TREATMENT OF LEACHATE BY *SCENEDESMUS* SP. VIA DIFFERENT MODES-TYPES OF BIOREACTORS AND ITS POTENTIAL FOR BIOETHANOL PRODUCTION

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2023

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by

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Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

August 2023

ACKNOWLEDGEMENT

I would like to express my gratitude to all those who contributed and supported me during my study for the Ph.D. program. First and always, all praise to Allah SWT, our lord and the creator of the universe, for His Blessed, mercy, guidance, and a chance for me to complete my study. Secondly, the Prophet Muhammad SAW, who the lead us to the light of this era, may the peace and blessings of Allah be upon him.

I would like to express my gratitude to my Adviser for this thesis, Associate Professor Dr. Husnul Azan Tajarudin for the opportunity to study and research under his guidance. I'm really grateful for his patience, encouragement, and support over my time to help me complete my thesis. I could not have imagined having a pleasant adviser, thanks are not enough for him but I ask Allah to protect him and put him in the highest ranking. and not forgotten to say thanks to my co-supervisors Prof. IR. Dr. Abdul Latif Ahmad, and Prof. Dr. Maznah Omar, also special thanks to my previous supervisor in my master's degree Dr. Yusuf Wibisono for his support and helpful guidance during my study. I am especially indebted to my thesis committee members for their input and valuable discussions. For my beloved parents, brothers, and sisters especially my late brother for their never-ending love and support. Without their prayers all the time, I could not finish my study. Also, I thank, my beloved wife and daughters for the help, support, and praying over time for me. For my laboratory mates, especially (Charles and Miza) thank you so much for all the moments you shared with me. They supported me in the easier adaption of my experiment. Not forgetting to say thanks a lot to my best friend in Indonesia Lita. And last, special thanks to all the students at USM who helped me, thanks a lot for their effort and support in my graduate study.

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LIST OF ABBREVIATIONS

˚C	Degree Celsius
%	Percentage
μ_{max}	Maximum specific growth rate
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CO_2	Carbon Dioxide
FID	Flame Ionization Detection
g	Gram
GC	Gas Chromatography
GHG	Greenhouse Gas
HCL	Hydrochloric Acid
He	Helium
H_2SO_4	Sulfuric Acid
kPa	Kilopascal
ks	Monod semi saturation constant
Min	Minute
mL	Milliliter
MSW	Municipal Solid Waste
NH3-N	Ammonium Nitrogen
Pb	Lead
Ni	Nickel
nm	Nanometer
OD	Optical Density
mg	milligram

MBR	Membrane bioreactor
Co	Cobalt
Zn	Zinc
Cr	Chromium
Pb	Lead
Ni	Nickel
nm	Nanometer
OD	Optical Density
S. cerevisiae	*Saccharomyces cerevisiae*
SS	Suspended Solids
TKN	Total Kjeldahl Nitrogen
v/v	Volume percent
YEPG	Yeast Extract Peptone Glucose

LIST OF SYMBOLS

μ	Specific growth rate (h^{-1})
P	Ethanol concentration (mg/L)
mg/L	Milligrams per liter
TD	Doubling Time (h)
q_s	Specific rate of COD uptake (mg COD/mg cell/day)
S	Substrate concentration (mg/L)
S_i	Inlet substrate concentration (mg/L)
m_s	Maintenance coefficient (mg COD/mg cell/day)
X	Biomass concentration (mg/L)
$Y_{P/S}$	Product yield coefficient (mg ethanol mg^{-1} substrate)
$Y_{x/s}$	Biomass yield coefficient (mg biomass g^{-1} substrate)

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RAWATAN UNTUK LARUT RESAPAN OLEH *SCENEDESMUS* SP. MELALUI MOD BERBEZA – JENIS BIOREAKTOR DAN POTENSINYA UNTUK PENGHASILAN BIOETANOL

ABSTRAK

Bagi memenuhi keperluan manusia di samping menjaga alam sekitar, penggunaan sumber bahan yang mampan adalah penting, kajian ini memfokuskan kepada penyiasatan *Scenedesmus* sp. yang dihidupkan dalam kepekatan polisakarida (kanji) yang berbeza-beza, diikuti dengan penggunaannya dalam rawatan bahan larut lesap. Objektif utama kajian ini adalah untuk menentukan kepekatan optimum polisakarida bagi pertumbuhan *Scenedesmus* sp., menilai dari kecekapannya dalam merawat bahan larut resapan tapak pelupusan perbandaran menggunakan mod pertumbuhan secara kelompok, berterusan dan membran, dan meneroka potensi untuk pengeluaran bioetanol. Metodologi melibatkan beberapa peringkat, termasuk pertumbuhan *Scenedesmus* sp. dengan kepekatan polisakarida yang berbeza untuk mengenal pasti keadaan pertumbuhan yang ideal. Selepas itu, pertumbuhan kelompok dijalankan dengan tahap pH yang berbeza-beza, manakala penapaian berterusan melibatkan pencairan yang berbeza. Pertumbuhan membran telah dijalankan pada pelbagai kadar aliran. Biojisim alga yang terhasil kemudiannya tertakluk kepada penapaian, dengan dan tanpa sonikasi, pada nilai pH yang berbeza untuk menghasilkan bioetanol. Keputusan menunjukkan bahawa kepekatan optimum 0.8 g polisakarida menghasilkan pertumbuhan tertinggi *Scenedesmus* sp. Dalam mod kelompok, pH 7.0 menunjukkan peratusan penyingkiran yang paling berkesan untuk COD, nitrogen, fosforus dan karbohidrat. Dalam mod berterusan, penyingkiran COD, karbohidrat, fosforus, dan nitrogen tertinggi berlaku pada kadar pencairan

