

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

**APPLICATION OF DIFFERENT STRATEGIES TO IMPROVE ANAEROBIC
DIGESTION FOR ORGANIC FRACTION OF MUNICIPAL SOLID WASTE**



PhD THESIS

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Department of Environmental Engineering

Environmental Science, Engineering and Management Programme

JUNE 2024

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

**KENTSEL KATI ATIKLARIN ORGANİK FRAKSİYONUNUN ANAEROBİK
ÇÜRÜTME PERFORMANSINI İYİLEŞTİRMEK İÇİN FARKLI
STRATEJİLERİN UYGULANMASI**

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To my beloved family,



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TABLE OF CONTENTS

	<u>Page</u>
FOREWORD	ix
TABLE OF CONTENTS	xi
ABBREVIATIONS	xiii
LIST OF TABLES	xv
LIST OF FIGURES	xvii
SUMMARY	xix
ÖZET	xxi
1. INTRODUCTION	1
1.1 Background	1
1.2 Purpose of Thesis	2
1.3 Outline of Thesis	2
2. LITERATURE SURVEY	3
2.1 MSW Characteristics	4
2.2 MSW Management	5
2.3 AD Improvement Strategies	7
2.3.1 Parameter optimization	7
2.3.2 Co-digestion	8
2.3.3 Additives	8
2.3.4 Bioaugmentation and biostimulation	8
2.3.5 Bioreactor design	9
2.3.6 Pretreatment applications	9
3. DETERMINATION OF WASTE COMPOSITION AND PHYSIOCHEMICAL CHARACTERISTICS FOR MECHANICALLY SEPARATED ORGANIC FRACTION OF MSW AT A FULL-SCALE ANAEROBIC DIGESTION PLANT	13
3.1 Introduction	13
3.2 Material and Methods	16
3.2.1 Plant description and sample processing	16
3.2.2 AD plant	16
3.2.3 Sampling of waste	17
3.2.4 Waste composition	17
3.2.5 Determination of physicochemical characteristics	18
3.2.6 Environmental benefits assessment	19
3.2.7 Description tool	19
3.2.8 Methodology	20
3.3 Results and Discussion	21
3.3.1 Waste composition	21
3.3.2 The physicochemical characteristics	22
3.3.3 Loss of environmental benefits	24
3.4 Conclusions	26

4. ENHANCEMENT OF METHANE PRODUCTION BY ELECTROHYDROLYSIS PRETREATMENT FOR ANAEROBIC DIGESTION OF OFMSW	29
4.1 Introduction	29
4.2 Materials and Methods	31
4.2.1 AD plant description and sample collection	31
4.2.2 Physicochemical characteristics of inoculum and OFMSW	32
4.2.3 Electrohydrolysis experimental setup	33
4.2.4 BMP Batch Tests.....	34
4.2.5 BMP experimental setup	34
4.2.6 Theoretical BMP calculations	35
4.2.7 BMP kinetics and model fitting	36
4.2.8 Analytical experiments.....	37
4.3 Results and Discussion.....	38
4.3.1 BMP batch tests results	38
4.3.2 Kinetics and model fitting.....	39
4.4 Conclusions	41
5. EFFECT OF DIRECT ENZYME ADDITION ON THE ANAEROBIC DIGESTION OF OFMSW	43
5.1 Introduction	43
5.2 Materials and Methods	45
5.2.1 MSW plant description and sampling	45
5.2.2 Inoculum and OFMSW characteristics	46
5.2.3 The BMP tests	47
5.2.4 The BMP setup and experimental procedure	47
5.2.5 Enzyme addition.....	48
5.2.6 The kinetics and model fitting for batch BMP tests.....	48
5.2.7 Analytical measurements	49
5.3 Results and Discussion.....	50
5.3.1 The BMP test results	50
5.3.2 Model fitting and kinetics	51
5.4 Conclusions	53
6. CONCLUSIONS.....	55
6.1 Overall Conclusions	55
6.2 Future Perspectives	56
REFERENCES.....	57
CURRICULUM VITAE	77

ABBREVIATIONS

AD	: Anaerobic Digestion
BMP	: Biochemical Methane Potential
C	: Carbon
C/N	: Carbon/Nitrogen
COD	: Chemical Oxygen Demand
DC	: Direct Current
EPA	: Environmental Protection Agency
EU	: European Union
GHG	: Greenhouse Gases
H	: Hydrogen
HRT	: Hydraulic Retention Time
KKA	: Kentsel Katı Atık
ms-OFMSW	: Mechanically Separated OFMSW
MRF	: Material Recovery Facility
MSW	: Municipal Solid Waste
N	: Nitrogen
O	: Oxygen
OBA	: Online Biogas Application
OFMSW	: Organic Fraction of Municipal Solid Waste
PEF	: Pulsed Electric Field
RMSE	: Root Mean Square Error
S	: Sulphur
SD	: Standard Deviation
S:I	: Substrate:Inoculum
TS	: Total Solids
VS	: Volatile Solids



LIST OF TABLES

	<u>Page</u>
Table 3.1 : The recyclable and non-recyclable waste distribution of OFMSW.....	22
Table 3.2 : The physicochemical characteristics for the ms-OFMSW and OFMSW.....	23
Table 3.3 : The elemental composition for the ms-OFMSW and OFMSW.	24
Table 3.4 : Input parameters for the Recyclator tool.	25
Table 3.5 : Environmental benefits and savings from the waste streams.	25
Table 4.1 : Physicochemical characteristics of OFMSW*.	33
Table 4.2 : Parameters and goodness of fit received from the evaluated modified Gompertz model.	40
Table 5.1 : The physicochemical characteristics of the OFMSW.	47
Table 5.2 : Parameters and goodness of fit obtained from the modified Gompertz model.	51



LIST OF FIGURES

	<u>Page</u>
Figure 3.1 : The schematic flow of the full-scale AD plant.	17
Figure 3.2 : The procedure for determination of waste composition.	18
Figure 3.3 : The system boundaries for the calculation.	20
Figure 3.4 : The waste composition of ms-OFMSW a) Organics and non-organics; b) Sub-categories of non-organics.	22
Figure 4.1 : The schematic flow of the whole treatment of MSW.	32
Figure 4.2 : The schematic view of the electrohydrolysis reactor configuration.	34
Figure 4.3 : (a) CH ₄ and CO ₂ % and (b) Total biogas amount for untreated, 30-min. treated, and 60-min. treated OFMSW samples.	38
Figure 4.4 : Modified Gompertz model fitting for cumulative CH ₄ yield by electrohydrolysis pretreatment for the untreated, 30-min. treated, and 60-min. treated OFMSW samples.	41
Figure 5.1 : The schematic flow of the full-scale MSW plant.	46
Figure 5.2 : The total biogas volume and distribution of the CH ₄ and CO ₂ and obtained for the untreated and enzyme treated OFMSW samples.	50
Figure 5.3 : The correlation of R_{max} . vs λ obtained for the untreated and enzyme treated OFMSW samples.	52
Figure 5.4 : Modified Gompertz model fitting for cumulative CH ₄ yield obtained for the untreated and enzyme treated OFMSW samples.	53



APPLICATION OF DIFFERENT STRATEGIES TO IMPROVE ANAEROBIC DIGESTION FOR ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

SUMMARY

Municipal Solid Waste (MSW) management is a worldwide issue with significant effects on public health, community welfare, environmental sustainability, and economic progress which includes items like packaging, food scraps, newspapers, and more, coming from homes, businesses, and industries. Effective MSW management is vital for sustainable urban development, emphasizing waste segregation, recycling, and treatment to minimize landfill reliance and harness waste-to-energy technologies. It is of great importance to plan waste management systems according to local conditions, considering factors like waste composition and financial resources.

