SIMULTANEOUS EFFECT OF MICROALGAE IN WASTEWATER TREATMENT AND MICROPLASTIC AGGLOMERATION

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A project report submitted in partial fulfilment of the requirements for the award of the degree of Bachelor of Engineering (Hons) Civil Engineering (Environmental)

Faculty of Engineering and Green Technology
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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled **"SUMULTANEOUS EFFECT OF MICROALGAE IN WASTEWATER AND MICROPLASTIC AGGLOMERATION"** was prepared by **HO HWEI NING** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons) Civil Engineering (Environmental) at Universiti Tunku Abdul Rahman.

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Specially dedicated to

my beloved family, supervisor, co-supervisor, and friends

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ABSTRACT

Microplastics as the emerging contaminants and excess nutrient content in the water bodies cause detrimental effects on the water quality. The microplastic and nutrients can be eliminated by using microalgae. In this study, PVC resin was used as the microplastic contaminant, being immersed in the synthetic wastewater that was added with fertilizer containing Nitrogen and Phosphorus contents. *Chlorella Vulgaris* was used to treat the contaminants in synthetic wastewater. The microalgae with microplastic were cultured in separate conical flasks for 11 days. Nutrient removal, dry biomass collection, microscopy observation, and FTIR spectroscopy were conducted after cultivation. This study also investigates the interaction between microplastic and microalgae in agglomeration. The *Chlorella Vulgaris* performed nutrient removal of 99.2% in synthetic wastewater. The PVC resin being introduced into microalgae did not inhibit the algal growth but promoted the growth showing a rise of dry biomass generated 2.81 g/L from 0.77 g/L. Microalgae and microplastic agglomerated and formed flocs were observed in physical observation. Some gel-like liquids were observed between PVC and *Chlorella Vulgaris* under the microscope. We hypothesized that the presence of extracellular polymeric substances (EPS) was secreted by microalgae to aggregate with microplastic. The functional group of microalgae such as protein (C=C bond, 1635 cm^{-1}) of primary amide, (N-O bond, 1540 cm^{-1}) of secondary amide, lipids (C-H bond, 2922 cm^{-1}), and carbohydrate (C-O bond, 1054 cm^{-1}) of polysaccharide were obtained through FTIR spectroscopy. Thus, microalgae was considered a feasible treatment to remove excess nutrients and microplastic in wastewater treatment, as the flocs sediment can be harvested from the wastewater.

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LIST OF SYMBOLS / ABBREVIATIONS

A^2O	Anaerobic-anoxic-aerobic system
BAF	Biologically active filter
BOD	Biological Oxygen Demand
COPD	Chronic obstructive pulmonary disease
COD	Chemical Oxygen Demand
DAF	Dissolved air floatation
DF	Disc filter
DO	Dissolved oxygen
MBR	Membrane bioreactor system
N	Nitrogen
NH_3-N	Ammoniacal Nitrogen
P	Phosphorus
PO_4^{3-}	Orthophosphate
PE	Polyethylene
PET	Polyethylene terephthalate
PS	Polystyrene
PVC	Polyvinyl Chloride
RSF	Rapid sand filter
WWTP	Wastewater treatment plant
kg	kilogram
m^3	cubic meter
mm	millimiter
µm	micrometer

CHAPTER 1

INTRODUCTION

1.1 Background

In the past few decades, environmental pollution such as ocean pollution has deteriorated due to several factors. Environmental pollution is mainly induced by anthropogenic activities such as agricultural runoff, industrial discharge, and domestic disposal. Many researchers found that there are countless plastic floats on the ocean surface. Oceanographers reported that approximately 15 trillion to 51 trillion microplastic particles were thought to be drifting in surface waters throughout the world in 2015 (Lim, 2021). According to UNEP (2023), due to the annual production of over 430 million tons of plastic, the globe is suffocating under the burden of plastic pollution. Production of plastic is abundant in industrial factories for multipurpose usage. Plastic can be used in a very broad range such as the production of plastic bags, car tires, cosmetic products, food containers, bottles, and so forth. The waste or byproduct produced during these good production processes will be discharged to the nearby waterways such as rivers and ponds. Plastic disposal without control as a result of water pollution among nations contributes to destructive negative impacts on our environment. Plastic is present in many stages and varies in shape and size. Plastic released into water can be further broken down to form microplastic.

Microplastics are small pieces of plastic fragments that are size of less than 5 mm in length that usually appear abundantly in the surrounding environment (US Department of Commerce, 2023). Microplastics exist in two modalities: primary microplastics and secondary microplastics. The primary microplastics are directly present in the environment in the form of extremely small pieces of plastic that are less than 5 mm. Primary microplastics include synthetic fibers, cosmetic goods, and plastic microbeads. In contrast, secondary microplastics exist in larger forms of plastics such as plastic bottles, plastic bags, straws, plastic containers, and so forth. These larger plastic pieces being exposed to weathering will then further break down into smaller pieces of plastics, as the result of secondary microplastics.

Microplastics are not only present in the water, but they are also found suspended in the air, drinking water, dust, soil, food, and so on. Microplastics may be derived from the breakdown of larger pieces of plastic, industrial manufacturing, microbeads, shedding of synthetic fibers, overflow of plastic pellets, tire wear, paint, and coating, and improper wastewater management (Horiba Scientific, n.d.). Once microplastic is released into the environment in abundance, the soil, water, air, food, and beverages, all organisms that live near the microplastic discharge spot will be directly exposed. Not only humans but aquatic and terrestrial organisms will also be affected by exposure to microplastics. Exposure of living organisms to microplastics tends to affect their health including ingestion, food poisoning, metabolism abilities, respiratory difficulties, bioaccumulation, reproductive system, and physical damage (Lee et al., 2023). Disposal of microplastics is not only detrimental to the health conditions of organisms but also lowers the quality of the environment such as air, water, and soil quality.

Wastewater treatment plants are mainly divided into four stages that are preliminary treatment, primary treatment, secondary treatment, and tertiary and advanced treatment. The purpose of wastewater treatment is to preserve the water quality of the incoming waterway through the elimination of dissolved solids, suspended solids, and pathogenic microorganisms. In addition, the offensive characteristics of wastewater such as color and foul odor are being resolved through the wastewater treatment process. The treated water by the wastewater treatment plant can be repurposed and reused in other domains.

Wastewater refers to the water that has been utilized for residential, commercial, industrial, and agricultural purposes (Tuser, 2021). Wastewater contains various components such as solids, dissolved and particulate matter, microorganisms, nutrients, micro-pollutants (microplastics), and heavy metals. The physical characteristics of wastewater include solids, odor, color, temperature, and turbidity. Meanwhile, the chemical characteristics of wastewater are pH value, dissolved oxygen (DO), hardness, nitrogen, and total organic carbon.

Preliminary treatment or (pretreatment) is to protect the wastewater treatment plant by removing debris, grit, and oily scum in the sewage. The bar rack, grit chamber, comminutor, and equalization basin are the four main components of the pretreatment process. These chambers are placed upstream of primary treatment to protect the wastewater treatment plant equipment. Primary treatment is followed by pretreatment. In primary treatment, suspended solids can be eliminated by floating and settled out by gravity sedimentation. Raw sludge is removed by mechanical scrapers and pumps. Floating materials such as grease and oil float at the surface that can be easily collected by a surface-skimming system. Approximately 50 to 60% of suspended solids are removed (Frankel, 2022), and 90% of settleable solids removed.

Unlikely primary wastewater treatment, secondary wastewater treatment is a biological treatment process that purposely minimizes the concentration of organic materials in the wastewater. Secondary wastewater treatment plays a vital role in removing the suspended solids that failed to be removed by primary settlement, it also dissolves BOD that is unable to be treated by primary treatment. Microorganisms are introduced into secondary wastewater treatment. Microorganisms can remove the biodegradable organics through the metabolism process. Secondary treatment is divided into three stages which are aerobic decomposition, anaerobic decomposition, and anoxic decomposition. Secondary treatment can treat 90% of the remaining suspended solids in wastewater (Frankel, 2022).

Microplastic can be removed through various stages of wastewater treatment plants, primary, secondary, and tertiary treatment. It was reported that primary treatment can remove 16.5 to 98.4% of microplastics from the wastewater treatment plant. This review demonstrated the very varying efficacy of the water treatment systems (Tang and Hadibarata, 2021). The overall microplastic removal efficiency for secondary treatment at wastewater treatment facilities ranges from 78.1 to 100%. Secondary treatment using activated sludge can remove 7% microplastic while membrane reactors can remove 99.9% microplastic (Tang and Hadibarata, 2021). A total of 87.3 to 99.9% of microplastics can be eliminated after tertiary treatment. (Tang and Hadibarata, 2021).

Microalgae are unicellular photosynthetic microorganisms that are invisible to the human naked eye. They are autotrophic microorganisms that inhabit freshwater, marine, and soil habitats and engage in photosynthesis to generate organic compounds (Dolganyuk et al., 2020). Microalgae are the primary producers in the aquatic food chain by producing their own food through the photosynthesis process. During photosynthesis, microalgae consume carbon dioxide, water, and the presence of sunlight to produce biomass as their food supply. Their existence of photosynthetic pigments enables them to photosynthesize. The pigments that predominate in an algal cell have an impact on the algae's color (Biology Online, 2021). Microalgae can be categorized into different colors, mainly green, red, or brown color. Although they have the same functionality as common green plants, microalgae are different from green plants, microalgae are absence of roots, stems, and leaves. Microalgae are prevalent in wastewater. Additionally, minerals found in wastewater include nitrogen and phosphorus, which are necessary for the development of plants (Li, 2019). Microalgae live in conditions of sufficient nutrients like nitrogen and phosphorus, exposure to sunlight, and carbon dioxide supply with the presence of water.

The specific relationship between microalgae and nutrients such as nitrogen and phosphorus is, that the nutrients can serve as food supplies for microalgae. According to Lu et al. (2023), injurious contaminants in wastewater have a propensity to be absorbed by microalgae and used as growth nutrients. Ammonia and total phosphorus levels are decreased by microalgae developing in wastewater, and both harmful pollutants are eliminated after 60 days of cultivation (Lu et al., 2023). Facilities for mitigating excess nutrients and energy recovery from wastewater are crucial, and they are built for this purpose (Lu et al., 2023). The development of microalgae-based systems allows for the absorption of nutrients from waste, and biomass can once more be employed for a variety of applications such as energy recovery (Lu et al., 2023).

According to Tuser (2021), eutrophication is a condition brought on by an abundance of nutrients like phosphorus and nitrogen, which can also be hazardous to aquatic life. Additionally, this encourages excessive plant growth and decreases oxygen availability, disrupting habitats and possibly putting some species in danger (Tuser, 2021). According to Chislock et al. (2013), the negative impact of the formation of eutrophication is the development of dense blooms of toxic, foul-smelling phytoplankton that damage water quality and impair water clarity. Algal blooms reduce light penetration, which inhibits plant growth and results in plant die-offs in maritime zones. They also make predators such as molluscivorous fish less effective because they need light to chase and catch food (Chislock et al., 2013). The excess nutrients, nitrogen, and phosphorus which will pose a threat to the aquatic ecosystem must be controlled by the growth of microalgae. The critical contaminants in water like microplastics which are nondegradable materials will also lead to water pollution and endanger marine life. Microplastics are potentially to be agglomerated together with the presence of microalgae in water. Hence, microplastics can be removed from water by collecting microalgae.

1.2 Problem Statements

Microplastics exist everywhere, contributing to microplastic pollution in aquatic and terrestrial ecosystems, as well as quality of life. Since microplastics are too small, they are easily ingested by various kinds of marine animals, which may pose to bioaccumulation as well as biomagnification within the food chain. Consuming microplastics also leads to adverse impacts on aquatic creatures and the wellness of ecosystems. In most studies, the mitigations of microplastics from water include physical filtration and chemical treatments. There is no denying that both physical filtration and chemical treatments can remove microplastics from water sources, but they must fit with some limitations and may pose some side impacts that could endanger the environment. Physical filtration is unlikely to completely remove all microplastics of any size; however, the chemical treatments might produce byproducts through the chemical reaction. The byproducts may be toxic to the environment, increasing the potential risk to marine life.

Ordinary wastewater treatment cannot remove all pollutants including microplastics in water. There might be some remaining pollutants still in the treated water such as microplastic particles. The remaining contaminants (microplastic) in water will contribute to water pollution if they are not fully removed from the water. Hence, developing alternative and effective treatment methods to remove microplastic in aquatic ecosystems is vital to preserve our environment. Microalgae has been proven to be an important character in the wastewater treatment process. It can remove organic materials from the wastewater through aerobic decomposition. In contrast, there are possible opportunities to treat microplastic as well. The usage of microalgae to treat microplastics needs to be examined. The efficiency and productivity of treatment need to be determined. For effective approaches to be developed to tackle microplastic pollution, it is essential to determine the behavior and fate of microplastics in the environment.

The purpose of this study is to examine the simultaneous effects of microalgae on wastewater treatment and their possible contribution to the agglomeration of microplastics. This study aims to contribute to more sustainable and effective strategies for reducing water pollution and microplastic contamination in aquatic ecosystems by filling knowledge gaps regarding the effectiveness of microalgae-based wastewater treatment and its interactions with microplastics

1.3 Aims and Objectives

The objectives of the thesis are shown as follows:

i) To study the performance of microalgae in nutrient-rich wastewater.

ii) To investigate the performance of microalgae in agglomerate microplastic.

iii) To study the interaction between microalgae and microplastic in promoting agglomeration.

CHAPTER 2

LITERATURE REVIEW

2.1 Review of Microplastics

According to Yang, H., et al. (2021), numerous earlier investigations have highlighted the pervasive distribution of microplastics in the soil, lakes, rivers, and oceans, as well as in foods, beverages, spices, and aquatic life. Microplastics are small pieces of plastic that are less than 5 mm in length which can be harmful to our ocean and aquatic ecosystem. Gradually increase in plastic requirement contributes to continuing plastic generation and production. Uncontrolled microplastic production results in a great abundant amount of microplastic in our mother earth, the concentration of microplastic also becomes unexpected and countless. The quantities and concentrations of microplastic are always a concern of environmental scientists and environmental researchers.

Microplastics are present anywhere from the surface of the ground to high altitudes due to the attraction of gravity (Yang, H., et al., 2021). However, the concentration of microplastics is higher at a lower level from the ground surface compared to a high level. This can be validated through simple tests and the collection of atmospheric microplastics at the ground surface and on the roofs of buildings in urban areas of Beijing (Yang et al., 2021). The concentration of atmospheric microplastics on the ground surface is higher than that on the roof.

2.2 Types of Microplastics

Microplastics are classified into two main types which are primary microplastics and secondary microplastics. According to Rogers, K. (2022), the primary microplastics include plastic fibers used in synthetic textiles (like nylon), plastic pellets used in industrial production, and microbeads found in cosmetic items. Primary microplastics may reach the ecosystem unintentionally through spills during production or transport, product use (such as household wastewater systems washing personal care goods into them) or scratching during the washing process by household or industrial washing machines (such as washing clothing made of artificial textiles) (Rogers, K., 2022).

When bigger plastics are subjected to weathering, such as when they are exposed to environmental factors like wind erosion, wave action, and UV radiation from sunlight, secondary microplastics are produced as a byproduct (Rogers, K., 2022). Used plastic water bottles, straws, plastic food containers, and plastic bags usually found in oceans, rivers, ponds, lakes, and other waterways, being thrown by humans are considered anthropogenic pollution sources to the marine system. These plastic products themselves carry toxic and hazardous properties, may pose a threat to the marine environment and marine creatures, harmful to their health, and result in some infections. These plastic-containing products are disposed of into waterways and then exposed to water waves. The combination of wave action and penetration of sunlight will initiate the degradation process of plastic goods. Plastic goods such as plastic bags degrade, decompose into smaller sizes, and further break down into even smaller fragments until their size ranges from 5 micrometers to 1 mm. At this point, the plastic fragment is considered secondary microplastic (Issac and Kandasubramanian, 2021).

2.3 Characteristics of Microplastics

The summary of a variety of microplastic characteristics is shown in **Table 2.3**. Microplastics are manufactured solid particles or polymers which insoluble in water and have an irregular shape with a size range of 1 micrometer to 5 mm. Under microscopic observation, there are 4 main shapes of microplastics observed and identified in Poyang Lake (Liu et al., 2019). There are microplastic fragments, films, fibers, and foam. According to Liu et al. (2019), various types of microplastics found in Poyang Lake in different grain sizes, fragments are smaller than 1 mm; film range from 1 to 2 mm; foaming ranges from 2 to 3 mm; and fiber range from 3 to 4 mm. The majority of the microplastics found in Poyang Lake were fragments, indicating that secondary sources like broken-down plastic waste and detritus were the secondary sources of microplastics. Different microplastics' surfaces were rougher in different Scanning Electron Microscopy (SEM) pictures, demonstrating that these materials had varied surface topography features that are typically characterized as rough, porous, cracked, or seriously damaged (Liu et al., 2019). According to their studies in Poyang Lake, fragmented microplastics were seen in a variety of colors and morphologies, and their surfaces were rough or uneven (Liu et al., 2019). However, film microplastics formed as irregular films with light and soft properties; fiber microplastics showed a smooth surface, linear shape, and blue in color; foam microplastics displayed in white and in different shapes.

Microplastics with a length of 5 mm and below have physical characteristics that enhance their bioavailability, such as size, density, color, and chemical composition (Liu et al., 2019). Due to features like buoyancy and great durability, microplastics have the potential to amass rivers, lakes, and the marine environment throughout the globe (Liu et al., 2019). Carbon and hydrogen atoms are linked in polymer chains to form microplastics (Rogers, K., 2022). Microplastic is the smaller version of plastics, which is persistent in the environment. Microplastics are extremely durable and resistant to degradation. Microplastics when disposed into an environment like the ocean, will never decompose, and persist there for a few decades. Microplastics are non-biodegradable. Biodegradation is a process of separation of any biodegradable materials in the environment by bacteria. Bacteria decompose the materials into organic or inorganic components that do little to no harm to the environment. Microplastic is non-biodegradable, it is unable to decompose by bacteria due to their specific chemical bonding. Hence, microplastics will permanently be in the sea and contribute to more pollution in the sea. More than 60% and 80% of the microplastic in both seawater and coral reef areas were smaller than 1 and 2 mm, whereas those larger than 3 mm represented the least to them ranging from 1.39 to 6.27% (Lei et al., 2021). Five different MP shapes, including fiber, granule, fiber bundle, fragment, and film, were found in the seawater and reef samples. However, fiber made up a large proportion of these in both seawater (77.18%) and corals (Lei et al., 2021). According to Lei et al., (2021), the most found microplastic in seawater is in black, however, transparent microplastics are found the most abundant in the coral region.

Table 2.3: Characteristics of Microplastics.

Shape	Size	Color	Surface morphology/ Composition*	Location	Reference
Fragment	< 1 mm	Various color	Rough	Poyang Lake, China	Liu et al. (2019a)
Films	1-2 mm	Various color	Light, soft		
Foam	2-3 mm	White	Rough		
Fiber	3-4 mm	Blue	Smooth		
Film	< 0.5 mm	Black	PP	Sanya Bay, China	Lei et al. (2021)
Fragment	0.5-1 mm	Blue	PET		
Fiber bundle	< 2 mm	Red	PE		
	< 3 mm	Green	PA		
Granule	>3 mm	Transparent	PS		
Fiber			CP		

* PA: Polyamide PE: Polyethylene, PET: Polyethylene terephthalate, PP: Polypropylene, PS: Polystyrene, CP: cellophane.

2.4 Source of Microplastics

Microbeads are considered as primary microplastics. Microbeads are microplastics that are mainly found in cosmetics, health, and beauty products. Microbeads present in these synthetic products work as exfoliants to perform cleansing the items such as body scrub, facial cleansing, toothpaste, and so forth. During the cleansing process, large amounts of microbeads collide and strike the surface of dirty items. Due to the high velocity of the crushing microbeads to the item surface, the residue that initially deposited on the surface dislodges and moves away from the target surface. For example, the body scrub is a type of cleansing product that contains microbeads that act as an exfoliator to clean the body's skin by scrubbing it. The variety of microplastic sources is shown in **Figure 2.4**.

