TREATMENT OF MATURE LANDFILL LEACHATE USING COMBINED COAGULATION, FILTRATION AND MICROALGAE PROCESSES

By
SWARNA KAMALA SUBRAMANIAM

A thesis/dissertation submitted to the Institute of Postgraduate Studies and Research,
Faculty of Science,
Universiti Tunku Abdul Rahman,
in partial fulfillment of the requirements for the degree of
Master of Science
October 2018

ABSTRACT

TREATMENT OF MATURE LANDFILL LEACHATE USING COMBINED COAGULATION, FILTRATION AND MICROALGAE PROCESSES

Swarna Kamala Subramaniam

Nowadays, water pollution issues have increased drastically, and these problems are major concern of our society. The research on landfill leachate is to explore the dual-purpose method to treat the leachate as well as microalgae culturing in mature leachate. In this research, 4 different filtration systems set up were used for filtration treatment. First 3 filtration systems were modified set up using activated carbon and the last filtration system set up act as a control. The mature leachate samples were undergone coagulation process by using aluminium ammonium sulphate 12-hydrate before the treatment using filtration system. The chemical analysis such as Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), turbidity, pH, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Organic Carbon (TOC), and heavy metals for mature leachate before treatment, after coagulation-filtration and after coagulation-filtration-microalgae treatment. The leachate samples after coagulation-filtration were polished using microalgae which are *Botryococcus sudeticus* and *Chlorella vulgaris* separately. The growth of *Chlorella vulgaris* and *Botryococcus sudeticus* were observed without dilution of pre-treated leachate sample. As a result, the chemical, physical and biological treatment achieved removal efficiencies of 76% TS, 98% TSS, 76% TDS, 95% turbidity, 77% conductivity, 94% BOD_5, 99% TOC, 99% COD using *Chlorella vulgaris* and 79% TS, 98% TSS, 66% TDS, 95% turbidity, 83% conductivity, 89% BOD_5, 99% TOC, 97% COD using *Botryococcus sudeticus*. After coagulation-filtration treatment, a significant drop in Na, Mg, Al, K and Ca has been identified which is more than 80% of metal reduction has been taken place after the combined treatment using *Chlorella vulgaris*. The results show that the deteriorative impact of leachate wastewater to the environment can be reduced with successful treatment of leachate.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to all those who have helped me to complete this thesis. First and foremost, I would like to express my heartfelt gratitude to my main supervisor, Dr. Sim Yoke Leng and co-supervisor, Dr. Tey Lai Hock for their constant guidance, enthusiastic encouragement and valuable suggestions throughout this project period. Their willingness to allocate their time so generously whenever I confront problems is very much appreciated.

Besides, I appreciate the help and assistance given by UTAR lab officers and would like to thank Mr.Leong, Mr.Patrick and Mr.Chin. I would also like to send my sincere gratitude to my Work Completion Seminar panels. They have given some suggestion and advice in order to improve my thesis.

Furthermore, I am thankful to SELEKTA SPEKTRA SDN BHD and Mr. Zulfadli Alias for allowing me to conduct on-site sample collection at the Papan Landfill in Papan district Ipoh, Perak.

Finally, I wish to thank to my beloved parents, siblings and friends for their undivided support and encouragement throughout the completion of this project.

APPROVAL SHEET

This thesis entitled **"TREATMENT OF MATURE LANDFILL LEACHATE USING COMBINED COAGULATION, FILTRATION AND MICROALGAE PROCESSES"** was prepared by SWARNA KAMALA SUBRAMANIAM and submitted as partial fulfillment of the requirements for the degree of Master of Science at Universiti Tunku Abdul Rahman.

Approved by:

____
(Dr. SIM YOKE LENG) Date: _______________
Associate Professor
Department of Chemical Science
Faculty of Science
Universiti Tunku Abdul Rahman

____
(Dr. TEY LAI HOCK) Date: _______________
Associate Professor
Department of Chemical Science
Faculty of Science
Universiti Tunku Abdul Rahman

INSTITUTE OF POSTGRADUATE STUDIES AND RESEARCH

UNIVERSITI TUNKU ABDUL RAHMAN

Date: __________________

SUBMISSION OF THESIS/DISSERTATION

It is hereby certified that Swarna Kamala Subramaniam (ID No: 16ADM06159) has completed this thesis entitled "TREATMENT OF MATURE LANDFILL LEACHATE USING COMBINED COAGULATION, FILTRATION AND MICROALGAE PROCESSES" under the supervision of Dr. Sim Yoke Leng (Supervisor) from the Department of Chemical Science, Faculty of Science, and Dr. Tey Lai Hock (Co-Supervisor) from the Department of Chemical Science, Faculty of Science.

I understand that the University will upload softcopy of my thesis/dissertation in pdf format into UTAR Institutional Repository, which may be made accessible to UTAR community and public.

Yours truly,

____

(SWARNA KAMALA SUBRAMANIAM)

DECLARATION

I Swarna Kamala Subramaniam hereby declare that the thesis/dissertation is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

____

(SWARNA KAMALA SUBRAMANIAM)
Date: _________________

TABLE OF CONTENTS

CHAPTER

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Municipal solid waste (MSW) has grown in volume as the increase in world's population, rapid industrialization, and changes in consumption pattern by people directly as well as indirectly. It results in the production of a large amount of industrial and municipal wastes, varying from synthetic to biodegradable and become more urbanized. The need of packaged goods has increased drastically as growing numbers of population have become more dependent upon others for basic needs of goods and services because people have valued leisure convenience and time. As the technology has accelerated, older durable goods have become obsolete at an accelerated pace. Hence, the amount of MSW and the nature has changed dramatically over time (Rees, 2007).

The existence of waste or unwanted material is due to the limitations of present technology to turn and treat waste into other useful means, for instance new source of raw material and energy. Unfortunately, every product which is produced by the industry during the very end of its life cycle will conceptually turn into waste as current technology cannot afford to transform the waste back to raw material and other useful means. Only a handful of waste can be turned back into new raw material which our current technology can afford which is the recycling of paper, plastics, glass and aluminium or metal.

Solid waste management is directly linked to landfill as landfilling is a technique to manage solid waste in a more appropriate way (Masirin et al., 2008). The strategic purpose of SWM is to make sure the public safety and health as well as to protect the environment. The system of SWM is shown based on the topography, food, economy, mixed culture and climate. MSW is becoming crucial as the increase in number of the population in cities, legal interventions, rising in public awareness about hygiene and sanitation as well as the availability of newer technology in waste treatment (Shekdar, 2009).

Sustainable landfilling is required to preserve the well-being of the environment and human health (Agamutu et al., 2011). Sanitary landfilling is one of the well-designed engineering landfill which makes the landfill sustainable and can be defined as “a method of disposing of refuse into land without creating hazards or nuisance to both the health and well-being of the environment and the people, by means of confining the refuse to a smaller area, to cover it with a layer of earth at the end of each day operation and to reduce it to the smallest practical volume as possible to provide more frequent intervals as may be necessary" (Raghab, Abd El Meguid, and Hegazi, 2013). To deal with this amount of waste generated globally and to keep urban centers clean, a proper solid waste management is one of the important services needed by the municipal authorities in each country (Asnani, 2006).

Sanitary landfilling is the most favorable procedure of solid waste disposal in numerous countries. There are many landfills throughout the world and almost all the landfills are open dumping grounds, and they lead to critical social and environmental threats (Manaf et al., 2009). Landfills were approved as the most environmental friendly and economical way for the solid waste disposal compared to the other disposal methods, such as gasification, composting, and incineration. Although landfills have been accepted, the production of leachate from the landfills is a major concern related to current disposal method (Manaf et al., 2009).

Leachate is a liquid that passes through a landfill by extracting suspended matter and dissolved matter from it is defined as leachate. It is generated through the waste layers in a landfill by excess rainwater percolation (Kjeldsen et al., 2002). Landfill leachate is a combination of chemical, physical, and microbial processes contained in waste transfer pollutants, which is from the waste material to the percolating water (Kjeldsan et al., 2002). Leachate production is a critical issue for municipal solid waste (MSW) landfills and it brings noticeable danger to groundwater and surface water (Raghab, Abd El Meguid, and Hegazi, 2013). The liquid phase which is leachate extracted from the bottom layer of a landfill is a costly, challenging as well as complex wastewater type to treat.

1.2 Problem Statement

Currently, most of the landfills are lack of a proper leachate treatment facilities and the landfills are surrounded by water streams and rivers. Thus, a proper treatment system is required to treat the leachate before severely pollute the environment. Biological treatment method is the most commonly used method for treating landfill leachate due to the nature of the treatment which is environmental friendly treatment with a simple, cost-effective and reliable treatment method to remove wide-range of contaminants in landfill leachate (Liu, 2013). However, the complex nature of landfill leachate inhibits the microalgae growth in raw leachate samples and requires a pre-treatment process (Klauson et al., 2014).

The most commonly used chemical treatment is coagulation which may be used successfully in treating mature and stabilized landfill leachates. Based on the studies, coagulation process is effective in the removal of organic solids and turbidity but could only remove 20-30% of COD in mature leachate (Shu et al., 2016). Therefore, coupling of coagulation with direct filtration system is required to eliminate the contaminants prior to biological treatment of mature leachate.

Moreover, activated carbon adsorption filtration systems have also been used previously in the landfill leachate treatments for the removal of organic compounds and they often must be combined with other leachate treatment technologies to attain desired results (Raghab, Abd El Meguid and Hegazi, 2013).

Hence, this research is to evaluate the combined treatments for mature leachate from Papan Landfill, Perak with coagulation, adsorption using filtration systems and biological treatment using two different microalgae species, *Botryococcus sudeticus* (BS) and *Chlorella vulgaris* (CV), in order to decrease the value of COD, BOD and other mineral contents. Furthermore, this research is cost effective, simple and reliable with combined chemical, physical and biological treatment. It serves as a dual-purpose treatment to reduce the contaminants as well as lipid production for biodiesel production.

1.3 Objectives

- To investigate the treatment efficiencies of mature landfill leachate after coagulation-filtration treatment and after coagulation-filtration-microalgae treatments.
- To study on the effective culture conditions for both the microalgae species of *Botryococcus sudeticus* (BS) and *Chlorella vulgaris* (CV) grown in pre-treated mature leachate.

- To identify the lipids present in both *Botryococcus sudeticus* (BS) and *Chlorella vulgaris* (CV) grown in pre-treated mature leachate.

1.4 Novelty of Study

Plenty of studies have been reported on the microalgae culture with landfill leachate prior to biofuel production. Previous researchers focused on finding the tolerant microalgae species and different dilution ratios of leachate were tested in most of the studies (Zhao et al., 2008). Higher removal efficiencies of contaminants achieved for microalgae cultivated in 10% leachate dilution (Sforza et al., 2015). However, to-date, there is still lacking of an optimized condition for microalgae growth in leachate without dilution. The principle applicable landfill leachate treatment methods comprise of chemical, physical and biological treatment methods. Typically, combination of chemical, physical and biological treatment methods is required for landfill leachate treatments because of the complications in getting satisfactory treatment effectiveness by using a single method (Aziz, 2011).

1.5 Scope of Study

The current research focused on mature landfill leachate from Papan Sanitary Landfill, Perak to explore the combined treatment efficiencies and dual-purpose method to treat the leachate as well as microalgae culturing in mature leachate. The mature landfill leachate was undergone coagulation, filtration and microalgae treatment using *Botryococcus sudeticus* and *Chlorella vulgaris* species separately. The mature leachate samples were analysed before treatment, after coagulation-filtration and after coagulation-filtration-microalgae treatment to study on the removal efficiencies of individual treatment stages.

1.6 Thesis Outline

There are five chapters in this thesis. The first chapter reveals the background of study, problem statement, objectives and scope of this research. Chapter two is on literature review from solid waste management, landfill leachate properties and compositions, and some crucial research on combined treatment methods of mature landfill leachate using chemical, physical and biological treatments. Chapter three is on methodology used throughout the research which includes sample collection, sample analysis, and experimental set-up and design. Chapter four is interpretation of results and discussed the key findings of this research outcome. Lastly, chapter five provides the conclusion for this research aligned with objectives and recommendations for further studies in future.

CHAPTER 2

LITERATURE REVIEW

2.1 Solid Waste Management (SWM)

SWM was privatized in Malaysia since 1996 and currently there are three solid waste concessionaries which operate at their own respective zones: southern regions by Southern Waste management, central regions are being managed by Alam Flora Sdn. Bhd and northern regions by Idaman Bersih Sdn Bhd respectively (Manaf et al., 2009). The government of Malaysia still has the role in municipal solid waste management as mentioned in Section 72 of the Local Government Act 1976 under the responsibility of the local authority. Solid waste in Malaysia are commonly grouped into three major categories namely:

i. Clinical waste
ii. Municipal solid waste
iii. Schedule waste or Hazardous waste

Different government department has their own responsible towards each category of the waste. Local Government and Ministry of Housing is responsible for municipal solid waste, clinical waste is under the responsibility of Ministry of Health (MOH) and hazardous is under the responsible of Department of Environment (DOE) (Manaf et al., 2009). According to review by Shekdar in year 2009, the author summarized the typical solid waste management system followed in Asian countries as shown in Figure 2.1 and Malaysia is having the same SWM system which is similar to the other Asian countries as well.

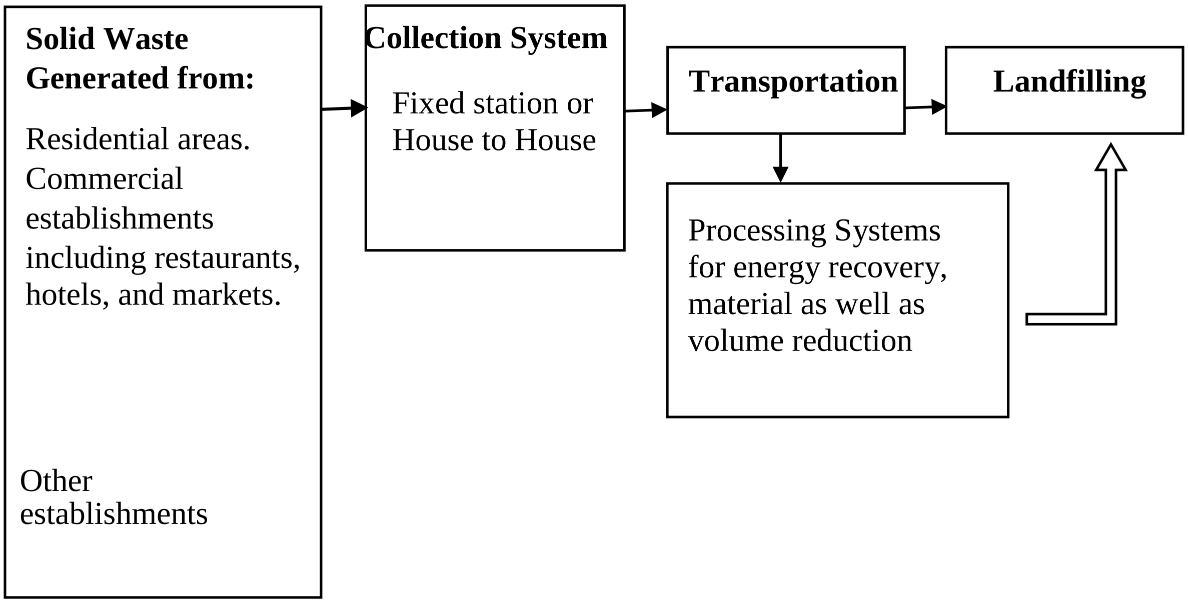


Figure 2.1 Typical Solid Waste Management in Asian Countries (Shekdar, 2009)

2.2 Landfilling

The Local Government and Ministry of Housing are under supervision of landfill sites in Malaysia. 4 different stages of continuous improvement of landfill has been listed in the Action Plan 1988 of Malaysia (Adnan et al., 2013; Fazeli et al., 2015):

- Level 0: Open dumpsite.
- Level 1: Controlled dumping site.
- Level 2: Sanitary landfill with daily cover.
- Level 3: Sanitary landfill with landfill leachate circulation.
- Level 4: Sanitary landfill with landfill leachate treatment.

Based on Manaf et al. in year 2009, there are 71 controlled dumping sites, 73 open dumping sites and 11 sanitary landfills is under operation in Malaysia. Table 2.1 summarizes the numbers and types of disposal site according to states where the site is operating in Malaysia.

Table 2.1 Types and Number of Disposal Site in Malaysia (Manaf et al., 2009)

State	Open dumping	Controlled dumping	Sanitary landfill	Total
Johor	12	14	1	27
Kedah	9	5	1	15
Kelantan	12	2	0	14
Melaka	2	3	0	5

Perak	15	11	4	30
Negeri Sembilan	8	6	0	14
Pahang	7	5	3	15
Perlis	0	1	0	1
Pulau Pinang	1	1	1	3
Selangor	5	15	0	20
Terengganu	2	8	1	11
Total	73	71	11	155

2.2.1 Level 0: Open Dump Site

Open dumping of municipal solid waste is the most commonly used method to discard in Malaysia. It is because compared to other solid waste disposal methods, this treatment procedure is the cheapest among the others for many years. Open dumping is still under operation in almost all municipalities up-to-date where the solid waste is being dumped in an uncontrolled manner which leads to major health and environmental issues (Tarmudi et al., 2012).

2.2.2 Level 1: Controlled Dump Site

Controlled dumpsite is similar to the open dumpsite because both controlled and open dumpsites are non-engineered disposal site without leachate circulation or treatment. The controlled dumping facilities were established due to need of the shutdown of open dump sites with the incorporation of few disposal facilities (UNEP, 2015). The controlled dump sites are also known as secure landfills that provides a more efficient disposal method of municipal solid waste within the protection standards and regulations of environment. It is due to the fact that the disposal is only allowed at several designated areas for controlled dumpsite and it has a planned capacity (USAID, 2016).

2.2.3 Level 2 – 4: Sanitary Landfills

Sanitary landfilling is distinct from open dumpsite method as well as controlled dumpsite in both facilities and planning. Sanitary landfilling has engineered disposal facilities in which the construction, operations manner, and design. Sanitary landfill facilities can minimize and protect the impacts to the public health and environment. Sanitary landfills go through proper and careful planning from the selection of the disposing site down to the closure of the landfill. Sanitary landfill provides all the facilities required to control the pollutants and hazards from the landfill. For instance, groundwater monitoring well, liner system in landfill, gas monitoring probe, leachate collections, treatment plants or systems, daily cover operations with waste and biogas management system (UNEP, 2015).

2.3 Landfill Leachate

Sanitary landfilling method is the most urban procedure in order to discard solid waste as the method has advantages such as simplicity, landscape restoration of holes from mining work such as gold mining and tin mining and low initial cost (Aziz et al., 2011). The formation of leachate from landfill is due to the penetration of rainwater through the garbage in the different layers of landfills and carries contaminants from the landfill (Aziz et al., 2014). Figure 2.2 describes the formation of landfill leachate (Shehzad et al., 2015). The garbage will undergo four different stages or phases of decay in the landfill, once the garbage has been dumped into the landfill. It includes the anaerobic acidic phase, initial aerobic phase, early methanogenic phase (initial phase) and lastly the stabilising methanogenic phase. The divergent phases of the waste decay can occur concurrently in separate or different layers of the landfill (Kuusik et al., 2014).

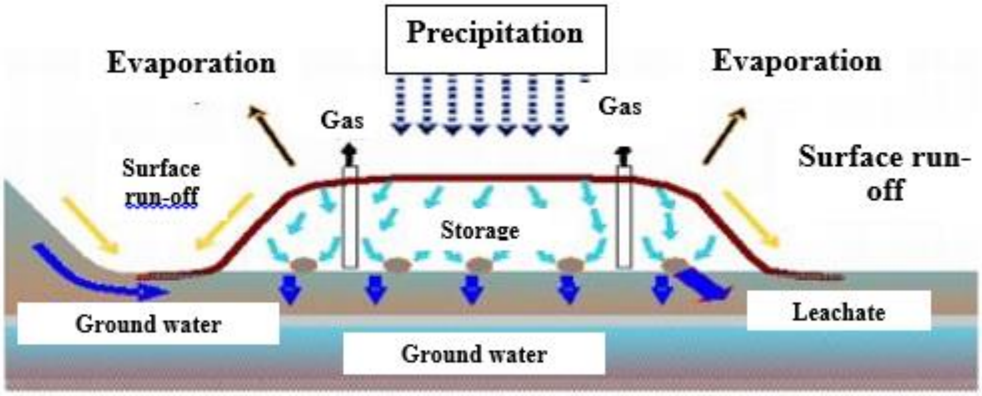


Figure 2.2 Landill Leachate Formation (Shehzad et al., 2015).

Leachates may consist huge amounts of organic matter, humic-type constituents, heavy metals, ammonia-nitrogen, chlorinated inorganic and organic salts (Renou et al., 2007). Landfill leachate is classified as a toxic wastewater which is contaminated with high values of total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), turbidity, colour, ammoniacal nitrogen (NH_3-N), pH, heavy metals and bad odour. It can cause severe environmental impact due to the large content of inorganic and organic pollutants generated in municipal landfills and if it is not properly controlled or treated (Raghab et al., 2013).

2.4 Types of Leachate

There are few factors influencing the quality of leachates. For instance, waste type, precipitation, age, seasonal weather variation, as well as composition. In specific, the composition of landfill leachates varies greatly based on the age of the landfill (Silva et al., 2004). There are three types of landfill leachates classified according to landfill age namely young, medium and mature leachate. The ammonia nitrogen concentration will rise and total organics concentration in leachate decreased as the age of landfill increased (Kulikowska and Klimiuk, 2008). Table 2.2 depicts the characteristics of leachate at different ages of landfills (Hector, Bruce and Simon, 2004).

2.4.1 Young Leachate

Young Leachate contains wide-range of biodegradable organic compound. The perplexing organic compounds are brewed anaerobically, producing mainly soluble organic acids, like low molecular weight compounds, free volatile fatty acids (VFAs), amino acids and gases like hydrogen and carbon dioxide (Bhalla et al., 2013).

2.4.2 Mature Leachate

Mature leachate is partially categorized by the lower concentration of free volatile fatty acids (VFAs). During second fermentation period, leachate is converted into methane and carbon dioxide gaseous as the end products. In return from the decrease in the content of free volatile fatty acids (VFAs) and biodegradable organic substances in the mature leachate, the refractory compound such as humic and fulvic acids will dominate the organic compounds in leachate (Bhalla et al., 2013).

Table 2.2 Characteristics of landfill leachate at different ages of landfill (Hector, Bruce and Simon, 2004).

Characteristics	Young	Medium	Mature
Age (Year)	< 1.0	1.0-5.0	>5.0

pH	<6.5	6.5-7.5	>7.5
Chemical Oxygen Demand, COD (g/L)	>15	3.0-1.5	<3.0
BOD_5/COD ratio	0.5-1.0	0.1-0.5	<0.1
TOC/COD ratio	<0.3	0.3-0.5	>0.5
Total Ammonia Content (mg/L)	<400	400	>400
Heavy Metals (mg/L)	>2.0	<2.0	<2.0
Organic Compound	80% Volatile Fatty Acids	5% to 30% Volatile Fatty Acid, Humic and Fulvic Acids	Humic Acids and Fulvic Acids

2.5 Compositions and Characteristics of Leachate

The leachate generation is basically caused by percolation of precipitation through waste deposited in layers of landfill. Different chemical, biological, and physical processes of the municipal solid waste in landfills combined with the particular waste composition will cause different compositions of the leachate to be generated (Azhar, 2008)**.** The characteristics of the landfill leachate generally be represented by the water analysis through basic parameters namely Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), pH measurement, ammonium nitrogen (NH_3-N), suspended solids (SS), heavy metals and Total Kjeldahl Nitrogen (TKN) (Renou et al., 2008).

Landfill leachate primarily composed of huge amounts of organic compounds including phenol, salinity, dissolved organic matter, hardness, phosphate, ammonical nitrogen, inorganic salts, heavy metals, acidity, sulphide, solids, alkalinity and other toxican (Foul et al., 2009; Renou et al., 2008; Wang et al., 2002; Aziz et al., 2009; Kang et al., 2002). The complex characteristics of landfill leachate makes the leachate even more complicated to treat or manage. Thus, the landfill leachate treatment constituents measurement prior to its drainage is a legal requirement to avoid both severe and continual toxicity as well as to prevent pollution of water bodies (Tatsi et al., 2003). Table 2.3 shows the characteristics of landfill leachate of a raw leachate in Malaysia.

Table 2.3 Characteristics of raw leachate in Malaysia (Zainol, Aziz and Yusoff, 2012).

No	Parameter	Kuala Sepetang Landfill Site		Kulim Landfill Site	
		Range	Average	Range	Average
1	pH	7.86-8.31	8.05	7.27-7.92	7.59
2	Conductivity (mS/cm)	5.71-22.52	11.90	2.57-3.54	2.92
3	Oxidation-Reduction Potential (mV)	-84.9-+91.4	-33.02	-33.3-+116.4	+17.8
4	Turbidity (NTU)	40.3-178	88.9	12.7 - 67.0	26
5	Colour (Pt Co)	1120-3100	2220	192.0 -440.0	326

6	Suspended Solids (mg/L)	151-278	233	11.0-99.0	47
7	Biochemical Oxygen Demand (mg/L)	97-184	158	7-69	29
8	Chemical Oxygen Demand (mg/L)	680.0-950.0	855	105.0-131.0	117
9	BOD_5/COD	0.11-0.26	0.19	0.060-0.520	0.24
10	Ammonia-N (mg/L)	410.0-1185.0	857.0	174.0-280.0	210
11	Sulphate (mg/L)	22.47-175.07	91.48	65.83-217.59	141.71
12	Chloride (mg/L)	837.60-3025.21	1800.46	194-292.35	243.18
13	Copper (mg/L)	0.002-0.15	0.08	0.02-0.07	0.03
14	Iron (mg/L)	0.96-3.65	2.18	0.24-0.71	0.38
15	Manganese (mg/L)	0.00-0.21	0.08	0.00-0.14	0.09
16	Nickel (mg/L)	0.09 - 0.23	0.16	0.06-0.09	0.07
17	Zinc (mg/L)	0.14 - 0.34	0.26	0.01-0.29	0.09

2.6 Impacts of Landfill Leachate and Discharge Limit

2.6.1 Environmental Impacts

In beginning, landfill was introduced to save and protect the society and environment from negative impacts of other more deleterious disposal of solid waste methods such as open-burning, river and ocean dumping. Landfilling leads to the production of hazardous leachate and gaseous which causes adverse effect on environmental issues like unpleasant odours, fire, explosion, air pollution and global warming as well as human health (El-Fadel et al., 2007).

The leachate from landfill mostly contains various heavy metals and xenobiotic organic compounds (XOCs) which makes leachate hazardous as heavy metals and XOCs may react within themselves and other compounds in the surrounding environment which contribute mutagenic, carcinogenic, flammable, eco- reactive, toxic and may be persistent or bio-accumulative (Slack et al., 2005). Due to the hazardous and toxicity characteristic of the leachate, run-off and the infiltration of leachate has the potential to cause negative effect by polluting groundwater to near-by surface water as well as vegetation which surrounds the landfill.

The containers which is being disposed into landfills may contain residual of harmful chemicals. For instance, poly-chlorinated biphenyl (PCB), insecticides, solvents, unused pesticides and pharmaceuticals, thus generating highly complex cancer-causing chemicals (Clarke et al., 2015). Ground water is being polluted when the leachate breached or seep through the bottom layer of the landfill and an impermeable layer or liner layer of the landfill. It causes leachate discharge to the ground's surface, thus reaching to the water until further diffuse while comtaminating the groundwater (El-Fadel et al., 2007). The landfill leachate leads to surface water and groundwater pollution. Thus, leachate quality deserved a stringent scrutiny and analysis in order to preserve and conserve the environment (Al-Yaqout and Hamoda, 2003).

2.6.2 Human Health Impacts

Leachate has a potential polluting liquid waste from landfill which possesses potential risk of health to the surrounding human populations and ecosystems. The biodegradation process in landfill which yields leachate in large concentration of ammoniacal-nitrogen (NH_3N) and heavy metals such as nickel, cadmium, mercury and others. They contaminate the ground water causing hazards to drinking water to people who rely on ground water for their day to day water use (Salem et al, 2008). Landfill site also poses serious health risk in terms of ground water pollution. (Klinck and Stuart, 2009). Table 2.4 shows the negative impacts of leachate heavy metals on human health by Kannan, (2013).

Table 2.4 Health Effects of Leachate on Humans (Kannan, 2013)

Type of pollutant	Health effects from exposure	
	Acute exposure	Long-term exposure
Lead	Diarrhoea, vomiting, confusion, abdominal pain, seizures, drowsiness	Hypertension, chronic nephropathy, anorexia, abdominal pain, constipation
Nickel	Gum disease, skin irritation, dermatitis, diarrhoea	NA

Mercury	Dehydration, renal failure, bloody diarrhoea	Memory loss, seizures, coma, decrease in platelets, tremors, irritability, anaemia that follows gastrointestinal bleed
Cadmium compounds	Cough, skin irritation, chest pain, nausea, metallic taste, diarrhoea	Kidney damage, possible prostate and lung problems
Phenols/cresols	Coma, vomiting, nausea, sweating and burning pain in mouth and throat	Renal failure
Toluene	Coma, convulsions, tremors	NA
Benzene	NA	Blood-related disorders

NA – Not Available

2.6.3 Standard Discharge Limit for Landfill Leachate

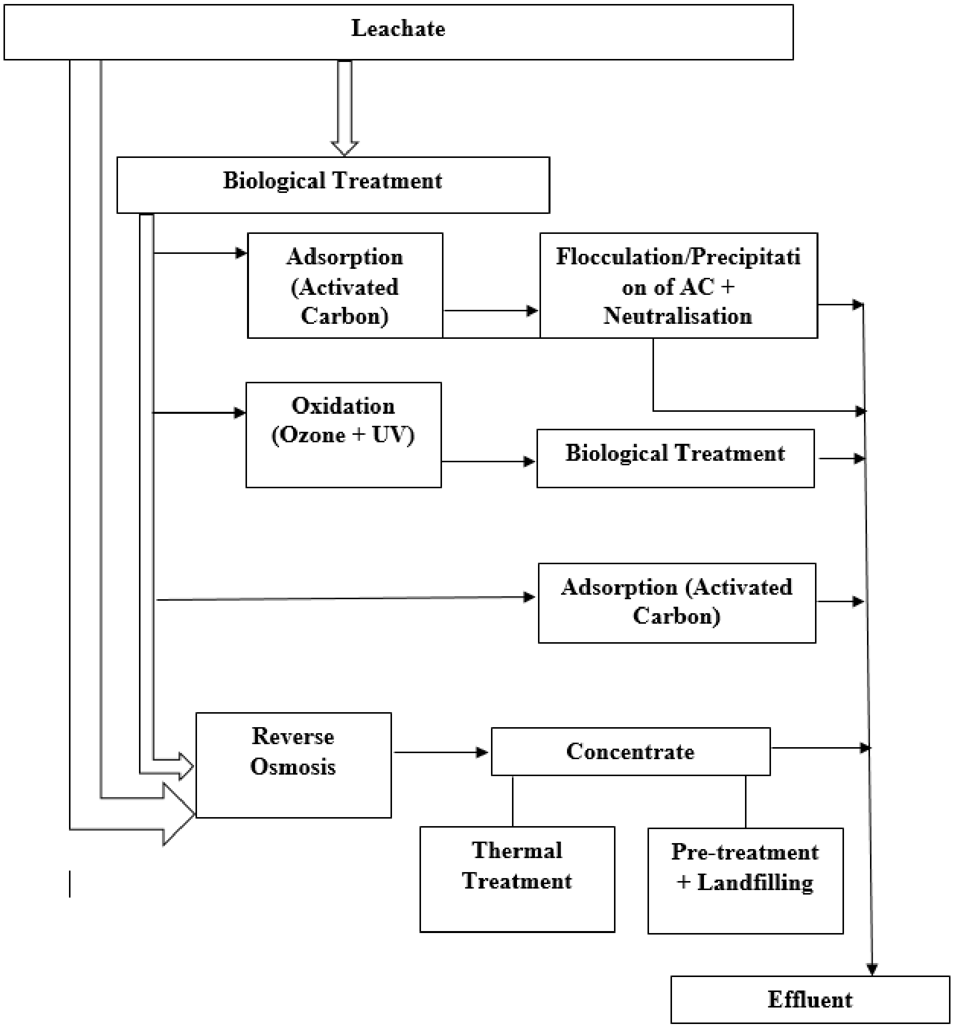
In order to minimize the hazardous consequences and to protect the well-being of the surrounding ecosystems, the treated leachate effluent must comply with the Environmental Quality Act 1974 Regulations 2009, in which Appendix P shows the discharge limit of parameters of treated effluent leachate in Malaysia. However, the parameters with the discharge limit range and values may vary from country to country.

2.7 Leachate Treatments

Leachate control is a vital process to maintain the long-term functionality of the water drainage system, to render possible high-tech treatment systems and to minimize the treatment costs. Treatment methods must consider the complex leachate composition and the relatively small flow rates which makes it distinct from sewage and other kinds of wastewater. The reduction of discharge limits values in most of the countries in past and future highlights the need of high-tech treatment technologies which often involves a combined treatment processes such as chemical, physical and biological processes (Stegmann et al., 2005).

Conventional treatment of leachate is categorized into three major categories by Renou et al., 2008, such as leachate transfer within the landfill which includes the combined treatment with domestic sewage and recycling of leachate; chemical and physical treatment which consist of chemical precipitation, chemical adsorption, coagulation or flocculation, air stripping and chemical oxidation; as well as biodegradation which uses anoxic, anaerobic and aerobic process to treat leachate.

Based on the research by Klauson et al. (2014), municipal waste leachate was subjected to different types of biological (BIO) and chemical (CHEM) treatment with the final aim of generating an effective treatment process with the combination of individual chemical, physical and biological processes (Klauson et al., 2014). Three kind of treatment methods (BIO-CHEM, CHEM-BIO and BIO-CHEM-BIO) were compared based on removal efficiencies and biodegradability results attained. The most effective combined process was with aerobic biological pre-treatment followed by Fenton reaction and biological post-treatment. It is due to high initial biodegradability of raw leachate. The effectiveness of combined treatment processes was studied at laboratory scale, with more than 90% of BOD_7 and COD removal (Klauson et al., 2014).



⇨: Potential leachate treatment from the methanogenic phase in landfill

AC: Activated Carbon

UV: Ultraviolet light

Figure 2.3 Schemes of often used methods and combinations for leachate treatment (Stegmann et al., 2005).

2.8 Leachate Treatment by Coagulation-Filtration

Coagulation is defined as the result of destabilization of colloids by neutralizing the forces which keep them apart. Cationic coagulants will produce the positive electric charges to lessen the zeta potential (negative charge) of colloid particles. Thus, the particles collide to form bigger particles (flocs) (Mbaeze, Agbazue and Orjioke, 2015). Coagulation is a chemical process used to eliminate colloid particles, suspended solids (SS), non-biodegradable organic compounds, and heavy metals from landfill leachate. It is efficient, simple as well as cost-effective. It is based on the use of aluminium salts or ferrous salts that flocculate the organic compounds or colloid particles into bulky floccules (Shu et al., 2016).

