



**ANAEROBIC CO-DIGESTION OF LANDFILL LEACHATE WITH
LIQUIDISED FOOD WASTE USING MODIFIED UP-FLOW
ANAEROBIC SLUDGE BLANKET REACTOR**

By

NUR AIN FITRIAH BINTI ZAMRISHAM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

August 2024

FK 2024 57

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Faculty : Engineering

Globally, growing concern over the management of landfill leachate (LFL) and food waste (FW) arises from methane (CH_4) and carbon dioxide (CO_2) emissions, which pose significant public health risks. However, LFL presents significant potential for biogas generation through the anaerobic co-digestion (ACoD) process. This technology provides significant advantages, enabling energy recovery while generating minimal residual sludge as a by-product. This study aimed to evaluate the co-digestion of LFL and liquidised food waste (LFW) under different mixing ratios and support carrier integration in an up-flow anaerobic sludge blanket reactor (UASB). A machine learning (ML) algorithm was employed to evaluate its performance for forecasting biogas production. The biomethane potential (BMP) tests 1, 2, and 3 were conducted for mono-digestion, co-digestion at various ratios, and different support carrier applications, using a total of 39 BMP test bottles. Both conventional and modified UASB reactors were operated at mesophilic temperature in a water bath with

organic loading rate (OLRs) of 1 to 7 g/L/day in semi-continuous study phase 1, while in phase 2, the modified reactors were run with OLRs of 1 to 8 g/L/day. The modified UASB system incorporates three types of support carriers, including Red Clay Bio Ring (RCBR), Ceramic Bio Ring (CBR), and Lava Rock (LR). To further evaluate biogas production, two activation methods, zinc chloride (ZnCl_2) and sodium hydroxide (NaOH), were employed to activate the support carriers. To evaluate potential tools for forecasting biogas generation, artificial neural networks (ANN) and support vector machines (SVM) were developed based on BMP test data. The ANN model was developed using feedforward backpropagation, while a linear function was applied to the SVM model. The ACoD of LFL and LFW with a ratio of 65:35 has been selected due to its superior performance in biogas production during BMP Tests 1 and 2, compared to the ratios of 50:50 and 80:20. The performance of the modified UASB reactor was successfully boosted by incorporating a co-digestion of LFL and LFW, along with the addition of NaOH-modified CBR support carrier, which resulted in higher biogas production (3275 mL/d), specific methane production (SMP) (459.95 mL $\text{CH}_4/\text{gCOD}_{\text{added}}$), and chemical oxygen demand (COD) removal (89.5%) at an OLR of 7 g/L/day during semi-continuous study phase 2. The ANN model produced a correlation coefficient (R^2) of 0.884, whereas the SVM model had a slightly higher R^2 value of 0.930. This indicates that the SVM model had superior predicted accuracy in biogas production from the anaerobic digestion (AD) process, and it was also found to be a more reliable system to serve as a preliminary tool in commercial applications.

Keywords: Anaerobic digestion; Landfill leachate; Liquidised food waste; Machine learning applications; Support carrier

SDG: GOAL 6: Clean Water and Sanitation, GOAL 7: Affordable and Clean Energy

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PENCERNAAN ANAEROBIK BERSAMA SISA LARUT RESAPAN
DAN SISA MAKANAN MENGGUNAKAN REAKTOR SELIMUT
LUMPUR ANAEROBIK ALIRAN NAIK YANG DIUBAHSUAI**

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Di peringkat global, kebimbangan yang semakin meningkat terhadap pengurusan sisa larut resapan (LFL) dan sisa makanan (FW) disebabkan daripada pelepasan metana (CH_4) dan karbon dioksida (CO_2), yang menimbulkan risiko kesihatan awam yang ketara. Walau bagaimanapun, LFL menunjukkan potensi besar untuk penjanaan biogas melalui proses penghadaman bersama anaerobik (ACoD). Teknologi ini memberikan kelebihan yang ketara, menghasilkan pemulihan tenaga di samping menjana sisa enapcemar yang minimum sebagai produk sampingan. Kajian ini bertujuan untuk menilai penghadaman bersama LFL dan sisa makanan cair (LFW) di bawah nisbah pencampuran yang berbeza dan aplikasi pembawa sokongan ke dalam reaktor lapisan enap cemar anaerobik aliran naik (UASB). Algoritma pembelajaran mesin (ML) digunakan untuk menilai kebolehlaksanaannya untuk meramalkan pengeluaran biogas. Ujian potensi biometana (BMP) 1, 2, dan 3 telah dijalankan untuk penghadaman tunggal, penghadaman bersama dengan pelbagai nisbah, dan aplikasi pembawa

sokongan yang berbeza, menggunakan sejumlah 39 botol ujian BMP. Kedua-dua reaktor UASB konvensional dan diubah suai dikendalikan pada suhu mesofilik di dalam 'water bath' dengan OLR 1 hingga 7 g/L/hari dalam kajian separa berterusan fasa 1, manakala dalam fasa 2, reaktor diubah suai dijalankan dengan OLR 1 hingga 8 g/L/hari. Sistem UASB yang diubah suai menggabungkan tiga jenis pembawa sokongan, termasuk 'Red Clay Bio Ring' (RCBR), 'Ceramic Bio Ring' (CBR), dan 'Lava Rock' (LR). Untuk menilai selanjutnya pengeluaran biogas, dua kaedah pengaktifan, zink klorida ($ZnCl_2$) dan natrium hidroksida (NaOH), telah digunakan untuk mengaktifkan pembawa sokongan. Untuk menilai alat yang berpotensi untuk meramalkan penjana biogas, rangkaian saraf tiruan (ANN) dan mesin vektor sokongan (SVM) telah dijalankan dengan menggunakan data ujian BMP. Model ANN dijana menggunakan perambatan belakang suapan, manakala fungsi linear digunakan pada model SVM. ACoD LFL dan LFW dengan nisbah 65:35 telah dipilih kerana prestasi unggulnya dalam pengeluaran biogas semasa Ujian BMP 1 dan 2, berbanding nisbah 50:50 dan 80:20. Prestasi reaktor UASB yang diubah suai telah berjaya ditingkatkan dengan menggabungkan pencernaan LFL dan LFW, bersama-sama dengan penambahan pembawa sokongan CBR yang diubah suai NaOH, yang menghasilkan pengeluaran biogas yang lebih tinggi (3275 mL/d), pengeluaran metana khusus (SMP) (459.95 mL CH_4 /gCOD_{added}), dan penyingkiran permintaan oksigen kimia (COD) (89.5%) pada OLR 7 g/L/hari semasa kajian separa berterusan fasa 2. Model ANN menghasilkan pekali korelasi (R^2) sebanyak 0.884, manakala model SVM mempunyai nilai R^2 yang lebih tinggi sedikit, iaitu sebanyak 0.930. Ini menunjukkan bahawa model SVM mempunyai ketepatan ramalan yang lebih baik dalam pengeluaran biogas daripada proses pencernaan anaerobik (AD), dan ia juga didapati

sebagai sistem yang lebih dipercayai untuk berfungsi sebagai alat awal dalam aplikasi komersial.

Kata Kunci: Pencernaan anaerobik; Sisa larut resapan; Sisa makanan cair; Aplikasi pembelajaran mesin; Pembawa sokongan

SDG: MATLAMAT 6: Air Bersih dan Sanitasi, MATLAMAT 7: Tenaga Mampu Milik dan Bersih



ACKNOWLEDGEMENTS

All praises to Allah, Lord of the Universe, the Merciful, for granting me the ability, knowledge, and strength to undertake this research study and complete it successfully.

Special thanks to my supervisor, Dr. Syazwani Idrus, for her undivided attention, advice, inspiring guidance, support, helpful suggestions, and inexhaustible fortitude throughout this research study. Since the commencement of my research, she has been kind enough to discuss my research study. Her feedback on the manuscripts for my thesis has been insightful. Also, I would like to express my gratitude to my co-supervisors, Associate Professor Ts. Dr. Mohd Razif bin Harun and Dr. Mohd Shahrizal bin Ab Razak, for their guidance and assistance in improving my research throughout my research.

My sincere thanks and appreciation are also extended to all lecturers, technical personnel, and those who have contributed directly or indirectly to this study. Lastly, I would like to thank my husband, parents, siblings, and companions, especially my research partner, Khairina binti Jaman @ Zaman, and Rohimatu Toyibah binti Masyhur, for their mental and physical support throughout my master's degree, which enabled me to complete this research. Undoubtedly, after overcoming numerous obstacles and difficulties to complete the task assigned to me, I have acquired a wealth of knowledge and experiences that I will cherish and apply in the future with the aim of achieving good and reaping benefits.

Thank you. May Allah SWT return all the good deeds with the ultimate reward.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

LFL	Landfill Leachate
FW	Food Waste
CH ₄	Methane
CO ₂	Carbon dioxide
ACoD	Anaerobic Co-Digestion
LFW	Liquidised Food Waste
UASB	Up-flow anaerobic sludge blankets
ML	Machine Learning
BMP	Biomethane potential test
OLR	Organic Loading Rate
RCBR	Red Clay Bio Ring
CBR	Ceramic Bio Ring
LR	Lava Rock
ZnCl ₂	Zinc chloride
NaOH	Sodium hydroxide
ANN	Artificial Neural Network
SVM	Support Vector Machine
SMP	Specific methane production
COD	Chemical Oxygen Demand
R ²	Correlation coefficient
AD	Anaerobic Digestion
MSW	Municipal Solid Waste
TAN	Total Ammonia Nitrogen
HRT	Hydraulic Retention Time
VS	Volatile solids

LIST OF ABBREVIATIONS

N ₂ O	Nitrous oxide
VFAs	Volatile Fatty Acids
TMEs	Trace Metal Elements
C/N	Carbon to Nitrogen
IA/PA	Intermediate alkalinity to partial alkalinity
C	Carbon
N	Nitrogen
EU	European Union
3R	Reduce, Reuse, and Recycle
5R	Refuse, Reduce, Reuse, Repurpose, and Recycle
DOE	Department of Environment
BOD	Biochemical oxygen demand
TN	Total nitrogen
SS	Suspended solids
SWCorp	Solid Waste Management and Public Cleansing Corporation
US EPA	United States Environmental Protection Agency
EPA	Environmental Protection Agency
US	United States
Mn	Manganese
TS	Total solids
H ₂ S	Hydrogen Sulphide
NH ₄ -N	Ammonium
NaHCO ₃	Sodium bicarbonate

LIST OF ABBREVIATIONS

SCOD	Soluble chemical oxygen demand
Na ⁺	Sodium
Cl ⁻	Chlorine
Cu	Copper
Zn	Zinc
TDS	Total dissolved solids
AF	Anaerobic filters
AnMBR	Anaerobic membrane bioreactors
AnSBR	Anaerobic sequencing batch reactor
TSS	Total suspended solids
TA	Total alkalinity
VFA/TA	Volatile fatty acid to total alkalinity
DIET	Direct electron transfer
IET	Interspecies electron transfer
K ₂ CO ₃	Potassium carbonate
KOH	Potassium hydroxide
CaCl ₂	Calcium chloride
H ₃ PO ₄	Phosphoric acid
H ₂ SO ₄	Sulphuric acid
BET	Brunauer-Emmett-Teller
HCl	Hydrochloric acid
SEM	Scanning electron microscopy
EPS	Extracellular polymeric substances
RM	Risk minimisation
SRM	Structural risk minimization

LIST OF ABBREVIATIONS

ERM	Empirical risk minimization
APHA	American Public Health Association
FAS	Ferrous ammonium sulphate
$K_2Cr_2O_7$	Potassium dichromate
DF	Dilution Factor
NO_3/N	Nitrate/nitrogen
O&G	Oil & Grease
FAU	Formazine Attenuation Units
NTU	Nephelometric Turbidity Unit
Fe	Ferum
PA	Partial alkalinity
PVC	Polyvinyl chloride
GC	Gas chromatography
ISO	International Organisation for Standardisation
USP	United States Pharmacopeia
ASTM	American Society for Testing and Materials
S/I	substrate-to-inoculum ratio
VSS	Volatile suspended solids
IA/TA	Intermediate to total alkalinity ratio

CHAPTER 1

INTRODUCTION

1.1 Research Background

Inadequate waste management can have adverse consequences for the environment and society. The waste management process encompasses many stages, starting with the collection, transportation, treatment, analysis, and disposal of waste products. Waste management practices contribute to greenhouse gas emissions, with landfills being the primary sources of concern due to the production of CH₄ during organic waste decomposition. According to Ahmad et al. (2021) and Nawaz et al. (2020), inadequate management of solid waste can result in the production of LFL at landfill sites. This has become a significant concern due to the expected global rise in solid waste generation. The inadequate handling of LFL has detrimental consequences for the environment and human health, as illustrated in Figure 1. The generation of LFL occurs due to precipitation and the infiltration of surface water or groundwater into the landfill facility. LFL is a contaminated liquid formed when excessive water flows within the layers of garbage in landfills (Ozbay et al., 2021). This process leads to the formation of a liquid that is dark in colour, extremely polluted, and emits unpleasant odours (Guo et al., 2010).

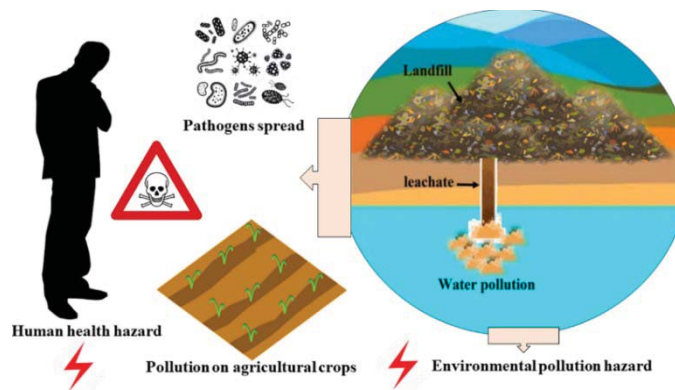


Figure 1: Environmental and health problems result from landfill leachate discharge

(Source: El-Saadony et al., 2023)

FW refers to any discarded food or inedible portions, primarily from cafeterias, restaurants, bakeries, households, and hotels. Negri et al. (2020) defined FW as the waste product resulting from producing, processing, and consuming household goods and catering services. The FW constitutes a significant portion of municipal solid waste (MSW) produced and appears to be a global challenge (Conrad et al., 2018). The FW mainly consisted of rice, grains, noodles, meats, fish bones, oils, vegetables, biscuits, eggshells, and other forms of waste. FW is a crucial worldwide issue affecting Malaysia, resulting in various adverse consequences such as escalating food production expenses, detrimental effects on human health, and destruction of the environment (Phooi et al., 2022).

Reduce, reuse, recycle, waste or wastewater treatment, and final disposal are all available waste management methods. The implementation of recycling as a waste management strategy poses challenges since not all types of waste can be recycled. For instance, including non-recyclable items, such as plastic bags, in the recycling process

can lead to contamination of recyclable materials and pose a risk to the functionality of recycling equipment. Shahril (2020) observed that the most often used technique for disposing of waste in Malaysia involves dumping waste into landfills. Landfilling causes air, water, and soil pollution, and odour contamination (Wanwari et al., 2018). Sin et al. (2013) also report that the majority of landfills in Malaysia are small-scale and inadequately maintained, resulting in significant environmental risks.

Hence, AD emerges as the preferred waste treatment technique. AD refers to the process of decomposing organic substances without the presence of oxygen (Lohani & Havukainen, 2017). The process is conducted by utilising a consortium of microorganisms, including fermentative bacteria, which facilitate the decomposition of organic material into biogas. There has been a growing need for anaerobic technology due to its efficient removal of contaminants from wastewater, generation of energy (CH_4), and production of a relatively small amount of sludge when compared to aerobic processes (Ahmad et al., 2020; Renou et al., 2008). Biogas is a promising source of fuel source, as stated by Wang et al. (2022). Besides, Alejo et al. (2018) observed that the production of biogas and the composition of effluent, specifically total ammonia nitrogen (TAN), can be accurately predicted through the utilisation of ML algorithms such as ANN and SVM.

However, mono-digestion, which involves using a single type of feedstock in the AD process, has several drawbacks, including an imbalanced nutrient profile and process instability due to inadequate buffering capacity, both essential for the efficient operation of AD systems (Pilarska et al., 2023; Tong et al., 2019). In contrast, ACoD, which utilises a combination of feedstocks, is widely regarded as a more effective waste treatment technology. ACoD addresses the limitations of mono-digestion by

promoting a more balanced nutrient composition, providing synergistic effects from the co-substrates, and ensuring process stability (Siddique & Wahid, 2018; Karki et al., 2021). According to Kunatsa and Xia (2021), co-digestion enhances microbial activity and digestion efficiency, leading to increased biogas production and contributing to a more sustainable energy source. This is further supported by a previous study, which demonstrated that ACoD of FW and fresh leachate maintained stable performance even under high OLRs of 8.3 g VS/L.d and a short hydraulic retention time (HRT) of 10 days. The process yielded 506.3 mL CH₄/g VS_{added} and achieved a high volatile solids (VS) removal rate of 78.0%, compared to FW single-digestion, which showed instability due to a lack of metal elements.

Furthermore, the output from the AD process, known as digestate, is also valuable. It refers to the residual substance resulting from the AD treatment, encompassing the non-digestible remnants of organic materials and deceased microorganisms. It is also abundant in nutrients and can serve as a fertiliser and soil conditioner.

1.2 Problem Statement

The presence of leachate originating from landfills is widely acknowledged as a significant issue for societies due to its composition, which consists of potentially harmful elements such as ammonia, nitrogen, and heavy metals (Abd El-Salam & I. Abu-Zuid, 2015; Hredoy et al., 2022). Furthermore, due to the elevated levels of ammonia nitrogen present in the LFL, methanogenic processes may be inhibited. In addition, LFL contains xenobiotic organic substances commonly used in agricultural, domestic, and industrial sectors, such as phthalates (Oliveira et al., 2014). According to Kawai et al. (2012) and Hredoy et al. (2022), LFL exhibits high salinity levels,

capable of inhibiting biological processes that can hinder the activity of anaerobic microorganisms. A high salinity level has the potential to induce significant changes in the microbial community and diversity, thereby influencing the digestive process's efficiency. Additionally, CH₄ and nitrous oxide (N₂O) emissions in leachate treatment systems are identified as the second most significant contributors to greenhouse gas emissions (Izhar & Xuan, 2021). Hence, the insufficient disposal or treatment of the LFL poses significant challenges for developing countries, as inappropriate urbanisation and industrialisation processes contribute to environmental degradation and societal issues.

Meanwhile, FW has excess organic acids accumulate during the acidogenesis stage, resulting in a sudden pH reduction and further methanogenic activity inhibition (Shen et al., 2013; Físgativa et al., 2016; Liu et al., 2023). The reason for this phenomenon is that the acidogenic phase is susceptible to a significant build-up of volatile fatty acids (VFAs), resulting in an impediment to the metabolic processes of methanogenic microorganisms and thus causing the collapse of the digestive system (Ye et al., 2018; Liang et al., 2022; Liu et al., 2023). Besides, FW has a lack of trace metal elements (TMEs) and a high moisture content, which contributes to the failure of the AD process (Zhang & Jahng, 2012; Song et al., 2021).

The majority of past research investigations have identified appropriate techniques for the management of LFL. The approaches include various biological processes, such as aerobic and anaerobic treatment, as well as leachate transfer and physical and chemical treatments (Renou et al., 2008; Bove et al., 2015; and Nawaz et al., 2020). However,

Karolinczak et al. (2021) stated that the aerobic treatment does not produce biogas, which is consequently unable to generate heat or electricity.

The use of AD of LFL as a sole substrate is limited due to the inherent characteristics of LFL, as Kawai et al. (2012) highlighted. According to Gao et al. (2022), the metagenomic evaluation of LFL revealed a reduction in the number of genes related to hydrolase and methanogenic enzymes that occurred during condition with high salinity. A weakening of the acetotrophic methanogenesis pathway is also found under extreme salinity levels of LFL (Gao et al., 2022).

Due to their practicability and low sludge formation, conventional AD reactors, such as UASB, have been widely used for diverse anaerobic waste treatments (Torres et al., 2009; Ye et al., 2011; Selvam et al., 2017). However, poor biogas output is a common problem with conventional reactors due to nitrogen build-up, ammonia inhibition, and obstruction in the single-phase reactor (Yang et al., 2019). To promote the production of CH₄, shorten the lag phase of microbial growth, and increase the decomposition of organic matter, the AD process could be enhanced through the incorporation of a support carrier (Luo et al., 2015; Ambaye et al., 2021; Wang & Lee, 2021). According to Mikheeva et al. (2022), the incorporation of support carriers into the AD system has been extensively acknowledged for its capacity to boost stability during operation, facilitate AD, and elevate biogas generation.

Insufficient forecasting of biogas generation could potentially compromise the reliability and accuracy of the study's outcome. The application of modelling techniques allows for the early anticipation of biogas generation, hence facilitating the

design of digesters, prediction of anaerobic system behaviour, optimisation of the digestion process, and enhancement of the methanogenesis process (Parra-Orobio et al., 2021; Li et al., 2022). The current kinetic equation lacks dynamism and does not account for the variations in input factors such as the carbon-to-nitrogen (C/N) ratio, OLR, and COD. Meanwhile, ANN and SVM have extensively identified ML approaches utilised for diverse applications, including classification, prediction, evaluation, and forecasting (Alejo et al., 2018; Cinar et al., 2022; Guo et al., 2021; Andrade Cruz et al., 2022). Besides, the AD processes are influenced by various parameters, including the C/N ratio, OLR, and COD, which, in the context of ML modelling, tools such as ANN and SVM can employed the input factors to estimate the biogas production of AD systems, which results in a more accurate prediction of biogas. Hence, ANN and SVM are viable approaches to predicting the amount of biogas produced by the AD process.

Applying ACoD of LFL with other substrates has demonstrated promising results in treating organic wastewater with high salinity while enabling energy recovery (Kawai et al., 2012; Gao et al., 2022). Therefore, to overcome the performance limitations of single digesting processes for LFL, it is suggested that LFL be used with substrates with complementary features. The primary focus of this research lies in the biological method, particularly the anaerobic process, which has received significant attention from researchers and engineers. Additionally, there is insufficient data regarding the ideal LFL and LFW substrates ratio in a semi-continuous study (LFL:LFW). In this study, the main substrate utilised was LFL, which was co-digested with LFW at three different ratios (50:50, 65:35, and 80:20). The impact of supplementing with support carriers in the UASB reactor was also evaluated. Furthermore, none of the previous

studies have compared and assessed SVM and ANN analysis regarding the ACoD of LFL with LFW, despite the benefits of both approaches. Thus, this study explored how effectively SVM and ANN estimate biogas generation from ACoD LFL and LFW in terms of R^2 .

1.3 Objective of the Research

The study aims to investigate the performance of mono-digestion of LFL and LFW, ACoD of LFL and LFW, ACoD of LFL and LFW with the integration of support carriers, and the application of ML as a forecasting tool for biogas production. The objectives are divided into sub-objectives as follows:

- i. To investigate biogas production from mono-digestion of LFL and LFW, ACoD of LFL and LFW with different ratios, and addition of support carrier to the substrate with the best ratio in the batch study.
- ii. To evaluate the performance of conventional and modified UASB reactors on the mono-digestion of LFL and the ACoD of LFL with LFW using the best ratio.
- iii. To assess the performance analysis of ANN and SVM in biogas forecasting.

1.4 Scope of Study

The primary purpose of this study is to investigate the efficiency of mono-digestion of LFL and LFW and co-digestion of LFL with LFW, at three different mixing ratios of 50:50, 65:35, and 80:20 (LFL:LFW) while incorporating support carriers. This mixing ratio represents the amount of COD in the substrate used for treatment processes. The mixing ratio of 80:20 was selected based on a previous study by Zhang et al. (2015),

which demonstrated high performance with 71.6% VS removal using a FW to LFL mixture (FW:LFL) mixture. However, in this study, LFL is the primary substrate, and thus the ratio has been adjusted to 80:20 (LFL:LFW). Meanwhile, the mixing ratio of 50:50 was selected, as it was also used in a previous study by Obulisamy et al. (2016) (FW:primary sewage sludge). Lv et al. (2021) also found that a 50:50 mixing ratio (FW:MSW leachate) increased the efficiency of AcoD by 12%–18% compared to single digestion. Furthermore, a different mixing ratio, 65:35, has been proposed to identify the optimal mixing ratio for the AcoD of LFL and LFW in order to maximise biogas production, as no prior studies have investigated LFL and LFW as substrates. The conventional and modified UASB reactors were run in mesophilic environments (37°C). The size of the UASB reactor employed in this study was a total volume of 5L. BMP Test 1 assessed the biogas output resulting from mono-digestion of LFL and LFW and co-digestion of LFL with LFW using various ratios. BMP Test 2 aimed to evaluate biogas production using the most favourable ratios, along with incorporating three different support carriers, the RCBR, CBR, and LR, into the modified UASB system. Next, BMP Test 3 was carried out by employing the most effective support carrier in a semi-continuous study phase 1 for biogas production. The support carrier employed in BMP Test 3 was subjected to physico-chemical activation.

After performing the BMP Tests, the ideal ratio, including a support carrier, was obtained and used in the semi-continuous study. The study involved a comparative analysis between conventional and modified UASB reactors. The performance of the reactor was evaluated by measuring biogas output, COD removal efficiency, and SMP. In addition, the study also investigates the correlation between the intermediate alkalinity to partial alkalinity (IA/PA) ratio, TAN, and pH value concerning AD system

stability. SVM and ANN models were applied to simulate the daily biogas output. The inputs for the ML model included COD_{in}, OLR, Carbon (C), Nitrogen (N), and C/N ratio. Both SVM and ANN models used MATLAB software to obtain the equation produced and the R² value. The ANN model was constructed through the feed-forward backpropagation approach, while the SVM model used a kernel function to estimate biogas production. The constraint of this study is the assumption of a constant temperature (37°C) and absolute pressure (1 atm).

1.5 Significance of the Research

This study aims to perform an in-depth and systematic investigation of the utilisation of LFL for the generation of biogas as a viable alternative method for wastewater management in Malaysia. To increase the generation of biogas's performance, the ACoD of LFL and LFW was conducted. The conventional UASB system was also modified by integrating support carriers, which can enhance the stability of the AD process by promoting the growth and activity of microorganisms within the AD system. Furthermore, to facilitate a more thorough evaluation of biogas production, the support carrier is treated with physico-chemical activation. This process improves the physical characteristics of the support carrier, thereby enhancing the carriers' pore size and forming biofilms that effectively eliminate pollutants from effluent. Therefore, the degradation of waste using a modified UASB system is expected to yield greater efficiency in COD removal, biogas production, and SMP compared to a conventional reactor. Next, this treatment method has the potential to be implemented on an industrial scale as it is low-cost. In addition to biogas, this study has the potential to meet the need for clean water, given the circumstances that require effective wastewater management to accommodate future development. Although the water

treated by the reactor may not be entirely appropriate for everyday consumption, it is deemed sufficient for reuse in various applications including industrial and irrigation processes. The biogas generated can also be used to generate an adequate amount of energy, thereby making a valuable contribution to the field of renewable energy. By consistently selecting appropriate treatment procedures, untreated leachate wastewater is expected to be effectively treated, reducing any unforeseen adverse impacts on the environment, public health, and safety.

1.6 Thesis Outline

This thesis evaluates and reports on the performance of conventional and modified UASB systems in treating LFL with LFW. The research comprises five chapters in total. The first chapter addresses the background and problem statement, encompassing the consequences of untreated LFL and LFW production, the existing waste management methods, and the suitability of AD as the most effective biological treatment for LFL and LFW. The research objectives are also outlined in this chapter. Next, this chapter also describes the scope of the study and the significance of the study on the ACoD of LFL and LFW.

Chapter 2 evaluates of the background study on wastewater originating from the landfill and food court. The main focus of this study is to investigate the problem of LFL and analyse the properties of LFL and LFW. The research goals are to explore the implementation of AD and evaluate the treatment processes. Adopting the various types of reactors often employed in AD was also addressed. Furthermore, this chapter clarifies the ACoD process involving various substrates, with particular emphasis on the co-digestion of LFL and FW. Next, this chapter discusses design parameters in AD

of LFL, limiting factors in LFL and LFW, and the role mixing ratio of ACoD on LFL and LFW. Besides, the differences between additive substances and support carriers were also explained in detail in this chapter. The explanation of the physico-chemical activation of the support carrier by two methods, which consist of the utilisation of ZnCl_2 and NaOH , was also provided. A thorough explanation of the function of the support carrier within the UASB reactor and a discussion of the geometric characteristics and biofilm formation on carriers were included. Additionally, this chapter offers an extensive understanding of the applications of ML algorithms, specifically ANN and SVM. This chapter primarily focuses on the recognised knowledge gap regarding the AD processes, specifically about the ACoD of LFL with LFW, the utilisation of support carriers to boost the AD process and the application of ANN and SVM for modelling the biogas output.

The third chapter presented a comprehensive depiction of the research flowchart and experimental setup, accompanied by detailed information about the study. The chapter extensively lists of all parameters used for the ACoD of LFL and LFW. A thorough explanation was provided regarding the setup procedure for both the conventional and modified UASB systems, along with detailed information on how the activation process supports carriers employed in the modified UASB. This chapter also encompasses a detailed discussion of the preparation of the sample and the inoculum, and the characteristics of the LFL and LFW. Furthermore, this chapter presents the ML techniques that have been applied in this study, which comprise ANN and SVM.

Chapter four contains an in-depth discussion of the findings gathered during the study, specifically examining the effectiveness of various OLRs in conventional and modified

UASB reactors. The evaluation considers the efficacy of biogas production, COD removal, and SMP. Furthermore, an assessment was conducted to evaluate the reactors' effectiveness and dependability by performing a comparative analysis of pH value, IA/PA ratio, and TAN parameters. Following the outcome of the continuous experiment phase 1, the support carrier undergoes an activation process to further boost the generation of biogas production from the ACoD of LFL with LFW. The BMP data were subsequently subjected to further analysis through the application of ML technologies.

The final chapter, titled "Chapter 5: Conclusions and Recommendations," serves as an exhaustive summary of the findings and discussions that were discussed in the previous chapter. Additionally, it offers valuable insights into potential approaches for future research that might effectively address and enhance the drawbacks identified within this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Landfill concept

2.1.1 Landfilling practice

There are significant distinctions in the landfilling practices of developed and developing countries concerning the management of MSW. Middle- and lower-income countries often exhibit unsatisfactory landfill management practices. This is because they have disposed of their waste through open dumping, with a total volume of 71.5 million tonnes (Hoornweg & Bhada-Tata, 2012). In India, the management of MSW indicates significant variances, with just 21% of the generated waste being adequately handled, while the remaining amount corresponds to uncontrolled and improper waste management practices (Mor & Ravindra, 2023). In 2022, Malaysia's recycling rate was approximately 31%, which is comparatively lower than that of its neighbouring countries such as Singapore, Korea, Taiwan, and Thailand. The Federal Government of Malaysia has set a goal of achieving a recycling rate of 40% by the year 2025. According to research carried out by Syed Ismail and Abd. Manaf (2013), they have observed that a significant proportion of MSW in developing Asian countries is being disposed of in landfills, ranging from 70% to 90%. These landfills would create leachate wastewater, also known as LFL, which comprises extremely toxic pollutants that can have severe effects on local habitats and public health problems. The generation of LFL has become a serious problem, and appropriate measures for waste management must be taken as Malaysia, Thailand, Vietnam, Indonesia, and

Philippines (Southeast Asian countries) rank among the top five nations globally in terms of generating substantial quantities of MSW, specifically at a rate of 1.14 kg/capita/day (Arumdani et al., 2021).

In contrast, the predominant method of garbage disposal in industrialised or most developed Asian countries such as Japan and Korea are incineration, which accounts for over 80% of the waste treatment process (Pheakdey et al., 2022). Ryu (2010) states that recycling rates in developed Asian countries, such as South Korea, recorded a significant rise of 57% in 2006, resulting in a corresponding decrease in landfill usage to 26%. Moreover, landfilling has emerged as the least preferred alternative in the majority of European states. This is due to recycling and composting serving as the primary waste disposal options in European Union (EU) countries, which include Austria (62.3%), Germany (71.1%), and Slovenia (60%), as illustrated in Table 1. This phenomenon can be attributed to the implementation of landfill regulations and the reinforcement of recycling and recovery strategies in these countries for the majority of their waste generation. According to Balwan et al. (2022), the new recycling hierarchy incorporates an expansion of the existing “Reduce, Reuse, Recycle” (3R) to include an additional two principles, resulting in a total of “Refuse, Reduce, Reuse, Repurpose, and Recycle” (5R). Hence, the concept of the 5R was proposed, which primarily encompasses the principles of waste management and reduction, which consequently minimises LFL generation, as illustrated in Figure 2. The goal of this plan is to effectively mitigate the environmental consequences resulting from waste products as shown in Figure 3.

Table 1: Waste disposal in European Union (EU) countries

Situation in EU countries			
	Municipal waste generated (kg/capita-2021)	Share of recycling and composting of municipal waste (2021)	Landfill rate of waste (excluding major mineral) (2020)
EU average	530	49.6%	18%
Austria	834	62.3%	9%
Luxembourg	793	55.3%	9%
Denmark	786	34.4%	1%
Belgium	759	53.3%	3%
Germany	646	71.1%	9%
Cyprus	644	15.3%	52%
Ireland	633	40.8%	18%
Malta	611	13.6%	73%
Finland	609	37.1%	7%
Czechia	570	43.3%	27%
France	561	45.1%	22%
Greece	524	21.0%	60%
Netherlands	515	57.8%	2%
Portugal	514	30.5%	46%
Slovenia	511	60.0%	6%
Slovakia	496	48.9%	31%
Italy	487	51.4%	15%
Lithuania	480	44.3%	17%
Spain	472	36.7%	43%
Latvia	461	44.1%	25%
Croatia	446	31.4%	34%
Sweden	418	39.5%	8%
Hungary	416	34.9%	35%
Bulgaria	408	65.5%	73%
Estonia	395	30.3%	83%
Poland	362	40.3%	11%
Romania	302	11.3%	51%

(Source : European Environment Agency, 2023)



Figure 2: 5R Concept in Waste Management Hierarchy

(Source: Leadsy, 2020)



Figure 3: Negative impact on various aspects of the environment due to poor waste disposal

(Source: Earth, 2021)

In Malaysia, the management of LFL is regulated by a framework of measures established by the Department of Environment (DOE). The DOE plays a pivotal role in monitoring compliance with environmental standards and enforcing regulations that restrict the discharge of leachate into water bodies. This regulatory framework encompasses the establishment of permissible limits for various pollutants, including pH value, biochemical oxygen demand (BOD), COD, total nitrogen (TN), heavy metals, and suspended solids (SS), as shown in Table 2. To ensure adherence to these standards, the DOE conducts regular inspections of landfill sites and mandates that landfill operators submit comprehensive monitoring reports. These reports must include detailed data on leachate quality and treatment efficiency, allowing the DOE to evaluate the effectiveness of leachate management practices and ensure the protection of environmental quality.

Table 2: Discharge limit of leachate (Second Schedule – Regulation 13)

	Parameter	Unit	Standard
	(1)	(2)	(3)
(i)	Temperature	°C	40
(ii)	pH Value	-	6.0-9.0
(iii)	BOD ₅ at 20°C	mg/L	20
(iv)	COD	mg/L	400
(v)	Suspended Solids	mg/L	50
(vi)	Ammoniacal Nitrogen		5
(vii)	Mercury	mg/L	0.005
(viii)	Cadmium	mg/L	0.01
(ix)	Chromium, Hexavalent	mg/L	0.05
(x)	Chromium, Trivalent	mg/L	0.20
(xi)	Arsenic	mg/L	0.05
(xii)	Cyanide	mg/L	0.05
(xiii)	Lead	mg/L	0.10
(xiv)	Copper	mg/L	0.20
(xv)	Manganese	mg/L	0.20
(xvi)	Nickel	mg/L	0.20
(xvii)	Tin	mg/L	0.20
(xviii)	Zinc	mg/L	2.0
(xix)	Boron	mg/L	1.0
(xx)	Iron (Fe)	mg/L	5.0
(xxi)	Silver	mg/L	0.10
(xxii)	Selenium	mg/L	0.02
(xxiii)	Barium	mg/L	1.0
(xxiv)	Fluoride	mg/L	2.0
(xxv)	Formaldehyde	mg/L	1.0
(xxvi)	Phenol	mg/L	0.001
(xxvii)	Sulphide	mg/L	0.50
(xxiii)	Oil and Grease	mg/L	5.0
(xxix)	Colour	ADMI*	100

(Source : Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009 (PU(A) 433))

According to Kamaruddin et al. (2017), the currently preferred technology for landfill treatment in Malaysia is the Fukuoka Method, also referred to as the semi-aerobic system. This approach was implemented at the Kuantan Jerangau-Jabor Landfill Site (Noh et al., 2020). Aziz and Ramli (2018) describe this system as utilising passive ventilation, which allows oxygen to enter the waste mass through leachate collection pipes, thereby fostering aerobic microbial activity and accelerating the decomposition of organic waste. This process not only expedites waste stabilisation but also significantly improves the quality of leachate. Furthermore, a systematic collection and

venting system facilitates the continuous discharge of both leachate and landfill gas. This method is an effective and cost-efficient solution for enhancing waste decomposition while mitigating the environmental impacts associated with LFL (Muchangos & Tokai, 2020).

Furthermore, the average global waste generation is approximately 0.74 kg/capita/day, with significant variations observed across countries, between 0.11 and 4.54 kg/capita/day (Mor & Ravindra, 2023). According to Patel (2023), the global annual generation of MSW amounts to approximately 2.01 billion tonnes. The expected quantity will probably rise to 3.40 billion tonnes by the year 2050, indicating growth rates of 19% and 40% for industrialised and developing countries, respectively (Kaza et al., 2018; Patel, 2023). The increase in the quantity of waste produced is resulting in an increasing generation of leachate that is very polluted, which then requires a high demand for more robust leachate treatment strategies. Efficient waste management practices not only serve to mitigate pollution but also contribute to the preservation of natural resources and help reduce greenhouse gas emissions.

2.1.2 Classification of Landfill

Landfills can be classified into three main types which are sanitary landfills, open-dumping landfills and semi-controlled landfills (Narayana, 2009; Kamaruddin et al., 2017). Open dumping is a prevalent waste management practice observed in less-industrialised countries, characterised by the unregulated disposal of waste in open and low-lying regions. This method poses significant environmental and health risks. Semi-controlled landfills are equipped with fundamental amenities such as waste sorting, segregation, shredding, and compaction, which are then followed by the

application of soil cover. Meanwhile, sanitary landfills are landfills that have been designed and constructed with superior engineering and technological features.

According to research by Yong et al. (2019), 50% of landfill sites in Malaysia operate as open-dumping sites. This waste disposal method is highly detrimental to both the environment and public health, as it often lacks essential containment and management practices. Moh & Abd Manaf (2017) and Fauziah & Agamuthu (2012) stated that landfill sites can be classified into four different levels, ranging from Level 1 (L1) to Level 4 (L4). However, according to Kamaruddin et al. (2017), the open dumpsite is also included, and it is classified as Level Zero (L0). Therefore, the landfill level can be categorised into five different levels (L0 until L4), as summarised in Table 3. Malaysia is expected to primarily focus on implementing landfill concepts at levels 3 and 4 due to the very few such landfills currently established in the country, as shown in Table 4 (Solid Waste Management and Public Cleansing Corporation (SWCorp), 2004). There were 162 landfills in Malaysia recorded in 2022, resulting in the generation of LFL around the country. According to SWCorp (2022) report, only 14 are classified as sanitary landfills, while 148 are classified as non-sanitary, as displayed in Table 5.

Table 3: Levels of landfills

Landfill levels	Description	Amenities provided
0	Open dumping	No facilities are available (the waste or garbage was just dumped in low-lying parts of open land)
1	Controlled tipping	Limited infrastructure, consisting of fences and perimeter drains
2	Sanitary landfill with a bund and daily cover soil	Consists of a gas removal system as well as distinct areas for unloading and working. These facilities also include a daily cover and an enclosing bund. Furthermore, it eradicated informal scavenging and provided environmental protection systems (Class I facilities)
3	Sanitary landfill with leachate recirculation	Has a leachate recirculation system for collecting, recirculating, and monitoring landfill leachate (Class II facilities)
4	Sanitary landfill with leachate treatment	Provide leachate treatment systems (Class III facilities)

(Source : Moh & Abd Manaf, 2017; Fauziah & Agamuthu, 2012; Kamaruddin et al., 2017)

Table 4: Concepts of each landfill

Landfill levels	Description of levels	Concept
0	-	-
1	Primitive level	Urban sanitation
2	Minimum level	- Urban sanitation - Protect a hygienic sanitary environment in and around the landfill - Enhanced stabilisation as a result of leachate
3	Basic level	- Urban sanitation - Protect a hygienic sanitary environment in and around the landfill - Reduce environmental repercussions - Enhanced stabilisation as a result of leachate - Reduced impact of leachate on the underlying water system
4	Advanced level	- Urban sanitation - Protect a hygienic sanitary environment in and around the landfill - Reduce environmental repercussions - Enhanced stabilisation as a result of leachate - Reduced impact of leachate on the underlying water system

(Source : SWCorp, 2004)

Table 5: Data on sanitary and non-sanitary landfills in Malaysia

WASTE DISPOSAL DATA BY STATE		
State	Sanitary	Non-sanitary
Johor	1	13
Kedah	1	6
Kelantan	0	11
Melaka	1	0
Negeri Sembilan	1	7
Pahang	2	14
Perak	0	16
Perlis	0	1
Pulau Pinang	1	1
Sabah	1	18
Sarawak	3	46
Selangor	3	5
Terengganu	0	9
WPKL	0	0
WP Labuan	0	1
Total	14	148

(Source : SWCorp, 2022)

2.2 Landfill Leachate

2.2.1 Landfill Leachate Generation

The predominant method for managing MSW is landfilling, mainly due to its economic advantages and operational simplicity. The MSW is an unavoidable by-product of routine human activity and comes from a wide variety of sectors, including the institutional, residential, commercial, municipal, industrial, and construction industries, which all contribute to the generation of leachate in landfills. These activities encompass the generation of diverse wastes, including FW, paper, plastic, metal, and glass (Chhay et al., 2018 and Chen, 2018). The United States Environmental Protection Agency (US EPA) (2016) stated that MSW, generally referred to as trash or rubbish, encompasses the everyday goods consumed by individuals and subsequently discarded, which originate from several sources, including residential households, educational institutions, medical facilities, and commercial establishments. According to a report by Ozbay et al. (2021), "open dumping" is used

as an option to handle MSW in about 70% of countries around the world. As per the 2017 report published by the Environmental Protection Agency (EPA), the United States (US) has established regulatory measures to prohibit the use of open dump landfills. LFL will be produced in large amounts if open dumping of solid garbage remains the main method used for waste management in developing countries.