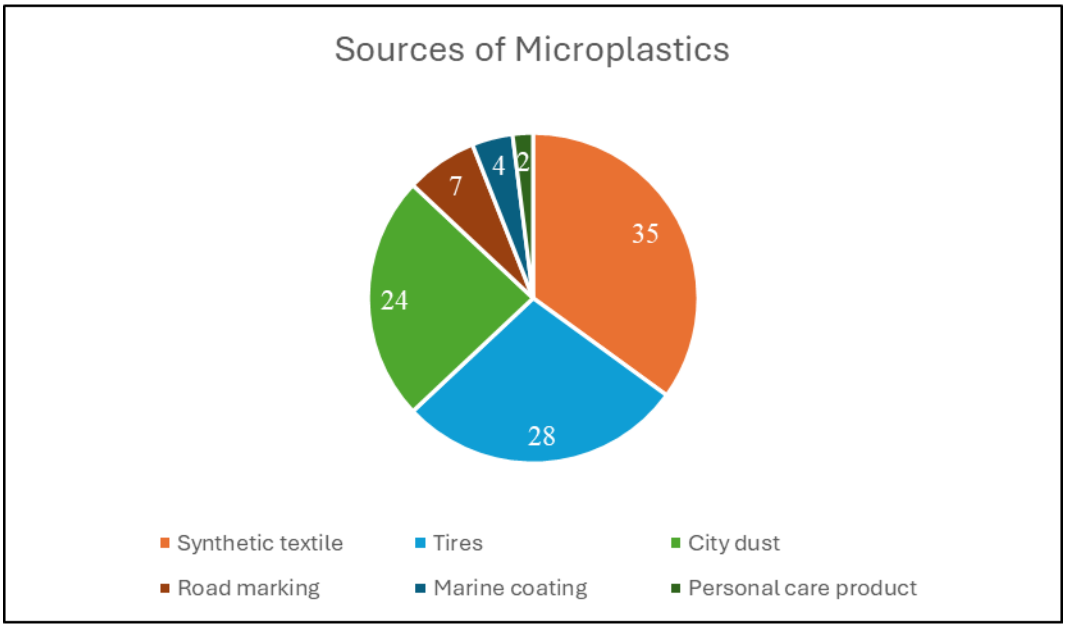

Figure 2.4: Sources of microplastics in the environment (Horiba Scientific, n.d.).

The largest single source of manufactured microplastics in the ocean, accounting for 35% of the total amount, is synthetic textiles (Horiba Scientific, n.d.). Our clothes are made up of synthetic fibers like polyester and nylon. These synthetic fibers are released into the environment through the washing process. The washing machines wash, rinse, spin, and dry our clothes with water. Synthetic fibers, dirt as well as dust from the clothes are washed away through the washing, rinsing, and spinning process. These potential microplastics are then transported by the outlet of the washing machine through the pipes and then directly disposed of into the waterway. Although there is an existing filtering system installed in the sewage system, since microplastics are too small, it is hard to be completely captured and filtered by the filtering system. Hence, microplastics will enter the environment through water transport systems.

Today, synthetic rubber, plastic polymer, and 19% natural rubber make up about 24 percent of a tire (Horiba Scientific, n.d.). Synthetic polymers are a matrix of microplastics that give the tire stiffness and traction (Horiba Scientific, n.d.). According to Moore (2023), tire wear is a key source of microplastics in rivers and oceans, and it may be up to four times more dangerous for the environment in cities than other microplastics. Tire decomposition results in the release of a variety of particles, including visible bits of tire rubber and nanoparticles. While tiny particles go airborne and are inhaled by humans or animals, larger particles are transported from the road by rain into rivers where they may release harmful compounds into the environment (Moore, 2023). Abrasion of tires when traveling on the road, small rubber particles that contain microplastics may release. The microplastics together with rubber particles will be washed into water bodies during rainfall. Washing away the microplastics into rivers and sea contributes to microplastic pollution and water pollution throughout all nations.

In addition to this, one major source of microplastic is the disposal of plastic pellets. Plastic pellets, also known as nurdles, are small particles that contain microplastic, they are usually produced in cylindrical pieces of plastic. Plastic pellets are made by manufacturers to produce several plastic products, such as plastic bottles, containers, plastic bags, and packaging bags. The pellets are subsequently delivered by these companies to factories that turn plastic into items. Inadvertent spills of pellets into the environment are possible during production, processing, transport, and recycling (Horiba Scientific, n.d.). Pesticides, PCBs, and mercury, among other harmful industrial and consumer toxins, can all be absorbed by plastic pellets (Horiba Scientific, n.d.). Plastic pellets combined with toxins in the environment, especially the sea, will carry destructive effects on the aquatic environment, increasing health issues, and infections among aquatic organisms.

Marine coating can contribute to exposure of microplastics to the aquatic environment. Marine coating, also known as painting, is to protect ships and boats. Marine coating applied to the boats could provide a barrier between water and the body of a boat. It can prevent or reduce interaction between water and the hull. Hence, the boat or ship can prevent corrosion and last longer. For marine coatings, designers employ a variety of plastics, primarily polyurethane and epoxy coatings, vinyl, and lacquers (Horiba Scientific, n.d.). Marine coating applied on the surface of hull, is directly exposed to seawater. The abrasion and constant friction among water movement during navigation leads to the degradation of these marine coatings. Erosion of marine coating certainly causes marine pollution and microplastic pollution that would endanger the ecosystem.

The human disposal of plastic products in the waterways is considered secondary microplastic. Examples of secondary microplastics are plastic bags, plastic bottles, and straws are the plastic products being dumped into the sea and river. They are the larger plastics that can be further breakdown into smaller plastic pieces when subjected to environmental factors such as sunlight penetration, wind, and water waves.

2.5 Impacts of Microplastics from Anthropogenic Activities

Microplastic pollution is induced by anthropogenic activities, such as improper discharge of sewage, dumping of plastic-made products into the river or sea, lack of proper wastewater treatment installations, discharge of domestic waste, industrial waste disposal, and spillage during industrial processes. Microplastic pollution poses significant impacts on various sectors, such as pollution of marine ecosystems, survival and health of marine life, human health, and microplastic deposition in the environment. As environmental levels of microplastic rise, so do the chances of ecosystem contact, interaction, ingestion, and harmful consequences on all levels of the food chain (Horton and Barnes, 2020).

2.5.1 Effects of Microplastics on the Aquatic Environment

Aquatic ecosystems are polluted by microplastics from a variety of sources, including sediment discharge from crops, the overflow of sewerages after heavy rains, and waste disposal from treatment facilities (Issac and Kandasubramanian, 2021). Microplastics are widely diffused in the aquatic environment as marine contaminants via ocean currents, acting as a carrier for the transfer of pollutants to animals present in water. Their floating and persistent characteristics cause them to be persistent as marine contaminants (Issac and Kandasubramanian, 2021).

The existence of microplastics in the natural world has been recognized to us since the early 1970s (Horton and Barnes, 2020). Plastics that reach waterways may linger there for hundreds or even thousands of years before becoming shattered by mechanical and photochemical processes to become microplastics that are less than 5 mm in length (Issac and Kandasubramanian, 2021). There are numerous ways that microplastics could reach the maritime environment, including ship transportation, fishing, and residence sewage discharges (Lei et al., 2021). This is particularly true for wastewater input from ships and research units to coastal waters, which are frequently not treated or not appropriately cleaned (Horton and Barnes, 2020).

Aquatic systems get polluted by microplastics through the discharge of waste from treatment plants, overflowing of sewers during heavy rains, and biosolid runoff from agricultural areas (Issac and Kandasubramanian, 2021). Microplastics are widely diffused in the aquatic environment as a marine contaminant via ocean currents, acting as a carrier for the transfer of pollutants to animals present in water. Their floating and persistent characteristics cause them to be a persistent and marine contaminant (Issac and Kandasubramanian, 2021).

Additionally, a variety of chemicals, including polymers, dyes, and plasticizers, are used to make plastics, some of which may be hazardous (Horton and Barnes, 2020). According to Issac and Kandasubramanian (2021), microplastics are a common marine contaminant in the aquatic environment due to their persistent and floatable properties, which serve as an agent for the transmission of contaminants to aquatic animals. Microplastic carry toxic and hazardous properties which will pollute our natural environment. The specific characteristics of microplastic enable them to absorb the existing chemicals and toxins in the environment. As a result, microplastic combined with the chemical pollutants create a highly toxic situation in the environment. Microplastics and declining oceanic pH due to rising carbon dioxide (CO_2) concentrations, are linked to comparable outcomes, including detrimental impacts on survival, growth, and reproduction (Horton and Barnes, 2020). Due to their small size and variety of impacts, microplastics serve as a habitat for developing microorganisms. The concentration of toxic organic pollutants like Dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers, and other manufacturing-related chemicals that already exist in water can be easily increased by microplastics through easy accumulation and emission (Issac and Kandasubramanian, 2021).

According to Horton and Barnes (2020), coral species found in warm waters and Antarctic systems are believed to consume microplastics, which have a substantially negative impact on their energy levels, growth, and pathogen rates. A critical issue that adversely impacts the socioeconomic sides of the shipping, trawling, and fish farming industries is eliminated by the presence of plastics in the aquatic environment (Issac and Kandasubramanian, 2021).

2.5.2 Effects of Microplastics on Human Health

Children and adults may consume anywhere from hundreds to more than 100,000 microplastic specks per day, according to small investigations of microplastics in the air, water, salt, and shellfish (Lim, 2021). Humans are primarily subjected to microplastics when they use various plastic products, such as packaging containers made of plastic, synthetic textiles, and personal hygiene products, as well as when they are exposed to paint flakes that have abrasively flown into the environment, such as air, water, and soil (Lee et al., 2023).

Given that people are the primary consumers of floating marine foods, which are severely impacted by microplastics, there is a significant likelihood that humans will become exposed to microplastics (Issac and Kandasubramanian, 2021). The presence of marine microplastics in seafood may endanger the safety of the food supply (Liu et al., 2019). Studies have shown that microplastics are able to reach the human body through a variety of routes, including tap water, sea salt, and bottled water (Issac and Kandasubramanian, 2021). Microplastics can reach the human body through direct ingestion, inhalation, and direct skin contact. The processes or ways of human beings exposed to microplastic are shown in **Figure 2.5.2**. According to Lee et al. (2023), microplastics can have an impact on a wide range of systems in the human body, including the digestive, respiratory, endocrine, reproductive, and immunological systems.

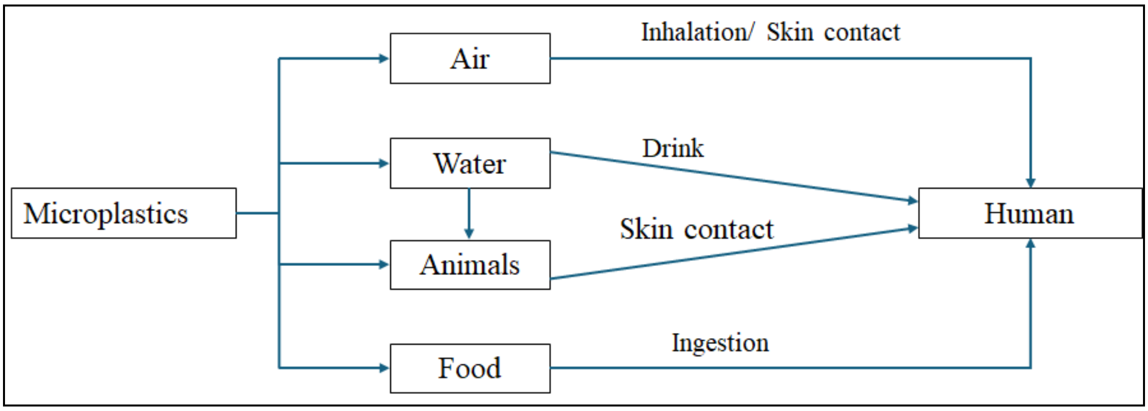

Figure 2.5.2: Route of human exposed to microplastics

2.5.2.1 Digestive issue

Ingesting microplastics poses a threat to the digestive system, and physical gastrointestinal discomfort may eventually lead to inflammation, which can produce a variety of gastrointestinal symptoms. Microplastics may alter the intestinal microbiota and lead to an imbalance of good and bad bacteria (Lee et al., 2023). This can produce a variety of gastrointestinal symptoms, including bloating and abdominal pain, (Lee et al., 2023). Microplastics can have chemical toxicity, which entails the absorption and buildup of environmental pollutants such as heavy metals, in addition to their physical effects on the digestive system (Lee et al., 2023). When microplastics are consumed, the harmful compounds may ingested into the body through the gastrointestinal tract, causing a variety of gastrointestinal symptoms such as vomiting and abdominal pain (Lee et al., 2023).

2.5.2.2 Respiratory and Cardiovascular issue

Humans may accidentally inhale the microplastics during respiration. The microplastics are adequately small and low density enabling them to float and suspend in the atmosphere. Microplastic is small enough to pass through the human nose, flow through the respiratory tract and trachea, be transported into the lungs and eventually enter our blood. The presence of microplastic leads to respiratory and cardiovascular issues. According to Lee et al. (2023), when humans breathe microplastic, it may result in oxidative stress in the lungs and air passages, causing inflammation and damage that can cause coughing, sneezing, and shortness of breath as well as weariness and lightheadedness from low blood oxygen levels. According to Dong et al. (2020), Microplastics have the potential to transmit other environmental pollutants, such as polystyrene (PS), which is harmful to human lung cells and raises the risk of chronic obstructive pulmonary disease (COPD). Thus, depending on personal vulnerability and particle properties, long-term exposure to low concentrations of microplastics in the air may be linked to cardiovascular and respiratory issues (Lee et al., 2023).

2.5.2.3 Endocrine issue

Sharp microplastic particles can physically stimulate the body and produce poisoning. Additionally, depending on the final application, various types of chemicals serve as the majority of endocrine disruptors that are employed when synthesizing plastic polymers (Lee et al., 2023). Endocrine disruptors, also known as hormonally active compounds, can impair a person's body by resulting in numerous malignancies and problems with the reproductive system. By encouraging the release of endocrine disruptors, microplastics can also have an impact on human health. Nonetheless, during adsorption, microplastics may transport other dangerous compounds such as heavy metals and organic contaminants that may be harmful to human health (Lee et al., 2023).

Nevertheless, microplastics may interfere with the endocrine system and result in a variety of endocrine disorders, including metabolic disorders, developmental disorders, and even reproductive disorders such as infertility, miscarriage, and congenital malformations (Vandenberg, Luthi and Quinerly, 2017). Microplastics can serve as a vehicle for environmentally damaging substances like bisphenol A, which are ingested by the body and contribute to several endocrine and reproductive system illnesses (Campanale et al., 2020). In animal studies, persistent microplastic exposure led to alterations in homeostasis and chronic inflammation. A study on human lung cells revealed that microplastics may stimulate immunity by controlling the expression of genes and proteins (Chiu et al., 2015).

2.5.3 Effects of Microplastics on Aquatic Life

The introduction of microplastics has had adverse effects on about 700 aquatic species worldwide, including sea turtles, penguins, and various crustaceans (Issac and Kandasubramanian, 2021). Various marine organisms can consume microplastics, which could then spread through the food chain (Lei et al., 2021). It has been shown that a variety of marine life mistakenly consumes microplastics as food, which causes physical harm, exposure to infections, and the spread of exotic species (Liu et al., 2019). Benthic species exposure and ingestion have been inevitably caused by the accumulation of microplastics in deep-sea sediments, particularly when this occurs concurrently with the deposition of debris (Horton and Barnes, 2020). The overall result of microplastic injections is a reduction in the nutrition of aquatic creatures; other difficulties include consequences for growth and population expansion (Issac and Kandasubramanian, 2021).

The degree of detrimental effects depends on particle sizes, dosages, and exposure conditions. In fish, microplastics may cause structural damage to the intestine, liver, gills, and brain, as well as impair metabolic balance, behavior, and fertility Zolotova et al. (2022). Due to the tiny size of microplastics, they are ingested by a variety of aquatic animals, disrupting their physiological processes, which then travel up the food chain and cause adverse health consequences in humans (Issac and Kandasubramanian, 2021).

However, the consequences of microplastic uptakes include less consumption of food, behavioral abnormalities, and developmental issues (Issac and Kandasubramanian, 2021). Microplastics without additives do not pose a chemical threat to aquatic creatures, but they can trigger physical issues like intestine blockages (Issac and Kandasubramanian, 2021). Various chemicals are integrated into virgin microplastics, depending on consumer demand for the product, which provide the additional property of adhering to pollutants already present in water and acting as vectors (Issac and Kandasubramanian, 2021).

According to Issac and Kandasubramanian (2021), the retention of plastic debris within the organisms may result in chemical leakages in the presence of additives, such as UV Stabilizers or absorbers, antioxidants, plasticizers, pigments, and surfactants. The presence of microplastic in the body could accumulate harmful effects. The nutrition, growth, spawning, and existence of aquatic organisms are impacted by microplastics, which are present in marine systems all over the world.

According to Sussarellu et al. (2016), their research demonstrated the negative effects of polystyrene microplastics on oyster ingesting and reproduction because of changes to their dietary intake and energy balance. Oysters produced fewer eggs, of lower quality, and with less sperm motility after being exposed to microsized polystyrene. Intake of micro polystyrene affects fertilization by reducing sperm speed and its quantity. Oysters discharge their eggs and sperms into the sea where they are released during external fertilization (Sussarellu et al., 2016). 6 micrometer polystyrene that the oyster had consumed was discovered in its feces, and the absence of a cumulation in the gut suggested a significant polystyrene ejection (Sussarellu et al., 2016). The production and growth of the progeny of oysters exposed to microplastic decreased by 41% and 18%, respectively (Sussarellu et al., 2016). In contaminated oysters, oocyte number was decreased by 38%, diameter reduced by 5%, and sperm velocity slowed down by 23%, all showed substantial reductions (Sussarellu et al., 2016). The study provided details on the detrimental effects of micro-sized PS on oyster development and reproduction, with significant consequences on offspring (Sussarellu et al., 2016).

Larger polystyrene (PS) particles with 5 μm in diameter, found in the gills, intestines, and livers of fish, favor fatty degeneration of hepatocytes and inflammatory responses in the liver and intestines, shift the qualitative and quantitative composition of the intestinal microbiome, disrupt with carbohydrate and lipid metabolism, and trigger changes in the expression of antioxidant protection genes related to oxidative stress (Lu et al., 2016; Wan et al., 2019).

Microplastic primarily builds up in the colon, while it can also happen in the liver, gills, and other organs, leading to pathological alterations in these tissues (Zolotova et al., 2022). The altered gut microbiota is a result of altered gene expression and protein production profiles, elevated levels of oxidative stress and inflammation, and reduced integrity of the intestinal epithelial barrier (Zolotova et al., 2022). The injured fish livers showed symptoms of oxidative stress, unbalanced lipid and carbohydrate metabolism, and other conditions (Zolotova et al., 2022). Furthermore, the toxins carried by the microplastic such as mercury, cadmium, phenanthrene, antibiotics, and polychlorinated biphenyls (Zolotova et al., 2022). These toxic substances carry serious toxic and dangerous effects on fish.

2.6 Treatment for Microplastics

2.6.1 Preliminary and Primary Treatment

Preliminary and primary treatment procedures like coarse and fine screening, grit and grease removal, skimming, and primary settling such as sedimentation are used for microplastic removal (Iyare, Ouki and Bond, 2020). Preliminary and primary treatment can filter varying sizes of suspended solids such as microplastics. Plastic particles larger than this are anticipated to be removed during preliminary treatment because screens of varied sizes, typically coarse particle size ranges from 6 to 150 mm, and fine particles size of less than 6 mm which retain suspended and floating solids (Iyare, Ouki and Bond, 2020). **Figure 2.6** shows that during the preliminary and primary wastewater treatment, an average of 72% (with a range of 32–93%) of the microplastic particles were eliminated. Talvitie et al. (2015), discovered that primary sedimentation removed most fibers, but secondary sedimentation and biological filtration barely eliminated none. Furthermore, Michielssen et al. (2016) observed that 84–88% of tiny anthropogenic debris was eliminated through screening and primary sedimentation.

According to Iyare, Ouki and Bond (2020), spherical microplastic particles with a diameter of 1.6 mm that have been discovered in wastewater treatment plants (WWTPs) are expected to either be eliminated by floatation (polymers with a density of less than 960 kg/m^3, such as expanded polystyrene, polypropylene) or sedimentation (remaining polymers with a density more than 1070 kg/m^3).

2.6.2 Secondary Treatment

Biological methods are frequently used in secondary treatment operations to further diminish suspended and dissolved particles that are still present in wastewater after the primary treatment (Iyare, Ouki and Bond, 2020). According to Iyare, Ouki and Bond (2020), various secondary treatments are widely used to remove microplastics such as activated sludge, biofiltration, trickling filtering, and solid contact tanks are found in various studies. Additional microplastics are removed during secondary treatment via trapping in solid flocs, sedimentation in secondary clarifiers, or even ingestion by existing microorganisms, such as protozoa (Iyare, Ouki and Bond, 2020). The formation of flocs and ferric sulfate employed in secondary sedimentation may help in the elimination of microplastics (Iyare, Ouki and Bond, 2020). According to Lee and Kim (2018), bigger microplastic particles with a size of greater than 300 µm have lower removal effectiveness, in contrast, the smaller microplastic particles size ranges from 106 µm to 300 µm have a higher removal rate because they not only conserved in the grit and grease removal stage but also readily adhere to sticky medium like biofilm or flocs. In comparison to preliminary and primary treatment, secondary treatment eliminated an additional 16% of microplastics, on average (0.2–52%) as shown in **Figure 2.6**. The average rate of elimination of microplastics from the activated sludge method of 16% range 0.2 to 52% (Iyare, Ouki and Bond, 2020). When removing microplastic, biofiltration performs a higher removal efficacy of 19% as compared to trickling filters and solids contact tanks which have a lower elimination rate of 7% (Iyare, Ouki and Bond, 2020). According to Talvitie et al. (2015), during secondary sedimentation, the removal of fibers was negligible compared to the synthetic particles, and elimination of both particles is higher in primary sedimentation compared to secondary. For instance, 92% and 32% of textile fibers and synthetic particles were removed by primary treatment respectively, while textile fibers and synthetic particles were eliminated with the removal efficiency of 0.2% and 52% respectively by secondary treatment (Talvitie et al., 2015). Although substantially greater removals have been reported by wastewater treatment plants, the average amount of microplastics retrieved during integrated preliminary/primary with secondary wastewater treatment was 88% shown in **Figure 2.6** (Iyare, Ouki and Bond, 2020).