0.220 j^{-1}, disertai dengan kadar pengambilan COD spesifik tertinggi. Begitu juga, kadar penyerapan zink, kobalt, nikel, plumbum dan kromium yang paling besar diperhatikan pada kadar pencairan ini. Produktiviti maksimum untuk penyingkiran logam berat dan nutrien dilaporkan pada 0.220 j^{-1}. Untuk mod pertumbuhan membran, kadar aliran 0.480 L/j menghasilkan penyingkiran COD, karbohidrat, nitrogen, fosforus dan logam berat yang paling tinggi, serta produktiviti puncak untuk kedua-dua logam berat dan nutrien. Produktiviti biojisim meningkat daripada 0.240 L/j kepada 0.480 L/j, mencapai titik tertinggi 137.729 mg/L pada kadar aliran 0.420 L/j. Walau bagaimanapun, apabila kadar aliran meningkat melebihi 0.420 L/j, produktiviti biojisim merosot kepada 118.096 mg/L/j. Bagi pengeluaran etanol daripada biojisim terawat, biojisim tanpa sonikasi dengan 9.026 mg/L etanol, kepekatan biojisim 3.300 µg/L, dan pH 5.0 adalah lebih tinggi daripada biojisim dengan sonikasi dengan 5.562 mg/L etanol, kepekatan biojisim 0.110 µg/L, dan pH 5.0. Oleh itu, adalah terbukti bahawa bahan larut lesap mempunyai potensi untuk dirawat oleh *Scenedesmus* sp. dan ditukar kepada bioetanol selaras dengan konsep bahan lestari.

TREATMENT OF LEACHATE BY *SCENEDESMUS* SP. VIA DIFFERENT MODES-TYPES OF BIOREACTORS AND ITS POTENTIAL FOR BIOETHANOL PRODUCTION

ABSTRACT

In order to address human needs while safeguarding the environment, the utilization of sustainable material sources is imperative, this study focuses on the investigation of *Scenedesmus* sp. cultivation with varying concentrations of polysaccharide (starch), followed by its application in leachate treatment. The main objectives of this study were to determine the optimal concentration of polysaccharide for *Scenedesmus* sp. growth, evaluate its efficiency in treating municipal landfill leachate using batch, continuous, and membrane cultivation modes, and explore its capacity for bioethanol production. The methodology involved several stages, including the cultivation of *Scenedesmus* sp. with different concentrations of polysaccharide to identify the ideal growth condition. Subsequently, batch cultivation was conducted with varying pH levels, while continuous cultivation involved different dilutions. Membrane cultivation was carried out at various flow rates. The resulting algal biomass was then subjected to fermentation, with and without sonication, at different pH values to produce bioethanol. The results indicated that an optimal concentration of 0.8 g of polysaccharide yielded the highest growth of *Scenedesmus* sp. In batch cultivation, a pH of 7.0 exhibited the most effective removal percentages for COD, nitrogen, phosphorus, and carbohydrates. In continuous cultivation, the highest removal of COD, carbohydrate, phosphorus, and nitrogen occurred at a dilution rate of 0.220 h^{-1}, accompanied by the highest specific rate of COD uptake. Likewise, the greatest

absorption rates of zinc, cobalt, nickel, lead, and chromium were observed at this dilution rate. The maximum productivity for removing heavy metals and nutrients was reported at 0.220 h^{-1}. For membrane cultivation, a flow rate of 0.480 L/h resulted in the highest removal of COD, carbohydrates, nitrogen, phosphorus, and heavy metals, as well as the peak productivity for both heavy metals and nutrients. Biomass productivity increased from 0.240 L/h to 0.480 L/h, reaching its highest point of 137.729 mg/L at a flow rate of 0.420 L/h. However, as the flow rate increased beyond 0.420 L/h, biomass productivity declined to 118.096 mg/L/h. As for ethanol production from treated biomass, biomass without sonication with 9.026 mg/L ethanol, a biomass concentration of 3.300 µg/L, and pH 5 were higher than biomass with sonication with 5.562 mg/L ethanol, a biomass concentration of 0.110 µg/L, and pH 5.0. Therefore, it is evident that the leachate has the potential to be treated by *Scenedesmus* sp. and converted to bioethanol in line with the concept of sustainable materials.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Globally, landfilling has taken over as the most popular and important method of getting rid of municipal solid waste. A landfill serves as a sizable anaerobic bioreactor that may accommodate a variety of chemical, physical, and biological systems (Lebron et al., 2021). Due to the production of leachate, which endangers groundwater, surface water, the environment, and human health, landfills have been an issue for most nations for decades. Leachate is a liquid that is obtained from the breakdown of solid waste and contains a wide variety of organic and inorganic substances (Wong, 2017), thanks to the combined action of rainwater and naturally occurring fermentation of the secreted waste (Gutiérrez et al., 2022). leachate It can be specified based on several factors, like pH, heavy metals, total dissolved solids (TDS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD). It may also contain heavy metals, organic contaminants, mineral salts, and nitrogen compounds (W. Chen et al., 2021). The leachate composition varies from dump to landfill and is influenced by factors like the age of the landfill and the type of garbage (Nabi et al., 2022). Leachate can also be classified as old, medium, or young depending on the age of the landfill (Costa et al., 2019).

Leachate treatment technologies have increased recently in several nations. These technologies, which can be divided into chemical, physical, and biological treatments, heavily depend on the physicochemical conditions of the leachate to decide the best treatment to use (Arij et al., 2018). In Malaysia, leachate treatment technologies that are effective and long-lasting are lacking, whereas conventional biological processes are costly, take a long time to complete, and produce a significant amount of carbon (Anis et al., 2019) . Young leachates with a high organic content have responded well to biological treatments, which have led to the production of useful products like biogas and fertilizer (Talebi et al., 2020). This sort of treatment uses certain microorganisms to increase and develop the leachate treatment ability (El Ouaer et al., 2020). Leachate treatment with algae is thus an alternative to traditional biological treatments.