This thesis focused on the characterization of MSW and increasing biogas production by applying different pretreatment strategies to organic fraction of MSW (OFMSW). Due to increasing population worldwide, it is essential to understand the MSW characteristics to apply efficient waste management strategies. The organic fraction of MSW (OFMSW) holds potential for energy recovery through anaerobic digestion (AD), offering municipalities economic opportunities besides environmental benefits.

In the first study, waste composition and physiochemical of the mechanically separated OFMSW (ms-OFMSW) at a full-scale AD plant in Türkiye was evaluated. Ms-OFMSW predominantly comprised of organic matter ($76.45 \pm 1.71\%$), alongside recyclable ($8.99 \pm 1.56\%$) and non-recyclable ($14.56 \pm 1.69\%$) components according to the findings of this study. Environmental assessment was conducted using Environmental Protection Agency's online tool (Recyclator tool) underscored the substantial energy and water savings associated with segregating recyclable materials (metal, glass and plastic) from the waste stream. Moreover, this study highlighted the importance of efficient pre-separation units in enhancing OFMSW digestibility and maximizing environmental benefits.

AD is recognized as an effective waste management strategy for different types of waste with the potential to mitigate greenhouse gas emissions (GHGs) while concurrently generating renewable energy. The physicochemical characteristics of OFMSW can significantly influence the AD process's efficiency and biogas production. Hydrolysis, a pivotal step in AD, is often rate-limiting for degradation of waste, particularly for substrates like OFMSW.

In the second and third studies, electrohydrolysis and enzyme pretreatment were explored to improve hydrolysis efficiency. Experiments showed that doubling electrohydrolysis treatment time (from 30 minutes to 60 minutes) led to notable improvements in methane production, with a significant reduction in the lag phase. Methane production increased by 3–10% following electrohydrolysis pretreatment, underscoring its potential to expedite the AD process and enhance biogas yields. While

in the enzyme pretreatment, anaerobic degradation of organic waste was investigated by employing alpha amylase enzyme which was obtained from *Aspergillus oryzae*. According to the results, a significant increase in the methane yield and a decrease in the lag phase was observed. Optimum results were obtained with the addition of 0.5 mg of enzyme per g volatile solids (VS) added, highlighting the effectiveness of alpha amylase in enhancing the biodegradability and biogas production of OFMSW.

In summary, this thesis sheds light on the importance of understanding and managing OFMSW characteristics to optimize AD efficiency and maximize environmental benefits. Efficient pre-separation units, electrohydrolysis and enzyme pretreatment emerge as promising strategies for enhancing OFMSW digestibility and biogas production in AD processes. However, further research is warranted to fully explore and optimize these approaches for practical implementation on a larger scale. By leveraging these advancements, the waste management sector can move towards more sustainable practices, reducing environmental impact and contributing to the transition to a circular economy.



KENTSEL KATI ATIKLARIN ORGANİK FRAKSİYONUNUN ANAEROBİK ÇÜRÜTME PERFORMANSINI İYİLEŞTİRMEK İÇİN FARKLI STRATEJİLERİN UYGULANMASI

ÖZET

Kentsel Katı Atık (KKA) yönetimi, evlerden, işletmelerden ve endüstrilerden gelen ambalaj, gıda atıkları ve gazete vb. maddeleri içeren, halk sağlığı, toplum refahı, çevresel sürdürülebilirlik ve ekonomik ilerleme üzerinde önemli etkileri olan dünya çapında bir konudur. Etkili KKA yönetimi, sürdürülebilir kentsel kalkınma için hayati önem taşımakta olup, depolama sahalarına bağımlılığı en aza indirmek ve atıktan enerji elde etme teknolojilerinden yararlanmada atık ayırma, geri dönüşüm ve etkin bertarafı vurgulamaktadır. Atık bileşimi ve mali kaynaklar gibi faktörler göz önünde bulundurulduğunda, atık yönetim sistemlerinin yerel koşullara göre planlanması büyük önem arz etmektedir.

Bu tez, KKA'ların organik fraksiyonun farklı ön işlem stratejileri uygulanarak atıklardan elde edilen biyogaz miktarının artırılmasını hedeflemektedir. Atık yönetimi uygulamalarının verimli olması için KKA'ların özelliklerinin bilinmesi önemlidir. KKA'ların organik fraksiyonu, anaerobik çürütme (AÇ) yoluyla enerji geri kazanımında potansiyel taşımakta olup belediyelere çevresel faydaların yanı sıra ekonomik fırsatlar da sunmaktadır.

Birinci çalışmada, Türkiye'deki tam ölçekli bir AÇ tesisinden alınan mekanik olarak ayrılmış organik atıkların bileşimi ve fizikokimyasal özellikleri incelenmiştir. Çalışma kapsamında 9 ay boyunca ayda bir alınan ortalama 48 kg numune kullanılmıştır. Numunelerde önce organik ve organik olmayan bileşenler olarak ayrılmıştır. Daha sonra organik olmayan bileşenler metal, cam, plastik, yapısal, tekstil ve inert olarak ayrılmış ve tartılmıştır. Tesisten alınan mekanik olarak ayrılmış organik atık numuneleri ve laboratuvarında elde ayrılan organik bileşim için pH, nem, toplam katılar, uçucu katılar, iletkenlik, üst ısı değeri ve elemental analiz (C, H, N, S) sonuçları değerlendirilmiştir. Çalışmadan elde edilen bulgulara göre, mekanik olarak ayrılmış organik atıkların içinde hala geri dönüştürülebilir (%8,99 ± 1,56) bileşenlerin bulunduğu tespit edilmiştir. Organik madde içeriği ise %76,45 ± 1,71'dir. Çevre Koruma Ajansı tarafından sunulan çevrimiçi program kullanılarak çevresel değerlendirme yapılmış ve geri dönüştürülebilir malzemelerin (metal, cam ve plastik) ayrılması durumunda önemli oranda enerji ve su geri kazanımının sağlanmasının yanı sıra depolama sahası ihtiyacının azalacağı ortaya konmuştur. Metaller (399.7 GJL) ve plastikler (403.7 GJL) neredeyse aynı miktarda enerji tasarrufu sağlarken, metaller en fazla su tasarrufunu sağlamıştır (421.8 kL) ve en büyük olumlu etki metaller için kaydedilmiştir. Sera gazı faydaları, her bir atık akışı için 3 ila 40 ton karbondioksit eşdeğeri arasındadır. Bu çalışma, ayrıca verimli ön ayırma ünitelerinin, organik atıkların çürütülebilirliğinin ve çevresel faydalarının artırılmasındaki önemini vurgulamakta, geri dönüştürülebilir atıkların AÇ'ye girişinin önlenmesi ile daha fazla çevresel fayda sağlanabileceğini göstermektedir. Bu çalışma ayrıca organik atığın

kariřık toplanan KKA'dan ayrılması için geliřmiř teknolojilere duyulan ihtiyaı ortaya koymaktadır.

