ANALYSIS OF LEACHING ADDITIVES FROM PLASTICS AND INVESTIGATION OF CHANGE IN PLASTIC PROPERTIES DURING SLUDGE PRETREATMENT PRIOR TO ANAEROBIC DIGESTION

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ABSTRACT

ANALYSIS OF LEACHING ADDITIVES FROM PLASTICS AND INVESTIGATION OF CHANGE IN PLASTIC PROPERTIES DURING SLUDGE PRETREATMENT PRIOR TO ANAEROBIC DIGESTION

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Microplastics (MPs) are small in size and have a high specific surface area, which makes them easily transported into soil, air, and water. Unfortunately, a large amount of MPs that end up in wastewater treatment plants are accumulated in sludge. Anaerobic digestion (AD) of sludge is known to be affected by MPs. Sludge disintegrations are applied before AD to enhance digestion efficiency by increasing biogas production and disrupting the complex structure of sludge. This study aimed to investigate the effects of different disintegration methods on polyethylene terephthalate (PET), polypropylene (PP) and polycarbonate (PC) MPs. Sludge samples spiked with MPs with 10 mg/g total solid (TS) dose were exposed to 0.5 M alkali for two days, thermally treated at 127°C for two hours and enzymatically disintegrated by pancreatin enzyme with 150 mg/g and 200 mg/g TS doses at 38°C for 24 hours, separately. Alkali-thermal and thermal-enzyme combined disintegrations were also applied. Phthalates and bisphenol-A (BPA) leaching after disintegrations were quantified using GC-MS following solid-phase extraction. Phthalates, mostly, did not leach upon disintegration. BPA concentration, on the

other hand, drastically increased 4000 times in PC-containing sludge after alkali-thermal combined disintegration. The carbonyl index decreased in PET and PC-MPs after both combined treatments, whereas that of PP-MPs was found to be negligible. The crystallinity of PET decreased by 10% after alkali-thermal disintegration and that of PP increased by 7% and 3% after alkali-thermal and thermal-enzyme treatments, respectively. For the first time, this study demonstrated the effects of sludge disintegrations on PP-MPs and PC-MPs.

Keywords: Microplastics, Anaerobic Digestion, Sludge Disintegrations, Plastic Additives.

ÖZ

PLASTİKLERDEN SIZAN KATKI MADDELERİNİN ANALİZİ VE ANAEROBİK ÇÜRÜTME ÖNCESİ ÖN ARITMA SIRASINDA PLASTİK ÖZELLİKLERİNDEKİ DEĞİŞİMLERİN İNCELENMESİ

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Mikroplastikler (MP'ler) küçük boyutları ve yüksek spesifik yüzey alanlarına sahip olmaları nedeniyle toprağa, havaya ve suya kolayca taşınabilmektedirler. Ne yazık ki, atıksu arıtma tesislerine ulaşan MP'lerin büyük bir kısmı çamurda birikmektedir. Çamurun anaerobik çürütücülerde işletilmesi MP'lerin varlığından etkilenmektedir. Çamurun çürütülmesini arttırmak ve karmaşık yapısını bozarak biyogaz üretimi sağlamak için anaerobik çürütme öncesinde çamura dezentegrasyon işlemleri uygulanmaktadır. Bu çalışma, farklı dezentegrasyon yöntemlerinin polietilen tereftalat (PET), polipropilen (PP) ve polikarbonat (PC) MP'ler üzerindeki etkilerini araştırmayı amaçlamıştır. 10 mg/g toplam katı madde (TKM) dozunda MP'lere sahip çamur numuneleri iki gün boyunca 0,5 M alkali, 120 dakika boyunca 127°C'de termal ve 38°C'de 24 saat boyunca 150 mg/g ve 200 mg/g TKM doz pankreatin enzimi ile enzimatik dezentegrasyonlara tabii tutulmuştur. Alkali-termal ve termal-enzim kombinasyonlu dezentegrasyon yöntemler uygulanmıştır. Dezentegrasyona maruz bırakılan çamurların üst suları katı-faz ekstraksiyon metodu ile ekstre edildikten sonra sızan fitalat ve bisfenol-A (BPA) derişimleri GC-MS ile tayin edildi.

Uygulanan dezentegrasyonlar sonrasında genel olarak fitalatların sızmadığı saptanmıştır. Öte yandan, alkali-termal kombine dezentegrasyonu sonrası PC içeren çamur örneklerinde BPA derişimi yaklaşık 4000 kat artmıştır. Karbonil indeks değerleri PET ve PC-MP'lerinde kombine işlemler sonrasında azalırken, PP-MP'lerinde bu değer tayin sınırlarının altında kalmıştır. Alkali-termal dezentegrasyonu sonrası PET-MP'lerinin kristalinitesinde %10 azalma, PP-MP'lerinde ise alkali-termal ve termal-enzim işlemlerinden sonra sırasıyla %7 ve %3 artışı tespit edilmiştir. Bu çalışma ile literatürde ilk kez çamur dezentegrasyon yöntemlerinin PP ve PC-MP'ler üzerindeki etkileri gösterilmiştir.

Anahtar Kelimeler: Mikroplastikler, Anaerobik Çürütücü, Çamur Dezentegrasyonu, Plastik Katkı Maddeleri.

To my family

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LIST OF ABBREVIATIONS

ABBREVIATIONS

ABS	Acrylonitrile-butadiene-styrene copolymer
AD	Anaerobic digestion
BPA	Bisphenol A
BBP	Butyl benzyl phthalate
CI	Carbonyl index
COD	Chemical oxygen demand
DEHP	Di-2-ethyl hexyl phthalate
DBP	Dibutyl phthalate
DDT	Dichlorodiphenyltrichloroethane
DEP	Diethyl phthalate
DSC	Differential scanning calorimetry
DiBP	Diisobutyl phthalate
DMP	Dimethyl phthalate
DPP	Dipentyl phthalate
EVA	Ethylenevinyl acetate copolymer
EPSs	Extracellular polymeric substances
FRs	Flame retardants
FTIR	Fourier-transform infrared spectroscopy
GC-MS	Gas chromatography coupled with mass spectrometry
Tg	Glass transition temperature
HPLC-MS	High purity liquid chromatography-mass spectrometry
HDPE	High-density PE
HIPS	High-impact polystyrene
Log Kow	Log Kow octanol-water partition coefficient
LDPE	Low-density polyethylene
MPs	Microplastics
MCE	Mixed cellulose ester
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
NPs	Nonylphenols
POPs	Persistent organic pollutants
PA	Polyamide
PAEs	Phthalic acid esters
PAH	Polycyclic aromatic hydrocarbons
PC	Polycarbonate

PCB	Polychlorinated biphenyls
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
Pyr-GC-MS	Pyrolysis gas chromatography- mass spectrometry
SEM	Scanning electron microscope
SPE	Solid-phase extraction
SS	Surrogate standard
TS	Total solid
TMCS	Trimethylchlorosilane
UV	Ultraviolet
VOC	Volatile organic compound
WWTP	Wastewater treatment plant

CHAPTER 1

INTRODUCTION

Plastics are synthetic organic polymers which have become an indispensable part of human life and are widely used in various industries such as agriculture, medicine, packaging, construction, textiles, and automotive. Plastics are popular because they are affordable, durable, lightweight and can be combined with other materials.[1]

As the population grows, the worldwide production of plastics has exceeded 390 million metric tons by 2021 due to increasing the usage of plastics. It has been reported that the waste will reach up to 53 million tons by 2030.[1] The constant growth in the production, consumption, and volume of mismanaged plastic waste is mirrored by the rising accumulation of these materials in the environment or dumped into the soil or aquatic systems.[2]

MPs with sizes of 1 μm to 5 mm can be discharged into the environment from a variety of sources due to inadequate handling of plastic waste.[3,4] MPs can be classified into two main categories. Primary MPs refer to the raw materials and they are utilized in household and personal care products. Secondary MPs are formed through the breakdown of primary plastic particles due to various environmental processes such as physical, chemical, and biological mechanisms.[5,6]

MPs are easily dispersed and transported between different environmental systems due to their diverse shape, small size, lightweight nature, and low density.[7] MPs have a high tendency to sorb and transport persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethane (DDT), and polycyclic aromatic hydrocarbons (PAH), due to their hydrophobic nature and high surface area-to-volume ratio.[5] MPs can degrade via environmental factors such as

sunlight, UV, temperature and pH.[8] Plastic additives such as phthalates and flame retardants are added during production to improve polymer properties and prolong their lifespan. While exposed to such physical or biological stressors, these additives can be leached from MPs.[9] Since they are not chemically bonded to the polymer structure, they can easily be released from the plastic when exposed to stress factors. The most commonly used additives are plasticizers, which can account for up to 70% of the polymer base. Phthalic acid esters (PAEs), also known as phthalates, are the most common plasticizers used.[10]

Wastewater treatment plants (WWTPs) are the final step in the water cycle created by human activities. A large number of MPs are usually collected from sources such as household and industrial wastewater, stormwater, and landfill leachate in WWTPs. While it has been claimed that WWTPs can remove up to 98% of MPs from wastewater, they are still accumulated in a by-product of the WWTP process called sewage sludge.[11,12] The most widely found MP types in sewage sludge are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polycarbonate (PC), and polyamide (PA).[13]

Many nations consider sewage sludge as a resource rather than waste due to its beneficial ingredients, including water, organic carbon and nutrients which can serve as an energy source, soil conditioner or fertilizer for plants.[14] However, sludge also contains some pathogenic microorganisms and toxic substances. Therefore, before it can be disposed of or used for sludge for land application, it must undergo stabilization.[15]

There are various methods to stabilize sludge, including physical, chemical, and biological processes.[16] Anaerobic digestion of sludge refers to a series of biochemical processes through which microorganisms decompose organic species in sewage sludge without oxygen. This method is cost-effective for recovering energy in the form of methane while having minimal environmental impact.[15,17] It also helps in reducing the final solids and eliminating most of the pathogenic microorganisms in the sludge. However, the hydrolysis of particulate organic matter to soluble

substances is the rate-limiting step during anaerobic digestion due to the complex floc structure of the sludge.[15] To address this issue, various sludge disintegration methods have been developed to enhance the rate of sludge solubilization and hydrolysis and improve the efficiency of the anaerobic digestion process. These methods include thermal, chemical, physical and biological disintegrations.[18]

Fragmentation of solid particles can be achieved through physical and mechanical disintegration methods, leading to the reduction of particle size. This reduction in size can promote the anaerobic digestion (AD) process by increasing the surface area of the particles. Chemical disintegration, on the other hand, increases biogas production by improving the biodegradability of cellular material. This method uses chemical reagents such as acids, bases, and oxidants to hydrolyze the sludge. Additionally, it is the most promising method for the destruction of complex organic waste. The thermal disintegration method involves elevated temperatures to promote hydrolysis and enhance the digestibility of sewage sludge and other wastes. Biological disintegrations are environmentally sustainable and employ enzymatic, aerobic, and anaerobic processes to predigest and augment the AD hydrolysis stages.[18,19]

Aforementioned disintegration methods can enhance sludge solubility and increase biogas production. However, these methods can also create stress factors for MPs accumulated in sludge. These stress factors may potentially change the properties of MPs; however, there are few studies on this topic in the literature.[20,21] This study aims to fill this gap in this area.

Aim of the Study

This study aims to investigate the effect of different sludge disintegration methods on the leaching of additives and the physicochemical and morphological properties of selected microplastics (MPs). PET, PP, and PC plastics were chosen for this study because they are commonly used in daily life and are known to be present in sludge. Several sludge disintegration methods were investigated and optimized in this study. There are various disintegration methods including alkali, thermal, enzyme, alkali-

thermal combined and thermal-enzyme combined disintegrations. The aim is to extract and analyze the additives leaching from the plastics. These additives are primarily plasticizers and antioxidants which increase flexibility, durability, and resistance to UV light. This study will focus on the phthalate group chemicals, which are typically used in plastics; namely diethyl phthalate (DEP), dibutyl phthalate (DBP), di-2-ethyl hexyl phthalate (DEHP), and bisphenol A (BPA), which is one of the monomers of PC and is commonly used as antioxidant in PET and PVC.

The following are the specific objectives of this study:

- The aim was to analyze leaching additives from plastics. Solid-phase extraction (SPE) and gas chromatography coupled with mass spectrometry (GC-MS) methods were established to extract and quantify PAEs (DEP, DBP and DEHP) and BPA.
- Another aim was to analyze the changes in surface morphology, crystallinity, or carbonyl index of PET, PP, and PC-MPs due to different sludge disintegration methods.

Our research group is investigating the effects of disintegration methods on improving biogas production and the properties of MPs during anaerobic digestion. We will monitor changes in biogas and the properties of MPs after 60 days of digestion. These findings will allow us to understand better the reasons for changes in biogas production and the properties of MPs during anaerobic digestion.

CHAPTER 2

LITERATURE REVIEW

2.1 Plastics

The concept of a world devoid of plastics, or synthetic organic polymers, appears inconceivable in the present era, even though their widespread manufacturing and utilization commenced about 1950. The emergence of synthetic plastics, exemplified by Bakelite, was observed during the initial years of the 20th century. However, plastics in non-military applications were not used until after the end of World War II. After the war, the manufacturing of plastics grew rapidly, exceeding that of most other synthetic materials.[22] Plastics exhibit a notable strength-to-weight ratio, high moldability, and impermeability to liquids. They are also resilient against physical and chemical deterioration, cost-effectiveness and potential for replacing various materials (e.g., glass, metal, wood, and natural fibers) in diverse applications. Furthermore, plastics can facilitate novel applications and services ranging from takeaway food to information technology. Due to these advantages, they have been widely employed and their usage is rising due to population growth.[23,24] Plastics are extensively utilized throughout many industrial domains such as packaging, construction, automotive, electronics, textiles, home goods, and toys.[23,25,26]

A diverse range of molecules are used during the production and processing stages of plastics.[27] They are synthesized from the repetition of monomer units.[28] Additives play a crucial role in enhancing the properties of plastics such as flexibility, fire resistance and protection against environmental conditions. Processing aids are also used to simplify the manufacturing or processing of plastics, such as polymerization catalysts, solvents, or lubricants. Plastics may also contain non-intentionally added

substances, such as byproducts, degradation products, and impurities.[29]Plastics can be divided into two major categories: thermoplastics and thermosets based on their composition.[30] When subjected to heat, thermoplastics retain their chemical composition, allowing for repeated molding processes.[27] They are highly recyclable, but they have poor heat resistance. Polytetrafluoroethylene (PTFE), polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) are a few examples of thermoplastics.[26] Thermoset plastics, on the other hand, undergo permanent solidification and remain in a solid state. The thermosetting procedure involves an irreversible chemical reaction. They are resistant to heat and weather, but they are difficult to recycle. Epoxy resin and silicone are examples of thermoset plastics.[27,31]

In 2021, the global production of plastic continued to increase, reaching 390.7 million metric tons (Mt) and 90.2% of global plastic production was derived from fossil-based sources, while post-consumer recycled plastics and bio-based plastics accounted for 8.3% and 1.5% of global plastics production, respectively. China is the largest producer of plastic, producing one-third of the world's total (32%) followed by North America (18%), other Asian countries (17%) and European nations (15%). Also, 44% of the annual plastic production in the world belongs to packaging, the following 18% to building and construction, 8% to automotive and the rest to electrical-electronics, household, leisure & sports, agriculture, farming & gardening.[32]

2.1.1 Plastic Waste

The plastic waste accumulation in the environment[33,34] can be attributed to various factors, including the extensive production of plastics[35] and inadequate waste management practices.[36] The waste may reach 53 million tons annually by 2030.[37]

Approximately 2500 Mt of plastics, accounting for 30% of the total plastics ever manufactured, are currently in use. Out of the plastic waste generated between 1950 and 2015, only 9% underwent recycling, while 12% was incinerated. The remaining 80% of plastic waste is either accumulating accumulated in landfills or natural environments. If the current pattern of waste production and management persists, about 12,000 Mt of plastic waste will be deposited in landfills or dispersed in the natural environment by the year 2050.[22]

Plastic waste incineration produces poisonous and dangerous gases, resulting in air pollution. Unfortunately, this is a common practice in many less developed and developing nations due to the lack of properly engineered and planned landfill sites to dispose of this waste. Furthermore, several improper disposal practices, such as open dumping, uncontrolled incineration, unscientific composting, and incorrect landfilling are commonly used in countries such as India, Nigeria and Cambodia.[8,38]

2.1.2 Environmental Consequences of Plastic Waste

Many researchers have conducted a comprehensive examination of the detrimental impacts of plastic waste on the environment which can be summarized as follows [8]:

- Plastic wastes in bulky form cause aesthetics problems, they have the potential to be carried to far distances by air and water transport and can cause transboundary pollution.
- The presence of plastics has been identified in animal habitats and was reported to be present in the digestive systems of animals.[39] The quantity of minuscule plastic particles, with a diameter of 20 μm, has exhibited a consistent upward trend since the 1960s. This phenomenon is ascribed to the process of shredding and grinding plastic waste before its disposal. The long-term implications of these small particles remain uncertain on ecosystems.
- The presence of toxic and hydrophobic pollutants in the environment, particularly in aquatic ecosystems, tends to adsorb plastic materials within that

environment. The bioaccumulation potential of hazardous pollutants increases due to the easy transfer from plastics to organisms.[40]

- The process of natural breakdown of polymeric plastics leads to the formation of smaller fragments and residual monomers. The leaching of plasticizers, additives, and monomeric wastes from landfills, soils, and sediments can lead to extensive contamination of surface and groundwater resources.[40] Plasticizers and antioxidants, such as BPA, a chemical known to damage the endocrine system, were found in the leachates of landfills in tropical Asian countries and Japan ranging from 180 ng/L to 4.3 mg/L. It was five orders of magnitude greater than the concentrations found in sewage. Approximately 50% of the estrogenic activity observed in the leachates can be related to BPA.
- Recently, degradable plastics have been marketed in developed nations with the expectation that they will degrade faster than conventional plastics. Nevertheless, the accelerated rate at which they visually vanish does not necessarily imply that they are harmless in terms of their environmental impact. The evaluation of the end products of their metabolism and their potential for bioaccumulation and endocrine disruption has yet to be conducted.[41]

2.1.3 Degradation of Plastics

Plastics are generally considered biologically and chemically inert substances which can maintain their stability in the environment for tens to hundreds of years. Nevertheless, the natural environment can lead to the degradation of these materials, despite their relatively inert nature. This degradation process is influenced by various environmental factors, including sunlight or UV exposure, temperature, pH conditions, nutrient availability (including oxygen), and the presence of microbes capable of biodegrading these polymers. Plastic degradation can take place abiotically or biotically.[8]

2.1.3.1 Abiotic Degradation

Abiotic degradation refers to the process of inducing alterations in the polymer at an intra-molecular level through physical and/or chemical processes.[42] These processes can lead to fragmentation and size reduction, as well as lower molecular weights of polymers. Additionally, they can result in a loss in the tensile strength of the material and cause visual disappearance.[41] The process of abiotic degradation facilitates the subsequent occurrence of biotic degradation since it aids in the transformation of inert substances into more readily biodegradable forms.

Several approaches have been employed to enhance the degradability of polymers. These involve changing polymer structure by adding functional groups (such as the carbonyl group), hydrolysable linkages and the use of photosensitizers and metal-organic complexes in the polymers.[41] Synthetic polymers including photosensitizers are also noted as oxo-biodegradable polymers. The photodegradation of low-density polyethylene (LDPE) and polypropylene (PP) films can be accelerated using metal oxides as catalysts. It results in a decrease in molar mass and the formation of oxygenated groups that are readily biodegradable, such as the carbonyl group. These polymers are more easily degraded under oxidative conditions.[43] Additional parameters can contribute to the degradation of plastics, such as the utilization of catalysts, exposure to ozone, autoclaving plastics, and the use of mechanical stress.

2.1.3.2 Biotic Degradation

Biotic degradation can occur through aerobic or anaerobic conditions.[8]

2.1.3.2.1 Aerobic Biodegradation

Aerobic bacteria use oxygen as an electron acceptor in this process, and plastics are degraded into CO_2 and H_2O (Equation 2.1). The organic degradation of pollutants at hazardous waste disposal sites.[44]

$C_{plastic} + O_2 \rightarrow CO_2 + H_2O + C_{residual} + Biomass$ Equation 2.1

2.1.3.2.2 Anaerobic Biodegradation

Anaerobic digestion refers to the decomposition of organic pollutants by microorganisms in the absence of oxygen. Anaerobic bacteria use nitrate, sulfate, iron, manganese, and carbon dioxide as electron acceptors to degrade organic molecules including plastics into smaller compounds, as in Equation 2.2.[44]

$C_{plastic} \rightarrow CH_4 + CO_2 + H_2O + C_{residual} + Biomass$ Equation 2.2

The majority of the plastic waste in the environment consists of non-biodegradable plastics such as PET, HDPE, LDPE and PS. These polymers consist of a long chain of carbon atoms with strong covalent bonds which require significant energy to break, typically more than naturally occurring microorganisms can provide. Moreover, these polymers do not possess functional groups such as esters or amides that can be easily identified and broken down by enzymes generated by microorganisms.[45] Therefore, although these methods exist theoretically, it is difficult to biodegrade the plastics that accumulate in nature due to their structures.

2.2 Microplastics (MPs)

Plastics can be divided into four categories based on their sizes: mega-plastics, macro-plastics, meso-plastics, micro-plastics, and nano-plastics (Figure 2.1). MPs are microparticles spanning in size from 5 mm to 1 μm.[3]

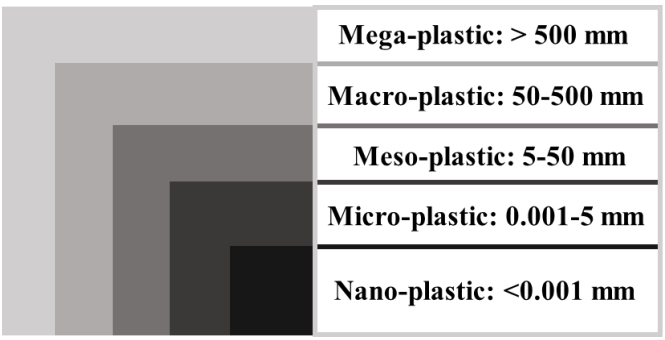


Figure 2.1. Classification of plastics based on their sizes.[18]

MPs can be discharged into the environment from a variety of sources due to inadequate handling of plastic waste.[4,5] MPs have been found in fresh waters[46], seawaters[47], ocean sediments[48] and urban and rural areas.[5,49] They are a subject of great worry for biologists and environmentalists due to their long-term durability, and their capacity to be easily transported between different environments.

2.2.1 Types and Sources of MPs

Microplastics can be classified into two main categories: primary and secondary MPs. Primary MPs refer to the raw materials that are utilized in household and personal care products. On the other hand, secondary MPs are formed through the breakdown of primary plastic particles due to various environmental processes such as physical, chemical, and biological mechanisms.[6] When plastics are exposed to UV or solar radiation, for example, they undergo weathering degradation and deterioration, resulting in the loss of their tensile abilities, discoloration, the formation of surface fractures, and ultimately becoming weak.[3]

2.2.1.1 Primary Sources of MPs

Some of the primary sources of MPs are plastic pellets, personal care products, rubber roads, vehicle tires and wastewater treatment plants and they are explained in the following sections.

2.2.1.1.1 Plastic Pellets

Plastic pellets refer to granular forms of plastic materials, typically exhibiting a regular shape and measuring between 2 and 5 mm in diameter.[50] These pellets are widely employed in the manufacturing processes of diverse plastic goods. Plastic pellets are often kept, transported, and processed in the state of semi-finished products. The majority of thermoplastics are composed of virgin plastic pellets, which are alternatively referred to as preproduction pellets or beads. Typically, these pellets are manufactured during the process of polymer synthesis or within recycling plants.[26]

2.2.1.1.2 Personal Care Products

The personal care and cosmetic industries play a significant role in the introduction of MPs into the environment. Among the microbeads utilized in personal care products, PE microbeads constitute 93% of the total MP beads. Plastic microbeads typically reach the sewage network along with washing wastewater due to their small size, insolubility in water, and slow degradation.[26] A preliminary study of plastic microbeads in facial cleansers and shower gels purchased from five Beijing supermarkets revealed that approximately 39 tons of microbeads are discharged into the environment each year in China due to the use of shower gel.[51] Moreover, Cheung & Fok reported that 306.9 tons of microbeads are released into the environment annually in China.[52] National regulations have been established to address the increasing number of tiny plastic particles in the ocean, which are

harming marine life and ecosystems. In 2014, The Netherlands was the first nation to implement a ban on the use of microbeads in cosmetic products. Australia, Canada, Italy, Korea, New Zealand, Sweden, the UK, and the US have all taken similar actions.[53]

2.2.1.1.3 Rubber Roads and Vehicle Tire Wear

Rubber asphalt road is a relatively new form of advanced road material. As waste tire rubber powder is the primary raw material used in rubber asphalt production, its use reduces the pollution produced by waste tires. However, during the use of rubber asphalt, rubber plastic particles are discharged into the environment due to friction between the road and vehicle tires. Rubber tires are one of the primary sources of environmental MPs in Norway and Denmark.[26]

2.2.1.1.4 Wastewater Treatment Plants (WWTPs)

One of the major collection and transfer points of MPs is WWTPs.[54,55] WWTPs receive MPs from various sources such as landfill leachate, surface runoff, industrial wastewater discharges and household wastewater.[3] Despite the high removal rates, an average of 10^5 -10^8 plastic particles are discharged into nature daily from the treated waters of WWTPs, depending on their capacity and type.[56]

2.2.1.2 Secondary Sources of MPs

2.2.1.2.1 Plastic Bags

Up to 5 trillion plastic bags which are generally composed of the following polymers PET, PE, high-density PE (HDPE), PVC, low-density PE (LDPE) and PP are used annually worldwide. Every year, 100 million plastic bags are used by consumers in the United States alone, but less than 10% of them are recycled. Some nations are

progressively banning the manufacture and use of plastic bags due to their long degradation cycle.[26]

2.2.1.2.2 Plastic Bottles

Plastic bottle containers are made of polymers such as PET, PE, and PP. Currently, it is estimated that one million plastic bottles are sold worldwide every minute. Nevertheless, as of 2016, less than 50% of plastic bottles sold were effectively recycled. Additionally, only 7% of the plastic bottles were recycled and transformed into new bottles in 2016.[26]

2.2.1.2.3 Plastic Packaging

Plastic packaging is utilized in all aspects, such as clothing, food, paper towels, etc. In 2017, 14.6 billion plastic containers for food and beverages were consumed in China. The oceans are a "plastic sink" in which millions of tons of plastic packaging waste are usually collected annually. Large volumes of so-called thin films of polymers are used for packaging such as PE, PP, and PS.[57]

2.2.2 Transport of MPs to the Environment

The wide-ranging transportation of MPs across long distances on land and within aquatic systems is attributed to their shape diversity, small size and low density.[7] The ones with larger dimensions and higher density sink easily and deposit in sediments.[58] Moreover, it has been observed that MPs with irregular shapes, characterized by jagged geometry and sharp ends, have a greater tendency to remain submerged in water rather than resurfacing. On the other hand, spherical particles tend to exhibit a stronger inclination to stay on the water's surface.[59,60]

The process by which MPs are transported through the atmosphere is not yet fully understood.[7] There are relatively fewer dispersal limits in the atmosphere compared

to aquatic systems. However, it should be noted that pollution in aquatic and terrestrial environments can affect the transportation of MPs in the atmosphere. Therefore, additional studies are required to understand better the underlying transportation mechanisms of MPs.

The hydrophobic nature and the high surface area-to-volume ratio of MPs make them susceptible to accumulating and transporting persistent organic pollutants such as polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethane (DDT), and polyaromatic polycyclic aromatic hydrocarbons (PAH). Consequently, these pollutants can be transferred to coastal areas and subsequently desorbed within living organisms.[61] So, organic pollutants in coastal regions are expected to increase significantly due to the transportation of pollutants facilitated via MPs. The form and distribution of MPs play a significant role in controlling the transportation of other contaminants in water.[62]

2.3 Plastics of Interest in This Study

In this study, as mentioned before, sludge disintegrations that may lead to trigger physical and morphological changes in plastics were applied. PET, PP and PC plastics were chosen to follow the behaviors of these plastics under various stress conditions. Some of the physicochemical properties of the three model plastics are listed in Table 2.1.

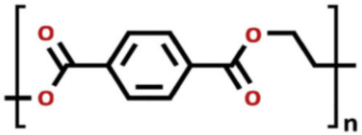
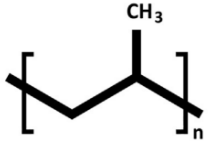
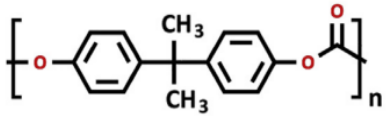
PET is a semi-crystalline polymer formed from ethylene glycol and terephthalic acid or dimethyl terephthalate monomer. It is known for its resistance to heat, abrasion, and aging. However, PET plastics undergo hydrolysis at elevated temperatures (>73-78°C) when exposed to water or humid conditions. Also, owing to the presence of the ester group in their composition, they can undergo hydrolysis wherein the ester bonds are cleaved in the presence of alkali solutions. For example, in a study, 1.0 M, 5.0 M and 10.0 M NaOH (temperature was not mentioned) were used to enhance the extraction of MPs from sludge. After alkali treatments, the mass and size of PET decreased by 30.2%-53.5% and 9.4%-16.7%, respectively.[63] Similarly, Hurley and

coworkers found that the mass of the PET particles decreased by 6.98-29.2% and the size decreased by 0.88-10.34%. Both studies concluded that the decrease in mass and size of MPs increase as the concentration of the alkali solution increases.[64]

PC is an amorphous polymer, and it is usually made up of BPA and phosgene. It is a solid and heat-resistant but corrosion-prone plastic. However, if PC is exposed to an aqueous solution in the presence of hydroxide, it will be hydrolyzed. Therefore, BPA may be released due to alkali-catalyzed ester hydrolysis. In a study, it was reported that the mass and size of the PC were reduced by 8.24-59.9% and 3.14-27.8%, respectively after exposing 1.0 and 10.0 M NaOH to the sludge.[64]

PP is a polyolefin type of polymer which is formed from propylene monomer, and it has semi-crystalline structure. They are resistant to many acids, bases and solvents. However, they can oxidize under UV radiation, and the surface peeling effect may increase. Arkatkar and his colleagues studied PP films that were treated with heat at 80°C for 10 days. They observed that the application of heat resulted in an approximately 10-fold increase in the mass loss of PPs.[65]

Table 2.1 Properties of PET, PP and PC (compiled from Crawford and Quinn [31]).