When the leachate wastewater is released or goes into the environment around it, things will get even worse. According to Ahmad et al. (2021), it is crucial to recognise the significant environmental implications associated with by-product waste, specifically the LFL, when it is not adequately managed or treated. When untreated LFL spills into the environment, it contributes to making things more dangerous, especially in tropical regions like Malaysia during the rainy season. To address this concern, the adoption of a well-designed leachate collection system, coupled with the implementation of robust leachate treatment methods, has played a crucial role in the sustainable management of LFL (Ogata et al., 2018). Moreover, it is advisable to construct landfills incorporating suitable landfill design strategies. These may include the incorporation of impermeable lining systems and the utilisation of engineering methodologies to mitigate the potential leaching of LFL. Such measures are crucial for protecting both human well-being and the ecological balance. Besides, in recent decades, there has been a significant expansion in the human population, coinciding with economic development. The observed phenomenon has resulted in a significant increase in MSW and LFL production, displaying a pattern of exponential growth. Thus, the management of waste has emerged as a pressing concern in recent years due to the necessity of effectively handling leachate wastewater.

Malaysia's MSW generation rate has exceeded the rate proposed by the Japan International Cooperation Agency study, which suggested a daily rate of 30,000 metric tonnes for the country by 2020 (Shahril, 2020). Moreover, according to the Department of Statistics Malaysia, the population in Malaysia has been experiencing significant growth, with a recorded total of 32.7 million individuals in 2021. The increase in population consequently led to a substantial generation of MSW, which directly led to higher production of LFL. Leachate refers to the liquid that becomes contaminated and is produced when rainwater permeates through the waste and the liquid leaches out of the waste. The daily generation of solid garbage in Malaysia was estimated to reach approximately 38,427 metric tonnes in 2021, equivalent to an average of 1.17 kg per capita per day (Yaser et al., 2022). Out of the total amount, 82.5% gets deposited in landfills. In addition, it was projected that the annual collection of MSW would reach 14 million metric tonnes by the year 2022 (Shahril, 2022). This quantity is sufficient to fill the Petronas Twin Towers every seven days. According to the Ministry of Economy's Department of Statistics Malaysia report, there will be about 33.2 million people in Malaysia in the first quarter of 2023, as shown in Figure 4. This figure represents a growth of 1.6% in comparison to the population of the first quarter of 2022, which was 32.6 million individuals. As a result, this demonstrates that an expanding human population drives both the generation of MSW and the production of leachate.

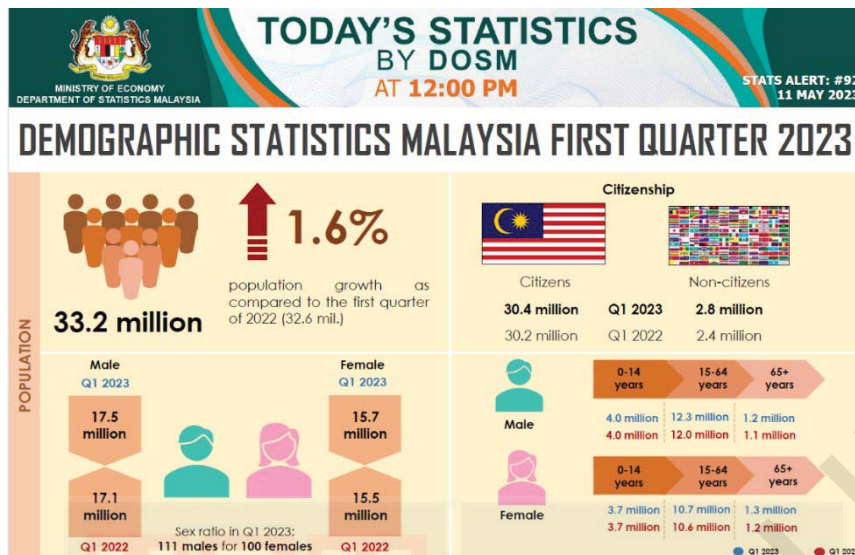


Figure 4: Demographic Statistics Malaysia First Quarter 2023
 (Source: Ministry of Economy Department of Statistics Malaysia, 2023)

2.2.2 Characteristics of Landfill Leachate

The composition of the LFL includes a significant concentration of both organic and inorganic pollutants, nitrogen, ammonia, humic compounds, and salts (Smaoui et al., 2017; Pasaalari et al., 2021). Nevertheless, LFL has great potential in biogas production through the ACoD process since single digestion has limitations due to insufficient nutrients in LFL (Liao et al., 2014; Kawai et al., 2012). According to the study done by Hussein et al. (2021), elevated levels of Manganese (Mn), Arsenic, Iron, Selenium, and Chromium were detected in the untreated LFL obtained from both sanitary and non-sanitary landfills. In addition, failure to properly handle LFL can result in significant environmental consequences, as the production and migration of heavy metals from landfills can potentially lead to soil and groundwater contamination, as well as subsequent surface water pollution, which directly impacts drinking water resources (Bashir et al., 2009; Smaoui et al., 2017; Nawaz et al., 2020; Hussein et al., 2021; Gu et al., 2022).

Leachate wastewater not only carries hazardous contaminants but also contains pathogenic microbes such as *Staphylococcus* and *Bacillus* genera, which have been associated with waterborne diseases (Kalwasinska & Burkowska, 2013; Nair, 2021). The study carried out by Bove et al. (2015), Ahmad et al. (2021), and Nawaz et al. (2020) reveals that the characteristics of LFL vary depending on age groups and the overall composition of LFL in the landfill site, as outlined in Table 6. Ahmad et al. (2021) stated that leachate can be classified into three distinct categories based on its age: young leachate, which refers to leachate that is less than one year old (<1); intermediate leachate, which encompasses leachate that is between five and ten years old (5–10); and old leachate, which pertains to leachate that is more than ten years old (>10). According to Cirik and Gocer (2020), it has been found that young LFL consists of a substantial quantity of biodegradable organic matter. As a result, conventional biological methods can be utilised for its treatment. On the other hand, intermediate and old LFL are characterised by a high concentration of recalcitrant compounds (Cirik and Gocer, 2020). They also have a low ratio (<0.1) of BOD to COD (BOD/COD) (Coppini et al., 2018). As a consequence, the management of LFL requires the implementation of integrated or combined technologies to eliminate the contaminants present in LFL (Youcai & Ziyang, 2017).

Table 6: Properties of landfill leachate

Types of landfill leachate	COD (g/L)	BOD ₅ (g/L)	BOD ₅ /COD	pH	Heavy Metals (mg/L)					Age (years)	References
					Zinc (Zn)	Copper (Cu)	Cadmium (Cd)	Nickel (Ni)	Chromium (Cr)		
Young	0.41-15	0.036-0.984	0.5-1.0	<6.5	<7.64 ^a	<2.42 ^b	<0.007 ^b	<0.66 ^b	<1.44 ^b	<1	Bove et al., 2015;
Intermediate	0.19-15	0.006-0.98	0.1-0.5	6.5-7.5	<1.43 ^b	<0.39 ^b	<0.03 ^b	<0.37 ^b	<0.28 ^b	1-5	Ahmad et al., 2021;
Old	0.70-10.4	1.5-3.0	<0.1	>7.5	<0.0003 ^b	<0.15 ^b	<0.04 ^b	<1.34 ^b	<0.002 ^b	>5	Nawaz et al., 2020

2.3 Liquidised Food Waste from Food Court

2.3.1 Liquidised Food Waste Generation

The generation of FW in Malaysia has been observed to exhibit a consistent upward trend, as stated in the evidence from the data given by SWCorp in 2012, which indicated that FW accounted for the biggest proportion (44.5%) of trash generated in Malaysia. In comparison, the percentages for paper (8.5%), plastics (13.2%), and garden waste (5.8%) were considerably lower, as shown in Figure 5. Based on the data presented by SWCorp, the daily generation of FW reached a total of 17,007 tonnes by Malaysian citizens in 2021. Furthermore, the report stated that the daily disposal of edible food was 4,046 tonnes, accounting for 24% of the total waste generated, which remains a significant amount.

Moreover, the SWCorp predicted that the generation of FW during the Ramadhan season in 2023 would increase by about 75,000 tonnes due to the trend of FW generated over the past few years remaining unchanged. Improper management of FW has the potential to exert significant adverse effects on the surrounding ecosystem, thereby influencing wildlife ecology, facilitating the dissemination of disease-causing bacteria, generating unpleasant odours, and compromising human health (Liu et al., 2023; Liang et al., 2022; Newsome & van Eeden, 2017). Moreover, with the constant discharge of FW into open dumping sites, it will keep on depending on landfills that are unable to be treated effectively. The disposal of a significant quantity of FW in landfill sites leads to environmental contamination due to the release of leachate, thus requiring the adoption of wastewater treatment technologies (Liang et al., 2022).

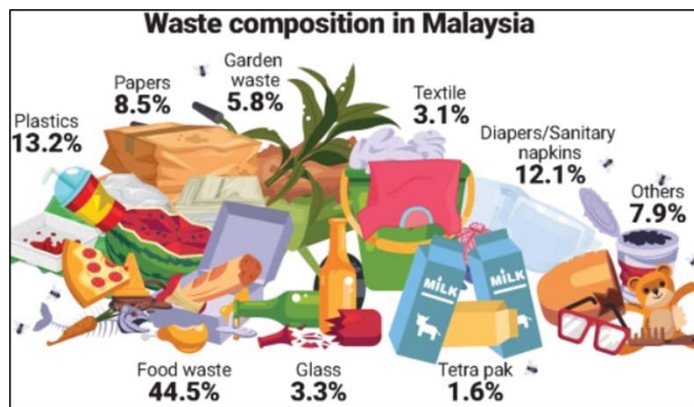


Figure 5: Waste Composition in Malaysia during 2012
(Source: SWCorp, 2012)

2.3.2 Characteristics of Liquidised Food Waste

The FW exhibits a large quantity of readily biodegradable organic material and possesses a high moisture content, as reported by Ma et al. (2018) and Ye et al. (2018). According to Behera et al. (2010), a large proportion of FW has been discarded along with MSW, highlighting the pressing requirement for effective treatment methods. The imbalance of AD systems in FW can occur during prolonged operation as a result of the limitations triggered by single digestion. Due to its abundance and high bioavailability, FW is considered a potential substrate for AD (Lee et al., 2019). However, it is frequently underutilised. FW has a high level of VFAs that are produced during the acidogenesis and acetogenesis phases. According to the findings by Szacherska et al., (2021), the primary constituents of VFAs consist of acetic acid, propionic acid, butyric acid, and pentanoic acid. The stability of the digestive system depends on the balance between the acid-producing and methanogenic phases, which is influenced by the composition of the substance in FW. This substance consists of carbohydrates (10.7–13.7%), lipids (1.4–6.5%), protein (2.4–3.6%), vitamins, minerals, total solids (TS) (16.7–30.9%), and volatile solids (VS) (15.3–26.4%)

(Mehariya et al., 2018). Liu et al. (2023) discovered that the FW has a higher C/N ratio and inadequate buffering capacity, which hinder the AD process. In addition, the lack of TMEs such as Fe and Ni in the FW has been found to lead to the instability of the AD treatment, resulting in a decrease in CH₄ output (Banks et al., 2012; Zhang & Jahng, 2012; Ye et al., 2018). Therefore, a supplementary addition of TMEs for AD in FW deserves significant consideration.

2.4 Anaerobic Digestion

Anaerobic treatment offers significant advantages over current technologies such as coagulation and flocculation, and aerobic treatment. These benefits are primarily due to the production of biogas, which mainly consists of CH₄ as a by-product, reduced sludge production compared to aerobic processes and coagulation & flocculation, cost-effectiveness, since it does not require chemicals or aeration and its high efficiency in treating leachate with a high organic content (Kheradmand et al., 2010; Ahmad et al., 2020; Chen et al., 2023). In contrast, the coagulation & flocculation process involves the addition of coagulants to form flocs from suspended particles, followed by sedimentation. This method incurs high chemical costs and generates large amounts of sludge, necessitating further treatment and disposal (Iwuzor, 2019). According to Irfan et al. (2013) and Abujazar et al. (2022), the coagulation and flocculation process is limited in its ability to remove organic matter, such as COD, due to improper selection of coagulant dosage, and it does not recover energy from the waste stream.

Similarly, aerobic treatment requires continuous aeration, leading to high energy consumption and substantial sludge production, which requires additional treatment (Skouteris et al., 2020). This method also lacks energy recovery and incurs high

operational energy costs (Skouteris et al., 2020; Show & Lee, 2016). While the membrane bioreactor integrates biological treatment with membrane filtration to produce high-quality effluent, it is prone to membrane fouling, which increases maintenance and operational costs (Neoh et al., 2015; Iorhemen et al., 2016; Al-Asheh et al., 2021). Therefore, AD, particularly using the UASB system, is a preferred wastewater treatment method for leachate and FW. AD not only facilitates energy recovery but also offers cost efficiency due to its simple design, low operational costs, and ability to withstand fluctuations in temperature and pH (Atelge et al., 2020; Dutta et al., 2018). The UASB system aligns with sustainable waste management goals by minimising sludge production and recovering valuable resources like biogas. It is particularly effective in treating wastewater with high COD levels, making it more suitable for leachate and LFW treatment compared to aerobic or chemical methods.

Throughout the AD process, bacteria function optimally in an oxygen-depleted environment to decompose organic matter that is abundant in various sources, including animal manure, biosolids generated during wastewater treatment, and FW. This is accomplished through microbial activities, as highlighted by Kurniawan et al. (2006) and Achinas et al. (2020). Through the synergistic interaction of diverse anaerobic bacteria within the reactor, biogas will be produced. Over the past few decades, there has been a significant increase in the use of anaerobic technology. This treatment approach holds great promise as it efficiently removes contaminants from wastewater, produces energy in the form of CH_4 , and minimises the production of sludge when compared to aerobic processes, as demonstrated by Renou et al. (2008) and Ahmad et al. (2020). AD is a highly efficient process that yields biogas primarily composed of CH_4 (50-75%), the main component found in natural gas (Li et al., 2019).

Besides, the sustainable energy source predominantly consists of CH₄, hydrogen sulphide (H₂S), CO₂, water vapour, and small quantities of various other gases (Li et al., 2019; Andriani et al., 2020). Moreover, the advantages of employing anaerobic treatment are its operational simplicity, superior organic removal performance, less odour risk, reduced greenhouse gas emissions, and high efficiency in bioenergy recovery (Agyeman & Tao, 2014; Meegoda et al., 2018; Yuan et al., 2022). Several extensive investigations have been carried out on the remarkable anaerobic process for biogas production, employing a diverse range of biomass as a sole substrate (Bi et al., 2019; Abid et al., 2021; Hao et al., 2022). However, the effective utilisation of a single substrate in direct applications in AD treatment can present certain challenges due to the lack of optimal nutritional balance, limited microbial diversity, and the influence of operational conditions.

The effluent resulting from the process of AD, known as digestate, can sometimes emit unpleasant odours due to the presence of various reduced compounds, including ammonia and H₂S (Bove et al., 2015; Chudnikova, 2018). However, this digestate possesses a significant abundance of valuable nutrients, making it an excellent resource for implementation as a fertiliser. According to the insightful research conducted by Stürmer et al. (2020) and Salomon et al. (2022), the digestate derived from biogas production has enormous promise as a valuable fertiliser. This is primarily due to its abundance of plant-available nitrogen (NH₄-N), which can significantly enhance crop yields. Nevertheless, the practical application of this resource remains quite limited in current practice. Salomon (2022) has observed that there is a growing demand for high-quality digestate among Swedish organic farmers. This particular form of organic waste is highly valued by farmers, with a great preference for raw manure or natural

fertilisers. In addition, the production of biogas with a significant CH₄ concentration presents an important benefit in terms of energy generation. However, it necessitates the implementation of suitable safety precautions to mitigate the risk of explosive CH₄ and oxygen mixtures (Zhou et al., 2003; Bove et al., 2015).

The effective application of the CH₄ component within biogas produced during AD presents a promising opportunity to support various energy-intensive applications, such as heat provision, electricity generation, and the operation of cooling systems. In the study conducted by Marzuki et al. (2021), it was observed that the AD treatment of chicken slaughterhouse wastewater resulted in the generation of 12.73 kWh/m³ of energy. Moreover, referring to the findings by Jaman et al. (2022), the study reveals the energy production associated with the anaerobic processing of FW, chicken manure, and the ACoD of FW with chicken dung is 122.96 kWh, 126.10 kWh, and 171.13 kWh, respectively. Moreover, complex waste originating from industrial processes, which contains harmful compounds, can be efficiently remediated through anaerobic treatment (Wijetunga et al., 2010). Hence, the adoption of anaerobic processes for treating leachate produced in landfills exhibits appealing potential in terms of its practicality. This approach offers advantages such as reduced space requirements, diminished greenhouse gas emissions, lower energy consumption due to the absence of aeration, minimal or negligible sludge generation, and CH₄ production that can be applied to renewable energy (Kumar et al., 2015; Nanda et al., 2016; Achinas et al., 2017).

2.4.1 Stages of the Anaerobic Digestion Process

AD is a biological process assisted by specific microorganisms that thrive in oxygen-depleted environments. Zaidi et al. (2018) and Bhatt & Tao (2020) mentioned that the process of organic matter breakdown occurs in four distinct stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis, as illustrated comprehensively in Figure 6. Every step within the AD system occurs through the activity of a particular microorganism. The imbalance in the bioavailability of these microorganisms can lead to instability within the AD system, as highlighted by Nie et al. (2021). The initial phase of the AD process is hydrolysis. This crucial stage involves the breakdown of complex and insoluble organic matter into simpler monomers. This breakdown is facilitated by the activity of hydrolytic microorganisms such as *Bacillus*, *Eubacterium*, and *Cellulomonas*. The proteins undergo a process of conversion wherein they are broken down into their constituent amino acids. Similarly, lipids will be converted into fatty acids while starch, a complex carbohydrate, is transformed into glucose, a simpler form of sugar. Additionally, carbohydrates will be converted into monomeric sugars. The hydrolysis reaction, as described in Equation 1, clarifies the transformative process by which organic waste undergoes conversion into a more readily accessible form of simple sugar (glucose) (Abdallah et al., 2016; Ahmad et al., 2020).

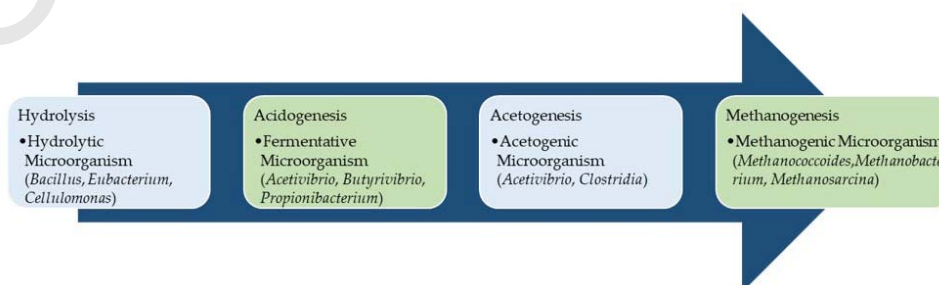
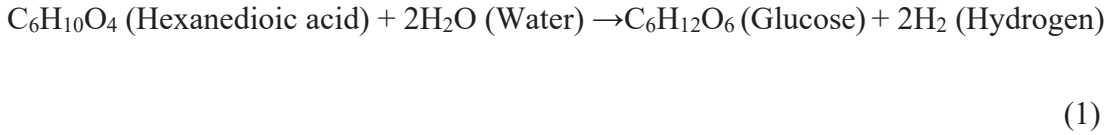
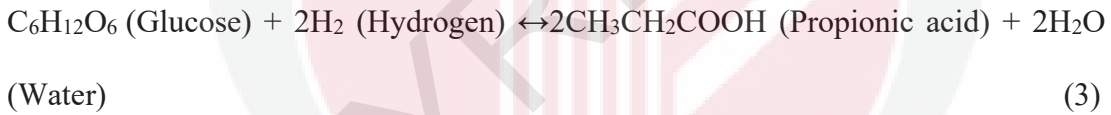


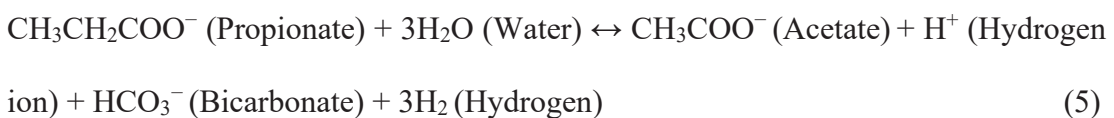
Figure 6: Phases of the Anaerobic Process
(Source: Zamrisham et al., 2023)



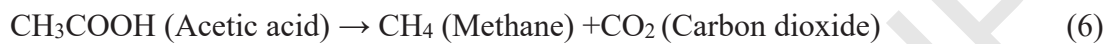
Following that, acidogenesis takes place to catalyse the transformation of simple sugars and acids into VFAs and alcohols through the reaction of acidogens. This is the quickest phase of the AD process, which involves complex bacterial consortiums including *Acetivibrio*, *Butyrivibrio*, and *Propionibacterium*. In the acidogenesis phase, butyric acid, formic acid, acetic acid, propionic acid, ethanol, lactic acid, and methanol are produced. Equations (2)-(4) illustrate three common acidogenesis reactions in which glucose is transformed into ethanol, propionic and acetic acid, respectively (Abdallah et al., 2016; Ahmad et al., 2020).



Then, the process of acetogenesis occurs, wherein the products generated during acidogenesis undergo transformation into CO_2 , hydrogen, and acetic acid, assisted by the presence of acetogenic bacteria such as *Acetivibrio* and *Clostridia*. The propionate to acetate conversion is depicted in Equation 5.



The final stage of the AD process is referred to as methanogenesis. In the process of methanogenesis, the acetic acid and hydrogen are transformed by methanogenic bacteria (*Methanococcoides*, *Methanobacterium*, and *Methanosarcina*) into CH₄ and CO₂, as indicated in Equation 6.



2.4.2 Design Parameters in Anaerobic Digestion

Optimising an AD process involves carefully considering important parameters consisting of temperature, pH, OLR, HRT, and the C/N ratio. Thus, it is crucial to take into account these parameters when conducting AD of LFL, as they greatly affect microbial activity, which in turn affects the production of biogas. These factors will be further explored below.

2.4.2.1 Temperature

Temperature is an important part of the AD process for keeping bacteria communities alive while producing biogas on a regular basis. The AD system operates at three temperature levels: psychrophilic (<20 °C), mesophilic (20-45 °C, ideal 35-37 °C), and thermophilic (50-60 °C, optimum 55 °C) (Madigou et al., 2019; Fernández-Rodríguez et al., 2016; Kovalovszki et al., 2020). This is because methanogenic bacteria are classified as psychrophiles, mesophiles, and thermophiles (Rabbani et al., 2023). Zhu et al. (2014) stated that when temperatures fall below the optimal range, the efficiency of removal will drop. Furthermore, temperature has an impact on the build-up of TAN,

which can impede the hydrolytic, acidogenic, and methanogenic processes, resulting in a reduction in CH₄ production (Bi et al., 2019). Most research has preferred a mesophilic temperature as it is the most suitable for studying various sample types in terms of CH₄ generation. Blasius et al. (2020) and Cao et al. (2020) have carried out AD experiments at mesophilic temperatures. Blasius et al. (2020) achieved a CH₄ production of 869 mL of CH₄/gTVS, while Cao et al. (2020) produced a CH₄ production of 470 L/kgVS feed. Thermophilic systems confront high operational costs due to the need for energy to sustain elevated operating temperatures. Additionally, they are susceptible to acidification and are very sensitive to toxins, as demonstrated by Mao et al. (2015) and Bhatt & Tao (2020). This acidity subsequently hampers the production of biogas. Several drawbacks associated with the use of thermophilic environments include reduced stability, the production of low-quality effluent, greater toxicity and financial investments and inadequate methanogenesis (García-Mancha et al., 2017). According to Bi et al. (2019), the production of CH₄ was much greater in mesophilic environments compared to thermophilic ones when a higher OLR was applied. This is because the accumulation of VFAs occurred more quickly in the thermophilic condition than in the mesophilic condition. Even though mesophilic reactors produce less biogas than thermophilic reactors, their strong microbial consortia can survive more environmental changes and are more stable and easier to maintain (Hu et al., 2018). Therefore, temperature is a crucial determinant that significantly impacts the metabolism of the microbial community and subsequently affects the effectiveness and consistency of the AD system.

2.4.2.2 pH

pH is a crucial factor in AD since microbial activity is highly responsive to variations in pH levels, which directly impact the efficiency of the digestion process and the resulting products. Despite numerous research studies, the search for the ideal pH range for the AD system remains challenging due to the variability of substrate features, inoculum classification, composition, and other operating conditions. This variability leads to unpredictable and inconsistent results. According to Dareioti et al. (2022), lower pH values typically lead to a reduction in hydrogenase activity, which is a significant component of the impact of pH on fermentative hydrogen generation. The study conducted by Nain et al. (2021) suggests that the ideal pH for AD in LFL would fall within the range of 5.56–7.58. The pH lowers during the initial phase of the AD process due to the hydrolysis and acidification stages. Subsequently, it gradually increases and reaches a rather stable level during the methanogenesis stage (Dai et al., 2016). Additionally, the pH in the anaerobic reactor observed a significant decrease as there was a rise in the overall concentration of VFAs (Sánchez et al., 2021). Therefore, maintaining accurate regulation of pH levels in LFL anaerobic digesters is a primary necessity (Bove et al., 2015). Ahmad et al. (2021) and Bi et al. (2019) discovered that NaOH and sodium bicarbonate (NaHCO_3) are effective in controlling pH levels in anaerobic treatment systems. The study performed by Mao et al. (2015) found that the presence of microbial species tends to increase from pH 4.0 to pH 7.0, in which the bacterial community that is most abundant at a pH of 6.0 is *Clostridium butyricum*. Jiang et al. (2013) reported that adjusting the pH level to 6.0 led to the highest concentration of VFAs and the largest ratio of VFAs to soluble chemical oxygen demand (SCOD), while simultaneously resulting in the lowest levels of VS. The study revealed that acetate and butyrate collectively constituted 77% of the total VFAs at a

pH of 6.0. At high OLR, VFAs can develop, which can lower pH and potentially cause AD to fail, as indicated by Zhang et al. (2014). It is important to highlight that both methanogenic and acidogenic microorganisms have specific pH levels that are most favourable for their growth and activity. The ideal pH range for the acidogenesis process to operate in is between 5.5 and 6.5, as determined by Mao et al. in 2015. Methanogenesis is highly robust within a pH range of 6.5–8.2, with the ideal pH being 7.0 (Lee et al., 2009; Zhang et al., 2014; Físgativa et al., 2016). Furthermore, Latif et al. (2017) discovered that a pH of 5.5 restricts the efficiency of methanogenesis in the AD process, resulting in a 50% decrease in CH₄ production and an elevation in propionic acid concentration to levels ranging from 370 to 430 mg/L. Zhou et al. (2016) stated that a pH of 7.0 in the reactor led to the highest biogas output, which can be used in large-scale biogas engineering, and the lowest amount of total organic carbon during mesophilic fermentation. Thus, choosing an appropriate pH for AD of LFL is very important, as microorganisms grow successfully at their optimal pH, which affects biogas production.

2.4.2.3 Organic Loading Rate (OLR)

The OLR affects AD process stability, system efficiency, microbial activity, biogas production, CH₄ content and digestate quality. Optimising AD system efficiency requires OLR management. Finding the right balance between the loading rate and HRT is one way to make sure the AD process works at its best (Liu et al., 2016). The consistency of the AD process relies on keeping the OLR at the right level. A high OLR indicates a high capability for treatment and CH₄ production. However, an excessively high OLR might overload the system, resulting in process imbalances, suppression of microorganisms, and reduced biogas production, including CH₄

formation (Musa et al., 2018; Duan et al., 2019). Sánchez et al. (2021) observed that when the OLR reached 7 g VS/(L d), there was a significant decline in the rate at which VS were removed in the control reactors. Additionally, if the OLR is excessively low, the system's efficiency may be compromised. The OLR has a proportional influence on the generation of biogas, as demonstrated by T.A.S. et al. (2020), who found that the mono-digestion of MSW resulted in a greater biogas output of 0.61 m³/kg VS reduced when the OLR was 6.2 VS/m³/day. The OLR plays a crucial function in AD as it governs the quantity of organic waste that can be efficiently digested and transformed into biogas. Ferguson et al. (2016) and Jung & Pandit (2019) declare that the OLR defines the quantity of organic matter introduced into a treatment system during a specific period, typically expressed as VS or COD and evaluated in terms of mass per day. In addition, by optimising the OLR, the operators can also achieve the primary objective of AD, which is to maximise biogas production. Reducing HRT and increasing OLR are advantageous in the treatment of acidic wastewater in a photobioreactor system, as they promote nutrient availability for the microorganisms (Liu et al., 2016). It is advisable to use a lower OLR when treating wastewater with high concentrations. This is to ensure that the substrate is fully biodegraded and to prevent sludge floating, which can result in low VFA concentrations (Zhu et al., 2014). An optimal OLR offers several benefits, including reducing the initial investment required for the plant, enhancing the production of biogas, and ensuring consistent operational performance (Jiang et al., 2020). Thus, it may be concluded that high OLRs can exert pressure on microorganisms, resulting in process instability, whereas low OLRs may not fully use the microbial population, thereby identifying the appropriate OLR is crucial for preserving a balanced and thriving microbial

environment in the AD process. The OLR is calculated using the COD concentration of a specific sample in the AD process, as expressed in Equation 7 below.

$$\text{OLR} = \frac{\text{COD}_{\text{in}} \times 50\% \text{ } W_v}{W_v} \quad (7)$$

Where;

COD_{in} = COD of influent (g/L/day)

W_v = Working volume of digester (L)

2.4.2.4 Hydraulic Retention Time (HRT)

HRT is a key part of the AD process for both LFL and LFW. It describes how long wastewater or feedstock stays in the digester to completely break down organic matter. It can have a major impact on how well AD systems work in terms of the removal of COD as well as the production of biogas (Mao et al., 2015; Kiyasudeen et al., 2016; Noraini et al., 2017). Lowering HRT and raising OLR allowed the nutrient removal to work better (Liu et al., 2016). In addition, the inverse of HRT is known as the dilution rate. If the dilution rate exceeds the growth rate of microbes, the microbes will be washed out of the treatment system (Ahmad et al., 2021). Besides, the reactor discharge occurs when the HRT is exceptionally low, signifying that every active microorganism escapes the reactor (David et al., 2019). The reduced efficiencies observed at extremely low HRTs may be attributed to the bacteria not having sufficient time to feed on the substrate, which can result in the accumulation of VFAs (Mao et al., 2015). Conversely, when HRT is longer than optimal, it leads to inadequate utilisation of the digester component. In addition, if the HRT is insufficient or the OLR is excessively high, there will be a significant build-up of VFAs, leading to inadequate waste

stabilisation (Dong et al., 2022). A shorter HRT accelerates nitrogen mineralisation which requires a higher OLR, as observed by Di Maria et al. (2014). Dhar et al. (2016) found that the production of biogas from the AD of the organic part of MSW was discovered to be 81% when the HRT was 7 days. In a study carried out by Dareioti et al. (2022), the researchers found that the highest rate of CH_4 production was 0.44 L/L.R.d when the HRT was set at 25 days and the CH_4 yield was measured at 295.3 $\text{mL/g VS}_{\text{added}}$. However, when the HRT was reduced to 20 days, the AD process experienced inhibition. The result was demonstrated by the accumulation of VFAs and a decrease in CH_4 productivity. In contrast, Huang et al. (2023) noticed a different outcome in their investigation, where decreasing the HRT from 4.4 days to 1.5 days increased CH_4 yield, specifically from 27% to 38%. Furthermore, another study found that a shorter HRT is optimal for high-strength organic waste, indicating that an HRT of 1 day is suitable for the ACoD of FW and dairy manure (Li et al., 2009).

Ahmad et al. (2021) stated that the accumulation of VFAs due to inappropriate HRT occurs as they cannot be completely broken down by heterotrophic microorganisms and methanogens. The HRT illustrates the period during which waste and bacteria come into contact (Reif et al., 2013). HRT is a crucial parameter for the continuous production of hydrogen and CH_4 (Dareioti et al., 2022). HRT also affects the microbial community, the effectiveness of nutrient removal, and the ability of biomass to settle, which can be converted to fuel (Chen et al., 2021). A prolonged HRT will need a higher digester capacity, which will increase the operational cost, whereas a shorter HRT can reduce the price of the digester operation but will eliminate the active bacterial community (Sravan et al., 2021). Hence, properly managing HRT can prevent process imbalances in the anaerobic digester. The optimum HRT is needed, where it is not too

short or too long, to get the best outcome from the AD in terms of biogas production, waste stabilisation and CH₄ yield.

2.4.2.5 C/N ratio

Having a well-balanced C/N ratio is crucial to providing sufficient C and N for microbial activity through the AD process. According to Morozova et al. (2020), the C/N ratio represents the nutritional availability of microorganisms. Therefore, if the C/N ratio is not properly balanced, it may result in disruptions to the microbial community and hinder the overall efficiency of the AD process. The characteristics of mature LFL have a low C/N ratio, which restricts the ability of AD (Nabi et al., 2022). An optimal balance of nutrients is extremely important to maintain CH₄ production without any negative impact on AD system. An optimal C/N ratio for the success of a biological process is typically regarded as being between 20 and 30 (Yan et al., 2015; Gil et al., 2018; Li et al., 2018; Xu et al., 2018; Ceron-Vivas et al., 2019). In an investigation conducted by Zhu et al. (2014), the biological treatment experiments were performed using different C/N ratios ranging from 1 to 7. Based on the study, it was found that with a C/N ratio of 5, the highest removal efficiency was observed for TN, nitrate-nitrogen, and COD. It was also found that the treatment efficiencies of the system were significantly influenced by the presence of a diverse microbial community, particularly at higher ammonia concentrations. Furthermore, a feedstock with an inadequate C/N ratio may hinder the methanogenesis process by causing a low pH (<6.8) and the build-up of VFAs, leading to a significant release of TAN and consequently negatively impacting CH₄ generation (Piątek et al., 2016; Hillion et al., 2018; Li et al., 2018; Lin et al., 2019). Anaerobic microorganisms desire an ideal nutritional equilibrium to facilitate growth and sustain a consistent habitat

(Zhang et al., 2014). According to Náthia-Neves et al. (2018), substrates with a greater C/N ratio have excess carbon, which leads methanogenesis to quickly consume nitrogen and reduce biogas generation. Additionally, when substrates have a high C/N ratio, it also results in low CH₄ generation (Li et al., 2019). However, low C/N ratios promote ammonia accumulation in the digester system, which affects the microbial community and reduces AD efficacy (Boonnorat et al., 2016). Therefore, co-digestion of feedstock with high and low C/N ratios is common for obtaining nutritional equilibrium, regulating C/N ratios, and accelerating biodegradation (Liang et al., 2021). Fazzino et al. (2021) found that matured LFL (has a low C/N ratio and source of nitrogen compound) may balance the higher C/N ratio of market waste and provide microorganisms with micro-nutrients with a CH₄ output of 0.302 NL/gVS_{added}. Thus, the C/N ratio is essential in AD since microbes need a balanced C and N composition to survive.

2.4.2.6 Summary of the optimal range of design parameters in anaerobic digestion

Table 7: Optimal range of design parameters in anaerobic digestion

Design parameters	Optimum range	References
Temperature	Mesophilic (20-45 °C, ideal 35-37 °C)	Bi et al. (2019); Hu et al., 2018); Kovalovszki et al., 2020; Fernández Rodríguez et al., 2016;
pH for Methanogenesis process	6.5–8.2 (ideal pH ~7.0)	Lee et al., 2009; Zhang et al., 2014; Físgativa et al., 2016
Organic Loading Rate (OLR)	- No specific range value of OLR. The best range for OLR in AD depends on several factors, such as the type of substrate, reactor design, and operating conditions. - Excessively high OLR can overload the system, disrupt microorganisms, and reduce biogas, including methane production. Conversely, low OLR can reduce efficiency. - Therefore, identifying the optimal OLR is essential for maintaining a balanced microbial environment in the AD process.	Musa et al., 2018; Duan et al., 2019
Hydraulic retention time (HRT)	- Balancing HRT with OLR is essential for optimal performance in AD systems. - An HRT of 1 day is recommended for high-strength organic wastes.	Di Maria et al., 2014; Dong et al., 2022; Li et al., 2009
C/N ratio	20:1 and 30:1 - In order to operate the anaerobic digester in an optimal ratio of C:N, biodegradable materials with a high ratio of C:N should be mixed with biodegradable materials with a low ratio of C:N.	Yan et al., 2015; Gil et al., 2018; Li et al., 2018; Xu et al., 2018; Ceron-Vivas et al., 2019

2.4.3 Limiting Factors in Anaerobic Digestion of Landfill Leachate

Treatment of leachate is required to minimise the pollution of water resources. AD has gained significant attention in recent years due to its cost-effectiveness and ability to generate high-energy biogas as a by-product. This biogas can be utilised as a renewable

energy source, contributing to environmental protection (Mao et al., 2015). Nevertheless, there are several considerations that must be taken into account when conducting AD treatment for LFL. According to a study carried out by Kawai et al. (2012), the Benowo LFL in Indonesia was found to have high levels of sodium (Na^+) and chlorine (Cl^-), which hinder the AD process. Moreover, the elevated concentration of Na^+ will have a detrimental effect on groundwater and subsequently contaminate surface water. This can have adverse effects on human health, including organ collapse, as well as destroy multiple enzymatic processes in the plant cytoplasm, leading to tissue dehydration and discoloration (Parvin & Tareq, 2021; Tester, 2003). In addition, LFL contains significant amounts of copper (Cu) and zinc (Zn), which act as inhibitors for the successful functioning of the AD system (Kurniawan et al., 2006; Guo et al., 2010; Zhang et al., 2013; Bove et al., 2015; A et al., 2017). These elevated levels of heavy metals can have detrimental effects on human health, such as kidney dysfunction and liver injury (Kurniawan et al., 2006).

Furthermore, the uncontrolled discharge of elevated levels of ammonium in LFL without undergoing proper treatment has been observed to have detrimental effects on the efficiency of biological treatment systems. This phenomenon also contributes to the acceleration of eutrophication, the destruction of dissolved oxygen, and increased toxicity for aquatic organisms in surface waters, as reported by Karadag et al. (2008).

The concentration of ammonium in leachate can reach levels of several thousand, as the previous LFL generally exhibits a significant concentration of ammonium, typically ranging from 1500 to 2500 g/m^3 , as reported by Wichitsathian et al. (2004) and Bove et al. (2015). The presence of high concentrations of ammonium is a significant characteristic of LFL conditions, as it can hinder the whole AD system. A

study conducted by Cabeza et al. (2007) aimed to investigate the removal of ammonium from two different types of LFL through electrochemical oxidation. The first type of leachate, referred to as raw LFL, had an initial ammonia concentration of 1900 mg/L, while the second type of leachate, which experienced biological and physico-chemical treatments, had an initial ammonia concentration of 860 mg/L. The results of the study showed that the LFL that was pre-treated was better at eliminating ammonium, with 95.8% of the ammonium removed. Nevertheless, Kawai et al. (2012) found that the ammonium ions in the LFL from the old landfill were within a range of values (237 mg/L), which was still lower than the inhibition level (4000–5000 mg/L) that did not prevent the AD process.

Additionally, elevated salinity levels may compromise the effectiveness of the AD process, thereby impeding the production of CH₄. According to Kawai et al. (2012), the accumulation of FW and intrusion of seawater could result in a salinity greater than 1% in the LFL of Benowo, Indonesia. A higher salinity (1.7%) of LFL Benowo was discovered to inhibit or even destroy the anaerobic microorganisms that are accountable for the digestion process. Additionally, Moradian et al. (2020) reported that the LFL of the Iranian coastal city of Bushehr has a high salinity with a 7.39 g/L total dissolved solids (TDS) value, which indicates that the leachate is saline. The results of the investigation show that the application of biological methods that deal with high salt levels is not feasible, as the high salinity disrupts the treatment process. Further investigations by Lee et al. (2009) and Wang et al. (2017) show that higher salinity levels affect biogas production, with salinity levels between 2 and 10 g/L being shown to moderately inhibit methanogenic activity, while salinity levels above 10 g/L strongly inhibit the process of methanogenesis.

LFL also possesses significant ammonia nitrogen and organic matter (Li et al., 2021). The high nitrogen content of LFL makes it difficult to remove nitrogen economically, and ammonia nitrogen concentration does not decrease with LFL age (Miao et al., 2019). Recently, LFL treatment research has focused on nitrogen removal. Since LFL has a high nitrogen content, Bove et al. (2015) concluded that appropriate treatment procedures and technologies have to be implemented to mitigate its adverse effects on the environment. On top of that, the old LFL exhibits a low C/N ratio and biodegradability (<0.3), both of which contribute to the instability of the AD system and ultimately result in the inability to generate biogas and CH_4 (Bove et al., 2015; Li et al., 2021). As a result, LFL is often co-digested with other substrates to compensate for its poor anaerobic digestibility and to balance the nutrients (Liao et al., 2014). This technique shows promise for enhancing digestion efficiencies.

2.4.4 Limiting Factors in Anaerobic Digestion of Liquidised Food Waste

AD of FW as a sole substrate is susceptible to imbalances over extended periods of operation. The utilisation of ACoD in FW with other substrates has been shown to address the performance constraints associated with single digestion processes. This approach enables the simultaneous utilisation of several waste streams and facilitates the recovery of valuable resources (Liu et al., 2023). According to the study performed by Jiang et al. (2012), fruit and vegetable waste possess a high potential for biodegradation, a similar characteristic of FW, making it suitable for energy recovery by conducting the AD process. Nevertheless, it is commonly distinguished by its substantial organic composition, as reported by Arvanitoyannis and Varzakas (2008) and Jiang et al. (2012), which includes over 95% of the total solid content. Additionally, it exhibits a significant moisture content, exceeding 80%. Also, the

organic portion of this FW consists of around 75% sugars and hemicellulose, 9% cellulose, and 5% lignin. FW is classified as a carbohydrate-rich substance, and it has been observed that substrates with high carbohydrate content often necessitate co-digestion with other feedstocks, the use of an alkaline buffer, or the use of additives to maintain the stable performance of the AD system (Ward et al., 2008; Ye et al., 2018). In addition, Xue et al. (2019) stated that FW which is rich in carbohydrates accelerates acidification and produces more hydrogen due to its imbalanced C/N ratio and lack of nutrients.

Furthermore, quick hydrolysis during mono-digestion of FW leads to significant acidity in anaerobic digesters, hence inhibiting the generation of biogas and CH₄ (Ward et al., 2008). The presence of trace metals in FW is insufficient; however, these metals are essential for the activation of methanogens and to maintain stable operation in AD of FW (Zhang & Jahng, 2012; Banks et al., 2012; Zhang et al., 2015; Ye et al., 2018). A possible solution to solve this problem involves employing co-digestion with LFL, as LFL is known to possess a significant concentration of trace elements (Naveen et al., 2017). Besides, Kim et al. (2011) observed that the initial pH for FW is 5, which is considered a low pH level that can cause inhibition of the AD system. Furthermore, it has been observed that the AD process, specifically the acidogenesis stage, is prone to instability and failure when operated solely with FW. This is primarily attributed to the accumulation of excessive organic acids, which impede the activity of methanogenic microorganisms (Fisgativa et al., 2016). Hence, the FW contains a build-up of VFAs generated during the acidogenic phase. This VFA accumulation could disrupt methanogenesis and collapse mono-digestion FW (Behera et al., 2010; Liu et al., 2023).

