MICROALGAE CULTIVATION IN STABILIZED LANDFILL LEACHATE FOR SIMULTANEOUS TREATMENT AND BIOMASS PRODUCTION

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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

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Date : 9-5-2023

Specially dedicated to

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

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MICROALGAE CULTIVATION IN STABILIZED LANDFILL LEACHATE FOR POLLUTANTS REMOVAL AND BIOMASS PRODUCTION

ABSTRACT

Landfill leachate is highly toxic and requires treatment before being released into the environment. Thus, an economical and eco-friendly solution is required. In recent years, microalgae treatment has emerged as an alternative to other conventional treatments as they have high nutrient recovery abilities and potential for bio-fuel production. However, high concentrations of inhibitory compounds and ammoniacal nitrogen in young landfill leachates require high dilutions for microalgae to thrive. This study aims to evaluate the performance of microalgae using stabilized landfill leachate instead with lower to no dilutions in nutrient removal, biomass and lipid production. Leachate concentration of 33v/v%, 44v/v%, 66v/v%, 89v/v% and 100v/v% were treated with the microalgae *C. vulgaris*. Parameters of Chemical Oxygen Demand (COD), ammoniacal nitrogen (NH_3–N), orthophosphate (PO_4^{3-}), total phosphorus (TP) and colour removal were evaluated. Highest removals of 43.67% for COD, >97.00% for NH_3–N, 79.26% for PO_3^{3-}, 77.64% for TP and 44.04% for colour were achieved. Highest biomass yield obtained was 220 mg/L by 89v/v% leachate concentration with 8.14% lipid yield. It is possible to treat stabilized landfill leachate without any dilutions using microalgae, as they can survive the conditions, perform nutrient removals and produce biomass simultaneously.

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COD	Chemical Oxygen Demand
NH_3–N	Ammoniacal Nitrogen
PO_4^{3-}	Orthophosphate
TP	Total Phosphorus
N	Nitrogen
P	Phosphorus
NH_4^+	Ammonium
NH_3	Ammonia
g	Grams
mg	Milligrams
L	Litre
cm	Centimeters
m	Meters

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In the 21st century, rapid expansion of the human population and urbanisation have led to a huge increase in the world's production of municipal solid waste. As landfill is one of the most convenient and economical method for municipal solid waste disposal, large amounts of solid waste are being disposed in landfills every day (Ren et al., 2022). In 2021, an estimated 38,427 metric tons of municipal solid waste were generated per day in Malaysia, where 82.5% of the waste were disposed in landfills. According to the data published, a total of 137 of the landfill sites are still in operation while 174 landfill sites were already closed in Malaysia (MIDA e-Newsletter, 2021). Only a total of 21 landfill sites out of the 137 operating landfills are sanitary landfills sites, which leads to the concern of improper landfill leachate treatment and management.

Landfill leachate is the liquid originating from landfill sites via rainwater infiltrations through the soil and waste layers, humidity from the disposed waste covered under the soil layers, and also through production via biological degradation processes (Peng, 2017). Leachate has highly variable composition affected by many different factors such as landfill age, composition of waste and humification degree (Liu et al., 2022). However, most landfill leachate share the common characteristics which are having high ammoniacal nitrogen (NH_3–N), organic compounds, chloride, sulfate and heavy metal concentrations (Lebron et al., 2021). Such characteristics contribute to high toxicity and is not safe to be released into the environment according

to the National Water Quality Standards for Malaysia in the Environmental Quality Act 1974, thus it is essential for leachate to undergo treatment.

The restrictions of conventional treatment methods for leachate such as high-energy requirements and environmental impacts caused by greenhouse gas emissions (Muga and Mihelcic, 2008) have led to an increased interest in phycoremediation or microalgae-based wastewater treatment as an alternative for an economic, sustainable and effective process (Morillas-España et al., 2022). Phycoremediation, where '*phyco'* means 'algae' in Greek, is a process of using algae including microalgae, macroalgae and cyanobacteria for treating wastewater to remove pollutants or to obtain products from wastewater such as algal biomass (Dayana Priyadharshini et al., 2021).

Microalgae has piqued interests in the recent years as a treatment system for wastewater and leachate, as it is a low-cost, ecological and a relatively novel alternative to other conventional treatment methods (Nawaz et al., 2020). Being considerably efficient in nutrient removal especially for nitrogen sources and also in heavy metal removal, it can be used as an alternative or even alongside other aerobic and anaerobic biological processes to enhance the treatment processes. Not only that, one of its major advantages is its ability for resource recovery, being able to produce economically valuable biomass that can be used for biofuel production, whilst treating waste such as leachate at the same time (Ray et al., 2019).

1.2 Problem Statement

Leachate production and discharge into water bodies can negatively impact aquatic ecosystem, thus it is important to have a suitable, economical and ecological solution for landfill leachate treatment. Microalgae is a suitable solution that does not release greenhouse gases compared to other conventional treatments such as the anaerobic biological treatment that releases methane gases. On the other hand, it is able to perform carbon capture, utilising carbon dioxide from the atmosphere for growth. Not only that, it is able to produce biomass products such as biofuel that contributes to

substituting non-renewable sources and other plant-based biofuels (Prabandono and Amin, 2015; Shay, 1993).

Microalgae is often studied for treatment of wastewaters, however research studies on leachate are comparatively lesser and almost none focused on stabilized landfill leachate. Most studies focus on using young or intermediate leachates with high nitrogen content and heavy metal concentration which causes high toxicity that inhibits growth of microalgae. Thus, dilution was frequently done to reduce the toxic levels, to a concentration of 5% to 10%, to have more effective nutrient recovery by microalgae and microalgal growth in young or intermediate leachates (el Ouaer et al., 2017; Porto et al., 2021; Nordin et al., 2017; Hernández-García et al., 2019).

On the other hand, stabilized landfill leachates naturally have lower heavy metal concentration and other nutrient contents due to its age as the landfill enters methanogenic stage. Thus, the necessity for diluting the leachate can be reduced. This study aims to evaluate such performance of microalgae in treating stabilized landfill leachate with lower to no dilutions (at least 30% leachate concentration), the optimum duration for treatment and biomass collection. For the purpose of evaluating its potential to be utilised for biofuel production, the biomass from the treatment will also be characterized for lipid yield to check for its suitability as biofuel. The data from this study has the potential to not only apply on stabilized landfills, but also on other scenarios such as polishing of treated young leachates.

1.3 Objectives

The objectives of this study are as following:

i. To evaluate the performance of microalgae in treating stabilized landfill leachate.
ii. To determine the optimum microalgae cultivation duration for treatment of stabilized landfill leachate and biomass harvesting.
iii. To determine the lipid yield of biomass samples.

1.4 Novelty

The novelty statements of this study are as following:

i. This study concentrates on using stabilized landfill leachate which is rarely focused in other studies. Stabilized landfill leachate has different characteristics compared to young and intermediate leachates mostly used in other literature.

ii. Previous literatures focus on high dilutions of leachate, up to 5-10v/v% leachate concentration. This study will test on the performance of microalgae in stabilized landfill leachate with lower dilution levels.

1.5 Scope of Study

This study revolves around the treatment efficiency of the microalgae *C. vulgaris* on stabilized landfill leachate using different dilutions in a duration of 19 days. Characterisation was done on untreated raw leachate and treated leachate to determine the treatment efficiency, which main parameters include COD, ammoniacal nitrogen, orthophosphate, total phosphorus, and colour. Obtained biomass was also measured to evaluate the survivability of microalgae in different landfill leachate dilutions. Data was collected 9 times throughout the experimental duration to determine the optimum duration of microalgae cultivation for best treatment efficiency and highest biomass yield. Optimal samples were determined and tested for lipid yield to determine its potential for biofuel production uses.

1.6 Thesis Outlines

The thesis outline starts from the current Chapter 1: Introduction of research, which elaborates on the background and issues of studies, novelty, objectives and scope of research. It is followed by Chapter 2: Literature Review which provides a deep understanding and a forecast on the outcome of the experiment based on past research studies. The flow of experiment with the standard procedures will be presented in Chapter 3: Methodology, followed by the experimental outcome with analysis and discussion in Chapter 4: Results and Discussion. Lastly, a final conclusive writing of the study along with recommendations will be outlined in Chapter 5: Conclusion and Recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Landfilling as Municipal Solid Waste Management in Malaysia

Municipal solid waste (MSW) is the solid waste that originates from municipal, residential, commercial, recreational and institutional activities that includes garbage, street cleanings, common medical waste and other wastes that are non-industrial (Speight, 2015). Typical composition of Malaysian MSW composes of a huge percentage of organic waste, followed by plastic, paper, etc, as shown in **Figure 2.1** (Salwa Khamis et al., 2019). Malaysians produced an estimated amount of 38,427 metric tons of municipal solid waste in the year 2021, of which 82.5% being disposed in landfills (MIDA e-Newsletter, 2021). Meanwhile according to the World Bank, the world generates an estimated of 2.01 billion tonnes of MSW every year, which is expected to reach 3.4 billion tonnes by the year 2050 (Silpa et al., 2018). With the increasing production of MSW, management systems need to be established properly for cleanliness and sanitary purposes.

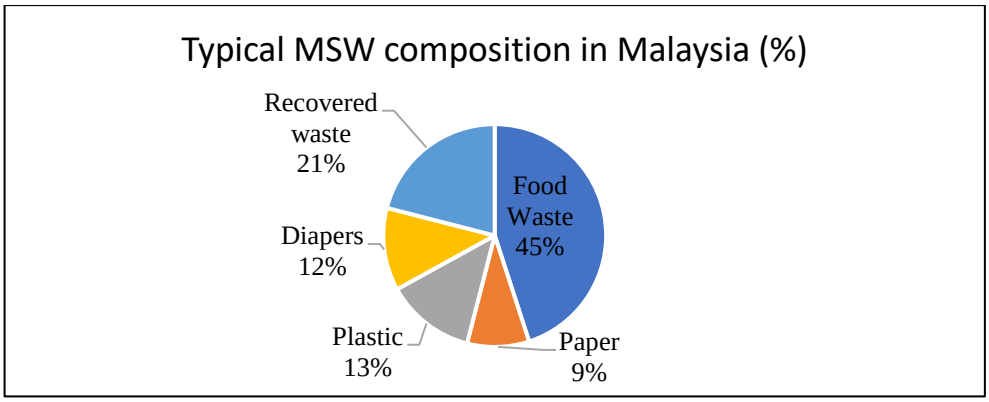

Figure 2.1 Typical MSW Composition in Malaysia, recovered waste includes incombustible materials and recycled waste such as rigid plastics, glass and metals (Salwa Khamis et al., 2019).

Landfilling and incineration are both common methods for handling MSW, however considering that incineration is more costly, releases large amounts of harmful gases and leaving toxic residues that also needs to be handled, landfilling has become the more popular option of the two even though incineration provides numerous benefits (Yu et al., 2022). To date, there are only 4 operating incineration plants in Malaysia located in Pulau Pangkor, Pulau Langkawi, Cameron Highlands and Labuan (Yong et al., 2019; Nathan, 2021), while there are 137 operating and 174 closed landfills in Malaysia (MIDA e-Newsletter, 2021). One of the reasons is that Malaysian MSW contains high moisture content which causes a challenge in finding an incineration technology capable of handling such waste with low operating cost, causing landfilling to be a more common disposal method of MSW in Malaysia (Samsudin and Don, 2013).

Landfilling has a lot of advantages such as being more economical, the disposal procedure being simple, able to solve large amount of waste generated per day and having effects of restoring landscapes on places such as mineral working holes (Bashir et al., 2015; Ngoc and Schnitzer, 2009). However, one of the main problems of MSW landfills is the generation of leachate that is toxic and hazardous. Leachate contains heavy metals, inorganic salts, microorganisms, persistent organic matters, xenobiotic compounds, and other toxic matters that can even be produced after landfill closure, posing as an environmental threat (Anna Tałałaj et al., 2021; Morello et al.,

2016; Kjeldsen et al., 2010). Thus, a leachate collection system and a proper leachate treatment must be done in order to reduce its adverse effects on the environment.

2.2 Landfill leachate and stabilized landfill leachate

Leachate is generally characterised by its high chemical oxygen demand (COD), biological oxygen demand (BOD), high levels of ammonia, organic and inorganic contaminants, heavy metals and refractory compounds (Poblete Chávez et al., 2019). The pollutants in a MSW landfill leachate can be divided into 4 groups: the first group including dissolved organic matter measured as COD or Total Organic Carbon (TOC), volatile fatty acids and refractory compounds including fulvic and humic-like compounds; the second group being inorganic macrocomponents such as calcium, magnesium, sodium, ammonium, chloride ions, etc; the third group being heavy metals such as cadmium, lead, copper, nickel and more; the fourth group being xenobiotic organic compounds (XOC) that are usually low in concentration including aromatic hydrocarbons, phenols, plasticizers, pesticides and more (Kjeldsen et al., 2010). Its generation depends on many factors such as the composition of waste, their particle size, temperature, moisture, degree of compaction on waste, site hydrology, oxygen availability and age of landfill (Shadi et al., 2020).

Generally, landfill leachates can be categorized into three categories based on their age and difference in composition, which are young, intermediate, and stabilized landfill leachate (Abuabdou et al., 2021). Young landfills lesser than 5 years old produce leachate with extremely high COD, BOD_5, and BOD_5/COD ratio, with strong colour and bad odor; whilst older landfills more than 10 years old produce stabilized leachates with moderately high COD and NH_3–N but lower in BOD_5/COD ratio (Shadi et al., 2020). **Table 2.1** below shows the comparison between young, intermediate and old landfills with pH, COD, BOD_5/COD ratio, NH_3–N, heavy metal (H.M), volatile fatty acids to humic and fluvic acid ratio (VFA/HFA) and biodegradability.

Table 2.1: Comparison between young, intermediate, and old landfills (Mojiri et al., 2021).

Age	Young	Intermediate	Old
Years	0-5	5-10	>10
pH	<6.5	6.5-7.5	>7.5
COD (mg/L)	>10,000	5,000-10,000	<5,000
BOD_5/COD	0.5-1.0	0.1-0.5	<0.1
NH_3–N (mg/L)	<400	N.A.	>400
Heavy Metals	Medium to low	Low	Low
VFA/HFA	VFA (80%)	VFA (5-30%) + HFA	HFA (80%)
Biodegradability	High	Medium	Low

VFA: Volatile fatty acids; HFA: Humic and fulvic acids; N.A.: Not Available.

Landfill can undergo at least 4 phases of decomposition: initial aerobic phase, anaerobic acid phase, initial methanogenic phase and finally stable methanogenic phase (Kjeldsen et al., 2010). Stabilized landfill leachates are produced when the landfill matures over a period of time and enters the methanogenic stage. Besides having lower COD value, stabilized landfills also have higher methane concentrations (Anna Tałałaj et al., 2021). This is due to methane being generated by methanogens that can go up to 55 to 60% of gas present in landfills (Shadi et al., 2020). Stabilized landfill leachate also consists of an increased concentration of recalcitrant compounds such as humic and fulvic acids that makes it difficult to treat, which are the main contributors to the COD in stabilized leachate (Anna Tałałaj et al., 2021; Gao et al., 2014). The low BOD_5/COD ratio also suggests that the leachate has low concentrations of volatile fatty acids and higher amounts of recalcitrant compounds (Kjeldsen et al., 2010).

Various compounds contribute to different parameters in leachate. The brown colour of leachate is contributed from humic and fulvic substances, also from oxidation of ferrous to ferric form, and the formation of ferric hydroxide colloids (Shadi et al., 2020; Shehzad et al., 2016), with the main contributor of colour to be humic substances (Naveen et al., 2016). Meanwhile, ammoniacal-nitrogen is released from waste when decomposition of proteins occurs, where decreasing trends are low and slow as there

are no mechanisms for its degradation inside the landfill during methanogenic conditions (Kjeldsen et al., 2010).

2.3 Current Leachate Treatment Technologies

Leachate treatment technologies include biological and physiochemical treatment methods, being used each on its own or even combined. Different methods perform differently, such as biological methods having higher efficiency in biodegradable material removal, adsorption processes for organic compounds and metals, advanced oxidation processes for complex organic compounds, and so on. Current treatment methods on leachate that are common will be further discussed below, with a summary of comparison between their advantages and disadvantages in **Table 2.2**.