Plastic Types	**PET**	**PP**	**PC**
Chemical Formula	$(C_{10}H_8O_4)_n$	$(C_3H_6)_n$	$(C_{16}H_{18}O_5)_n$
Structure			
Monomer(s)	Terephthalic acid, Ethylene glycol	Propylene	Bisphenol A (BPA), Phosgene
Classification	Thermoplastic	Thermoplastic	Thermoplastic
Molecular Orientation in Solid Phase	Semi-crystalline	Semi-crystalline (isotactic, syndiotactic) Amorphous (atactic)	Amorphous
Density (g/ cm^3)	1.4	0.9	1.2
Melting Point (°C)	255-265	160-166	-
Glass Transition Temperature- T_g(°C)	73-78	(-20)-(-10)	140-150
General Properties	Visible light and microwave transparency. Outstanding resistance to heat, corrosion, and aging. Lightweight, impact and fracture resistance. Excellent gas and moisture resistance.	Resistant to a variety of solvents, acids, and alkalis. Low density, high stiffness, heat resistance, good transparency, and a favorable ratio of impact strength to rigidity	Naturally transparent with light transmissibility similar to that of glass. Tough, strong, heat resistant and excellent dimensional stability
Typical Applications	Beverage bottles, food containers, film and sheeting, filling material	Bottle caps and container lids, packaging tape, pipes, rope, automotive applications, outdoor furniture	CDs, bullet-proof glass, carboy, baby bottles, roofing

2.4 Additives in Plastics

Additives enhance the performance of polymers during shaping processes such as injection molding, extrusion, blow molding, and vacuum molding. Additionally, they contribute to improving the functionality and ageing characteristics of the polymer. Plasticizers, flame retardants, antioxidants, acid scavengers, light and heat stabilizers, lubricants, pigments, antistatic agents, slip compounds, and thermal stabilizers are often employed as additives in various polymeric materials. It is important to emphasize that additives, in almost all instances, are not chemically bonded to the plastic polymer. However, the inclusion of reactive organic additives, such as some flame retardants, results in their polymerization with the plastic molecules, thereby integrating them into the polymer chain.[10]

The additives can be primarily classified into four distinct types:[30,66]

- Functional additives (plasticizers, stabilizers, antistatic agents, flame retardants, lubricants, slip agents, curing agents, foaming agents, etc.)
- Colorants (pigments, soluble azocolorants, etc.)
- Fillers (mica, talc, kaolin, clay, calcium carbonate, barium sulfate)
- Reinforcements (e.g., glass fibres, carbon fibres).

The amount of additives (w/w%) used in plastics is listed in Table 2.2. Among the additives, plasticizers are mostly used in plastics and they can reach up to 70% of the polymer base.

Table 2.2 Amount of commonly used additives in plastics.[67]

Type of Additives	(w/w%) in the polymer matrix
Functional Additives	
Plasticizers	10-70
Flame Retardants	3-25 (for brominated)
Stabilizers, Antioxidants and UV agents	0.05-3
Slip Agents	0.1-3
Colorants	0.001-10
Fillers	Up to 50
Reinforcements	15-30

2.4.1 Plasticizers

Plasticizers are frequently employed to enhance the flexibility, durability, and stretchability of polymeric films, while simultaneously lowering the melt flow of thermoplastic polymers. They are able to migrate freely within the resin. It is reported that compatible plasticizers form close associations with the polymer chains, thereby increasing the free volume in the bulk plastic matrix. In this way, the glass transition temperature Tg (°C) of polymers decreases, which is inversely proportional to the fractional free volume in the polymer.[67,68] Several important plasticizers are commonly used in various applications. One example is phthalic esters (PAEs), specifically DEHP, which is extensively utilized in PVC formulations. PAEs account for approximately 80% of the total plasticizer volume in PVC production. Other commonly used plastic PET may include dipentyl phthalate (DPP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), and dibutyl phthalate (DBP) as plasticizers.[10]

2.4.2 Antioxidants

Polymers undergo a process called autooxidation in which they react with molecular oxygen. This process is triggered by heat, UV exposure, or mechanical stress. As a result of autooxidation, free radicals are formed, which then react with oxygen to create peroxy radicals. Antioxidants are added to various synthetic plastics such as PE and PP to prevent the formation of free radicals.[69] PE and PP account for approximately 60% of the worldwide demand for antioxidant additives. Arylamines are frequently employed as antioxidants in plastic food packaging. Phenolics and organophosphates are employed to decrease the formation of hydroperoxides during the process of oxidizing alcohols. BPA and nonylphenols (NPs) are commonly used as antioxidants or plasticizers in PE, PP and PVC. Annually, over three million tons of BPA was used in the production of plastics.[10]

2.4.3 Flame Retardants

Flame retardants (FRs) minimize fire danger and delay the combustion of plastics.[70] They have environmental concerns because of their relatively high concentrations (10–20%) in flame retardant products. Polybrominated diphenyl ethers (PBDEs) are popular FRs used in construction, transportation, and textile sectors. PBDEs are employed as FRs in high-impact polystyrene (HIPS), resin PE, ethylene vinyl acetate copolymer (EVA), and acrylonitrile-butadiene-styrene copolymer (ABS).[67]

2.4.4 Toxicity of Additives

The potential toxicity of microplastics stems from the release of unreacted monomers, oligomers, and chemical additives. They can be released from the plastic over an extended period. Additives are the main threat to human health.[71] The majority of additives are carcinogenic and can disturb hormonal balance. Their health effects are listed in Table 2.3.

Table 2.3 Plastic additives, their applications and their associated health effects.

Additives	Applications	Health Effects	References
BPA	Packaging materials, aluminum cans, bottles	Coronary illness, reproductive abnormalities, breast cancer, hormonal disorders	[72–74]
PAEs	Rubber, paints, inks, synthetic perfumes, toys	Genetic mutation, allergic, psoriasis, reproductive disorders	
Flame retardants	Textiles, electronic devices, housewares	Infertility, developmental consequences, thyroid hormone levels imbalance, prostate cancer	

2.4.5 Leaching of Additives from MPs

Leaching of additives from MPs results in the discharge of chemicals into the environment; including persistent organic pollutants (POPs) which are adsorbed on the particles' surface and additives utilized in the production of plastics. In MP production, organic additives are added to polymers to improve their physical and chemical properties. Plastic additives typically do not form covalent bonds with polymers, keeping their unique chemical properties.[75] These additives can leach into the environment.[9,75] Some additives observed to leach from MPs in aquatic environments and reported in the literature are listed in Table 2.4.

Table 2.4 Leaching additives from common MPs in aquatic environments.

MP Type(s)	Type of Additive(s) Leached	References
PC	BPA	[76]
PE, PVC and PET	DEHP, DBP	[77]
PE, PVC and PET	BPA, DEP, BBP[1]	[78,79]
PVC, PE	DIOP[2], DMP[3]	[80]
PVC	DINP[4], BPA	[81]

[1] Butyl benzyl phthalate, [2] Diisooctyl phthalate, [3] Dimethyl phthalate, [4] Diisononyl phthalate.

Leaching can have two distinct forms: bioleaching and abiotic leaching. Bioleaching is the process facilitated by the release of enzymes and chemicals by specific living organisms.[82] These substances aid in the dissolution and extraction of substances. In contrast, abiotic leaching is predominantly influenced by physical variables, including temperature, wind erosion and UV radiation.[83]

The molecules in plastics can be released into environments via various mechanisms, such as erosion, desorption, and diffusion. In diffusion, additives migrate from high to low concentration between the interior and exterior of the plastic. In desorption, adsorbed molecules are released from the surface of plastics to the nearby environment. This procedure may lead to the leaching of preservatives, including chloroform and phenol. Erosion takes place when the physical deterioration of the plastic surface results in the release of additives such as aldehydes and phenols. In addition, plastic surfaces may contain additional environmental contaminants such as heavy metal ions and pesticide residues.[84]

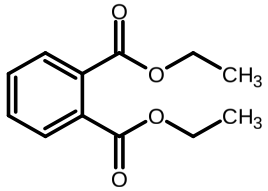
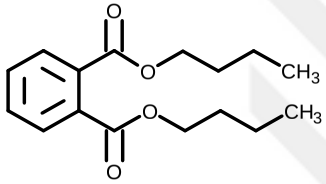
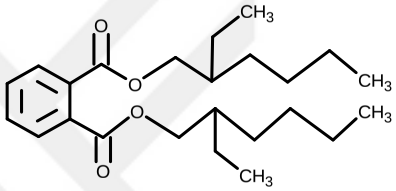
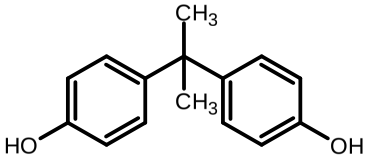
Prolonged exposure of MPs to aqueous environments may result in the release of the additives. The overall leaching of additives is determined through internal diffusion in the plastic matrix or diffusion in the aqueous boundary layer based on their chemical properties.[85] For additive-polymer systems, diffusion within the polymer is typically regulated by Fick's diffusion law and it is influenced by the concentration gradient and diffusion coefficient of organic additives. Several variables affect the diffusion coefficient of organic additives, including their size, molecular weight, and interaction of additives with the polymer matrix. The rate of additive loss from plastic particles slows down, with an increase in hydrophobicity or the partition coefficient between plastic and water. In this case, the diffusion of the aqueous boundary layer becomes more dominant.[84]

When analyzing these processes, the partition constant (K_p) between plastic and water is a crucial parameter. The equilibrium ratio between the concentration of the additive in the plastic phase (C_p) and that in water (C_w) determines K_p as shown in Equation 2.3.

$$Kp = \frac{Cp}{Cw} \qquad \text{Equation 2.3}$$

The majority of organic plastic additives have a strong affinity for the solid MP phase rather than the water phase owing to their intrinsic lipophilic characteristics, as evidenced by their broad log K_{ow} values Log K_{ow}, i.e. octanol-water partition coefficient is the ratio of the concentration of a chemical in the n-octanol phase to that in the water phase. Having high log K_{ow} value (log $K_{ow} \geq 5.0$)[84] of an additive indicates low solubility in water hence it is hydrophobic. Physicochemical properties of common leaching additives from MPs are given in Table 2.5.

Table 2.5 Physicochemical properties of additives that are examined for their leaching from MPs in this study.

Compound	DEP	DBP	DEHP	BPA
Molecular Structure				
Molecular Weight (g/mol)	222.2	278.4	390.6	228.3
Boiling Point (°C)	295	340	384	360
Density (g/mL)	1.23	1.04	0.98	1.2
Solubility in Water (mg/L at 25 °C)	591	9.9	2.5×10^{-3}, 0.041[86], 0.27[87], 0.023-0.34[88]	210
Vapor Pressure (mm Hg at 25 °C)	6.48×10^{-2}	4.73×10^{-3}	2.52×10^{-5}	4.0×10^{-8}
Log K_{ow}	2.54	4.27	7.73	3.64
References	[89,90]			[91]

2.4.5.1 Factors Affecting Leaching of Additives from MPs

Some of the factors affecting additive leaching from MPs are explained in the following sections such as size of MPs, temperature, UV radiation and pH.

2.4.5.1.1 Size of MPs

The size of MPs and the amount of additives leached from plastics is correlated by the mathematical model of equilibrium distribution and desorption kinetics. Smaller MPs exhibit a higher surface-to-volume ratio in comparison to larger MPs. The larger surface area facilitates greater interaction with the surrounding environment. Consequently, smaller MPs undergo a faster and extensive leaching of additives. This occurs because more additive molecules come in contact with the surrounding environment and are released from the polymeric material into the surrounding medium.[92,93]

2.4.5.1.2 Temperature

An increase in temperature will speed up the deterioration of MPs. Additionally, their resistance to high temperatures decreases and they decompose faster as the aging of MPs increases. Toxic substances, including phthalates[94], BPA[95,96] and organophosphorus esters[97] are released into the environment when MPs are exposed to environmental factors. Also, as the temperature increases, brominated flame retardants (PBDEs leach into water. Lin & Su reported that the leaching of chemicals from MPs can be influenced by temperature due to their potential impact on several factors.[98] For leaching of additives, overcoming an energy barrier at the interface between the plastic and the surrounding medium is required. Increased temperatures can lower this barrier, known as the reflection activation energy, making it easier for additives to escape to the environment. Raising the temperature of plastic materials increases the motion of molecules, including those of the additives and polymer

chains present in the material. This causes the additives to diffuse faster within the plastic and to the surface, which in turn increases the release of these additives into the surrounding environment. Also, high temperatures enhance the mobility and fluidity of the polymer chains within the MP. This increased mobility allows additive molecules to move freely within the plastic, facilitating their release into the environment. Additives and polymer itself can experience photodegradation when exposed to UV-light. This degradation is frequently accelerated by temperature which leads to breakdown of the polymer chains and release of additives. In another study by Yousif & Haddad, elevated temperatures increase the thermal breakdown of freshly created peroxides, resulting in the generation of free radicals and a loss in molecular weight. Such temperature-induced weathering is more noticeable in aquatic environments.[99]

2.4.5.1.3 UV Radiation

The degradation of plastic polymer chains by UV radiation can result in the formation of surface fissures and microcracks. This increases the surface area in contact with the surrounding environment, allowing for opportunities to release additives. Chemical reactions within the polymer, such as cross-linking or oxidation, can be initiated by UV radiation. The aforementioned reactions have the potential to modify the plastic's structure and functions, so affecting its interactions with additives and possibly promoting their release.[100]

2.4.5.1.4 pH

The pH influences the electrostatic interactions between polymer matrix and additives. Changes in pH can alter their interactions, affecting the mobilization of additives inside the plastic and their subsequent release into the environment. Depending on the acidity or alkalinity of the environment, certain additives may exist in various forms with distinct properties. For instance, an amine group attached to

an additive can undergo protonation in an acidic environment, thereby increasing its solubility and leaching rate. Furthermore, the pH can affect the surface charge of both MPs and the surrounding medium. This may alter the leaching characteristics of additives as well as their adsorption capacity on the MP surface. As an example, it has been observed that acidic chemicals, such as phthalates, exhibit increased solubility and leaching potential in acidic medium.[101] Likewise, basic compounds like benzotriazoles may exhibit increased solubility and enhanced leaching capabilities in basic environments.

2.4.6 Analysis of Additives Leaching from MPs in Environmental Samples

Solid-phase extraction is one of the extraction methods for separation and preconcentration of the phthalates and BPA additives from plastics. Afterwards the isolated molecules are analyzed using liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), pyrolysis GC-MS (Pyr-GC-MS) or high purity LC-MS (HPLC-MS) coupled MS techniques.

2.4.6.1 Solid-Phase Extraction (SPE)

Solid-phase extraction (SPE) entails the separation of analytes between a liquid phase (sample matrix or solvent) and a solid phase (sorbent). This method concentrates and purifies analytes by adsorbing them onto a solid sorbent and purifying the resulting extract. It has been widely used for the extraction of phthalates and BPA from water, surface water, and wastewater samples.[102–105]

The choice of a suitable SPE extraction sorbent relies on the interaction between the sorbent and the target analyte. The predominant retention mechanisms utilized in SPE involve van der Waals forces, hydrogen bonding, dipole-dipole forces, and ionic interactions. Each sorbent has a unique mixture of these characteristics to address a diverse range of extraction challenges. There are three main sorts of interactions: reversed phase, normal phase, and ion exchange.

i) A polar or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase comprise the reversed phase (RP). Generally, the analyte of interest exhibits a mid- to nonpolar character. The primary mechanism underlying the retention of organic analytes from polar solutions onto these SPE materials is the attraction between the functional groups on the sorbent surface and the carbon-hydrogen bonds in the analyte. The attractive forces between nonpolar molecules, known as van der Waals forces or dispersion forces, are primarily responsible for this phenomenon. A nonpolar solvent is employed to elute a compound adsorbed onto an RP SPE cartridge or disk, as it can disrupt the intermolecular interactions between the sorbent and the substance. The sorbents commonly employed for reversed phases include carbon-based, polymer-based, polymer-coated and bonded silica media. Carbon-based media are composed of non-porous graphitic carbon and exhibits a strong affinity for polar or nonpolar organic molecules in polar or nonpolar matrices. Polymer-based sorbents refer to styrene/divinylbenzene. It is utilized to maintain hydrophobic chemicals that possess some hydrophilic characteristics, particularly aromatics.

ii) The normal phase consists of a polar analyte, a moderately to nonpolar matrix, and a polar stationary phase. The main factor responsible for the retention of an analyte in normal phase is the interaction between the polar functional groups of the analyte and the polar groups on the surface of the sorbent, which involves hydrogen bonding. A molecule that is adsorbed by these methods can be removed by using a solvent that breaks the binding mechanism. Typically, a solvent that is more polar than the matrix of the sample is used for this purpose.

iii) Ion exchange SPE is applicable for the isolation of chemicals present in a liquid solution. Aliphatic quaternary amine groups attached to the silica surface can be used to isolate negatively charged molecules. Cationic

chemicals are separated by employing silica that has aliphatic sulfonic acid groups attached to surface. The principal retention mechanism of the compound is predominantly determined by the electrostatic attraction between the charged functional group in the compound and the charged group bound to the silica surface.[106]

For the environmental samples, RP SPE has been commonly used in literature. RP SPE applications in literature for environmental samples are listed in Table 2.6.

Table 2.6 RP SPE applications in the literature for environmental samples.

Analyte of Interest	Matrix	SPE Adsorbent	Reference
Nonylphenols, BPA, phthalate esters (DMP, DEP, DBP, BBP, DEHP)	River water	C18	[107]
Plastic additives (PAEs and BPA), surfactants, lubricants	River water	HLB*	[108]
Herbicides, phthalates, alkylphenols, BPA	Source water	HLB	[109]
Organophosphate esters (OPEs), phthalates (PAEs), bisphenols (BPs), perfluorinated compounds (PFCs), organochlorinated compounds (OCs)	Sea water	HLB	[110]
BPA and its chlorinated derivatives, alkylphenols, phthalates esters (DMP, DEP, DBP, DOP, BBP, DEHP)	Wastewater	C18	[111,112]
Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalates, BPA	Wastewater	HLB	[113]
Phthalates esters (DMP, DEP, DBP, BBP, DOP, DEHP)	Wastewater	C18	[114]

*Hydrophilic Lipophilic Balance

The typical procedure involves applying a solution onto the SPE solid phase, eliminating unwanted components through washing, and subsequently eluting the target analytes into a collecting tube using a different solvent. Figure 2.2 shows the main steps of the SPE.

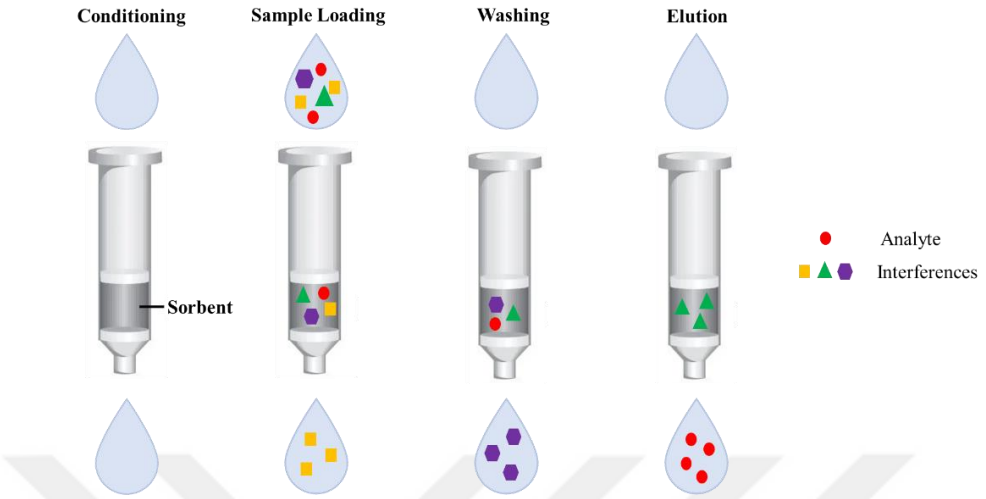


Figure 2.2 Illustration of main steps of SPE using cartridge format.

First, the cartridge is conditioned with a solvent or mixture of solvents. Conditioning the SPE sorbent eliminates any contaminants that may persist from the manufacturing process and activates the surface of the sorbent to enhance interaction with the analyte. Some of the solvents and their volumes used in the conditioning process of SPE of extraction for PAEs and BPA in environmental samples are listed in Table 2.7. After conditioning, the sample is loaded into the SPE cartridges, with the amount depending on the cartridge volume and sorbent amount. Then, the cartridge is washed with a solvent or solvents to eliminate interferences, salts, and other matrix constituents. The analytes retained on the SPE sorbent are collected by introducing a suitable solvent(s) which disrupt the interaction between analyte and adsorbent.[106]

Table 2.7 Conditioning solvents used for the PAEs and BPA.

Conditioning	Cartridge Capacity	Reference
5.0 mL diethyl ether, 5.0 mL methanol, 5.0 mL deionized water (DI)	500 mg/ 3.0 mL	[111,112]
10.0 mL hexane, 10.0 mL dichloromethane (DCM), 10.0 mL methanol, 15.0 mL DI.	200 mg/ 6.0 mL	[109]
7.0 mL n-hexane, 7.0 mL DCM, 7.0 mL methanol, 10.0 mL DI	1000 mg/ 6.0 mL	[114]
10.0 mL DCM, 10.0 mL methanol, and 10.0 mL DI	200 mg/ 6.0 mL	[108]

2.4.6.2 Gas Chromatography- Mass Spectrometry (GC-MS)

GC-MS is used to separation technique that uses a column to separate compounds based on both their volatility and polarity. Mass spectrometry can then identify these compounds by measuring their mass-to-charge (m/z) ratios. The pollutants in the environmental samples such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), phthalic acid esters (PAEs), polychlorinated biphenyls (PCBs), and pesticides can be detected by GC-MS. Also, it is a useful technique to find the source of the pollutants. For example, GC-MS is particularly effective in detecting phthalates and BPA, which are common organic pollutants in the environment. There are several studies listed in Table 2.8 that demonstrate the usefulness of this technique.

Table 2.8 Examples of literature studies using GC-MS to analyze additives and other pollutants in environmental samples.

Sample Matrices	Analytes	Technique	Reference
Bottled water	PAEs (DNOP, DMP, DEP, DBP, BBP, DEHP) and BPA	GC-MS	115,116
Sea water	Phthalates and BPA	GC-MS	117
Beach plastic waste (BPW)	Short-chain fatty acid compounds, phthalate and non-phthalate additives	Pyrolysis GC-MS	118
Car tire waste	MPs (PE, PP, PS, PET, PA, PMMA, SBR)	Thermal Extraction Desorption (TED) GC-MS	119
Food packaging waste	Phthalates, alkylphenols, BPA and di(2-ethylhexyl) adipate	GC-MS	120

In GC-MS, the sample is injected into the gas chromatograph (GC). The sample is vaporized and carried through a column by an inert gas, such as helium. The components in the sample interact differently with the stationary phase on their volatility and polarity. The separated components are then eluted from the column and transferred into the mass spectrometer (MS). The ion source ionizes the components by bombarding with electrons having 70 eV energy. When electrons collide with the molecules, they eject an electron and create a positively charged ion. Then, these ions are separated based on their mass-to-charge (m/z) ratios and detected.

2.5 MPs in WWTPs

Water is the primary means by which microplastics are introduced into the environment. Domestic and industrial plastics can enter sewage systems, which collect wastewater from homes, businesses and industries and transport it to WWTPs for cleaning before it is discharged into water bodies for reuse or disposal.

Water is the primary means by which MPs are introduced into the environment. Domestic and industrial plastics can enter sewage systems which collect wastewater from homes, industries and businesses transport it to WWTP for cleaning wastewater before it is discharged into water bodies for reuse and disposal.[121]

Main units of wastewater treatment can be summarized as follows:

1- Bar screens: During the first stage of wastewater treatment, a screening step is used to remove any large objects that that may have inadvertently entered the sewer system. These large objects can cause damage pumps and reduce the water flow. A bar screen is typically used to extract these objects from the influent. Once removed, the objects are transported to a landfill.

2- Grit chamber: Grit (sand and small rock particles) removal is achieved by passing the influent through a grit chamber.

3- Primary clarifier: Solid matter is first separated from wastewater which is the solids on the bottom of the clarifier, is periodically removed to prevent it from interfering with the separation process. Once the water phase has been removed, the sludge is disposed of and often utilized as fertilizer.

4- Biological treatment: Air is introduced into the aeration tank or basin in the form of fine bubbles to promote the conversion of organic matter to CO_2 and H_2O and NH_3 to NO_3. The air also provides oxygen to aerobic bacteria that degrade wastewater and continue to grow.

5- Secondary clarifier: This stage of the wastewater treatment process is similar to the primary clarifier. Its function is to help settle the solids, which consists of a mass of microorganisms generated during biological treatment to the bottom of the tank. This mass of particulate matter is commonly called activated sludge and consists primarily of a high concentration of bacteria that are actively metabolizing. Part of the activated sludge is reintroduced into the aeration tank to increase the bacterial concentration, facilitate their proliferation, and accelerate the decomposition of organic matter. The excess of sludge is disposed of.

2.5.1 Sewage Sludge and MPs

Recent studies demonstrate that WWTP effluent is one of the major sources of MPs. Synthetic textile fibers from clothing laundering and facial scrubs, toothpaste, and other personal care products, as well as microbeads utilized in toothpaste, are conveyed from raw wastewater to WWTPs. However, these fibers can pass through the WWTPs and penetrate to the aquatic environment due to their small dimensions. A WWTP discharges approximately 65 million MPs into the receiving waters daily[2] An estimated 209.7 trillion microbeads (306.9 tons) are discharged into the aquatic environment annually in China from WWTPs.[52] Indeed, the efficacy of WWTPs in eliminating MPs from municipal effluents is demonstrated by a removal percentage of 98.4%.[2] In other words, the majority of MPs in the unprocessed wastewater are retained in sewage sludge another product of WWTPs.[11] PE, PP, PS, PET, PC, and PA are the most common MPs found in sewage sludge.[13]

Sewage sludge can be a potential fertilizer for plants since it contains nutrients such as nitrogen, phosphorous, and potassium.[14] Moreover, it is also rich in pathogenic microorganisms, and toxic substances.[15] It has a high level of biochemical oxygen demand and is commonly characterized by putridity and stench.[14] Therefore, physical, chemical, or biological stabilization methods are required before ultimate disposal or for land application to remove pathogen and toxic chemicals.[15]

2.6 Sludge Stabilization Methods

Sludge stabilization methods can be grouped under three main categories: physical, chemical and biological as shown in Figure 2.3.[122]

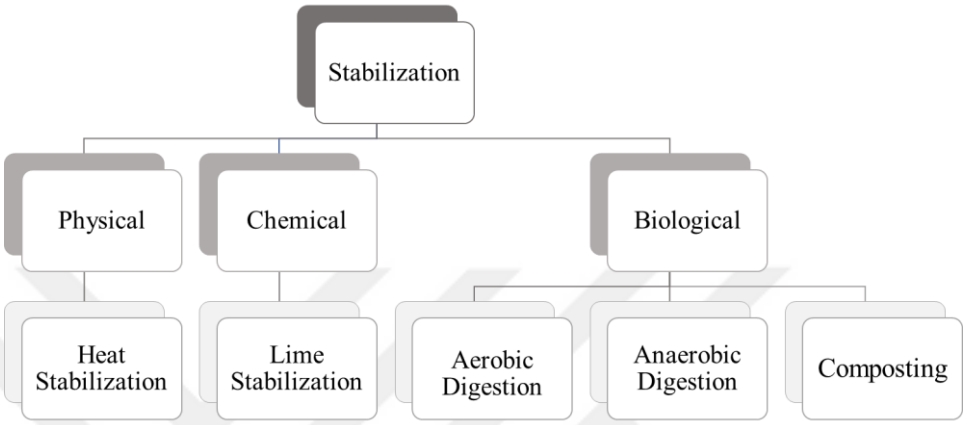


Figure 2.3 The most commonly used methods for sludge stabilization.

2.6.1 Heat Stabilization

Heat stabilization is a method of physically stabilizing sludge that is generated by both industrial and municipal wastewater treatment plants. This process involves increasing the temperature of the reactor holding the sludge from 180 °C to 200 °C for a period of 30 to 60 minutes. This high temperature is very effective in killing pathogens and the breakdown of the organic matter in the sludge. Nevertheless, this method of heat stabilization is quite expensive and requires a significant amount of thermal energy.[123]

2.6.2 Lime Stabilization

Lime is a commonly used chemical in the process of stabilizing of sludge. In this process, lime (calcium hydroxide) is added to the sludge to increase the pH level and suppress odors, while also destroying most of the microbial activity. The process of

lime stabilization of sludge is considered easy to operate and cost-effective. The method can be utilized for the treatment of sludge generated from both municipal and industrial wastewater treatment plants. However, lime stabilization does not effectively decrease the organic content of sludge, which is a crucial factor in sludge stabilization. Additionally, lime stabilization does not decrease the sludge volume unlike biological stabilization methods. The addition of lime to sludge may result in the precipitation of some components, leading to a potential increase in the overall amount of sludge. Lime reduces nutrient contents of sludge and it results sludge to have less agricultural value.[124]

2.6.3 Aerobic Digestion

Digestion is a process in which large molecules are broken down into smaller ones in the existence or absence of oxygen.[125] In aerobic digestion, oxygen is used to break down the organic matter in the sludge.[126] Aerobic digestion involves the rapid consumption of organic matter by microbes, which subsequently convert it into carbon dioxide, water, and various organic compounds with lower molecular weights [125,127]. Sewage treatment employs this method to reduce the volume of sewage sludge in preparation for its subsequent use. The capital costs of aerobic digestion are lower compared to anaerobic digestion and it has quicker rate of operation. Also, aerobic digestion is considerably simpler to manage and operates at room temperature, making it significantly less complicated.[125] One notable drawback of aerobic digestion is its high energy demand due to continuous aeration requirement and it predominantly produces carbon dioxide instead of methane as the principal gaseous byproduct.[126]

2.6.4 Anaerobic Digestion

Anaerobic digestion (AD) refers to a series of biochemical processes in which microorganisms decompose organic substances present in sewage sludge in the

absence of oxygen. This process produces gases, such as carbon dioxide and methane, as well as a moist mixture called digestate Even though there are other ways to stabilize these materials, anaerobic digestion is often preferred because it is considered a sustainable solution that can generate renewable energy and reduce the volume of sludge or organic waste.[128,129] There are numerous applications for the two primary by-products of AD of organic waste as shown in Figure 2.4.