Leachate treatment with algae has drawn a lot of interest from academics due to its many benefits, including its high energy generation capacity, particularly biogas, as well as its fast growth rate and simplicity of cultivation. Algae has higher production rates and can grow 5 to 10 times under ideal conditions faster than terrestrial materials (Dogaris et al., 2020). Additionally, it is inexpensive because algae are abundant and resilient to harsh settings (Al-Dahhan et al., 2018), also algae may grow in arid environments, like deserts and coastal plains (de Souza & Lima, 2021). Additionally, because microalgae don't contain lignin, it is simple to convert them into monosaccharides for ethanol synthesis (Surendhiran et al., 2019). Algae serve the dual purpose of concurrently digesting contaminants and producing beneficial bioproducts while treating leachate (Nawaz et al., 2020). These characteristics make algae a suitable medium for the synthesis of ethanol and the treatment of leachate.

Scenedesmus sp. is one of the most promising algal species utilized as a feedstock for ethanol production. According to Sami (Abobaker et al., 2022), among some microalgae, *Scenedesmus* sp. can grow to the highest biomass concentration even when grown in a heterotrophic environment. *Scenedesmus* sp. is chosen for microalgae investigations because of other advantageous traits such as quick growth, CO2 fixation, the capacity to grow in wastewater, and the capacity to accumulate lipids. Another helpful microorganism for ethanol synthesis from algae-based leachate remediation is yeast. It is well known that yeast may remove organic material, obviating the need for additional nutrient removal procedures (Walls et al., 2019). Additionally, yeast is playing essential for the fermentation process that turns carbohydrates into ethanol.

Numerous research has discussed on the use of combined the potential microalgae cultivation and yeast for wastewater treatment and the production of useful products. Examples of applications of this strategy include the pilot-scale treatment of urban wastewater and production of biodiesel (Iasimone et al., 2018), yeast industry liquid digestate treatment and biofuel production (Qin et al., 2019), the production of ethanol from synthetic wastewater and municipal wastewater (Romero-Frasca et al., 2021), and the production of bioethanol from municipal wastewater (Walls et al., 2019). However, little study has been done on the use of microalgae cultivation and yeast fermentation for enhanced leachate treatment and ethanol generation. Valuable products, such as ethanol, can be extracted coupled with waste generation reduction and resource recovery to assure the process sustainability.

In this study, to determine the impact of the concentration of carbohydrates on *Scenedesmus* sp. growth before treatment of leachate by *Scenedesmus* sp., starch was selected as the medium due to starch being a polysaccharide (complex carbohydrate), and leachate contains a lot of polysaccharides. Five samples with various starch concentrations were selected for *Scenedesmus* sp. cultivation. After that *Scenedesmus* sp. was used to treat landfill leachate by *Scenedesmus* sp. cultivation via three different modes batch, continuous, and membrane. Then subsequently treated leachate was used as a substrate for fermentation by the yeast *Saccharomyces cerevisiae* to produce ethanol. Finding a long-term solution for landfill leachate that can advance energy research was the goal of this project. A sustainable method of manufacturing ethanol from renewable energy sources may be possible if algae and yeast are combined with leachate as a substrate.

1.2 Problem Statement

Leachate is one of the dangerous wastes that are removed from the collection of solid waste and are in a liquid form system (Lin et al., 2022). Consequently, a method for treating this kind of waste is required to safeguard our ecosystem and subsurface water due to the danger posed by this substance. The earlier researcher demonstrates the results of their successful study to treat leachate using a biological approach, such as using algae or bacteria. But this process also results in biomass and supernatant.

The current leachate management problem is the main objective for all countries, most of them are trying to find the best way to treat leachate and take advantage, also reduce the contamination. Some of the studies tried to treat the leachate using physical and chemical treatment, but these methods could not completely replace the biological treatment due to the operational cost constraint. landfill leachate especially old ones are very difficult to treat using conventional biological processes during batch bioreactors only, leachate consists of complex carbohydrates as polysaccharides, which must know its effect on the growth of *Scenedesmus* sp. before the treatment. To this end, continuous and membrane bioreactors have proven to be promising alternatives, membrane bioreactors offer many potential advantages over batch and continuous in that they allow the removal of end products from the culture while retaining the cells in the reactor, which increase the ability to remove nutrients and heavy metals. Most contemporary treatments merely concentrate on the treatment and do not take into account any useful products that may come from their process, which makes focusing on the products which can come from the treatment process essential to benefit from them.

1.3 Objective

1) To investigate the growth of *Scenedesmus* sp. during its cultivation with different concentrations of polysaccharide (starch) before cultivating it in leachate to know the effect of polysaccharide on *Scenedesmus* sp. growth.

2) To study the treatment of leachate by *Scenedesmus* sp. and compare the productivity of biomass, nutrients, and heavy metals during different modes of cultivation via a batch bioreactor, continuous bioreactor, and membrane bioreactor.

3) To investigate ethanol production from treated leachate (Sonicated biomass & Biomass without sonication) using fermentation process by *Saccharomyces cerevisiae* and compare kinetic growth and productivity of *Saccharomyces cerevisiae* and ethanol in two type of substrates fermentation for biomass of *Scenedesmus sp.* without sonication and biomass of *Scenedesmus* sp. with sonication.

1.4 Scope of Study

This research is conducted on a laboratory scale. The study focused on following step by step the effect of carbohydrate concentration on *Scenedesmus* sp. growth during its cultivation, which starch was selected as polysaccharide and *Scenedesmus sp.* was cultivated with different concentrations of starch, also treatment leachate by *Scenedesmus* sp. using different cultivation modes, besides investigating ethanol production from treated biomass with sonication and without sonication after the fermentation of treated leachate with *Saccharomyces cerevisiae* in the complete absence of oxygen.