Coagulation is a vital process in water or industrial wastewater treatment. Several studies were reported on the research of coagulation for the landfill leachate treatment by targeting at performance optimization. For instance, selection of the most suitable coagulant, assessment of pH effect, determination of experimental conditions and investigation of coagulant addition. Ferrous sulfate, aluminum sulfate (alum), ferric chloride and ferric chloro-sulfate are the most commonly used coagulants (Vaezi et al., 2005).

The incorporation of coagulation process is also essential in leachate treatment plants to remove organic solids. The proposed research method can be utilized as an additional treatment procedure in combination with biological treatments, for young, intermediate and mature leachates. Chemical coagulation could be applied as a pre-treatment step for leachates, prior to their biological treatment (Tatsi et al., 2013). The implementation of an appropriate physical and chemical treatment method, before the application of a biological treatment gives an efficient outcome in the partial removal of organic particles and of possible existing heavy metal contaminants. Thus, it can reduce the initial loading of pollutants while permitting the application of a more effective secondary biological treatment (Tatsi et al., 2003).

Undesirable compositions in landfill leachate, for instance, heavy metals, organic halides, polychlorinated biphenyl and others are being eliminated commercially using this method. Coagulation is more effective in treating matured or stabilized leachate with COD removal up to 75% from the initial value compared to young leachate with COD removal up to 25 – 38% (Wiszniowski et al., 2006). The addition of coagulant into leachate initiates the coagulation process due to the negatively charged colloidal particles, in order to minimize and neutralize the negative-negative repulsive forces in between the particles (Liu, 2013).

Aluminium sulphate, is the well-known chemical coagulants in waste water treatment was studied with the aim of determining its coagulation efficiencies. The parameter levels of a cloudy or turbid water were identified and evaluated before and after treatment using alum at different ranges of coagulant dosages from 1 to 10 g per 3 litres of turbid water. The leachate analysis was done namely pH, total suspended solids (TSS), fluoride, dissolved oxygen (DO), turbidity, biochemical oxygen demand (BOD_5), phosphate, chloride and chemical oxygen demand (COD) (Mbaeze, Agbazue and Orjioke, 2015). The coagulation with alum as the coagulant has achieved the removal efficiencies of pH (44.92%), DO (90.10%), Turbidity (98.70%), TSS (98.71%), BOD_5 (100%), Phosphate (80%), fluoride (100%), chloride (100%), COD (100 %) and Copper (0.00%) (Mbaeze, Agbazue and Orjioke, 2015).

Based on the studies, the chemicals generally used as primary coagulants for direct filtration, physical treatment, are iron salts, aluminum salts and cationic polymers. Gregory and Duan, (2001) stated that aluminum salts are one of the most efficient and economical coagulants to use. The pilot scale direct filtration system (physical treatment) produced sustainable finished water when alum was used as the coagulant with a polymer as a coagulant aid (Simate, 2015).

Huan-jung et al. (2006) analyzed the liquid output of three different kinds of landfills. They pointed out the active landfill leachates with high concentrations of the volatile suspended solids, chemical oxygen demand, total solids, total organic carbon, conductivity and high contents of iron, chromium and nickel. A study was carried out by Aziz et al. (2009) to investigate the effectiveness of flocculation and coagulation processes for eliminating turbidity from a semi-aerobic landfill leachate in Malaysia. Four types of coagulant namely ferrous (II) sulphate, aluminium (III) sulphate (alum), ferric (III) sulphate and ferric (III) chloride were used to investigate by standard jar test apparatus. Turbidity analysis has been done for the leachate sample. The effect of different coagulant dosages on turbidity removal showed similar trend as for turbidity, COD and suspended solids (Aziz et al., 2009).

Physical filtration systems may be classified into two different types which are gravity and pressure. Pressure filters consist of closed vessel containing beds of sand or other granular material through which water is forced under the pressure. The initial cost of pressure filters is high due to the expensive component parts. A gravitational filter consist of an open-topped box, partly filled with filtering medium which is usually sand and drained at the bottom. Raw wastewater is admitted to the space above the sand and it flows downward under the gravitational force. Purification takes places during this downward passage and the treated effluent is discharged though the under-drains (Manning, 2003).

Proper filter media specifications depend on the performance and filter design. Selection of a filter medium depends on the durability of the filter media, desired degree of purification, ease of filter wash and length of filter run to remove suspended solids from the media (Gregory and Duan, 2001). In a research of direct filtration plants, dual media filters consist of a coal layer of 35 to 50 cm with an effective size of 0.98 mm and a sand layer ranging from 15 to 38 cm with an effective size of 0.46 mm (John, 2002). John (2002) reported that mixed media filters have the greater advantage in providing storage for floc in the media bed while increasing the length of filter runs. The performance of rapid sand filters was studied and evaluated in three tertiary treatment plants in the State of Kuwait. The results obtained indicated drastic improvements, at 95% and 99% significance levels, in solids (TDS and TSS) and organics (COD, BOD) removal by using sand filtration (Hamoda, Al-Ghusain and Al-Mutairi, 2004). Different kinds of filter media and filtration methods are available for wastewater treatment. The availability, practicality, accessibility, ease of use and reliability of these filtration media and methods vary widely. It is often depends on the local factors. The efficiency of these filtration methods in eliminating microbes also varies widely, depending on the type and quality of the filtration medium or system and the type of microbes used (Berk, 2013). Sand filters have been used as a cost-effective alternative to conventional septic tank for domestic wastewater (Healy et al., 2007). A study comparing single-pass sand filters and bio-filtration systems for the treatment of septic tank effluent has shown that single-pass sand filters have the greatest organic and nutrient removal efficiency (Healy et al., 2007).

Landfill leachate is a complex refractory wastewater which consist large amount of ammonia and organic compounds. Based on the studies, the adsorption technology exploited on activated carbon has become promising importance in the landfill leachate treatment due to its low preparation cost and simplicity in design of activated carbon in addition to high treatment effectiveness (Shehzad et al., 2015). Activated carbon is an adsorbent with large porous surface area, thermostability, controllable pore structure and low base or acid reactivity which makes it beneficial in leachate treatment. It has an ability for removal of a wide variety of inorganic and organic contaminants dissolved in aqueous media as well as from gaseous environment (Foo and Hameed, 2009).

The removal and transformation of natural organic matter were monitored in the different stages of the drinking water treatment train. Several methods to measure the quantity and quality of organic matter were used. The full-scale treatment sequence consisted of coagulation, flocculation, floatation, disinfection with chlorine dioxide, activated carbon filtration and post-chlorination. Four of the activated carbon filters were monitored over the period of 1 year. Activated carbon filtration was most effective in the removal of organic matter (Matilainen, Vieno and Tuhkanen, 2006).

Lately, a separate studies using granular activated carbon (GAC), ferric chloride and granular activated alumina for the treatment of heavy metals such as copper, cadmium, manganese, chromium, zinc and lead. The results indicate that granular activated carbon was the most competent adsorbent with the reduction of 80–96%, at a pH range of 6–7.7 with an initial concentration of 184 mg/L (Foo and Hameed, 2009). A comparative study for the removal of ammonium nitrogen has been undertaken by Aziz et al. in Malaysia by using limestones granular activated carbons and in Burung Island landfill. About 40% of ammonium nitrogen (NH_3-N) with an initial concentration of 1909 mg/L was removed with 42 g/L of Granular Activated Carbon while 19% only removal was achieved by using 56 g/L of limestone under the same concentration.

Azmi et al. (2014) evaluated the performance of sugarcane-based activated carbons in treating leachate samples. The removal of colour, COD, and ammoniacal nitrogen were described by Langmuir isotherm model. The optimum experimental conditions resulted in 83.61%, 94.74%, and 46.65% removal of COD, colour and ammoniacal nitrogen, respectively.

2.9 Landfill leachate Treatment using Microalgae

Microalgae are eukaryotic or prokaryotic photosynthetic microorganisms which can grow rapidly in harsh conditions due to their simple multicellular or unicellular structure. Phytoremediation using microalgae as a way to control and lessen nutrient concentrations in landfill leachate is a low cost and environmentally sustainable method. Accumulated nutrients in the plants can then be eliminated by harvesting and anaerobically digesting the biomass. Landfill leachate compositions are depending and vary on a number of factors but generally are characterized by high levels of salts, ammonia nitrogen, certain metals and an extensive array of organic compounds. However, certain species of microalgae may use some of these pollutants as a source of nutrients (Strom, 2010). Microalgae culture offers a cost-effective approach to remove nutrients from landfill leachate. Microalgae cultures is an elegant solution to tertiary and quaternary treatments due to the ability of microalgae to use phosphorus and inorganic nitrogen for their growth and their capacity in removing heavy metals (Raouf et al., 2012). Strom (2010) reported the ability of *Chlorella vulgaris* in removal of nutrients and studied a nutrient removal efficiency of 86% for inorganic nitrogen and 78% for inorganic phosphorus.

The performance of microalgae aquaculture wastewater treatment system predominated mainly by *Chlorella* and *Scenedesmus* was assessed. Treatment induced a tremendous reduction in both BOD and COD to values below the discharge limits. The average percentage of total COD reduction was 89% and 91.7% in the batch and continuous systems respectively. The applied system has achieved 84% of BOD removal from the wastewater.

Renou et al. (2008) has tested the leachate with microalgae. In this study, the raw leachate was fed to a microalgae treatment system as the sole nutrient source to determine nitrogen removal over a wide range of environmental and system conditions, including influent ammonia levels well above concentrations currently believed to inhibit algal activity. The obtained results showed that the ammonia and nitrogen removal rates as high as 8.43 and 9.18 mg of nitrogen (L/day), respectively. Biomass grew at a maximum rate of 25.6 mg/(L/day) with a maximum concentration of 480 mg/L. Overall, ammonia concentrations above 80 mg NH_3/L cause an inhibition of ammonia removal, but the biomass growth was not affected (Renou et al., 2008). The toxicity of landfill leachate to many microalgae as well as other organism is well recognized (Paskuliakova et al., 2016). The elemental compositions of landfill leachate have been compared with Bold Basal Medium (BBM) in Table 2.5 to evaluate the potential of landfill leachate as a cultivation medium for supplying both fertilizer and water for algae biomass production. The substitution of medium with leachate will be a very promising method for microalgae growth.

Table 2.5 Elemental composition of Landfill Leachate compared with BBM (Edmunson and Wilkie, 2013).

Component		LL	BBM
Macronutrients (mg/L)	Nitrogen (N)	980	41.2
	Ammonia-N (NH_3-N)	980	-
	Nitrate-N	-	41.2
	Phosphorus (P)	13.2	53.2
	Potassium (K)	980	106.0
	Magnesium (Mg)	88.0	7.4
	Calcium (Ca)	110	6.8

	Iron (Fe)	16	1.0
	Sodium (Na)	3700	86.3
	Chloride (Cl)	1800	27.5
Micronutrients (mg/L)	Manganese (Mn)	0.11	0.5
	Copper (Cu)	0.17	0.4
	Zinc (Zn)	0.06	2.0
	Cobalt (Co)	0.07	0.1
Toxic metals (mg/L)	Arsenic (Ar)	0.13	-
	Antimony (Sb)	<0.06	-
	Cadmium (Cd)	<0.0032	-
	Chromium (Cr)	0.12	-
	Lead (Pb)	<0.013	-
	Selenium (Se)	<0.068	-

Sforza et al., (2015) conducted a study on exploitation of leachate from urban landfill as a nutrient source for microalgae biomass production. This study has been carried out to test the possibility of utilizing nutrients from landfill leachate. *Acutodesmus obliquus* is a microalgal species which has been isolated from a pond. The pond contains pre-treated leachate from an urban landfill which is located in Lazio (Italy). Microalgae were cultured in landfill leachate sample from an Italian landfill in Lazio, Italy. There was no sterilization treatment conducted during the cultivation of microalgae. The microalgal uptake from different leachate dilutions vary from 30% to 97% for nitrogen, and it was constantly more than 90% for phosphorus. Specifically, the highest ammonia (NH_3) uptake was measured at 216 mg L^{-1} with 97 % removal for 10 % leachate dilution, while the phosphorus utilization was almost total in every situation. The growth and nutrient elimination efficiency of a newly segregated microalgae *Scenedesmus sp.* were surveyed when the strain grew in landfill leachate at higher dilution, which includes 2%, 5%, 10% and 20% treatment. The COD removal was less than 16% for all dilutions (Cheng and Tian, 2013). The microalgae species, *Chlorella Vulgaris sp.* was cultivated to analyze its capacity for growth and nutrient removal in leachate which was isolated from Häradsudden landfill, Sweden. The results of the first cultivation run clearly showed that the *Chlorella Vulgaris sp.* cannot grow in leachate from Häradsudden landfill except that some dilution takes place first. In the second cultivation run, *Chlorella vulgaris sp.* showed growth but in greater dilution of leachate which was 10% of leachate (Strom, 2010). A system dynamic was approached to analyze the efficiency of using mixed microalgae populations such as *Pavlova lutheri, Nanochloropsis, Tetraselmis chuii and Chaetoceros muelleri* in order to treat leachate–hypersaline water. The uptake kinetics of the metals which were removed from the landfill leachate are modelled using basic adsorption kinetics. After 10 days, the microalgae population was observed to have 95% of the metals uptake from the solution. The metal selective adsorption rates were higher for the metals namely cerium, lanthanum, iron, and aluminium which were completely, or almost completely, taken out from the leachate (Richards and Mullins, 2013). Landfill leachate is rich in metal content and the metal content must be removed in order to treat the leachate. Algae is very good in metal absorption as the uptake of metals as nutrient is essential for algae to grow. Excessive metal content in landfill leachate is sensitive for algae and also causes inhibitory effect for algae growth (Liu et al., 2011). The pre-treatment of leachate may decrease excess metal content and the remaining metal content could help in algae growth (Liu et al., 2011).

2.10 Lipid Extraction from Microalgae for Bio-fuel Production

Microalgae are known as photosynthetic microorganisms with simple growing requirements (sugars, CO_2, light, P, N, and K) which can yield proteins, carbohydrates and lipids in large amounts over short time of period. These products can be treated into both valuable co-products and biofuels. (Brennan et al., 2009) Biofuels derived from microalgae have been recommended as an alternative approach that does not affect agriculture. Microalgae have been predicted to generate higher biomass productivity compared to plant crops in terms of land area needed for cultivation, are estimated to provide lower cost per yield, as well as have the potential to lower Green House Gas emissions through the replacement of fossil fuels. (Chiu et al., 2015)

Several microalgal population are able to accumulate high level of lipid quantities, and they are characterized as oleaginous. In widespread species belonging to the genera of *Chlorella, Dunaliella, Porphyridium, Nannochloropsis, Isochrysis,* *Tetraselmis, Schizochytrium* and *Phaeodactylum* a lipid content varies between the range of 20% -50%. However, higher lipid accumulation depends on the culture conditions of microalgae species. Factors such as irradiance, temperature, and, mostly, nutrient availability were shown to affect lipid content and composition in microalgal cells (Bellou et al., 2014). Microalgae species do not need to be cultivated on agricultural crop areas but undesirable agricultural land can be used instead. It is because they can generate extra biodiesel oil compared to oilseed crops by using minimum mainland and water (Bellou et al., 2014). On top of that, lipid recovery studied for many microalgae species greatly surpass the oil recovery of the best producing oil crops. It demonstrates that microalgae give the optimum biodiesel oil yield. Therefore, microalgae may be able to generate up to 200 times of the amount of oil per unit of surface than soybeans (Chisti, 2007). The particular properties make microalgae the most effective and promising organisms on earth which have the potential to displace the petroleum-based diesel fuel oil completely without adversely affecting other crop products and supply of food (Bellou et al., 2014).

2.11 Summary of Microalgae Treatment

Numerous studies have been conducted on the leachate by using specific microalgae species as a biological treatment. Table 2.6 shows a summary of different types of microalgae and its nutrients uptake at various landfills sites. It shows nitrogen content uptake, chemical oxygen demand, and heavy metal adsorption in different dilution levels of landfill leachate. The studies claimed that leachate is highly contaminated and causes inhibitory effect for microalgae growth. The nutrients in leachate are equal as a media for microalgal growth but require dilution in order to microalgae to grow. The higher the dilution of leachate, the greater the algae growth in leachate and it causes a lot of consumption of water. The pre-treatment of leachate may decrease excessive contaminants which could help in microalgae growth.

Table 2.6: Comparison of different types of microalgae and its nutrients uptake at various landfills.

Landfill	Types of microalgae	Dilution level of landfill leachate (%)	Nitrogen content uptake (%)	COD (%)	Heavy metal Adsorption by microalgae (%)	Ref
Urban Landfill in Lazio (Italy)	*Acutodesmus obliquus*	10	97	-	-	(Sforza et al., 2015)
		25	65	-	-	
		34	-	-	-	
		50	30	-	-	
		100	NG	-	-	
Landfill in Northern Ireland	*Chlamydomonas*	10	90.7	-	-	(Paskuliakova et al., 2016)
	Scenedesmus	2	75	< 16	-	

Landfill in Hangzhou, China		5	70.6	< 16	-	(Cheng and Tian, 2013)
		10	69.7	< 16	-	
		20	11.8 (NG)	< 16	-	
Landfill in Alachua County	*Scenedesmus cf. Rubescens*	100 (with pH adjustment)	-	-	-	(Edmundson and Wilkie, 2013)
Landfill in Alachua County	*Chlorella cf. ellipsoidea*	100 (with pH adjustment)	-	-	-	(Edmundson and Wilkie, 2013)
Landfill in West Australia	*Nanochloropsis, Pavlova lutheri, Tetraselmischuii and ChaetocerosMuelleri*	-	-	-	95% (Al and Fe)	(Edmundson and Wilkie, 2013)
Landfill in Guangzhou, China	*Chlorella pyrenoidosa*	10	78	70	-	(Lin et al., 2007)
		30	70	72	-	
		50	50	45	-	
		80	25	25	-	
		100	18	20	-	
Landfill in Guangzhou, China	*Chlamydomonassnowiae*	10	80	80	-	(Lin et al., 2007)
		30	70	70	-	
		50	25	25	-	
		80	10	15	-	
		100	5	5	-	
Landfill in Haradsudden, Sweden	*Chlorella vulgaris*	100 (NG)	-	-	-	(Strom, 2010)
		10	90	-	-	
	Ankistrodesmus Convolutes, Euglena	25	-	-	-	(Mustafa et al., 2012)
		50	-	-	-	

Landfill in Selangor, Malaysia	*Gracilis, Scenedesmusquadricauda, Chlorella vulgaris*	75	-	-	-	
		100	-	-	-	
Landfill in Shanghai, China	*Consortium*	5	84	-	-	(Zhao et al., 2008)
		10	84	-	-	
		15	84	-	-	
		20	55	-	-	

**Note:* NG (No Growth)*

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Methodology Flow

Figure 3.1 shows the methodology flowchart which includes sample collection, leachate analysis, chemical treatment, physical treatment, biological treatment and lipid extraction. Initially, mature leachate sample was collected from Papan Landfill, Perak. Raw mature leachate sample was tested for initial characteristics.

Leachate analysis was carried out in 4 separate phases which were before treatment, after coagulation-filtration treatments only, after combined coagulation-filtration and biological treatment using *Chlorella vulgaris*, and after combined coagulation-filtration and biological treatment using *Botryococcus sudeticus*. The leachate analysis was done to measure the characteristics such as pH, Conductivity, Total Dissolved Solids (TDS), Total Solids (TS), Total Suspended Solids (TSS), Chemical oxygen demand (COD), Turbidity, Total Organic Carbon (TOC), Biochemical oxygen demand (BOD), heavy metals using Inductively coupled plasma mass spectrometry (ICP-MS) and Total Ammonia Determination.

The chemical treatment of mature leachate was done by coagulation process using aluminium ammonium sulphate-12 hydrate as a coagulating agent. The mature leachate samples from the chemical treatment were undergone physical treatment immediately using modified filtration systems. 3 sets of modified filtration systems were incorporated with activated carbon and one control set similar to commercial filtration set-up were used for filtration. The samples from coagulation-filtration treatment were stored inside the fridge for leachate analysis and biological treatment. Later, the biological treatment was conducted by using *Chlorella vulgaris* and *Botryococcus sudeticus* separately on the mature leachate samples from coagulation-filtration treatment. A bioassay was conducted to study on the growth and nutrient removal by two microalgal strains, namely *Botryococcus sudeticus* and *Chlorella vulgaris* in pre-treated mature landfill leachate samples. Finally, hydrocarbons were extracted in hexane from *Botryococcus sudeticus* and *Chlorella vulgaris* cell biomass to study the lipid profiles of algal biomass. All field and laboratory analysis were done according to APHA standard methods for the examination of waste and wastewater.

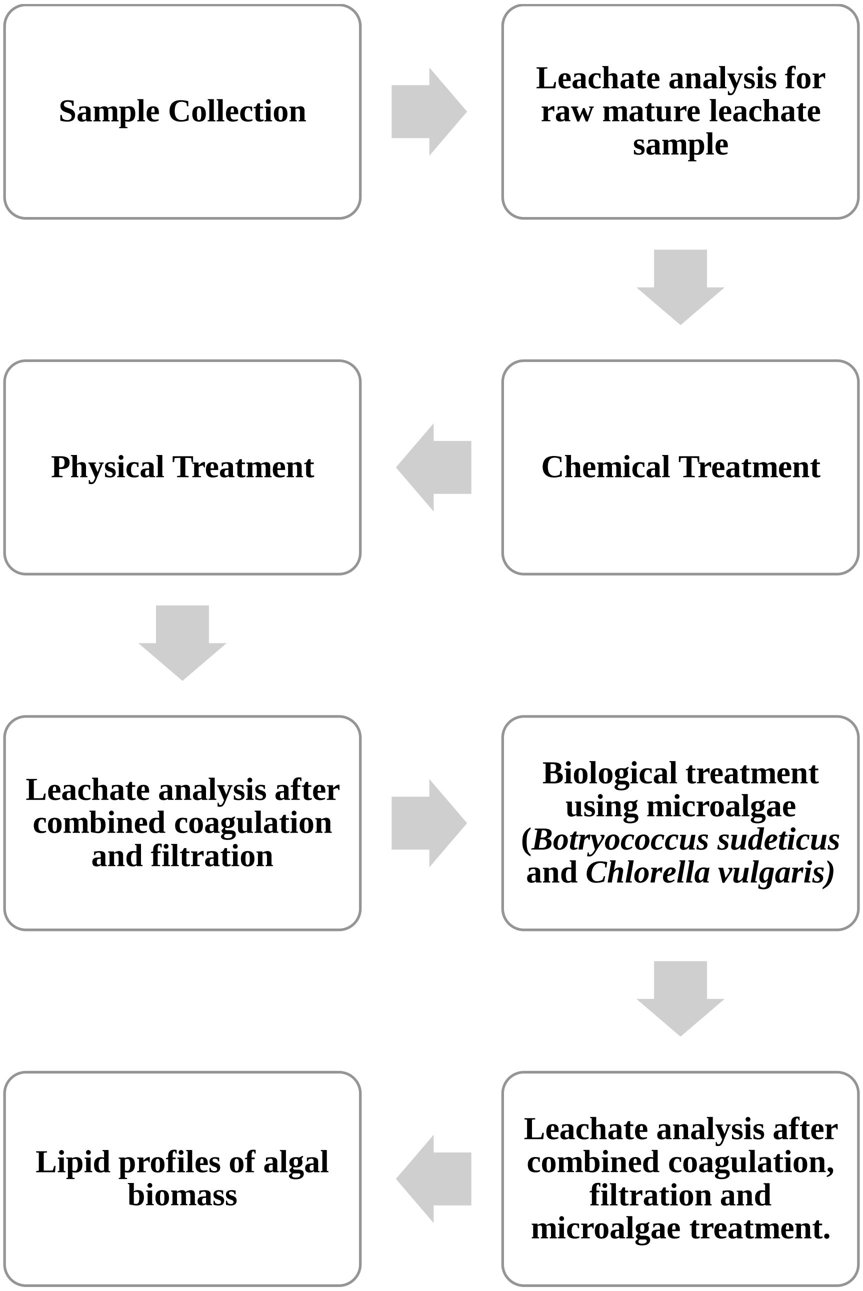


Figure 3.1 Methodology Flowchart

3.2 Leachate Collection and Site Location

The mature leachate samples were collected from “Tapak Pelupusan Sisa Pepejal, Wilayah Ulu Johan Papan” (Papan Sanitary landfill) at Papan district, Perak, Malaysia. The landfill site location is shown in Figure 3.2.

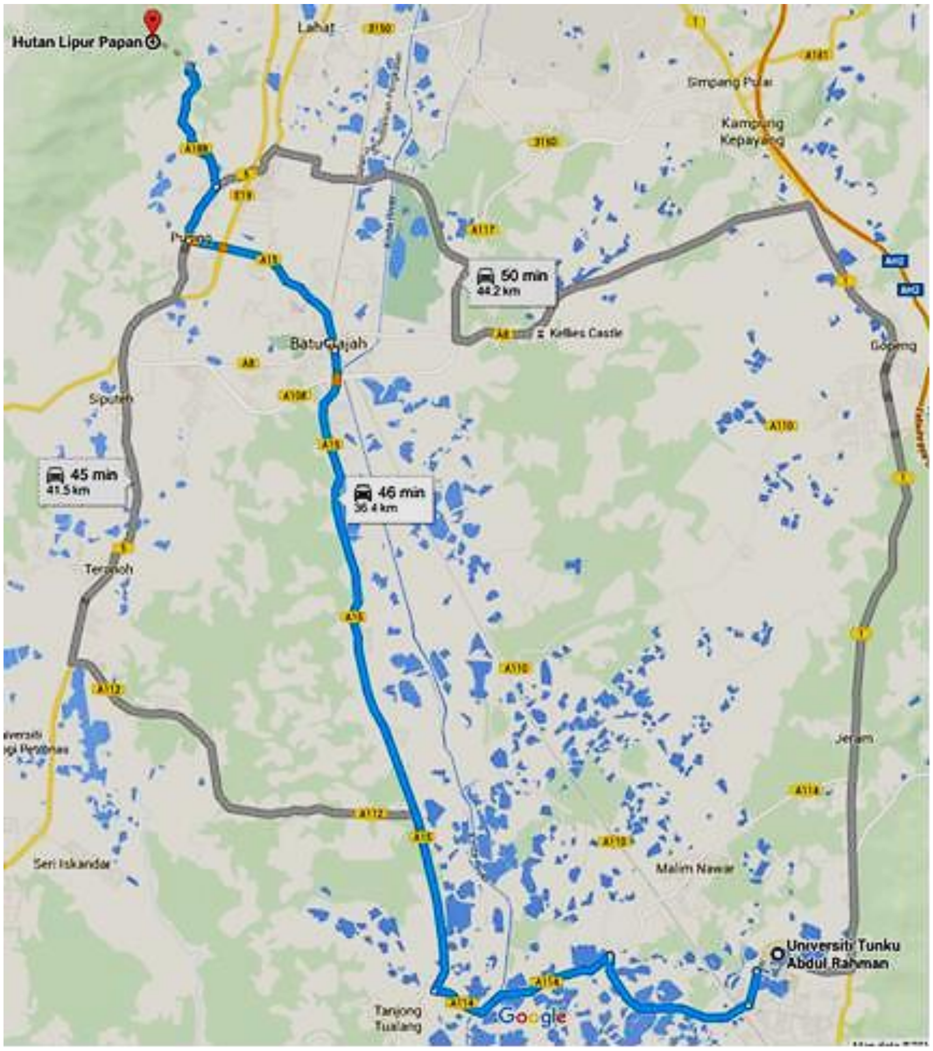


Figure 3.2 Papan Sanitary Landfill Site Location from Universiti Tunku Abdul Rahman, UTAR Perak Kampus (extracted from Google Maps, Accessed on 10th July 2016)

Papan Sanitary Landfill is a level 3 sanitary landfill with leachate collection and treatment systems, landfill bio-gas collection and electric generation systems. Since July 2015, Papan sanitary landfill has been under operation with 10 acres of land and has a bigger area of 560.24 acres or 226.73 hectares compared to the older landfill which has only 96 acres in size. Papan landfill serves more capacity to receive dumps at a rate of 800 tonnes a day as the new site has 53,516 residents and 34 housing estates within a 5 km radius from the Papan Sanitary Landfill. Apart from that, the lifespan of the landfill is projected to prolong for 35 years (Negeri Perak, 2013). The mature leachate samples were collected in fixed volume with 2 different batches during one-year duration. The 1st batch of leachate samples were collected in June 2016 and the 2nd batch of leachate samples were collected in December 2016 from Papan landfill site.

Figure 3.3 Image of Leachate sample at Papan Landfill.

Figure 3.4 Sample collection of mature leachate in Papan Landfill.

3.3 Coagulation-Filtration Treatment

3.3.1 Coagulation

A simple coagulation research was done using 3 different types of coagulants including ammonium iron (III) sulphate 12-hydrate, aluminium ammonium sulphate 12 –hydrate and aluminium sulphate to study on the suitable coagulant for mature leachate samples with 8g of coagulant for 1L of leachate sample. The samples were tested using COD and Turbidity analysis (Mao Rui and Bin Daud, 2011).

The coagulation process, chemical treatment was done for mature leachate samples before undergoing the modified filtration system. Aluminium ammonium sulphate 12- hydrate was used as a coagulant since a clear layer of solution has formed on the top of the leachate sample after adding the aluminium ammonium sulphate 12-hydrate. Coagulants make the smaller particles to stick together to be expelled from water through physical filtration. A clear solution indicated the coagulation of solid content in the leachate sample. The ratio was 8:1 which means 8g of coagulant for 1L of leachate sample (Mao Rui and Bin Daud, 2011).

3.3.2 Physical Filtration

The coagulated leachate samples were undergone physical filtration. Filtration of leachate samples were carried out and the filtrates were collected in separate bottles. 2.0 L of leachate sample was used for each filtration. Filtrates were kept in the fridge prior to water analysis and biological treatment to minimize any possible means on any chemical reactions and the biodegradation of the leachate sample. Figure 3.5 shows the arrangement of materials for filtration set-up with the capacity of 4L container. Table 3.1 shows the combination of materials in 4 different filtration systems. Figure 3.6 shows the example of physical filtration set up.

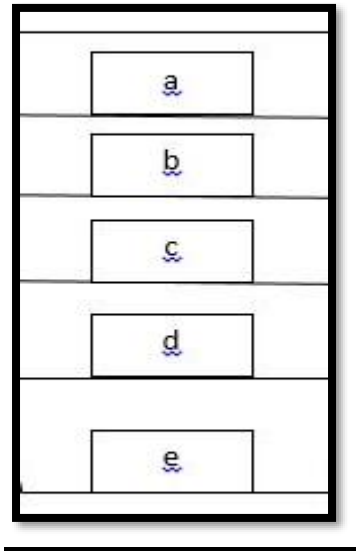


Figure 3.5 Arrangement of materials for filtration set-up

Table 3.1 Combination of materials in 4 different filtration systems.

Set/ Materials	a	b	c	d	e
1	Fine sand	Activated Carbon	Coarse sand	-	-
Ratio of Set 1	3	1	1		
2	Fine sand	Small pebbles	Activated Carbon	Coarse sand	-
Ratio of Set 2	3	1	1	1	
3	Fine sand	Activated Carbon	Small pebbles	Coarse Sand	-
Ratio of Set 3	3	1	1	1	
4	Fine sand	1.2 – 2.4 mm sand	Coarse sand	Small pebbles	Big pebbles
Ratio of Set 4	3	1	1	1	1

*Set 4 is the commercial sand filtration set up for waste water treatment.

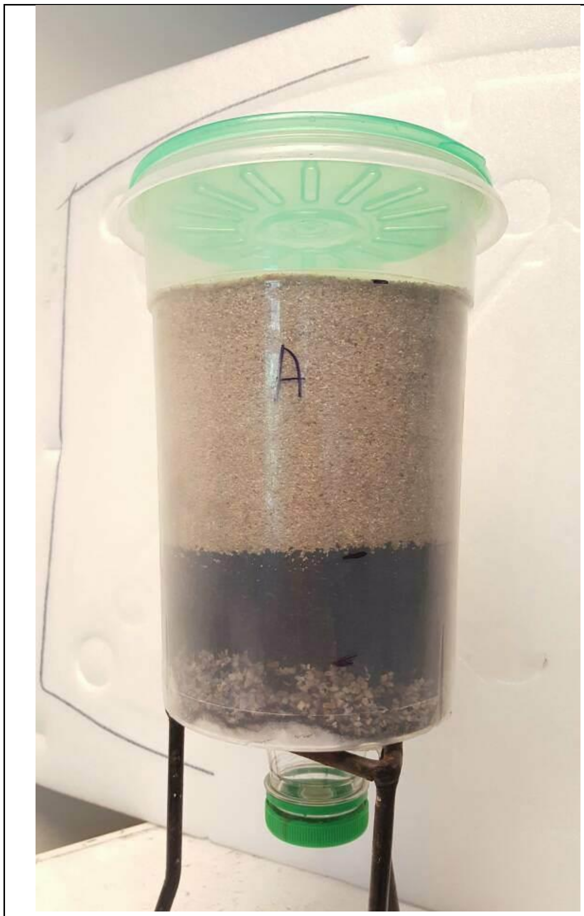


Figure 3.6

3.4 Biological Treatment Using *Botryococcus sudeticus* and *Chlorella vulgaris*

Botryococcus sudeticus and *Chlorella vulgaris* were cultivated separately with zero dilutions in pre-treated mature leachate samples with four different filtration systems. The biological treatment was done using *Chlorella vulgaris* and *Botryococcus sudeticus* with the aeration set-up for the samples by using BB-8000 aquarium air pump. The microalgae growth has been monitored for a month by measuring the chlorophylls and carotenoids content of both *Botryococcus sudeticus* and *Chlorella vulgaris* species separately.

Figure 3.7 ***Chlorella vulgaris*** **in mature leachate samples with aeration set-up.**

3.4.1 Cultivation of *Botryococcus sudeticus* and *Chlorella vulgaris in Bold's Basal Medium (BBM)*

Both *Botryococcus sudeticus* and *Chlorella vulgaris* microalgae species were cultured separately in Bold's Basal Medium (BBM) into 500ml conical flask as a stock to cultivate in treated mature leachate samples. Conical flasks were sent for autoclave. Bunsen burner was lighted up and the apparatus were sterilized. 40ml of BBM, 40ml of sterile tap water and 20 ml of sample were added into 500 ml conical flask. The conical flask was covered using aluminium foil and put under sunlight for growth (Gitelson, Gritz and Merzlyak, 2003).

3.4.2 Cultivation of microalgae in Pre-treated Mature Leachate samples

Both *Botryococcus sudeticus* and *Chlorella vulgaris* microalgae species were grown in pre-treated mature leachate samples separately. Conical flasks were sent for autoclave. The Bunsen burner was lighted up and the apparatus were sterilized. 30ml of microalgae stock in BBM and 270 ml of treated mature leachate sample were added into 500 ml conical flask for growth. The conical flask was covered using aluminium foil and put under sunlight for growth. *Botryococcus sudeticus* and *Chlorella vulgaris* were cultivated separately in pre-treated mature leachate sample using filtration Set 1 to 4 (Gitelson, Gritz and Merzlyak, 2003).