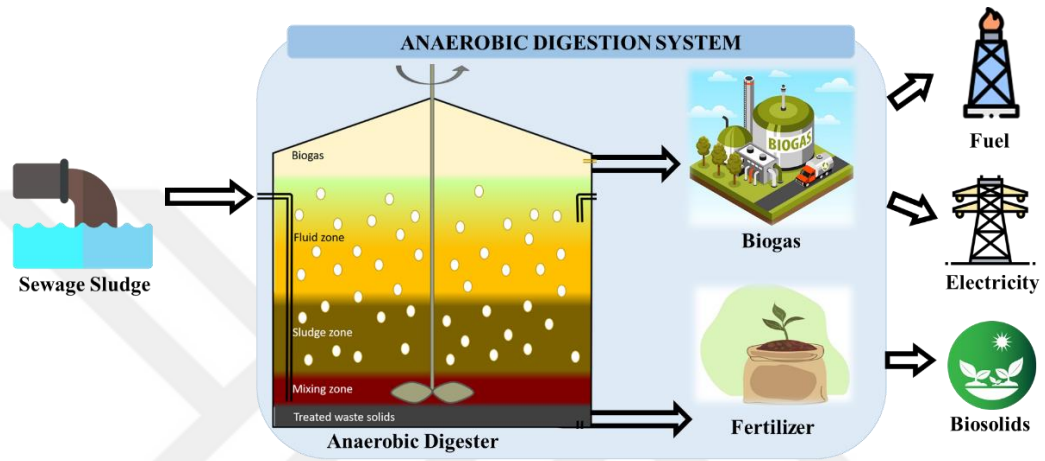


Figure 2.4 Anaerobic digestion system (adapted from Cambi, 2022).

The first product is biogas which consists primarily of carbon dioxide and methane, with trace amount of water vapor and other gases. Some bacteria execute distinct enzymatic reactions during the process of converting organic substances to methane in effluent.[18] Refining or purifying biogas to biomethane can increase its commercial value. Biogas is a renewable energy source that can be utilized for heat and/or electricity production or fueling. Biogas generated from sludge can be utilized by the sewage industry to partially compensate for the energy expenditure associated with wastewater treatment.[130] Another product of AD is digested sludge, also known as digestate. These can be reused as fertilizer in sustainable agriculture to cultivate plants.[131]

The anaerobic digestion process consists of four primary phases, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These sequential processes are carried out by a diverse microbial community in an oxygen-deprived environment.

During the hydrolysis process, compounds break down in organic feed prior to their utilization as microbial food. Proteins, carbohydrates, lipids and other complex polymers must be decomposed into their smaller components. The process by which polymers degrade in digester is facilitated by hydrolytic microorganisms releasing hydrolase enzymes.[18] In acidogenesis, acidogenic or fermentative bacteria produce volatile fatty acids, ammonia, hydrogen sulfide, carbon dioxide and other by-products.[132] During acetogenesis, acetate is produced from the short-chain fatty acids released during acidogenesis, along with hydrogen and carbon dioxide. Finally, methanogenic bacteria present in the digester metabolize the readily available intermediates generated during acidogenesis and acetogenesis, namely acetate, hydrogen, and carbon dioxide, resulting in the production of methane. Carbon dioxide is the second most abundant gas generated during this final stage after methane.

The hydrolysis stage of anaerobic digestion is the slowest step, and it can be attributed to the existence of various factors, including microbial cells, floc structure, aggregation of extracellular polymeric substances (EPSs), resistant compounds of lipids and proteins, and resilient cell walls.[133] These factors cause longer retention time during anaerobic digestion. Therefore, developing methods to improve the efficiency of digestion by promoting the hydrolysis step becomes an interest among researchers.[134–136] Pilli and Khanal and coworkers (2007) reported that sludge disintegration can be carried out to reduce retention periods during AD.[17,137]

2.6.5 Sludge Disintegration

Sludge disintegrations can be classified into under four groups: physical, chemical, thermal and biological as listed in Figure 2.5. It was reported that an effective disintegration method can accelerate the solubilization of sewage sludge.

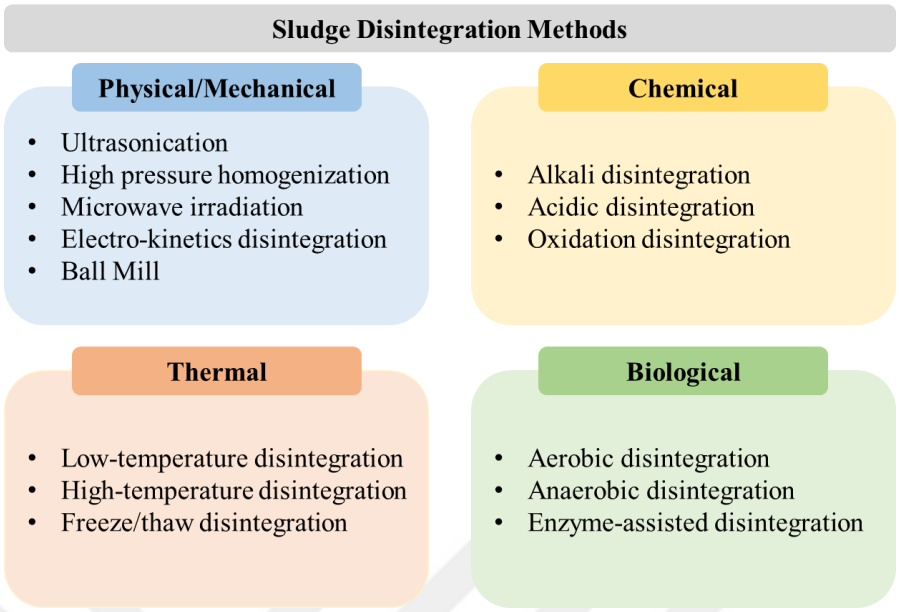


Figure 2.5 Sludge disintegration methods.[18]

The use of physical and mechanical disintegration methods results in the fragmentation of solid particles, leading to a reduction in their size. This reduction in size subsequently increases the surface area of the particles, hence promoting the anaerobic digestion (AD) process.[134] Several studies showed that the presence of larger particles is associated with a decrease in chemical oxygen demand (COD) and a reduction in biogas generation.[138,139]

Chemical disintegration is the most promising method for decomposing complex organic waste. It increases biogas production by enhancing the biodegradability of cellulose. This method uses chemical reagents such as acids, bases, and oxidants to hydrolyze the sludge.[19,130,140]

Thermal disintegration is used to improve AD hydrolysis.[141,142] This method involves subjecting sewage sludge and other wastes to elevated temperature, which promotes hydrolysis and enhances their digestibility.[143] Thermal disintegration has several advantages including pathogen reduction, reduced sludge volume, odor elimination, and improved sludge dewaterability.[142,144,145]

Biological disintegration is an environmentally sustainable method that employ enzymatic, aerobic and anaerobic processes to augment the AD hydrolysis. It employs a complex matrix of microorganisms to break down the sludge floc structure and other organic compounds, in this way hydrolysis step can be accelerated. Although economical and environmentally favorable, biological disintegration method is time-consuming and requires optimal conditions for microbial proliferation.[136]

The disintegration methods shown in Figure 2.5, as well as the ones used in this thesis study, are explained in the following sections in detail.

2.6.5.1 Alkali Disintegration

Alkali disintegration disrupts EPS and sludge structure and subsequently helps in the increase in solubility of organic matter in sludge,[146] while preventing the formation of harmful residues that may hinder subsequent processes. The energy demands are moderate, and the reaction can be carried out at room temperature and atmospheric pressure.[19,136] COD solubility can be effectively achieved through alkali disintegration. Nevertheless, solubility depends on concentration and types of chemicals employed in the treatment process. The decreasing order of efficiency of these chemicals are from largest to smallest NaOH > KOH > $Mg(OH)_2$ > $Ca(OH)_2$.[18] Evidently, monobasic salts solubilize at a faster rate than dibasic ones. This is likely due to the fact that dibasic alkali salts dissolve in sludge only partially.[19,147] Sodium hydroxide is the most frequently employed base in alkali treatment and is regarded as the most effective alkali due to its high solubilization rate using relatively low dosages.[148] A study conducted by Tulun and Bilgin[149] showed that using NaOH in the treatment increases biogas production by more than 43% compared to that of untreated sludge. In another study by Uma and coworkers,[150] pH 12.0 was the optimal condition. An excessively high pH level results in the cell's disruption, incapability to maintain a suitable turgor pressure, and subsequent loss of viability.[151]

It is advantageous to use high NaOH concentration such as 0.5 M to dissolution of the complex structures such as proteins and carbohydrates in the sludge; nevertheless, excessive sodium ion concentration inhibits the growth of methanogens. A preliminary study by Hatinoglu & Sanin, (2022) and McCarty (1964) revealed that 0.5 M NaOH inhibits anaerobic microorganisms at a moderate sodium ion concentration.[21,152] Additionally, they reported that antagonistic effect of one ion can substantially mitigate the toxicity of another ion. Therefore, it is suggested to decrease the sodium ion concentration below the inhibitory threshold, KOH and NaOH can be combined by keeping the concentration same for each base in the matrix.[21]

2.6.5.2 Thermal Disintegration

Thermal treatment, which encompasses both low-temperature (≤100°C) and high-temperature (>100 °C) treatments, [153] is extensively employed due to its efficacy and absence of chemical additives.

The application of thermal disintegration to sludge at elevated temperatures has been shown to be remarkably efficient in terms of diminishing organic matter, resulting in favorable outcomes for biological processing, such as increased biogas production and elimination of pathogens.[145,154–156] Thirty wastewater treatment facilities have effectively implemented thermal hydrolysis as a treatment method for sludge stabilization at full scale.[157] Although various techniques exist for thermal pretreatment, thermal hydrolysis has gained significant recognition and acceptability in the scientific and industrial communities.[158,159] Thermal hydrolysis is typically carried out at a temperature ranging from 160 to 180°C, under a pressure of 600–2500 kPa, for a maximum of 60 minutes.[156,160] These conditions may facilitate the disruption of bacterial floc cell walls, thereby accelerating the rate-limiting hydrolytic process in biological processing. However, the formation of recalcitrant compounds, such as melanoidins, can occur at temperatures exceeding 170–190°C.[160,161] In addition, an increased discharge of inhibitory substances, including

metals and ammonia, is anticipated, which will have adverse impacts on the process of digestion.[133,161]

Additionally, it has been documented those low-pressure conditions (1300 kPa), a broad temperature range (100–175°C) can cause partial disruption of the cell walls; nevertheless, the cell contents may remain viable for biological treatment.[162] Additionally, it has been noted that under these pretreatment conditions, the viscosity of the sludge decreases while its dewaterability increases. Aside from these, substances such as endogenous nonbiodegradable organic matter are unaffected by thermal hydrolysis. Nevertheless, Burger and Parker (2013) reported that heterotrophic biomass decomposes into two distinct components with 64% of the organic matter decomposing slowly and 36% decomposing.[163]

A previous study conducted in group showed that a temperature of 127°C and a pressure of 1.7 bar were optimal for the solubilization of sludge.[21]

Significant increases in the biogas potential of anaerobic reactors are achievable through thermal hydrolysis. Perez-Elvira and coworkers showed that thermal hydrolysis of mixed sludge increased the methane yield of an anaerobic system by as much as 40%.[164] In other studies, it was reported that the overproduction of electricity from biogas resulting from anaerobic digestion of mixed sludge was 20% greater when thermal hydrolytic treatment was implemented, as opposed to anaerobic digestion without disintegration.[162,165] This indicates that biogas is an effective source for generating electricity.

2.6.5.3 Enzyme Disintegration

The use of enzymes in sludge disintegrations has received attention for improving AD hydrolysis[166] because it is environmentally friendly, pollution-free and does not require special equipment compared to chemical or physical disintegration methods.[167] Dissolution of extracellular polymeric substances may be the key mechanism for enzyme treatment of sludge.[168] It is required to evaluate and optimize

parameters such as activity, specificity, amount of enzyme, stability, temperature, and pH to ensure effective enzymatic treatment.[169] The principal constituents of sludge in wastewater treatment facilities are carbohydrates, proteins, with trace amounts of lipids. Sludge consists of flocs rich in EPS and have a reduced capacity for disintegration[135]; consequently, the primary enzymes employed for enzymatic sludge disintegration are carbohydrates, proteases, and lipases.[136] Various enzymes, including protease, amylase, and glycosidase, have been reported to enhance anaerobic digestibility and biogas production, thereby accelerating the anaerobic degradability of sludge. Through the use of protease treatment, biogas production increased by 26%.[166] Chen and coworkers conducted a study to the suitability of lysozyme, protease, and α-amylase for the enhancement of waste activated sludge hydrolysis and degradability. Their findings indicated that lysozyme exhibited superior effectiveness in comparison to the other enzymes examined.[170]

According to Odnell and coworkers, certain enzymes are better adapted to sludge environments and are necessary for large-scale disintegrations. Enzyme activity, lifetime, and biogas production were evaluated for a variety of enzymes, including cellulose, α-amylase, protease, lysozyme, subtilisin, and trypsin. Their findings demonstrated that the activity longevity of each enzyme evaluated in waste activated sludge and anaerobic digester sludge was constrained to less than 24 hours. Subtilisin was the only enzyme among those which were evaluated to yield a significantly higher biogas output at 37%.[171] The effects of endogenous enzymes including amylase, protease, and amylase/protease mixtures were assessed for sludge disintegration. They concluded that the utilization of a combined enzymatic treatment yielded superior results in terms of biogas production while amylase outperformed protease or mixed enzymes for sludge solubilization and acidification.[168] These studies indicate that enzymatic treatment can increase biogas production and sludge AD.

2.6.5.4 Alkali and Thermal Combined Disintegration

One type of thermo-chemical disintegration is the addition of base (NaOH, KOH, etc.) either at high temperature (115-170˚C) or low temperature (50-90˚C). A study by Park and coworkers showed the addition of NaOH at 121˚C and pH 12.0 yields 154% increase in biogas production compared to single disintegration.[172] Although promising outcomes have been reported for alkali-thermal combined treatment at high temperatures, their practical use is hindered by the constraints of elevated temperatures and excessive consumption of chemical reagents.[19] Consequently, as an alternative method for reducing energy consumption, some studies have been conducted on the implementation of reduced temperatures (50–90˚C).[122] Kim and coworkers found that using 0-0.2 M NaOH at 60-90˚C increased methane production by 23.4-70.6% during anaerobic digestion. Optimum methane production occurred at 0.1 M NaOH and 73.7˚C.[173] Another study compares alkali-thermal procedures in various orders; thermal-alkali pretreatment (TAP) and alkali-thermal pretreatment (ATP).[148] It was found that alkali pretreatment followed by thermal pretreatment (ATP) improves sludge treatment solubility positively affects methane production. Also, the thermal effect improved the performance of alkali pretreatment using a mixture of NaOH and $Ca(OH)_2$ at 80˚C, methane production increased by 308.7%.[148]

2.6.5.5 Thermal and Enzyme Combined Disintegration

The effect of thermal and enzyme combination disintegration on sludge has not yet been studied. However, we have been using these two methods to improve AD efficiency in our group. Pancreatin was used as an enzyme to ensure the successful identification of MPs and their elimination from environmental matrices.[174] Experiments were conducted to find the optimum enzyme concentration and optimum period and 150 mg Pancreatin enzyme (PEN)/g TS dose and 24 hours were found to be optimum conditions. It was observed that sludge solubility and methane gas production increased by approximately 38% and 18% with using these two

combined methods, respectively. Hence, it was concluded that employing enzymatic pre-treatment after thermal treatment would yield enhanced efficacy, it was confirmed by the aforementioned findings.

It has been hypothesized that the aforementioned disintegration techniques, which enhance the solubility of sludge and the production of biogas, also create stress factors for MPs accumulated in sludge. Such stress factors could potentially lead to alterations in the structure of MPs and physicochemical properties, as evidenced by limited studies.[20,21] Therefore, this study aimed to both measure the additives leaching from plastics after different sludge disintegrations and to reveal the changes, if any, in the structures of these MPs. Although structural changes after alkali+thermal combined disintegration for PET were noted in a study conducted by our group[21], analysis of the additives leaching after this disintegration was not performed. In addition, additive leaching after disintegrations for PP-MPs and PC-MPs and the resulting changes in the structures of the mentioned plastics have not been reported in any study in the literature. This study takes a step toward filling this gap in literature.

CHAPTER 3

MATERIALS AND METHODS

The experimental work conducted in this study can be grouped into three main parts: (i) developing a method for quantification of leaking chemicals from polyethylene terephthalate (PET), polypropylene (PP), and polycarbonate (PC) during sludge disintegration; (ii) establishing a proper extraction method prior to GC-MS analysis; and (iii) characterization of the MPs exposed to different sludge disintegration methods. First, a GC-MS method was developed so that all the target phthalate esters, namely, diethyl phthalate (DEP), di-n-butyl phthalate (DBP) and di-2-ethyl hexyl phthalate (DEHP); as well as bisphenol A (BPA) can be separated from each other and quantified. Afterward, a solid-phase extraction method was established to extract and preconcentrate the target analytes from disintegrated sludge samples prior to GC-MS analysis. In the final part of the study, microplastics (MPs) subjected to alkali, thermal, enzyme, alkali-thermal combined, and thermal-enzyme combined disintegrations were collected and characterized with additional analytical techniques FT-IR, SEM and DSC.

3.1 Materials

3.1.1 Chemicals and Reagents

Phthalate ester mixtures were prepared by dissolving dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), di-2-ethyl hexyl phthalate (DEHP), and di-n-octyl phthalate (DOP) in hexane at a concentration of 2000 μg/mL and bisphenol-A in methanol were used to prepare calibration standards GC-MS. Benzyl benzoate (BBz) was used as an internal standard. As surrogate standards (SS), bisphenol-F (BPF) and dibenzyl phthalate

(DBzP) were used for the extraction efficiency of BPA and phthalates, respectively. All the analytes, internal and SS were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Before GC-MS measurement, bisphenols (BPA and BPF) were derivatized using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide in 1% trimethylchlorosilane (BSTFA+TMCS, 99:1) supplied from Supelco Analytical (Sigma Aldrich Co. LLC, USA).

All the GC-grade solvents methanol, hexane, dichloromethane (DCM), and acetonitrile used for the extraction steps were from Supelco (Germany).

Sodium hydroxide (NaOH), potassium hydroxide (KOH) and pancreatin enzyme which consists of amylase, lipase, protease, trypsin, and ribonuclease from Merck (Germany) were used for alkali and enzymatic disintegrations.

Anhydrous sodium sulfate (Na_2SO_4) was obtained from Merck (Darmstadt, Germany) to remove moisture during the solid phase extraction.

For the pH adjustment of the samples, analytical grade of 37% (w/w) hydrochloric acid (HCl) and NaOH from Merck were used.

Extra pure 65% (w/w) HNO_3 acetone, and alconox (White Plains, NY, USA) from Merck, were used for cleaning the glassware.

3.1.2 Apparatus and Materials

Sludge samples from WWTP were taken into 5-liter glass jars. Glass flasks, pipettes, and test tubes were used to prepare solutions, and the extraction processes. Crucibles or aluminum weighing dishes were used to find the total solid amount per liter of sludge.

Filtrations of the sludge samples were done using vacuum filtration sets. A 1.2 μm pore-sized glass microfiber and 0.45 μm pore-sized mixed cellulose ester (MCE) filter papers were employed for the filtration experiments.

OASIS HLB (hydrophilic and lipophilic balanced) SPE cartridges from Waters Corporation, USA with 200 mg bed weight, 6 mL tube volume with 30 μm particle diameters were obtained for the preliminary SPE experiments. LiChrolut RP-18 (reversed phase) SPE cartridges from Merck with 200 mg bed weight, 3.0 mL tube volume, and 40-63 μm particle size were used for the extraction experiments.

The corrosion-resistant VacElut cartridge manifold with a 12-port rack from Agilent was used to get a constant flow rate of the solutions in the extraction experiments.

3.1.3 Sludge Samples and Model Plastics

Sludge samples were collected from the Central Wastewater Treatment Plant (WWTP) of Ankara located in Tatlar village. It is an urban WWTP treating municipal wastewater with a capacity of 765,000 m^3 /day which is the largest WWTP in Turkey (ASKI). Sludge samples were supplied from the sludge return line of the secondary clarifier by grab sampling method as shown in Figure 3.1. Samples were kept at 4°C.

Figure 3.1 Sludge sample (on the left) and its source in WWTP (on the right).

Three different types of MPs were used in this study: PET, PP, and PC. Physical properties of these plastics are given in Table 3.1. PET, PC and PP samples were obtained from Erikli water bottles, pink plastic straws without bellows, and PC

pellets (Rainbow Polycarbonate Corporation), respectively and they can be seen from Figure 3.2. Plastics were shredded with clean scissors, and then passed through a series of sieves to obtain MPs in 250-500 μm size range.

Table 3.1 Properties of MPs.

Type of MP	Size (μm)	Density (g/cm^3)	Shape
PET	250-500	1.38- 1.45	Film
PP		0.89- 0.92	Film
PC		1.20- 1.22	Rod

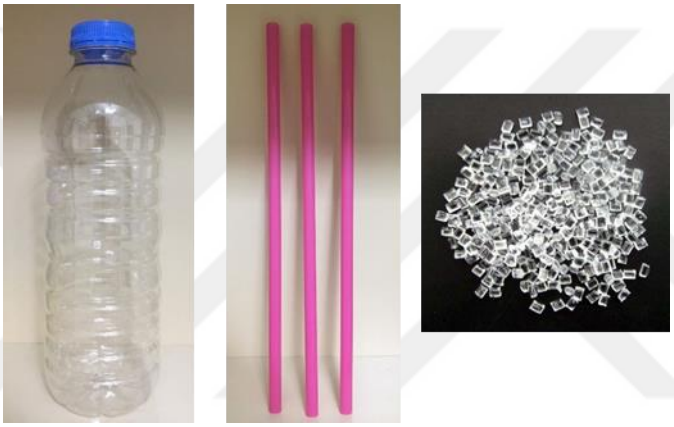


Figure 3.2 MP sources used in this study.

3.2 Experimental Procedures

The experimental step used in this study is shown in Figure 3.3. Following sludge sampling, samples were added the desired amount of different MPs and subjected to different disintegration processes. Then, the liquid fraction of samples was collected and analyzed using GC-MS following SPE procedure. In addition, MPs subjected to different disintegrations were recovered and characterized.

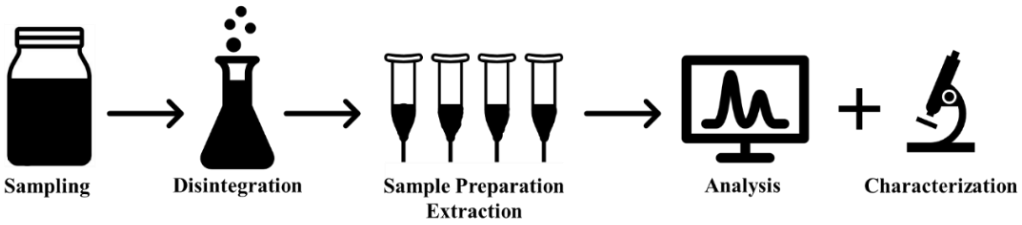


Figure 3.3 Experimental procedure developed for the analysis of leaching additives and MPs characterization.

The sludge samples were collected in glass jars in WWTP and brought to the laboratory. They were centrifuged at 4000 rpm for 3 minutes, then the supernatants were discarded. This process was repeated until the desired solids concentration was achieved. In this study, 20 g total solid (TS) per liter sludge, i.e., 2.0% TS was calculated by subtracting the tare weight of the crucible from 10.0 mL sludge dried in that crucible at 105°C overnight. Afterwards, disintegrations were applied to sludge samples containing added MPs in them. The dose of the MPs in the sludge was 10.0 mg MPs/g TS and each MP was added separately for sample.

3.2.1 Sludge Disintegrations

Disintegration methods were applied to sludges containing PET, PP, and PC MPs separately to follow the effect of each treatment to each plastic. Additionally, one MP control sludge sample was prepared without spiking extra MPs to identify the contribution of MPs already present in the sludge to additive leaching (sludge background identification). Therefore, four samples were used for each disintegration, as shown in Figure 3.4. Each sample contained 400.0 mL of sludge with 2.0% TS by weight and 0.08 g MPs (10.0 mg MPs/ g TS dose) which was selected as an environmentally relevant dose.[175]

In alkali disintegration, 16.4 mL of 10 M NaOH and 4.6 mL of 10.0 M KOH were added to each 400.0 mL sludge sample in Erlenmeyer flask to get 0.5 M alkali concentration, and samples were incubated at 25 °C for 2 days.

For thermal disintegration, sludges were prepared in bottles (400.0 mL) and they were exposed to 127°C for 2 hours in an autoclave.

In enzymatic disintegration, 1.2 g and 1.6 g enzyme for 150 and 200 mg Pancreatin enzyme/g TS were added to 400.0 mL sludge sample, and they were incubated at 38°C for 24 hours.

In alkali+thermal combined disintegration, first alkali disintegration to 400.0 mL of sludge as in previously described, then thermal disintegration was applied.

The last disintegration method was the combination of thermal and enzyme treatment methods. Here, 400.0 mL of samples were exposed to thermal treatment as described above, then the pancreatin enzyme was added to each of them and incubated as in the single enzyme disintegration process.

The applied doses of alkali and enzyme and the conditions of thermal treatment were all selected based on their effectiveness for sludge solubilization in the previous part of the project.

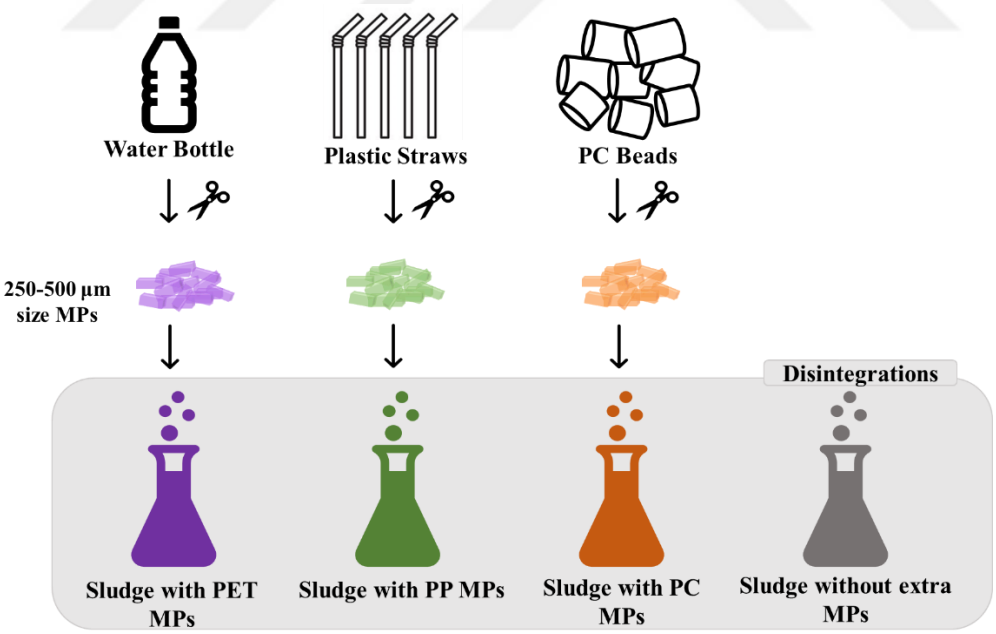


Figure 3.4 Schematic of sludge disintegration procedure.

After disintegration methods were applied to the samples, sample preparation is required before their extraction through SPE. The pH of the disintegrated samples containing MPs was adjusted to 3.0 using 12.0 M HCl to stabilize and avoid precipitation of metals in the sludge. pH-adjusted disintegrated sludge samples were centrifuged at 4000 rpm for 5 minutes.

The supernatant (liquid) part was collected into a glass container and the supernatant was filtered through first 1.2 µm pore-sized glass microfiber and then, 0.45 µm pore-sized mixed cellulose ester (MCE) filters. In Figure 3.5, an untreated sludge sample and centrifuged and filtered sludge supernatant are presented. The final step before the extraction of the sample was the addition of SS to determine extraction efficiency in the further steps.

Figure 3.5 Picture of sludge sample (left) and its centrifuged and filtered supernatant (right).

After the sludge sample was prepared, they were extracted through a solid phase cartridge, in this way, analytes from the complex matrix can be separated then, preconcentrated before quantifying them in GC-MS.

3.2.2 Extraction of PAEs and BPA from Liquid Phase of Sludge

3.2.2.1 Selection of SPE Sorbent Type for PAEs and BPA Analysis

Preliminary studies for the extraction of phthalates (DEP, DBP, DEHP) and BPA from the liquid phase of sewage sludge were optimized using two cartridges having different sorbent materials (LiChrolut RP-18 and OASIS HLB), various solvent combinations for elution with spiked samples.

For the conditioning step, a mixture of 10.0 mL of hexane, 10.0 mL of dichloromethane, 10.0 mL of methanol, and 10.0 mL of distilled water was used. As a sample 100.0 mL DI water spiked by 10.0 µL of 2.0 $x10^4$ µg/L of SS (DBzP and BPF) to make the final concentration of SSs in solution would be 100 µg/L. 10.0 mL of distilled water was utilized for washing the SPE cartridges. Finally, hexane, methanol, and acetonitrile were used sequentially at different volumes to collect the analytes from the cartridges and the volumes are listed in Table 3.2. Recovery of each target analyte was determined by comparing the measured concentration of the surrogate to the spiked concentration as shown in Equation 3.1.

$$Surrogate\ Recovery\ \% = \frac{Cexp}{Cthe}\ x\ 100 \qquad \text{Equation 3.1}$$

where:

C_{exp} is the measured concentration of the surrogate in the spiked sample.

C_{the} is the theoretical concentration of the surrogate added to the sample.

Table 3.2 Elution conditions used for sorbent selection.

Matrix	Sample Volume (mL)	Concentration of DBzP and BPF in Solution (ppb)	Elution (mL)		
			Hexane	Methanol	Acetonitrile
DI water	100	100	5.0	5.0	5.0
DI water	100	100	3.0	3.0	3.0
DI water	100	100	4.0	4.0	2.0

3.2.2.2 Surrogate Standards Determination in Sludge Supernatant

A 20.0 mL of sludge supernatant was used to check the existence of SS in sludge. Supernatant was passed through C18 cartridge and collected with a solvent combination of 4.0 mL hexane, 4.0 mL methanol and 2.0 mL acetonitrile to follow SS in untreated sludge supernatant The conditioning and washing steps were the same as listed in Section 3.2.2.1.