1.5 Novelty of Research

Mostly, the treatment of leachate at landfill uses a combination method which is physical, chemical, and biological methods. Normally, the biological method is in the secondary stage. However, the application of chemicals contributes to the production of scheduled waste. At this moment not too many reports about the combination of physical and biological methods only and most of the recent treatments just only focus on treatment and do not consider valuable product produces from their process. This research focuses to treat leachate using *Scenedesmus* sp. which is from a fresh algae group. Until now, not too many documents elaborate on this species to treat leachate. Then, this research also investigates leachate treatment and biomass production capability by batch, continuous, and membrane bioreactors. Then, as added value for the treatment of leachate, this research explores about the application of biomass of *Scenedesmus* sp. for ethanol production in the batch bioreactor.

1.6 Thesis Outline

This thesis comprises five chapters. Chapter 1 Introduction, it discussed the background for the research was outlined together with the problem statements and objectives of this research project. In Chapter 2, background information on landfill leachates, characteristics, and ways of treatment were thoroughly discussed. Also, the cultivation, metabolism, and harvesting of *Scenedesmus* sp. were explained. Thereafter, the cultivation of microalgae was reviewed in different bioreactors. This was followed by the metabolism of *Saccharomyces cerevisiae* and its fermentation activities towards ethanol production were reviewed. In Chapter 3 Material and Methods, and the overall experimental design was outlined. Also, all the methods and materials involved in this research were described in detail. In Chapter 4 Results and Discussion, all results and discussion about the effect of polysaccharide on *Scenedesmus* sp. growth, and the treatment of leachate by *Scenedesmus* sp. during different modes via batch, continuous, and membrane bioreactors. Also compare the productivity of biomass, nutrients, and heavy metals during different modes of cultivation. In addition, study the kinetic growth and productivity of *Saccharomyces cerevisiae* for ethanol in two types of substrates: fermentation for biomass of *Scenedesmus* sp. without sonication and biomass of *Scenedesmus* sp. with sonication. In Chapter 5 Conclusion and Recommendations, the research project's findings were summarized, along with suggestions for more investigation in relation to this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, background information on landfill leachates, characteristics, and ways of treatment were thoroughly discussed. Also, the cultivation, metabolism, and harvesting of *Scenedesmus* sp. were explained. Thereafter, the cultivation of microalgae was reviewed in different bioreactors. This was followed by the metabolism of *Saccharomyces cerevisiae* and its and fermentation activities towards ethanol production were reviewed.

2.1.1 Leachate

Leachate is a hazardous liquid produced during the degradation of solid waste biochemically in a bioreactor landfill when it is semi-solid or solid (Mohammad et al., 2022). Landfill leachate is contaminated aqueous effluent from a landfill caused by rain percolation, moisture in deposited waste, groundwater runoff, and biodegradation. Organic materials, ammoniacal nitrogen compounds and toxic metals are commonly found in landfill leachate (Elmaadawy et al., 2020). Ongoing urbanization, combined with the unchecked increase in population in urban areas and higher living standards, has resulted in massive amounts of municipal solid waste generation (Vyas et al., 2022). Municipal solid waste (MSW) generation is expected to rise significantly by 19 percent in developed countries and 40 percent in developing nations, respectively, culminating in a total estimated generation of over 3 billion tones. by 2050, a billion tones (Kaza et al., 2018).

Decomposition of waste composite generally occurs in landfills via a variety of biological and chemical processes that follow the inceptive aerobic stage, the anaerobic acidogenic aspect, the methanogenic process, and the stabilization phase (Qiao et al., 2018).

However, the basic parts of a landfill, as shown in Figure 2.1, are:

- Bottom liner system - separates waste from groundwater and any resulting leachate.
- Cells (old and new) - where the garbage is kept within the landfill.
- Storm water drainage system - assembles rainwater falling on the landfill.
- Leachate collection system - assembles water that has percolated through the landfill itself and has contaminating substances (leachate).
- Methane collection system - assembles methane gas produced by the decomposition of rubbish.
- Covering or cap - closes off the landfill's top.

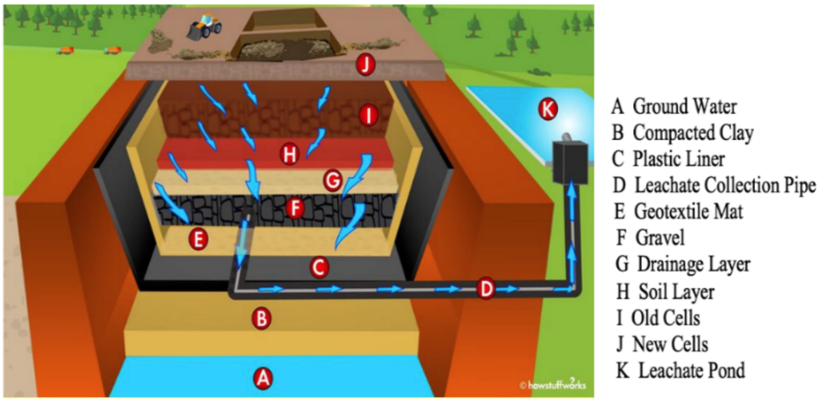

Figure 2.1 The structure of a municipal solid waste landfill.

2.1.2 The characteristic and impact of leachate

Rain, evaporation and transpiration, surface runoff, subsurface infiltration, and the relative density within the landfill are all factors that impact the amount of leachates produced (Miao et al., 2019). Because irregular landfill leachate is not maintained properly, stored, or managed, the underlying soil ecosystem is directly affected, providing long-term harm to ecological systems, agricultural processes, and human health. (Gu et al., 2022). Different approaches, such as cover layers, and water layers, have been utilized to control water entry into the landfill to help in the reduction of leachates. (Costa et al., 2019).

Samples of leachates collected from various landfill sites and subjected to diverse environmental conditions over the course of several years reveal significant variations in components and volume of water (Sackey et al., 2020). However, multiple physico-chemical indices such as suspended solids (SS), , chemical oxygen demand (COD), pH, ammonia, total nitrogen (TN), chloride, phosphorus, heavy metals, biochemical oxygen demand (BOD) and alkalinity can be used to define the quality of landfill leachates (Khasawneh et al., 2022).