3.4.3 Extraction of Chlorophylls and Carotenoids

The growth of *Botryococcus sudeticus* and *Chlorella vulgaris* were observed and monitored separately for a month and the extraction of chlorophylls and carotenoids were done weekly until the 4th week from the date of culture. 3 ml of microalgae sample was pipette into 15 ml centrifuge tube for ethanol extraction. Firstly, the microalgae sample was centrifuged at 3000 rpm for 30 minutes and the supernatant was removed. The pellet was kept aside. 1-2 ml of 95% ethanol was then added into centrifuge tube. The centrifuge tube was placed into the 60-70 ^{o}C water bath for 10-

15 minutes until the solvent turns into green colour. The supernatant was now kept to measure the absorbance at wavelengths of 664.1 nm, 648.6 nm, and 470 nm for 95% ethanol extract (Gitelson, Gritz and Merzlyak, 2003).

3.5 Lipid Extraction

After 1 month of cultivation period in pre-treated mature leachate samples, both *Botryococcus sudeticus* and *Chlorella vulgaris* samples were harvested to produce the microalgal biomass. Sterile tap water was added into microalgae sample and sonicated to breakdown the cell wall to allow the lipid release from the cell. After that, the sample was transferred into the separating funnel. N-hexane was used to extract lipid that released from the microalgae. The ratio of microalgae and hexane is 1:2. The funnel was stoppered tightly and shook for few times. The pressure was released simultaneously while shaking until no further pressure. The separating funnel was placed on the retort stand and left for the obvious separation of 2 immiscible layers to occur. The 2 immiscible layers were organic layer and aqueous layer. The lower aqueous layer was flowed into the conical flask and upper organic layer was kept into another dried conical flask. Second extraction was done for aqueous layer using another potion of n-hexane. The aqueous layer was collected to obtain wet weight. The organic layer was then added with anhydrous sodium sulphate in order to eliminate any water residue and the organic layer was filtered into a pre-weighed dried round bottom flask. The organic solvent was evaporated using rotary evaporator roughly at temperature of 45^{o}C and pressure of 150 mbar. The flask was dried in an oven overnight and cooled to room temperature to weigh the flask. The weight of lipid was obtained (Samori et al., 2013).

3.6 Leachate Analysis

The leachate analysis was done by referring to standard methods for water and wastewater analysis by American Public Health Association (APHA) (Baird and Bridgewater, 2017). Table 3.2 Types of test with standard test methods by APHA. Leachate analysis was repeated after coagulation-filtration treatments only, after combined coagulation-filtration and biological treatment using *Chlorella vulgaris*, and after combined coagulation-filtration and biological treatment using *Botryococcus sudeticus*.

Table 3.2 Types of test with standard test methods by APHA (Baird and Bridgewater, 2017).

Type of Test	**Standard Test Methods**
pH	APHA 4500
Conductivity	APHA 2510B
Total Solids	APHA 2540 B (Total Solids Dried at 103 °C – 105°C)

Total Suspended Solids	APHA 2540 D (Total Suspended Solids Dried at 103 °C – 105°C)
Total Dissolved Solids	APHA 2540 C (Total Dissolved Solids Dried at 103 °C – 105°C)
Turbidity	APHA 2130 B
Biochemical Oxygen Demand (BOD5)	APHA 5210 B 5 Day BOD Test
Chemical Oxygen Demand (COD)	APHA 5220 C

3.6.1 Total Organic Carbon

Raw mature leachate samples were diluted with 10 dilution factors. The samples were undergone filtration by using 0.45µm pore size filter membrane with syringe. 50ml of the filtrates were collected and sent for Total Organic Carbon analysis by using TOC analyzer. The data was duplicated for average reading. TOC analysis was repeated after coagulation-filtration treatments only, after combined coagulation-filtration and biological treatment using *Chlorella vulgaris*, and after combined coagulation-filtration and biological treatment using *Botryococcus sudeticus*.

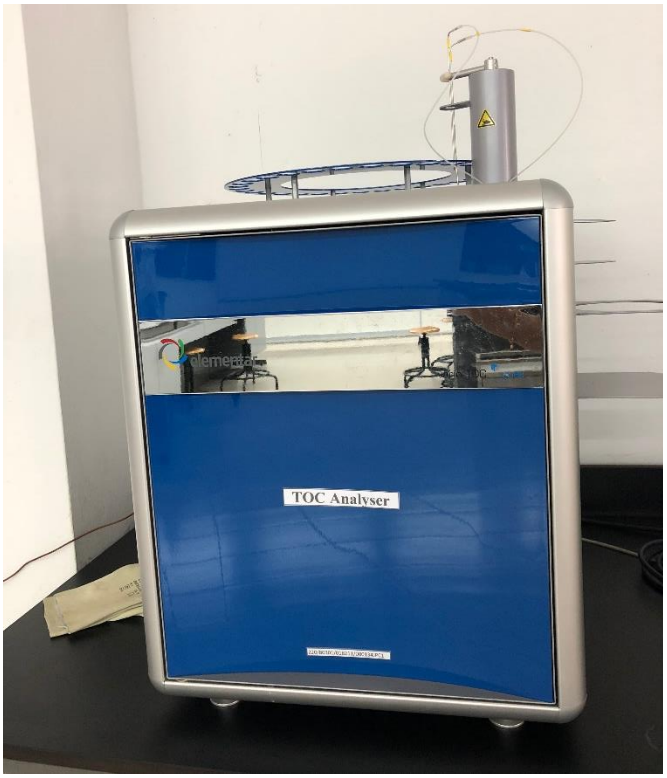


Figure 3.8 TOC Analyser

3.6.2 Heavy metals using Inductively coupled plasma mass spectrometry (ICP-MS)

The calibration solutions were diluted to different concentrations such as 100 ppb, 200 ppb, 300 ppb, 400 ppb, 500 ppb and 1000 ppb from 10000 ppb solution. The calibration solutions were prepared in 50 ml centrifuge tubes. All the solutions were diluted using deionized water. Raw mature leachate samples were diluted with 100 and 1000 dilution factor. The samples were prepared in 15 ml centrifuge tubes. All the samples were diluted using deionized water and 1% of HNO_3 was added to the samples. The samples were filtered using 0.45 µm filter before sending for heavy metals analysis. Heavy metals analysis was repeated after coagulation-filtration treatments only, after combined coagulation-filtration and biological treatment using *Chlorella vulgaris*, and after combined coagulation-filtration and biological treatment using *Botryococcus sudeticus*.

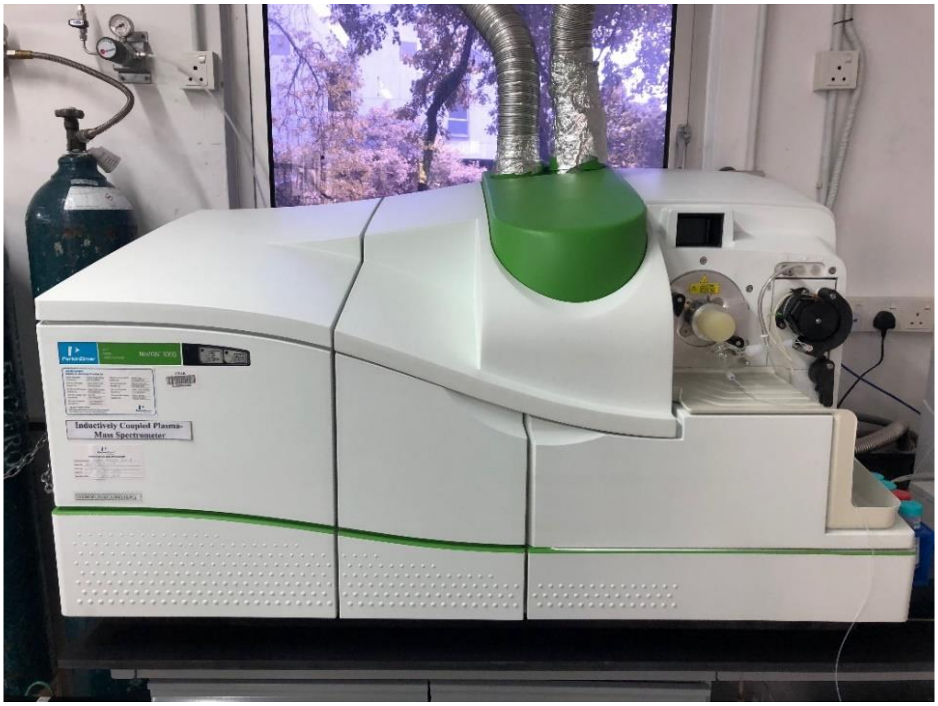


Figure 3.9 Inductively coupled plasma mass spectrometry (ICP-MS)

3.6.10 Total Ammonia Determination

A. Preparation of Ammonium Solution

About 2L of ammonia free water was prepared by simple distillation. Anhydrous NH_4Cl was dried in oven at 100 ^oC for 1 hour. Standard ammonium NH_3-N solution (1000ppm) was prepared by weighing 3.819g of anhydrous ammonium chloride ((NH_4Cl) and diluted in 1000 ml of distilled water.

B. Preparation of Rochelle salt solution

50g of potassium sodium tartrate was dissolved in 100 ml distilled water.

C. Nessler Reagent

50g of potassium iodide was dissolved in 35 ml of distilled water and added saturated solution of mercuric solution until a slight precipitate persists. 400ml of potassium hydroxide (50% aqueous) was then added. The solution was diluted to 1000 ml by the addition of distilled water. The solution allowed to settle for one week, decanted supernatant liquid, and stored in tightly stoppered bottle.

D. Total Ammonia Determination (NH3-N)

The standards were prepared using standard ammonium NH_3-N solution as shown below

From 50mg/L	5 ml/ 50 ml dH2O	5 mg/L
	4 ml/ 50 ml dH2O	4 mg/L
	3 ml/ 50 ml dH2O	3 mg/L
	2 ml/ 50 ml dH2O	2 mg/L
	1 ml/ 50 ml dH2O	1 mg/L

The mature leachate samples were diluted to 10 times dilution factor. 1 ml of potassium sodium tartrate and Nessler's reagent were added into 50 ml of each standard, 50 ml of distilled water (free ammonia as blank) and 50 ml of leachate samples. The absorbance readings were recorded at the wavelength of 425nm of the samples and standards to plot the standard curve. The total ammonia values were determined from the standard curve in mg/L. The total ammonia determination analysis was repeated after coagulation-filtration treatments only, after combined coagulation-filtration and biological treatment using *Chlorella vulgaris*, and after combined coagulation-filtration and biological treatment using *Botryococcus sudeticus*.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Mature leachate characteristics

Mature leachate samples from Papan Sanitary Landfill were collected and analyzed for various parameters before the chemical, physical and biological treatment to estimate its pollution potential. It has been found that leachate samples contain high concentration of organic and inorganic constituents beyond the permissible limits. The color of leachate samples were dark brown. Associated with the leachate is a malodorous smell, due mainly to the presence of organic acids, which come from the high concentration of organic matter when decomposed (Bhalla, Saini and Jha, 2013). Table 4.1 shows the characteristics of raw mature leachate from Papan Landfill. The total solids, total suspended solids, and total dissolved solids were 8526 mg/L, 1602 mg/L and 5045 mg/L respectively. The pH, conductivity, turbidity values were 8.37, 17.62 ms/cm and 235.05 Ntu. Organics in leachate are characterized by different levels of biodegradability. In this study, the BOD_5/COD ratio for the collected leachate samples of the Papan Sanitary Landfill site was 0.1.

Based on the Table 2.2, the characteristics of mature leachate samples is consistent with the previous studies and within the range of mature leachate sample characteristics (Hector, Bruce and Simon, 2004).

Table 4.1 Characteristics of raw mature leachate from Papan Landfill

Parameters	**Values**
Total solids	8526 ± 510 mg/L
Total suspended solids	1602 ± 810 mg/L
Total dissolved solids	5045 ± 1001 mg/L
Conductivity	17.62 ± 0.63 ms/cm
Turbidity	235.05 ± 48.28 Ntu
pH	8.37 ± 0.04
TOC	583.69 ± 510.00 mg/L
COD	2377.5 ± 759.9 mg/L
Total ammonia content	394.37 ± 43.07 mg/L
BOD_5	428.5 ± 424.9 mg/L

4.2 Combination of chemical, physical and biological treatment

Treatment of mature landfill leachate from Papan Sanitary landfill by combined chemical, physical and biological methods was investigated in the present study. The coagulation and filtration treatment serve as a pre-treatment and no further dilution of mature leachate sample required growing the two different types of microalgae species, namely, *Botryococcus sudeticus* and *Chlorella vulgaris*.

Raw mature leachate samples were undergone coagulation process by using aluminium ammonium sulphate 12-hydrate (chemical treatment) before the treatment using filtration system (physical treatment). First 3 modified filtration systems were found to be more efficient compared to the control filtration system which is similar to the commercial system used in wastewater industry. The modified filtration system assisted by activated carbon as a physical treatment of mature leachate samples was employed and found to be highly effective in removing a large amount of refractory inorganic and organic compounds in the leachate (Ahmad et al., 2014).

The biological treatment using *Botryococcus sudeticus* and *Chlorella vulgaris* was capable of further polishing the water quality of leachate effluent to the discharge standard. Both *Botryococcus sudeticus* and *Chlorella vulgaris* species were cultured in pre-treated mature leachate samples separately as the inhibition of growth occurs in raw mature leachate samples due to the different level of contamination in raw leachate and pre-treated mature leachate samples.

The growth of *Botryococcus sudeticus* and *Chlorella vulgaris* species were monitored for 1-month period with aeration system set-up. Comparing both microalgae species in biological treatment, *Chlorella vulgaris* species managed to grow easily in 100% pre-treated mature leachate samples compared to *Botryococcus* *sudeticus* because *Chlorella vulgaris* species managed to show growth in all pre-treated mature leachate using modified filtration set up.

4.2.1 Coagulation

A simple coagulation research was done using 3 different types of coagulants including ammonium iron (III) sulphate 12-hydrate, aluminium ammonium sulphate 12 –hydrate and aluminium sulphate to study on the suitable coagulant for mature leachate samples with 8g of coagulant for 1L of leachate sample. The samples after coagulation were compared based on the COD and turbidity removal. Figure 4.1 and Figure 4.2 show COD and turbidity removal from mature leachate samples. Based on the graph of turbidity, aluminium ammonium sulphate 12 –hydrate effectively removed 48% of cloudiness in mature leachate sample while ammonium iron (III) sulphate 12-hydrate and aluminium sulphate have shown lesser removal efficiency in turbidity.

Based on the Figure 4.2, aluminium ammonium sulphate 12 –hydrate effectively removed 39% of COD for mature leachate. Comparing with the other two coagulants, aluminium ammonium sulphate 12 –hydrate has shown greater removal efficiencies because ammonium iron (III) sulphate 12-hydrate and aluminium sulphate removed less than 20% of COD. Therefore, coagulation using aluminium ammonium sulphate 12 –hydrate is more suitable for treating mature leachate.

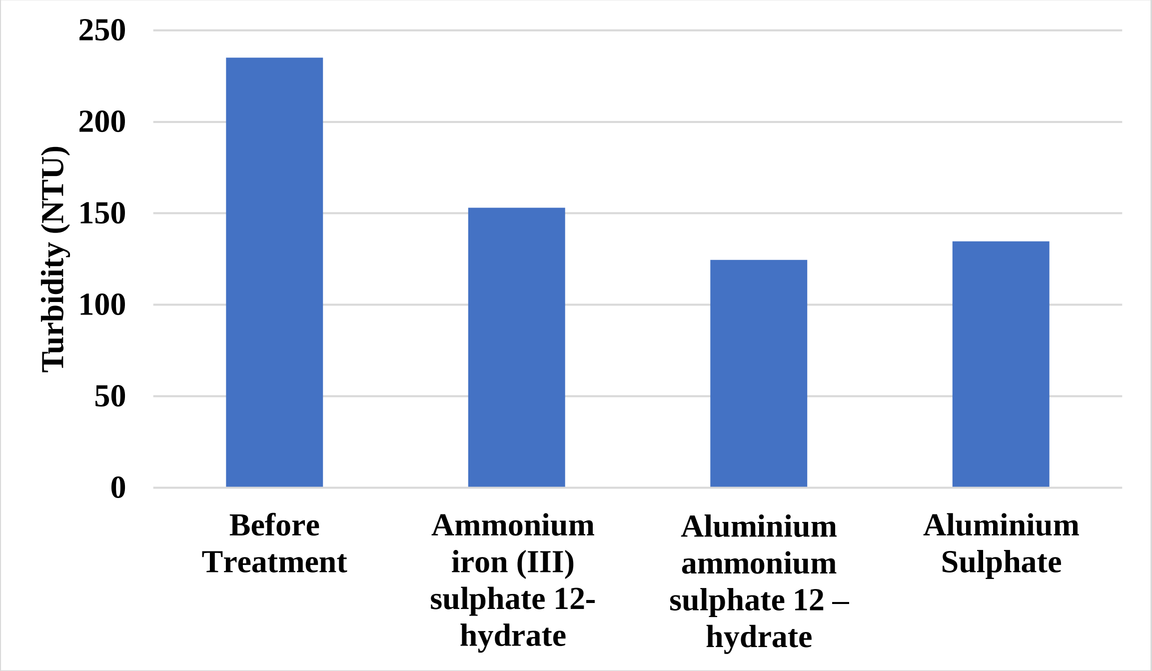


Figure 4.1 Graph of turbidity of mature leachate samples before and after coagulation.

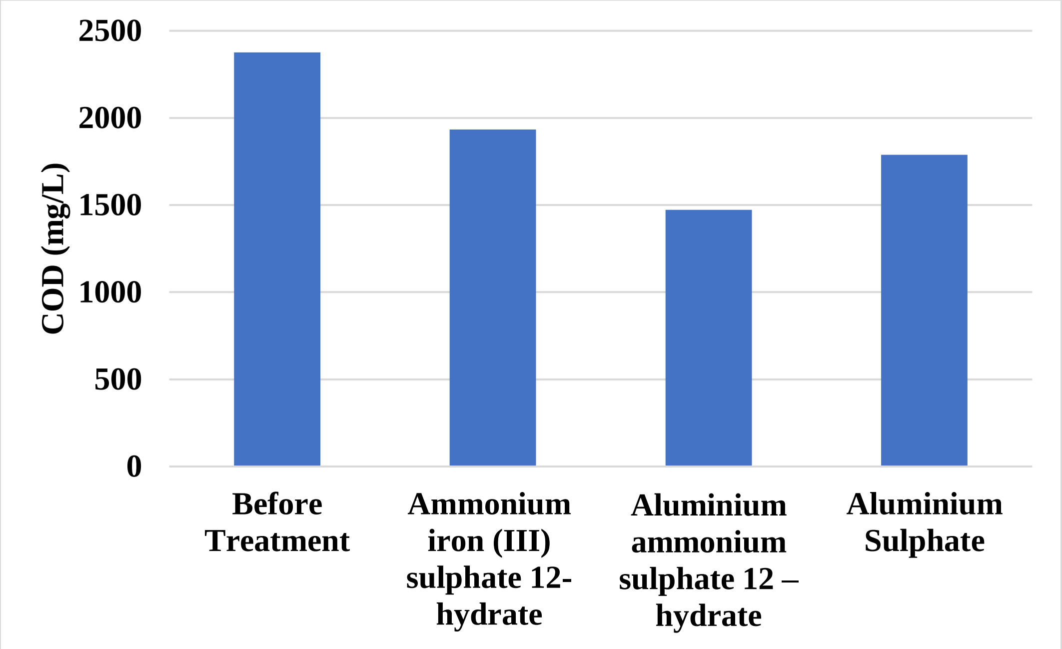


Figure 4.2 Graph of Chemical Oxygen Demand (COD) versus mature leachate samples before and after coagulation.

Aluminium ammonium sulphate 12–hydrate results in charge neutralization and particle destabilization because the colloids contained in the mature leachate samples are negatively charged (Kamaruddin et al., 2017). These colloid particles are highly stable due to the repellent forces between the negative charges. These colloid particles are destabilized by positively charged aluminium ions (Al^{3+}) formed from the hydrolysis of coagulants (Shu et al, 2016). Destabilization of colloids influenced by the adsorption, charge neutralization, double layer compression, interparticle bridging and entrapment in precipitates. The polymeric hydrolyzed component possess highly positive charges and adsorbed onto the surface of the negative colloid particles. It results in a reduction of the zeta potential to a level where the colloids particles are destabilized. The destabilized colloidal particles aggregate by interparticulate Van der Waals forces along with their adsorbed hydro-metallic hydroxometallic complexes (Vaezi et al., 2015). These forces are aided by the gentle mixing in mature leachate.

4.2.2 Physical Treatment by Filtration

Referring to the results obtained in Figure 4.1 and Figure 4.2 for coagulation process, this method may result in only moderate removals of COD and turbidity and coupling with filtration system is essential. The commercial filtration set-up using various types of sand has been proven to be less efficient in current studies. The commercial filtration set-up has been modified by incorporating activated carbon and the effective filtration set up removed 75% TS, 68%, TDS, 90% turbidity, 89% TSS, 90% COD, 92% BOD_5, and 97% of TOC. The combined process of coagulation and adsorption using activated carbon can ameliorate the drawback of coagulation process using aluminium sulphate 12–hydrate. Coagulation can eliminate moderate COD and reduce the COD burden for subsequent filtration process. On the other hand, it reduces the amount of activated carbon usage in the filtration system.

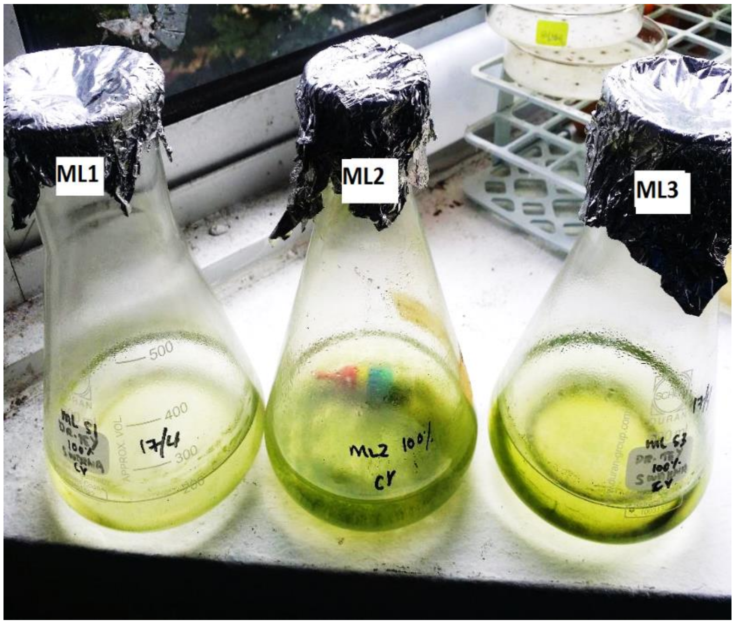
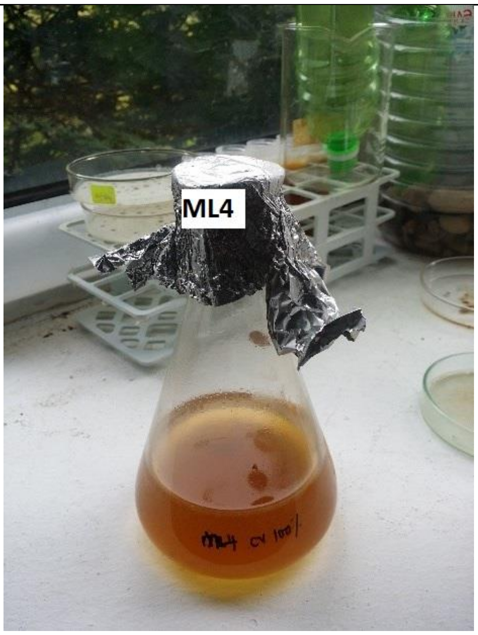
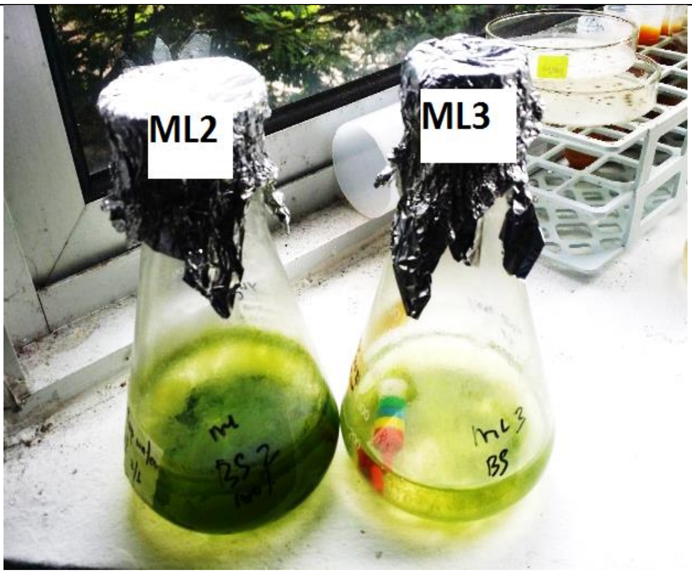
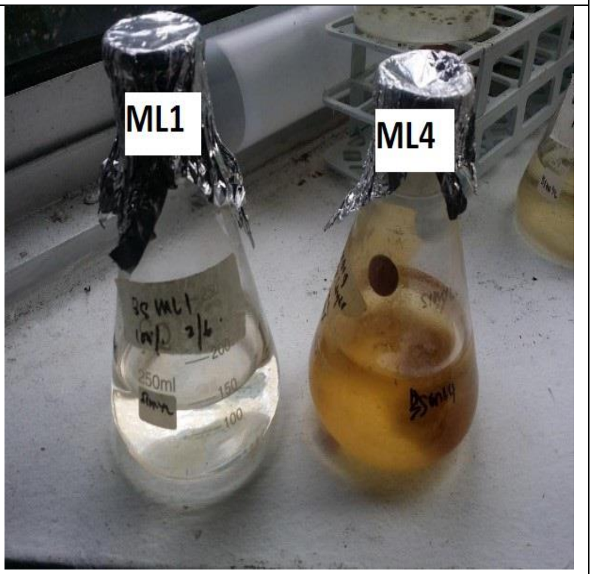
In this research, 3 sets of multilayer sand filtration have been used for single pass of coagulated mature leachate by incorporating activated carbon. The coagulated leachate samples have been loaded through the gravel layers at decremental depths in the layered filter column. Activated carbon has microporous structure, large surface area and surface reactivity. Thus, activated carbon adsorption in filtration process has been the most attractive in the removal of recalcitrant compounds from mature leachate. The adsorption of pollutants onto activated carbon provided great reduction in contaminants, whatever the initial organic matter concentration (Lim et al., 2010).

4.2.3 Biological Treatment using *Botryococcus sudeticus* and *Chlorella vulgaris*

The pre-treated mature leachate from the coagulation-filtration treatment using four different filtration set-ups was subjected for further treatment using *Botryococcus sudeticus* and *Chlorella vulgaris* with aeration separately. *Chlorella vulgaris* species effectively grown in set 1 to set 3 of pre-treated mature leachate while *Botryococcus sudeticus* species could only grow in set 2 and set 3 of pre-treated mature leachate. Table 4.2 shows the pictures of *Chlorella Vulgaris* (CV) and *Botryococcus sudeticus* (BS) grown in pre-treated mature leachate. Both *Botryococcus sudeticus* and *Chlorella vulgaris* did not show the growth in pre-treated mature leachate filtration using set 4 which is commercial filtration set-up. The high turbidity of landfill leachate samples treated using set 4 causes inhibitory effect for microalgae growth in it. Thus, the lower transparency prevents the light penetration to reach to microalgae as light is essential for microalgae to carry out photosynthesis process for growth (Paskuliakova et al., 2016). Microalgae can grow in highly diluted or pre-treated leachate and the different level of contamination in the pre-treated leachate is the cause of inhibitory effect.

Table 4.2 Pictures of *Chlorella Vulgaris* (CV) and *Botryococcus sudeticus* (BS) grown in pre-treated mature leachate.

***Chlorella Vulgaris* (CV) and *Botryococcus sudeticus* (BS) in pre-treated mature leachate**	
CV grown in ML1[a], ML2[b] and ML3[c]	**CV in ML4[d] (No growth observed)**

	
***Botryococcus sudeticus* (BS) in pre-treated mature leachate**	
BS grown in ML2 and ML3	**BS in ML1 and ML4 (No growth observed)**
	

[a] Microalgae grown in pre-treated mature leachate using modified filtration system, Set 1.

[b] Microalgae grown in pre-treated mature leachate using modified filtration system, Set 2.

[c] Microalgae grown in pre-treated mature leachate using modified filtration system, Set 3.

[d] Microalgae grown in pre-treated mature leachate using control filtration system, Set 4.

Based on the results, further treatment using *Botryococcus sudeticus* and *Chlorella vulgaris* more than 95% of reduction in TS, TSS, TDS, turbidity, COD, BOD_5, and TOC were noticed. Moreover, according to ICP-MS results, landfill leachate is rich in heavy metal content and the metal content must be removed in order to treat the leachate. Microalgae is very good in metal absorption and has uptake the metals as nutrient for it to grow (Liu et al., 2011).

Mature leachate treatment using *Botryococcus sudeticus* and *Chlorella vulgaris* effectively removed high concentrations of BOD_5 with its simplicity, reliability and high cost-effectiveness. Microalgae used high levels of salts, ammonia nitrogen, certain metals and an extensive array of organic compounds in mature leachate as a source of nutrients to grow (Paskuliakova et al., 2016). *Botryococcus sudeticus* and *Chlorella vulgaris* produce oxygen which can be used by aerobic bacteria to biodegrade the pollutants while, in return, consume the carbon dioxide released from bacterial respiration (Oswald, 2008). Thus, providing a cheaper and safer alternative for carbon dioxide mitigation (Guieysse et al., 2002).

4.3 Pigment Extractions

Figure 4.3 shows pigments extraction with ethanol from *Chlorella vulgaris* for 1-month period. The absorbance readings were taken weekly once for a month and the growth of *Chlorella vulgaris* has been monitored and compared with tap water. Based on the results, *Chlorella vulgaris* managed to survive in mature leachate treated using Set 1 to 3. It shows that *Chlorella vulgaris* easily grow in pre-treated mature leachate using modified filtration systems. No growth has been observed for the first week and consistently grow from second week onwards in pre-treated mature leachate samples. The chlorophyll content and carotenoid content increases from week 2 to week 4. Both chlorophyll and carotenoids content were higher for the *Chlorella vulgaris* grown in tap water compared to pre-treated leachate.

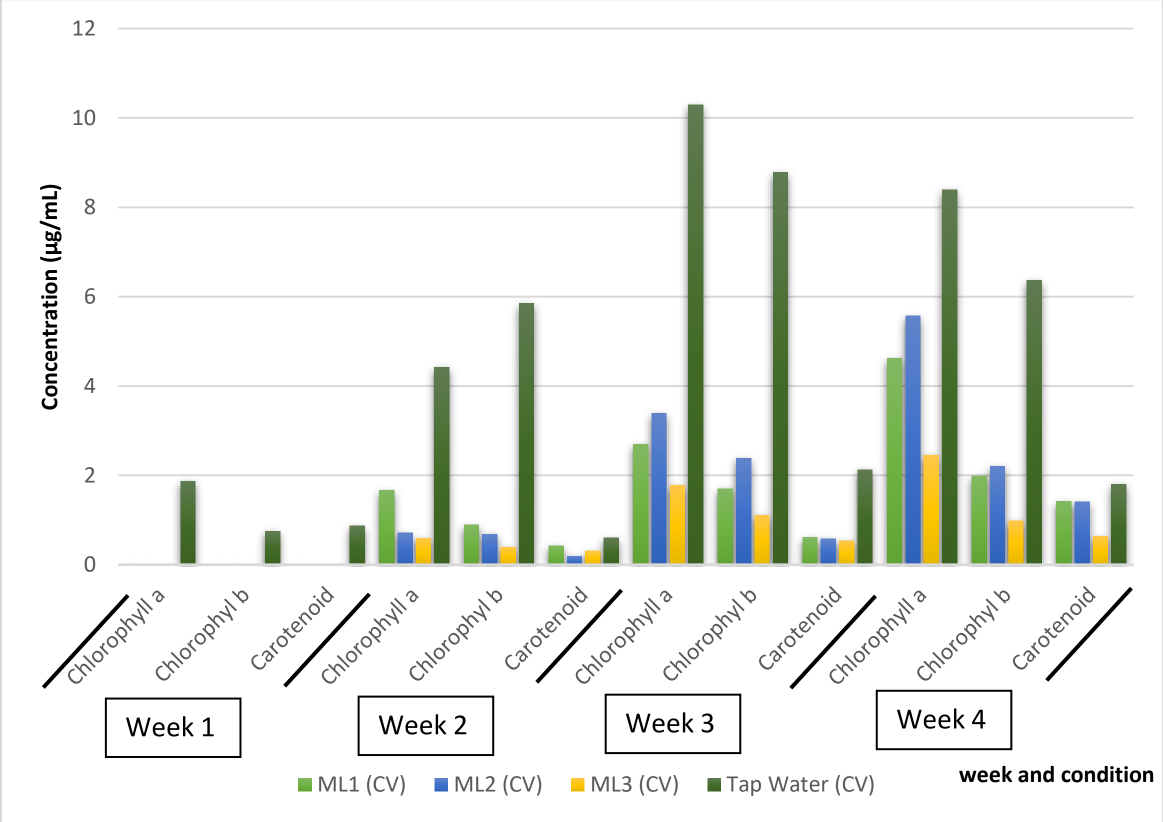


Figure 4.3 Pigments extraction with ethanol from *Chlorella vulgaris* for a month.

Figure 4.4 shows ethanol extract of the pigments from *Botryococcus sudeticus* for 1-month period. The absorbance readings were taken weekly once for a month and the growth *Botryococcus sudeticus* has been monitored and compared with tap water. Based on the results, *Botryococcus sudeticus* managed to survive in mature leachate treated using Set 2 and Set 3. No growth has been observed in pre-treated mature leachate using Set 1 and Set 4 filtration system. No growth has been observed for the first week and consistently grow from second week onwards in mature leachate samples. The chlorophyll and carotenoid contents increase from week 2 to week 4. Both chlorophyll content and carotenoid were higher for the *Botryococcus sudeticus* grown in tap water compared to pre-treated leachate.