A, farklı türdeki atıklar için sera gazı emisyonlarını azaltma ve yenilenebilir enerji üretme potansiyeline sahip etkili bir atık yönetim stratejisi olarak kabul edilmektedir. Organik atıklar yüksek biyolojik paralanabilirlikleri sayesinde A için iyi bir hammaddestir. Organik atığın fizikokimyasal özellikleri A'nin verimliliğini ve biyogaz üretimini önemli ölçüde etkilemektedir. Ancak, organik atıkların hidroliz işlemi ile kompleks makro moleküllerden (protein, karbonhidrat, yağ) basit moleküllere (aminoasit, glikoz, yağ asidi) dönüřtürülmesi A için sınırlayıcı bir faktördür. Hidroliz, A'de önemli bir aşama olup, özellikle organik atık gibi substratlar için atığın bozunma hızını sınırlamaktadır.

İkinci ve üçüncü alışmalarda, hidroliz verimliliğini iyileřtirmek üzere elektrohidroliz ve enzim ön işlemleri araştırılmıştır. Deneysel alışmalarda, elektrohidroliz ön işlem süresinin iki katına ıkarılması (30 dakikadan 60 dakikaya) ile metan üretiminde kayda değer iyileřmelerin yanı sıra lag fazının azaldığı tespit edilmiştir. Ön işlem uygulaması ile birlikte metan üretiminin %3-10 oranında arttığı tespit edilmiş ve bu duruma baėlı olarak A sürecinin hızlandırılması ile biyogaz veriminin artırılabilceėi öngörülmüřtür.

Elektrohidroliz ön işleminin, organik atıkların hidrolizi üzerindeki etkilerini belirlemek için, otomatik biyokimyasal metan potansiyeli (BMP) test sistemi kullanılarak termofilik (55°C) kořullarda alışılmıştır. Özel tasarlanmış (1 L kapasiteli, silindirik boyutlandırılmış) bir reaktörde, iç kısmında katot ve dış kısmında anot elektrodu konumlandırılarak elektrohidroliz işlemi gerekleştirilmiştir. Bu reaktörde 20 V gerilimde, 30 ve 60 dakika boyunca elektrohidroliz ön işlemi organik atıklara uygulanmıştır. Ön işlem uygulanmamış ve uygulanmış (30 dakika ve 60 dakika) organik atık numunelerinin g uucu katı madde başına metan üretimleri sırasıyla 225±2 mL, 231±4 mL ve 248±7 mL olarak ölçülmüřtür. Elektrohidroliz ön işlem uygulandığında, hidroliz aşamasındaki lag fazı, 30 dakika uygulama için %43, 60 dakika uygulama için ise %40 azalmıştır.

Bu tez kapsamında, ayrıca organik atıklardaki kompleks makro moleküllerden basit moleküllere dönüřtürülmesini artırmak için enzim ön işlemi araştırılmıştır. Enzim ön işlem uygulamasında ise *Aspergillus oryzae*'den elde edilen alfa amilaz enzimi, organik atıkların bozunmasının incelenmesi amacıyla kullanılmıştır. Alfa amilaz enziminin altı farklı konsantrasyonu (0.15, 0.3, 0.5, 1.0, 1.5 ve 3.0 mg/g UKM_{eklenen}) doğrudan toz olarak ilave edilmiş ve otomatik biyokimyasal metan potansiyeli (BMP) testleri gerekleştirilmiştir. alışmadan elde edilen BMP test sonuçlarına göre metan veriminde yaklaşık %17,5 artış elde edilirken, lag fazında da %19,3'lük azalma gözlemlenmiştir. 0,5 mg/g UKM_{eklenen} enzim ilave edildiğinde optimum sonuçlar elde edilmiş ve alfa amilazın organik atıkların biyolojik paralanabilirliğini ve biyogaz üretimini artırmadaki verimliliėi ortaya konmuřtur.

Elektrohidroliz ve enzim ön işlem uygulamalarında BMP testi sonuçları Modifiye Gompertz modeli ile deėerlendirilmiştir. Her iki ön işlem için, deneysel sonuçlar model sonuçlar ile örtüřmüřtür. BMP testi sonuçları, özellikle enzim ön işlem uygulamasında deneysel ve simüle edilmiş deėerler arasında güçlü bir ilişki olduğunu göstermiştir.

Özetle, bu tez, AÇ verimliliğini optimize etmek ve çevresel faydaları en üst düzeye çıkarmada KKA'ların organik fraksiyonunun bileşimini ve özelliklerini anlamının ve yönetimin önemine ışık tutmaktadır. Mekanik ayırma ünitelerinin verimliliği, elektrohizroliz ve enzim ön işlemlerinin uygulanması, KKA'ların organik fraksiyonunun anaerobik çürütülmesinde biyogaz üretimini arttırmada umut verici stratejiler olarak ortaya çıkmaktadır. Bununla birlikte, bu yaklaşımların büyük ölçekte uygulanabilmesi için daha fazla araştırma yapılması ve optimizasyonu gerekmektedir. Atık yönetimi sektörü, bu gelişmelerden yararlanarak daha sürdürülebilir uygulamalara yönelebilir, çevresel etkiyi azaltabilir ve döngüsel ekonomiye geçişte katkıda bulunabilir.





1. INTRODUCTION

1.1 Background

Municipal solid waste (MSW) management is a global concern threatening public health, community well-being, environmental sustainability, and economic development, necessitating immediate actions at individual and governmental levels (Kaza et al., 2018). The treatment and repurposing of waste components, especially organic waste, offer economic and environmental benefits in addressing these concerns. The organic fraction of municipal solid waste (OFMSW) is a promising source for biogas generation and renewable energy production (Woon and Lo, 2016). Anaerobic digestion (AD) stands out as a highly promising and sustainable method for treating food and yard wastes (Bandini et al., 2022). AD can also be used to turn OFMSW into renewable fuel (biogas) and digestate by microbial consortium in the absence of oxygen (Uddin and Wright, 2022). In contrast to incineration and landfilling, AD offers significant advantages as a renewable energy source, with the biogas produced serving as a valuable fuel that contributes to reducing carbon emissions and minimizing air pollution, thus presenting an environmentally friendly alternative (Kumar and Samadder, 2017; Van Fan et al., 2018).