Previously some researchers demonstrated that the total properties of the leachate would be influenced by (i) the type of physico-biochemical process, such as dissolution, precipitation, adsorption, dilution due to rainwater infiltration, and volatilization of substances that take place in the Bioreactor landfill (ii) climatic and hydrogeological situations that influence the actions of microbes, (iii) rainwater infiltration through the top valve, (iv) prevalent lifestyle of the populace in terms of waste management practice (Baettker et al., 2020; Vaccari et al., 2019; Wdowczyk & Szymańska-Pulikowska, 2020; Yang et al., 2019). There are four types of leachate contaminants, as shown in Table 2.1

Table 2.1 Types of leachate contaminants.

	Type of leachate pollutant	Examples	References
1.	Dissolved organic matter	volatile fatty acids, as well as more resistant organic compounds like humic and	(Kumar et al., 2023)

		fulvic acid.	
2.	Macro inorganic components	Ammonia (NH_4^+-N), Sodium (Na^+), Potassium (K^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Manganese (Mn^{2+}), Iron (Fe^{2+}), Chloride (Cl^-), Sulphate (SO_4^{2-}), and Hydrogen carbonate (HCO_3^-)	(Qiao et al., 2018)
3.	Heavy metals	Chromium (Cr^{3+}), Cadmium (Cd^{2+}), Copper (Cu^{2+}), Lead (Pb^{2+}), Mercury (Hg^{2+})	(Robinson, 2017)
4.	Xenobiotic organic compounds	Aromatic hydrocarbons, phenols, pesticides, and plasticizers.	(Xaypanya et al., 2018)

The majority of the pollutants listed above have aggregated, endangering, and deleterious impacts on water organism growth, ecosystems, and food chains resulting in massive public health challenges such as neurotoxicity, genotoxicity and carcinogenicity (Budi et al., 2016; Baderna et al., 2019).

The constitution of the leachate affects the leachate's efficacy system of collection. Even in enclosed landfills, leachate can threaten soil and water systems. Geotextiles, for example, are commonly used to keep landfills contained. Nonetheless, clogging of geotextile, which happens due to solid particle build-up, precipitation of minerals, and biofilms, may result in alterations in the geotextile's performance (Bandala et al., 2021). According to a previous study, the effect of when Municipal Solid Waste (MSW) is present, geotextile clogging might be aggravated. MSW and bottom ash from MSW incineration are disposed of simultaneously (W. Wu et al., 2018).

The discharge of sludge left over from the degradation of organic pollutants into landfills adds to the problem. Many previous investigations have found that the accruing quality of water metrics frequently surpasses local and WHO criteria for table water and farm irrigation. (Sauve & Van Acker, 2020) conducted research that measured the implications of MSW in landfills around Europe, considering a variety of criteria such as waste composition, meteorological parameters, and landfill management strategies. The study's findings show that removing sludge produced from leachate treatment into landfills has had a massive effect on freshwater resources and other ecological systems, and the study attributes these effects to the discharge of sludge produced from the treatment of leachate into landfills.

Research has uncovered a common problem, which is that whereas metals in landfill leachate can damage soil, surface waterways, and groundwater around landfills, groundwater has a higher risk of becoming unsafe to drink due to restricted flushing and replenishment by fresh inflows (Alemayehu et al., 2019; Rezapour et al., 2018)

2.2 Ways of Leachate Treatment

Numerous technologies have been investigated and applied in the leachate's treatment. Regular landfill leachate treatments are broadly divided into three main categories: (1) biological (aerobic or anaerobic) processes; (2) physical/chemical processes; and (3) simultaneous biological and physical-chemical means.

2.2.1 Biological treatment

Because it is simple, reliable, and cost-effective, biological treatment is often employed to remove leachates containing substantial levels of organic compounds (Luo et al., 2020). Microbes decompose organic substances to carbon (IV) oxide (CO2) and sludge in aerobic settings and biogas in anaerobic conditions (e.g., CO2 and methane). Biodegradation effectively removes organic and nitrogenous compounds in leachates with an increased BOD/COD proportion (Miao et al., 2019).

Biological procedures can be classified as aerobic or anaerobic, depending on the amount of oxygen present. Microorganisms convert organic compounds to inorganic compounds under aerobic circumstances. They transform organic materials into biogas under certain conditions.

2.2.1(a) Aerobic treatment

Aerobic treatment results in the nitrification of NH_4-N and the elimination of some biodegradable organic contaminants. So far, a wide range of aerobic biological processes have been successful in treating landfill leachates, including: (i) aerated lagoons; (ii) aerobic activated sludge; (iii) sequencing batch reactors (SBR); (iv) rotating biological contactors (RBC); (v) trickling filters; (vi) moving-bed biofilm reactor (MBBR); (vii) fluidized-bed biofilm reactor (FBBR); (viii) membrane biological reactor (MBR); (ix) constructed wetlands; and (x) fungal treatment and phytoremediation (F. Torretta et al., 2016)

In a wastewater treatment facility, activated sludge is often used in the treatment of municipal wastewater or in the co-treatment of leachate and home effluent. (Lebron et al., 2021). The aerated activated sludge is a blend of microbes grown in a reactor that consumes organic waste and then changes into novel biological biomass, CO_2, water, and nutrients via metabolic activities (Figure 2.2). When compared to stabilization ponds, the activated sludge system provides a more intensive treatment by combining a larger number of acclimated bacteria with a more great and forceful aeration (Luo et al., 2020). The blended liquor routinely submerges from the biological tank to the isolation stage, which is commonly a settling tank. The biomass/sludge is filtered and taken back to the biological reactor, while the cleared effluent is gathered from the top and discharged or treated further.