2.3.1 Physiochemical Treatment

Physiochemical treatment is effective for removal of refractory compounds, ammonia, and organic compounds with low biodegradable organic fraction. It generally has a higher cost and lower effectiveness compared to biological treatment processes, thus often only used as a subsequent polishing step in combination with biological treatments for better treated effluent quality (Loukidou and Zouboulis, 2001).

2.3.2 Coagulation-Flocculation Process

The coagulation-flocculation process is one of the most common treatment methods especially in wastewater industries. Its concept involves using a coagulant to destabilize the colloidal suspension of particles and using a flocculant to agglomerate the destabilized particles, forming flocs that are easier to settle, which are often

separated from the leachate treatment using a clarifier (Lee, et al., 2012). Examples of coagulants and flocculants include alum, aluminium sulphate, polyaluminium chloride (PAC), polyacrylamide (PAM), ferric chloride and so on.

2.3.3 Adsorption

Adsorption is also one of the commonly used treatment methods, with common absorbents being activated carbon, chitosan, zeolite, plant-based clay minerals and even industrial waste (Yu and Han, 2015). Its mechanism is attracting and attaching the fluid mixture which will be the wastewater or leachate to the surface of the adsorbent through physical or chemical bonds (Reshadi et al., 2020). One of the factors that determine the efficiency of pollutant removal of an absorbent is the porosity which increases the total surface area of the absorbent, thus which is why the forementioned absorbents being quite popular.

2.3.4 Membrane technology

Membrane filtration technology includes processes such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). Its concept is simple, which is by using the membrane to separate solid immiscible particles from liquid or gaseous streams. In leachate treatment, these processes are promising, with reverse osmosis process reportedly being able to remove more than 99% of organic macromolecules and colloids, while nanofiltration removing 60-70% COD and about 50% ammonia from leachate (Anand & Singh, 2014). This technique has advantages of not requiring chemical additions and being able to reuse the membranes, however has a major disadvantage of membrane fouling (Ezugbe and Rathilal, 2020).

2.3.5 Ion Exchange

The ion exchange method is commonly used to remove organic and toxic substances from wastewater and even used in water treatment processes. The process involves exchange of undesirable ionic contaminants with another less or non-objectionable ionic substance from the ion exchanger which is a resin (Khan and Mondal, 2021). It is also useful to remove low concentrations of heavy metal but the downside is that it is non-selective (Khan, 2021).

2.3.6 Oxidation and Advanced oxidation processes (AOP)

Oxidation process in leachate is a process of mineralization of contaminants to inorganics, water and carbon dioxide or other less harmful products (Zhou et al., 2010). Oxidants such as chlorine, ozone and potassium permanganate are common substances being used in wastewater and leachate treatment for oxidation. On the other hand, advanced oxidation process (AOP) is a process of using strong oxidants combinations, irradiation and catalysts to further enhance the treatment efficiency.

2.3.7 Biological treatment

Biological treatment involves the use of microorganism to convert organic compounds into carbon dioxide and biomass in aerobic conditions or biogas in anaerobic conditions for via biodegradation (Renou et al., 2008). It is a process that is efficient in removing organic and nitrogenous matters in leachate. However, high refractory compounds are known to limit biological treatment processes effectiveness.

Common biological treatment examples used in leachate include suspended growth process and attached growth process. Large scale treatments involve uses of lagoons, activated sludge processes and bioreactors.

Table 2.2: Summary table of common physio-chemical and biological treatment processes for landfill leachate.

Type of Treatment	Example Process or Material	Advantages	Disadvantages	References
Coagulation-flocculation	• Ferric & alum, aluminium sulphate.	• Efficient for stabilize and old leachate, economic.	• Sludge produced, increase of aluminium or iron in liquid phase.	(Assou, et al., 2015)
Adsorption	• Activated carbon, zeolite.	• Efficient in removal of dissolved organic matter and NH_4^+–N.	• Frequent regeneration and high absorbent consumption.	(Teng et al., 2021)
Membrane-based filtration	• Microfiltration, ultrafiltration, nanofiltration, reverse osmosis.	• Highly effective in reducing organic and inorganic substance. • Generate high quality effluent.	• Membrane fouling increases operation costs. • The pollutant concentrated membrane is even more difficult to treat.	(Teng et al., 2021)
Ion exchange	• Cationic Resin, Anionic resin.	• Efficient in removing dissolved inorganics and ammonium nitrogen.	• Need to regularly regenerate ion exchange resin and high operation cost.	(Bashir, et al., 2015)

Oxidation	• Advance Oxidation Process, ozonation, Fenton process.	• Reach complete mineralization, effective for degradation of recalcitrant organic pollutant.	• High energy, cost and dosage, and produce toxic intermediate products.	(Teng et al., 2021)
Attached-growth biomass systems	• Trickling filter, moving-bed biofilm reactor, rotating biological contractors.	• Active biomass conservation, less sensitive to low temperature and toxicity, better endurance in inflow variability.	• Potential clogging, unwanted fungal growth. • Odour generation.	(Nath and Debnath, 2022; Saxena et al., 2022)
Suspended growth Biomass Process	• Aerobic: aerated lagoons, activated sludge. • Anaerobic: digester, sequencing batch reactor (SBR), Up-flow anaerobic sludge blanket (UASB).	• Effective removal of organic carbon, nutrients, and ammonia content. • Low cost and short retention time (UASB).	• Sensitive to temperature, pH, and chemicals. • Poor sludge settleability, excess sludge produced and large settling tank volume. • Skilled labour required for anaerobic processes.	(Nath and Debnath, 2022; Saxena et al., 2022)

2.4 Microalgae and their General Applications

The term ‘microalgae’ refers to algae that are too small for the naked eye to see, including a diverse group of both eukaryotic autotrophic protists and prokaryotic cyanobacteria (Mohd Udaiyappan et al., 2017). They are usually unicellular and in sizes of 5 to 50 μm, characterized by a simple morphology (Singh and Saxena, 2015). Microalgae exist at almost everywhere, green microalgae growing in fresh water and seawater, some species growing in extreme saline environments such as the Dead Sea in Israel, some growing 200-300 metres below water surface at photic zone limits, some grows in rich humus soil, desert sand, snowfields, rocks, in fur of animals, and even in 2000m height air ((Sharma et al., 2007; Singh and Saxena, 2015). Over 40,000 species of microalgae are known and recorded in current databases, however only around 100 species are used for studies and research in green algae, diatoms and cyanobacteria (Pulz and Gross, 2004; Oncel et al., 2015).

Microalgae has been used in many commercial applications including protein supplements as food, poultry feed, health food products, therapeutics, pigments for food colour, fine chemical source, biofuel, bio-fertilizer, soil conditioner and waste treatment (Becker, 1995). Using microalgae as feed for animals has received much praise, and are already being used as food additives, meal, oil replacement, enhancer of nutrition value for feed such as zooplankton feed and so on in the aquaculture (Guedes et al., 2015). For humans, microalgae also serve as health supplements as they are able to produce secondary metabolites like pigments and phytosterols, and also carbohydrates, protein and polyunsaturated fatty acids, having high potential in the medicinal field than how much they are already being exploited currently (Barreira et al., 2015). However, as microalgae is used to treat toxic leachate in this project, their applications would be more focused on biofuel and their ability to treat waste.

As non-renewable energy sources are getting depleted over time, the research on the utilization of microalgae as biofuel has also been increasing. Biofuel products from algae include biodiesel, bioethanol and biomethane. For biodiesel production, microalgae have potential as a raw material for fuel production as not only they have high growth rate, but they also provide the lipid fraction for biodiesel production, with their lipids being mostly neutral lipids with lower degree of saturation (Singh and

Saxena, 2015). Besides that, microalgae having higher photosynthetic efficiency and higher productivity rates, up to 12.5 $kgm^{-2}year^{-1}$ compared to other crops for biomass production (Prabandono and Amin, 2015; Shay, 1993), such as oil palm that only produces oil of 0.595 $Lm^{-2}year^{-1}$ (Chisti, 2007). Therefore, there is potential for microalgal lipids to not only replace fossil fuels, but also other energy crops for biofuel production.

For bioethanol production, there is potential to produce bioethanol using microalgae for self-fermentation or as feedstock for other bacteria to undergo fermentation. Being rich in proteins and carbohydrates (**Table 2.3**), microalgae is suitable to act as a carbon source for the fermentation process, while not affecting the supply of food and crop products, unlike other crops. Not only that, using microalgae for biodiesel and bioethanol production may be a good alternative for oil crops used for biodiesel and sugarcane used for bioethanol production that are unsustainable when produced in large quantities (Chisti, 2008). Bioethanol production can be accompanied with biomethane production, where the remaining microalgae biomass slurry can be used in the anaerobic digestion to produce methane (Singh and Saxena, 2015). Microalgae has low cellulose and lignin content, being a good candidate for high conversion efficiency and process stability in anaerobic digestion processes (Vergara-Fernández et al., 2008).

Table 2.3: Table of protein and carbohydrate content in various species of microalgae (Singh and Saxena, 2015)**.**

Algae Strain	Protein, % dwt	Carbohydrate, % dwt
Anabaena cylindrical	43 – 56	25 – 30
Chlamydomonas rheinhardii	48	17
Chlorella pyrenoidosa	57	26
C. vulgaris	51 – 58	12 – 17
Dunaliella bioculata	49	4
Dunaliella salina	57	32
Euglena gracilis	39 – 61	14 – 18
Porphyridium cruentum	28 – 45	40 – 57
Prymnesium parvum	28 – 45	25 – 33
Scenedesmus dimorphus	8 – 18	21 – 52
Scenedesmus obliquus	50 – 56	10 – 17
Scenedesmus quadricauda	47	N.A.
Spirogyra sp.	6 – 20	33 – 64
Spirulina maxima	28 – 39	13 – 16
Spriulina platensis	52	8 – 14
Synechoccus sp.	46 – 63	15
Tetraselmis maculate	52	15

Microalgae has the ability to treat wastewater along with biofuel production, with its process being a comparatively easy approach, at the same time being able to remove heavy metal and nutrients, also converting them into easily removable forms of carbons, lipids and other simple elements (Mohd Udaiyappan et al., 2017). Wastewater as growth medium for microalgae cultivation has a huge advantage of being rich in nutrients and inexpensive as they are unwanted, whilst being able to remove pollutants at the same time (Ray et al., 2019). Compared to common biological processes in wastewater or leachate treatment which produces sludge that needs to be handled as scheduled or non-scheduled wastes, it is a promising source of treating wastewater as the generated biomass has other uses of producing biofuel.

2.5 Growth and Mechanisms of Pollutant Removal in Microalgae

2.5.1 Growth Kinetics of Microalgae

As a living microorganism, microalgae have a growth curve with lag phase, exponential phase, linear phase, declining phase, stationary phase and death phase. The ideal shape of the growth curve is as in **Figure 2.2**, however different parts of the growth curve may be different due to different conditions such as nutrient concentrations, light intensity, temperature, etc (Becker, 1994). In the lag phase (1), growth rate is low due to the different environment where the microalgae adjust themselves to adapt to the new environment; exponential phase (2) is where light intensity and nutrient concentration are not the limiting factor as the microalgal cells grow and divide; linear phase (3) is where cell division is affected by limiting factors such as nutrients and light intensity due to the cells shading each other thus going into a constant rate; declining growth phase (4) is where cell division rate is decreased due to limiting factors such as nutrient exhaustion, which however can remain over a longer time if the culture is nutrient rich; stationary phase (5) is where storage are accumulated and synthesized substances are broken down, and can be described as the maximum attainable concentration of biomass; death phase (6), also known as crash phase is where microalgal cells die, releasing organic and even growth inhibiting materials into the medium (Becker, 1994; Lee et al., 2015).

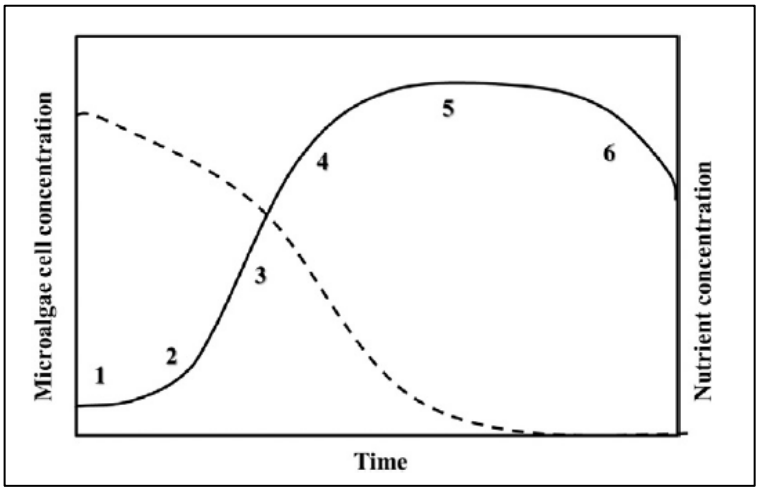

Figure 2.2: Growth Phase Curve of Microalgal Culture (Lee et al., 2015; Mata et al., 2010a; Becker, 1994)

2.5.2 Algal Nutrition of Microalgae

There are 2 major forms of algal nutrition which are autotrophy and heterotrophy. Autotrophs are all those organisms that obtain all the elements for growth form inorganic compounds only such as carbon dioxide, whereas heterotrophs are organisms that need organic substrates synthesised by other organisms (Becker, 1995). Mixotrophy is the combination of both, being able to utilise organic and inorganic forms of carbon.

2.5.3 Nitrogen Removal

Common nitrogenous pollutants in wastewater are in forms of ammonia, ammonium, organic nitrogen, nitrate and nitrite. One of the pathways for nitrogen removal is via assimilation. Microalgae utilize nitrogen sources and assimilate them into proteins, enzymes, peptides, chlorophylls, glutamine, genetic materials such as DNA and RNA and also energy transfer molecules such as ATP and ADP (Ruiz et al., 2013). They prefer NH_4^+–N as the N source and can readily take up almost all NH_4^+–N for usage (Khan et al., 2014; Wang et al., 2017).

Besides that, microalgae can also cause abiotic removal of ammoniacal nitrogen by changing the pH of its surroundings. The photosynthesis process can increase the pH of the solution especially when the photosynthesis rate is limited by carbon sources, causing a pH shift which would shift the NH_4^+ /NH_3 equilibrium towards NH_3 formation instead (Alcántara et al., 2015). This would enhance ammonia volatilization and aid in ammonia removal.

2.5.4 Phosphorus Removal

Phosphorus can also be taken up via assimilation and accumulated as polyphosphate (Alcántara et al., 2015). The amount being taken up depends on a few factors, such as light intensity, dissolved phosphate concentration and the nitrogen content, which is one of the major factors. A study done by Beuckels et al. (2015) has concluded that *Chlorella* and *Scenedesmus* species can adjust the nitrogen and phosphorus concentration in their biomass based on the supply provided, however the phosphorus uptake would highly depend on the nitrogen concentration.

Phosphorus removal is also possible via other indirect routes, similarly with nitrogen removal, when the pH of the solution increases due to photosynthetic activity of microalgae. At high pH, phosphate precipitation will occur with calcium ions (Ca^{2+}) as shown in the equation below (Alcántara et al., 2015):

$$3HPO_4^{2-} + 5Ca^{2+} + 4OH^- \rightarrow Ca_5(OH)(PO_4)_3 + 3H_2O \qquad (2.1)$$

2.5.5 Heavy metal Removal

The cell wall of microalgae is covered with negatively charged ions such as the carboxyl and hydroxyl group, which can be used to attract metals that are positively charged to bind onto its surface, aiding in removal of heavy metals from polluted wastewaters via adsorption, chelating and bioaccumulation (Ray et al., 2019). For physical adsorption, there are ion exchange with protons from R–COOH, R–OH and R–NH, and chemisorptions, which are considered passive mechanisms that happen at the cell surface (Alcántara et al., 2015; Chojnacka et al., 2005).

2.6 Microalgae as a Treatment Process

Microalgae as a treatment process has attracted more and more attention in the recent years, being one of the most promising technologies for nutrient recovery and advanced treatment with its high efficiencies in municipal, agricultural and industrial wastewaters (Li et al., 2019). Using microalgae for treatment can generally be referred as phycoremediation. Phycroremediation is a type of biological treatment of waste where algae remove simple organic and inorganic compounds for their own growth while also causing biotransformation of some complex compounds (Paskuliakova et al., 2018). Microalgae is attractive as a treatment step as not only it is able to treat waste, it has the potential to be subsequently harvested and used as fertiliser, feed or biofuel, and not be treated as another waste that needs to be handled.