2.6.3 Tertiary Treatment

In most cases, tertiary treatment methods are applied in accordance with a specific discharge permission or reuse condition to remove specific inorganic and organic pollutants to levels unattainable by traditional secondary treatment processes (Iyare, Ouki and Bond, 2020). Tertiary treatment significantly lowered the content of microplastics by 5-20% beyond the secondary treatment elimination. Talvitie et al. (2015), investigated the effectiveness of reducing microplastics greater than 20 μm from tertiary treatment methods frequently used in Finland: Membrane Bioreactor System (MBR) serving primary effluent, Dissolved Air Floatation (DAF), Rapid Sand Filter (RSF), and disc-filter (DF) processing secondary effluent. The maximum percentage removal was achieved by MBR (99.9%), while RSF, DAF, and DF removed 97%, 95%, and 40-98.5% of the microplastic respectively (Iyare, Ouki and Bond, 2020). According to Iyare, Ouki and Bond (2020), microplastic particles greater than 190 µm were eliminated by ultrafiltration and reverse osmosis (RO), although the small-size microplastics (< 190 µm) were found to be the most prevalent following tertiary treatment.

The quantitative research showed that filteration treatment techniques had the highest removal effectiveness for microplastics among the important treatment methods. Large particle size fibers and microplastics which 0.5 to 5 mm were easily segregated via the primary settling. Microbe in the activated sludge of the bioreactor system were easily able to capture small-particle size of 0.5 mm (Liu et al., 2020). The removal of microplastic treatment methods in wastewater treatment plants found that these technologies unable to eliminate entirely microplastics from wastewater. The total concentration reduced by 6%, 68%, 92%, and 96%, respectively, following the preliminary and primary, secondary, and tertiary treatment in wastewater treatment (Blair, Waldron and Gauchotte-Lindsay, 2019); 99% of the microplastics transported to wastewater treatment plants were removed by mechanical, chemical, and biological treatment methods (Ziajahromi, Neale and Leusch, 2016).

Study	Primary	Secondary	Tertiary & advanced	Primary + secondary	Primary + secondary + tertiary
35	87–93	4–9		96–97	
5				99.9	
36	69	19		88	
8	32–92	0.2–52	5–14		97–98
21					99.9
9	78	20		98	
24				72	
11					95.5
12					99.9
13[a]					90
14	92	7		98	
15				76	
16				98.3	99.4
37				98.5	
38				99.3	
39				98.2	
17				91.4	
34	41	24		64	
18				90.5	
19				53.6	82.1
20	NA	NA	20		84
Mean (*n*)	72% (6)	17% (6)	15% (2)	88% (15)	94% (8)

Figure 2.6: Percentage of microplastic elimination during wastewater treatment in selected studies (Iyare, Ouki and Bond, 2020)

The effectiveness of reducing microplastics varied amongst different wastewater treatment plant technologies. For instance, an anaerobic-anoxic-aerobic system (A^2O), aeration grit chambers, and advanced oxidation (UV and O_3) were used to trap microplastics in a Beijing wastewater treatment plant, and their respective microplastics removal efficiencies were 54.47%, 58.84%, and 71.67%, (Yang et al., 2019). However, the identical treatment technologies of microplastic elimination rates in a Shanghai wastewater treatment plant fell to 26.0%, 49.56 %, and 0.7%, respectively (Jia et al., 2019). The summary of elimination of microplastics through wastewater treatment is shown in **Table 2.6**.

Table 2.6: Microplastic removal and treatment (Tang and Hadibarata, 2021)

Treatment	Efficiency of treatment	Advantages/ disadvantages	Microplastic removed	Citation
Primary settling with flocculation	99% (Polyethylene) 97% (Polyester fiber)	- Remove floating MPs by aggregating them to form flocs - Easy to remove flocs	15 µm (Polyethylene) 140 µm (Polystyrene) 90 µm (polyester fiber)	Lapointe et al. (2020); Liu et al. (2020)
MBR technology	99.9%	- Very high removal efficiency - Membrane pore size of 0.1 µm	>0.1 µm	Liu et al. (2020)
A^2O system	Low	- Poor removal efficiency - Possible backflow	Various types of microplastics	Liu et al. (2020)
Granular filtration	86.9 to 99.9%	- Applicable to most sizes of MP except 10-20 µm (<86.9% removal)	1 µm to 125 µm	Zhang et al. (2020)
Ozone	99.2% (overall) 89.9% (stage-wise)	- Rapid reaction(60 min) - Energy save (35-45°C)	Various types of microplastics	Hidayaturrahman and Lee (2019)
Membrane disc-filter	99.1% (overall) 79.4% (stage-wise)	-A pore size of 10 µm, enables to separate of MPs - Large quantities of MP clog the	Various types of microplastics	

		membrane leading to backwash and reducing the efficiency		
Rapid sand filtration	98.9% (overall) 73.8% (stage-wise)	- No energy is involved, only use gravity - Not applicable to small sizes of MP (< 65 µm)	>65 µm or size greater than sand diameter	
Coagulation	Average 94.4% (overall) Average 60.8% (stage-wise)	-Use Al-based and Polyaluminum chloride (PAC) as coagulants with different dosages.	Various types of microplastics	
Advanced treatment (denitrification, ultra-filtration, ozonation and ultraviolet)	71.67%	Not mentioned	Microparticles (681.5 µm) Microfibers (1110.7 µm) Except microparticles (< 50 µm)	Yang et al. (2019)
Aerated grit chamber, primary sedimentation tank	58.84%	Not mentioned		
A^2O treatment	54.47%	Not mentioned		

Ozonation and granular activated carbon filtration	56.8-60.9% (stage-wise) 82.1-88.6% (overall)	- Strong shear force split off the MP, causing negative removal	>100 to 1 µm	Wang, Lin and Chen (2020)
Coagulation and sedimentation	40.5 to 54.5%	- Applicable to a wide range of MPs Higher removal for MP (>10 µm)	>100 to 1 µm	
Sand filtration	29- 44.4%	-Effectively remove larger size MP of >50 µm and 5-10 µm	Fibers (30.9-49.3% removal) Fragments (18.9-27.5% removal)	

Primary treatment processes such as primary settling treatment, grit and grease treatment were applied for microplastic removal; secondary treatment methods including A^2O, biofilters, and other bioreactors, and tertiary treatment processes consist of UV, O3, chlorination, biologically active filters (BAFs), disc filters (DFs), and rapid sand filters (RSFs) were among the various treatment technologies used in the wastewater treatment plants (Liu et al., 2020).

2.6.4 Primary Settling with Floculation

According to Lapointe et al. (2020), flocs are associated with microplastics during the flocculation process via hydrogen bonds, van der Waals forces, as well as electrostatic forces. Microplastic particles stay stable and float on the water surface due to their like-charge properties. Since microplastic particles carry the same charges, they tend to repel each other due to the inter-particle electrostatic forces (Liu et al., 2020). The repulsive force between microplastic particles was successfully decreased using flocculants that had opposing charges to the microplastics. Microplastics and flocs could aggregate because of the Brownian motion and mechanical agitation becoming active (Larue et al., 2003).

During the elimination of microplastic through flocculation, iron-based salt such as aluminum salt are commonly utilized in wastewater treatment plants (Liu et al., 2020). The schematic of microplastic removal through flocculation is demonstrated in **Figure 2.6.4**. The binding of iron hydroxide aggregates was what led to the flocculation of microplastics with iron (Larue et al., 2003). In low pH conditions, small aggregates with a high positive charge were regionally deposited on the surfaces of microplastics. In this instance, flocs reduced the repulsive forces between the microplastics and neutralized their charges. The size of the floc aggregates grew in neutral and basic pH conditions, and they accumulated to create bridges between the microplastics (Larue et al., 2003).

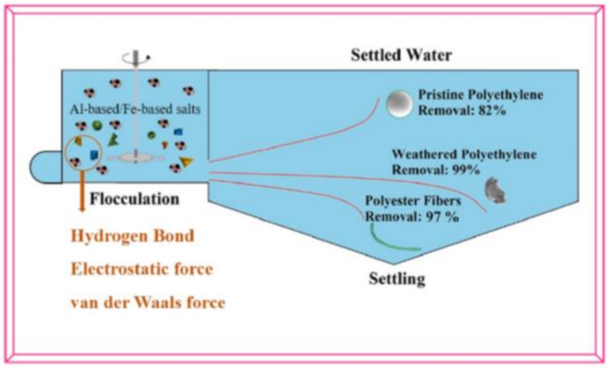

Fig. 5. The schematics of primary settling with flocculation technologies in microplastics removal (Lapointe et al., 2020).

Figure 2.6.4: The primary settling with flocculation process (Lapointe et al., 2020).

According to Lapointe et al. (2020), hydrogen bonds were involved when microplastics interact with aluminum-based flocculants. Additionally, anionic carboxyl groups in the weathered microplastic particles were electrostatically linked by cationic aluminum flocculant. The weathered microplastic surface's new functional groups, like carboxyl, hydroxyl, and carbon-carbon double bonds facilitated interactions between formation of flocs and microplastics (Lapointe et al., 2020).

According to Liu et al. (2020), the primary settling method removed the settable components in the suspended microplastics in the majority. The majority of the floating, non-sinkable microplastics were attached to the flocs and precipitated as a group, while others were skimmed off as scum (Lee et al., 2012). These plastic particles were disposed of as main sludge.

2.6.5 Activated sludge

The bioreactor system for microplastic removal involves an Anaerobic- Anoxic-Aerobic filter tank (A^2O), activated sludge, membrane bioreactor (MBR) system, and biofilter. The primary methods of the bioreactor system to remove microplastic were microbe ingestion and sludge aggregate development as shown in **Figure 2.6.5**.

Particularly, domesticated activated sludge was probably going to encourage the accumulation of microplastics in wastewater treatment facilities (Liu et al., 2020).

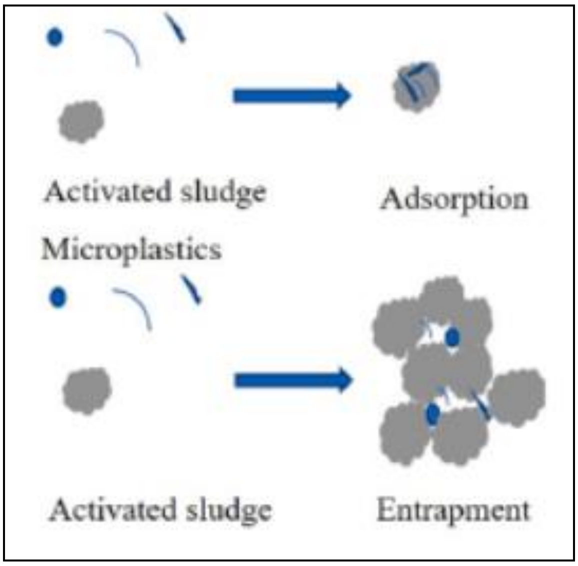

Figure 2.6.5: Activated sludge process (Zhang et al., 2020)

2.6.6 Membrane bioreactor system (MBR)

The membrane bioreactor (MBR) system is an integral technology among the secondary treatment systems being widely applied to wastewater treatment plants. It has an outstanding effectiveness in the removal of microplastics up to 99.9% resulting in high concentrations of mixed liqueur suspended solids which range from 6000 mg/L to 10000 mg/L (Talvitie et al., 2017a). According to Liu et al. (2020), the MBR system combined membrane separation and the conventional activated sludge process, as seen in Fig. 2.4 The biofilm carrier side of the MBR system was where the majority of the microplastics remained (Liu et al., 2020). This showed that one of the main mechanisms for the MBR system's removal of microplastics was the adsorption phenomenon (Liu et al., 2020). The microplastics that have tiny size of less than 5 mm can be effectively captured by MBR system since the MBR system has pore size of 0.1 µm. The components in MBR system as shown in **Figure 2.6.6**.

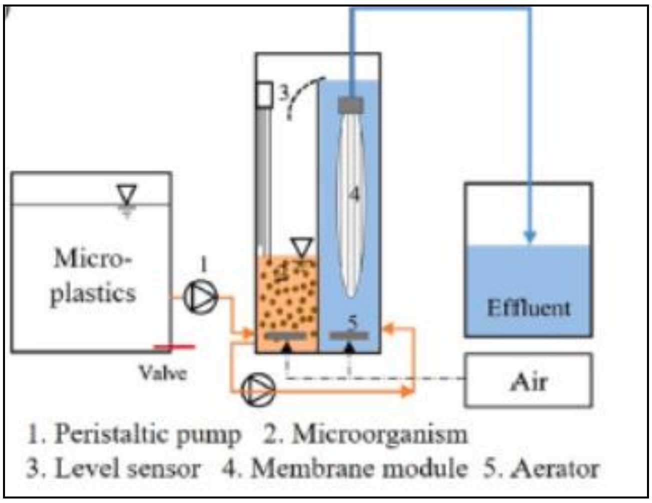

Figure 2.6.6: MBR system (Li et al., 2020)

2.6.7 Biofilter system

The biofilter system is also considered one of the most feasible methods of the microplastic reduction process. The microplastics that were introduced to the biofilter treatment unit are less dense and have smaller particle sizes. These made the removal of microplastics more challenging. However, according to **Figure 2.6.7**, biofilter technology still has the best removal capacity for microplastics. The primary methods for removing microplastics from biofilters were biofilm filtration and adsorption (**Figure 2.6.7**), which combined physical and biological purification processes. The microbe film that was developing on the inert filter material's surface came into touch with microplastics, increasing the surface area in which they may encounter microbes. Backwashing in the rising water movement made it simple to remove extra microbes and leftover microplastics (Rocher et al., 2012).

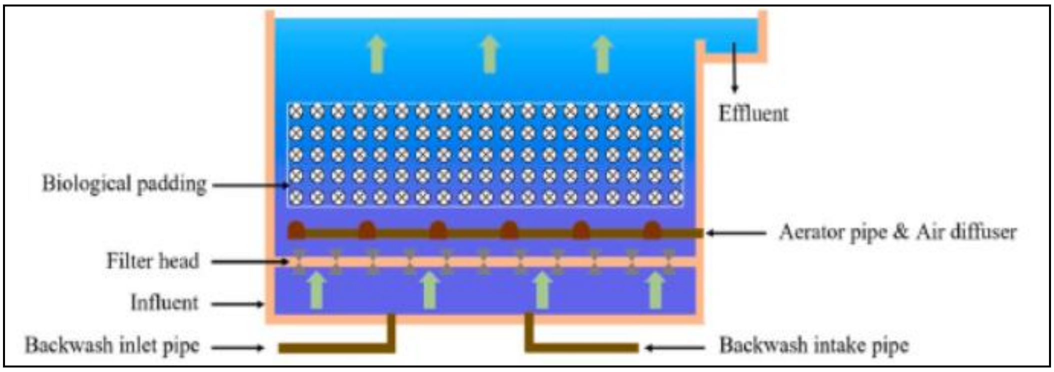

Figure 2.6.7: Process of biofilter system (Liu et al., 2020)

2.6.8 Anaerobic-Anoxic-Aerobic system (A^2O)

The Anaerobic-Anoxic-Aerobic system (A^2O) is implemented in wastewater treatment plants that effectively perform microplastic removal as shown in **Figure 2.6.8**. Due to the sludge return, it had a relatively low efficacy in cleaning up microplastics (Liu et al., 2020). A portion (20%) of the microplastics that were introduced into the sludge would return to the aqueous phase, causing the rate of microplastic breakdown in A^2O to be quite low (Liu et al., 2020). According to Auta et al. (2018), the current work assesses how exposure to polypropylene (PP) microplastics affects the development response and the mechanism of PP breakdown by *Bacillus sp. strain 27* and *Rhodococcus sp. strain 36* isolated from mangrove sediments. The decrease in polymer mass demonstrated that both bacterial strains could use PP microplastic for growth. After 40 days of incubation, weight loss induced by *Rhodococcus sp. strain 36* was 6.4% and by *Bacillus sp. strain 27* was 4.0% (Auta et al., 2018).

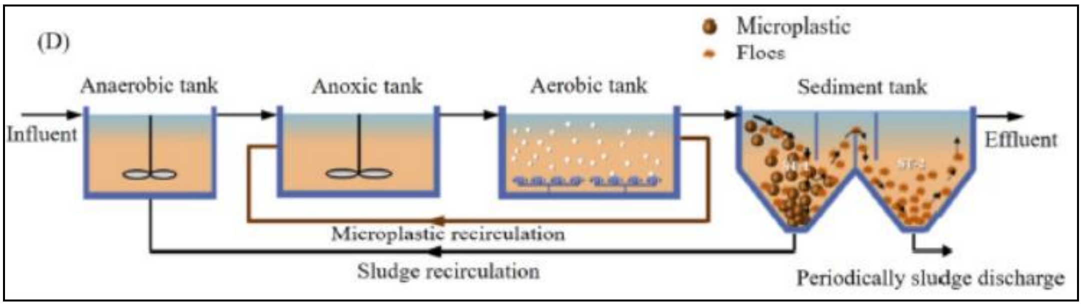

Figure 2.6.8: Process of A^2O system (Liu et al., 2020)

2.7 Introduction to Microalgae

Microalgae are unicellular microorganisms, that literally live in aquatic environments. Microalgae are very small size in diameter and are invisible to the human naked eye. Microalgae can only be observed under a microscope, considered microscopic algae. According to Dolganyuk et al. (2020), microalgae are a class of autotrophic microorganisms that inhabit freshwater while engaging in photosynthesis to generate organic compounds. Microalgae are the primary producers in the aquatic food chain. Autotrophic organisms such as microalgae generate biomass from inorganic substances in the presence of light that is converted during photosynthesis, in contrast to heterotrophic organisms that need a variety of organic components for growth (Dolganyuk et al., 2020). For instance, microalgae are autotrophic microorganisms that are able to produce their own food (biomass) by consuming carbon dioxide and water with sunlight penetration, while animals such as tigers are heterotrophic organisms that are unable to produce their own food, they depend on hunting other animals for their food supply. Additionally, microalgae biomass production systems may take up space unsuitable for growing agricultural products without deteriorating the environment, consuming carbon dioxide while producing oxygen, consuming relatively little water, and not damaging the environment (Dolganyuk et al., 2020).

2.8 Characteristics of microalgae

Microalgae are photosynthetic microorganisms that fall within the prokaryote and eukaryote taxonomy groups. Blue-green algae are prokaryotic microalgae like cyanobacteria, and diatoms and green algae are eukaryotic microalgae (Khavari et al., 2021). Microalgae are a big group of microorganisms that differentiate with various sizes, their size ranges from 1 μm to 100 μm or 0.1 mm. Although microalgae have similar functions to green plants which are the ability to generate their own food through photosynthesis, they are physically different. Microalgae do not have conventional root systems, green leaves, and stems. Microalgae carry out photosynthesis to produce their own food by utilizing sunlight energy, inorganic components like carbon dioxide, and water. Photosynthesis generates organic compounds that serve as their food supply, with oxygen as the byproduct. Various nutrients such as proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, pigments, phycobiliproteins, enzymes, and other biologically active components are abundant in microalgae (Dolganyuk et al., 2020).

According to Silva et al. (2020), microalgae have three different types of pigments: phycobiliproteins, carotenoids, and chlorophylls. Phycobilin appeared in red or blue; carotenoids in orange color, while chlorophyll shown in green color. As an instant quencher of reactive oxygen species and in the thermal dissipation of surplus energy in the photosynthetic machinery, carotenoids are crucial for oxygen photosynthesis (Dolganyuk et al., 2020). Besides, Cyanobacteria, Rhodophyta, Glucophyta, and some cryptomonads have phycobilins (phycocyanin and phycoerythrin) in their stroma. They are frequently employed as dyes in the food market and as fluorescent tags in molecular biology because they are readily soluble in water (Dolganyuk et al., 2020). Green pigments called chlorophylls, which are soluble in fat, are essential to photosynthesis (Dolganyuk et al., 2020). Natural pigments are one of the most pertinent categories to be researched out of the large range of chemicals from microalgae. In addition to their coloring capabilities, natural microalgal pigments provide health advantages including antioxidant, anticancer, and anti-inflammatory benefits; pigments can take the place of synthetic colorants (Rodrigues et al., 2015).

According to Dolganyuk et al. (2020), depending on the cultured species, an ultimate culture for microalgae compose of inorganic components like nitrogen (N) and phosphorus (P). With levels ranging from 1% to 14% in the dry mass, nitrogen comes in second to carbon, which makes up about 50% of the elemental component in the biomass of microalgae. It can be absorbed either inorganically as NO_3, NO_2, NO, and NH_4, or organically as urea or amino acids, or in some situations as Nitrogen (Dolganyuk et al., 2020). According to Sacristán de Alva et al. (2018), from 0.05% to 3.3% of phosphorus can be found in the dry biomass of microalgae. Various agricultural fertilizers, such as phosphates and superphosphates made of phosphorites, can be used to soak ecosystems where microalgae are grown with phosphorus (Dolganyuk et al., 2020). Trace elements are additional nutrients that must be present in the growing environment for the microalgae to reproduce properly such as Mg, S, Ca, Na, Cl, Fe, Zn, Cu, Mo, Mn, B, and Co, Mg, S, and Fe (Markou, Vandamme and Muylaert, 2014).

