of different sizes and filtration to separate these materials. The pore size of the mesh depends on the size distribution and volume of the sample collected. Sampling methods are classified in three categories [74] [75] for floating microplastics:

1. *Selective sampling:* material collection is in situ and the samples are directly extracted as they are recognizable to the human eye. This method is mostly suitable for macroplastics

due to their size and volume. However, incorrect spotting when plastic is mixed with other rubbish can lead to a significant human error [76].

2. *Bulk sampling:* it is a non-volume-reduce collection technique. Mostly applicable when plastic is mixed with other materials at the sampling site and filtration is not feasible [74].

3. *Volume-reduced sampling:* this method reduces the initial volume of the sample, preserving the fraction used for further analysis [77]. This method is mainly used for water sampling, followed by filtration through nets, commonly outside of the sampling site [76].

2.6. Sample contamination and quality control

There are clear difficulties associated to the reproducibility and comparability of data on microplastic concentrations in water [73]. Reliability in experimental data is essential to obtain valid and comparable data on the concentration, particle size and polymer type across the environment. Thus, with the increasing occurrence of microplastic waste in water bodies, the need of standardized methodologies when sampling and detecting is crucial. Moreover, defined protocols of analytical methods will lead to an effective technique validation and quality control.

Prior to the identification of samples, potential sources of cross-contamination need to be considered. Contamination of microplastics samples may occur due to insufficient isolation or scarce air quality. The use of cotton-protective equipment (gloves and lab coat) is employed to avoid synthetic fibers coming into the samples. Metal or glass laboratory wear is recommended for satisfactory sample storage instead of plastic wear. Other scenarios of contamination can be unsealed containers with samples, paint particles broken off from the side of the vessel by samplers, or particles of synthetic nets used for water sampling [78].

Proper sample control includes validation trials and blank samples for contamination monitoring [79]. Inadequate sample monitoring could result in underestimation or overestimation of microplastics pieces. When working with microplastics, it is highly recommended to perform experimental procedures in the shortest time lapse to reduce the risk of impurities [80].

2.7. Microplastics separation methods

Microplastic pieces must be isolated from water prior to their quantification and characterization. The separation process includes a reduction of the sample volume to allow the concentration of plastics followed by density separation, filtration, and sieving.

2.7.1. Density separation

This method uses density differences to separate microplastics from heavier materials present in the sample. The specific density of most plastics ranges from 0.8 to 2.3 g/cm^3 and generally, the density of sand and other deposits is 2.65 g/cm^3 [78]. Separation occurs by placing the sample in a solution saturated with salt and mixing it for a certain period of time. After mixing, the heavier materials settle out, while the less dense particles remain suspended at the surface of the solution where they will be collected for further processing [81]. Density separation is preferred 65% of the times by researchers studying the recovery of microplastics from sediments [82]. A list of the most common separating solutions for microplastics recovery and their respective density is shown in Table 2.1.

Table 2.1. The density of the most common separating solutions for microplastic recovery by density separation [1]

Solution	Density (g/cm^3)
Deionized water	1
Sodium Chloride	1.2
Sodium tungstate dihydrate	1.4
Potassium formate	1.57
Zinc Chloride	2.91
Sodium Iodide	1.566

The density separation technique is normally carried out with a NaCl-based separating solution. Within the advantages of using NaCl are: cost efficiency, availability and no toxicity. Microplastics separation through this method is adequate only to plastics with lower density to enhance material flotation. Some of the plastic types able to be separated by density are polypropylene (PP), polystyrene (PS), PE, and polyamide. However, more dense plastics cannot be recovered such as polyvinyl chloride (PVC) and polyethylene terephthalate (PET) which make up 17% of global plastic demand.

Even though flotation can accomplish effective separation in the millimeter size range, is seldom used for plastic particles that are too small. Furthermore, flotation is incompatible with the smallest size fractions of plastic since the buoyant force is low and surface fouling can significantly change the particle density. Another concern is that the attachment of bubbles to non-plastic particles can carry denser particles to the air−liquid interface [81].

2.7.2. Filtration

Vacuum filtration is commonly performed to microplastic samples at the density separation stage. Paper, fiber-glass, silicone, and polycarbonate membranes are the most frequent materials

employed during filtration. The pore size is always adjusted to retain microplastics of a desired size and it can vary from 1 to 1.6 μm. However, filter obstruction regularly caused by organic matter represents a challenge. In water, the reduction step usually is related to sample collection (e.g. manta nets) followed by filtration or sieving with pore or mesh sizes varying from 0.45 to 55500 mm.

Filtration is also used to isolate microplastics from bulk water samples. In this case, filters up to 15 cm in diameter and with a pore size of up to 47 μm are used. It is possible to collect microplastic particles from the surface of the solution with tweezers. For separation of large particles, samples before filtration can be passed through a sieve with a mesh size of 500 μm [20]. An important point is the process of moving the float- ing particles from the surface of the solution to the fil- ters and sieves. To prevent any loss of analyte associated with the adherence of particles to the walls of the laboratory ware, it is recommended to sequentially wash the walls of glassware directly on the filter [76].

2.7.3. Sieving

Microplastics can be isolated from samples by sieving the latter through sieves with different mesh sizes. The material on the sieves is further sorted, and the remainder that has passed through the sieve is dis- carded. The use of sieves with meshes of various sizes makes it possible to separate microplastic particles into several size groups. A cascade of several sieves (one to six) with sizes of 0.038 to 4.75 mm is usually used. The sieve material is usually stainless steel or copper. Filters and sieved material is dried at room temperature or in desiccators. The temperature in the desiccators varies greatly in different methods (from 60 to 90°C), but the standard [24] sets the conditions for preparing plastic samples before testing and recommends not to exceed the temperature of 50 ± 2°C, with a drying time of 24 h with subsequent bringing the temperature to normal conditions in a desiccator. This is established to prevent changes in the composition and physicochemical properties of the plastics.

The most common separation techniques previously discussed have their distinctive challenges. Moreover, to achieve an efficient recovery, they frequently add other pollutants that contaminate the water. This project aims to study an environmentally friendly approach to separate microplastics from water using biosurfactants.

2.8. Biosurfactants

Surfactants are surface-active compounds able to modify the interface between several phases. Their properties on the interface are due to their ability to orient themselves depending of the polarities of the phases. The lyophilic group (polar) in the molecule is oriented towards the hydrophilic (more polar) phase [83]. Between two liquids or a solid and a liquid; surfactant

molecules will migrate and orientate to reduce the interaction of their hydrophobic groups and water [83]. Likewise, the aggregation or micellization process is given when micelles of surfactant with polar head orient towards the polar phase. Micelles are formed at a very low concentration known as the critical micelle concentration (CMC).

2.8.1. Properties

Due to their capacity to mimic their synthetic counterparts, biosurfactants represent high-quality alternatives for chemical surfactants used in a wide variety of industries. Some of the advantages they exhibit over other surfactants are lower toxicity, biodegradability, environmental compatibility, greater surface activity and sustainability [84]. They are active at extensive environmental conditions, resistant to extreme temperatures, salinity and pH as well, and can be produced by renewable sources such as agricultural waste or microbial fermentation [85].

Even though, the cost of producing biosurfactants is significantly higher, they display no additional cost required for their disposal [13]. Currently, biosurfactants are produced in the small-scale, with rhamnolipids, surfactin, emulsan and phospholipids being the main types produced. The basic characteristics of some of the most common biosurfactants are shown in Table 2.2.

Table 2.2. Characterization of the most common biosurfactants including their surface tension (ST), critical micelle concentration (CMC) and Emulsification index (E_{24})

Biosurfactant	ST (mN/m)	CMC (mg/L)	E_{24} (%)	Reference
Rhamnolipids	28.3-30.6	1-400	26.1-98.7	[29][30][31][32][33]
Sophorolipids	27.2-48	48.76-90.6	71.2	[34]
Surfactin	22-41	10-50	98	[35][36][26][37]
Emulsan	32-56.4	80	66	[38]

Comparing the above biosurfactants, surfactin and rhamnolipids are the most effective biosurfactants, as both can reach a 98% emulsification index with the lowest surface tension (ST). Also, the low CMC shows that less quantity of biosurfactant will be needed to produce the emulsion. Therefore, both are good options regarding yield and efficiency.

As shown in the Table 2.2, rhamnolipids can have numerous property variations, for example, the large range of CMC and E_{24} data. This behavior occurs due to their different types that can be diverse in structure and microbial sources. Hence, their effectiveness for different industrial application can be also affected [63][64].

Rhamnolipids are extensively studied glycolipids that consist of one (mono) or two (di) rhamnose groups, associated with one or two fatty acids alkyl chain [86] [87] [88]. Mono-rhamnolipids and

di-rhamnolipids, differ in physiochemical properties. Compared with mono-rhamnolipids, di-rhamnolipids demonstrate advanced surface-activity properties, higher hydrophilic–lipophilic-balance value (HLB) [89] and higher efficiency in enhancing inhibition of the proliferation of human breast cancer cells [90].

Rhamnolipids constitute one of the most produced biosurfactants in the market [91] [92]. They are produced by several Pseudomonas species [93] [94] and Burkholderia genera. However, *Pseudomonas aeruginosa* represents the top rhamnolipids producer with titers over 100 g/L obtained mainly by the process of fermentation [17]. Rhamnolipids can be produced in a well-defined manner by controlling conditions such as fermentation conditions, substrate used and the microbial strain. Under the right pH conditions, the hydrophilic head has a carboxylic acid group which provides the rhamnolipid an anionic character. They have a polar and a non-polar region in the same molecule making them surface active [95].

2.8.2. Biosurfactant Structure

The so-called biosurfactant precursors like fatty acids, fatty alcohols, sugars, glycerides, and phospholipids are not the subject of this chapter as are glycolipids and amino acid containing lipids with small and great quantities of amino acids [84]. Also lipopolysaccharides and lipoteichoic acids are excluded [96]. Fig 2.3 illustrated the chemical structure of some commonly used biosurfactants.

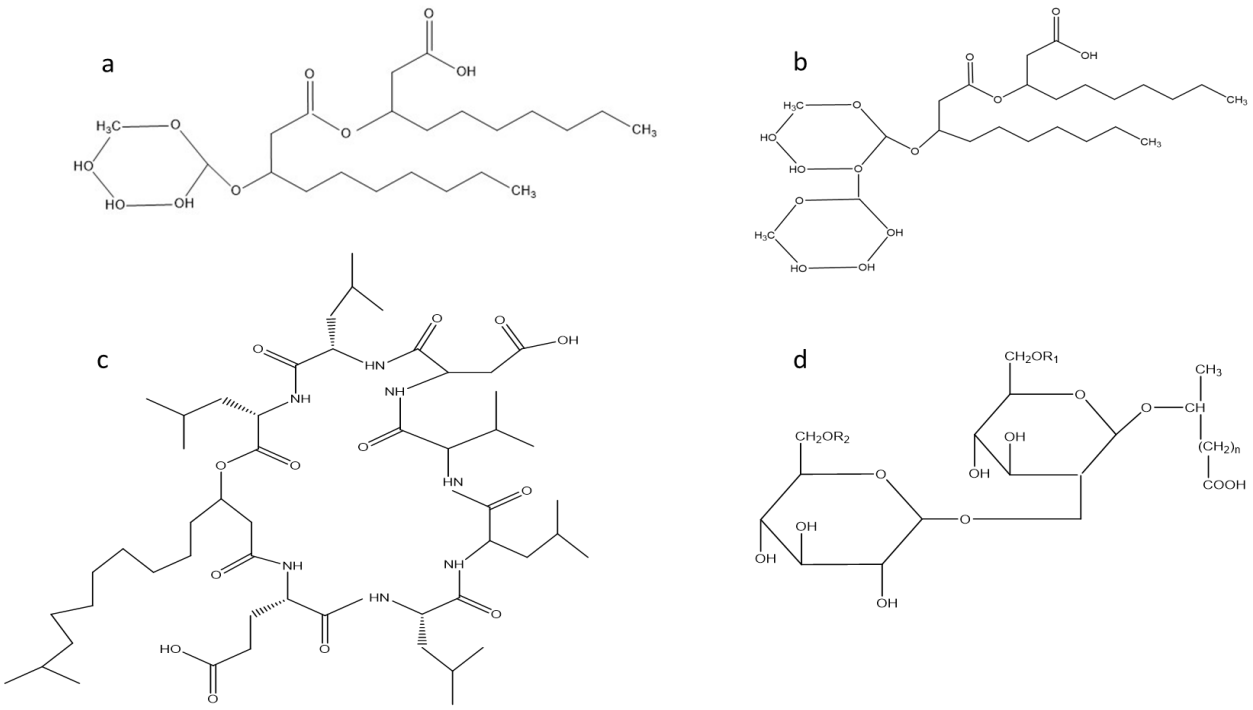

Figure 2.3. Chemical structure of mainly used biosurfactants. (a) Mono-rhamnolipid; (b) Di-rhamnolipid; (c) Surfactin and (d) Sophorolipid.

When a pure glycolipid has been obtained, proved by thin-layer chromatography, and followed by detection with sugar-and lipid specific reagents, the substance is hydrolyzed under alkaline and acidic conditions.

The structure of monosaccharide or disaccharide can be determined by comparison with authentic samples using thin-layer, gas, and high-pressure liquid chromatography or by enzymatic proof. For the structure elucidation of the fatty acid, the combination of H-, C-NMR, and gas chromatography/mass spectroscopy is very helpful.

The location of the acyl groups on the sugar molecule is a problem that can be attacked in different ways. One way is the irreversible blocking of the free hydroxyl groups of the sugar moiety by methylation. then permethylated glycolipid is hydrolyzed in acidic medium, giving poly-O-methyl monosaccharide [97]. The places where the fatty acids were linked to the sugar moiety in the initial glycolipid are pointed out by the alcohol groups that remain free in the methylated sudar. Another way begins with the reversible protection of the free hydroxyl groups by acetylation and continues with deacylation of the ester groups in alkaline medium [98]. After methylation of these few hydroxyl groups the other hydroxyl groups that had been protected by acetylation were liberated by acid hydrolysis [97].

When alkaline saponification of a natural glycolipid results in a fatty acid and a new, more hydrophilic glycolipid, this fact points to an ester bond and a O-glycosidic bond between a sugar and a hydroxyl group of a lipid moiety, respectively[99]. After application of specific chemical or spectroscopic methods mentioned above, the lipid moiety can be separated by acidic hydrolysis and further converted with chromic acid to a fatty acid the chain length of which is reduced in comparison to the original [11].

Lipopeptides were treated first with HCl leading to a mixture of amino acids and of fatty acids. The amino acid sequence was determined using the method of Edman by using trifluoroacetic acid in hydrolysis and analyzing the amino acids in peptides remaining after hydrolysis [97]. The optical configuration was investigated enzymatically also using D-amino acid oxidase by comparing the amino acid composition of hydrolyzates of peptide fragments before and after the enzyme action. The fatty acid structure studied by H-NMR and mass spectroscopy [85]. That the fatty acid was bound to the amide group of glutamic acid was confirmed by isolation of dimethyl-N-acyl glutamate as a methanolysis product of surfactin [99].

There are several surfactants and different microorganisms that produce them. However, the best known and chemically characterized biosurfactants molecules are: rhamnolipids synthesized through convergence of the biosynthesis of rhamnose, cellobiolipids synthesized by *Ustilago*

maydis, trehalose lipids synthesized by *Rhodococcus sp*, sophorolipids synthesized by *Cobacterium sp*, and mannosylerythriol lipids synthetisized by *Pseudozyma antarctica*. [100]

Moreover, biosurfactants constituted by polysaccharides bound to proteins are called polymeric biosurfactants. They are also known as bioemulsifiers and include liposan, mannan lipid protein, emulsan and biodispersan [84].

2.8.3. Industrial applications

Currently, biosurfactants are frequently used in industries such as agriculture, wastewater, bioremediation, oil recovery, textile, food, cosmetics, pharmaceutical [101].

2.8.3.1. Environmental

2.8.3.1.1. Bioremediation

Different kinds of biosurfactants have a potential use in environmental factors such water, soil and bioremediation because of its high emulsifying property, forming efficiency, surface active properties, biodegradability and non- toxicity nature [102].

Study showed that biosurfactants played a major role in bioremediation for oil spills by distributing hydrophobic oil toxins in the aqueous phase and controlling the biodegradation rate for those hydrophobic compounds by increasing their bioavailability [103]. The living microorganisms are boosted by the biosurfactants, increasing the hydrophobic nature of the cell membrane and change in membrane permeability, which increases the intake of hydrocarbon compounds and utilizing those compounds, thus resulting in biodegradation of hydrocarbon compounds [103].

2.8.3.1.2. Oil spill

Oil spills are considered as major disasters where petroleum hydrocarbons are accidentally discharged into the environments especially the marine environment [104]. Biosurfactants are capable of emulsifying hydrocarbons that can further dissolve in water [105]. Emulsan and alasan are examples of bioemulsifiers that are produced from *Acinetobacter* spp. used for microbial enhanced oil recovery. The bioemulsifier emulsan can degrade substances that are poorly soluble in water by coating those substrates [106]. Since there are equal ratios of pure and mixed forms of aliphatic and aromatic hydrocarbons, emulsan can emulsify mixtures of aliphatic and aromatic hydrocarbons and not the pure form of aliphatic and aromatic hydrocarbons [106].

2.8.3.1.3. Heavy metal removal

Heavy metals due to their low bioavailability, carcinogenic nature, teratogenic nature are considered harmful for humans [107]. Smelting, volcanic activities, mining, forest fires, ore processing, automotive exhausts, electroplating, leather tanning, weathering of minerals are some of the ways through which soil contamination can take place with heavy metals [107].

Bacteria called rhizobacteria produce biosurfactants that weaken the strong bonds of metal and soil and encourages desorption, or reduction of interfacial tension, via the solid-solution interface that permits biosurfactants to take in metal in solid solution [107]. The feasibility of metal removal with the help of biosurfactant because of its anionic character has led to many research studies [108]. For instance, heavy metals like lead and cadmium can be removed with biosurfactants produced from *pseudomonas aeruginosa*. Rhamnolipids produced from pseudomonas aeruginosa can decrease toxicity and allow microbial activity to keep up the soil quality [108].

The interaction between biosurfactants and heavy metals in the soil is carried out by van der Waals forces and an electrostatic interaction where ion exchange, precipitation-dissolution, ion binding also tend to occur [107]. Some other interactions that take place between the heavy metals and biosurfactant are precipitation-dissolution, ion exchange, and counter ion bonding [107]. Biosurfactants form a complex with heavy metals that can reduce the solution phase activity and encourages desorption of metals [107].

3.2.1.1. Medical and pharmaceutical industry

3.2.1.1.1. Anti-adhesive activity

Microorganisms group especially bacteria cells, stick to form bio-film and this phenomenon is commonly known as a microbial biofilm [109]. Biofilms are formed by the process of adhesion [110]. The two-step procedure for any pathogenic bacteria begins with the attachment to the surface to form biofilm is that the bacteria form a weak physiochemical bond to the surface by acting like an inert colloidal particle followed by constructing an electrostatic repulsion within the surface of the biofilm using the flagella or pili which is highly hydrophobic in nature when compared with the cell wall of bacteria which results in the displacement of water due to its hydrophobic nature [111]. Microbial surface charge and adhesion can be stopped with biosurfactants [112]. As biosurfactants have the capability forming micelles consisting of hydrophilic head and a hydrophobic tail, biosurfactants can attach to the hydrophobic surface of the bacterial biofilm lowering the strength of hydrophobic interaction thus making them less adhesive [111].

Biosurfactants that are produced from the species *Bacillus, Pediococcus acidilactici, Lactobacillus plantarum* have anti-adhesive, anti-microbial, anti-fungal, anti-bacterial and anti-oxidant capabilities [112] [113] [109]. Study showed that the anti-microbial activity of a biosurfactant

against bacteria *Staphylococcus aureus* was high [113]. *Pediococcus acidilactici* and *Lactobacillus plantarum* are the two kinds of bacteria that produce biosurfactant that possess an anti-adhesive property [113]. The concentration of the biosurfactant plays a major role as biosurfactants exhibit antibiofilm opposing pathogens nonetheless, the spectrum was different for different microorganisms [109].

3.2.1.1.1. Drug delivery

Various diseases can be treated with the discovery of new medicines and their drug delivery system [114]. Increase bioavailability of drug and the ability for it to reach the specific target and releasing the drug in a controlled environment are most important characteristics of a drug delivery system and this can be achieved by introducing a pharmaceutical carrier like polymeric particulate, macromolecular and cellular carrier [114]. Currently, there is research going on microemulsions for drug delivery system for different routes like transdermal, nasal, intravenous ocular, etc. [8]. With the introduction of this new system there is the need of more care required for the formulation of self-microemulsifing drug delivery system since microemulsions systems are thermodynamically stable [114].

3.2.1.1.2. Cosmetics

Glycolipids can be used as an additive in the cosmetic industry as a moisturizer that can keep the outer layer of the skin softer [115]. Water content of the skin is increased and can retain skin and hair properties [115]. *Pseudozyma* species produce a glycolipid called mannosylerythritol lipid that is a current trend in the cosmetic industry [107]. In contrast to sodium dodecyl sulfate, mannosylerthritol lipids could recover feasibility of cells [107]. Other cosmetic formulation where rhamnolipids and mannosylerythriol lipids are used is antacids, insect repellants, anti-dandruff, contact lens solution, toothpastes, deodrants, etc [107]. Sophorolipid is converted to glycolipid ester and has potential in cosmetic composition [115]. Sophorolipids are commonly used in lip cream, eye shadows, anti-radical, anti-elastic properties [116] [115]. They are also used in collagen neosynthesis and increase the stimulation of dermal fibroblast metabolism [115].

Most commonly used chemical surfactant in cosmetics is polyethylene glycol ester and can have many harmful effects such as skin irritation, allergies and environmental contamination [101]. Therefore, based on their properties like Hydrophile-lipophile balance (HLB) value, CMC, ionic nature biosurfactants can be formulated in cosmetics [101]. Biosurfactants like rhamnolipids, sophorolipids, surfactins, MELs can replace chemical surfactants because they serve in product formulations such as moisturizers, restoring an important protein called collagen and improving elastin content in the skin. Other formulations as toothpaste, anti-aging, deodorants, lipsticks, acne treatment, de-pigmenting agents, and nail treatments. On the other hand, saponins have shown a potential application in shampoos because of their physiochemical properties like foaming,

solubilization, and emulsification and biological properties like anti-inflammatory, antimicrobial, anti-tumor, analgesic, anti-diabetic and anti- obesity [101].

3.2.1.1.3. Nanoparticle drug carrier

Researchers are working on finding new methods to efficiently deliver drugs to a specific target and hence nanoparticles of the biopolymer emulsan have been used as a drug carrier [117]. Studies show that nanoparticle drug carrier that consists of emulsan shell with a hydrophobic oil core into which a specific drug called pheophorbide was loaded and the uptake of nanoparticle resulted in faster drug delivery and killed the tumor cells after laser irradiation [117].

2.9. Conclusion

Microplastics define as plastic pieces smaller than 5mm long, are a matter of emerging concern due to their impact and abundance in the environment. Although multiple research studies have reported their occurrence, the investigation and development of efficient microplastic removal techniques is still lacking.

Biosurfactants are of significant interest for their high-quality functions such as non-toxic to the environment, highly biodegradable and having to serve the purpose of reducing surface tension, low CMC value, properties like adaptability at extreme temperature and pH, and many more. Currently, rhamnolipids and surfactin are considered the powerful biosurfactants used. Hence numerous industries like oil and gas, pharmaceutical, cosmetic, environmental, agricultural, etc. are starting to explore and incorporate biosurfactants in their processes as a replacement for their chemical counterparts. Biosurfactants are mainly effective in bioremediation of oil spills at a small scale, as well as the removal of heavy metal from soils. Due to their wide variety of applications and properties, one can say that these molecules could potentially be used to capture and remove polymer particles.

CHAPTER 3

3. Investigation of polyethylene microplastic removal with rhamnolipid biosurfactants using response surface methodology

3.1. Introduction

Microplastics generated from the breakdown of larger plastic pieces, are an emerging contaminant in marine ecosystems and freshwater. With a particle size of less than 5 mm [118], microplastics pose problems in the aquatic environment based on their presence in various environments [119] [120], their non-biodegradable nature [121] [122], and their ingestion by a variety of marine organisms [123] [124]. In addition to the physical effects after ingestion, microplastics can potentially carry other toxic pollutants [2] such as plasticizers and organic contaminants that harm wildlife once they are released [125].

Since microplastics are abundant in surface waters where plankton are found, they can potentially be bioavailable through the food web. Even though, there is no evidence of plastic consumption, with humans as top predators, there is high probability rate of human plastic intake [6]. While conventional wastewater treatment techniques such as sedimentation and filtration have shown an ability to remove a portion of microplastics [126], it is estimated that between 3 and 23 billions of microplastic particles smaller than 300 μm are still released into rivers, lakes and the ocean [127]. Other techniques such as electrocoagulation and biological degradation, have shown to be an effective solution in microplastic separation [128] [129]. However, they may not be the best option for removing microplastics from water due to their limited effectiveness and potential drawbacks [130] [131].

Some potential approaches include the use of biosurfactants as foaming agents in the removal of mineral compounds [85] [132] and in the degradation of microplastics [133], [134]. Since biosurfactants can remove heavy particles and have affinity for both hydrophilic and hydrophobic phases, one can say they are capable of potentially removing microplastic particles [135]. Microbial surfactants have the capacity of changing the surface layer properties of water, creating a suitable environment for air bubbles to trap microplastic particles and take them successfully to the surface [136]. However, to the best of our knowledge, no study has evaluated the potential role of biosurfactant based-foam for microplastic separation.

The purpose of this study was to investigate the removal of microplastics in water using rhamnolipid biosurfactant as a foaming agent. Polyethylene particles of size 40-48 µm were used as a model for microplastics in the study due to their common use in the industry and occurrence in water [137]. Response surface methodology was adopted to study the effect of six operating variables, namely rhamnolipid concentration, PE concentration, operating time, air flow rate, NaCl concentration and system configuration, and their impact on the PE removal efficiency of a microplastic removal system. The experimental runs were performed using a two-level factorial Placket Burman design for the identification of key factors in PE removal. Bubble size analysis of rhamnolipids foam through image processing was conducted.

3.2. Materials and methods

Rhamnolipid biosurfactant purified from *Pseudomonas aeruginosa* as the microbial source was used. The biosurfactant with a purity of 90% was purchased from Sigma Aldrich, Oakville, Canada. The rhamnolipids obtained are a mixture of mono-rhamnolipids and di-rhamnolipids containing L-rhamnose and β-hydroxyl fatty acids.

Polyethylene (PE) microplastic powder, with a particle size range of 40-48 µm was selected as a microplastic source for microplastic removal in the context of the present study. The powder, which was purchased from Sigma Aldrich, Oakville, Canada, has a particle density of 0.94 g/mL at 25℃.

Artificial seawater was prepared using the enriched seawater, artificial water medium (ESAW). The components of the ESAW media were as follows: NaCl (3.63×10^{-1} M), Na_2SO_4 (2.50×10^{-2} M), KCl (8.03×10^{-3} M), $NaHCO_3$ (2.07×10^{-3} M), KBr (7.25×10^{-4} M), H_3BO_3 (3.72×10^{-4} M), NaF (6.67×10^{-5} M), $MgCl_2.6H_2O$ (4.71×10^{-2} M), $CaCl_2.2H_2O$ (9.14×10^{-3} M), $SrCl_2.6H_2O$ (8.18×10^{-5} M), $NaNO_3$ (5.49×10^{-4} M), $NaH_2PO_4.H_2O$ (2.24×10^{-5} M), $Na_2SiO_3.9H_2O$ (1.06×10^{-4} M), $Na_2EDTA.2H_2O$ (6.56×10^{-6} M), $FeCl_3.6H_2O$ (6.55×10^{-6} M), $Na_2EDTA.2H_2O$ (8.30×10^{-6} M), $ZnSO_4.7H_2O$ (2.54×10^{-7} M), $CoSO_4.7H_2O$ (5.69×10^{-8} M), $MnSO_4.4H_2O$ (2.42×10^{-6} M), $Na_2MoO_4.2H_2O^g$ (6.12×10^{-9} M), $Na_2SeO_3^g$ (1.00×10^{-9} M), $NiCl_2.6H_2O^g$ (6.27×10^{-9} M). The media was autoclaved at 15 psig for 20 min and cooled to 21℃ before use.