With the Zero Waste Project, which started in 2017, there have been significant improvements in the separation and recycling of packaging waste at the source in Türkiye. However, separation of organic waste at source is not yet implemented. For this reason, mechanical separation is utilized in the design of biomethanization plants for removing the impurities from the MSW to increase the efficiency of AD. Even though the organic fraction is separated during mechanical separation, AD still lacks pure OFMSW content. In the case of Türkiye, existing biomethanization plants that are used for MSW are insufficient in terms of waste separation therefore there is a need to improve these plants within the framework of waste-to-energy production.

1.2 Purpose of Thesis

The purpose of this thesis was to investigate application of different strategies to improve anaerobic digestion performance for the OFMSW. For this purpose electrohydrolysis and enzyme pretreatment were applied. In addition, waste composition and physiochemical characteristics of mechanically separated OFMSW (ms-OFMSW) were determined at the full-scale AD plant.

1.3 Outline of Thesis

This thesis contains 5 chapters as following:

Chapter 1 includes the introduction, problem statement and the aim of this thesis.

Chapter 2 reviews the literature focusing on the OFMSW characterization, management and improvement of AD.

Chapter 3 explains the OFMSW characteristics, OFMSW properties, full scale AD plant description.

Chapter 4 encompasses the results obtained from the electrohydrolysis pretreatment study that was carried out as part of this thesis. Anaerobic digestibility of OFMSW was investigated by evaluating the methane enhancement.

Chapter 5 includes findings of enzyme pretreatment study that was conducted. Similar to electrohydrolysis pretreatment study, methane production was evaluated to assess the enzyme application on the anaerobic digestibility of OFMSW.

Chapter 6 provides an overview of the general conclusions derived from this thesis in addition to future perspectives.

2. LITERATURE SURVEY

MSW is a heterogeneous mix of various materials that its composition varies based on factors such as location, lifestyle, and economic status (Bahukhandi and Ollemman, 2022; Heidari-Maleni et al., 2023). MSW, often referred to as trash or garbage, comprises the discarded items that are used in daily life, such as product packaging, grass clippings, bottles, clothing, furniture, newspapers, food scraps, paint, appliances, and batteries (EPA, 2016). These materials are originated from residential, commercial, institutional, and industrial sources (Rhyner et al., 2017). MSW management is indeed a critical global challenge, exacerbated by rapid urbanization, population growth, and immense consumption patterns (Sharma and Jain, 2020). The volume of waste generated within the cities has increased exponentially, particularly in developing countries, because of high migration rates from rural areas to urban areas.

High waste generation poses significant environmental, social, and economic implications for communities worldwide (Zambrano-Monserrate et al., 2021). Improper disposal of waste leads to environmental pollution because of greenhouse gases resulting from landfills and contamination soil and water sources from leachate (EPA, 2024). The proliferation of waste in urban areas also contributes to the spread of diseases and poses health risks to residents, particularly to the population who are living in close proximity to dumpsites or are exposed to untreated waste (Kaza et al., 2018). Waste handling has another impact on the municipalities by forcing them to use their resources for managing the waste rather than for the fundamental services or infrastructure (UNEP, 2020).

Efficient MSW management plays a pivotal role in tackling challenges to foster sustainable urban development. Through the adoption of comprehensive waste management practices, cities can significantly alleviate pollution while conserving valuable resources and protecting public health and well-being. Eriksson et al. (2015)

underscored the importance of proper waste segregation, recycling, or treatment in reducing the burden on landfills, to allow minimizing environmental degradation and optimizing resource recovery. On the other hand, Cointreau-Levine et al. (2017) highlight the importance of waste-to-energy technologies that present promising avenues for renewable energy generation, thus further bolstering environmental sustainability and contributing to climate change mitigation efforts. However, it is better for each country to tailor its own waste management system to its unique circumstances by considering factors such as waste characteristics, financial resources, and environmental impact. As noted by Demir (2020), blindly transferring a technology without considering these factors would be an ineffective way of managing waste, particularly in developing countries.

The characteristics of MSW play a crucial role in determining the convenient waste management (Zambrano-Monserrate et al., 2021). Understanding the composition, physical properties, and sources of MSW is essential for designing efficient waste management and implying strategies for waste reduction, recycling, and treatment (Sharma and Jain, 2020). The OFMSW, which is consisted of food waste, yard waste, and other biodegradable materials, holds significant potential for conversion of waste into renewable energy sources such as biogas through AD (Ravindranath et al., 2016). The biodegradability properties of the OFMSW are becoming increasingly important as they provide energy recovery and economic benefits (Ranieri et al., 2018). In this essence, this brings an opportunity to municipalities not only to manage the waste in a more sustainable way but also to generate revenue or offset energy costs through the biogas production.

2.1 MSW Characteristics

Different factors such as geographical location, population density, economic status, and cultural practices influence the composition of MSW, thereby enriching its complexity and making it essential to comprehend its characteristics (Fisgativa et al., 2016; Taghipour et al., 2016). According to the World Bank report and Zari (2024), understanding the sources of waste, waste generation rates, waste types, and waste

composition is crucial for characterization, monitoring, and fostering sustainable waste management practices (Kaza et al., 2018).

Tchobanoglous et al. (1993) stated that the major components of MSW were organic, inorganic, recyclable, and hazardous materials. While Ravindranath et al. (2016) highlighted that organic waste was the major fraction of the MSW. Characterization studies provide valuable insights into MSW composition (Wilson et al., 2012). Sharma and Jain (2020) noted that the composition and amount of the waste are influenced by seasonal variations. For instance, food waste generation is typically increased during holiday seasons (Sharma and Jain, 2020). According to Albanna (2013), MSW contains a significant amount of organic fraction, comprising food residues and garden waste, which constitutes 70% of the waste composition, with a moisture content ranging from 85% to 90%.

2.2 MSW Management

Waste management includes the handling of waste, such as collecting, transporting, and processing the waste (Tchobanoglous et al., 1993). Ramli et al. (2021) stated that both the quantity and composition of the MSW are crucial in implementing efficient policies for the MSW management while promoting environmentally sustainable options for protecting the public health besides conserving resources in urban areas (Sharma and Jain, 2020). Collection and transportation play a crucial role in MSW management, ensuring the timely and efficient removal of waste from residential, commercial, and institutional sources (Singh et al., 2024; Das and Bharti, 2023). Waste collection can be categorized in two main types: a) source-separated (or separate) collection, and b) mixed collection (Liu et al., 2020). Source-separated collection is defined as the separate collection of different types of waste at the point of where they were generated (Zhang et al., 2022). This type of collection method facilitates recycling and composting of waste materials easily and leads to higher rates of material recovery while reducing the contamination (Zeng et al., 2016). On the other hand, in mixed collection systems, different types of waste, such as organic waste, recyclables, and non-recyclable waste, are collected together in a single stream. This method is mostly common in areas where sorting facilities are available to

separate recyclable materials from non-recyclables after they are collected. Mixed collection requires more sophisticated sorting and processing infrastructure than source-separated collection to effectively recover recyclable materials (Tanguay-Rioux et al., 2022).