2.9 Application of Microalgae in Industry

According to Camacho, Macedo and Malcata (2019), microalgae have been successfully used in a variety of industrial applications, such as the creation of food, feed, cosmetics, health goods, and fertilizers. They are widely engage in wastewater treatment equipment and biofuel generation.

2.9.1 Pharmaceutical Industry

According to Khavari et al. (2021), microalgae are essential to the biomedical and pharmaceutical industries because they have prospective applications as antioxidant, anti-inflammatory, antitumor, anticancer, antibacterial, antiviral, and anti-allergy medicines. Microalgae-based nanoparticles have been utilized in medicine or drug delivery systems for the past ten years (Aw et al., 2012). Low toxicity, biodegradability, and wide surface area are these nanoparticles' key benefits over alternative carriers (Khavari et al., 2021). For the manufacture of growth factors, hormones, antibodies, vaccines, and immunological regulators, microalgae are essential in medical and pharmaceutical biotechnology (Yan et al., 2016). According to Rizwan et al. (2018), microalgae can produce bioactive substances that are difficult to chemically synthesize, such as antibiotics, subunit vaccines, monoclonal antibodies, hepatotoxic and neurotoxic substances, hormones, enzymes, and other substances with pharmacological and medicinal uses. Additionally, microalgae pigments provide health advantages including the ability to resist cancer, heart disease, neurological disorders, and eye ailments (Khavari et al., 2021).

Biosurfactants additionally exhibit strong bioactivities, such as antibacterial, antifungal, and anti-tumor properties (De Luca et al., 2021). Microalgae contain polyunsaturated fatty acids (PUFAs) which provide antioxidant and anti-inflammatory functions, that help to prevent heart disease and block the development of cancerous cells (De Luca et al., 2021). PUFAs have demonstrated efficacy in the treatment and prevention of a wide range of illnesses, including malignancies, atherosclerosis, thrombosis, arthritis, and inflammatory diseases (De Luca et al., 2021).

2.9.2 Functional Food

Functional foods (or nutraceutical food ingredients) have positive impacts on biological processes, enhance consumer health and wellness, and lower the risk of sickness (Camacho, Macedo and Malcata, 2019). As a result, adequate consumption of functional foods improves the life quality and adequately lowers the cost of health care for the general populace (Plaza et al., 2009). Microalgae used as food have high levels of proteins, polyunsaturated fatty acids, polysaccharides, vitamins, minerals, and sterols, which give them a nutritional benefit (Andrade, 2018). The omega 6 family and omega 3 family can be manufactured by microalgae (Camacho, Macedo and Malcata, 2019). Docosahexaenoic acid (DHA) and Eicosatetraenoic acid (EPA) have essential hypolipidemic action, for lowering triglycerides and boosting high-density lipoprotein cholesterol as well as reducing problems in cardiovascular effusions, arthritis, and hypertension (Jacob-Lopes et al., 2019). The growth and operation of the neurological system depend on DHA. In addition to the chemotactic activity of neutrophils, arachidonic acid (ARA) and Eicosatetraenoic acid (EPA) are also responsible for the aggregative platelets and the anti-aggregative and vasodilator effects in the endothelium (Jacob-Lopes et al., 2019).

2.9.3 Food Colorant

Pigments of microalgae can serve as food colorants. Carotene is a highly colored orange pigment that is found in large amounts in many vegetables and fruits, including carrots and pumpkins, as well as green leafy plants like parsley, spinach, and broccoli (Camacho, Macedo and Malcata, 2019).

2.9.4 Biofuel

Besides, microalgae contain a tremendous amount of lipids, protein, and carbohydrates, which are significant in the production of biofuels (Goh et al., 2019). Microalgae have the potential to produce a variety of biofuels, including bioethanol, biodiesel, bio-oil, biomethane, biohydrogen, and others (Brennan and Owende, 2010).

Figure 2.9.4 shows the flow of microalgae in the production of biofuel. (Camacho, Macedo and Malcata, 2019). According to Tiwari and Kiran (2018), their biofuels are both highly biodegradable and safe. They represent the pinnacle of structural component reduction and are virtually free-living chloroplasts. They effectively trap large amounts of carbon dioxide, which lowers greenhouse gas emissions. The biomass's carbohydrate element is used to make bioethanol, while algal oil is utilized for biodiesel and the leftover biomass can be used to make methane, fuel gas, or fuel oil (Tiwari and Kiran, 2018). Triacylglycerols (TAGs) are transesterified with an acid or alkali catalyst throughout the production process to generate biodiesel and glycerol (Johnson and Wen, 2009).

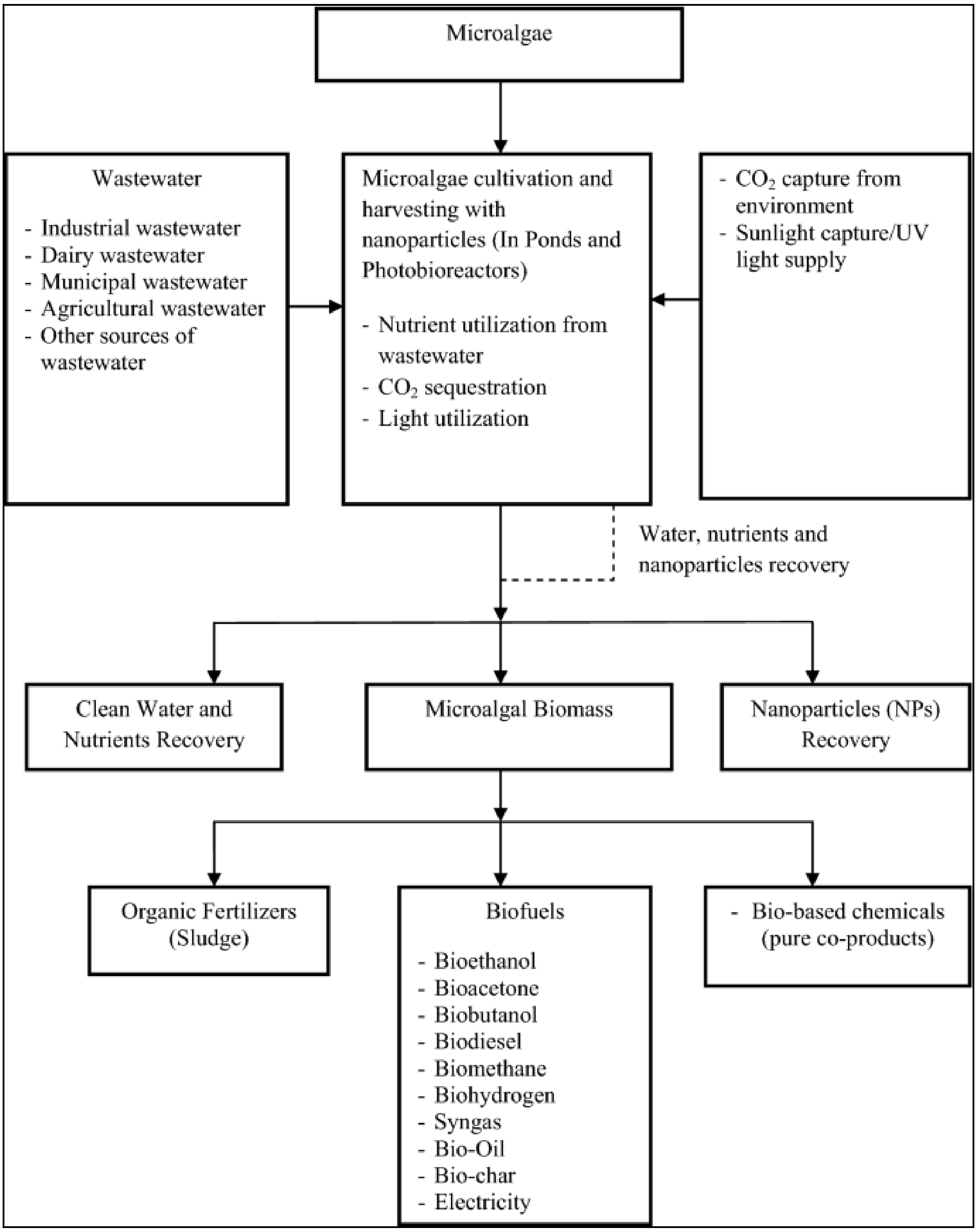

Figure 2.9.4: Flow diagram of processing microalgae for general uses through harvesting of microalgal biomass (Hossain, Mahlia and Saidur, 2019).

2.9.5 Cosmetic Products

Many microalgal species are classified as oleaginous because they may collect significant amounts of fat (De Luca et al., 2021). Lipids and their byproducts are one of the key components in cosmetic compositions. Different lipid classes are excellent moisturizers, emollients, softeners, serve as emulsifiers and surfactants, provide product consistency, carry color and smell, act as preservatives to preserve the quality of the product, and can be a component of a molecule's transport mechanism (De Luca et al., 2021). Numerous secondary metabolism products produced by microalgae have anti-inflammatory, anti-blemish, and antibacterial properties (Flament et al., 2013). *Chlorella vulgaris,* among other microalgal extracts, can be used to treat and prevent wrinkles as well as slow down skin aging (Enamala et al., 2018). Polyunsaturated fatty acids (PUFAs), phytosterols, and carotenoids are among the lipids with beneficial properties that the cosmetic and pharmaceutical industries are becoming more interested in (Cezare-Gomes et al., 2019). Additionally, oils are typically made into creams or emulsions for dermatological delivery agents to enable more uniform, efficient use and conveyance of active agents (De Luca et al., 2021).

Fatty acyls from oil seeds are the main source of bio-based surfactant material in the cosmetics industry (Fahy et al., 2005). Further subclassification of the fatty acyls category includes fatty acids and conjugates, eicosanoids, fatty alcohols, and esters (De Luca et al., 2021). A hydrocarbon chain with a carboxyl group at one end makes up fatty acids (FA). FA, which are regarded as vital oily raw ingredients in cosmetic applications, can be employed as softeners, detergents, and lighteners in addition to serving as emulsifiers (De Luca et al., 2021). They are thin, oily particles that can temporarily accumulate between the desquamating corneocytes, making the skin smooth, silky, and more radiant (Draelos, 2018). FAs are also essential skin elements that contribute to maintaining the skin barrier's regular function (Yang, Zhou and Song, 2020).

Microalgae contain PUFAs. Wax products belong to the fatty acyl category's fatty ester subclass (Fahy et al., 2005). For instance, waxes are essential ingredients in lipsticks because they give the stick the proper rigidity, hardness, stability, and texture (De Luca et al., 2021).

All lipids containing glycerol fall within the lipids group, which is made up of glycerolipids, except for glycerophospholipids, which are much more common and play more significant roles (De Luca et al., 2021). The most well-known group of glycerolipids, tri-substituted glycerol, called triacylglycerol (TAG) (De Luca et al., 2021). Since they behave as emollients, TAGs derived from vegetable oils are also incorporated into the formulation of bath and body products, cleansers, scents, foot powders, facial cosmetics, personal hygiene, suntan, and other skin care products (De Luca et al., 2021). Triacylglycerol (TAGs) maintain a high degree of skin hydration by creating an occlusive barrier that slows down the rate of water loss from the skin (H. Birjandi Nejad et al., 2020).

2.10 Application of Microalgae in the Environment

Microalgae are a class of autotrophic microorganisms that inhabit freshwater, conducting photosynthesis to generate organic compounds. They can be applied as a source of biologically valuable products are expanding quickly because of their great metabolic flexibility, tolerance to a variety of conditions, and potential for rapid development (Dolganyuk et al., 2020). Microalgae consist of specific characteristics; they contain green pigments such as chlorophyll, which enable the microalgae to undergo photosynthesis and produce nutrients by the inclusion of sunlight energy, and absorption of carbon dioxide (CO_2) and water. Photosynthesis mechanisms produce organic compounds and release oxygen (O_2) as the byproducts. The growth of microalgae is critical in preserving environmental quality by reducing carbon dioxide concentrations and raising oxygen emissions to the environment.

Because they balance sustainable vectors by reusing pollutants like carbon, nitrogen, and phosphorus present in wastes produced by industry and creating microalgal sludge, nutrient cycling by microalgae arises as an exciting method for environmental applications (Santos et al., 2019). By absorbing and redistributing dissolved organic matter and inorganic nutrients in the oceans, microalgae play important roles in oceanic energy exchanges and nutrient cycles (Arrigo, 2004).

2.10.1 Carbon Adsorption

Through the photosynthetic processes of autotrophic microalgae, carbon can be recovered from the environment and industrial exhaust gases in the form of carbon dioxide (Santos et al., 2019). The capability of microalgae to reduce carbon footprint by absorbing carbon dioxide (CO_2) released through the burning of fossil fuel. If microalgae are utilized to produce biofuel, minimize the net emission of CO_2 since the generation of carbon dioxide from facilities could be trade off with the fixation of CO_2 through photosynthetic activity (Rizwan et al., 2018). The amount of CO_2 produced during the fuel life cycle (the combustion of fossil fuels) increases with the process' energy intensity (Rizwan et al., 2018). Compared to terrestrial plants, which typically only absorb up to 0.06% of CO_2 from the environment, microalgae enable an efficient CO_2 content capture (5–15%) from the flue and flaring gases (Hsueh, Chu and Yu, 2007). Typically, microalgae may absorb the atmospheric carbon dioxide CO_2 generated by power plant operations and soluble carbonates (Rizwan et al., 2018). Many types of soluble carbonates (Na_2CO_3 and $NaHCO_3$) can be consumed by microalgae to capture CO_2 (Wang et al., 2008). According to Zeiler et al. (1995), the algae *Monoruphidium minutum* can effectively use flue gas that contains a high concentration of CO_2, coupled with sulfur and nitrogen oxides, to produce a sizable amount of biomass. In comparison to terrestrial plants, *Chlorophyta*, green algae, has demonstrated 10–50 times greater solar energy absorption efficiencyn(Wang et al., 2008).

2.10.2 Uptake Excess nutrients

The most prevalent type of industrial pollutants is rich in nutrients like nitrogen, phosphorus, and organic materials. Most of the nitrogen in wastewater is present as inorganic nitrogen, microalgae are crucial for both nitrogen fixation and assimilation (Santos et al., 2019). These species have the capacity to fix nitrogen, either by releasing it into the environment or integrating it into amino acids, proteins, and chlorophyll (Santos et al., 2019). The most prevalent measures to eliminate phosphorus and nitrogen from wastewater include biological processes like anaerobic digestion, nitrification and denitrification (Santos et al., 2019). Microalgae are a desirable alternative for wastewater treatment system because of their ability to concurrently eliminate inorganic nitrogen and phosphorus to minimize contaminants by harvesting beneficial biomass (Santos et al., 2019).

Another macronutrient, phosphorus, helps microalgae thrive. It is found in cytoplasmic solutes such as phospholipids, lipopolysaccharides, and nucleic acids (Peccia et al., 2013). Nitrogen and phosphorus can be essentially removed by microalgae. Some microalgae exhibit heterotrophic behavior, utilizing organic carbon sources like those found in wastewater to produce ATP through respiration (Santos et al., 2019).

2.10.3 Heavy Metals Adsorption

Their potential use in purifying wastewater comprising dissolved metallic ions is made possible by the microalgal affinity for polyvalent metals (Bashan and Bashan, 2010). Up to 10% of the biomass that microalgae produce can be retained as metals (Santos et al., 2019). According to Santos et al. (2019), both passive and active metal uptake by microalgal species is feasible. The association of metal ions to the functional surface ligands of the cell wall structure is what drives passive absorption the most. The presence of lipids, proteins, or polysaccharides on the surface of cell walls, which in turn contained functional groups like sulphate, carboxyl, hydroxyl, and amino that could operate to sequester heavy metal ions (Priya et al., 2014). The active mechanism is based on bioaccumulation processes, in which living things called biosorbents remove iron biologically (Santos et al., 2019). Using cyanobacteria and microalgae that are present in abundance naturally, biosorption is a technique to eliminate heavy metals from wastewater (Priya et al., 2014).

Additionally, through metabolic activity, microalgae can biodegrade, or bio-transform organic contaminants. Accumulation and degradation, which includes both transformation and mineralization, are the mechanisms for elimination (Wang et al., 2019).

2.11 Microalgae-Based Wastewater Treatment

Wastewater comprises a variety of substances, some of which are extremely hazardous to organisms at certain doses. High chemical oxygen demand (COD) and biological oxygen demand (BOD) represent the considerable amounts of inorganic and organic nutrients that are discharged into the environment (Abdelfattah et al., 2022). Microalgae-based systems may eliminate 45–65% of BOD and COD from wastewater, which is a significant improvement over the traditional bioremediation method (Al-Jabri et al., 2021).

The most effective bioremediating agents for many toxins are microalgae because of their high surface-to-volume ratios and high biosorption capabilities (Abdelfattah et al., 2022). Using wastewater as a nutrition supply for algal growth enhances the circular economy and environmental sustainability making microalgal-based wastewater treatment feasible (Srimongkol et al., 2022). Because microalgae can thrive in arid environments like wastewater and only require a small amount of land for production, they compete less for land with other uses like agriculture, zoos, industries, and human residential areas (Abdelfattah et al., 2022). Microalgae can survive and grow in various types of wastewater such as domestic waste, urban waste, industrial waste, and agricultural effluent.

2.11.1 Types of wastewaters and their composition

2.11.1.1 Municipal wastewater

Wastewater can be generated from a wide range of sources, wastewater classified as municipal wastewater, agricultural wastewater, and industrial wastewater. Municipal wastewater, also known as domestic wastewater, refers to the used water from houses, kitchens, bathrooms, and washing machines. Municipal wastewater typically has a relatively low Chemical Oxygen Demand (COD) (< 300 mg/L), Nitrogen (15-90 mg/L), and Phosphorus (5-20 mg/L) (You et al., 2022). Municipal wastewater may be classified into four groups based on the content of the wastewater including raw sewage (primary wastewater), wastewater after primary treatment, the effluent of aeration tank, and the residue from sludge sedimentation (You et al., 2022). Numerous studies have shown that adding high concentrations of CO_2 (5–15%) can promote the removal of nutrients from municipal wastewater, as well as boost microalgae growth and lipid synthesis (Liu and Hong, 2021).

2.11.1.2 Agricultural wastewater

Agricultural wastewater includes animal manure and farmland drainage wastewater that is discharged during the growing of crops, raising livestock and processing of agricultural goods (You et al., 2022). Animal manure excrement from agricultural sources exhibits high turbidity, a significant amount of nutrients, and high rates of insoluble organic compounds, currently seldom utilizing microalgae to remediate animal waste (Liu and Hong, 2021).

2.11.1.3 Industrial wastewater

The wastewater produced by the paper manufacturing, petroleum industry, sugar mill, metal-contaminated wastewater, drugs, textile colorant, palm oil mill effluent (POME), metal plating, and farming equipment manufacturing industries are all considered forms of industrial wastewater (Mohd Udaiyappan et al., 2017; Srimongkol et al., 2022). Industrial wastewater contains a variety of toxins, including heavy metals, pharmaceuticals, fat, and oil, as well as other chemical components (Mohd Udaiyappan et al., 2017; Srimongkol et al., 2022). *Scenedesmus* and *Cholorella* are efficient microalgae for cleaning industrial wastewater and olive oil (Tao et al., 2017). Chemical substances and heavy metals such as copper, lead, manganese, mercury, nickel, and zinc are typically present in industrial wastewater (Ahmed et al., 2022).

The removal of organic pollutants, chemicals, heavy metals, and pathogens from the sewage as well as the creation of biofuel can be accomplished by using microalgal development and wastewater treatment (Rizwan et al., 2018). The most often used microalgal species for nutrient removal include *Chlorella*, *Scenedesmus*, and *Spirulina* species. Similarly, *Botryococcus braunii*, *Phormidium bohneri*, and *Nannochloris* are also utilized to treat wastewater (Rizwan et al., 2018).

2.11.2 Heavy Metals Removal

Microalgae cells are capable of removing heavy metal by uptaking heavy metal ions from wastewater through the processes of biosorption (Srimongkol et al., 2022) and bioaccumulation (Ahmed et al., 2022). Bioaccumulation is the process of heavy metals entering the microalgae cell and penetrating the cell membrane, which is gradual intracellular diffusion and accumulation (Srimongkol et al., 2022). Through the processes of ion exchange and micro-precipitation, the heavy metal ions are captured and bound to the functional groups of the microalgae cell surface. (Srimongkol et al., 2022). Metal ions are physically deposited onto the surface of cells that carry functional groups such as amino group, carboxyl, and hydroxyl through the biosorption process. (Ayele and Godeto, 2021). *Scenedesmus, Chlorella, Botryococcus, Phormidium, Limnospira, and Chlamydomonas* are just a few of the microalgal species that have been proven to be excellent in bioremediating nutrients, heavy metals, emerging contaminants, and pathogens from wastewater (Ahmad et al., 2021).