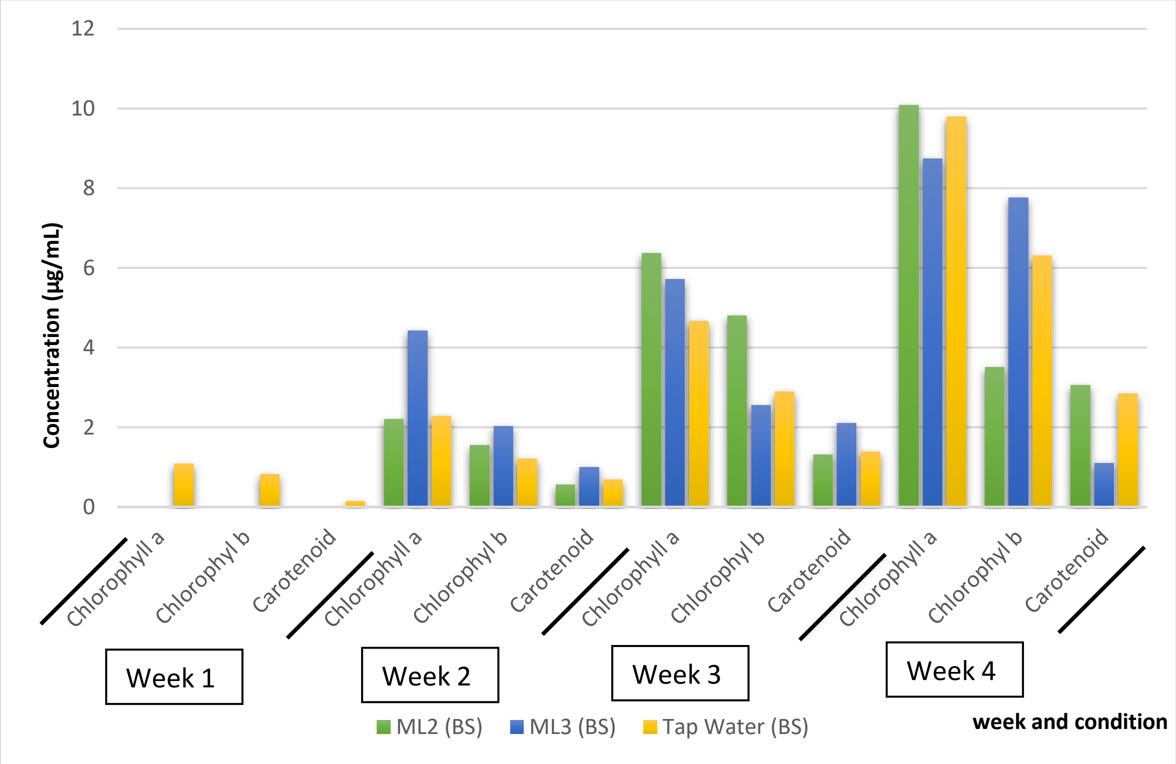


Figure 4.4 Pigments extraction with ethanol from *Botryococcus sudeticus* for a month.

4.4 Leachate Analysis

In order to make comparison of before and after analysis in percentage, the data of chemical analysis has been analyzed and computed into bar graphs. Optimum operating conditions for each treatment units were evaluated and identified to offer an overall good presentation of the combined treatment system that was found to provide an efficient and economical alternative for dealing with the leachate. The values obtained have been analyzed if the treated leachate effluent comply with the Environmental Quality Act 1974 Regulations 2009 in Table 2.7.

4.4.1 Total Solids

From both Figure 4.5 and Figure 4.6, a significant drop is shown between before and after coagulation-filtration treatment while a slight decrease was shown after the coagulation-filtration-microalgae treatment for mature leachate samples. The most effective filter set for mature landfill leachate sample using coagulation-filtration treatment is set 1 in which the sample has been treated from 8526 mg/L to 2200 mg/L which means a total reduction of 75% in total solids. Mature leachate sample has been effectively treated by using Set 1 and Set 2 with combined coagulation-filtration-microalgae treatment using *chlorella vulgaris* which showed a sharp decrease from 8526 mg/L to 1989 mg/L and 2007 mg/L for Set 1 and 2 respectively with almost 76% reduction in total solids. However, the combined coagulation-filtration-microalgae treatment using *botryococcus sudeticus* is effective with Set 3 resulting in 79% of total solids removal. Thus, the combination of materials from Set 1 to 3 is effective to remove total solids in landfill leachate samples. The modified set ups are all shown to be more effective in removing total solids compared to control set.

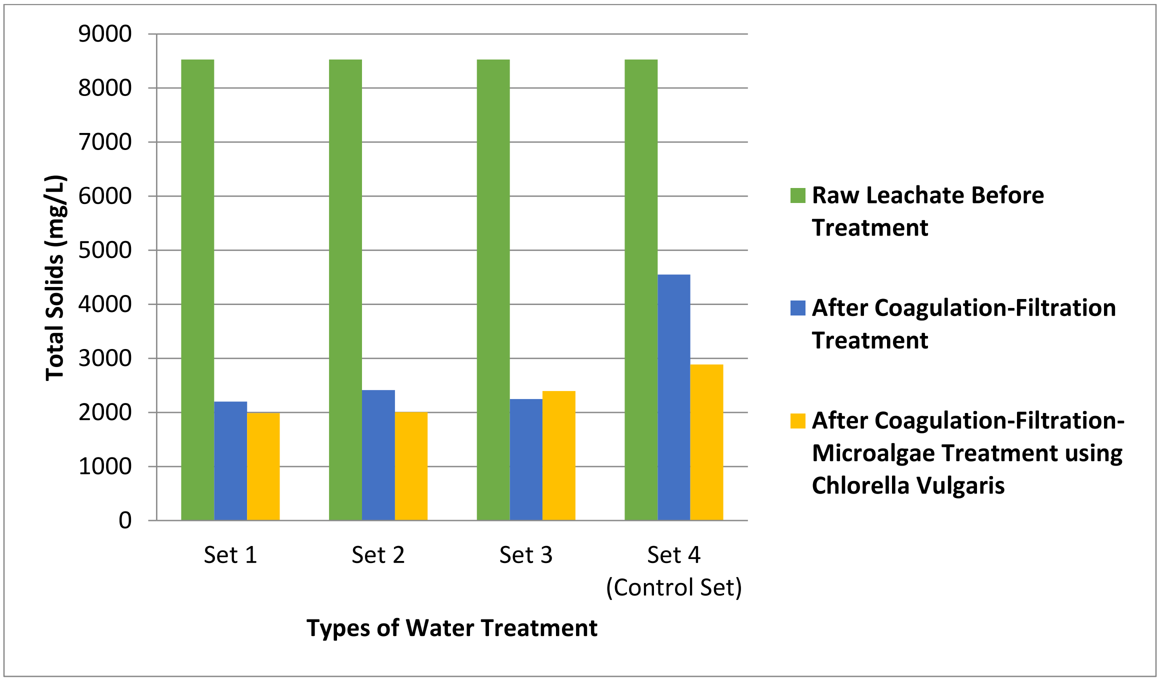


Figure 4.5 Total Solids versus Types of Treatment for Mature leachate using *Chlorella Vulgaris*.

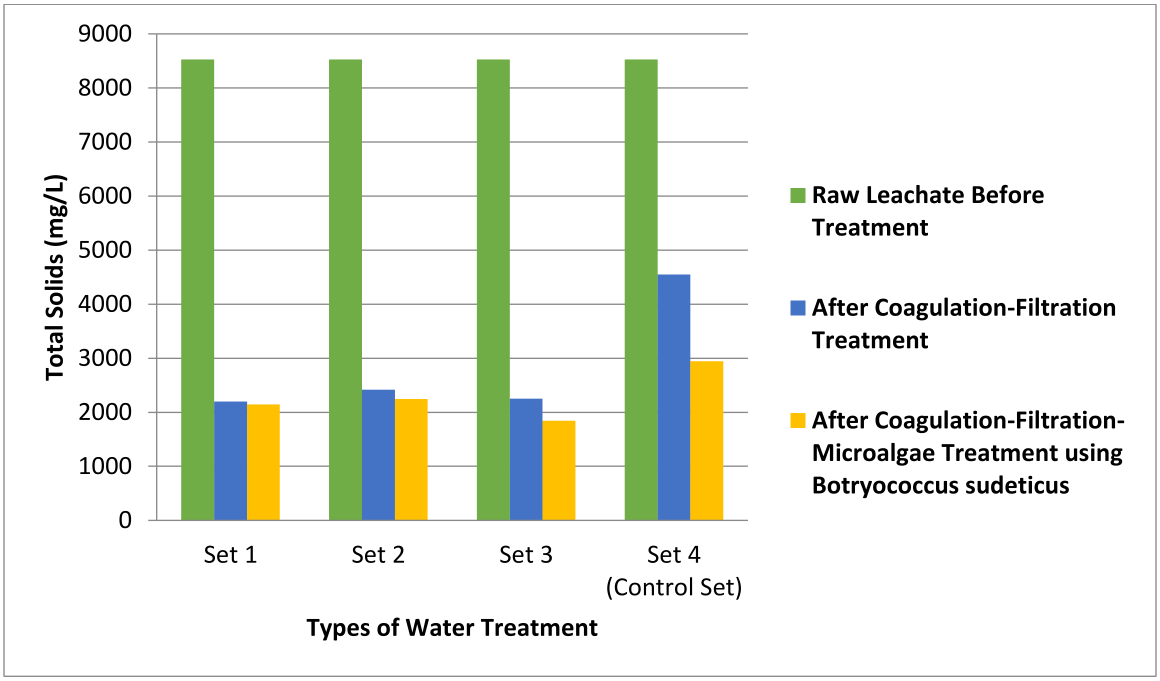


Figure 4.6 Total Solids versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.2 Total Suspended Solids

From Figure 4.7 and Figure 4.8, a clear drop is shown between before and after coagulation-filtration treatment while a slight decrease has been shown after the coagulation-filtration-microalgae treatment for landfill leachate samples. The most effective filter set for mature leachate sample after coagulation-filtration treatment is set 2 in which the sample has been treated from 1602 mg/L to 169 mg/L with a total reduction of 89% in total suspended solids. The materials in set 2 effectively filter the suspended solids in landfill mature leachate. The fine sand traps the bigger particles on of the filtration set up while the activated carbon further traps the smaller particles in second layer by providing adsorption surface. The mature leachate samples have been effectively treated by Set 3 after coagulation-filtration-microalgae treatment using *Chlorella vulgaris* and *Botryococcus sudeticus* which showed a sharp decrease with almost 98% reduction in total suspended solids. Thus, the combination of materials shows that Set 3 is effective to remove total suspended solids in mature leachate samples in the end of biological treatment using both microalgae species.

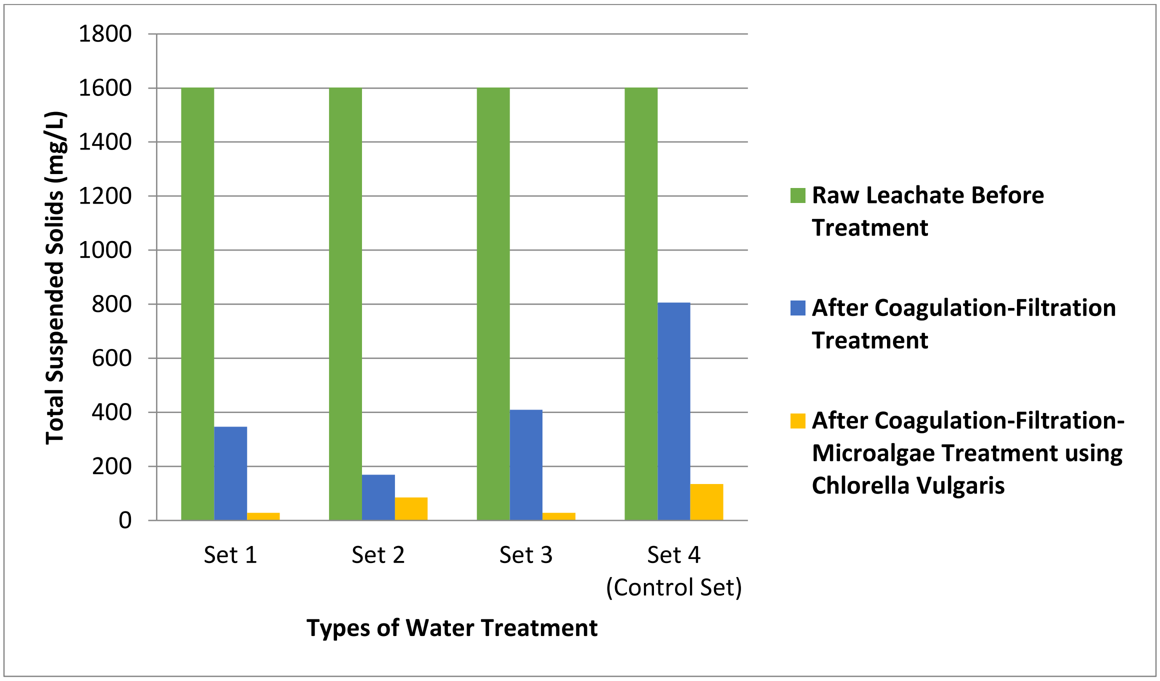


Figure 4.7 Total Suspended Solids versus Types of Treatment for Mature leachate using *Chlorella Vulgaris.*

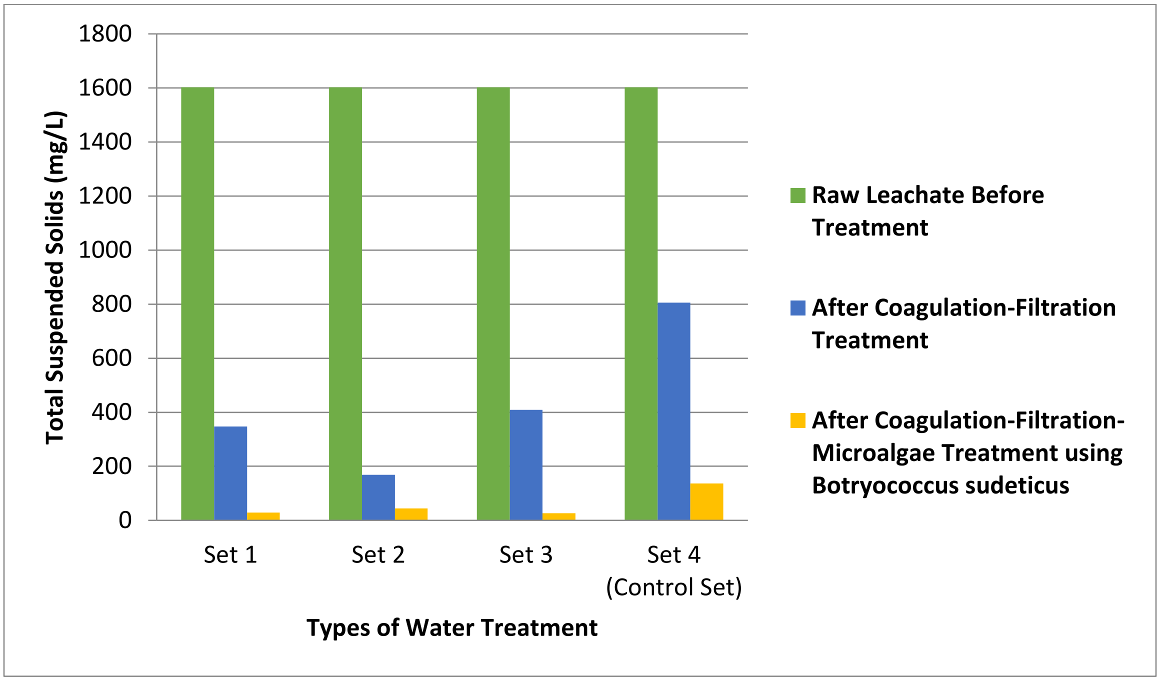


Figure 4.8 Total Suspended Solids versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.3 Total Dissolved Solids

From Figure 4.9 and Figure 4.10, a significant drop is shown between before and after coagulation-filtration treatment while a slight decrease has been shown after combined coagulation-filtration-microalgae treatment for mature leachate samples. The most effective filter set after coagulation-filtration treatment is set 1 in which a total reduction of 68% in total dissolved solids was observed. Mature leachate has been effectively treated by Set 2 after combined coagulation-filtration-microalgae treatment with 76% and 66% reduction in total dissolved solids using *Chlorella vulgaris* and *Botryococcus sudeticus* respectively. Thus, the combination of materials of Set 2 is effective to remove total dissolved solids in mature leachate samples. Overall, when comparing the control set with modified filtration set ups, the removal of total solids, total suspended, and total dissolved solids were more effective for all modified filtration set up compared to control set which is normally being used as a commercial filtration system in waste water industry.

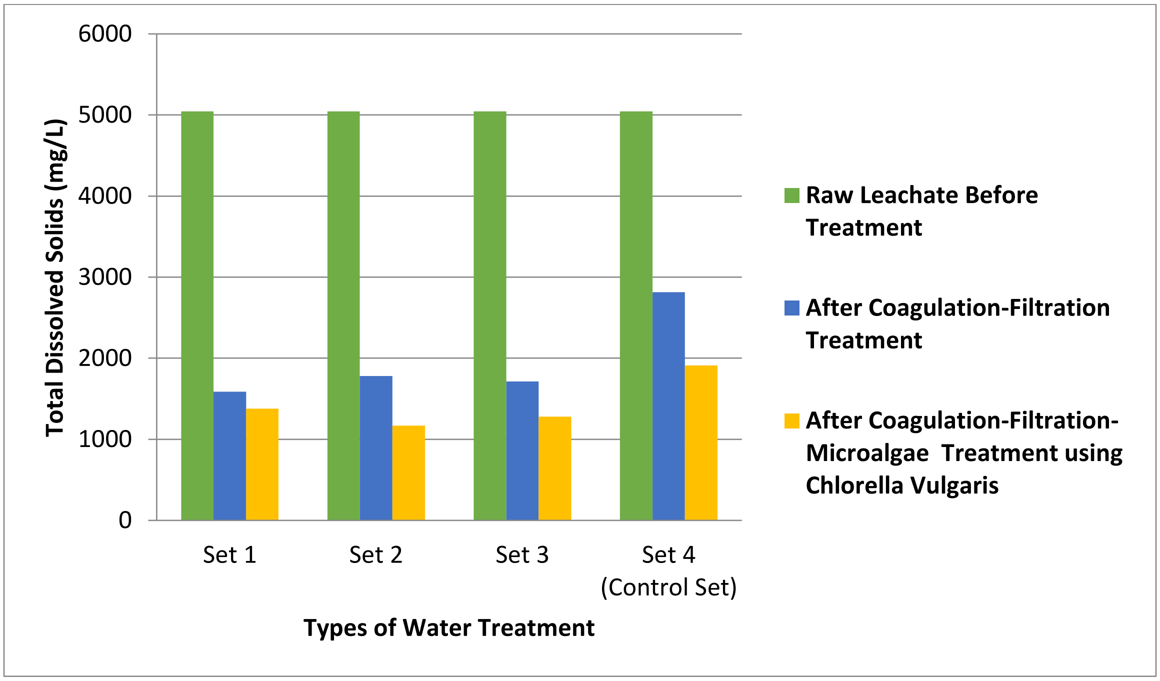


Figure 4.9 Total Dissolved Solids versus Types of Treatment for Mature leachate using *Chlorella Vulgaris*.

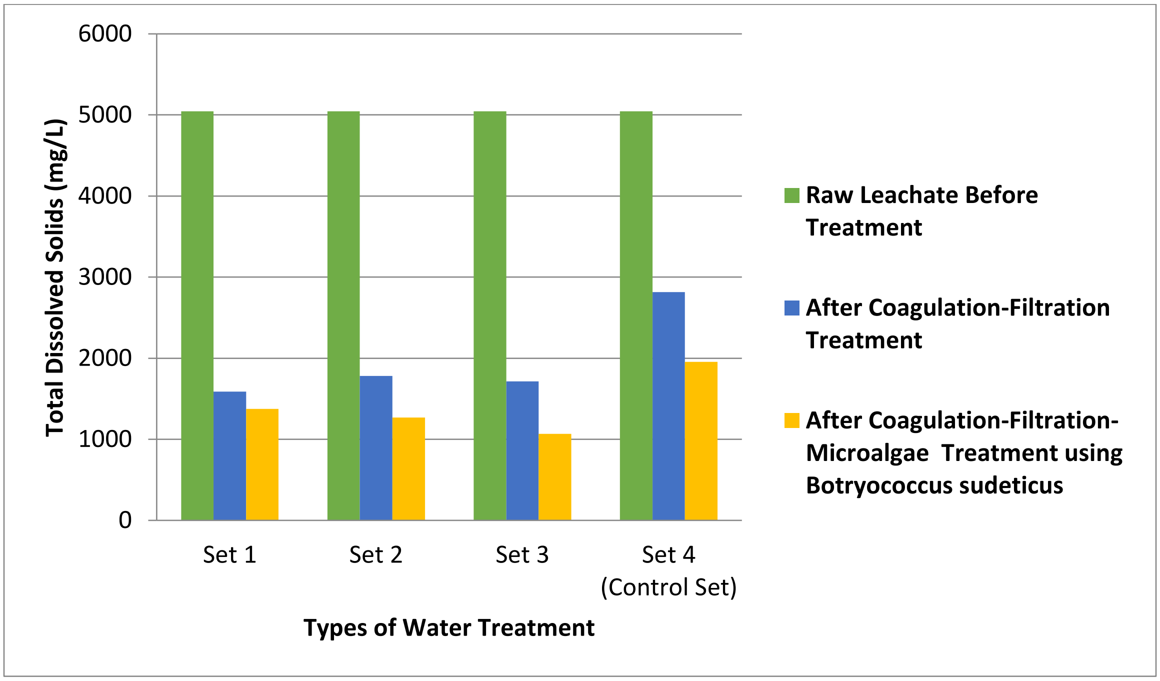


Figure 4.10 Total Dissolved Solids versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.4 Conductivity

The conductivity has decreased drastically after the coagulation-filtration treatment and coagulation-filtration-microalgae treatment for mature leachate samples. The conductivity has been effectively reduced by set 1 which is about 47% after coagulation-filtration treatment**.** Subsequently, the combined coagulation-filtration-microalgae treatment was efficient using Set 1 filtration set up also with both *Chlorella vulgaris* and *Botryococcus sudeticus* which is about 77% and 83% in reduction using 100% of treated leachate with aeration set up for microalgae growth. Conductivity measurement is related to the number of electrons in mature leachate sample before and after the treatment. Thus, the concentration of ions decreases eventually after the coagulation-filtration treatment and coagulation-filtration-microalgae treatment which can be related to the adsorption of ions during filtration and microalgae treatment. The modified filtration set ups could remove conductivity effectively while the control set is not effective and no microalgae growth has been observed during the biological treatment.

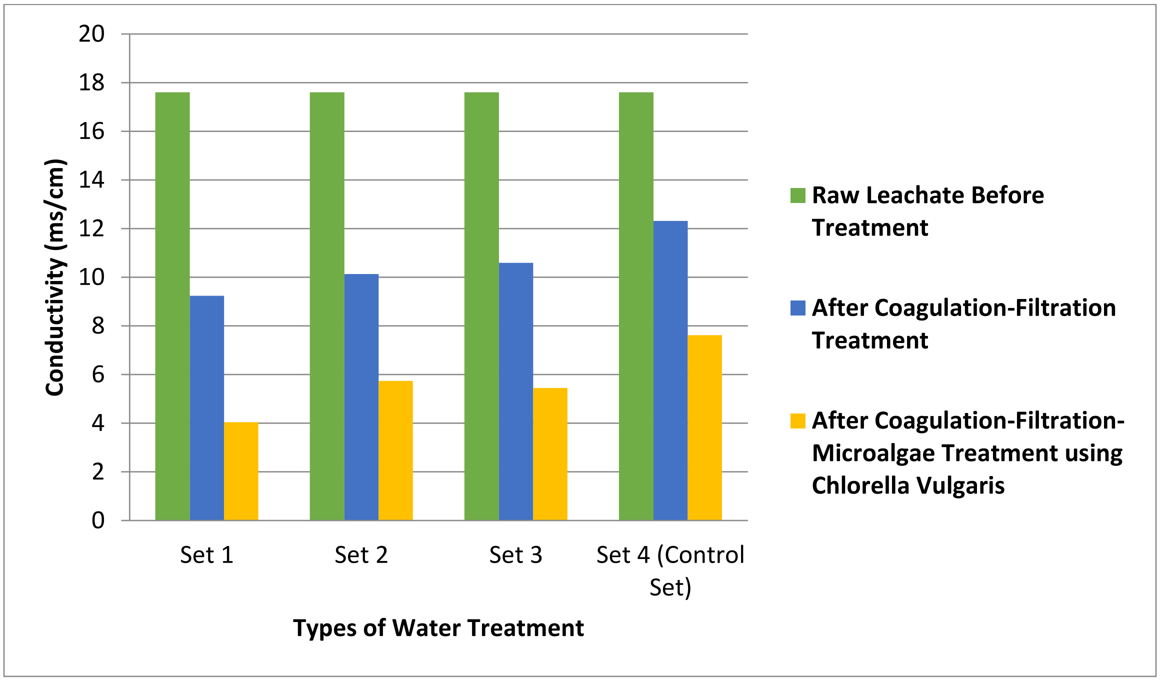


Figure 4.11 Conductivity versus Types of Treatment for Mature leachate using *Chlorella Vulgaris.*

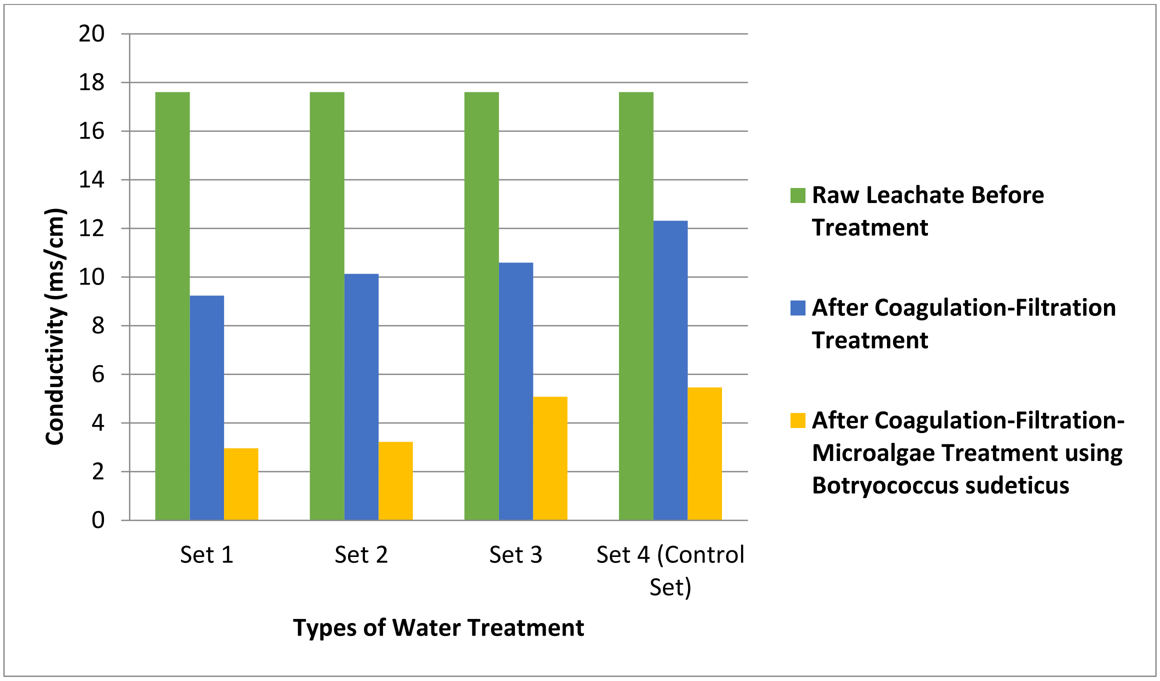


Figure 4.12 Conductivity versus Types of Treatment for Mature leachate using *Botryococcus sudeticus*.

4.4.5 Turbidity

Based on the graphs of turbidity, after the coagulation-filtration treatment, the modified filtration set ups are effective in removing the turbidity compared to control. It is effective as until 95% of cloudiness or haziness of a fluid caused by total solids has been removed. The elimination of turbidity is assisted by the pre-treatment process which is coagulation. The modified filtration set ups removed up to 90% of turbidity after coagulation-filtration treatment and more than 95% after the combined coagulation-filtration-microalgae treatment in mature leachate samples. The least effective set is control set due to the cloudiness and no algae growth has been observed.

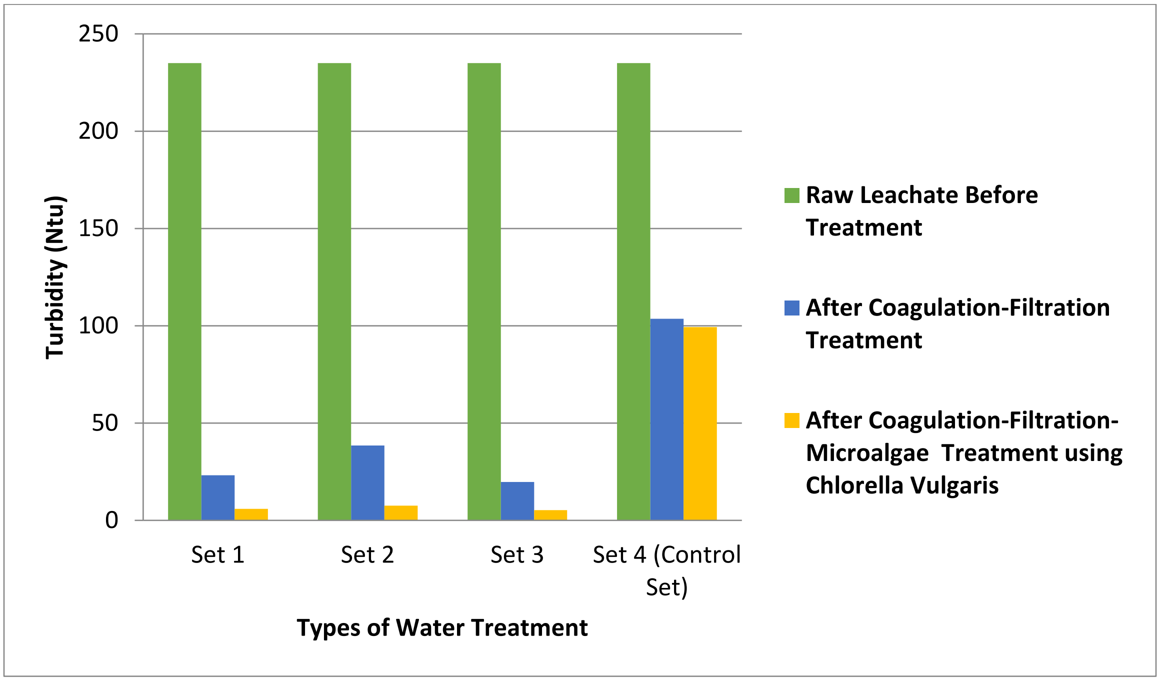


Figure 4.13 Turbidity versus Types of Treatment for Mature leachate using *Chlorella Vulgaris*.

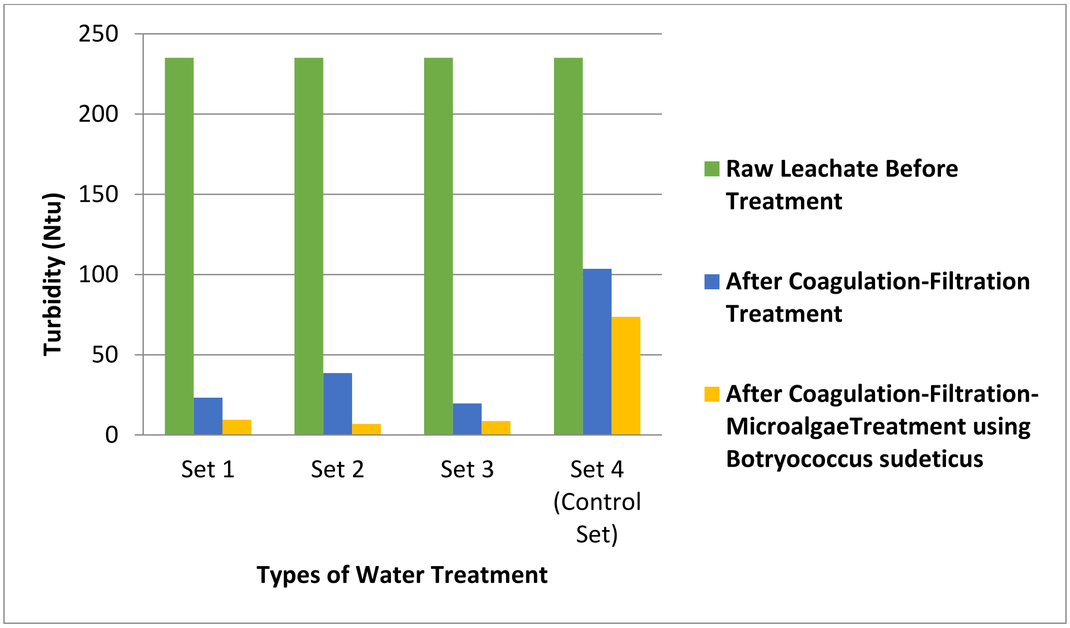


Figure 4.14 Turbidity versus Types of Treatment for Mature leachate using *Botryococcus sudeticus*.

4.4.6 pH

The pH versus types of water treatment graphs shows the pH for mature landfill leachate is slightly basic before the treatment. The pH dropped slightly after the coagulation-filtration treatment for leachate samples due to the coagulation process using aluminium ammonium sulphate 12-hydrate as a coagulant. The coagulant is a weakly acidic substance contributing the decrease in pH to neutralize the mature leachate samples (Mbaeze et al., 2015). The growth of microalgae will not be affected when the pH is in control (Edmunson and Wilkie, 2013). The pH after the coagulation-filtration-microalgae treatment remains near to pH 7 which means no further pH adjustment is needed.