2.5 Anaerobic Digestion of Landfill Leachate

2.5.1 Anaerobic Reactor

Multiple types of anaerobic reactors have been widely investigated for their ability to remove pollutants from LFL and produce energy in the form of CH₄. Common anaerobic reactors are UASB, fluidised and expanded bed reactors, anaerobic filters (AF), and completely mixed anaerobic digesters (Ersahin et al., 2011). In addition, other types of anaerobic reactors used to treat LFL include anaerobic membrane bioreactors (AnMBR) and anaerobic contact reactors, as highlighted by Ahmad et al. (2021). Table 8 displays different types of anaerobic reactors used in the treatment of LFL. According to Kennedy's (2000) study, it was shown that continuous UASB reactors had superior performance at higher OLRs compared to anaerobic sequencing batch reactors (AnSBR). The study found that the COD removal efficiency remained consistently within 77-91% across all HRTs, while the CH₄ percentage exceeded 84%. Furthermore, Ye et al. (2011) demonstrated that the UASB reactor exhibited a high efficiency in removing 82.4% of COD from fresh leachates obtained from incineration plants. Additionally, Li et al. (2020) reported that the utilisation of a UASB reactor, which was modified based on the Bardenpho method, demonstrated enhanced efficacy in the treatment of fresh LFL. The UASB reactor exhibited outstanding results in terms of COD removal, with removal rates ranging from 97.5% to 99.5%, as well as ammonium removal, with rates ranging from 99.3% to 99.7%. This finding suggests that the implementation of UASB reactors is a viable approach for treating LFL.

Table 8: Performance of various anaerobic reactors in treating LFL

Type of anaerobic reactors	Chemical Oxygen Demand, COD content (mg/L)	Hydraulic Retention Time, HRT (days)	Organic Loading Rate, OLR	% removal	Methane production	Remarks	References
Up-flow anaerobic sludge blanket reactors (UASB) (Old LFL)	14640	30	-	• COD removal: 74% • TP removal: 89% • TSS removal: 81% • BOD removal: 64% • TN removal: 50%	-	• Excellent at eliminating TP and TSS from LFL • Support material and concentrated biological growth are unnecessary • Inefficient for (TN) removal	(Ridzuan et al., 2018; Zhang et al., 2022)
Anaerobic fluidised bed reactors (AFBR) (Young LFL)	Average: 35000	1	12 g COD L-1 day-1	COD removal: 90%	75%	• Accumulates significant biomass by natural attachment • Short retention times • High flow rates • High stability performance	(Gulsen et al., 2004)
Anaerobic Filter (AF) (Young LFL)	15200	4.5	3.3 g COD L-1 day-1	COD removal: 74.72%	19.24 d ⁻¹	• Optimises high-load systems and remains stable during transient conditions with fluctuating effluent compounds and toxic substances • Exhibits high efficiency in COD removal and biogas production	(Zayen et al., 2016)

Table 8: Cont.

Type of anaerobic reactors	Chemical Oxygen Demand, COD content (mg/L)	Hydraulic Retention Time, HRT (days)	Organic Loading Rate, OLR	% removal	Methane production	Remarks	References
Anaerobic baffled reactors (Old LFL)	3966-5090	0.73	3.58 g COD L ⁻¹ day ⁻¹	COD removal: 66%	-	The addition of sulphate has been shown to enhance the performance of anaerobic baffled reactors (ABRs) in the treatment of leachate	(Burbano-Figueroa et al., 2015)
Anaerobic membrane bioreactor (Middle/Old LFL)	7014 ± 250	1	-	COD removal: 83%	0.35 L methane/g COD _{removed}	Air stripping as a pre-treatment effectively removed high levels of ammonia from LFL, thereby enhancing biodegradability in the anaerobic membrane bioreactor	(Cirik & Gocer, 2020)

Besides, UASB reactors are also used as anaerobic digesters for the treatment of wastewater with high organic content (Rajagopal et al., 2019). The UASB reactor, as described by Chernicharo et al. (2015) and Behera & Varma (2018), utilises a three-phase separator to effectively separate gas, water, and sludge mixtures, as shown in Figure 7. This separation process occurs under high-turbulence conditions, enabling the reactor to be designed in a cost-effective and relatively simple manner when compared to other types (Nabi et al., 2022). The digester facilitates the extraction of biogas by separating it from the liquid effluent and residual sludge particles. This biogas can then be used as an alternative source of electricity (Tauseef et al., 2013; Ferreira et al., 2019). The UASB is a highly efficient anaerobic reactor known for its ability to effectively remove a large amount of COD while simultaneously producing

biogas (Bakraoui et al., 2020). This remarkable performance is achieved despite the compact size of the reactor. The UASB reactor employs an up-flow configuration, wherein wastewater is introduced into the reactor from the bottom and flows in an upward direction. This design allows for the wastewater to come into contact with the highly concentrated biomass present in the sludge bed initially before moving upwards through the sludge blanket, which contains a lower concentration of biomass (Adhikari & Lohani, 2019; Mainardis et al., 2020). The sludge blanket is composed of an anaerobic microbial community, in which this community is responsible for decomposing the organic substance present in the substrate and producing biogas, as described by Ngema et al. (2023). The removal of pollutants from wastewater is facilitated by the microflora that is attached to the sludge particles (Abbasi & Abbasi, 2012). Therefore, the success of a UASB reactor is dependent on the level of contact between the sludge and wastewater.

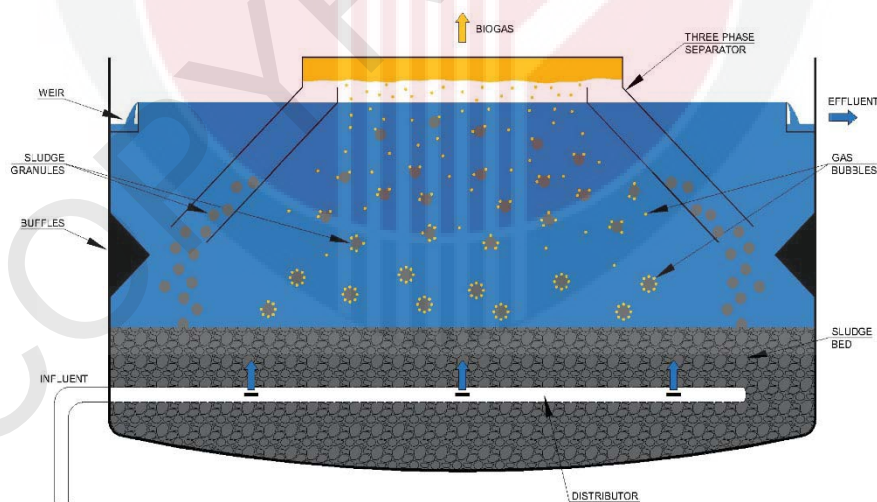


Figure 7: UASB reactor process
(Source: Mainardis et al., 2020)

In addition, the UASB reactor demonstrates an outstanding treatment capacity, enabling the removal of up to 90% of COD, as identified by Musa et al. (2019), and Marzuki et al. (2021). This significant capability serves as a major advantage. The utilisation of a UASB reactor results in a significantly reduced generation of biosolids waste when compared to a reactor operating under aerobic conditions (Yoo et al., 2012). This is due to the conversion of a significant amount of the energy present in the wastewater into a gaseous form, leaving minimal energy available for the growth of new cells. The system is also capable of effectively managing high OLRs and is suitable for the treatment of various types of wastewater influents (Daud et al., 2018). In addition, the UASB system necessitates minimal energy due to the absence of aeration and low nutrient requirements (Berni et al., 2014; Bobade et al., 2015; Mainardis et al., 2020). Apart from that, the UASB system also offers effective operational control and has a small footprint due to its compact design, making it well-suited for locations with space constraints (Rattier et al., 2022). However, the UASB reactor has been recognised for its long start-up time, and recovery time may be long after stress conditions (Jung et al., 2013 and Mainardis et al., 2020).

2.6 Anaerobic Co-Digestion

The application of biological treatment is commonly employed for the treatment of LFL substances, as stated by Kurniawan et al. (2006). Nevertheless, the effectiveness of biological treatment in removing refractory substances is limited due to the presence of high levels of toxic compounds in LFL (Bhatt and Tao, 2020). Hence, the implementation of co-digestion is necessary to optimise the degradation of organic compounds in LFL. The process known as ACoD involves the decomposition of multiple substrates with complementary characteristics, as stated by Takeda et al.

(2020). Its purpose is to address issues associated with mono-digestion, enhance the efficiency of the AD system, and provide economic benefits through biogas production, particularly CH₄, which can be used for heating and power generation (Hagos et al., 2017; Rabii et al., 2019; Takeda et al., 2020). Besides, ACoD is a process that involves the combination and treatment of diverse organic materials in an anaerobic digester. These materials typically exhibit different C/N ratios and possess distinct properties.

According to a previous study conducted by Begum et al. (2018), it was observed that the anaerobic treatment of LFL as a sole substrate led to a decrease in the entire AD treatment efficiency. This situation occurred due to the changes in the properties of the LFL, as well as the presence of inorganic salts, dissolved organic substances, and heavy metals. In contrast, the ACoD of multiple substrates has been observed to enhance the availability of macronutrients and micronutrients and promote microbial development (Simioni et al., 2020). This process also improves the distribution of hazardous or inhibitory substances, maintains water balance, offers a greater balance of the C/N ratio and enhances the buffering capacity of the mixture (Silvestre et al., 2015; He et al., 2017; Guven et al., 2018; Takeda et al., 2020; Thakur et al., 2023). Furthermore, previous studies have been conducted to investigate the combination of various substances, such as LFL with crude glycerol, acid mine drainage, FW, and sewage sludge, in the AD process (Takeda et al., 2020; Zhou et al., 2020; Liao et al., 2014; Hombach et al., 2003). Table 9 presented below illustrates the complementary characteristics that result from combining two substrates. The goal of this combination is to enhance the removal of organic material, increase biogas production, and improve CH₄ generation.

Hence, ACoD is a sustainable and useful solution that can manage organic waste, generate renewable energy, and recover nutrients while lowering waste disposal's environmental impact. ACoD also increases biodegradable components, the digesting microbial community, and active biomass (Tyagi et al., 2018). In order to enhance the outcome of co-digestion, it is imperative to implement several precautionary measures. The implementation of these precautions is crucial in order to optimise biogas production, maintain the stability of AD system operation, and mitigate potential issues that may impede the AD process. Singh et al. (2021) suggested that the selection of an appropriate mixing system and the implementation of an effective management approach can create favourable conditions for the highly sensitive microbial community in an anaerobic digester. This, consequently, can guarantee process stability and enhance AD efficiency. In addition, it is necessary to establish appropriate monitoring parameters, such as pH and temperature, to effectively control and optimise the ACoD digester during its operation, ensuring efficient performance in the AD system. Rabii et al. (2019) mentioned that the implementation of suitable monitoring procedures during the execution of the ACoD process enables the complete utilisation of system capacity while mitigating the risk of overflow. By adhering to the necessary precautions and implementing best practices, the results of ACoD can be enhanced, leading to improved biogas production and sustained process stability.

Table 9: Properties of substrate and co-substrate in anaerobic co-digestion

Anaerobic Co-Digestion of Leachate with Industrial Wastewater		
Leachate	Glycerol	Reference
• Low phosphorus concentration • High water content • Rich in macro and micronutrients • High levels of non-biodegradable and recalcitrant substances • High amount of ammoniacal nitrogen content	• Excessive phosphorus concentration • Low moisture level (toxic compound) • Insufficient macro and micronutrient content • High biodegradability / provides easily biodegradable organic material • Low level of nitrogen content	Takeda et al., 2020 Guven et al., 2018; Takeda et al., 2020 Silvestre et al., 2015; Guven et al., 2018; Takeda et al., 2020 He et al., 2017; Begum et al., 2018; Takeda et al., 2020 Silvestre et al., 2015; He et al., 2017; Takeda et al., 2020
Leachate	Acid mine drainage	Reference
• pH-modifying agent • High carbon content	• Low pH • High sulphate concentration	Zhou et al., 2020
Anaerobic Co-Digestion of Leachate with Solid Waste		
Leachate	Food waste	Reference
• Rich water content • Low biodegradability • pH modifier agent (high salinity-act as buffering capacity) • Low C/N ratio • High content of ammonia nitrogen	• Low water content • High biodegradability • Low pH • High C/N ratio • High levels of VFAs	Liao et al., 2014 Jiang et al., 2012; Liao et al., 2014; Kim et al., 2011; Liao et al., 2014 Bove et al., 2015; Li et al., 2021 Miao et al., 2019; Li et al., 2021

(Source : Zamrisha et al., 2023)

2.6.1 Anaerobic Co-Digestion of Landfill Leachate with Food Waste

LFL has been widely employed as a substrate for AD in numerous previous research studies (Kawai et al., 2012; Gao et al., 2022; Ahmad et al., 2021; Bonu et al., 2023).

Based on the findings of Kawai et al. (2012), AD has been identified as a promising treatment to generate renewable energy and a cost-effective treatment option for LFL.

Therefore, it is recommended that AD be considered as a suitable approach for implementation in developing countries such as Malaysia, China, and Vietnam in treating LFL. Moreover, it has been observed that LFL exhibits promising

characteristics as a substrate for AD since it can enhance the production of biogas by effectively mitigating the negative effects of acidic inhibition caused by the accumulation of VFA (Liao et al., 2014; Ahmad et al., 2021). Consequently, the utilisation of LFL in AD systems can affect the stability and efficiency of the AD process. Nevertheless, the current studies show that the mono-digestion of LFL predominantly results in inhibition rather than efficient CH₄ production (Berenjkar et al., 2018; T.A.S. et al., 2020; Takeda et al., 2020). Like LFL, FW also presents challenges when used as a sole substrate. These problems include VFA build-up, inhibition at high OLR, and deficiency of mass transfer of substrate to the microbe community, all of which result in the system being unstable and the reactor being less efficient (Ye et al., 2018; Lee et al., 2019; Liu et al., 2023). Thus, the co-digestion of LFL and FW has the potential to improve the AD process while maintaining nutritional balance and microbial interaction.

A study done by Liao et al. (2014) investigated the ACoD FW and LFL in single-phase batch reactors. The main components of the FW substrate used in the study consisted of meat, rice, and vegetables, which were thoroughly mixed. The experiment was carried out at a mesophilic temperature of 35 ± 1 °C, using 8 different mixing ratios of LFL. The quantity of FW was kept at 40 mL for reactors 1–7 (R1–R7) and 0 mL for reactor 8 (R8), while LFL ranged from 0, 142, 284, 426, 568, 710, 852, and 1200 mL for all 8 reactors (R1–R8). The results indicated by the co-digestion of LFL and FW in the reactors helped alleviate the acidic inhibition caused by VFA accumulation. The system demonstrated consistent stability, achieving the highest biogas generation (878 mL/g VS) and CH₄ yield (466 mL/g VS) by reactor 5 (R5) (40 mL FW: 568 mL LFL). The biogas yield achieved almost 0 mL in the mono-digestion of LFL, which is

in contrast to the co-digestion with FW. Liao et al. (2014) also highlighted the effectiveness of the AD system due to the characteristic of LFL, which can supplement water addition and act as a pH modifier in AD systems. Additionally, it is essential to observe that the introduction of TAN into the AD systems serves two purposes. Firstly, it provides nitrogen for bacterial growth. Secondly, it creates a buffer system with VFA, which helps maintain a sensitive biochemical balance between the acidogenic and methanogenic microbes. Therefore, combining FW and LFL can increase biogas production by promoting methanogenic activity.

In a different study performed by Zhang et al. (2015), outstanding results were observed in the production of biogas, with a CH_4 yield of 506.3 mL/g VS_{added} . This was achieved through the ACoD process, which involved a combination of fresh LFL and FW (77.3% FW and 22.7% fresh LFL). The fresh LFL was obtained from the waste storage bunker of an MSW incineration plant located in Dalian, China, while the FW was collected from a campus restaurant at Dalian University of Technology, China. The study consisted of three phases, with HRT running for 20 days in phase 1, 15 days in phase 2, and 10 days in phase 3. Throughout the extended operation, the ACoD of FW with fresh LF demonstrated significantly superior performance in comparison to mono-digestion. The AD system remained stable during co-digestion, with a consistent pH of 7.5 and no occurrence of VFA inhibition. It has been observed that fresh leachate can provide additional TMEs such as Fe and Nickel, which are lacking in FW. Therefore, the increased levels of TMEs in LFL can be effectively managed by combining it with FW in an AD system, resulting in a synergistic effect through the co-digestion approach. The AD system remains stable even under high OLR (8.3 gVS/L Day) and short HRT (10 days). In addition, the removal of VS

reached 71.6% with a CH₄ gas of 64.3% during phase 3. It is shown that the performance and stability of anaerobic mono-digestion of LFL are greatly affected by an imbalance of essential metal elements. Therefore, this issue can be resolved by co-digesting with FW, which provides the balance of TME contents within the substrates.

2.6.2 The Role of Mixing Ratio

The mixing ratio is a critical factor that greatly influences the efficiency and effectiveness of the digestion process in AD. Based on a study conducted by Latha et al. (2019), an incorrect mixing ratio in the AD system can result in poor biogas production, lower SMP, and reduced CH₄ content. According to a study carried out by Chaher et al. (2021), it was discovered that choosing the right mixing ratios can significantly increase biogas yield. The study observed that the biogas yield improved from 2–3.6 kg VS/m³.d. During ACoD, it requires the simultaneous digestion of multiple substrates in a single digester, in which the mixing ratio of the substrates being digested is a crucial factor (Rabii et al., 2019). The mixing ratio in ACoD determines the proportion of different substrates in the feedstock, which can greatly affect the efficiency of the AD operation (Hagos et al., 2017). The optimal mixing proportion of substrates in ACoD is influenced by multiple criteria. These factors encompass the characteristics of the substrates, such as a balanced C/N ratio to ensure adequate nutrient supply for microorganisms, the CH₄ potentials of each substrate, and the specific microbial community present in the substrate, all of which contribute to optimising biogas production (Siddique & Wahid, 2018; Shah et al., 2019).

In addition, optimising the mixing ratio is essential for achieving the desired pH levels in the digester system, enhancing anaerobic reaction rates, and maximising the removal of organic matter to boost biogas production (Liu et al., 2022). During the carry-out of

the ACoD system, it's crucial to select substrates that are compatible and can be co-digested without any negative impact on the AD process (Cucina et al., 2021). Moreover, the mixture ratio may be influenced by the substances and proportions of organic matter present in the feedstock. For example, when a high concentration of TMEs (for example, LFL) is combined with a low concentration of TMEs (such as FW) with a suitable mixing ratio, it can lead to a more balanced feedstock (Zhang et al., 2015). Besides, the mixing ratio can have a major impact on the production of biohythane. Biohythane is a gaseous product that is formed through the degradation of organic matter in a two-stage AD process (Brémond et al., 2018). The constitution of biohythane consists of a mixture of hydrogen and CH₄, with the hydrogen content ranging from 10–30% v/v (David et al., 2019).

Maintaining an ideal mixing ratio of substrates in ACoD is very important for achieving optimal biogas outputs and enhancing the stability of the digestion process (Dhungana et al., 2022). Once the mixing ratio is optimised, the ACoD system can generate biogas and other valuable products more efficiently and sustainably (Ibro et al., 2022). Various feedstocks possess unique characteristics, necessitating the adjustment of the mixing ratio to enhance the performance of AD. Hence, it is vital to ensure proper mixing and a well-balanced mixing ratio to prevent any inhibition in the AD system, which may have an impact on the generation of biogas and CH₄.

2.7 Application of Support Carriers and Additive Substances into the Anaerobic System

The extensive implementation of AD has encountered obstacles such as inadequate system stability, diminished methanogenic activity, and excessive accumulation of

VFAs, thereby increasing the operational expenses associated with the treatment process (Nabi et al., 2022). Furthermore, the operation and performance of AD systems are often affected by several issues, including ammonia inhibition, inadequate CH₄ production, inefficient microbe growth rates, and slow mass transfer (Jadhav et al., 2021). These challenges necessitate appropriate measures for modification. Hence, the incorporation of support carriers and additive substances into the AD digester can enhance the performance of AD in LFL treatment systems.

2.7.1 Differences between Support Carriers and Additive Substances

Support carriers, also referred to as support media or biofilm carriers, are solid surfaces or materials that facilitate the attachment of microorganisms to form biofilm, thereby enhancing biogas production (Al-Amshawee & Yunus, 2021). The carriers also provide a suitable environment for biofilm formation, allowing anaerobic microbes to colonise and establish a stable microbial community on their surface. The main objective of employing support carriers is to boost the biomass concentration in the anaerobic reactor. This can be achieved by immobilising bacteria on carrier surfaces, allowing for a longer period of operation and thus improving treatment efficiency (Zhang et al., 2019; Al-Amshawee & Yunus, 2021). In order for biogas to be produced efficiently, it relies on the presence of anaerobic bacteria within the anaerobic digester. These bacteria are responsible for converting organic matter into biogas. Thus, by using support carriers, it is desirable to create an attached growth system for the growth of biomass and the formation of a biofilm (Sehar & Naz, 2016). This biofilm may effectively eliminate organic pollutants and nitrogenous materials at a rapid rate in the process of wastewater treatment (Chattopadhyay et al., 2022). Additionally, the overall stability of the AD reactor plays a crucial role in the wastewater treatment process.

This can be accomplished by utilising a microbial biofilm that is attached to surface carriers in supporting media, including rock, ceramic, or plastic, as shown in Figure 8 below (Chanathaworn and Dussade, 2017). Table 10 below indicates the different sizes and pore sizes of support carriers that can be utilised in an AD system based on previous studies.

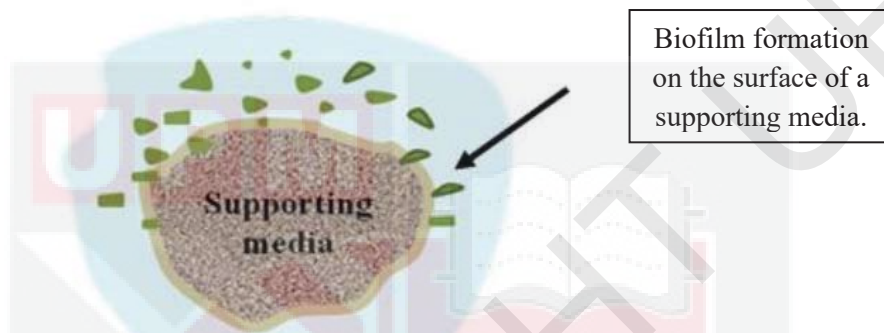


Figure 8: Attachment of biofilm to support media
(Source: Chanathaworn and Dussade, 2017)

Table 10: Sizes and Pore sizes of various support carriers

Type	Pore size/size	References
Ceramic rods	Pore size: 0.6–100 μm	Muter et al., 2017
Porous Ceramic	Pore size: 20 μm	Wan et al., 2012
Vegetal Carbon	Pore size: 1.9 μm	Silva et al., 2006
Polyvinyl chloride (PVC) tube	Size (Length): 1 inch	Chanathaworn and Dussade, 2017
Nylon fiber	Size (Length): 10 cm	Chanathaworn and Dussade, 2017
Polyvinyl chloride cylinder	Size (Height): 2.5-3.0 mm	Wang et al., 2005
	Size (Diameter): 2.5 mm	

In the study done by Chan et al. (2012), a support carrier called a CBR was used to treat palm oil mill effluent through an integrated anaerobic-aerobic bioreactor. The goal of integrating with this support carrier is to serve as a biomass immobiliser in biological treatment. This CBR is a vertical hollow channel within the packing media, which effectively mitigates the issue of clogging caused by the high concentration of total suspended solids (TSS) in palm oil mill effluent. Based on the obtained result, the BOD, TSS, and COD all exceeded 90% at an OLR of 10.5 gCOD/L day. The VFA to total alkalinity (TA) ratio (VFA/TA) also discovered that the AD system is operating stably, with a value of less than 0.3. In addition, a CH₄ production rate of 64% was achieved, with a CH₄ yield of 0.24 L CH₄/g COD_{removed}. Therefore, the findings demonstrate that the anaerobic treatment utilising a hollow-centred packed bed (CBR) holds promise as a technically viable solution. However, a comprehensive assessment of its long-term effectiveness is required to ascertain its ability to enhance CH₄ content through the conversion of organic matter into biomass.

In a different investigation conducted by López-López et al. (2013), volcanic rock (Tezontle), also known as LR, was employed as the packing medium to facilitate the production of biofilm in the treatment of domestic wastewater. The reactor employed is an up-flow anaerobic filter with different HRTs of 12, 18, and 24 hours and temperatures ranging from 20 to 35 °C. The temperature has been identified as the primary determinant for achieving high COD elimination efficiency. The study also discovered that volcanic rock is economically advantageous, abundant, and has a high level of porosity. The interstitial porosity of the supporting medium is 0.65. Furthermore, the significant porosity, accounting for approximately 65% of the pore volume for a grain size ranging from 10 to 20 mm, is favourable for the colonisation of microorganisms, hence promoting elevated levels of biological activity. Through

the incorporation of volcanic rocks, it is demonstrated that organic matter can be effectively eliminated at a rate above 80% when the HRT is 24 hours and the temperature is maintained at 35 °C. Furthermore, the result shows that the effluent meets the standards set by Mexican legislation.

In addition, the integration of a clay carrier in wastewater treatment was investigated by Nguyen et al. (2022). Incorporating this carrier into aquaculture wastewater treatment systems showed promising results by enhancing bacterial attachment and biofilm formation, leading to a nitrogen removal efficiency of over 90%. The clay support carrier was modified by coating it with alginate and essential nutrients, which were crucial in promoting bacterial adhesion and cohesion, ultimately fostering bacterial growth. Adding nano-nutrients to the surface of the modified carrier significantly improved its bacteria-holding capacity compared to the unmodified carrier. This can be demonstrated by the nitrogen removal rates, with the modified carrier achieving an efficiency of 91.62%, in contrast to 79.87% for the unmodified carrier. Hence, these findings suggest that the modified clay support carrier like RCBR creates an optimised environment for biofilm formation, thereby enhancing nitrogen removal efficiency and improving wastewater treatment performance in aquaculture systems.

Conversely, additive substances are substances that are added to the system to improve the treatment process or solve particular concerns associated with the AD system. The application of additive substances, such as conductive materials (Nabi et al., 2022) and conductive nanoparticles (Jadhav et al., 2021) in AD systems has several benefits. These additives enhance microbial colonisation, eliminate toxic compounds, expedite

direct electron transfer (DIET), and stimulate biogas production (Zhao et al., 2017; Cheng & Call, 2016; Kumar et al., 2017). Figure 9 below illustrates the integration of conductive material in the AD process, demonstrating the mechanism of DIET. The conductive materials can be classified into three categories, which are bio-based carbon materials (such as biochar and carbon cloth), iron-based materials (for instance, magnetite), and carbon-based materials (such as granular activated carbon and graphite). According to a study by Zhao et al. (2017), carbon-based additives have the ability to facilitate efficient interspecies electron transfer (IET) between anaerobic organisms in the AD system. The enhanced performance of AD is related to the chemical stability, conductivity, biocompatibility, porous structure, and lightweight composition of carbon-based conductive materials. The selection of additives and their respective dosages is dependent upon the specific demands of the anaerobic process and the characteristics of the wastewater or organic material that is being treated, as shown in Table 11.

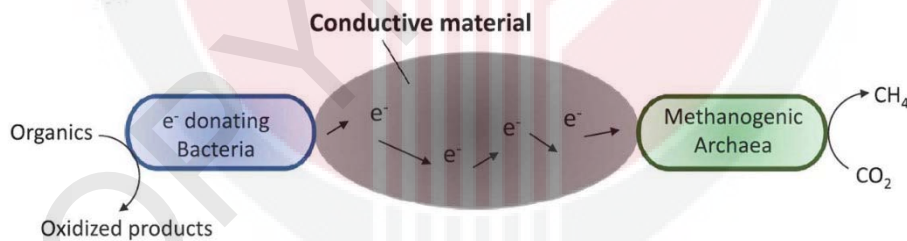


Figure 9: DIET mechanism
(Source: Park et al., 2018)

In another study by Madondo et al. (2023), the addition of magnetite nanoparticles, a carbonaceous additive substance, to the AD process of wastewater (specifically sewage sludge), produced a synergistic impact that could also improve DIET. This is because, during the DIET process, it is the most powerful strategy for CH₄ production

when compared to IET (Park et al., 2018). IET incorporates electron carriers, such as hydrogen, which is a crucial pathway for CH₄ production in conventional AD (Park et al., 2018). In contrast, DIET employs syntrophic metabolism, in which free electrons are transferred between cells via shared physical and electrical connections, eliminating the need for electron carriers (Gahlot et al., 2020). Furthermore, Madondo et al. (2023) stated that the inclusion of conductive materials in the AD process can assist in the promotion of the DIET process for microbes that lack the ability to generate nanowires for IET. In addition, the use of additive substances in the AD system offers several benefits, including increased conversion of biomass to renewable energy, enhanced elimination of hazardous pollutants, and improved electrochemical efficiency (Kamali et al., 2019; Song et al., 2021; Madondo et al., 2023).

Table 11: Conductive materials and conductive nanoparticles employed in the AD system

Additives Substance (Conductive materials)		
Type	Concentration	Performance on AD process
Bio-based carbon material	• 5 g/L	• Biochar boosts methane production by 16% in UASB system-treated sludge and wastewater (Zhao et al., 2016).
	• 1000 cm² and 1.5 pieces/L	• Carbon cloth increases methane production by 60% when treated with leachate from MSW incineration using a UASB reactor (Lei et al., 2016).
Iron-based	• 25 g/L	• Magnetite can improve methane production by 34% in anaerobic digestion of complex organic waste (Zhao et al., 2017).
	• 10 g/L	• Using magnetite to treat fresh leachate from a MSW incineration plant with a UASB reactor resulted in a 32% rise in methane production (Lei et al., 2018).
Additives Substance (Conductive nanoparticles)		
Zero-valent metals	• 1000 mg/L	• Biogas production rises by 105.46% when Fe is used to treat domestic sludge (Amen et al., 2017).
	• 5–10 mg/kgVS	• Using nickel to treat sewage sludge leads to a 10% rise in methane production (Baniamerian et al., 2019).
Metal oxides	• 5, 10, or 20 mg/L	• Using Fe₃O₄ will boost biogas output by 66% and methane generation by 96% of the feedstock in a biogas plant (Asam et al., 2011).
		• The amount of methane produced increases by 38% when Fe₂O₃ is used to treat sewage sludge (Mustapha et al., 2020).
Carbon-based conductive nanoparticles	• 0.5–2 g/L	• Using graphene to treat activated sludge increases the production of biogas by 19.5% and the amount of methane by 25% (Li et al., 2020).

2.7.2 Advantages of the support carrier

The performance of support carriers in AD might vary based on the particular system, the type of carrier material employed, and the operational parameters. Effective design and regular maintenance are crucial for optimising the advantages of utilising support carriers in AD processes. Support carriers facilitate microbial attachment, leading to

the production of a biofilm that promotes the retention of microorganisms in the reactor, eliminates microbial washout, and maintains a steady microbial population (Ercan & Demirci, 2013; Raj Deena et al., 2022). Anaerobic digesters integrated with support carriers can enhance biogas production from organic matter by maintaining a stable and robust microbial population, which leads to increased energy recovery in the form of CH₄ (Cayetano et al., 2022). Employing a support carrier has numerous advantages, particularly in the treatment of high-strength wastewater, as indicated in Table 12. Currently, there is a lack of research on the use of support carriers, such as CBR, RCBR, and LR, for the treatment of ACoD in LFL and LFW.

Table 12: Advantages of support carriers in a variety of treatments

Support carrier	Advantages	References
CBR	- Excellent media for the submerged biological aerated filter and outstanding results for the treatment of effluent wastewater (from the primary sedimentation tank) - Very powerful media that is able to remove: COD (83.93%), BOD (83.65%), and TSS (85.60%) with HRT = 72 mins	(El-Halafawy et al., 2023)
	- Show a great interest in purifying water from ages. - Utilisation of this media has led to a significant enhancement in membrane performance. - Adsorbs heavy metals very well. - Efficient in the removal of oil from water	(Dutt et al., 2020)
RCBR	Clays can be used in the removal of colour from textile wastewater.	(Rai et al., 2005)
	- Acts as adsorbents that are commercially available - Non-toxicity and low cost - Has potential for ion exchange - The clay base can adsorb organic dyes in wastewater with a removal efficiency of 88%. - Low cost - Resistance to toxic substances - Simple operation	(Vimonses et al., 2009) Zheng et al., 2012; Zhou et al., 2014)

Table 12: Continued

Support carrier	Advantages	References
LR	• LR is environmentally friendly and can be found in rural areas • Capable of treating swine wastewater with high removal efficiency for COD (86–93%), TSS (91–97%), and NH₃-N (86–87%)	(Escalante-Estrada et al., 2019)
	• Exhibits high porosity (66%)	(Huang et al., 2013; Méndez-Romero et al., 2011)
	• Inexpensive, abundant, and high porosity • Effective for the treatment of domestic wastewater by achieving COD removal (57-86%)	(López-López et al., 2013)
	• Act as a filter medium • Capable of removing COD and TSS from grey water effluent by 85-88%, respectively	(Katukiza et al., 2014)
	• Can promote the maturation of manure compost • Enhanced diversity of microbial communities attached to the carrier	(Ma et al., 2023)

2.8 Physico-chemical activation of support carrier

The increased concentration of organic matter in high-strength leachate wastewater reduces the efficiency of anaerobic treatment systems. Therefore, an activated support carrier is necessary to improve biogas generation by facilitating the growth of biofilms on its porous structure. The method of physico-chemical activation is used in the adsorption process that commonly used to enhance the properties of material, such as its ability to remove both organic and inorganic particles, suspended molecules, heavy metals, and pathogens (Enaime et al., 2020; Dehkordi et al., 2022). According to Kwiatkowski & Broniek (2017), the physico-chemical activation method is growing and being used in modern activated technologies. This method of activation produces materials with varying porosity, specific surface area, electron transfer, and pore volume in the final product, which has been found to enhance biogas production and promote the AD process compared to individual activation methods such as physical or chemical treatment, as demonstrated by studies conducted by Panwar & Pawar (2020), Deng et al. (2021), Jjagwe et al. (2021), and Nabi et al. (2022). Furthermore,

Farias et al. (2019) stated that the use of physico-chemical activation shows promise for treating LFL by degrading organic matter and removing colour.

In the physical-chemical activation method, a two-stage process is utilised, including thermal or dry activation followed by chemical activation, in which both approaches have an impact on the physical characteristics of the final product (Panwar & Pawar, 2020). Throughout the process of dry activation, the raw material undergoes exposure to temperatures $<800^{\circ}\text{C}$ to enhance the morphological characteristics of the material (Panwar & Pawar, 2020). Furthermore, through the process of physical activation, it will generate pores and voids within the material structure, which results in an increased specific surface area and a high microporous structure (Sakhiya et al., 2020). Following that, the material undergoes chemical activation. Activation of chemicals occurs through the addition of activating reagents, typically acids or bases, to the material in which they will react together, which leads to the modification of the physical properties of the material, such as surface area and pore volume (Jaya Rajan & Indira Anish, 2022). During this stage, the material undergoes impregnation with a chemical activating agent at a high temperature range of $400\text{--}800^{\circ}\text{C}$ (Venkatesan et al., 2023). This process is referred to as carbonisation (Sharma et al., 2019). During chemical treatment, the intercalation effect took part as a result of the activating reagent's (alkali or acid) interaction with the material structure, which then created a porous structure (Liew et al., 2018). Several commonly used activating agents include ZnCl_2 , NaOH , potassium carbonate (K_2CO_3), potassium hydroxide (KOH), calcium chloride (CaCl_2), phosphoric acid (H_3PO_4), and sulphuric acid (H_2SO_4) (Mendoza-Carrasco et al., 2016; Liew et al., 2018; Jjagwe et al., 2021; Venkatesan et al., 2023). The selection of the activating agent has a significant impact on the surface properties

and porosity of the material being activated (Togibasa et al., 2021). Therefore, the activation method requires a high activation temperature in order to achieve a material with a highly porous structure and a large surface area. In the study performed by Khedr et al. (2014), chemical activation was found to have the advantages of low energy costs and a more effective development of porous structures. Therefore, through the combination of physical and chemical methods, there is potential to enhance the structure of the support carrier and improve the performance of the AD system.

2.8.1 Activation with zinc chloride

The carbonisation temperature and concentration of the activating reagent have significantly influenced the surface areas and pore volumes of the activated support medium or support carrier. The study conducted by Üner et al. (2019) evaluated five impregnation ratios for activating the activated carbon from waste watermelon using ZnCl_2 . These ratios were 0.5:1, 1:1, 2:1, 3:1, and 4:1 (ZnCl_2 /dried watermelon rind) (w/w). The study found that the optimal impregnation ratio for the production of activated carbon from waste watermelon rind was 3:1. This optimal ratio resulted in the highest total pore volume, including the maximum volume of mesopores, of $1.41 \text{ cm}^3/\text{g}$. Another study also discovered that the impregnation ratio of 3:1 is the most favourable condition for producing activated carbon from sour cherry stones, as opposed to ratios of 1:1, 2:1, and 4:1 (Angin, 2014). Next, according to the Brunauer-Emmett-Teller (BET) result, the impregnation ratio of 3:1 yielded the largest pore volume of $1.566 \text{ cm}^3/\text{g}$, making it the most optimal (Angin, 2014).

Furthermore, the temperature of carbonisation also plays a crucial role in the activation mechanism. Previous studies have carried out carbonisation investigations at temperatures ranging from 400 to 900°C (Rajbhandari et al., 2013; Kumar & Mohan Jena, 2015; Üner et al., 2019). The study conducted by Üner et al. (2019) and Angin (2014) revealed that a carbonisation temperature of 700°C resulted in the greatest surface area of support medium, measuring 1156 m²/g and 1704 m²/g, respectively. Following the carbonisation process, it is necessary to wash the mixture using hydrochloric acid (HCl) in order to extract the activating agent (ZnCl₂). Next, the sample was rinsed with distilled water until it reached a pH level of 6.5-7 to remove any remaining activating agent residue and other inorganic compounds that were produced throughout the process. Hence, the suitable impregnation ratio and activation temperature will impact the physical characteristics of the activated substance or material, including surface area, pore volume, and pore size.

2.8.2 Activation with sodium hydroxide

The implementation of NaOH as an activating agent has demonstrated significant improvements in both surface area and pore volume. This can be proved by the research conducted by Cazetta et al. (2011), which investigated the properties of activated carbons derived from coconut shells using NaOH activation at impregnation ratios of 1:1, 2:1, and 3:1. The study revealed that impregnation ratios of 3:1 resulted in a significant increase in surface area, making it an effective adsorbent for the elimination of methylene blue from aqueous solutions. The BET results indicate that the impregnation ratio of 3:1 exhibits the maximum surface area for impregnation, which is 2825 m²/g, followed by 1842 m²/g for the impregnation ratio of 2:1, and 783 m²/g for the impregnation ratio of 1:1. In addition, it was also discovered that the

activating agent must be eliminated before proceeding with further study. To accomplish this, the activated support media should be washed by rinsing it with a 0.1 M solution of HCl and afterwards with distilled water until the pH becomes neutral (Tan et al., 2014; Liew et al., 2018).

Hafizuddin et al. (2021) found that altering the surface with NaOH can produce highly microporous activated carbon, which effectively enhances the surface properties. The surface-modified activated carbons generated from palm kernel shells and coconut shells exhibited superior physical characteristics in comparison to unactivated carbon, as indicated by the obtained results. The surface area values of the activated carbons are higher than those of the unactivated carbons, in which the activated palm kernel shells have a surface area of 427.64 m²/g, while the unactivated palm kernel shells have a surface area of 135.97 m²/g. Similarly, the active coconut shells have a surface area of 356.87 m²/g, while the unactivated coconut shells have a surface area of 204.64 m²/g. The activated carbons exhibit a smaller pore size (1.99 nm for palm kernel shells and 2.24 nm for coconut shells) and an increased pore volume (0.30 cm³/g for palm kernel shells and 0.34 cm³/g for coconut shells) compared to the untreated activated carbon. The outcome is in accordance with the concept that a greater fraction of micropores in the support media is associated with an increased specific surface area. Lastly, surface modifications using NaOH also enhance the adsorption capacity by enhancing the active functional groups on the support media's surface. The maximum adsorption capacity was attained at a temperature of 700°C, as reported by Cazetta et al. (2011) and Hafizuddin et al. (2021). Therefore, by employing NaOH activation with a suitable impregnation ratio and carbonisation temperature as a surface modification

approach on the support carrier or media, it will be possible to create a highly microporous structure, leading to an increased surface area.

2.9 The Role of Support Carrier in UASB Reactor

The ideal volume of support carriers integrated into an anaerobic reactor plays a significant role in the success of AD operations. The volume of support carriers applied in the operational experiment was determined by the carrier volume, as studied by Wang et al. (2005), which recommended a range of 10% to 75%. Besides, the geometry of the biofilm carrier and biofilm formation on the support carrier are also important to evaluate while conducting the AD treatment, as discussed in the detailed section below.

2.9.1 Geometry of biofilm carrier

2.9.1.1 Pore size

Choosing the right support carrier with an ideal pore size is essential for the successful performance of wastewater treatment in AD. Loucks et al. (2012) classified pore size into three types, as outlined in Table 13. The optimal pore size of a support carrier will enhance the growth of microorganisms that stick to its surface. This microorganism, or bacteria, is responsible for AD by adhering to a carrier surface and subsequently forming a biofilm to eliminate pollutants in wastewater. The study demonstrated by Al-Amshawee & Yunus (2021) found that maintaining an optimally sized pore structure is crucial for biofilm carriers to create an ideal habitat for microbe attachment and evolution. However, there is a lack of information on the specific range for ideal pore size that can lead to highly efficient wastewater treatment using the AD process,

as indicated by the previous study. In general, the presence of large pores in the support carrier or media allows for greater microbial immobilisation and growth, leading to the formation of biofilms that are not tightly packed with biofilms and have uneven surfaces (Yang et al., 2015). But if the pores are considerably larger than the immobilised cells, there is a possibility of leakage into the environment, which can impede AD activity, as stated by Dzionek et al. (2016).

Table 13: Types of pore sizes

Classification of pores sizes	Size (nm)
Micropores	<2
Mesopores	2-50
Macropores	>50

(Source : Loucks et al., 2012)

Sun et al. (2019) showed that the pore structure has a crucial influence in determining the specific surface area of the support carrier. The relationship between pore size and the area of the support carriers is inversely proportional, meaning that as the pore size increases, the area of the support carriers decreases (Murphy & O'Brien, 2010). Pores are integral to the topography of biofilm carriers, assisting in the immobilisation of biomass and providing protection to biofilm colonies against leakage and sloughing (Al-Amshawee & Yunus, 2021). The employment of suitable pore sizes can offer resistance against shear forces, ensuring the stability of the biofilm and preventing its loss by the fluid circulation in the digester. Additionally, the mass transfer within the biofilm is influenced by the pore size, in which optimal pore sizes facilitate the diffusion of nutrients and substrates, thereby enabling microorganisms to access them.