3.2.2.3 Effects of Different Ratios of Elution Solvents on PAEs and BPA Recoveries

The sample volume (supernatant of nondisintegrated sludge) was used as 10.0 mL to eliminate the blockage in case of excessive loading of the cartridges. 10.0 mL of DI and 10.0 mL of 10% methanol in DI were used to investigate whether they affect the analyte recovery for the washing of SPE columns. Finally, hexane, methanol, and acetonitrile were used sequentially at various ratios to collect the analytes from the cartridges. The volume of the solvents used for the elution step is listed in Table 3.3. The optimum value was selected based on the percent recoveries of the analytes as explained in Chapter 4.

Table 3.3 Volume ratios used for elution of PAEs and BPA.

Elution Condition (mL)				Washing Solvent
#	Hexane	Methanol	Acetonitrile	
1	6.0 mL EtOAc: MeOH (9:1)			DI
2	4.0	3.0*	5.0	DI
3	4.0	3.0*	5.0	10% MeOH
4	4.0	3.0	5.0	DI
5	4.0	3.0	5.0	10% MeOH

*3.0 mL of EtOAc:MeOH (9:1)

Elution of solvents was carried out with the help of a vacuum manifold since the elution rate of each substance (solvent and sample) to be passed through the cartridges in the solid-phase extraction will significantly affect the recovery efficiency of analytes. The flow rate was 1.0 mL/min. Figure 3.6 shows the experimental setup for SPE.

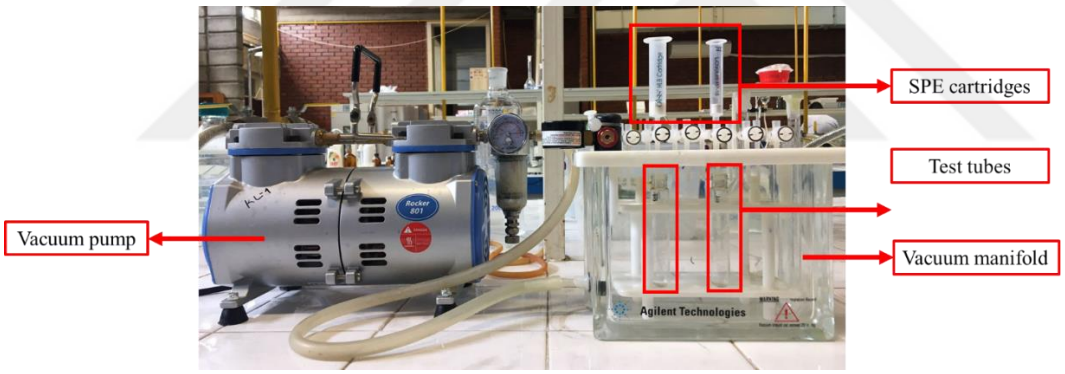


Figure 3.6 Experimental set-up for SPE.

3.2.2.4 SPE Method for Extraction of PAEs and BPA

The optimized SPE protocol is listed in Table 3.4. Sludge supernatants obtained after each disintegration (alkali, thermal, enzyme and combined alkali+thermal and thermal+enzyme disintegrations) were extracted using this optimized procedure.

Table 3.4 Optimized SPE method for extraction of PAEs and BPA.

Conditioning	10.0 mL hexane, 10.0 mL DCM, 10.0 mL methanol, 10.0 mL DI water (solvent flow rate: 1.0 mL/min)
Sample Loading	10.0 mL sample (1.0 mL/min)
Washing	10% by 10.0 mL MeOH
Elution	4.0 mL hexane, 3.0 mL methanol, 5.0 mL acetonitrile (1.0 mL/min)

After the solid-phase extraction step was completed, a 12.0 mL solvent mixture (4.0 mL hexane, 3.0 mL methanol, 5.0 mL acetonitrile) carrying target analytes (DEP, DBP, DEHP and BPA) collected from the elution step was evaporated under a gentle nitrogen stream in a water bath at 50-55°C until complete dryness. Then, 2.0 mL acetonitrile was added to dried samples and then mixed using vortex for 30 seconds. Later, the acetonitrile layer was passed through anhydrous Na_2SO_4 columns to dehydrate the sample before GC-MS analysis. Dehydrated samples were then separated into two: one group for phthalate analysis and the other for BPA analysis. For phthalate analysis, 1.0 mL of the sample was transferred into GC vials and 5.0 µL of internal standard from $10x10^3$ ppb stock solution was added. For the determination of BPA, 50.0 µL of the sample was dried under N_2 first, then after complete dryness, 50.0 µL of BSTFA:TMCS (99:1) was added and the sample was vortexed and allowed to derivatize in a water bath at 75°C for 1 hour. Afterward, the mixture was dried under nitrogen flow and then, 50.0 µL acetonitrile, and 1.0 µL internal standard (benzyl benzoate, BBz) were added. Finally, these samples were analyzed using GC-MS.

3.2.3 Identification and Quantification of PAEs and BPA

3.2.3.1 Optimization of GC-MS

Several methods with different oven programs were used with GC-MS to identify and quantify phthalates and BPA. Firstly, the methods used in the literature listed in Table 3.5 for these chemicals were used to develop a method with high separations of analytes within a short time, and high repeatability.

Table 3.5 Studies in the analysis of the additives in the literature using GC-MS.

Compounds	Instrument	Column	Oven Program	Reference
BPA, its chlorinated derivatives, alkylphenols, phthalates esters (DMP, DEP, DBP, DOP, BBP, DEHP)	6890 GC - 5976 MSD (Agilent)	ZB-5 MS Zebron (30 m length × 0.25 mm I.D.; 0.2 µm film thickness)	120 °C for 1 min, 120 °C to 230 °C at 15 °C/min, 230 °C to 260 °C at 30 °C/min held for 8 min Injector Port Temp.: 250 °C MS Transfer line temp.: 270 °C Ion Source Temp.: 250 °C	111
Phthalate esters (DEHP, DEP, DBP, DMP, BBP, DNOP) and BPA	Trace GC ultra, Thermo Finnigan Electron Corporation coupled with an ion trap MS (Polaris Q, Thermo Finnigan)	Rtx-5MS (30 m × 0.25 mm I.D.; 0.2 µm film thickness)	60 °C for 1.5 min, 60 °C to 180 °C at 20 °C/min, 180 °C to 230 °C at 5 °C/min, 230 C to 310 C at 20 C/min held for 5 min Injector Port Temp.: 280 °C MS Transfer line temp.: 300 °C Ion Source Temp.: 200 °C	115
Herbicides, phthalates, alkylphenols, BPA	GC-MS (Thermo Electron Corporation) Trace GC 2000 instrument	DB-5MS (30 m × 0.25 mm I.D.; 0.2 µm film thickness)	70 °C for 2.0 min, 70 °C to 135 °C at 10 °C/min, 135 °C to 160 °C at 3 °C/min, 160 °C to 175 °C at 1 °C/min 175 °C to 195 °C at 3 °C/min 195 °C to 310 °C at 10 °C/min held for 5 min Injector Port Temp.: 280 °C MS Transfer line temp.: 280 °C Ion Source Temp.: 200 °C	109
Phthalates esters (DMP, DEP, DBP, DOP, BBP, DEHP)	7890A GC- 5975C MSD (Agilent)	DB-5MS (dimensions are not mentioned)	80 °C for 0.5 min, 80 °C to 220 °C at 10 °C/min, 220 °C to 290 °C at 30 °C/min held for 4 min Injector Port Temp.: 300 °C MS Transfer line temp.: 270 °C Ion Source Temp.: 230 °C	176
Phthalates esters (DMP, DEP, DBP, DOP, BBP, DEHP)	7890B GC- 5977B MSD (Agilent)	DB-5 (30 m × 0.25 mm × 0.25 µm)	70 °C for 1.0 min, 70 °C to 280 °C at 75 °C/min, held for 15 min Injector Port Temp.: n.m. MS Transfer line temp.: 280 °C Ion Source Temp.: 230 °C	177

3.2.3.2 GC-MS Method for PAEs and BPA

GC-MS (7890A GC- 5975C MS with, Agilent) was used for identification and quantification of phthalates and BPA in disintegrated sludge samples. The GC operating parameters are given in Table 3.6.

Table 3.6 GC-MS parameters for determination of phthalates and BPA.

GC-MS Method	
Column	HP-5MS 5% Phenyl Methyl Siloxane
Carrier Gas	Helium (1 mL/min)
Injection Volume	1.0 µL
Injection Mode	Splitless
Injection Temperature	280 °C
MS Interface Temperature	280 °C
MS Source Temperature	230 °C
MS Quadrupole Temperature	150 °C
MS Mode	SIM
Oven Program	120°C for 0.5 min, 25 °C/min to 170 °C, 15 °C/min to 260°C hold for 5 min, 260°C 15 °C/min to 310 °C hold for 3.17 min
Total Run Time	20 min

The sample (1.0 µL) was injected in the splitless mode at 280 °C into the GC column. The MS system was routinely used in Selective Ion Mode (SIM) to quantify the analytes. The mass spectrometer works based on the electron impact ionization (EI) mode and the energy of the electrons was selected as 70 eV in all measurements. MS interface, source and analyzer (quadrupole) temperatures were kept at 280°C, 230°C, and 150°C, respectively. Each analyte was quantified based on peak area using one target and two qualifier ion(s).

3.3 Quality Assurance/ Quality Control (QA/QC)

3.3.1 Glassware

In order to meet QA/QC standards, all glassware used in analyses was cleaned with Alconox detergent for 1 h in hot water and then rinsed consecutively with: i) distilled water; ii) 10% by weight nitric acid, iii) distilled water, and iv) acetone (technical grade). All glassware was dried in an oven at 105°C for an hour, then in a muffle furnace at 450°C for 15 minutes.

3.3.2 Linearity

The linearity of the response of phthalates and BPA was found by creating 7-point calibration curves in the 1.0-250 µg/L concentration range. The ratio of the peak area of each analyte to the area of internal standard versus the concentration of the analytes was plotted and linear relationships with the correlation coefficients, R^2 >0.99 were obtained.

Th stock solution was prepared as a mixture of 1000 µg/L phthalates (DEP, DBP and DEHP) and BPA prepared in acetonitrile and stored at 4 °C until the measurements. This stock solution was used to prepare calibration standards at different concentrations. Triplicate injections of each standard were performed in calibration curves.

An external calibration method was employed to determine the concentration of the analytes in the sludge samples. A 10.0 mL (pH 3.0) sludge supernatant was spiked with the standard solution to get a final concentration range of 25-250 µg/L and 5 different concentrations were prepared. After they were extracted through SPE cartridges based on the experimental conditions in Section 3.2.2.3, they were measured as triplicate in GC-MS.

3.3.3 Instrument Detection and Quantification Limits (IDL and IQL)

The stock solution with the smallest concentration (1.0 μg/L) used in chemical calibrations was injected repeatedly (n = 7) and the standard deviations of these measurements were calculated. Three times the standard deviation of concentration obtained from external calibrations was used as IDL, and ten times as instrumentation quantification limit (IQL). IDL and IQL values are listed in Chapter 4.

3.3.4 Method Detection Limit (MDL) and Method Quantification Limit (MQL)

Method detection and quantification limits were calculated using Equation 3.2 and Equation 3.3, respectively using the standard deviation and calibration curve slope obtained from procedural calibration.

$$MDL = 3.3 \times \frac{SY}{S} \qquad \text{Equation 3.2}$$

$$MQL = 10 \times \frac{SY}{S} \qquad \text{Equation 3.3}$$

S_Y: standard deviation of the regression line
S: slope of the calibration curve.

3.4 Characterization of MPs

3.4.1 Recovery of MPs

The recovery procedure of MPs in disintegrated sludge is shown in Figure 3.7. Sludge samples containing MPs were first filtered through a 1.2 μm filter, and then MPs in the sludge were collected with tweezers. The sorted plastics were kept in 15% H_2O_2 solution for a night to clean the impurities adhering to the plastic surface.

After cleaning, the plastics were characterized using ATR-FTIR, SEM and DSC described in the following sections.

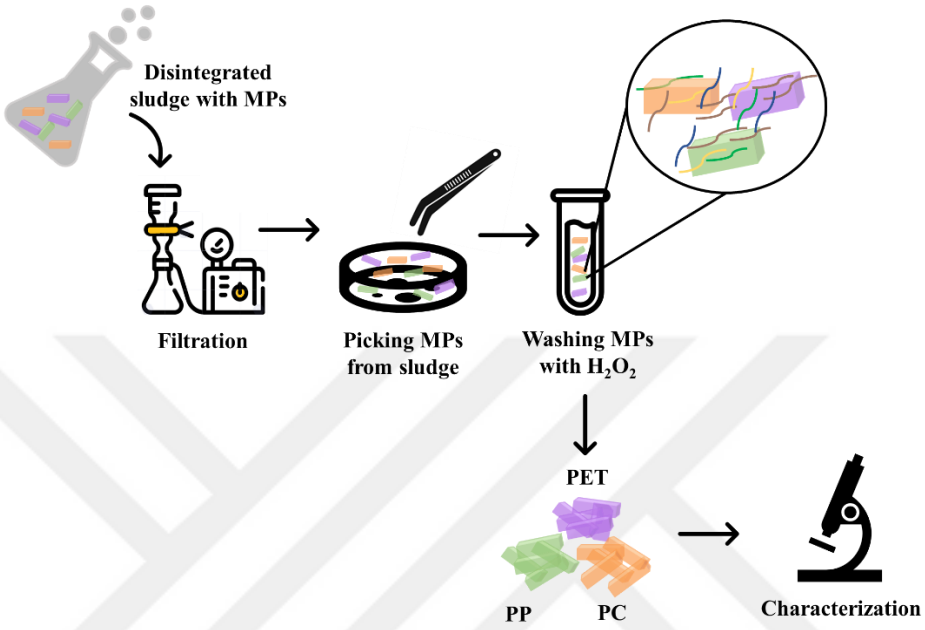


Figure 3.7 Collection scheme of MPs from sludge samples.

3.4.2 Attenuated-Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR was used to determine carbonyl index (CI) which is defined as the ratio of the carbonyl absorbance intensity in the range of 1870-1650 cm^{-1} obtained from FTIR measurements to that of a band selected as a reference. CI is a parameter used to show oxidation and wear on the surface of MPs. The infrared used for CI calculations were recorded by using the ATR-FTIR (Thermoscientific Nicolet iS10 FTIR Spectrometer), with 128 scans and a spectral resolution of 4 cm^{-1} in the wave number range of 600 and 4000 cm^{-1}. Five replicates were used. The carbonyl and reference peak values for PET, PP and PC are listed in Table 3.7.

Table 3.7 Peaks used in CI determination.

Types of MP	Carbonyl Peak (cm^{-1})	Reference Peak (cm^{-1})	Reference
PET	1712	1505	[178]
PP	1712	1472	[179]
PC	1773	2970	[180]

3.4.3 Differential Scanning Calorimetry (DSC)

The change in the crystal structures of PET, PP and PC MPs after disintegration was measured with a differential scanning calorimeter (DSC) (TA, DSC250). The temperature range was 50-300°C with a heating rate of 10°C/min for PET, 20-200°C with a heating rate of 10°C/min for PP and PC with a heating rate of 10°C/min. Dry nitrogen was used as the experimental atmosphere for all polymers. The crystallinity of the MPs was calculated with Equation 3.4:

$$X_c = \frac{\Delta Hm}{\Delta H°m} * 100 \qquad \text{Equation 3.4}$$

ΔHm, enthalpy of melting is calculated by dividing the area of the melting peak with y sample weight (J/g) and ΔH°m is the standard melting enthalpy (J/g) for 100% crystallinity for the polymer. ΔH°m values are given in Table 3.8.

Table 3.8 Standard melting enthalpy values of PET and PP.

Types of MP	ΔH°m (J/g)	Reference
PET	135.8	[181,182]
PP	207	[182]
PC	Amorphous	[183]

3.4.4 Scanning Electron Microscope (SEM)

The scanning electron microscope (SEM) can magnify the samples under examination by a factor of up to 100.000. The surface morphology examination of PET, PP, and PC MPs that were recovered from the sludge in the reactors was conducted at the METU central laboratory. This study examined specimens under various magnification levels utilizing the QUANTA 400F Field Emission SEM instrument.

3.5 Solids and pH Analyses in Sludge Samples

The TS (Total solid) values of the sludges were adjusted to 2% for disintegration after sampling by first siphoning the clear supernatant of the sludges and then, gently centrifuging (4000 rpm, 4 min) sludges to reach the required concentration. The characterization of sludges was performed after adjusting the TS value. Within 24 hours of sampling, TS, volatile solid (VS), total suspended solids (TSS), volatile suspended solid (VSS), and pH were measured. All characterization parameters were measured in triplicates, as shown in Table 3.9.

Table 3.9 Characteristics of concentrated sludge.

Parameter	**WAS**
TS (g/L)	20.1±0.1
VS (g/L)	13.0±0.3
TSS (g/L)	16.67±0.11
VSS (g/L)	13.03±0.2
pH	7.3

3.6 Statistical Analyses

Two sample t-test was used in order to determine whether there is a significant difference between the control and experimental groups, considering the significance level as 0.05. The tests were conducted using R-studio.

CHAPTER 4

RESULTS AND DISCUSSION

This study focused on the leaching of additives from MPs and changes in the characteristics of MPs resulting from different sludge disintegration methods. The first part of the thesis involved evaluating the results of extraction and analysis of additive leaching. The second part examined and discussed the characteristics of MPs upon disintegration.

4.1 Development of GC-MS Methods

In Chapter 3.2.3, it was mentioned that GC-MS oven programs for the analysis of PAEs and BPA were optimized using a stock solution with a known concentration. Examples of chromatograms are shown in Figures 4.1 and 4.2. The aim was to separate the following analytes: DEP, DBP, DEHP, BPA; their SS, DBzP and BPF and internal standard, BBz in a relatively short GC run time with satisfying abundances.

Column bleeding results from the polymer that forms the stationary phase in the column. It was observed as shown in Figure 4.1 using Bono-Blay and coworkers and Amiridou and Voutsa due to high temperatures employed. DEHP, which is one of the target analytes, was detected in the bleeding region. However, the increasing background signal may affect the accuracy of the area under the DEHP peak, leading to erroneous calibration curve results. Additionally, the duration of the method in Figure 4.1 (a) is about 55 min which would be very time-consuming during further analysis. Therefore, these two methods were found not suitable for the analysis.

A method developed by Mersal and coworkers showed column bleeding after the 15^{th} minute as shown in Figure 4.2 (a). In the chromatogram, the baseline started at low levels and as the temperature program progressed. As mentioned earlier, this increase in the background level indicates bits of column reaching the detector. As a result, siloxane ions (m/z 73, 207 and 281) were observed for the region after the BPF peak. In Figure 4.2 (b), a chromatogram obtained by using the Ballesteros' method is shown. Here, no bleeding occurred at high temperatures and unlike Mersal's method, siloxane ions did not interfere with analyte ions. Also, all analytes of interest were separated within 20 minutes. Therefore, it was the best option to work within the analysis.

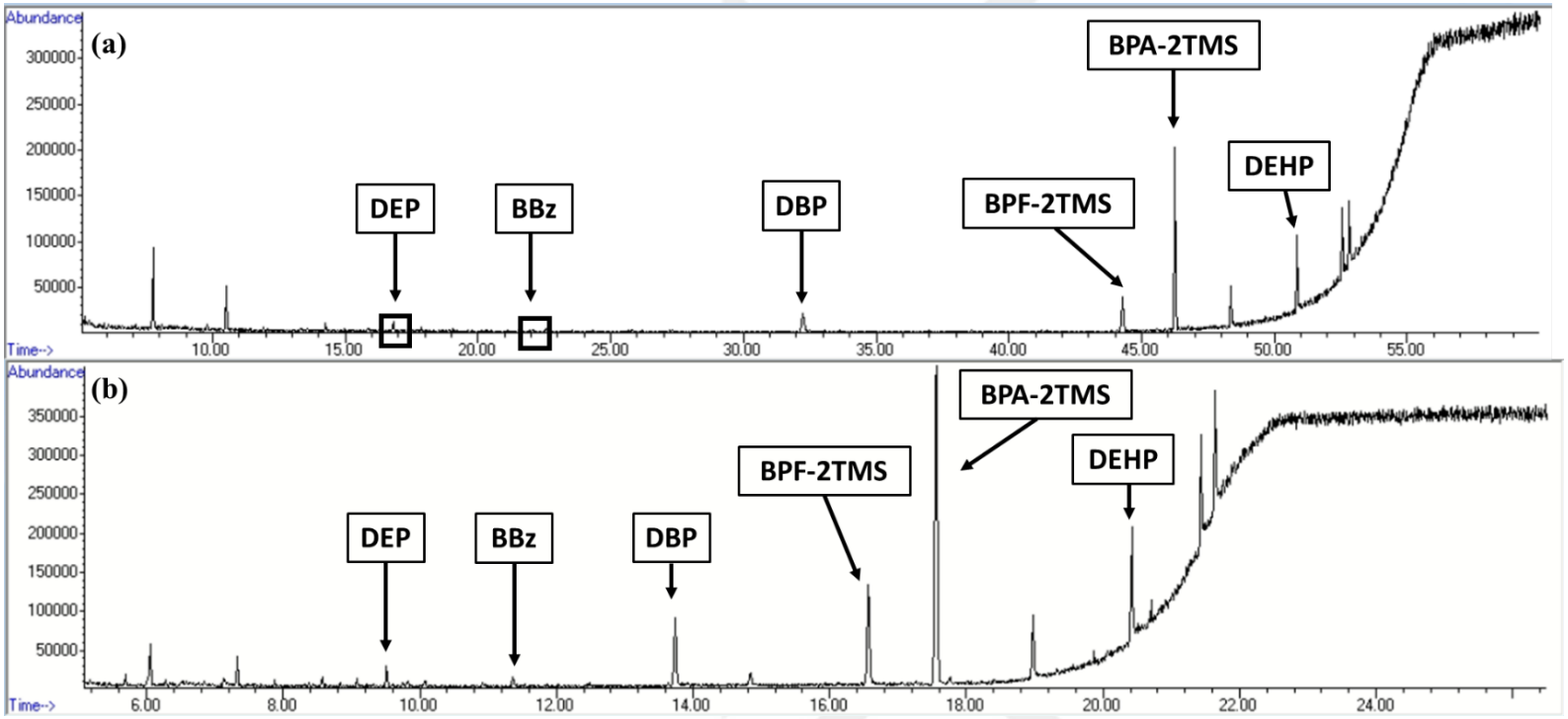


Figure 4.1 GC-MS chromatograms of stock solution (containing DMP, DEP, DBP, BBP, DEHP, BPF, BPA and DBzP) with a concentration of 1000 μg/L using methods by (a) Bono-Blay and coworkers (2012) and (b) Amiridou and Voutsa (2011) mentioned in Table 3.5.

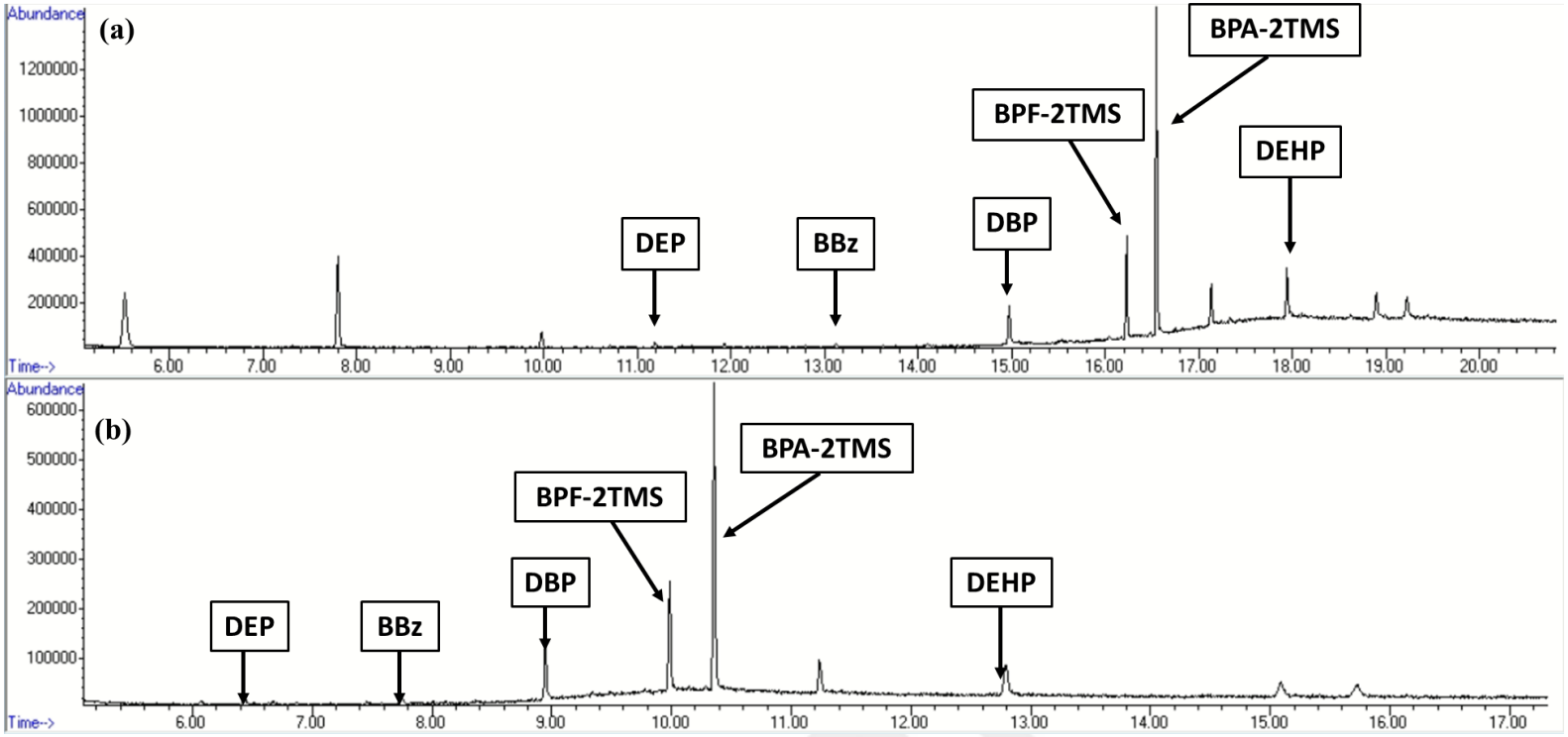


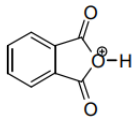
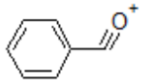
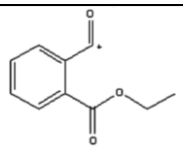
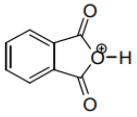
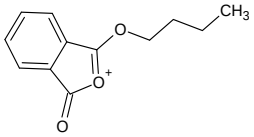
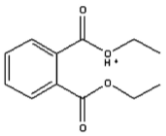
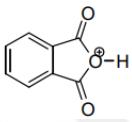
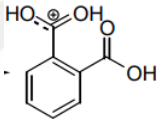
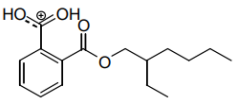
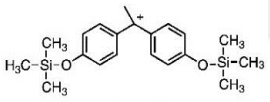
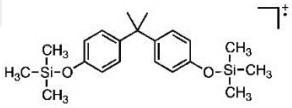
Figure 4.2 GC-MS chromatograms of stock solution (containing DMP, DEP, DBP, BBP, DEHP, BPF, BPA and DBzP) with a concentration of 1000 µg/L using methods by (a) Mersal and coworkers (2022) and (b) Ballesteros and coworkers (2006) mentioned in Table 3.5.

It should be noted that the DBzP peak was not seen in any of the chromatograms, as shown in Figures 4.1 and 4.2. Therefore, a method similar to the method but without any bleeding (Ballesteros') was developed. In Figure 4.3, the chromatogram obtained from the developed method is shown. The details of the method are listed in Table 3.6. Unlike the Ballesteros' method, the oven temperature was increased to 310°C instead of 260°C to remove impurities in the column. The procedure resulted in the following retention times: DEP 4.7 min, BBz 6.1 min, DBP 7.1 min, BPF 8.4 min, BPA 8.8 min, DEHP 11.15 min, and DBzP 13.6 min. The retention times of analytes and fragments of ions of target analytes are represented in Tables 4.1 and 4.2, respectively.

Table 4.1 SIM parameters and retention times of analytes obtained from optimized GC-MS method.

Compound	**Retention Time (min)**	**m/z**
DEP	4.9	105, 149, 177, 222
DBP	7.2	149, 205, 223
DEHP	11.3	149, 167, 279
BPA-2TMS	8.8	73, 357, 372

Table 4.2 Fragment of ions of target analytes (DEP, DBP, DEHP and BPA).

Analytes	m/z		
DEP	**149**	**105**	**177**
			
DBP	**149**	**205**	**223**
			
DEHP	**149**	**167**	**279**
			
BPA	**357**	**372**	
			

Then, a mixture of stock solution (including DEP, DBP, DEHP, BPA, BPF and DBzP) in acetonitrile was used to prepare 7-point calibrations for DEP, DBP, DEHP and 6-point calibrations for BPA, BPF and DBzP. The reason for preparing a 6-point calibration for DBzP, BPA and BPF is that there were no peaks for these chemicals at 1 µg/L concentration. The calibration curves are depicted in Figure 4.4 and LOD/LOQ values were determined as listed in Table 4.3. All calibrations showed a minimum R^2 value of 0.9913. The LOD values for DEP, DBP, DEHP, and BPA were calculated to be 1.4, 1.4, 0.5, and 0.7 ppb, respectively.