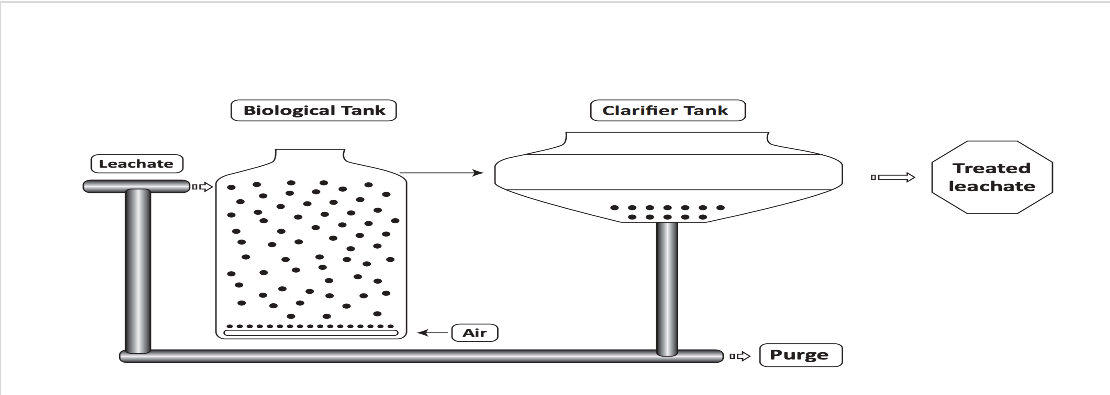

Figure 2.2 The aerated activated sludge (Lebron et al 2021).

The SBR is an activated sludge system that treats wastewater and landfill leachates using organic carbon oxidation and nitrification. Feeding, aeration, and discharge are common operators in aerobic situations (Aziz et al., 2018) . When the influent ammonium concentration grew, the COD removal rate dropped when using aerobic granular sludge SBR for treatment of leachate (Wei et al., 2012). Dissolved oxygen (DO) was a valuable process control measure for removing N and organic materials at little NH_{4+} input. Pretreatment by ultrasonic treatment has been used to increase the efficacy of the SBR process for treating leachate (Yin et al., 2019), resulting in removal efficiency of 90% and beyond, and ammonia removal of nearly 70%.

The MBBR system comprises an aerated biological tank and unique supporting growth media that offer a surface contact area for biofilm growth. In the biological tank, the aeration system mixes this support medium, ensuring substrate (In the wastewater) and the biomass in the support medium to closely interact. Significant biomass levels, shorter settling time, reduced reactivity to hazardous chemicals, and high organic and NH_3 reductions in a single operation are all perks of MBBR over activated sludge (Renou et al., 2008).

Microorganisms affix to media that has been glitzed and create a biofilm on the top in an attached-growth process known as FBBR (Nelson, 2017). FBBR technologies are often simple to operate since they are not involved in filtering particulates from the incoming flow, and the fiercely developing biomass in the enlarged bed may be amassed easily (Torretta et al., 2016). The bioreactor could be set up as a one or two column system with respect to the treatment process. If the process involves aeration, the moving wastewater and/or the air stream create fluidization in the column (Bello et al., 2017).

Phytoremediation and fungal therapy Fungi and their extracellular enzymes offer effortless breakdown of unstabilized organic material (e.g. cellulose, hemicellulose, and lignin) found in fresh-faced landfill leachate, as well as the breakdown of stable, refractory organic matter (e.g. humic and fulvic acids) found in mature landfill leachate, which is beneficial throughout the landfill's lifecycle (Ghosh & Thakur, 2017). *Dichomitus squalens*, a white-rot fungus, could grow in prime landfill leachate and use the organic debris as a carbon source. Following fungal treatment, scientists noticed a 60% reduction in BOD and COD removal and a decrease in leachate toxicity to the bacterium *Aliivibrio fischeri* (Kalciková et al., 2014).

Phytoremediation could be seen as an environmentally tolerable, cost-effective, and decent alternative for treating leachates with loading rates of 250–500m^3/ha/year with appropriate management (Jones et al., 2006). Phytoremediation systems can detoxify, degrade, and inactivate potentially hazardous components in leachate by leveraging the potential of natural or actively managed soil-plant systems (Lavagnolo et al., 2017) More long-term empirical field tests and durable prediction remediation approaches are needed in the future for the phytoremediation of landfill leachate.

2.2.1(b) Anaerobic treatment

Anaerobic treatment is frequently utilized for treating wastewater, particularly landfill leachate. Anaerobic biological procedures have various advantages over aerobic biological processes, namely: (1) massively diminished sludge generation; (2) reduced organics stabilization; and (3) power generation, as a result of enhanced gas restoration. Anaerobic digestion (AD), anaerobic filter (AF), up-flow anaerobic sludge blanket (UASB), and anaerobic ammonium oxidation (Anammox) are all examples of anaerobic treatment of landfill leachate (Luo et al., 2020).

Even though biological systems (activated sludge, filters, aerobic and anaerobic ponds) are well-known for their ease of use and favorable cost-benefit ratios, their application is limited to landfills with a biobased fraction significantly larger than 10 g/mL, which conforms to landfills that have been up and running for about 2 years or less, in tropical countries and 0–10 years in clement territories. Nonetheless, excessive amounts of cyanide, chromium, nickel, and zinc can hinder the bacteria that removes NH_3 (Brennan et al., 2016; Lebron et al., 2021)

The process is still used because of it is cheap and has low operational facility. Notwithstanding, this procedure has been tested in modern times due to inhibitory substances with low ability to be biodegraded and trace metals, which can lessen the biological wastewater treatment efficiency (Çeçen & Aktaş, 2004). As a result, it is critical to understand the attributes of leachate while attempting to establish the most suitable type for its treatment.

In general, physico-chemical methodologies are employed to remove NH_3 when used pre-treatment and remove resistant chemicals in post-treatment phases (Miao et al., 2019). The biological treatment methods may fail to achieve the maximum values established in regulation for its release into water bodies as landfill age increases because of excessive concentrations of NH_{4+}-N. As a result, combining physicochemical and biological procedures emerges to be an intriguing option for reaping the benefits of both technologies.