According to Crini and Lichtfouse (2018), primary treatment focuses on removing solids, and secondary treatment uses microbial activities to break down organic material, but these procedures result in comparatively significant operating and maintenance expenses. Iron and aluminum salts are frequently used in chemical procedures for precipitation, producing a sizable volume of sludge that needs to be disposed of or treated further. Large infrastructures are needed for biological approaches, which also generate a lot of activated sludge that needs to be handled. This increases the need for energy input, the overall budget, and the complexity of the process (Kalra, Gaur and Goel, 2020). As a result, microalgae-based bioremediation is regarded as an effective substitute for modernizing the old wastewater treatment systems since it provides a dependable means of handling liquid or solid wastes generated by conventional procedures and turning them into products with commercial value (Abdelfattah et al., 2022). For this purpose, small to medium-sized municipal wastewater treatment facilities or maturation ponds can be used such as Advanced Integrated Wastewater Pond Systems (AIWPS) Technology (Oswald, 1991).

2.11.3 Carbon Fixation

Microalgae that grow autotrophically consume carbon dioxide (CO_2) as a source of carbon, lowering the atmospheric concentration. Each microalgal biomass converts roughly 1.8 pounds of CO_2 (Mustafa et al., 2021). P and N are also absorbed and transformed into proteins, carbohydrates, lipids, and other value-added compounds in addition to CO_2 (Mastropetros et al., 2022). Due to the typically high nutritional content of wastewater, adding microalgae to wastewater treatment saves production costs and minimizes the overall carbon footprint (Abdelfattah et al., 2022).

2.11.4 Nutrient Removal

Utilizing microalgae for wastewater treatment and aquaculture systems may be the greatest solution for both microalgal growth and bioremediation. Nitrogen and phosphorus, which are typically found in manufacturing wastes, can be used by microalgae (Rizwan et al., 2018). Microalgae can also mitigate the detrimental effects of sewage and industrial wastewater including nitrogenous waste from water treatment or fish farming (Rizwan et al., 2018). Eutrophication can be addressed by microalgae by eliminating nitrogen and phosphate from wastewater (Rizwan et al., 2018).

Chlorella vulgaris has an average removal effectiveness of 72% for nitrogen and 28% for phosphorus (Aslan and Kapdan, 2006).

2.12 Relationship between Microplastic and Microalgae

Microplastic is the ubiquitous substance being disposed into the environment, it tends to accumulate and condense in the aquatic environment due to its persistence characteristics. Microplastic cannot be decomposed and degraded naturally by decomposers such as bacteria. Abundance microplastic accumulates in the aquatic environment, pollutes it, and tends to put the health of aquatic life in danger. Meanwhile, microalgae are microorganisms that may appear naturally, and grow in the aquatic environment. Microalgae are able to survive under harsh conditions. They are autotrophic organisms that do not depend on harvesting food, they can produce their own food by converting carbon dioxide, sunlight, and water into microalgal biomass.

The discharge of microplastic into the ocean, and naturally found microalgae in the ocean has driven a potential relationship between these substances. Microplastic and microalgae could be present in the aquatic ecosystem at the same time. The coexistence of microplastic and microalgae is prevalent in the aquatic environment. Microalgae contain attachment capability to microplastic. Microalgae is smaller than microplastic, the microalgae tend to attach and aggregate onto the surface of microplastic. Su et al. (2023) demonstrated that microalgae can initially adhere to the pores or protrusions of microplastics and then highly collect in the local area, leading to the formation of multi-layer aggregation. Different types of microalgae affect the aggregation of microplastics. Size, shape, and types of microplastics are the main factors affecting the heterogeneous aggregation by microalgae (Su et al., 2023). When the microalgae successfully colonize and aggregate on the microplastic surface, the density of the microplastic increases. According to the cell density and number of the attached microalgae, the density of aggregates which included microplastic and microalgae was higher than that for virgin microplastics (Su et al., 2023).

The microplastic particles can affect the growth of microalgae. The studies by Su et al. (2023) show that by restricting the mass and gas movement between microalgal cells and the extracellular environment, microplastics also prevented the development of microalgae, especially when they were aggregating.

CHAPTER 3

METHODOLOGY

3.1 Experimental Flow

The summary of experimental procedures is shown in **Figure 3.1**.

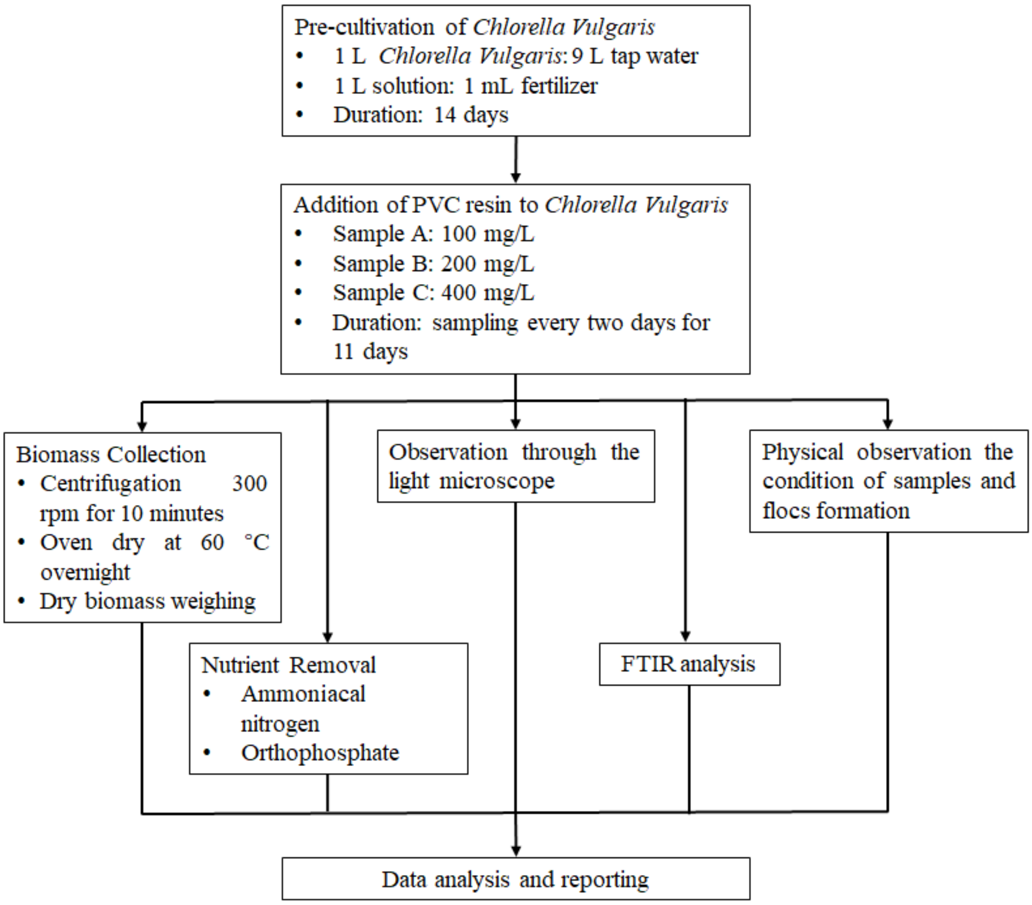

Figure 3.1: Experimental flowchart

Before starting the experiment, microalgae pre-cultivation was conducted. *Chlorella vulgaris* stock was diluted with tap water with a ratio of 1 L stock: 1 L tap water. The fertilizer was added to the microalgae stock solution with a ratio of 1 L stock solution: 1 mL fertilizer. This is to prepare the synthetic wastewater. The pre-cultivation of *Chlorella vulgaris* stock lasted for 14 days for the microalgae to grow mature. After the pre-cultivation, different concentrations of microplastic (PVC resin) were added to the solution to produce 100 mg/L, 200 mg/L, and 400 mg/L samples. The samples were taken every two days and lasted for 11 days.

200 mL of each sample were extracted to perform biomass collection. Centrifugation of 200 mL samples at 3000 rpm for 10 minutes. After centrifugation, the samples let dried overnight in oven at 60 °C. The supernatants were collected to examine the orthophosphate and ammoniacal nitrogen concentrations in the samples by Hach methods. The changes in the samples such as color change and flocs formation were observed through physical observations. The microscopic changes can be observed under a light microscope. The FTIR analysis aimed to determine the functional groups of the microplastic and microalgae. All results collected were further investigated and analyzed in report writing.

3.2 Microalgae stock *Chlorella Vulgaris* Pre-cultivation

Microalgae cell, *Chlorella vulgaris* was obtained from Universiti Tunku Abdul Rahman Petrochemical workshop (Block J). 1 L of water is diluted with 1 ml of fertilizer. The cultivation of microalgae is prepared by extracting 1 L of sample microalgae, *Chlorella vulgaris* then diluting with 9 L of tap water, and 10 mL of fertilizer added into the medium, total of 10 L of microalgae medium is stored in a 10 L container. The microalgae medium grows under continuous illumination (Toh et al., 2014) and is left at room temperature at 25 °C. The microalgae medium is also maintained under continuous aeration (Toh et al., 2014) by air pumping into it to ensure sufficient oxygen for microalgae growth. The microalgae medium was left to grow for 14 days. The regular observation of microalgae medium every 2 days to ensure the growth and survival of microalgae.

3.3 Concentration of Microplastics

PVC resin is weighted 0.025 g, 0.05 g, and 0.1 g by using electrical weight balance, Shimadzu AUX320 Analytical Balance. The weighted PVC resin is added to the *Chlorella vulgaris* solution to prepare different concentrations of the solution, 100 mg/L, 200 mg/L, and 400 mg/L respectively.

3.4 Preparation of nutrient-rich wastewater

Fertilizer was added to the microalgae culture with the ratio of 1 ml fertilizer to 1 L of culture medium. A total of 10 ml of fertilizer was added into the 10 L of culture medium. The fertilizer contents included ammoniacal nitrogen, nitrite, nitrate, and nitrogen (N), phosphorus (P) as the nutrients for the growth of *Chlorella vulgaris.* Synthetic wastewater consists of inorganic nutrients that serve as contaminants in water. Inorganic compounds such as ammoniacal nitrogen, nitrate, nitrogen, and phosphorus are the pollutants in wastewater, meanwhile, they also serve as the nutrient source of the algae. Algae consume nitrogen and phosphorus for their growth.

3.5 Microalgae and Microplastic Coexistence

After 14 days of cultivation, *Chlorella vulgaris* reached maturity. 250 mL of microalgae sample was taken from the culture medium and then added into the 500 mL borosilicate conical flask. Addition of microplastic (PVC resin) with different concentrations into the conical flask. The concentration of PVC resin 100 mg/L, 200 mg/L, and 400 mg/L is prepared by measuring 0.025 g, 0.25g, and 0.5 g then added into the 250 mL of microalgae solutions in the conical flask. Different concentrations of microalgae solution with PVC are triplicated to ensure consistency. A control sample of 250 mL of *Chlorella vulgaris* was prepared for testing. The mixing of PVC and microalgae solution and the control samples were left for 12 days, and tests of the samples were taken every 2 days. The setup of the coexistence of *Chlorella vulgaris* and PVC resin is shown in **Figure 3.5.1**. The solution was aerated with a pipe connected to the aeration pumps. The conical flasks were covered with cotton balls and aluminum foils to prevent evaporation.

There are three concentrations of samples, each concentration was triplicated, and one control sample of algae. A total of 54 conical flasks were throughout the experiment, the first day of the experiment was only for samples, one sample for each concentration with one control sample without the addition of PVC resin. Three concentrations of samples were triplicated, with one control sample per day. All 54 samples were placed accordingly on the rack shown in **Figure 3.5.2**. The microalgae cultivation lasts for 14 days. After the cultivation, the microalgae stock was transferred into 54 conical flasks for the addition of PVC resin.

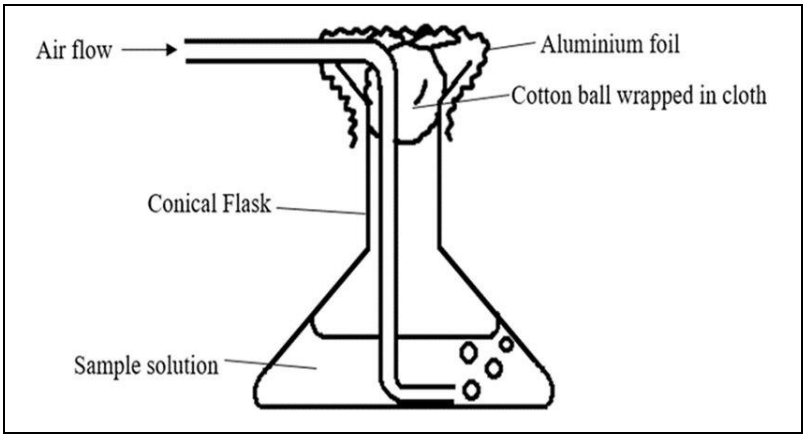

Figure 3.5.1: Setup of microalgae and microplastic coexistence in Block J.

Figure 3.5.2: The overall experimental setup in Block J, Engineering Workshop, UTAR.

3.6 Nutrient Concentration

The supernatants obtained from the centrifugation were stored below 6°C for nutrient tests. The supernatants were allowed to cool to room temperature before the analysis. The nutrient contents in the samples include nitrogen and phosphorus. Nitrogen concentrations of the samples were determined in the form of ammoniacal nitrogen. The ammoniacal nitrogen concentration was determined by using the USEPA Nessler Method 8038. The ammoniacal nitrogen removal calculated by

$$NH_3 - N(\%) = \frac{NH_3 - N_{Day\,X} - NH_3 - N_{Day\,0}}{NH_3 - N_{Day\,0}} \times 100\% \quad (3.1)$$

where X= 0, 2, 4, 7, 9, and 11.

The phosphorus concentration is determined in the form of orthophosphate. The concentration of orthophosphate was obtained by using the USEPA PhosVer 3 Ascorbic Acid Method. The removal of orthophosphate calculated by

$$PO_4^{3-}(\%) = \frac{PO_4^{3-}{}_{Day\,X} - PO_4^{3-}{}_{Day\,0}}{PO_4^{3-}{}_{Day\,0}} \times 100\% \quad (3.2)$$

where X= 0, 2, 4, 7, 9, and 11.

Both ammoniacal nitrogen and orthophosphate determination are examined by using a DR3900 Laboratory VIS Spectrophotometer.

Figure 3.6: Hach DR3900 Laboratory VIS Spectrophotometer

3.7 Biomass collection

The microalgae solution was shaken by hand to ensure homogeneity and prevent attachment of biomass on the wall of the conical flask. The microalgae solution was put into the 50 mL centrifuge tubes and then put into the centrifuge machine. To obtain 100% cell separation efficiency, centrifugation at 3000 rpm for 10 minutes was applied (Toh et al., 2017). After that, the biomass was collected and further dried overnight in an oven at 60 °C. Microalgal biomass was obtained through centrifugation by using Velocity 14 Pro Versatile Centrifuge. The model of oven used is Universal Oven XU032. The dried biomass was weighted by the electrical weight balance, Shimadzu AUX320 Analytical Balance. The biomass production can be calculated by

$$Biomass\ Production\ (g/L) = \frac{Mass\ of\ biomass\ (g)}{Volume\ of\ supernatant\ (L)} \qquad (3.3)$$

The biomass increment calculated by

$$Biomass\ Increment = Biomass_{Day\ X}\ (g/L) - Biomass_{Day\ 0}(g/L) \qquad (3.4)$$

where X= 2, 4, 7, 9, 11.

Figure 3.7.1: Velocity 14 Pro Versatile Centrifuge Machine

Figure 3.7.2: Universal Oven XU032

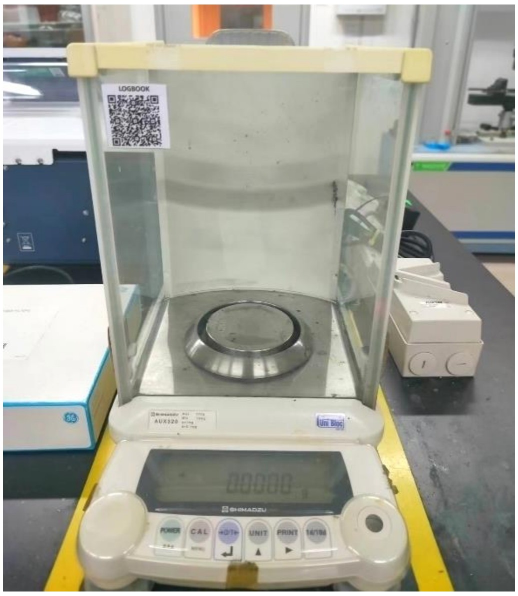

Figure 3.7.3: Shimadzu AUX320 Analytical Balance

3.8 Observation by using a light microscope

An optical microscope Leica DM500 was used in the experiment. The optical microscope was used to observe the initial shapes and sizes of microplastic and microalgae in the experiment. The microscope with a camera was used to observe the condition of microalgae with the PVC resin. The color change can also be observed under the microscope. The initial condition of microalgae compared to microalgae after being attached with PVC resin on the surface. The size of *Chlorella vulgaris* can be observed under 100X and 400X magnification with the scale bar inserted.

Figure 3.8: Leica DM500 microscope

3.9 FTIR

The FTIR spectroscopy analyzed the type of material being tested by the machine. The FTIR analysis was based on the absorbance of infrared light by the material, and the wavelength of the material. The infrared section of the electromagnetic spectrum, which has a longer wavelength and a lower frequency than visible light, is what is measured by FTIR analysis (Mathias, 2022). The fundamental idea at play is that different atoms' bonds absorb infrared light at various frequencies (Mathias, 2022). FTIR evaluation involves measuring the light with an infrared spectrometer, which generates an infrared spectrum as its output. The FTIR spectrum is a graph that plots the frequency (wavelength) on the horizontal axis and the amount of infrared light that the substance absorbs on the vertical axis (Mathias, 2022). The sample's capacity to absorb energy from infrared light at various wavelengths is examined to ascertain the molecular composition and structure of the material (Mathias, 2022). One can discover unknown components, additives within polymers, surface contamination on a material, and more using Fourier Transform Infrared Spectroscopy Analysis (Mathias, 2022). The wave number in the infrared spectrum is depicted between 4,000 and 400 cm^{-1} for mid-range IR (Mathias, 2022).

An accurate and quick detection method for analyzing polymers that have been separated from water and biological samples is ATR-FTIR (Tirkey and Upadhyay, 2021). Surface contact analysis is a type of measurement used in ATR-FTIR (Tirkey and Upadhyay, 2021). The infrared spectra were acquired using attenuated total reflectance (ATR) (Moura et al., 2023). The equipment involved is an FTIR spectrometer and an Attenuated Total Reflection accessory. With the ATR, the IR spectrum of a substance can be easily obtained by pressing the sample up against a transparent crystal, often a diamond. Infrared light enters the sample through the crystal, where it is captured by the sample and then reflected into the crystal to produce a spectrum. The Attenuated Total Reflectance (ATR) Spectroscopy of model PerkinElmer Spectrum Two FTIR Spectrometer was used to determine the functional groups of microalgae, microplastic, and the flocs formed.

Figure 3.9: PerkinElmer Spectrum Two FTIR- ATR Spectrometer

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Performance of Microalgae in Nutrient-rich Wastewater

Microalgae such as *Chlorella Vulgaris* can exist in the water body naturally or purposely used in wastewater treatment systems to remove contaminants. Inorganic substances such as nitrogen and phosphorus are the main components found in water that cause water pollution. However, high concentrations of inorganic substances in the water lead to eutrophication. The inorganic contaminants can be eliminated by microalgae.

4.1.1 The Removal of Ammoniacal Nitrogen from Synthetic Wastewater

Figure 4.1.1 (a) presents the concentration of nitrogen content in different PVC concentrations of the sample for 11 days. **Figure 4.1.1 (b)** shows the Ammoniacal Nitrogen removal rate for 11 days.

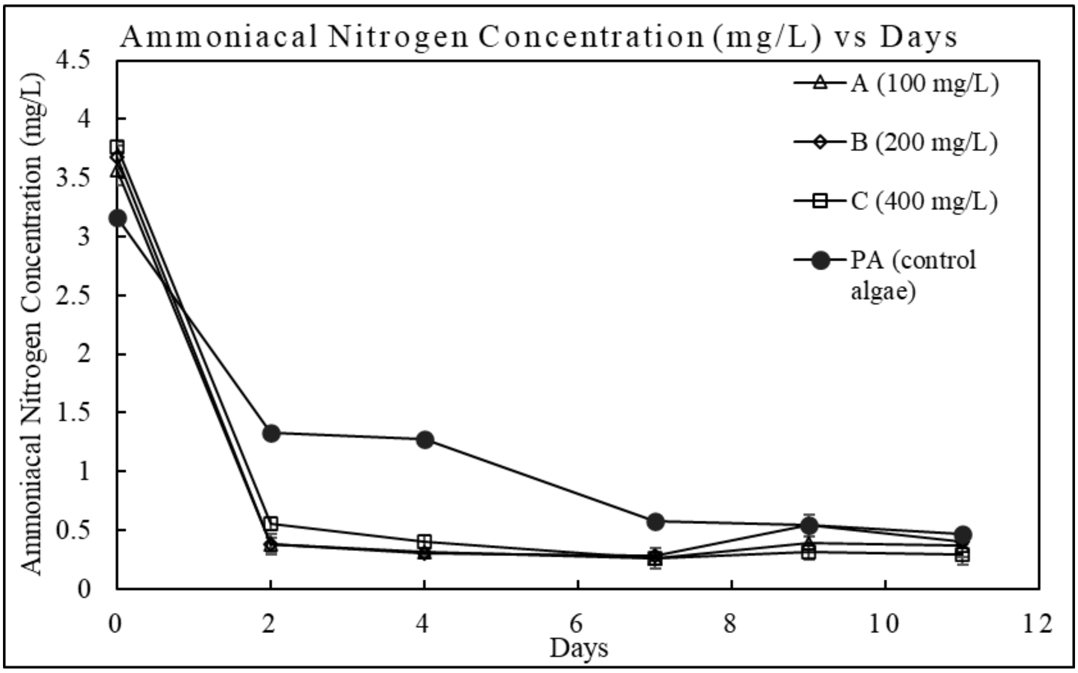

Figure 4.1.1 (a): Ammoniacal Nitrogen Concentration of *Chlorella Vulgaris* under different concentrations of PVC resin: A (100 mg/L), B (200 mg/L), C (400 mg/L), and PA for the microalgae control group for 11 days.