Based on a study performed by Kaushik et al. (2015), it was discovered that pore blockage significantly reduces the pore space available for nutrient transmission to microorganisms, hence reducing hydraulic conductivity in the treatment of LFL. The clogging of the pore can occur as a result of an excessively tiny pore size. Therefore, when a concentrated influent of wastewater is applied and an unsuitable pore (small pore) of a support carrier is used, it can lead to clogging caused by the excessive accumulation of biomass inside the carrier (Al-Amshawee & Yunus, 2021). This clogging negatively impacts the overall efficiency of AD. Moreover, it can result in the formation of a dense biofilm layer on the solid surface of the support carrier, which is not suitable for the AD system to work with. Feng et al. (2015) found that nanoporous surfaces with a size range of 15 to 100 nm can effectively decrease bacterial attachment, thereby leading to a reduction in biofilm formation.

Furthermore, the pore structure plays a role in determining the total surface area of the support carrier. The optimal pore size of the support carrier promotes the survival and reproduction of microbes while also promoting the transfer of biomass for their evolution and maturation (Ahmad et al., 2017). The pore structure within the biofilm carrier can be effectively investigated and evaluated using scanning electron microscopy (SEM) and BET techniques (Al-Amshawee & Yunus, 2021). Hence, the application of optimal pores in biofilm carriers creates an appropriate environment for the robust growth and development of microorganisms, thereby enhancing the AD system for the production of biogas.

2.9.1.2 Shape

The morphology of the support carrier is crucial in influencing the formation of biofilms and the diffusion of substrates or nutrients. A study by Tan et al. (2014) stated that the shape of the material plays a significant role in determining its adsorption capacity. This can be enhanced by applying different treatments, such as alkali impregnation, heat treatment, concentrated acid washing, and thermal treatment with ammonia. An ideal surface should offer sufficient area for microbial attachment to promote biofilm formation and enable a larger microbial population, hence enhancing the efficiency of organic matter decomposition. Choosing the appropriate carrier can effectively reduce blockages when dealing with high loading rates (Raj Deena et al., 2022). In addition, the surface of a material that has been modified by NaOH activation has different pore sizes that exhibit a higher level of compatibility for the adsorption process (Tan et al., 2014). In another study, Hafizuddin et al. (2021) discovered that the activated carbon, when treated with NaOH alteration, produced a surface texture that was uneven with the creation of micropores, resulting in improved adsorption capabilities. Furthermore, Sharma et al. (2019) found that the utilisation of NaOH impregnation in the production of activated carbon material resulted in a roughness of the material's outer surface, as observed by SEM analysis. This surface characteristic will promote the attachment of microorganisms to the carrier surface in order to break down the organic material during the wastewater treatment process (Al-Amshawee et al., 2020).

2.9.2 Biofilm formation on a support carrier

Biofilms are essential for the decomposition of both organic and inorganic substances in wastewater treatment processes as they function as grouped communities of microbes, particularly in AD systems. Zhao et al. (2019) conducted a study on the application of inorganic materials, such as ceramics, volcanic rock, and activated carbon, as biofilm carriers. These materials are commonly employed due to their numerous benefits, including high mechanical strength, easy accessibility, and large specific surface areas. Besides, Zhang et al. (2019) stated that the microorganisms adhere to the solid surface of a support carrier and grow, forming the biofilm formation that works synergistically to carry out metabolic activities that lead to the complete degradation of substances in wastewater treatment. This process helps to maintain the stable performance of the AD reactor over an extended period of operation. The stickiness of bacteria to carriers is essential for holding them inside the system, disabling them from washing out, and maintaining a stable and active microbial community (Cayetano et al., 2022). According to Di Martino (2018) and Karygianni et al. (2020), biofilms are comprised of microbial communities that are enclosed within a three-dimensional extracellular matrix that consists of extracellular polymeric substances (EPS). Additionally, biofilms can promote the attachment of various microbial communities in an aqueous environment, enveloped inside an EPS matrix (Zhao et al., 2023). This arrangement enables the formation of a comprehensive structure of interactions, hence improving the efficiency of the biological wastewater treatment process.

The presence of biofilm on support carriers stimulates the colonisation of microorganisms. Each individual piece of biofilm functions as a hydrodynamic

structure, effectively improving the transfer of mass and promoting the sustainability of biofilm (Al-Amshawee & Yunus, 2021). In addition, the biofilm, which is a complex grouping of microbes that attach to a solid surface and are surrounded by a viscous material, requires a smaller area within the reactor while effectively eliminating pollutants (Al-Amshawee et al., 2020). Based on a study by Sauer (2017), biofilms must be preserved against potential resistance since they consist of several densely packed microbial cells that live together on a carrier surface and are entrapped in a slimy matrix. Nevertheless, a study carried out by Habouzit et al. (2011), Ahmad et al. (2017), and Huang et al. (2018) have determined that the effectiveness of biofilm carriers cannot be accurately assumed by solely evaluating the mass and thickness of the biofilm, but the crucial factor found is the roughness or texture of the surfaces, as well as the size and shape of the porous carrier, as these properties significantly influence the initial adherence and growth of the biofilm. Besides, the surface texture of the carrier has a role in the initial attachment and retention of microorganisms before the formation of a biofilm (Huang et al., 2018).

According to the findings by Freedman et al. (2017) and Moreno Osorio et al. (2021), the resistance of biofilm formed on support carrier surfaces to sudden environmental fluctuations, including changes in wastewater composition, temperatures, and pH, can be as high as 500 times greater than that of suspended microorganisms in terms of survival mechanisms. As a result, the structure of biofilms is not stagnant and homogeneous; rather, it undergoes dynamic changes in response to the conditions of the surrounding environment. Therefore, biofilms play a crucial role in the AD process of wastewater treatment since they represent the adherence of microbes to the carrier

surface. This attachment of microbes to the carrier surface makes it feasible for them to grow, which is essential to efficiently remove pollutants from wastewater.

2.10 Machine Learning (ML) applications

Every industry across the world looks for an intelligent system proficient in effective forecasting, especially in industries where waste generation is a significant concern. The conversion of waste into biogas has many benefits due to its economic value and environmentally sustainable aspects. Several widely used ML applications for tasks such as classification, prediction, assessment, and forecasting are ANN and SVM models (Alejo et al., 2018; Guo et al., 2021; Cinar et al., 2022). Both types of models involve a training and learning phase whereby existing data is employed to create a model. Then an optimisation strategy is employed to automatically adjust this model in order to fit the target data. The objective is to allow the learned model to exhibit good generalisation when displaying unobserved data (Alejo et al., 2018). Both ANN and SVM models employ the principle of risk minimisation (RM) to identify the most optimal data separation, hence enhancing their capacity for generalisation (Alejo et al., 2018). Currently, the most widely used approach for forecasting biological processes is the ANN model, but recent studies have revealed the predictive capabilities of the SVM model. Alejo et al. (2018) and Guo et al. (2021) observed that the SVM model exhibited better predictive accuracy when compared to the ANN model. In addition, Guo et al. (2015) applied both ANN and SVM models to forecast effluent contents in a wastewater treatment plant for FW, and the results indicated that SVM outperformed ANN in terms of accuracy.

2.10.1 Artificial Neural Network (ANN) analysis

ANN has the capability to make a substantial impact on the AD process. It can serve as a suitable model for improving the accuracy of predictions and optimising CH₄ production, as well as assisting in determining the appropriate size of biogas plants (Simeonov et al., 2004; Mohamed et al., 2018). ANN is a type of ML model that resembles the structure and processes of the human brain (Mouloudi et al., 2021). Furthermore, ANN is utilised in a diverse range of applications, encompassing image and audio identification, natural language processing, and many types of data forecasting (Galbusera et al., 2019). Yang & Wang (2020) also mentioned that an ANN is a computational framework consisting of multiple artificial neurons that perform non-linear operations on their respective inputs. Sathya and Abraham (2013) have indicated that there are two distinct approaches to training an ANN, which include supervised and unsupervised learning. The concept of supervised learning involves providing detailed examples of a target concept in order to acquire the ability to identify class members or construct a regression model with regard to descriptive attributes. The unsupervised learning method incorporates the use of a dataset that lacks a predetermined classification, and its primary objective is to identify related trends and patterns within the dataset, which is typically attained by identifying groups of examples with similar characteristics. Meanwhile, according to Abbasi et al. (2021), ML can be classified into four classes, consisting of supervised learning, semi-supervised learning, unsupervised learning, and reinforcement learning.

The fundamental structure of an ANN typically comprises three layers, which are an input layer, an output layer, and hidden layers (Jaman et al., 2023), as shown in Figure

10 below. The hidden layer plays a crucial role as it transfers the processed information to the output layer, which is responsible for generating the final output (Xie et al., 2023). The neuron, which serves as the fundamental processing unit of an ANN, collects inputs for information processing and generates outputs or results (Wu & Feng, 2017). An ANN provides a viable option that can effectively handle faults and failures, process imprecise and complex information, and demonstrate the capability to generalise (Abiodun et al., 2018). Moreover, an ANN possesses substantial features in the area of data processing and analysis, allowing it to effectively handle complex and non-linear data (Wang et al., 2018). ANN has been employed to tackle complicated problems in diverse areas, including forecasting, modelling, cluster analysis, trend detection, and simulation (Mougari et al., 2021). Furthermore, an ANN is a ML system that uses variables to create mathematical connections between designated input and output datasets with the purpose of predicting the yield of biogas.

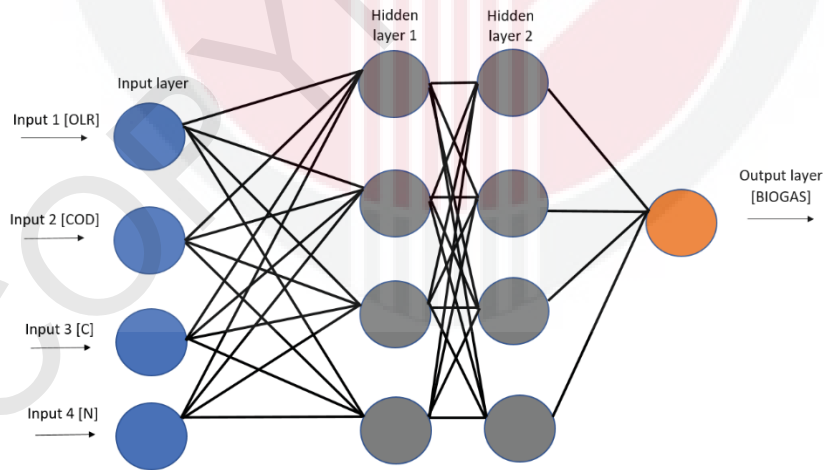


Figure 10: Fundamental structure of the ANN model

Several research investigations have demonstrated the advantageous application of ANN in resembling the mechanisms of the AD system. In a study conducted by Wang

et al. (2018), the back propagation-feedforward neural network was employed to estimate the biogas production from an ACoD system consisting of cow manure, maize straw, and fruit and vegetable waste. The ANN model yielded a biogas forecast with an R^2 value of 0.9948. Seo et al. (2021) employed linear regression to forecast the biogas generation of AD from dry FW in a separate investigation. The R^2 value for the validation data was 0.82, while the R^2 value for the test data was 0.85, indicating strong linear associations between the datasets. Besides, in a study conducted by Kannah et al. (2022), the R^2 value obtained was 0.97 through the utilisation of a three-layer backpropagation ANN model. This model was constructed to evaluate the optimum substrate concentration and greatest biogas yield from LFL. However, ANN does possess significant disadvantages. A limitation to consider in the ANN model is the potential impact of the random distribution of the data, which might make them prone to overfitting (Guo et al., 2021).

2.10.2 Support Vector Machine (SVM) analysis

SVM is widely acknowledged as an outstanding ML technique. It has gained recognition for its superior performance in prediction and classification operations (Cinar et al., 2022; Kazemi et al., 2020; Pisner & Schnyer, 2020). In the SVM model, the technique of linear regression is employed within the feature space, and by employing this mapping procedure, it is feasible to solve the non-linear problem within a linear space, as represented in Figure 11. Kazemi et al. (2020) have reported that the SVM is capable of constructing predictive models for a range of important factors influenced by AD treatment. The factors encompassed in the AD process comprise the rate at which biogas is generated, the yield of CH_4 , the pH level, the presence of VFAs, and other important variables. In SVM, the input data is commonly converted into a

feature space with a high number of dimensions or multiple dimensions. This is achieved by utilising kernel functions (Alejo et al., 2018; Padierna et al., 2014). The process commences with the identification of support vectors, which are data points positioned in very close proximity to the decision boundary. Following that, the intent is to optimise the margin, which refers to the distance between the support vectors and the decision boundary (Cervantes et al., 2020; Ling & Lu, 2021). As a consequence, Otchere et al. (2021) have demonstrated the production of a robust classifier that exhibits the capability to effectively handle messy data and is insensitive to overfitting can be obtained by employing the SVM model.

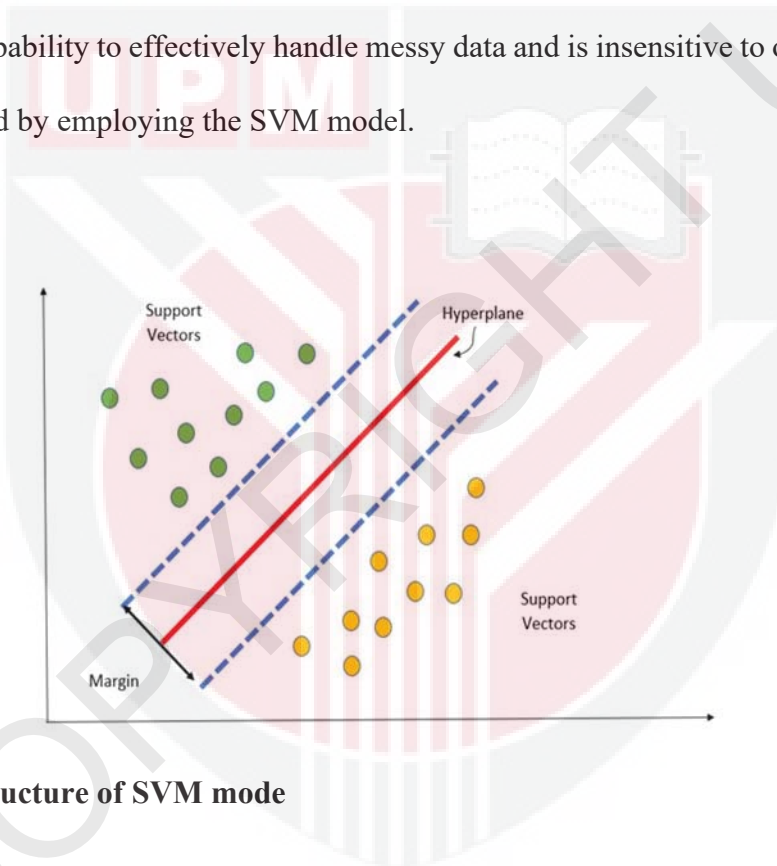


Figure 11: Structure of SVM mode

The kernel functions that are commonly used include the linear, polynomial, radial basis, exponential radial basis, multilayer perceptron, spline, and sigmoid functions (Alejo et al., 2018; Guo et al., 2021; Kazemi et al., 2020). The SVM is based on the principle of structural risk minimisation (SRM), which seeks to minimise an upper bound of the generalisation error. This is in contrast to the empirical risk minimisation (ERM) approach observed in ANN-based learning methods, which aims for a minimal

average error over the training set of data. (Cheng et al., 2023). The concept of SRM is to minimise an upper bound on the expected risk for the purpose of providing a model that is not overfitted (Guo et al., 2021). The fundamental of the SVM technique, as described by Al-Mejibli et al. (2020), is to identify the hyperplane in the feature space that efficiently separates groups of data points into distinct classifications. Thus, SVMs have demonstrated significant efficacy in tackling non-linear, multi-dimensional problems, especially those that exhibit an inadequate amount of available data.

According to Alejo et al. (2018), SVM demonstrates considerable promise in the prediction of non-linear and AD processes in upcoming investigations. Hence, the research performed by Guo et al. (2021) indicates that SVM has exhibited superior performance when compared to ANN. Based on the findings conducted by Alejo et al. (2018), the R^2 for SVM in the analysis of AD in poultry manure (TAN value) was 0.90, whereas ANN achieved an R^2 value of 0.88. In the study done by Cinar et al. (2022), it was reported that the SVM model exhibited the greatest R^2 value of 0.93 in predicting biogas output compared to other ML algorithms such as linear regression, logistic regression, K-NN, decision trees, random forest, and XGBoost. This performance was observed under varying intensities of feeding and temperature fluctuations. Furthermore, in a study carried out by Baek et al. (2023), the efficacy of SVM in predicting the rate of CH_4 generation was proven. The findings revealed that the R^2 obtained was 0.97. In a separate investigation, Wang et al. (2023) employed SVM to forecast the SMP of a varied set of lignocellulosic biomass samples (277 samples). The forecast model was highly precise, as shown by an R^2 value of 0.83 for the SVM model

compared to an R^2 value of 0.80 for the ANN model. However, the use of SVM in the field of ML is associated with significant limitations.

2.11 Summary of Literature Review

The ACoD LFL and LFW, with the integration of support carriers, can be improved for the production of CH_4 gas and COD removal through the treatment of the anaerobic process using a UASB reactor. The co-digestion of LFL with other substrates is required to balance the nutrients and therefore can avoid the failure of AD LFL as a sole substrate. The support carriers provide a surface for microbial attachment and promote the formation of biofilms in the reactor. Without proper treatment, the LFL may affect the environment as it contains high organic and inorganic matter. Besides, due to the significantly high production of MSW in Malaysia, it consequently creates a large amount of LFL, which requires an efficient treatment method, specifically anaerobic treatment, to fully utilise the leachate generation from landfills. The relationship between AD treatment and the high production of CH_4 gas has been proven in multiple studies that justify its capability to provide adequate quality greenhouse gases.

The UASB reactor is the most reasonable and promising approach to treating LFL, with numerous advantages compared to other methods. This includes a low energy requirement for the application, the ability to handle medium to high OLR, effectively removing a significant amount of COD, and producing biogas. However, the UASB reactor requires a long start-up system before it can degrade efficiently. Therefore, a modified UASB system that is integrated with a support carrier is required to improve the stability of the AD process, which will then result in a higher biogas yield. None

of the previous studies have investigated the integration of different types of support carriers that consist of RCBR, CBR, and LR in the UASB reactor for treating LFL and LFW.

The performance analysis using ML applications, specifically ANN and SVM, was also evaluated in terms of R^2 . Therefore, this literature has determined the knowledge gap and recognised several measures that can be taken. In addition, this literature also provides the basic information needed for the analysis of the AD process once the study has been carried out and will be focused on filling the knowledge gap by conducting experimental work.

CHAPTER 3

MATERIALS AND METHODS

3.1 General

The experiment was carried out using the AD process, with the substrate consisting of LFL and LFW. Two types of studies are conducted, including batch studies and continuous studies. The batch studies employ the principle of one-off feeding, and the system operates under mesophilic conditions for 32–36 days. Meanwhile, semi-continuous study is conducted using the conventional UASB reactor and a modified UASB reactor. The modified reactors have incorporated the integration of support carriers. The reactors were initially put through a period of acclimatisation with LFL and LFW substrate for two weeks to enhance the inoculum. The OLR was consistently maintained at a low level of 0.5 g/L/day throughout the acclimation process. Subsequently, the genuine substrate was introduced for semi-continuous study purposes. The semi-continuous system operated in mesophilic conditions at 37°C for 24 hours (1 day).

The substrate comprises a mixture of raw LFL, obtained from Forward Energy Sdn Bhd, and LFW, sourced from a food court situated in the 10th College of Universiti Putra Malaysia, with various ratios. The inoculum is obtained from the treatment facility situated at the 10th college of Universiti Putra Malaysia. Prior to the beginning of the feeding process, thorough rinsing of the reactor's apparatus and equipment was conducted using deionised water. This meticulous procedure was executed to prevent any potential contamination that could compromise the reliability and accuracy of the

subsequent results. The outcome of the BMP tests was then used as input for the modelling process, which consists of ANN and SVM ML algorithms. The selection of the best-performing bioreactors among the 7 UASB reactors (2 conventional UASB reactors, UASB-RCBR support carrier, UASB-CBR support carrier, UASB-LR support carrier, UASB-activated CBR with ZnCl_2 support carrier, and UASB-activated CBR with NaOH support carrier) will be based on the criteria of achieving the highest SMP, biogas production, COD removal, pH, IA/PA and TAN.



3.1.1 Research Flowchart

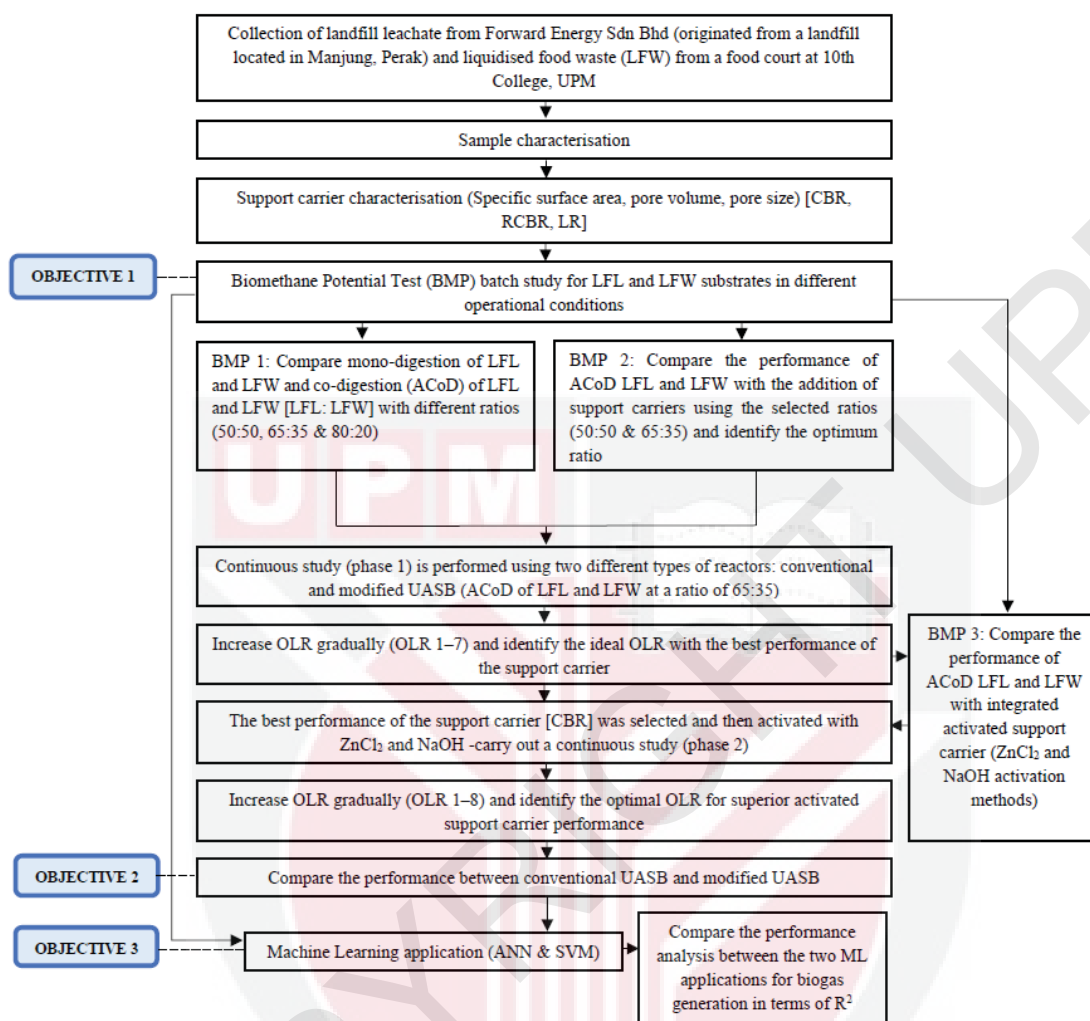


Figure 12: Research Flowchart

3.2 Analytical method for characterisation of landfill leachate and liquidised food waste

All analytical methods employed for the characterisation of LFL and LFW in this research adhere to the guidelines established by the American Public Health Association (APHA). This approach ensures that the methodologies utilised are standardised and reliable, facilitating accurate and reproducible results. By following the protocols outlined by APHA, the research maintains a high level of credibility, allowing for effective comparisons with existing literature and enhancing the overall quality of the data obtained.

3.2.1 Chemical Oxygen Demand (COD)

The closed reflux titrimetric technique was employed in this study. In situations where the concentration of the tested sample exceeds the quantifiable range of 10 mg/L to 500 mg/L, it becomes necessary to employ the process of dilution with deionised water. The procedure for the experiment necessitates the use of a H_2SO_4 reagent, a ferrous ammonium sulphate (FAS) titrant with a concentration of 0.10M, a ferroin indicator, and a potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) digesting solution with a concentration of 0.01667 M. The required equipment consists of 10 mL vials, a COD reactor heater (Model HACH 45600-00), a pipette, a beaker, a glass dropper, and a burette with a retort stand. The volumes of the FAS utilised at the end of the experiment for each titration were recorded to calculate the COD concentration, as shown in Equation 8.

$$\text{COD (mg O}_2\text{ /L)} = \frac{(A-B) \times M \times 8000}{\text{ml sample}} \quad (8)$$

Where;

A = mL FAS used for blank

B = mL FAS used for sample

M = molarity of FAS = 0.1 M

3.2.2 Total Ammonia Nitrogen (TAN)

In addition to alkalinity, measuring TAN in the effluent is crucial for assessing AD processes' stability. The test can be easily performed using a calorimeter (HACH DR 900, Agilent, Santa Clara, CA, USA), and the method adopted was Powder Pillows (Ammonia Salicylate and Ammonia Cyanurate Powder Pillow) Method 8155. If the concentration of the sample is excessively high, it is necessary to dilute it. This study employed a range of dilution factors (DF) between 100 and 500, depending on the effluent concentration. Following the dilution process, a volume of 10 mL from the diluted sample was carefully placed into a designated sample cell. Another sample cell was also prepared by pipetting 10 mL of deionised water, serving as the blank.

After that, one sachet containing ammonia salicylate powder was placed into each sample cell. The cells were then capped and vigorously shaken to facilitate the dissolution of the powder. The beginning of a three-minute interval for reaction was started. The solution exhibited a noticeable colour change when adding ammonia salicylate, transitioning to a pale-yellow colour. Subsequently, an additional sachet of ammonia cyanurate powder was added to each sample cell. The cells were then capped and vigorously shaken again to facilitate the dissolution of the powder. Following this, the cells were left for 15 minutes to allow for the completion of the reaction. The colour gradually transitions from yellow to green, with stronger colours of green representing a greater concentration of TAN and lighter colours indicating a lower concentration of

TAN. After the timer beeps, the blank is placed into the designated cell holder of the calorimeter. The sample cell was securely covered with the instrument cap. Afterwards, the 'Zero' button was pressed on the device calorimeter to set the neutral 0 value. Following this, the prepared sample was placed into the cell holder of the calorimeter, and the 'Read' button was pressed to record the TAN reading in mg/L.

3.2.3 Total Nitrogen (TN)

The TN concentration was determined using the NANOCOLOR® Gesamt-Stickstoff TN_b 22 analysis system. The measurement of TN can be efficiently conducted by inserting the sample vial into the high-tech electronic device called Spectrophotometers NANOCOLOR® UV/VIS II and VIS II. Firstly, 5 mL of the waste was transferred into the vial of Gesamt-Stickstoff TN_b 22, obtained from box A. Then, the mixture was vigorously shaken to mix the powder and the sample thoroughly. The vial was then placed into the heating blocks at a temperature of 120 °C for 30 minutes. After that, the vial was removed from the heating block and allowed to cool until it attained a temperature of 20–25 °C. Once the vial is cool, the compensation reagent tablet is put inside it. Then the reagent was dissolved in the solution, and the vial was labelled vial A.

Once vial A has been set up, the vial labelled Gesamt-Stickstoff TN_b 22 from box B is taken out. 500 µL of the solution from vial A was transferred into vial B. Next, 500 µL of the nitrate/nitrogen (NO₃/N) solution in box B was added into the vial B and allowed for complete mixing for 10 minutes. Once the period ended, the TN reading was obtained by inserting the vials into the spectrophotometers NANOCOLOR® UV/VIS II and VIS II. The concentration of TN is measured in mg/L.

3.2.4 Total Solids (TS)

A well-mixed sample was put into the clean dish, and the dish was heated in the oven at 103°C-105°C until dry. The clean dish was weighed before use (Reading A). After the sample was dried in the oven, the dish was taken out of the oven. The dish was then put in the desiccator to let it cool. The dish containing the residue was weighed using the analytical balance, which refers to Reading B. The equation representing the calculation of the TS is outlined in Equation 9.

$$TS \left(\frac{\text{mg}}{\text{L}} \right) = \frac{(\text{Reading B} - \text{Reading A}) \text{ in mg} \times 1000}{\text{ml sample}} \quad (9)$$

3.2.5 Total Dissolved Solids (TDS)

The TDS values were determined for both the sample LFL and LFW using ISOLAB TDS METERS - table top. To ensure proper homogenisation of the solution, the TDS electrode was submerged in the sample while the sample was being stirred. The TDS electrode was rinsed carefully with deionised water before and after each use to avoid inaccurate data. The TDS concentration is expressed in mg/L.

3.2.6 Volatile Solids (VS)

The filter paper was adequately placed within the vacuum filtration apparatus, and subsequently, the power of the vacuum filtration device was turned on to start the filtration process. The measured sample was poured over the filter paper and allowed to drain fully. Afterwards, the filter paper was transferred onto an evaporating dish and dried in an oven at a temperature range of 103 °C-105 °C for 1 hour. The dish was

removed from the oven and allowed to cool inside the desiccator. The residue on the filter paper was weighed using an analytical balance, denoted as Reading D.

Next, the filter paper was placed into a ceramic dish and heated to a temperature of 550 °C in a furnace for 15-20 minutes. The ceramic dish was removed from the furnace and cooled in a desiccator. The residue on the filter paper was weighed again and recorded as reading G. Equation 10 denotes the computation of the VS value.

Calculation:

$$VS \left(\frac{\text{mg}}{\text{L}} \right) = \frac{(\text{Reading D} - \text{Reading G}) \text{ in mg} \times 1000}{\text{ml sample}} \quad (10)$$

3.2.7 Oil and Grease (O&G)

The sample's oil and grease (O&G) content was calculated using the Partition Gravimetric Method. A sample volume of 500 mL was collected in a beaker and acidified with 1:1 H₂SO₄ to a pH value of 2 or below. Next, the sample was put into a separating funnel with a capacity of 1 L. The sample undergoes a rinsing process using 30 mL of n-Hexane, followed by adding a liquid soap solvent into the funnel. Following the secure closing of the mouth of the funnel apparatus, vigorous shaking for 2 minutes. Subsequently, the solution was allowed to rest undisturbed, facilitating the separation of its constituent layers.

After the separation process, the lower portion of the layer, known as the aqueous layer, was gradually transferred into a beaker, allowing just the remaining upper layer (the solvent layer) to be collected and put in a glass dish that was previously weighed as A.

The glass dish with the solvent layer was drained in an oven operating at 85°C for 60 minutes. The dish was removed from the oven and allowed to cool down to room temperature before being weighed as a B. Equation 11 below will determine the oil and grease content of the sample.

$$\text{Oil and grease content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{(\text{Weight B} - \text{Weight A}) \text{ in mg} \times 1000}{\text{ml sample}} \quad (11)$$

3.2.8 Colour

The measurement of colour intensity in the effluent sample was conducted using the HACH DR900 Colorimeter. The sample was diluted if required before analysis, considering the darkness of the colour, to enable the calorimeter to measure the intensity of the colour accurately. Then, the samples were transferred into the allocated sample cells and promptly inserted into the hole of the calorimeter. The programme had set to 'Zero' reading under neutral conditions with a sample cell containing deionised water as blank. Then the 'Read' button was pressed to obtain the sample result to be measured in unit Pt-Co.

3.2.9 Salinity

Salinity is an essential parameter for assessing the efficiency of an AD system. The salinity values were measured using ISOLAB TDS METRES - table top devices. In order to achieve optimal homogeneity of the solution, the sample was stirred using a magnetic stirrer, and the conductivity electrode was immersed in the sample. To ensure

the accuracy of the data, the salinity electrode was thoroughly cleaned with deionised water before taking the salinity reading. The salinity concentration is reported in mg/L.

3.2.10 Turbidity

The measurement of turbidity was conducted using the absorptometric method. The test can be readily performed using a spectrophotometer (HACH DR900 Colorimeter) by choosing a programme called 745 Formazine Attenuation Units (FAU). A volume of 10 mL of deionised water was poured into the sample cell and denoted as the blank. Next, the blank was inserted into the designated cell holder, and the 'Zero' button was pressed. As a result, the display will show the value 'Zero' FAU. After that, 10 mL of the sample was poured into a second sample cell, and the prepared sample was thoroughly cleansed before being inserted into the cell holder. Next, the 'Read' button was pressed to obtain the results, which will be displayed in the FAU unit. The unit of measurement known as 1 FAU is considered to be equivalent to 1 Nephelometric Turbidity Unit (NTU).

3.2.11 Carbon to Nitrogen ratio (C/N)

To ensure optimal microbial performance, a well-balanced nutrient ratio is required. C and N are the two most vital of the nutrients. The C value was measured from the COD analysis while the N value was obtained from the TN analysis, as explained in Sections 3.2.1 and 3.2.3. The C/N was obtained by dividing the C value by the N value. The C/N is crucial in the process of treating wastewater since it is one of the determining constraints for the effective digestion of substrate and biogas generation. Too high of a C/N ratio typically prevents microorganism growth, while too low of a ratio prevents adequate N removal and results in an effluent with a concentration of N that cannot

meet the discharged requirement. According to Rajlakshmi et al. (2023), the ACoD process offers the benefit of maintaining a balanced C/N ratio during digestion. This, in turn, leads to increased CH₄ production. The optimal C/N ratio for AD typically falls within the range of approximately 20:1 to 30:1 (Wang et al., 2023).

3.2.12 pH

In order to assess the stability of the AD process within the conventional and modified UASB reactors, daily measurements of the effluent's pH were conducted following its HRT. The pH of the systems was determined using an ISOLAB pH METERS - table top. The electrode was immersed in the sample while simultaneously stirring it to achieve adequate homogenisation of the solution. To ensure the integrity of the data and minimise potential contamination, the pH electrode was thoroughly washed with deionised water both before and following each usage. The pH meter was calibrated weekly using buffer solutions with pH values of 4.01, 7.00, and 10.00 to represent acidity, neutrality, and alkalinity, respectively.

3.2.13 Copper (Cu)

The concentration of Cu was determined using the Bicinchoninate method, specifically method 8506 (CuVer 1) powder pillows. The Cu concentration of the effluent sample was quantified using the HACH DR900 Calorimeter, with appropriate dilution performed if required. A volume of 10 mL of the sample was introduced into the sample cell, followed by the addition of CuVer 1 Cu reagent in the form of a powder pillow. Next, the mixture was gently swirled to facilitate thorough mixing, and a reaction time of 2 minutes was allowed to begin. After that, the blank was prepared by

introducing the second sample cell containing 10 mL of the sample. Next, the blank was inserted into the designated cell holder after ensuring that the blank sample cell had been well-cleaned beforehand. Subsequently, the 'Zero' button was pressed, displaying a reading of 0.00 mg/L Cu. After the 30-minute timer had expired, the prepared sample was inserted into the cell holder, and the 'Read' button was pressed, resulting in the display of the concentration of Cu in mg/L.

3.2.14 Ferum (Fe)

The concentration of Ferum (Fe) was determined using the FerroVer method, specifically employing the 8008 powder pillows method. The content of Fe in the effluent sample was evaluated using the HACH DR900 Calorimeter. The sample was diluted if deemed required. A volume of 10 mL of the sample was put into a sample cell to prepare the sample. Then, the FerroVer Iron Reagent Powder Pillow was introduced into the sample cell and swirled to facilitate the dissolution of the solution. The experiment commences with a reaction time of 3 minutes. The blank was prepared by adding 10 mL of sample to the second sample cell. The blank was inserted into the cell holder, and the 'Zero' button was pressed. The sample cell was cleaned before being inserted into the cell holder. Then, the button for reading was pressed, resulting in the display of the Fe concentration in mg/L.

3.2.15 Manganese (Mn)

The concentration of Mn was determined using the 1-(2-Pyridylazo)-2-Naphthol (PAN) method, specifically method 8149 powder pillow. The HACH DR900 Calorimeter was employed to measure Mn concentration. The cells were filled with 10

mL of deionised water for the blank and 10 mL of the sample of LFL and LFW for the sample. Subsequently, an ascorbic acid powder pillow was introduced into each individual sample cell. A stopper was placed on both samples, which were subsequently inverted to promote the dissolution of the powder. Each cell was added to the alkaline cyanide reagent solution, with a total of 12 drops being applied. After that, both cells were stirred to mix the solution, followed by adding 12 drops of PAN indicator solution, with a concentration of 0.1%, to each cell. The cell was once again stirred to achieve mixing, and the resulting sample exhibited an orange colour in the presence of Mn. A reaction of 2 minutes duration took place, following which the blank and prepared sample cells were cleaned before being inserted into the cell holder. The 'Zero' button was pressed, resulting in a 0.00 mg/L value. Next, the prepared sample was placed into the cell holder, and the 'Read' button was pressed to measure the concentration of Mn.

3.2.16 Zinc (Zn)

The content of Zn was determined using the Zincon method (method 8009 powder pillows). The HACH DR900 Calorimeter is used for the measurement of Zn content. A volume of 20 mL of the sample was added into a graduated mixing cylinder with a capacity of 25 mL. After that, the ZincoVer 5 Reagent Powder Pillow was added to the mixing cylinder, and the cylinder was securely closed. The cylinder was vigorously shaken to achieve total dissolution of the powder. It is important to consider that if all particles are not fully dissolved, it may lead to inconsistent readings. Next, the blank was prepared by pouring 10 mL of the solution into a sample cell, whereas the prepared sample was prepared by adding 0.5 mL of cyclohexanone to the solution in the mixing cylinder. The experiment began with the initiation of a 30-second reaction time.

Then, the mixing cylinder was tightly closed and vigorously stirred to ensure thorough mixing of the prepared sample throughout the reaction. The observed colour of the sample varies between reddish-orange, brown, or blue, depending upon the concentration of Zn. The 3-minute reaction time was scheduled to start. Afterwards, the prepared sample solution obtained from the mixing cylinder was transferred into a secondary sample cell. The blank was introduced into the cell holder. The 'Zero' button was pressed, and the prepared sample was subsequently placed into the cell holder. Then, the Zn content in mg/L will be displayed by pressing the 'Read' button.

3.3 Analytical Method for Semi-continuous study

The AD process facilitates the treatment of low-strength and high-strength wastewater in both conventional and modified UASB reactors. This process involves inserting the influent wastewater into the reactor, producing treated effluent and biogas that can be used as an alternative energy source. To assess the effectiveness of the reactor in wastewater treatment, various parameters were measured and reported on a daily basis. Additionally, some data collection was also observed twice weekly and once for each OLR during the experiment, enabling a comprehensive evaluation of the AD system. Biogas production and effluent pH parameters were monitored and recorded daily, while COD and the IA/PA ratio were measured twice per week. In addition, the TAN and gas chromatograph were tested for analysis on each OLR basis.

Daily monitoring of biogas production is crucial for tracking any immediate fluctuations in CH₄ generation, allowing for timely adjustments to optimise the digestion process's effectiveness in converting organic material into CH₄. Monitoring

effluent pH daily is essential to maintain an optimal range (pH 6.7 to 7.8), as it indicates the acidity or alkalinity within the digester, impacting microbial health (Widyarani et al., 2018). Acidic conditions may inhibit methanogenic bacteria, potentially reducing biogas production and destabilising the AD process (Alavi-Borazjani et al., 2020). Additionally, measuring COD twice weekly helps evaluate degradation efficiency by indicating the extent of organic material converted to biogas. Regular IA/PA monitoring twice weekly also provides early detection of imbalances; a low IA/PA ratio signals acidification risks, while an optimal ratio (<0.4) suggests a balanced acid-to-buffer capacity (Astals et al., 2012; Martín-González et al., 2013). Furthermore, monitoring TAN with each OLR ensures ammonia levels are maintained within a range supportive of microbial health, while gas chromatograph measurements at each OLR allow for precise adjustments to optimise gas quality and production efficiency by providing a detailed breakdown of biogas composition, particularly CH_4 content (Akindele & Sartaj, 2017; Wu et al., 2018).

In addition, BET and SEM were also conducted to evaluate the physical characteristics of the support carriers. Once the wastewater treatment system has attained stability, an analysis is conducted on the collected biogas to measure its CH_4 content. This analysis is crucial for calculating the SMP for each OLR. In the previous section 3.2, the measurement of COD, TAN, and pH were thoroughly discussed.

3.3.1 Intermediate alkalinity to partial alkalinity (IA/PA) Ratio

The evaluation of AD process stability was performed by employing the IA/PA as an alternative to the VFA/TA, as stated by Ferrer et al. (2010) and Astals et al. (2012). The IA/PA value is of significant importance in evaluating a system's ability to endure

variations in pH caused by the release of organic acids (Sakar et al., 2009). IA/PA was determined using the titrimetric method. The test was conducted using a pH meter, a burette with a retort stand, a 250 mL beaker, and a magnetic stirrer. The alkalinity of the effluent was measured by titrating the sample with a 0.02N solution of H₂SO₄ until the pH reached 5.7. The volume of acid solution used was recorded and labelled as partial alkalinity (PA). Subsequently, the titration process was resumed until a pH value of 4.3 was attained, which was subsequently recorded as the TA. Lastly, the alkalinity (α) value was determined by using Equation 12.

$$\alpha = \frac{IA}{PA} = \frac{|TA-PA|}{PA} = \frac{\text{Vol.of TA}-\text{Vol.of PA (mL)}}{\text{Vol.of PA (mL)}} \quad (12)$$

Where;

PA = Partial Alkalinity (pH = 5.7)

TA = Total Alkalinity (pH = 4.3)

IA = Intermediate Alkalinity

3.3.2 Specific Methane Production (SMP)

The biogas was collected directly from the conventional and modified UASB reactors to determine the CH₄ content in the collected biogas. A 60 mL syringe with a small flexible Polyvinyl chloride (PVC) tube was used to collect the biogas from the reactor's outlet. The obtained biogas was then securely sealed by wrapping the syringe tip and PVC tube tip with parafilm. The syringe was then sent to the Material Characterization Laboratory, Department of Chemical and Environmental Engineering, located in the Faculty of Engineering. The gas chromatography (GC) technique (Agilent Technology 6890N Network Gas Chromatography System) was utilised to conduct an analysis of the CH₄ content within the collected biogas sample. The sample was injected into the device configured with a Plot 1010 column, while argon was employed as the carrier

gas at a flow rate of 3 mL/min. After gathering the outcome, the CH₄ yield was calculated in terms of SMP with the unit of mL of CH₄/gCOD_{added}. The quantity of COD_{added} is equivalent to the initial amount (influent) of COD present in the substrate that is being introduced or fed to the reactor. The following equation, denoted as Equation 13, was applied to compute the SMP values.

$$\text{SMP} \left(\frac{\text{mL CH}_4}{\text{g COD}_{\text{added}}} \right) = \frac{\left(\frac{\% \text{CH}_4}{100} \right) \times \text{biogas produced (mL)}}{\text{COD}_{\text{in}} (\text{g/L/day}) \times \text{Vol. of substrate (L)}} \quad (13)$$

3.3.3 Brunauer, Emmett and Teller (BET)

The BET theory is frequently employed to assess gas adsorption data and determine a specific surface area value, typically given in units of area per mass of the sample (m²/g). This approach has been recommended by multiple standard organisations, including the International Organisation for Standardisation (ISO), the United States Pharmacopeia (USP), and the American Society for Testing and Materials (ASTM). Before doing the analysis, it is necessary to place the sample in a pre-conditioning step to eliminate physically bound impurities that may be present on the surface of the material. This pre-conditioning process is commonly referred to as degassing or outgassing. The typical method involves subjecting the sample to extreme temperatures while applying a vacuum or constantly flowing inert gas. In this study, a degassing temperature of 90 °C for 60 minutes and 150 °C for 240 minutes were employed. To get the most exact and repeatable results, this process needs to be carefully controlled.