2.2.2 Phisyco-chemical treatment

Coagulation-flocculation, chemical precipitation, adsorption, membrane filtration, ion-exchange, air stripping, chemical oxidation/advanced oxidation processes (AOPs); and electrochemical treatment are the most common physical-chemical treatments. These techniques are typically used to remove substances that cannot be biodegraded (e.g humic and fulvic acids) and unpleasant compounds from landfill leachates (Kurniawan et al., 2006; Wang et al., 2016). Among many of the various physical-chemical processes, those that are most commonly used in treating landfill leachate can be identified.

2.2.2(a) Coagulation-flocculation

The following steps are involved in this technique, including using chemical substances to destabilize and raise the particles' size and the visible separation of the solids from the liquid phase. The coagulation-flocculation process (Figure 2.3) oversees disrupting small, dispersed phases to amalgamate and form flocs, which can be eliminated using sedimentation, flotation, or filtration techniques. As a result, these mechanisms are required to add to the expulsion of non-biodegradable materials and metallic ions (Lebron et al., 2021). Nonetheless, they are systems whose capacity depends on the characteristics of the raw leachate, and operating conditions may need to be adjusted regularly. Coagulation-flocculation leachate treatment studies revealed that iron salts are more effective than aluminum salts at lessening COD achieving elimination efficiencies of close to 50% and 10–40%, respectively (Alfaia et al., 2019).

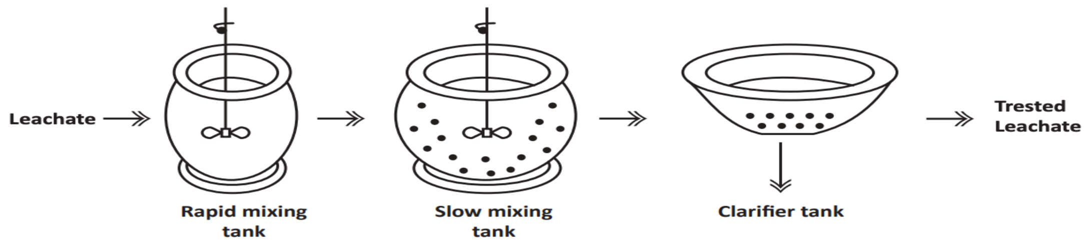

Figure 2.3 Coagulation-flocculation mechanism in the leachate treatment process (Lebron et al., 2021).

2.2.2(b) Air stripping

The methodology of moving an enormous volume of air through the leachate to cause the mass transfer of certain chemical contaminants from the liquid phase to the gaseous phase is known as air stripping (M.-H. Yuan et al., 2016). Air stripping is capable of removing CH_4, NH_{4+}, and volatile organic compounds (VOCs) as shown at

(Figure 2.4). The effectiveness of this procedure is significantly expanded by increasing the pH, temperature, and retention time values. Because of its effectiveness when compared to biological processes, ammonium stripping is the commonly used treatment for removing ammoniacal nitrogen from landfill leachates (Costa et al., 2019).

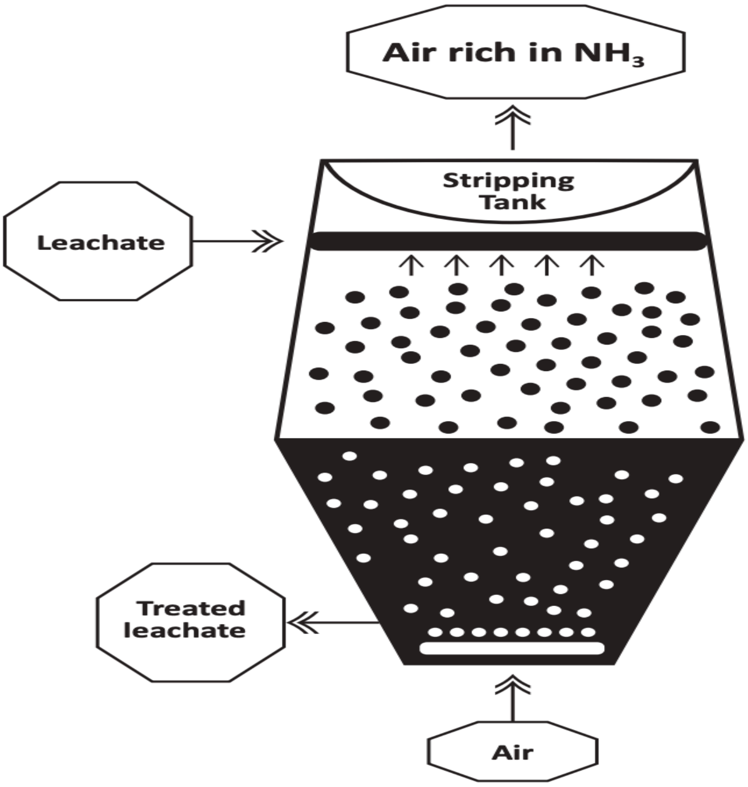

Figure 2.4 Air stripping in leachate treatment (Lebron et al., 2021).

Stripping can be used successfully to pre-treat the biological process when combined with other technologies. In high concentrations of ammonium, the approach grants its restoration as a by-product, which benefits the procedure. Stripping is frequently used as anomalous procedure in leachate treatment in developing countries.

2.2.2(c) Chemical precipitation

Chemical precipitation (CP) is the best method for removing non-biodegradable and resistant contaminants from leachates, like heavy metals, Sulphur, and ammonia/ammonium compounds (Kurniawan et al., 2006). Because of its low operating expense, ease of operation, and efficiency, this technology has been frequently used over the decades (Kabadsli et al., 2000; Luo et al., 2020). CP transforms soluble ionic pollutants into insoluble precipitates in the CP process. After that, the insoluble precipitates were removed either by gravity separation or filtration (Luo et al., 2020). The CP method was also employed to extract NH_4+-N from leachate with a raw NH_4-N content of 640 mg/L. The study discovered that using Mg, N, and P in the targeted medium in a 1:1:1 ratio produced 88 at an optimum pH of 10. The recovery of NH_{4+}-N is 88 percent when Mg, N, and P are present in the specific medium in a 1:1:1 ratio (Chen et al., 2013). Another study employed treated leachate wastewater from the Ultrasound and Fenton oxidation processes to use the CP procedure to remove more NH_3- N.