3.2.1. Activity assessment of biosurfactants

The performance of rhamnolipids in a water-biosurfactant system was assessed by the estimation of the surface tension of the liquid. The potential ability of rhamnolipids to remove microplastic particles was investigated using foam stability tests over time.

3.2.1.1. Surface tension measurements

The surface tension (ST) was measured using a drop shape analyzer (DSA30E, Kruss Scientific, Surface Science Western, Canada). The DSA30E is equipped with a uniform LED lightning unit and high-quality optical components. The instrument calculates surface tension by analyzing the shape of a dispensed drop, using the pendant drop method [138]. The density of the liquid, along with the droplet volume and contact angle of the liquid interface are used to calculate the surface tension of the liquid.

Seven different solutions were prepared by adding rhamnolipids to distilled water. The concentrations of rhamnolipids in the solutions were 10, 20, 40, 60, 80, 100 and 120 mg/L. To guarantee homogeneous solutions, dry rhamnolipids were dissolved in distilled water with a constant agitation of 300 rpm for 4 minutes. The suspension was heated at 60℃ and then, cooled to 21°C.

The procedure used in the interfacial tension measurements consisted of various steps: 1) A small amount of the solution was placed onto the analyzer surface 2) A camera in the instrument captured an image of the droplet from underneath, while a light source illuminated the droplet from above 3) The image was analyzed by Kruss software to determine the shape of the droplet and the contact angle between the droplet and the surface it is sitting on and 4) The contact angle, along with other parameters such as the droplet volume and the density of the liquid, was used to calculate the surface tension of the liquid [139].

3.2.1.2. Foam stability measurements of rhamnolipids foam

Four different solutions were prepared by adding rhamnolipids to distilled water. The concentrations of rhamnolipids in the solutions were 0.5 g/L, 1 g/L, 2 g/L and 4 g/L, respectively. To guarantee homogeneous solutions, dry rhamnolipids were dissolved in distilled water with a constant agitation of 300 rpm for 4 minutes. The suspension was heated at 60℃ and then cooled to 21°C before analysis. Each solution was transferred into a falcon tube and then agitated for 3 minutes using a vortex. The stability of foam-based rhamnolipids was estimated as the foam height over the duration of 60 min. Measurements of the height of the foam film were taken, starting at 0 min, and reported in 10 min increments. Each experiment was carried out in triplicates.

3.2.2. Experimental set up

A microplastic removal unit was developed for a water-polymer-biosurfactant system. Fig 3.1 shows a schematic diagram of the experiment set-up used in the present work for microplastic removal analysis.

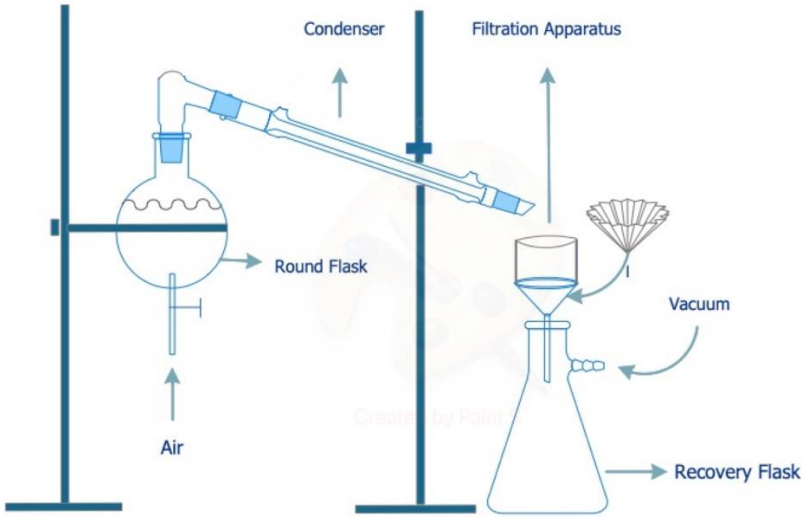

Figure 3.1. Diagram of microplastics separation unit

The microplastic removal unit consists of four pieces of glassware: a round flask (500 ml), a condenser (300 mm length), a filtration apparatus, and a recovery flask (1 L), all securely attached to ring stands. The filtration system contained a vacuum filtration assembly clamped to a filtration flask with a filter paper of 25 µm pore size for the collection of microplastic particles. The round bottom flask contained the liquid phase with the PE microplastic particles. Air was introduced into the system as a gas stream, acting as a mixing agent to formulate bubbles. The inlet gas flow rate was measured and controlled with a flowmeter (VWR, Mississauga, Canada).

3.2.3. Experimental approach

Several investigations were carried out to assess the potential efficiency of rhamnolipids to remove microplastics in a water system. These studies were carried out at a constant temperature of 21°C in the microplastics removal apparatus. Aqueous solutions containing different concentrations of rhamnolipids were prepared in distilled water as follows. To guarantee homogeneous solutions, dry rhamnolipid powder was dissolved in distilled water with a constant agitation of 300 rpm for a minimum of 4 minutes. The suspension was heated at 60°C and cooled to 21°C. The rhamnolipid was directly added to the solution with no further purification.

A typical experimental run was as follows: initially the spherical flask contained 1g/L of microplastics in 100 mL of rhamnolipid solution. and air was introduced into the system to create foam. The air flow rate was 1 L/min. The concentrations of rhamnolipid biosurfactant in the solutions varied from 0.5 g/L to 5 g/L, combined with a PE concentration of 1 g/L in each solution. Once bubbles started forming and foam was created, the foam traveled up the flask and through the condenser reaching the filtration apparatus by gravity (Fig 3.1). In the filtration system, the PE particles were collected, washed, placed in an oven overnight at 80°C to remove residual water.

To avoid PE residue on the condenser walls, the condenser was rinsed with distilled water after every sampling run to collect any PE particles in the condenser line. Samples were taken at 10 min intervals for a total of 40 min and the microplastic collected was weighed. The cumulative removal of microplastics was determined and the efficiency of removal (based on Equation 1 below) was estimated.

$$PE\ removal\ efficiency\ (\%) = \frac{mass\ of\ PE_f}{mass\ of\ PE_i}\ x\ 100\% \quad (1)$$

Where PE_i and PE_f are the initial and final concentration of the polyethylene microplastic.

3.2.4. Response surface methodology

Response surface methodology was applied to study the removal of PE. A two-level factorial Plackett Burman (PB) design was selected to study the PE removal efficiency (Y) of the system [140]. The PB design is a statistical method employed to identify the most important factors influencing the removal of PE in a water system, while minimizing the number of experiments required. The PB design is a two level design for studying k variables in N runs where N is a multiple of 4 and N $\geq$ k+1 [141]. In a PB design, each dependent variable varies at two levels, typically high (+1) and low (-1) [142]. The response variable is measured for each experiment, and the data is analyzed to identify the significant variables that affect the response.

The design screened parameters based on a first-order model expressed in Eq. (2).

$$Y = \beta_0 + \sum \beta_i X_i \quad (2)$$

In Eq. 2, Y represents the outcome of interest being measures, β_0 is the intercept of the response variable when all factors are at zero, β_i is the regression coefficient of each parameter being varied and X_i corresponds to each factor in the experiment.

To perform the analysis of the experimental data, the statistical software Minitab was used. The overall design included 6 factors with their respective units, codes, and levels (Table 3.1). The independent parameters rhamnolipid concentration (X_1), PE concentration (X_2), operating time (X_3), air flow rate (X_4), NaCl concentration (X_5) and system configuration (X_6) were selected according to previous studies on flotation method [143] [144] and microplastics occurrence in water systems [15] [19]. The variable X_5 refers to the NaCl concentration of 0 M and 0.5 M in fresh and marine water respectively. For the system configuration factor X_6, the level -1 refers to a lateral air inlet and the level 1 represents a bottom air inlet, both located in the round flask.

Table 3.1. Coded factors and levels selected in the Plackett Burman design for response surface analysis of PE removal using rhamnolipids

Factors	Unit	Code	Level	
			-1	1
Rhamnolipid concentration	g/L	X_1	1	5
PE concentration	g/L	X_2	0.5	1.5
Operating time	min	X_3	20	40
Air Flow Rate	Lpm	X_4	1	2
NaCl concentration	M	X_5	0	0.5
System configuration*		X_6	1	2

* For factor coded as X_6, level -1 refers to a lateral air inlet and level 1 to a bottom air inlet, both located in the round flask of the microplastics removal unit

Based on the statistical analysis a response surface method was developed. The model was assessed by analysis of variance (ANOVA) with a confidence level of 95%. A linear regression was carried out to obtain the regression coefficients. A Pareto plot was used as a significance test to identify main factors that influence the PE recovery. An area where the predicted means of the PE recovery variable were acceptable was estimated through an overlaid contour plot. A surface plot displayed the interaction between the response and the independent variables.

3.2.5. Image processing and bubble size analysis

Images of the bubbles in the rhamnolipids foam were processed in the ImageJ software. All the images were taken with a charge-coupled device (CCD) video camera. The analysis was carried to take into consideration combinations between only the significant factors. The images were opened in their native format and transformed into 8-bit images into gray images to remove noise. A LUT conversion profile was applied for better delimitation of the bubbles followed by an enhancement of contrast. A threshold of 50 was used for easier identification.

3.3. Results and discussion

3.3.1. Surface tension measurements of rhamnolipids

According to Fig. 3.2, the surface tension of the rhamnolipid solutions decreased from 72 mNm^{-1} to a minimum value of 24.11 mNm^{-1} with a surfactant concentration of 0 and 120 mg/L, respectively.

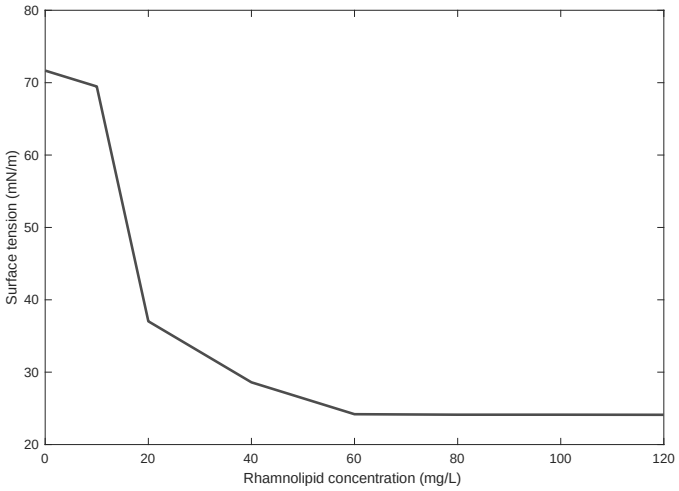

Figure 3.2. Surface tension measurements of water-biosurfactant systems as a function of time when using rhamnolipid biosurfactant

The surface tension of water decreased gradually as the rhamnolipid concentration increased, until the critical micelle concentration (CMC) was reached. Once the CMC was achieved, the surface tension value remained constant. The average CMC value of rhamnolipids obtained in this study was 60 mgL^{-1}, which is in agreement with other reported studies that analyzed the surface active properties of rhamnolipids [97], [145].

A decrement in the surface tension of water, indicates surface active properties that characterized a biosurfactant [146]. A relationship between the CMC and the foam stability of biosurfactants has been established by previous authors [97]. A low CMC value is associated with a stable foam that serves not only as a surfactant but as a bioemulsifier [147]. For the purpose of this study, a stable foam is one of the properties one should take into account when using foam-based surfactant as a separation alternative.

3.3.2. Foam stability measurements of rhamnolipids in water

The results for rhamnolipids foam film height as a function of time are shown in Fig 3.3. As it is seen in the figure, the lowest height of the foam film at 5.44 cm is observed with a concentration of rhamnolipids of 0.5 g/L, while the highest foam film was obtained when the concentration of rhamnolipids increased to 4g/L. When the concentration of rhamnolipids was 0.5 g/L the maximum level of foam decay of 100% was reached at 60 min. On the other hand, the lowest level of foam decay of 76.10% was reached at 60 min when the concentration of rhamnolipids is 4g/L.

Numerous studies have found that a lower decrement on the foam film thickness of a biosurfactant over time is an indication of stable foam. Foam stability has been found to be affected by both, the concentration, and the surface tension of a biosurfactant [148]. A stable foam is often associated

with low foam collapse and less bubble rupture, favouring the separation of desired particles [148] [149]. Foam collapse is mainly caused due to a liquid drainage in the foaming of biosurfactants [150]. In the case of rhamnolipids, the hydrophilic sugar-based portion has been shown to provide better foaming abilities than oil-based substrates [149].

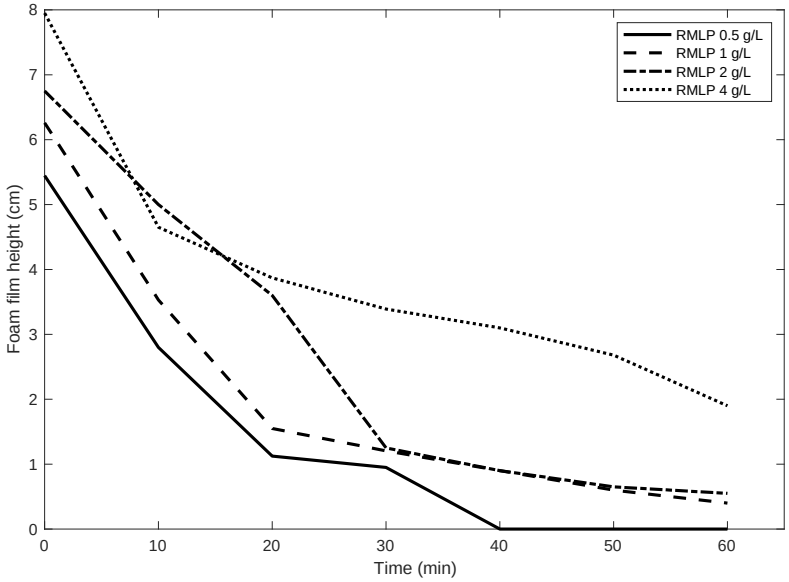

Figure 3.3. Foam stability of rhamnolipids based-foam in distilled water as a function of time

3.3.3. PE removal efficiency of rhamnolipids foam in a water-polymer-biosurfactant system

In order to assess the role of rhamnolipids biosurfactant in the removal of PE microplastic particles, a series of experiments were conducted without the inclusion of rhamnolipids in the mixture. These experiments were carried out to investigate the impact of the absence of rhamnolipids on the removal efficiency of PE microbeads. The results revealed that in the absence of rhamnolipids biosurfactant, the system exhibited no removal of PE. This finding suggests that rhamnolipids play a significant role in facilitating the effective removal of PE microplastics in the ternary system. The lack of rhamnolipids in the liquid mixture led to an absence of PE particle removal, highlighting the crucial role of their presence in achieving effective removal of polymer particles.

Several variables can affect the removal of microplastics using the rhamnolipid biosurfactant foam. These include all the variables listed in Table 3.1 previously. First, forty-eight batch removal experiments were carried out by varying the rhamnolipid concentration to study polyethylene microplastic removal as a function of time (twelve experiments for one rhamnolipid concentration over time, repeated four times). Figure 3.4 shows the PE microplastic removal efficiency at different concentrations of rhamnolipids as a function of the operating time.

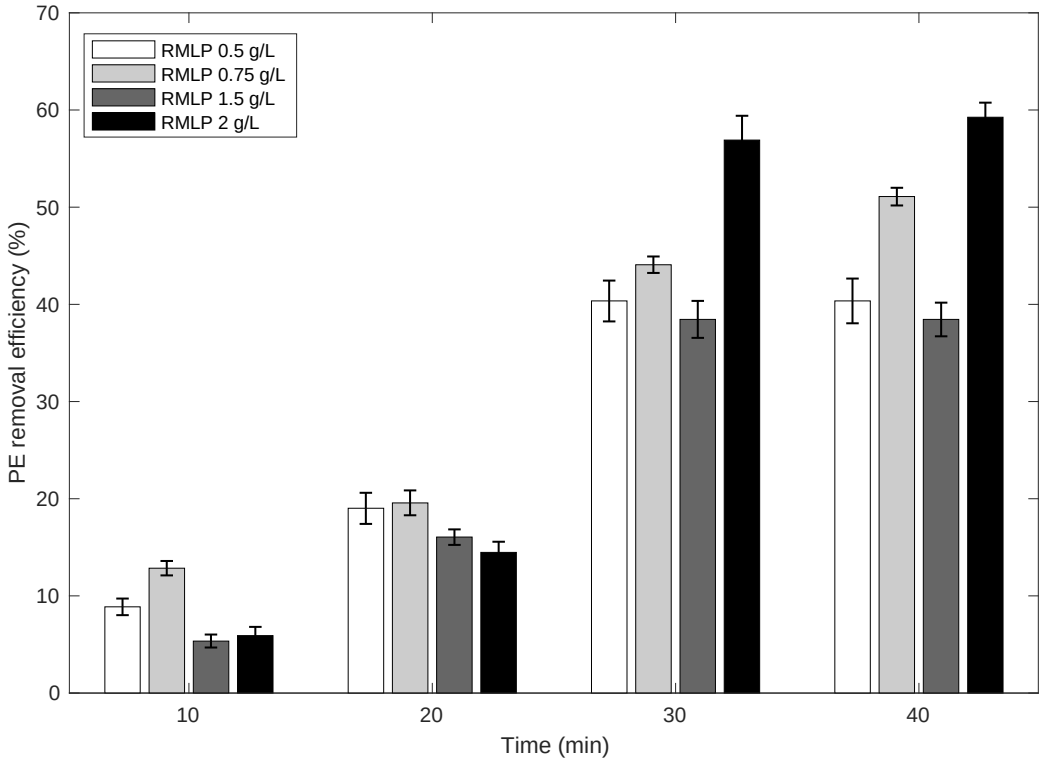

Figure 3.4. PE removal efficiency as a function of time using rhamnolipid biosurfactant based-foam

As shown in Figure 3. 4, there is an increase in the PE removal efficiency over time. When 0.5 g/L of rhamnolipids are added to the mixture, the PE removal efficiency increases from 8.87% at 10 min to 40.35% at 40 min. When the concentration of rhamnolipids in the system increases to 0.75 g/L a similar behavior is observed starting with a PE removal efficiency of 12.85% at 10 min to a PE removal efficiency of 51.09% at 40 min. One can observed the same increasing trend for a rhamnolipids concentration of 1.5 g/L, in which the PE removal efficiency reaches a removal of 38.45% of PE particles at 40 min. When the highest concentration of rhamnolipids of 2 g/L is used, the system is able to remove the highest amount of PE microplastic particles being this 59.26%. The foam stability studies (Figure 3) indicated that more stable foam is obtained at 2 g/L, leading to a high PE removal efficiency.

It is important to mention that lower concentrations of rhamnolipid exhibited higher PE removal efficiencies when compared to higher concentrations of rhamnolipids at 10 and 20 min. Specifically, at rhamnolipid concentrations of 0.5 g/L and 0.75 g/L, the system achieved removal efficiencies of 8.87% and 12.85%, respectively. However, the PE removal efficiency decreased to 5.35% when the concentration of rhamnolipids increased to 1.5 g/L. A decrease in the PE removal (5.91%) was also observed for 2 g/L of rhamnolipids in the system.

As it is observed in Fig 3.4, the use of biosurfactant in the system allows for an effective removal of PE microplastic particles over time. A higher PE removal at lower concentrations of rhamnolipids at 10 and 20 min can be explained by the different behavior of the surfactant that influences its ability to remove microplastics. This means that it is possible that during the initial stages of the experiment, the biosurfactant experienced a greater detachment of PE particles from the surface due to reduced interfacial activity at lower concentrations. However, as the experiment progressed, higher concentrations of biosurfactants removed more microplastics, suggesting that the surfactant molecules formed a stronger surface layer capable of removing more PE microplastic particles.

In the next set of experiments, the PE concentration was varied while keeping the rhamnolipid concentration at 2 g/L. Fig 3.5 shows the effect of the PE concentration on the PE removal efficiency as a function of time. Overall, the results show that the PE removal efficiency of the system increases over time as the concentration of microplastics in suspension increases. When the operating time is 10 min, the PE removal efficiencies achieved for different concentrations of PE have similar values of 5.33 % (0.5 g/L), 6.17 % (0.75 g/L) and 3.22 (1 g/L), with an increase to 12.39% for a PE concentration of 1.5 g/L. As the operating time increases, the system with a PE concentration of 0.5 g/L reaches a PE removal efficiency of 58.77% at 40 min. The same behavior occurs with a PE concentration of 0.75 g/L, 1 g/L and 1.5 g/L, in which the maximum PE removal efficiency obtained were 41.05%, 76.64% and 81.54%, respectively, with an operating time of 40 min.

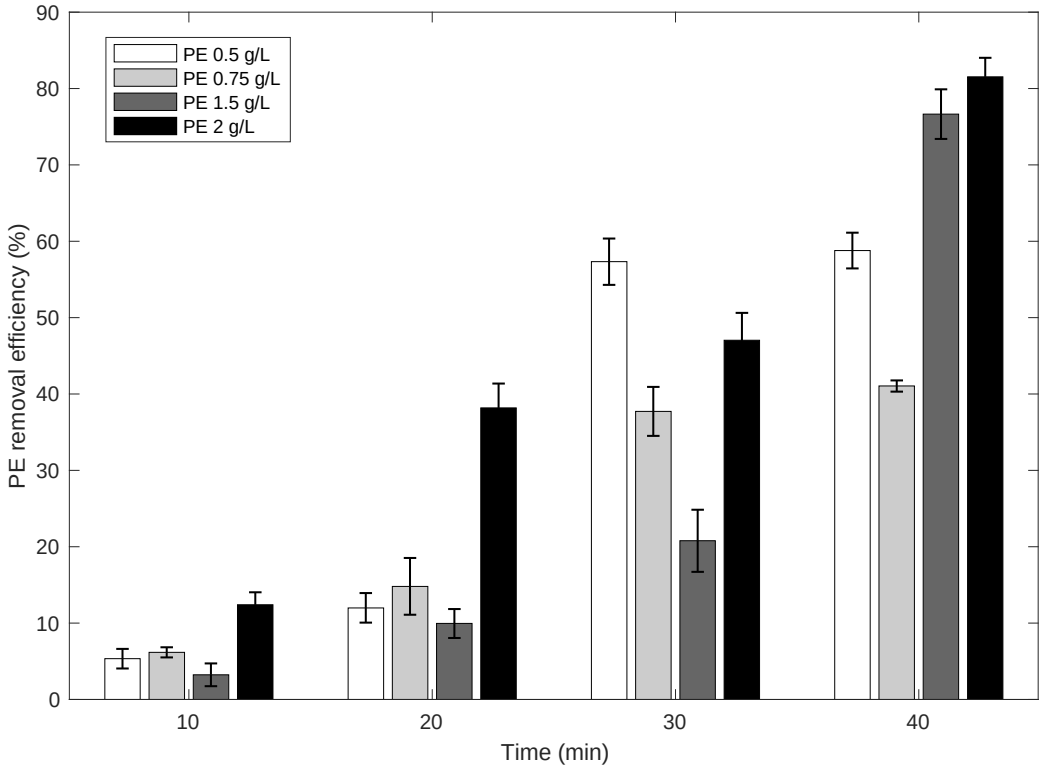

Figure 3.5. Effect of PE microplastic concentration on the PE removal efficiency of a as a function of time using rhamnolipids biosurfactant. The error bars are standard deviation based on triplicate experiments.

3.3.4. Plackett Burman design analysis for main factors

The initial set of experiments demonstrated that a number of variables and their interactions between variables can affect the removal efficiency of the microplastic with the rhamnolipid foam. Therefore, the efficacy of PE removal using rhamnolipids was further evaluated with a Plackett Burman (PB) design. The design matrix and response values for each of the experimental runs are presented in Table 3.2 as X_1 to X_6.. This experimental design approach was helpful to minimize the number of experiments and was able to estimate the effects of the independent variables X_1, X_2, X_3, X_4, X_5 and X_6, with as few as 24 data points.

Table 3.2. Effect of variables on PE removal using an experimental matrix of the Plackett Burman design

Run	X_1	X_2	X_3	X_4	X_5	X_6	PE removal efficiency %
1	+	-	-	-	+	-	82.54
2	+	-	+	+	-	+	58.58
3	-	+	-	+	+	-	54.23
4	-	+	+	-	+	+	84.63
5	-	-	-	+	-	+	60.36
6	+	-	+	+	+	-	85.21
7	-	+	+	+	-	-	48.36
8	-	-	+	-	+	+	53.42
9	+	+	+	-	-	-	22.59
10	+	+	-	+	+	+	58.42
11	+	+	-	-	-	+	28.11
12	-	-	-	-	-	-	25.64
13	+	-	-	-	+	-	83.42
14	+	-	+	+	-	+	59.05
15	-	+	-	+	+	-	57.94
16	-	+	+	-	+	+	90.32
17	-	-	-	+	-	+	56.36
18	+	-	+	+	+	-	92.42
19	-	+	+	+	-	-	49.32
20	-	-	+	-	+	+	58.49
21	+	+	+	-	-	-	36.68
22	+	+	-	+	+	+	54.34
23	+	+	-	-	-	+	18.62
24	-	-	-	-	-	-	12.41

The results show that the highest percentage of PE removal efficiency achieved was 92.42% (run 18) when the system operated for 40 min with bottom air inlet configuration as in Table 3.2., with a rhamnolipid concentration of 5 g/L, PE concentration of 0.5 g/L, air flow rate of 2 Lpm and NaCl concentration of 0.5 M. The lowest PE removal of 12.41% (run 24) was observed when all the predictors performed at the lowest level.

A linear regression equation was obtained for the PE removal efficiency and is represented as below:

$$Y = -16.73 + 8.33X_1 + 0.47X_2 + 1.455X_3 + 2.07X_4 + 2.06X_5 + 0.28X_6 \quad (3)$$

In Eq 3., each dependent variable is multiplied by their respective coefficient, showing the contribution of these coefficients to the value of the dependent variable. The regression

coefficients indicate that all parameters X_1, X_2, X_3, X_4, X_5 and X_6 have positive effects by increasing Y. According to this equation, the highest coefficient 8.33 for X_1, shows that 1 unit increment in this variable contributes to an increase in Y of 8.33 units when all other parameters are constant. On the other hand, variable X_6, with a coefficient of 0.28 units, has the smallest contribution on the response variable when compared to the other parameters in the equation.

As part of the regression analysis, an ANOVA is presented in Table 3.3. The statistical significance of the parameters and the model was determined based on a p-value less than 0.05.

Table 3.3. ANOVA for PE removal efficiency

Term	Degrees of freedom	Sum of squares	F-Value	P-Value
X_1	1	6660.67	277.09	<0.0001*
X_2	1	1.3	0.06	0.817
X_3	1	5079.7	211.32	<0.0001*
X_4	1	25.7	1.07	0.316
X_5	1	101.8	4.24	0.055
X_6	1	1.8	0.08	0.787
X_1*X_3	1	1.7	0.07	0.799
Model	6	1187.11	82.31	<0.0001*

R^2=0.92

The first column of the ANOVA table contains the terms and interactions that may have an effect in the PE removal efficiency. The second column shows the degrees of freedom, which represent the number of observations which are free to vary in the final calculation of a statistic [151]. The third column shows the sum of squares, representing the sum of squared deviations of the data from the mean [152]. The fourth column shows the F-statistic, which is widely used to test the significance of the experimental results [153]. Finally, the last column reports the p-values associated with the F-statistic, indicating the statistical significance of the results [154].

The quality of the linear model expressed by the coefficient of R^2 of 0.92 accurately predicted the effects of the parameters on the PE removal efficiency. This value indicates that even though the sum of the squares of the overall model is high at 1187.11, the model has a proper adjustment of 92.46% and provides a correct association of the variables.

According to the results of the ANOVA in Table 3.3, both the biosurfactant concentration and operating time parameters were found to significantly influence the PE removal efficiency. The model was also verified to be significant due to the adequate F values of 82.31 for the removal of microplastics. As a general rule of thumb in regression models, a model is considered to be significant when the F value > 2.5 [155]. The lack of fit of the model was not significant due to a p-value of 0.482. Once the critical parameters were identified, the interaction between the

rhamnolipid concentration and operating time factors (X_1*X_3) was included in the analysis of variance. Even though both parameters X_1 and X_3 are significant, the interaction between the two factors does not influence the PE removal efficiency.