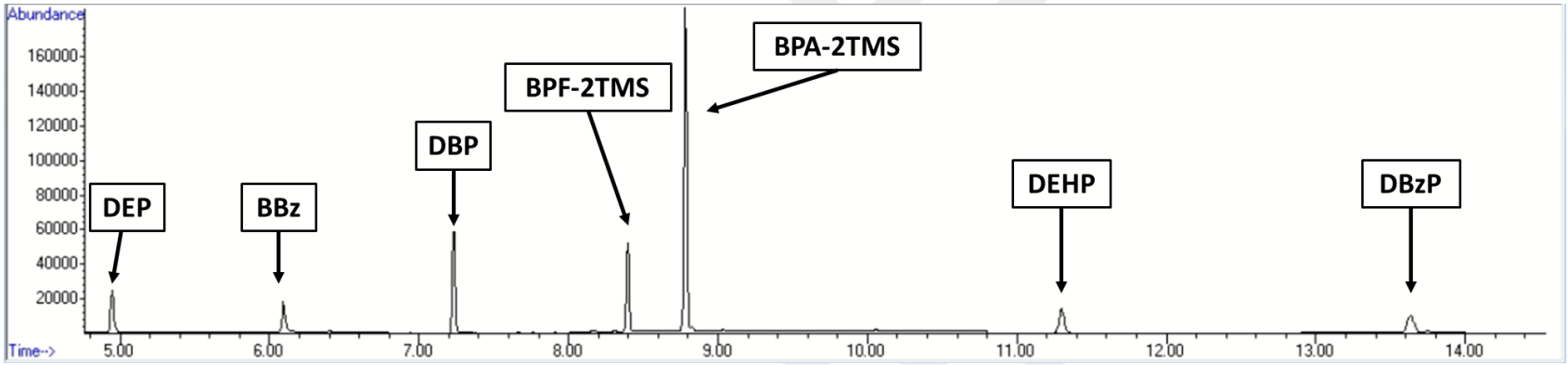


Figure 4.3 Chromatogram of a stock solution (containing DEP, DBP, DEHP, BPF, BPA and DBzP) with a concentration of 1000 µg/L using the developed GC-MS method detailed in Table 3.6.

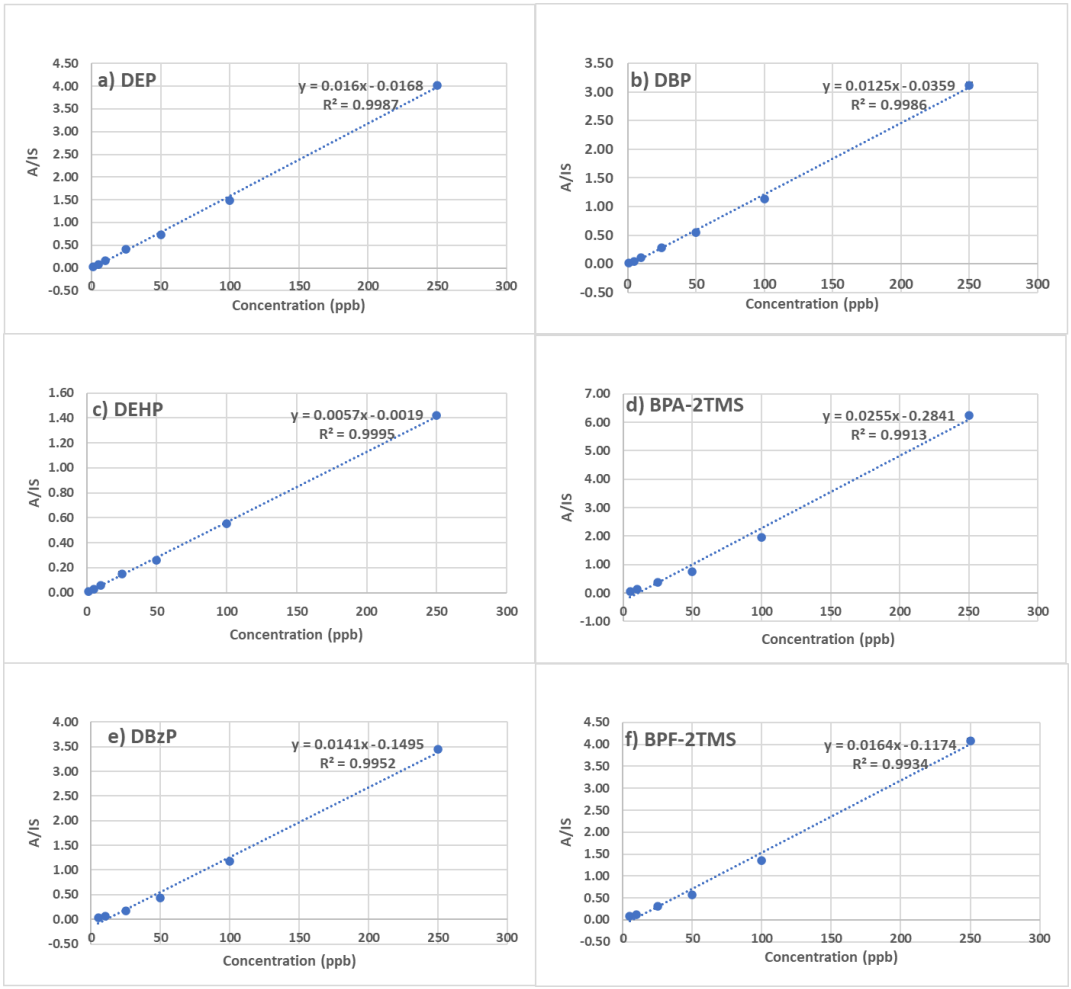


Figure 4.4 External calibrations between 1-250 µg/L of DEP (a), DBP (b), DEHP (c), BPA (d), DBzP (e) and BPF (f).

Table 4.3 LOD and LOQ values of analytes (calculated as explained in Section 3.3.3).

Compounds	LOD (ppb)	LOQ (ppb)
DEP	1.4	4.6
DBP	1.4	4.8
DEHP	0.5	1.8
BPA	0.7	2.3

4.2 Development of SPE Method

4.2.1 Extraction from C18 Sorbent versus HLB Sorbent

In Chapter 2 (Table 2.6), it was mentioned that C18 and HLB type sorbents are commonly used for analyzing PAEs and BPs from environmental samples. Table 4.4 compares the recovery efficiencies between C18 and HLB type of SPE cartridges. A solution including 100 μg/L SS (DBzP and BPF) for SPE studies with sludge was added to 100.0 mL DI. Different solvent ratios were used as mobile phases for C18 and HLB SPE cartridges, as shown in Table 4.4. Various solvents were used during the extraction process to recover two different groups of chemicals, namely PAEs and BPA. These two groups have different polarities, so solvents such as hexane (H) were used to extract phthalates, which have a nonpolar structure, while methanol (M) was used to extract bisphenols, which have a polar structure. Additionally, acetonitrile (AN) was used to extract both types of chemicals. These solvents were also used to prepare calibration standards and extract analytes from the cartridges. However, the recoveries of analytes from SPE can differ depending on several factors such as the sample matrix, the number of compounds being analyzed simultaneously and the analytical instrumentation. The extraction method was accepted as adequate if the average percent recovery is within the range of 50 to 150% according to the United States Environmental Protection Agency (US-EPA) 8270E method.[184]

Table 4.4 Percent recoveries of DBzP and BPF obtained from C18 and HLB cartridges.

Matrix	Sample Volume (mL)	Conc. of DBzP and BPF in Solution (ppb)	Elution (mL)			Recovery (%)			
						C18		HLB	
			H	M	AN	DBzP	BPF	DBzP	BPF
DI water	100	100	5	5	5	79.1	140.2	90.8	193.5
DI water	100	100	3	3	3	68.7	83.0	50.6	55.2
DI water	100	100	4	4	2	65.3	132.5	51.2	87.7

In the first experiment, 15.0 mL of solvent was used for elution, consisting of 5.0 mL hexane, 5.0 mL methanol and 5.0 mL acetonitrile. The solvents passed were collected in a receiving vessel as they passed through the cartridges in the vacuum manifold and elution was only possible with limited volumes. Therefore, the amount of elution solvent used in the first experiment was very close to the tube volume (15.0 mL), which created a risk of overflow. Also, it was observed that it took a long time during the solvent evaporation step. In the case of using 5.0 mL of each solvent (hexane, methanol and acetonitrile), the recovery for DBzP was found to be 79.1% and 90.8% using C18 and HLB, respectively. Meanwhile, 140.2% and 193.5% recoveries were obtained for BPF using C18 and HLB, respectively. Even though the recoveries of DBzP were satisfactory for both cartridges, the HLB cartridge showed high BPF recoveries when 5.0 mL of hexane, methanol, and acetonitrile (1:1:1 v/v) were used. Positive errors in SPE was measured when the recovery of the target analyte exceeded 100%. This may occur for a variety of reasons listed below:

i. Impurity coextraction occurs when substances other than the target analytes are extracted, which can interfere with detection and lead to overestimation of analyte concentration.

ii. Measuring errors is also a common source of errors. This could include inaccuracies in measuring the sample volume, eluent volume or the amount

of the internal standard. Such errors can result in erroneous calculations and overstated recovery values.

iii. Non-specific interaction with SPE sorbent is another source of errors. Occasionally, the SPE sorbent may interact with additional components present in the sample, resulting in the removal of unwanted compounds. This can also result in a positive recovery error.

In the second attempt, the elution solvent volume was reduced to 9.0 mL with a ratio of 1:1:1 (hexane, methanol and acetonitrile). The C18 cartridge was used to determine the yields for PAEs and BPA which were found to be 68.7% and 83.0%, respectively. Therefore, the low recoveries of DBzP and BPF of 50.6% for and 55.2%, respectively, obtained using HLB cartridges showed that the specified elution system was unsuitable for this cartridge.

The volume of hexane and methanol was increased to 4.0 mL to increase DBzP and BPF recoveries compared to the second trial while 2.0 mL of acetonitrile was used to keep the total volume as low as possible. Although the BPF yield increased from 55.2% to 87.7% when the HLB cartridge was used, the DBzP yield did not change. Poor recovery of DBzP from HLB cartridges may result from the hydrophilic nature of the sorbent. Phthalates are known to be lipophilic substances, and HLB cartridges are specifically made to capture substances that dissolve in water. Consequently, the capacity of HLB cartridges to retain phthalates is limited, resulting in inadequate recovery. Therefore, it is recommended to utilize reversed-phase solid-phase extraction (RP-SPE) cartridges, specifically designed to keep lipophilic substances such as C18, to enhance the recovery of DBzP and phthalates. Consequently, based on the results of these three experiments, only C18 type cartridges were used in the further extraction experiments.

4.2.2 Optimization of Elution Conditions

US-EPA has a procedure (3535-A) for separating certain organic compounds from water samples using SPE. The method describes the conditions for extracting various organic compounds from water-based substances such as groundwater and wastewater. This method stated that it is not enough to rely only on organic-free reagent water for performance evaluations. The validity of performance evaluations must be supported by data obtained from real sample matrices (EPA 3535-A). Therefore, optimizations were made to achieve optimal recoveries using sludge supernatant as a sample rather than pure water.

4.2.2.1 Surrogate Standards in Sludge Supernatant

It is essential to avoid SS in the sludge samples to prevent interference with the spiked SS in the samples, which can lead to higher yields than expected.

20.0 mL of sludge supernatant was extracted through a C18 cartridge and collected with a solvent combination of 4.0 mL hexane, 4.0 mL methanol and 2.0 mL acetonitrile to extract the SS in the supernatant left from the previous experiment. As a result, SS were not detected in the sludge supernatant.

Known concentrations (100 μg/L) of SS were spiked into the sludge supernatant to determine the efficiency of detecting with the solvent combination used for subsequent elution. However, the results in Table 4.5 showed unsatisfactory recovery values. The recovery of DBzP was as low as 42.0% and the recovery of BPF was inconsistent between replicates with one replicate having very high recovery of 143.0%. Therefore, it was concluded that the volume of solvent combination was not effective in sludge supernatant extraction.

Table 4.5 Percent recoveries of SS spiked in the sludge supernatant.

Elution (mL)			Replicate #	Recovery (%)	
				C18	
H	M	AN		DBzP	BPF
4	4	2	1	41.6	143.1
			2	42.3	106.7

Several factors can cause poor recovery in SPE, which can result in imprecise quantification of desired analytes. Below are some of the most common causes of low recovery:

i. Insufficient wetting of the sorbent bed prior to sample loading can lead to inadequate binding of the analytes and obtain recoveries less than expected.

ii. The presence of other constituents in the sample matrix, such as proteins, lipids, humic acids, or salts can hinder the extraction process. These matrix constituents may compete with analytes for binding sites on the sorbent or disrupt the elution process.[185]

iii. Overloading the cartridge with too much sample can exceed the sorbent's binding capacity, resulting in analyte breakthrough and insufficient extraction.

4.2.2.2 Efficiency of Different Ratios of Elution Solvents on SS Recoveries

To improve the efficiency of the analyte recovery from the SPE cartridges, various ratios of the solvents were tested as shown in Table 4.6.

In the first attempt (#1), a mixture of 6.0 mL ethyl acetate (EtOAc):methanol (MeOH) (9:1) mixture was used in the elution phase, based on the study by

Ballesteros and coworkers (2006). The average BPF recovery was 179.0%, while the recovery of DBzP was approximately 60.0% on average. In the second attempt (#2), hexane and acetonitrile were added to improve DBzP recovery since its recovery was low and that of BPF was too high. The average recoveries were 130% and 113% for DBzP and BPF, respectively.

In the literature, it was suggested that using a low percent (5-10%) methanol-water solution rather than only DI water in the washing step is important, especially when extracting environmental samples. Using a methanol-water solution removes humic and fulvic acids from the sorbent, improving the quality of the chromatogram.[185] Humic and fulvic acids already exist in sludge to sink pollutants, and they can be easily retained on the SPE sorbent which leads to undesirable recovery results. Based on this information, in the third trial (#3), the same elution condition was utilized as in #2 after washing the cartridges with 10% methanol. The efficiency of PAEs recovery under this condition improved compared to previous investigations and was very close to the expected concentration.

Table 4.6 Percent SS recoveries obtained from different solvent ratios used in elution.

Elution Condition (mL)				**Replicates**	**Recoveries (%)**		**Washing**
#	**H**	**M**	**AN**		**DBzP**	**BPF**	
1	6.0 mL EtOAc: MeOH (9:1)			**1**	62.9	185.7	DI
				2	56.8	171.5	
2	4.0	3.0*	5.0	**1**	120.0	110.3	DI
				2	139.6	116.1	
3	4.0	3.0*	5.0.	**1**	110.1	123.2	10% MeOH
				2	100.9	113.6	
4	4.0	3.0	5.0	**1**	65.8	168.4	DI
				2	66.4	173.6	
5	4.0	3.0	5.0	**1**	98.9	116.2	10% MeOH
				2	100.1	125.4	
				3	100.9	113.8	
				4	99.8	122.2	

*3.0 mL of EtOAc:MeOH (9:1)

In the fourth attempt (#4), we tried a mixture of 3.0 mL methanol with 4.0 mL hexane and 5.0 mL of acetonitrile in the elution instead of a combination of ethyl acetate and methanol. Although the recovery results obtained from cartridges washed using DI water were consistent, the average was 66.0% for DBzP and 171.0% for BPF. Humic and fulvic acids in the sludge may have been retained in the cartridge, since these acids have -OH groups in their structures and they may have been derivatized when combined with the derivatization agent (BSTFA:TMCS, 99:1). This could be the reason for obtaining very high BPF recovery.

To see if there would be an improvement in the recoveries, as in experiments #2 and #3, the same conditions were applied to the cartridges, which were washed with 10% methanol after sample loading (#5). The experiment (#5) was repeated four times and the DBzP recovery achieved almost 100% and BPF recovery was nearly 119.0% on average. Washing the SPE cartridges with a solvent combination having extra polar solvent (methanol) eliminated humic contaminants that may have adhered to the cartridge. It ensured that the BPF efficiency reached the expected levels. Consequently, a mixture of 4.0 mL hexane, 3.0 mL methanol and 5.0 mL acetonitrile with 10.0 mL of 10% methanol washing have been provided to recover almost all of the DBzP and BPF. Therefore, it would be the best condition for determining the PAEs (DEP, DBP and DEHP) and BPA from the disintegrated sludge samples.

4.2.3 Calibration Curves for PAEs and BPA

Procedural calibration focuses on measuring the analyte through analytical method, including all steps in the method (i.e. sample preparation, SPE). To eliminate matrix effect from the results, calibration curves were prepared by spiking stock mixture solution to sludge supernatant. The added solution had a final concentration of 25-250 μg/L in the 10.0 mL of sludge supernatant. Then, the samples were extracted through SPE applying the conditions decided in the previous section and 5-point calibration curves were prepared. Calibration curves for DEP, DBP, DEHP, BPA, DBzP and BPF can be seen in Figure 4.5.

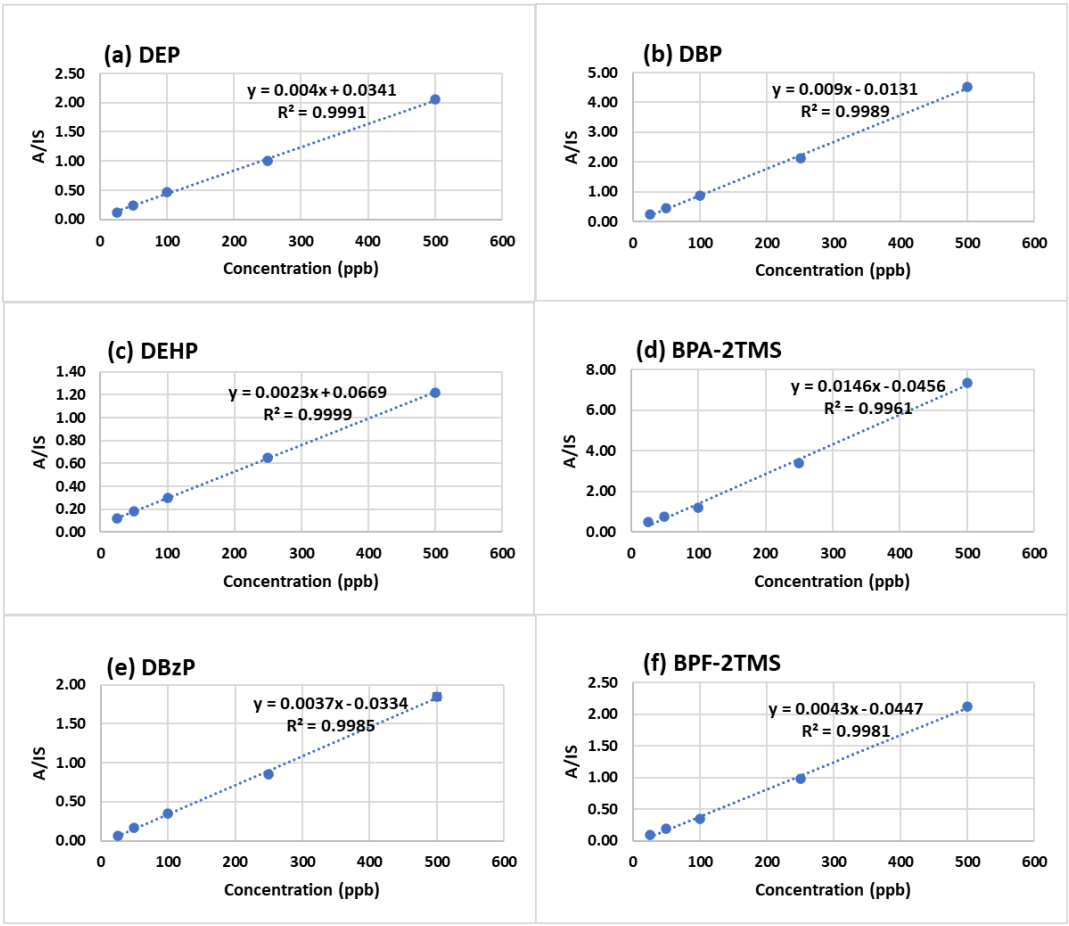


Figure 4.5 Calibration curves of (a) DEP, (b) DBP, (c) DEHP, (d) BPA and their SSs (e) DBzP and (f) BPF.

After the SPE of sludge supernatants spiked with different concentrations, all analytes and their SS showed good linearity with a minimum R^2 value of 0.9961. MDL and MQL values derived from these calibration curves are listed in Table 4. 7.

The slopes of the procedural calibration curves were found to be different from the external calibration slopes shown in Figure 4.4. The slope in the procedural calibration takes into account the combined effect of the analyte's chemical properties, sample matrix, and sample preparation. Therefore, any changes in the slope compared to that of external calibrations indicate the matrix and procedure (SPE) effect on the signals. Due to these changes between the calibration curves, a difference was also observed between IDL/QL and MDL/QL. Additional variables

such as matrix and the sample preparation procedure were added to the measurements in procedural calibrations. Therefore, it was expected that MDL/QL values would be higher than IDL/QL values.

Table 4.7 MDL and MQL values of analytes (calculated from Equation 3.2 and 3.3).

Compounds	MDL (ppb)	MQL (ppb)
DEP	3.2	9.6
DBP	4.9	14.9
DEHP	1.6	4.8
BPA	9.4	28.4

4.2.3.1 Analytes in Sludge Supernatant

Sludge supernatant samples were analyzed using the optimized elution parameters listed in Section 4.2.2.2. This was done to determine the amount of target analytes in these samples. The average concentration of each analyte, namely DEP, DBP, DEHP and BPA was below MQL in the four separate sludge supernatants taken from different days.

4.3 Impact of Sludge Disintegrations on the Concentrations of Leaching Additives

Alkali, thermal and alkali+thermal combined disintegration results were obtained on 5 days (n=5), using different SPE cartridges. Enzyme and thermal+enzyme combined disintegration results were repeated twice on two different enzyme dosages (150 mg enzyme/ g TS and 200 mg enzyme/ g TS). Each dosage was repeated twice on different days (n=2) using different SPE cartridges. Procedural calibration curves were used for the quantitative analysis of chemicals. The

efficiency of SS, i.e. surrogate recovery (SS%) was calculated based on the procedural calibrations. The results are given in the following sections.

4.3.1 Alkali Disintegration

In this section, the leaching of any additives of interest due to alkali disintegration of sludge samples having PET, PP and PC-MPs separately were examined. The tests also contained a control group without added plastic present. Figure 4.6 shows the percent recoveries of SS namely, DBzP and BPF, obtained from five replicates in the SPE after alkali disintegration. Each SS was added to each sample before SPE with a final concentration of 100 µL/g. Figure 4.6 reveals that the target chemicals could be collected with high recoveries. The reason for high standard deviations might be that a different SPE cartridge was used for each measurement. After each use, the SPE cartridge was washed through all SPE steps except sample loading and made ready to be used for another sample. Using each cartridge on different samples may have resulted in high standard deviations.

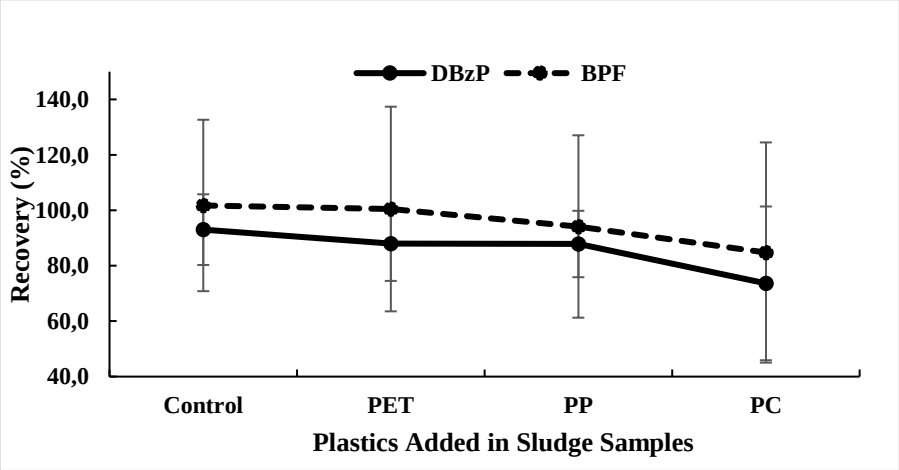


Figure 4.6 Percent recovery of SS in the samples exposed to alkali disintegration.

Table 4.8 shows the concentrations of target chemicals (ppb) leaching into the sludge supernatant after alkali disintegration was applied in the presence of additional PET, PP and PC-MPs. Each column in the table shows the average of three injections into

GC-MS and the results reported for replicates, showing negligible variability (coefficient of variance (CV) < 10%).

Concentration of additives below the MDL was reported with a "–" sign in Table 4.8. The analytes' concentrations that were detected at or above the MDL were shown with numbers and those above the MQL were shown in bold.

In control samples, DEP, DBP and BPA concentrations were detected either below MDL or between MDL and MQL values. On the other hand, in two replicates, concentrations of DEHP exceeded the MQL value and were found to be 10.7 ppb and 23.9 ppb. DEP could not be detected in two replicates of the control and plastic-containing samples. The first three replicates (#1, #2 and #3) of plastic-contained samples showed an increase in DEP concentration compared to the control sample. The largest leaching of DEP was detected in the supernatant of PET and PC-contained sludge at 13.1 ppb and 14.6 ppb, i.e. above the MQL, respectively. In the fourth (#4) and fifth (#5) replicates, although it was present in the control, DEP was not detected in the samples containing plastic.

Although DBP was not measured above the MQL value for any sample, the results found were between MDL and MQL values and were close to that of control in the first two replicates for each sample.

DEHP was measured above the MQL as 10.7 ppb and 23.9 ppb in two replicates of the control sample. All values detected in the samples containing PET were found above the MQL and between 4.8-15.1 ppb. In samples containing PP, 5.6 ppb and 12.5 ppb DEHP were found in two replicates and in the PC-contained sample, 6.3 ppb DEHP, higher than MQL, was found in one replicate. No consistent results was noticed among the replicates, similar to the findings in DEP.. For example, in the first and fifth replicates, it was observed that the DEHP concentrations in all the plastic-included samples decreased compared to the control. However, in the third replicate, DEHP concentration in the samples containing PET and PP increased.

In three replicates of samples including PET and PP, BPA concentration values were between MDL and MQL and they were very close to the one in the control sample. In the PC-contained samples, BPA was found in every replicate, and all of them were higher than the BPA concentration in the control sample.

Table 4.8 Concentration of leached additives in samples exposed to alkali disintegration.

Analyte	**Sample**	**Concentrations in Samples (ppb)**				
		#1	**#2**	**#3**	**#4**	**#5**
DEP	**C**	6.1	-	-	9.1	6.3
	PET	**13.1**	7.6	6.6	-	-
	PP	9.1	9.3	6.1	-	-
	PC	7.5	5.4	**14.6**	-	-
DBP	**C**	5.5	4.8	-	-	-
	PET	5.5	5.3	-	-	-
	PP	5.8	5.1	-	-	-
	PC	5.4	5.4	-	-	-
DEHP	**C**	**10.7**	3.9	-	-	**23.9**
	PET	**4.9**	**4.8**	**15.1**	-	-
	PP	**5.6**	3.9	**12.5**	-	-
	PC	2.9	**6.3**	-	-	-
BPA	**C**	9.6	-	-	13.6	-
	PET	10.3	-	-	-	-
	PP	9.7	-	12.9	-	-
	PC	**33.3**	24.5	10.8	19.2	17.3

-: below MDL.

Figure 4.7 shows the percentage changes in concentration of each additive from each plastic compared to its control ($C_{leached}/C_{control}$ %). For each additive, the percentage was calculated by averaging the concentrations above the MDL. The concentration of leached additives from the control sample of each plastic was normalized to 100% and the amount of additives leached was expressed as a percentage. '*' sign shows the significant changes in concentration ($p<0.05$) compared to control based on the t-test.

After single alkali disintegration, DEHP concentration decreased in PP and PC-added samples. BPA increased only in the sample containing PC-MP and the change in BPA concentration in the samples containing PET and PP-MPs was not significant ($p>0.05$).

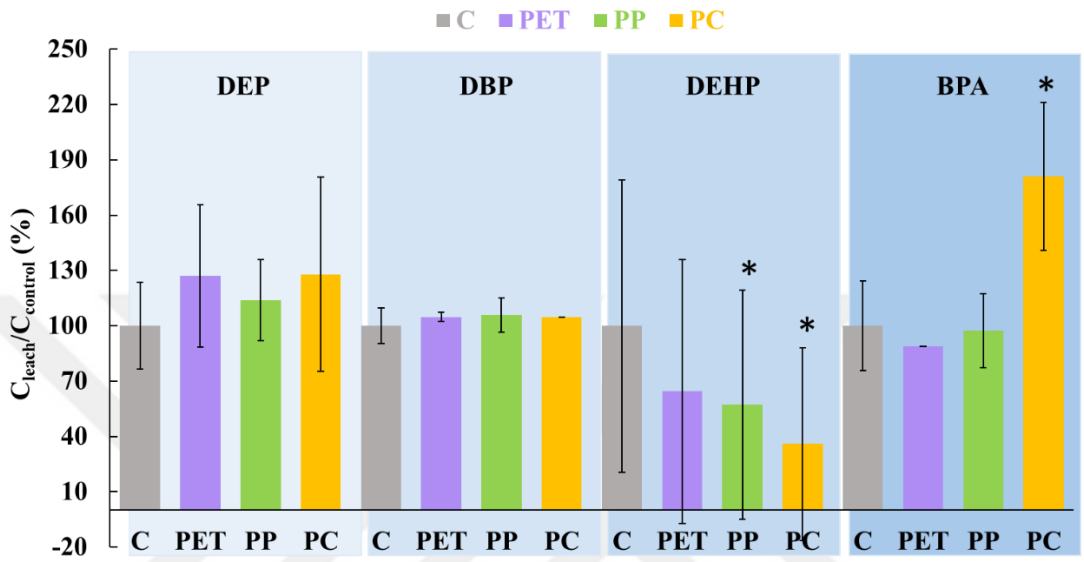


Figure 4.7 Percent change in each additive relative to their concentration in the control sample after alkali disintegration.

The physicochemical properties of additives and plastics play a role in the leaching of additives from MPs or the adsorption of chemicals into MPs. For example, the low water solubility of DEHP can result in adsorption on MPs or sludge. In addition, the surrounding environment including temperature, pH, medium of the surroundings affects the migration of additives. The presence of PC-MPs in basic medium may lead to hydrolysis of the polymer. Comparisons with literature studies and detailed explanations are given below.

DEHP was found in the samples without extra MPs added to it, but its concentration decreased upon addition of MPs to the sludge. The main reason for this decrease was related to its low solubility in water. Studies showed that DEHP is a high molecular weight phthalate (MW: 390.6 g/mol) with a log K_{ow} value of 7.0, which means it is highly hydrophobic. Even when DEHP is released due to alkali disintegration, its

physicochemical properties cause it to adsorb to the surfaces of MPs. Thus, DEHP could not pass into the liquid part of the sludge and its concentrations may be lower compared to the control sample.