The recommended quantity of the sample is between 2-5g. The analytical cell that is frequently employed reveals a cylindrical shape like a test tube, featuring a 9 mm opening at its upper end and a bulb structure at its lower end. The sample must travel through the tube, which has a diameter of approximately 9 mm, and then sit in the bottom of the bulb (2 mL, 4 mL, or 7 mL in size). Physical adsorption of a gas (typically nitrogen, krypton, or argon) onto the surface of the 2 g sample at cryogenic temperatures (typically liquid nitrogen or liquid argon temperatures) was then used to determine the specific surface area of a material. The nitrogen gas and liquid nitrogen were chosen for this investigation.

3.3.4 Scanning electron microscopy (SEM)

The SEM is a very powerful and efficient imaging device used to gather precise details about the surface topography of a specimen. It offers high-resolution images, enabling researchers to thoroughly examine specimens' detailed characteristics at the nanoscale. Additionally, it is recognised as a technique for obtaining detailed surface images using electrons. Different surface topographies generate different quantities of electrons, contributing to the contrast shown in electron micrographs. This contrast is indicative of the surface's topography and composition. Moreover, the SEM employs a beam of high-energy electrons to produce diverse signals on the surface of specimens. The signals obtained from interactions between electrons and the specimens provide valuable information such as texture or exterior morphology, chemical composition, and crystalline structure and orientation. The SEM analysis was performed in this study to determine the surface topography of the most effective support carrier (CBR).

3.4 Experimental Setup

3.4.1 Biomethane Potential Tests (BMP)

In the BMP test, three different operating conditions were implemented. These conditions included a system that did not have a support carrier (BMP Test 1), a system that was integrated with a support carrier (BMP Test 2), and a system that was integrated with an activated support carrier (BMP Test 3), as depicted in Figures 13, 14, and 15, respectively. The support carriers that have been used in this study include RCBR, CBR, and LR. All operational parameters were adjusted to a substrate-to-inoculum (S/I) ratio of 1:4, as a lower S/I ratio is conducive to the methanogenesis process (Li et al., 2022). Okewale et al. (2018) reported that the water displacement method was used to figure out the amount of biogas that was produced. Therefore, in this study, a graduated measuring cylinder was used to collect the biogas generated. Besides, the inoculum employed in this study had 116.54 g/L of volatile suspended solids (VSS), which originated from the Universiti Putra Malaysia sewage treatment plant. The sample calculation of the VSS of the inoculum is provided below.

$$\begin{aligned} VSS \left(\frac{g}{L} \right) &= Volume \text{ of sludge} \times \{VSS \text{ of sludge} \left(\frac{g}{L} \right) \times 100\% \} \\ &= 450 \times 0.259\% \text{ (g/L)} \\ &= 116.54 \text{ (g/L)} \end{aligned}$$

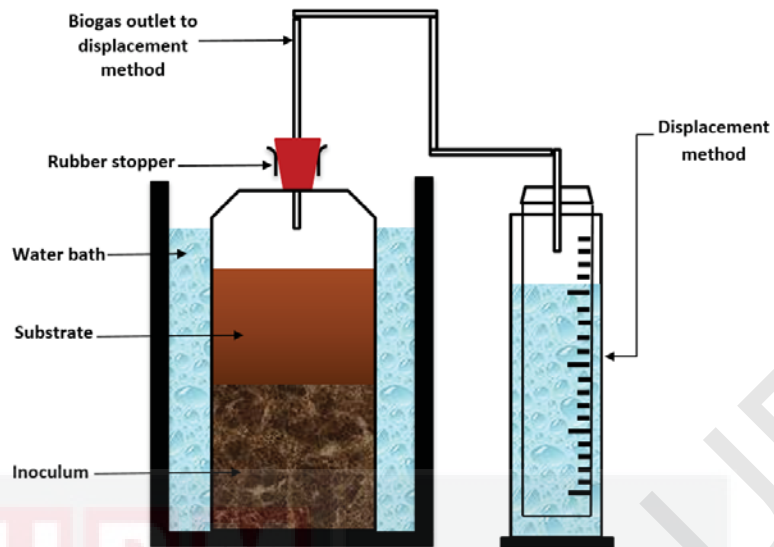
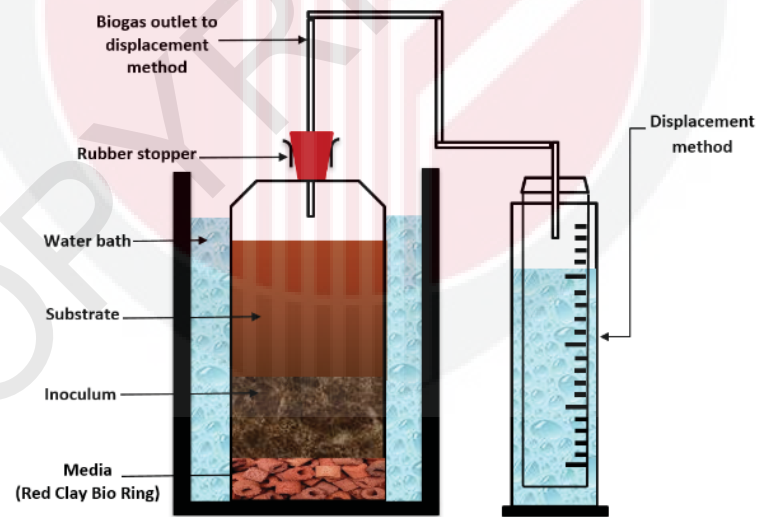
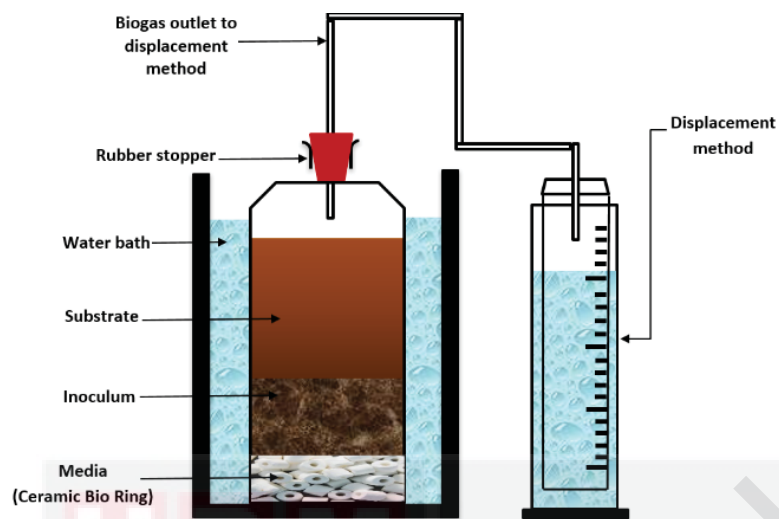


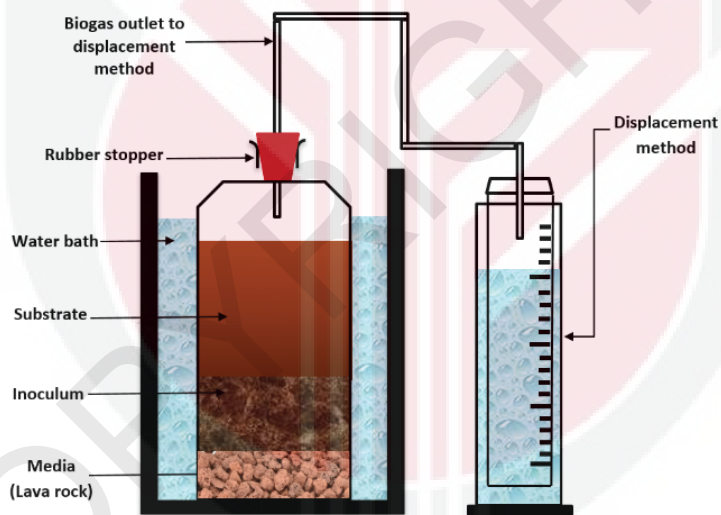
Figure 13: Biomethane Potential (BMP) Test without a support carrier (BMP Test 1)



a)



b)



c)

Figure 14: Biomethane Potential (BMP) Test integrated with a support carrier (BMP Test 2): (a) addition of RCBR; (b) addition of CBR; (c); addition LR

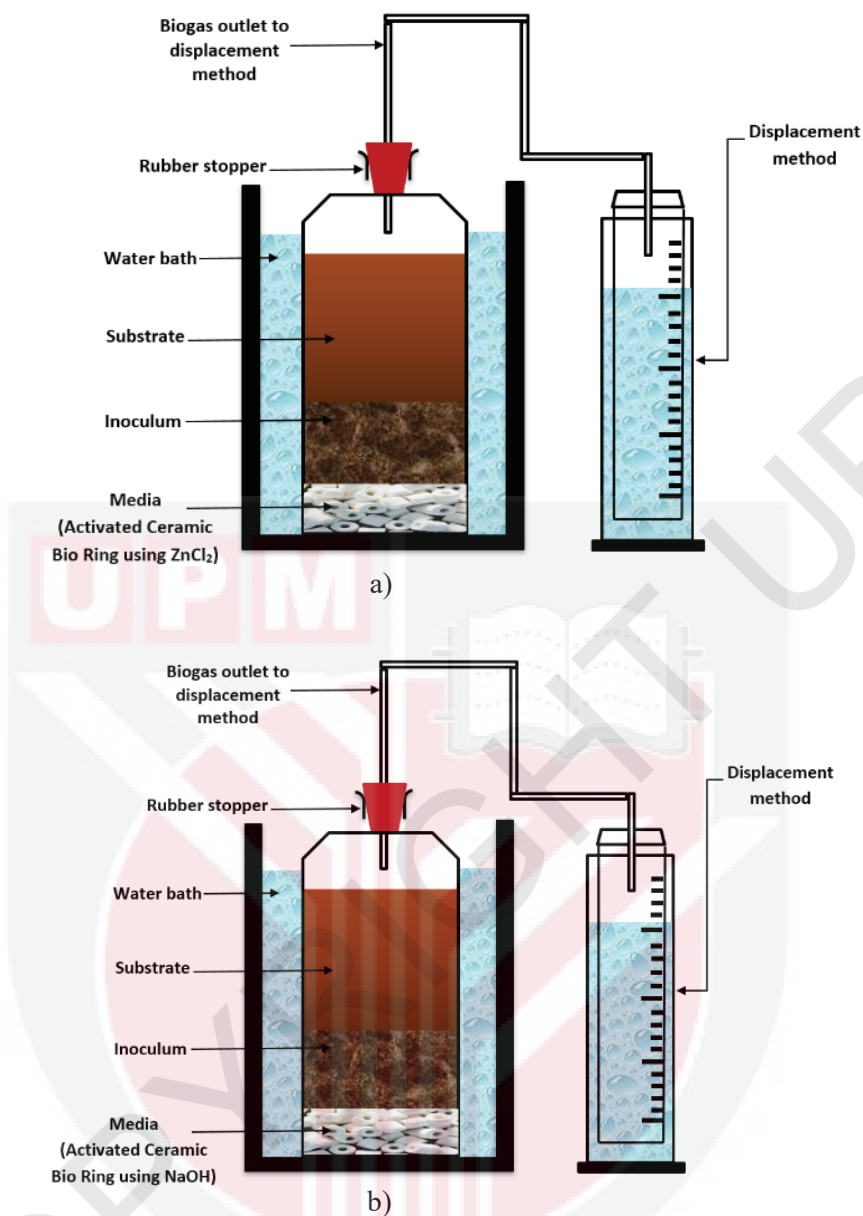


Figure 15: Biomethane Potential (BMP) Test integrated with activated CBR support carrier (BMP Test 3): (a) ZnCl₂ activation; (b) NaOH activation

30% of the wastewater volume designed for biological treatment was assigned to the support carrier in the modified UASB reactor. The objective is to improve the performance of ACoD LFL with LFW by investigating it in BMP Tests 2 and 3. The BMP Test 3 was conducted after obtaining the outcome of the best support carrier's performance in terms of biogas production and SMP in a semi-continuous study phase 1. Through continuous evaluations, it has been observed that CBR's support carrier

exhibits superior performance compared to RCBR and LR in BMP Test 2 and semi-continuous study phase 1. Therefore, the CBR was subjected to additional evaluation by employing ZnCl_2 and NaOH activation techniques in BMP Test 3. The operating parameters were carefully selected to simulate the environmental and functional conditions of both a conventional UASB reactor (without a support carrier) and modified UASB reactors (with a support carrier). This approach ensures that the experimental setup of BMP tests closely mirrors the actual operational characteristics and performance behaviours of conventional and modified UASB reactors during semi-continuous study, thereby enabling a more accurate assessment of their efficiencies, microbial interactions, and overall reactor system.

Furthermore, the COD values of the co-digestion of LFL with LFW employed for the BMP Tests 1, 2, 3, and the continuous studies were computed based on the mixing ratio. There are four distinct ratios for BMP Test 1, which include 100:0, 50:50, 65:35, and 80:20 for LFL, LFW, and ACoD of LFL with LFW (LFL:LFW). In BMP Test 2, two ratios were evaluated, including 65:35 and 50:50 (LFL:LFW). Meanwhile, in BMP Test 3, two activation methods of the support carrier were analysed with a ratio of 65:35.

All the BMP tests were conducted at 37 °C, which was maintained by placing the BMP bottles in a water bath. The temperature was continuously monitored using an automated digital panel. In this study, 39 samples were analysed using a 1 L BMP bottle; specifically, 15 samples were analysed for BMP Test 1, 18 for BMP Test 2, and 6 for BMP Test 3. Rubber stoppers were used to cap all BMP bottles, ensuring no air

was left in the headspace. The setup for operating BMP bottles in the analysis of BMP Tests 1, 2, and 3 is presented in Tables 14, 15, and 16, respectively.

Table 14: Biomethane Potential (BMP) Test 1 operation setup

Bottle ID	Substrate/sample	Operating ratio
1,6,11	LFL	100% LFL
2,7,12	LFW	100% LFW
3,8,13	LFL + LFW	50:50
4,9,14	LFL + LFW	65:35
5,10,15	LFL + LFW	80:20

Table 15: Biomethane Potential (BMP) Test 2 operation setup

Bottle ID	Substrate/sample	Support carrier	Operating ratio
1,7,13,	LFL + LFW	Red Clay Bio Ring (RCBR)	50:50
2,8,14,	LFL + LFW	Ceramic Bio Ring (CBR)	50:50
3,9,15,	LFL + LFW	Lava rock (LR)	50:50
4,10,16	LFL + LFW	Red Clay Bio Ring (RCBR)	65:35
5,11,17	LFL + LFW	Ceramic Bio Ring (CBR)	65:35
6,12,18	LFL + LFW	Lava rock (LR)	65:35

Table 16: Biomethane Potential (BMP) Test 3 operation setup

Bottle ID	Substrate/sample	Type of activation	Support carrier	Operating ratio
1,3,5	LFL + LFW	ZnCl ₂ activation	Ceramic Bio Ring (CBR)	65:35
2,4,6	LFL + LFW	NaOH activation	Ceramic Bio Ring (CBR)	65:35

3.4.2 Semi-continuous study

In general, two types of reactors were employed: conventional UASB and modified UASB, in a semi-continuous study phase 1, as illustrated in Figure 16. However, the modified UASB reactor was further evaluated in semi-continuous study phase 2 by incorporating the activation of the highest-performing support carrier in biogas and SMP production during semi-continuous study phase 1. The activation methods chosen for the CBR support carrier in this study were ZnCl₂ and NaOH activation.

In this research, the adaptation method was employed to facilitate a semi-continuous study as shown in Tables 17-19, aimed at enhancing the efficiency and stability of the AD process. According to Musa et al. (2018), this approach is founded on the recognition that microbial communities in anaerobic digesters can demonstrate varying levels of tolerance and metabolic adaptability in response to different OLR. By gradually increasing the OLR, the microbial populations can acclimate to the altered conditions, thereby optimising the degradation of substrates. The process of adaptation is crucial in AD treatment, as abrupt changes in substrate concentration can lead to disturbances in microbial activity, potentially resulting in system failure or reduced biogas production. Hence, to mitigate such risks, the gradual increment of OLR serves to familiarise the microbial communities with the characteristics of the new substrates.

The UASB reactors are made of cylindrical tubes fabricated from PVC. These reactors were operated at a temperature of 37°C under mesophilic conditions. Furthermore, a thermometer was placed inside the water bath to monitor its temperature. The heating coil was located between the two reactors, with one endpoint submerged in the water bath. The reactors had an internal diameter of 115 mm and a total volume of 5L. In addition, the headspace volume for both reactors were 0.5 L, while the working volume was 4.5 L. This volume was divided into two equal parts, which 2.25 L for the inoculum and another 2.25 L for the substrate. Besides, each reactor was supplied with a Teddler bag with a capacity of 10L, that was connected to the water displacement method through the gas outflow tube placed at the top of the reactors.

Both conventional and modified UASB reactors were working in an up-flow configuration. In addition, a peristaltic pump operating at a consistent flow rate of 10

revolutions per minute was set up to feed both reactors. This was performed in order to provide sufficient duration for the substrate and inoculum to come into contact with each other. The substrate was pumped through the sludge blanket from the bottom of the reactors. Both reactors were subjected to 2 weeks of acclimatisation using LFL and LFW. The OLR was consistently kept at a low level of 0.5 g/L/d throughout the acclimatisation phase. The biogas was consistently monitored until it reached a state of equilibrium. Following that, the introduction of the genuine substrate to feed in a semi-continuous study could begin.

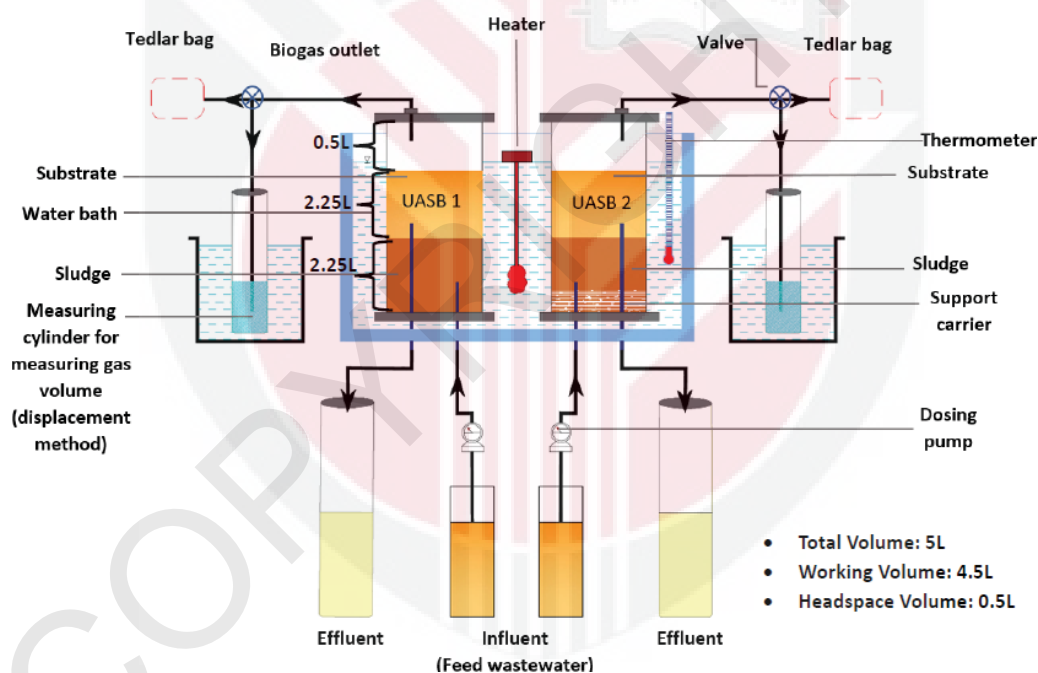


Figure 16: Conventional and modified up-flow anaerobic sludge blanket (UASB) diagram

3.4.2.1 Conventional up-flow anaerobic sludge blanket (UASB)

The study aimed to investigate the semi-continuous study (phase 1) on the AD process of mono-digestion of LFL and the ACoD of LFL and LFW. The ratio of 65:35

(LFL:LFW) was adopted for co-digestion of LFL and LFW. In this semi-continuous study employing a conventional UASB reactor, the addition of a support carrier is not incorporated. The OLR was increased systematically and gradually during each phase, with a period of 9–15 days between each increase. The experimental design for the semi-continuous study on the mono-digestion of LFL and ACoD of LFL and LFW using the conventional UASB reactor is summed up in Table 17.

Table 17: Experimental setup and operating condition of semi-continuous study for conventional up-flow anaerobic sludge blanket (UASB)

Operational condition	Operating period (days)	OLR (g/L/Day)	Operating ratio
Mono-digestion of LFL	15	1	100% LFL
	12	2	
	13	3	
	9	4	
	9	5	
	11	6	
	9	7	
Co-digestion of LFL and LFW	15	1	65% LFL + 35% FW
	12	2	
	13	3	
	9	4	
	9	5	
	11	6	
	9	7	

3.4.2.2 Modified up-flow anaerobic sludge blanket (UASB)- integration with support carrier

By integrating support carriers, the modified UASB reactor was utilised to treat the co-digestion of LFL and LFW. The ratio of 65:35 selected for the continuous studies (phases 1 and 2) of ACoD LFL and LFW is identical to that implemented in a conventional reactor. This ratio was chosen based on its superior biogas generation performance during BMP Tests 1 and 2. Three unique support carrier types have been integrated for each set of co-digestion samples: CBR, RCBR, and LR. In the modified

UASB, 30% of the volume of influent (wastewater) to be treated is chosen to fill with the support carrier. The OLR exhibited a gradual and consistent increase throughout each phase, similar to the conventional UASB, over 9–15 days. The experimental design for the modified UASB reactor integrating a support carrier in treating ACoD in LFL and LFW is described in Table 18.

Table 18: Experimental setup and operating conditions of semi-continuous study for a modified up-flow anaerobic sludge blanket (UASB) without the integration of an activated support carrier

Operational condition	Operating period (days)	OLR (g/L/Day)	Support Carrier	Operating co-digestion
Co-digestion of LFL and LFW	15	1	RCBR	65% LFL + 35% LFW + RCBR
	12	2		
	13	3		
	9	4		
	9	5		
	11	6		
	9	7		
Co-digestion of LFL and LFW	15	1	CBR	65% LFL + 35% LFW + CBR
	12	2		
	13	3		
	9	4		
	9	5		
	11	6		
	9	7		
Co-digestion of LFL and LFW	15	1	LR	65% LFL + 35% LFW + LR
	12	2		
	13	3		
	9	4		
	9	5		
	11	6		
	9	7		

3.4.2.3 Modified up-flow anaerobic sludge blanket (UASB)- integration with an activated support carrier

A further investigation (semi-continuous study phase 2) was carried out to evaluate the performance of ACoD of LFL and LFW. This evaluation involved the incorporation of an activated support carrier in a modified UASB reactor. Two separate methods of

activating support carriers have been employed, which comprise ZnCl_2 activation and NaOH activation. During the second semi-continuous study (phase 2), it incorporates an activated support carrier that accounts for 30% of the volume of wastewater to be treated, similar to the support carrier volume in the semi-continuous study phase 1. The OLR exhibited a gradual and steady increase throughout each phase, similar to the pattern of behaviour observed in both the conventional and modified UASB reactors in the semi-continuous study phase 1. This semi-continuous study of phase 2 was carried out for a period of 9 to 15 days. Table 19 summarises the experimental setup for the semi-continuous study of the ACoD of LFL and LFW, incorporating an activated support carrier in the modified UASB reactor.

Table 19: Experimental setup and operating conditions of semi-continuous study for modified up-flow anaerobic sludge blanket (UASB) with the integration of an activated support carrier

Operational condition	Operating period (days)	OLR (g/L/Day)	Support Carrier	Activation method	Operating co-digestion
Co-digestion of LFL and LFW	13	1	CBR	ZnCl_2 activation	65% LFL + 35% LFW + CBR
	15	2			
	12	3			
	11	4			
	9	5			
	9	6			
	9	7			
	10	8			
Co-digestion of LFL and LFW	13	1	CBR	NaOH activation	65% LFL + 35% LFW + CBR
	15	2			
	12	3			
	11	4			
	9	5			
	9	6			
	9	7			
	10	8			

3.4.3 Water Displacement Method for Biogas

To monitor the daily biogas production from the AD process, a pipeline had been set up to connect the gas outflow tube located at the top of both the conventional and modified UASB reactors to a graduated cylinder. The water displacement method was employed to measure the volume of biogas generated in both reactors. This was achieved by observing the displacement of water in the graduated cylinder caused by the inflow of biogas. The cylinder was initially filled with tap water up to the designated 0 level, resulting in only biogas within the cylinder. Besides, to prevent biogas discharge into the atmosphere, it was important to cover the gas output pipe before connecting it to the syringe to carry out gas composition analysis. Once the gas output was connected to a 60-mL syringe, the gas output tube could be left uncovered to extract the biogas.

3.5 Preparation of Landfill Leachate and Liquidised Food Waste as Substrate

The experimental substrate employed in this study consists of LFL and LFW. The LFL was obtained from Forward Energy Sdn Bhd, a privately owned company based in Ipoh, Perak, Malaysia. The LFL sample was obtained from a sanitary landfill facility in Manjung, Perak, Malaysia. On the other hand, the LFW sample was collected from a food court located at the 10th College of Universiti Putra Malaysia. The FW sample contained both solid components, such as bones, meat, and eggshells, and liquids, including soup, curry sauce, and rich coconut gravy. To facilitate feeding into the UASB reactor, the FW sample was blended to achieve a uniform liquid consistency, transforming the waste into a more digestible form for AD systems. LFW enhances microbial access, as solid waste, particularly larger or tougher particles, can create

physical barriers that impede microbial activity. Additionally, the blending process increases the surface area of the waste, allowing microorganisms in the digester to more effectively access and decompose the organic material (Kang & Yuan, 2017). The resulting liquid mixture also enables uniform particle distribution, promoting consistent conditions throughout the digestion process. A 50L container was used to collect the wastewater for LFL, while four 10L bottles were used to collect the LFW. To mitigate the degradation of the samples at room temperature, both samples were stored at a temperature of 4 °C.

The calculation of OLR was performed during each feeding phase to standardise the substrates' loading (Table 20). Prior to initiating the feeding process, the raw LFL and LFW were appropriately diluted according to the OLR. This dilution was necessary to gradually increase the loading within the conventional and modified UASB reactors. The OLR was calculated using Equation 14, as provided below. Equation 15 displayed the calculation for the volume of the raw sample required, denoted as X, which was then added with tap water to get a total volume of 2.25 L. This mixture was then pumped into the anaerobic digester.

$$\text{OLR}(\text{gCOD/L/day}) = \frac{\text{COD}_{\text{in}} (\text{g/L/day}) \times \text{Volume of substrate}(\text{L})}{\text{Working volume of reactor (L)}} \quad (14)$$

Volume of raw LFL + LFW needed to produce x volume of substrate:

$$X (\text{L}) = \frac{\text{COD}_{\text{in}} (\text{g/L/day}) \times \text{Volume of Substrate (L)}}{\text{COD}_{\text{raw}} \text{ of LFL+LFW (g/L)}} \quad (15)$$

Table 20: OLR in each phase of LFL+LFW feeding

OLR (g/L/day)	COD _{in} (g/L)	Volume of raw sample needed, x (mL)
1.0	2.0	19.57
2.0	4.0	39.13
3.0	6.0	58.70
4.0	8.0	78.26
5.0	10.0	97.83
6.0	12.0	117.39
7.0	14.0	136.96
8.0	16.0	156.52

Example Calculation:

$$OLR \text{ (gCOD/L/day)} = \frac{2 \text{ (g/L/day)} \times 2.25 \text{ (L)}}{4.5 \text{ (L)}}$$
$$= 1 \text{ g/L/day}$$

$$x \text{ (L)} = \frac{2 \text{ (g/L/day)} \times 2.25 \text{ (L)}}{0.2299 \text{ (g/L)}}$$
$$= 19.57 \text{ L}$$

3.6 Preparation of activated support carriers

Based on the semi-continuous study phase 1 results, the CBR was selected as the most suitable support carrier for facilitating biogas production from the ACoD of LFL and LFW. The physical and chemical activation using ZnCl₂ and NaOH was proposed to enhance the performance of a support carrier in terms of effective physical characteristics for biofilm formation, which in turn could enhance biogas production from the co-digestion of LFL and LFW. The physical characteristics of the activated CBR were evaluated through the analysis of adsorption isotherms (using nitrogen gas) at a temperature of -195.98°C (analysis bath temperature). This isotherm quantifies the amount of gas adsorbed onto a material's surface and pores. Therefore, by employing

the BET analysis method, the parameters such as surface area, pore volume, and pore size of a support carrier can be determined.

3.6.1 Zinc chloride (ZnCl_2) activation of a ceramic bio ring

675g of support carrier (CBR) was activated, which is equivalent to 30% of the wastewater to be treated for each reactor. This activated CBR will be evaluated for its biogas production performance in batch and continuous studies. A total of 675g was introduced into the flasks, followed by the addition of ZnCl_2 solutions with impregnation ratios (ZnCl_2/CBR) of 3:1 (w/w). This specific impregnation ratio of 3:1 (w/w) was chosen based on recommendations from previous studies, as it has been shown to optimise activation conditions and enhance material properties (Üner et al., 2019; Angin, 2014). The purpose of this addition was to activate the support carrier. The mixture was then placed under magnetic stirring for 2 hours to allow for the completion of the reaction between ZnCl_2 solution and CBR. Subsequently, the mixture was dried at a temperature of 130°C for 1.5 hours. Following the drying process, the carbonisation of the mixtures was carried out by placing them in a furnace at a temperature of 700°C for 2 hours. After that, the samples were removed from the furnace and cooled before being treated with 0.1 M HCl solutions. The activated CBR samples were washed using deionised water until a neutral pH range of 6.5-7 was achieved. This step was undertaken to remove any residual activating agents and other inorganic species that may have been generated during the activation process (Williams & Reed, 2006; Lu et al., 2014). Finally, the activated support carrier undergoes a drying process at a temperature of 110°C for a period of 24 hours. Afterwards, it was kept in a desiccator for further analysis. The procedure for support carrier activation employing the ZnCl_2 method is illustrated in Figure 17.

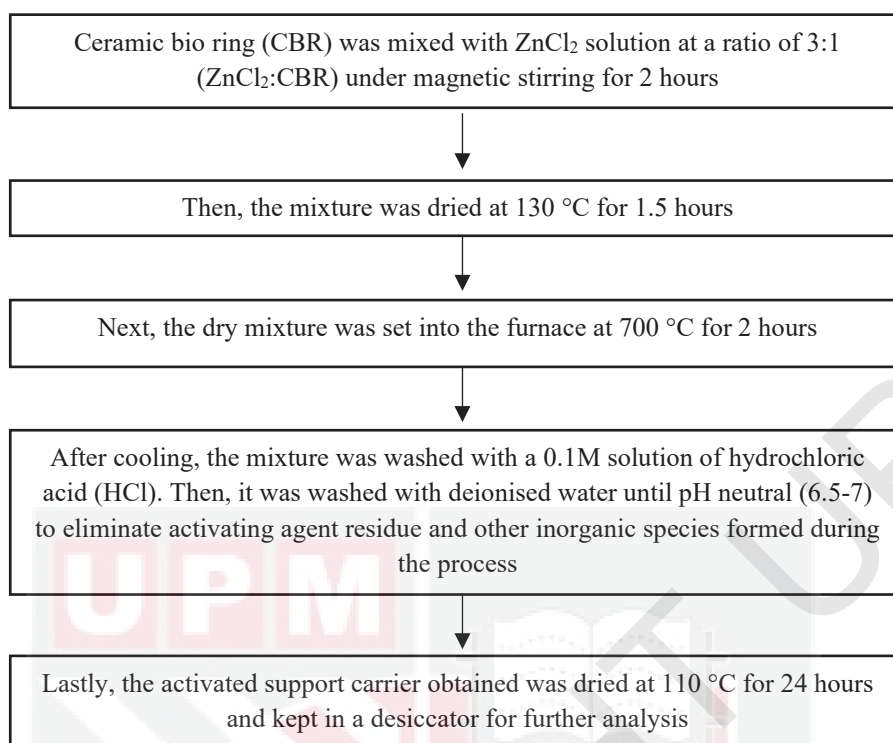


Figure 17: Activation of CBR using ZnCl₂ activation

(Source: Lu et al., 2014; Kumar & Mohan Jena, 2015; Üner et al., 2019)

3.6.2 Sodium hydroxide (NaOH) activation of a ceramic bio ring

A total of 675g of support carrier (CBR) was activated using NaOH activation. This activated CBR will undergo evaluation in both batch and continuous studies, identical to the evaluation of the ZnCl₂ activation method. A total weight of 675g was introduced into the flasks, and NaOH solutions with impregnation ratios (NaOH/CBR) of 3:1 (w/w) were added to the flask to initiate the activation of the support carrier. Cazetta et al. (2011) reported that an impregnation ratio of 3:1 (w/w) yields the highest surface area for activation. Therefore, this study applied this ratio to further examine the characteristics of CBR activated with NaOH. Following that, the mixture was put through magnetic stirring for 2 hours to promote the reaction involving NaOH solution and CBR support carrier. Next, the mixture went through a drying process at a

temperature of 130°C for a period of 4 hours. After the completion of the drying process, the mixtures were subjected to carbonisation by being placed inside a furnace set at a temperature of 700°C for an entire period of 1.5 hours. Then, the samples were removed from the furnace and went through a cooling process prior to their treatment with 0.1 M HCl solutions. The activated CBR samples undergo a washing process with deionised water until a neutral pH range of 6.5-7 is attained to eliminate any remaining activating agents and other inorganic species that could have been developed during the activation process. The activated CBR is put through a drying process at a temperature of 110°C for a duration of 24 hours, and it is kept in a desiccator for further analysis. The process of activating support carriers using NaOH is depicted in Figure 18.

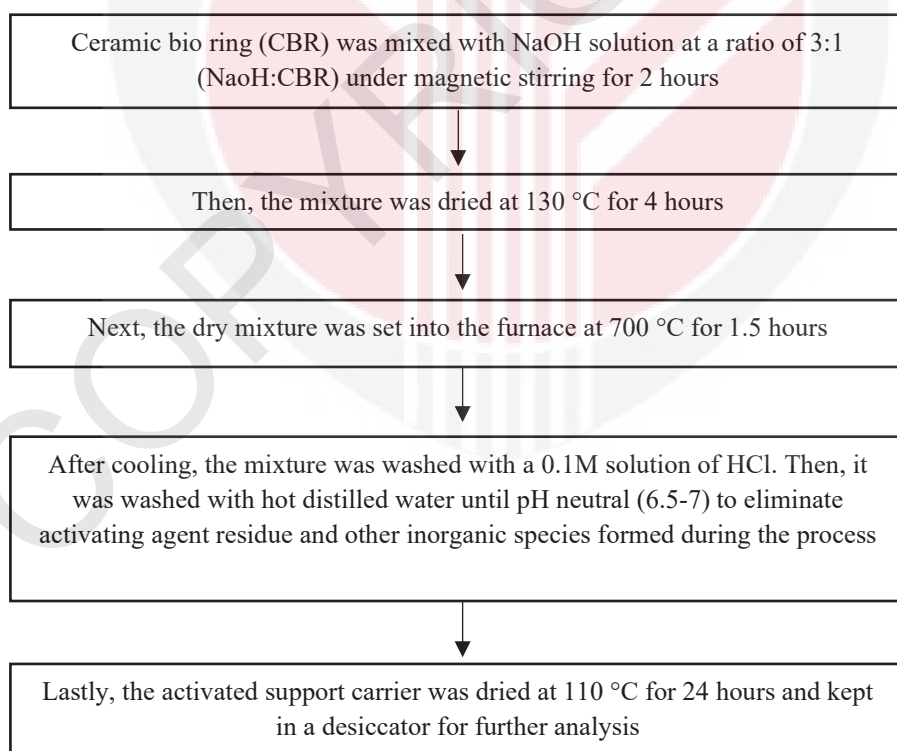


Figure 18: Activation of CBR using NaOH activation

(Source: Cazetta et al., 2011)

3.6.3 Details of support carrier

During phase 1 of the semi-continuous study, the support carriers employed were RCBR, CBR, and LR. In contrast, phase 2 integrated support carriers consisting of activated CBR, treated with NaOH and the ZnCl_2 activation method, as depicted in Figure 19 below.

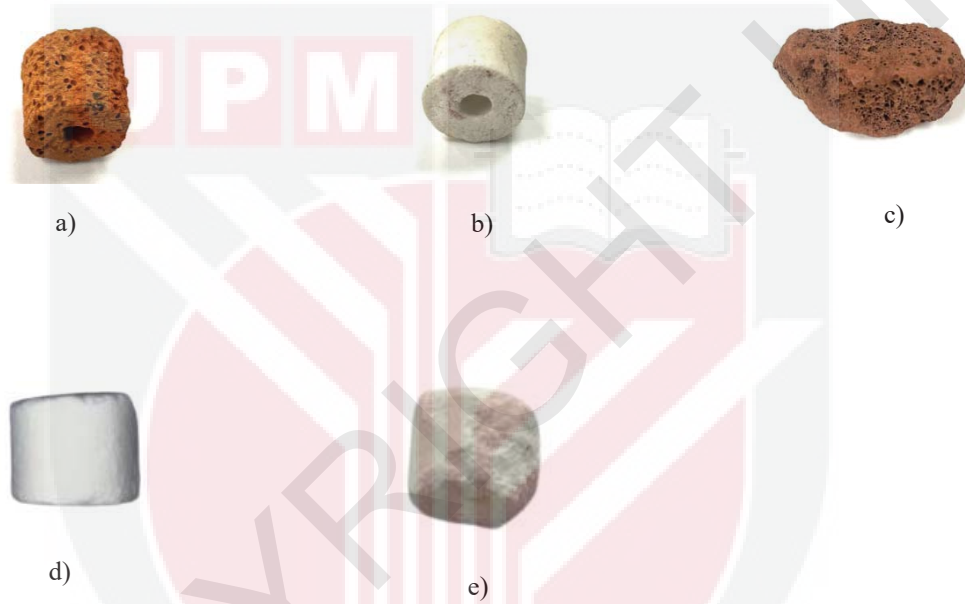


Figure 19: Types of support carriers: (a) Red Clay Bio Ring (RCBR); (b) Ceramic Bio Ring (CBR); (c) Lava Rock (LR); (d) Sodium hydroxide (NaOH)-modified CBR; (e) Zinc chloride (ZnCl_2)-modified CBR

3.7 Machine Learning Application

3.7.1 Artificial Neural Network (ANN) Analysis

Before conducting any database analysis in ANN modelling, the input data required normalisation using Equation (16) to ensure their suitability for the activation function in the hidden and output layers (Mohamed, 2019). During the training stage, the input dataset was selected to have values within the range of -1 and 1, as stated by

Rahimikhoob (2010) and Mohamed (2019). The results are subsequently multiplied by the sigmoid activation function, which can be either the Log-Sigmoid or the Tan-Sigmoid. These activation functions are represented by Equations (17) and (18), respectively (Mougari et al., 2021). The values produced by either of these two sigmoid functions are transmitted to the output layer, which has an equivalent number of neurons and output variables, or to the other hidden layers and determined by a pure-linear activation function (Equation 19) (Mougari et al., 2021).

$$X_{nor} = \frac{3(X_i - X_{min})}{2(X_{max} - X_{min})} - 1 \quad (16)$$

Where;

X_i = original value,

X_{min} = minimum value of original values

X_{max} = maximum value of original values

X_{nor} = is the normalised value

$$g(a) = \text{Tanh}(a) = \frac{\exp(a) - \exp(-a)}{\exp(a) + \exp(-a)} \quad (17)$$

$$g(a) = \log(a) = \frac{1}{1 + e^{-a}} \quad (18)$$

$$g(a) = \text{Purelin}(a) = a \quad (19)$$

This study employed ANN as a computational tool to predict the daily biogas production in BMP Tests 1, 2, and 3. This ANN model was developed using a dataset structured by triplicating the data from the BMP tests, which originally comprised 39 individual data. This approach expanded the dataset to a total of 117 data. The estimation process involved the consideration of several input variables, including OLR, C/N ratio, and C and N concentrations as input. Meanwhile, one parameter is employed for output, known as biogas. The design of the ANN network is presented in Table 21, with two hidden layers. The first hidden layer comprises 25 neurons and employs either Tan-sigmoid or Log-sigmoid activation functions.

Similarly, the second hidden layer comprises 25 neurons and uses Log-sigmoid or Tan-sigmoid activation functions. The dataset was employed to train the neural network using the MATLAB programme. The MATLAB function 'nntool' was employed in this analysis.

Table 21: Detailed information about the developed ANN models

Parameters	ANN 1	ANN 2
No. of layers	2	2
No. of neurons first hidden layer	25	25
No. of neurons second hidden layer	25	25
Activation function (first hidden layer)	Tan-sigmoid	Log-sigmoid
Activation function (second hidden layer)	Log-sigmoid	Tan-sigmoid

3.7.2 Support Vector Machines (SVM) Analysis

The primary goal of this SVM model is to determine the optimal hyperplane that maximises the margin between the hyperplane and the nearest data point in a high-dimensional space (Guo et al., 2021; Cinar et al., 2022). This SVM model utilised a dataset comprising triplicates of the 39 data obtained from BMP tests, resulting in a total of 117 data under similar conditions to those applied in the ANN model. This approach ensures consistency in the data used across models, thereby allowing for a more reliable comparison of predictive accuracy and model performance. The SVM algorithm also uses the maximum margin classification technique to determine the hyperplane that produces the most favourable results (Cervantes et al., 2020). The regression formula is used in order to enable the SVM model to map the input vector to high-dimensional feature space, as shown in Equation 20 (Kazemi et al., 2020). In

addition, Alejo et al. (2018) stated that the SVM uses Equation 21 to create a hyperplane that separates two datasets for classification or regression tasks. The input data also required normalisation similar to the ANN model using Equation 16, as mentioned in Section 3.7.1.

$$y(x) = \sum_{i=1}^1 w_i \cdot K(x, x_i) + b \quad (20)$$

Where;

$y(x)$ = Predicted value

$K(x, x_i)$ = Kernel function for input features

w_i and b = Coefficients

$$z = \phi(x) \quad (21)$$

where;

x = Input vector of the SVM

z = Feature space vector

This study employed linear kernel functions, as presented in Equation 22. The SVM model utilised a total sample of 117, triplicating the data from the BMP Tests result. The SVM technique was utilised to predict the daily biogas production from AD of LFL and LFW, and the ACoD of LFL with LFW in BMP Tests 1, 2, and 3. The prediction was formulated using four parameters as input, including OLR, C/N ratio, C concentration, and N concentration, and one output parameter, biogas production. The SVM analysis was conducted using MATLAB software, which is similar to that employed in ANN analysis. The developed SVM model is presented in Table 22.

$$f(x) = \omega \cdot \phi(x) + b \quad (22)$$

Where;

$f(x)$ = linear function in the feature space

ω = flatness

$\cdot$ = inner product

b = bias.