Chemical precipitation processes are designed to produce non-soluble compounds that can be removed from the aqueous phase. Aside from persistent organic pollutants and heavy metals, the procedure aids in separating ammoniacal nitrogen. If the complex formed is appropriately regained and free of other pollutants, it could be used as a fertilizer. Metal precipitation from solution is typically favored by calcium oxide, whereas ammoniacal nitrogen expulsion occurs with magnesium and/or phosphorus salts as the precipitating agent (Lebron et al., 2021) The ammonia precipitation method outperforms other methods like stripping, biological denitrification mechanisms, and electrochemical modification because it is less expensive and quicker (Chen et al., 2013).

Regular chemical precipitation procedures primarily include hydroxide and sulphide precipitation, which have a heavy metal removal performance of 92–100% (Fu & Wang, 2011). Struvite (Magnesium Ammonium Phosphate (MAP)) and lime have lately been utilized as precipitants to remove ammonia or heavy metals from landfill leachates (Huang et al., 2014). Previous studies show that pH, the molar ratio of magnesium, ammonium, phosphate, and the feeding order of precipitating reagents all influenced the efficiency of struvite precipitation (Zhang et al., 2020). According to investigations, waste phosphoric acid can remove 82 percent of ammonia from landfill leachate (COD = 4295 mg/L, NH_4^+-N = 1750 mg/L) and save 68 percent of the cost of struvite precipitation, using low-cost Phosphorus and Magnesium sources, 68 percent of the cost of struvite precipitation was saved.

The basic description of some physisco-chemical leachate treatment processes is summarized in Table 2.2.

Table 2.2 Basic description of some physico-chemical leachate treatment processes.

Physico-chemical treatments	Basic description	References
Electrochemical process	Electrocoagulation/electro flocculation, electro-flotation, and electrooxidation are electrochemical techniques for leachate treatment. The electrodes in the leachate can be aluminum or iron, and when an electric current is applied, coagulants are produced at the anode. Oxidation of organic compounds, such as ammonia–	(Du et al., 2013)

	nitrogen, can overtly or covertly from the solution's degradable component.	
Chemical oxidation	The electron acceptors are oxygen molecules or a substance that contains oxygen. Electron acceptors react directly with pollutants to mineralize them. The loss of one or more electrons from the element that oxidizes with an electron acceptor is oxidation.	(Huang et al., 2015)
Ion exchange	Ion exchange is the interchange of anions or cations between pollutants and the exchange medium to eliminate ions from an aqueous medium. Ion exchange substances are often synthetic organic materials with ionic functional groups, largely inorganic or natural polymeric materials.	(Mazloomi & Jalali, 2016)
Sand filtration	The movement of effluent through high-quality sand media with certain particle sizes, such as 0.8 and 1.7 mm, is known as sand filtration. Water is pushed downhill by gravity or pressure. The suspended particles in water passing through a fixed bed sand filter, commonly graded sand, are trapped and removed.	(Yunnen et al., 2016)

Membrane filtration	Membrane technology separates water from leachate using a concentration gradient and pressure force. The majority of the leachate chemicals are well maintained in this method. However, based on the membrane variety of filtration utilized, such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis, the extent of retention varies (RO).	(Xue et al., 2018)
Advanced Oxidation Processes	Powerful oxidizing agents like hydrogen peroxide (H_2O_2), ozone (O_3), and hydrated ferrous sulphate ($FeSO_4.7H_2O$) are used with ultraviolet (UV) ionizing radiation to breakdown pollutants into harmless molecules like CO_2 and H_2O. To cause contaminant mineralization, AOPs produce a wide range of reactive species, including sulphate (SO_4) and hydroxyl (OH) radicals.	(Luo et al., 2020)

2.3 Algae

At the microscopic level, algae are classified as microalgae or phytoplankton ('Phyto' = plant; 'planktons' = wandering) depending on their physical traits and size (Barsanti et al., 2021), as well as macroalgae that grow in water (Rajkumar, 2014). Macroalgae, sometimes known as 'seaweeds' are multicellular prehistoric plants called Thallophytes, without root, stem and leaves, which are tiny plants that range in size from 1/1000 of a millimeter to 2 millimeters, floating in the top 200 meters of the ocean, believed to be anaerobic (Barsanti et al., 2021).

Microalgae are called one of the most antiquated life forms and most abundant living organisms on Earth (Rajkumar, 2014) because they are amenable to genetic alteration and can live in a wide range of ecological environments. They feature a basic reproductive and cell development system that allows for rapid multiplication and long-term survival in a variety of hostile settings (Brennan et al., 2016; Suparmaniam et al., 2019), including fresh water, salt, ice, and hot springs (Rajkumar, 2014) . On the other hand, microalgae are single-celled microscopic organisms that use chlorophyll as their primary material for photosynthesis to convert energy.

On the other hand, microalgae are microscopic unicellular organisms with chlorophyll as their primary photosynthetic pigment. Even though microalgae are marine plants, scientists have difficulty recognizing and measuring their large population of one to ten million algal species (Suparmaniam et al., 2019). Algae is the generic term for eukaryotic macro and prokaryotic microorganisms that can quickly catch solar energy and fix CO_2 for growth and survival (Salama et al., 2016). *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (containing diatoms), and *Chrysophyceae* (including golden algae) are the four main categories of microalgae. Microalgae like *Arthrospira* (Spirulina), Chaetoceros, *Chlorella*, *Dunaliella*, and *Isochyris*, are the most economically grown (Rajkumar, 2014).