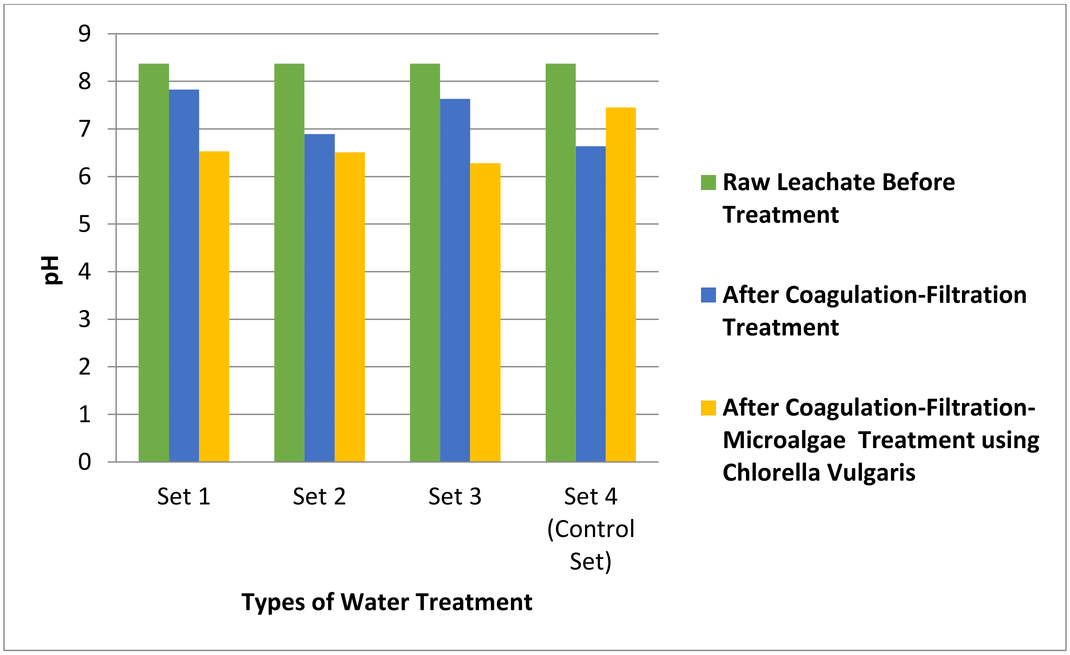


Figure 4.15 pH versus Types of Treatment for Mature leachate using *Chlorella Vulgaris.*

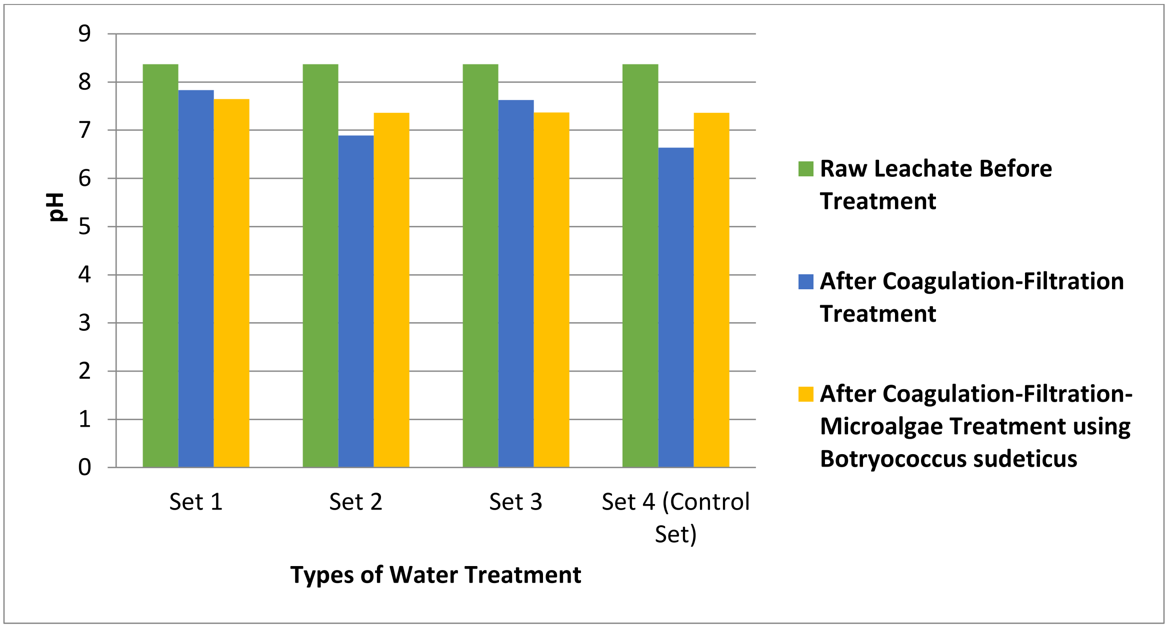


Figure 4.16 pH versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.7 Total Organic Carbon (TOC)

From Figure 4.17 and Figure 4.18, a significant drop is shown between before and after coagulation-filtration treatment while a slight decrease is shown after combined coagulation-filtration-microalgae treatment for mature leachate samples. The coagulation-filtration treatment shows the most effective filter set for leachate treatment is set 3 in which leachate has been treated up to 97% in total organic carbon reduction. After the combined coagulation-filtration-microalgae treatment, both Set 1 and 3 are effective using *chlorella vulgaris* and Set 1 using *botryococcus* *sudeticus*. On average, all modified filtration set ups were efficient in removing TOC with almost 99% of removal have been attained.

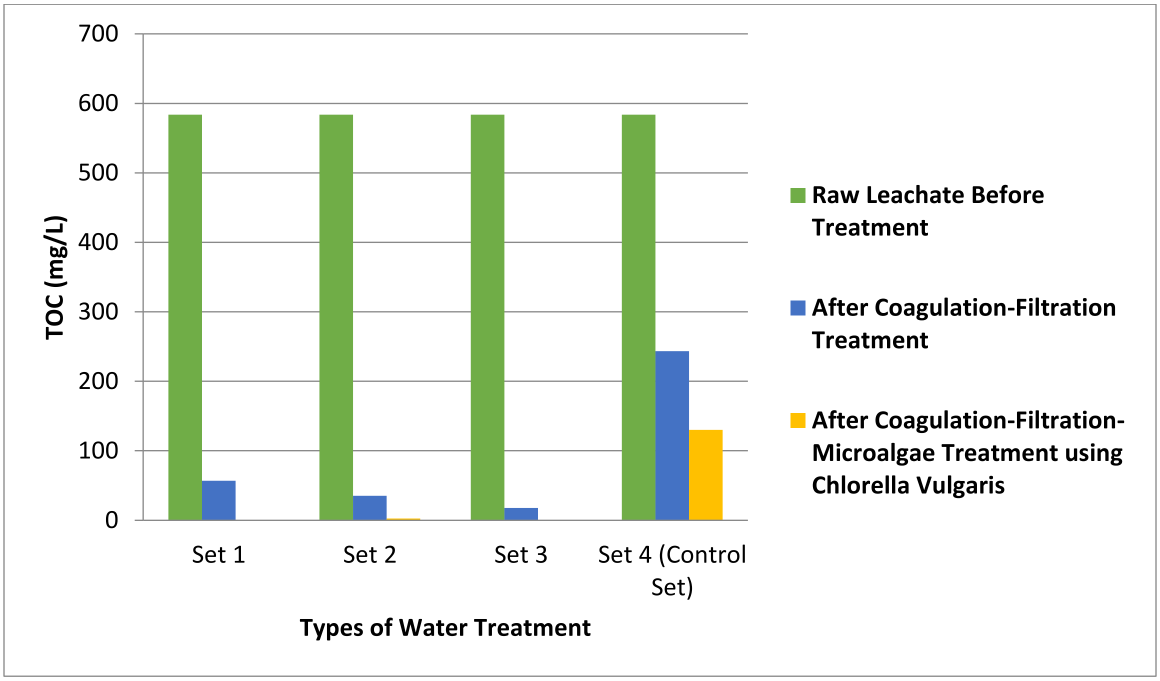


Figure 4.17 TOC versus Types of Treatment for Mature leachate using *Chlorella Vulgaris.*

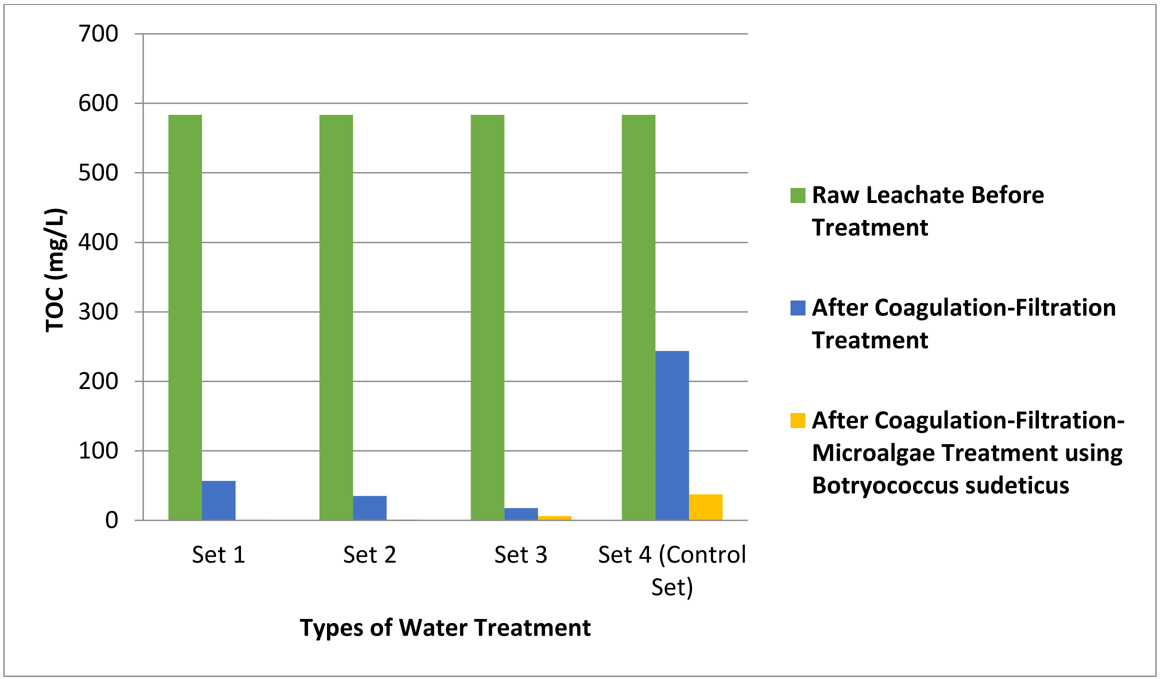


Figure 4.18 TOC versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.8 Chemical Oxygen Demand (COD)

Effectiveness is shown in the COD graphs (Figure 4.19 and Figure 4.20) comparing readings before and after the mature leachate treatment. About 90% of COD has been removed for sample after the coagulation-filtration treatment and about 95% of the COD has been removed further after the combined coagulation-filtration-microalgae treatment. It shows that all the modified filtration set up effectively removes the COD in mature leachate samples. The further reduction after the biological treatment shows that microalgae have ability to reduce the COD level in mature leachate.

The COD level reflects the organic substances in mature leachate samples. The COD test measures the amount of oxygen required for chemical oxidation of organic compounds in the sample to convert to carbon dioxide and water (Moreno et al., 2007). The complete oxidation of organic compounds under such strong oxidizing conditions produces carbon dioxide (CO_2) and water (H_2O). The equation 4.1 represents the oxidation process of Chemical Oxygen Demand (COD):

$\text{Organics} + Cr_2O_7^{2-} + H^+ \rightarrow CO_2 + H_2O + 2Cr^{3+}$ Eq (4.1) (Moreno et al., 2007)

From the graphs of COD using *Chlorella vulgaris* and *Botryococcus sudeticus,* a significant decline in Chemical Oxygen Demand (COD) has been observed after the coagulation-filtration treatment and slight decrease following the coagulation-filtration-microalgae treatment. For coagulation-filtration treatment, the most effective filter set for is set 3. The reading decreased from 2377.5 mg/L to 140.0 mg/L which is total of 94% reduction in COD. For combined coagulation-filtration-microalgae, all the 3 sets treated using *Chlorella vulgaris* and *Botryococcus sudeticus.* Set 2 is also noticeably most effective set up when treated using *Chlorella vulgaris.* There is a difference of 99% reduction in COD which reduced from 2377.5 mg/L to 34.7 mg/L. However, when using *Botryococcus sudeticus,* set 3 is identified as the most efficient set up. This is because the measurement declined from 2377.5 mg/L to 58.3 mg/L which is around 97% reduction in COD.

Overall, the COD results depict that the organic substances have been successfully removed by coagulation, filtration and microalgae treatment in mature leachate samples. The permissible discharge limit of Chemical Oxygen Demand (COD) set by Department of Environment under Environmental Quality Act is 400 mg/L and the values have reduced to the permissible level after coagulation-filtration treatment itself and eventually reduced further after biological treatment for all modified filtration set-ups.

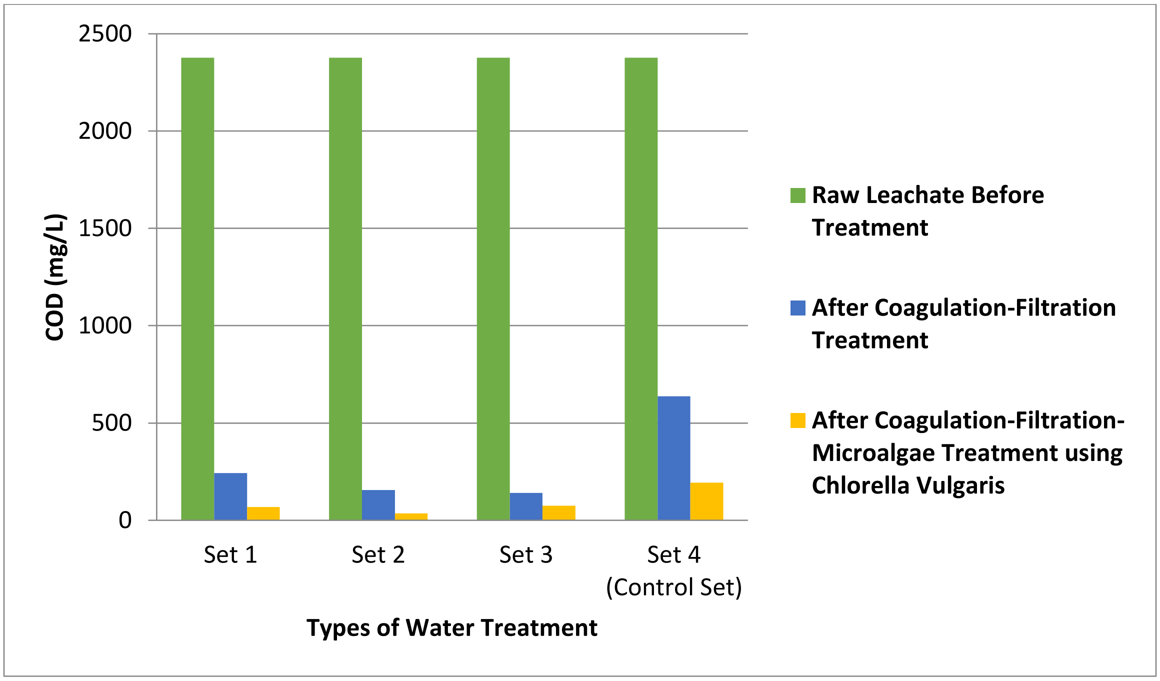


Figure 4.19 COD versus Types of Treatment for Mature leachate using *Chlorella Vulgaris.*

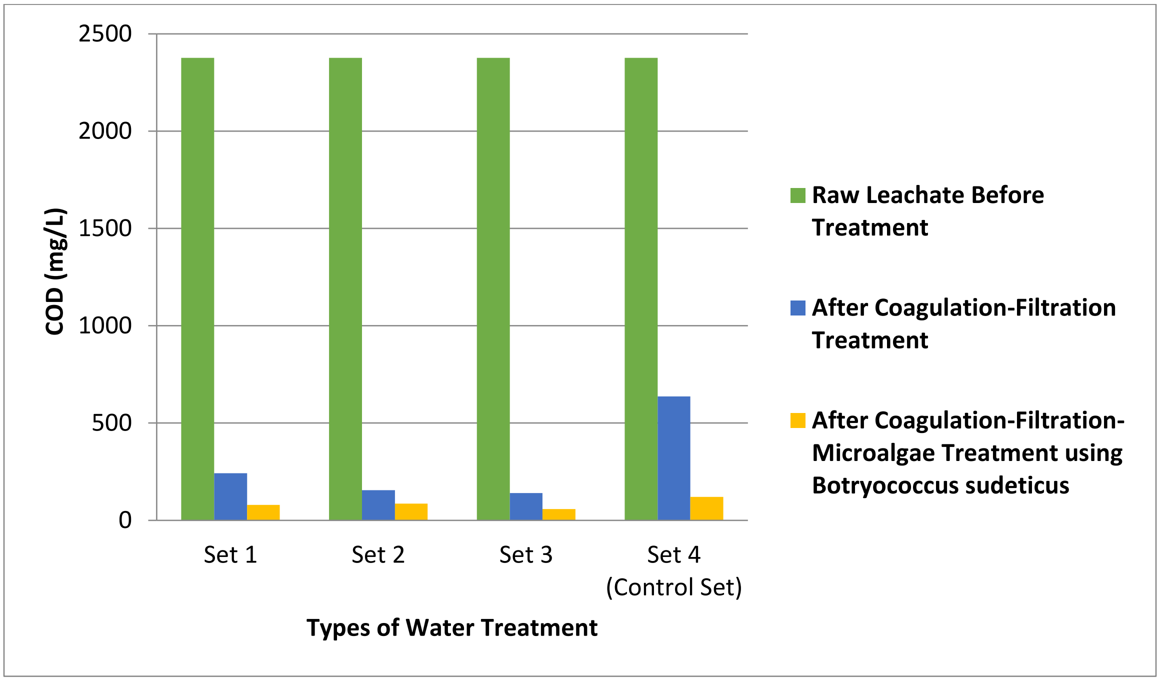


Figure 4.20 of COD versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.9 Total Ammonia Content (TAC)

From the graphs of TAC for mature leachate samples, a notable drop in Total Ammonia Content (TAC) has been identified after the coagulation-filtration treatment and a slight decrease following the coagulation-filtration-microalgae treatment has been noticed. The most effective filter set for landfill leachate by coagulation-filtration treatment is Set 2. This is due to big drop in reading with 394.37 mg/L before the treatment and 144.40 mg/L after the treatment which is around 63.4% of reduction in Total Ammonia Content.

After the combined coagulation-filtration-microalgae treatment, the most efficient filtration set-up for *Chlorella vulgaris* species to survive and further treat is Set 1 because the reading before treatment was 394.4 mg/L and after the treatment is 123.05 mg/L. This has around 68.9% reduction. Additionally, biological treatment using *Botryococcus sudeticus,* set 1 is noticeably the most effective filter set up. This is because after the treatment, the reading drops to 139.68 mg/L which is around 64.6% of reduction.

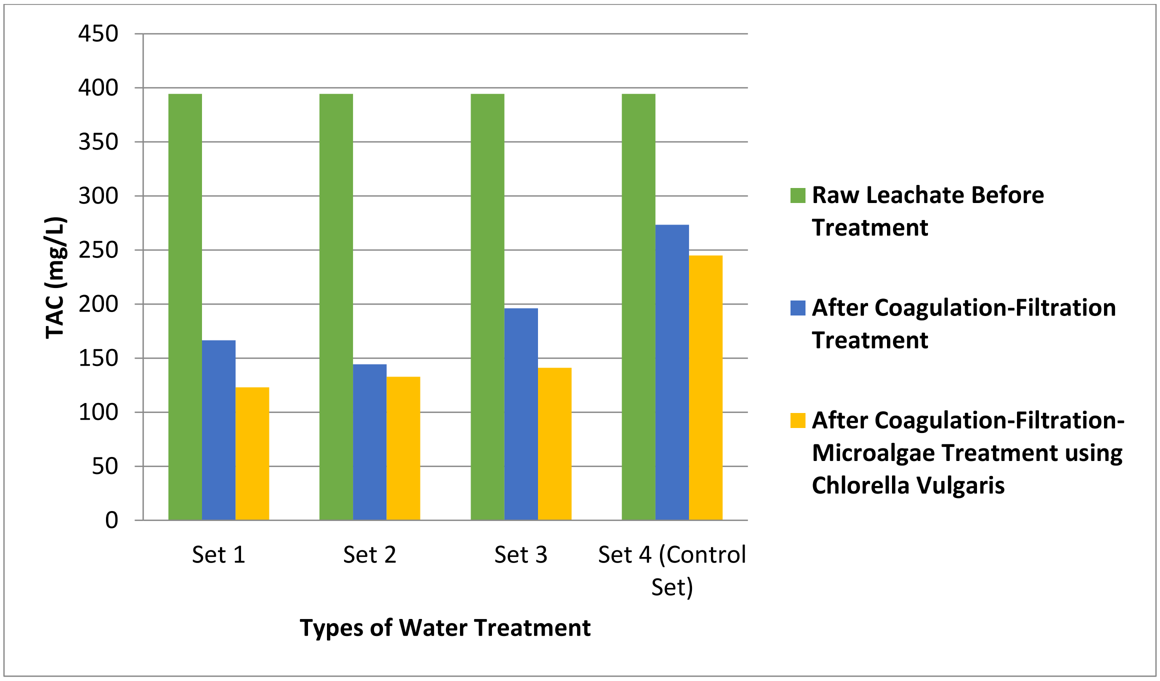


Figure 4.21 TAC versus Types of Treatment for Mature leachate using *Chlorella Vulgaris.*

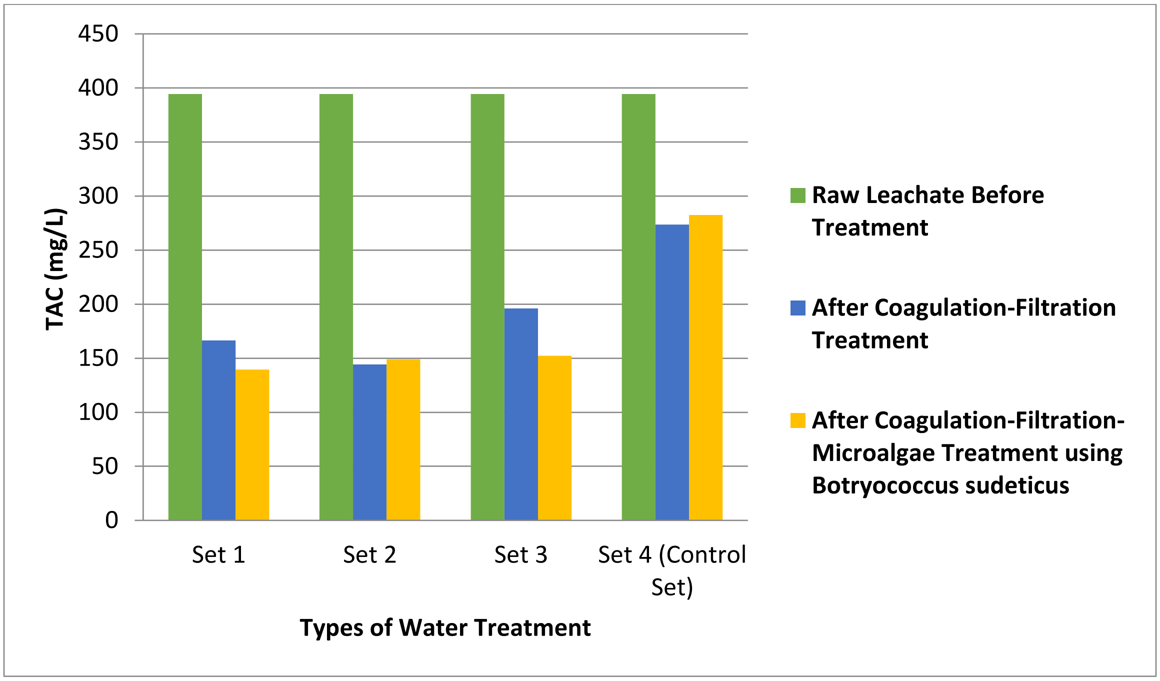


Figure 4.22 TAC versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.10 Biochemical Oxygen Demand (BOD)

From the graphs of BOD, a notable drop in Biochemical Oxygen Demand (BOD) has been identified after the coagulation-filtration treatment and a slight fluctuation following the coagulation-filtration-microalgae treatment has been noticed. Set 2 turn out to be the most effective filter set up for coagulation-filtration treatment. The reading was 428.5 mg/L before the treatment and 31.0 mg/L after the treatment which is around 92% of reduction in Biochemical Oxygen Demand.

For coagulation-filtration-microalgae using *Chlorella vulgaris* species, set 1 is noticed as most effective filter because the reading before treatment was 428.5 mg/L and after the treatment is 22.5 mg/L. This has around 94% reduction. For coagulation-filtration-microalgae treatment using *Botryococcus sudeticus*, set 3 is noticeably the most effective filter set up. This is because after the treatment, the reading drops to 47.0 mg/L which is around 89% of reduction.

According to Environmental Quality Act 1974, the acceptable discharge limit of leachate for BOD is 20 mg/L. The coagulation-filtration-microalgae treatment with *Chlorella vulgaris* using Set 3 filtration set-up managed to achieve up to 22.5 mg/L with Set 1 which is very near to the permissible limit discharge standard by Department of Environment (DOE).

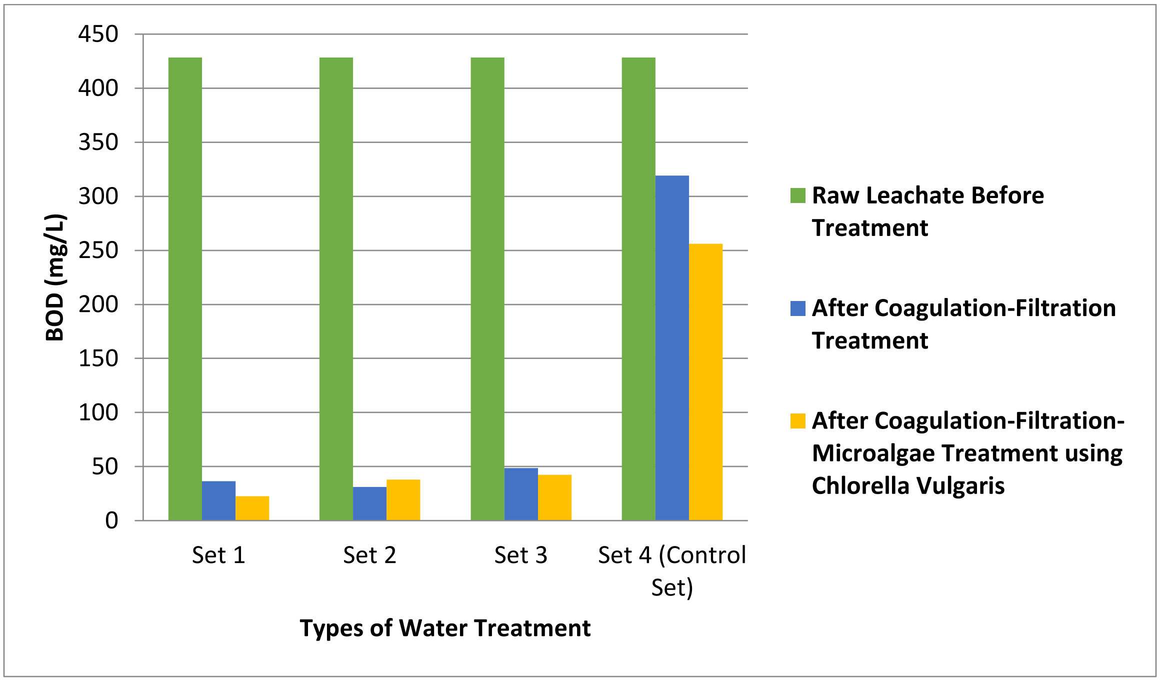


Figure 4.23 BOD versus Types of Treatment for Mature leachate using *Chlorella Vulgaris*.

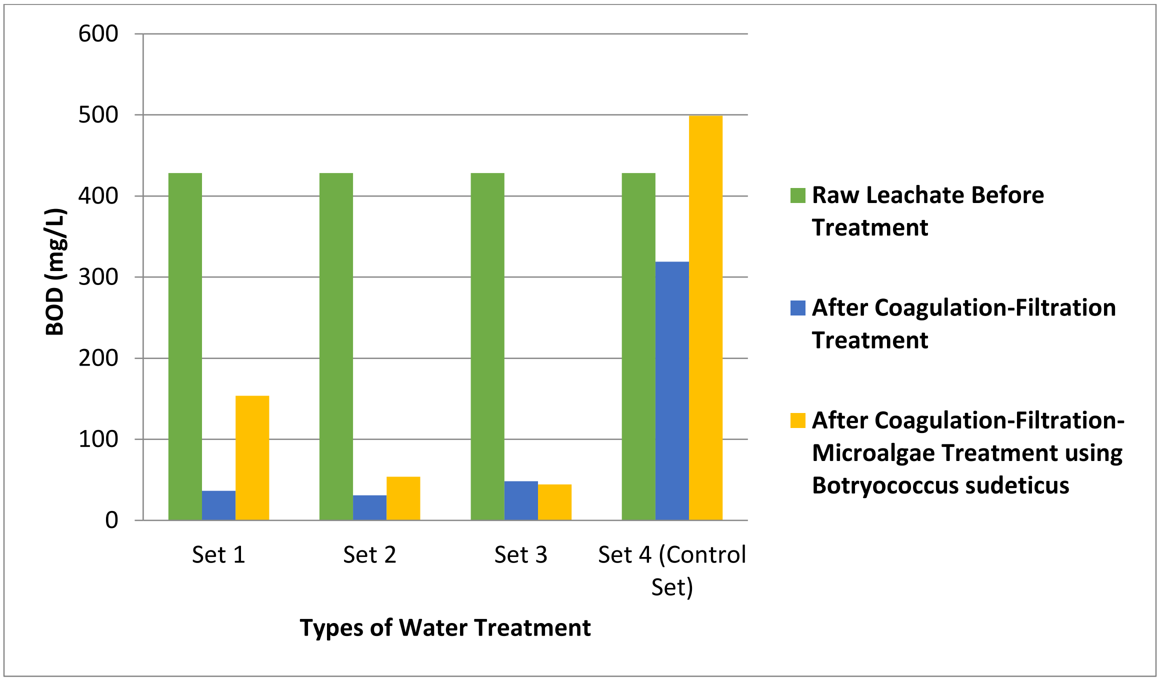


Figure 4.24 BOD versus Types of Treatment for Mature leachate using *Botryococcus sudeticus.*

4.4.11 Heavy Metal Content using ICP-MS

Figure 4.25 to Figure 4.27 shows the types and concentrations of heavy metals present in mature leachate before as well as after the treatments. The mature leachate samples were analyzed using ICP-MS instrument to obtain the heavy metal contents present. The adsorption of heavy metals namely Sodium (Na), Magnesium (Mg), Aluminium (Al), Potassium (K), Calcium (Ca), Iron (Fe), Manganese (Mn), Zinc and Copper (Cu) were observed. The values were analyzed and depicted using bar graphs as shown in Figure 4.25 to Figure 4.27 and the metal adsorption after the coagulation-filtration treatment and combined coagulation-filtration-microalgae treatment can be clearly seen.

The removal efficiencies by modified filtration set-ups, microalgae treatment using *Chlorella Vulgaris* and microalgae treatment *Botryococcus sudeticus* have been identified from the graphs. The filtration systems using activated carbon have been effective as a metal adsorbent (Wasay et al., 2009). Subsequent microalgae treatment using *Chlorella Vulgaris* and *Botryococcus sudeticus* microalgae species removed the heavy metals further up to 98%. Microalgae has potential to adsorb the metal contents and use up as a nutrient source for the growth (Richards and Mullins, 2013).

Besides that, the major heavy metal contents such as Iron (Fe), Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Manganese (Mn), Zinc and Copper (Cu) are the essential nutrients for the microalgae to grow referring to Table 2.5 which could be found in Bold's Basal Medium (Edmunson and Wilkie, 2013). Growth has been observed in Set 1 to Set 3, pre-treated leachate using all modified filtration set-ups, for mature leachate sample using *Chlorella Vulgaris* while *Botryococcus sudeticus* managed to grow in pre-treated coagulation-filtration using Set 2 and Set 3 mature leachate samples.

Higher metal removal efficiencies were attained in the pre-treated leachate samples after biological treatment with microalgae growth. The leachate effluent without the growth of *Chlorella Vulgaris* and *Botryococcus sudeticus* have lower metal removal efficiencies. No growth has been observed in leachate samples treated using control set-up filtration system. The metal uptake still has taken place in pre-treated leachate using control set but the removal efficiencies were lower compared to modified-filtration set-ups.

Among the four filtration set-ups, Set 3 filtration system has removed 57% of Na content in mature leachate samples after coagulation-filtration treatment. The subsequent treatment using *Chlorella Vulgaris* shows the metal uptake up to 96% of Na in mature leachate sample using Set 3 filtration system too. However, mature leachate in Set 1 and Set 2 filtration system also shows Na uptake by *Chlorella Vulgaris* up to 92%. *Botryococcus sudeticus* could only grow in pre-treated mature leachate using Set 2 and Set 3 filtration system for and the Na metal removal efficiency was up to 98% after combined coagulation-filtration-microalgae treatment.

The Mg removal have been effectively achieved by using Set 2 filtration set-up for mature leachate samples after coagulation-filtration treatment. Set 2 filtration set-up achieved 90% removal of Mg concentration from the initial of 112.185 ppb to 24.014 ppb using *Chlorella Vulgaris.* About 90% of Mg removal achieved in Set 2 and Set 3 filtration system for mature leachate using *Botryococcus sudeticus.* Around 70% and 95% of Al and K have been removed by Set 2 for mature leachate after coagulation-filtration treatment. The Al and K uptake were more than 90% in ML1, ML2 and ML3 using *Chlorella Vulgaris*. 98% of Al and K have been effectively removed by ML2 and ML3 using *Botryococcus sudeticus* after combined coagulation-filtration-microalgae treatment.

Ca has been effectively adsorbed during combined coagulation-filtration-microalgae treatment using *Chlorella Vulgaris* with Set 1 filtration system. *Botryococcus sudeticus* has removed more than 95% of Ca, Fe, Mn, Zn and Cu in pre-treated mature leachate sample using Set 2 and 3. Physical treatment has only removed Fe, Zn and Cu up to 25% on average using modified filtration set-ups. *Chlorella Vulgaris* used 88% of Fe for its growth in pre-treated mature leachate using Set 1 filtration system. Even though the initial concentration of Mn is lower compared to the other heavy metals in mature leachate sample before the treatment, the combined coagulation-filtration with modified filtration system has removed up to 70% of Mn and the subsequent treatment with *Chlorella vulgaris* effectively removed 85% of Mn in mature leachate using Set 3 filtration set-up.