Fig 3.6 shows the pareto plot, overlaid contour plot and the response surface 3D plot for the PE removal efficiency of the system. As it is observed in the Pareto Plot (Fig 3.6a), the parameters rhamnolipid concentration and operating time, are confirmed to influence the response variable significantly. The diagram includes all the variables from largest to smallest and it verifies the most frequent causes of PE removal. The chart elaborated by Minitab sorts the variables in descending order in the y axis and records the frequency of occurrences in the x axis.

The bars in Fig 3.6a that represent rhamnolipid concentration (X_1) and operating time (X_3) cross the reference line, meaning they are statistically significant at the 0.05 level. The reference line presented in the graph identifies their significant effect with a confidence level of 95%. Thus, most of the effect in PE recovery efficiency is found to come from only both factors X_1 and X_3. This is in accordance with a previous study that indicated the significant effect of biological surfactant concentration and time on PE recovery in a froth flotation system [156].

As shown earlier in Figure. 3.4, higher concentration of rhamnolipids and a longer operating time, favor the removal efficiency of PE in floated products. The comparison between the rhamnolipid performance at 1 g/L and 5 g/L can be explained from their wettability properties [157]. At higher concentrations, the biosurfactant is capable of wetting on hydrophobic surfaces more rapidly. Moreover, the contact angle between biosurfactant molecules and plastics is a function of the surfactant concentration [158]. At low concentrations of rhamnolipids, the molecules adsorbed onto the surface with a horizontal configuration. As the concentration of the surfactant rises, their tails start to interact and their orientation changes from horizontal to vertical [159]. The surface tension decreases on water along with a decrease in the contact angle between the molecules of the biosurfactant and plastics [160]. This causes a rapid hydrophilization of the surface, due to the surface adsorption of the tails which also causes a decrease in the free energy of the entire system. Moreover, other studies have found that adhesion tension values of rhamnolipid solutions on plastics decrease as the concentration of rhamnolipid increases [161] [162].

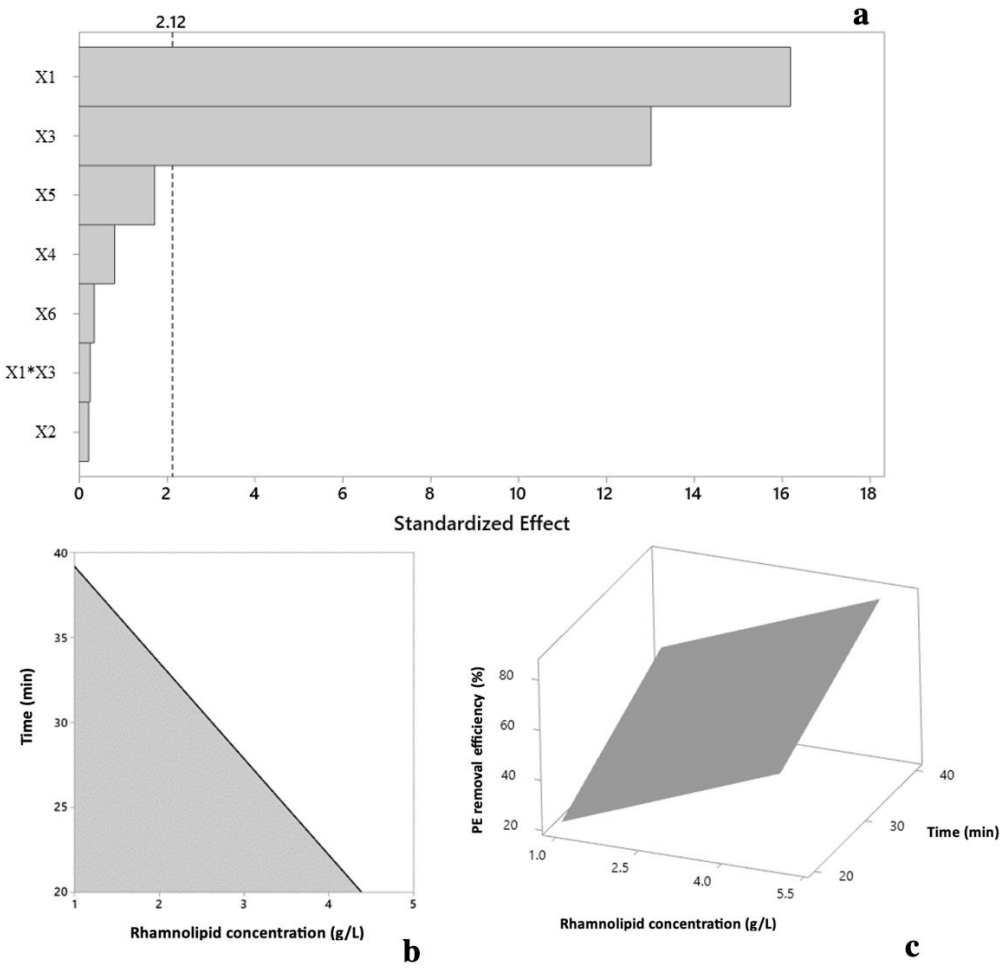

Figure 3.6 a) Pareto chart of the standardized effects obtained for PE removal efficiency using six dependent factors at the 0.05 statistically significant level b) Overlaid contour plot of PE recovery efficiency at varying levels of rhamnolipid concentrations and time c) Response Surface 3D plot of PE recovery efficiency using rhamnolipid concentration as a function of time

An overlaid contour plot presented in Figure 3.6b was constructed to identify the feasible area of both biosurfactant concentration and time. The graph illustrates the variables necessary to achieve a PE recovery efficiency range between 50% and 98%. The graph displays contours for these bounds vs the continuous variables X_1 and X_3 on both axis and presents a white region where both parameters are in the specified range. According to this graph, rhamnolipid concentrations greater than 4.2 g/L can remove 50-98% of the PE particles in the system despite of the conditioning time. Below this concentration, rhamnolipids' performance affecting PE removal varies and is highly dependent on the time variable. In addition, an operating time greater than 39 min can achieve an effective removal of PE despite of the biosurfactant concentration. The combination of the variables X_1 and X_3 that lay in the gray area do not produce PE recovery values within the boundaries of the fitted response.

In combination with the contour plot, a surface plot of PE recovery with the biosurfactant concentration and time variables is presented in Figure 3.6c. The surface plot displays a response surface with the form of a trapezoid in reference to the model containing linear parameters that are statistically significant. The highest values of PE recovery are in the upper right corner of the graph, which corresponds with high values of both rhamnolipid concentration and time. The lowest values of PE removal are found in the lower left corner of the plot, which corresponds with low values of both parameters.

The surface plot in Fig. 3.6c shows the relationship between the operating time and rhamnolipid concentration settings and the PE removal efficiency. Low concentrations of rhamnolipids biosurfactant and shorter time intervals resulted in low PE removal efficiencies. However, higher concentrations of biosurfactants combined with high time intervals resulted in high PE removal efficiencies. The shape of the plot illustrates a linear relationship between the significant variables rhamnolipid concentration and operating time, and the response variable PE removal efficiency. The peak on the plot corresponds with the highest PE removal efficiency value of 92.42%, and occurs at a concentration of rhamnolipids of 5 g/L and operating time of 40 min.

The difference between the results in PE removal efficiency, can be attributed to the positive effect of rhamnolipids in the mixture. As the concentration of biosurfactant in the mixture increased, the removal of plastics also increased. On the contrary, the PE removal efficiency of the system decreased with an increment in the air flow rate [29].

3.3.5. Bubble size analysis

Fig 3.7 shows the average bubble diameter of rhamnolipids foam as a function of time for two different concentrations of rhamnolipids of 1 and 5 g/L. An average of 7 measurements of the bubble diameters were calculated for each combination of the statistically significant predictors of the response variable. The bubble diameter used for this analysis was the average diameter of the main size of the bubbles after visual observation. Bubble size diameters are presented in Appendix D. Table D.1.

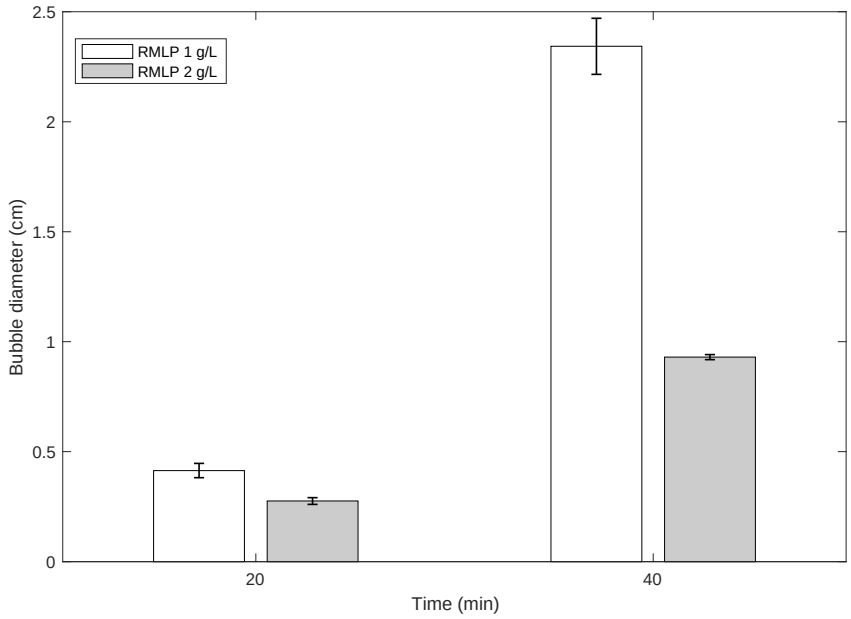

Figure 3.7. Average bubble diameter as a function of time for concentrations of rhamnolipids of 1 g/L and 5 g/L

According to Figure 3.7, the bubble diameter with a rhamnolipid concentration of 1 g/L had a decreasing tendency from 0.46 mm at a time of 20 min to 2.4 mm at 40 min. At a higher surfactant concentration of 5 g/L, the bubble diameter presented a smaller value of 0.27 cm at 20 min and increased to 0.94 cm when the time reached 40 min. Our results show that a higher concentration of rhamnolipids is characterized by lower bubble size throughout time along with high PE removal. On the contrary, lower concentration of rhamnolipids led to a bigger size of the bubbles and a lower recovery of microplastics. This demonstrates that bubble diameter is inversely correlated to rhamnolipid concentration. Other studies have stated that bubble size is a crucial variable in flotation, since it represents a suitable surface for particle attachment [163]. Studies have shown than in flotation tanks, frother concentration is one of the operating variables that affect bubble size [163], [164]. These observations are consistent with the fact that, when the biosurfactant concentration increases, there is also an increase in the overall surface area and its capacity to transport particles up to the floated product [165]. Smaller bubble size has been also confirmed to contribute to foam stability. A higher rhamnolipid concentration exhibits smaller bubble size resulting in higher foam stability values and higher flotation recovery of the desired product. Although froth stability has been shown to be a critical variable in the flotation process, there have been no studies that report the effect of rhamnolipid to remove microplastic particles [166].

3.4. Conclusions

In this study, the microplastics removal efficiency was successfully demonstrated using rhamnolipids based foam. A two-level factorial design with 24 data points was carried out to evaluate the performance of a microplastic removal system that uses rhamnolipids for the removal of PE. The independent factors studied in this investigation were rhamnolipid concentration (X_1), PE concentration (X_2), operating time (X_3), air flow rate (X_4), NaCl concentration (X_5) and system configuration (X_6). PE removal efficiency (Y) was selected as the response variables. A first order model was fitted with an R^2 of 0.92. The highest removal efficiency of PE observed was 92.42% and the lowest recovery was 12.41%. This helped to elucidate that the microplastic removal system can remove PE microplastic particles using foam from a biological source.

An ANOVA analysis was performed to determine the critical parameters that affect the PE removal efficiency. The rhamnolipid concentration and operating time were found to have a significant effect in the PE removal efficiency. Although these parameters have a positive effect in PE removal, their interaction between those do not significantly influence the response variable. The study revealed that other parameters such as PE concentration, air flow rate, NaCl concentration and system configuration do not have an effect on the efficiency of the system to remove PE particles.

Once the significant parameters were identified, it was possible to determine a bubble size variation related to rhamnolipid concentration. Images of the combinations between the factors biosurfactant concentration and operating time were processed and measurements of the bubble size diameters were taken. The results show an inversely proportional correlation between the concentration of rhamnolipids and the bubble size. Moreover, when rhamnolipid concentration increased the bubble size decreased, increasing the ability of the bubbles to remove PE particles. Thus, the microplastic removal unit along with the use of foam from a biological source may be a major solution to remove small plastic particles in water systems without adding more pollutants to water bodies. Since the nature of rhamnolipids is a non-toxic-biodegradable compound, one could say that the addition of this substance to water at lower concentrations of less than 4g/L would not harm aquatic ecosystems. However, it is important to conduct specific toxicity assessments to determine their impact on aquatic creatures including various factors such like the rhamnolipids concentration, and exposure duration, among others.

CHAPTER 4

4. Investigation of the use of rhamnolipids foam to remove polyethylene microplastics of various sizes

This chapter reports a series of experiments designed and conducted to study the effect of four operating variables, namely rhamnolipid concentration, operating time, PE microplastic size and PE concentration and their impact on PE microplastic removal. The studies were carried out at 21°C in a microplastic removal system using several size ranges of PE microplastic particles.

PE was selected as a model for microplastics in the study for the following reasons: PE is currently one of the most commonly used polymers in the plastic industry and its low and high density forms are found in water bodies ubiquitously [167] [168]. Among its applications in the industry, PE is used to make packaging products, primarily single used products that range from clear plastic bags to plastic films. Due to the extensive use of plastics and their persistence in the environment, numerous studies have reported the negative impact of plastic pollution in marine life, human health, among others [169].

In the aquatic environment, PE particles are fragmented into pieces that are of ecological concern as they are a vector for organic pollutants through absorption [170], [171]. These synthetic polymer is characterized to have an average size greater than 20 microns [172]. To replicate this material, a size diameter of PE particles in the range of 53-300 microns was selected.

Although there is evidence about microplastic degradation as a biological approach to reduce microplastic contamination, in our view, no study has been conducted to evaluate the efficiency of their biproducts to remove microplastic particles [173] [174]. Since it was shown in the previous chapter that rhamnolipids biosurfactant can effectively remove PE microplastics, the focus of this chapter is to investigate the potential of rhamnolipid biosurfactant to remove PE microplastic particles at different concentrations and operating conditions.

4.1. Materials and methods

Rhamnolipid biosurfactant purified from *Pseudomonas aeruginosa* as the microbial source was used. The biosurfactant with a purity of 90% was purchased from Sigma Aldrich, Oakville, Canada. The rhamnolipids obtained are a mixture of mono-rhamnolipids and di-rhamnolipids containing L-rhamnose and β-hydroxyl fatty acids.

Polyethylene microplastic powder samples with an average particle size of 53-59 microns and 125 microns were both obtained from Sigma Aldrich. On the other hand, Polyethylene powder, low density with an average particle size of 500 microns was purchased from Thermo Scientific Chemicals Canada.

4.1.1. Experimental Approach

Several investigations were carried out to assess the potential efficiency of rhamnolipids to remove PE microplastic particles of several sizes in a water system. These studies were carried out at a constant temperature of 21℃ in the microplastics removal apparatus. Aqueous solutions of 100 ml were prepared by adding distilled water, PE, and rhamnolipids at various concentrations. To guarantee homogeneous solutions, dry rhamnolipids were dissolved in distilled water with a constant agitation of 300 rpm for 4 minutes. The suspension was heated at 60℃ and cooled to 21℃. Rhamnolipids were directly added to the solution with no further purification.

The solution containing PE, rhamnolipids, and distilled water was added to the round flask and air was introduced into the system, acting as a mixing agent. Once bubbles started forming and foam was created, the foam traveled up the flask and through the condenser reaching the filtration apparatus by gravity. In the filtration system, the PE product was collected, placed in an oven overnight and heated at 80℃ to remove any traces of water. To avoid PE residue on the condenser walls, the instrument was rinsed with distilled water after every run and the product was filtrated.

4.1.2. Assessment of the PE removal efficiency using rhamnolipids foam

The potential ability of rhamnolipids based-foam to remove several sizes of PE microplastic particles was assessed using different settings of the rhamnolipids concentration variable combined with variations in the operating time. For the evaluation of the PE removal efficiency aqueous solutions of 100 ml were prepared. Each solution contained distilled water, rhamnolipids and PE microplastic particles. The concentrations of rhamnolipid biosurfactant in the solutions were 1 g/L, 2 g/L and 4 g/L, combined with variations of the PE concentration of 1 g/L, 1.5 g/L and 2 g/L. The experiments were carried out in the microplastic removal system with a bottom air inlet.

For the calculation of the PE removal rate, the mass of PE initially added to the system and the final mass of PE particles collected in the paper filter were recorded as defined in Eq. (1). The mass of PE microplastic particles present in deionized water was neglected.

4.1.3. Response surface methodology

Response surface methodology was applied to study the removal efficiency of PE. A full factorial design with 4 factors, and 3 levels was selected to study the microplastic removal efficiency (Y) of the system. The overall design of factors with their respective units, codes and levels is listed in

Table 4.1. The independent parameters rhamnolipid concentration (X_1), operating time (X_2), PE size (X_3), and PE concentration (X_4) were selected as part of the investigation according to previous studies on microplastic occurrence in water systems [15] [19]. The response variable was measured for each experiment, and the data was analyzed to identify the significant variables that affect Y. A total number of 81 data points with 1 replica were used as a part of this investigation.

Table 4.1. Coded factors with their units and levels for response surface analysis of PE removal using rhamnolipids

Factors	**Unit**	**Code**	**Level**		
			-1	**0**	**1**
Rhamnolipid concentration	g/L	X_1	1	2	4
Operating time	min	X_2	12	24	36
PE size	μm	X_3	53-59	125	300
PE concentration	g/L	X_4	1	1.5	2

Microplastic products were analyzed using Matlab software for technical computing. This software integrates computation and programming to analyze and design mathematical models, among other applications. Polyfitn, an extension of the polyfit package, was used to develop a model that correlates the response variable with more than one independent variable. Using experimental data, polyfitn was able to fit a polynomial regression model with 4 independent variables and a singular dependent variable for each type of the studied microplastics. A p-value smaller than 5% was used to identify main factors that influence microplastic recovery. The significance of the interaction between the factors was also assessed.

4.1.4. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy was used as a qualitative analysis to identify the polymer nature of collected plastics. This approach helps to rule out any other contaminants in the microplastic product that could lead to incorrect estimations. For each tested microplastic, the characteristic wave numbers were determined based on the FTIR spectrum.

Measurements were performed on a Bruker Tensor II system FTIR spectrometer with a Hyperion 2000 microscope at Surface Science Western, Canada. The system employs an Attenuated Total Reflectance accessory mod. Smart Performer. All spectra were obtained with a Ge crystal cell (maximum depth 0.8 mm).

4.1.5. Bubble size analysis of rhamnolipids

Images of the bubbles produced using rhamnolipids were processed in the image analysis software ImageJ. The bubble sizes were obtained from the ternary mixture containing water PE microplastic particles and rhamnolipids.

For the evaluation of the effect of polymer and rhamnolipid concentration in the bubble size, the variables rhamnolipid concentration, PE size and PE concentration were considered as part of the analysis. The experiments were carried out in the microplastics removal unit with an air flow rate of 2 LPM. Aqueous solutions (100 ml) containing rhamnolipids and distilled water were prepared. The concentrations of rhamnolipid biosurfactant in the solutions were 0.5 g/L, 1 g/L and 2 g/L. To guarantee homogeneous solutions, dry rhamnolipids were dissolved in distilled water with a constant agitation of 300 rpm for a minimum of 4 minutes. The suspension was heated at 60℃ and cooled to 21℃. Then, PE microplastics was added to the mixture in concentrations of 0.5 g/L, 1 g/L and 2 g/L.

Bubble photographs were captured using a NIKON D5600 camera with a AF-S NIKKON 18-140 mm lens. The images were opened in their native format and transformed into 8-bit images into gray images to remove noise. An LUT conversion profile was applied for better delimitation of the bubbles followed by an enhancement of contrast. A threshold of 50 was used for easier identification.

4.2. Results and discussions

The efficacy of PE removal was evaluated using rhamnolipids. The effect of rhamnolipid concentration, microplastic concentration, operating time and microplastic size were studied in order to determine the critical parameters that influence the PE removal efficiency.

4.2.1. PE removal efficiency of rhamnolipid based foam in a water-microplastic-biosurfactant system

The removal of polyethylene powder using rhamnolipids- foam is influenced by various factors, which are listed in Table 4.1. The initial experiments involved changing the concentration of rhamnolipids to determine how the removal of polyethylene microplastics changes over time. Fig 4.1 illustrates the efficiency of polyethylene microplastic removal for different concentrations of rhamnolipids and operating times, with a microplastic size of 53 μm in the system.

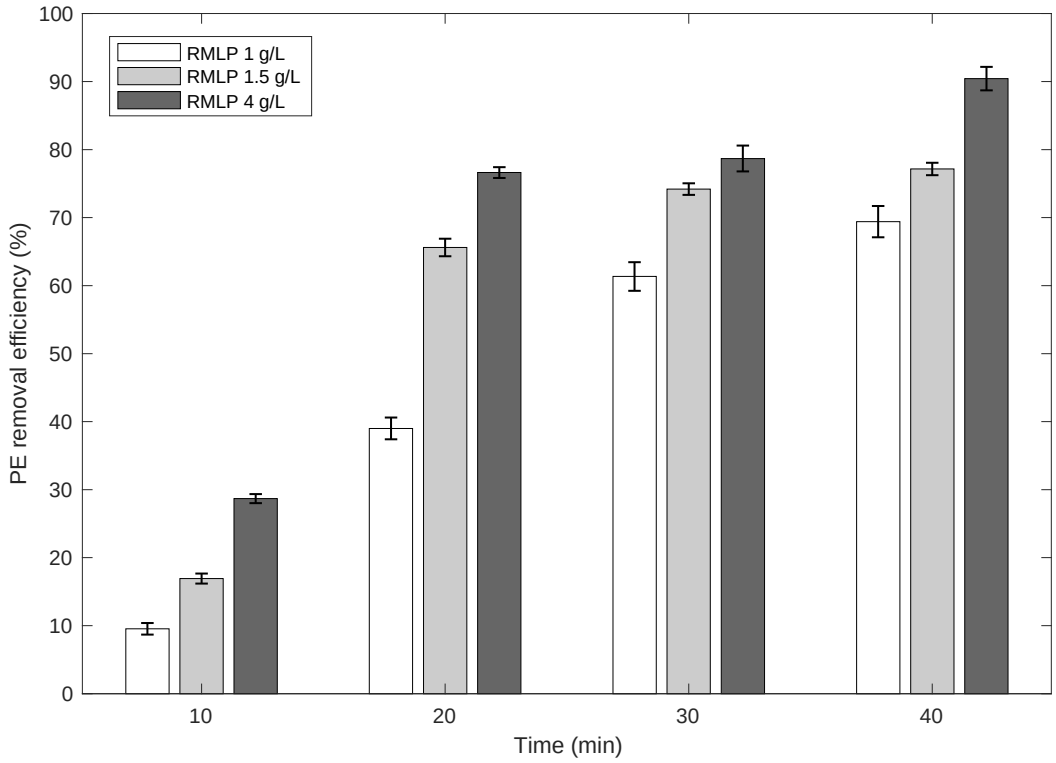

Figure 4.1 Effect of rhamnolipids concentration on the PE removal efficiency of a water-biosurfactant-polymer system as a function of time when the PE size is 53 μm

As shown in Fig. 4.1, there was an increment in the PE removal efficiency in a water- polymer-biosurfactant system when higher concentrations of rhamnolipids were used over time. When 1 g/L of rhamnolipids were added to the mixture, the PE removal efficiency increases from 9.86% at 10 min to 67.02% at 40 min. When the concentration of rhamnolipids in the system increased to 1.5 g/L a similar behavior was observed starting with a PE removal efficiency of 15.37% at 10 min to a PE removal efficiency of 78.9% at 40 min. When the highest concentration of rhamnolipids of 4 g/L was used, the system was able to remove the highest amount of PE microplastic particles being this 88.92 %.

A similar behavior occurred when the PE microplastic size increased to 125 μm. Figure 4.2 shows the effect of the rhamnolipids concentration variable on the PE removal efficiency of a water-biosurfactant-polymer system as a function of time when the PE size is 125 μm.

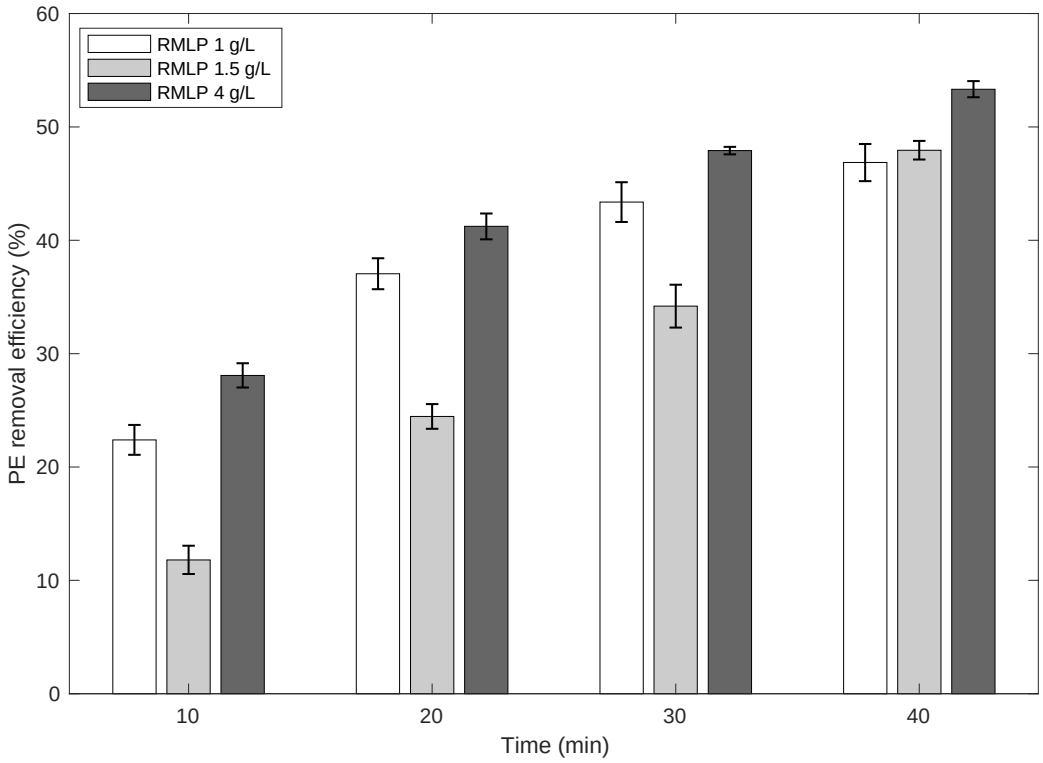

Figure 4.2. Effect of rhamnolipids concentration on the PE removal efficiency of a water-biosurfactant-polymer system as a function of time when the PE size is 125 μm

As shown in Fig. 4.2, when the concentration of PE microplastic size in the system increased to 125 μm, the PE removal efficiency followed a similar increasing behavior compared to the PE removal efficiency when the PE size in the system was smaller. According to this graph, there was an increment in the PE removal efficiency in the ternary system when higher concentrations of rhamnolipids were used over time. When 1 g/L of rhamnolipids were added to the mixture, the PE removal efficiency increased from 21.31% at 10 min to 44.87% at 40 min. When the concentration of rhamnolipids in the system increased to 1.5 g/L a similar behavior was observed starting with a PE removal efficiency of 10.43% at 10 min to a PE removal efficiency of 46.98% at 40 min. When the highest concentration of rhamnolipids of 4 g/L was used, the system was able to remove the highest amount of PE microplastic particles being this 52.76%. Although the increased in the rhamnolipid concentration had a positive impact in the PE removal efficiency of the system, the PE particle size, on the contrary suggests an inverse trend.