Waste separation, an essential step for material recycling, becomes crucial due to the abundant presence of valuable resources like metals, paper, plastic, and glass within MSW (Nzihou, 2020). There are two common practices to separate the waste: a) segregating the waste at the source where it is generated, and b) processing and sorting the waste after it is mixedly collected from the household etc. (Nzihou, 2020). In Türkiye, MSW is collected as mixed waste, therefore the organic and non-organic parts of MSW are then separated by mechanical processing and sorted.

This process relies on the size sorting and mechanical principles, utilizing equipment such as shredders, rotary drums, and trommel screens (Dehkordi et al., 2020). As a result, the effectively separated organic material is ready to be processed further. The organic fraction which is obtained through mechanical separation then serves as feedstock for AD plants, with mechanical separation being the primary method for producing OFMSW (Dehkordi et al., 2020).

Treatment and recycling of MSW are essential to recover valuable resources, to reduce landfill dependency, and to mitigate environmental pollution (Shah et al., 2024). Treatment technologies such as composting, AD, and thermal processing (e.g., incineration, pyrolysis) convert organic waste into renewable energy and organic fertilizers while recovering materials for recycling (Baskar et al., 2022).

The high organic content, moisture content, and biodegradability potential of food wastes emphasize bioenergy recovery as a waste management approach which enhances the applicability of biological processes (Liang, 2022). This high organic content also enhances the applicability of biological processes. Furthermore, the low calorific value, along with the high organic and moisture content of MSW, make biological processes more suitable than thermal methods, particularly considering the high energy demand associated with thermal processes. Unlike incineration or

landfilling, AD does not present a potential pollution risk if the produced biogas is effectively utilized (Baskar et al., 2014).

In waste management, aerobic and anaerobic processes are distinct biological methods; the former method requires oxygen, whereas the latter method does not (Ramli et al., 2021). The biodegradation of food waste offers several advantages, including volume reduction, biogas production, water recovery, and soil conditioning (Usman et al., 2020). However, challenges related to AD still exist due to difficulties in initiating the process, prolonged stabilization time, and inhibition caused by the formation of toxic compounds (Vögeli, 2014). In the anaerobic degradation of food waste, hydrolysis is often the rate-limiting step due to the presence of complex macromolecules which are resistant to biodegradation or degrading slowly (Prabhakaran et al., 2016; Usman et al., 2020; Assis and Gonçalves, 2022).

2.3 AD Improvement Strategies

AD is a promising technology to obtain renewable energy sources from organic waste. Various strategies have been implied to AD to improve digestibility were as following: 1) parameter optimization, 2) co-digestion, 3) additives, 4) bioaugmentation and biostimulation, 5) bioreactor design, and 6) pretreatment applications (Aworanti et al., 2023; Simioni et al., 2022).

2.3.1 Parameter optimization

To ensure the efficiency of digestion, optimizing AD process parameters such as temperature, pH, and HRT are crucial (Mao et al., 2017). According to Wang et al. (2023), understanding operational factors, reactor design, and optimization methods are essential in AD for the treatment of organic wastes that have high-solid content. In the study of Kazimierowicz et al. (2021) the impact of temperature and organic loading rates were investigated on methane concentration in biogas production from food waste AD was investigated for of food waste. The authors observed an increase in the methane concentration under both mesophilic (methane content increased from $59.5 \pm 2.1\%$ to $61.4 \pm 1.7\%$) and thermophilic (methane content increased from $66.6 \pm 2.5\%$ to $68.6 \pm 1.8\%$) conditions.

2.3.2 Co-digestion

Co-digestion of multiple substrates is a well-known method for optimizing nutrient balance and enhancing stability of AD process (Angelidaki et al., 2018). Wang et al. (2022) stated that co-digestion performance is influenced by factors such as process parameters, substrate optimization, microbial community dynamics, and methane purification technologies. Perin et al. (2020) investigated the effects of co-digestion of food waste mixed with garden waste. Authors discovered that the co-digested mixed waste had a methane content of 67% which was 24% higher than when only food waste was digested.

2.3.3 Additives

Additives can be supplements such as nanoparticles, composite additives, metal oxides, carbon-based additives, and zeolite. All additives have shown promising potential for enhancing biogas production and overall process efficiency in AD (Liu et al., 2021; Abdelwahab and Fodah, 2022; Manikandan et al., 2023). Juntupally et al. (2023) showed that introducing nanoparticles or composite additives in the AD of food waste increased the biogas yield ranging from 15% to 63%. Dompara et al. (2023) demonstrated that the biogas yield was increased by 12% (TiO₂) and 44% (ZnO/Ag) after adding metal oxides of food waste during digestion. According to authors, metal oxides not only improved the biogas yield but also increased the volatile solids (VS) and chemical oxygen demand (COD) removal efficiencies. On the other hand, Zhang et al. (2023) showed that zeolite addition effectively reduced the ammonia levels and stabilized AD performance.

2.3.4 Bioaugmentation and biostimulation

Angelidaki et al. (2018) proposed that manipulating microbial communities through bioaugmentation and immobilization promotes microbial activity and substrate degradation. Wang et al. (2024) showed that bioaugmentation by *Clostridium thermopalmarium* HK1 and *Bacillus thermoamylovorans* Y25, enhanced the hydrolytic activity (increased by 14.54 %) and the methane production (increased by 19.79%). On the other hand, Altamirano-Corona et al. (2021) used conductive materials such as granular activated carbon, biochar, and magnetite to perform

biostimulation. The authors demonstrated that the substances magnetite (Fe_3O_4) at 0.1 g/L of concentration and biochar at 1 g/L of concentration not only improved buffering capacity besides promoting microbial colonization but also significantly increased methane yield (30.1% and 20.3% increase in methane production for Fe_3O_4 and biochar, respectively).

2.3.5 Bioreactor design

Technological innovations, including high-rate digestion systems and integrated biorefinery concepts, offer compact and efficient solutions for improving AD process (Kusch-Brandt et al., 2023). Zhang et al. (2022) provided an overview of structural advancements for the AD reactors such as electrochemical AD reactors, microbubble reactors, multistage AD reactors, and zero-valent iron AD reactors (Zhang et al., 2022). Amui Khorshidi et al. (2024) compared the performance of a novel portable horizontal continuous bioreactor to the conventional batch reactors by evaluating the biogas production from sugar beet waste and showed that methane production was increased by 55.1%.