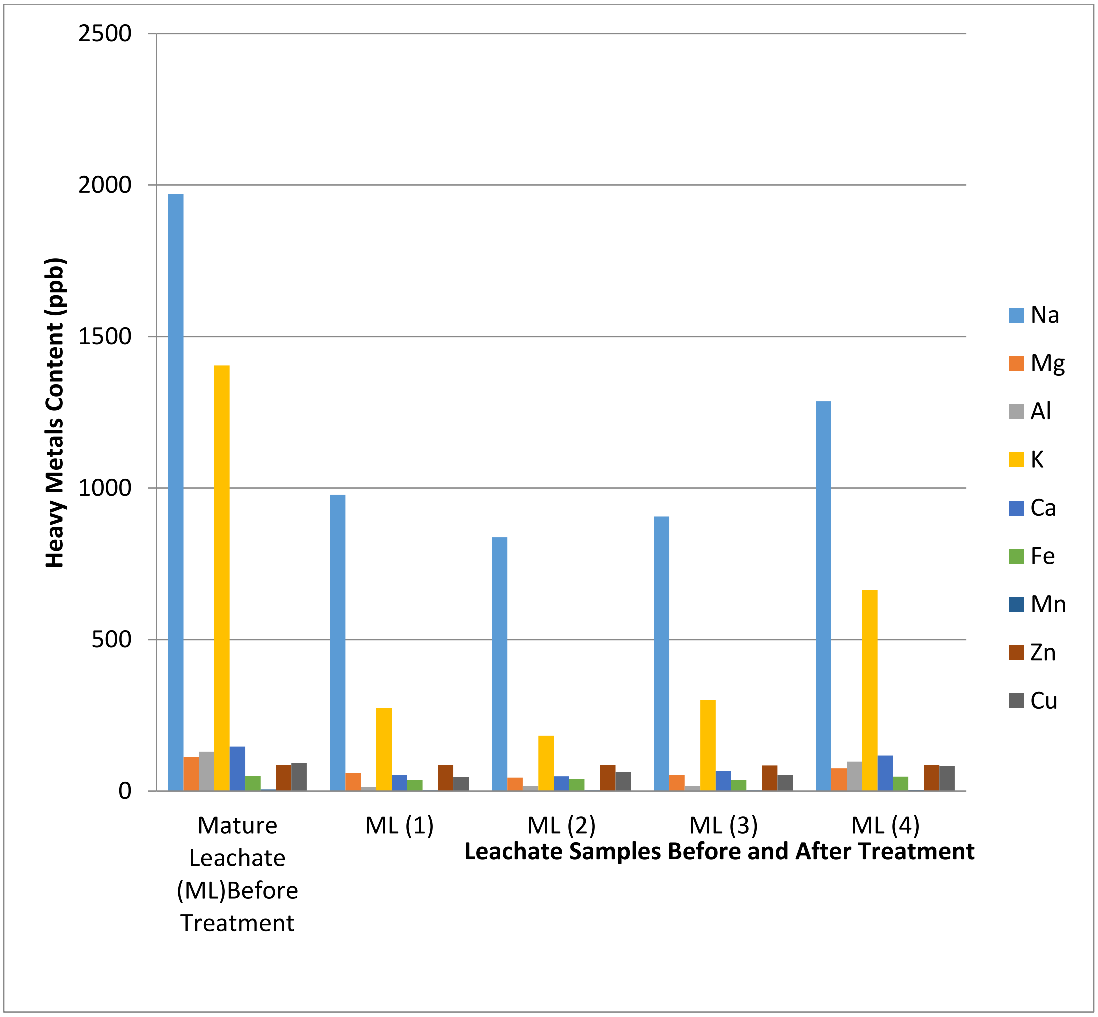


Figure 4.25 Heavy Metal Contents versus Mature Leachate samples Before and After Coagulation-Filtration Treatment

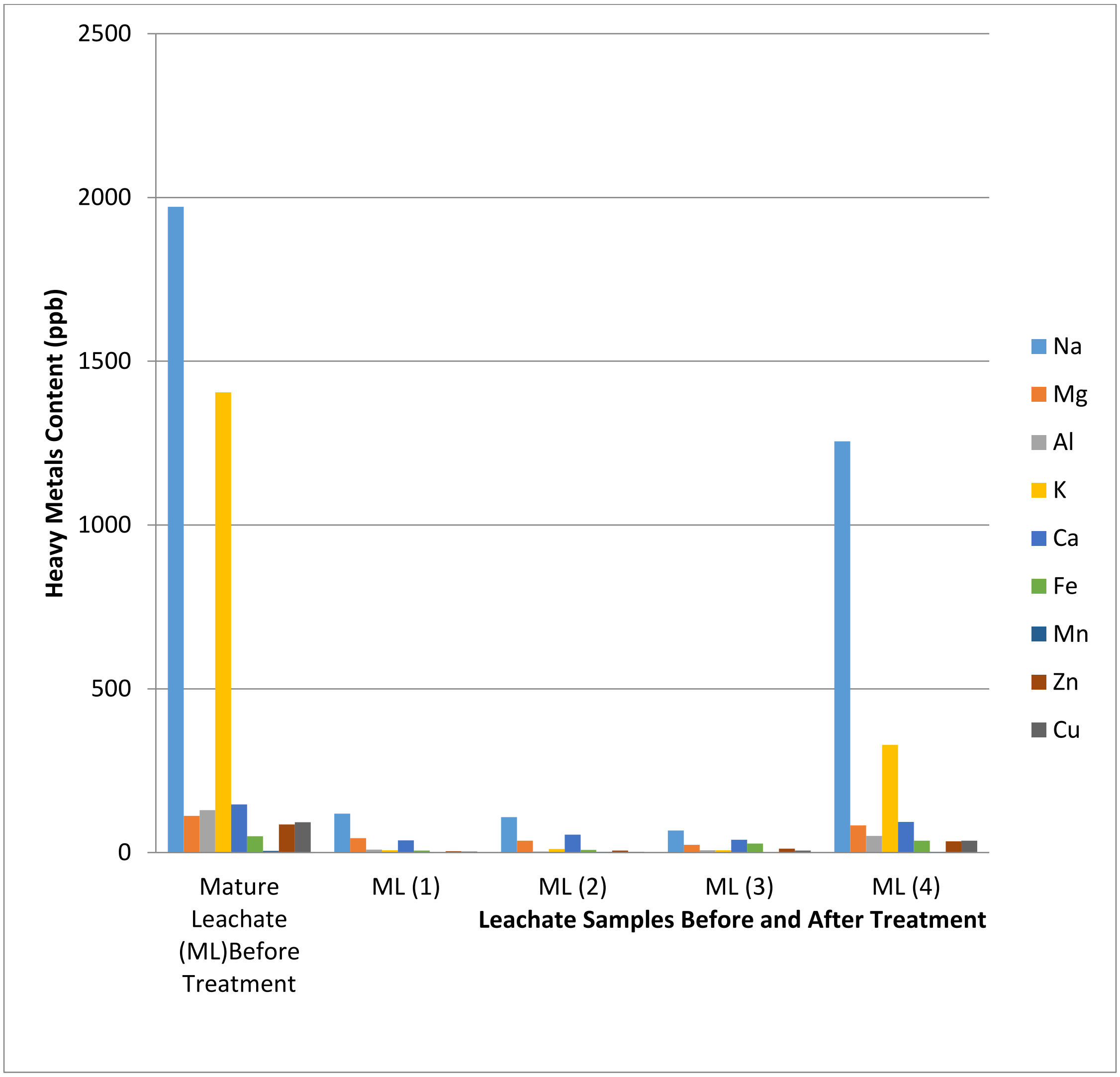


Figure 4.26 Heavy Metal Contents versus Mature Leachate samples Before and After Coagulation-Filtration-Microalgae Treatment using *Chlorella Vulgaris*.

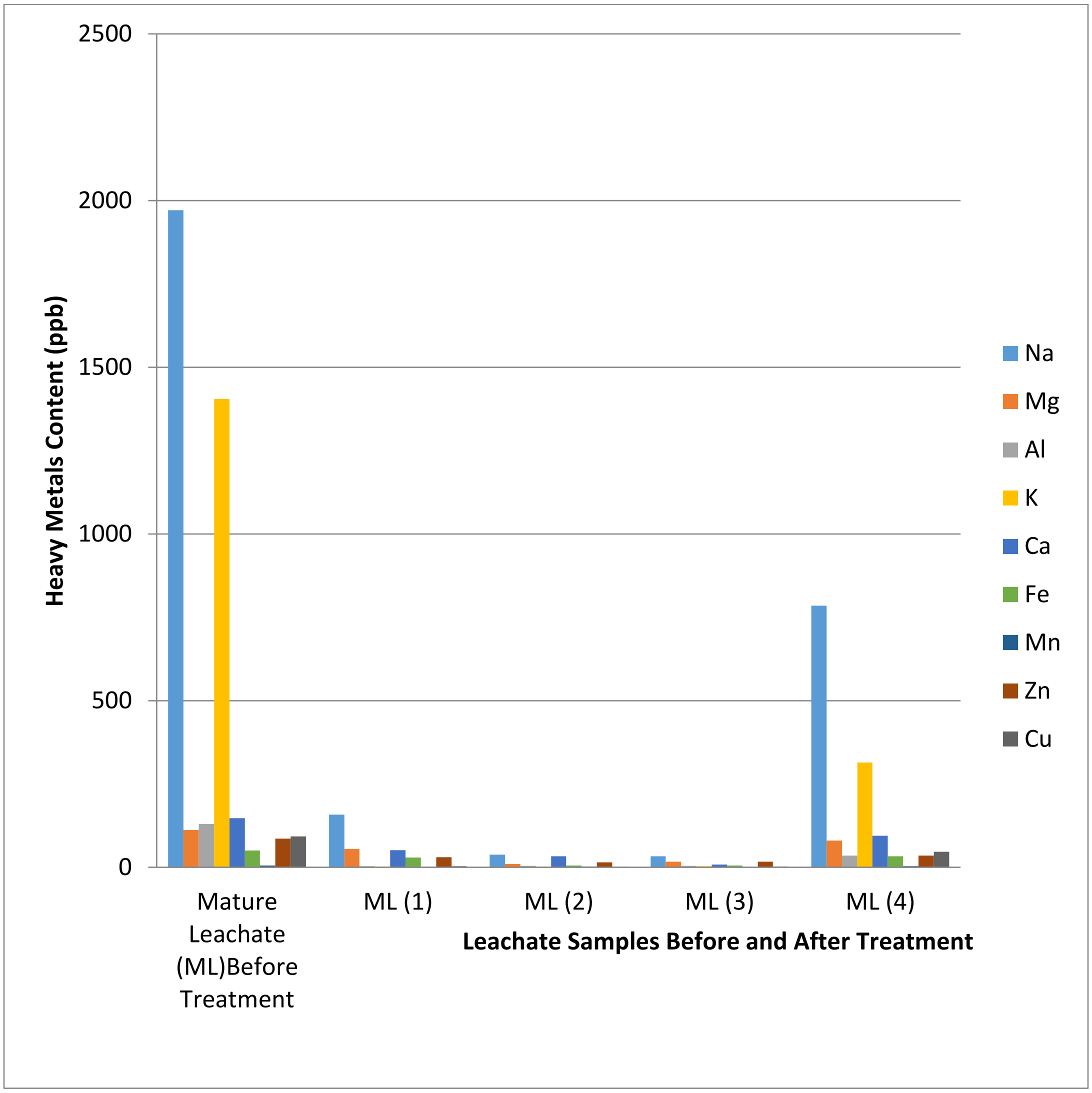


Figure 4.27 Heavy Metal Contents versus Mature Leachate samples Before and After Coagulation-Filtration-Microalgae Treatment using *Botryococcus sudeticus.*

4.4.12 Summary of Water Analysis

Table 4.3 and 4.4 show the summary of water analysis with percentage of uptake before treatment, after coagulation-filtration and after combined coagulation-filtration-microalgae treatment using *Chlorella vulgaris* and *Botryococcus sudeticus*. The overall results were tabulated with standard deviation errors and compared the values standard discharge limit for leachate by Department of Environment. The COD and pH results achieved the standard discharge limits by Department of Environment.

Table 4.5 shows effective filtration set up with percentage of uptake after combined coagulation-filtration-microalgae treatment using *Chlorella vulgaris* and *Botryococcus sudeticus.* Higher removal percentage of contaminants treated using particular filtration set ups were tabulated and identified the effective filtration set up on average. Set 1 filtration system was the most effective set up for *Chlorella vulgaris* while Set 3 for *Botryococcus sudeticus.*

Table 4.3 Summary of water analysis with percentage of uptake before treatment, after coagulation-filtration and after combined coagulation-filtration-microalgae treatment using *Chlorella vulgaris*.

Water Analysis	Water Analysis Before and After Treatment with Percentage of Uptake									Standard Discharge Limit for Leachate by Department of Environment [c]
	Before Treatment	Set 1[b]		Set 2		Set 3		Set 4		
		Coagulation-filtration Treatment [a]	Combined coagulation-filtration-microalgae Treatment using Chlorell	Coagulation-filtration Treatment	Combined coagulation-filtration-microalgae Treatment using Chlorell	Coagulation-filtration Treatment	Combined coagulation-filtration-microalgae Treatment using Chlorell	Coagulation-filtration Treatment	Combined coagulation-filtration-microalgae Treatment using Chlorell	

			a vulgaris		a vulgaris		a vulgaris		a vulgaris	
Total Solids (mg/L)	**8526 ± 510**	**2200 ± 668 [d] (75%) [e]**	**1989 ± 760 (76%)**	**2416 ± 694 (99%)**	**2007 ± 278 (76%)**	**2250 ± 361 (73%)**	**2397 ± 384 (71%)**	**4550 ± 758 (47%)**	**2888 ± 632 (66%)**	**-**
Conductivity (mS/cm)	**17.61 ± 0.63**	**9.24 ± 2.20 (47%)**	**4.04 ± 2.13 (77%)**	**10.13 ± 4.05 (42%)**	**5.74 ± 0.30 (67%)**	**10.59 ± 4.49 (39%)**	**5.45 ± 0.69 (69%)**	**12.32 ± 5.45 (30%)**	**7.62 ± 1.83 (56%)**	**-**
Turbidity (NTU)	**235.05 ± 48.28**	**23.15 ± 2.26 (90%)**	**5.95 ± 0.50 (97%)**	**38.50 ± 2.72 (83%)**	**7.55 ± 1.86 (96%)**	**19.72 ± 2.62 (91%)**	**5.30 ± 1.56 (97%)**	**103.52 ± 11.21 (55%)**	**99.37 ± 17.37 (57%)**	**-**
Total Organic Carbon (mg/L)	**583.69 ± 510.00**	**56.80 ± 4.63 (90%)**	**0 ± 0 (100%)**	**35.40 ± 18.12 (94%)**	**2.41 ± 3.40 (99%)**	**17.61 ± 7.54 (97%)**	**0 ± 0 (100%)**	**243.57 ± 196.22 (58%)**	**130.13 ± 164.82 (78%)**	**-**
Chemical Oxygen Demand (mg/L)	**2377.5 ± 759.9**	**242.5± 170.5 (90%)**	**69.0 ± 1.8 (97%)**	**155.0 ± 20.8 (93%)**	**34.7± 4.4 (99%)**	**140.0 ± 70.2 (94%)**	**74.7 ± 3.40 (97%)**	**637.5 ± 160.2 (73%)**	**193.5 ± 105.8 (92%)**	**400**

Total Ammonia Content (mg/L)	**394.37 ± 43.07**	**166.45 ± 15.03 (58%)**	**123.05 ± 21.31 (69%)**	**144.40 ± 15.00 (63%)**	**132.9 ± 41.97 (66%)**	**196.07 ± 13.47 (50%)**	**141.07 ± 36.13 (64%)**	**273.52 ± 45.90 (30%)**	**244.9 ± 28.54 (37%)**	**5**
Biochemical Oxygen Demand (mg/L)	**428.5 ± 424.9**	**36.5 ± 13.4 (91%)**	**22.5 ± 0.7 (94%)**	**31.0 ± 0 (93%)**	**38.0 ± 24.0 (91%)**	**48.5 ± 4.9 (89%)**	**42.5 ± 17.6 (90%)**	**319.0 ± 373.3 (25%)**	**256.0 ± 110.3 (40%)**	**20**
pH	**8.37 ± 0.04**	**7.83 ± 0.19**	**6.53 ± 0.42**	**6.89 ± 0.21**	**6.51 ± 0.44**	**7.63 ± 0.16**	**6.28 ± 0.15**	**6.64 ± 0.30**	**7.45 ± 0.68**	**6.0-9.0**

[a] Coagulation-filtration treatment is the sequential treatment of coagulation and filtration.

[b] Set stands for filtration system used in the present study.

[c] Department of Environment of Malaysia.

[d] Error limits are standard deviations.

[e] Value in parenthesis represents the percentage of removal compared to raw leachate sample before treatment.

Table 4.4 Summary of water analysis with percentage of uptake before treatment, after coagulation-filtration and after combined coagulation-filtration-microalgae treatment using *Botryococcus sudeticus*.

Water Analysis	Water Analysis Before and After Treatment with Percentage of Uptake									Standard Discharge Limit for Leachate by Department of Environment [c]
	Before Treatment	Set 1[b]		Set 2		Set 3		Set 4		
		Coagulation-filtration Treatment [a]	Combined coagulation-filtration-microalgae Treatment using *Botryococcus sudeticus*	Coagulation-filtration Treatment	Combined coagulation-filtration-microalgae Treatment using *Botryococcus sudeticus*	Coagulation-filtration Treatment	Combined coagulation-filtration-microalgae Treatment using *Botryococcus sudeticus*	Coagulation-filtration Treatment	Combined coagulation-filtration-microalgae Treatment using *Botryococcus sudeticus*	

Total Solids (mg/L)	**8526 ± 510**	**2200 ± 668** [d] **(75%)** [e]	**2146 ± 317** **(74%)**	**2416 ± 694** **(99%)**	**2247 ± 513** **(74%)**	**2250 ± 361** **(73%)**	**1843 ± 259** **(79%)**	**4550 ± 758** **(47%)**	**2496 ± 508** **(71%)**	**-**
Conductivity (mS/cm)	**17.61 ± 0.63**	**9.24 ± 2.20** **(47%)**	**4.04 ± 2.13** **(77%)**	**10.13 ± 4.05** **(42%)**	**5.74 ± 0.30** **(67%)**	**10.59 ± 4.49** **(39%)**	**5.45 ± 0.69** **(69%)**	**12.32 ± 5.45** **(30%)**	**7.62 ± 1.83** **(56%)**	**-**
Turbidity (NTU)	**235.05 ± 48.28**	**23.15 ± 2.26** **(90%)**	**5.95 ± 0.50** **(97%)**	**38.50 ± 2.72** **(83%)**	**7.55 ± 1.86** **(96%)**	**19.72 ± 2.62** **(91%)**	**5.30 ± 1.56** **(97%)**	**103.52 ± 11.21** **(55%)**	**99.37 ± 17.37** **(57%)**	**-**
Total Organic Carbon (mg/L)	**583.69 ± 510.00**	**56.80 ± 4.63** **(90%)**	**0 ± 0** **(100%)**	**35.40 ± 18.12** **(94%)**	**2.41 ± 3.40** **(99%)**	**17.61 ± 7.54** **(97%)**	**0 ± 0** **(100%)**	**243.57 ± 196.22** **(58%)**	**130.13 ± 164.82** **(78%)**	**-**
Chemical Oxygen Demand (mg/L)	**2377.5 ± 759.9**	**242.5± 170.5** **(90%)**	**69.0 ± 1.8** **(97%)**	**155.0 ± 20.8** **(93%)**	**34.7± 4.4** **(99%)**	**140.0 ± 70.2** **(94%)**	**74.7 ± 3.40** **(97%)**	**637.5 ± 160.2** **(73%)**	**193.5 ± 105.8** **(92%)**	**400**
Total Ammonia	**394.37 ± 43.07**	**166.45 ± 15.03**	**123.05 ± 21.31**	**144.40 ± 15.00**	**132.9 ± 41.97**	**196.07 ± 13.47** **(50%)**	**141.07 ± 36.13** **(64%)**	**273.52 ± 45.90** **(30%)**	**244.9 ± 28.54** **(37%)**	**5**

Content (mg/L)		**(58%)**	**(69%)**	**(63%)**	**(66%)**					
Biochemical Oxygen Demand (mg/L)	**428.5 ± 424.9**	**36.5 ± 13.4** **(91%)**	**22.5 ± 0.7** **(94%)**	**31.0 ± 0** **(93%)**	**38.0 ± 24.0** **(91%)**	**48.5 ± 4.9 (89%)**	**42.5 ± 17.6 (90%)**	**319.0 ± 373.3 (25%)**	**256.0 ± 110.3 (40%)**	**20**
pH	**8.37 ± 0.04**	**7.83 ± 0.19**	**6.53 ± 0.42**	**6.89 ± 0.21**	**6.51 ± 0.44**	**7.63 ± 0.16**	**6.28 ± 0.15**	**6.64 ± 0.30**	**7.45 ± 0.68**	**6.0-9.0**

[a] Coagulation-filtration treatment is the sequential treatment of coagulation and filtration.

[b] Set stands for filtration system used in the present study.

[c] Department of Environment of Malaysia.

[d] Error limits are standard deviations.

[e] Value in parenthesis represents the percentage of removal compared to raw leachate sample before treatment.

Table 4.5 Effective Filtration Set with percentage of uptake after combined coagulation-filtration-microalgae treatment using *Chlorella vulgaris* and *Botryococcus sudeticus.*

Water Analysis	**Effective Filtration Set with percentage of uptake**	
	Chlorella Vulgaris	***Botryococcus sudeticus***
Total Solids	**1 (76%)**	**3 (79%)**
Total Suspended Solids	**3 (99%)**	**3 (99%)**
Total Dissolved Solids	**2 (75%)**	**2 (80%)**
Conductivity	**1 (80%)**	**1 (80%)**
Turbidity	**3 (98%)**	**2 (97%)**
Total Organic Carbon	**1 (99%)**	**1 (99%)**
Chemical Oxygen Demand	**2 (98%)**	**3 (98%)**
Total Ammonia Content	**1 (60%)**	**1 (60%)**
Biochemical Oxygen Demand	**1 (94%)**	**3 (89%)**

Effective Filtration Set on Average	**1**	**3**

4.5 Lipid Contents

The lipid content was extracted in hexane and analysed using Liquid Chromatography Mass Spectrometry (LC-MS) from both *Chlorella vulgaris* and *Botryococcus sudeticus* microalgae species grown in pre-treated mature leachate (ML) samples separately. The possible hydrocarbon compounds present in *Chlorella vulgaris* and *Botryococcus sudeticus* were tabulated in Table 4.6 and summarized the carbon ranges in Table 4.7. The lipids with broad carbon range of (C-14 to C-36) were identified in *Chlorella vulgaris* (ML1), *Chlorella vulgaris* (ML2), *Chlorella vulgaris* (ML3), *Botryococcus sudeticus* (ML2) and *Botryococcus sudeticus* (ML3).

Based on the research, the compounds such as ketones, fatty acid methyl ester (FAME) and fatty acids were found in the oil extracted from *Chlorella vulgaris* and *Botryococcus sudeticus* grown in pre-treated mature leachate. The most interesting part is the presence of naturally occurring fatty acid methyl ester in both *Chlorella vulgaris* and *Botryococcus sudeticus*. Fatty acid methyl esters (biodiesel) is considered a very attractive, renewable and non-toxic fuel (Herrera et al., 2011), which can be used with existing technology for diesel consumption. Fatty acid methyl esters (biodiesel) are often produced by esterification of free fatty acids with methanol in the presence of strong acids (Herrera et al., 2011).

Five possible FAMEs have been identified in *Chlorella Vulgaris* and 3 possible FAMEs in *Botryococcus sudeticus.* Interestingly, FAMEs can be recovered naturally from the plant cells which has been studied previously for Jatropha curcas by Annarao et al. in year 2008. The lipid contents in developing seeds of Jatropha curcas revealed the existence of FAMEs in hexane extracts of very young seeds (Annarao et al., 2008). In the case of microalgae, the existence of naturally occurring FAMEs have been reported previously in the freshwater grown green microalgae *Eudorina unicocca* and *Volvox aureus* species by Zhang et al. in year 2009. The current results revealed the natural occurrence of FAMEs in microalgae at the end of the growth phase of *Chlorella vulgaris* and *Botryococcus sudeticus* cultures. These new insights will pave the way for future research in these microalgae species regarding the biosynthesis of FAMEs, and their potential use as biofuels.

Table 4.6 Possible Compounds (hydrocarbons) present in *Chlorella vulgaris* and *Botryococcus sudeticus*.

Microalgae grown in pre-treated mature leachate (ML) samples	Possible Compounds (hydrocarbons) present in microalgae	Molecular Weight of Compounds (g/mol)	Number of Carbons with Functional Group
Chlorella Vulgaris (ML1)	Methyl eicosa-5,8,11,14,17-pentaenoate- $C_{21}H_{32}O_2$	316	C-21 (Fatty Acid Methyl Ester-FAME)
	Heneicosanoic acid, 18-propyl-, methyl ester- $C_{25}H_{50}O_2$	382	C-25 (Fatty Acid Methyl Ester-FAME)
	5,9-Pentacosadienoic acid, methyl ester- $C_{26}H_{48}O_2$	392	C-26 (Fatty Acid Methyl Ester-FAME)
	Z,Z-6,26-Pentatriacontadien-2-one- $C_{35}H_{66}O$	502	C-35 (Ketone)
	Tetratriacontanedioic acid, dimethyl ester- $C_{36}H_{70}O_4$	566	C-36 (Fatty Acid Methyl Ester-FAME)
Chlorella Vulgaris (ML2)	Butanedioic acid, hydroxy-, bis[1-methylbutyl] ester- $C_{14}H_{26}O_5$	274	C-14 (Fatty Acid Methyl Ester-FAME)
	Methyl eicosa-5,8,11,14,17-pentaenoate- $C_{21}H_{32}O_2$	316	C-21 (Fatty Acid Methyl Ester-FAME)
	Heneicosanoic acid, 18-propyl-, methyl ester- $C_{25}H_{50}O_2$	382	C-25 (Fatty Acid Methyl Ester-FAME)
	5,9-Pentacosadienoic acid, methyl ester- $C_{26}H_{48}O_2$	392	C-26 (Fatty Acid Methyl Ester-FAME)
	Z,Z-6,26-Pentatriacontadien-2-one- $C_{35}H_{66}O$	502	C-35 (Ketone)
	Tetratriacontanedioic acid, dimethyl ester- $C_{36}H_{70}O_4$	566	C-36 (Fatty Acid Methyl

			Ester-FAME)
Chlorella Vulgaris (ML3)	Methyl eicosa-5,8,11,14,17-pentaenoate- $C_{21}H_{32}O_2$	316	C-21 (Fatty Acid Methyl Ester-FAME)
	Heneicosanoic acid, 18-propyl-, methyl ester- $C_{25}H_{50}O_2$	382	C-25 (Fatty Acid Methyl Ester-FAME)
	5,9-Pentacosadienoic acid, methyl ester- $C_{26}H_{48}O_2$	392	C-26 (Fatty Acid Methyl Ester-FAME)
	Z,Z-6,26-Pentatriacontadien-2-one- $C_{35}H_{66}O$	502	C-35 (Ketone)
	Tetratriacontanedioic acid, dimethyl ester- $C_{36}H_{70}O_4$	566	C-36 (Fatty Acid Methyl Ester-FAME)
Botryococcus sudeticus (ML2)	Octanedioic acid, 2,2,7,7-tetramethyl- $C_{12}H_{22}O_4$	230	C-12 (Fatty Acis)
	Methyl eicosa-5,8,11,14,17-pentaenoate- $C_{21}H_{32}O_2$	316	C-21 (Fatty Acid Methyl Ester-FAME)
	Heneicosanoic acid, 18-propyl-, methyl ester- $C_{25}H_{50}O_2$	382	C-25 (Fatty Acid Methyl Ester-FAME)
	5,9-Pentacosadienoic acid, methyl ester- $C_{26}H_{48}O_2$	392	C-26 (Fatty Acid Methyl Ester-FAME)
	Tetratriacontanedioic acid, dimethyl ester- $C_{36}H_{70}O_4$	566	C-35 (Ketone)
Botryococcus sudeticus (ML3)	Methyl eicosa-5,8,11,14,17-pentaenoate- $C_{21}H_{32}O_2$	316	C-21 (Fatty Acid Methyl Ester-FAME)
	5,9-Pentacosadienoic acid, methyl ester- $C_{26}H_{48}O_2$	392	C-26 (Fatty Acid Methyl Ester-FAME)

Table 4.7 Summary Table of Hydrocarbons Compositions Carbon range

Microalgae grown in pre-treated mature leachate (ML) samples	**Hydrocarbons Compositions Carbon range**
Chlorella vulgaris (ML1)	**(C-21 to C-36)**
Chlorella vulgaris (ML2)	**(C-14 to C-36)**
Chlorella vulgaris (ML3)	**(C-21 to C-36)**
Botryococcus sudeticus (ML2)	**(C-12 to C-36)**
Botryococcus sudeticus (ML3)	**(C-21 to C-28)**

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

The water quality analysis of the mature leachate before treatment, after coagulation-filtration treatment and after coagulation-filtration-microalgae treatment were compared. The combined coagulation-filtration-microalgae treatments achieved removal efficiencies of more than 90% of total solids, total suspended solids, total dissolved solids, turbidity, conductivity, COD, BOD_5, total ammonia content, and heavy metals. Combined coagulation, filtration and microalgae treatments using *Chlorella vulgaris* and *Botryococcus sudeticus* effectively removed high concentrations of contaminants in complex wastewater (landfill leachate) with its simplicity, reliability and high cost-effectiveness approach.

The most effective filtration set-ups have been identified for the growth as well as nutrient removal efficiency of *Chlorella vulgaris* and *Botryococcus sudeticus* in pre-treated leachate samples. Set 1 filtration system was the most effective set up for *Chlorella vulgaris* while Set 3 for *Botryococcus sudeticus.* No dilution of leachate was required to culture the *Chlorella vulgaris* and *Botryococcus sudeticus* for biological treatment. Microalgae has utilized high levels of salts, ammonia nitrogen, certain metals and an extensive array of organic compounds in leachate as a source of nutrients to grow (Paskuliakova et al., 2016). Biological treatment using *Chlorella vulgaris* and *Botryococcus sudeticus* have proven to be a promising method for further polishing of leachate after coagulation-filtration treatment.

The results revealed the natural presence of FAMEs at the end of the growth phase of *Chlorella vulgaris* and *Botryococcus sudeticus* cultures. The lipids with broad carbon range of (C-14 to C-36) were identified in *Chlorella vulgaris* (ML1), *Chlorella vulgaris* (ML2), *Chlorella vulgaris* (ML3), *Botryococcus sudeticus* (ML2) and *Botryococcus sudeticus* (ML3). The new insights from this research will pave the way for further research in harvesting the microalgae after leachate treatment and the biosynthesis of FAMEs to be used as biofuels.

5.2 Recommendations for Future Research

The use of zeolites in filtration systems will further improve the efficiency of current filtration system as it is a low cost and effective adsorbent of contaminants. A number of studies were carried out on different types of zeolites. Zeolites are used in a few applications such as catalysts, adsorbents, solar energy storage, and thermal adsorption storage due to their unique porous properties. Zeolites showed good adsorption capacities for removal of heavy metals and organic pollutants from wastewater.

The use of bio-coagulants in leachate treatment must be investigated in future research. The conclusion is drawn that bio-coagulants have been used in many countries with great benefits. In an era of increasing environmental concerns, water scarcity, the draw backs of chemical coagulants and poor sanitary facilities in most low-income earning countries, the need to further develop natural coagulants as alternative environmentally favourable water purifying chemicals is exigent.

REFERENCES

Adnan, S.N.S.M., Yusoff, S. and Chua, Y.P., 2013. Soil chemistry and pollution study of a closed landfill site at Ampar Tenang, Selangor, Malaysia. *Waste Management & Research*, 31 (6), pp. 599-612.

Agamuthu, P. and Fauziah, S.H., 2011. Challenges and issues in moving towards sustainable landfilling in a transitory country-Malaysia. *Waste Management and Research*, 29(1), pp. 13-19.

Ahmad, M., Rajapaksha, A., Lim, J., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S. and Ok, Y., 2014. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere*, 99, pp. 19-33.

Al-Yaqout, A. and Hamoda, M., 2003. Evaluation of landfill leachate in arid climate-A case study. *Environment International*, 29(5), pp. 593-600.

Annarao, S., Sidhu, O., Roy, R., Tuli, R. and Khetrapal, C., 2008. Lipid profiling of developing Jatropha curcas L. seeds using 1H NMR spectroscopy. *Bioresource Technology*, 99(18), pp. 9032-9035.

Asnani, P.U., 2006. Solid waste management. *Journal of India Infrastructure*, 2(4), pp. 570-573.

Azhar, H., Aziz, H., Johari, M., Ariffin, K. and Hung, Y., 2008. Removal of ammoniacal nitrogen and COD from semi-aerobic landfill leachate using low-cost activated carbon zeolite composite adsorbent. *International Journal of Environment and Waste Management*, 4(3), pp. 399-401.

Aziz, H., Alias, S., Adlan, M., Faridah, Asaari, A. and Zahari, M., 2009. Colour removal from landfill leachate by coagulation and flocculation processes. *Bioresource Technology*, 98(1), pp. 218-220.

Aziz, S.Q., Aziz, H.A., Mojiri, A., Bashir, M.J. and Amr, S.S.A., 2014. Landfill Leachate Treatment Using Sequencing Batch Reactor (SBR) Process: Limitation of Operational Parameters and Performance. International Journal Science Resources, 1, pp. 34-43.

Aziz, S.Q., Aziz, H.A., Yusoff, M.S., Bashir, M.J.K. and Umar, M., 2011. Leachate characterization in semi-aerobic and anaerobic sanitary landfills: A comparative study, *Journal of Environmental Management*, 91(12), pp. 2608–2614.

Azmi, N., Bashir, M., Sethupathi, S. and Ng, C., 2014. Anaerobic stabilized landfill leachate treatment using chemically activated sugarcane bagasse activated carbon: kinetic and equilibrium study. *Desalination and Water Treatment*, 57(9), pp. 3916-3927.

Baird, R. and Bridgewater, L., 2017. *Standard methods for the examination of water and wastewater*, 23rd ed. Washington: American Public Health Association.

Bellou, S., Baeshen, M., Elazzazy, A., Aggeli, D., Sayegh, F. and Aggelis, G., 2014. Microalgal lipids biochemistry and biotechnological perspectives. *Biotechnology Advances*, 32(8), pp. 1476-1493.

Berk, Z., 2013. Filtration and Expression. *Food Process Engineering and Technology,* 8, pp. 217-240.

Bhalla, B., Saini, M. and Jha, M., 2013. Effect of age and seasonal variations on leachate characteristics of municipal solid waste landfill. *International Journal of Research in Engineering and Technology*, 2(8), pp. 223-232.

Brennan, L. and Owende, P., 2010. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products, *Renewable and Sustainable Energy Reviews*, 14(2), pp. 557–577.

Cheng, H. and Tian, G., 2013. Preliminary Evaluation of A Newly Isolated Microalgae Scenedesmus sp. CHX1 for Treating Landfill Leachate, China, *IEEE Xplore,* 112, pp. 323-326.

Chisti, Y., 2007. Biodiesel from microalgae. *Advance Biotechnology*, 25, 294–306.

Chiu, S.Y., Kao, C.Y., Chen, T.Y., Chang, Y.B., Kuo, C.M. and Lin, C.S., 2015. Cultivation of microalgal Chlorella for biomass and lipid production using wastewater as nutrient resource, *Bioresource Technology*, 184, pp. 179–189.

Clarke, B.O., Anumol, T., Barlaz, M. and Snyder, S.A., 2015. Investigating landfill leachate as a source of trace organic pollutants. *Chemosphere*, 127, pp. 269-275.

Edmundson, S.J and Wilkie, A.C., 2013. Landfill leachate- A water and nutrient resource for algae-based biofuels. *Environmental Technology*, 34(14), pp. 1849-1857.

El-Fadel, M., Findikakis, A.N. and Leckie, J.O., 2007. Environmental impacts of solid waste landfilling. *Journal of environmental management*, 50(1), pp.1-25.