As shown in Fig. 4.3, for a higher PE microplastic size of 300 μm, one can observe an increase in the PE removal efficiency in a water- polymer-biosurfactant system over time. When 1 g/L of rhamnolipids were added to the mixture, the PE removal efficiency increases from 26.2 % at 10 min to 52.36%% at 40 min. When the concentration of rhamnolipids in the system increased to 1.5

g/L the PE removal efficiency followed the same increasing trend, starting with a removal of 9.92% at 10 min to a removal of 47.18% at 40 min. When the highest concentration of rhamnolipids of 4 g/L was used, the system achieved the highest removal of PE particles, being this 56.30%.

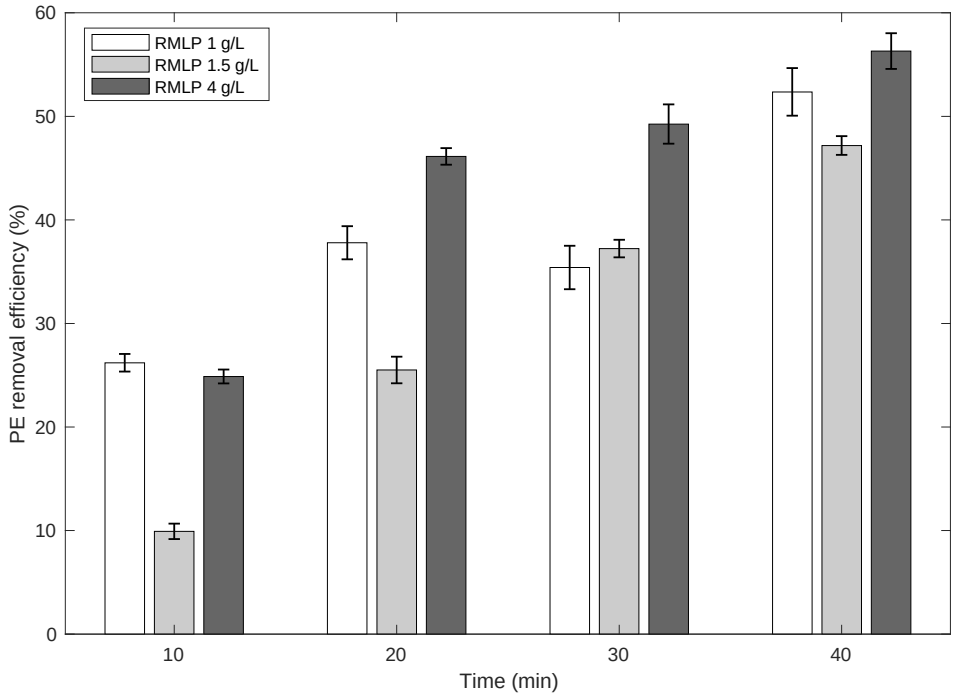

Figure 4.3. Effect of rhamnolipids concentration on the PE removal efficiency of a water-biosurfactant-polymer system as a function of time when the PE size is 300 μm

The results obtained, suggested that the removal of PE particles of various sizes could be influenced by several parameters. For this reason, further analysis of the parameters that significantly influenced the PE removal efficiency was conducted.

4.2.2. Experimental design and modelling

In order to calculate the PE removal efficiency in water using rhamnolipids foam, a least squares quadratic fit was used. The coefficients of each parameter and interaction, along with the standard error of the mean and pvalue are included in Table 4.1. The matrix of the full factorial design with the predictors and response variable is reported in Appendix A: Full factorial design for the investigation of PE removal efficiency.

Table 4.1. Polynomial coefficients with their p-value, standard error of the mean and tStat for the model predicting the PE removal efficiency using rhamnolipids

Estimated coefficients				
Variables	**Coefficients**	**pValue**	**tStat**	**SE**
X_1	-12.491	0.26677	-1.12	11.153
X_2	3.393	0.0005382*	3.6389	0.93242
X_3	0.051089	0.50237	0.67447	0.075747
X_4	-1.6552	0.95582	-0.055613	29.762
X_1X_2	0.26604	0.0028927*	3.0943	0.085978
X_1X_3	-0.017311	0.042167	-2.0721	0.0083541
X_1X_4	1.5462	0.45634	0.7493	2.0635
X_2X_3	-0.0049685	3.5009e-05*	-4.4403	0.001119
X_2X_4	-0.1919	0.48992	-0.69432	0.27638
X_3X_4	-0.0044804	0.86801	-0.16684	0.026855
X_1^2	2.7238	0.18006	1.3549	2.0103
X_2^2	-0.032176	0.052373*	-1.9757	0.016286
X_3^2	0.0001013	0.51232	0.6588	0.00015376
X_4^2	-1.9956	0.83219	-0.21273	9.3807
Intercept	0.13067	0.99638	0.0045505	28.715

The results in Table 4.1 represent the coefficients of a multiple regression model with one response variable and four predictor variables. The model included all dependent variables X_1, X_2, X_3, and X_4, as well as their interactions and quadratic terms. The information provided includes the estimated coefficients, their standard errors (SE), t-statistics, and associated p-values for testing the null hypothesis. In this case, the null hypothesis stated that there is no significant relationship between the independent factors and the response variable.

In terms of the p-value, some of the predictors and their interactions are significant at the 5% level. This means, that the p-values of less than 5% have a significant effect on the response variable. Among the independent variables, X_2 and some of its interactions ($X_1.X_2$ and $X_2.X_3$) are statistically significant (p-values < 0.05), while X_1 and X_3 are not (p-values > 0.05). The quadratic term for X_2 could also be considered as significant since its p-value of 0.05237 is very close to the limit of 0.05, while the other quadratic terms are not. The intercept term is statistically insignificant, with a p-value of 0.99638, which means that it is less likely to have an effect in Y. In the context of this study, the intercept was not eliminated from the model, as it represents the efficiency of PE removal when all the variables are zero.

The values obtained in Table 4.1 include a coefficient of determination (R^2) 0.82. This suggests that the model explains a large portion of the variability in the PE removal efficiency [175]. The model also has an adjusted R^2 value of 0.782. This means, that approximately 78.2% of the variation in Y is explained by the model. The adjusted R^2 is a modification of R^2 that has been adjusted for the number of predicting factors in the model and an indication of good fit [176]. The

F-statistic of 21.5 with a very low p-value suggests that the model is significantly better than the constant model, which has no predictors [177].

The root mean squared error (RMSE) of the model calculated through the ANOVA analysis was found to be 9.95. This value represents the absolute error measure of the residuals, which indicates the degree to which the model's predictions deviate from the actual observations [178]. A lower RMSE indicates a better accuracy of the model [179] [180]. Nevertheless, an RMSE value of 9.95 is not extremely high, suggesting that the model has a reasonable predictive power, which is confirmed by the R^2 value of 0.82. The F-statistic of the model (21.15) with a p-value of 2.74e-19 suggests that the overall model is statistically significant, meaning that at least one of the predictors has a non-zero effect on the response variable.

The graph presented in Figure 4.4, shows a scatter plot of the adjusted predicted values in y versus the adjusted response values y for the linear regression model. The plot, assesses the goodness of fit of the model, showing a reasonably straight line with some deviating points that do not fit the general pattern of the data.

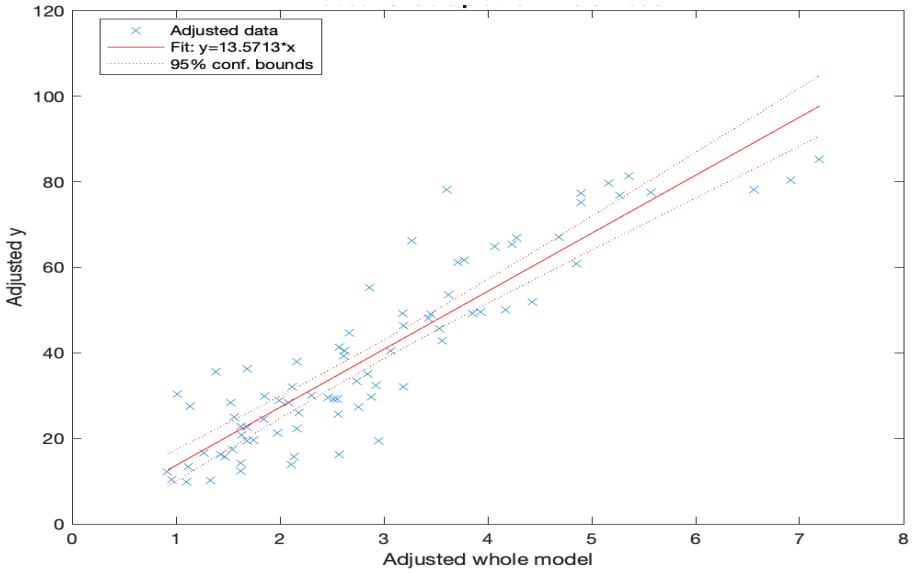

Figure 4.4. Added plot for the whole model

The plot is in accordance with the model presented in Table 4.1, where the regression obtained, explains 82% of the variations in the model, with some residual error that is not explained by the predictor variables.

4.2.3. FTIR spectra of collected microplastics

FTIR spectra of collected microplastic particles is shown in Fig. 4.5. The FTIR spectra plot, displays the wavenumber in cm^{-1} of the infrared radiation in the x-axis and in the y-axis, the

absorbance of the radiation. The absorption bands in Fig. 4.2 at 2916 and 2849 cm^{-1} are attributed to aromatic and aliphatic -C-H bond stretching. The absorption band in the FTIR spectrum at around 1471 cm^{-1} is often attributed to the bending of the CH_2 group in the PE structure [181] [182]. Even though this peak is not as prominent as the aromatic and aliphatic stretching at around 2900 cm^{-1}, it is a complimentary peak to confirm the presence of PE in the sample. Absorption peaks at around 729 to 718 cm^{-1} are also characteristics peaks of the PE spectra [181] [182]. These peaks are associated by the rocking of the CH_2 group.

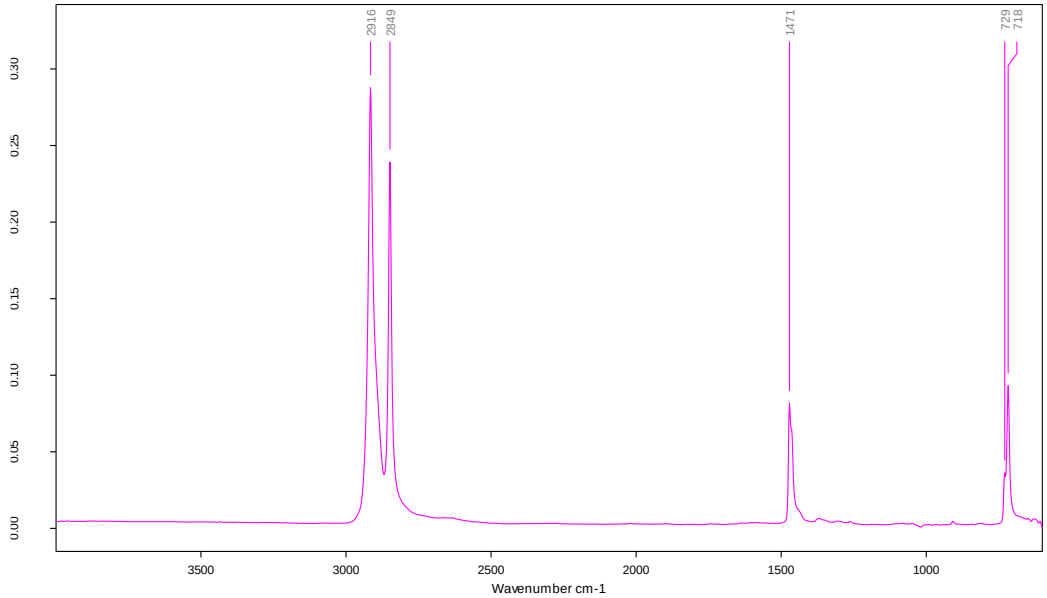

Figure 4.5. FTIR spectra of collected microplastics

Overall, the FTIR spectrum presented in Fig. 4.5 showed that the functional groups identified in the spectra, correspond to the presence of PE in the collected sample. PE is characterized by strong absorption peaks in the 2900-2839 cm^{-1} range, which are indicative of the aromatic group in the PE structure [183]. Thus, the spectra confirms there are no other peaks that provide information of other content or material in the collected product.

4.2.4. Bubble size analysis of rhamnolipids

Fig 4.6 shows the effect of the rhamnolipid concentration and PE concentration upon the bubble size of the produced foam. Bubble size diameters for PE microplastic particles are presented in Appendix D. Table D.2. According to Figure 4.6a, when the C_{PE} in the ternary system is 0 g/L, the variation in the bubble diameter has a decreasing tendency from 0.23 cm (C_{RMLP} = 0 g/L) to 0.065 cm (C_{RMLP} = 2 g/L). This decreasing trend in the bubble size is also observed for all three PE sizes when polymer was added to the system. Specifically, as indicated in Fig 4.6b, when the smallest PE size range of 53 μm was present in the liquid mixture, the bubble diameter experienced a reduction from 0.165 cm when the C_{RMLP} was 0.5 g/L, to a size of 0.086 cm when the C_{RMLP}

increased to 2 g/L. Similarly, in Fig 4.6c, when the PE size increased to 125 μm, the bubble diameter decreased from 0.123 cm when C_{RMLP} is 0.5 g/L to 0.04 cm at C_{RMLP} is 2 g/L. Finally in Fig 4.6d, the bubble size decrement was also favored when the PE size is 300 μm. Here, the bubble diameter reached 0.141 cm when the concentration of rhamnolipids is 0.5 g/L and 0.078 for a concentration of rhamnolipids of 2 g/L.

Our findings indicate that with the addition of polymer solute to the system, one can observe a reduction in the bubble diameter. Moreover, when the polymer concentration increases in a water-polymer-biosurfactant system, the formation of smaller bubbles is exhibited.

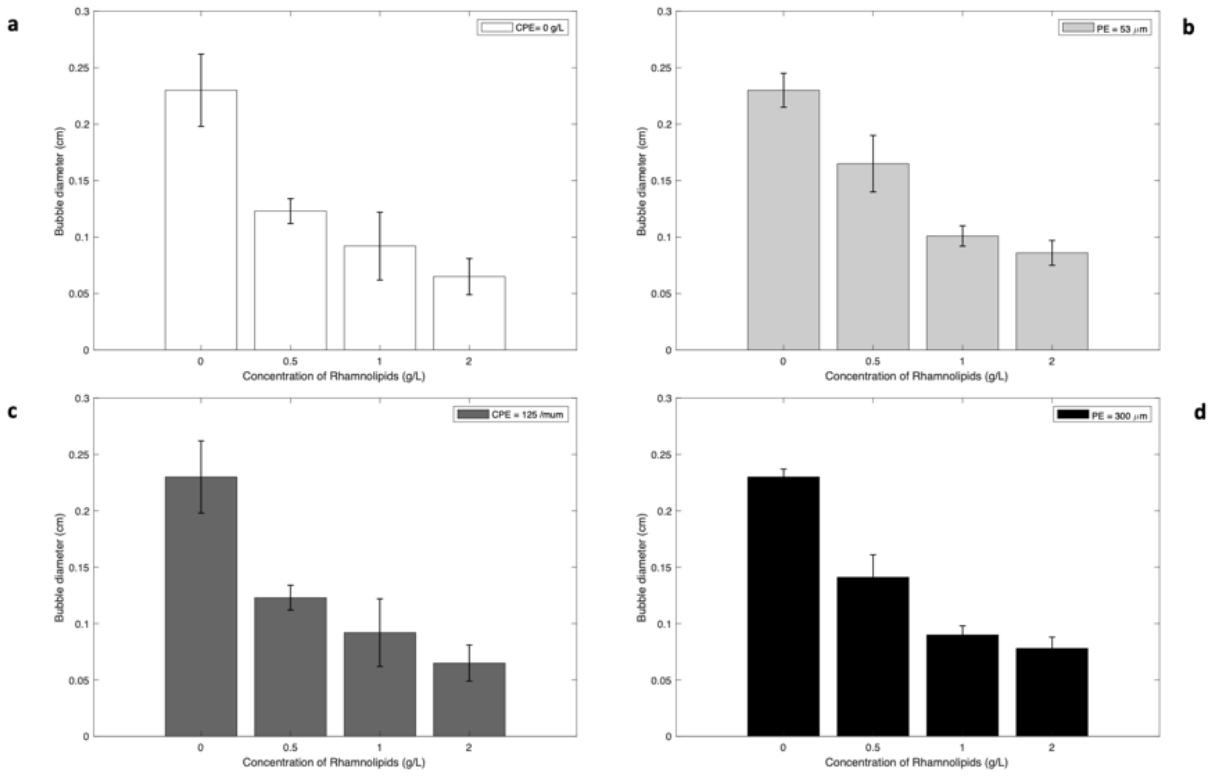

Figure 4.6. Effect of rhamnolipids concentration and PE concentration upon bubble's size. a) C_{PE} = 0 g/L, b) C_{RMLP} = C_{PE} when PE size is 53 μm, c) C_{RMLP} = C_{PE} when PE size is 125 μm, c) C_{RMLP} = C_{PE} when PE size is 300 μm

Our results on the bubble diameter of rhamnolipids based-foam agree with the findings of a recent study that investigated the bubble size and bubble distribution in a polymer-surfactant-water system. According to the study, when gas was introduced into the liquid phase, larger bubbles were produced at higher polymer concentrations in the absence of surfactant. However, increasing the polymer concentration also led to the rupture of large bubbles, generating smaller-sized bubbles and increasing the gas-liquid interfacial area at high polymer concentrations [160].

According to several investigations, the occurrence of small bubbles is due to the rupture of larger bubbles caused by the viscosity of the aqueous solution [184]. Moreover, the presence of surfactant in the ternary mixture increases the gas-liquid interfacial area compared to solutions without surfactant.

4.3. Conclusions

The study's findings suggest that rhamnolipids biosurfactant is able of effectively capturing and removing polyethylene microplastic particles of different sizes. The study also developed a model that correlates the factors rhamnolipid concentration (X_1), operating time (X_2), plastic size (X_3), and plastic concentration (X_4) and the response variable PE removal efficiency (Y), achieving a multiple regression with an R^2 of 0.82, indicating a good fit of the model. Among the independent variables, the operating time variable and its interactions with both the rhamnolipid concentration and plastic size parameters were statistically significant at the 5% level.

Additionally, the investigation found that in a ternary system, the PE concentration had a decreasing influence on the bubble diameter of rhamnolipids based-foam. The results in the bubble size revealed that an increase in the concentration of rhamnolipids within the system led to a decrease in the bubble size when no polymer was present in the system. However, with the addition of PE solute there is a slight decrement in the bubble diameter regardless of the PE particle size. This behavior can be explained by the foam collapse and bubble rupture, favoring the generation of a considerable number of bubbles of less than 1mm size [160].

CHAPTER 5

5. Investigation of car tire residue removal using rhamnolipids based-foam

This chapter reports a set of experiments that were conducted to investigate the influence of different parameters on the removal of car tire waste using rhamnolipids biosurfactant. The factors selected in the study were rhamnolipid concentration, operating time, car tire residue (Ctr) size and Ctr concentration. The experiments were carried out at 21°C in a microplastic removal system using Ctr microplastic particles.

Car tire residue was selected as a model for microplastics in the study due its occurrence in different environments. Automotive car tires are composed of a mixture of synthetic materials such as rubber, polyester, nylon fiber, additives and fillers [27] [185]. These materials shed as car tire particles, characterized as airborne and road wear particles generated by the rolling shear of tread against a surface [186]. Tire particles can aggregate to other road wear particles such as pavement, brake dust and atmospheric deposition, contaminating aquatic environments [187]. Several studies have found that car tire emissions contribute nowadays to water pollution in rivers, estuaries, among others [29]. A recent study from the University of British Columbia found that more than 50 tonnes of tire and road wear particles are released into waterways annually in the Okanagan area [28]. Another study, reported auto tire emissions in surface waters are greater than 8.500 tonnes annually [25].

Car tire residue pollution in water is a matter of emerging concern since it not only affects aquatic ecosystems but can also harm them. Car tire microplastic particles contain toxic chemicals such as 6ppd quinone, metals, persistent organic pollutants, among other substances, that can negatively impact and kill aquatic organisms [188] [189]. With humans as top predators, Ctr can pose risks to human health caused by the ingestion of microplastic particles in food sources or from drinking water [190] [191]. For this reason, different techniques that include physical and chemical treatments have been used to mitigate the amount of auto tire particles in water streams [192] [193]. However, these treatments have limited effectiveness to remove Ctr particles.

Some potential approach uses microbial surfactant for the degradation of microplastic particles and removal of mineral compounds [85] [132] [133], [134]. Since biosurfactants can remove metal particles and have affinity for both hydrophilic and hydrophobic phases, one can say they are capable of potentially remove microplastic particles [135]. Although recent studies have used different methods to remove Ctr from water, in our view, no study has been conducted to evaluate the efficiency of rhamnolipids biosurfactant to remove Ctr microplastic particles [173] [174]. The focus of this chapter is to investigate the potential of rhamnolipid biosurfactant to remove Ctr microplastic particles at different concentrations and operating conditions.

5.1. Materials and methods

Rhamnolipid biosurfactant purified from *Pseudomonas aeruginosa* as the microbial source was used. The biosurfactant with a purity of 90% was purchased from Sigma Aldrich, Oakville, Canada. The rhamnolipids obtained are a mixture of mono-rhamnolipids and di-rhamnolipids containing L-rhamnose and β-hydroxyl fatty acids.

Automotive car tire residue was obtained from Sparton Enterprises Inc in Ohio with a density of 1.2 g/ml. The composition of the tire waste product was as follows: natural rubber (17.3%), synthetic rubber (17.9%), carbon black (28%), steel (14.5%), fillers (16.5%), glass (5.8%), ash content (5.1%) A vibratory sifter was used to separate Ctr particles into different sizes using vibrating motions. These allowed for the Ctr dry powder to be separated into samples of 53, 90 and 300 microns. Car tire waste is now considered a common type of microplastic found in water streams according to several investigations [194], [195].

5.1.1. Experimental Approach

Several investigations were carried out to assess the potential efficiency of rhamnolipids to remove Ctr in a water system. These studies were carried out at a constant temperature of 21℃ in the microplastics removal apparatus. Aqueous solutions of 100 ml were prepared by adding distilled water, Ctr, and rhamnolipids at various concentrations. To guarantee homogeneous solutions, dry rhamnolipids were dissolved in distilled water with a constant agitation of 300 rpm for 4 minutes. The suspension was heated at 60℃ and cooled to 21℃. Rhamnolipids were directly added to the solution with no further purification.

The solution containing Ctr, rhamnolipids, and distilled water was added to the round flask and air was introduced into the system, acting as a mixing agent. Once bubbles started forming and foam was created, the foam traveled up the flask and through the condenser reaching the filtration apparatus by gravity. In the filtration system, the Ctr product was collected, placed in an oven overnight and heated at 80℃ to remove any traces of water. To avoid Ctr residue in the condenser walls, the instrument was rinsed with distilled water after every run and the product was filtrated.

5.1.2. Response surface methodology

Response surface methodology was applied to study the removal efficiency of Ctr. A full factorial design with 4 factors, and 3 levels was selected to study the Ctr removal efficiency (Y) of the system. The overall design of factors with their respective units, codes and levels is listed in Table 5.1. The independent parameters rhamnolipid concentration (X_1), operating time (X_2), Ctr size (X_3), and Ctr concentration (X_4) were selected as part of the investigation according to previous studies on microplastic occurrence in water systems [15] [19]. The response variable was measured

for each experiment, and the data was analyzed to identify the significant variables that affect Y. A total number of 81 data points with 1 replica were used as a part of this investigation.

Table 5.1. Coded factors with their units and levels for response surface analysis of Ctr removal using rhamnolipids

Factors	Unit	Code	Level		
			-1	**0**	**1**
Rhamnolipid concentration	g/L	X_1	1	2	4
Operating time	min	X_2	12	24	36
Ctr size	μm	X_3	53	90	300
Ctr concentration	g/L	X_4	1	1.5	2

Analysis of microplastic products was carried out using Matlab, which is a computational and programming tool. The extension polyfitn was used to create a multiple regression model that connects the predictors with the dependent variable. By using experimental data, a polynomial regression model with four independent variables and one dependent variable was developed. To determine the factors that affect microplastics recovery, a p-value less than 5% was used.

Using experimental data, polyfitn was able to fit a polynomial regression model with 4 independent variables and a singular dependent variable for each type of the studied microplastics. A p-value smaller than 5% was used to identify main factors that influence microplastic recovery. The significance of the interaction between the factors was also assessed.

5.1.3. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy was used as a qualitative analysis to identify the polymer nature of collected plastics. This approach helps to rule out any other contaminants in the microplastic product that could lead to incorrect estimations. For each tested microplastic, the characteristic wave numbers were determined based on the FTIR spectrum.

Measurements were performed on a Bruker Tensor II system FTIR spectrometer with a Hyperion 2000 microscope at Surface Science Western, Canada. The system employs an Attenuated Total Reflectance accessory mod. Smart Performer. All spectra were obtained with a Ge crystal cell (maximum depth 0.8 mm).

5.1.4. Bubble size analysis of rhamnolipids

Images of the bubbles produced using rhamnolipids were processed in the image analysis software ImageJ. The bubble sizes were obtained from the ternary mixture containing water, Ctr microplastic particles and rhamnolipids.

For the evaluation of the effect of polymer and rhamnolipid concentration in the bubble size, the variables rhamnolipid concentration, Ctr size and Ctr concentration were considered as part of the analysis. The experiments were carried out in the microplastics removal unit with an air flow rate of 2 LPM Aqueous solutions (100 ml) containing rhamnolipids and distilled water were prepared. The concentrations of rhamnolipid biosurfactant in the solutions were 0.5 g/L, 1 g/L and 2 g/L. To guarantee homogeneous solutions, dry rhamnolipids were dissolved in distilled water with a constant agitation of 300 rpm for a minimum of 4 minutes. The suspension was heated at 60℃ and cooled to 21℃. Then, Ctr microplastics was added to the mixture in concentrations of 0.5 g/L, 1 g/L and 2 g/L.

Bubble photographs were captured using a NIKON D5600 camera with a AF-S NIKKON 18-140 mm lens. The images were opened in their native format and transformed into 8-bit images into gray images to remove noise. An LUT conversion profile was applied for better delimitation of the bubbles followed by an enhancement of contrast. A threshold of 50 was used for easier identification.

5.2. Results and discussions

The efficacy of Ctr removal was evaluated using rhamnolipids. The effect of rhamnolipid concentration, microplastic concentration, operating time and microplastic size were studied in order to determine the critical parameters that influence the Ctr removal efficiency.

5.2.1. Ctr removal efficiency of rhamnolipid based foam in a water-microplastic-biosurfactant system

The removal of car tire residue of various sizes using rhamnolipids based-foam can be impacted by numerous variables. Table 5.1 includes all the variables that could impact the removal process. This study conducted initially batch removal experiments altering the concentration of rhamnolipids to measure the Ctr removal efficiency over time. Fig.5.1 illustrates the Ctr microplastic removal efficiency as a function of time when Ctr microplastics were 53 microns in size and various concentrations of rhamnolipids were used.