Table 22: SVM model details

Parameters	SVM 1	SVM 2
Kernel function	Linear	Polynomial
No. of inputs	4	4
No. of output	1	1

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The AD of LFL as a single substrate and in co-digestion with LFW was thoroughly evaluated using two different types of UASB reactors. The BMP test was conducted to analyse LFL and LFW's mono-digestion and LFL and LFW's co-digestion. Various operational conditions were considered, including different mixing ratios, incorporating support carriers, and using activated support carriers through ZnCl_2 and NaOH activation methods. Before commencing the semi-continuous study, a batch study of LFL and LFW mono-digestion and LFL and LFW co-digestion was conducted to evaluate their respective performances. Subsequently, a semi-continuous study was conducted to evaluate the performance of the AD system in treating LFL and LFW with and without the integration of a support carrier. After obtaining the results from the semi-continuous study phase 1, the activated support carrier was employed in the AD system to evaluate its performance further and compare it with the unactivated support carrier. The OLRs involved in conducting a semi-continuous study vary from 1 g/L/day to 8 g/L/day. The biogas production, COD removal, pH, IA/PA and TAN values were also measured and analysed for all OLRs. The percentage of CH_4 is also assessed to calculate the SMP value. The BET and SEM analyses also were carried out to evaluate the physical characteristics of the support carriers.

4.2 Physiochemical characteristics of raw sample LFL and LFW

Both samples, LFL and LFW, were analysed for various parameters, including COD, TN, VS, TS, TDS, O&G, colour, salinity, turbidity, C/N ratio, pH, and metals such as Fe, Mn, Cu, and Zn. Table 23 displays the physiochemical properties of the raw samples of LFL and LFW.

Table 23: Physiochemical characteristics of raw sample LFL and LFW

Parameters	Unit	LFL	LFW
COD	mg/L	32,000	72,000
TN	mg/L	1,100	1,600
VS	mg/L	5,340	74,255
TS	mg/L	11,325	90,125
TDS	mg/L	34,500	14,425
O&G	mg/L	203.4	3,928.8
Colour	Pt-Co	22,000	78,750
Salinity	mg/L	26,250	10,925
Turbidity	NTU	4,500	13,000
C/N ratio	-	29.09	45.00
pH	-	8.74	5.77
Copper (Cu)	mg/L	8	7
Ferum (Fe)	mg/L	11	7.4
Manganese (Mn)	mg/L	6	2.2
Zinc (Zn)	mg/L	27.5	19.2

4.3 Summary of BMP Tests

4.3.1 BMP Tests 1 of AD for Landfill Leachate, Liquidised Food Waste, and co-digestion of Landfill Leachate and Liquidised Food Waste with various ratios

The BMP test 1 was conducted on samples of LFL, LFW, and co-digestion LFL and LFW to assess the potential of biogas production under various operational scenarios. This study aims to identify whether mono-digestion of LFL or co-digestion of LFL and LFW is great for biogas production. This batch study was also performed to find out

the best mixing ratio for ACoD of LFL and LFW to be used in a semi-continuous system using the UASB reactor at mesophilic temperatures of 37 °C. The operation for BMP Test 1 is a system without a support carrier. In this study, 15 samples were analysed for 36 days, as shown in Table 24. Four different mixing ratios were employed for BMP test 1, which consist of 100:0, 50:50, 65:35, and 80:20 for LFL, LFW, and ACoD of LFL with LFW (LFL:LFW). Regarding BMP Test 1 (Figure 20), it was indicated that the ideal mixing ratio is 65:35 with the highest biogas production (204 mL), followed by ratios of 50:50 (203 mL) and 80:20 (108 mL), respectively. The biogas production from LFL is typically higher than that from LFW, as LFL generally contains higher concentrations of organic components, which promote microbial activity and lead to increased biogas output compared to LFW (Abdel-Shafy et al., 2024). Additionally, LFL provides more readily available organic material that anaerobic bacteria can efficiently convert into biogas, resulting in faster and more effective CH₄ production (Abuabdou et al., 2020).

According to research conducted by Siddique & Wahid (2018) and Karki et al. (2021), it is concluded that co-digestion generally outperforms mono-digestion, as combining different substrates yields a more balanced nutrient profile. This is because the co-digestion often results in higher biogas output due to the synergistic effects of mixing substrates with varying compositions (Zhang et al., 2015). Furthermore, Liao et al. (2014) reported that mono-digestion is susceptible to challenges such as acidification and inhibition, especially when the substrate is unbalanced or contains inhibitory compounds. By contrast, co-digestion mitigates these issues by diluting potential inhibitors and balancing the feedstock, contributing to a more stable AD process (Qi et al., 2023). At a high LFL proportion (80:20) (LFL:LFW), there may be an excess of

certain nutrients or organic compounds that overwhelm the microbial community in AD process. This improper mixing ratio can create an imbalance, leading to the accumulation of intermediate products, such as VFAs, which inhibit microbial activity and result in lower biogas output (Latha et al., 2019). Therefore, establishing an optimal co-digestion ratio of LFL and LFW is essential for creating a more favourable environment for microbial activity, thereby enhancing biogas production (Chaher et al., 2021; Liu et al., 2022).

Table 24: BMP Test 1 performance

Substrate/sample	Operating ratio	Biogas production (mL)
LFL	100% LFL	192
LFW	100% LFW	124
LFL + LFW	50:50	203
LFL + LFW	65:35	204
LFL + LFW	80:20	108

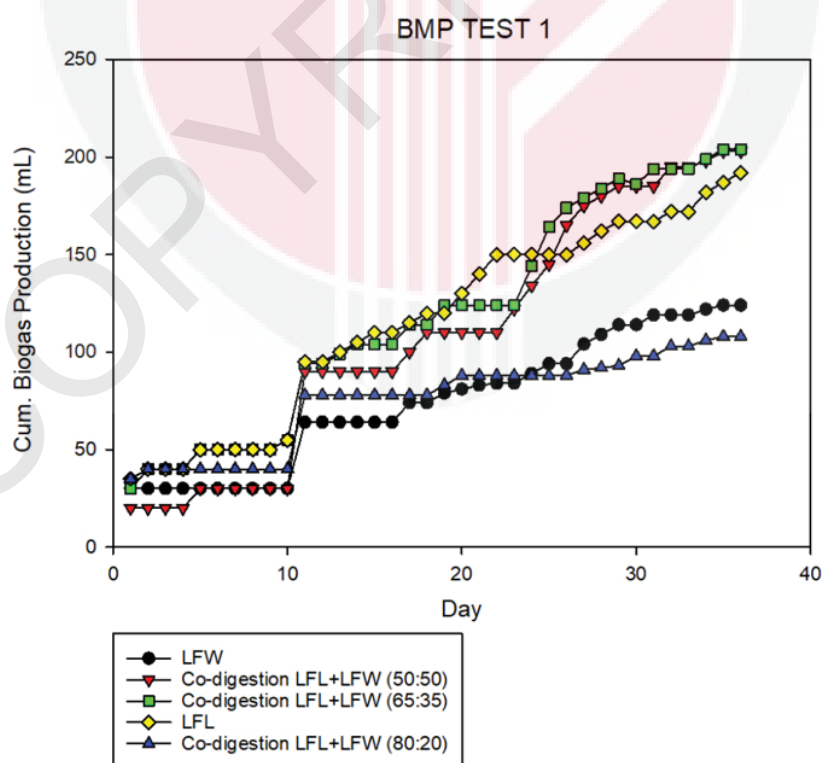


Figure 20: Biogas production for BMP Test 1

4.3.2 BMP Test 2 of co-digestion of Landfill Leachate and Liquidised Food Waste with integrated support carriers at ratios of 50:50 and 65:35

The second phase of BMP testing was conducted using a UASB system that was integrated with a support carrier at 37 °C, under mesophilic temperatures. BMP Test 2 utilises three support carriers: RCBR, CBR, and LR. 30% of the wastewater volume to be treated was assigned as the support carrier volume in a UASB reactor to enhance the performance of ACoD LFL with LFW. Chanathaworn and Dussadee (2017) reported that incorporating a support carrier in the BMP test boosts biogas production by providing an ideal environment for microbial colonisation. The ratios of 65:35 and 50:50 showed great efficiency in BMP Test 1. They were then evaluated in BMP Test 2, including three distinct support carrier types, to achieve maximum performance in biogas production. The mixing ratio for co-digestion of LFL and LFW (80:20) was not selected since it resulted in inefficient biogas generation performance during BMP Test 1, in comparison to co-digestion with ratios of 65:35 and 50:50. A total of 18 samples were carried out in BMP Test 2 for a total of 36 days, as indicated in Table 25. The results of BMP Test 2, as depicted in Figure 21, demonstrate that the integration of CBR as a support carrier greatly enhances the biogas production (4925 mL) of ACoD of LFL and LFW with a ratio of 65:35, compared to a system employing RCBR (2480 mL) and LR (3050 mL) support carriers. According to study conducted by Moujanni et al. (2018), the highest biogas achieved from co-digestion of LFL with domestic wastewater (including FW) without adding a support carrier was 450 mL. The results show that incorporating a suitable support carrier into the anaerobic digester improves biogas production. This can be proved based on the study done by Elhalafawy et al. (2023), which found that the performance of the biological system is improved by the presence of a CBR support carrier due to the larger porosity and a unique cylindrical

shape with exterior fines, enable the movement of wastewater across the surface of the CBR material and through its central hole.

Additionally, it was observed that the CBR creates an optimal environment for the active growth of biofilm due to its larger pore size of 435.53 nm. In contrast, the RCBR and the LR have significantly smaller pore sizes of 28.86 nm and 51.61 nm, respectively, as determined by BET analysis in Section 4.4. The CBR support carrier facilitates the entrapment of microorganisms on its surface and provides ample space for biofilm development, allowing microorganisms to attach more easily to the available surfaces and ensuring better access to nutrients and substrates for microbial colonisation, owing to its ideal porosity (Al-Amshawee et al., 2020). The physical characteristics of the support carrier are critical for supporting microbial communities, as an ideal pore size promotes more effective attachment and retention of microorganisms which create biofilm formation, leading to greater biogas production and overall enhanced efficiency of the AD process (Yang et al., 2015; Chanathaworn and Dussade, 2017). Therefore, integrating the CBR as a support carrier in an anaerobic digester treating LFL and LFW creates an ideal environment for biofilm growth through its larger pore size, enhanced nutrient accessibility, effective microbial attachment and retention, and promotion of stable microbial communities, all of which contribute to increased biogas production in AD processes.

Table 25: BMP Test 2 performance

Substrate/sample	Support carrier	Operating ratio	Biogas production (mL)
LFL + LFW	Red Clay Bio Ring (RCBR)	50:50	2610
LFL + LFW	Ceramic Bio Ring (CBR)	50:50	2040
LFL + LFW	Lava rock (LR)	50:50	2760
LFL + LFW	Red Clay Bio Ring (RCBR)	65:35	2480
LFL + LFW	Ceramic Bio Ring (CBR)	65:35	4925
LFL + LFW	Lava rock (LR)	65:35	3050

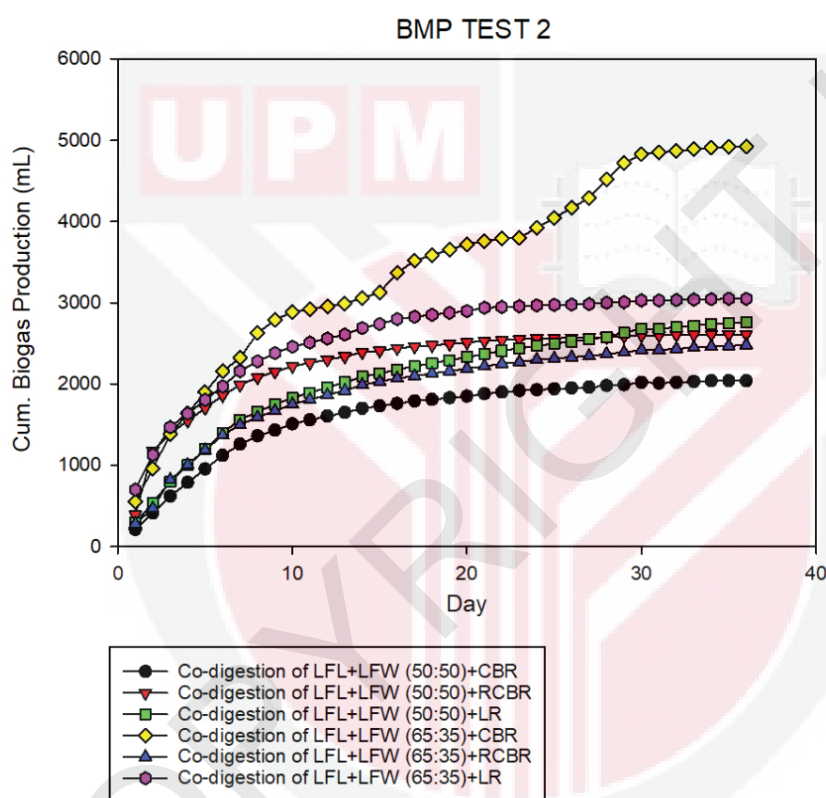


Figure 21: Biogas production for BMP Test 2

4.3.3 BMP Test 3 of co-digestion of Landfill Leachate and Liquidised Food Waste with integrated activated support carriers at ratios of 65:35

BMP Test 3 was conducted to investigate the impact of the support carrier, CBR, further, by activating it using the ZnCl_2 and NaOH activation methods in the UASB system for the treatment of LFL and LFW. The mixing ratio of LFL and LFW 65:35 was chosen as it performed better in biogas production during BMP Test 2. Through

semi-continuous study phase 1, it has been found that the ACoD of LFL and LFW in a ratio of 65:35, with the incorporation of a support carrier known as CBR, resulted in the highest production of biogas (2150 mL/d) and SMP (293.30 mL CH₄/gCOD_{added}). However, the alkalinity (IA/PA) during OLR 6 exceeded 0.6, reaching 1.2 during OLR 7, indicating system instability (Astals et al., 2012; Martín-González et al., 2013). Hence, the purpose of conducting BMP Test 3 is to optimise the functionality of CBR as a support carrier for the growth of biofilm by activating it, thereby ensuring the stability of the AD system and promoting the microbial population. Biofilms play a crucial role by promoting the retention of microbes in the anaerobic reactor, preventing their escape, and maintaining a continuous microbial population. The selection of suitable pore size and surface characteristics for the support carrier used in the AD system can enhance microbial activity by allowing attachment to the carrier surface. Following that, it can improve organic matter removal and increase biogas production from the ACoD of LFL and LFW. The results of BMP Test 3 (Table 26) and (Figure 22) demonstrated that the activation with ZnCl₂ resulted in the maximum biogas output, measuring 819 mL. Meanwhile, the biogas generation for the system utilising activated CBR with NaOH activation only reached 292 mL.

According to BET analysis, ZnCl₂-activated CBR possesses a pore volume of 0.37 cm³/g and a pore size of 20107.37 nm, which is sufficient for microbial attachment under a lower S/I ratio in the BMP test. This is because an ideal pore size provides a surface area for microbial communities to attach and grow and breakdown the organic matter to produce biogas. Despite the comparatively smaller physical characteristics of ZnCl₂-activated CBR in term of specific surface area, pore volume, and pore size, it outperformed the NaOH-activated CBR in biogas production during BMP test.

Furthermore, Hock and Zaini (2018) highlight that ZnCl_2 activation is widely utilised in industry to form a porous structure. In contrast, NaOH activation is also a commonly preferred chemical activation agent, as it results in a higher specific surface area and enhances adsorption capacity, which refers to the adhesion of microbes or particles to the surface of the carrier material (Jais et al., 2020 and Lu et al., 2022). There are 6 samples in total, which were conducted over 32 days at a constant temperature of 37 °C.

Table 26: BMP Test 3 performance

Substrate/sample	Activation method	Support carrier	Operating ratio	Biogas production (mL)
LFL + LFW	ZnCl_2	Ceramic Bio Ring (CBR)	65:35	819
LFL + LFW	NaOH	Ceramic Bio Ring (CBR)	65:35	292

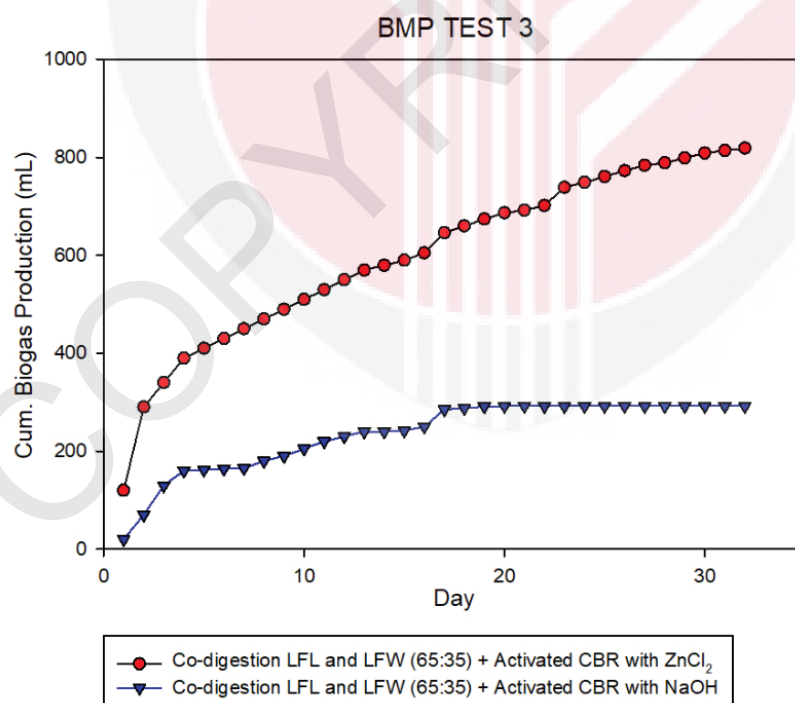


Figure 22: Biogas production for BMP Test 3

4.4 Physical characteristics of support carriers

Table 27 details the characteristics of the support carrier used in the ACoD of LFL with LFW in a modified UASB reactor for semi-continuous study phase 1. The support carriers utilised in semi-continuous study phase 1 are composed of RCBR, CBR, and LR, while in semi-continuous study phase 2, they consist of activated CBR using NaOH and the ZnCl_2 activation method. Integrating support carriers into anaerobic reactors is generally recognised for its effectiveness in improving operational stability and thus enhancing biogas production, as Mikheeva et al. (2022) mentioned. Incorporating the support carrier into the anaerobic reactor allows for the formation of a biofilm, effectively removing pollutants from wastewater (Murshid et al., 2023). Based on the findings, the BET analysis reveals that the CBR exhibits a significantly larger pore size of 435.53 nm, in contrast to the RCBR with 28.86 nm and the LR with 51.61 nm. Hence, integrating CBR as a support carrier in an anaerobic digester can greatly improve AD performance due to its larger pore size. The larger pore size may promote the attachment of microbes to the support carrier, as discovered by Yang et al. (2015). Furthermore, RCBR and LR have demonstrated a smaller pore volume of $0.07 \text{ cm}^3/\text{g}$ and $0.12 \text{ cm}^3/\text{g}$, respectively. This can result in tightly packed biofilms forming compared to CBR, which has a larger pore volume of $0.56 \text{ cm}^3/\text{g}$.

Table 27: Physical characteristics of support carriers employed in semi-continuous study phase 1

Parameters	Red Clay Bio Ring (RCBR)	Ceramic Bio Ring (CBR)	Lava Rock (LR)
Specific surface area (m^2/g)	4.99	2.56	4.52
Pore Volume (cm^3/g)	0.07	0.56	0.12
Pore Size (nm)	28.86	435.53	51.61

Meanwhile, the properties of the activated support carrier (CBR) used in semi-continuous study phase 2 are indicated in Table 28. Two different activation processes have been investigated in this study, ZnCl_2 activation and NaOH activation. Based on the BET result, it is demonstrated that the CBR activated with NaOH has a significantly larger specific surface area ($0.53 \text{ m}^2/\text{g}$) compared to the CBR activated with ZnCl_2 ($0.04 \text{ m}^2/\text{g}$). However, despite showing that CBR activated with NaOH has a greater specific surface area than CBR activated with ZnCl_2 , it was smaller than unactivated CBR. According to Auta et al. (2013), the reduction in specific surface area after the activation process may occur due to the entrapment of NaOH in the macropore area, which induces structural alterations in the support carrier. On the other hand, the total pore volume of CBR increased due to structural alterations resulting from NaOH activation modification, as shown in Table 28. The CBR activated with NaOH exhibited a higher pore volume of $0.57 \text{ cm}^3/\text{g}$ with a pore size of 2159.03 nm, compared to the CBR activated with ZnCl_2 . The BET results prove that an activation method on a support carrier can significantly enhance the pore size, as Tan et al. (2014) and Angin (2014) stated. The unactivated CBR has a pore size of 435.53 nm, while the activated CBR shows larger pore sizes of 20107.37 nm for the ZnCl_2 activation and 2159.03 nm for the NaOH activation method. Raj Deena et al. (2022) found that, the suitable biomedica carriers chosen with uneven biofilm can efficiently use in wastewater treatment as it can form the attached microbes able to access nutrients, and larger pore ($>1\text{mm}$) spaces leads to unrestricted mass diffusion, resulting in a high rate of substrate and oxygen transport to the inner regions and a high degradation efficiency.

Table 28: Physical characteristics of activated support carriers employed in semi-continuous study phase 2

Parameters	ZnCl ₂ activation	NaOH activation
Specific surface area (m ² /g)	0.04	0.53
Pore Volume (cm ³ /g)	0.37	0.57
Pore Size (nm)	20107.37	2159.03

4.5 Semi-continuous study of AD for LFL, ACoD of LFL + LFW, ACoD LFL + LFW + support carrier and ACoD LFL + LFW + activated support carrier with increasing OLR

4.5.1 Biogas Production

Biogas production was systematically monitored during the continuous study to evaluate the AD performance at varying OLRs. In Subtopic 4.5.1.1, biogas production was measured during Phase 1 of the continuous study, where OLRs ranged from 1 to 7 gCOD/L/d. Similarly, in Subtopic 4.5.1.2, biogas production was recorded during Phase 2 of the continuous study, with an extended OLR range of 1 to 8 gCOD/L/d.

4.5.1.1 Biogas production at different OLRs for AD for LFL, ACoD of LFL + LFW, and ACoD LFL + LFW + support carrier

The conventional UASB and modified UASB reactors were operated in semi-continuous mode for a period of 84 days. After achieving stability in biogas production, an increased OLR was introduced, as shown in Figure 23. The investigation consisted of performing mono-digestion of LFL and co-digestion of LFL with LFW in a conventional UASB reactor. In addition, the ACoD of LFL and LFW was also carried out in a UASB reactor that had been modified to include support carriers consisting of RCBR, CBR, and LR. Both reactors were run at a similar OLR, beginning at an OLR of 1 gCOD/L/day and continuously rising until reaching an OLR of 7 gCOD/L/day.

Overall, the AD system exhibits good performance in biogas generation when co-digesting LFL with LFW, as compared to the mono-digestion of LFL.

The AD systems experienced a slight decrease in biogas production once the OLR transitioned to a subsequent phase of OLR, as represented in Figure 23. The mono-digestion of LFL employing conventional UASB showed a severe decrease in biogas production performance when the OLR exceeded 4 gCOD/L/d (< 390 mL), reflecting inhibition of the system. This might happen due to an imbalance of nutrients in the mono-digestion of LFL. The introduction of OLR 5 to 7 gCOD/L/d to the LFL system's mono-digestion significantly deteriorated the AD system's performance in treating LFL using conventional UASB. This was shown in the very low daily biogas output, almost zero at OLR 5-7 gCOD/L/d. The failure of the AD process with LFL as the sole substrate may be attributed to its higher salinity level ($26,250 \text{ mg/L} = 26.25 \text{ g/L}$), as presented in Table 23, Section 4.2. These elevated salt levels have been observed to impede the functioning of anaerobic bacteria (Kawai et al., 2012). Based on the finding by He et al. (2024), high salinity can alter the microbial community in the reactor, favouring salt-tolerant microorganisms over AD microorganisms, which can then disrupt the synergy needed for efficient biogas production. This is because salinity within the range of 2–10 g/L has been observed to impede the activity of methanogens moderately, whereas a salinity concentration above 10 g/L strongly hinders the process of methanogenesis (Wang et al., 2017; Lee et al., 2009). Furthermore, according to Wang et al. (2017) and Zhao et al. (2020), AD is often hindered and may even fail as a result of elevated salt levels. The presence of elevated levels of inorganic salts in the sample to be treated can have detrimental effects on microorganisms and significantly hinder the effectiveness of treatment processes.

Nevertheless, when the identical reactor, which is conventional UASB, was employed for ACoD of LFL and LFW, the system demonstrated enhanced effectiveness and resistance to sudden increases in organic load, resulting in biogas generation rates of 940, 1072, and 736 mL at an OLR of 5 to 7 gCOD/L/d, respectively. Furthermore, the application of ACoD involving the combination of LFL and LFW, and the integration of RCBR and CBR exhibits a remarkable enhancement in biogas generation. The biogas production rates reached 2100 mL (integrated with the RCBR support carrier) and 2150 mL (integrated with the CBR support carrier), respectively, when operating at an OLR of 6 gCOD/L/d. The biogas production is enhanced by employing a support carrier or media that stimulates biofilm growth, hence improving the anaerobic process. Murshid et al. (2023) emphasised the effectiveness and advantages of using biofilm to solve the issue of wastewater contamination, in which the utilisation of microbial biofilms for wastewater treatment is a very efficient and environmentally friendly approach. In addition, a study executed by Elhalafawy et al. (2023) demonstrated that ceramic rings have excellent efficacy in treating wastewater through the biological technique, as these rings effectively retain biofilm, which assists in removing pollutant substances.

In addition, the integration of LR support carrier in the AD system decreases biogas production (<500 mL) when the OLR is increased to 4–7 gCOD/L/d. This phenomenon results from the larger pore sizes of LR (453.91 nm) compared to the immobilised cells, as mentioned in Table 27, Section 4.4, which could potentially lead to their release into the AD environment. Dzionek et al., (2016) and Bleve et al. (2011) found that if the pore size of the media is too large, the AD system becomes useless and inefficient for microbes to attach to its surface while extracting nutrients. Besides, Al-

Amshawee and Yunus (2021) discovered that the improper choice of carrier pore size hinders the activity of attached microbes on the carrier to receive nutrients, which limits the efficiency of the treatment process.

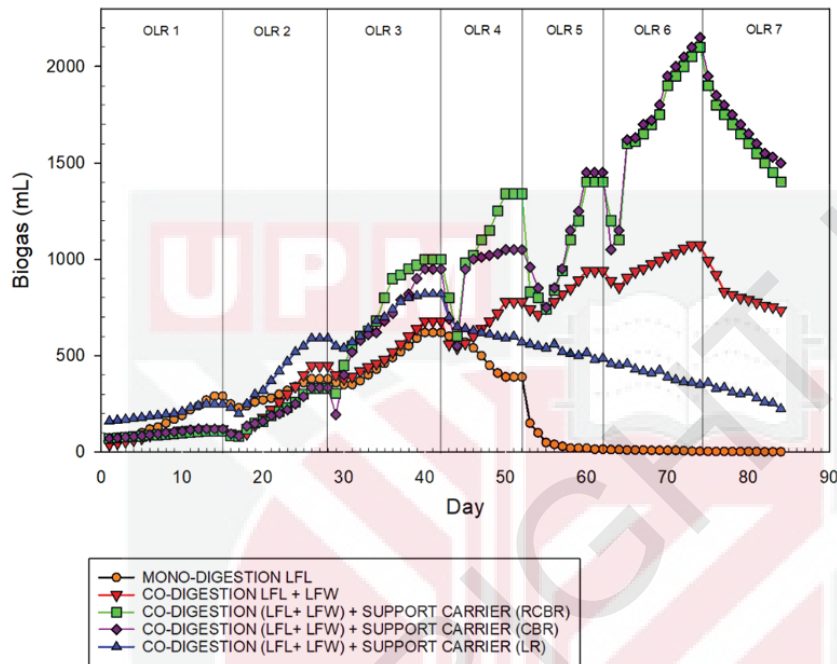


Figure 23: Biogas generation in the semi-continuous system (phase 1) of conventional and modified UASB reactors

4.5.1.2 Biogas production at different OLRs for ACoD LFL + LFW + activated support carrier

Further investigation was conducted to enhance the physical characteristics of the support carrier by employing physico-chemical activation methods consisting of ZnCl_2 and NaOH activation. The biogas production was demonstrated in Figure 24, where the modified UASB reactor was integrated with two different types of activated support carriers. The UASB reactor that incorporates the activated CBR using NaOH activation demonstrates superior performance compared to the UASB reactor that utilises ZnCl_2 activation of the support carrier. This result was opposed to the BMP

Test 3. It might be due to the implementation of CBR, which, when activated with ZnCl_2 , can only perform well at lower OLR. This is because, the BET result shows that the physical properties of the activated CBR with ZnCl_2 have a poor specific surface area and pore volume. The specific surface area for activated CBR with NaOH ($0.53 \text{ m}^2/\text{g}$) is higher than that of activated CBR with ZnCl_2 ($0.04 \text{ m}^2/\text{g}$). The maximum biogas production, obtained by integrating activated CBR with NaOH activation, reached a volume of 3275 mL/d at an OLR of 7 gCOD/L/d . From this result, it was shown that the incorporation of CBR, activated by the NaOH method, significantly improves biogas production from ACoD of LFL and LFW, as it has a pore size and pore volume of 2159.03 nm and $0.57 \text{ cm}^3/\text{g}$, respectively. Meanwhile, the UASB system, which employs ZnCl_2 activation of CBR, only reaches a biogas generation of 2230 mL at an OLR of 7 gCOD/L/d . The UASB system integrated with the CBR activated with the ZnCl_2 method produces biogas inefficiently due to its too-large pore size of 20107.37 nm . This large pore size decreases specific surface area, making it undesirable for bacteria to attach to the carrier surfaces. This can be proved by the finding of Dzionek et al. (2016), which indicates that if the pores are significantly larger than the immobilised cells, there is a risk of escape into the surrounding environment, which can hinder the activity of AD.

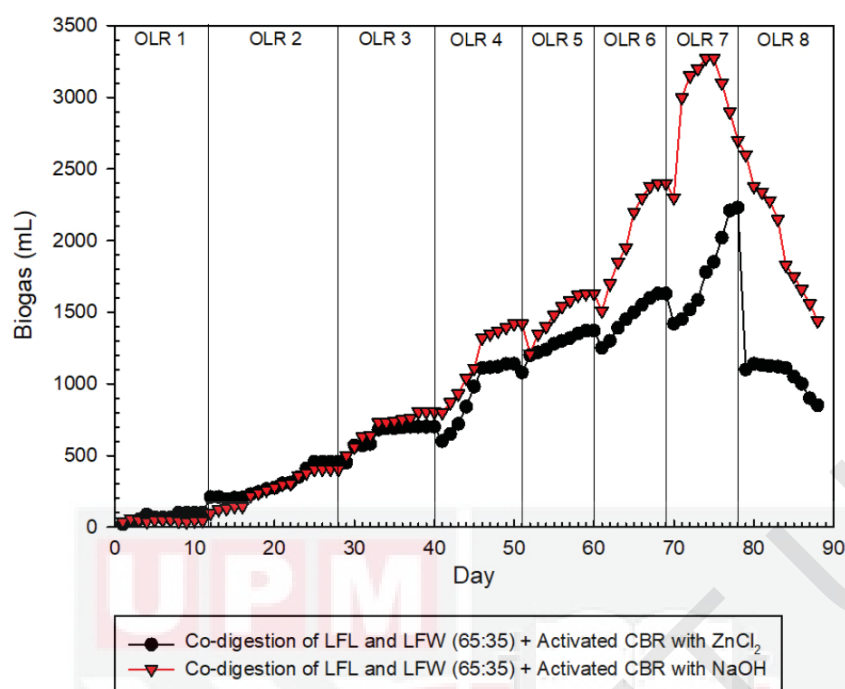


Figure 24: Biogas generation in the semi-continuous system (phase 2) of modified UASB reactors (integrated with the activated support carrier)

4.5.2 Chemical Oxygen Demand (COD) removal Efficiency

The removal of COD was evaluated at each OLR to determine the performance of the AD process. In Subtopic 4.5.2.1, COD removal was analysed during the Phase 1 continuous study, with OLRs ranging from 1 to 7 gCOD/L/d. In addition, in Subtopic 4.5.2.2, COD removal was observed during the Phase 2 continuous study, with OLRs ranging from 1 to 8 gCOD/L/d. These evaluations provided critical insights into the UASB system's efficiency in degrading organic matter under varying loading rates.

4.5.2.1 COD removal Efficiency at different OLRs for AD for LFL and LFW, ACoD of LFL + LFW, and ACoD LFL + LFW + support carrier

The effectiveness of both the conventional and modified UASB systems was determined by evaluating the COD removals at various OLRs, as shown in Figure 25. During the entire duration of phase 1 of the study, the OLR varied between 1 and 7

gCOD/L/d. The modified UASB reactor demonstrated outstanding performance, employing a substrate composed of LFL and LFW with integrated CBR and RCBR as support carriers. It achieved a COD removal effectiveness exceeding 80% when subjected to an OLR of 6 gCOD/L/d. In contrast, the UASB system that incorporated LR only achieved less than 40% COD removal. The study conducted by Kaushalya et al. (2018) determined that incorporating support media effectively reduces COD levels. The results demonstrate that the application of CBR resulted in an 84% decrease in COD, significantly surpassing the 83% reduction obtained by employing RCBR as a supporting carrier. The larger pore size of CBR ($435.53 \text{ m}^2/\text{g}$) leads to more microorganisms adhering to the support carrier. Hence, the attachment of numerous bacteria results in the creation of biofilm carriers, which play a crucial role in the functioning of wastewater treatment processes. Moreover, the CBR can be recognised by its cylindrical shape and the presence of exterior fines, which facilitate the movement of wastewater across the surface of the CBR material and through its central hole. This remarkable feature encourages the formation of a biofilm, hence enhancing the AD process.

The mono-digestion process of LFL achieves a maximum COD elimination efficiency of 48% when operated at an OLR of 3 gCOD/L/d. Additionally, it has been observed that the removal of COD experiences a significant decrease, dropping to below 40% as the OLR gradually increases to a range of 4-6 gCOD/L/d. Moreover, when operating at an OLR of 7 gCOD/L/d, the removal efficiency significantly decreases, approaching almost 0%. The microbial community's reaction to a sudden shift in the rate of substrate supply and the nutritional imbalance is the reason that causes inefficiency in the conventional UASB reactor for the mono-digestion of LFL. These factors

influenced the AD system's instability, leading to degradation process disruptions (Marzuki et al., 2021; Kainthola et al., 2020). The modified UASB system, which incorporates CBR as support carriers, showed endurance to a higher OLR and achieved excellent COD removal efficiency with an HRT of 24 hours. Therefore, the comparative analysis of conventional and modified UASB systems indicates that the modified UASB reactor, which includes a CBR, displayed improved efficiency in COD removal compared to a conventional UASB reactor. The findings of Elhalafawy et al. (2023) suggested that the integration of CBR into biological aerated filters led to a significant reduction in COD values, achieving a maximum reduction of 83.93%.

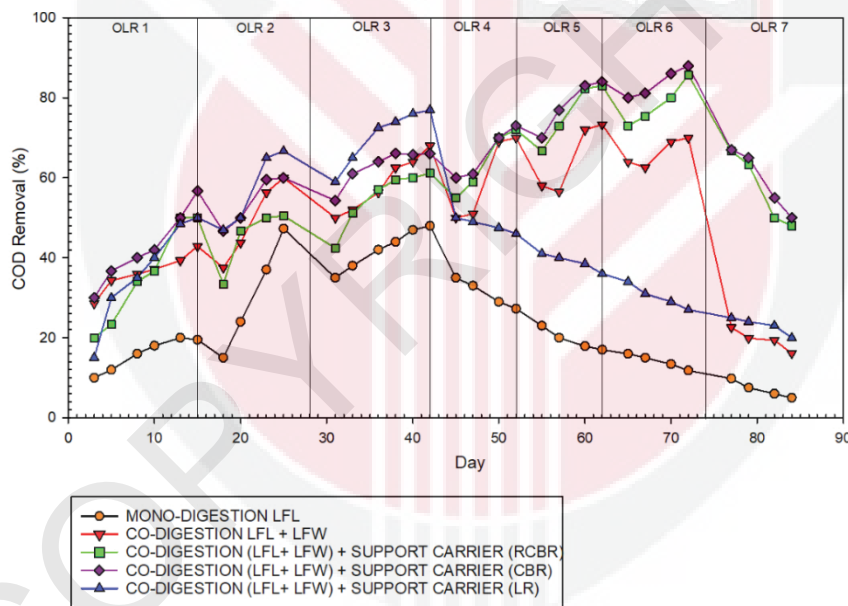


Figure 25: Chemical Oxygen Demand (COD) removal in conventional and modified UASB for semi-continuous system (phase 1)

4.5.2.2 COD removal Efficiency at different OLRs for ACoD LFL + LFW + activated support carrier

Additional research was carried out to improve AD's performance by activating the support carrier using physico-chemical activation methods. The COD elimination

during the semi-continuous study phase 2 (Figure 26) exhibited remarkable results as compared to the semi-continuous study phase 1 (Figure 25). The UASB reactor that was modified by incorporating a NaOH activation support carrier exhibits improved performance compared to the UASB reactor that employs ZnCl_2 activation of the support carrier. The system, especially when combined with NaOH-modified CBR, obtained a COD removal rate of up to 89.5% at an OLR of 7 gCOD/L/d. However, the UASB system that was combined with ZnCl_2 -modified CBR only achieved a maximum COD removal of 86.24%. This is because the activated CBR treated with NaOH exhibits a greater specific surface area of $0.53 \text{ m}^2/\text{g}$, which is higher than the specific surface area of activated CBR treated with ZnCl_2 , which is only $0.04 \text{ m}^2/\text{g}$. In addition, the NaOH-modified CBR exhibits a significantly greater pore size of 2159.03 nm, whereas the unactivated CBR has a smaller pore size of 435.53 nm. The suitable surface area and higher pore size allow greater microbial attachment, hence enhancing the degradation of pollutants in wastewater. This finding demonstrates that incorporating CBR, activated using the NaOH technique, greatly enhances COD removal from the ACoD of LFL and LFW. This can be proved by the previous study, which performed an ACoD process on LFL and FW without the inclusion of a support carrier, in which this approach only achieved a COD removal efficiency of up to 80% (Liu et al., 2022). Moreover, in anaerobic reactors, stability and performance can often be enhanced through the growth and conservation of the microbial community. Therefore, it has been observed that the process of microbial population adaptation to the surrounding environment can be improved by implementing of a modified UASB reactor, which ultimately results in enhanced COD removal (Musa & Idrus, 2020).

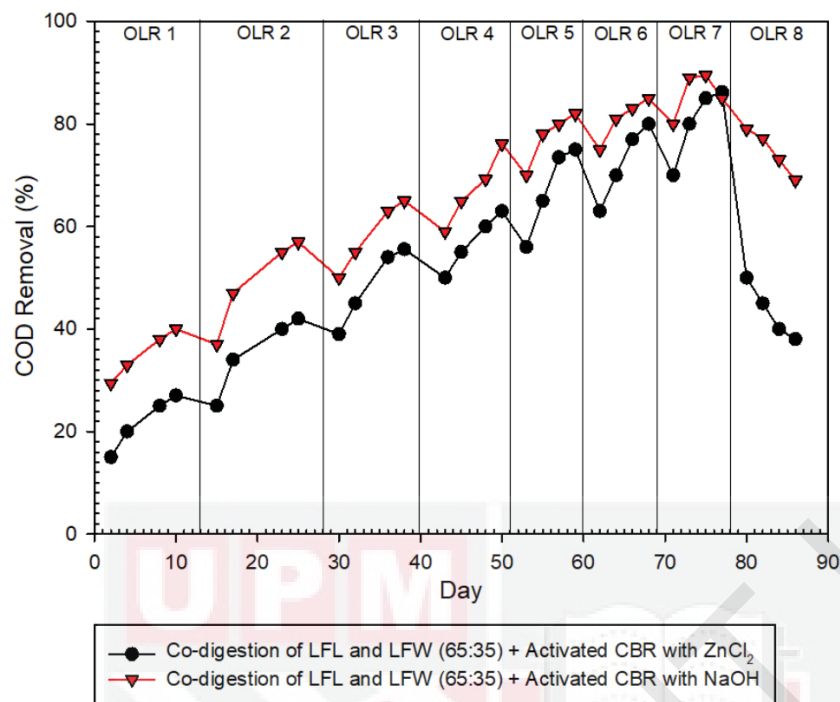


Figure 26: Chemical Oxygen Demand (COD) removal in the semi-continuous system (phase 2) of modified UASB reactors (integrated with the activated support carrier)

4.5.3 Specific Methane Production (SMP)

This section provides an evaluation of the SMP at each OLR during the continuous studies conducted in both Phase 1 and Phase 2. Section 4.5.3.1 presents the analysis of SMP during Phase 1, where OLRs varied from 1 to 7 gCOD/L/d. A further evaluation of SMP under OLRs ranging from 1 to 8 gCOD/L/d during Phase 2 is provided in Section 4.5.3.2.