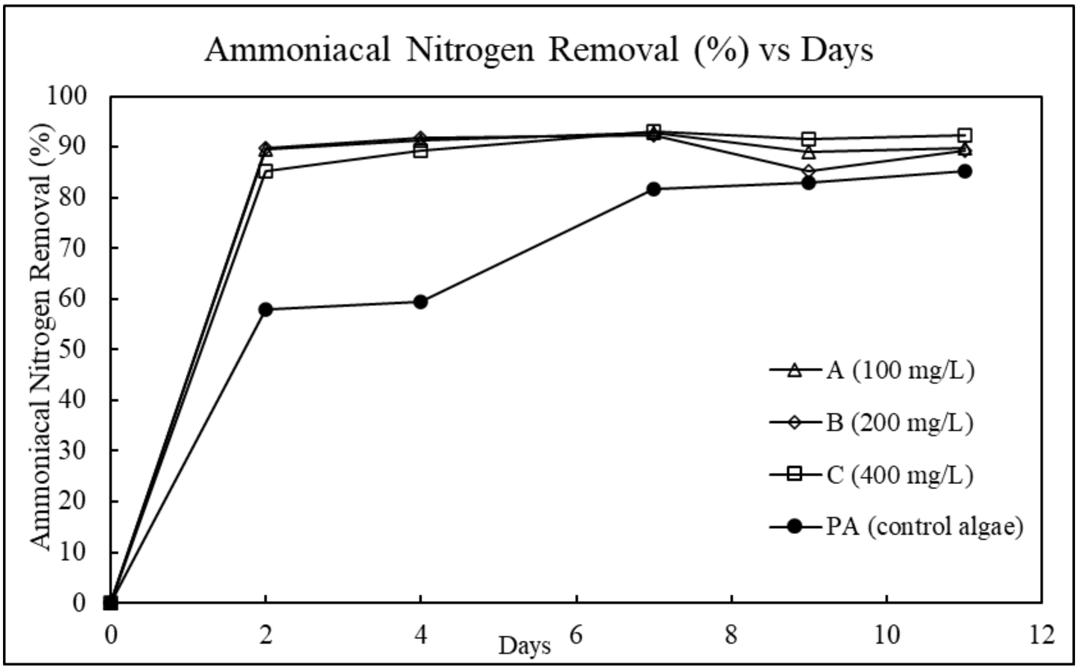

Figure 4.1.1 (b): Graph of Ammoniacal Nitrogen Removal by *Chlorella Vulgaris* under different concentrations of PVC resin: A (100 mg/L), B (200 mg/L), C (400 mg/L), and PA for the microalgae control group for 11 days

According to **Figure 4.1.1 (a)**, overall Ammoniacal Nitrogen concentrations of the samples show decreasing trends throughout the experiment. Microalgae (*Chlorella Vulgaris*) consume Nitrogen and Phosphorus as their nutrients for growth. The reduction of Nitrogen and Phosphorus concentrations indicated that *Chlorella Vulgaris* grew by ingesting the nutrients throughout 11 days. Samples A, B, and C showed the strike reduction of nitrogen from Day 0 to Day 2, this means that *Chlorella Vulgaris* had grown well by uptaking and removing the nitrogen component in the water. The control sample of *Chlorella Vulgaris* showed a gradual decrease in ammoniacal nitrogen concentrations throughout 11 days.

Four samples experienced a decrease in the ammoniacal nitrogen concentration throughout 11 days of the experiment shown in **Figure 4.1.1 (a)**. The PA (microalgae control group) showed a gradual reduction of concentrations from 3.16 mg/L to 0.47 mg/L on Day 0 and Day 11 respectively. Sample A reduced from 3.57 mg/L to 0.37 mg/L; sample B fell from 3.67 mg/L to 0.39 mg/L, while sample C declined from 3.76 mg/L to 0.29 mg/L on Day 0 and Day 11 respectively.

The removal of ammoniacal nitrogen by *Chlorella Vulgaris* was shown in **Figure 4.1.1 (b)**. All samples experienced a significant reduction on Day 2. Sample A and B achieved 89% removal, sample C 85% removal, and the microalgae control group had a lower 57% removal rate on Day 2. Samples A, B, and C had higher removal efficiency of ≥90%. The microalgae control group experienced a slightly lower removal rate of ≥80% compared to other samples.

According to Nguyen et al. (2022), through the processes of phosphorylation and nitrogen assimilation, microalgae extracted nutrients from the growth medium. In this case, the growth medium referred to synthetic wastewater which was composed of fertilizers and microplastics. The fertilizer contained nitrogen (N), phosphorus (P), and potassium (K). Inorganic substances such as nitrogen and phosphorus serve as nutrients for the microalgae, meanwhile, they were also contaminants in the wastewater. Extremely high levels of nitrogen and phosphorus induced eutrophication in the water body and caused algae bloom. Inorganic nitrogen, such as nitrate, nitrite, ammonium, and ammonia, must be converted into its organic form through a process known as nitrogen assimilation. This organic form is the basis for peptides, proteins, enzymes, chlorophylls, and energy transfer molecules like adenosine triphosphate (ATP) and adenosine diphosphate (ADP), as well as genetic materials like RNA and DNA (Nguyen et al., 2022). With the aid of nitrate and nitrite reductase, nitrate and nitrite were eventually reduced to ammonium during assimilation. Glutamate (Glu) and ATP then helped transform ammonium into the intracellular amino acid glutamine (Nguyen et al., 2022).

4.1.2 The Removal of Orthophosphate from Synthetic Wastewater

Microalgal cells require phosphorus in order to produce phospholipids, DNA, RNA, and ATP for the processes of metabolism that include the transfer of energy and the synthesis of nucleic acids (Yaakob et al., 2021). Polyphosphate or orthophosphate are two common phosphorus that are consumed by microalgae in promoting algal growth and nutritional value in the cells (Yaakob et al., 2021). Intracellular organic molecules, such as proteins, lipids, and nucleic acids, incorporate inorganic phosphorus ($H_2PO_4^-$ and HPO_4^{2-}) via phosphorylation (Nguyen et al., 2022). Multiple phosphate transporters at the microalgae's plasma membrane absorbed inorganic phosphorus for cellular phosphorous transformation. The process of transformation under light conditions, or photosynthesis, involves the synthesis of polyphosphate (such as acid-soluble and acid-insoluble polyphosphate) by polyphosphate kinase and the production of ATP from ADP.

The orthophosphate concentration exhibited in the microalgae solution in addition to concentration of PVC resin of A (100 mg/L), B (200 mg/L), C (400 mg/L), and PA for the microalgae control group are shown in **Figure 4.1.2 (a)**. Where **Figure 4.1.2 (b)** demonstrates the removal rate of orthophosphate by microalgae.

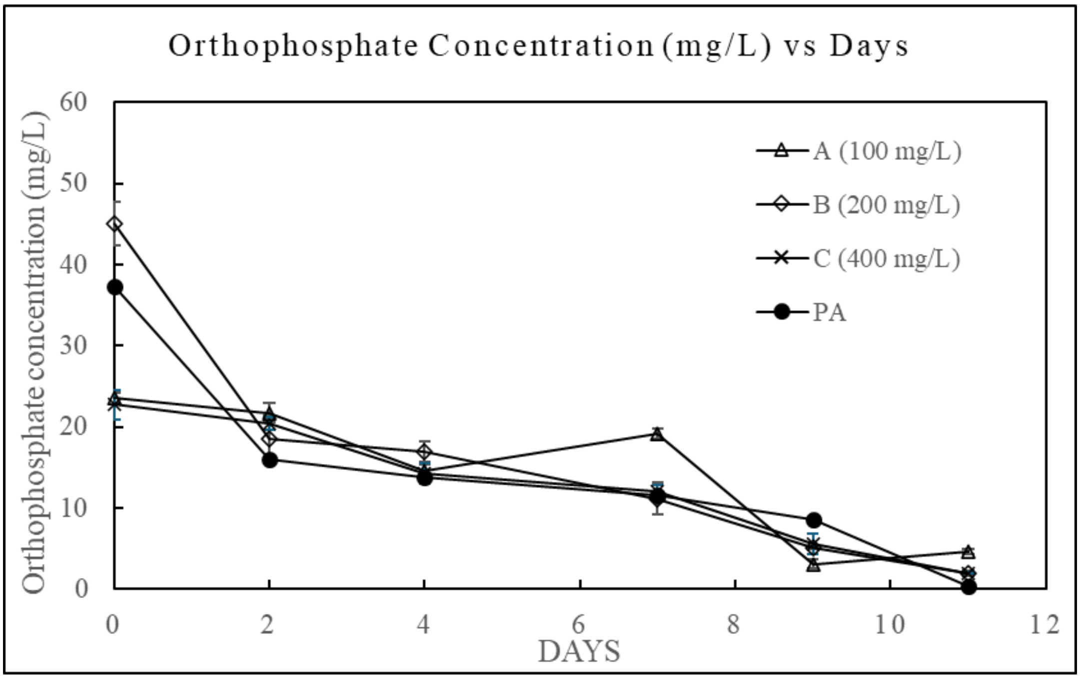

Figure 4.1.2 (a): Graph of Orthophosphate concentration *Chlorella Vulgaris* under different concentrations of PVC resin: A (100 mg/L), B (200 mg/L), C (400 mg/L), and PA for the microalgae control group for 11 days

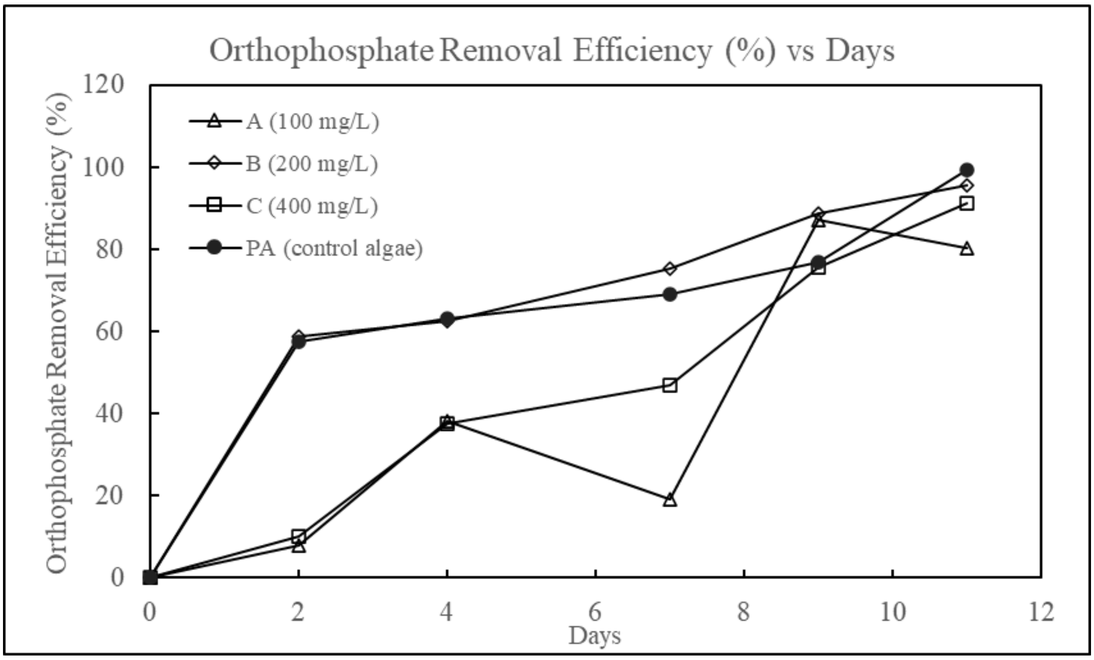

Figure 4.1.2 (b): Graph of Orthophosphate Removal by *Chlorella Vulgaris* under different concentrations of PVC resin: A (100 mg/L), B (200 mg/L), C (400 mg/L), and PA for the microalgae control group for 11 days

The overall orthophosphate concentration in four samples demonstrated decreasing trends as shown in **Figure 4.1.2 (a)**. The orthophosphate concentration of samples A, B, C, and microalgae control samples were initially high, 23.6 mg/L, 44.9 mg/L, 22.7 mg/L, and 37.3 mg/L respectively. On Day 11, 4.61 mg/L, 1.94 mg/L, 1.97 mg/L, and 0.3 mg/L orthophosphate concentrations in samples A, B, C, and the control algae group respectively. The microalgae control group had the highest orthophosphate removal rate of 99% on Day 11 shown in **Figure 4.1.2 (b)**. Sample A had 87% removal, sample B had 95% removal, and sample C had 91% removal. *Chlorella Vulgaris* removes 100% of phosphorus and 62% of nitrogen removal in wastewater (Rinna et al., 2017). Microalgae collect more phosphorus in the form of orthophosphate to promote their growth by transforming into ATP under poor nutritional supplements (Yaakob et al., 2021). When compared to the ideal concentrations required for their growth, microalgae are highly efficient at absorbing inorganic phosphate from wastewater by rapid absorption in the range of 70% to 90% according to Mulbry et al. (2008); Solovchenko et al. (2016). A study by Chu et al. (2013) showed that has higher lipid productivity up to 58.39 mg/L/day under phosphorus-sufficient conditions. These findings suggested that phosphorus concentrations considerably impacted growth, although nitrogen deprivation had minimal effect on biomass production (Chu et al., 2013). According to Nguyen et al. (2022), the rate of nutrient removal is directly correlated with the rate of biomass production. The more nutrients are removed by photosynthesis, the more biomass is produced.

4.1.3 The Growth of Microalgae in Synthetic Wastewater

The introduction of microplastic into algae could potentially affect algal growth. The microplastic size and algae species features such as cell walls affect algal growth considerably (Podbielska and Szpyrka, 2023). Different types of microplastic could variously affect algae growth. Song et al. (2020) verified that various microplastics with different sizes and different concentrations of microplastic cause different effects on various microalgae species. **Figure 4.1.3 (a)** shows the total dry weight of

Chlorella Vulgaris and different concentrations of PVC resin throughout the experiments.

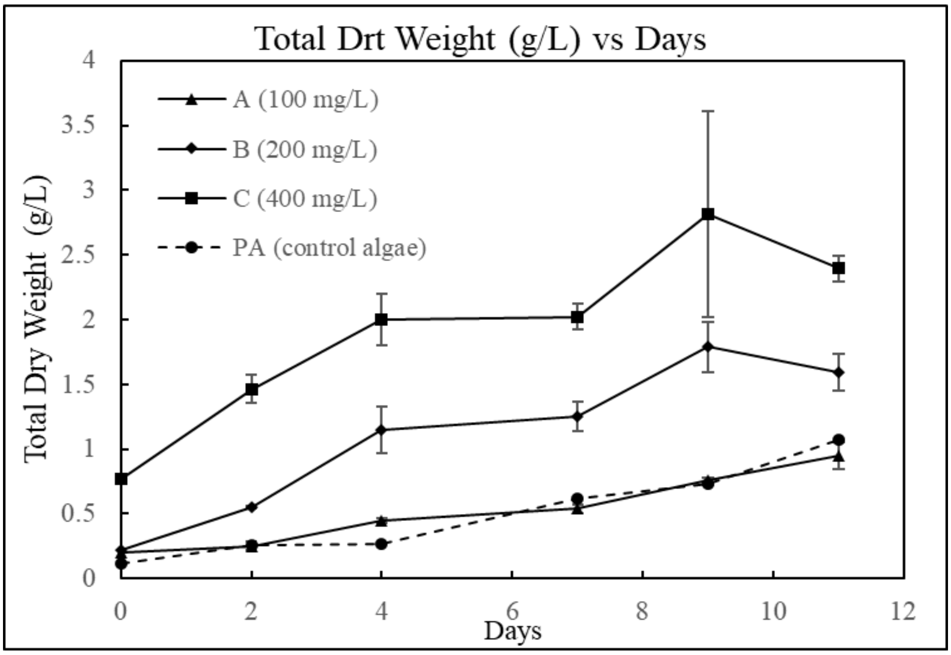

Figure 4.1.3 (a): Graph of Total Dry Weight (g/L) of various concentrations of PVC: A (100 mg/L), B (200 mg/L), C (400 mg/L), and PA for the microalgae control group in 11 days.

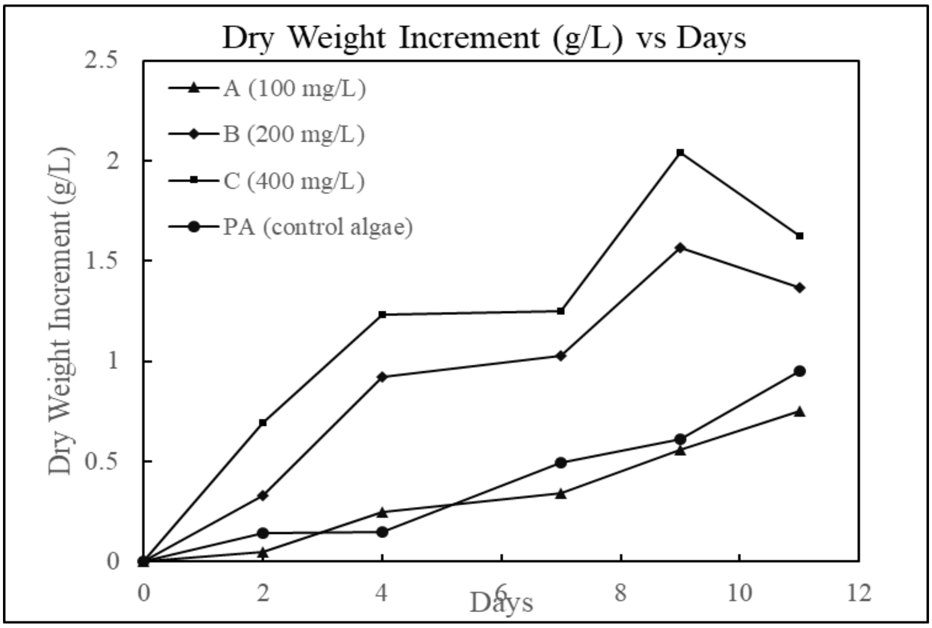

Figure 4.1.3 (b): Graph of Dry Weight Increment (g/L) of different concentrations of PVC resin: A (100 mg/L), B (200 mg/L), C (400 mg/L), and PA for the microalgae control group concentrations for 11 days.

The overall biomass production showed an upward trend throughout the experiment. This means that the microalgae (*C. Vulgaris*) kept growing throughout the experiment for 11 days. The microalgae grew in the absence and presence of microplastic as shown in **Figure 4.1.3 (a)**. However, the dry biomass includes the PVC resin added into the *C. Vulgaris*. The sequence of biomass production (from high to low) is sample C (400 mg/L) generates the most biomass, followed by sample B (200 mg/L), sample A (100 mg/L), and *C. Vulgaris* (control algae) produces the least biomass. The growth curve demonstrates that the addition of PVC resin shows a positive effect on the growth of *C.Vulgaris*. According to Prata et al. (2022), higher microplastic concentration (100 mg/l) enhances algae growth. Additional microplastics to the microalgae can be utilized as the substrates for the algal growth (Prata et al., 2022) thus, showing the enhancement of algal growth in the presence of microplastic.

The pure algae sample (control sample) shows the increasing trend of biomass generated, proving that *Chlorella Vulgaris* has grown constantly throughout the experiment. Sample A with the addition of the low microplastic concentration (100 mg/L) grew constantly throughout the experiments, proving that the low microplastic concentration does not affect the algae growth over a longer period. At a high dosage (100 mg/L) on Day 17 (exponential phase), algal growth was greatly enhanced by 0.1 µm and 1.0 µm Polystyrene with growth rates of 74.71% and 35.87% respectively (Jiao et al., 2022).

On Day 9, both samples B (200 mg/L) and C (400 mg/L) have a higher biomass production, 1.79 g/L and 2.81 g/L respectively. However, both samples B and C have a reduction of biomass generation to 1.59 g/L and 2.39 g/L on Day 11.
The growth of the *Chlorella Vulgaris* with the addition of different concentrations of PVC resin is shown in **Figure 4.1.3 (b)**. Control sample and sample A (addition of 100 mg/L PVC resin) grew continuously throughout the experiment. The Microalgae control group and sample A had the highest 0.95 g/L and 0.75 g/L increments on Day 11. Sample B (200 mg/L) and sample C (400 mg/L) grew from Day 0 to Day 9 but showed a slight reduction of biomass production upon Day 9. Sample B and C had the highest increment of 1.565 g/L and 2.042 g/L on Day 9 respectively. The biomass

production of samples B and C reduced on Day 11 (B, 1.365 g/L) and (C, 1.627 g/L) due to the experience of the death phase. This is consistent with studies by Islam et al. (2021), which show that *Chlorella sp.* reaches the death phase in 7-10 days.