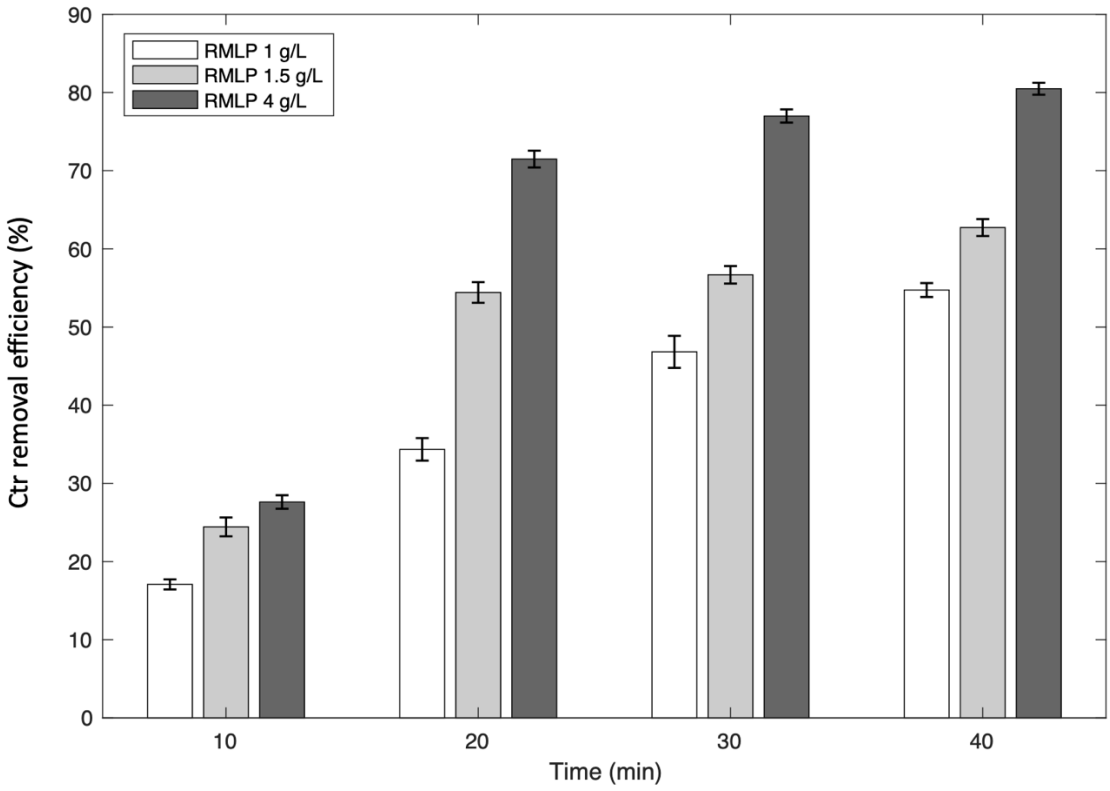

Figure 5.1. Effect of rhamnolipids concentration on the Ctr removal efficiency of a water-biosurfactant-polymer system as a function of time when the Ctr size is 53 μm

As shown in Fig. 5.1, there was an increment in the Ctr removal efficiency in a water- polymer-biosurfactant system when higher concentrations of rhamnolipids were used over time. When 1 g/L of rhamnolipids were added to the mixture, the Ctr removal efficiency increases from 17.08% at 10 min to 54.73% at 40 min. When the concentration of rhamnolipids in the system increased to 1.5 g/L a similar behavior was observed starting with a Ctr removal efficiency of 24.43% at 10 min to a Ctr removal efficiency of 62.725% at 40 min. When the highest concentration of rhamnolipids of 4 g/L was used, the system was able to remove the highest amount of Ctr microplastic particles being this 80.48%.

A similar behavior occurred when the Ctr microplastic size increases to 90 μm. Figure 5.2 shows the effect of the rhamnolipids concentration variable on the Ctr removal efficiency of a water-biosurfactant-polymer system as a function of time when the Ctr size was 90 μm.

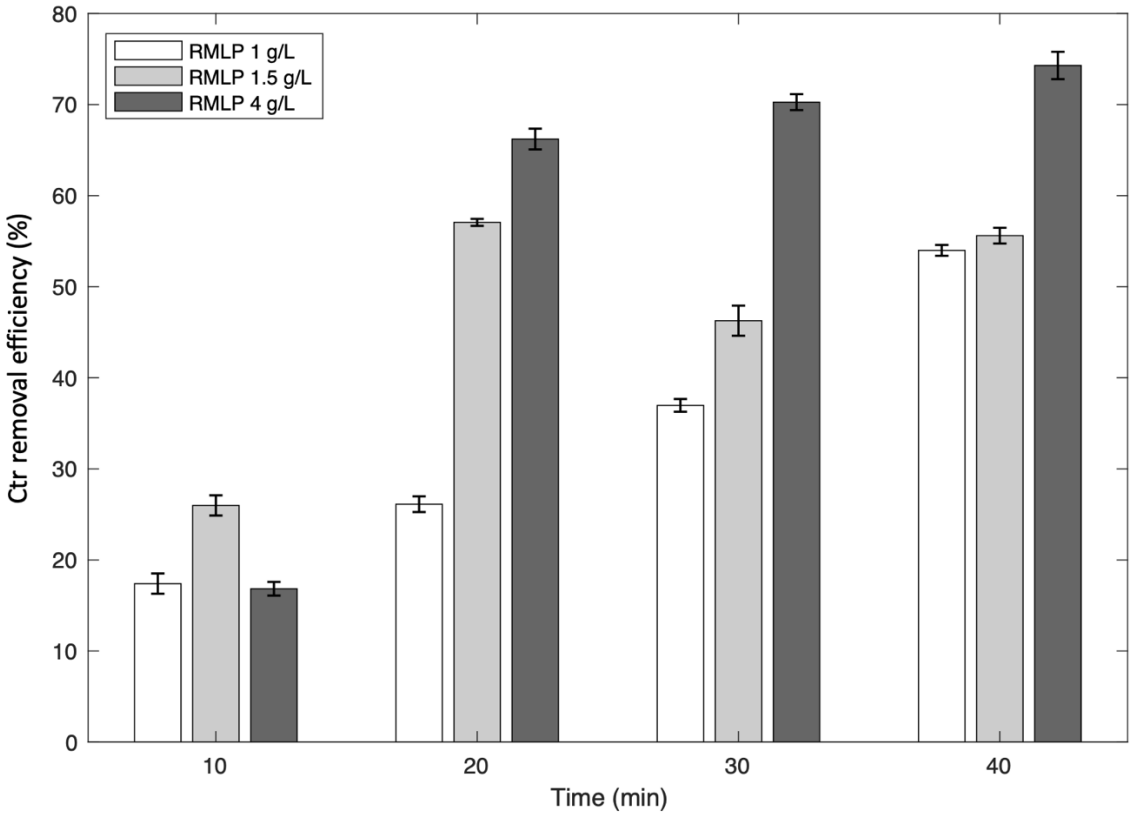

Figure 5.2. Effect of rhamnolipids concentration on the Ctr removal efficiency of a water-biosurfactant-polymer system as a function of time when the Ctr size is of 90 μm

As shown in Fig. 5.2, when the concentration of Ctr microplastic size in the system increased to 90 μm, the Ctr removal efficiency followed a similar increasing behavior compared to the Ctr removal efficiency when the Ctr size in the system was smaller. According to this graph, there is an increment in the Ctr removal efficiency in the ternary system when higher concentrations of rhamnolipids are used over time. When 1 g/L of rhamnolipids were added to the mixture, the Ctr removal efficiency increased from 17.40% at 10 min to 53.9925% at 40 min. On the contrary, when the concentration of rhamnolipids in the system increased to 1.5 g/L, the Ctr removal efficiency increased from 25.98% (10 min) to 57.06% (20 min), but experienced a decrement to 46% at 30 min, with a slight increment to 55.61% at 40 min. When the highest concentration of rhamnolipids of 4 g/L was used, the system was able to remove the highest amount of Ctr microplastic particles being this 74.29%. Although the increased in the rhamnolipid concentration had a positive impact in the Ctr removal efficiency of the system, the Ctr particle size, on the contrary suggests an inverse trend.

As it is observed in Fig. 5.3, for a higher Ctr microplastic size of 300 μm, one can observe an increase in the Ctr removal efficiency in a water- polymer-biosurfactant system over time. When 1 g/L of rhamnolipids were added to the mixture, the Ctr removal efficiency increases from 11.83% at 10 min to 29.87% at 40 min. When the concentration of rhamnolipids in the system increased

to 1.5 g/L the Ctr removal efficiency followed the same increasing trend, starting with a removal of 32.92% at 10 min to a removal of 48.83% at 40 min. When the highest concentration of rhamnolipids of 4 g/L was used, the system achieved the highest removal of Ctr particles, being this 48.83%.

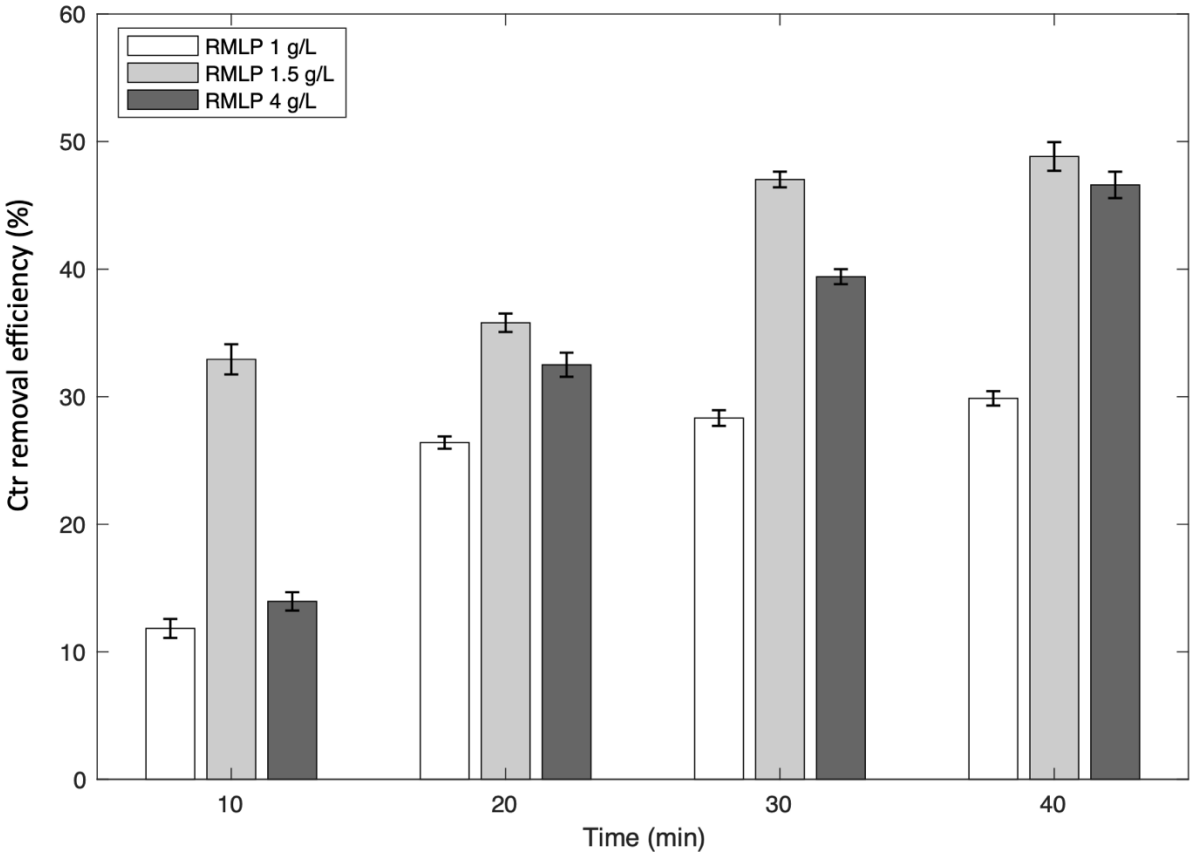

Figure 5.3. Effect of rhamnolipids concentration on the Ctr removal efficiency of a water-biosurfactant-polymer system as a function of time when the Ctr size is of 300 μm

The results obtained, suggested that the removal of Ctr particles of various sizes could be influenced by several parameters. For this reason, further analysis of the parameters that significantly influenced the Ctr removal efficiency was conducted.

5.2.2. Experimental design and modelling

In order to calculate the Ctr removal efficiency in water using rhamnolipids foam, a least squares quadratic fit was used. The coefficients of each parameter and interaction, along with the standard error of the mean and pvalue are included in Table 5.2. The matrix of the full factorial design with the predictors and response variable is reported in Appendix B: Full factorial design for the investigation of Ctr removal efficiency.

Table 5.2. Polynomial Coefficients with their p-value, standard error of the mean and tStat for the model predicting the Ctr removal efficiency using rhamnolipids

Estimated coefficients				
Variables	**Coefficients**	**pValue**	**SE**	**tStat**
X_1	39.811	1.5761e-06*	7.546	5.2757
X_2	5.6315	5.8775e-13*	0.63089	8.9263
X_3	0.16243	0.0023173*	0.051252	3.1692
X_4	3.4741	0.86356	20.137	0.17252
X_1X_2	0.25277	4.9117e-05*	0.058174	4.3451
X_1X_3	-0.025317	3.0502e-05*	0.0056526	-4.4788
X_1X_4	0.19785	0.88774	1.3962	0.14171
X_2X_3	-0.0039264	2.2202e-06*	0.0007571	-5.186
X_2X_4	-0.092685	0.6218	0.187	-0.49563
X_3X_4	-0.010157	0.57805	0.018171	-0.55901
X_1^2	-7.273	1.1986e-06*	1.3602	-5.347
X_2^2	-0.088089	2.7137e-11*	0.011019	-7.994
X_3^2	-0.00022272	0.035986*	0.00010404	-2.1408
X_4^2	-2.2304	0.90311	6.3472	-0.3514
Intercept	-77.628	0.00016519*	19.429	-3.9954

The results of the multiple regression model presented in Table 5.2 aim to explain the correlation between four independent variables (X_1, X_2, X_3, X_4) and one response variable (Y). The model includes 15 terms with their coefficients, including the four predictors with their combinations and their squared terms. The standard errors, t-statistics, and associated p-values are also presented for testing the null hypothesis that the corresponding coefficient is equal to zero. In this case, the null hypothesis stated that there is no significant relationship between the independent variables and the response.

The SE column represents the standard error of the estimated coefficients, which indicates the precision of the estimate. The tStat column represents the t-statistic for testing whether the estimated coefficient is significantly different from zero. The pValue column represents the pValue associated with the t-statistic and indicates the level of significance of the coefficient estimate.

The results of the regression model suggest that the four predictor variables (X_1, X_2, X_3) have a significant impact on the response variable (Y). Specifically, we can see that X_1 has the strongest effect on Y with an estimated coefficient of 39.811 and a very low p-value of 1.5761e-06. This means that the factor rhamnolipid concentration is statistically significant at the 0.05 level, indicating that an increase in the concentration of rhamnolipid is associated with an increase in the Ctr removal efficiency, holding all other variables constant. Additionally, X_2 and X_3 have moderate effects on Y and p-values less of than 0.05. The coefficient for X_2 (5.6315) is positive and statistically significant, indicating that an increase in the operating time of the experiment is associated with an increase in the Ctr removal efficiency. The coefficient of X_3 of 0.16243 is also

positive and significant, meaning that the microplastic size has an effect in the response. On the other hand, X_4 has a coefficient of 3.4741 but its p-value is greater than 0.05, which suggests that the microplastic concentration does not appear to be a significant predictor of Y in this model.

The interaction terms in the model ($X_1.X_2$, $X_1.X_3$, $X_1.X_4$, $X_2.X_3$, $X_2.X_4$ and $X_3.X_4$) represent the effect of the interaction between two independent variables on the dependent variable. The coefficient for $X_1.X_2$ of 0.25277 is positive and statistically significant, indicating that the effect of X_1 on Y depends on the level of X_2. The coefficient for $X_1.X_3$ (-0.025317) is negative and statistically significant, showing that the interaction between X_1 and X_3 influences the response variable Y. The coefficient for $X_1.X_4$ (0.19785) is positive but not significant and may not have a strong or consistent effect on the Ctr removal efficiency. The coefficient for $X_2.X_3$ (-0.0039264) is negative and significant, which tell us that the interaction between these factors influence Y. The coefficient for $X_2.X_4$ and $X_3.X_4$ are not statistically significant, indicating that the interactions between X_2 and X_4, and between X_3 and X_4 are not good predictors of the Ctr removal efficiency.

The squared term X_1^2 has a negative coefficient of -7.273, indicating that the effect of X_1 on Y is not linear, but instead decreases as X_1 increases beyond a certain point. The squared term X_2^2 has a negative coefficient of-0.088089, suggesting a similar non-linear effect on X_2 on Y. The squared term X_3^2 has a negative coefficient of -0.00022272, but its p-value of 0.035986 suggests that it is only marginally statistically significant in this model. The squared term X_4^2 has a coefficient of -2.2304, but its p-value of 0.72641 suggests that it is not statistically significant predictor of Y in this model.

The intercept term (-77.628) represents the expected value of Y when all predictor variables are equal to zero. The coefficients of the predictor variables indicate the change in Y for one-unit increase in each variable, holding all other variables constant.

The R^2 value (0.895) obtained through the ANOVA analysis represents the proportion of variation in the response variable that is explained by the predictor variables. In this case, the model explains 89.5% of the variance in the response variable, which is considered a good fit. The adjusted R^2 value (0.873) adjusts for the number of predictor variables in the model.

The F-statistics tests the overall significance of the model, which compares the model with all predictor variables to a model with only the intercept term. The p-value associated with the F-statistic indicates that the model is statistically significant ($p < 0.001$).

Finally, the root mean squared error (RMSE) indicates the average difference between the observed and predicted values of the response variable. In this case, the RMSE is 6.73, which means that the model's predictions are on average within 6.73 units of the actual values.

The graph presented in Figure 5.3, shows a scatter plot of the adjusted predicted values in y versus the adjusted response values y for the linear regression model. The plot, assesses the goodness of fit of the model, showing a reasonably straight line with some deviating points that do not fit the general pattern of the data.

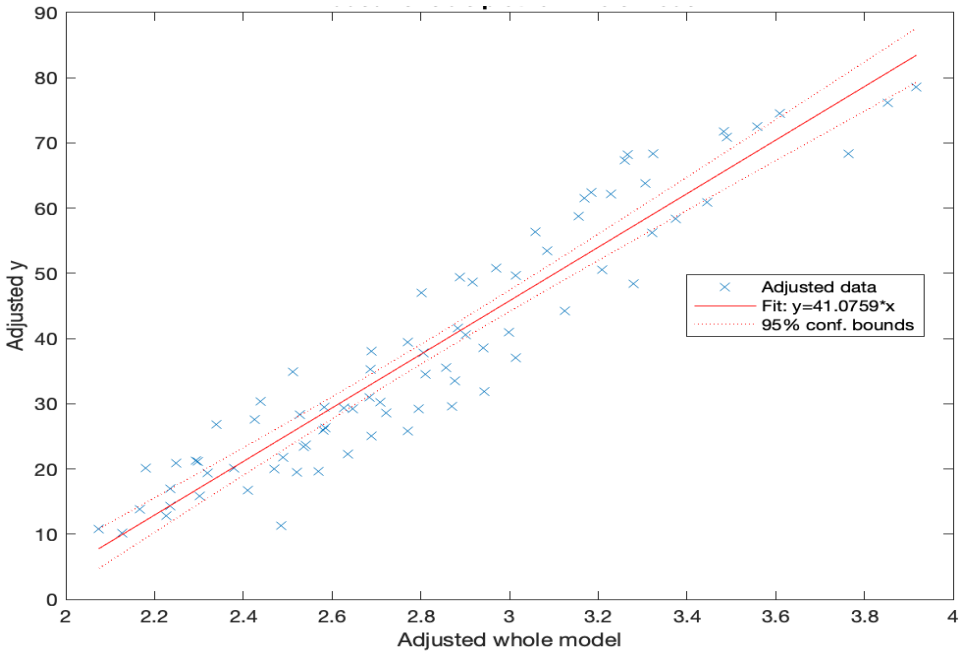

Figure 5.4. Added plot for the whole model

5.2.3. FTIR spectra of collected Ctr microplastic particles

Data obtained from recovered car tire powder spectra was used to identify the presence of rubber compounds [196]. An image of the recovered Ctr microplastic particles that were selected for FTIR analysis can be found in Appendix C: Car tire residue.

The FTIR plot displayed in Figure 5.5 shows the absorption of infrared radiation as a function of wavenumber in cm^{-1}. According to FTIR spectra presented in Figure 5.5., our findings indicate that one of the materials present in the collected microplastic sample corresponds to the absorption spectra of calcium carbonate ($CaCO_3$). This common material is naturally abundant, inorganic, and inexpensive [197]. In tires, $CaCO_3$ is used primarily as a reinforcing agent to improve properties of tires [198]. It also helps to improve the wear properties and reduces the rolling resistance of the tire, which can improve fuel efficiency and reduce carbon emissions [199].

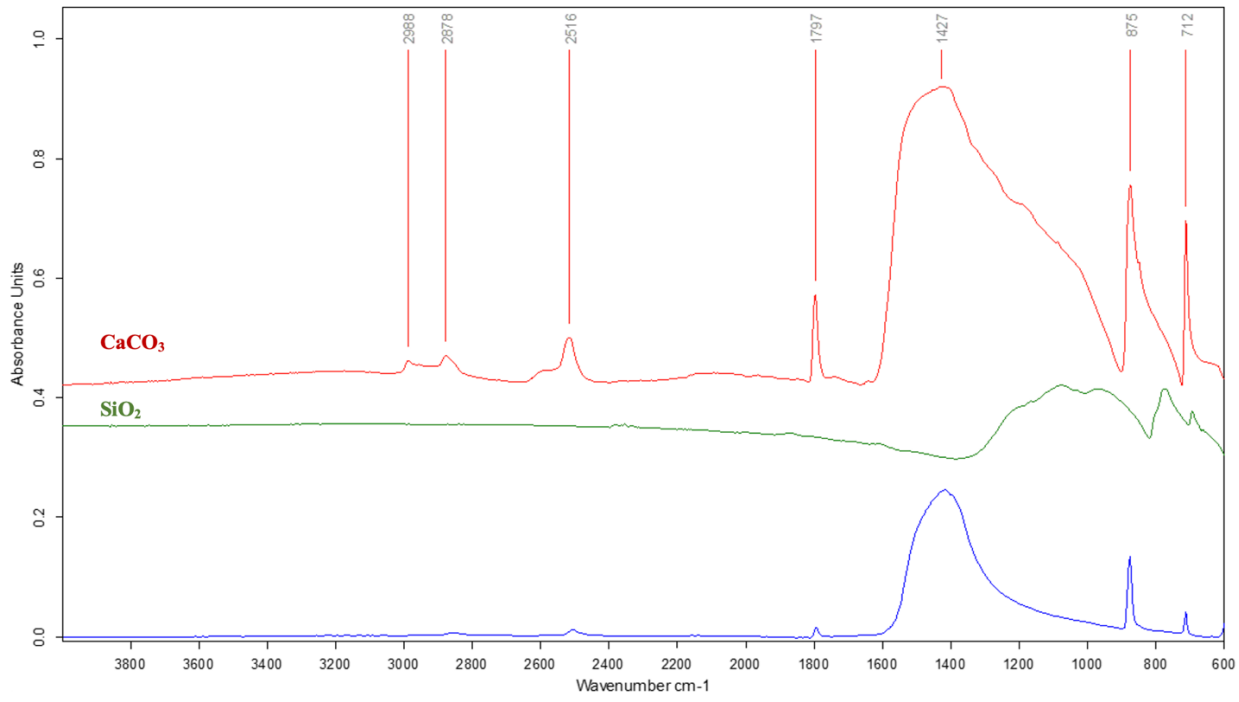

Figure 5.5. FTIR spectra of $CaCO_3$ and SiO_2

Typical FTIR spectra of PET is shown in Figure 5.6. The absorption bands at 3100 to 2800 cm^{-1} are attributed to aromatic and aliphatic -C-H bond stretching. A sharp absorption peak around 1720 cm^{-1}, corresponds the C=O stretching vibration in the ester group (-COO-) of PET. There is a relative weak absorption band in the FTIR spectrum at around 1412 cm^{-1} and it is often attributed to the deformation of the aromatic ring in the PET structure [200] [201] [202]. Even though this peak is not as prominent as the C=0 stretching at 1720 cm^{-1}, it is a complimentary peak to confirm the presence of PET in the sample. Absorption peaks at around 1270 cm^{-1} also show the asymmetry stretch of the C-C-O group bonded to the aromatic ring. The spectral absorption displayed at 1085 cm^{-1}, corresponds to the C-O stretching and C-H bending vibrations, respectively. Additional weak absorption peaks at around 2950 cm^{-1} which correspond to the symmetric CH_2 stretching vibrations of the PET backbone [203].

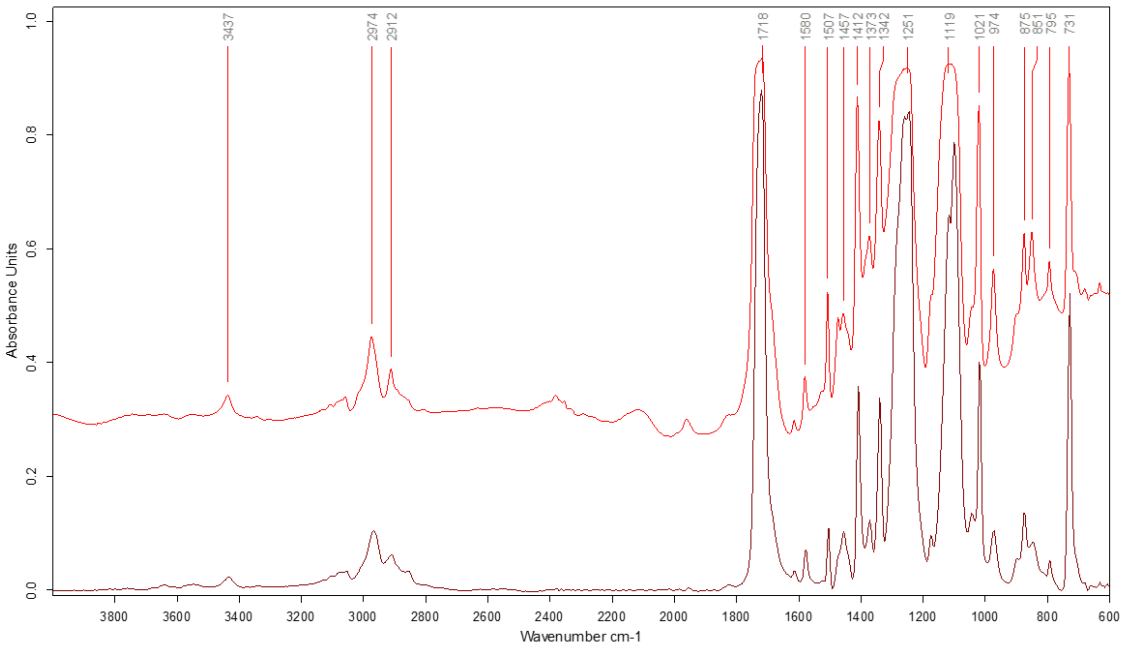

Figure 5.6. FTIR spectra of collected PET

Overall, the FTIR spectrum presented in this study show that the functional groups identified in the spectra, correspond to the presence of PET in the collected sample. PET is characterized by strong absorption peaks in the 1700-1750 cm^{-1} range, which are indicative of the ester group in the PET structure [183]. The other absorption peaks can be used to identify and confirm the presence of PET in a sample [204]. Thus, there are no other peaks that provide information of other content or material in the sample [205].

The FTIR spectra presented in Figure 5.7 shows absorption bands that characterized a rubber sample. The FTIR spectra of rubber can be verified due to several broad absorption bands in the infrared region that range from 4000 to 400 cm^{-1}. The most prominent absorption peak in the spectrum of rubber is commonly found around 2900 cm-1 and corresponds to the CH stretching vibrations of the CH groups in rubber [206] [207]. This peak is in accordance with the peak showed in Figure 5.5 at 2919 cm^{-1}. Another important peak of rubber was observed at around 1428 cm^{-1}, typically attributed to the CH_2 bending vibrations, while the band at around 1360 cm^{-1} is a characteristic of CH_3 vibrations. The FTIR spectra also exhibit absorption bands around 1000 to 1100 cm^{-1} associated with the presence of ether and alcohol groups, respectively [208]. In addition, the presence of sulfur in rubber can be identified by the presence of absorption bands at around 600 to 650 cm^{-1} [209].