To fully utilise microalgae as a treatment step, both using microalgae in the treatment and microalgae harvesting are important to treat wastewater and remove the suspended microalgal cells from the effluent for subsequent usage. A brief view of the process is shown in **Figure 2.3**, with both the treatment step and biomass separation step to be further discussed in Section **2.6.1** and **2.6.2**.

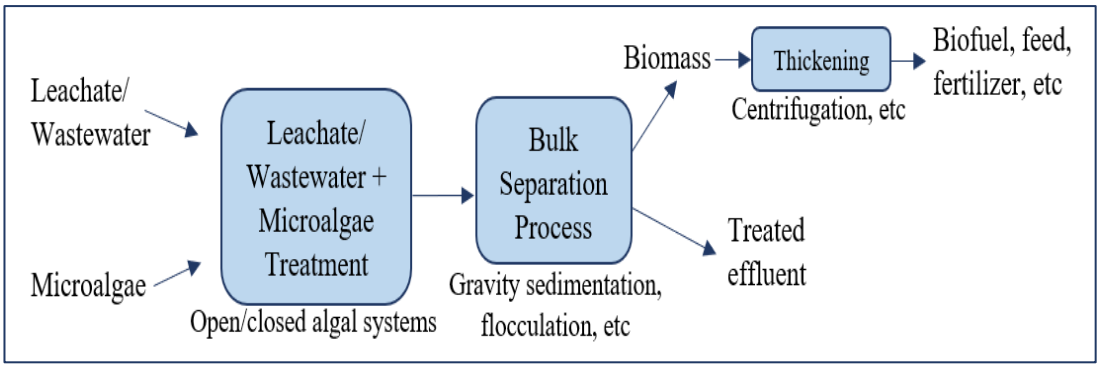

Figure 2.3: Brief view of microalgae as a treatment step.

2.6.1 Microalgae in Wastewater and Leachate Treatment

Cultivation of microalgae in wastewater has gained importance in the recent years and is widely accepted as a wastewater treatment system that is as effective as conventional systems (Sen et al., 2013), being used for treatment, pollution control and production of energy. It can remove a wide variety of pollutants, from biodegradable components and nutrients to heavy metals and pathogens. **Table 2.4** shows a summary of major pollutants found in wastewater and the comparison of conventional treatment and microalgae-based wastewater treatment mechanisms.

Table 2.4: Summary of major pollutants found in wastewater and the comparison of conventional treatment and microalgae-based wastewater treatment mechanisms (Alcántara et al., 2015).

Pollutants (associated risks)	Conventional treatment mechanisms	Microalgae-based WWT
Suspended solids (reduce water clarity, clog waterways, many solids are biodegradable organic pollutants as well)	Gravity settling prior to or during biological treatment	Microalgae-based WWT systems are well mixed and not designed for the removal of inert or nonbiodegradable solids. Prior primary settling of suspended solids may be required often to avoid operational issues and improve light penetration into the influent.
Biodegradable organic pollutants (uncontrolled biodegradation triggers oxygen depletion in wastewater discharge recipients,	Aerobic or anaerobic biodegradation by heterotrophs, the former is often preferred for treating low-strength effluents such as domestic wastewater and inhibitory and/or recalcitrant influents	Aerobic biodegradation by heterotrophs, microalgae provide additional metabolic abilities and support aerobic heterotrophic degraders via exchange of substrates.

certain organics are also hazardous).	such as industrial wastewater.	
Nutrients (trigger uncontrolled algae growth in recipient ecosystems)	Microbial assimilation, nitrification/ denitrification processes, biological phosphate removal via accumulation of poly-P in bacterial cells, chemical phosphate precipitation.	Microalgae photosynthesis enhances nutrient assimilation by boosting biomass productivity. Environmental conditions also favour N volatilization and P precipitation. Certain algae are capable of intracellular P accumulation as stable poly-P.
Pathogens (active vectors of human and animal diseases).	Disinfection in maturation ponds, chemical disinfection (e.g., UV irradiation, chlorine, ozone).	Microalgae photosynthesis can promote pathogen disinfection by contributing to raise of pH and dissolved oxygen concentration. The high-illuminated surface / volume ratio also favours disinfection via UV radiation.
Heavy metals (acute and chronic toxicity, bioaccumulation).	Chemical precipitation (lime), electrochemical precipitation, ion exchange, adsorption onto GAC or various biomaterials (e.g., waste wood chips, bacteria and yeasts from industrial fermentations).	Microalgae-based WWT has the potential to generate significant amounts of cost-effective biosorbent. Algae photosynthesis can also favour heavy metal precipitation at high pH.

Numerous studies have been done using microalgae to treat wastewater and leachate, achieving high removal efficiencies and producing a good amount of biomass. **Table 2.2** shows a summary on a few research of microalgae treating different types of wastewaters and leachate, the type of microalgae species used and their

performances. Most research focus on the removal of total nitrogen, ammonia or ammonium nitrogen, nitrate, phosphorus, COD and also on the biomass produced.

As microalgae assimilate nitrogen and phosphorus for production of cell components, they tend to have a high removal rate of nitrogen and phosphorus when in optimum condition. el Ouaer et al. (2017) has achieved 90% of ammonium-nitrogen (NH_4^+–N) removal efficiency within 13 days using *Chlorella sp.* on undiluted raw leachate, with the 10% diluted leachate having a lower removal efficiency of only 60% within 13 days. Meanwhile Li et al. (2011) has also achieved a high removal efficiency of 93.9% ammoniacal-nitrogen (NH_3–N), 89.1% total nitrogen (TN) and 80.9% total phosphorus (TP) in undiluted concentrated municipal wastewater. Using a tubular photobioreactor (PBR) which has advantages of being able to control the environment, Porto et al. (2021) managed to achieve a high nitrogen (NO_3–N and NO_2–N) removal efficiency of 91.1% and orthophosphate removal of 70.1% with *C. vulgaris* in 5% diluted landfill leachate.

There are many who emphasize the importance on the ratio of nitrogen and phosphorus in order for best nutrient removal efficiencies. A study by Paskuliakova et al. (2016) has concluded that phosphate is a limiting factor in microalgae based phycoremediation, with addition of phosphate being able to enhance removal of ammoniacal nitrogen from 51.7% to 90.7%. However, another study by Beuckels et al. (2015) has a slightly different approach, where it was stated the 2 species *Chlorella* and *Scenesdesmus* are capable of adjusting nitrogen and phosphorus in their biomass, with the degree of adjusting depending on the nitrogen concentration, indicating high concentrations of nitrogen being a pre-requisite for effective phosphorus removal. Regardless, both studies amplify the fact that nitrogen and phosphorus ratios are important for maximum removal of both of them and for biomass production. A study by (Pereira et al., 2016a) has shown that N:P ratio of 35:1 could achieve orthophosphate removal of 54-100%, being the highest compared to other ratios (12:1 and 23:1), whilst for NH_4^+–N and NO_3–N removal the N:P ratios having not much of an obvious effect, somehow supporting the study by Beuckels et al. (2015). However, for samples with no phosphorus addition, the removal efficiency of NH_4^+– N decreases dramatically, thus also supporting the study by Paskuliakova et al. (2016).

Microalgae are also effective or semi-effective in removing organic pollutants or COD in some cases. Heterotrophic and mixotrophic microalgae are able to utilise organic compounds as carbon and energy sources, thus being able to remove COD and organic pollutants (Alcántara et al., 2015; Gualtieri and Barsanti, 2006). However, for inert organic materials, it would be difficult for microalgae and other simultaneously existing bacteria to utilize them, causing limited uptake of organic materials (Wang et al., 2017). Regardless, there are many studies on the performances of microalgae in COD removal. A study by Li et al. (2011) has a high COD removal efficiency of 90.8% using *Chlorella sp.* in municipal wastewater centrate in 14 days, while el Ouaer et al. (2017) achieved 50.7% COD removal in undiluted raw leachate in 24 days, 60% COD removal in 10% diluted leachate in 13 days.

In microalgae cultivation, it is important to have sufficient nutrients, balance between nutrients, and low presence of inhibitory compounds such as high concentration of ammonia and heavy metals. Dilutions are often done as in previously mentioned studies to lower the concentration of inhibitory substances; however, this action might also decrease the amount of available nutrients. As such, there are studies that attempt to dilute leachate with wastewater or to mix different types of wastewaters together to achieve the optimum nutrient balance and reduce the concentration of inhibitory compounds. A study by Lu et al. (2015) was done by mixing wastewater from different processes of meat processing plant and managed to reach removal efficiency of 68.65–90.48% NH_4^+–N, 30.64–50.94% total nitrogen removal and 44.95–63.51% total phosphorus removal. Another study by Hernández-García et al. (2019) diluted leachate using municipal wastewater (MW), with the best performance in 9 days excluding raw MW being 7% leachate, achieving 79% and 82% NH_4^+ removal efficiency, 74% and 77% TN removal efficiency, 43% and 41% PO_4^{3-} removal efficiency, 64% and 67% COD removal efficiency for *S. obliquus* and *Desmodemus* spp., respectively.

Unlike laboratory scale, microalgae as a mode of treatment at pilot scale or large scales has two types of systems: closed systems and opened systems. Closed systems are more complexed photobioreactor systems such as vertical tubular photobioreactor (PBR), horizontal PBR, flat panel PBR and soft frame PBR (**Figure 2.4**) that have an advantage of being able to manipulate the environment more easily,

having lesser chances of contaminations and producing higher biomass yields compared to open systems (Morillas-España et al., 2022). It has a major disadvantage of complexity, high energy consumption and high costs, thus only used for high value products (Morillas-España et al., 2022; Li et al., 2019). However, there are still studies being carried out using PBR to treat leachate, one of them by Porto et al. (2021) that aims to further improve nutrient removal efficiencies, who managed to achieve nitrogen removal efficiency of 91.1% using *C. vulgaris* and 88.7% using *T. obliquus*.

Open systems on the other hand, are simpler to operate, have much lower fixed and operational costs. Majority of open systems are raceway ponds (**Figure 2.4**), which are also known as high-rate algal ponds (HRAP), where the ponds have a raceway configuration with paddle wheels for mixing and circulation (Li et al., 2019). Most of them grow under sunlight as the main light source and have a depth of generally 0.10 – 0.40m (Li et al., 2019; Acién et al., 2017) to ensure light penetration. Studies also have been done using raceway ponds, one by Posadas et al. (2015) achieving removal efficiencies of 79% in total nitrogen and 84% in COD using *Scenedesmus* sp. Due to uncontrolled environments, open systems tend to have lower biomass yields when compared to PBRs. There are also other systems such as membrane photobioreactors and microalgae biofilms but are newer and less common.

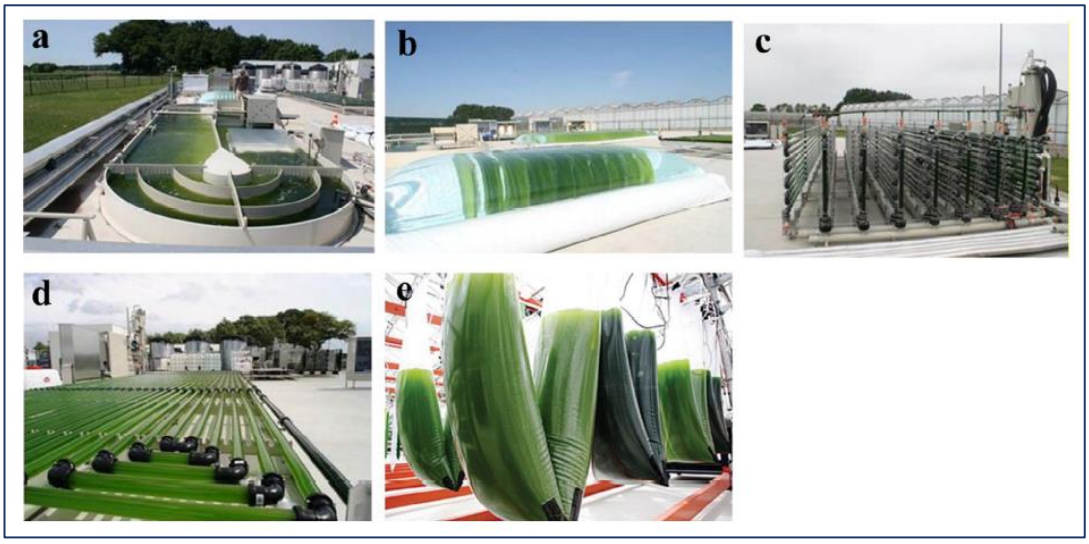

Figure 2.4: Microalgae cultivation systems: (a) Raceway Pond, (b) Flat Panel PBR, (c) Vertical Tubular PBR, (d) Horizontal Tubular PBR, (e) soft frame PBR (Li et al., 2019; De Vree et al., 2015; Koller, 2015; Vo et al., 2019)

Table 2.2 Summary of Various Treatments on Wastewater or Landfill Leachate using Microalgae.

Sample	Microalgae species	Treatment Condition	Performance: Removal Efficiency (RE), lipid productivity (LP)	Reference
Concentrated Municipal wastewater from activated sludge thickening process	*Chlorella sp.*	• Cultivation volume: 100 mL • Light condition: 24 h, 50 μmol $m^{-2}s^{-1}$ • Mixing: Carbonator pump 1.8 L/min • Experiment duration: 14 days	• NH_3 RE: 93.9% • TN RE: 89.1% • TP RE: 80.9% • COD RE: 90.8% • Biodiesel yield: 0.12g-biodiesel/L-algae culture	(Li et al., 2011)
Meat processing plant wastewater (mixed from different processing steps of plant)	*Chlorella sp.*	• Mixing wastewater from different processing steps of the meat processing plant. • Experiment duration: 9 days	• NH_4^+–N RE: 68.65 – 90.48% • TN RE: 30.64 – 50.94% • TP RE: 44.95 – 63.51% • COD RE: 7.95 – 43.91% • Biomass yield: 0.675 – 1.538 g/L	(Lu et al., 2015)
Leachate diluted with municipal wastewater (MWW)	*Scenedesmus obliquus* (S), *Desmodesmus spp.* (D)	• Diluting leachate with wastewater (Leachate concentration of 0%, 7%, 10%, 15%) • Cultivation volume: 4 L • Light condition: 12 h, 53 μmol $m^{-2}s^{-1}$ • Mixing: Manual shaking • Experiment duration: 28 days	• NH_4^+–N RE: 22 – 95% (S), 20 – 96% (D). • TN RE: 25 – 90% (S), 20 – 93% (D). • PO_4^{3-}–P RE: 5 – 60% (S), 6 – 57% (D). • COD RE: 26 – 86% (S), 24 – 86% (D). • Nitrates RE: 13 – 50% (S), 14 – 60% (D). • Highest biomass yield in 7% leachate: 1.2 ± 0.07 g/L (S), 1.3 ± 0.1 g/L (D).	(Hernández-García et al., 2019)

3 types of landfill leachate from aerated stabilization pond	*C. vulgaris*	• Addition of phosphorus to achieve N:P molar ratios of 12:1, 23:1 and 35:1. • Cultivation volume: 1 L • Light condition: 24 h, 32-42 μmol $m^{-2}s^{-1}$ • Mixing: Air injection 90 L/h • Experiment duration: 12 days.	• 35:1 ratio: 64-100% NH_4^+–N RE, 100% PO_4^{3-} - P RE • no phosphorus addition: 36-100% N-NH_4^+ RE	(Pereira et al., 2016)
Landfill leachate	*Chlorella sp.*	• Using sample of raw leachate and diluted 10% leachate. • Cultivation volume: 500 mL • Light condition: 16 h, 75 μmol $m^{-2}s^{-1}$ • Experiment duration: 13 & 24 days	• Raw leachate: up to 50.7% COD RE (24 days), 90% NH_4^+–N RE (13 days). • 10% leachate: up to 60% COD RE (13 days), 60% NH_4^+–N RE (13 days), 80% NH_4^+-N RE (24 days) • Considerably increased cell density for 10% leachate. • Lipid productivity of 4.74 mg/L.d for 10% leachate.	(el Ouaer et al., 2017)

Landfill leachate from biological treatment effluent	*C. vulgaris* & *Tetradesmus obliquus*	• Dilution of v/v 5%, 10%, 15%, 20% and 25% was done to determine optimum dilution. • Optimum dilution sample was experimented with tubular photobioreactor (PBR) • Dilution cultivation volume: 1L in 1-L flasks • PBR cultivation volume: 520 mL with flow rate at 50L/h • Light condition: 24 h, 55-65μmol $m^{-2}s^{-1}$ • Mixing: Air injection 90 L/h • Experiment duration: 11 days (1-L flasks), 7 days (TBR)	• 5% in 1-L flasks had most favourable microalgal growth and nitrogen uptake. • 5% leachate had better performance in TBR than in 1-L flask. • In TBR, NO_3 – N + NO_2 – N RE was 91.1% (*C. vulgaris*) and 88.7% (*T. obliquus*); PO_4 – P RE was 70.1% (*C. vulgaris*) and 56.0% (*T. obliquus*); biomass of *C. vulgaris* improved significantly compared to 1-L flasks, no improvement for *T. obliquus.*	(Porto et al., 2021)
Nitrified Landfill leachate	*C. vulgaris, Scenedesmus sp., Oscillatoria sp.*	• Dilution of v/v 10%, 15%, 20%, 25%, 30% was done. • Cultivation volume: 350 mL • Light condition: 12 h, 2000-3000 lux intensity • Mixing: Aeration • Experiment duration: 14 days	• Max NO_3^- RE: 77.4% in 20% leachate (*C. vulgaris*), 66.34% in 20% leachate (*Scenedesmus sp*), 84% in 20% leachate (*Oscillatoria sp.*). • Max biomass: were 428.66 mg/L in 20% leachate (*C. vulgaris*), 237.04 mg/L in 30% leachate (*Scenedesmus sp)* and 805.96 mg/L in 20% leachate (*Oscillatoria sp).*	(Nordin et al., 2017)

2.6.2 Microalgae Harvesting Methods

Microalgae harvesting is an expensive but necessary process in order to utilise microalgae as a source of biodiesel while completing the treatment process, with its efficiency determining the quality of the biodiesel (Ray et al., 2019). Not only that, microalgae harvesting also has the additional advantage of acting as a treatment process as well, as most separation techniques are also commonly used in wastewater or leachate treatment processes.