BPA can leach from PC-MPs into a liquid through two distinct processes. The first is the diffusion of residual BPA, present in PC following the manufacturing process. The second is the hydrolysis of the polymer when exposed to aqueous solutions catalyzed by hydroxide.[186] Hydrolysis of PC is the predominant mechanism by which BPA is liberated from the surface of the polymer into aqueous media.[187] Therefore, the increase in the BPA concentration in the PC-MPs contained sludge sample under the alkali process can be explained by the hydrolysis that occurred, according to the reaction shown in Figure 4.8.

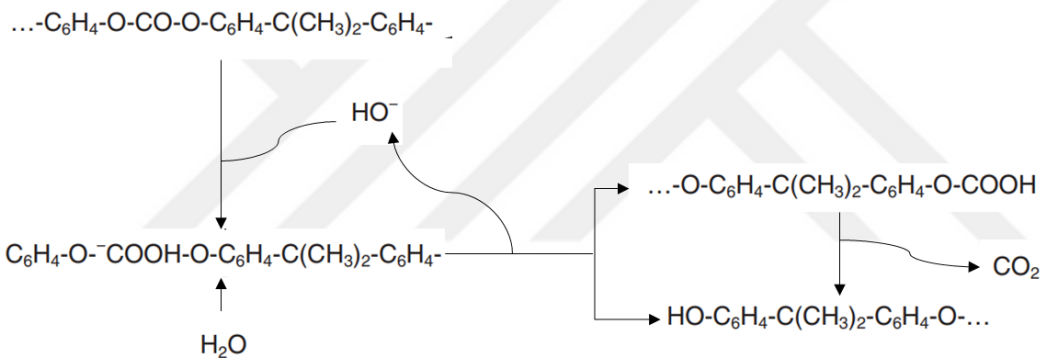


Figure 4.8 Hydrolysis reaction scheme of PC.[186]

4.3.2 Thermal Disintegration

The average SS recoveries after thermal disintegration in SPE, were shown in Figure 4.9. The recoveries were almost 100% indicating that the target analytes could be collected from SPE efficiently.

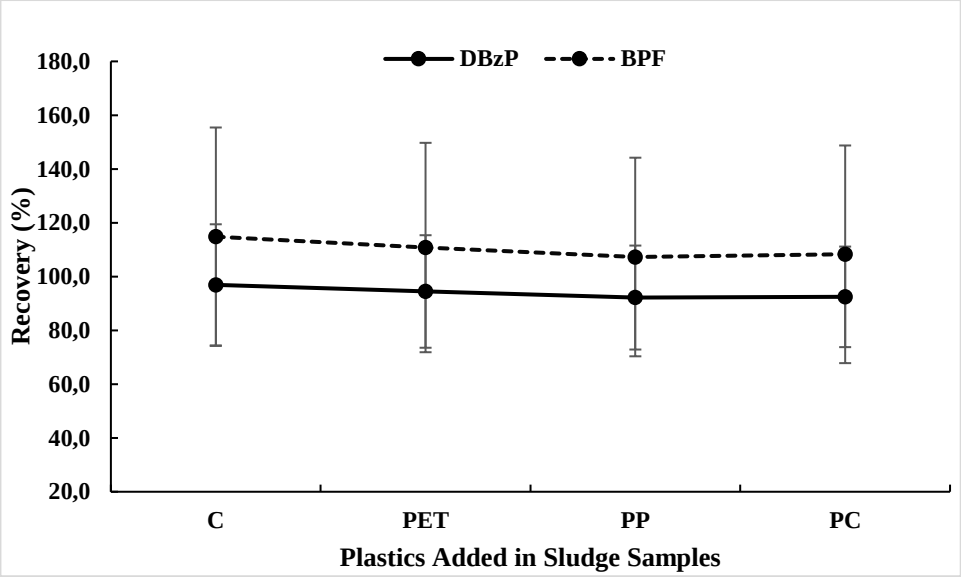


Figure 4.9 Percent recovery of SS in the samples exposed to thermal disintegration.

Table 4.9 shows the target chemical concentrations of target chemicals (ppb) detected in the supernatant of sludge with and without the addition of plastic particles. The sludge was divided into separate samples including PET, PP and PC-MPs.

In the first replicate, the concentration of DEP was below MDL for all samples. However, in the second replicate, DEP was measured between MDL and MQL in all the samples that contained extra MPs. In the third replicate, no DEP was detected in the control or PC-containing samples. In the fourth and fifth replicates of the PET-included sample, DEP concentrations were above the MQL in the control sample, but they decreased to 3.2 ppb and 4.2 ppb, respectively. Also, DEHP could not be detected in the sludges with additional PP and PC-MPs.

None of the replicates in any sample could detect DBP levels above the MQL, and the concentrations were either at the control level or between the MDL and MQL values. DEHP was found above the MQL value in most replicates of control samples and also samples containing PC-MPs. In two of the sludge samples containing PET-MPs, concentrations above MQL were detected and higher than the control sample.

In PP-MPs containing sludge, DEHP was detected at the MDL level, and they were lower than concentrations in the control sample.

BPA was detected only in the first two replicates, and the concentrations found were nearly the same as the control. All other concentrations were below the MQL value.

Table 4.9 Concentration of leached additives in samples exposed to thermal disintegration.

Compounds	**Sample**	**Concentrations in Samples (ppb)**				
		#1	**#2**	**#3**	**#4**	**#5**
DEP	**C**	-	-	-	**11.5**	**13.5**
	PET	-	8.9	**11.0**	3.2	4.2
	PP	-	5.3	9.4	-	-
	PC	-	7.1	-	-	-
DBP	**C**	5.5	6.3	-	-	-
	PET	5.0	5.8	-	-	-
	PP	5.7	5.6	-	-	-
	PC	-	5.8	-	-	-
DEHP	**C**	**5.2**	**6.0**	**8.3**	**5.9**	-
	PET	2.6	**10.0**	**13.6**	-	-
	PP	1.8	4.0	2.4	2.4	-
	PC	**6.1**	**5.3**	**17.4**	-	-
BPA	**C**	9.4	10.0	-	-	-
	PET	9.0	12.6	-	-	-
	PP	9.4	9.0	-	-	-
	PC	9.3	8.9	-	-	-

-: below MDL.

Figure 4.10 shows the percentage changes in concentration of each additive from each plastic compared to its control ($C_{leached}/C_{control}$ %) after thermal disintegration.

The average DEP concentration in the samples decreased significantly ($p<0.05$) after thermal disintegration.

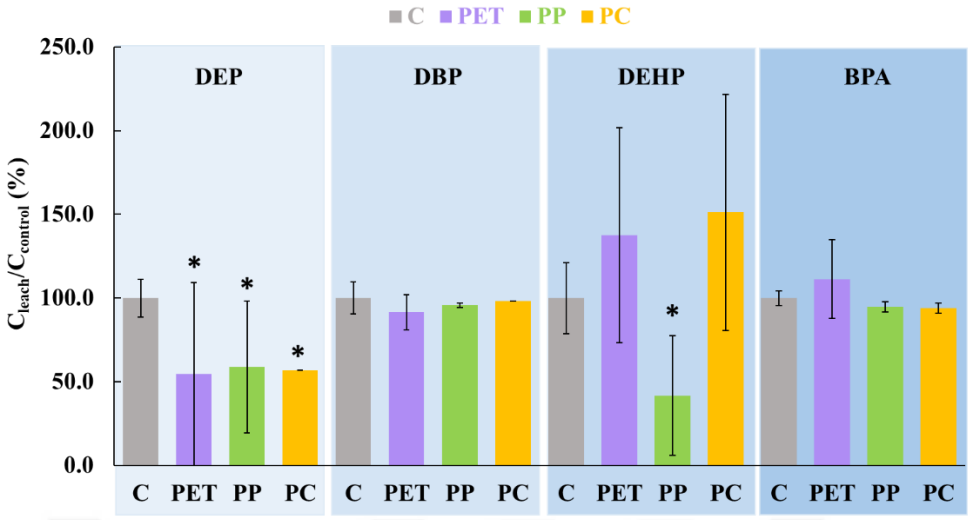


Figure 4.10 Percent change in each additive relative to their concentration in the control sample after thermal disintegration.

It is known that DEP and DBP are both low molecular weight phthalates. In a study by Dimassi and coworkers,[188] an interpretation was made for the decreasing concentration of DBP, which was the closest chemical targeted to be detected in this current study. They stated that DBP could not be detected under marine conditions (high salinity, pH, sunlight and temperature) at 20±2 °C, even though it was found in raw sea water. They explained that the reason for the decrease in concentration was due to the additive adsorption to the microplastics. This means that the dispersant may have adsorbed to the microplastics in the sludge sample when there were other microplastics present. [188]. Also, the concentration of DBP was consistently found to be very similar to the control group for all types of plastics in alkali and thermal disintegrations. This can be due to the size of the pores in the polymer being a good match for the size of DBP. When the sizes of the polymer and the additive are very close to each other, it becomes very difficult for the additive to leak from the plastic.

4.3.3 Alkali and Thermal Combined Disintegration

Figure 4.11 reveals the average percentage of SS recovery achieved in the SPE following alkali+thermal combined disintegration treatment. All the recoveries were within the recommended range of 50-150% SS recovery by EPA.

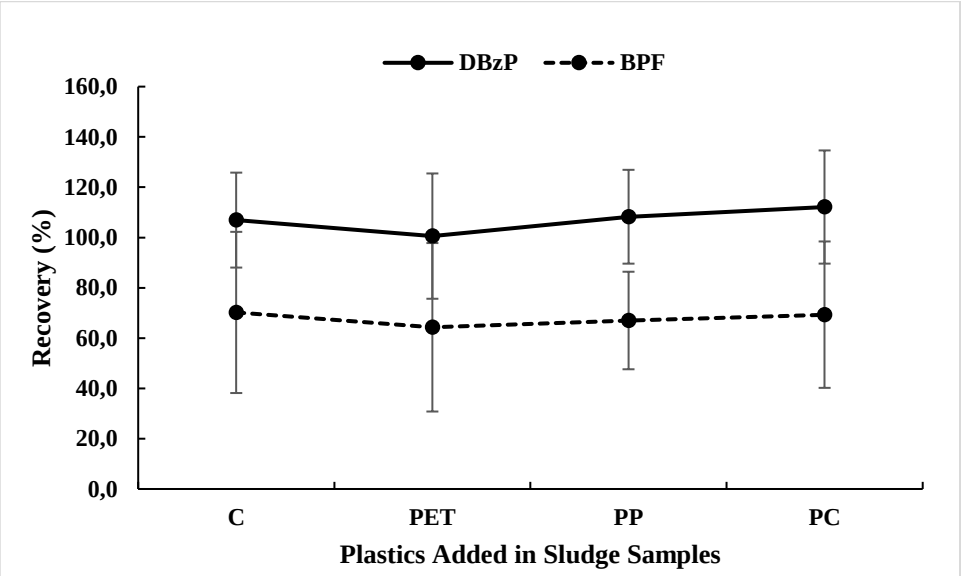


Figure 4.11 Percent recovery of SS in the samples exposed to alkali+thermal disintegration.

Table 4.10 shows the concentration of leached additives after alkali+termal combined disintegration. DEP was not detected in the control sample and PET-containing sludge samples. On the other hand, DEP was found in the two replicates of PP and PC including samples with concentrations of 4.8 ppb and 5.5 ppb for PP, 5.3 ppb and 4.2 ppb for PET containing samples. However, none of these concentrations were above the MQL.

DBP concentrations showed comparable results with alkali and thermal single disintegration processes and were measured in two replicates for each sample. The DBP concentrations in samples including MPs were almost the same as the control sample, and all results were below the MQL.

In the three replicates of the control sample, DEHP concentrations were found as 8.1 ppb, 33.7 ppb and 14.1 ppb all of which were above the MQL. Nevertheless, the concentration of DEHP decreased in the samples with extra plastic compared to the control sample. This trend was not seen in alkaline or thermal disintegrations alone. For example, the DEHP concentration measured as 33.7 ppb in the second replicate of the control sample decreased to 2.9 ppb and 10.0 ppb in the sludges containing PET and PC-MPs, respectively.

BPA was detected in the two replicates of the control sample with concentrations of 14.6 ppb and 12.7 ppb. BPA was detected in all replicates of other samples containing extra MPs. Unlike alkaline or thermal single disintegrations, BPA concentrations in the samples with PET were higher than in the control sample and the values were 18.0 ppb, 16.2 ppb, 12.3 ppb, 11.0 ppb and 11.8 ppb. In all the replicates of samples containing PC, BPA concentrations were above the MQL with an average of 4024.1 ppb. In only alkali disintegration, BPA concentrations increase relative to the control sample; nonetheless, it did not change in the thermal only disintegration. When comparing the concentrations of BPA in the samples with PC during single and combined disintegrations, it was found that the combined process resulted in more significant stress factor for the PC, leading to more leaching.

Table 4.10 Concentration of leached additives in samples exposed to alkali+thermal disintegration.

Analyte	Sample	Concentrations of Analytes in Samples (ppb)				
		#1	#2	#3	#4	#5
DEP	C	-	-	-	-	-
	PET	-	-	-	-	-
	PP	-	4.8	-	-	5.5
	PC	-	5.3	4.2	-	-
DBP	C	7.8	7.8	-	-	-
	PET	6.1	6.1	-	-	-
	PP	5.2	6.0	-	-	-
	PC	5.3	5.8	-	-	-
DEHP	C	**8.1**	**33.7**	**14.1**	-	-
	PET	**4.8**	2.9	-	-	-
	PP	**4.8**	-	2.1	-	-
	PC	-	**10.0**	**7.5**	**5.2**	-
BPA	C	14.6	12.7	-	-	-
	PET	18.0	16.2	12.3	11.0	11.8
	PP	12.8	11.0	9.9	13.5	9.4
	PC	**4188.7**	**4564.3**	**3577.7**	**4551.0**	**3259.0**

-: below MDL.

The graph in Figure 4.12 shows the percentage changes in concentration of each additive from each plastic compared to its control ($C_{leached}/C_{control}$ %) after alkali+thermal combined disintegration.

The concentration of BPA was drastically increased to ppm levels in the sludge of the PC after alkali+thermal combined disintegration.

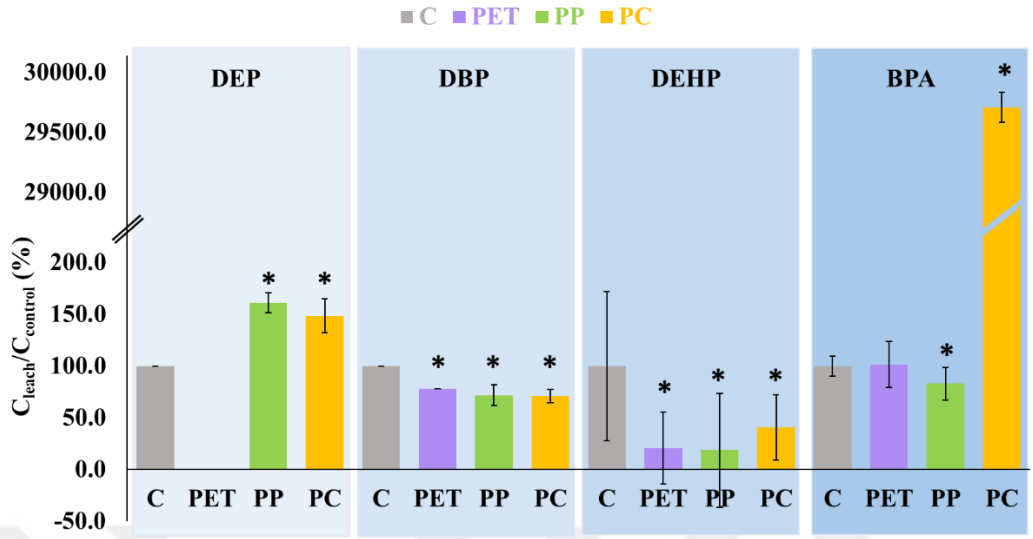


Figure 4.12 Percent change in each additive relative to their concentration in the control sample after alkali+thermal disintegration.

The size of pores in the polymer and the size of the additives are important factors for the migration of additives in plastics. Additives with low molecular weights can pass through larger pores in a polymer. Additionally, additives that fit precisely within the pores exhibit a modest but significant tendency for migration.[93] Therefore, depending on the sizes of additives and pores of polymers, leaching can be increased in alkali+thermal treatment and this could be one of the explanations for DEP leaching. Li and coworkers studied the extraction of MPs from sludge by using 1.0 M, 5.0 M and 10.0 M NaOH doses and they examined the effects of the treatment on the physicochemical properties of MPs. They observed moderately rough and broken surfaces belonging to PP and PET plastics. Also, they reported a decrease in the mass and size of PET and PP. They concluded that alkali pretreatments alter the mass and size of plastics and induce surface aging.[63] In another study, 1.0 M and 10.0 M NaOH were applied to the soil and sludge to remove complex organic substances before analyzing MPs. They observed a decrease in the mass and size of the PET and PC plastics.[64] Although the alkali doses used in these studies were much higher than those used in our study, it was stated that even after the lowest concentration alkali treatment, the properties of the plastics such as mass and size changed, and the

surfaces of the plastics were degraded. However, these studies focused only on the physical properties of plastics and did not measure additives that could leach into the environment. Therefore, the concentrations of leached additives could not be directly compared with these studies. These studies found that alkali disintegration damages the surface structure of plastic, which can cause physically bound additives to easily leak into the environment, potentially explaining the leaching of DEP from plastics after alkali treatment. Hongyan and coworkers investigated the reason of the substantial discharge of BPA due to combined disintegration.[189] It is known that BPA is more soluble in alkaline solutions than in acidic solutions.[186,190] Therefore, the increase and release of BPA from PC-MPs at high pH can be readily explained. Hydrolysis occurs more rapidly in alkaline solutions and is inhibited at or below pH 5.0. Additionally, carbonate linkages as shown in the circle in the structure of PC (Figure 4.13) are susceptible to hydrolysis at high temperatures.[187]

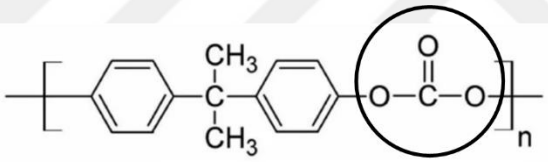


Figure 4.13 Chemical structure of PC.

Since the PC source used in this study was beads, a 3D printer was used to obtain the desired MP dimensions. Then, with the help of heat, the bead PCs were turned into fishing lines. To investigate BPA leaching, PC plastic was printed before disintegrations and the same experiment was conducted using bead and ground carboy forms of PC obtained locally. The concentration of BPA using different types of PC after alkali+thermal disintegration is listed in Table 4.11.

Table 4.11 Concentrations of BPA leaching by alkali+thermal treatment from different types and forms of PC.

Form of PC	BPA (ppb)
Fishing Line	4188.7±143.8
Bead	7606.4±479.7
Ground Carboy	2334.8±131.0

The source of the PCs in bead and fishing line forms was the same, and these PCs were produced for use in signboard and plate making. The concentration of BPA leaching from PC in bead form was higher than that from PC in fishing line form. Therefore, this may indicate that some BPA might be lost or more firmly bound to fishing lines during 3-D printing of PC beads. It was expected that less BPA would leak from the ground carboy since it is produced to come into direct contact with drinking water for human consumption. There is no regulation for BPA concentration in drinking water in Turkey. On the other hand, the highest concentration of BPA allowed in the drinking water is 100 μg/L in regulations by Japan which is one of the highest concentrations of BPA regulated. These results were enough to verify that regardless of the form of PC, the BPA concentrations leached after the alkali+thermal combined process reached levels from μg/L to mg/L. All the detected BPA concentrations from Table 4.11 were in the toxicity range of BPA (1.0 ppm to 20.0 ppm) in aquatic environments.[113]

Additionally, to determine whether this BPA leakage from PC-MPs depends on the plastic dose, the combined process was repeated with doses of 1.0 mg MPs/g TS and 3.0 mg MPs/g TS. Figure 4.14 shows the correlation between PC-MP doses and BPA concentration. The amount of leached BPA was found to increase proportionally with the added dose of PC.

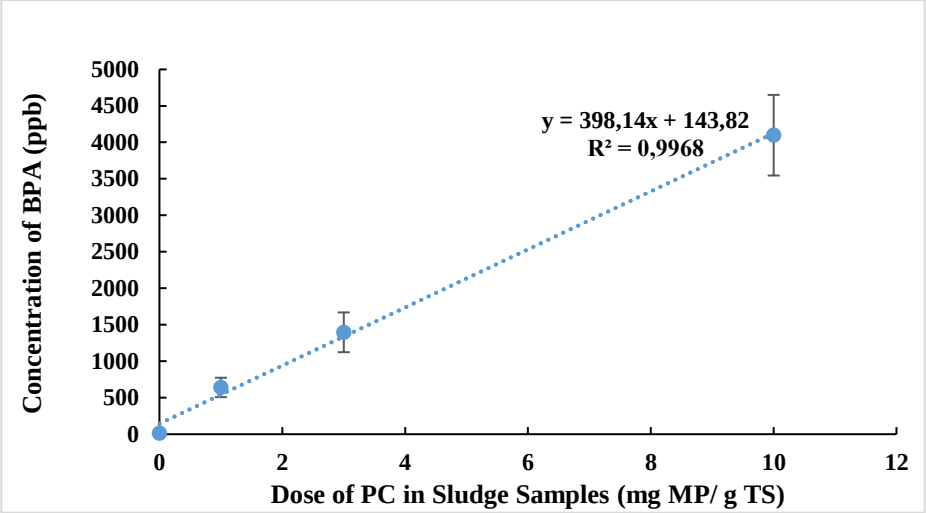


Figure 4.14 Leaching BPA concentration using sludge samples without PC-MP (0 mg/g TS) and 1.0 mg MPs/g TS, 3.0 mg MPs/g TS and 10.0 mg MPs/g TS PC-MPs doses.

After alkali+thermal combined treatment, the concentration of DBP and DEHP decreased in sludges containing PET and PP plastic, even in samples with extra plastic, as shown in Figure 4.12.It is stated in Section 2.3 that PET is sensitive to both alkaline and thermal conditions, and PP is especially sensitive to thermal conditions. On the other hand, a decrease in phthalate concentration was observed even though extra PET and PP were added to the sludge environment and exposed to alkali+thermal conditions. One of the main reasons for this is that the selected model plastics are used for different purposes and must comply with legal restrictions to ensure that any additives used do not threaten human health and that the plastic materials are resistant to environmental factors.

In this current study, PET water bottles and PP straws were used to investigate whether there was phthalate leaching in the applied disintegrations. PET and PP-MPs were added to the sludge at a higher dose (100 mg MPs/g TS), than typically detected amounts in WWTPs and used in this study (10 mg MPs/g TS). Then, the alkali+thermal combined disintegration method was applied to follow if there would

be an increase in phthalate leaching depending on the MP concentration in the sludge. The corresponding concentrations are shown in Table 4.12.

After repeating the experiment twice, it was found that DEP and DEHP were mostly below MQL in both types of plastic irrespective of the amount of plastic used. DBP was not detected in either type of plastic. On the other hand, the concentration of BPA leaching from PET increased in both replicates when compared to the 10 mg MPs/g TS dose. As a result of this experiment, it was concluded that PAEs did not leach from PET and PP plastics under the combined conditions, even when added in exaggerated amounts.

Table 4.12. Concentrations of additives leaching from plastic at a dose of 100 mg/g TS with alkali+thermal disintegration.

Additives	**Concentration of Additives (ppb)**			
	PET		**PP**	
	#1	**#2**	**#1**	**#2**
DEP	5.8	6.1	-	3.6
DBP	-	-	-	-
DEHP	**6.6**	3.8	**23.7**	2.0
BPA	**59.1**	**81.9**	**27.8**	8.2

-: below MDL.

In the rest of this section, the results of the leaking experiment conducted using enzyme and combined thermal+enzyme disintegration are presented. First, individual enzyme disintegration results are presented (thermal only disintegration results are included in section 4.3.2), and then results for the combined method are given.

4.3.4 Enzyme Disintegration

This section present results for two different pancreatin enzyme (PEN) doses, 150 mg PEN/ g TS and 200 mg PEN/ g TS. Figures 4.15 and 4.16 show the percent

recoveries of SS after SPE in enzyme disintegration with the aforementioned doses. The SS recoveries were in the acceptable range for both doses of enzymes.

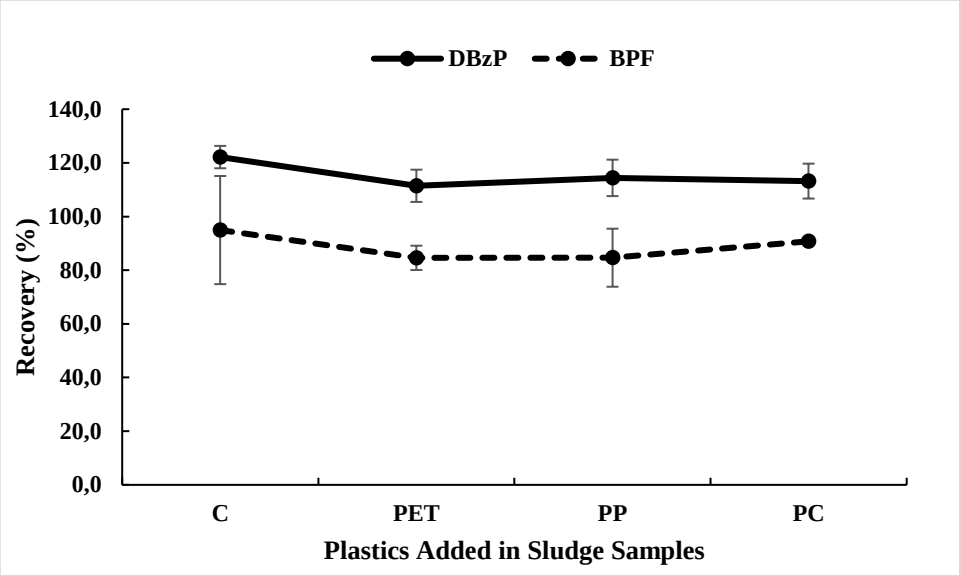


Figure 4.15 Percent recovery of SS in the samples exposed to enzyme disintegration with a dose of 150 mg PEN/g TS.

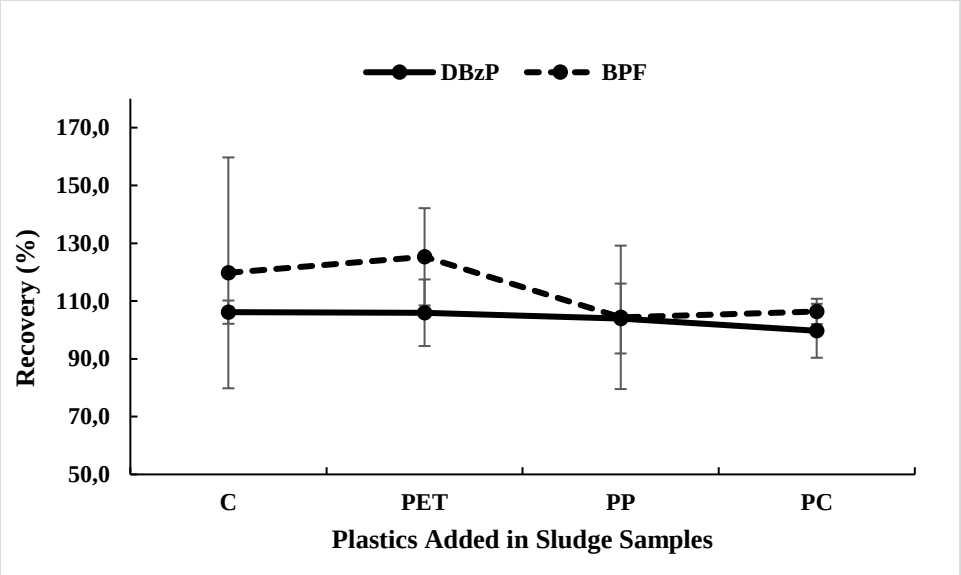


Figure 4.16 Percent recovery of SS in the samples exposed to enzyme disintegration with 200 mg PEN/g TS dose.

Table 4.13 demonstrates the target chemical concentrations (ppb) that leached into the sludge supernatant when two different enzyme doses were applied to the sludge samples with and without additional MPs. The results for replicates in the columns are the average of three injections into GC-MS and the variability between injections was acceptable as coefficient of variance (CV) was below 10%. Unlike previous disintegrations, each dose was repeated in two replicates due to the limited amount of enzyme available. The t-test was not applied to the enzyme and enzyme+thermal combined disintegration results since the concentrations in both replicates were not above MDL.

When using 150 mg PEN/g TS, no additives were detected above the MDL value in control and plastic-containing samples. However, in control samples containing 200 mg PEN/g TS, 11.3 ppb and 12.4 ppb DEP which were above MQL were found. DEP was also detected in both replicates of the sample containing PET at 6.5 ppb and 4.9 ppb. In the first replicate of PP and PC-MPs, DEP was found to be 8.5 ppb and 8.2 ppb, respectively.

DEHP was detected in both replicates of the control samples with concentrations of 1.5 ppb and 3.3 ppb which were very close to the MDL. In PET samples, the DEHP concentration was reported as 7.6 ppb and 7.1 ppb.

Table 4.13 Concentrations of additives leaching due to enzyme disintegration.

Analyte	**Sample**	**Concentrations in Samples (ppb)**			
		150 mg PEN/g TS		**200 mg PEN/g TS**	
		#1	**#2**	**#1**	**#2**
DEP	**C**	-	-	**11.3**	**12.4**
	PET	-	-	6.5	4.9
	PP	-	-	8.5	-
	PC	-	-	8.2	-
DBP	**C**	-	-	-	-
	PET	-	-	-	-
	PP	-	-	-	-
	PC	-	-	-	-
DEHP	**C**	-	-	1.5	3.3
	PET	-	-	**7.6**	**7.1**
	PP	-	-	-	1.9
	PC	-	-	-	-
BPA	**C**	-	-	-	-
	PET	-	-	-	-
	PP	-	-	-	-
	PC	-	-	-	-

Figure 4.17 shows the percentage changes in concentration of each additive from each plastic compared to its control ($C_{leached}/C_{control}$ %) after enzyme disintegration for 200 mg PEN/g TS dose.