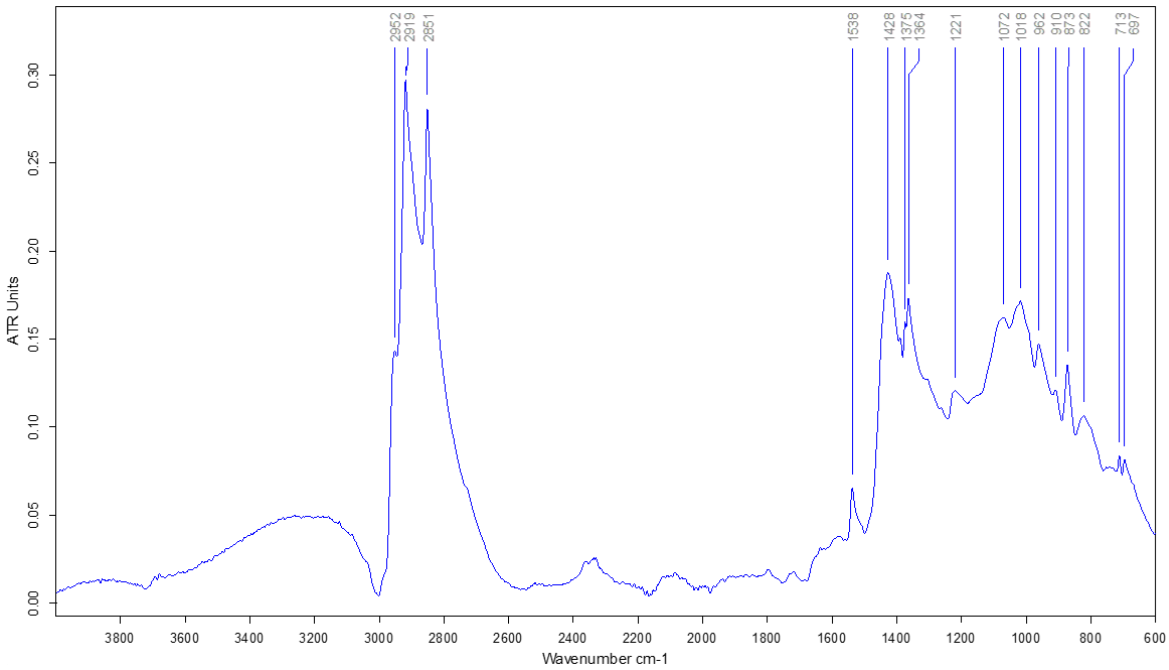

Figure 5.7. FTIR spectra of rubber

5.2.4. Bubble size analysis of rhamnolipids

Fig 5.8 shows the effect of the rhamnolipid concentration and Ctr concentration upon the bubble size of the produced foam. Bubble size diameters for Ctr microplastic particles are presented in Appendix D. Table D.3. According to the graph in Fig 5.8a, when the C_{Ctr} in the ternary system is 0 g/L, the variation in the bubble diameter has a decreasing tendency from 0.23 cm (C_{RMLP} = 0 g/L) to 0.065 cm (C_{RMLP} = 2 g/L). This decreasing trend in the bubble size is also observed for all three Ctr sizes when polymer was added to the system.

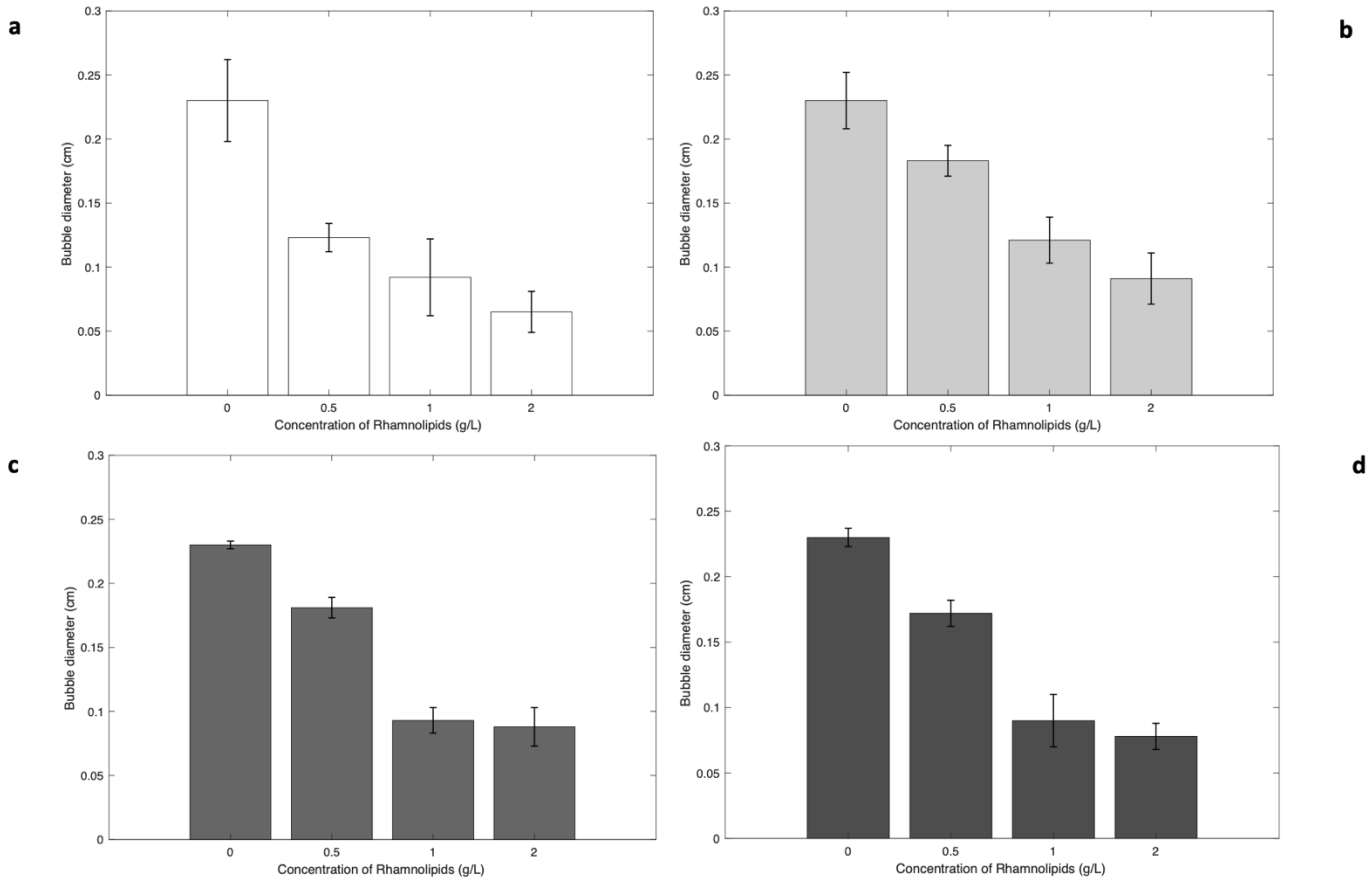

Figure 5.8. Effect of rhamnolipids and Ctr concentration upon bubble's size. a) C_{Ctr} = 0 g/L, b) $C_{RMLP} = C_{Ctr}$ when Ctr size is 53 μm, c) $C_{RMLP} = C_{Ctr}$ when Ctr size is 90 μm, d) $C_{RMLP} = C_{Ctr}$ when Ctr size is 300 μm

Specifically, as illustrated in Fig 5.8b, when the smallest Ctr size of 53 μm was present in the liquid mixture, the bubble diameter experienced a reduction from 0.183 cm when the C_{RMLP} was 0.5 g/L, to a size of 0.091 cm when the C_{RMLP} increased to 2 g/L. Similarly, as observed in Fig 5.8 c, when the Ctr size was increased to 90 μm, the bubble size showed a decreased, reaching a value of 0.088 when 2 g/L of rhamnolipids were used. Finally in Fig 5.8d, the bubble size decrement was also favored, when the Ctr size was 300 μm. Here, the bubble diameter reached 0.172 cm when the concentration of rhamnolipids is 0.5 g/L and 0.078 for a concentration of rhamnolipids of 2 g/L.

Our findings indicate that with the addition of polymer solute to the system, one can observe a reduction in the bubble diameter. Moreover, when the polymer concentration increases in a water-polymer-biosurfactant system, the formation of smaller bubbles is exhibited. This behavior has been also observed in a recent study in a water-polymer-surfactant system, in which a decay in the foam, followed by bubble rupture, and a production of smaller bubbles were found with an increment in the concentration of surfactant.

The results of the investigation related to the size of bubbles in the rhamnolipids based-foam support the conclusions of a recent study that explores bubble distribution and size in a polymer-surfactant-water system [160]. The study revealed that introducing gas into the liquid phase led to the production of larger bubbles at higher polymer concentrations when surfactant was absent. However, higher polymer concentrations also caused a decay in the foam, resulting in the generation of smaller-sized bubbles and increasing the gas-liquid area [160].

Multiple investigations suggest that the presence of smaller bubbles is the result of larger bubble rupture induced by the viscosity of the aqueous solution [184]. Additionally, the study concluded that adding surfactant to the ternary mixture enhances the gas-liquid interfacial area compared to solutions that lack surfactant [184].

5.3. Conclusions

This study revealed the potential of rhamnolipids foam in removing Ctr microplastic particles in a ternary system. The study revealed that the microplastic removal system can successfully remove Ctr particles of sizes ranging from 53-300 μm using foam from a biological source. Moreover, a response surface method was developed for the removal of Ctr at various concentrations using rhamnolipids. The model evaluated the performance of the response variable Ctr removal efficiency based on the predictors rhamnolipid concentration, operating time, plastic size, and plastic concentration. The model's R^2 was 0.89. The parameters X_1, X_2, X_3, the interactions $X_1.X_2$, $X_1.X_3$, $X_2.X_3$, and the squared terms X_1^2, X_2^2 , were found significant with pValues of less than 0.05.

For the first time, research revealed that rhamnolipids based foam can remove various types of materials in a ternary system. The investigation found that silicon dioxide, calcium carbonate, PET and rubber particles were collected products in the removal process.

6. Conclusions and recommendations

This research work studied the effect of microbial biosurfactant for the removal of microplastic particles. Specifically, the potential of rhamnolipids biosurfactant was investigated for the removal of polyethylene and car tire residue microplastic particles. The effect of the variables rhamnolipid concentration, operating time, microplastic size, microplastic concentration, air flow rate, system configuration and NaCl concentration on the microplastic removal efficiency was also reported.

On this basis, the following are the major findings of the present study:

- A microplastic removal unit was developed for a water-polymer-biosurfactant system. The microplastic removal unit consists of four pieces of glassware: a round flask, a condenser a filtration apparatus, and a recovery flask.

- A two-level factorial design with 24 data points was carried out to evaluate the performance of the microplastic removal system. The independent factors studied in this investigation were rhamnolipid concentration, PE concentration, operating time, air flow rate, NaCl concentration and system configuration. PE removal efficiency was selected as the response variable. A first order model was fitted with an R^2 of 0.92. The highest PE removal efficiency for a PE size range of 40-48 μm observed was 92.42% and the lowest recovery was 12.41%.

- Through response surface methodology, the critical parameters that affect the PE removal efficiency for a PE size range of 40-48 μm were determined. The rhamnolipid concentration and operating time were found to have a significant effect in the PE removal efficiency of the ternary system.

- For a PE size range of 40-48 μm, the results showed that a higher concentration of rhamnolipids is characterized by lower bubble size throughout time along with high PE removal. On the contrary, lower concentration of rhamnolipids led to a bigger size of the bubbles and a lower recovery of microplastics. Among the independent variables, the factor operating time and its interactions with both the rhamnolipid concentration and plastic size variables were statistically significant at the 5% level.

- The microplastic removal system can successfully remove PE particles of sizes ranging from 40-300 μm using foam from a biological source.

- A response surface method that correlates 4 factors and 3 levels was developed for the removal of PE at various concentrations using rhamnolipids. The model evaluated the

performance of the response variable PE removal efficiency based on the predictors rhamnolipid concentration, operating time, plastic size, and plastic concentration. The model's R2 was 0.82, which is a measurement of good fitting.

- The microplastic removal system can successfully remove Ctr particles of sizes ranging from 53-300 μm using foam from a biological source.

- A response surface method that correlates 4 factors and 3 levels was developed for the removal of Ctr at various concentrations using rhamnolipids. The model evaluated the performance of the response variable Ctr removal efficiency based on the predictors rhamnolipid concentration, operating time, plastic size, and plastic concentration. The model's R2 was 0.89. The parameters X_1, X_2, X_3, the interactions $X_1.X_2$, $X_1.X_3$, $X_2.X_3$, and the squared terms X_1^2, X_2^2 , were found significant with pValues of less than 0.05.

Recommendations

- It is recommended that future work further investigates the use of biological foam for the capture and the removal of various types of microplastic particles.

- It is recommended to investigate the use of rhamnolipids foam-based for the removal of microplastic particles present in real-life water samples. It is anticipated that this will provide a closer prediction of the removal efficiency of microplastic particles when using the microplastic removal unit.

- It is recommended to evaluate different biosurfactant types for the removal of PE and Ctr microplastic particles using the microplastic removal unit.

7. References

[1] Statista, "Global plastic production 1950-2018," *Production of plastics worldwide from 1950 to 2018 (in million metric tons)**, 2019. https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/ (accessed Apr. 12, 2019).

[2] M. A. Browne, "Rochman 2013_Classify Plastic Waste as hazardous_Nature," pp. 6–8, 2013.

[3] M. Cole, P. Lindeque, C. Halsband, and T. S. Galloway, "Microplastics as contaminants in the marine environment: A review," *Mar. Pollut. Bull.*, vol. 62, no. 12, pp. 2588–2597, 2011, doi: 10.1016/j.marpolbul.2011.09.025.

[4] M. Claessens, S. D. Meester, L. V. Landuyt, K. D. Clerck, and C. R. Janssen, "Occurrence and distribution of microplastics in marine sediments along the Belgian coast," *Mar. Pollut. Bull.*, vol. 62, no. 10, pp. 2199–2204, Oct. 2011, doi: 10.1016/J.MARPOLBUL.2011.06.030.

[5] S. Rapagna *et al.*, "Quantification of human bone microarchitecture damage in press-fit femoral knee implantation using HR-pQCT and digital volume correlation," *J. Mech. Behav. Biomed. Mater.*, vol. 97, no. December 2018, pp. 278–287, 2019, doi: 10.1016/j.jmbbm.2019.04.054.

[6] M. Liboiron *et al.*, "Low plastic ingestion rate in Atlantic cod (Gadus morhua) from Newfoundland destined for human consumption collected through citizen science methods," *Mar. Pollut. Bull.*, vol. 113, no. 1–2, pp. 428–437, 2016, doi: 10.1016/j.marpolbul.2016.10.043.

[7] S. Nalini and R. Parthasarathi, "Optimization of rhamnolipid biosurfactant production from Serratia rubidaea SNAU02 under solid-state fermentation and its biocontrol efficacy against Fusarium wilt of eggplant," *Ann. Agrar. Sci.*, vol. 16, no. 2, pp. 108–115, 2018, doi: 10.1016/j.aasci.2017.11.002.

[8] D. Sharma and B. S. Saharan, "Functional characterization of biomedical potential of biosurfactant produced by Lactobacillus helveticus," *Biotechnol. Rep.*, vol. 11, pp. 27–35, 2016, doi: 10.1016/j.btre.2016.05.001.

[9] F. Anjum, G. Gautam, G. Edgard, and S. Negi, "Biosurfactant production through Bacillus sp. MTCC 5877 and its multifarious applications in food industry," *Bioresour. Technol.*, vol. 213, pp. 262–269, 2015, doi: 10.1016/j.biortech.2016.02.091.

[10] M. A. Díaz De Rienzo, I. M. Banat, B. Dolman, J. Winterburn, and P. J. Martin, "Sophorolipid biosurfactants: Possible uses as antibacterial and antibiofilm agent," *New Biotechnol.*, vol. 32, no. 6, pp. 720–726, 2015, doi: 10.1016/j.nbt.2015.02.009.

[11] A. P. Karlapudi *et al.*, "Role of biosurfactants in bioremediation of oil pollution-a review," *Petroleum*, vol. 4, no. 3, pp. 241–249, 2018, doi: 10.1016/j.petlm.2018.03.007.

[12] Y. B. Zhao, X. D. Lv, and H. G. Ni, "Solvent-based separation and recycling of waste plastics: A review," *Chemosphere*, 2018, doi: 10.1016/j.chemosphere.2018.06.095.

[13] A. L. Andrady and M. A. Neal, "Applications and societal benefits of plastics," *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 364, no. 1526, pp. 1977–1984, 2009, doi: 10.1098/rstb.2008.0304.

[14] J. Zalasiewicz *et al.*, "The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene," *Anthropocene*, vol. 13, pp. 4–17, Mar. 2016, doi: 10.1016/J.ANCENE.2016.01.002.

[15] J. P. da Costa, "Micro- and nanoplastics in the environment: Research and policymaking," *Curr. Opin. Environ. Sci. Health*, vol. 1, pp. 12–16, 2018, doi: 10.1016/j.coesh.2017.11.002.

[16] OECD, "OECD Global plastics outlook: Economic drivers, environmental impacts and policy options 2022 Organisation for Economic Co-operation and Development (OECD)," Feb. 2022. [Online]. Available: https://www.oecd.org/environment/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm

[17] J. C. Prata, "Microplastics in wastewater: State of the knowledge on sources, fate and solutions," *Mar. Pollut. Bull.*, vol. 129, no. 1, pp. 262–265, 2018, doi: 10.1016/j.marpolbul.2018.02.046.

[18] T. O'Brine and R. C. Thompson, "Degradation of plastic carrier bags in the marine environment," *Mar. Pollut. Bull.*, vol. 60, no. 12, pp. 2279–2283, 2010, doi: 10.1016/j.marpolbul.2010.08.005.

[19] M. Pivokonsky, L. Cermakova, K. Novotna, P. Peer, T. Cajthaml, and V. Janda, "Occurrence of microplastics in raw and treated drinking water," *Sci. Total Environ.*, vol. 643, pp. 1644–1651, 2018, doi: 10.1016/j.scitotenv.2018.08.102.

[20] A. Bakir, I. A. O'Connor, S. J. Rowland, A. J. Hendriks, and R. C. Thompson, "Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life," *Environ. Pollut.*, vol. 219, pp. 56–65, 2016, doi: 10.1016/j.envpol.2016.09.046.

[21] L. D. K. Kanhai, R. Officer, O. Lyashevska, R. C. Thompson, and I. O'Connor, "Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean," *Mar. Pollut. Bull.*, vol. 115, no. 1–2, pp. 307–314, 2017, doi: 10.1016/j.marpolbul.2016.12.025.

[22] X. Liu *et al.*, "Sorption behaviors of tris-(2,3-dibromopropyl) isocyanurate and hexabromocyclododecanes on polypropylene microplastics," *Mar. Pollut. Bull.*, vol. 135, no. June, pp. 581–586, 2018, doi: 10.1016/j.marpolbul.2018.07.061.

[23] J. Gigault *et al.*, "Current opinion: What is a nanoplastic?," *Environ. Pollut.*, vol. 235, pp. 1030–1034, 2018, doi: https://doi.org/10.1016/j.envpol.2018.01.024.

[24] H. Du and J. Wang, "Characterization and environmental impacts of microplastics," *Gondwana Res.*, vol. 98, pp. 63–75, 2021, doi: https://doi.org/10.1016/j.gr.2021.05.023.

[25] B. Baensch-Baltruschat, B. Kocher, C. Kochleus, F. Stock, and G. Reifferscheid, "Tyre and road wear particles - A calculation of generation, transport and release to water and soil with special regard to German roads," *Sci. Total Environ.*, vol. 752, p. 141939, 2021, doi: https://doi.org/10.1016/j.scitotenv.2020.141939.

[26] P. T. Harris, "The fate of microplastic in marine sedimentary environments: A review and synthesis," *Mar. Pollut. Bull.*, vol. 158, p. 111398, 2020, doi: https://doi.org/10.1016/j.marpolbul.2020.111398.

[27] T. Buadit, A. Ussawarujikulchai, K. Suchiva, S. Papong, H. Ma, and C. Rattanapan, "Environmental impact of passenger car tire supply chain in Thailand using the life cycle assessment method," *Sustain. Prod. Consum.*, vol. 37, pp. 156–168, 2023, doi: https://doi.org/10.1016/j.spc.2023.02.013.

[28] H. R. Mian, G. Chhipi-Shrestha, K. McCarty, K. Hewage, and R. Sadiq, "An estimation of tire and road wear particles emissions in surface water based on a conceptual framework," *Sci. Total Environ.*, vol. 848, p. 157760, 2022, doi: https://doi.org/10.1016/j.scitotenv.2022.157760.

[29] A. Wik and G. Dave, "Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment," *Environ. Pollut.*, vol. 157, no. 1, pp. 1–11, 2009, doi: https://doi.org/10.1016/j.envpol.2008.09.028.

[30] Y. H. Fung *et al.*, "Different sorption behaviours of pyrene onto polyethylene microplastics in a binary system with water and a ternary system with water and sediment," *Environ. Technol. Innov.*, vol. 30, p. 103086, 2023, doi: https://doi.org/10.1016/j.eti.2023.103086.

[31] F. Yu *et al.*, "Potential impact of polyethylene microplastics on the growth of water spinach (Ipomoea aquatica F.): Endophyte and rhizosphere effects," *Chemosphere*, vol. 330, p. 138737, 2023, doi: https://doi.org/10.1016/j.chemosphere.2023.138737.

[32] M. S. Kabir, H. Wang, S. Luster-Teasley, L. Zhang, and R. Zhao, "Microplastics in landfill leachate: Sources, detection, occurrence, and removal," *Environ. Sci. Ecotechnology*, vol. 16, p. 100256, 2023, doi: https://doi.org/10.1016/j.ese.2023.100256.

[33] K. A. Adegoke *et al.*, "Microplastics toxicity, detection, and removal from water/wastewater," *Mar. Pollut. Bull.*, vol. 187, p. 114546, 2023, doi: https://doi.org/10.1016/j.marpolbul.2022.114546.

[34] N. A. Khan *et al.*, "Microplastics: Occurrences, treatment methods, regulations and foreseen environmental impacts," *Environ. Res.*, vol. 215, p. 114224, 2022, doi: https://doi.org/10.1016/j.envres.2022.114224.

[35] Y. Pan *et al.*, "Removing microplastics from aquatic environments: A critical review," *Environ. Sci. Ecotechnology*, vol. 13, p. 100222, 2023, doi: https://doi.org/10.1016/j.ese.2022.100222.

[36] P. G. Ryan, C. J. Moore, J. A. Van Franeker, and C. L. Moloney, "Monitoring the abundance of plastic debris in the marine environment," *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 364, no. 1526, pp. 1999–2012, 2009, doi: 10.1098/rstb.2008.0207.

[37] J. G. B. Derraik, "The pollution of the marine environment by plastic debris: a review," *Mar. Pollut. Bull.*, vol. 44, no. 9, pp. 842–852, Sep. 2002, doi: 10.1016/S0025-326X(02)00220-5.

[38] E. R. Graham and J. T. Thompson, "Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments," *J. Exp. Mar. Biol. Ecol.*, vol. 368, no. 1, pp. 22–29, Jan. 2009, doi: 10.1016/J.JEMBE.2008.09.007.

[39] Y. Liu, H. Shi, L. Chen, X. Teng, C. Xue, and Z. Li, "An overview of microplastics in oysters: analysis, hazards, and depuration," *Food Chem.*, p. 136153, 2023, doi: https://doi.org/10.1016/j.foodchem.2023.136153.

[40] A. Dąbrowska, M. Mielańczuk, and M. Syczewski, "The Raman spectroscopy and SEM/EDS investigation of the primary sources of microplastics from cosmetics available in Poland," *Chemosphere*, vol. 308, p. 136407, 2022, doi: https://doi.org/10.1016/j.chemosphere.2022.136407.

[41] K. Duis and A. Coors, "Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects," *Environ. Sci. Eur.*, vol. 28, no. 1, p. 2, Jan. 2016, doi: 10.1186/s12302-015-0069-y.

[42] J. Kalman, A.-B. Muñiz-González, M.-Á. García, and J.-L. Martínez-Guitarte, "Chironomus riparius molecular response to polystyrene primary microplastics," *Sci. Total Environ.*, vol. 868, p. 161540, 2023, doi: https://doi.org/10.1016/j.scitotenv.2023.161540.

[43] A. L. Andrady, "Microplastics in the marine environment," *Mar. Pollut. Bull.*, vol. 62, no. 8, pp. 1596–1605, Aug. 2011, doi: 10.1016/J.MARPOLBUL.2011.05.030.

[44] B. Xia *et al.*, "Secondary PVC microplastics are more toxic than primary PVC microplastics to Oryzias melastigma embryos," *J. Hazard. Mater.*, vol. 424, p. 127421, 2022, doi: https://doi.org/10.1016/j.jhazmat.2021.127421.

[45] M. Huber, V.-M. Archodoulaki, E. Pomakhina, B. Pukánszky, E. Zinöcker, and M. Gahleitner, "Environmental degradation and formation of secondary microplastics from packaging material: A polypropylene film case study," *Polym. Degrad. Stab.*, vol. 195, p. 109794, 2022, doi: https://doi.org/10.1016/j.polymdegradstab.2021.109794.

[46] J. Wang, Z. Tan, J. Peng, Q. Qiu, and M. Li, "The behaviors of microplastics in the marine environment," *Mar. Environ. Res.*, vol. 113, pp. 7–17, Feb. 2016, doi: 10.1016/j.marenvres.2015.10.014.

[47] M. Zbyszewski, P. L. Corcoran, and A. Hockin, "Comparison of the distribution and degradation of plastic debris along shorelines of the Great Lakes, North America," *J. Gt. Lakes Res.*, vol. 40, no. 2, pp. 288–299, Jun. 2014, doi: 10.1016/J.JGLR.2014.02.012.

[48] L. C.-M. Lebreton, S. D. Greer, and J. C. Borrero, "Numerical modelling of floating debris in the world's oceans," *Mar. Pollut. Bull.*, vol. 64, no. 3, pp. 653–661, Mar. 2012, doi: 10.1016/J.MARPOLBUL.2011.10.027.

[49] M. J. Doyle, W. Watson, N. M. Bowlin, and S. B. Sheavly, "Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean," *Mar. Environ. Res.*, vol. 71, no. 1, pp. 41–52, Feb. 2011, doi: 10.1016/J.MARENVRES.2010.10.001.

[50] N. Maximenko, J. Hafner, and P. Niiler, "Pathways of marine debris derived from trajectories of Lagrangian drifters," *Mar. Pollut. Bull.*, vol. 65, no. 1–3, pp. 51–62, Jan. 2012, doi: 10.1016/J.MARPOLBUL.2011.04.016.

[51] J. M. Restrepo-Flórez, A. Bassi, and M. R. Thompson, "Microbial degradation and deterioration of polyethylene - A review," *Int. Biodeterior. Biodegrad.*, vol. 88, pp. 83–90, 2014, doi: 10.1016/j.ibiod.2013.12.014.

[52] H. Kaczmarek, K. Bajer, P. Gałka, and B. Kotnowska, "Photodegradation studies of novel biodegradable blends based on poly(ethylene oxide) and pectin," *Polym. Degrad. Stab.*, vol. 92, no. 11, pp. 2058–2069, Nov. 2007, doi: 10.1016/J.POLYMDEGRADSTAB.2007.07.019.

[53] G. Berenstein, E. A. Hughes, A. Zalts, S. Basack, S. M. Bonesi, and J. M. Montserrat, "Environmental fate of dibutylphthalate in agricultural plastics: Photodegradation, migration and ecotoxicological impact on soil," *Chemosphere*, vol. 290, p. 133221, 2022, doi: https://doi.org/10.1016/j.chemosphere.2021.133221.

[54] R. K. Singh, B. Ruj, A. K. Sadhukhan, and P. Gupta, "Thermal degradation of waste plastics under non-sweeping atmosphere: Part 1: Effect of temperature, product optimization, and degradation mechanism," *J. Environ. Manage.*, vol. 239, pp. 395–406, 2019, doi: https://doi.org/10.1016/j.jenvman.2019.03.067.

[55] P. Das and P. Tiwari, "Thermal degradation kinetics of plastics and model selection," *Thermochim. Acta*, vol. 654, pp. 191–202, 2017, doi: https://doi.org/10.1016/j.tca.2017.06.001.