Microalgae harvesting can be divided into 2 steps: bulk harvesting to separate the biomass from suspension, and subsequently thickening of the slurry to concentrate the biomass (Brennan and Owende, 2010). The second step of thickening is commonly the more energy intensive and expensive step compared to bulk harvesting. Most common microalgae harvesting methods include flocculation, gravity sedimentation, centrifugation, filtration, microscreening, flotation and electrophoresis. Gravity sedimentation is one of the common techniques as it is economical and requires less energy. It can be coupled with flocculation to increase its efficiency, however is not desirable as adding organic or inorganic flocculants could increase costs (Schenk et al., 2008). There is also a method of autoflocculation, where the algal system is disrupted from its carbon dioxide supply which would cause the microalgae to flocculate on its own (Demirbas, 2010).

For methods like filtration is only suitable for laboratory scale, as filtration of microalgae on large scale would cause membrane clogging problems and high maintenance costs (Prabandono and Amin, 2015). Conventional filtration processes are only appropriate for larger sizes of microalgae >70μm such as *Coelastrum* and *Spirulina*, being unsuitable for microalgae <30μm such as *Dunaliella*, *Chlorella*, and *Scenedesmus* which require microfiltration and ultra-filtration (Brennan and Owende, 2010). Centrifugation is also only suitable for laboratory scale and expensive to apply for biomass bulk harvesting. However, it is useful for the second step which is slurry thickening, being able to concentrate slurry from 10 – 20g/L to algal paste of 100 – 200g/L, and has potential to be combined along with oil extraction steps (Schenk et al., 2008; Prabandono and Amin, 2015).

2.7 Chlorella vulgaris

Since ancient times there were reported utilizations of algae in Greek and China, whilst the *Chlorella vulgaris* was reportedly being the first unialgal culture achieved in the year 1890 by Beijerinck (Prabandono and Amin, 2015; Oncel et al., 2015). In today's era, the *C. vulgaris* is more commonly used as dietary supplements and feedstocks, even becoming a health supplement in Japan (Heimann and Huerlimann, 2015).

2.7.1 Identification, Morphology and Characteristics

The freshwater microalgae *C. vulgaris* is classified as a Plantae for the type of kingdom, as Viridiplantae for subkingdom and Chlorophyta for infrakingdom (Heimann and Huerlimann, 2015). It is a mixotroph that can shift between autotrophy and heterotrophy, being capable of growing on carbon dioxide under light conditions and on certain form of organic carbons such as acetic acids in the dark (Becker, 1994).

The *C. vulgaris* have sizes of 2 – 10 μm (Illman et al., 2000; Yamamoto et al., 2004), being a fairly nondescript round cell as shown in **Figure 2.5** (Heimann and Huerlimann, 2015; Safi et al., 2014). It has a single chloroplast, where starch granules composed of amylose and amylopectin can be formed within especially during unfavourable growth, whilst also containing a single pyrenoid that contains high levels of ribulose-1,5-bisphosphtae carboxylase oxygenase (RuBisCO) for carbon dioxide fixation (Safi et al., 2014). Lipid globules would accumulate in the chloroplast besides the cytoplasm when there is nitrogen stress (Edward Lee, 2008; Safi et al., 2014). The *C. vulgaris* is a nonmotile reproductive autospore that undergoes asexual reproduction rapidly, with one cell being able to multiply into 4 daughter cells via autosporulation within 24 hours in optimal conditions (Safi et al., 2014).

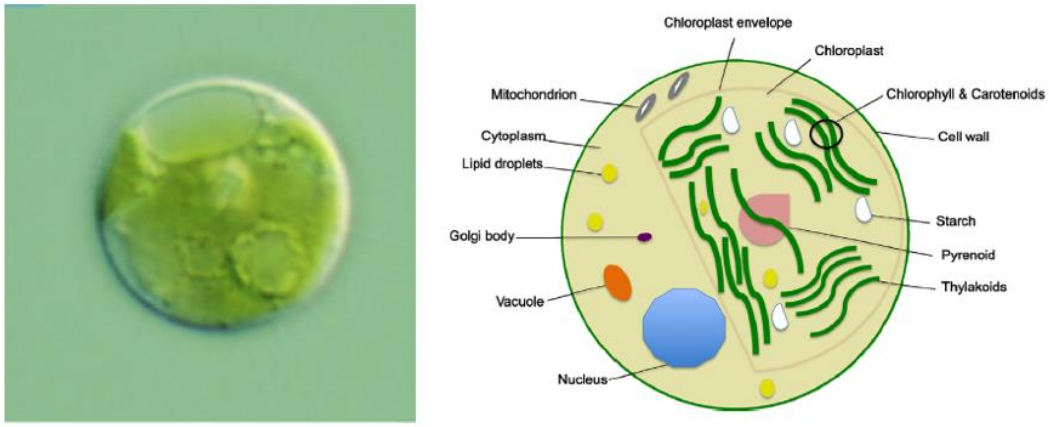

Figure 2.5: Left: Micrograph of *Chlorella sp.* (Orandi and Lewis, 2013); Right: Schematic ultrastructure of *C. vulgaris* (Safi et al., 2014).

2.7.2 Growth and Production

The *C. vulgaris* being a mixotroph, is able to carry out both autotrophic and heterotrophic processes. For autotrophy, it would rely on photosynthesis for growth, thus open pond systems and closed photo-bioreactors support autotrophy by providing sufficient sunlight and carbon dioxide. For heterotrophic growth, the *C. vulgaris* would not require any light and will rely on organic carbon sources. Production via this mode would provide carbon sources in forms of glucose, acetate, glycerol and glutamate, with glucose being the most effective for maximum specific growth rate (Safi et al., 2014). For mixotrophic growth, the cells will not be strictly dependent on light nor organic substrate to grow, being able to combine both autotrophic and heterotrophic processes, with the main advantage being able to limit the biomass loss during dark respiration and limiting the amount of organic substrates required for growth (Yeh and Chang, 2012a; Safi et al., 2014).

2.7.3 Suitability for Leachate Treatment

C. vulgaris is frequently used in wastewater treatment studies as it has high adaptability, survivability under adverse conditions and tolerance towards heavy metals (Nguyen et al., 2019; Suparmaniam et al., 2020; Silva et al., 2022; Xaaldi Kalhor et al., 2017). It has demonstrated high removals of nutrients in various wastewaters especially for nitrogen and phosphorus as shown in **Table 2.2**. Not only that, but it is also able to tolerate high heavy metal concentrations and even carry out removals, with a study by Cheng et al. (2016) showing the *C. vulgaris* removing 96.8% of cadmium, with the concentration of cadmium in the cells being 16.34 mg Cd (II) g^{-1}. Such properties exhibit suitability for leachate treatment while being able to produce valuable biomass for potential bio-fuel production. Furthermore, *Chlorella* species were found to be high biomass producers, have high nutrient removals and have strong anti-pollution abilities (Ray et al., 2019; Suparmaniam et al., 2020).

CHAPTER 3

METHODOLOGY

3.1 Experimental Flow

A brief summary on the experiment flow is shown in **Figure 3.1**.

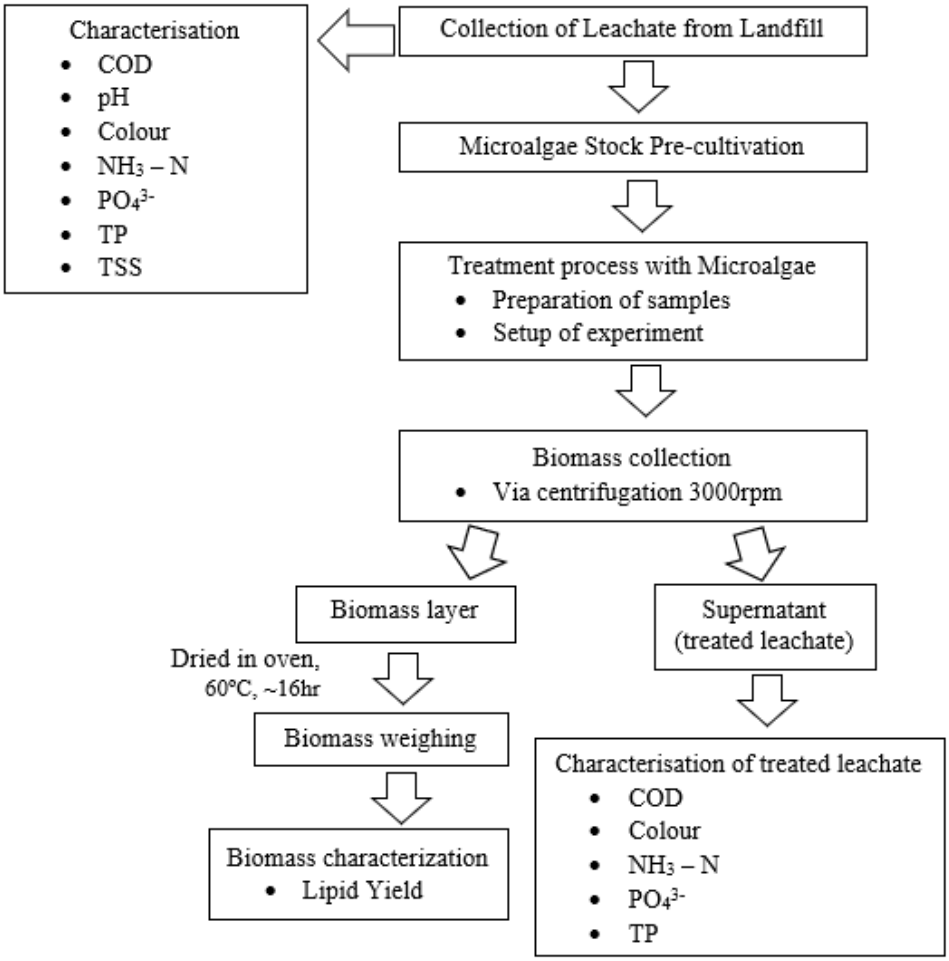

Figure 3.1: Experimental flowchart

3.2 Landfill Site Description and Leachate Collection

Sungai Siput Selatan landfill is located at Jalan Sahom, Kampar, Perak, Malaysia with coordinates of 4º 23’ 25” N, 101º 10’ 57” E (**Figure 3.2**). It has operated since 1992 with approximately 37 acres of landfilling area. The Sungai Siput Selatan landfill is an anaerobic landfill that receives an average of 100 tonnes of municipal solid waste from the Kampar district that has a recorded population service of 101,183 people in the year 2017 (Bashir et al., 2017). Sungai Siput Selatan landfill is an anaerobic landfill equipped with a composting centre, leachate collection system, and a leachate treatment pond (**Figure 3.3**). However, the treatment pond at the time of collection only acted as a leachate collection pond as the treatment system had stopped working.

The leachate sample was collected from the Sungai Siput Selatan landfill leachate pond into pre-cleaned high-density polyethylene (HDPE) plastic bottles. The sample was stored at 4ºC to minimize biodegradation and chemical reactions of the sample in the Environmental Laboratory at the Faculty of Engineering and Green Technology in Universiti Tunku Abdul Rahman (UTAR), Kampar. The raw leachate was characterized on the parameters of COD, pH, ammoniacal nitrogen, orthophosphate, total phosphorus, colour and total suspended solids.

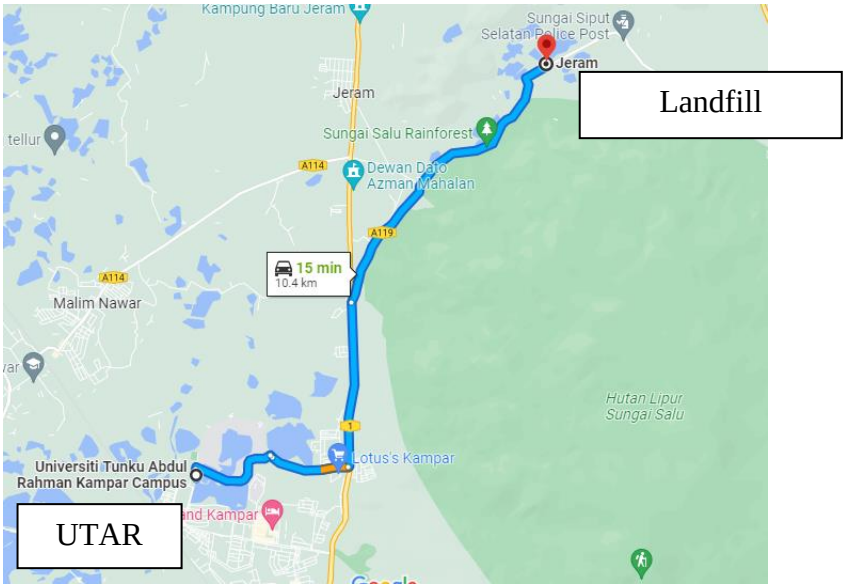

Figure 3.2: Location of Sungai Siput Selatan Landfill, Perak (Image extracted from Google Map, accessed 2nd September 2022)

Figure 3.3: Leachate collection pond.

3.3 Microalgae Stock Pre-cultivation

The *C. vulgaris* microalgae was pre-cultivated in 10 L cultivation medium at the ratio of water : fertilizer of 1 L : 0.5 mL in a 10 L glass bottle. The microalgae stock was cultivated under continuous light illumination and air bubbling system of ~1.5 L/min (Pereira et al., 2016). The microalgae stock was left in room temperature of 25 ºC with no temperature adjustments. After pre-cultivation, the stock was used for the experiment where 500 mL conical flasks would be used. The initial biomass for the stock solution was 489 mg/L.

3.4 Treatment Process with Microalgae

3.4.1 Preparation of samples

500 mL borosilicate conical flasks were used for the experiment. Each conical flask would contain a total of 250 mL solution consisting varying amounts of leachate sample, microalgae stock solution and distilled water for dilution. Prior to being used, the collected leachate sample and the microalgae stock solution were respectively mixed thoroughly via stirring to ensure homogeneity.

In this experiment, 5 groups of different dilutions were used, resulting in leachate concentrations (v/v%) of 33v/v%, 44v/v%, 66v/v%, 89v/v% and 100v/v%. A constant amount of 63 mL microalgae stock solution was used in every sample. Amount of leachate and distilled water varied in every sample to create the desired dilution, as shown in **Table 3.1**. The leachate concentrations were calculated as follows:

$$\text{Leachate concentration (v/v\%)} = \frac{Leachate\ volume\ (mL)}{Distilled\ water + Leachate\ volume\ (mL)} \qquad (3.1)$$

For each sample, there would be duplicates to determine the harvested biomass on the initial day and desired days. The experiment was carried out for 19 days. Therefore, to harvest biomass on day 0, 2, 5, 7, 9, 12, 14, 16, and 19, nine duplicates of the same sample was used.