2.3.6 Pretreatment applications

Different pretreatment methods were applied to OFMSW to enhance the biogas production and to improve the digestibility of the substrate. These can be classified as mechanical, thermal, chemical, biological, or combination of methods (Panigrahi and Dubey, 2019). A successful pretreatment method should meet several criteria: 1) reserve the organic materials in the biomass; 2) enhance the progress of hydrolysis; 3) avoid the formation of any toxic and/or inhibitory compounds; 4) be environmentally friendly; and 5) be economically viable (Derman et al., 2018; Choi et al., 2019).

Mechanical pretreatment involves size reduction, shredding, and screening of MSW to increase the surface area of the substrate to enhance the biodegradability. Zhang et al. (2019) investigated the effect of mechanical pretreatment on MSW composition and methane production. Authors reported that shredding MSW into smaller particles improved the biogas yield by facilitating substrate availability for the microbes.

Electrohydrolysis and pulsed electric field (PEF) are often used for treating biomass to enhance AD process or extracting colorants, bioactive compounds etc. in food processing (Mazumdar et al, 2022). Although both utilize electricity, their operational mechanisms and applications differ. Electrical field is used to disrupt the cell structure of biomass in electrohydrolysis pretreatment method. The application of electric current can cause electroporation or other physical disruptions to the cells, making the organic compounds more accessible for microbial degradation during AD (Kainthola et al., 2019). In electrohydrolysis, continuous electric current is applied to induce both chemical and physical changes, making the substrate available for further degradation (Kainthola et al., 2019). In contrast, short and high-voltage pulses are used in PEF to create transient pores in cell membranes and cause physical disruptions without chemical alteration (Rahman et al., 2022). This makes PEF ideal for applications requiring non-destructive cell disruption, such as in food processing and extraction.

Microwave irradiation, steam explosion, and hydrothermal treatment were employed as thermal pretreatment to disrupt lignocellulosic structure of MSW to enhance its digestibility. According to Scherzinger and Kaltschmitt (2021), it is important to choose the appropriate operating conditions for thermal pretreatment (Scherzinger and Kaltschmitt, 2021). Dasgupta and Chandel (2019), showed that the hydrothermal pretreatment (140 °C for 30 minutes) improved the biodegradability of the OFMSW and increased the methane production (31.9%) while reduced the digestion time (29.9%) required for AD.

Chemical pretreatment involves the use of acids, alkalis, and oxidizing agents to break down complex organic compounds into smaller molecules to improve biodegradability. Junoh et al. (2016) investigated the effect of calcium hydroxide addition (6.1 g/liter sample) to food waste and reported a 20% increase in the methane production compared to untreated food waste (720.37 mL/g VS_{destroyed}). Linyi et al. (2020) reported that by applying 1% CaO not only increased the stability of the reactor but also increased the biogas production while exhibiting significant variations in the relative abundance of bacterial groups under various conditions.

In biological pretreatment, complex organic macromolecules such as protein polymers, lipids, and carbohydrates are sequentially hydrolyzed into simpler

molecules like sugars, long-chain fatty acids, and amino acids (Venturin et al., 2019). It is facilitated by microbes, enzymes, or fungi (Atelge et al., 2020). Biological pretreatment aims to enhance substrate accessibility for microbial degradation and promote the growth of beneficial microbial species. Different studies explored enzyme-based treatment strategies to improve biogas yield (Güelfo et al., 2011; Zhao et al., 2019; Çakmak and Ugurlu, 2020). According to Bala and Mondal et al. (2020), the use of lignin degrading fungal strains *Phanerochaete chrysosporium* and *Pleurotus ostreatus* in the biological pretreatment of OFMSW resulted in an increase of 43.1% and 25.6% in the biogas production, in order compared to the untreated OFMSW (246 mL/g COD_{added}).

Several studies investigated combined application of different pretreatment methods to synergistically enhance methane production from MSW. For instance, the study conducted by Shanthi et al. (2019) on fruit and vegetable waste using chemical-ultrasonic pretreatment showed that this method resulted in a significant increase of in methane production (287.7%) and a reduction in suspended solids (17%) while achieving a yield of 0.190 m³ CH₄/kg COD. On the contrary, Shahriari et al. (2012) reported that the combined microwave heating for digestion of OFMSW with hydrogen peroxide (H₂O₂) addition increased the time (from 8 days to 20 days) required for degrading OFMSW. Li et al (2015) investigated single-phase or two-phase AD application to the hydrothermally pretreated MSW (including waste activated sludge (WAS), fruit and vegetable residues, and kitchen wastes). Authors showed that two-phase AD applied MSW produced significantly more biogas (0.71 L/g VS_{added}) compared to one-phase systems (0.53-0.55 L/g VS_{added}).



3. DETERMINATION OF WASTE COMPOSITION AND PHYSIOCHEMICAL CHARACTERISTICS FOR MECHANICALLY SEPARATED ORGANIC FRACTION OF MSW AT A FULL-SCALE ANAEROBIC DIGESTION PLANT¹

3.1 Introduction

On a global scale, population growth combined with a linear economy, as opposed to a circular economy, would result in increased energy demands and waste, becoming major challenges for humanity. Globally, solid waste management accounts for nearly 5 % of total greenhouse gas (GHG) emissions (Tyagi et al., 2018). If the sector did not improve, it is predicted that this amount of waste would cause a total emission of 2.6 billion tons of carbon dioxide (CO₂) equivalent per year by the end of 2050 (Kaza et al., 2018). In addition, global waste production is projected to reach 3.4 billion tons per year by 2050 (Kawai and Tasaki, 2016). These alarming projections highlight the urgent need for effective waste management strategies and sustainable practices. Without significant improvements in waste management, the environment will continue to suffer from increased greenhouse gas emissions and pollution. It is crucial for governments, industries, and individuals to prioritize waste reduction, recycling, and proper disposal methods to mitigate these challenges and ensure a sustainable future for all.