[56] W. C. LI, H. F. TSE, and L. FOK, "Plastic waste in the marine environment: A review of sources, occurrence and effects," *Sci. Total Environ.*, vol. 566–567, pp. 333–349, Oct. 2016, doi: 10.1016/J.SCITOTENV.2016.05.084.

[57] A. L. Andrady *et al.*, "Oxidation and fragmentation of plastics in a changing environment; from UV-radiation to biological degradation," *Sci. Total Environ.*, vol. 851, p. 158022, 2022, doi: https://doi.org/10.1016/j.scitotenv.2022.158022.

[58] S. Kumagai *et al.*, "A comprehensive study into the thermo-oxidative degradation of sulfur-based engineering plastics," *J. Anal. Appl. Pyrolysis*, vol. 168, p. 105754, 2022, doi: https://doi.org/10.1016/j.jaap.2022.105754.

[59] M. Gigli *et al.*, "Enzymatic hydrolysis studies on novel eco-friendly aliphatic thiocopolyesters," *Polym. Degrad. Stab.*, vol. 98, no. 5, pp. 934–942, 2013, doi: https://doi.org/10.1016/j.polymdegradstab.2013.02.019.

[60] F. Luzi *et al.*, "Combined effect of cellulose nanocrystals, carvacrol and oligomeric lactic acid in PLA_PHB polymeric films," *Carbohydr. Polym.*, vol. 223, p. 115131, 2019, doi: https://doi.org/10.1016/j.carbpol.2019.115131.

[61] A. S. C. de Bomfim, M. M. Á. D. Maciel, H. J. C. Voorwald, K. C. C. de C. Benini, D. M. de Oliveira, and M. O. H. Cioffi, "Effect of different degradation types on properties of plastic waste obtained from espresso coffee capsules," *Waste Manag.*, vol. 83, pp. 123–130, 2019, doi: 10.1016/j.wasman.2018.11.006.

[62] F. Pinto, P. Costa, I. Gulyurtlu, and I. Cabrita, “Pyrolysis of plastic wastes. 1. Effect of plastic waste composition on product yield,” *J. Anal. Appl. Pyrolysis*, vol. 51, no. 1, pp. 39–55, 1999, doi: 10.1016/S0165-2370(99)00007-8.

[63] I. Jakubowicz, “Evaluation of degradability of biodegradable polyethylene (PE),” *Polym. Degrad. Stab.*, vol. 80, no. 1, pp. 39–43, Jan. 2003, doi: 10.1016/S0141-3910(02)00380-4.

[64] S. Pant and R. B. Valapa, “Chapter 15 - Microbial attachment studies on ‘plastic-specific’ microorganisms,” in *Biodegradability of Conventional Plastics*, A. Sarkar, B. Sharma, and S. Shekhar, Eds., Elsevier, 2023, pp. 309–337. doi: https://doi.org/10.1016/B978-0-323-89858-4.00009-9.

[65] J. Colwell, S. Pratt, P. Lant, and B. Laycock, “Hazardous state lifetimes of biodegradable plastics in natural environments,” *Sci. Total Environ.*, vol. 894, p. 165025, 2023, doi: https://doi.org/10.1016/j.scitotenv.2023.165025.

[66] F. Hua, H. Q. Wang, Y. Li, and Y. C. Zhao, “Trans-membrane transport of n-octadecane by Pseudomonas sp. DG17,” *J. Microbiol.*, vol. 51, no. 6, pp. 791–799, Dec. 2013, doi: 10.1007/s12275-013-3259-6.

[67] A. A. Shah, F. Hasan, A. Hameed, and S. Ahmed, “Biological degradation of plastics: A comprehensive review,” *Biotechnol. Adv.*, vol. 26, no. 3, pp. 246–265, 2008, doi: https://doi.org/10.1016/j.biotechadv.2007.12.005.

[68] N. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre, and J.-E. Nava-Saucedo, “Polymer biodegradation: Mechanisms and estimation techniques – A review,” *Chemosphere*, vol. 73, no. 4, pp. 429–442, 2008, doi: https://doi.org/10.1016/j.chemosphere.2008.06.064.

[69] D. G. Shaw and R. H. Day, “Colour- and form-dependent loss of plastic micro-debris from the North Pacific Ocean,” *Mar. Pollut. Bull.*, vol. 28, no. 1, pp. 39–43, Jan. 1994, doi: 10.1016/0025-326X(94)90184-8.

[70] M. S. Savoca, C. W. Tyson, M. McGill, and C. J. Slager, “Odours from marine plastic debris induce food search behaviours in a forage fish,” *284*, Aug. 2017.

[71] J. F. DING *et al.*, “Separation and Identification of Microplastics in Digestive System of Bivalves,” *Chin. J. Anal. Chem.*, vol. 46, no. 5, pp. 690–697, 2018, doi: 10.1016/S1872-2040(18)61086-2.

[72] J. P. da Costa, A. C. Duarte, and T. A. P. Rocha-Santos, *Microplastics – Occurrence, Fate and Behaviour in the Environment*, vol. 75. Elsevier Ltd, 2017. doi: 10.1016/bs.coac.2016.10.004.

[73] M. G. J. Löder and G. Gerdts, “Methodology Used for the Detection and Identification of Microplastics---A Critical Appraisal,” in *Marine Anthropogenic Litter*, M. Bergmann, L. Gutow, and M. Klages, Eds., Cham: Springer International Publishing, 2015, pp. 201–227. doi: 10.1007/978-3-319-16510-3_8.

[74] A. B. Silva, A. S. Bastos, C. I. L. Justino, J. P. da Costa, A. C. Duarte, and T. A. P. Rocha-Santos, “Microplastics in the environment: Challenges in analytical chemistry - A review,” *Anal. Chim. Acta*, vol. 1017, pp. 1–19, 2018, doi: 10.1016/j.aca.2018.02.043.

[75] Kyoung Y. et al., “Large Accumulation of Micro-Sized Synthetic Polymer Particles in the Sea Surface Microlayer,” vol. 7, pp. 1–8, 2014.

[76] M. B. Zobkov and E. E. Esiukova, “Microplastics in a Marine Environment: Review of Methods for Sampling, Processing, and Analyzing Microplastics in Water, Bottom Sediments, and Coastal Deposits,” *Oceanology*, vol. 58, no. 1, pp. 137–143, 2018, doi: 10.1134/S0001437017060169.

[77] M. T. V. Hidalgo-Ruz, L. Gutow, R.C. Thompson, “Microplastics in the marine environment: a review of the methods used for identification and quantification,” *Env. Sci Technol*, vol. 46, p. 3060e3075, 2012.

[78] J. C. Prata, J. P. da Costa, A. C. Duarte, and T. Rocha-Santos, “Methods for sampling and detection of microplastics in water and sediment: A critical review,” *TrAC - Trends Anal. Chem.*, vol. 110, pp. 150–159, 2019, doi: 10.1016/j.trac.2018.10.029.

[79] T. Wang *et al.*, “Preliminary study of the source apportionment and diversity of microplastics: Taking floating microplastics in the South China Sea as an example,” *Environ. Pollut.*, vol. 245, pp. 965–974, 2019, doi: S0269749118329737.

[80] D. Yang, H. Shi, L. Li, J. Li, K. Jabeen, and P. Kolandhasamy, “Microplastic Pollution in Table Salts from China,” *Environ. Sci. Technol.*, vol. 49, no. 22, pp. 13622–13627, 2015, doi: 10.1021/acs.est.5b03163.

[81] B. Nguyen, D. Claveau-Mallet, L. M. Hernandez, E. G. Xu, J. M. Farner, and N. Tufenkji, “Separation and Analysis of Microplastics and Nanoplastics in Complex Environmental Samples,” *Acc. Chem. Res.*, vol. 52, no. 4, pp. 858–866, 2019, doi: 10.1021/acs.accounts.8b00602.

[82] B. Quinn, F. Murphy, and C. Ewins, “Validation of density separation for the rapid recovery of microplastics from sediment,” *Anal. Methods*, vol. 9, no. 9, pp. 1491–1498, 2017, doi: 10.1039/c6ay02542k.

[83] F. Dell’Anno, C. Sansone, A. Ianora, and A. Dell’Anno, “Biosurfactant-induced remediation of contaminated marine sediments: Current knowledge and future perspectives,” *Mar. Environ. Res.*, vol. 137, no. March, pp. 196–205, 2018, doi: 10.1016/j.marenvres.2018.03.010.

[84] I. Anic, A. Nath, P. Franco, and R. Wichmann, “Foam adsorption as an ex situ capture step for surfactants produced by fermentation,” *J. Biotechnol.*, vol. 258, no. March, pp. 181–189, 2017, doi: 10.1016/j.jbiotec.2017.07.015.

[85] X. Vecino, R. Devesa-Rey, J. M. Cruz, and A. B. Moldes, “Evaluation of biosurfactant obtained from Lactobacillus pentosus as foaming agent in froth flotation,” *J. Environ. Manage.*, vol. 128, pp. 655–660, 2013, doi: 10.1016/j.jenvman.2013.06.011.

[86] S. Vijayakuma and V. Saravanan, “Biosurfactants-Types, Sources and Applications,” *Res. J. Microbiol.*, 2015, doi: 10.3923/jm.2015.181.192.

[87] B. G. Freitas *et al.*, “Formulation of a commercial biosurfactant for application as a dispersant of petroleum and by-products spilled in oceans,” *Front. Microbiol.*, vol. 7, no. OCT, pp. 1–9, 2016, doi: 10.3389/fmicb.2016.01646.

[88] R. Geys, W. Soetaert, and I. Van Bogaert, “Biotechnological opportunities in biosurfactant production,” *Curr. Opin. Biotechnol.*, vol. 30, pp. 66–72, 2014, doi: 10.1016/j.copbio.2014.06.002.

[89] M. M. Müller *et al.*, “Rhamnolipids-Next generation surfactants?,” *J. Biotechnol.*, 2012, doi: 10.1016/j.jbiotec.2012.05.022.

[90] K. Rahimi, T. B. Lotfabad, F. Jabeen, and S. Mohammad Ganji, “Cytotoxic effects of mono- and di-rhamnolipids from Pseudomonas aeruginosa MR01 on MCF-7 human breast cancer cells,” *Colloids Surf. B Biointerfaces*, vol. 181, no. June, pp. 943–952, 2019, doi: 10.1016/j.colsurfb.2019.06.058.

[91] N. Charalampous, G. Grammatikopoulos, C. Kourmentza, M. Kornaros, and S. Dailianis, “Effects of Burkholderia thailandensis rhamnolipids on the unicellular algae Dunaliella tertiolecta,” *Ecotoxicol. Environ. Saf.*, vol. 182, p. 109413, Oct. 2019, doi: 10.1016/J.ECOENV.2019.109413.

[92] A. B. Abeer Mohammed, A. A. Tayel, and N. M. Elguindy, “Production of new rhamnolipids Rha C16-C16 by Burkholderia sp. through biodegradation of diesel and biodiesel,” *Beni-Suef Univ. J. Basic Appl. Sci.*, vol. 7, no. 4, pp. 492–498, Dec. 2018, doi: 10.1016/J.BJBAS.2018.05.003.

[93] S. Nalini and R. Parthasarathi, “Optimization of rhamnolipid biosurfactant production from Serratia rubidaea SNAU02 under solid-state fermentation and its biocontrol efficacy against Fusarium wilt of eggplant,” *Ann. Agrar. Sci.*, vol. 16, no. 2, pp. 108–115, 2018, doi: 10.1016/j.aasci.2017.11.002.

[94] J. Du, A. Zhang, X. Zhang, X. Si, and J. Cao, “Comparative analysis of rhamnolipid congener synthesis in neotype Pseudomonas aeruginosa ATCC 10145 and two marine isolates,” *Bioresour. Technol.*, 2019, doi: 10.1016/j.biortech.2019.121380.

[95] L. Bai and D. J. McClements, “Formation and stabilization of nanoemulsions using biosurfactants: Rhamnolipids,” *J. Colloid Interface Sci.*, vol. 479, pp. 71–79, Oct. 2016, doi: 10.1016/j.jcis.2016.06.047.

[96] X. Wang, T. Cai, W. Wen, and Z. Zhang, “Effect of biosurfactant on biodegradation of heteroatom compounds in heavy oil,” *Fuel*, vol. 230, no. January, pp. 418–429, 2018, doi: 10.1016/j.fuel.2018.05.047.

[97] R. Cohen and D. Exerowa, “Surface forces and properties of foam films from rhamnolipid biosurfactants,” *Adv. Colloid Interface Sci.*, vol. 134–135, pp. 24–34, 2007, doi: 10.1016/j.cis.2007.04.018.

[98] M. J. Chaprão, R. de C. F. Soares da Silva, R. D. Rufino, J. M. Luna, V. A. Santos, and L. A. Sarubbo, “Formulation and application of a biosurfactant from Bacillus methylotrophicus as collector in the flotation of oily water in industrial environment,” *J. Biotechnol.*, vol. 285, no. June, pp. 15–22, 2018, doi: 10.1016/j.jbiotec.2018.08.016.

[99] A. H. Ali Khan *et al.*, “Role of nutrients in bacterial biosurfactant production and effect of biosurfactant production on petroleum hydrocarbon biodegradation,” *Ecol. Eng.*, vol. 104, pp. 158–164, 2017, doi: 10.1016/j.ecoleng.2017.04.023.

[100] P. C. Martins and V. G. Martins, “Biosurfactant production from industrial wastes with potential remove of insoluble paint,” *Int. Biodeterior. Biodegrad.*, vol. 127, no. November 2017, pp. 10–16, 2018, doi: 10.1016/j.ibiod.2017.11.005.

[101] S. Akbari, N. H. Abdurahman, R. M. Yunus, F. Fayaz, and O. R. Alara, “Biosurfactants—a new frontier for social and environmental safety: a mini review,” *Biotechnol. Res. Innov.*, 2018, doi: 10.1016/j.biori.2018.09.001.

[102] W. C. Chen, R. S. Juang, and Y. H. Wei, “Applications of a lipopeptide biosurfactant, surfactin, produced by microorganisms,” *Biochemical Engineering Journal*. 2015. doi: 10.1016/j.bej.2015.07.009.

[103] R. Patowary, K. Patowary, M. C. Kalita, and S. Deka, “Application of biosurfactant for enhancement of bioremediation process of crude oil contaminated soil,” *Int. Biodeterior. Biodegrad.*, vol. 129, no. January, pp. 50–60, 2018, doi: 10.1016/j.ibiod.2018.01.004.

[104] B. Doshi, M. Sillanpää, and S. Kalliola, “A review of bio-based materials for oil spill treatment,” *Water Res.*, vol. 135, pp. 262–277, 2018, doi: 10.1016/j.watres.2018.02.034.

[105] A. P. Karlapudi *et al.*, "Role of biosurfactants in bioremediation of oil pollution-a review," *Petroleum*. 2018. doi: 10.1016/j.petlm.2018.03.007.
[106] S. M. P. J. N. Karve, "Production, characterization, and applications of bioemulsifiers (BE) and biosurfactants (BS) produced by Acinetobacter spp.: A review," *J. Basic Microbiol.*, vol. 59, 2019.
[107] S. Lal, S. Ratna, O. Ben Said, and R. Kumar, "Biosurfactant and exopolysaccharide-assisted rhizobacterial technique for the remediation of heavy metal contaminated soil: An advancement in metal phytoremediation technology," *Environmental Technology and Innovation*. 2018. doi: 10.1016/j.eti.2018.02.011.
[108] C. N. Mulligan, "Recent advances in the environmental applications of biosurfactants," *Curr. Opin. Colloid Interface Sci.*, vol. 14, no. 5, pp. 372–378, 2009, doi: 10.1016/j.cocis.2009.06.005.
[109] D. Sharma and B. S. Saharan, "Functional characterization of biomedical potential of biosurfactant produced by Lactobacillus helveticus," *Biotechnol. Rep.*, vol. 11, pp. 27–35, 2016, doi: 10.1016/j.btre.2016.05.001.
[110] T. Janek, M. Łukaszewicz, and A. Krasowska, "Antiadhesive activity of the biosurfactant pseudofactin II secreted by the Arctic bacterium Pseudomonas fluorescens BD5," *BMC Microbiol.*, vol. 12, 2012, doi: 10.1186/1471-2180-12-24.
[111] A. Splendiani, A. G. Livingston, and C. Nicolella, "Control of membrane-attached biofilms using surfactants," *Biotechnol. Bioeng.*, vol. 94, no. 1, pp. 15–23, 2006, doi: 10.1002/bit.20752.
[112] S. S. Giri, E. C. Ryu, V. Sukumaran, and S. C. Park, "Antioxidant, antibacterial, and anti-adhesive activities of biosurfactants isolated from Bacillus strains," *Microb. Pathog.*, vol. 132, pp. 66–72, 2019, doi: https://doi.org/10.1016/j.micpath.2019.04.035.
[113] X. Yan *et al.*, "Antimicrobial, anti-adhesive and anti-biofilm potential of biosurfactants isolated from Pediococcus acidilactici and Lactobacillus plantarum against Staphylococcus aureus CMCC26003," *Microb. Pathog.*, 2019, doi: 10.1016/j.micpath.2018.11.039.
[114] E. J. Gudiña, V. Rangarajan, R. Sen, and L. R. Rodrigues, "Potential therapeutic applications of biosurfactants," *Trends in Pharmacological Sciences*, vol. 34, no. 12. pp. 667–675, Dec. 2013. doi: 10.1016/j.tips.2013.10.002.
[115] D. Kitamoto, T. Morita, T. Fukuoka, M. a. Konishi, and T. Imura, "Self-assembling properties of glycolipid biosurfactants and their potential applications," *Current Opinion in Colloid and Interface Science*. 2009. doi: 10.1016/j.cocis.2009.05.009.
[116] M. Inès and G. Dhouha, "Glycolipid biosurfactants: Potential related biomedical and biotechnological applications," *Carbohydr. Res.*, vol. 416, pp. 59–69, 2015, doi: 10.1016/j.carres.2015.07.016.
[117] G. Yi, J. Son, J. Yoo, C. Park, and H. Koo, "Emulsan-based nanoparticles for in vivo drug delivery to tumors," *Biochem. Biophys. Res. Commun.*, vol. 508, no. 1, pp. 326–331, 2019, doi: 10.1016/j.bbrc.2018.11.106.
[118] C. Arthur, J. Baker, and H. Bamford, "Proceedings of the International Research Workshop on the Occurrence , Effects , and Fate of Microplastic Marine Debris," *Group*, no. January, p. 530, 2009.
[119] H. LI, L. ZHU, M. MA, H. WU, L. AN, and Z. YANG, "Occurrence of microplastics in commercially sold bottled water," *Sci. Total Environ.*, vol. 867, p. 161553, 2023, doi: https://doi.org/10.1016/j.scitotenv.2023.161553.
[120] H. Kye, J. Kim, S. Ju, J. Lee, C. Lim, and Y. Yoon, "Microplastics in water systems: A review of their impacts on the environment and their potential hazards," *Heliyon*, vol. 9, no. 3, p. e14359, 2023, doi: https://doi.org/10.1016/j.heliyon.2023.e14359.
[121] C. Ioakeimidis *et al.*, "The degradation potential of PET bottles in the marine environment: An ATR-FTIR based approach," *Sci. Rep.*, vol. 6, no. March, pp. 1–8, 2016, doi: 10.1038/srep23501.
[122] A. Turner, R. Arnold, and T. Williams, "Weathering and persistence of plastic in the marine environment: Lessons from LEGO," *Environ. Pollut.*, vol. 262, p. 114299, 2020, doi: 10.1016/j.envpol.2020.114299.
[123] A. L. Lusher, M. McHugh, and R. C. Thompson, "Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel," *Mar. Pollut. Bull.*, vol. 67, no. 1–2, pp. 94–99, 2013, doi: 10.1016/j.marpolbul.2012.11.028.
[124] E. Of, M. I. N. The, and A. G. Assessment, "Science for Sustainable Oceans", [Online]. Available: www.imo.org
[125] J. Oehlmann *et al.*, "A critical analysis of the biological impacts of plasticizers on wildlife," *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 364, no. 1526, pp. 2047–2062, 2009, doi: 10.1098/rstb.2008.0242.
[126] S. A. Carr, J. Liu, and A. G. Tesoro, "Transport and fate of microplastic particles in wastewater treatment plants," *Water Res.*, vol. 91, pp. 174–182, 2016, doi: 10.1016/j.watres.2016.01.002.
[127] S. A. Mason *et al.*, "Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent," *Environ. Pollut.*, vol. 218, pp. 1045–1054, 2016, doi: https://doi.org/10.1016/j.envpol.2016.08.056.

[128] M. Luo *et al.*, “Removal and toxic forecast of microplastics treated by electrocoagulation: Influence of dissolved organic matter,” *Chemosphere*, vol. 308, p. 136309, 2022, doi: https://doi.org/10.1016/j.chemosphere.2022.136309.
[129] R. Amanna, M. Samavi, and S. K. Rakshit, “Chapter 12 - Biological degradation of microplastics and nanoplastics in water and wastewater,” in *Current Developments in Biotechnology and Bioengineering*, R. D. Tyagi, A. Pandey, P. Drogui, B. Yadav, and S. Pilli, Eds., Elsevier, 2023, pp. 293–314. doi: https://doi.org/10.1016/B978-0-323-99908-3.00002-6.
[130] B. Thakur, J. Singh, J. Singh, D. Angmo, and A. P. Vig, “Biodegradation of different types of microplastics: Molecular mechanism and degradation efficiency,” *Sci. Total Environ.*, vol. 877, p. 162912, 2023, doi: https://doi.org/10.1016/j.scitotenv.2023.162912.
[131] F. Liu *et al.*, “A systematic review of electrocoagulation technology applied for microplastics removal in aquatic environment,” *Chem. Eng. J.*, vol. 456, p. 141078, 2023, doi: https://doi.org/10.1016/j.cej.2022.141078.
[132] P. Dhar *et al.*, “Toward green flotation: Interaction of a sophorolipid biosurfactant with a copper sulfide,” *J. Colloid Interface Sci.*, vol. 585, pp. 386–399, 2021, doi: https://doi.org/10.1016/j.jcis.2020.11.079.
[133] N. Taghavi, W.-Q. Zhuang, and S. Baroutian, “Effect of rhamnolipid biosurfactant on biodegradation of untreated and UV-pretreated non-degradable thermoplastics: Part 2,” *J. Environ. Chem. Eng.*, vol. 10, no. 1, p. 107033, 2022, doi: https://doi.org/10.1016/j.jece.2021.107033.
[134] U. Anand *et al.*, “Biotechnological methods to remove microplastics: a review,” *Environ. Chem. Lett.*, Feb. 2023, doi: 10.1007/s10311-022-01552-4.
[135] S. Wang and C. N. Mulligan, “Rhamnolipid biosurfactant-enhanced soil flushing for the removal of arsenic and heavy metals from mine tailings,” *Process Biochem.*, vol. 44, no. 3, pp. 296–301, 2009, doi: https://doi.org/10.1016/j.procbio.2008.11.006.
[136] T. Gaudin *et al.*, “Impact of the chemical structure on amphiphilic properties of sugar-based surfactants: A literature overview,” *Adv. Colloid Interface Sci.*, vol. 270, pp. 87–100, 2019, doi: https://doi.org/10.1016/j.cis.2019.06.003.
[137] H. Yu, M. Liu, D. Gang, J. Peng, C. Hu, and J. Qu, “Polyethylene microplastics interfere with the nutrient cycle in water-plant-sediment systems,” *Water Res.*, vol. 214, p. 118191, 2022, doi: https://doi.org/10.1016/j.watres.2022.118191.
[138] J. D. Berry, M. J. Neeson, R. R. Dagastine, D. Y. C. Chan, and R. F. Tabor, “Measurement of surface and interfacial tension using pendant drop tensiometry,” *J. Colloid Interface Sci.*, vol. 454, pp. 226–237, 2015, doi: https://doi.org/10.1016/j.jcis.2015.05.012.
[139] Kruss Scientific, “Drop Shape Analyzer.” Kruss Scientific. [Online]. Available: https://www.kruss-scientific.com/en/products-services/products/dsa30e
[140] S. Izadyar, S. Fatemi, and T. Mousavand, “Synthesis and modification of nano-sized TiO2 for photo-degradation process under visible light irradiation; a Placket–Burman experimental design,” *Mater. Res. Bull.*, vol. 48, no. 9, pp. 3196–3203, 2013, doi: https://doi.org/10.1016/j.materresbull.2013.04.087.
[141] M. Zribi, B. Samet, and S. Baklouti, “Screening of Factors Influencing Phosphate-Based Geopolymers Consolidation Time, Using Plackett-Burman Design,” in *Advances in Materials, Mechanics and Manufacturing*, F. Chaari, M. Barkallah, A. Bouguecha, B. Zouari, M. T. Khabou, M. Kchaou, and M. Haddar, Eds., Cham: Springer International Publishing, 2020, pp. 115–122.
[142] H. T. A. El-Hamid, A. E. AlProl, and M. A. Hafiz, “The efficiency of adsorption modelling and Plackett-Burman design for remediation of crystal violet by Sargassum latifolium,” *Biocatal. Agric. Biotechnol.*, vol. 44, p. 102459, 2022, doi: https://doi.org/10.1016/j.bcab.2022.102459.
[143] R. Prakash, S. K. Majumder, and A. Singh, “Chemical Engineering & Processing : Process Intensi fi cation Flotation technique : Its mechanisms and design parameters,” vol. 127, no. February, pp. 249–270, 2018, doi: 10.1016/j.cep.2018.03.029.
[144] D. Salerno, H. Jordão, F. La, and M. T. Carvalho, “Using factorial experimental design to evaluate the separation of plastics by froth flotation,” *Waste Manag.*, vol. 73, pp. 62–68, 2018, doi: 10.1016/j.wasman.2017.12.001.
[145] I. E. Kłosowska-Chomiczewska *et al.*, “Rhamnolipid CMC prediction,” *J. Colloid Interface Sci.*, vol. 488, pp. 10–19, 2017, doi: 10.1016/j.jcis.2016.10.055.
[146] S. Varjani and V. N. Upasani, “Evaluation of rhamnolipid production by a halotolerant novel strain of Pseudomonas aeruginosa,” *Bioresour. Technol.*, vol. 288, no. May, p. 121577, 2019, doi: 10.1016/j.biortech.2019.121577.