Table 3.1 Leachate concentration and its proportions

Leachate Concentration (v/v%)	Ratio (Leachate: Microalgae + Distilled water)	Proportions (mL)		
		Leachate	Microalgae solution	Distilled water
33	1:3	63	63	124
44	1:2	83	63	104
66	1:1	125	63	62
89	2:1	167	63	20
100	3:1	187	63	0

3.4.2 Setup of Experiment

The setup of each conical flask is shown in **Figure 3.4**. Each conical flask was supplied with air bubbling (~0.3 L/min). The mouth of the conical flask was stoppered and wrapped with a layer of aluminium foil.

The overall setup is shown in **Figure 3.5**. The setup was located at the Petrochemical Workshop (J001) in block J, Faculty of Engineering and Green Technology, University Tunku Abdul Rahman. A rack was used to accommodate the apparatus, with each layer installed with a fluorescent lamp. Air flow was supplied using aquarium air pumps (SOBO SB-948), with each pump capable of supplying 4 air flows through major air tubes having an inner diameter of 4.5 mm, where each major tube would supply air flow to 5 conical flasks using a 5-way air flow control splitter (**Figure 3.6**) into 5 minor tubes with inner diameter of 2 mm. Each minor tube connection was wrapped with parafilm to ensure tight connection with minimum air escape.

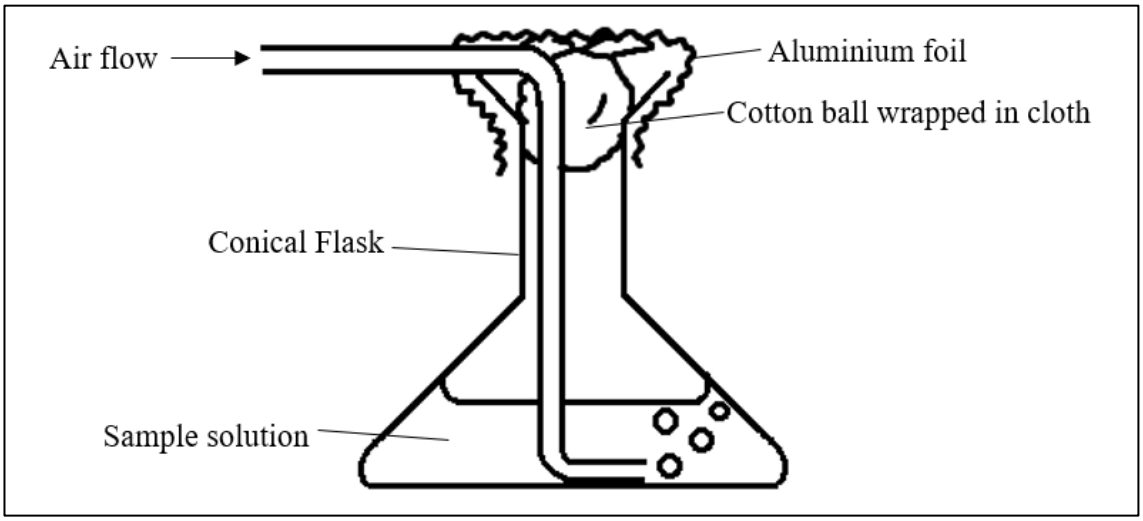

Figure 3.4: Setup of each conical flask.

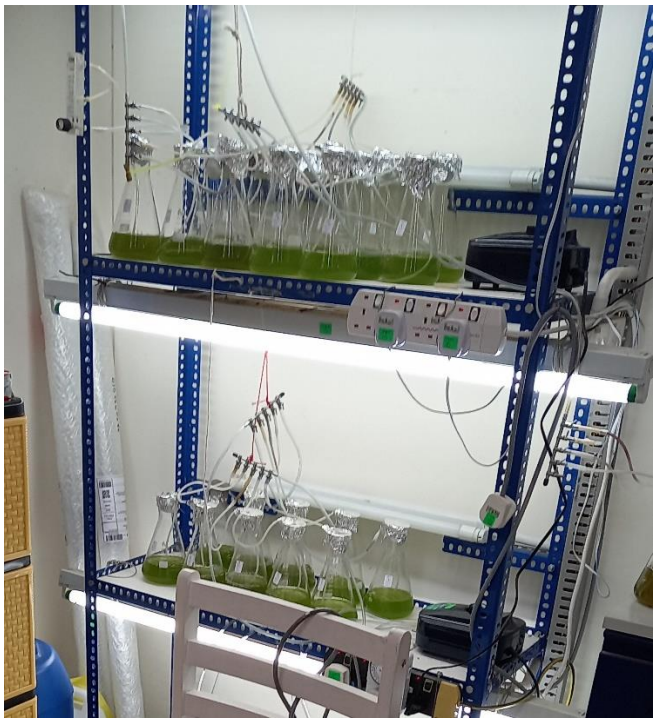

Figure 3.5: Overall experimental setup

Figure 3.6: 5-way air flow control splitter

When the samples reached the duration where biomass harvesting should be performed, the conical flasks would be transported from the workshop to the Environmental Laboratory for biomass harvesting, treated sample collection and sample characterisation.

3.5 Biomass Collection

Biomass collection was done using centrifugation for 10 minutes at 3000 rpm ((Becker, 1994; Toh et al., 2018) in 50 mL centrifuge tubes. The centrifuge machine model was Z326K Laboratory Centrifuge (Hermle Labortechnik, Germany). The conical flasks were shaken and stirred to ensure homogeneity of sample and biomass not adhering to the flask walls or base. 200 mL otherwise the whole amount if insufficient were centrifuged for each sample. Part of the sample was centrifuged each round, drained and subsequently adding the same sample into the same centrifuge tube to accumulate all of the biomass, while the drained supernatant was filtered and collected for characterisation. The accumulated biomass was dried in oven at 60ºC for ~16 hours using the XU032 Universal Oven (France Etuves, France) and weighed on the AUX320 Analytical Balance (Shimadzu, Japan). To calculate biomass generation, the following formula is used:

$$\text{Biomass Generated (mg/L)} = [\text{Mass on Day X (mg)} - \text{Mass on Day 0 (mg)}] / \text{volume of centrifuged sample (L)} \quad (3.2)$$

Where X = 2, 5, 7, 9, 12, 14, 16 and 19.

The temperature was ensured to not exceed 60ºC as it is the optimum temperature to retain the best concentration triacylglycerol (TAG) and lipid yield (Brennan and Owende, 2010; Widjaja et al., 2009).

Figure 3.7: Z326K Laboratory Centrifuge (Hermle Labortechnik, Germany).

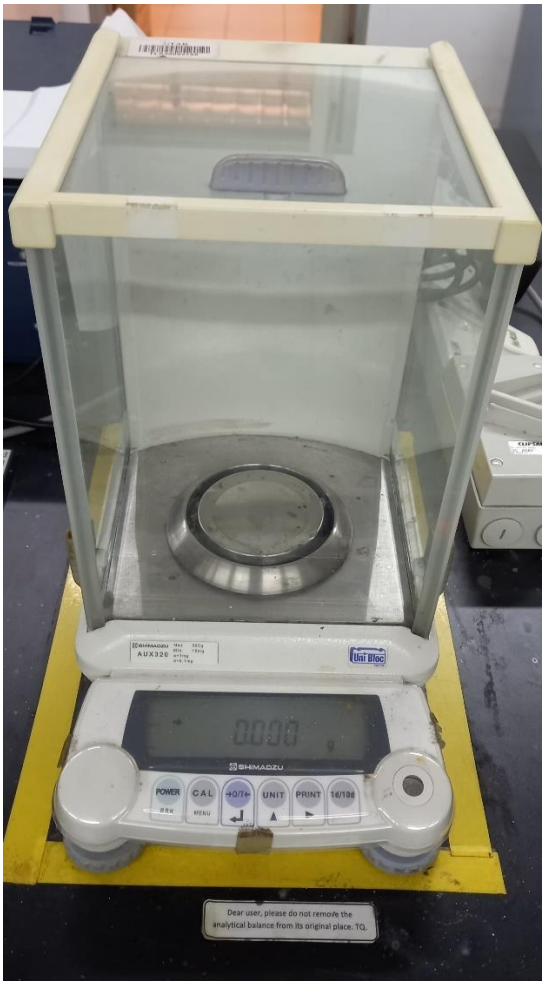

Figure 3.8: AUX320 Analytical Balance (Shimadzu, Japan).

3.6 Analytical Methods

Characterisation was done on raw leachate collected from the landfill and treated samples from the experiment. The treated samples from the experiment (centrifuged and filtered supernatant) were preserved at 4ºC to minimise occurrence of any biological or chemical processes that might alter the characteristics of the samples.

3.6.1 Chemical Oxygen Demand (COD)

COD was measured in mg/L COD using the USEPA Reactor Digestion Method 8000. 2 mL of leachate samples were each added into “High Range (HR)” (20-1500 mg/L COD) digestion vials, shaken via inversion and inserted into a pre-heated COD reactor DRB 200 (Hach, USA) at 150ºC for 2 hours. The vials were inverted again while still warm and placed in a dark place to cool. The cooled vials were cleaned and tested using the DR 890 Portable Colorimeter (Hach, USA) with the program code ‘17’. A blank sample was prepared using the same procedure by replacing the sample with deionised water. Each sample were tested with 2 vials to ensure the accuracy of the data. COD removal was calculated using the formula:

$$COD\ removal\ (\%) = \frac{COD\ (Day\ X) - COD\ (Day\ 0)}{COD\ (Day\ 0)} \times 100\%, \quad (3.3)$$

$$where\ X\ =\ 2, 5, 7, 9, 12, 14, 16\ and\ 19.$$

Figure 3.9: COD reactor DRB 200 (Hach, USA)

3.6.2 pH

pH was measured using the HI2550 pH/ORP & EC/TDS/NaCl Benchtop Meter (Hanna Instruments, UK). The pH meter was calibrated each time before use, using standard solutions of pH 4.01, 7.01 and 10.01 respectively.

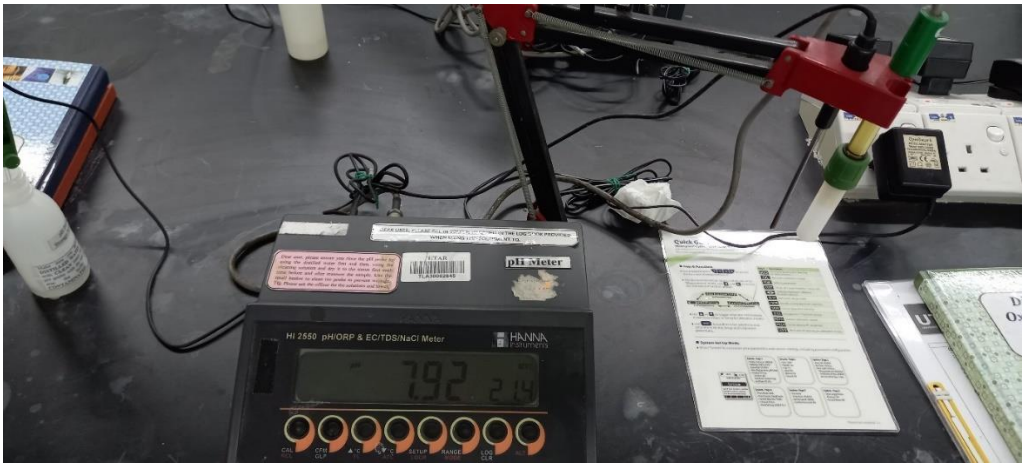

Figure 3.10: HI2550 pH/ORP & EC/TDS/NaCl Benchtop Meter (Hanna Instruments, UK).

3.6.3 Colour

Colour was measured in the unit of Platinum Cobalt (Pt/Co) using the APHA Platinum-Cobalt Standard Method 8025. A blank sample using deionised water and leachate sample was prepared in 2 sample cells respectively and tested using the DR 890 Portable Colorimeter (Hach, USA) with the program code '19'. Colour removal was calculated using the formula:

$$Colour\ removal\ (\%) = \frac{Colour\ (Day\ X) - Colour\ (Day\ 0)}{Colour\ (Day\ 0)} \times 100\%, \quad (3.4)$$

$$where\ X\ =\ 2, 5, 7, 9, 12, 14, 16\ and\ 19.$$

Figure 3.11: DR 890 Portable Colorimeter (Hach, USA)

3.6.4 Ammoniacal Nitrogen (NH_3–N)

Ammoniacal nitrogen (NH_3–N) concentrations were tested using the USEPA Nessler Method 8038 with a measurement range of 0.02 to 2.50 mg/L in units of NH_3–N. 25 mL of diluted sample is added with 3 drops of mineral stabilizer, 3 drops of polyvinyl alcohol dispersing agent and 1.0 mL Nessler Reagent, with the solution mixed each time after addition of each chemical. A blank sample was prepared with the same method by substituting the sample with 25 mL of deionized water. After 1-minute reaction time, the samples were tested in a sample cell using the DR3900 Laboratory Spectrophotometer (Hach, USA) with the program code '380'. Ammoniacal nitrogen removal was calculated using the formula:

$$NH_3 - N\ removal\ (\%) = \frac{NH_3{-}N\ \ (Day\ X) - NH_3{-}N\ \ (Day\ 0)}{NH_3{-}N\ (Day\ 0)} \times 100\%, \tag{3.5}$$

$$where\ X\ =\ 2, 5, 7, 9, 12, 14, 16\ and\ 19.$$

Figure 3.12: DR3900 Laboratory Spectrophotometer (Hach, USA)

3.6.5 Reactive Phosphorus, Orthophosphate (PO_4^{3-})

Reactive phosphorus, orthophosphate was measured using the USEPA PhosVer 3 (Ascorbic Acid) Method 8048, which can measure reactive phosphorus in a range of 0.02 to 2.50 mg/L PO_4^{3-}. A sample cell was filled with 10 mL of samples, subsequently being added with 1 PhosVer 3 Phosphate Reagent Powder Pillow and shaken vigorously for 20-30 seconds. The sample cell was then left for 2 minutes for reaction to occur. The blank sample was prepared by adding 10 mL of the same sample into a sample cell. The sample cells were cleaned before being inserted into the cell holder of the DR3900 Laboratory Spectrophotometer (Hach, USA) with program code '490' for results. Orthophosphate removal was calculated using the formula:

$$\mathrm{PO_4^{3-}}\ removal\ (\%) = \frac{\mathrm{PO_4^{3-}}\ (Day\ X) - \mathrm{PO_4^{3-}}\ (Day\ 0)}{\mathrm{PO_4^{3-}}\ (Day\ 0)} \times 100\%, \qquad (3.6)$$

$$where\ X\ =\ 2, 5, 7, 9, 12, 14, 16\ and\ 19.$$

3.6.6 Total Phosphorus (TP)

Total phosphorus (TP) was measured using the USEPA Method 8190, which measures total phosphorus in a range of 0.00 to 3.50 mg/L PO_4^{3-}. 5 mL of samples were added

into the Test N' Tube vials (Total and Acid Hydrolyzable Test Vial), with one Potassium Powder Pillow for Phosphonate subsequently added into the vials. The vials were capped tightly and shaken to dissolve the powder, before being placed in the preheated DRB 200 COD Reactor (**Figure 3.9**) for 30 minutes at 150 ºC. After removed from the reactor, the vials were cooled to room temperature. 2 mL of sodium hydroxide solution were added into a vial and mixed. The vial was placed in the DR3900 Laboratory Spectrophotometer (**Figure 3.12**) for zero reading. One PhosVer 3 Phosphate Reagent Powder Pillow was added into the vial and shaken for 10 – 15 seconds. After a 2-minute reaction time, the vial was placed again in the spectrophotometer to get the concentration of total phosphorus reading. The same process was repeated for each vial. Total phosphorus removal was calculated using the formula:

$$\text{TP } removal\ (\%) = \frac{\text{TP } (Day\ X) - \text{TP } (Day\ 0)}{\text{TP } (Day\ 0)} \times 100\%, \tag{3.7}$$

$$where\ X\ =\ 2, 5, 7, 9, 12, 14, 16\ and\ 19.$$

3.6.7 Total Suspended Solids (TSS)

Total suspended solids were measured in the unit of mg/L using USEPA Method 160.2. Preparation of fibre filters involved washing glass-fibre filter disks with 3 successive portions of >20 mL distilled water in the vacuum filter, subsequently dried in the XU032 Universal Oven (France Etuves, France) at 104ºC for more than 1 hour, cooled and weighed. The preparation was considered adequate when the manufacturer's weight and measured weight had difference of less than 0.5mg. The prepared fibre filters were kept in the desiccator. For measuring TSS of the samples, the leachate sample of a fixed volume was passed through the fibre filter with applied vacuum and again washed with 3 successive portions of >10 mL distilled water to eliminate dissolved materials. The filter was then carefully placed on a weighing dish using forceps and dried in the oven at 104 ºC for at least 1 hour. The filter was weighed again after cooled to ambient temperature.