4.5.3.1 SMP at different OLRs for AD for LFL and LFW, ACoD of LFL + LFW, and ACoD LFL + LFW + support carrier

Upon comparing the conventional and modified UASB systems, it was noticed that the modified UASB system demonstrated a greater SMP. The UASB system, which was modified by integrating a CBR, attained the maximum SMP value of 293.30 mL

$\text{CH}_4/\text{gCOD}_{\text{added}}$ at an OLR of 6 gCOD/L/d . The superiority of the SMP values for the modified UASB system with CBR as a support carrier over those obtained with other substrates is demonstrated in Figure 27. The CBR has a larger pore size ($435.53 \text{ m}^2/\text{g}$) in comparison to the other support carriers, namely the RCBR ($28.86 \text{ m}^2/\text{g}$) and the LR ($51.61 \text{ m}^2/\text{g}$). The increased porosity enables the efficient removal of organic matter, hence enhancing the AD process (El-Halafawy et al., 2023). As a result, the methanogenesis process is optimised, resulting in increased biogas output. Besides, the results of this research revealed that the SMP of the modified UASB system, which was combined with RCBR, CBR, and LR, exceeded $150 \text{ mL CH}_4/\text{gCOD}_{\text{added}}$ when the OLR reached 3 gCOD/L/d . However, the SMP of the conventional UASB reactors was less than $150 \text{ mL CH}_4/\text{gCOD}_{\text{added}}$ compared to the modified UASB reactors under the same conditions (OLR 1-7 gCOD/L/d). During the initial stages of the mono-digestion of LFL, the SMP showed a steady increase as the OLR increased from 1 to 3 gCOD/L/d . The maximum SMP, reaching $94.60 \text{ mL CH}_4/\text{gCOD}_{\text{added}}$, was recorded at an OLR of 3 gCOD/L/d . Nevertheless, there was a significant decrease in SMP production when the OLR was raised to 4 to 7 gCOD/L/d , resulting in nearly 0 $\text{mL CH}_4/\text{gCOD}_{\text{added}}$. When comparing the performance of ACoD systems without the integration of a support carrier, it is observed that ACoD systems exhibit better performance than AD systems using solely a single LFL at high OLRs, starting from an OLR of 4 gCOD/L/d , achieved highest SMP value of $99.4 \text{ mL CH}_4/\text{gCOD}_{\text{added}}$ at an OLR of 6 gCOD/L/d . Conversely, at lower OLRs (1–3 gCOD/L/d), ACoD systems without the integration of a support carrier produce less biogas than single LFL digestion. This outcome is due to the superior performance of mono-digestion at lower OLRs, where the readily degradable feedstock creates stable conditions that support rapid decomposition and higher CH_4 yields (Kumar et al., 2022; Luo and Pradhan,

2024). Under low OLR conditions, co-digestion may experience competition for microbial resources, such as proteins, which can hinder CH_4 production efficiency (Hagos et al., 2016). Nevertheless, the performance of ACoD systems without a support carrier is still lower than those integrated with a support carrier. Hence, these findings indicate that the ACoD of two substrates (LFL and LFW) with the integration of support carriers shows remarkable efficiency since it produces a higher SMP value.

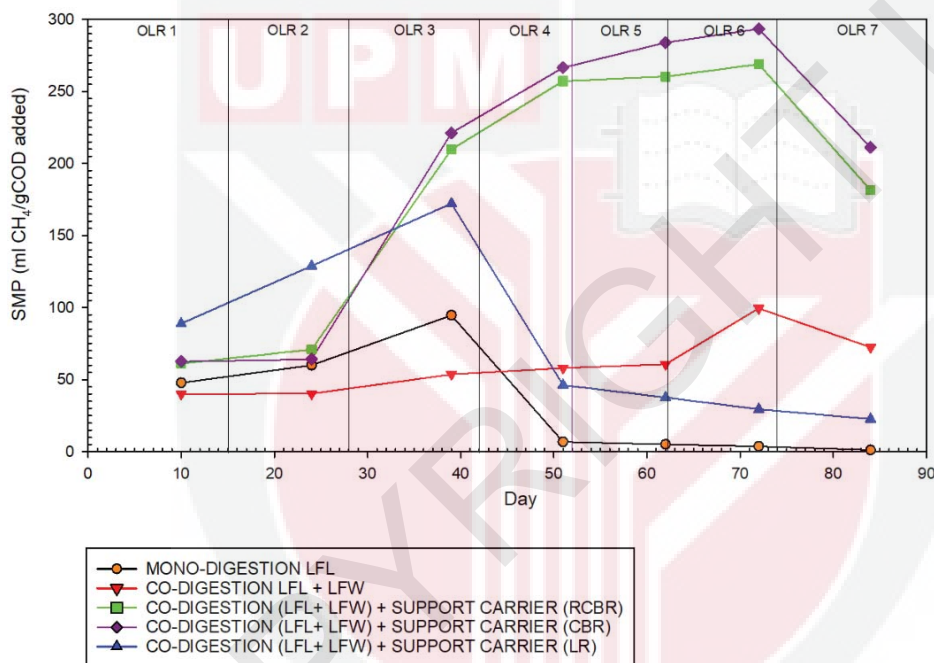


Figure 27: Specific Methane Production (SMP) in conventional and modified UASB for semi-continuous system (phase 1)

4.5.3.2 SMP at different OLRs for ACoD LFL + LFW + activated support carrier

A further study was performed to improve AD performance by implementing a UASB system with an activated support carrier. The SMP values exhibited superior performance in the semi-continuous study phase 2 in comparison to the semi-continuous study phase 1, as illustrated in Figure 28. The UASB reactor, which was incorporated using NaOH-modified CBR, showed outstanding results compared to the

UASB reactor integrated with an unactivated CBR support carrier. The highest SMP value achieved by NaOH-modified CBR was 459.95 mL CH₄/gCOD_{added} at an OLR of 7 gCOD/L/d, while the unactivated CBR only achieved an SMP value of 293.30 mL CH₄/gCOD_{added} at an OLR of 6 gCOD/L/d. This situation may have occurred because the NaOH-modified CBR has a larger pore size (2159.03 nm) than the unactivated CBR (435.53 nm). Meanwhile, the UASB system, integrated with ZnCl₂-modified CBR only produced an SMP value of 305.00 mL CH₄/gCOD_{added} at an OLR of 7 gCOD/L/d.

The increased surface area and pore size enable more microbial attachment to the support carrier. The efficient production of biogas depends on the presence of anaerobic bacteria within the anaerobic digester. Therefore, it is advantageous to utilise appropriate support carriers to establish an attached growth system that promotes biomass growth and biofilm creation. This system aids in decomposing of pollutants in wastewater, resulting in the efficient generation of biogas, mainly CH₄, in the digester. Liao et al. (2014) found that combining LFL and LFW in the AD system successfully minimised acidification caused by the build-up of VFAs. This resulted in the system effectively maintaining stability without any adverse effects on biogas production, promoting a good performance for SMP generation. Implementing a modified UASB reactor that includes a NaOH-modified CBR as a support carrier in treating ACoD of LFL and LFW offers an appealing alternative to the conventional UASB reactor for practical application. The primary reason for this is because it was able to generate more biogas during the second phase of the semi-continuous study (as shown in Figure 24), which consequently led to an increase in the production of SMP (Figure 28) compared to the semi-continuous study phase 1.

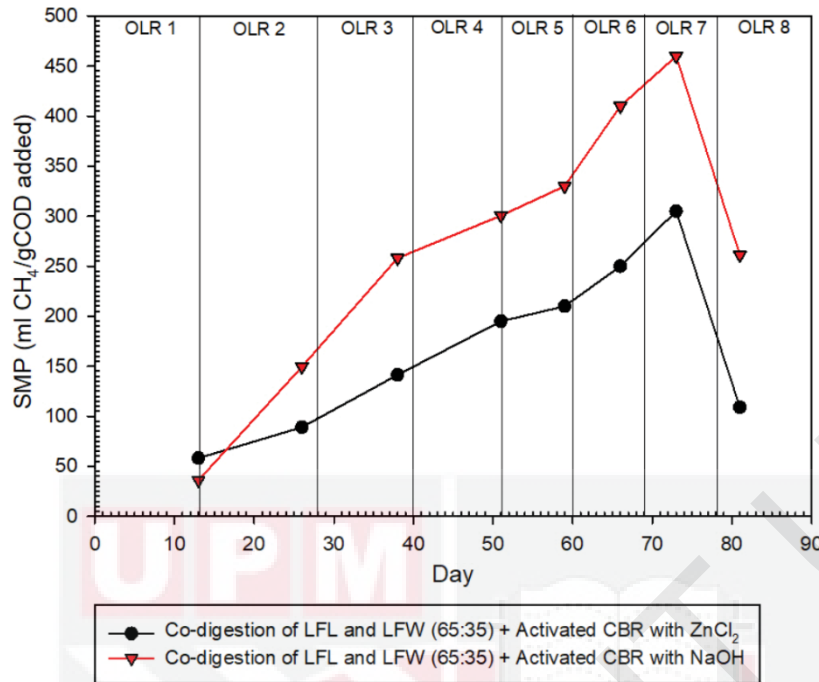


Figure 28: Specific Methane Production (SMP) in the semi-continuous system (phase 2) of modified UASB reactors (integrated with the activated support carrier)

4.5.4 pH Value

pH was monitored during the continuous study to evaluate the AD performance at varying OLRs. In Subtopic 4.5.4.1, pH was measured during Phase 1 of the continuous study, while in Subtopic 4.5.4.2, pH was recorded during Phase 2 of the continuous study. In an AD process, the pH level can vary depending on the microbial stages involved, as each phase of organic matter breakdown affects the pH in different ways. Initially, during hydrolysis and acidogenesis, the pH decreases due to the accumulation of acids (Tang et al., 2016). If acidogenesis exceeds the rates of acetogenesis and methanogenesis, the pH may continue to decline, resulting in process imbalances. As methanogenesis becomes more dominant, it neutralises the acids, causing the pH to rise and stabilise, thereby supporting the efficiency of the AD process and leading to higher biogas production (Alavi-Borazjani et al., 2020 and Vítězová et al., 2020).

4.5.4.1 pH Value at different OLRs for AD for LFL and LFW, ACoD of LFL + LFW, and ACoD LFL + LFW + support carrier

The pH profile of conventional and modified UASB reactor systems at different OLRs is depicted in Figure 29. At an OLR of 6 gCOD/L/d, the pH values of the ACoD process involving LFL and LFW with the addition of RCBR and CBR as support carriers were 6.50 and 6.23, respectively. This suggests that the system continues to operate within the optimal pH range, which is generally considered to be 6.8–7.2 (Gonde et al., 2023; Cioabla et al., 2012; Adekunle & Okolie, 2015). The pH values between 6.12 and 7.71 remained constant in both reactor types (conventional and modified UASB) over the entire OLR range of 1 to 3 gCOD/L/d. Unfortunately, as the influent concentration increased, the pH values of the conventional UASB system utilised for the mono-digestion of LFL exhibited a significant rise, resulting in 8.9 at an operating level of 7 gCOD/L/d. Increasing the influent concentration of LFL can overload the UASB system, exceeding the capacity of acidogenic bacteria to produce acids. As the acidogenesis phase is overwhelmed, acid production declines, and acetogenic and methanogenic processes increase. This shift towards methanogenesis can promote alkalinity, especially if the system cannot supply sufficient acidogenic intermediates, causing a pH rise (Kabir et al., 2022; Alavi-Borazjani et al., 2024). On the contrary, the pH value of the modified UASB system, which integrated LR, decreased significantly and peaked at 4.10 under identical conditions. This outcome may be attributed to the modified UASB system integrated with LR has potentially lacks adequate buffering capacity. As a result, it may be unable to neutralise the acids produced during high OLR, leading to a rapid decline in pH Masebinu et al. (2019). Widyarani et al. (2018) stated that a methanogenic community's metabolism may be significantly inhibited at pH levels below 5. Additionally, elevated pH levels have been

observed to inhibit AD progression potentially. The rapid conversion of ionised ammonia nitrogen to free ammonia nitrogen has been described as this phenomenon (Ceron-Vivas et al., 2019). Therefore, a second phase of continuous investigation took place to examine the stability of AD further.

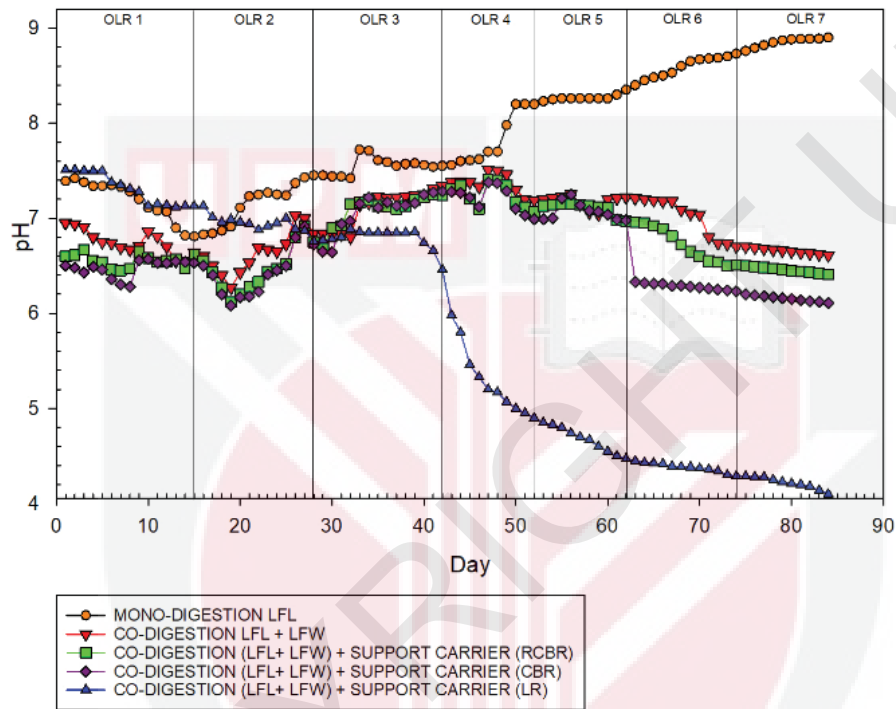


Figure 29: pH values in conventional and modified UASB for semi-continuous system (phase 1)

4.5.4.2 pH Value at different OLRs for ACoD LFL + LFW + activated support carrier

Phase 2 of the semi-continuous study evaluated the function of CBR as a support carrier in facilitating the AD process for the ACoD of LFL and LFW. In this phase, the CBR was chosen due to its superior performance in dealing with the AD process when compared to other support carriers that were applied. The results indicate that activated CBR containing NaOH and $ZnCl_2$ improves pH values ranging from 6.50 to 7.67 at an

OLR of 1 to 8 gCOD/L/d. The pH result indicates the stability of the AD system, as the AD process occurs within a specific pH range. As mentioned in Section 4.4.4.1, the maximum ideal pH for the AD process is 7.2. Nevertheless, Cioabla et al. (2012) stated that the system can still function within the pH range of 6.5 to 8. According to a study conducted by Yacob et al. (2006), a decrease in pH below 6.8 may potentially result in the inhibition of methanogens and the acid phase, thereby preventing any potential countermeasures. Additionally, based on the findings of Morales-Polo et al. (2018), fermentation bacteria demonstrate their most efficient growth in the pH range of 4 to 8.5. In contrast, according to Widyarani et al. (2018), methanogenic microbes exhibit optimal growth and activity within the pH range of 6.7 to 7.8. Based on Figure 30, the pH value for ZnCl₂-modified CBR at the beginning of OLR 7 gCOD/L/d was 7.14, and it increased to 7.63 by the end of OLR 7 gCOD/L/d. For NaOH-modified CBR, the pH value was 7.20 at the beginning of OLR 7 gCOD/L/d, and it rose to 7.67 by the end of OLR 7 gCOD/L/d. Thus, in OLR 7, the methanogenic bacteria thrive at an optimal pH value of around 7, allowing them to easily operate within the UASB system that is integrated with activated support carriers. This configuration results in the maximum levels of biogas production, COD removal, and SMP, as depicted in Figures 24, 26, and 28, respectively when integrating with activated support carrier in AD system. Rajagopal et al. (2013) stated that to reduce the negative effects of free ammonia nitrogen on the AD process, it is advisable to ensure pH levels close to 7. Therefore, this finding improved the ACoD for LFL and LFW compared to semi-continuous study phase 1.

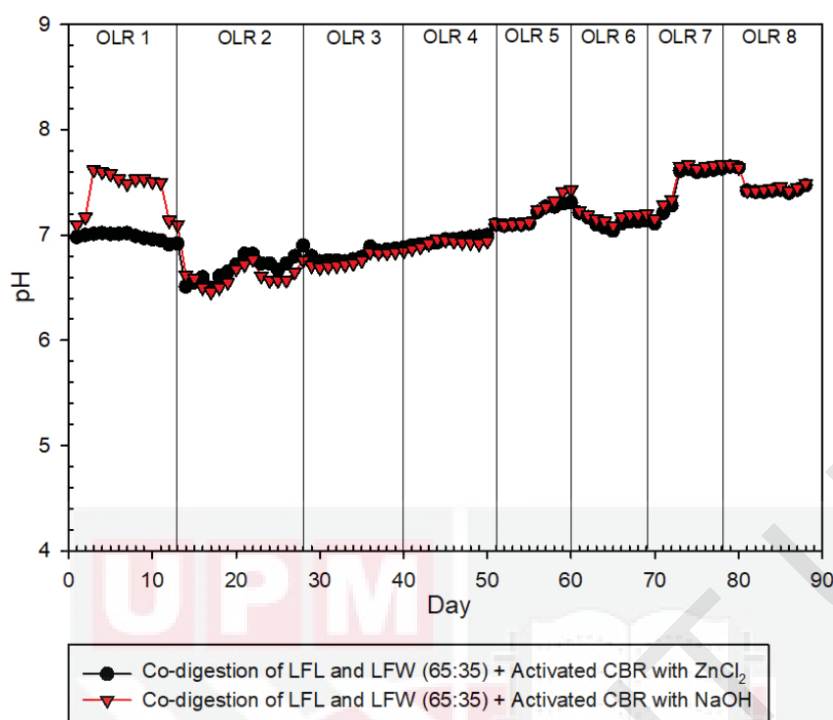


Figure 30: pH values in the semi-continuous system (phase 2) of modified UASB reactors (integrated with the activated support carrier)

4.5.5 Intermediate alkalinity to partial alkalinity (IA/PA) Ratio

This subtopic evaluates the stability of the UASB reactor by measuring the IA/PA value during the continuous studies conducted in both Phase 1 and Phase 2. The results of the IA/PA values are further discussed in Section 4.5.5.1 and Section 4.5.5.2.

4.5.5.1 IA/PA Ratio at different OLRs for AD for LFL and LFW, ACoD of LFL + LFW, and ACoD LFL + LFW + support carrier

The evaluation of AD process stability was performed by utilising the IA/PA ratio as opposed to the VFA/TA ratio, as suggested in previous research (Ferrer et al., 2010; Astals et al., 2012). In addition, the previous studies conducted by Fernández (2001) have investigated the stability of digesters by employing the intermediate-to-total alkalinity ratio (IA/TA). However, it has been indicated that the IA/TA ratio exhibits

a lower level of responsiveness than the IA/PA ratio, making it unsuitable for systems marked by elevated alkalinity levels (Astals et al., 2012). Figure 31 illustrates the IA/PA ratio of both conventional and modified UASB systems while conducting the AD process of LFL, ACoD LFL and LFW, and ACoD LFL and LFW with the inclusion of support carriers. The IA/PA ratio of the modified UASB system, which incorporates a CBR, demonstrates values that fall below the specified threshold of 0.4. This is observed during operation at an OLR that ranges from 1 to 5 gCOD/L/d. The outcome suggests that the working environment is stable and possesses an adequate level of alkalinity, which is significantly lower than the inhibitory threshold. The alkalinity value obtained through conventional UASB (where mono-digestion of LFL and ACoD of LFL and LFW are performed) exceeded 0.4 when the OLR was above 4 gCOD/L/d. This observation suggests that the system was not in a stable state. The study revealed that raising the OLR to 7 gCOD/L/d in conventional UASB reactors increased the IA/PA ratio (>1). The alkalinity levels observed were 1.5 for the mono-digestion of LFL and 1.4 for the ACoD LFL and FW. The elevated IA/PA value represents abnormal activity within the system.

Moreover, integrating a CBR into the UASB system slightly enhances the system's buffering capacity. This improvement is observed at an OLR of 6 and 7 gCOD/L/d, where the buffering capacity reaches a value of 1.1 and 1.18, respectively. This is because, the integration of this support carrier into an UASB system providing a surface for microbial attachment, including for acidogenic and methanogenic bacteria hence can slightly enhance the system's buffering capacity (Ercan & Demirci, 2013 and Raj Deena et al., 2022). This CBR can create microenvironments that foster gradual acid consumption and conversion to CH_4 , thereby stabilising pH fluctuations

within the UASB reactor (Freedman et al., 2017; Moreno Osorio et al., 2021; Al-Amshawee & Yunus, 2021).

In addition, the inhibition observed in both conventional and modified UASB systems can be caused to the accumulation of long-chain fatty acids in the solid biomass and the build-up of VFAs (Marzuki et al., 2021; Astals et al., 2012). Based on the findings of Astals et al. (2012) and Martín-González et al. (2013), it is crucial to maintain the IA/PA ratio of the reactor below 0.4 to avoid the possibility of system instability. When the IA/PA ratio exceeds 0.4, there is an increased probability of encountering unstable conditions. Boe et al. (2010) observed that using alkalinity ratios is a more reliable approach for assessing AD's stability than relying solely on pH values. Moreover, it has been stated by Fuentes et al. (2021) that relying solely on the pH value is insufficient when it comes to interpreting and analysing the stability caused by the buffer capacity of wastewater.

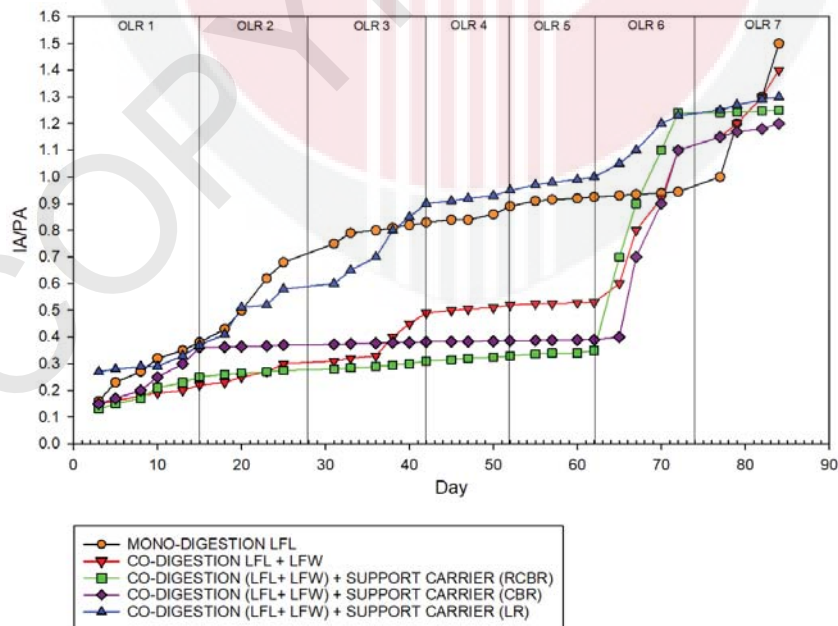


Figure 31: Intermediate alkalinity to partial alkalinity (IA/PA) ratio values in conventional and modified UASB for semi-continuous system (phase 1)

4.5.5.2 IA/PA Ratio at different OLRs for ACoD LFL + LFW + activated support carrier

Further investigation was carried out to determine approaches to ensuring the stability of the AD system throughout the AD process. Based on the research conducted by Marzuki et al. (2021) and Sakar et al. (2009), the IA/PA amount is essential for evaluating how well the system can tolerate variations in pH caused by the release of organic acids. From the results obtained in this study, the stability of the AD system was achieved by incorporating the activated CBR in the UASB system. The CBR was chosen for semi-continuous study phase 2 as it demonstrated superior AD stability in semi-continuous study phase 1 (Figure 31) (1.1 at an OLR of 6 gCOD/L/d) compared to other types of support carriers used (RCBR = 1.25, LR = 1.3 at an OLR of 6 gCOD/L/d). According to the findings shown in Figure 32, the use of activated CBR with NaOH impregnation resulted in an enhancement of IA/PA values, reaching 0.25 at an OLR of 7 gCOD/L/d and 0.39 at an OLR of 8 gCOD/L/d. Meanwhile, the IA/PA for the system utilising ZnCl₂ impregnation for activating CBR exhibited a value of 0.31 and 0.49 at an OLR of 7 and 8 gCOD/L/d, respectively, which is slightly higher than the system utilising NaOH-modified CBR. Thus, it could be concluded that the system that incorporates CBR activation via the NaOH approach has effectively attained stability in the AD process, as it falls below the threshold value of 0.4. Thus, it is observable that a stable AD system yields superior results when incorporated with the NaOH-modified CBR in terms of biogas production (Figure 24), COD elimination (Figure 26), and SMP value (Figure 28).

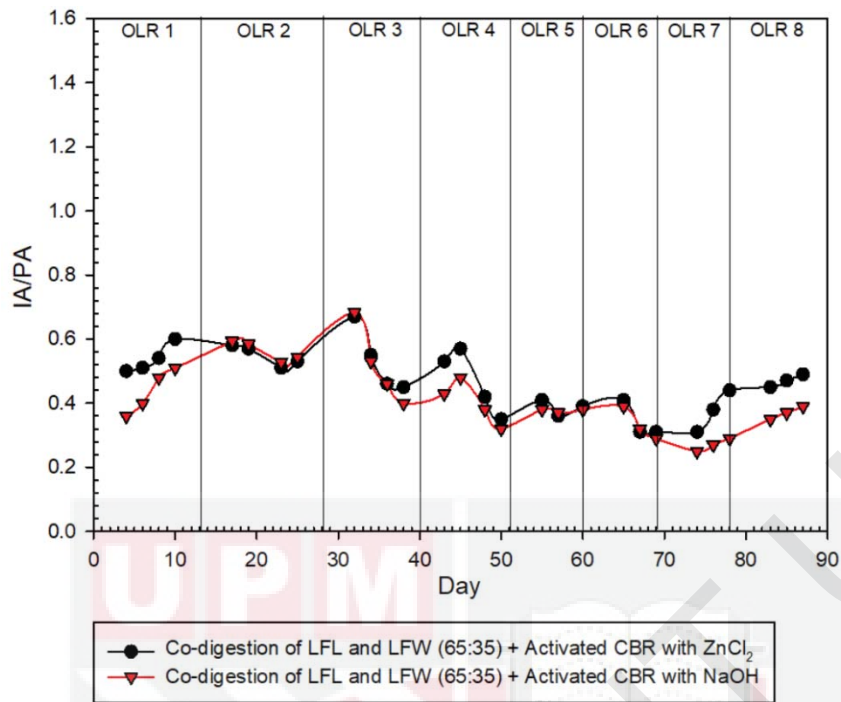


Figure 32: Intermediate alkalinity to partial alkalinity (IA/PA) ratio values in the semi-continuous system (phase 2) of modified UASB reactors (integrated with the activated support carrier)

4.5.6 Total Ammonia Nitrogen (TAN)

The TAN concentration was assessed during both Phase 1 and Phase 2 of the continuous studies. Section 4.5.6.1 presents the analysis of TAN concentration during Phase 1, where OLRs ranged from 1 to 7 gCOD/L/d. A more detailed evaluation of TAN concentration under OLRs ranging from 1 to 8 gCOD/L/d, using a UASB system integrated with activated CBR, is provided in Section 4.5.6.2.

4.5.6.1 TAN at different OLRs for AD for LFL, ACoD of LFL + LFW, and ACoD LFL + LFW + support carrier

The TAN concentration profiles of conventional UASB and modified UASB systems at various OLRs, running from 1 to 7 gCOD/L/d, are illustrated in Figure 33. Previous research has established that protein hydrolysis in AD generally produces a significant

quantity of TAN (Marzuki et al., 2021; Labatut et al., 2014). The results displayed in Figure 33 indicate that the concentration of TAN in the conventional UASB reactor during the mono-digestion of LFL was 120 mg/L, 190 mg/L, and 880 mg/L, respectively, at OLRs of 1, 2, and 3 gCOD/L/d. However, as the OLR was increased to 7 gCOD/L/d, the TAN concentration was 600 mg/L. In contrast, the observed concentrations of TAN during co-digestion of LFL and LFW in conventional UASB were 140, 230, and 301 mg/L, respectively, at OLRs of 1, 2, and 3 gCOD/L/d. The TAN concentrations exhibited constant values within the range of 4 to 6 gCOD/L/d OLRs but declined to 190 mg/L at 7 gCOD/L/d OLR. The highest TAN concentrations for ACoD of LFL and LFW were 728 mg/L and 512 mg/L at an OLR of 6 gCOD/L/d, respectively, when integrated with a support carrier consisting of RCBR and CBR. The TAN value peaked at 6 gCOD/L/d for the modified UASB system (integrated with the support carrier). In the case of LFL mono-digestion, it has been discovered that sudden increased concentrations of TAN can impede the process of methanogenesis. This is because an excessive concentration of TAN may induce the release of ammonia from proteins (Musa & Idrus, 2020). The subsequent increase in alkalinity caused by this ammonium discharge makes the AD system unstable. A greater TAN concentration during single digestion of LFL reflects that a substantial quantity of microbial biomass rapidly degrades the substrate. Conversely, a lower TAN value at elevated OLR shows that protein degradation is not taking place due to inadequate quantities of acetogens and methanogens (Jaman et al., 2023; Musa & Idrus, 2020).

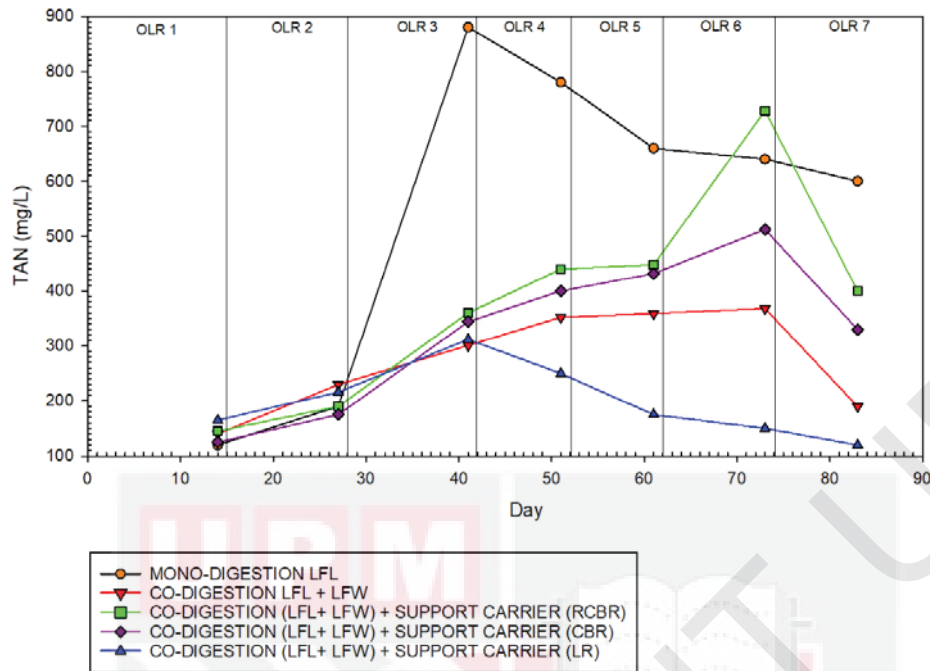


Figure 33: Total Ammonia Nitrogen (TAN) in conventional and modified UASB for semi-continuous system (phase 1)

4.5.6.2 TAN at different OLRs for ACoD LFL + LFW + activated support carrier

In order to improve the methanogenesis process in the ACoD of LFL and LFW, a further investigation was carried out. This involved using physico-chemical activation methods, specifically ZnCl_2 and NaOH activation of the support carrier, during the semi-continuous study phase 2. Figure 34 demonstrates the TAN values of different systems. The UASB system that was integrated with a CBR support carrier activated with NaOH showed the highest TAN value of 580 mg/L at an OLR of 7 gCOD/L/d. This value was higher than the TAN value obtained in the system integrated with a CBR activated with ZnCl_2 (535 mg/L) and the unactivated CBR system in phase 1 (512 mg/L). Xiao et al. (2021) found that the inhibitory levels of TAN fall within the range of 1500 to 3000 mg/L. Therefore, all reactors that performed continuous studies in phases 1 and 2 are within the acceptable limit. The UASB reactor that uses the NaOH activation method for the CBR support carrier has greater performance in comparison

to the UASB reactor that employs ZnCl_2 activation of the support carrier. Lee et al. (2016), Yang et al. (2017), and Johnravindar et al. (2020) have found that the addition of activated material can greatly enhance CH_4 production. The use of activated material creates an environment that enhances microbe growth and increasing the CH_4 production rate. This can be observed through surface morphology examination using SEM. The SEM analysis will be further explained in Section 4.5. Therefore, the adoption of NaOH-modified CBR would increase CH_4 generation in the ACoD of LFL and LFW since it resulted in the greatest TAN value compared to ZnCl_2 -modified CBR.

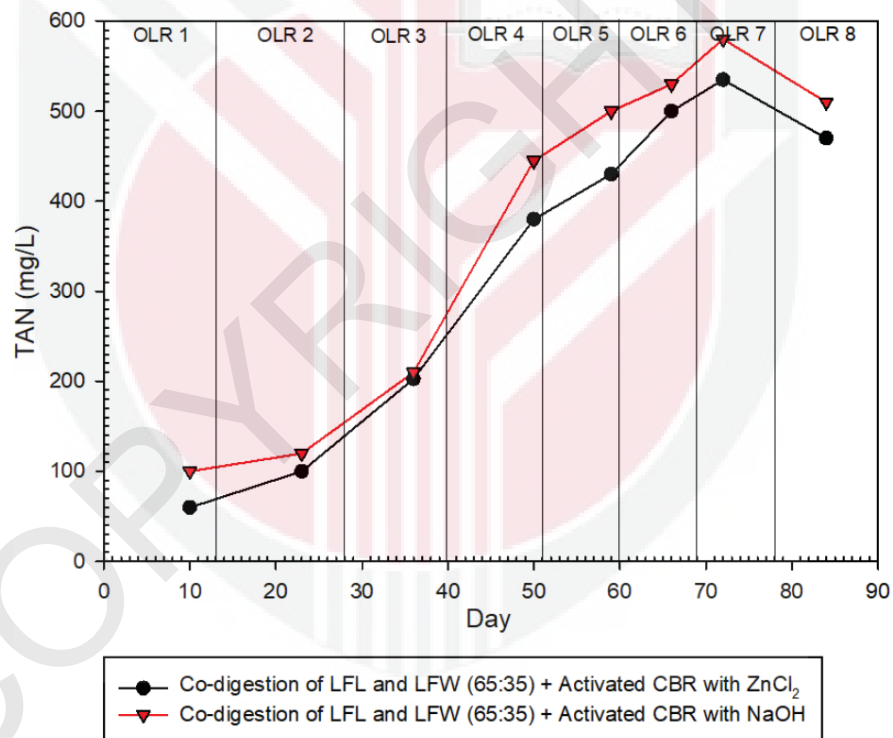


Figure 34: Total Ammonia Nitrogen (TAN) in the semi-continuous system (phase 2) of modified UASB reactors (integrated with the activated support carrier)

4.5.7 Summary of conventional and modified UASB reactor in a semi-continuous study

The summary of the conventional and modified UASB reactors during the semi-continuous study phases 1 and 2 is presented in Table 29 below. A total of eight parameters were assessed to evaluate the performance of each reactor. These parameters include operating ratio, type of support carrier used, biogas production, COD removal, SMP, pH, IA/PA, and TAN.

Table 29: Summary of conventional and modified UASB reactor in a semi-continuous study phase 1 and 2

Operational condition	Operating ratio	Types of support carrier integration	OLR (g/L/Day)	Biogas production (mL)	COD removal (%)	SMP (mL CH ₄ /g COD added)	pH	IA/PA	TAN (mg/L)
Semi-continuous study phase 1									
Mono-digestion of LFL	100% LFL	-	3	620	48	94.60	7.55	0.80	880
Co-digestion of LFL and LFW	65% LFL + 35% FW	-	6	1072	70	99.40	6.70	1.10	368
Co-digestion of LFL and LFW	65% LFL + 35% LFW + RCBR	RCBR	6	2100	83	268.92	6.51	1.25	728
Co-digestion of LFL and LFW	65% LFL + 35% LFW + CBR	CBR	6	2150	84	293.30	6.23	1.10	512
Co-digestion of LFL and LFW	65% LFL + 35% LFW + LR	LR	3	820	77	172.22	6.46	0.90	312
Semi-continuous study phase 2									
Co-digestion of LFL and LFW	65% LFL + 35% LFW + CBR	ZnCl ₂ - modified CBR	7	2230	86.24	305.00	7.60	0.31	535
Co-digestion of LFL and LFW	65% LFL + 35% LFW + CBR	NaOH-modified CBR	7	3275	89.50	459.95	7.63	0.25	580

4.6 Surface morphologies of the activated support carrier

To improve the AD performance of LFL and LFW, the surface modification technique carried out in this study primarily modifies the structure of CBR, including specific surface area, pore volume, and pore size. The SEM images of the modified CBR implementing two distinct activation methods, namely, ZnCl_2 and NaOH , are depicted in Figure 35. The SEM images of the activated CBR were examined at increasing levels of magnification (100x, 500x, and 1000x), as illustrated in Figures 35 (a, b, c, d, e, and f). The SEM analysis of the morphological structures indicated that CBR activated with NaOH formed a rough surface, as demonstrated in Figure 35 (a, c, and e). The observed phenomenon can be attributed to the intercalation effect of NaOH interaction with the CBR structure. During thermal treatment, Na atoms react with the CBR structure and penetrate the innermost layer of the activated CBR (Liew et al., 2018). This would lead to the formation of a rugged surface, which is indicative of a well-developed porous structure. Meanwhile, the 100x (b), 500x (d), and 1000x (f) magnification images reveal that the CBR activated with ZnCl_2 had an undesirable surface for microbe attachment due to its amorphous and moderately smooth surface. In addition, the result clearly shows that the activated CBR with NaOH exhibits a rough surface as opposed to the activated CBR treated with ZnCl_2 . This rough surface provides an optimal environment for microbial attachment (Al-Amshawee et al., 2020). Therefore, when the support carrier offers an ideal surface for microbial attachment, biofilm formation occurs, which can assist microbial retention and enable the support carrier to decompose organic compounds in effluent while enhancing biogas production efficiently. Hence, the CBR that has been activated with NaOH can potentially to boost the AD process.

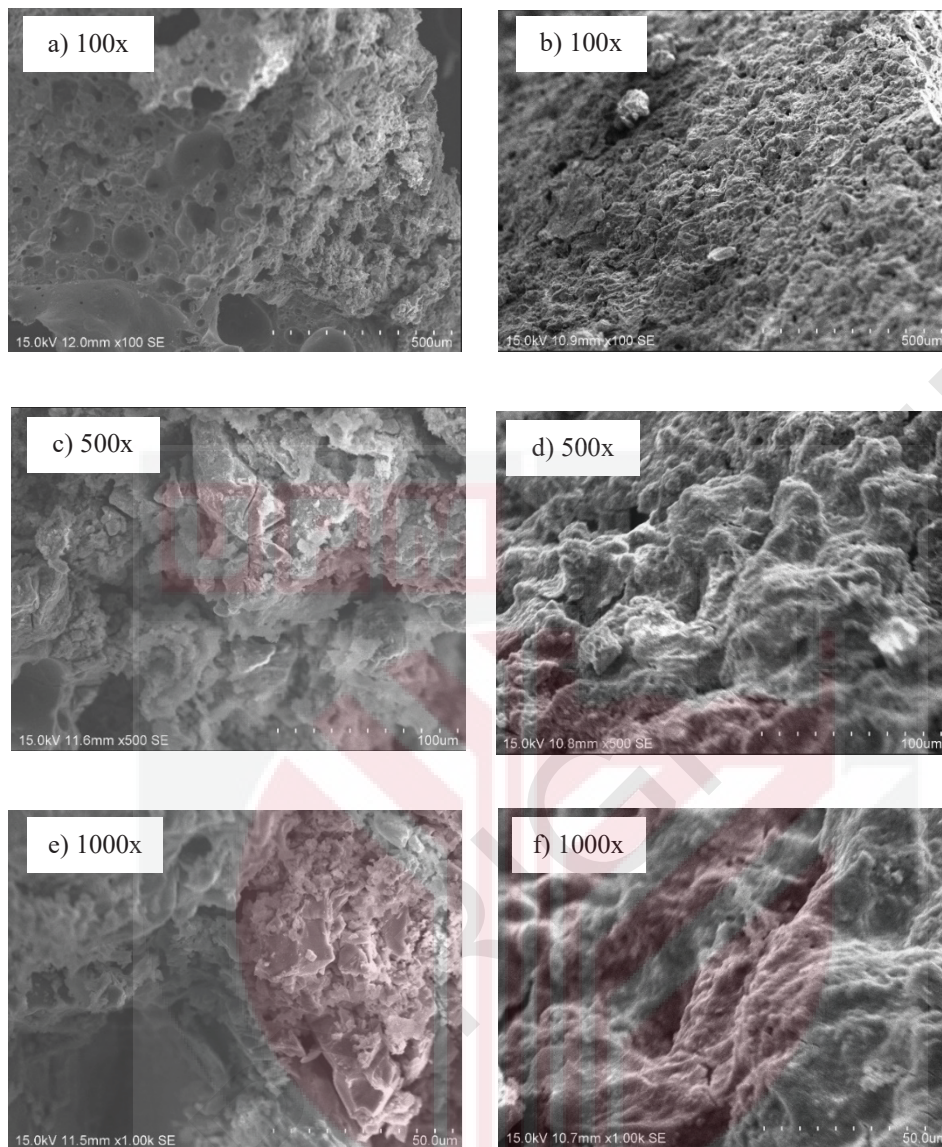


Figure 35: SEM micrographs of activated CBR: (a) activated CBR with NaOH (magnification= 100x), (b) activated CBR with ZnCl₂ (magnification= 100x), (c) activated CBR with NaOH (magnification= 500x), (d) activated CBR with ZnCl₂ (magnification= 500x), (e) activated CBR with NaOH (magnification= 1000x), (f) activated CBR with ZnCl₂ (magnification= 1000x)

4.7 Machine Learning (ML) application

4.7.1 ANN analysis of biogas production from BMP Tests 1, 2 and 3

The ANNs model was developed with the objective of forecasting biogas output in BMP tests. The R^2 values generated from the MATLAB software employing the ANN model were 0.659 for ANN 1 and 0.884 for ANN 2, as depicted in Figures 36 and 37, respectively. Both ANN 1 and 2 models use 25 neurons for both the first and second hidden layers. The ANN 1 model was generated by incorporating the tan-sigmoidal equation for the first hidden layer and the log-sigmoidal equation for the subsequent hidden layer. In the case of ANN 2 model, the combination of a log-sigmoidal and a tan-sigmoidal equation was applied. The biogas output performance analysis of BMP Tests 1, 2, and 3 using the ANN 2 model was selected, as it demonstrates higher accuracy in biogas production (0.884). These results are presented in Figures 38, 39, and 40, respectively.