Fazeli, A., Bakhtvar, F., Jahanshaloo, L., Sidik, N.A.C. and Bayat, A.E., 2016. Malaysia’s stand on municipal solid waste conversion to energy: A review. *Renewable and Sustainable Energy Reviews*, 58, pp.1007-1016.

Foo, K. and Hameed, B., 2009. An overview of landfill leachate treatment via activated carbon adsorption process. *Journal of Hazardous Materials*, 171(1), pp. 54-60.

Foul, A.A., Aziz, H.A., Johari, M.A.M., Ariffin, K. S. and Hung, Y. T., 2009. Primary treatment of anaerobic landfill leachate using activated carbon and limestone: Batch and column studies. *International Journal of Environment and Waste Management, 4,* pp. 282-298.

Gitelson, A., Gritz, Y. and Merzlyak, M., 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology*, 160(3), pp. 271-282.

Gregory, J. and Duan, J., 2001. Hydrolyzing metal salts as coagulants. *Pure and Applied Chemistry*, 73(12), pp. 2017-2026.

Guieysse, B., Borde, X., Munoz, R., Hatti Kaul, R. and Nugier Chauvin, C., 2002. Influence of the initial composition of algal bacterial microcosms on the degradation of salicylate in fed batch culture. *Biotechnology Letters*, 24, pp. 531-538.

Hamoda, M., Al-Ghusain, I. and Al-Mutairi, N., 2004. Sand filtration of wastewater for tertiary treatment and water reuse. *Desalination,* 164(3), pp. 203-211.

Healy, M., Rodgers, M. and Mulqueen, J., 2004. Recirculating Sand Filters for Treatment of Synthetic Dairy Parlor Washings. *Journal of Environment Quality,* 33(2), pp. 713.

Hector, A.V., Bruce, J. and Simon, J., 2004. Membrane bioreactors vs conventional biological treatment of landfill leachate: A brief review. *Journal Chemistry Technology Biotechnology,* 79, pp. 1043-1049.

Herrera, V., Vazquez, V.R., Larque, S.F. and Barahona, P.L., 2011. Naturally occurring fatty acid methyl esters and ethyl esters in the green microalga Chlamydomonas reinhardtii. *Annals of Microbiology,* 62(2), pp. 865-870.

Huan-jung, F., Shu, H.Y., Yang, H.S. and Chen, W.C., 2006. Characteristics of landfill leachates in central Taiwan. *Taiwan Science Total Environment*, 361, pp. 25–37.

John, H., 2002. Suspended Solids Removal. *Journal of Design and Operating Guide for Aquaculture Seawater Systems,* 33, pp. 137-149.

Kamaruddin, M., Abdullah, M., Yusoff, M., Alrozi, R. and Neculai, O., 2017. Coagulation-Flocculation Process in Landfill Leachate Treatment: Focus on Coagulants and Coagulants Aid. *IOP Conference Series: Materials Science and Engineering,* 209, pp. 12-23.

Kannan, R., 2013, *What contaminated ground water can do to your health?* [Online]. Available at: http://www.thehindu.com/news/cities/chennai/whatcontaminated-ground-water-can-do-to-your-health/article5019514.ece [Accessed on 11 July 2018].

Kang, K. H., Shin, H. S. and Park, H., 2002. Characterization of humic substances present in landfill leachates with different landfill ages and its implications. *Water Research, 36,* pp. 4023-4033.

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A. and Christensen, T. H., 2002. Present and Long-Term Composition of MSW Landfill Leachate: A Review. *Critical Reviews in Environmental Science and Technology, 32*(4), pp. 297-336.

Klauson, D., Kivi, A., Kattel, E., Klein, K., Viisimaa, M., Bolobajev, J., Velling, S., Goi, A., Tenno, T. and Trapido, M., 2014. Combined processes for wastewater purification: treatment of a typical landfill leachate with a combination of chemical and biological oxidation processes. *Journal of Chemical Technology and Biotechnology*, 90(8), pp. 1527-1536.

Klinck, B.A. and Stuart, M.E., 2009. Human health risk in relation to landfill leachate quality. *British Geological Survey*, 17, pp. 99-103.

Kulikowska, D. and Klimiuk, E., 2008. The effect of landfill age on municipal leachate composition. *Bioresource technology, 99*(13), pp. 5981-5985.

Kuusik, A., Pachel, K., Kuusik, A. and Loigu, E., 2014. Landfill runoff water and landfill leachate discharge and treatment. *Environmental Engineering. Proceedings of the International Conference on Environmental Engineering*, 9 (1), pp. 1-2.

Lim, P.E., Lim, S.P., Seng, C.E. and Noor, A.M., 2010. Treatment of landfill leachate in sequencing batch reactor supplemented with activated rice husk as adsorbent. *Chemical Engineering Journal*, 159(1), pp. 123-128.

Lin, L., Chan, G.Y.S., Jiang, B.L. and Lan, C.Y., 2007. Use of ammoniacal nitrogen tolerant microalgae in landfill leachate treatment. *Waste Management*, 27(10), pp. 1376–1382.

Liu, G., Chai, X., Shao, Y., Hu, L., Xie, Q. and Wu, H., 2011. Toxicity of copper, lead, and cadmium on the motility of two marine microalgae Isochrysis galbana and Tetraselmis chui. Journal of Environmental Sciences, 23, pp. 330–335.

Liu, S., 2013, *Landfill leachate treatment methods and evolution of Hedeskoga and Masalycke landfills* [Online]. Available at: http://www.vateknik.lth.se/exjobbR/E709.pdf [Accessed: 4 June 2018].

Mao Rui, L. and Bin Daud, Z., 2011. Efficiency of the Coagulation-Flocculation for the Leachate Treatment. *International Journal of Sustainable Development*, 2(10), pp. 85-90.

Manaf, L.A., Samah, M.A.A. and Zukki, N.I.M., 2009. Municipal solid waste management in Malaysia: Practices and challenges. *Waste Management, (4)29*, pp. 2902-2906.

Manning, J., 2003. Water Supplies and Water Treatment. *Encyclopedia of Food Sciences and Nutrition*, pp. 6105-6111.

Masirin, M., Idrus, M., Ridzuan, M.B. and Mustapha, S., 2008. An overview of landfill management and technologies: A Malaysian case study at Ampar Tenang. *Journal of Environment, Development and Sustainability*, 3(1), pp. 157-165.

Matilainen, A., Vieno, N. and Tuhkanen, T., 2006. Efficiency of the activated carbon filtration in the natural organic matter removal. *Environment International*, 32(3), pp. 324-331.

Mbaeze, M.C., Agbazue, V.E. and Orjioke, N.M., 2015. Comparative Assessment of Performance of Aluminium Sulphate (Alum) and Ferrous Sulphate as Coagulants in Water Treatment. *Modern Chemistry and Applications*, 5(4), pp. 1-14.

Moreno, A., Rueda, O., Cabrera, E. and Luna-del-Castillo, J.D., 2007. Standarization in wastewater biomass growth. *Ig Mod*, 94 (1), pp. 24– 32.

Mustafa, E.M., Phang, S.M. and Chu, W.L., 2011. Use of an algal consortium of five algae in the treatment of landfill leachate using the high-rate algal pond system. *Journal of Applied Phycol*, 24(4), pp. 953-963.

Negeri Perak, 2013. *Maklumat Asas: Pihak Berkuasa Tempatan (NEGERI PERAK DARUL RIDZUAN)* [Online]. Available at: https://www.perak.gov.my/images/menu_utama/ms/rakyat/dataasaspbt2013.pdf [Accessed: 10 July 2018]

Oswald, W.J., 2008. *Micro-Algae and Waste-water Treatment*, 1st Ed, Cambridge, Borowitzka.

Paskuliakova, A., Tonry, S. and Touzet, N., 2016. Phycoremediation of landfill leachate with chlorophytes: Phosphate a limiting factor on ammonia nitrogen removal, *Water Research*, 99, pp. 180–187.

Raghab, S.M., Abd El Meguid, A.M. and Hegazi, H.A., 2013. Treatment of leachate from municipal solid waste landfill, *HBRC Journal*, 9(2), pp. 187–192.

Raouf, N.A., Al-Homaidan, A.A. and Ibraheem, I.B.M., 2012. Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences*, 19(3), pp. 257–275.

Rees, J., 2007. Optimisation of methane production and refuse decomposition in landfills by temperature control. *Journal of Chemical Technology and Biotechnology*, 30(1), pp. 458-465.

Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F. and Moulin, P., 2007. Landfill leachate treatment: Review and opportunity. *Journal of Hazardous Materials*, 150(3), pp. 468–493.

Richards, R.G. and Mullins, B.J., 2013. Using microalgae for combined lipid production and heavy metal removal from leachate, *Ecological Modelling*, 249, pp. 59–67.

Salem, Z., Hamouri, K., Djemaa, R. and Allia, K., 2008. Evaluation of landfill leachate pollution and treatment. *Desalination*, 220(1), pp. 108-114.

Samori, C., Lopez Barreiro, D., Vet, R., Pezzolesi, L., Brilman, D., Galletti, P. and Tagliavini, E., 2013. Effective lipid extraction from algae cultures using switchable solvents. *Green Chemistry*, 15(2), pp. 353-359.

Sforza, E., Khairallah, A.E.M.H., Sharif, A. and Bertucco, A., 2015. Exploitation of Urban Landfill Leachate as Nutrient Source for Microalgal Biomass Production, *Chemical Engineering Transactions*, 43, pp. 373–378.

Shehzad, A., Bashir, M., Sethupathi, S. and Lim, J., 2015. An overview of heavily polluted landfill leachate treatment using food waste as an alternative and renewable source of activated carbon. *Process Safety and Environmental Protection*, 98, pp. 309-318.

Shekdar, A.V., 2009. Sustainable solid waste management: an integrated approach for Asian countries. *Waste management*, 29(4), pp. 1438-1448.

Shu, Z., Lü, Y., Huang, J. and Zhang, W., 2016. Treatment of compost leachate by the combination of coagulation and membrane process. *Chinese Journal of Chemical Engineering*, 24(10), pp. 1369-1374.

Silva, N.F.P., Gonçalves, A.L., Moreira, F.C., Silva, T.F.C.V., Martins, F.G., Alvim, F. M.C.M., Boaventura, R.A.R., Vilar, V.J.P. and Pires, J.C.M., 2014. Towards sustainable microalgal biomass production by phycoremediation of a synthetic wastewater: A kinetic study. *Algal Research*, 11, pp. 350–358.

Simate, G., 2015. The treatment of brewery wastewater for reuse by integration of coagulation/flocculation and sedimentation with carbon nanotubes ‘sandwiched’ in a granular filter bed. *Journal of Industrial and Engineering Chemistry*, 21, pp. 1277-1285.

Slack, R.J., Gronow, J.R. and Voulvoulis, N., 2005. Household hazardous waste in municipal landfills: contaminants in leachate. *Science of the total environment*, 337(1), pp. 119-137.

Stegmann R., Heyer K. U. and Cossu R., 2015. Leachate treatment. *Proceedings of the Tenth International Waste Management and Landfill Symposium*, 3(1), pp. 21-28.

Strom, E., 2010. *Leachate treatment and anaerobic digestion using aquatic plants and algae.* Msc thesis, Linkoping University, Sweden.

Tarmudi, Z., Abdullah, M.L. and Tap, A.O.M., 2012. A Review of Municipal Solid Waste Management in Malaysia. *Jurnal Teknologi*, 57(1), pp. 41-56.

Tatsi, A. A., 2013. A field investigation of the quantity and quality of leachate from municipal solid waste in mediterranean climate. *Advances in Environmental Research, 6,* pp. 207-219.

United Nations Environment Programme (UNEP), 2005. Training Module: Closing of an Open Dumpsite and Shifting from Open Dumping to Controlled Dumping and to Sanitary Land Filling. Department of Environment and Natural Resources. Republic of the Philippines.

USAID, 2016. Chapter 6: Landfilling and Open Dump Closure. (Solid Waste Management Privatization Procedure Manual, Egyptian Environmental Policy Program) Available at: http://pdf.usaid.gov/pdf_docs/Pnacy344.pdf [Accessed on 10th July 2018]

Vaezi, F., Mohagheghian, A., Nouri, J., Eshraghian, M. R. and Ghasri, A., 2005. Improvement of NOM Removal from Water Resources Modifying the Coagulation Process. *Iranian Environmental Health Sciences Engineering*, 2, pp. 43-49.

Wang, Z.P., Zhang, Z., Lin Y.J., Deng, N.S., Tao, T. and Zhuo, K., 2002. Landfill leachate treatment by a coagulation-photooxidation process. *Journal of Hazardous Materials, 95*, pp. 153-159.

Wasay, S. A., Barrington, S.F. and Tokunaga, S. 2009. Remediation of soils polluted by heavy metals using salts of organic acids and chelating agents. Environmental Technology, 19, pp. 369-380.

Wiszniowski, J., Robert, D., Surmacz, G.J., Miksch, K. and Weber, J.V., 2006. Landfill leachate treatment methods: A review. *Environmental Chemistry Letters*, 4(1), pp. 51-61.

Zainol, N.A., Aziz, H.A. and Yusoff, M.S., 2012. Characterization of Leachate from Kuala Sepetang and Kulim landfills: A comparative study. *Energy and Environment Research*, 2(2), pp. 102-108.

Zhang, Q., Tian, B., Zhang, X., Ghulam, A., Fang, C. and He, R., 2013. Investigation on characteristics of leachate and concentrated leachate in three landfill leachate treatment plants. *Waste Management*, 33(11), pp. 2277-2286.

Zhang, Z., Sachs, J. and Marchetti, A., 2009. Hydrogen isotope fractionation in freshwater and marine algae: II. Temperature and nitrogen limited growth rate effects. *Organic Geochemistry*, 40(3), pp. 428-439.

Zhao, X., Zhou, Y., Huang, S., Qiu, D., Schideman, L., Chai, X. and Zhao, Y., 2008. Characterization of microalgae-bacteria consortium cultured in landfill leachate for carbon fixation and lipid production, *Bioresource Technology*, 156, pp. 322–328.

APPENDIX A

Table A1 Turbidity values after coagulation of leachate using different types of coagulants.

Coagulant	Turbidity (Ntu)		
	1st Trial	2nd Trial	Mean Average
Aluminium iron (III) sulphate 12-hydrate	**153.0**	**145.3**	**149.1± 5.4**
Aluminium ammonium sulphate 12 –hydrate	**124.5**	**112.5**	**118.5 ± 8.4**
Aluminium Sulphate	**134.7**	**140.2**	**137.4 ± 3.8**

Table A2 COD values after coagulation of leachate using different types of coagulants.

Coagulant	COD (mg/L)		
	1st Trial	2nd Trial	Mean Average
Aluminium iron (III) sulphate 12-hydrate	**1933.5**	**1825.3**	**1879.4 ± 76.3**
Aluminium ammonium sulphate 12 –hydrate	**1473.0**	**1412.0**	**1442.5 ± 43.1**
Aluminium Sulphate	**1788.0**	**1812.0**	**1800.0 ± 16.9**

APPENDIX B

Pigments Extraction in Bold's Basal Medium (BBM)

Table B1 Absorbance Reading of CV and BS in BBM (1St Trial)

Week	Ethanol					
	Absorbance *(Chlorella vulgaris)*			Absorbance *(Botryococcus sudeticus)*		
	664 nm	**648 nm**	**470 nm**	**664 nm**	**648 nm**	**470 nm**
1	**0.131**	**0.069**	**0.175**	**0.184**	**0.098**	**0.260**
2	**0.344**	**0.161**	**0.432**	**0.336**	**0.176**	**0.453**
3	**0.465**	**0.206**	**0.564**	**0.409**	**0.191**	**0.532**
4	**1.214**	**0.554**	**1.464**	**0.561**	**0.260**	**0.741**

Table B2 Absorbance Reading of CV and BS in BBM (2nd Trial)

Week	Ethanol					
	Absorbance *(Chlorella vulgaris)*			Absorbance *(Botryococcus sudeticus)*		
	664 nm	**648 nm**	**470 nm**	**664 nm**	**648 nm**	**470 nm**
1	**0.065**	**0.033**	**0.083**	**0.107**	**0.052**	**0.140**
2	**0.167**	**0.084**	**0.222**	**0.243**	**0.114**	**0.331**
3	**0.211**	**0.119**	**0.305**	**0.296**	**0.137**	**0.390**
4	**0.256**	**0.122**	**0.382**	**0.316**	**0.160**	**0.395**

Table B3 Concentration of pigments of CV and BS for ethanol extract in BBM

Week	Pigments		Concentration (µg/ml)				Mean Average
		CV (1st Trial)	CV (2nd Trial)	Average value	BS (1st Trial)	BS (2nd Trial)	
1	Chl A	1.392	0.6971	1.0445 ± 0.4913	2.848	1.1697	2.0088 ± 1.1867
	Chl B	0.829	0.3774	0.6032 ± 0.3193	1.512	0.5575	1.0347 ± 0.6749
	Caro	0.436	0.2137	0.3249 ± 0.1572	0.876	0.3975	0.6367 ± 0.3384
2	Chl A	3.7603	1.7952	2.7777 ± 1.3895	3.5755	2.6548	3.1152 ± 0.6510
	Chl B	1.6230	0.9481	1.2855 ± 0.4772	2.0994	1.1539	1.6267 ± 0.6685
	Caro	1.2704	0.6009	0.9356 ± 0.4734	1.1502	1.0176	1.0839 ± 0.0937
3	Chl A	5.1433	2.2014	3.6724 ± 2.0802	4.4729	3.2435	3.8582 ± 0.8693
	Chl B	1.8747	1.5509	1.7128 ± 0.2289	1.9181	1.3544	1.6362 ± 0.3986
	Caro	1.7703	0.7123	1.2413 ± 0.7481	1.6038	1.2002	1.4020± 0.2853
4	Chl A	13.3438	2.7870	8.0654 ± 7.4647	6.1456	3.3914	4.7685 ± 1.9475
	Chl B	5.3385	1.2677	3.3031 ± 2.8785	2.5765	1.8229	2.1997 ± 0.5328
	Caro	4.3748	1.2071	2.7909 ± 2.2399	2.2791	1.0038	1.6415 ± 0.9018

APPENDIX C

Pigments Extraction in Treated Landfill Leachate

Table C1 The Absorbance Reading of CV and BS in Mature Leachate (1st Trial)

Sample	Absorbance Reading in Ethanol (A)											
	664 nm				648 nm				470 nm			
	Week				Week				Week			
	1	2	3	4	1	2	3	4	1	2	3	4
ML1 100% (CV)	**NG**	**0.217**	**0.276**	**0.394**	**NG**	**0.099**	**0.142**	**0.154**	**NG**	**0.240**	**0.343**	**0.389**
ML2 100% (CV)	**NG**	**0.069**	**0.118**	**0.177**	**NG**	**0.049**	**0.060**	**0.095**	**NG**	**0.118**	**0.159**	**0.192**
ML3 100% (CV)	**NG**	**0.056**	**0.168**	**0.223**	**NG**	**0.031**	**0.090**	**0.102**	**NG**	**0.105**	**0.223**	**0.234**

ML2 100% (BS)	**NG**	**0.300**	**0.575**	**1.1012**	**NG**	**0.152**	**0.368**	**0.394**	**NG**	**0.329**	**0.820**	**1.153**
ML3 100% (BS)	**NG**	**0.224**	**0.413**	**1.040**	**NG**	**0.134**	**0.256**	**0.625**	**NG**	**0.287**	**0.5995**	**1.139**
Tap Water (CV)	**0.168**	**0.623**	**1.174**	**0.903**	**0.082**	**0.388**	**0.901**	**0.513**	**0.259**	**0.691**	**1.436**	**0.921**
Tap Water (BS)	**0.062**	**0.144**	**0.461**	**0.983**	**0.034**	**0.077**	**0.232**	**0.767**	**0.087**	**0.208**	**0.568**	**1.133**

Table C2 Concentration of pigments for ethanol extract of CV and BS in Mature Leachate (1st Trial)

Week	Pigment	Concentration of pigments (µg/mL)									
		ML1 100% (CV)	ML2 100% (CV)	ML3 100% (CV)	ML4 100% (CV)	ML1 100% (BS)	ML2 100% (BS)	ML3 100% (BS)	ML4 100% (BS)	Tap Water (CV)	Tap Water (BS)
1	Chl A	NG	NG	NG	NG	NG	NG	NG	NG	1.8189	1.3736
	Chl B	NG	NG	NG	NG	NG	NG	NG	NG	0.8851	1.3014
	Caro	NG	NG	NG	NG	NG	NG	NG	NG	0.8072	0.0527
2	Chl A	2.3853	0.6675	0.5873	NG	NG	3.2191	2.2972	NG	6.3096	3.0431
	Chl B	0.9535	0.7838	0.3956	NG	NG	1.7334	1.8567	NG	5.5941	1.4762
	Caro	0.6785	0.1916	0.3116	NG	NG	0.7316	0.4824	NG	0.6285	0.8248
3	Chl A	2.9504	1.2651	1.773	NG	NG	5.7721	4.1890	NG	12.6465	4.3789
	Chl B	1.6539	0.6876	1.1045	NG	NG	5.4252	3.6685	NG	9.6956	3.1647
	Caro	0.8384	0.4266	0.5329	NG	NG	1.3301	1.0904	NG	2.2185	1.3334

4	**Chl A**	**3.6629**	**1.8717**	**2.4499**	**NG**	**NG**	**11.4755**	**10.6507**	**NG**	**9.4016**	**9.3908**
	Chl B	**1.5121**	**1.1686**	**0.9871**	**NG**	**NG**	**2.5899**	**8.6989**	**NG**	**6.7392**	**5.0465**
	Caro	**1.1175**	**0.3536**	**0.6335**	**NG**	**NG**	**4.1898**	**1.2773**	**NG**	**1.1625**	**3.9051**

Table C3 The Absorbance Reading of CV and BS in Mature Leachate (2nd Trial)

Sample	Absorbance Reading in Ethanol (A)											
	664 nm				648 nm				470 nm			
	Week				Week				Week			
	1	2	3	4	1	2	3	4	1	2	3	4
ML1 100% (CV)	NG	0.094	0.234	0.511	NG	0.058	0.133	0.241	NG	0.119	0.257	0.610
ML2 100% (CV)	NG	0.073	0.531	0.835	NG	0.043	0.306	0.365	NG	0.097	0.565	0.850
ML3 100% (CV)	NG	0.099	1.019	1.240	NG	0.067	0.466	0.555	NG	0.115	1.101	1.299
ML2 100% (BS)	NG	0.123	0.657	0.806	NG	0.906	0.347	0.400	NG	0.214	0.695	0.851
ML3 100% (BS)	NG	0.590	0.635	0.685	NG	0.255	0.241	0.450	NG	0.548	0.8100	0.871
Tap Water (CV)	0.172	0.312	0.798	0.721	0.073	0.315	0.523	0.432	0.259	0.721	1.212	1.111

Tap Water (BS)	**0.137**	**0.218**	**0.421**	**0.875**	**0.088**	**0.137**	**0.240**	**0.443**	**0.141**	**0.323**	**0.597**	**1.308**

Table C4 Concentration of pigments for ethanol extract of CV and BS in Mature Leachate (2nd Trial)

Week	**Pigment**	**Concentration of pigments (µg/mL)**									
		ML1 100% (CV)	**ML2 100% (CV)**	**ML3 100% (CV)**	**ML4 100% (CV)**	**ML1 100% (BS)**	**ML2 100% (BS)**	**ML3 100% (BS)**	**ML4 100% (BS)**	**Tap Water (CV)**	**Tap Water (BS)**
1	**Chl A**	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**1.919**	**0.7855**
	Chl B	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**0.606**	**0.3479**
	Caro	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**NG**	**0.937**	**0.2457**
2	**Chl A**	**0.9548**	**0.7521**	**0.9749**	**NG**	**NG**	**1.1969**	**6.5589**	**NG**	**2.533**	**1.5242**

	Chl B	**0.8277**	**0.5867**	**1.0339**	**NG**	**NG**	**1.3602**	**2.2039**	**NG**	**6.107**	**0.9428**
	Caro	**0.1729**	**0.1824**	**0.0573**	**NG**	**NG**	**0.3763**	**1.5255**	**NG**	**0.570**	**0.5392**
3	**Chl A**	**2.4359**	**5.5060**	**11.1953**	**NG**	**NG**	**6.9766**	**7.2328**	**NG**	**7.947**	**4.9549**
	Chl B	**1.7481**	**4.0819**	**4.5081**	**NG**	**NG**	**4.1834**	**1.4509**	**NG**	**7.866**	**2.6204**
	Caro	**0.3882**	**0.7403**	**3.0478**	**NG**	**NG**	**1.2999**	**3.1241**	**NG**	**2.040**	**1.4430**
4	**Chl A**	**5.5761**	**9.2613**	**13.6859**	**NG**	**NG**	**8.6922**	**6.8161**	**NG**	**7.390**	**10.1902**
	Chl B	**2.4613**	**3.2318**	**5.1549**	**NG**	**NG**	**4.4273**	**6.7813**	**NG**	**5.995**	**7.5709**
	Caro	**1.7119**	**2.4628**	**3.6676**	**NG**	**NG**	**1.9149**	**0.9299**	**NG**	**2.439**	**1.7802**

Table C5 Concentration of pigments for ethanol extract of CV and BS in Mature Leachate with Mean Average and Standard Deviation

Week	Pigment	Concentration of pigments Mean Average and Standard Deviation (μg/mL)									
		ML1 100% (CV)	ML2 100% (CV)	ML3 100% (CV)	ML4 100% (CV)	ML1 100% (BS)	ML2 100% (BS)	ML3 100% (BS)	ML4 100% (BS)	Tap Water (CV)	Tap Water (BS)
1	Chl A	NG	NG	NG	NG	NG	NG	NG	NG	1.8689 ± 0.0707	1.0796 ± 0.4158
	Chl B	NG	NG	NG	NG	NG	NG	NG	NG	0.7456 ± 0.1974	0.8247 ± 0.6742
	Caro	NG	NG	NG	NG	NG	NG	NG	NG	0.8721 ± 0.0918	0.1492 ± 0.1365
2	Chl A	1.6701 ± 1.0115	0.7098 ± 0.0598	0.7811 ± 0.2741	NG	NG	2.2080 ± 1.4299	4.4281 ± 3.0135	NG	4.4213 ± 2.6705	2.2837 ± 1.0740
	Chl B	0.8906 ± 0.0889	0.6853 ± 0.1394	0.7148 ± 0.4513	NG	NG	1.5469 ± 0.2638	2.0303 ± 0.2455	NG	5.8506 ± 0.3627	1.2095 ± 0.3772
	Caro	0.4257 ± 0.3575	0.1870 ± 0.0065	0.1845 ± 0.1798	NG	NG	0.5539 ± 0.2512	1.0039 ± 0.7376	NG	0.5993 ± 0.0414	0.6820 ± 0.2019

3	**Chl A**	**2.6932 ± 0.3638**	**3.3856 ± 2.9988**	**6.4845 ± 6.6629**	**NG**	**NG**	**6.3744 ± 0.8517**	**5.7109 ± 2.1523**	**NG**	**10.2968 ± 3.3230**	**4.6669 ± 0.4073**
	Chl B	**1.7010 ± 0.0666**	**2.3848 ± 2.4001**	**2.8063± 2.4067**	**NG**	**NG**	**4.8043 ± 0.8781**	**2.5597± 1.5681**	**NG**	**8.7808 ± 1.2937**	**2.8926 ± 0.3849**
	Caro	**0.6133 ± 0.3183**	**0.5834 ± 0.2218**	**1.7904 ± 1.7783**	**NG**	**NG**	**1.3150 ± 0.0213**	**2.1073 ± 1.4380**	**NG**	**2.1293 ± 0.1262**	**1.3882 ± 0.0775**
4	**Chl A**	**4.6195 ± 1.3528**	**5.5665 ± 5.2252**	**8.0679 ± 7.9451**	**NG**	**NG**	**10.0839 ± 1.9681**	**8.7366 ± 2.7159**	**NG**	**8.3958 ± 1.4224**	**9.7905 ± 0.5653**
	Chl B	**1.9867 ± 0.6712**	**2.2002 ± 1.4589**	**3.0710 ± 2.9471**	**NG**	**NG**	**3.5086 ± 1.2992**	**7.7575 ± 1.3313**	**NG**	**6.3671 ± 0.5262**	**6.3087 ± 1.7850**
	Caro	**1.4147 ± 0.4203**	**1.4082 ± 1.4914**	**2.1506 ± 2.1454**	**NG**	**NG**	**3.0524 ± 1.6086**	**1.1036 ± 0.2456**	**NG**	**1.8008 ± 0.9026**	**2.8427 ± 1.5025**

APPENDIX D

Table D1 Total Solids of Mature Leachate

Samples	Volume of sample used (ml)	Mass of petri dish (g)	Time taken (min)	Mass of petri dish + sample (g)	Total solids (mg/L)	Total Solids Mean Average with Standard Deviation (mg/L)
Before Treatment 1F	25	57.7462	0 60	82.7717 57.9463	8004	**8526 ± 510**
Before Treatment 2F	25	57.3799	0 60	82.0342 57.5851	8208	
Before Treatment 1S	25	56.1150	0 60	80.2158 56.3345	8780	
Before Treatment 2S	25	57.7469	0 60	81.8585 57.8747	9112	
After Coagulation-Filtration Treatment						
Set 1 1F	25	54.7696	0 60	79.7094 54.8291	2380	**2200 ± 668**

Set 1 **2F**	25	50.9389	0 60	75.9551 51.0129	2960	
Set 1 **1S**	25	54.7696	0 60	79.7894 54.8021	1300	
Set 1 **2S**	25	50.9389	0 60	75.9898 50.9929	2160	
Set 2 **1F**	25	56.1150	0 60	81.4551 56.1914	3056	**2416 ± 694**
Set 2 **2F**	25	57.7615	0 60	81.7799 57.8359	2976	
Set 2 **1S**	25	56.1150	0 60	81.8651 56.1614	1856	
Set 2 **2S**	25	57.7615	0 60	81.7867 57.8059	1776	
Set 3 **1F**	25	56.4255	0 60	80.6632 56.4951	2784	**2250 ± 361**
Set 3 **2F**	25	57.5206	0 60	81.9914 57.5729	2092	

Set 3 **1S**	25	56.4255	0 60	80.4678 56.4789	2136	
Set 3 **2S**	25	57.5342	0 60	81.9914 57.5839	1988	
Set 4 **1F**	25	57.1831	0 60	82.1353 57.2939	4432	**4550 ± 758**
Set 4 **2F**	25	55.4356	0 60	80.0024 55.5687	5324	
Set 4 **1S**	25	57.1831	0 60	82.1863 57.2719	3552	
Set 4 **2S**	25	55.4376	0 60	80.0754 55.5599	4892	
After Coagulation-Filtration-Microalgae treatment using *Chlorella vulgaris* in 100% Treated Leachate						
Set 1 **1F**	25	54.7661	0 60	72.0989 54.8145	1936	**1989 ± 760**
Set 1 **2F**	25	50.9384	0 60	73.7980 50.9976	2208	
Set 1	25	54.7661	0	69.0872	992	

1S			60	54.7909		
Set 1 **2S**	25	50.9384	0 60	79.6623 51.0089	2820	
Set 2 **1F**	25	56.1146	0 60	80.8624 56.1723	2308	**2007 ± 278**
Set 2 **2F**	25	57.7456	0 60	79.1112 57.7908	1808	
Set 2 **1S**	25	56.1146	0 60	79.9903 56.1690	2176	
Set 2 **2S**	25	57.7456	0 60	82.7564 57.7890	1736	
Set 3 **1F**	25	56.2915	0 60	79.9075 56.3444	2116	**2397 ± 384**
Set 3 **2F**	25	57.3795	0 60	81.9236 57.4431	2544	
Set 3 **1S**	25	56.2915	0 60	81.7534 56.3633	2872	
Set 3 **2S**	25	57.3795	0	80.0119	2056	

			60	57.4309		
Set 4 **1F**	25	57.1801	0 60	83.0442 57.2689	3552	**2888 ± 632**
Set 4 **2F**	25	55.4326	0 60	80.8232 55.5011	2740	
Set 4 **1S**	25	57.1801	0 60	81.0001 57.2321	2080	
Set 4 **2S**	25	55.4326	0 60	81.8167 55.5121	3180	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate						
Set 1 **1F**	25	54.7661	0 60	75.0219 54.8211	2200	**2146 ± 317**
Set 1 **2F**	25	50.9384	0 60	79.6632 50.9811	1708	
Set 1 **1S**	25	54.7661	0 60	74.1176 54.8213	2208	
Set 1 **2S**	25	50.9384	0 60	79.1904 51.0001	2468	

Set 2 **1F**	25	56.1146	0 60	79.0043 56.1661	2060	**2247 ± 513**
Set 2 **2F**	25	57.7456	0 60	80.9066 57.7999	2172	
Set 2 **1S**	25	56.1146	0 60	81.7601 56.1890	2976	
Set 2 **2S**	25	57.7456	0 60	83.2256 57.7901	1780	
Set 3 **1F**	25	56.2915	0 60	79.9999 56.3321	1624	**1843 ± 259**
Set 3 **2F**	25	57.3795	0 60	79.0944 57.4321	2104	
Set 3 **1S**	25	56.2915	0 60	79.6645 56.3422	2028	
Set 3 **2S**	25	57.3795	0 60	79.0823 57.4199	1616	
Set 4 **1F**	25	57.1801	0 60	83.1122 57.2698	3588	**2946 ± 508**

Set 4 **2F**	25	55.4326	0 60	80.9712 55.5101	3100	
Set 4 **1S**	25	57.1801	0 60	80.2389 57.2411	2440	
Set 4 **2S**	25	55.4326	0 60	80.9954 55.4990	2656	

APPENDIX E

Table E1 Total Suspended Solids

Samples	Mass of petri dish (g)	Mass of filter paper (g)	Time taken (min)	Mass of petri dish + sample (g)	Total Suspended solids (mg/L)	Total Suspended Solids Mean Average with Standard Deviation (mg/L)
Before Treatment 1F	50.9389	0.5653	0 60	52.1671 51.5393	1404	**1602 ± 810**
Before Treatment 2F	54.7696	0.5536	0 60	56.3241 55.3492	1040	
Before Treatment 1S	50.9389	0.5150	0 60	52.9076 51.4831	1168	
Before Treatment 2S	54.7696	0.5469	0 60	56.3265 55.3864	2796	
After Coagulation-Filtration Treatment						

Set 1 **1F**	50.9393	0.5418	0 60	52.1948 51.4891	320	**347 ± 73**
Set 1 **2F**	54.7665	0.5448	0 60	56.0178 55.3187	296	
Set 1 **1S**	50.9393	0.5674	0 60	52.8041 51.5146	316	
Set 1 **2S**	54.7665	0.5512	0 60	56.0177 55.3291	456	
Set 2 **1F**	56.1150	0.5679	0 60	57.7804 56.6879	200	**169 ± 196**
Set 2 **2F**	57.7462	0.5643	0 60	58.7020 58.3108	12	
Set 2 **1S**	56.1150	0.5612	0 60	57.7769 56.6838	304	
Set 2 **2S**	57.7462	0.5609	0 60	58.9845 58.3180	436	