TSS would be calculated using the following formula:

$$TSS, mg/L = \frac{(A-B)\times 1000}{sample\ volume, ml} \quad (3.8)$$

Where A = final weight of dried filter with residue (mg), B = initial weight of dried filter (mg)

Figure 3.13: XU032 Universal Oven (France Etuves, France)

3.7 Biomass Characterisation

The obtained dried biomass was characterised to obtain more data on the biomass. In this study, the lipid yield of the biomass was tested because biomass obtained from treatment of landfill leachate is more suitable to be used for bio-fuel production than feedstock due to the toxic substances present in landfill leachate.

3.7.1 Lipid Yield

For lipid extraction, the method of Bligh and Dyer (1959) was used and modified as follows: the weighed dried cells were added 2.25 mL of distilled water and underwent sonication for 5 minutes. Subsequently chloroform and methanol were added at a volume ratio of 5.5:6:6 (distilled water: chloroform: methanol). The samples were placed in 60ºC water bath equipped with magnetic stirrers at 500rpm for 2 hours, the duration recommended by Ahmad et al. (2014) as an optimum reaction time for biodiesel production using *Chlorella sp*. The samples were then centrifuged at 3000rpm for 2 minutes to form 2 separate layers. The bottom layers that consist of chloroform and lipid were transferred to a pre-weighed vial and evaporated in a water bath of 85 ºC. Finally, the dry lipid was weighed again, the lipid yield being calculated using the formula (Bligh and Dyer, 1959):

$$Lipid\ Yield, wt\% = \frac{Weight\ of\ total\ lipid\ (g)}{Weight\ of\ dry\ biomass\ (g)} \times 100\% \qquad (3.9)$$

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Characterisation of Raw Landfill Leachate

Raw landfill leachate samples taken from Sungai Siput Selatan Landfill were characterized in the lab, the results displayed in **Table 4.1**.

Table 4.1: Characterization of Leachate from Sungai Siput Selatan Landfill.

Parameter	Reading	Standard Discharge Limit
pH	7.90 ± 0.23	6.0 – 9.0
Colour	957 ± 228.5 Pt Co	100 Pt Co
COD	337 ± 47 mg/L	400 mg/L
NH_3–N	145 ± 40 mg/L	5 mg/L
Orthophosphate	7.50 ± 0.9 mg/L PO_4^{3-}	-
Total Phosphorus	9.2 ± 1.1 mg/L PO_4^{3-}	-
TSS	8.65 ± 1.45 mg/L	50 mg/L

Based on the Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations, 2009 (**Appendix A**), the leachate had exceeded the standard discharge limit in colour and ammoniacal nitrogen, indicating further treatment is required before release into natural water bodies.

4.2 Treatment performance

5 different leachate concentrations (33v/v%, 44v/v%, 66v/v%, 89v/v%, 100v/v%) were treated with a fixed volume of microalgae stock solution for 19 days, with sampling done on selected 9 times throughout the experimental duration (Day 0, 2, 5, 7, 9, 12, 14, 16, 19). Each sample (45 samples in total) collected from the experiment were tested for their COD removal, ammoniacal-nitrogen removal, orthophosphate removal, total phosphorus removal and colour removal. Removal percentage of the aforementioned parameters are shown and discussed below.

4.2.1 Chemical Oxygen Demand (COD) Removal

The Chemical Oxygen Demand (COD) of each sample were tested, with the results being shown in **Figure 4.1**.

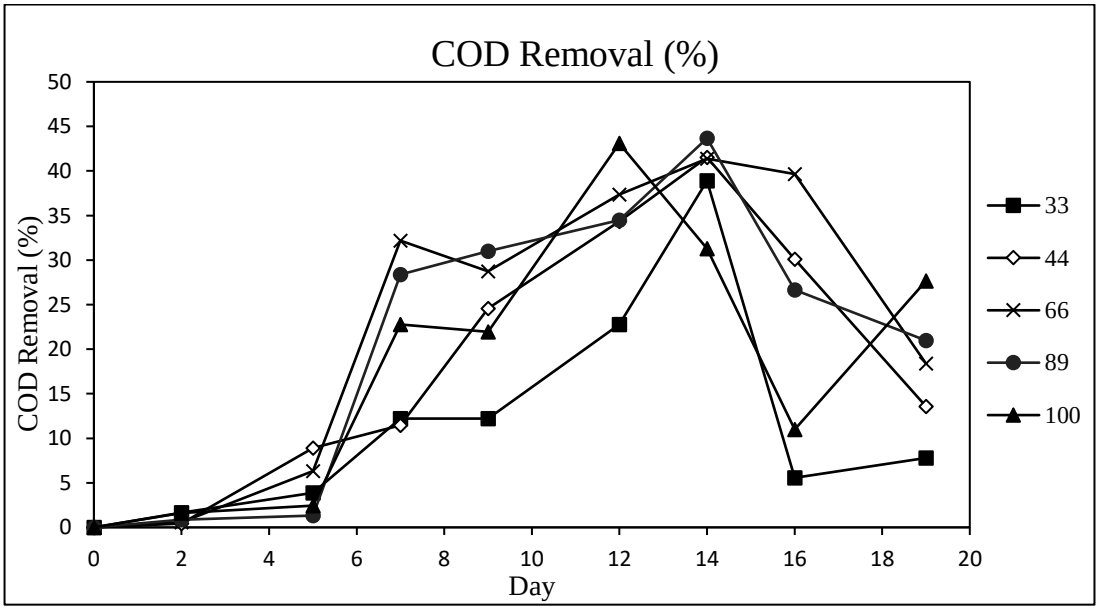

Figure 4.1: COD removal (%) of different leachate concentrations (33%, 44%, 66%, 89%, 100%) by *C. vulgaris* in the function of day.

The optimal COD removal was achieved by 89v/v% leachate concentration at 43.67% on day 14, followed by 43.09% by 100v/v% leachate concentration on day 12. Peak COD removal for other concentrations were all on day 14, being 38.89v/v% (33v/v% leachate) 41.53% (44v/v% leachate) and 41.38% (66v/v% leachate). Although the 2 higher leachate concentrations displayed better peak performances than other concentrations, the removal efficiency of other lower concentrations were not far off from the higher ones. However, all the removal efficiencies were below 50%. The COD removals are observed to have a steep drop right after the day of optimal COD removal in all ranges of leachate concentrations. The decrease of COD removal in the later stage is due to the mixotrophic nature of the *C. vulgaris*, which would use CO_2 as the carbon source when organic substances in the solution that can be utilized are no longer available, causing them to release organic substances back into the solution in the process, such as glycolic acid (Merrett & Lord, 1973).

The overall low COD removal efficiency (<50%) is due to humic substances that exist as the majority portion in stabilised landfill leachate, which are difficult to be bio-degraded (Mojiri et al., 2021). However, COD removals were not high as well in other studies using young or intermediate landfill leachate. A study by el Ouaer et al. (2017) on microalgae treatment on young landfill leachate had shown COD removal of 60% within 13 days using 10v/v% leachate, whilst undiluted leachate shown only 50.7% of COD removal at 24 days. Hu et al. (2021) had reported approximately 60% COD removal in 5% leachate for monocultures of microalgae, namely *C. vulgaris* and *S. dimorphus* whilst 73.6% in their co-cultures; approximately 80% for all cultures in 10v/v% leachate; <40% for all cultures at 15v/v% leachate. The study had shown high removals for co-cultures, indicating that monocultures as was used in this experiment (only *C. vulgaris*) might not be sufficient for good COD removals. Not only that, in this study they had carried out pre-treatment of the leachate using NaClO, which is a strong oxidising agent. This indicates that using a chemical pre-treatment such as oxidation or advanced oxidation processes might help in COD removal by microalgae as these processes are able to break down complex and recalcitrant compounds that exists in landfill leachate.

4.2.2 Ammoniacal Nitrogen (NH_3–N) Removal

Ammoniacal nitrogen includes un-ionized ammonia (NH_3) and ammonium ions (NH_4^+), where free NH_3 mainly contributes to ammonia toxicity while NH_4^+ contributes to a certain degree of toxicity when in high concentration (Zhou and Boyd, 2016; Liew et al., 2013). **Figure 4.2** shows graph of ammoniacal nitrogen removal efficiency vs time of all leachate concentrations. For this parameter, there are control samples where pure leachate samples without addition of microalgae were also run to determine the NH_3–N removal due to aeration, with the results shown in **Figure 4.2** as well.

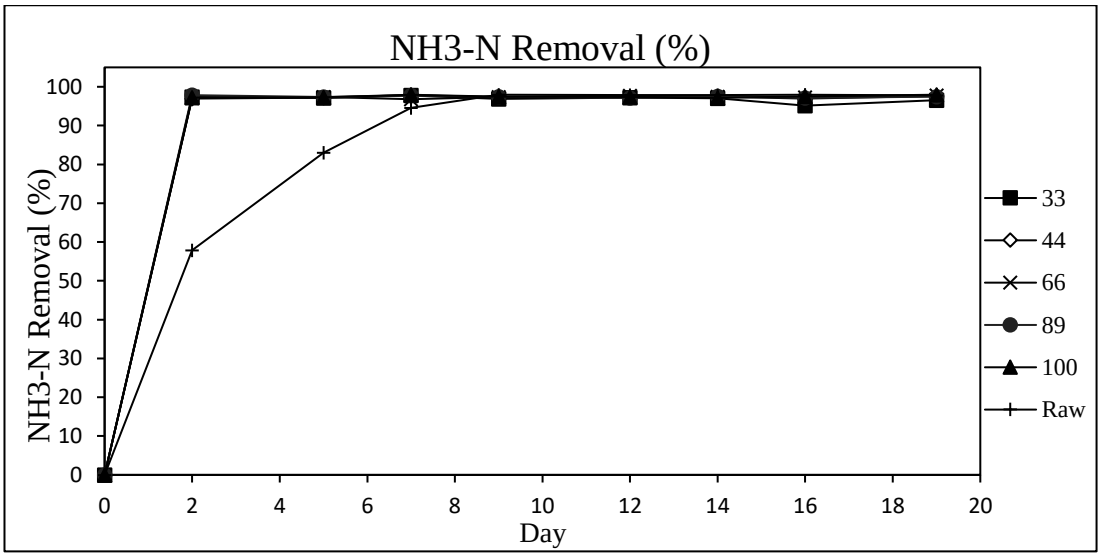

Figure 4.2: NH_3–N Removal (%) of different leachate concentrations (33v/v%, 44v/v%, 66v/v%, 89v/v%, 100v/v%) by *C. vulgaris* and without *C. vulgaris* (Raw) in the function of day.

The ammoniacal nitrogen removal efficiency of every leachate concentration was extremely high, achieving ≥97% removal efficiency on Day 2, whilst maintaining a removal efficiency of >95% throughout the entire duration. This can be explained by the microalgae utilising ammonium as its nitrogen source. Algae species prefer ammonium as the nitrogen source as it can be directly used to incorporate into amino acids, whilst other inorganic nitrogen sources have to be converted into ammonium form first during assimilation (Cai et al., 2013; Pereira et al., 2016). Pure raw leachate

samples also however had high removals, achieving 57.88% on day 2 and >97% removal efficiency on day 9, indicating there are other means of ammoniacal nitrogen removal. One of them being ammonia stripping that happens when aeration is provided, causing volatilization of ammonia from the solution (Tam and Wong, 1989; Lin et al., 2007). Volatilization of NH_3 would have contributed to the rapid removal of ammoniacal-nitrogen and the survival of the microalgae, as NH_3 causes toxicity. Another means of ammoniacal nitrogen removal in raw leachate might be due to bacterial activity from microorganisms present in the leachate as the solutions did not undergo autoclaving.

4.2.3 Orthophosphate (PO_3^{3-}) Removal

Orthophosphate concentrations were tested in all samples, the data shown in **Figure 4.3**.

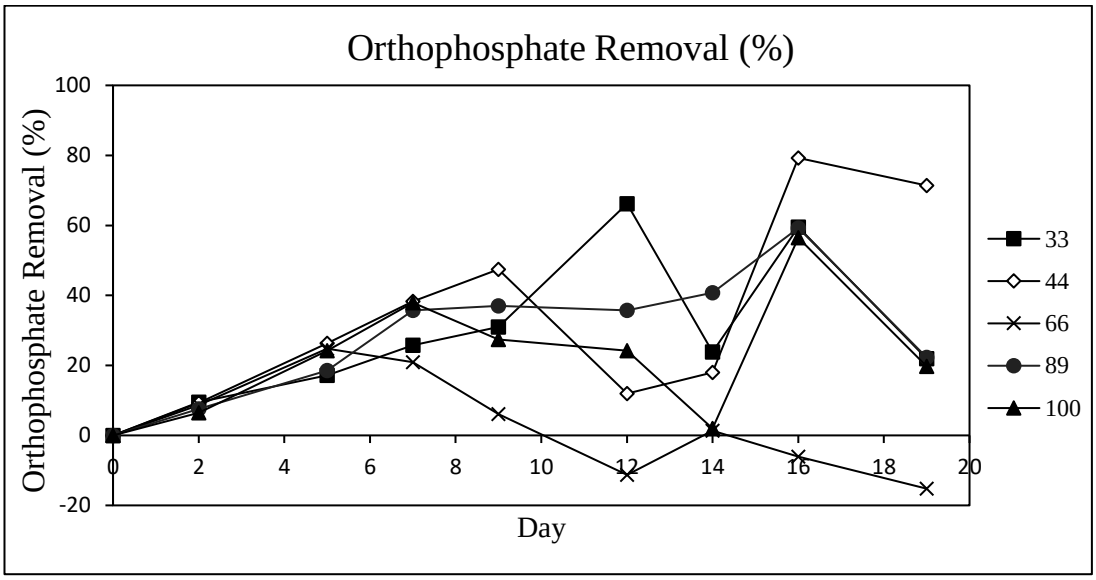

Figure 4.3: Orthophosphate Removal (%) of different leachate concentrations (33v/v%, 44v/v%, 66v/v%, 89v/v%, 100v/v%) by *C. vulgaris* in the function of day.

Microalgae can directly uptake orthophosphate as a phosphorus source for cell growth and nutritional content (Azrina Yaakob et al., 2021). From **Figure 4.3**, highest

orthophosphate removal was achieved by 44v/v% leachate concentration on day 16 at 79.26%. Peak removal for other concentrations were 66.19% (day 12), 24.78% (day 5), 59.24% (day 16) and 56.45% (day 16) for concentrations of 33v/v%, 66v/v%, 89v/v% and 100v/v% respectively. 66v/v% leachate had the worst performance, even having negative orthophosphate removal. This can be correlated with the biomass production performance of 66v/v% leachate (**Figure 4.6**), which also had the worst performance in terms of biomass growth, as orthophosphate is used for cell growth. Negative removal can be caused by the release of materials by the microalgae and efflux by dead cells into the medium, which occurs in stationary and death phase of the microalgal growth curve (Becker, 1994).

The inability of the microalgae in this experiment to have high orthophosphate removals might be due to being limited by the concentration of nitrogen in the leachate. A study by Beuckels et al. (2015) had concluded that *Chlorella* sp. and *Scenedesumus* sp. can adjust the nitrogen and phosphorus concentration in their biomass based on the supply, however the degree of adjustment had depended on the nitrogen concentration, implying that high nitrogen concentration is a prerequisite for effective phosphorus removal. In a study by Pereira et al. (2016), the data have shown complete removal of PO_4^{3-} and NH_4^+ in one of the assays at a ratio of 35:1, while other ratios of 12:1 and 23:1 did not have a complete removal. Other assays also had shown more favourable PO_3^{3-} removal at higher ammoniacal nitrogen concentrations. In this experiment the raw leachate shows a NH_3–N to PO_3^{3-} ratio of about 16:1, which indicates the ammoniacal nitrogen concentration might not be high enough for complete orthophosphate removal.

4.2.4 Total Phosphorus (TP) Removal

The total phosphorus removal efficiency was tested for all samples, the data shown in **Figure 4.4**.