A study by Fu et al. (2019) clarifies that the inhibition ratio (IR) declines with the increasing concentration of PVC ranging from 10 mg/L, 100 mg/L, and 1000 mg/L. Results show that the highest inhibition ratio is up to 28.25% by 10 mg/L of virgin and aged microplastic polyvinyl chloride (PVC) (Fu et al., 2019). In a study by Song et al. (2020), the PE, PET, and PVC with 74 µm in size and 200 mg/L promote the growth of *Chlorella sp.*. Song et al. (2020) demonstrated *Chlorella sp.* L38 grew more when microplastics were added, and microplastics might be utilized as algal growth substrates. A positive effect on algae growth with the addition of microplastics by Chae, Kim and An (2019). According to Chae, Kim and An (2019), the addition of high-concentration microplastic (200 to 350 mg/L) stimulates 125 - 140% of algal growth. This condition caused by leaching out of additive chemicals (UV stabilizer, antioxidants, and hydrophobic organic chemicals) raises the algae growth and boosts photosynthetic activities via a process called hormesis (Chae, Kim and An, 2019). In contrast, research on microplastics' ability to limit growth has shown inconsistent results, with larger particle sizes and greater concentrations frequently exhibiting growth augmentation (Prata et al., 2022).

4.2 Performance of Microalgae in Agglomerate Microplastic

4.2.1 Physical Observation of the PVC resin and *Chlorella Vulgaris*

The images were taken from the bottom of the conical flasks to observe the sedimentation of *Chlorella Vulgaris* and PVC resin throughout the experiment as shown in **Figure 4.2.1**.

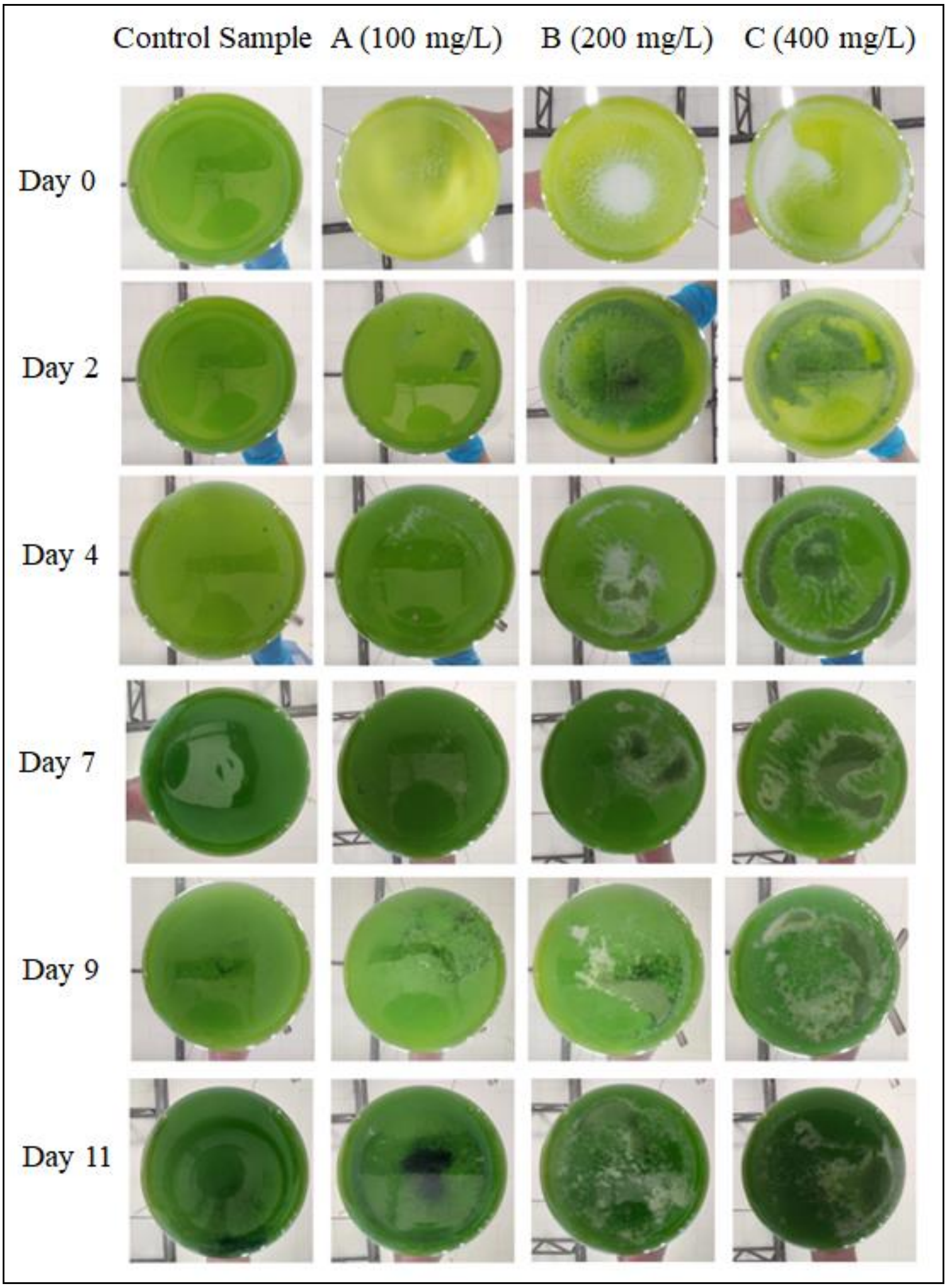

Figure 4.2.1: The physical observation of the interaction between *Chlorella Vulgaris* and different concentrations of PVC resins from day 0 to day 11.

Figure 4.2.1 shows the color change of *Chlorella Vulgaris* from Day 0 to Day 11. On Day 0, four samples, control *Chlorella Vulgaris*, samples A, B, and C showed a light green color. In the following days, the samples became darker compared to the previous day. On Day 11, four samples changed to dark green in color. The color changed from light green to dark green on the last day of the experiment indicating the growth of *Chlorella Vulgaris*.

On Day 0, the addition of PVC resin to the *Chlorella Vulgaris* did not show any interaction between them. The white PVC resin being introduced into the *Chlorella Vulgaris* was separated and settled down at the bottom of the conical flask. The sedimentation of PVC resin indicated that there was no instant reaction or interaction between PVC and algae on Day 0.

On Day 2, microplastic was found attached to the microalgae and deposited on the bottom of the flask. Meanwhile, there was some free-floating white PVC resin dispersed in the solution without clumping together with the cell. The flocs formed by aggregating *Chlorella Vulgaris* with PVC were movable when manually shaking the conical flask. The flocs formed were small and a small quantity of PVC was deposited to the bottom of the flasks. With higher concentrations of PVC resins in the medium, the larger flocs can be formed and deposited on the bottom of the flask, this situation is visible during Day 4 observation.

On Day 7, the flocs formed were deposited at the bottom of the flask immobilized and stuck to the bottom of the flask. The flocs cannot be removed by manually shaking the conical flask. Sample C formed the flocs larger than the flocs of samples A and B. This is due to the reason that higher concentration of PVC resin in sample C so that more PVC was readily available to agglomerate with the *Chlorella Vulgaris*. The results were consistent with the study by Fu et al. (2019) and Lagarde et al. (2016).

Results from Fu et al. (2019) which addition of microplastic PVC (mPVC) with different concentrations, 10 mg/L, 100 mg/L, and 1000 mg/L showed that the low concentration (10 mg/L) tended to scatter or evenly spread through the microalgae solution. The 10 mg/L mPVC well dispersed in the microalgae solution, increases the contact surface area between the cell, while 100 mg/L and 1000 mg/L of mPVC tended to agglomerate and accumulate at the bottom of the flask (Fu et al., 2019). High concentrations of microplastic that is larger than 400 µm in size do not inhibit algae growth before 63 days (Lagarde et al., 2016). It shows a reduction of cell density up to 18% after 78 days (Lagarde et al., 2016). The formation of aggregate was observed in 20 days in the presence of polypropene in the microalgae medium (Lagarde et al., 2016).

4.2.2 Observation of PVC resin and *Chlorella Vulgaris* via optical light microscope

Figure 4.2.2 shows the observation between *Chlorella Vulgaris* and different concentrations, 100 mg/L, 200 mg/L, and 400 mg/L of PVC resins under the light microscope. The images of the samples were taken under 400X magnification using a light microscope. The round shape of the green cell was *Chlorella Vulgaris* while the black with non-uniform shape was the PVC resin were observed under the light microscope.

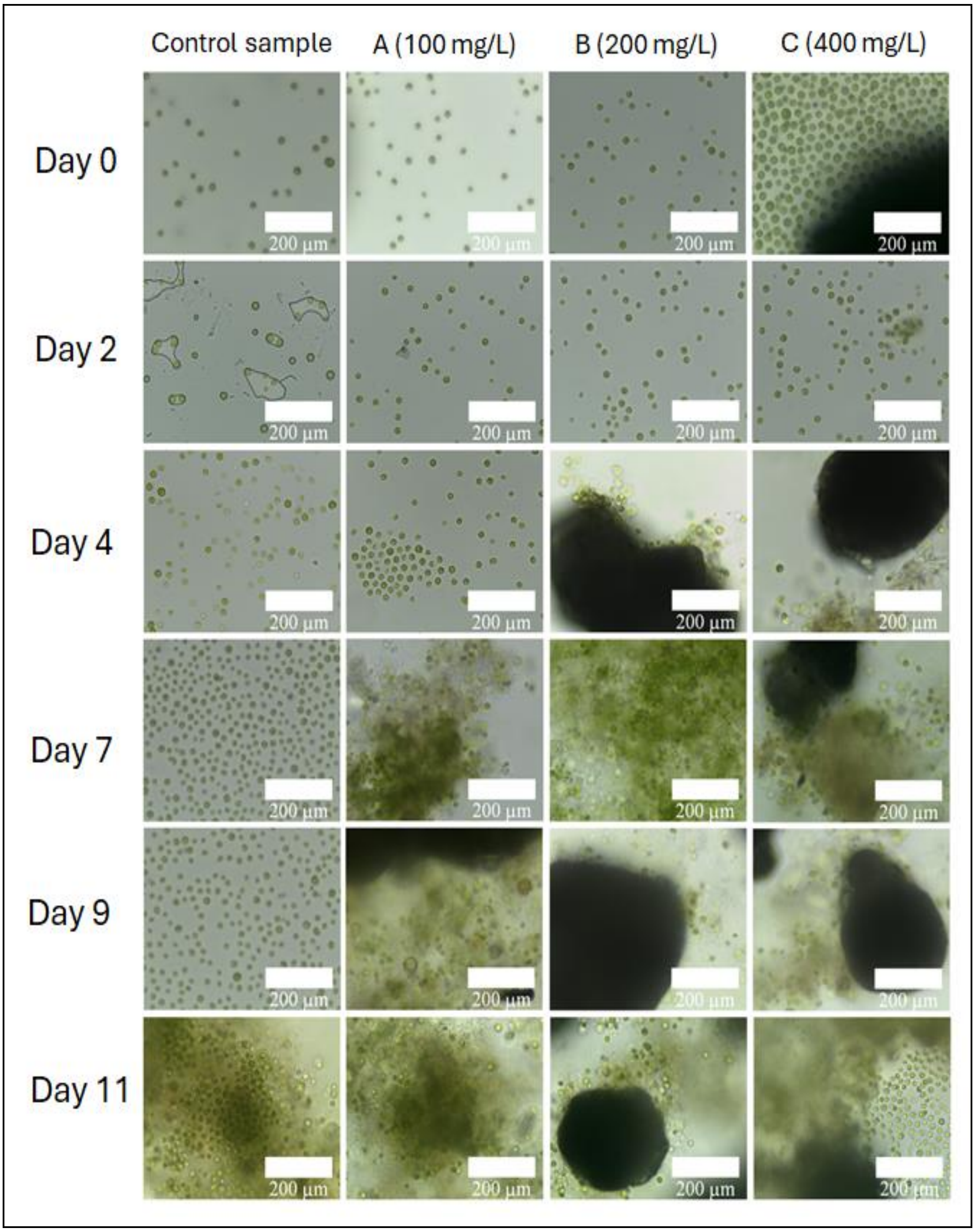

Figure 4.2.2: Observation of the interaction between *Chlorella Vulgaris* and different concentrations of PVC resins using a light microscope under 400X magnification.

The size of the PVC resin was around 200 µm and above as shown in the microscope images. The *Chlorella Vulgaris* cells were observed at approximately 14 µm based on the 200 µm scale bar as shown in **Figure 4.2.2**. Hence, the PVC resin particles are larger than the *Chlorella Vulgaris* cells in this experiment.

The control sample (pure *Chlorella Vulgaris*) grew from Day 0 to Day 11 as can be seen in the density of algae increased and the number of cells increased during the experiment. The control sample on Day 11 was observed to flocculate and clump together due to the generation of gel-like liquid, which we hypothesized as extracellular polymeric substances (EPS).

All three samples A, B, and C moved freely from Day 0 to Day 2. Sample B and C started to attach to the surface of PVC resin on Day 4. The bigger size of the black piece indicated the PVC resins that are attached by the small green cells. The outer layers of the PVC resins were coated with a layer of semitransparent liquid, known as extracellular polymeric substances (EPS), and the PVC particles were surrounded by the microalgae cell. The agglomeration of PVC and *Chlorella Vulgaris* cells aligned with the results reported (Fu et al. ,2019), which observed that the *C. vulgaris* cells were adsorbed on the mPVC surface.

The EPS generated was less on Day 4, more EPS were observed in the following days, Day 7, 9, and 11. On Day 11, it was observed that a significant number of EPS was excreted in all samples when compared to the previous days. According to Lagarde et al. (2016), microalgae tend to excrete more EPS when microplastics (such as polypropylene and high-density polyethylene) are introduced into the medium.

4.2.3 Study on the Functional group of PVC resin and *Chlorella Vulgaris* via FTIR

Figure 4.2.3.1 presents the observed functional groups of PVC and *Chlorella Vulgaris* on Day 0. **Figure 4.2.3.2** and **Figure 4.2.3.3** shows the functional groups of samples A, B, and C, microalgae control sample and PVC control sample on Day 0 and Day 11 respectively.

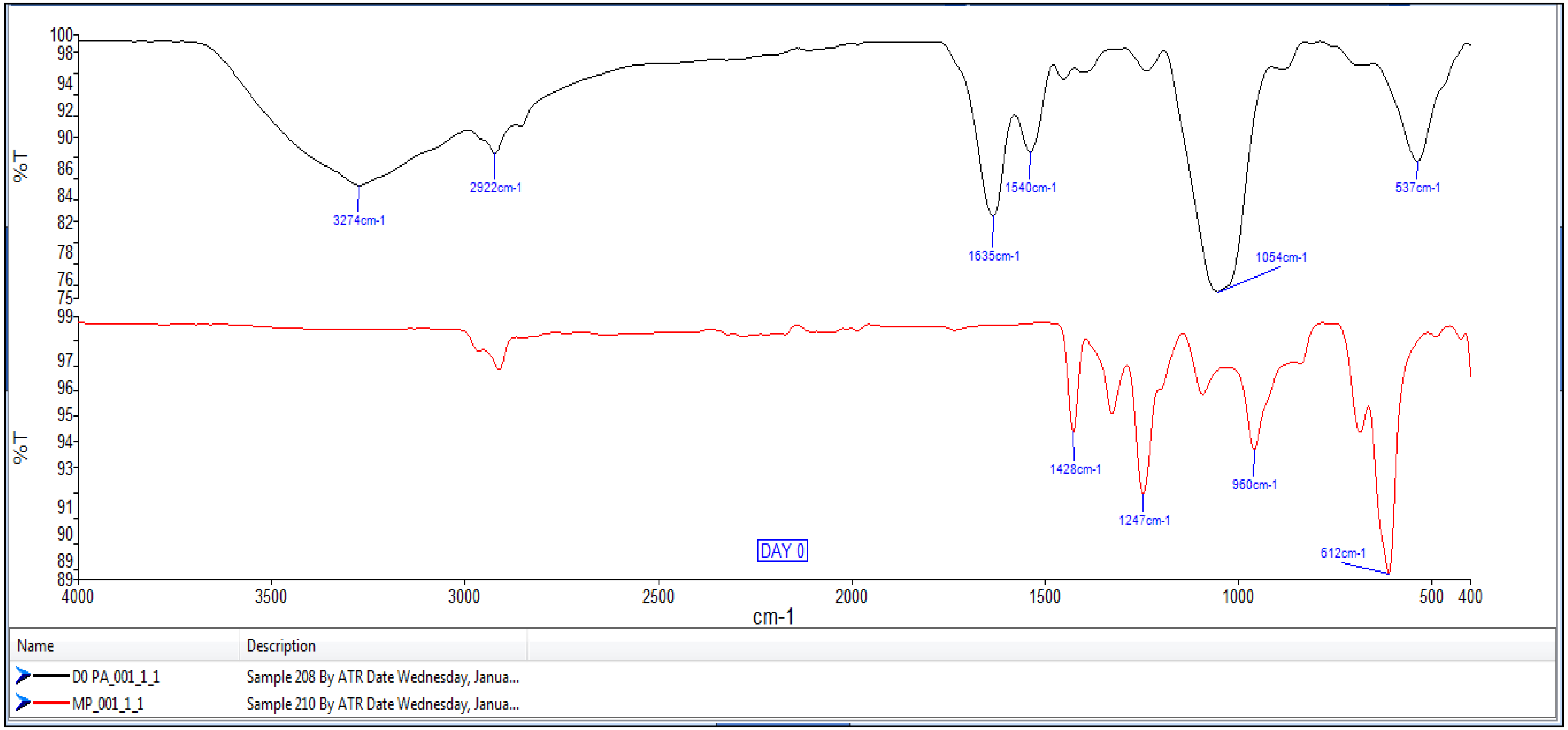

Figure 4.2.3.1: FTIR spectra of microplastic and microalgae on Day 0, black graph: *Chlorella Vulgaris*; red graph: PVC resins.

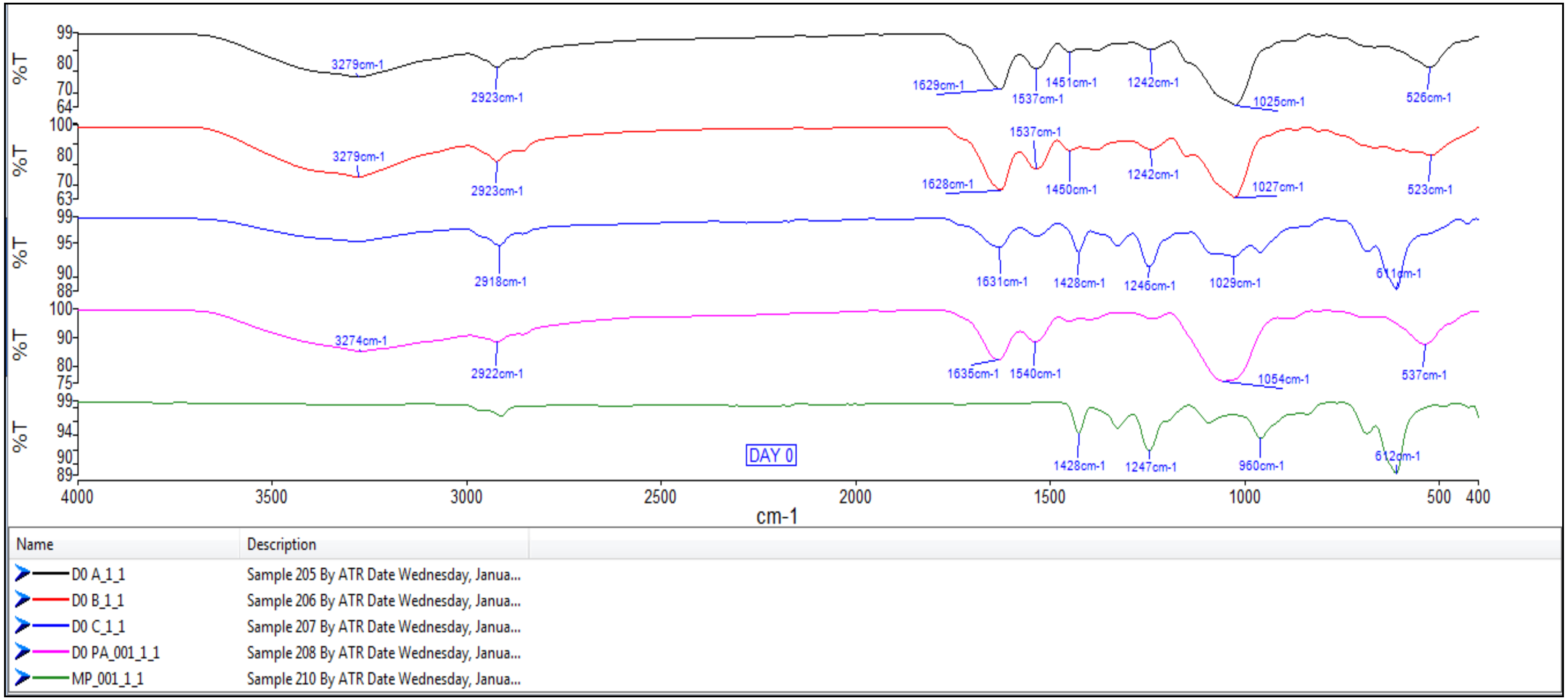

Figure 4.2.3.2: FTIR spectra of sample A (100 mg/L) in black, B (200 mg/L) in red, C (400 mg/L) in blue, control microalgae in pink, and PVC resin in green on Day 0.