Set 3 **1F**	50.9355	0.5668	0 60	52.1713 51.5097	296	**409 ± 205**
Set 3 **2F**	54.7696	0.5577	0 60	56.0050 55.3327	216	
Set 3 **1S**	50.9355	0.5356	0 60	52.5741 51.4821	440	
Set 3 **2S**	54.7696	0.5421	0 60	56.0941 55.3288	684	
Set 4 **1F**	56.1150	0.5572	0 60	58.0123 56.6899	708	**806 ± 175**
Set 4 **2F**	57.7462	0.5602	0 60	58.6208 58.3266	808	
Set 4 **1S**	56.1150	0.5489	0 60	58.0645 56.6902	1052	
Set 4 **2S**	57.7462	0.5600	0 60	58.9063 58.3226	656	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate						
Set 1	50.9393	0.5581	0	52.1901	36	**28 ± 11**

1F			60	51.4983		
Set 1 **2F**	54.7665	0.5384	0 60	56.1087 55.3053	16	
Set 1 **1S**	50.9393	0.5747	0 60	52.1041 51.5150	40	
Set 1 **2S**	54.7665	0.5621	0 60	56.0632 55.3291	20	
Set 2 **1F**	56.1150	0.5497	0 60	57.7403 56.6658	44	**85 ± 52**
Set 2 **2F**	57.7462	0.5334	0 60	58.9072 58.2810	56	
Set 2 **1S**	56.1150	0.5721	0 60	57.1785 56.6891	80	
Set 2 **2S**	57.7462	0.5390	0 60	58.3925 58.2892	160	
Set 3 **1F**	50.9355	0.5186	0	52.1454	20	**28 ± 6**

			60	51.4546		
Set 3 **2F**	54.7696	0.5899	0 60	56.2359 55.3604	36	
Set 3 **1S**	50.9355	0.5265	0 60	52.2326 51.4627	28	
Set 3 **2S**	54.7696	0.5311	0 60	56.2003 55.3014	28	
Set 4 **1F**	56.1150	0.5523	0 60	57.3767 56.6712	156	**135 ± 20**
Set 4 **2F**	57.7462	0.5420	0 60	58.9883 58.2913	124	
Set 4 **1S**	56.1150	0.5931	0 60	57.1907 56.7118	148	
Set 4 **2S**	57.7462	0.5622	0 60	59.4803 58.3112	112	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate						
Set 1 **1F**	50.9393	0.5329	0 60	52.1724 51.4729	28	**29 ± 16**

Set 1 **2F**	54.7665	0.5901	0 60	56.6609 55.3568	8	
Set 1 **1S**	50.9393	0.5371	0 60	52.4674 51.4772	32	
Set 1 **2S**	54.7665	0.5691	0 60	56.6534 55.3368	48	
Set 2 **1F**	56.1150	0.5432	0 60	57.2829 56.6597	60	**44 ± 14**
Set 2 **2F**	57.7462	0.5723	0 60	58.7582 58.3191	24	
Set 2 **1S**	56.1150	0.5213	0 60	57.3653 56.6375	48	
Set 2 **2S**	57.7462	0.5908	0 60	58.9734 58.3381	44	
Set 3 **1F**	50.9355	0.5864	0 60	52.9125 51.5220	4	**26 ± 21**

Set 3 **2F**	54.7696	0.5685	0 60	58.8125 58.3393	48	
Set 3 **1S**	50.9355	0.5433	0 60	52.8747 51.4798	40	
Set 3 **2S**	54.7696	0.5290	0 60	56.8035 55.2989	12	
Set 4 **1F**	56.1150	0.5189	0 60	58.0009 56.6359	80	**137 ± 55**
Set 4 **2F**	57.7462	0.5588	0 60	58.9350 58.3090	160	
Set 4 **1S**	56.1150	0.5923	0 60	58.0703 56.7099	105	
Set 4 **2S**	57.7462	0.5577	0 60	58.9111 58.3090	204	

APPENDIX F

Table F1 Total Dissolved Solids

Samples	Volume of sample used (ml)	Mass of petri dish (g)	Time taken (min)	Mass of petri dish + sample (g)	Total Dissolved solids (mg/L)	Total Dissolved Solids Mean Average with Standard Deviation (mg/L)
Before Treatment 1F	25	56.2915	0 60	80.2964 56.4184	5076	**5045 ± 1001**
Before Treatment 2F	25	57.3799	0 60	81.9782 57.5259	5840	
Before Treatment 1S	25	56.2915	0 60	80.2009 56.3821	3624	
Before Treatment 2S	25	57.3799	0 60	81.8757 57.5209	5640	
After Coagulation-Filtration Treatment						
Set 1	25	56.1129	0	80.6225	1324	**1588 ± 264**

1F			60	56.1460		
Set 1 **2F**	25	57.7444	0 60	81.9131 57.7933	1956	
Set 1 **1S**	25	56.1129	0 60	79.8748 56.1512	1532	
Set 1 **2S**	25	57.7444	0 60	75.8998 57.7829	1540	
Set 2 **1F**	25	57.1750	0 60	80.9225 57.2198	1792	**1782 ± 198**
Set 2 **2F**	25	55.4309	0 60	80.8836 55.4822	2052	
Set 2 **1S**	25	56.1123	0 60	80.1432 56.1520	1588	
Set 2 **2S**	25	57.7438	0 60	80.7136 57.7862	1696	
Set 3 **1F**	25	56.4255	0 60	80.6632 56.4715	1840	**1714 ± 156**

Set 3 **2F**	25	57.5206	0 60	81.9914 57.5591	1540	
Set 3 **1S**	25	56.4255	0 60	80.3271 56.4718	1852	
Set 3 **2S**	25	57.5206	0 60	81.1498 57.5612	1624	
Set 4 **1F**	25	57.1771	0 60	81.9133 57.2408	2548	**2815 ± 309**
Set 4 **2F**	25	55.4304	0 60	79.6595 55.5056	3008	
Set 4 **1S**	25	57.1771	0 60	82.3518 57.2410	2556	
Set 4 **2S**	25	55.4304	0 60	80.4508 55.5091	3148	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate						
Set 1 **1F**	25	54.7661	0 60	65.2453 54.7954	1172	**1379 ± 613**
Set 1	25	50.9384	0	63.8197	1140	

2F			60	50.9669		
Set 1 1S	25	54.7661	0 60	65.9054 54.7891	920	
Set 1 2S	25	50.9384	0 60	69.5532 50.9955	2284	
Set 2 1F	25	56.1146	0 60	80.8765 56.1497	1404	**1169 ± 344**
Set 2 2F	25	57.7456	0 60	79.3362 57.7761	1220	
Set 2 1S	25	56.1146	0 60	79.9724 56.1492	1384	
Set 2 2S	25	57.7456	0 60	82.0722 57.7623	668	
Set 3 1F	25	56.2915	0 60	79.5723 56.3211	1184	**1279 ± 212**
Set 3 2F	25	57.3795	0 60	80.6354 57.4113	1272	
Set 3 1S	25	56.2915	0	80.9876	1576	

			60	56.3309		
Set 3 **2S**	25	57.3795	0 60	79.1829 57.4066	1084	
Set 4 **1F**	25	57.1801	0 60	81.0856 57.2110	1236	**1911 ± 578**
Set 4 **2F**	25	55.4326	0 60	79.2332 55.4892	2264	
Set 4 **1S**	25	57.1801	0 60	79.8012 57.2211	1640	
Set 4 **2S**	25	55.4326	0 60	78.0965 55.4952	2504	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate						
Set 1 **1F**	25	54.7661	0 60	74.2309 54.7912	1004	**1377 ± 330**
Set 1 **2F**	25	50.9384	0 60	77.3267 50.9709	1300	
Set 1 **1S**	25	54.7661	0 60	74.6712 54.8011	1400	

Set 1 **2S**	25	50.9384	0 60	79.1870 50.9835	1804	
Set 2 **1F**	25	56.1146	0 60	77.4302 56.1321	700	**1270 ± 544**
Set 2 **2F**	25	57.7456	0 60	81.9076 57.7720	1056	
Set 2 **1S**	25	56.1146	0 60	79.1818 56.1643	1988	
Set 2 **2S**	25	57.7456	0 60	8.7655 57.7790	1336	
Set 3 **1F**	25	56.2915	0 60	78.9893 56.3123	832	**1067 ± 313**
Set 3 **2F**	25	57.3795	0 60	79.4510 57.4076	1124	
Set 3 **1S**	25	56.2915	0 60	79.5467 56.3287	1488	
Set 3 **2S**	25	57.3795	0 60	79.3209 57.4001	824	

Set 4 1F	25	57.1801	0	81.2015	1960	**1956 ± 290**
			60	57.2291		
Set 4 2F	25	55.4326	0	80.7913	2336	
			60	55.4910		
Set 4 1S	25	57.1801	0	80.9322	1632	
			60	57.2209		
Set 4 2S	25	55.4326	0	80.4598	1896	
			60	55.4800		

APPENDIX G

Table G1 Conductivity

Samples	Conductivity of Mature Leachate (ms/cm)	Conductivity Mean Average with Standard Deviation (ms/cm)
Before Treatment 1F	17.11	**17.62 ±0.63**
Before Treatment 2F	17.02	
Before Treatment 1S	18.21	
Before Treatment 2S	18.12	
After Coagulation-Filtration Treatment		
Set 1 1F	7.68	**9.24 ±2.20**
Set 1 2F	7.02	
Set 1 1S	11.02	
Set 1	11.24	

2S		
Set 2 **1F**	6.68	**10.13 ±4.05**
Set 2 **2F**	6.57	
Set 2 **1S**	13.61	
Set 2 **2S**	13.67	
Set 3 **1F**	6.68	**10.59 ±4.49**
Set 3 **2F**	6.72	
Set 3 **1S**	14.35	
Set 3 **2S**	14.63	
Set 4 **1F**	7.11	**12.32 ±5.45**
Set 4 **2F**	8.12	
Set 4 **1S**	17.04	
Set 4 **2S**	17.02	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate		

Set 1 **1F**	5.85	**4.04 ±2.13**
Set 1 **2F**	5.92	
Set 1 **1S**	2.05	
Set 1 **2S**	2.35	
Set 2 **1F**	5.44	**5.74 ±0.30**
Set 2 **2F**	5.52	
Set 2 **1S**	5.98	
Set 2 **2S**	6.03	
Set 3 **1F**	5.99	**5.45 ±0.69**
Set 3 **2F**	6.12	
Set 3 **1S**	4.81	
Set 3 **2S**	4.89	
Set 4 **1F**	9.30	**7.63 ±1.83**

Set 4 2F	9.12	
Set 4 1S	6.03	
Set 4 2S	6.05	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate		
Set 1 1F	2.70	**2.97 ±0.28**
Set 1 2F	2.74	
Set 1 1S	3.21	
Set 1 2S	3.23	
Set 2 1F	2.65	**3.23 ±0.59**
Set 2 2F	2.78	
Set 2 1S	3.67	
Set 2 2S	3.80	
Set 3 1F	4.90	**5.08 ±0.16**
Set 3	5.01	

2F		
Set 3 **1S**	5.23	
Set 3 **2S**	5.21	
Set 4 **1F**	4.98	**5.47 ±0.54**
Set 4 **2F**	5.02	
Set 4 **1S**	5.90	
Set 4 **2S**	5.98	

APPENDIX H

Table H1 Turbidity

Samples	Turbidity of Landfill Leachate (Ntu)	Turbidity Mean Average with Standard Deviation (Ntu)
Before Treatment **1F**	192.3	**235.05 ±48.28**
Before Treatment **2F**	194.2	
Before Treatment **1S**	275.5	
Before Treatment **2S**	278.2	
After Coagulation-Filtration Treatment		
Set 1 **1F**	21.0	**23.15 ±2.26**
Set 1 **2F**	21.4	
Set 1 **1S**	24.9	
Set 1 **2S**	25.3	

Set 2 **1F**	35.4	**38.50 ±2.72**
Set 2 **2F**	37.1	
Set 2 **1S**	40.2	
Set 2 **2S**	41.3	
Set 3 **1F**	20.5	**19.72 ±2.62**
Set 3 **2F**	23.1	
Set 3 **1S**	17.5	
Set 3 **2S**	17.8	
Set 4 **1F**	90.6	**103.52 ±11.52**
Set 4 **2F**	97.7	
Set 4 **1S**	112.4	
Set 4 **2S**	113.4	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate		
Set 1	5.7	**5.95 ±0.50**

1F		
Set 1 **2F**	6.7	
Set 1 **1S**	5.8	
Set 1 **2S**	5.6	
Set 2 **1F**	5.7	**7.55 ±1.86**
Set 2 **2F**	6.2	
Set 2 **1S**	9.4	
Set 2 **2S**	8.9	
Set 3 **1F**	3.9	**5.30 ±1.56**
Set 3 **2F**	4.0	
Set 3 **1S**	6.5	
Set 3 **2S**	6.8	
Set 4 **1F**	84.2	**99.37 ±17.37**
Set 4	85.3	

2F		
Set 4 1S	119.0	
Set 4 2S	109.0	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate		
Set 1 1F	4.3	**9.55 ±5.56**
Set 1 2F	5.2	
Set 1 1S	14.8	
Set 1 2S	13.9	
Set 2 1F	4.4	**6.95 ±2.58**
Set 2 2F	5.1	
Set 2 1S	9.6	
Set 2 2S	8.7	
Set 3 1F	6.6	**8.58 ±2.76**
Set 3 2F	5.8	

Set 3 1S	11.2	
Set 3 2S	10.7	
Set 4 1F	80.6	**73.58 ±8.46**
Set 4 2F	81.2	
Set 4 1S	66.0	
Set 4 2S	66.5	

APPENDIX I

Table I1 pH

Samples	**pH of Landfill Leachate**	**pH Mean Average with Standard Deviation for Landfill Leachate**
Before Treatment 1F	8.32	**8.37 ± 0.04**
Before Treatment 2F	8.42	
Before Treatment 1S	8.38	
Before Treatment 2S	8.36	
After Coagulation-Filtration Treatment		
Set 1 1F	7.97	**7.83 ± 0.19**
Set 1 2F	8.02	
Set 1 1S	7.73	
Set 1 2S	7.61	
Set 2	6.96	**6.89 ± 0.21**

1F		
Set 2 2F	7.17	
Set 2 1S	6.74	
Set 2 2S	6.71	
Set 3 1F	7.76	**7.64 ±0.16**
Set 3 2F	7.79	
Set 3 1S	7.55	
Set 3 2S	7.45	
Set 4 1F	6.35	**6.65 ± 0.30**
Set 4 2F	6.43	
Set 4 1S	6.83	
Set 4 2S	6.97	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate		
Set 1 1F	6.16	**6.53 ± 0.42**

Set 1 2F	6.18	
Set 1 1S	6.92	
Set 1 2S	6.89	
Set 2 1F	6.15	**6.51 ± 0.44**
Set 2 2F	6.13	
Set 2 1S	6.98	
Set 2 2S	6.81	
Set 3 1F	6.15	**6.28 ± 0.15**
Set 3 2F	6.18	
Set 3 1S	6.31	
Set 3 2S	6.50	
Set 4 1F	8.08	**7.45 ± 0.68**
Set 4 2F	8.01	

Set 4 **1S**	6.81	
Set 4 **2S**	6.90	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate with Aeration		
Set 1 **1F**	7.70	**7.66 ± 0.05**
Set 1 **2F**	7.69	
Set 1 **1S**	7.64	
Set 1 **2S**	7.59	
Set 2 **1F**	7.11	**7.36 ± 0.30**
Set 2 **2F**	7.09	
Set 2 **1S**	7.66	
Set 2 **2S**	7.59	
Set 3 **1F**	7.34	**7.37 ± 0.08**
Set 3 **2F**	7.28	
Set 3	7.43	

1S		
Set 3 2S	7.44	
Set 4 1F	6.83	**7.37 ± 0.64**
Set 4 2F	6.80	
Set 4 1S	7.90	
Set 4 2S	7.93	

APPENDIX J

Table J1 Total Organic Carbon (TOC)

Samples	Raw Data of TOC of Landfill Leachate (Ntu)	Average of TOC	TOC Mean Average with Standard Deviation for Landfill Leachate (Ntu)
Before Treatment 1F	320.97	324.91	**583.69 ± 510.00**
Before Treatment 2F	323.87		
Before Treatment 3F	329.89		
Before Treatment 1S	845.65	842.47	
Before Treatment 2S	842.73		
Before Treatment 3S	839.04		
After Coagulation-Filtration Treatment			
Set 1 1F	50.79	53.52	**56.80 ± 4.63**
Set 1 2F	53.67		
Set 1 3F	56.09		
Set 1 1S	61.29	60.08	
Set 1 2S	59.91		
Set 1 3S	59.03		

Set 2 1F	47.93	48.22	**35.40 ± 18.12**
Set 2 2F	46.93		
Set 2 3F	49.80		
Set 2 1S	24.65	22.59	
Set 2 2S	21.66		
Set 2 3S	21.47		
Set 3 1F	22.73	22.95	**17.61 ± 7.54**
Set 3 2F	21.83		
Set 3 3F	24.29		
Set 3 1S	13.02	12.28	
Set 3 2S	13.06		
Set 3 3S	10.76		
Set 4 1F	100.99	104.82	**243.57 ± 196.22**
Set 4 2F	103.34		
Set 4 3F	110.15		
Set 4 1S	382.46	382.32	
Set 4 2S	383.37		
Set 4 3S	381.14		
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate			
Set 1 1F	0	0	**0 ±0**
Set 1 2F	0		
Set 1 3F	0		
Set 1 1S	0	0	
Set 1 2S	0		

Set 1 3S	0		
Set 2 1F	4.16	4.82	**2.41 ± 3.40**
Set 2 2F	5.95		
Set 2 3F	4.34		
Set 2 1S	0	0	
Set 2 2S	0		
Set 2 3S	0		
Set 3 1F	0	0	**0 ±0**
Set 3 2F	0		
Set 3 3F	0		
Set 3 1S	0	0	
Set 3 2S	0		
Set 3 3S	0		
Set 4 1F	245.93	246.68	**130.13 ± 164.82**
Set 4 2F	248.72		
Set 4 3F	245.39		
Set 4 1S	13.58	13.58	
Set 4 2S	13.46		
Set 4 3S	13.70		
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate with Aeration			
Set 1 1F	0	0	**0.84 ±1.18**
Set 1 2F	0		
Set 1 3F	0		
Set 1 1S	1.23	1.67	

Set 1 2S	1.98		
Set 1 3S	1.81		
Set 2 1F	2.52	2.54	**1.27 ±1.79**
Set 2 2F	2.81		
Set 2 3F	2.29		
Set 2 1S	0	0	
Set 2 2S	0		
Set 2 3S	0		
Set 3 1F	0	0	**6.06 ±8.56**
Set 3 2F	0		
Set 3 3F	0		
Set 3 1S	12.46	12.11	
Set 3 2S	11.98		
Set 3 3S	11.90		
Set 4 1F	14.53	14.65	**37.42 ± 32.20**
Set 4 2F	14.55		
Set 4 3F	14.86		
Set 4 1S	59.60	60.19	
Set 4 2S	60.43		
Set 4 3S	60.54		

APPENDIX K

Table K1 Chemical Oxygen Demand (COD)

Samples	COD of Landfill Leachate (mg/L)	COD Mean Average with Standard Deviation for Landfill Leachate (mg/L)
Before Treatment 1F	1760	**2377.5 ±759.9**
Before Treatment 2F	1680	
Before Treatment 1S	3030	
Before Treatment 2S	3040	
After Coagulation-Filtration Treatment		
Set 1 1F	380	**242.5 ±170.5**
Set 1 2F	400	
Set 1 1S	90	
Set 1 2S	100	

Set 2 1F	180	**155.0 ±20.8**
Set 2 2F	160	
Set 2 1S	150	
Set 2 2S	130	
Set 3 1F	90	**140.0 ±70.2**
Set 3 2F	70	
Set 3 1S	210	
Set 3 2S	190	
Set 4 1F	510	**637.5 ±160.2**
Set 4 2F	490	
Set 4 1S	800	
Set 4 2S	750	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate		
Set 1	70	**69.0±1.8**

1F		
Set 1 **2F**	71	
Set 1 **1S**	67	
Set 1 **2S**	68	
Set 2 **1F**	30	**34.7 ±4.4**
Set 2 **2F**	32	
Set 2 **1S**	38	
Set 2 **2S**	39	
Set 3 **1F**	70	**74.7 ± 3.4**
Set 3 **2F**	78	
Set 3 **1S**	75	
Set 3 **2S**	76	
Set 4 **1F**	97	**193.5 ±165.8**
Set 4	107	

2F		
Set 4 **1S**	280	
Set 4 **2S**	290	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate		
Set 1 **1F**	83	**79.2 ±5.7**
Set 1 **2F**	85	
Set 1 **1S**	76	
Set 1 **2S**	73	
Set 2 **1F**	82	**86.5 ±5.8**
Set 2 **2F**	81	
Set 2 **1S**	91	
Set 2 **2S**	92	
Set 3 **1F**	64	**58.3 ±5.6**
Set 3 **2F**	62	

Set 3 1S	53	
Set 3 2S	54	
Set 4 1F	116	**121.5 ±5.9**
Set 4 2F	120	
Set 4 1S	120	
Set 4 2S	130	

APPENDIX L

Table L1 Total Ammonia Content (TAC)

Samples	TAC of Landfill Leachate (mg/L)	TAC Mean Average with Standard Deviation for Landfill Leachate (mg/L)
Before Treatment **1F**	351.5	**394.37 ± 43.07**
Before Treatment **2F**	363.1	
Before Treatment **1S**	432.3	
Before Treatment **2S**	430.6	
After Coagulation-Filtration Treatment		
Set 1 **1F**	159.9	**166.45 ± 15.03**
Set 1 **2F**	148.3	
Set 1 **1S**	178.3	
Set 1 **2S**	179.3	
Set 2	130.9	**144.40 ± 15.00**

1F		
Set 2 **2F**	132.5	
Set 2 **1S**	153.3	
Set 2 **2S**	160.9	
Set 3 **1F**	180.9	**196.07 ± 13.47**
Set 3 **2F**	190.2	
Set 3 **1S**	201.2	
Set 3 **2S**	212.0	
Set 4 **1F**	224.8	**273.52 ± 45.90**
Set 4 **2F**	243.9	
Set 4 **1S**	312.3	
Set 4 **2S**	313.1	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate		
Set 1 **1F**	112.1	**123.05 ± 21.31**

Set 1 2F	100.2	
Set 1 1S	148.6	
Set 1 2S	131.3	
Set 2 1F	103.7	**132.90 ± 41.97**
Set 2 2F	90.2	
Set 2 1S	172.3	
Set 2 2S	165.4	
Set 3 1F	121.9	**141.07 ± 36.13**
Set 3 2F	100.7	
Set 3 1S	179.4	
Set 3 2S	162.3	
Set 4 1F	219.7	**244.90 ± 28.54**
Set 4 2F	220.8	

Set 4 **1S**	272.1	
Set 4 **2S**	267.0	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate		
Set 1 **1F**	170.3	**139.68 ± 32.84**
Set 1 **2F**	161.2	
Set 1 **1S**	129.0	
Set 1 **2S**	98.2	
Set 2 **1F**	195.3	**148.88 ± 44.51**
Set 2 **2F**	178.6	
Set 2 **1S**	109.3	
Set 2 **2S**	112.3	
Set 3 **1F**	196.8	**155.25 ± 28.81**
Set 3 **2F**	151.3	
Set 3	140.9	

1S		
Set 3 2S	132.0	
Set 4 1F	267.8	**282.60 ± 25.25**
Set 4 2F	255.0	
Set 4 1S	307.9	
Set 4 2S	299.7	

APPENDIX M

Table M1 Biochemical Oxygen Demand (BOD)

Samples	BOD of Landfill Leachate (mg/L)	BOD Mean Average with Standard Deviation for Landfill Leachate (mg/L)
Before Treatment 1F	128	**428.5 ± 424.9**
Before Treatment 1S	729	
After Coagulation-Filtration Treatment		
Set 1 1F	46	**36.5 ± 13.4**
Set 1 1S	27	
Set 2 1F	31	**31.0 ± 0**
Set 2 1S	31	
Set 3 1F	45	**48.5 ± 4.9**
Set 3 1S	52	
Set 4	55	**319.0 ± 373.3**

1F		
Set 4 **1S**	583	
After Coagulation-Filtration-Microalgae treatment using *Chlorella Vulgaris* in 100% Treated Leachate		
Set 1 **1F**	22	**22.5 ± 0.7**
Set 1 **1S**	23	
Set 2 **1F**	21	**38.0 ± 24.0**
Set 2 **1S**	55	
Set 3 **1F**	30	**42.5 ± 17.6**
Set 3 **1S**	55	
Set 4 **1F**	334	**256.0 ±110.3**
Set 4 **1S**	178	
After Coagulation-Filtration-Microalgae treatment using *Botryococcus sudeticus* in 100% Treated Leachate		
Set 1 **1F**	152	**153.5 ± 2.1**
Set 1 **1S**	155	

Set 2 **1F**	58	**54.0 ± 5.7**
Set 2 **1S**	50	
Set 3 **1F**	42	**44.5 ± 3.5**
Set 3 **1S**	47	
Set 4 **1F**	488	**499.0 ± 15.6**
Set 4 **1S**	510	

APPENDIX N

Table N1 Heavy Metal Contents using ICP-MS (1st Trial)

Sample	Heavy metal contents in Mature Leachate								
	Na (ppb)	Mg (ppb)	Al (ppb)	K (ppb)	Ca (ppb)	Fe (ppb)	Mn (ppb)	Zn (ppb)	Cu (ppb)
Mature Leachate (ML) Before Treatment	1809.668	143.888	178.921	1015.667	156.723	61.502	3.134	50.723	88.742
Mature Leachate After Coagulation-Filtration Treatment									
ML (1)	1362.747	75.453	19.900	86.271	64.060	51.408	2.538	49.401	88.645
ML (2)	1271.789	64.088	5.726	56.634	94.756	51.196	2.602	50.375	88.659
ML (3)	1129.778	62.669	27.208	90.731	129.848	53.770	1.390	49.284	88.549
ML (4)	1784.576	100.866	158.877	672.005	147.170	59.193	3.019	50.614	88.362
Mature Leachate After Coagulation-Filtration-Microalgae Treatment using *Chlorella vulgaris* (CV)									
ML1 CV	103.903	19.550	13.435	4.418	6.692	4.062	0.293	5.860	5.533
ML2 CV	152.986	15.747	2.560	3.211	58.700	7.773	1.505	12.869	1.466
ML3 CV	90.301	18.809	12.759	5.048	42.343	3.594	0.722	3.654	10.350
ML4 CV	1523.569	91.193	98.857	643.055	108.158	51.650	1.259	48.626	70.171
Young Leachate and Mature Leachate After Coagulation-Filtration-Microalgae Treatment using *Botryococcus sudeticus* (BS)									

ML1 BS	**265.444**	**74.607**	**12.037**	**4.019**	**52.327**	**50.983**	**1.022**	**40.344**	**2.380**
ML2 BS	**35.001**	**12.169**	**3.499**	**2.645**	**5.608**	**3.190**	**0.353**	**10.263**	**0.322**
ML3 BS	**40.190**	**20.802**	**5.989**	**2.570**	**4.559**	**4.616**	**0.670**	**13.907**	**0.417**
ML4 BS	**1543.661**	**100.475**	**148.308**	**61.525**	**127.571**	**53.238**	**3.003**	**49.086**	**67.249**

Table N2 Heavy Metal Contents using ICP-MS (2nd Trial)

Sample	Heavy metal contents in Mature Leachate (ppb)								
	Na (ppb)	Mg (ppb)	Al (ppb)	K (ppb)	Ca (ppb)	Fe (ppb)	Mn (ppb)	Zn (ppb)	Cu (ppb)
Mature Leachate (ML) Before Treatment	2132.911	80.482	80.261	1793.923	137.309	38.372	7.130	121.503	97.117
Mature Leachate After Coagulation-Filtration Treatment									
ML (1)	593.454	44.168	8.131	463.322	41.720	20.119	2.330	121.528	5.046
ML (2)	402.399	24.631	25.142	308.628	1.970	29.328	1.502	120.423	35.868
ML (3)	681.726	43.887	6.563	511.450	38.967	19.097	1.849	119.751	16.476
ML (4)	787.872	49.777	35.274	653.891	86.562	35.179	3.405	121.160	77.909
Mature Leachate After Coagulation-Filtration-Microalgae using *Chlorella vulgaris* (CV)									
ML1 CV	134.154	69.285	4.733	9.980	67.335	7.884	2.401	1.788	0.330
ML2 CV	63.911	56.731	3.446	17.841	49.832	7.577	3.443	0.086	2.399
ML3 CV	44.624	29.220	1.961	8.290	35.207	3.755	0.984	20.469	1.214
ML4 CV	987.708	74.352	3.483	14.727	79.534	20.495	3.111	20.551	2.879
Mature Leachate After Coagulation-Filtration-Microalgae using *Botryococcus sudeticus* (BS)									

ML1 BS	**657.819**	**36.607**	**2.037**	**1.072**	**51.327**	**7.983**	**1.922**	**20.344**	**3.802**
ML2 BS	**41.599**	**7.145**	**6.105**	**1.829**	**59.805**	**6.802**	**3.703**	**20.286**	**2.873**
ML3 BS	**25.700**	**12.802**	**5.989**	**3.243**	**11.559**	**5.616**	**0.320**	**19.907**	**4.166**
ML4 BS	**25.252**	**60.475**	**8.308**	**15.695**	**60.571**	**13.238**	**3.393**	**20.086**	**24.927**

Table N3 Heavy Metal Contents using ICP-MS Mean Average with Standard Deviation

Sample	Heavy metal contents Mean Average with Standard Deviation (ppb)								
	Na (ppb)	Mg (ppb)	Al (ppb)	K (ppb)	Ca (ppb)	Fe (ppb)	Mn (ppb)	Zn (ppb)	Cu (ppb)
Mature Leachate (ML) Before Treatment	1971.289 ± 228.567	112.185 ± 44.834	129.591 ± 69.763	1404.795 ± 550.310	147.016 ± 13.727	49.937 ± 16.355	5.132 ± 2.825	86.113 ± 50.049	92.929 ± 5.922
Mature Leachate After Coagulation-Filtration Treatment									
ML (1)	978.100 ± 543.972	59.810 ± 22.121	14.015 ± 8.321	274.796 ± 266.615	52.89 ± 15.796	35.763 ± 22.124	2.434 ± 0.147	85.464 ± 51.001	46.845 ± 59.113
ML (2)	837.094 ± 614.751	44.359 ± 27.900	15.434 ± 13.729	182.631 ± 178.186	48.363 ± 65.609	40.262 ± 15.463	2.052 ± 0.777	85.399 ± 49.531	62.263 ± 37.328
ML (3)	905.752 ± 316.820	53.278 ± 13.280	16.885 ± 14.598	301.090 ± 297.493	65.909 ± 90.423	36.433 ± 24.517	1.619 ± 0.324	84.517 ± 49.827	52.512 ± 50.963
ML (4)	1286.224 ± 704.776	75.321 ± 36.125	97.075 ± 87.400	662.948 ± 12.808	116.866 ± 42.856	47.186 ± 16.980	3.212 ± 0.272	85.887 ± 49.883	83.135 ± 7.391
Mature Leachate After Coagulation-Filtration-Microalgae using *Chlorella vulgaris* (CV)									
ML1 CV	119.028 ± 21.390	44.417 ± 35.167	9.029 ± 6.075	7.199 ± 3.932	37.013 ± 42.881	5.973 ± 2.702	1.347 ± 1.490	3.824 ± 2.879	2.931 ± 3.679
ML2 CV	108.448 ± 62.985	36.239 ± 28.980	3.003 ± 0.626	10.526 ± 10.344	54.266 ± 6.270	7.675 ± 0.138	2.474 ± 1.370	6.477 ± 9.038	1.932 ± 0.659

ML3 CV	**67.462 ± 32.298**	**24.014 ± 7.361**	**7.36 ± 7.635**	**6.669 ± 2.292**	**38.775 ± 5.045**	**27.622 ± 33.980**	**0.853 ± 0.185**	**12.061 ± 11.890**	**5.782 ± 6.460**
ML4 CV	**1255.638 ± 378.910**	**82.772 ± 11.908**	**51.17 ± 67.439**	**328.891 ± 444.294**	**93.846 ± 20.240**	**36.072 ± 22.029**	**2.185 ± 1.309**	**34.588 ± 19.852**	**36.525 ± 47.582**
Mature Leachate After Coagulation-Filtration-Microalgae using *Botryococcus sudeticus* (BS)									
ML1 BS	**157.819 ± 152.204**	**55.607 ± 26.870**	**3.028 ± 1.401**	**2.545 ± 2.083**	**51.827 ± 0.707**	**29.483 ± 30.405**	**1.472 ± 0.636**	**30.344 ± 14.142**	**3.091 ± 1.005**
ML2 BS	**38.300 ± 4.665**	**9.655 ± 3.554**	**4.375 ± 2.446**	**2.237 ± 0.576**	**32.706 ± 38.323**	**4.996 ± 2.554**	**2.028 ± 2.368**	**15.274 ± 7.087**	**1.597 ± 1.803**
ML3 BS	**32.945 ± 10.245**	**16.802± 5.656**	**4.279 ± 2.417**	**2.906 ± 0.475**	**8.059 ± 4.949**	**5.116 ± 0.707**	**0.495 ± 0.247**	**16.907 ± 4.242**	**2.291 ± 2.650**
ML4 BS	**784.456± 1073.677**	**80.475 ± 28.284**	**34.916 ± 37.630**	**314.11 ± 422.022**	**94.071 ± 47.376**	**33.238 ± 28.284**	**3.198 ± 0.275**	**34.586 ± 20.506**	**46.088 ± 29.926**

APPENDIX O

Table O1 List of Formula

Analysis	Formula
Total Solids	$\frac{(A-B)\times 1000}{sample\ volume, mL}$ A - Mass of petri dish + sample (mg) B- Mass of petri dish (mg) Sample Volume- 25 mL
Total Suspended Solids	$\frac{(A-B-C)\times 1000}{sample\ volume, mL}$ A - Mass of petri dish + dried residue (mg) B- Mass of petri dish (mg) C- Mass of filter paper (mg) Sample Volume- 25 mL
Total Dissolved Solids	● $\frac{(A-B)\times 1000}{sample\ volume, mL}$ A - Mass of petri dish + sample (mg) B- Mass of petri dish (mg) Sample Volume- 25 mL

Chlorophyll and Carotenoid Contents	Ethanol with 5% (v/v) of water : Chlorophyll a, $C_a\ (\frac{\mu g}{mL}) =$ $13.36A_{664} - 5.19A_{648}$ Chlorophyll b, $C_b\ (\frac{\mu g}{mL}) =$ $27.43A_{648} - 8.12A_{664}$ Sum of leaf carotenoid, $C_{(x+c)}\ (\frac{\mu g}{mL})$ $= \frac{1000A_{470} - 2.13Ca - 97.64Cb}{209}$