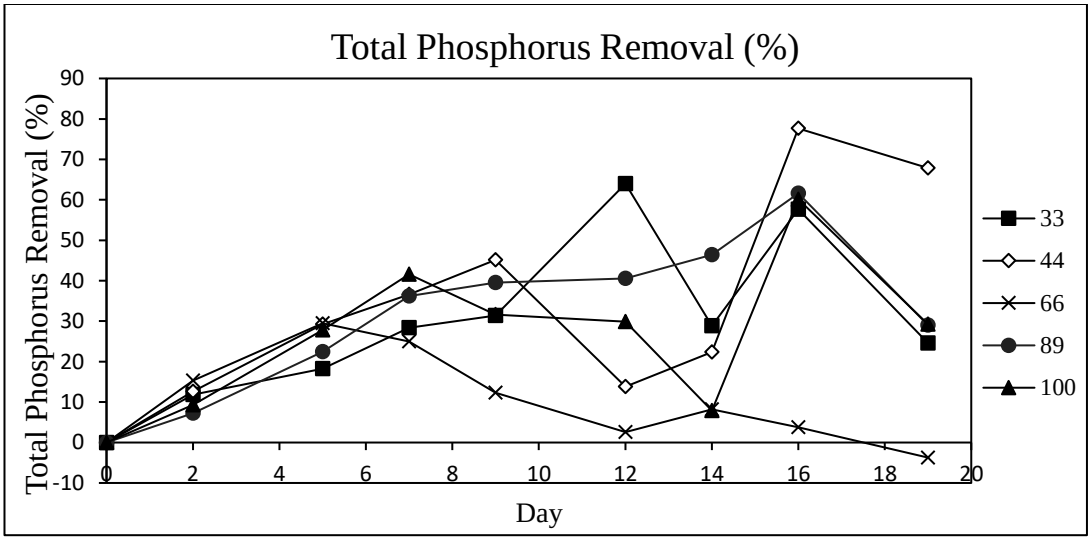

Figure 4.4: Total Phosphorus Removal (%) of different leachate concentrations (33v/v%, 44v/v%, 66v/v%, 89v/v%, 100v/v%) by *C. vulgaris* in the function of day.

From **Figure 4.4**, the overall trend of total phosphorus removal is extremely close with the trend for orthophosphate removal (**Figure 4.3**), with the highest removal also being 44v/v% leachate on day 16 at 77.64%. Highest removal for other concentrations were 63.98% (day 12), 29.48% (day 5), 61.59% (day 16) and 60.14% (day 16) for concentrations of 33v/v%, 66v/v%, 89v/v% and 100v/v% respectively. The initial non-orthophosphate content was <15% of total phosphorus for all samples, and with the trend in the orthophosphate (**Figure 4.3)** being highly similar to total phosphorus in **Figure 4.4**, it can be said that the main component affecting total phosphorus removal by microalgae was orthophosphate in the stabilized landfill leachate. 33v/v% had also negative removal on the last day, mostly likely caused by efflux of dead cells as mentioned in the previous section. Similar to orthophosphate removal, complete or high TP removal did not happen due to insufficient nitrogen concentration. Study by Hu et al. (2021) shows an average of approximate 80% TP removal for 3 dilutions with an N:P ratio in the leachate of approximately 47:1,

showing a high concentration of nitrogen source. Similar high TP removals of >87% was achieved by Tang et al. (2023) with a N:P ratio of 53:1, with the initial concentration of phosphorus being 6.21 mg/L.

4.2.5 Colour Removal

As stabilized landfill leachate had high colour which exceeds the standard discharge limit, colour removal was tested on all samples, the results showed in **Figure 4.5**.

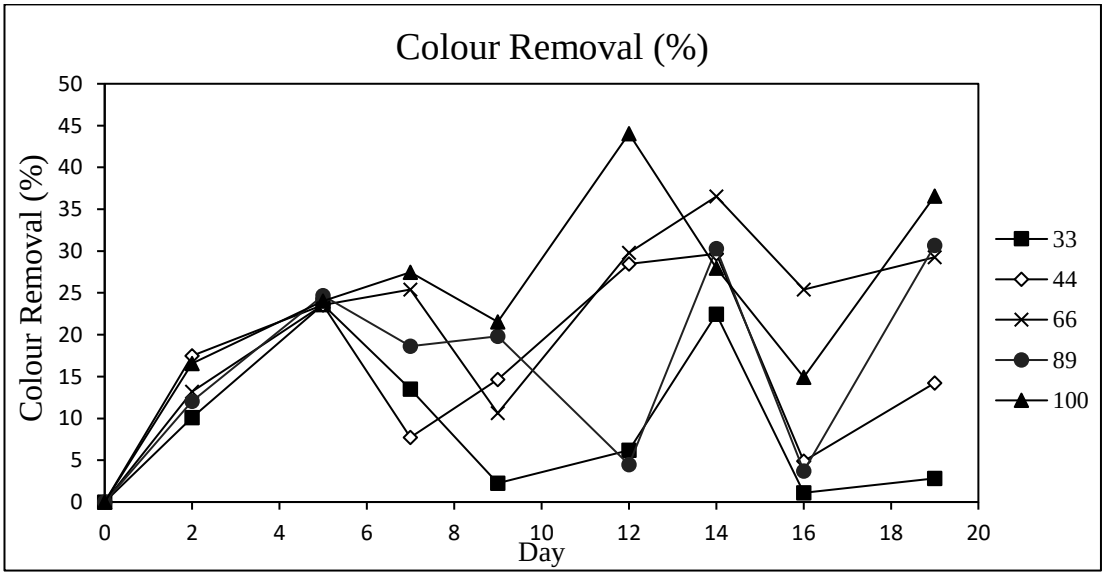

Figure 4.5: Colour Removal (%) of different leachate concentrations (33v/v%, 44v/v%, 66v/v%, 89v/v%, 100v/v%) by *C. vulgaris* in the function of day.

The main contributor to the brown colour of stabilized landfill leachate is from humic and fulvic substances, with other side contributors being ferric hydroxide colloids (Shadi et al., 2020; Shehzad et al., 2016; Naveen et al., 2016). As previous literatures have suggested humic and fulvic substances being the main contributor to organic substances in stabilized landfill leachate (Kjeldsen et. al., 2010; Mojiri et. al., 2021), it can be said that colour removal of stabilized landfill leachate is closely related to the COD removal as well. Based on **Figure 4.5**, the trend of colour removal is

observed to have some resemblance to the trend of COD removal (**Figure 4.1**). The highest colour removal is 44.04% by 100v/v% leachate on day 12. Coincidently, the highest COD removal for 100% leachate was also on day 12. The highest colour removal by 44v/v%, 66v/v% and 89v/v% leachate were on day 14, and highest COD removal for these concentrations were also on day 14. Colour and COD removals are associated with each other for landfill leachate, as shown in studies by Abd Aziz and Ramli (2014) and Veli et al. (2021) using advanced oxidation processes where condition variations would result in similar trends of increasing and decreasing in both colour and COD removal rates. Thus, it can be said that if COD removals were improved via methods such as having a pre-treatment or using co-cultures, the colour removal efficiency would improve as well.

4.3 Biomass Production

Biomass of each sample were obtained through centrifugation and were subsequently dried and weighed. The generation of the biomass were calculated by taking the mass of the dried sample deducting the mass of the initial biomass on Day 0. Data of biomass generated of each sample is shown in **Figure 4.6**.

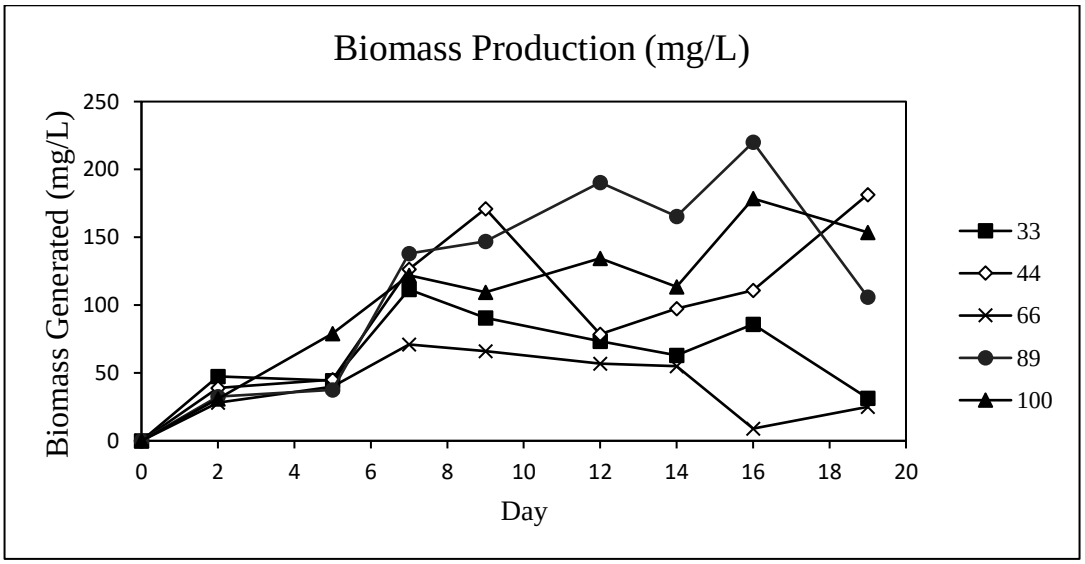

Figure 4.6: Graph of Biomass Generated (mg/L) vs Days.

From the data, it was shown that 89v/v% leachate concentration had the highest biomass production of 220 mg/L on day 16 compared to other concentrations. Study by other researchers reported that *C. vulgaris* can have biomass production of 2-5 $gL^{-1}d^{-1}$ (Yeh and Chang, 2012) and up to 3.86 ± 0.03 gL^{-1} in controlled solutions (Silva et al., 2022). Under optimum conditions and sufficient nutrients, microalgae can produce high concentrations of biomass. For macronutrients, besides carbon in the form of CO_2 or organic carbon, nitrogen is the second most important element in their growth (Becker, 1998). For micronutrients, certain metals such as magnesium, sodium, potassium, manganese, nickel, copper, iron and zinc are important for the cell growth and lipid production (Mulgund, 2022; Becker, 1998), which may be an advantage of using landfill leachate. However, high concentrations of heavy metals in landfill leachate cause toxicity and can greatly inhibit enzyme activity and synthesis of chlorophyll, affecting microalgal growth (Battah et al., 2014). A paper by Qian et al. (2009) had demonstrated 78.55% of *C. vulgaris* cell inhibition with the solution containing only 1.5 µM copper and 2.0 µM cadmium.

In landfill leachates, authors have reported a much lower *C. vulgaris* biomass production range: 0.81-1.71 gL^{-1} in pre-treated young landfill leachate (Pereira et al., 2016); about 0.1-0.25 gL^{-1} in diluted landfill leachate (Hu et al., 2021); 0.283 ± 0.034 gL^{-1} in 10% leachate (Tang et al., 2023). The amount of biomass generated in the present study is close to the aforementioned studies, which is lower than microalgae cultivated in normal solutions, most likely caused by inhibition by toxic compounds in landfill leachate and the lack of certain nutrients.

The overall trend of biomass production for 89v/v% leachate was also higher than the rest, with its biomass production on day 7, 12, 14, 16 being the highest compared to other leachate concentrations. The trend lines in the graph resemble that of a microalgae growth curve. Peak of biomass production varies between different concentrations, with 33v/v% and 66v/v% leachate peaking on day 7 (111.5 mg/L and 71.0 mg/L), 44v/v% on day 9 (171.0 mg/L) and seemingly again on day 19 (181.5 mg/L), 89v/v% and 100% on day 16 (220.0 mg/L and 178.5 mg/L). The trend shows that lower concentrations tend to reach to optimal concentrations earlier while higher concentrations of leachate tend to reach later. This is due to different conditions the

microalgae are being exposed to, which in this case is the nutrients and toxicity concentrations due to the dilutions, resulting in different lengths of each phase in the growth curve for different dilutions (Becker, 1994). Similar patterns of having maximum biomass on different days for different concentrations were also observed in other studies. In a study by Hu et al. (2021), the diluted biological landfill effluent of concentration of 10v/v% and 15v/v% had seemingly peaked on Day 6, while 5v/v% kept increasing until the end of the experiment period which was 10 days. It is to be noted that Hu et al. (2021) had used pre-treated young leachate which still had high nutrient concentrations: 2114 ± 221 mg/L NH_4^+, 7063 ± 221 mg/L COD, 54 ± 11 mg/L TP, and from the results it was prominent 5v/v% had the best results for *C. vulgaris* growth. It is shown that under favourable conditions, the biomass kept increasing until day 10, whilst for non-favourable conditions, which in their case is the higher leachate concentration, the biomass production reached the highest at an earlier stage. Similar behaviour of *C. vulgaris* of decreasing biomass production in unfavourable concentrations at earlier stages compared to favourable concentrations was also observed in a study by Nordin et al. (2017).

Both higher concentrations of leachate, 89v/v% and 100v/v% are observed to have similar trends. From **Figure 4.6**, 100% had a similar trend with 89v/v%, gradually increasing in biomass production reaching day 12, decreased production on day 14 and peaking on day 16, lastly dropping after the peak. Although higher leachate concentrations tend to reach the optimal concentration in the later period, it is to be noted even in the early period, higher concentrations produce biomass amounts that are close with those of lower concentrations. On day 7, 89v/v% had the highest production (138 mg/L) with 100v/v% being close as the 3rd highest (122.0 mg/L); on day 9, 89v/v% was the 2nd highest production (147.0 mg/L) followed by 100v/v% (109.5 mg/L). As this happened even in the early stage where lower concentrations were reaching their optimal, this proves that lower concentrations reaching their optimal concentrations earlier is due to having lesser nutrients, but not due to being able to adapt quicker in lower toxicity concentrations, as higher concentrations had a close amount of biomass produced in early stage.

Unlike biomass data reported in some studies (Pereira et al., 2016; Lu et al., 2015; Nordin et al., 2017; Tan et al., 2022) that have smooth increasing trendlines, the

biomass trendline in this experiment does not smoothly increase or decrease, instead having pits in between increasing trends. This might be due to the aforementioned studies applying sterilization such as autoclaving or pre-treatment processes on the wastewater such as nitrification, centrifugation, coagulation-flocculation, biological oxidation and photo-oxidations processes. Studies by Lin et al. (2007), Silva et al. (2022) and Paiva et al. (2021) with no sterilization or major pre-treatments applied had shown similar trendlines to the present study, where the graph shows multiple peaks and pits in between increasing growth. In a study by Li et al. (2011), they had compared biomass concentration between autoclaved municipal centrate and raw centrate, in which they found out algae growth in autoclaved centrate has similar rate and patterns with the "ideal" TAP medium, while raw centrate showed different growth rates with a sooner declining phase, which they deemed algal growth in raw centrate is complicated due to existing bacterial growth. Similar opinions were stated in studies by Silva et al. (2022) and Abid et al. (2017), where other microorganisms such as endogenous bacteria in the existing wastewater would cause progressive adjustment and growth periods.

4.4 Process Optimization and Lipid Yield

Treatment performance and biomass production were analysed for determining optimum sets of high treatment performance with high or acceptable biomass productivity and acceptable time frame. These samples will be subsequently tested for lipid yield. For COD, 89v/v% leachate achieved the highest removal (43.67% - day 14) followed by 100v/v% leachate (43.09% - day 12). For colour, 100v/v% achieved highest removal (44.04% - day 12), followed by 100v/v% as well (36.59% - day 16) and 66v/v% (36.53 - day 12). For NH_3–N, removal was >97% for all samples on day 2. For orthophosphate, 44v/v% achieved the highest (79.26% - day 16), followed by 33v/v% (66.19% - day 12). Similar rankings were observed for total phosphate, 44v/v% being the highest (77.64%) followed by 33v/v% (63.98%). Lastly for biomass, the highest production was by 89v/v% leachate (220.0 mg/L - day 16), followed by also 89v/v% (190.5 mg/L – day 12), 44v/v% (181.5 mg/L – day 19), and closely by 100v/v% (178.5 mg/L – day 16).