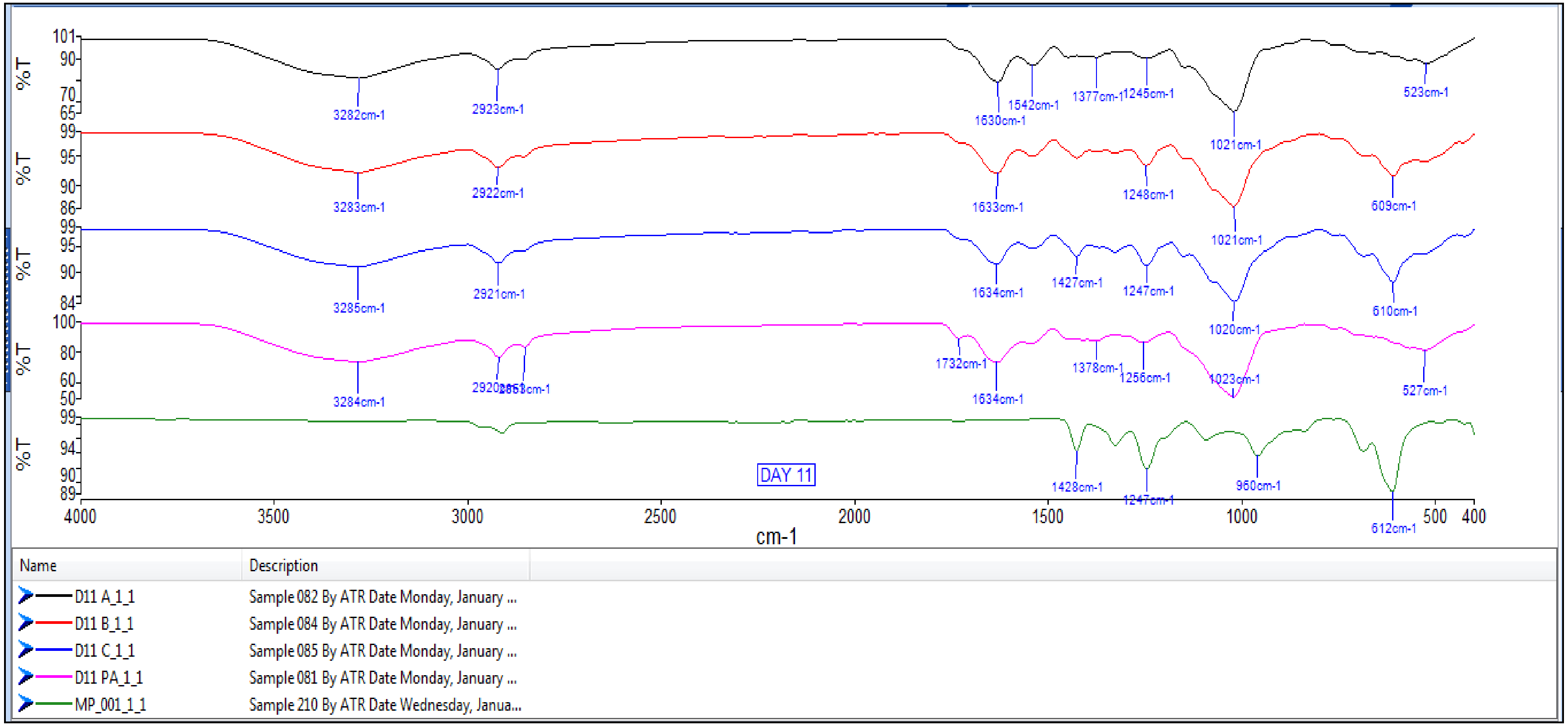

Figure 4.2.3.3: FTIR spectra of sample A (100 mg/L) in black, B (200 mg/L) in red, C (400 mg/L) in blue, control microalgae in pink, and PVC resin in green on Day 11.

4.2.3.1 Initial characterization of PVC resin and *Chlorella Vulgaris*

The dry biomass was used to conduct FTIR spectroscopy during the experiment. The samples were analyzed by using Perkin-Elmer Spectrum Two with Universal ATR. The peak absorption wavelength and correlated functional groups of *Chlorella Vulgaris* are shown in **Table 4.1**. The peak absorption wavelength and correlated functional groups of PVC are shown in **Table 4.2**. Determination of functional groups for *Chlorella Vulgaris* and PVC is shown in **Figure 4.2.3.1**.

Table 4.1: Peak absorption wavelength (cm^{-1}) with corresponding functional group of *Chlorella Vulgaris* observed on Day 0.

Peak Absorption Wavelength (cm^{-1})	Functional Groups
3274	Strong O-H and -COOH Stretching
2922	Medium C-H Stretching
1635	Medium C=O Stretching
1540	Strong N-H Bending and C-N Stretching
1054	Strong C-O Stretching
537	Strong C-I/ Strong C-Br Stretching

The infrared spectra of *Chlorella Vulgaris* have absorption peaks at wavelengths of 537 cm^{-1}, 1054 cm^{-1}, 1540 cm^{-1}, 1635 cm^{-1}, 2922 cm^{-1}, and 3274 cm^{-1} as shown in **Table 4.1**. The adsorption wavelength of 3274 cm^{-1} indicates the presence of strong O-H stretching bonds in *Chlorella Vulgaris*, confirming the presence of carboxylic acid or alcohol group in the cell. A wavelength of 2922 cm^{-1} refers to medium C-H stretching bonds which are found in lipids, and carbohydrates, 1635 cm^{-1} indicates medium C=C stretching bonds which indicate the alkene group indicates the primary amide group, 1540 cm^{-1} indicates the strong N-O stretching bonds referred to secondary amide group, 1054 cm^{-1} indicates the Strong C-O stretching bonds in carbohydrate of polysaccharide, 537 cm^{-1} indicates the strong C-I / C-Cl stretching bonds represent halo compound. Thus, the peak absorption bands found by FTIR indicate the presence of cellulose (C=O, O-H, and -COOH), amide group of protein (C=O and C-N, and N-H), lipids (-CH), and carbohydrates (C-O).

These peaks of wavelengths can be verified by Mishra and Mukherji (2012), where 3324 cm^{-1} indicates the presence of carboxylic -OH stretching, ~2925 cm^{-1} represents -CH stretching, amide group with 1652 cm^{-1}, C=O stretching and 1540 cm^{-1} N-H stretching bonds. Research by Tattibayeva et al. (2022) study on the *Chlorella Vulgaris* showed the -OH group by peak 3448 cm^{-1}, with wavelengths of 2923 cm^{-1} indicates CH groups, while the amine group with 1654 cm^{-1}.

Table 4.2: Peak absorption wavelength (cm^{-1}) with corresponding functional group of PVC observed on Day 0.

Peak Absorption Wavelength (cm^{-1})	Functional Groups
612	Strong C-Br / C-Cl stretching
960	Strong C=C Bending (class, alkene)
1247	Strong C-O Stretching
1428	Medium O-H Bending
2915	Medium C-H Stretching

The functional group of pure *Chlorella Vulgaris* and PVC resin were observed as shown in **Figure 4.2.3.1**. The infrared spectra of PVC resin have absorption peaks at wavelengths of 612 cm^{-1}, 960 cm^{-1}, 1247 cm^{-1}, 1428 cm^{-1}, and 2915 cm^{-1} as shown in **Table 4.2**. The peak wavelength of 2915 cm^{-1} refers to the medium C-H stretching, 1428 cm^{-1} indicates the medium O-H bending, 1247 cm^{-1} strong C-O stretching, strong C=C bending of 960 cm^{-1}, and 612 cm^{-1} strong C-Br / C-Cl stretching. The absorption bands can be verified by Fu et al. (2019) studies, showing 3417 cm^{-1}, 2912 cm^{-1}, 1633 cm^{-1}, 1139 cm^{-1}, and 603 cm^{-1} in virgin microplastic PVC.

4.2.3.2 Comparison of samples A, B, C, PVC resins and *Chlorella Vulgaris* between Day 0 and Day 11

All observed functional groups are listed and analyzed shown in **Table 4.3**.

Table 4.3: Comparison of the functional group of samples A, B, and C between Day 0 and Day 11.

Day 0	Samples	Day 11
3279 (-OH and -COOH) 1629 (C=O) amide I 1537 (CH and NH)-amide II 1451 (CH) 1242 (CN) amine 1025 (C-O-C) carbohydrate 526 (C-Br) halo compound	A	3282 (-OH and -COOH) **2923 (CH, OH, NH)** 1630 (C=O) amide I 1542 (CH and NH)-amide II **1377 (CH and OH)** 1245 (CN) amine 1021(C-O-C) carbohydrate 523 (C-Br) halo compound
3279 (-OH and -COOH) 2923 (CH, OH, NH) 1628 (C=O) amide I **1537 (CH and NH)-amide II** **1450 (CH)** 1242 (CN) amine 1027 (C-O-C) carbohydrate 523 (C-Br) halo compound	B	3283 (-OH and -COOH) 2922 (CH, OH, NH) 1633 (C=O) amide I 1248 (CN) amine 1021 (C-O-C) carbohydrate 609 (C-Br) halo compound
2918 (CH, OH, NH) 1631 (C=O) amide I 1428 (CH and OH) 1246 (CN) amine 1029(C-O-C) carbohydrate 611 (C-Br) halo compound	C	**3285 (-OH and -COOH)** 2921 (CH, OH, NH) 1634 (C=O) amide I 1427 (CH and OH) 1247 (CN) amine 1020 (C-O-C) carbohydrate 610 (C-Br) halo compound

Figure 4.2.3.2 and **Figure 4.2.3.3** show the peak absorption of the samples between Day 0 and Day 11. All the observed peak absorption bands are listed in **Table 4.3**. There are some minor differences between samples A, B, and C between Day 0 and Day 11 as shown in **Table 4.3**. **Table 4.3** also indicates the functional groups corresponding to their peak wavelength. The differences between Day 0 and Day 11 are due to the production of extracellular polymeric substances (EPS) of the microalgae.

In sample A, the identification of the 2923 cm^{-1} absorption peak represents the C-H bonds in polysaccharides, the wavelength of 1377 cm^{-1} was identified as C-H and O-H bonds in the protein amine group, and the lack of 1451 cm^{-1} wavelengths of C-H bonds. Polysaccharides were present at 2937 cm^{-1} peak absorption wavelengths by Li et al. (2021). For sample B, C-H and N-H bonds at 1537 cm^{-1} and 1450 cm^{-1} were observed initially but absent at the end. The peak for EPS at 1650 cm^{-1} that represented the C=O vibration in protein amide I vanished both before and after adsorption, suggesting that protein amide I molecules were involved in the adsorption (Li et al., 2021). For sample C, identification of O-H and COOH bonds at peak wavelength 3285 cm^{-1} at a later stage (Day 11) which represents the formation of carboxylic acid for the protein. This is consistent with the study of Li et al. (2021), in which 3300 cm^{-1} indicated the N-H and O-H stretching bonds in EPS.

Different absorption bands represent different functional groups. According to Wang et al. (2018), the peak absorption of wavelength in 1800 to 600cm^{-1} is separated into six regions, where the protein amide I is represented by peaks in the range of 1700 –1600 cm^{-1}, protein amide II peaks in the range of 1600 to 1500 cm^{-1}, carboxyl groups and hydrocarbons with peaks in the range of 1500 – 1300 cm^{-1}, protein amide III by peaks in the ranges of 1600 – 1500 and 1300 −1200 cm^{-1}, polysaccharides and nucleic acids by peaks in the range of 1200 to 900 cm^{-1} and the fingerprint region is denoted by peaks in the range of 900 to 600 cm^{-1}. These six ranges of absorption bands are validated in the study by Yuan et al. (2010) as well. All the peak absorption with corresponding functional groups conform to the existence of EPS components such as proteins, polysaccharides, and lipids.

4.3 Interaction between Microalgae and Microplastic

Microplastic being introduced to the microalgae did not inhibit the algal growth, as the microplastic acts as the substrate for microalgae to deposit and grow well. A variety of microplastic polymers serve as a substrate that can be extensively occupied by a wide range of microalgae communities (Nava et al., 2021). The deposition of microalgae on microplastic surfaces is known as surface colonization. Studies by Nava et al. (2021) show that through biofouling processes, a diverse range of organisms including microalgae can colonize surfaces comprised of microplastic.

In this study, we found that microalgae aggregate with microplastic by adhering to the microplastic surface. Gel-like liquids were observed under a light microscope, this was hypothesized of extracellular polymeric substances (EPS). We hypothesize that the production of extracellular polymeric substances (EPS) facilitates the adsorption and aggregation of microalgae and microplastic. The samples were analyzed by FTIR spectrum to determine the existence of functional groups. We evaluated the presence of proteins, carbohydrates, lipids, and polysaccharides in the experiments. This is consistent with Wang et al. (2018) study that several absorption bands appeared in nearly all six FTIR regions, suggesting the functional groups associated with complex proteins, lipids, and polysaccharides as well as the composition of EPS. The main components of extracellular polymer substances (EPS), polysaccharides, aromatic proteins, and humic acids, facilitate the adsorption and aggregation of particles or microalgae (Song et al., 2023). The contents in EPS such as polysaccharides and proteins are involved in the aggregation and attachment of microalgae to microplastics. According to Song et al. (2023), protein components are the major parameter to agglomerate and adsorb to microplastics because they have a higher concentration of hydrophobic groups, while polysaccharides provide lower effect in the microplastics and microalgae aggregation.

Cell aggregates and individual cells are attached to biotic or abiotic surfaces by the attractive attraction that extracellular polymeric substances (EPS) give (Lagarde et al., 2016). The generation of extracellular polymeric substances (EPS) was not species-specific, and the EPS produced by a strain of the same species can vary based on the strain's age and environmental factors (Lagarde et al., 2016). The physical characteristics of EPS, such as cohesion and stickiness, are determined by their composition (Lagarde et al., 2016).

The interaction and forces between biotic cells and abiotic particles or polymeric surfaces involve van der Waals force, electrostatic force, and Lewis acid-base interactions (Toh et al., 2014). The electrostatic force displays the main attraction between *Chlorella sp.* and iron oxide nanoparticles (IONPs) (Toh et al., 2014). This is due to the negatively charged microalgae and positive zeta potential at the surface of IONPs. The opposite charge of two surfaces tends to adhere and attract together based on electrostatic attraction. However, the IONPs can have positive zeta potential due to modified surface charge, the bare IONP are observed as zeta negative (Toh et al., 2014). The negative zeta potential nanoparticles tend to repel negatively charged microalgae due to electrostatic repulsion. Extracellular polysaccharide components facilitate the attachment of biofilms to negatively charged polymer surfaces, such as microplastics (Song et al., 2023).

Extracellular polymeric material (EPS) may adhere to particles through electrostatic or hydrogen bonding interactions, which promotes microalgae and particle hetero-aggregation (Fu et al., 2019). The aminde, aldehydes, hydroxyls, and carboxyls groups of the EPS and the microplastic surface coating may form hydrogen bonds, and facilitate interactions (Chen et al., 2012).

Microalgae carry negative zeta potential (Podbielska and Szpyrka, 2023), it tends to adhere to positively charged surfaces such as positively charged microplastics, without inhibiting algal growth. According to Podbielska and Szpyrka (2023), compared to neutral MPs, negatively charged MPs stick to cell surfaces less and damage cells less. Positively charged microplastics result in a higher algal growth inhibition ratio compared to negative zeta potential microplastics (Podbielska and Szpyrka, 2023). While the inhibition ratio of algae growth can be affected by the concentration of microplastics, Podbielska and Szpyrka (2023) reported that higher concentrations of microplastics raise the inhibition ratio of algae growth.

The interactions between two substances that are subjected to a liquid medium are van der Waals forces either attraction or repulsion interactions (Toh et al., 2014). The microalgae aggregate and sediment due to van der Waals force between the microalgae cells (Joung Sook Hong et al., 2022).

EPS are complex compounds made up of inorganic materials, proteins, lipids, polysaccharides, and nucleic acids (Podbielska and Szpyrka, 2023). Additionally, they have a lot of polar functional groups, hydrophobic chains in the polysaccharide portions, and aliphatic monomers in the protein fractions (Podbielska and Szpyrka, 2023). EPS can coagulate due to water turbulences, making them sticky and making it easier for nanoparticles to adhere to the surface of algae (Podbielska and Szpyrka, 2023).

The polar interaction plays an important role that involves biological substances which is Lewis acid-base interactions (Toh et al., 2014). The hydrophilic nature of microalgae and nanoparticles poses a higher affinity to water and facilitates binding through hydrogen bonding (Toh et al., 2014).

In short, the presence of extracellular polymeric substances (EPS) displays complex roles of physical and chemical properties between the interaction of microalgae and microplastics. The components, protein, polysaccharides, and lipids facilitate the interaction of microalgae and microplastics effectively. The aggregation of these two components can facilitate microplastic removal in wastewater treatment systems by harvesting biomass.

4.4 Sustainability of the project

Integration of microalgae in wastewater treatment aimed to address environmental issues. The growing microplastic pollution in the environment become a significant concern around the world. This project has the potential to be a sustainable measure in wastewater treatment. The aggregation of microplastics with microalgae evaluated in this project can be applied to the wastewater treatment process to treat microplastics. Microplastics can be removed when microalgae agglomerate with them, forming flocs and sediment. By harvesting the biomass, microplastic can be removed from wastewater.

Integrated microalgae-based wastewater treatment not only removes microplastics and other contaminants in wastewater. Biomass production can be repurposed for various applications such as biofuel production. This can promote a circular economy and enhance resource utilization.

Elimination of microplastics from water bodies can improve and enhance water quality which aligns with the UN-17 Sustainable Development Goals (SDGs). SDG 3, SDG 6, SDG 12, and SDG 14 can be achieved. Microplastic and other contaminants eliminated from the water aim to provide clean and safe water access to the public which aligns with SDG 6 Clean Water and Sanitation. Good water management is crucial to ensure human access to clean water from water sources. Human health and well-being can be ensured when clean water is provided. Human access to clean water can effectively reduce the risk of water-borne diseases and infections to achieve SDG 3 Good Health and Well-Being. Advanced water treatment and wastewater treatment facilities safeguard human health and the environment. Microplastic removal by microalgae method during wastewater treatment plants can effectively maintain water quality, and make sure that no microplastic is released into the environment. Hence, reduces the exposure of aquatic organisms to microplastics. This can achieve SDG 14 Life below water, where aquatic organisms are free from contamination by microplastics. The biomass generated can be repurposed for biofuel production which aligns with SDG 12 Responsible Consumption and Production. The consumption of microalgae and biomass production facilitates a circular economy that is sustainable for the environment and the ecosystem.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study has demonstrated the nutrient removal and microplastic elimination in synthetic wastewater by using *Chlorella Vulgaris*. The experiments were conducted through the coexistence of *Chlorella Vulgaris* and PVC resin. *Chlorella Vulgaris* is mixed with different concentrations of PVC resin, 100 mg/L, 200 mg/L, and 400 mg/L. The investigation on the interaction between *Chlorella Vulgaris* and PVC resin through nutrient removals, FTIR analysis, physical observation, and microscopy observation. The algal growth is monitored by harvesting biomass produced via centrifugation.

Objective 1 about the performance of microalgae in nutrient-rich wastewater is demonstrated through the nutrient removal and growth curve of *Chlorella Vulgaris*. The microalgae perform high orthophosphate removal up to 99.2%, which significantly reduces the orthophosphate concentration in the wastewater. Biomass production shows increasing trends, highest biomass generation at 2.81 g/L at a later stage in sample C. The FTIR analysis and physical and microscopy observation of the interaction between *Chlorella Vulgaris* and PVC resin aligned with Objective 2. The FTIR shows the functional groups of *Chlorella Vulgaris* and PVC resin. It evaluates the presence of proteins, carbohydrates, and lipids are present in the experiments. The physical and microscopy observations show the aggregation and sedimentation of PVC and *Chlorella Vulgaris*. The excretion of extracellular polymeric substances (EPS) was hypothesized and observed under microscopy determination. The aggregation and adsorption of microalgae on the surface of microplastics were demonstrated in the experiment. Thus, utilization of microalgae in wastewater treatment contributes to clean and safe environment by eliminating microplastics and unnecessary nutrients from the wastewater. This can contribute to Sustainable Development Goals (SDGs), creating a sustainable environment with clean and safe water. Human health and animals well-being are preserved.

5.2 Recommendations

- To obtain accurate biomass production, separation of microplastic from microalgae is required before conducting the centrifugation and drying process to obtain the yield of biomass from microalgae.
- To further understand the extracellular polymeric substances (EPS), the colorimetric method can further analyze the EPS.
- Suggest determining the interaction of microalgae using different species such as *Scenedesmus sp.*, and *Spirulina sp.*, whereas different types of microplastic such as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET).
- Determination of remaining microplastic concentrations after the biomass harvesting to evaluate the removal efficiency of microplastics.

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