The most common method of waste disposal is to dump it on a site or send it to a landfill (Kaur et al., 2021). However, it is not applied in the same way in every

¹ This chapter is based on “Kabakci, Y., Kosar, S., Dogan, O., Uctug, F. G., & Arıkan, O. A. “.2024. Determination of characteristics for mechanically separated organic fraction of MSW at a full-scale anaerobic digestion plant. *Waste Disposal & Sustainable Energy*, 1-9. DOI: 10.1007/s42768-023-00183-x

country, and it varies according to the wealth of the population. In general, low-income countries prefer dumping; nearly 93 % of waste is treated in this manner, whereas only 2 % of waste in high-income countries is treated in this way (Kaza et al., 2018). Upper-middle-income countries send 54 % of their waste to landfills, while high-income countries send 39 %, with the remainder recycled (35 %), or incinerated (22 %), which is a common application (Fei et al., 2018). Despite the fact that many countries have made the separate collection of recyclable waste fractions a priority, a significant amount of municipal solid waste (MSW) is still mixedly collected (Sholokhova et al., 2022). According to the European Commission (European Commission, 2008), sorting waste at the source is critical for increasing recycling rates; however, its applicability is limited to the region's developmental level. According to Backes (Backes, 2020), recyclable materials can be recovered from MSW before it is disposed of in landfills or incinerators within the framework of the circular economy. According to Kaza et al. (2018), only 19 % of generated waste is recycled and composted, with the remainder being incinerated (11 %), landfilled (37 %), and open dumped (33%) globally. MSW has the highest organics, at 48 % on average, according to Osra et al. (2021), followed by plastics, paper, and metals at 25 %, 20 %, and 4 %, respectively. The impurities in MSW, as highlighted by Ouigmane et al. (2021), are often linked to living standards and should be carefully sorted. It is important to note that the organic fraction of MSW, which can range from 42 to 75% according to López-Gómez et al. (2019), is significantly influenced by seasonal variations and geographical factors (Seruga et al., 2020).

Waste management for the OFMSW is critical, and if it is not managed properly, serious problems may be encountered. OFMSW can be treated using composting, incineration, AD, or landfilling. Composting, for example, requires a larger area and more time to produce a high-quality product, whereas incineration results in emissions and ash residue management (Pour and Makkawi, 2021). AD is regarded as a low-cost and environmentally friendly method of treating OFMSW while producing biogas and nutrient-rich digestate suitable as fertilizer. Energy savings, methane gas production, waste stabilization, waste volume reduction, and a relatively small footprint are some of the advantages of AD over other waste treatment technologies.

In addition, the AD process can also generate valuable by-products such as biogas and nutrient-rich digestate, which can be used for renewable energy production and as a natural fertilizer respectively (Nzihou, 2020). This not only contributes to the overall sustainability of waste management but also provides economic benefits by creating additional revenue streams.

The United Nations promotes circular economy as a means of involving governments and societies in the creation of a sustainable world by a sustainable world by minimizing waste, maximizing resource efficiency, and promoting sustainable consumption and production. As a result, encouraging Agenda 2030 for transition in the context of the circular economy necessitates better waste management and increased separate collection (UN Agenda, 2023). In the context of the circular economy, the European Commission established several goals, including mandatory separate collection of biowaste by 2023 and a 65 % recycling rate for MSW by 2035 (European Commission, 2020). These goals aim to ensure that valuable resources are not lost in the waste stream and can be reused or recycled, reducing the need for extracting new raw materials. Additionally, they promote a shift towards a more sustainable and circular approach to waste management, which is crucial for achieving the objectives of Agenda 2030 and creating a more sustainable future (Tyagi et al., 2018; Sezer and Arikan, 2011; Thiriet et al., 2020). To reach the goals set by the European Commission (European Commission, 2008), waste sorting has become the most efficient way to increase resource or energy recovery from MSW, which includes at least three stages, including collection and transportation (C&T), pretreatment, and resource utilization (Nzihou, 2020). Fiscal and non-fiscal tools have emerged for authorities to use in this regard: 1) to develop the circular economy and promote a life cycle perspective to be adopted by economic individuals in an attempt to decouple economic development from resource use and related environmental impacts; and 2) to promote the growth and adoption of circular economy-related technologies and services by changing the behaviors and habits of natural resource producers and consumers (Brears, 2018). The Recyclator, a non-fiscal online tool, calculates the environmental benefits of large-scale recycling schemes across 21 pre-defined material types (Brears, 2018; NSW, 2023), with the goal of assisting decision-makers

in forecasting the full environmental benefits of recycling, expanding resource recovery activities, and interacting with these benefits by determining greenhouse gas benefits, energy, water, and landfill savings.

In recent years, the number of AD facilities for OFMSW has been increasing in many countries. The most important issue for these facilities is the content of the waste entering. The lower the inorganic fraction in the incoming waste, the higher the efficiency of the facility. Reducing the inorganic fraction can be achieved either by separation at the source or by pre-separation at the facility entrance. The latter is possible by knowing the characterization of the waste, and there is limited information on full-scale facilities in the literature. The main aim of this study was to shed light on the physicochemical properties of the mechanically separated OFMSW (ms-OFMSW) and OFMSW at a full-scale AD plant, as well as highlight the environmental benefits of recyclable materials using the Recyclator tool and to promote highly advanced separation technologies and/or source separation.

3.2 Material and Methods

3.2.1 Plant description and sample processing

3.2.2 AD plant

The AD plant is located in the northwest of Turkey. It serves a population of 400,000 people and receives 300 tons/day mixed collected MSW. The facility includes a pre-separation unit, that can be referred as simple mechanical separation (Dehkordi, 2020), in which waste is screened through a trommel having a screen size of 80 mm. Undersize materials are sent to an anaerobic digester after a magnetic separator, which accounts for approximately 50 % of incoming waste, is referred to as ms-OFMSW. Oversize materials are directed to a magnetic separator followed by a hand sorting unit in order to separate recyclable materials. The schematic flow of the full-scale AD plant is shown below in Figure 3.1.

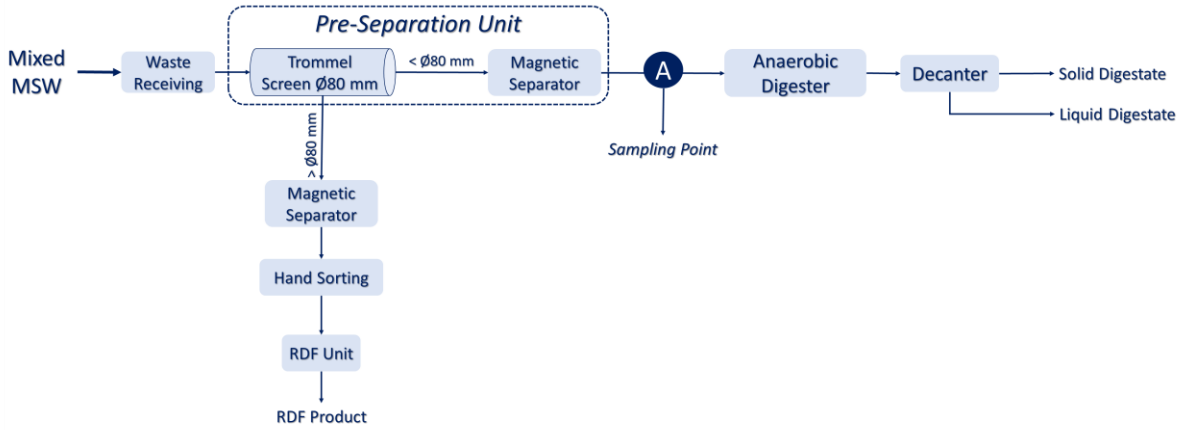


Figure 3.1 : The schematic flow of the full-scale AD plant.