From the study by Tighiri and Erkurt (2019) that treated landfill leachate in a sequencing batch mode, they had determined the end period of each cycle according to any nutrient removal that was approximately greater than 95%. This indicates the focus on treatment performance for cultivation of microalgae. Thus, from the data obtained in this study, 89v/v% and 100v/v% leachate concentration had achieved high removals in COD and colour, along with high biomass production. Thus, these 2 concentrations were selected for lipid yield testing. Day 12 was selected for 100v/v% leachate as it had the highest colour removal, whilst day 14 for 89v/v% leachate for its highest COD removal. Both samples also exhibited high ammoniacal nitrogen removal.

As biomass produced from landfill leachate is only most suitable for biofuel production due heavy metals and other toxic substances present in landfill leachate, lipid yields of selected samples were tested to evaluate the potential for biofuel. Optimum samples were picked in terms of nutrient removals and highest biomass generated from the previous results. For highest biomass generated, the peak production in the middle of the growth trends were selected. Data on the samples picked are shown in **Table 4.2**, with the leachate concentration, selected day, biomass generated, and lipid yield stated in the table.

Table 4.2 Lipid Yield (%).

	Leachate Concentration	**Day**	**Biomass Generated**	**Lipid Yield**
	v/v%		**mg/L**	**%**
Optimum treatment performance	89	14	165.5	13.96
	100	12	134.5	11.01
Highest biomass	33	7	111.5	9.30
	44	9	171.0	11.84
	66	7	71.0	27.69
	89	16	220.0	8.14
	100	16	178.5	13.58

C. vulgaris is reported to be able to reach 5-40% lipids per dry weight under optimal growth (Becker, 1994). However, under unfavourable conditions it was reported to even reach 58% (Becker, 1994; Mata et al., 2010b; Stephenson et al., 2014;

Safi et al., 2014). **Table 4.2** shows a lipid yield of 8.14-27.69%, which is in range of reported yields. For samples picked for optimum nutrient removals, the lipid yield was 13.96% and 11.01% respectively for 89v/v% and 100v/v% leachate. Study by Hu et al. (2021) shows lipid yield in a range of 21-28% (biomass about 0.1-0.25 gL^{-1}) for 5-15% diluted leachate, whilst (Tang et al., 2023) reported lipid yield of 27.60% (biomass 0.283 ± 0.034 gL^{-1}) on day 10 with 10% leachate. Lipid accumulation in microalgae is often encouraged by stress conditions, such as temperature and nutrient starvation, however would in turn reduce biomass productivity (Monfet and Unc, 2017; Mulgund, 2022). For these 2 samples at the instance, they had high removals of COD and colour along with ammoniacal nitrogen, and in the biomass generation trend they were both before the peak of biomass production, indicating they were not under high nutrient starvation, which explains the lower lipid yield compared to other studies.

For highest biomass production, the lipid yield results for 66v/v% and 89v/v% can also be explained by biomass yields which indicates stressful conditions for the microalgae. 66v/v% had extremely low biomass, indicating higher stress conditions, which resulted in highest lipid yield (27.69%). Meanwhile 89v/v% which had the highest biomass yield indicated lower stress conditions, thus resulting in lowest lipid yield (8.14%).

4.5 Cost Analysis

To evaluate the economical suitability of using microalgae as a treatment of landfill leachate, a cost analysis was conducted. The cost analysis method was based on a study by Nagarajan et al. (2013) that calculated the cost required to produce 1 L of biodiesel using microalgae in an area of 4,000,000 m^2. The cost includes operational costs, harvesting costs and biofuel extraction costs. The study assumes an open pond system of 0.3 m depth with paddle wheels for mixing and aeration. Operational activities and estimations were modified according to Malaysian prices and the conditions of the experiment as discussed below.

In this study, the data for the undiluted sample on day 12, 212.5 mg/L biomass was used for calculations. For ease of calculations, it is converted into the unit of per

m^2 by multiplying with the assumed pond depth of 0.3 m, obtaining a value of 63.75 g/m^2, which is close to the referred paper's value of 60 g/m^2. Power for groundwater extraction and flue gas supply, and nutrients in form of N and P from the referred paper were omitted from the calculation in the present study, as no water, flue gas nor nutrients were additionally supplied. Power rates were assumed to be RM 0.35/kWh for industrial tariff rates, being $0.08 when converted to USD. Price for methanol and sodium hydroxide (NaOH) were assumed at a rate of $405/MT and $600/MT respectively according to newest sources (Methanax Corporation, 2023; Procurement Resource, 2023). Labour costs were estimated to be RM28.57/hr or $6.43/hr according to technician operator rates (Salary Expert, 2023). Other costs such as polyelectrolyte and waste disposal were adapted from the paper using a cost inflation index of 1.655. Maintenance, tax and insurance were assumed to be 15% of the total operating cost. The final analysis by using the method based on Nagarajan et al. (2013) modified as aforementioned is shown in **Table 4.3**. The cost to treat $1m^3$ leachate is calculated to be ~$0.03. For this study, the cost to treat $1m^3$ leachate was used instead of the cost required to produce 1L of biodiesel, which was the focus of the study by Nagarajan et al. (2013). However, the cost was calculated regardless to provide a value for comparison with the paper. The value obtained was $1.50 per litre of biodiesel.

Table 4.3: Cost required to treat $1m^3$ of leachate using microalgae.

Operating cost ($/ha/yr)	**Price (RM)**	**Price ($)**
Power for paddle wheels	3,769.31	848.94
Power (ultrasonic + centrifugation + mixer)	2,558.65	576.28
Power, Other	554.20	124.82
Methanol ($405/MT)	83,418.90	18,788.04
NaOH ($600/MT)	30,895.89	6,958.53
Polyelectrolyte ($910.25/MT)	3,057.59	688.65
Labour	6,372.64	1,435.28
Waste disposal	13,622.83	3,068.20
Maintenance, tax, insurance (@15% of total cost)	21,637.50	4,873.31
Total operating cost ($/ha/yr)	**165,887.49**	**37,362.06**
Cost per m^3 leachate ($/$m^3$)	**0.15**	**0.03**
Cost per $100m^3$ leachate ($/$100m^3$)	**15.15**	**3.41**
Cost per L biodiesel ($/L)	**6.66**	**1.50**

For biodiesel production, the data for the undiluted sample obtaining 11.01% lipid yield is used, unlike 50% lipid yield used in the referred paper. Assuming the biodiesel produced will have an 89% conversion efficiency from the lipid yield and a density of 862kg/m^3 (Arun et al., 2022), the biodiesel yield for each m^3 of treated leachate would be 0.0242 L/m^3 (Refer to **Appendix B** for detailed calculation steps). The cost per L biodiesel is shown in **Table 4.3**, whilst the potential earning from biodiesel is shown in **Table 4.4**. The calculated potential earning value is found to be not significant, being only $0.01 per m^3 of treated leachate. This is because the lipid yield used for calculation is only 11.01%, compared to the study of using 50%. The high lipid yields greatly reduced their cost of producing 1L of biodiesel, being $0.42/L in year 2012, $0.70/L if inflated using the inflation cost index, much lower compared to the value of $1.50/L obtained in **Table 4.3**.

Table 4.4: Potential earnings from biodiesel sold.

Biomass produced per m^3 leachate (g/m^3)	212.50
Biodiesel produced per m^3 leachate (L/m^3)	0.0242
Potential earning for biodiesel sold per m^3 treated leachate ($/m^3) (selling price: $0.52/L)	**0.01**
Potential earning for biodiesel sold per 100m^3 treated leachate ($/100m^3) (selling price: $0.52/L)	**1.26**

The market price of biodiesel in Malaysia for B10 Biodiesel blend of $0.52/L in August 2021 (Wahab, 2021) was used for calculation in **Table 4.4**. Biodiesel prices of other countries are shown in **Table 4.5**, with potential earnings according to the values being calculated as well. The higher price of biodiesels in other countries indicates the potential for higher earnings in export markets.

Table 4.5: Biodiesel prices in different countries.

Country	**Biodiesel Blend**	**Price ($/L)**	**Potential earning ($/m^3 leachate)**	**Source**
Malaysia	B10	0.52	0.01	(Wahab, 2021)
United States	B20	4.46	0.11	(U.S. Department of Energy, 2023)
	B99/B100	5.22	0.13	
Japan	B100	~4.74	0.11	(Sasatani, 2021)

Another potential earning from the microalgae treatment is via selling carbon credits. Carbon credit price is estimated at \$15.38/ton CO_2 in the year 2015 in a study by (Tan et al., 2015). Carbon dioxide fixation is assumed to be 0.318g CO_2/L wastewater/day according to a study on carbon dioxide fixation using *C. vulgaris* by (Ayatollahi et al., 2021). The potential earnings were calculated in **Table 4.6**, the value also being insignificant when in small amounts, being \$0.49 for 100m^3 of leachate treated per day.

Table 4.6: Potential Earnings from Carbon Credit

Carbon credit price (\$/ton CO2)	15.38
CO_2 fixation rate (g/L/d)	0.318
CO_2 fixation rate (g/m^3/d)	318
Carbon credit per m^3 leachate per day (\$/m^3)	**0.005**
Carbon credit per 100m^3 leachate per day (\$/100m^3)	**0.49**

The final cost required to treat 1m^3 and 100m^3 of landfill leachate using microalgae cultivation after the deduction of potential earnings was calculated in **Table 4.7**. The cost to treat 1m^3 of landfill leachate is found to be \$0.02 or RM0.08. Another table was constructed on operational costs required for the treatment of landfill leachate with other methods in **Table 4.8**, which is shown to be in a wide range of values due to the different conditions of each place and the variations of landfill leachate. The calculated treatment cost of \$0.02/m^3 leachate is found to be in an acceptable range to be incorporated in landfill leachate treatment.

Table 4.7: Final cost of treatment for landfill leachate using microalgae cultivation.

	1m^3	**100m^3**
Cost of treatment (\$/m^3)	0.03	3.41
Potential earning for biodiesel sold (\$/m^3)	-0.01	-1.26
Potential earning from carbon crediting (\$/m^3)	-0.005	-0.49
Final cost of treatment (\$/m^3)	**0.02**	**1.66**
Final cost of treatment in RM (RM/m^3)	0.08	7.38

Table 4.8: Leachate treatment cost estimation of other methods.

Location	Type of leachate	Treatment Method	Operational Cost ($/m^3)	Reference
Brazil	Young	Reverse Osmosis	0.132-0.265	(de Almeida, Bila, et al., 2020)
Malaysia	Young	N.A.	8.48	(Rangga et al., 2019)
China	N.A.	Conventional; Enhanced	7.60; 4.78	(Zhang et al., 2023)
Brazil	N.A.	Coagulation-Flocculation/Air Striping with nanofiltration	10.5-12.80	(de Almeida, Oroski, et al., 2020)

The estimated cost of $0.02/m^3 leachate treatment is found to be in an acceptable range, while also having the potential for more earnings if the market were to be widened to exportations. However, it is to be noted that the calculations done only serve to view microalgae as a potential treatment method to be used on a large scale, thus having a few shortcomings. One of them being the capital costs of the treatment not calculated. Besides that, unlike the referred paper which assumed biomass collection on every day, the experiment in the present study collected the biomass on day 12 of the undiluted leachate for best treatment, and it is unclear on the biomass production performance if the experiment were to be carried out with continuous flow and hydraulic retention time of 12 days. However, the cost estimation is expected to not be greatly affected as the cost of biodiesel extraction would decrease as well if the biomass yields were to be decreased. Besides that, as mentioned in the previous sections, the COD and colour removal of using microalgae alone were not high enough for the safe release of the landfill leachate, indicating other pre-treatments or modifications towards the cultivation methods are required, which may increase the treatment cost.

4.6 Summary on Findings

Cultivation of microalgae in high concentration (89v/v%) and undiluted stabilized landfill leachate yielded better results in some parameters than other lower concentrations, thus indicating cultivation of microalgae in high concentration or undiluted stabilized landfill leachate is possible and feasible. High ammoniacal nitrogen removal (>95%) was achieved in all concentrations. Highest removals for each parameter were 43.67% for COD (89v/v% leachate day 14), >97% for NH_3–N (all concentrations day 2), 79.26% for PO_3^{3-} (44v/v% leachate day 16), 77.64% for TP (44v/v% leachate day 16) and 44.04% for colour (100v/v% leachate day 12). Poor COD and colour removals due to recalcitrant compounds indicated that pre-treatments might be required for higher removal in these parameters.

Microalgae cultivation for treatment of stabilized landfill leachate reduces the negative impact on the environment, firstly by treating the leachate before being released into the environment. It also performs carbon capture from the atmosphere by using CO_2 in its autotrophic processes and can be a strategy for CO_2 mitigations (Li et al., 2023). Not only that, but it is also able to produce biofuel that is valuable as fossil fuels are being depleted, with a smaller area occupied compared to other crop-based production (Prabandono and Amin, 2015).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In line to objective (i), the study had demonstrated the performance of microalgae in high ammoniacal nitrogen removals and moderate removals of COD, colour, orthophosphate and total phosphate in stabilized landfill leachate. It was shown high concentrations (89v/v%) and undiluted stabilized landfill leachate had performed well compared to other dilutions in major parameters such as ammoniacal nitrogen, COD and colour. For objective (ii), optimum cultivation duration for treatment and biomass harvesting were between day 12 to 16 where there were highest nutrient removals and biomass production. For objective (iii), lipid yield testing had given results of 8.14-27.69% lipid yield, which are within range of lipid content of the *C. vulgaris*. The lipid yield is also shown to be affected by biomass production.

Cultivation of microalgae in undiluted stabilized landfill leachate had yielded good results compared to other dilutions, thus indicating feasibility for microalgae treatment without any dilutions. 100v/v% was the most desirable concentration as it did not require any dilutions and had good treatment performances compared to other dilutions. 100v/v% had demonstrated highest colour removal on day 12, which is the parameter that did not meet standard discharge limits, and second highest COD removal with close to complete removal in ammoniacal nitrogen as well, thus being the most optimum sample in treatment. However, as demonstrated colour removal performances were poor and did not meet the standard discharge limits, indicating improvements in the treatment system is required.

5.2 Recommendations

- To improve colour removal, pre-treatment of the leachate can be carried out via methods such as oxidation or advanced oxidation processes that are effective in breaking down recalcitrant compounds. Symbiosis of the algal culture with other algal species or bacteria can also be used to improve the performance.
- To test the feasibility of using microalgae for stabilized landfill leachate treatment, it is recommended to carry out the experiment in pilot scale and on stabilized landfill leachate samples sourced from other stabilized landfills.

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APPENDICES

Appendix A: Acceptable Conditions for Discharge of Leachate (Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations, 2009))

	Parameter	Unit	Standard
i	Temperature	°C	40
ii	pH Value	-	6.0 -9.0
Iii	BOD_5 at 20°C	mg/L	20
Iv	COD	mg/L	400
V	Suspended Solids	mg/L	50
Vi	Ammoniacal Nitrogen	mg/L	5
vii	Mercury	mg/L	0.005
Viii	Cadmium	mg/L	0.01
Ix	Chromium, Hexavalent	mg/L	0.05
X	Chromium, Trivalent	mg/L	0.20
Xi	Arsenic	mg/L	0.05
Xii	Cyanide	mg/L	0.05
Xiii	Lead	mg/L	0.10
Xiv	Copper	mg/L	0.20
Xv	Manganese	mg/L	0.20
Xvi	Nickel	mg/L	0.20
Xvii	Tin	mg/L	0.20
Xviii	Zinc	mg/L	2.0
Xix	Boron	mg/L	1.0

Xx	Iron	mg/L	5.0
Xxi	Silver	mg/L	0.10
Xxii	Selenium	mg/L	0.02
Xxiii	Barium	mg/L	1.0
Xxiv	Fluoride	mg/L	2.0
Xxv	Formaldehyde	mg/L	1.0
Xxvi	Phenol	mg/L	0.001
Xxvii	Sulphide	mg/L	0.50
Xxviii	Oil and Grease	mg/L	5.0
Xxix	Colour	ADMI	100

Appendix B: Calculation steps for biodiesel yield per m^3 treated landfill leachate.

Lipid Yield = 11.01%

Biodiesel conversion efficiency = 89% (Arun et al., 2022)

Density of biodiesel from *C. vulgaris* = 862kg/m^3 = 862g/L (Arun et al., 2022)

Biomass Productivity = 212.5mg/L = 212.5g/m^3

Lipid Productivity in g per m^3 = 212.5g/m^3 × 11.01% = 23.3963g/m^3

Biodiesel Productivity in g per m^3 = 23.3963g/m^3 × 89% = 20.8227g/m^3

Biodiesel Productivity in L per m^3 = 20.8227g/m^3 ÷ 862g/L = **0.0242L/m^3**