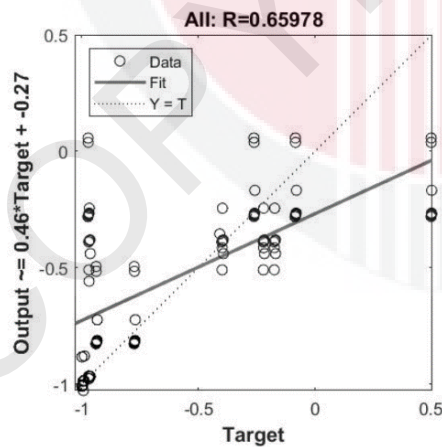


Figure 36: R^2 value for ANN 1 (0.659)

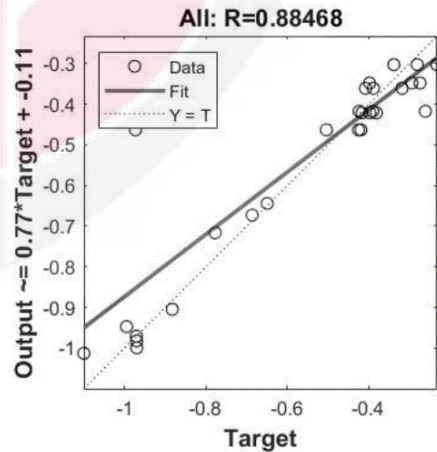


Figure 37: R^2 value for ANN 2 (0.884)

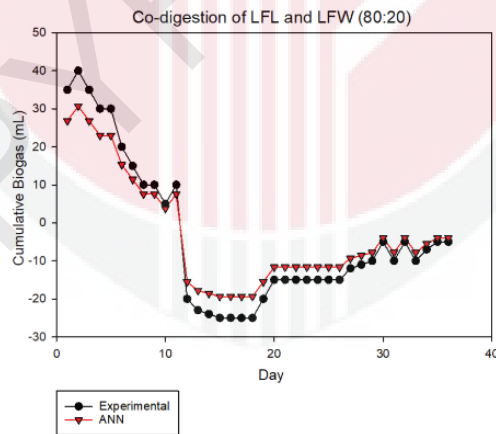
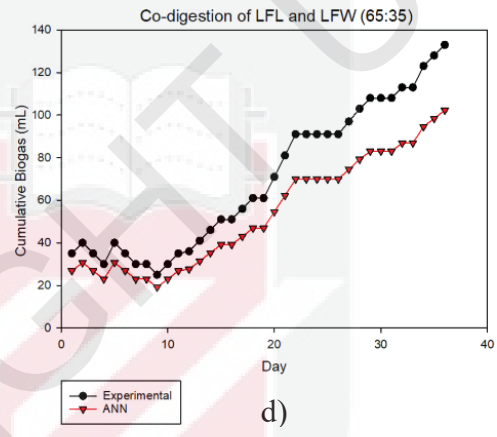
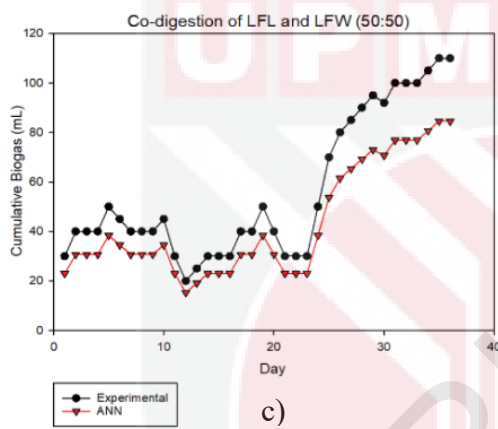
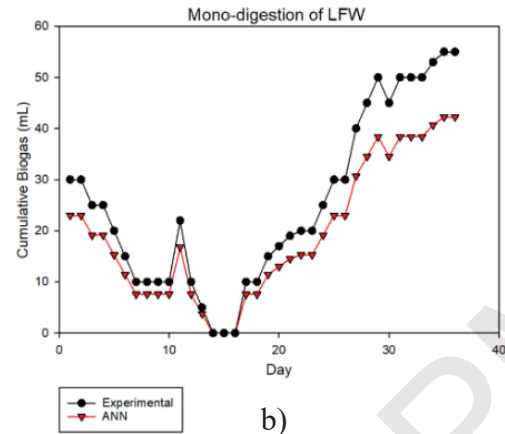
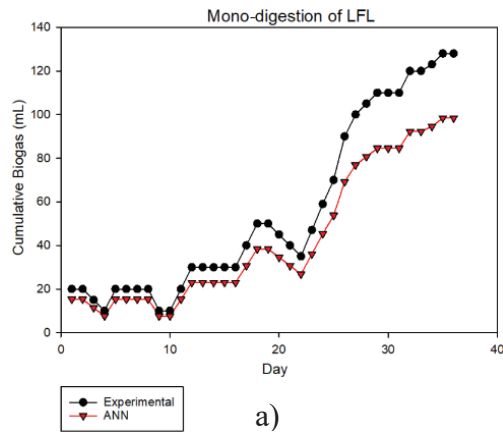


Figure 38: Comparison of the ANN model and measured biogas from BMP Test 1 for AD LFL and LFW, and ACoD LFL + LFW with different ratios: (a) Mono-digestion LFL; (b) Mono-digestion LFW; (c) LFL + LFW (50:50); (d) LFL + LFW (65:35); (e) LFL + LFW (80:20)

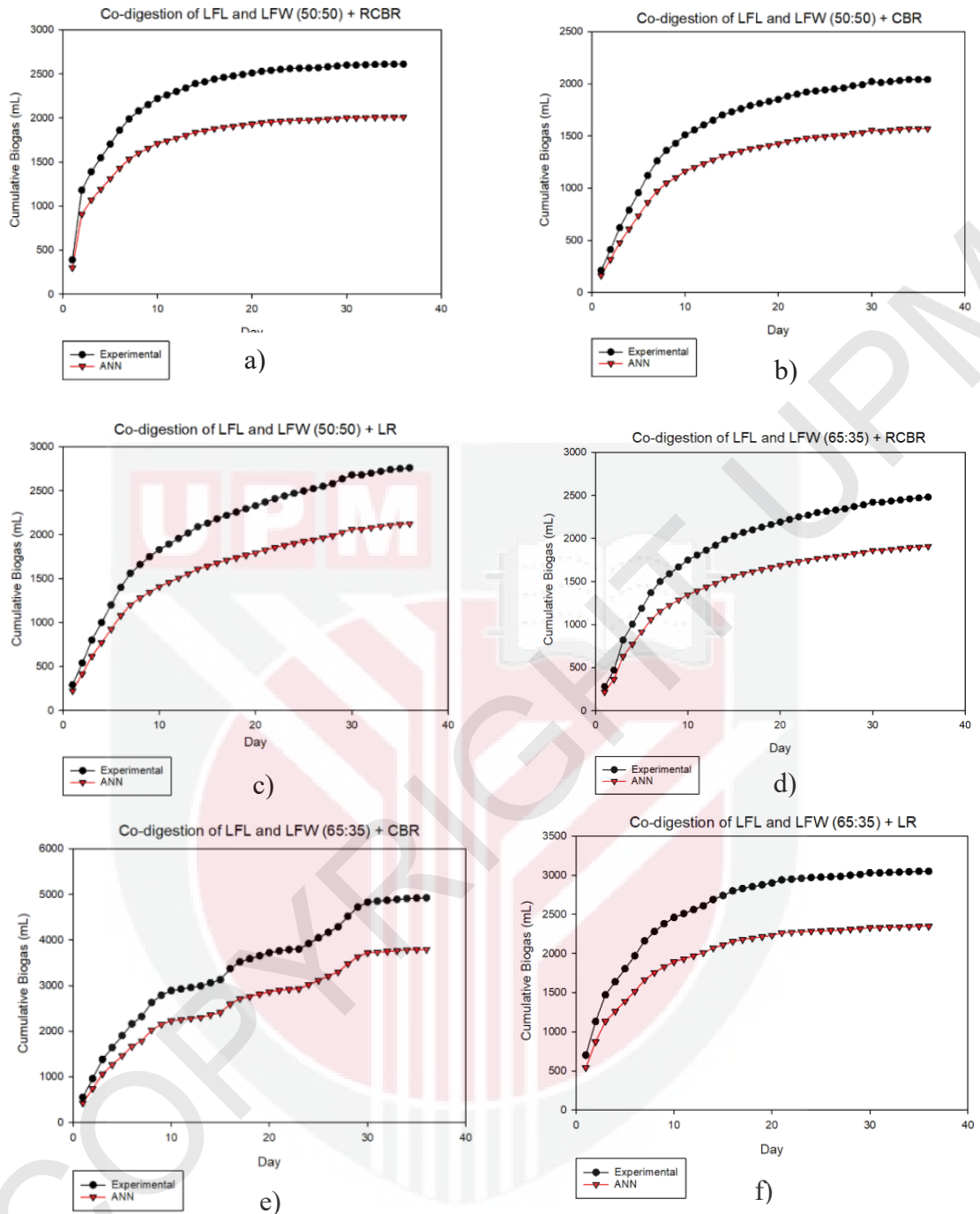


Figure 39: Comparison of the ANN model and measured biogas from BMP Test 2 for AcoD LFL + LFW (50:50) and (65:35) with different support carrier integration: (a) LFL + LFW (50:50) + RCBR; (b) LFL + LFW (50:50) + CBR; (c) LFL + LFW (50:50) + LR; (d) LFL + LFW (65:35) + RCBR; (e) LFL + LFW (65:35) + CBR; (f) LFL + LFW (65:35) + LR

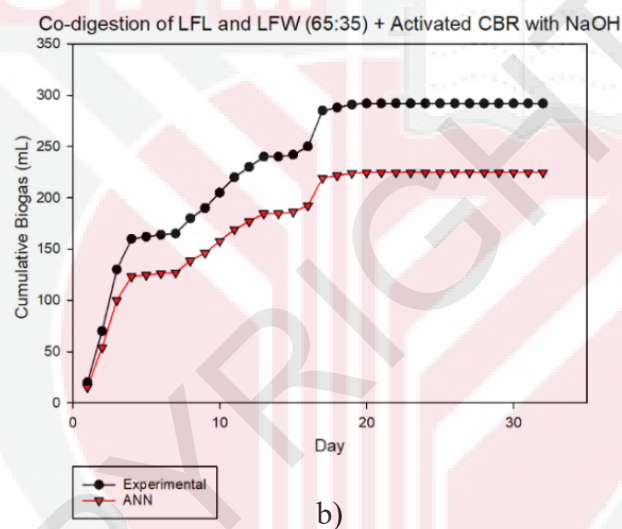
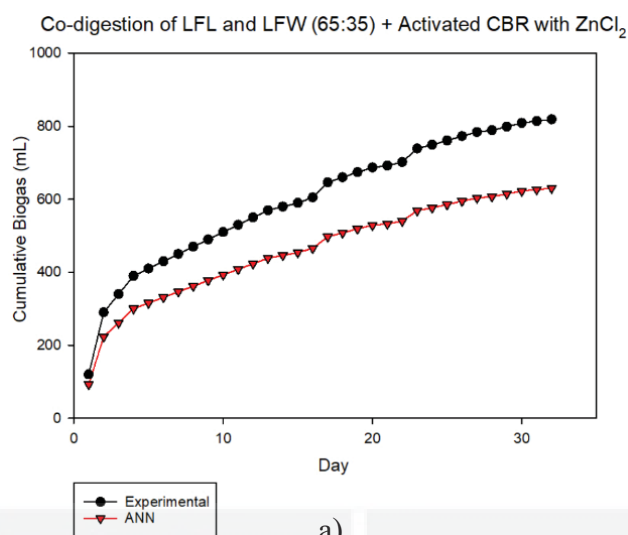


Figure 40: Comparison of the ANN model and measured biogas from BMP Test 3 for AcoD LFL + LFW (65:35) with different types of support carrier activation: (a) LFL + LFW (65:35) + activated CBR with ZnCl_2 ; (b) LFL + LFW (65:35) + activated CBR with NaOH

4.7.2 SVM analysis of biogas production from BMP Tests 1, 2 and 3

The SVM 1 model employed a linear function to estimate the generation of biogas in BMP tests 1, 2, and 3. The R^2 value obtained from the SVM 1 model (0.930) was slightly greater than the R^2 value obtained from the SVM 2 model (0.929) and therefore was chosen to predict the biogas generation as illustrated in Figures 41 and 42. The SVM 2 model was developed with a polynomial function. The findings of this study indicate that the SVM model exhibits higher accuracy in the performance analysis of biogas output compared to the ANN models, as explained in the previous section (Section 4.7.1), which is aligned with the findings made by Alejo et al. (2018) and Guo et al. (2015). Figures 43, 44, and 45 demonstrate the biogas production in correlation with its actual and projected values for BMP Tests 1, 2, and 3, as observed by the implementation of the SVM 1 model. As the R^2 generated by the SVM model 1 was close to 1, the results indicate a strong relationship between the predicted biogas output and the measured data.

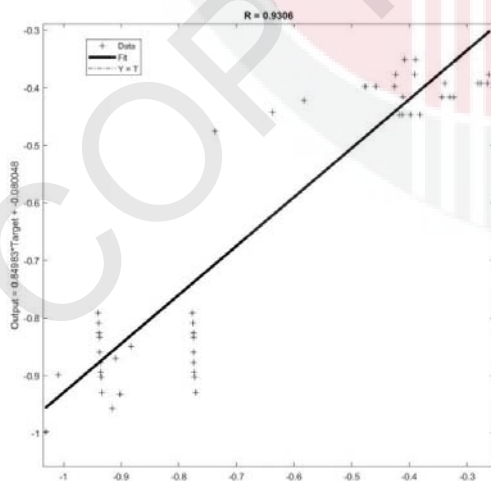


Figure 41: R^2 value for SVM 1 (0.930)

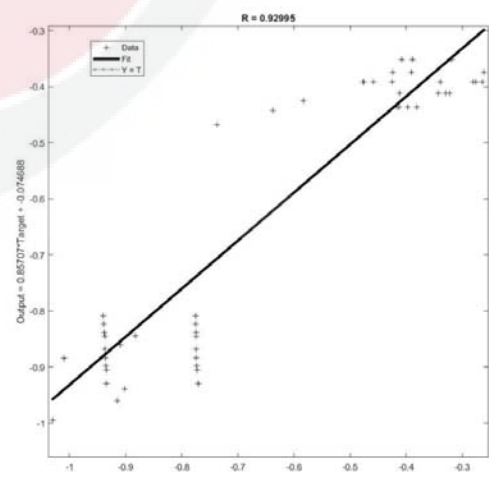


Figure 42: R^2 value for SVM 2 (0.929)

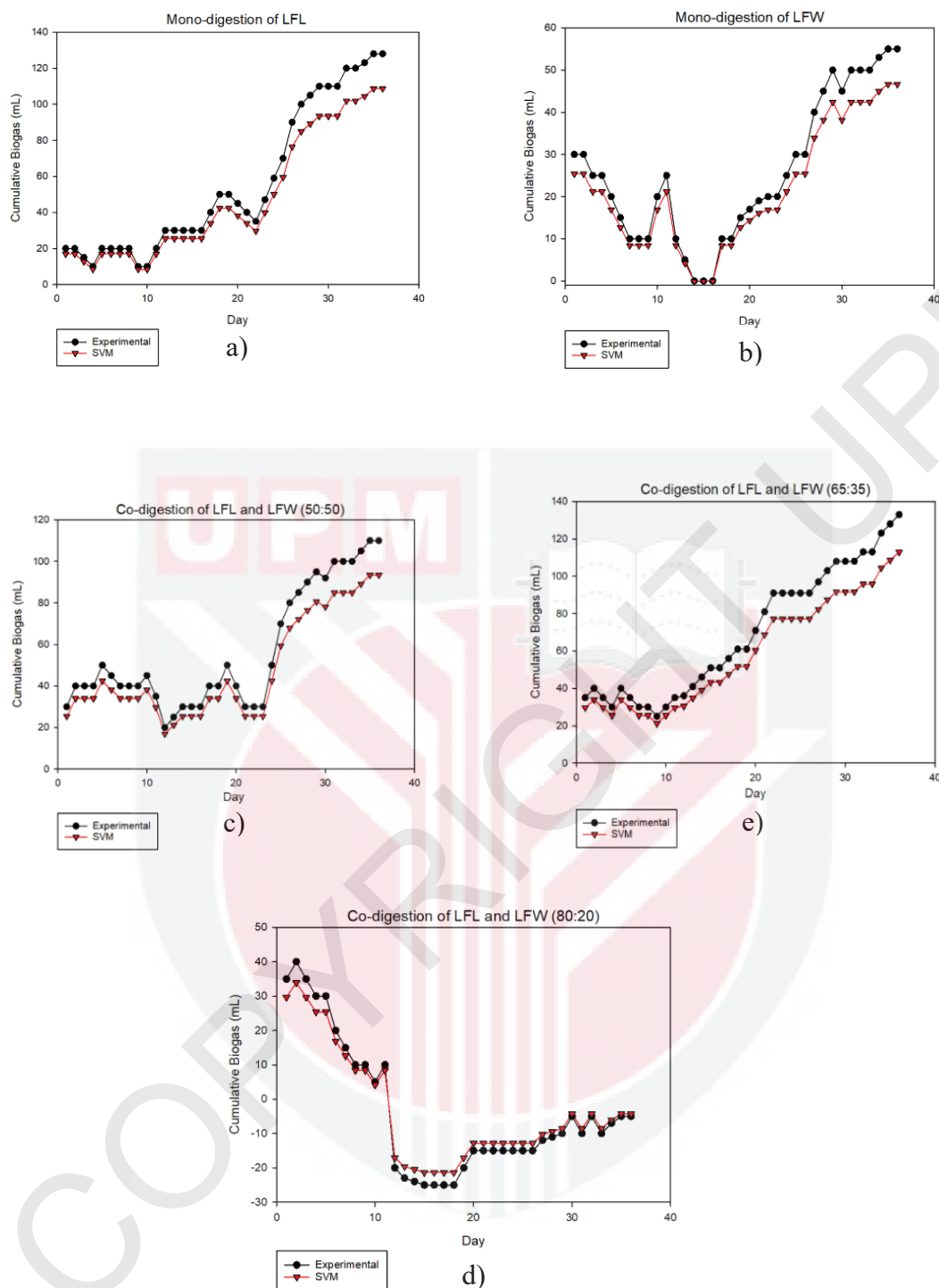


Figure 43: Comparison of the SVM model and measured biogas from BMP Test 1 for AD LFL and LFW, and ACoD LFL + LFW with different ratios: (a) Mono-digestion LFL; (b) Mono-digestion LFW; (c) LFL + LFW (50:50); (d) LFL + LFW (65:35); (e) LFL + LFW (80:20)

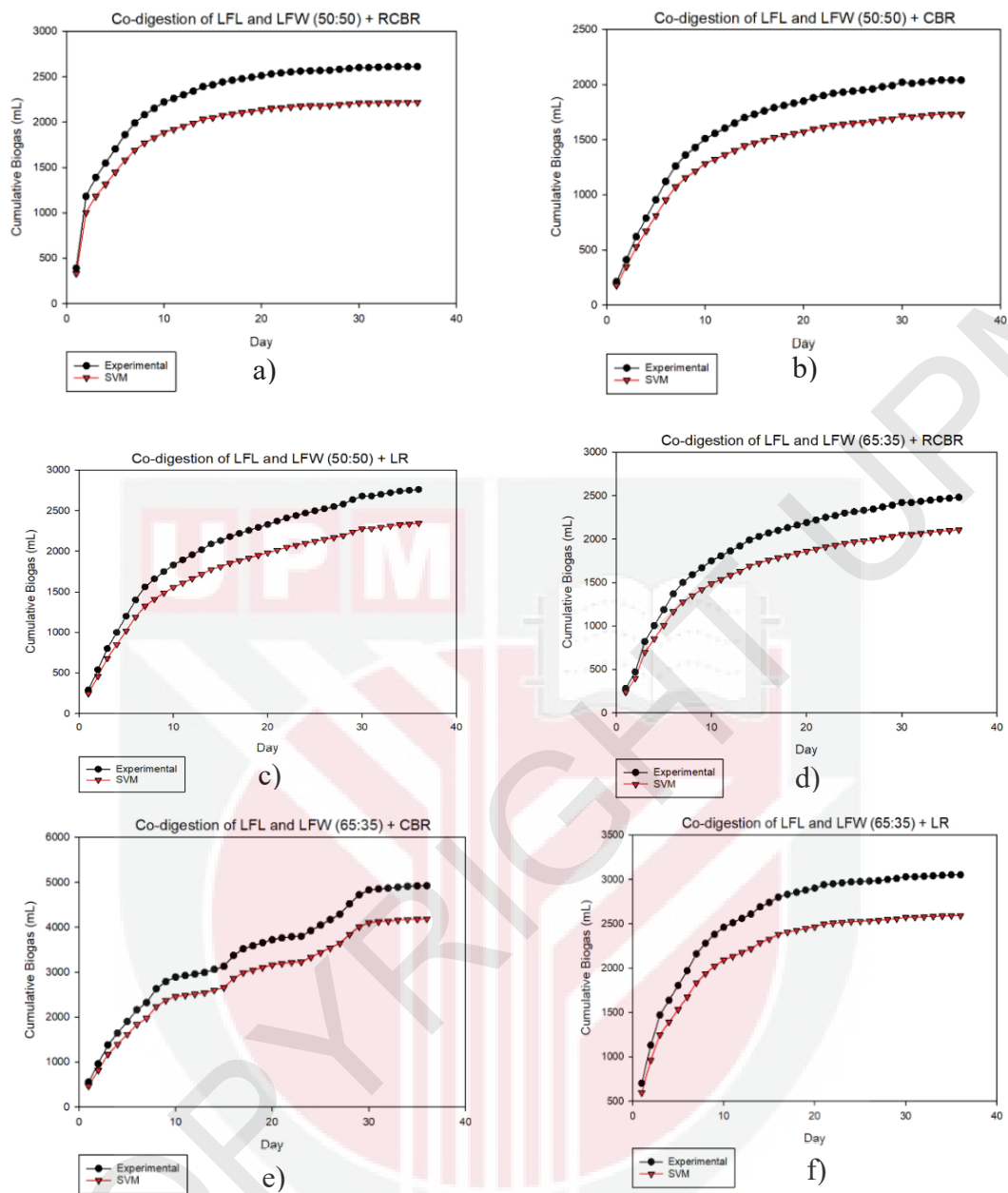


Figure 44: Comparison of the SVM model and measured biogas from BMP Test 2 for ACoD LFL + LFW (50:50) and (65:35) with different support carrier integration: (a) LFL + LFW (50:50) + RCBR; (b) LFL + LFW (50:50) + CBR; (c) LFL + LFW (50:50) + LR; (d) LFL + LFW (65:35) + RCBR; (e) LFL + LFW (65:35) + CBR; (f) LFL + LFW (65:35) + LR

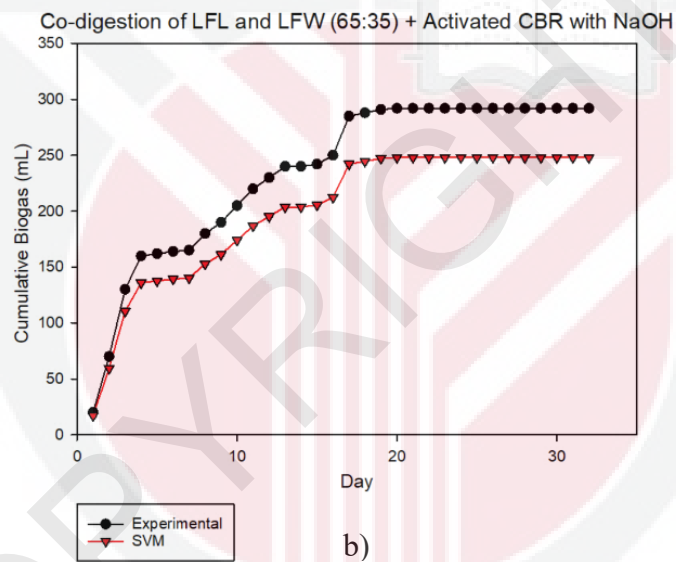
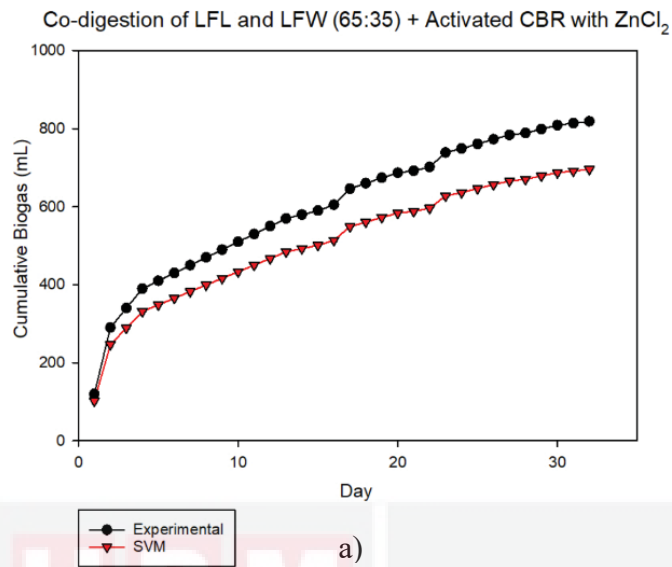


Figure 45: Comparison of the SVM model and measured biogas from BMP Test 3 for ACoD LFL + LFW (65:35) with different types of support carrier activation: (a) LFL + LFW (65:35) + CBR activated with ZnCl_2 ; (b) LFL + LFW (65:35) + CBR activated with NaOH

CHAPTER 5

CONCLUSION AND RECOMMENDATION

AD is a widely recognised technique that can potentially serve as an alternative approach to wastewater treatment. It also offers the benefits of reducing odours, minimising greenhouse gas emissions, and generating renewable energy. This study involved batch and continuous studies to further evaluate the performance of ACoD LFL and LFW using conventional UASB reactors and modified UASB reactors integrated with the support carriers. All the objectives of the study have been successfully achieved. This chapter will provide a clear summary of the findings obtained through this study and recommendations for further research.

5.1 Conclusions

1. Three batch studies were conducted to investigate the performance of mono-digestion of LFL and LFW and ACoD of LFL and LFW using three different ratios (batch study 1), to evaluate the performance of ACoD LFL and LFW using the best ratios with the integration of three types of support carriers (batch study 2), and to compare the performance of ACoD of LFL and LFW using the best ratio by integrating the two types of activated support carriers, which consist of ZnCl_2 activation and NaOH activation (batch study 3). The results revealed that ACoD of LFL and LFW with activated CBR as a support carrier is the most stable and performs best compared to mono-digestion of LFL. The first batch of the study demonstrated that the ACoD of LFL and LFW, with a ratio of 65:35 and 50:50, performed outstandingly in biogas production of 204 mL and 203 mL, respectively. These two best ratios were thus chosen for

additional evaluation in BMP Test 2. The ACoD of LFL and LFW with a ratio of 65:35 that integrated with CBR produced 4,925 mL of biogas, whereas the ACoD of LFL and LFW with a ratio of 50:50 that integrated with CBR only produced 2040 mL. Next, BMP Test 3 revealed that the ACoD of LFL and LFW integrated with ZnCl_2 -activated CBR generated the highest biogas production (819 mL), which was greater than the UASB system that integrated with NaOH-activated CBR, which only produced biogas of 292 mL.

2. Two phases of continuous studies were conducted to analyse the performance of conventional and modified UASB reactors. The obtained result indicates that the modified UASB system, with a ratio of 65:35 of LFL and LFW and the integration of CBR as a support carrier, achieved good AD performance compared to the conventional UASB reactor. The system produced 2150 mL of biogas, had an SMP of 293.30 mL $\text{CH}_4/\text{gCOD}_{\text{added}}$, and achieved a COD removal efficiency of 84% at an OLR of 6 gCOD/L/day. However, the reactor seems unstable since the IA/PA ratio is higher than the threshold value (>0.4), which achieved 1.1 at OLR 6 gCOD/L/day. Thus, a second semi-continuous study is needed to further evaluate the role of support carriers in the stability of the AD system. In the second phase of the semi-continuous study, it was found that the modified UASB system that was integrated with activated CBR exhibited outstanding stability and efficiency in contrast to the first phase of the semi-continuous study. The modified UASB system in the semi-continuous study phase 2, consisting of ACoD of LFL and LFW integrated with NaOH-activated CBR, demonstrates great performance. It achieved the highest biogas production of 3275 mL, SMP of 459.95 mL $\text{CH}_4/\text{gCOD}_{\text{added}}$, and COD removal

of 89.5% with an IA/PA of 0.25 at an OLR of 7 gCOD/L/day. This finding is opposed to the outcome of the BMP test 3. This is because the NaOH-activated CBR shows a great physical characteristic compared to the ZnCl₂-activated CBR, which is a more suitable support carrier to be used while conducting a semi-continuous study of the ACoD of LFL and LFW.

3. An evaluation of the performance analysis of two ML applications (ANN and SVM) was conducted to assess biogas potential. SVM exhibits superior accuracy in comparison to ANN. Particularly, SVM model 1, which employed a linear function, yielded a higher R² value (0.930) than ANN model 2, which used the log-sigmoidal equation for the initial hidden layer and the tan-sigmoidal equation for the subsequent hidden layer (0.884).

5.2 Recommendations

The outcomes of this research suggest that the optimisation of biogas generation from LFL and LFW can be achieved by integrating an activated CBR support carrier. This integration promotes the development of microbial populations by providing an ideal surface area, pore volume, and pore size for the microbes. However, this research study does have certain limitations and restraints. There are some recommendations stated below aimed at improving the current study in consideration of upcoming research endeavours:

1. Perform further research on the performance of a modified UASB reactor using the same wastewater samples that integrate with different proportions of support carriers.
2. Further research should be conducted on similar wastewater samples employing the combination of various types of support carriers in the UASB system to evaluate the effectiveness as well as stability of the ACoD of LFL and LFW.
3. Additional investigation can be conducted to assess the effectiveness of the AD process and energy recovery by using thermophilic conditions and different HRT.
4. A further study might be carried out to explore the optimisation and scale-up analysis of the modified UASB reactor for the treatment of ACoD of LFL with LFW at a commercial scale.

5. Post-treatment of the effluent is necessary to comply with the guidelines for wastewater effluent discharge standard.
6. It is imperative to implement other types of ML techniques that not only forecast biogas production but are also able to predict the generation of other substances or operational variables involving COD removal, SMP, TAN, alkalinity, and pH value.



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APPENDICES

Appendix A

SAMPLES



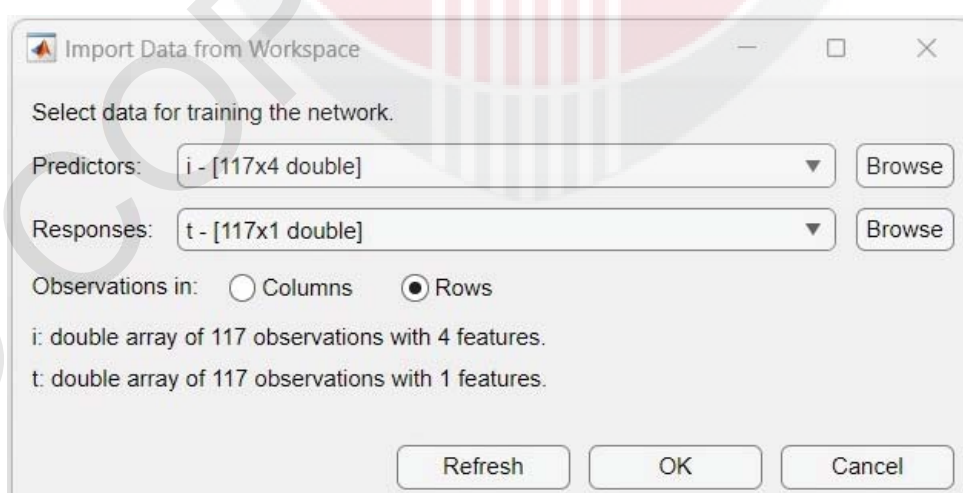
Landfill leachate



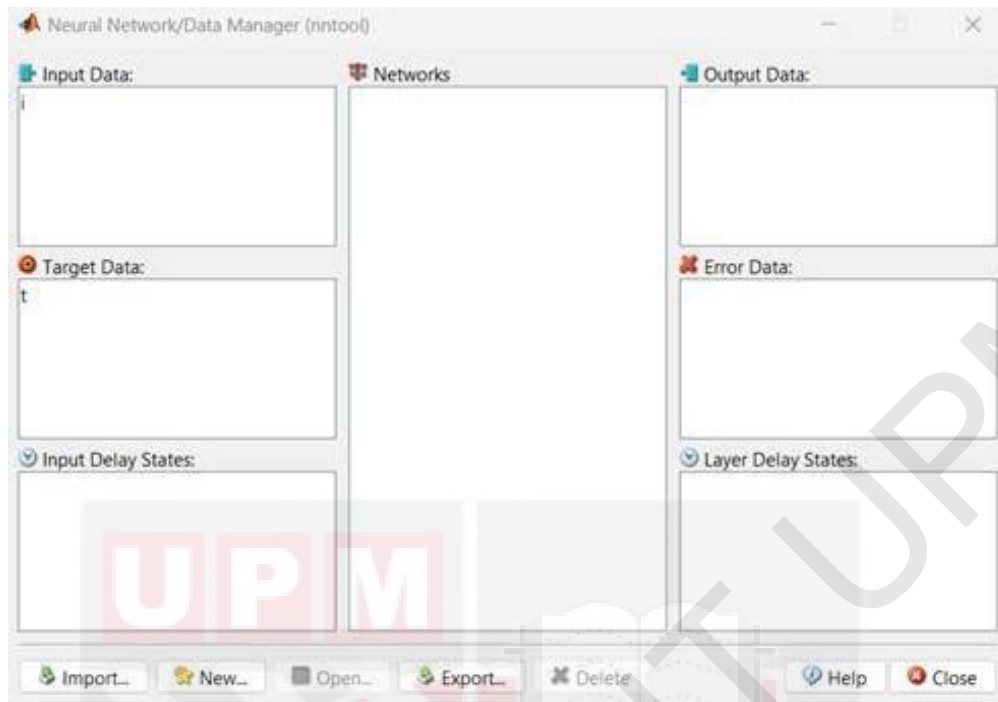
Liquidised food waste

Appendix B

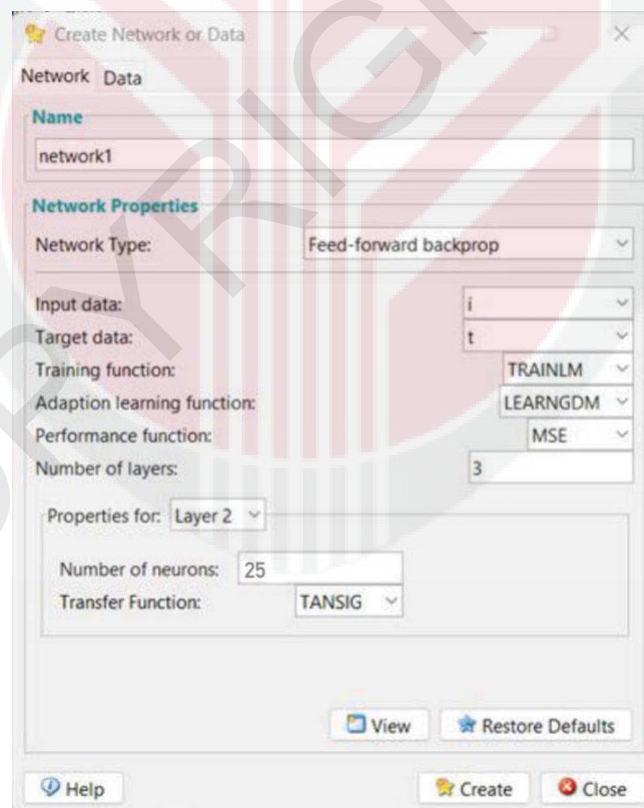
ARTIFICIAL NEURAL NETWORK



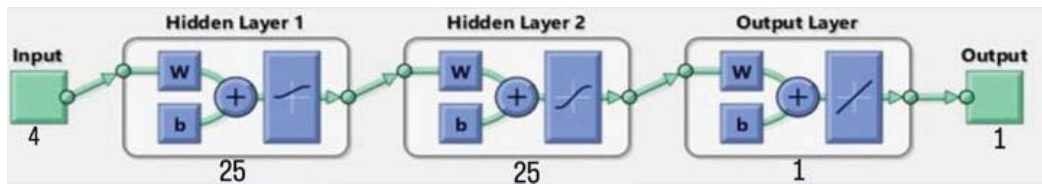
Data input for training ANN network



Data manager- nntool of ANN



Network configuration of ANN



Visualisation of ANN

Training Results

Training finished: Met validation criterion ✓

Training Progress

Unit	Initial Value	Stopped Value	Target Value
Epoch	0	17	1000
Elapsed Time	-	00:00:00	-
Performance	1.87	0.12	0
Gradient	5.3	0.00131	1e-07
Mu	0.001	0.0001	1e+10
Validation Checks	0	6	6

ANN network training

Appendix C

SUPPORT VECTOR MACHINES

```

svm5.m  x  +
1 function [trainedModel, validationRMSE] = svm5(trainingData)
2 % [trainedModel, validationRMSE] = trainRegressionModel(trainingData)
3 % Returns a trained regression model and its RMSE. This code recreates the
4 % model trained in Regression Learner app. Use the generated code to
5 % automate training the same model with new data, or to learn how to
6 % programmatically train models.
7 %
8 % Input:
9 %   trainingData: A matrix with the same number of columns and data type
10 %   as the matrix imported into the app.
11 %
12 % Output:
13 %   trainedModel: A struct containing the trained regression model. The
14 %   struct contains various fields with information about the trained
15 %   model.
16 %   trainedModel.predictFcn: A function to make predictions on new data.
17 %
18 %   validationRMSE: A double containing the RMSE. In the app, the Models
19 %   pane displays the RMSE for each model.
20 %
21 %

```

Command Window

New to MATLAB? See resources for [Getting Started](#).

Starting parallel pool (parpool) using the 'threads' profile ...
Connected to parallel pool with 4 workers.

f >>

Ready Zoom: 100% UTF-8

Matlab tools used for SVM model

New Session from Workspace

Data set

Data Set Variable

svm5 117x6 double

☒ Use columns as variables
☐ Use rows as variables

Response

☒ From data set variable
☐ From workspace

column_6 double -1.0301...

Predictors

	Name	Type	Range
☒	column_1	double	-1.00062 .. 0.500004
☒	column_2	double	-1 .. 0.5
☒	column_3	double	-1 .. 0.5
☒	column_4	double	-0.999839 .. 1.03779

[How to prepare data](#)

Validation

Validation Scheme

Cross-Validation

Protects against overfitting. For data not set aside for testing, the app partitions the data into folds and estimates the accuracy on each fold.

Cross-validation folds 5

[Read about validation](#)

Test

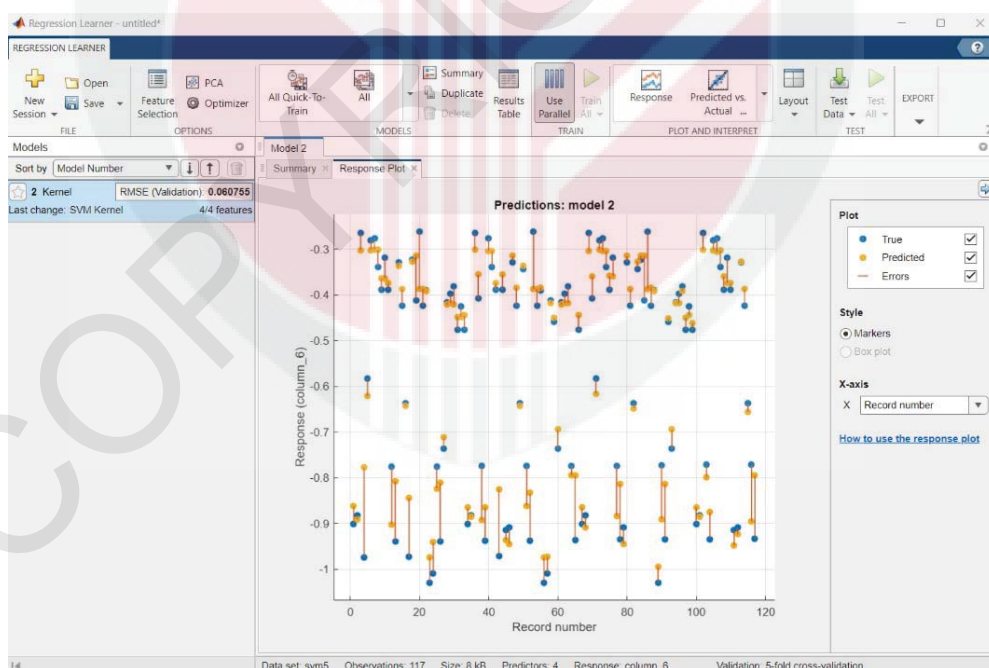
☐ Set aside a test data set

Percent set aside 10

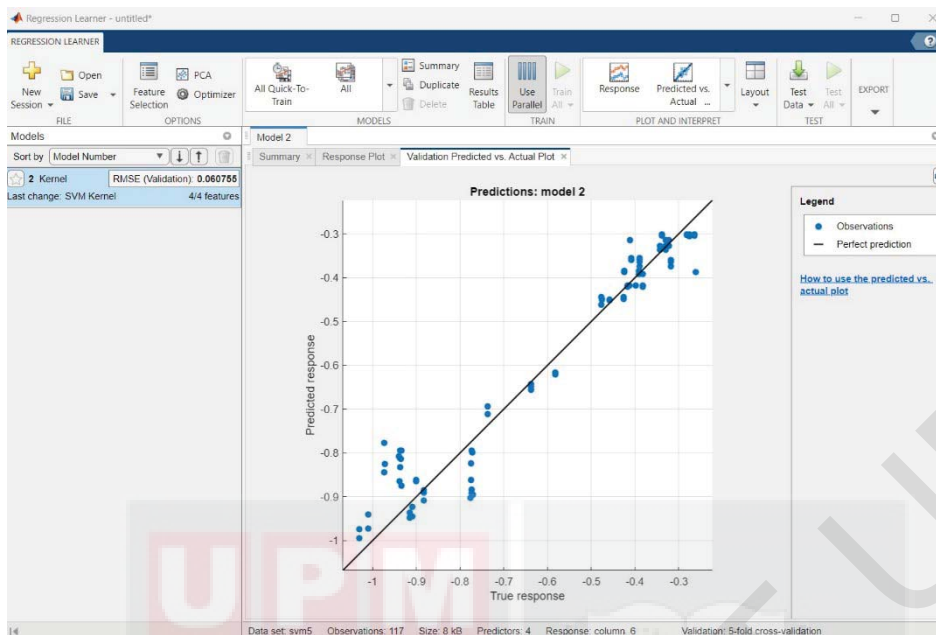
Use a test set to evaluate model performance after tuning and training models. To import a separate test set instead of partitioning the current data set, use the Test Data button after starting an app session.

[Read about test data](#)

Data input for SVM (kernel function: Linear)



Regression learner training (kernel function: Linear)



Validation predicted vs. Actual plot (kernel function: Linear)

BIODATA OF STUDENT

The author, Nur Ain Fitriah binti Zamrisham, born on February 6, 1998, in Kota Tinggi, Johor, at Hospital Kota Tinggi, completed her pre-university education in 2017 under the Science PST Module 1 at Kolej Matrikulasi Pahang. She then pursued an undergraduate degree in Civil Engineering at Universiti Putra Malaysia, Serdang, where she was awarded a Bachelor's Degree with Honours on October 14, 2021, after four years of study. Following her graduation, she gained professional experience as a project engineer at Enpro Engineering Sdn Bhd in Kuala Lumpur for a period of 1 year and 2 months. In October 2022, she advanced her education by enrolling in a Master's degree program in Environmental Engineering. As of March 2024, she is employed as a Green Building Consultant at Allied Environmental Consultant Sdn Bhd, Kuala Lumpur, specialising in environmental consultancy.

LIST OF PUBLICATIONS

Journal

Zamrisham, N. A., Wahab, A. M., Zainal, A., Karadag, D., Bhutada, D., Suhartini, S., Musa, M. A., & Idrus, S. (2023). State of the art in anaerobic treatment of landfill leachate: A review on Integrated System, additive substances, and Machine Learning Application. *Water*, 15(7), 1303 <https://doi.org/10.3390/w15071303>

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