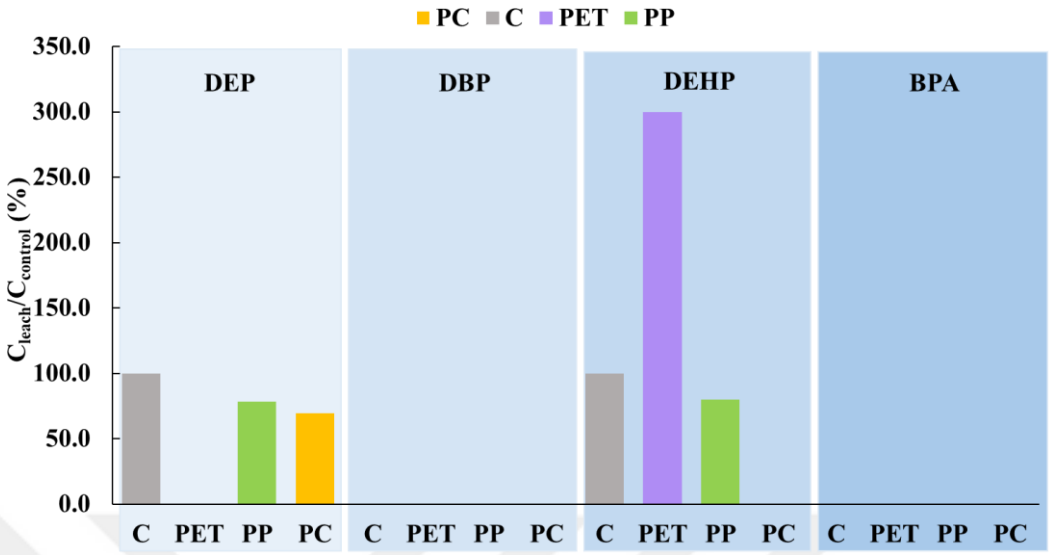


Figure 4.17 Percent change in each additive relative to their concentration in the control sample after enzyme disintegration (200 mg PEN/ g TS).

It could not be determined whether the increase in DEHP concentration was statistically significant. However, it has been stated in the literature that the ester bonds of PET can be hydrolyzed and degraded especially in the presence of lipase enzyme.[191] It is possible that the concentration of leached DEHP has increased as a result of interaction between the enzyme and PET. Another study has demonstrated that the lipase enzyme breaks down phthalates (DEP, DBP, and DEHP) and that the extent of this degradation is dependent on the water solubility of the additives. However, it has been stated that DEHP is difficult to degrade since it has very low solubility in water.[192] In other words, although DEHP leaked after the effect of the enzyme on the plastic, due to the physicochemical properties of DEHP, the enzyme could not degrade DEHP and remained in the water.

BPA and DBP were not detected in any of the control and MP-contained sludge samples for both enzyme doses after enzyme treatment.

4.3.5 Thermal and Enzyme Combined Disintegration

Figures 4.18 and 4.19 show the surrogate recovery efficiencies obtained from the SPE stage after thermal+enzyme combined disintegration using 150 mg PEN/g TS and 200 mg PEN/g TS, respectively.

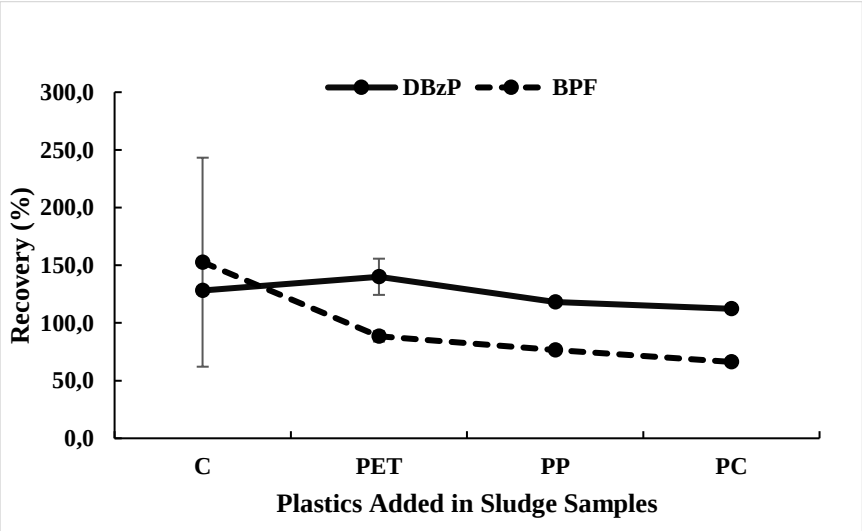


Figure 4.18 Percent SS recoveries from thermal+enzyme disintegration (150 mg PEN/ g TS).

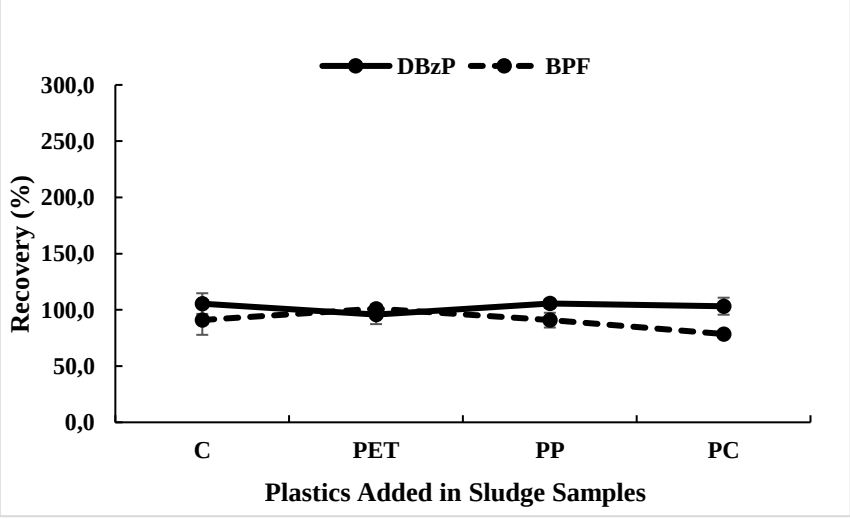


Figure 4.19 Percent SS recoveries from thermal+enzyme disintegration (200 mg PEN/ g TS).

After combining thermal and enzyme disintegration, it was observed that the majority of the concentrations of all chemicals were lower than MDL from Table 4.14. For the 150 mg PEN/g TS dose, DEHP concentrations increased compared to the control in both replicates of the sludge sample containing PP-MPs. The DEHP concentration was measured as 4.4 ppb and 4.9 ppb for these replicates. In the first replicate of samples containing 200 mg PEN/g TS dose, 6.3 ppb DEP was found in the control while the concentration of DEP was found as 7.5 ppb in PET, 4.9 ppb in PP and 7.4 ppb in PC-contained sludge samples. However, in the second replicate, no DEP additive was detected. Also, no DEHP was detected in any of the control samples for the 200 mg enzyme/g TS dose. Nevertheless, 2.0 ppb and 3.3 ppb DEHP were detected in the samples with PET and PP-MPs, respectively, in one of the replicates. Similar to single enzyme disintegration, BPA and DBP were not detected in any MP-contained sludge samples. In the combination of thermal and enzyme, almost none of the leached chemicals exceed the MQL.

Table 4.14. Concentrations of additives leaching due to thermal+enzyme disintegration.

Analyte	Sample	Concentrations in Samples (ppb)			
		150 mg PEN/g TS		200 mg PEN/g TS	
		#1	#2	#1	#2
DEP	C	-	-	6.3	-
	PET	-	9.2	7.5	-
	PP	-	-	4.9	-
	PC	-	-	7.4	-
DBP	C	-	-	-	-
	PET	-	-	-	-
	PP	-	-	-	-
	PC	-	-	-	-
DEHP	C	-	-	-	-
	PET	-	-	-	2.0
	PP	4.4	**4.9**	-	3.2
	PC	-	-	-	-
BPA	C	-	-	-	-
	PET	-	-	-	-
	PP	-	-	-	-
	PC	-	-	-	-

-: below MDL.

The amounts of DEP, DBP, DEHP and BPA found in sludge treated with thermal, enzyme and their combinations are shown in Figure 4.20. Green, orange and purple colors represent thermal, enzyme and thermal+enzyme combined processes. Figure 4.20 (a) depicts the disintegration results with a dose of 150 mg PEN/g TS. The concentrations detected in the thermal disintegration fell below the MDL when subjected to only enzyme disintegration (150 mg PEN/ g TS dose). Additionally, most of the chemicals after the thermal+enzyme combination were not detected. In Figure 4.20 (b), the concentrations of analytes resulting from disintegrations with 200 mg PEN/ g TS are compared. It has been concluded that, mainly due to the addition of enzymes to the medium after thermal treatment, the amounts of chemicals that leached out is less than those that leaked in thermal disintegration or that cannot be detected. The decrease in the additive concentration in the combined process was

explained in a study as follows. A study has shown that DEP, DBP and DEHP known as PAEs are not degraded during thermal disintegration of sludge at 70°C.[18] However, it has been stated that adding esterase or lipase enzymes to the sludge after thermal treatment can help degrade PAEs present in the sludge. Furthermore, the solubility of these phthalates in water plays a crucial role in their degradation, the more soluble they are the easier they break down.[192] It was stated that among the phthalates examined, DEHP was found to be the most difficult to degrade in both thermal and combined thermal and enzyme disintegration due to its low solubility in water.[192] Overall, our findings are consistent with previous studies that have found phthalates are often not detected after combined thermal+enzyme disintegration.

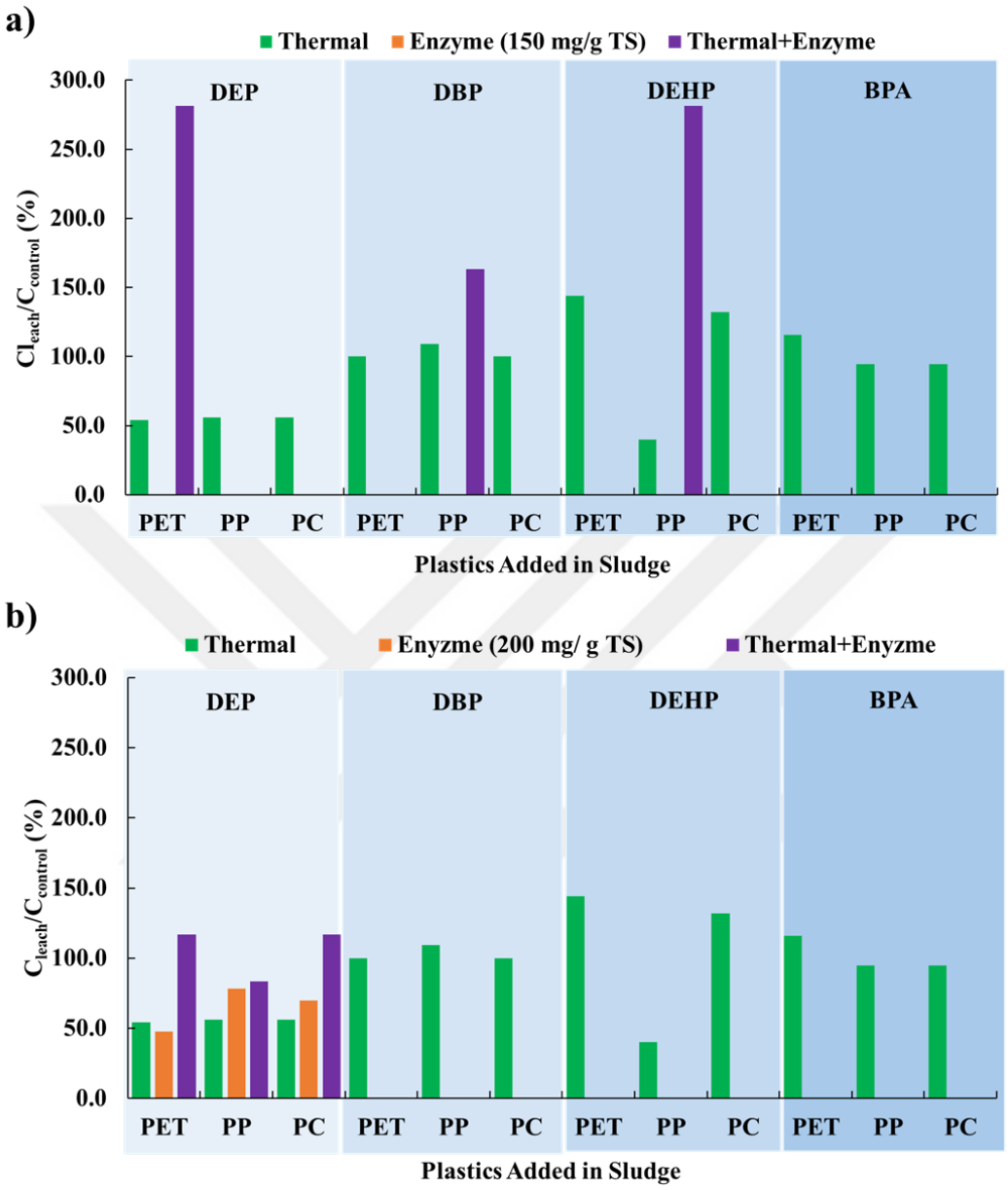


Figure 4.20 Leaching concentration of analytes from disintegration with enzyme dose 150 mg PEN/g TS (a) and enzyme dose 200 mg/g TS (b).

4.4 Characterization of MPs Recovered from Disintegrated Sludge

In this section, we analyzed three plastics that were recovered from sludge samples subjected to combined disintegrations namely, alkali+thermal and thermal+enzyme.

The first step of the experiment involved evaluating the FTIR spectra of untreated MPs collected from sludge after undergoing various disintegrations. Based on this evaluation, carbonyl index values were assigned. The second step was to examine the changes in crystallinity values using information obtained from the DSC graphs. Finally, the surface morphologies of the plastics were analyzed by SEM.

4.4.1 Analysis of PET-MPs from Combined Disintegrations

4.4.1.1 Carbonyl Index Analysis of PET-MPs from Samples

FTIR spectra of untreated, alkali+thermal and thermal+enzyme treated PET are shown in Figure 4.21. Each measurement was repeated five times for all MPs (PET, PP and PC), and the spectra shown in Figure 4.21 are the averages of five spectra.

Among the absorption bands of PET presented in these spectra, the ones specific to PET at 1020 to 1120 cm^{-1} are assigned to C-O stretching and methylene group vibrations, 1240 cm^{-1} to ester group stretching, 1712 cm^{-1} to ester carbonyl bond stretching and they were assigned on the Figure 4.21.[193,194]

When spectra of the disintegrated PET were compared with those of non-disintegrated, there was no formation of a new bond or disappearance of the existing bond in the PET after disintegrations. The outcome of this study is consistent with the study conducted by Li and his colleagues. They found that even though they used a much higher dosage of alkali than what was utilized in this study, there was no noticeable alteration in the surface functional groups of the PET material when compared to non-alkali-exposed PET.[63] Since the alkali dose used in our study was much lower, it may not have triggered any changes in the surface groups.

Also, it is known that the lipase enzyme, one of the components of the pancreatin enzyme used in this study, can degrade PET. However, for this enzyme to degrade PET, the chain segments in the PET structure must first become mobile. This situation is directly related to the melting temperature of the plastic.[191] PET, which

has a melting temperature of approximately 250°C, may not gain enough mobility during the thermal treatment applied at 127°C in this study, and the enzyme could not cause any change in the structure of PET. Therefore, no effect such as the formation of a new bond or the disappearance of an existing bond was observed in FTIR analysis after thermal+enzyme disintegration.

It was observed that all absorbance values in the PET (control) spectrum kept in undisintegrated sludge were higher than those with disintegration. However, this decrease did not affect CI calculations since the ratio of peak intensities was used.

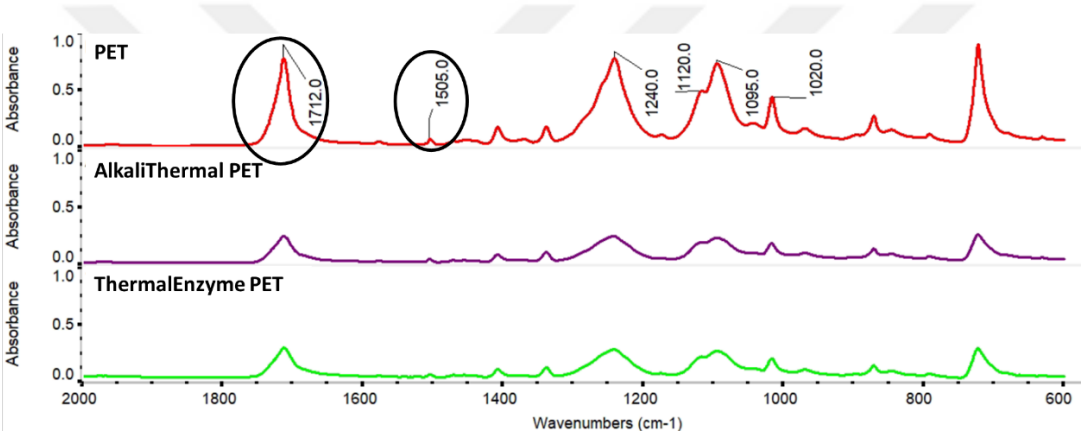


Figure 4.21 FTIR spectra of PET-MPs.

Change in carbonyl index of PET-MPs, determined by diving the absorbance of the carbonyl peak (1715 cm^{-1}, C=O stretching) by that of a reference peak (1505 cm^{-1}, ring CH in-plane bending) is shown in Figure 4.22.

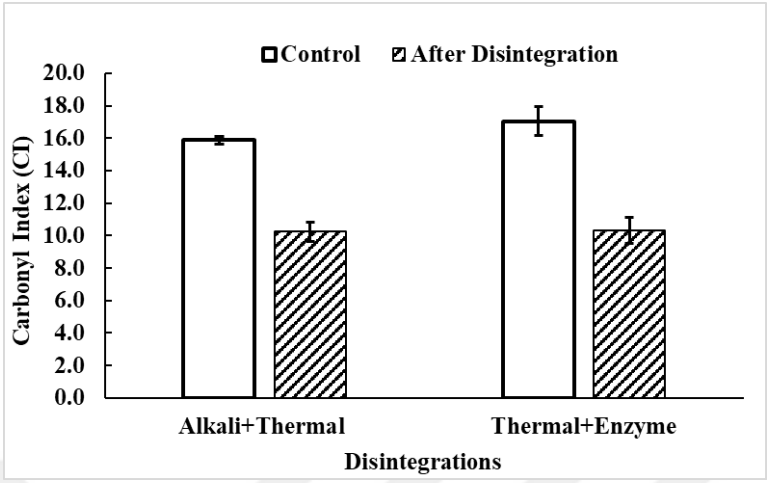


Figure 4.22. CI values of PET-MPs.

As seen in Figure 4.22, the carbonyl index value of PET decreased compared to non-disintegrated PET, as a result of combined disintegrations. The decrease observed after alkali + thermal treatment was also seen in another study conducted by our group using the same alkali dose and temperature. The reduction in the absorbance of the carbonyl stretching band may be attributed to the degradation of the ester carbonyl bond in PET by implementing alkali processes[21,178] according to the hydrolysis reaction[195] shown in Figure 4.23.

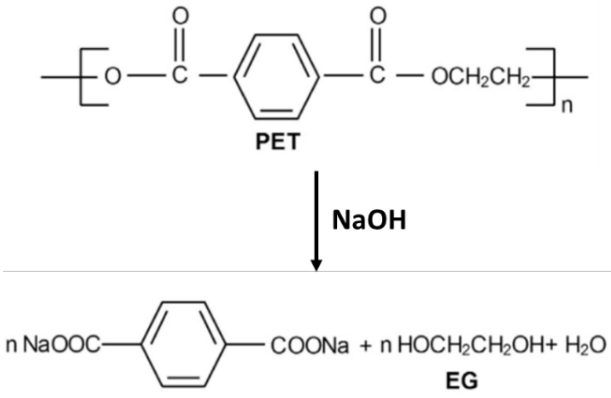


Figure 4.23 Hydrolysis of PET under basic conditions (EG: ethylene glycol).

4.4.1.2 Crystallinity Analysis of PET-MPs

The differential thermograms of PET after alkali+thermal and thermal+enzyme treatment are shown in Figures 4.24 (a) and 4.24 (b). The crystallinity percentages which were obtained by dividing the enthalpy value obtained from the melting curve in the first heating cycle to 135.8 J/g, which is the melting enthalpy for PET, are shown in Figure 4.25. The first heating cycle indicates the thermal history of the sample which helps to draw conclusions about the processing of the polymer. Therefore, it was used for crystallinity calculations. The second heating curve depicts the properties of the pure material, unaffected by processing once the cooling phase is complete. Although there is a cooling and second heating cycle in the thermograms, the enthalpy values obtained from them were not used in this study.

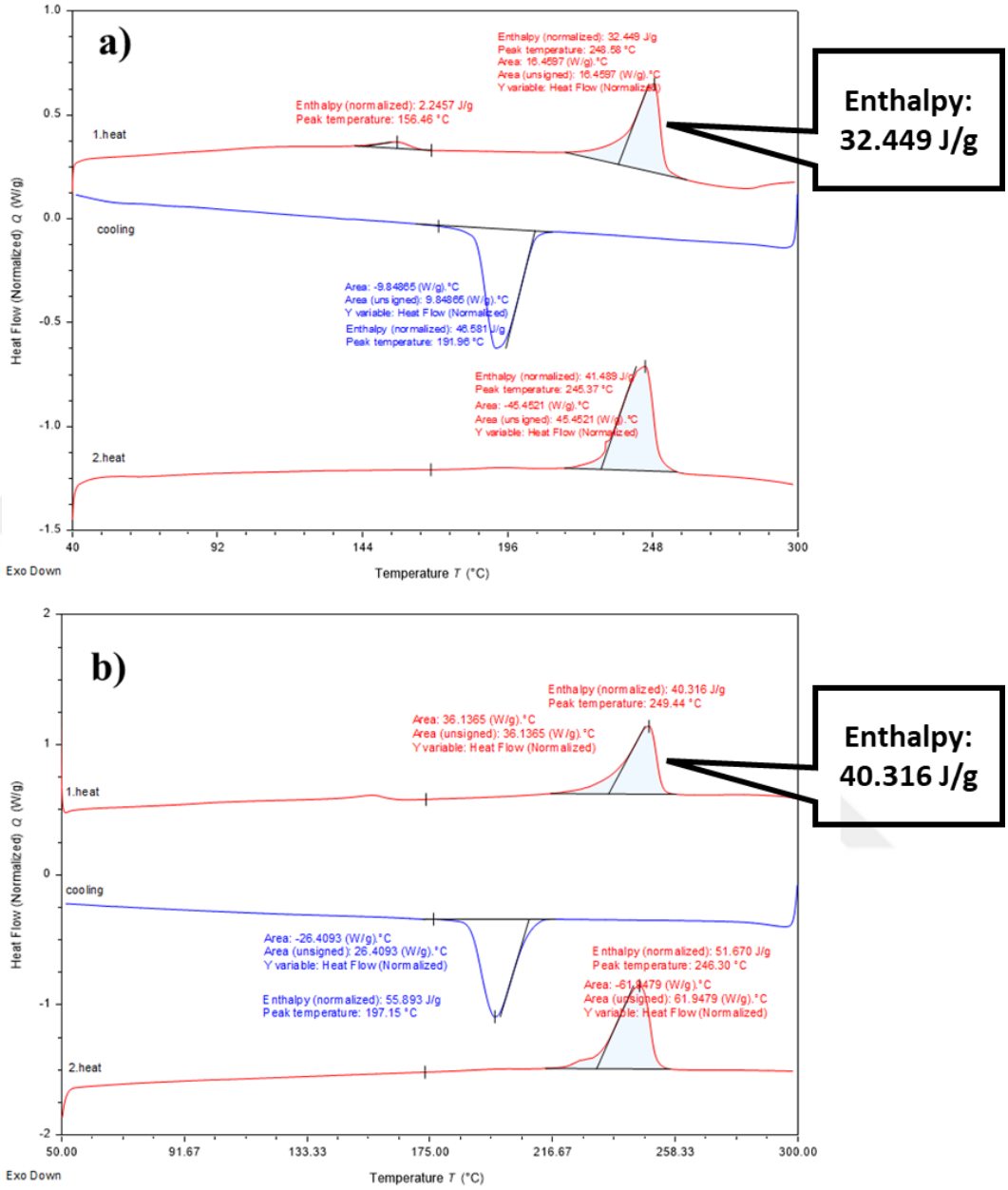


Figure 4.24. DSC thermograms of PET-MPs obtained from alkali+thermal (a) and thermal+enzyme (b) disintegrations (Applied heating rate: 10°C/min and temperature range: 50-300°C).

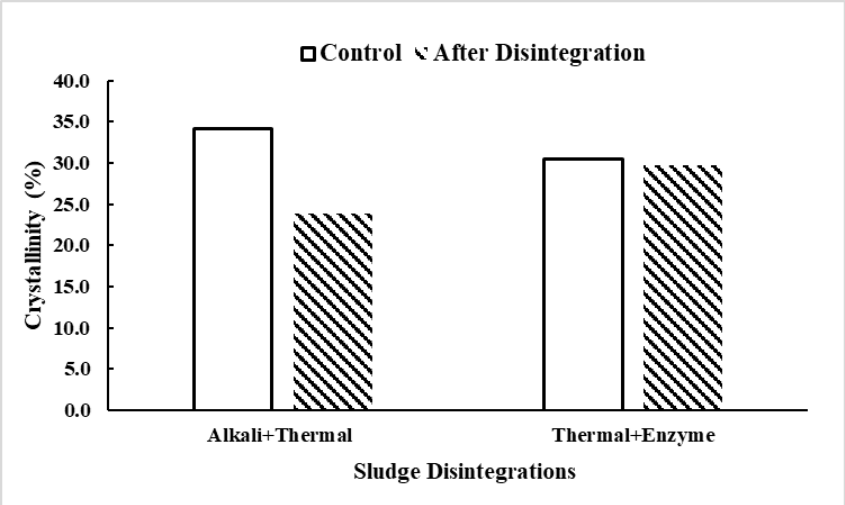


Figure 4.25 Crystallinity (%) of PET obtained from alkali+thermal and thermal+enzyme disintegrations DSC curves.

DSC analysis was performed for all types of MPs. In Figure 4.25, the crystallinities of PET in non-disintegrated sludge and PET in treated sludge were compared. The comparison showed that the percentage of crystallinity decreased from 34% to 24% after combined alkali + thermal treatment, but there was a negligible decrease after combined thermal + enzyme disintegration compared to the control.

The decrease in the PET's crystallinity may result from the aging of PET due to alkali and thermal processes as found in CI results. Similar results and conclusions exist some studies in the literature.[196,197] Another reason might be that the presence of foreign bisphenols in PET decreased in the degree of crystallinity. This can be attributed to the formation of intermolecular hydrogen bonds and a reduction in structural regularity. With the increase in bisphenols, the rate of crystallization and the degree of crystallinity of PET were further decreased.[198]

4.4.1.3 Surface Morphology of PET-MPs

Changes in the morphology of PET-MPs after combined disintegrations were analyzed using SEM. Figure 4.26 compares the untreated PET-MPs and those recovered from alkali+thermal disintegrated sludge. The SEM images showed that the PET-MPs after the alkali+thermal combined disintegration process experienced openings at the edges.

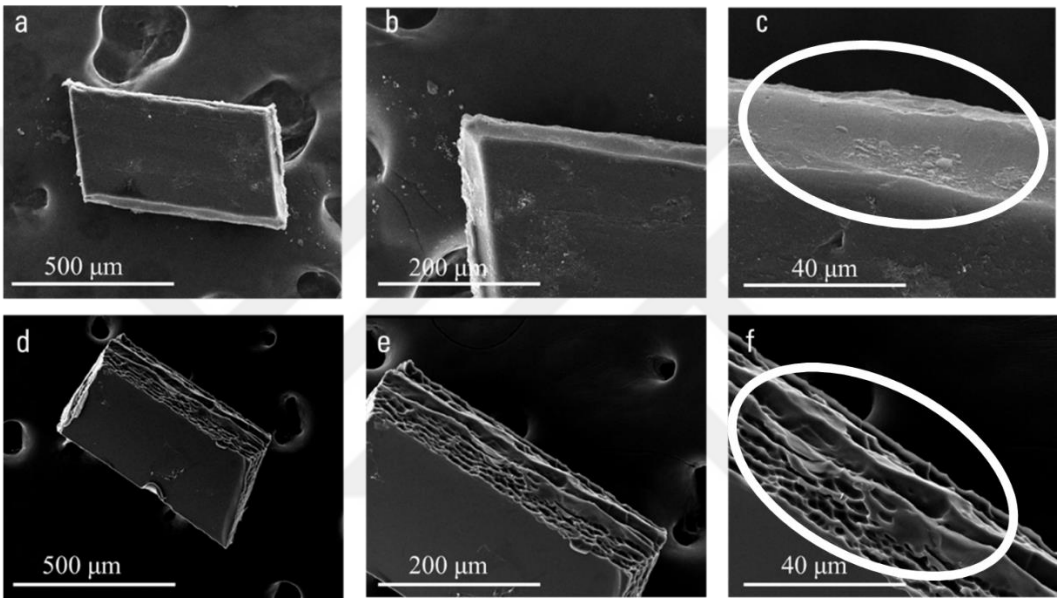


Figure 4.26. SEM images of untreated PET-MPs at x250 (a), x500 (b) and x2500 (c); alkali+thermal treated PET-MPs at x250 (d), x500 (e) and x2500 (f). The circles on the (c) and (f) show the openings on the edge of the PET.

Figure 4.27 shows untreated and thermal+enzyme treated PET-MPs. Similar to alkali+thermal treatment results, openings and wear were noticed on the edges of the PET-MPs.