[147] J. Du, A. Zhang, X. Zhang, X. Si, and J. Cao, “Comparative analysis of rhamnolipid congener synthesis in neotype Pseudomonas aeruginosa ATCC 10145 and two marine isolates,” *Bioresour. Technol.*, 2019, doi: 10.1016/j.biortech.2019.121380.
[148] I. Priyadarshini, A. Chowdhury, A. Rao, B. Roy, and P. Chattopadhyay, “Assessment of bimetallic Zn/Fe0 nanoparticles stabilized Tween-80 and rhamnolipid foams for the remediation of diesel contaminated clay soil,” *J. Environ. Manage.*, vol. 325, p. 116596, 2023, doi: https://doi.org/10.1016/j.jenvman.2022.116596.
[149] S. Gaur, A. Sahani, P. Chattopadhyay, S. Gupta, and A. Jain, “Remediation of Waste Engine Oil Contaminated Soil using Rhamnolipid based Detergent Formulation,” *Mater. Today Proc.*, vol. 77, pp. 31–38, 2023, doi: https://doi.org/10.1016/j.matpr.2022.08.452.
[150] I. Terziyski, L. Alexandrova, I. Stoineva, N. Christova, R. Todorov, and R. Cohen, “Foam and wetting films from rhamnolipids produced by Pseudomonas aeruginosa BN10,” *Colloids Surf. Physicochem. Eng. Asp.*, vol. 460, pp. 299–305, 2014, doi: https://doi.org/10.1016/j.colsurfa.2013.12.075.
[151] R. A. Rodríguez *et al.*, “Degrees of freedom: Definitions and their minimum and most meaningful combination for the modelling of ecosystem dynamics with the help of physical principles,” *Ecol. Model.*, vol. 392, pp. 226–235, 2019, doi: https://doi.org/10.1016/j.ecolmodel.2018.11.021.
[152] C. J. Willmott, K. Matsuura, and S. M. Robeson, “Ambiguities inherent in sums-of-squares-based error statistics,” *Atmos. Environ.*, vol. 43, no. 3, pp. 749–752, 2009, doi: https://doi.org/10.1016/j.atmosenv.2008.10.005.
[153] G. I. Neel and P. R. L. (Lackland A. F. Base (Tex.)), *Estimation of Probabilities Associated with the F Statistic by Digital Computer Techniques*. in PRL-TDR. Personnel Research Laboratory, Aerospace Medical Division, Air Force Systems Command, 1963. [Online]. Available: https://books.google.ca/books?id=xNTfAt7SHHMC
[154] S. Einav and M. O’Connor, “P-values and significance: The null hypothesis that they are not related is correct,” *J. Crit. Care*, vol. 54, pp. 159–162, 2019, doi: https://doi.org/10.1016/j.jcrc.2019.08.020.
[155] R. Kissell and J. Poserina, “Chapter 2 - Regression Models,” in *Optimal Sports Math, Statistics, and Fantasy*, R. Kissell and J. Poserina, Eds., Academic Press, 2017, pp. 39–67. doi: https://doi.org/10.1016/B978-0-12-805163-4.00002-5.
[156] Y. Zhao *et al.*, “Application of biosurfactant tea saponin in fl otation separation for ternary plastic mixtures : Statistical optimization and mechanism analysis,” *J. Clean. Prod.*, vol. 232, pp. 499–507, 2019, doi: 10.1016/j.jclepro.2019.06.002.
[157] S. Costa *et al.*, “Wettability of Aqueous Rhamnolipids Solutions Produced by Pseudomonas aeruginosa LBI,” *J. Surfactants Deterg. - J SURFACTANTS DETERG*, vol. 12, pp. 125–130, May 2009, doi: 10.1007/s11743-008-1102-6.
[158] E. A. Vogler, “Practical Use of Concentration-Dependent Contact Angles as a Measure of Solid-Liquid Adsorption. 1. Theoretical Aspects,” *Langmuir*, vol. 8, no. 8, pp. 2005–2012, 1992, doi: 10.1021/la00044a022.
[159] E. Hallmann and K. Mędrzycka, “Wetting properties of biosurfactant (rhamnolipid) with synthetic surfactants mixtures in the context of soil remediation,” *Gdań. Univ. Technol.*, vol. 70, pp. 29–37, 2015.
[160] A. García-Abuín, D. Gómez-Díaz, M. Losada, and J. M. Navaza, “Bubble column gas–liquid interfacial area in a polymer+surfactant+water system,” *Chem. Eng. Sci.*, vol. 75, pp. 334–341, 2012, doi: https://doi.org/10.1016/j.ces.2012.03.054.
[161] A. Zdziennicka and B. Jańczuk, “Wetting and adhesion properties of rhamnolipid and surfactin,” *Int. J. Adhes. Adhes.*, vol. 84, no. April, pp. 275–282, 2018, doi: 10.1016/j.ijadhadh.2018.04.005.
[162] D. Mańko, A. Zdziennicka, J. Krawczyk, and B. Jańczuk, “Wettability prediction of such polymers as polyethylene and polytetrafluoroethylene by aqueous solutions of classical surfactants and biosurfactants,” *Colloids Surf. Physicochem. Eng. Asp.*, vol. 506, pp. 409–415, 2016, doi: 10.1016/j.colsurfa.2016.06.061.
[163] D. Mesa, A. J. Morrison, and P. R. Brito-parada, “The e ff ect of impeller-stator design on bubble size : Implications for froth stability and fl otation performance,” *Miner. Eng.*, vol. 157, no. June, p. 106533, 2020, doi: 10.1016/j.mineng.2020.106533.
[164] R. A. Grau and K. Heiskanen, “Bubble size distribution in laboratory scale flotation cells,” *Miner. Eng.*, vol. 18, no. 12, pp. 1164–1172, 2005, doi: 10.1016/j.mineng.2005.06.011.
[165] B. K. Gorain, J. P. Franzidis, and E. V. Manlapig, “The empirical prediction of bubble surface area flux in mechanical flotation cells from cell design and operating data,” *Miner. Eng.*, vol. 12, no. 3, pp. 309–322, 1999, doi: 10.1016/s0892-6875(99)00008-4.
[166] S. J. Neethling, “Predicting fl otation behaviour – The interaction between froth stability and performance,” *Miner. Eng.*, vol. 120, no. December 2017, pp. 60–65, 2018, doi: 10.1016/j.mineng.2018.02.002.

[167] W. P. de Haan, A. Sanchez-Vidal, and M. Canals, “Floating microplastics and aggregate formation in the Western Mediterranean Sea,” *Mar. Pollut. Bull.*, vol. 140, pp. 523–535, Mar. 2019, doi: 10.1016/j.marpolbul.2019.01.053.
[168] G. Suaria, C. G. Avio, A. Mineo, G. L. Lattin, M. G. Magaldi, and G. Belmonte, “The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters,” Scientific, 2016. [Online]. Available: https://www.nature.com/articles/srep37551.pdf
[169] M. M. Sari *et al.*, “Plastic pollution in the surface water in Jakarta, Indonesia,” *Mar. Pollut. Bull.*, vol. 182, p. 114023, 2022, doi: https://doi.org/10.1016/j.marpolbul.2022.114023.
[170] J. Zhang *et al.*, “Combined effects of polyethylene and organic contaminant on zebrafish (Danio rerio): Accumulation of 9-Nitroanthracene, biomarkers and intestinal microbiota,” *Environ. Pollut.*, vol. 277, p. 116767, 2021, doi: https://doi.org/10.1016/j.envpol.2021.116767.
[171] V. Budhiraja, A. Urh, P. Horvat, and A. Krzan, “Synergistic Adsorption of Organic Pollutants on Weathered Polyethylene Microplastics,” *Polymers*, vol. 14, no. 13, p. 2674, Jun. 2022, doi: 10.3390/polym14132674.
[172] A. C. Vivekanand, S. Mohapatra, and V. K. Tyagi, “Microplastics in aquatic environment: Challenges and perspectives,” *Chemosphere*, vol. 282, p. 131151, 2021, doi: https://doi.org/10.1016/j.chemosphere.2021.131151.
[173] S. Dey *et al.*, “Microbial strategies for degradation of microplastics generated from COVID-19 healthcare waste,” *Environ. Res.*, vol. 216, p. 114438, 2023, doi: https://doi.org/10.1016/j.envres.2022.114438.
[174] Q. Liu, Y. Chen, Z. Chen, F. Yang, Y. Xie, and W. Yao, “Current status of microplastics and nanoplastics removal methods: Summary, comparison and prospect,” *Sci. Total Environ.*, vol. 851, p. 157991, 2022, doi: https://doi.org/10.1016/j.scitotenv.2022.157991.
[175] L. Ricci, “Adjusted R-squared type measure for exponential dispersion models,” *Stat. Probab. Lett.*, vol. 80, no. 17, pp. 1365–1368, 2010, doi: https://doi.org/10.1016/j.spl.2010.04.019.
[176] S. Deilami, K. Abbasi, A. Houshyar, H. Izadneshan, and H. M. Beni, “Study the effect of temperature variation and intrinsic layer thickness on the linear response of a PIN photodetector: A finite element method approach,” *Results Eng.*, vol. 17, p. 100810, 2023, doi: https://doi.org/10.1016/j.rineng.2022.100810.
[177] J. M. Wooldridge, “A note on computing r-squared and adjusted r-squared for trending and seasonal data,” *Econ. Lett.*, vol. 36, no. 1, pp. 49–54, 1991, doi: https://doi.org/10.1016/0165-1765(91)90054-O.
[178] D. S. K. Karunasingha, “Root mean square error or mean absolute error? Use their ratio as well,” *Inf. Sci.*, vol. 585, pp. 609–629, 2022, doi: https://doi.org/10.1016/j.ins.2021.11.036.
[179] C. Draper, R. Reichle, R. de Jeu, V. Naeimi, R. Parinussa, and W. Wagner, “Estimating root mean square errors in remotely sensed soil moisture over continental scale domains,” *Remote Sens. Environ.*, vol. 137, pp. 288–298, 2013, doi: https://doi.org/10.1016/j.rse.2013.06.013.
[180] N. (Klaas) M. Faber, “Estimating the uncertainty in estimates of root mean square error of prediction: application to determining the size of an adequate test set in multivariate calibration,” *Chemom. Intell. Lab. Syst.*, vol. 49, no. 1, pp. 79–89, 1999, doi: https://doi.org/10.1016/S0169-7439(99)00027-1.
[181] P. A. Athulya and N. Chandrasekaran, “Interactions of natural colloids with microplastics in aquatic environment and its impact on FTIR characterization of polyethylene and polystyrene microplastics,” *J. Mol. Liq.*, vol. 369, p. 120950, 2023, doi: https://doi.org/10.1016/j.molliq.2022.120950.
[182] S. Krimm, C. Y. Liang, and G. B. B. Sutherland, “Infrared Spectra of High polymers. II. Polyethylene,” *The Journal of Physics*, vol. 3, no. 3, Sep. 1956.
[183] Z. Chen, J. N. Hay, and M. J. Jenkins, “FTIR spectroscopic analysis of poly(ethylene terephthalate) on crystallization,” *Eur. Polym. J.*, vol. 48, no. 9, pp. 1586–1610, 2012, doi: https://doi.org/10.1016/j.eurpolymj.2012.06.006.
[184] Z. Deng, T. Wang, N. Zhang, and Z. Wang, “Gas holdup, bubble behavior and mass transfer in a 5 m high internal-loop airlift reactor with non-Newtonian fluid,” *Chem. Eng. J. - CHEM ENG J*, vol. 160, pp. 729–737, Jun. 2010, doi: 10.1016/j.cej.2010.03.078.
[185] Kautsch and G. Kunsts, “Emissions from tires in dedicated use,” vol. 51, pp. 182–186, 1998.
[186] W. F. Rogge, L. M. Hildemann, M. A. Mazurek, G. R. Cass, and B. R. T. Simoneit, “Sources of fine organic aerosol. 3. Road dust, tire debris, and organometallic brake lining dust: roads as sources and sinks,” *Environ. Sci. Technol.*, vol. 27, no. 9, pp. 1892–1904, Sep. 1993, doi: 10.1021/es00046a019.
[187] F. J. Charters, T. A. Cochrane, and A. D. O’Sullivan, “Particle size distribution variance in untreated urban runoff and its implication on treatment selection,” *Water Res.*, vol. 85, pp. 337–345, 2015, doi: https://doi.org/10.1016/j.watres.2015.08.029.

[188] B. Cunningham, B. Harper, S. Brander, and S. Harper, “Toxicity of micro and nano tire particles and leachate for model freshwater organisms,” *J. Hazard. Mater.*, vol. 429, p. 128319, 2022, doi: https://doi.org/10.1016/j.jhazmat.2022.128319.
[189] L. Kim, T.-Y. Lee, H. Kim, and Y.-J. An, “Toxicity assessment of tire particles released from personal mobilities (bicycles, cars, and electric scooters) on soil organisms,” *J. Hazard. Mater.*, vol. 437, p. 129362, 2022, doi: https://doi.org/10.1016/j.jhazmat.2022.129362.
[190] E. M. F. Kallenbach, E. S. Rødland, N. T. Buenaventura, and R. Hurley, “Microplastics in Terrestrial and Freshwater Environments,” in *Microplastic in the Environment: Pattern and Process*, M. S. Bank, Ed., Cham: Springer International Publishing, 2022, pp. 87–130. doi: 10.1007/978-3-030-78627-4_4.
[191] Z. Yuan, R. Nag, and E. Cummins, “Human health concerns regarding microplastics in the aquatic environment - From marine to food systems,” *Sci. Total Environ.*, vol. 823, p. 153730, 2022, doi: https://doi.org/10.1016/j.scitotenv.2022.153730.
[192] M. Venturelli, E. Falletta, C. Pirola, F. Ferrari, M. Milani, and L. Montorsi, “Experimental evaluation of the pyrolysis of plastic residues and waste tires,” *Appl. Energy*, vol. 323, p. 119583, 2022, doi: https://doi.org/10.1016/j.apenergy.2022.119583.
[193] P. Wiśniewska, S. Wang, and K. Formela, “Waste tire rubber devulcanization technologies: State-of-the-art, limitations and future perspectives,” *Waste Manag.*, vol. 150, pp. 174–184, 2022, doi: https://doi.org/10.1016/j.wasman.2022.07.002.
[194] M. B. Sathicq *et al.*, “PET particles raise microbiological concerns for human health while tyre wear microplastic particles potentially affect ecosystem services in waters,” *J. Hazard. Mater.*, vol. 429, p. 128397, 2022, doi: https://doi.org/10.1016/j.jhazmat.2022.128397.
[195] C. Johannessen, J. Liggio, X. Zhang, A. Saini, and T. Harner, “Composition and transformation chemistry of tire-wear derived organic chemicals and implications for air pollution,” *Atmospheric Pollut. Res.*, vol. 13, no. 9, p. 101533, 2022, doi: https://doi.org/10.1016/j.apr.2022.101533.
[196] A. Al-Sabagh, T. El-Sawy, and A. Hegazy, “Effect of Filler Type and Concentration on the Mechanical Properties of Tire Tread Compounds,” *Journal of Polymers and the Environment*.
[197] S. Wongwat, R. Yoksan, and M. S. Hedenqvist, “Bio-based thermoplastic natural rubber based on poly(lactic acid)/thermoplastic starch/calcium carbonate nanocomposites,” *Int. J. Biol. Macromol.*, vol. 208, pp. 973–982, 2022, doi: https://doi.org/10.1016/j.ijbiomac.2022.03.175.
[198] E. Papirer, J. Schultz, and C. Turchi, “Surface properties of a calcium carbonate filler treated with stearic acid,” *Eur. Polym. J.*, vol. 20, no. 12, pp. 1155–1158, 1984, doi: https://doi.org/10.1016/0014-3057(84)90181-2.
[199] K. Longkaew, W. Tessanan, P. Daniel, P. Phinyocheep, and A. Gibaud, “Using sucrose to prepare submicrometric CaCO3 vaterite particles stable in natural rubber,” *Adv. Powder Technol.*, vol. 34, no. 1, p. 103924, 2023, doi: https://doi.org/10.1016/j.apt.2022.103924.
[200] S. K. Bhatia, S. K. Sharma, and P. V. Joshi, “Investigation of polyethylene terephthalate (PET) waste for chemical recycling to monomers,” *Polymer-Plastics Technology and Engineering*, vol. 38, no. 6, pp. 969–979.
[201] S. S. Ray and M. Okamoto, “Polymer/layered silicate nanocomposites: a review from preparation to processing,” *Progress in Polymer Science*, vol. 28, pp. 1539–1641.
[202] J. A. Deanin, “Application of infrared spectroscopy to the study of polyesters,” vol. 4, pp. 1303–1313.
[203] M. Mecozzi and L. Nisini, “The differentiation of biodegradable and non-biodegradable polyethylene terephthalate (PET) samples by FTIR spectroscopy: A potential support for the structural differentiation of PET in environmental analysis,” *Infrared Phys. Technol.*, vol. 101, pp. 119–126, 2019, doi: https://doi.org/10.1016/j.infrared.2019.06.008.
[204] E. Mendiburu-Valor *et al.*, “Valorization of urban and marine PET waste by optimized chemical recycling,” *Resour. Conserv. Recycl.*, vol. 184, p. 106413, 2022, doi: https://doi.org/10.1016/j.resconrec.2022.106413.
[205] T. R. Shojaei and S. Azhari, “Chapter 16 - Fabrication, functionalization, and dispersion of carbon nanotubes,” in *Emerging Applications of Nanoparticles and Architecture Nanostructures*, A. Barhoum and A. S. H. Makhlouf, Eds., in Micro and Nano Technologies. Elsevier, 2018, pp. 501–531. doi: https://doi.org/10.1016/B978-0-323-51254-1.00016-6.
[206] S. Gunasekaran, R. K. Natarajan, and A. Kala, “FTIR spectra and mechanical strength analysis of some selected rubber derivatives,” *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.*, vol. 68, no. 2, pp. 323–330, 2007, doi: https://doi.org/10.1016/j.saa.2006.11.039.

[207] A. N. Chaudhry and N. C. Billingham, “Characterisation and oxidative degradation of a room-temperature vulcanised poly(dimethylsiloxane) rubber,” *Polym. Degrad. Stab.*, vol. 73, no. 3, pp. 505–510, 2001, doi: https://doi.org/10.1016/S0141-3910(01)00139-2.
[208] M. Yoshizawa, E. Marwanta, and H. Ohno, “Preparation and characteristics of natural rubber/poly(ethylene oxide) salt hybrid mixtures as novel polymer electrolytes,” *Polymer*, vol. 41, no. 26, pp. 9049–9053, 2000, doi: https://doi.org/10.1016/S0032-3861(00)00277-9.
[209] H. Kang, M. Kang, and K.-H. Han, “Identification of Natural Rubber and Characterization of Rubber Biosynthetic Activity in Fig Tree,” *Plant Physiol.*, vol. 123, pp. 1133–42, Aug. 2000.

8. Appendices

Appendix A. Full factorial design for the investigation of PE removal efficiency

This appendix reports the PE removal efficiency (Y) for a full factorial design. The overall design included the following factors rhamnolipid concentration (X_1), operating time (X_2), PE size (X_3) and PE concentration (X_4).

Table A. 1. Full factorial design matrix for PE removal efficiency using rhamnolipids biosurfactant

X1	X2	X3	X4	Y
4	36	300	2	49.01
4	36	300	1.5	49.15
4	36	300	1	50.12
4	36	125	2	75.08
4	36	125	1.5	76.9
4	36	125	1	77.47
4	36	53-59	2	78.21
4	36	53-59	1.5	80.34
4	36	53-59	1	85.18
4	24	300	2	29.65
4	24	300	1.5	46.37
4	24	300	1	48.08
4	24	125	2	61.65
4	24	125	1.5	64.9
4	24	125	1	66.95
4	24	53-59	2	77.32
4	24	53-59	1.5	79.63
4	24	53-59	1	81.32
4	12	300	2	22.81
4	12	300	1.5	24.43
4	12	300	1	28.94
4	12	125	2	21.34
4	12	125	1.5	25.93
4	12	125	1	30.1
4	12	53-59	2	29.14
4	12	53-59	1.5	33.36
4	12	53-59	1	35.17

1.5	36	300	2	14.18
1.5	36	300	1.5	37.99
1.5	36	300	1	40.48
1.5	36	125	2	44.65
1.5	36	125	1.5	49.27
1.5	36	125	1	53.55
1.5	36	53-59	2	49.5
1.5	36	53-59	1.5	51.91
1.5	36	53-59	1	60.9
1.5	24	300	2	20.79
1.5	24	300	1.5	28.34
1.5	24	300	1	29.53
1.5	24	125	2	15.78
1.5	24	125	1.5	25.59
1.5	24	125	1	32.41
1.5	24	53-59	2	55.28
1.5	24	53-59	1.5	66.15
1.5	24	53-59	1	78.21
1.5	12	300	2	10.27
1.5	12	300	1.5	10.23
1.5	12	300	1	12.43
1.5	12	125	2	12.23
1.5	12	125	1.5	16.51
1.5	12	125	1	17.37
1.5	12	53-59	2	9.87
1.5	12	53-59	1.5	16.25
1.5	12	53-59	1	19.51
1	36	300	2	24.98
1	36	300	1.5	32.04
1	36	300	1	39.22
1	36	125	2	29.12
1	36	125	1.5	40.49
1	36	125	1	45.71
1	36	53-59	2	61.24
1	36	53-59	1.5	65.32
1	36	53-59	1	67.04
1	24	300	2	22.59
1	24	300	1.5	22.34
1	24	300	1	41.29

1	24	125	2	13.85
1	24	125	1.5	16.21
1	24	125	1	19.4
1	24	53-59	2	27.41
1	24	53-59	1.5	32.05
1	24	53-59	1	42.76
1	12	300	2	27.45
1	12	300	1.5	28.32
1	12	300	1	29.82
1	12	125	2	30.32
1	12	125	1.5	35.62
1	12	125	1	36.24
1	12	53-59	2	13.45
1	12	53-59	1.5	15.79
1	12	53-59	1	19.55

Appendix B. Full factorial design for the investigation of Ctr removal efficiency

This appendix reports the PE removal efficiency (Y) for a full factorial design. The overall design included the following factors rhamnolipid concentration (X_1), operating time (X_2), Ctr size (X_3) and Ctr concentration (X_4).

Table B. 1. Full factorial design matrix for PE removal efficiency using rhamnolipids biosurfactant

X1	X2	X3	X4	Y
4	36	300	2	35.23
4	36	300	1.5	37.81
4	36	300	1	40.64
4	36	90	2	63.76
4	36	90	1.5	63..47
4	36	90	1	70.91
4	36	53	2	68.32
4	36	53	1.5	76.21
4	36	53	1	78.59
4	24	300	2	25.04
4	24	300	1.5	29.21
4	24	300	1	33.47
4	24	90	2	61.53
4	24	90	1.5	67.28
4	24	90	1	68.36
4	24	53	2	71.78
4	24	53	1.5	72.47
4	24	53	1	74.57
4	12	300	2	10.81
4	12	300	1.5	13.84
4	12	300	1	14.27
4	12	90	2	16.69
4	12	90	1.5	21.74
4	12	90	1	23.73
4	12	53	2	26.34
4	12	53	1.5	29.17
4	12	53	1	30.99
1.5	36	300	2	29.45
1.5	36	300	1.5	30.21

1.5	36	300	1	34.54
1.5	36	90	2	36.99
1.5	36	90	1.5	44.21
1.5	36	90	1	50.58
1.5	36	53	2	48.46
1.5	36	53	1.5	58.35
1.5	36	53	1	60.9
1.5	24	300	2	39.42
1.5	24	300	1.5	41.55
1.5	24	300	1	50.78
1.5	24	90	2	56.32
1.5	24	90	1.5	58.71
1.5	24	90	1	62.11
1.5	24	53	2	62.37
1.5	24	53	1.5	68.21
1.5	24	53	1	56.21
1.5	12	300	2	26.81
1.5	12	300	1.5	30.41
1.5	12	300	1	34.94
1.5	12	90	2	11.3
1.5	12	90	1.5	19.67
1.5	12	90	1	29.37
1.5	12	53	2	20.03
1.5	12	53	1.5	23.43
1.5	12	53	1	25.89
1	36	300	2	21.2
1	36	300	1.5	27.59
1	36	300	1	28.28
1	36	90	2	38.1
1	36	90	1.5	47.02
1	36	90	1	49.36
1	36	53	2	48.72
1	36	53	1.5	49.71
1	36	53	1	53.46
1	24	300	2	19.47
1	24	300	1.5	22.25
1	24	300	1	28.62
1	24	90	2	25.76
1	24	90	1.5	29.6

1	24	90	1	31.84
1	24	53	2	35.52
1	24	53	1.5	38.62
1	24	53	1	40.96
1	12	300	2	10.19
1	12	300	1.5	12.83
1	12	300	1	15.79
1	12	90	2	17.01
1	12	90	1.5	19.32
1	12	90	1	20.11
1	12	53	2	20.19
1	12	53	1.5	20.85
1	12	53	1	21.29

Appendix C. Car tire residue

An image of the Ctr microplastic product used for FTIR analysis is shown in Figure

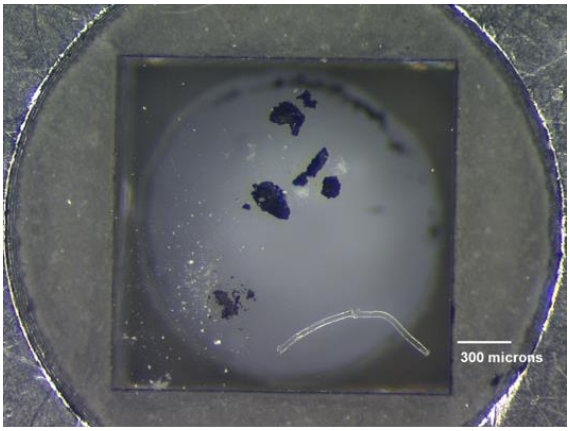

Figure C.1. Image of collected Car tire residue used for FTIR analysis

Appendix D. Bubble sizes of rhamnolipids based-foam

This appendix reports the bubble size of bubble formulated in the microplastic removal from the ternary mixture containing water, microplastic particles and rhamnolipids biosurfactant.

Table D. 1. Bubble diameter of rhamnolipids based-foam for the removal of PE as a function of time

Bubble diameter (cm)	Time (min)	Rmlp (g/L)
0.414	20	1
2.343	40	1
0.276	20	5
0.930	40	5

Table D. 2. Bubble diameter of rhamnolipids based-foam using various PE sizes and PE concentrations in the ternary mixture

PE particle size (μm)	**C_{RMLP} (g/L)**	**Bubble Diameter (cm)**
	0	0.23
	0.5	0.123
	1	0.092
	2	0.065
	C_{RMLP} =C_{PE} (g/L)	**Bubble Diameter (cm)**
	0	0.23
53-59	0.5	0.165
	1	0.101
	2	0.086
	C_{RMLP} =C_{PE} (g/L)	**Bubble Diameter (cm)**
	0	0.23
125	0.5	0.123
	1	0.092
	2	0.04
	C_{RMLP} =C_{PE} (g/L)	
	0	0.23
300	0.5	0.141
	1	0.09
	2	0.078

Table D. 3. Bubble diameter of rhamnolipids based-foam using various Ctr sizes and Ctr concentrations in the ternary mixture

Ctr particle size (μm)	**C_{RMLP} (g/L)**	**Bubble Diameter (cm)**
	0	0.23
	0.5	0.123
	1	0.092
	2	0.065
	$C_{RMLP} = C_{Ctr}$ (g/L)	**Bubble Diameter (cm)**
53	0	0.23
	0.5	0.183
	1	0.121
	2	0.091
	$C_{RMLP} = C_{Ctr}$ (g/L)	**Bubble Diameter (cm)**
90	0	0.23
	0.5	0.181
	1	0.093
	2	0.088
	$C_{RMLP} = C_{Ctr}$ (g/L)	**Bubble Diameter (cm)**
300	0	0.23
	0.5	0.172
	1	0.09
	2	0.078

Curriculum Vitae

Ana M. Giron

EDUCATION

- **Master of Engineering Science in Chemical Eng.) |** University of Western Ontario **| 2016**
- **Bachelor of Science in Chemical Engineering|** University of Los Andes **| 2012**

RELEVANT EXPERIENCE

Research Assistant |University of Western Ontario | Sep 2018 – Present

- Investigated the use of biosurfactant-based foam as an alternative for microplastic removal
- Produced, extracted and purified different biosurfactants
- Carried out experiments to identify the parameters that influence the microplastic removal efficiency
- Designed a microplastics removal unit
- Developed a mathematical model to predict the removal of plastics from water

Researcher |KMW Energy Inc | Sep 2016 – May 2017

- Studied the effect of operational and non-operational parameters in steam biomass gasification using a novel Riser Simulator Reactor.
- Optimized the process
- Prepared and characterized the materials to load the reactor
- Carried out laboratory runs and process simulations
- Prepared reports

Chemical Engineer | RECAT Technologies | Aug 2014 – Aug 2016

- Developed standard operating procedures for product testing using GC, HPLC, TOC and mass spectrometer.
- Conducted mass and energy balance calculations for yield optimization of biomass systems for

a reactor unit.

- Assisted with trouble shooting of scale-up problems and developed solutions.
- Worked on optimizing a thermal biomass gasification process and its application to electricity and fuel production.
- Dried, weighed, and characterized biomass feedstocks to produce samples and small-scale batches for experimental trials.
- Assisted with technology transfer from R&D batch scale to commercial facilities.

Process Engineer | Pacific Rubiales Energy | Jan 2012 – Dec 2013

- Designed a wastewater treatment plant, including hydraulic modeling and piping system design (PFDs and P&IDs)
- Evaluated the cost of the plant including equipment, valves, pipes, etc.
- Effectively coordinated with Quality Engineering teams for reduction of pollutants throughout production.
- Evaluated equipment and processes to ensure compliance with safety and environmental regulations

Process Engineer| Fundacion Cardio Infantil | Jan 2011 – Dec 2011

- Worked on the development of an Acellular hemoglobin blood substitute.
- Assisted in continuous improvement of a laboratory model of the circulatory system in humans with emphasis on problem detection and clogged prevention.
- Developed competency to perform operations and operate equipment as assigned.
- Prepared project evolution reports weekly including future work.