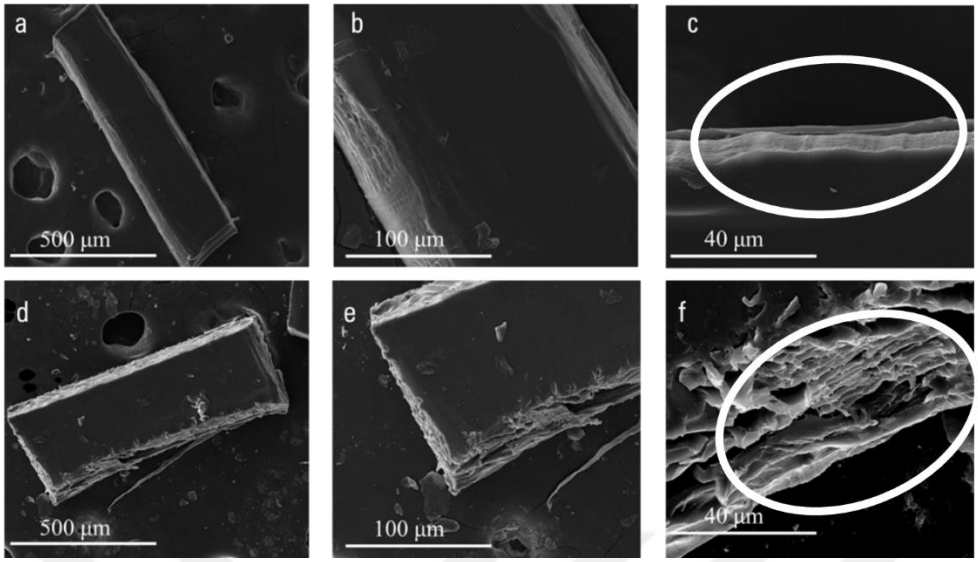


Figure 4.27. SEM images of untreated PET-MPs at x250 (a), x1000 (b) and x2500 (c); thermal+enzyme treated PET-MPs at x250 (d), x1000 (e) and x2500 (f). The circles on the (c) and (f) show the openings on the edge of the PET.

4.4.2 Analysis of PP-MPs from Combined Disintegrations

4.4.2.1 Carbonyl Index Analysis of PP-MPs from Samples

FTIR spectra of PP-MPs are shown in Figure 4.28. The order of untreated, alkali+thermal and thermal+enzyme treated PP from top to bottom is displayed. The results showed that no new peaks were formed or disappeared after combined treatments, suggesting that the applied disintegrations did not trigger any changes in the surface groups.

Among the absorption bands of PP presented in these spectra, the ones specific to PP at 1456 cm^{-1} and 1377 cm^{-1} are assigned to symmetrical bending of CH_3 and 1166 cm^{-1} to wagging of C-H and they are shown with the circles in Figure 4.28.

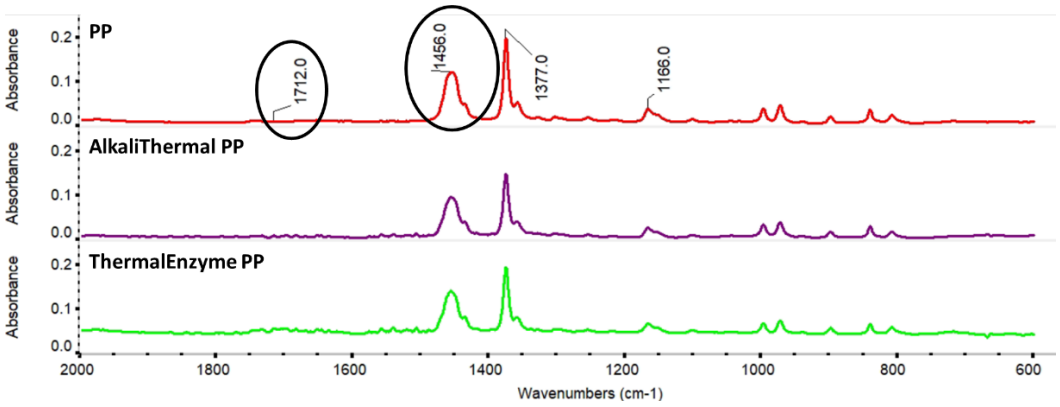


Figure 4.28 FTIR spectra of PP-MPs.

Carbonyl index values were found to be zero since there was no change in the carbonyl region. In studies examining PP affected by environmental factors such as photo-oxidation or thermal oxidation, CI values ranged from 0.18[199], 0.20-0.28[200] to 0.74.[201] For PP to undergo oxidation, free radicals must first be formed and this generally occurs in the presence of high temperatures or UV radiation. As the samples in this study were not exposed to UV radiation and the temperature did not exceed the melting point of PP (Tm of PP: 170 °C), it was expected that there would be no increase in CI.

4.4.2.2 Crystallinity Analysis of PP-MPs

The differential thermograms of PP after alkali+thermal and thermal+enzyme treatment are shown in Figures 4.29 (a) and 4.29 (b). Figure 4.30 depicts crystallinity percentages obtained from the melting curve' enthalpy value during the first heating cycle divided to 207.0 J/g (theoretical melting enthalpy for PP).

Figure 4.30 compares the crystallinity of PP in non-disintegrated sludge and treated sludge. The results showed that the crystallinity increased by 7% and 4% with respect to the control group after alkali+thermal and thermal+enzyme treatment. When the level of crystallinity increases, it indicates that the material is degrading due to chain scission.[202] Chain scission leads to increased mobility of polymer chains, allowing for rearrangement and ultimately enhancing polymer crystallinity.[203] The increase in

crystallinity of PP-MPs may result from chain scission during the combined disintegrations.

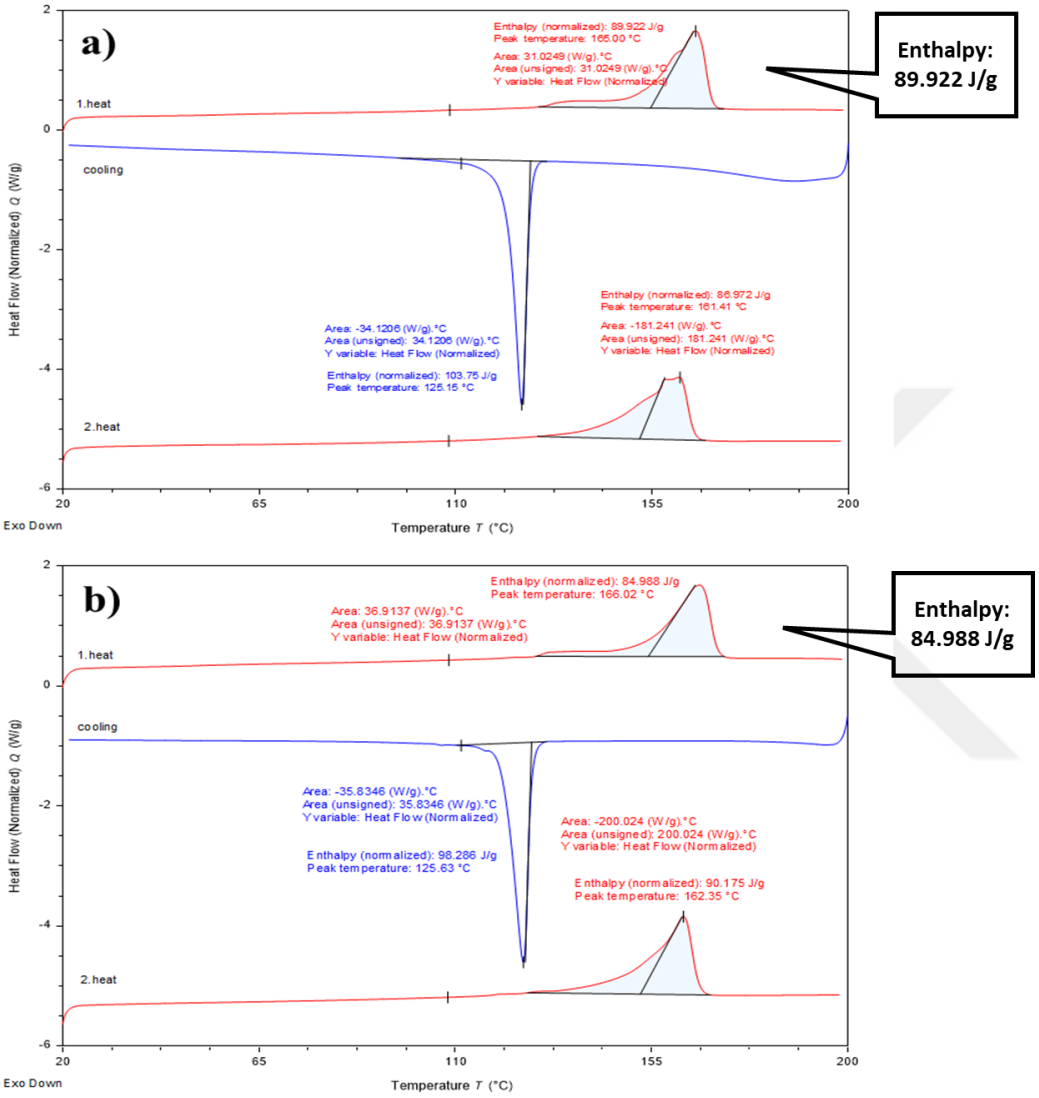


Figure 4.29. DSC thermograms of PP-MPs obtained from alkali+thermal (a) and thermal+enzyme (b) disintegrations. (Applied heating rate: 10°C/min and temperature range: 20-200°C).

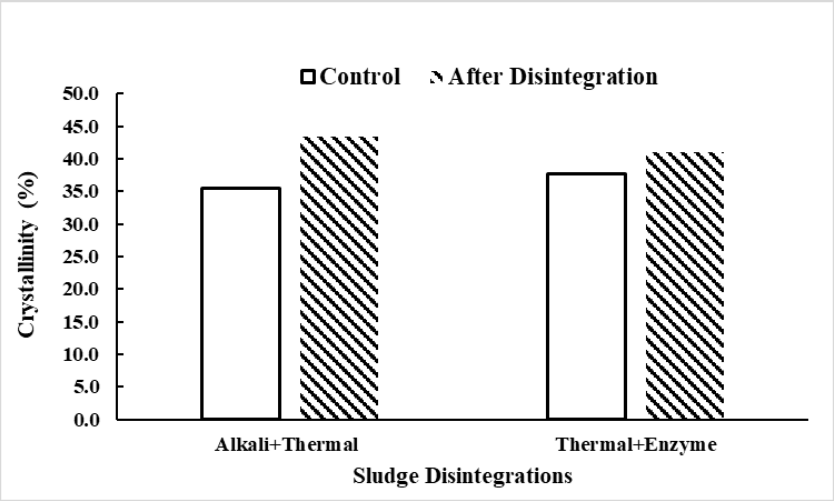


Figure 4.30 Crystallinity (Xc %) of PP obtained from DSC curves.

4.4.2.3 Surface Morphology of PP-MPs

Figures 4.31 and 4.32 depict how the surface of PP-MPs changed after applying different types of combined disintegrations. Figure 4.31 shows SEM images of the PP-MPs recovered from untreated and alkali+thermal disintegrated sludge. Surface peeling and deformation on the edges of the PP-MPs were observed. However, Figure 4.32 reveals that no surface or edge deformation in PP-MPs underwent enzyme+thermal disintegration.

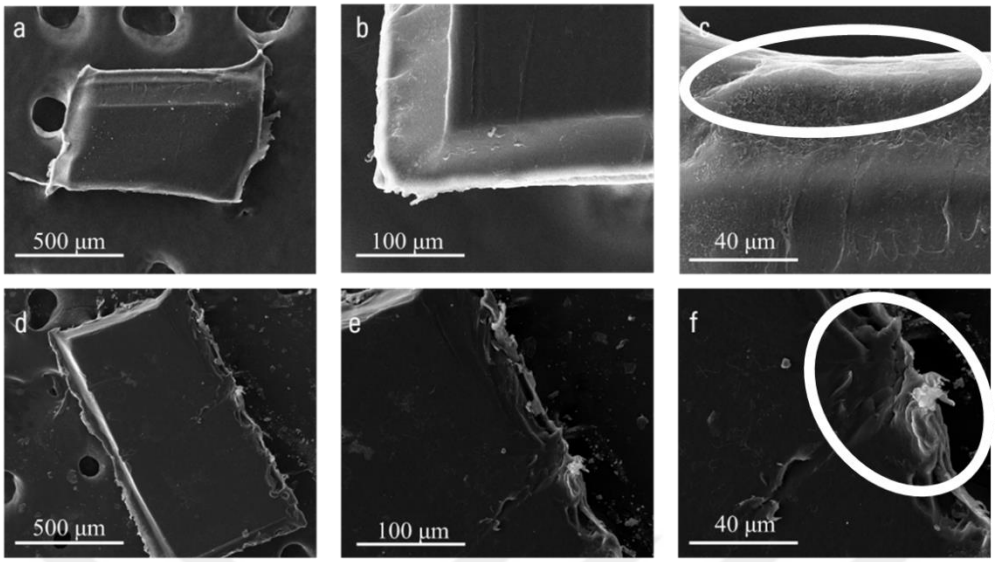


Figure 4.31. SEM images of untreated PP-MPs at x250 (a), x1000 (b) and x2500 (c); alkali+thermal treated PP-MPs at x250 (d), x500 (e) and x2500 (f). The circles on the (c) and (f) show the openings on the edge of PP.

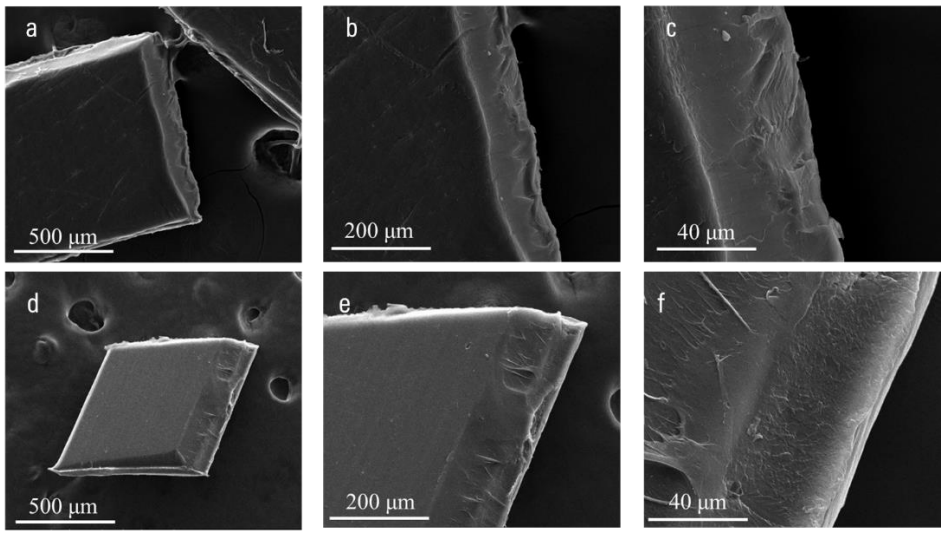


Figure 4.32. SEM images of untreated PP-MPs at x250 (a), x500 (b) and x2500 (c); thermal+enzyme treated PP-MPs at x250 (d), x1000 (e) and x2500 (f).

4.4.3 Analysis of PC-MPs from Combined Disintegrations

4.4.3.1 Carbonyl Index Analysis of PC-MPs from Samples

FTIR spectra of PC-MPs are shown in Figure 4.33, listed from top to bottom as untreated, alkali+thermal and thermal+enzyme treated. The principal peaks in the PC are caused by the following vibrations: C–H aromatic ring deformations around 3000 cm^{-1}; C=O carbonate group deformations near 1770 cm^{-1}; C=C vibrations at 1506 cm^{-1}; asymmetric O–C–O carbonate group deformations in the range 1232–1164 cm^{-1} and symmetric O–C–O carbonate group deformations near 1015 cm^{-1}.[204]

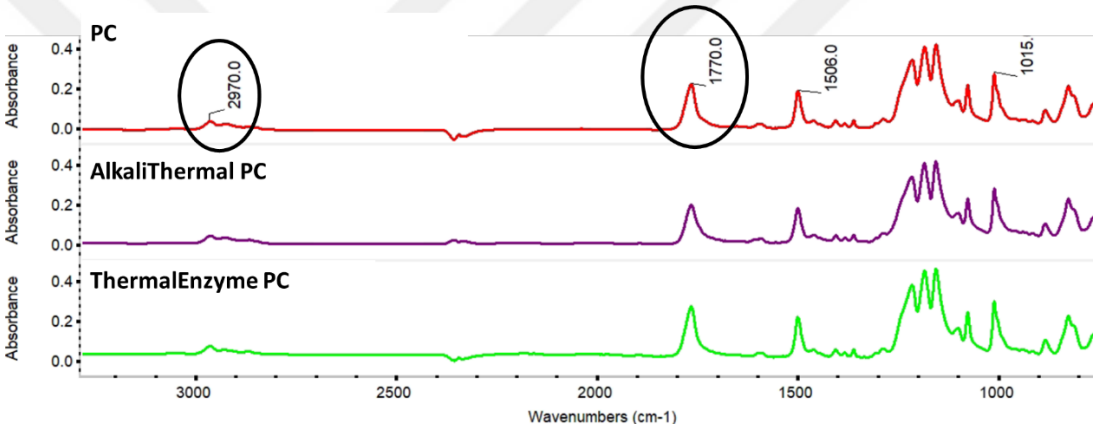


Figure 4.33 FTIR spectra of PC-MPs.

Carbonyl index values were found by taking the ratio of the values signed in the spectra (2970 cm^{-1} and 1770 cm^{-1}). Changes in these values were compared to the controls are shown in Figure 4.34.

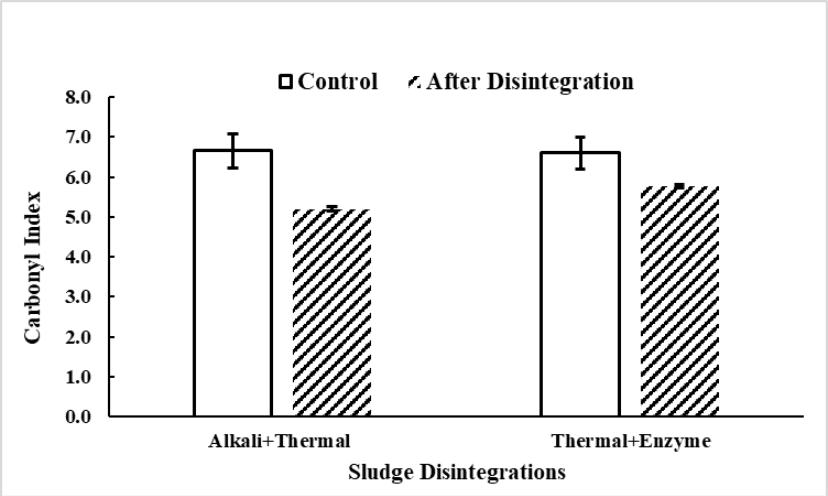


Figure 4.34 CI values of PC-MPs.

After both alkali+thermal and thermal+enzyme disintegrations, the CI values of PC decreased. As explained in previous sections, the combination of alkali+thermal disintegration causes hydrolysis of PC, resulting in the release of very high amounts of BPA. This decrease in CI values can also be explained by hydrolysis. When the polymer hydrolyzes, carbonyl groups generally turn into alcohol groups as shown in Figure 4.35, therefore, carbonyl index decreases.

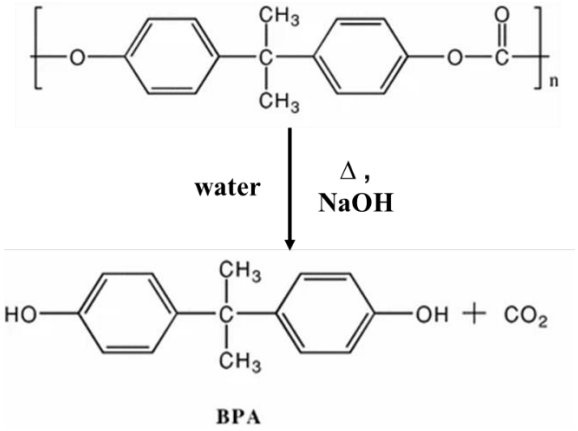


Figure 4.35 Hydrolysis of PC under alkali+thermal conditions.

Applying thermal+enzyme disintegration may lead to biodegradation of PC. As earlier research revealed, carbonyl groups are selectively degraded by

microorganisms which could result in a noticeable reduction in CI, as noted by Campanaro and coworkers.[205]

4.4.3.2 Crystallinity Analysis of PC-MPs

Figure 4.36 shows an example DSC thermogram of PC-MPs. It should be noted that there is no melting peak in the thermogram. Since PC is a completely amorphous polymer, the increase in its crystallinity depends on high temperatures and pressure. However, in the applied disintegrations, the temperature is 127°C, far below the melting point of PC, which is 280°C. Therefore, no melting peak was observed in PC.

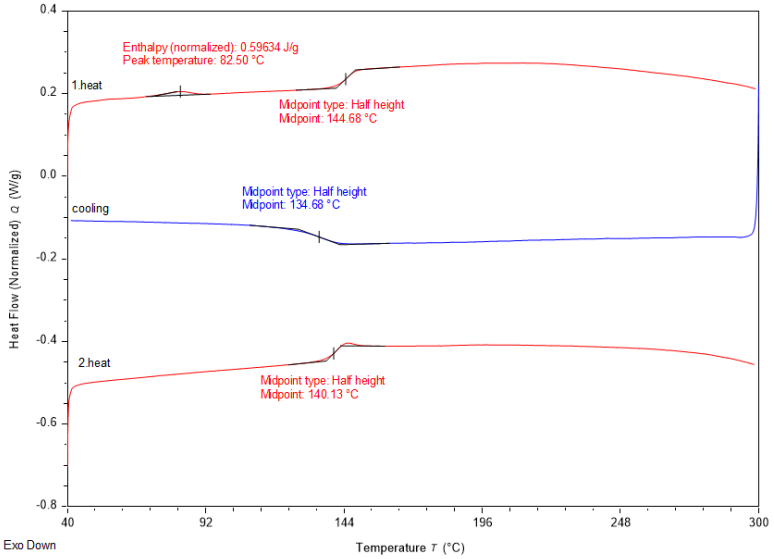


Figure 4.36 An example DSC curve for PC-MP (Applied heating rate: 10°C/min and temperature range: 50-300°C).

4.4.3.3 Surface Morphology of PC-MPs

Changes in the morphology of PC-MPs after combined disintegrations were shown in Figures 4.37 and 4.38. The surfaces of control PC-MPs were smooth, on the other hand, after alkali + thermal treatment, the surfaces gained a porous appearance and became rougher. This is due to the hydrolysis of PC during alkali and thermal treatment, which can create pits and cracks on the surface. However, there was no visible change on the PC surfaces that were exposed to thermal+enzyme disintegration.

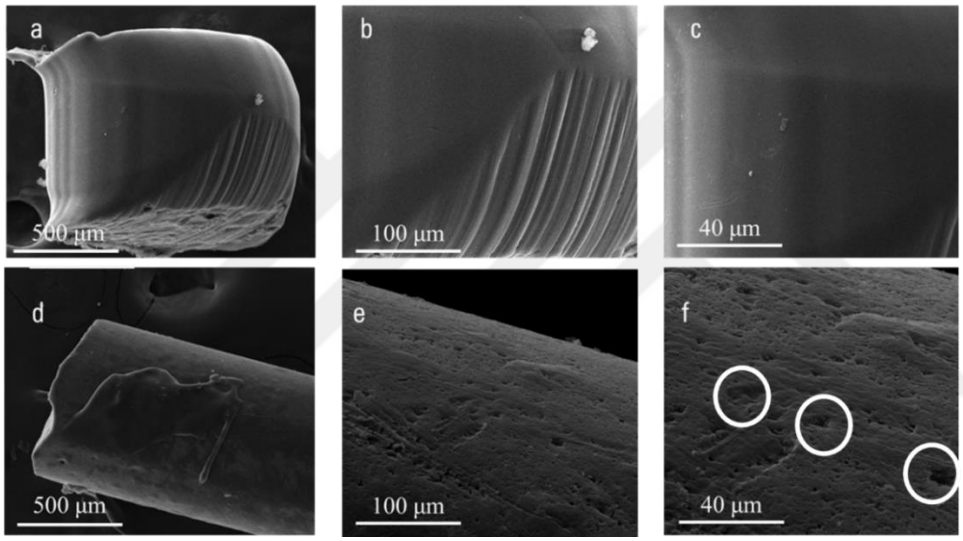


Figure 4.37 SEM images of untreated PC-MPs at x250 (a), x1000 (b) and x2500 (c); alkali+thermal treated PC-MPs at x250 (d), x500 (e) and x2500 (f).

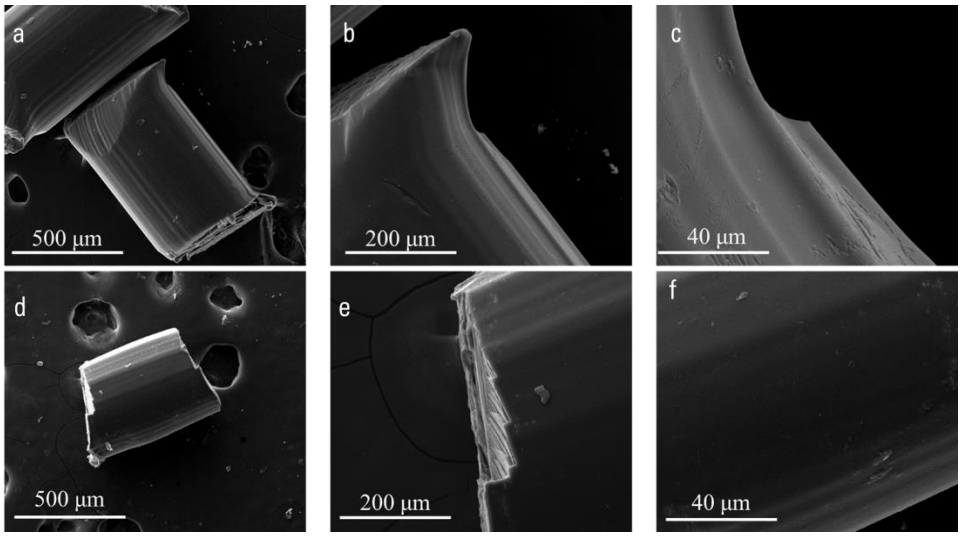


Figure 4.38 SEM images of untreated PC-MPs at x250 (a), x500 (b) and x2500 (c); thermal+enzyme treated PC-MPs at x250 (d), x1000 (e) and x2500 (f)

CHAPTER 5

CONCLUSION

The aim of this study was to investigate the impact of five different sludge disintegration methods on the leaching of additives from PET, PP and PC-MPs found in the sludge. To achieve this, a solid-phase extraction method was developed, and the amount of leached additives was determined using GC-MS. Additionally, the carbonyl index, crystallinity and surface morphologies of PET, PP and PC-MPs collected from the combined disintegrated sludge were analyzed using FTIR, DSC and SEM, respectively.

The following conclusions were made based on the results obtained from the experiments on leaching of additives:

- After single alkali disintegration, DEHP concentration decreased by 35-64% in all samples. As stated in the literature, the low water solubility of DEHP can result in adsorbing on MPs[188] or sludge. The sample containing PC-MPs showed an 80% increase in BPA concentration relative to the control, which indicates hydrolysis of PC under alkali conditions.
- After thermal disintegration, the average DEP concentration detected in the samples decreased by 45% with respect to the control sample.
- DBP concentration was very close to the control sample for all plastics and disintegrations. This shows that DBP may not be present in the plastics used in the study, and even if there is, DBP did not leach under the applied disintegration conditions.
- No BPA leaching was observed in sludges containing PET and PP that were subjected to disintegration.
- After the alkali + thermal combination process, the amount of BPA in the sludge containing PC-MP, increases drastically, nearly 4000 times higher

than that of the control sample. In these conditions, BPA leaching in the presence of PC plastic is an expected result because studies in the literature indicate that PC hydrolyzes in an alkaline medium and thermal treatment accelerates this hydrolysis.[189] Thus, residual BPA in the PC structure can easily leach into the liquid phase.

- Using doses of 150 mg PEN/g TS and 200 mg PEN/g TS enzyme+thermal combined treatment, almost all of the additives could not be detected. This result was consistent with the study of Gavala and coworkers.[192] As stated, the enzyme, especially lipase, may have degraded phthalates and these chemicals could not be detected after disintegration.

After analyzing PET, PP and PC-MPs from disintegrated sludge samples, the following conclusions were drawn:

- There was a significant decrease in carbonyl indexes of PET-MPs, which were collected after undergoing alkali+thermal and thermal+enzyme combined disintegrations. Degradation of the ester bond in PET by alkali processes or hydrolysis of ester bonds by lipase in PEN could cause a reduction in the carbonyl index of PET.
- The crystallinity of PET-MPs exposed alkali+thermal combined disintegrations decreased, as compared to the control. From the leaching results, it was concluded that the adsorption of target additives, such as DEHP, on PET-MPs could take place. Therefore, as Fang Wang and colleagues stated, the crystallinity of PET may have decreased as adsorption increased.[206]
- After undergoing alkali+thermal and thermal+enzyme combined disintegrations, openings were observed at the edges of PET MPs in SEM images. Wear was observed on the edges of PP-MPs exposed to alkali+thermal treatment, however no change was observed in the surfaces and edges of PP-MPs examined after thermal+enzyme.

- Pores were formed on the surface of PC-MPs after exposure to alkali+thermal treatment, but no noticeable change was observed on the surfaces of PC-MPs after thermal+enzyme treatment which might be correlated with the results of leaching additives.

This study comprehensively examined the effects of sludge disintegrations on MPs by applying disintegrations that can be used to increase the biogas and energy efficiency obtained from sludge. The conditions were developed in our laboratory, making it the first attempt of this kind in literature.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE STUDIES

Based on the literature review and the the laboratory work carried out in this study, the following recommendations can be made for future studies.

In this study, SPE cartridges were used to extract and preconcentrate phthalate and BPA leaking into sludge supernatant from the matrix. Moreover, although these cartridges are disposable, they were washed with the specified SPE method and reused for the following sample. Therefore, the extraction process was a long and used a lot of solvents. To shorten this labor-intensive method and use less solvent, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, which has recently gained importance in the literature, can be used. Wang and coworkers used this method to find the DBP concentration leached from PET-MPs after anaerobic digestion of sludge.[207] The QuEChERS method can be developed and extracted for phthalate or BPA measurement after disintegration.

It has been stated in the literature that biofilm formation on MPs can affect the leaching behavior of additives and surface properties of MPs, in addition to conditions such as UV, size, temperature, and pH. One study revealed that biofilm formation makes PE-MP less hydrophobic. It would interesting to investigate how biofilm formation is affected with or without disintegration. If there is a biofilm on MPs, its effect could be used both to investigate the leaching behavior of additives and to interpret plastic properties.

In this study, when the pancreatin enzyme was used after thermal treatment, no additives were observed in the sludge supernatant. In the literature, it has been stated that lipase, one of the components of pancreatin, breaks down DEP, DBP and DEHP. To determine if phthalates are degraded by the pancreatin enzyme, it can be spiked into the sludge with added phthalates. Additionally, the effect of other individual

enzymes that exist in pancreatin (amylase, lipase, protease, trypsin, and ribonuclease) may be tested. This will help to better understand the impact of enzymes on phthalates.

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