3.2.3 Sampling of waste

The ms-OFMSW samples were collected monthly from the conveyor belt before the anaerobic digester (Point A), as depicted in Figure 3.1. Approximately 48 kg/day composite samples (2 kg per hour) were taken after mechanical separation once in a month over a nine-month period from September 2021 to May 2022. To preserve the characteristics of the waste, samples were immediately brought to the laboratory.

3.2.4 Waste composition

Approximately 48 kg samples were separated into subcategories by hand sorting in the laboratory using the standard method (ASTM D5231-92, 2016). The procedure is described in Figure 3.2 to categorize the non-organics from the ms-OFMSW. Subcategories were defined as organics, plastics, metals, glass, textiles, inert, and miscellaneous. The separated samples were weighed on a balance. After the removal of non-organics, the material is referred to as OFMSW. Based on the formula given below, the percentage of composition for each waste type was calculated:

$$\text{Composition of Waste, \%} = \frac{\text{Weight of separated waste}}{\text{Total mixed waste sample}} \times 100 \% \quad (3.1)$$



Figure 3.2 : The procedure for determination of waste composition.

The mean and standard deviation for the characterization samples of nine months were evaluated by using MS Excel 2019, Microsoft, USA.

3.2.5 Determination of physicochemical characteristics

The physicochemical characteristics were determined for the ms-OFMSW and OFMSW. All parameters were performed in triplicates for physicochemical characteristics. Total solids (TS) and volatile solids (VS) were determined using Standard Methods 2540G (APHA, 2017). A multimeter was used to measure pH and conductivity (Mettler Toledo – S400, Mettler Toledo, USA). The upper calorific value was determined using an Isoperibol oxygen bomb calorimeter (Parr 6200, Parr Instrument Company, USA) in accordance with the ASTM standard method (ASTM D5865/D5865M-19, 2019). 0.5 g of pellets were prepared using a manual press for upper calorific measurement.

The elemental composition of the samples was determined using an elemental analyzer in accordance with the ASTM 5291-21 standard method (Elementar Vario Macro Cube) for carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content (ASTM D5291-21, 2010). For elemental analysis, sample size was reduced to 0.6 mm, and was dried for 3 hours at 103 ± 2 °C prior to analysis. The oxygen content of the samples was calculated using the equation below (ASTM D3176-159, 2016):

Total Oxygen Weight %

$$\begin{aligned} &= 100 - (\text{Total C Weight \%} \\ &+ \text{Total Hydrogen Weight \%} \\ &+ \text{Total Nitrogen Weight \%} + \text{Total Sulfur Weight \%} \\ &+ \text{Total Ash Weight \%}) \end{aligned} \quad (3.2)$$

The empirical and chemical formulas for the OFMSW were calculated using the methodology described by Komilis et al. (2012). The empirical formula was derived, and the chemical formula was calculated using the mass of the waste to determine the elemental composition of the OFMSW.

3.2.6 Environmental benefits assessment

3.2.7 Description tool

The Recyclator online interactive tool, which aims to calculate large-scale recycling initiatives for 21 material types, was used to assess environmental benefits (NSW, 2023). These 21 types are divided into six major categories, which are as follows: a) Metals; b) Concrete, brick, and asphalt; c) Paper and cardboard; d) Organics; e) Glass; and f) Plastics. All of the types have subcategories predefined in the tool, allowing more detailed classification. It aims to broaden resource recovery by improving understanding of the benefits from waste.

This tool assists authorities in calculating the benefits of GHGs while also measuring energy, water, and landfill savings. In this method of calculation, system boundaries (Figure 3.3) begin at the point of waste generation and extend to the processes required to recycle waste (ISO, 2006; ISO 2006). Furthermore, the system boundary is taken into account when producing new materials from raw materials and producing/using fertilizers (EPA, 2010). Thus, the inclusion of additional items in the system allows for the quantification of the system's benefits.

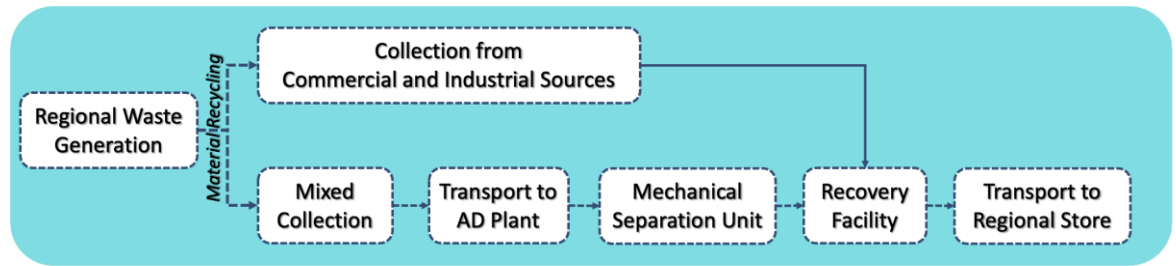


Figure 3.3 : The system boundaries for the calculation.

3.2.8 Methodology

The loss of environmental benefits for inefficient mechanical separation was calculated (EPA, 2010). Thus, in the equation, recycling operations included collection, transportation, and sorting of recycled materials, as well as reprocessing of recycled materials. The recycling operations excluded the production of raw materials or fertilizers, as well as collection, transportation, and landfill treatment. As a result, the net benefit of recycling can be calculated using the method given in the equation below.

Collection, transport and sorting of recycled materials

+ Reprocessing to recycled materials

– Production of virgin material OR Production and use of fertilizers (for organics)

– Collection, transport and treatment in landfill

= Net benefit of recycling

3.3 Results and Discussion

3.3.1 Waste composition

Waste composition reveals organics, recyclable materials, inert and miscellaneous substances in waste streams and is important for proper waste management. The distribution of ms-OFMSW fractions for the organics and non-organics including its sub-categories were shown in Figure 3.4. Organics accounted for $76.5 \pm 1.7\%$ as a nine-months average and represented the majority of the ms-OFMSW. It also showed that ms-OFMSW consisted of considerable non-organics ($23.6 \pm 1.7\%$) which is not suitable for AD. The ms-OFMSW had recyclable (glass, plastics and metals) and non-recyclable (textiles, inert, and miscellaneous) sub-categories which accounted for $9.0 \pm 1.7\%$ and $14.6 \pm 1.7\%$ of the ms-OFMSW, respectively (Table 3.1). Plastics, glass and metals were $4.3 \pm 1.0\%$, $3.3 \pm 0.7\%$ and $1.4 \pm 0.7\%$, respectively while miscellaneous, textiles and inert were $6.6 \pm 1.0\%$, $6.0 \pm 1.2\%$ and $1.9 \pm 0.5\%$, respectively. These results are consistent with the results of the study conducted by Sezer and Arikan (2011). Authors reported the results for the Istanbul region that organics, paper-cardboard, glass and textile occupied 73.9 %, 9.4 %, 4.2 % and 3.9 % of the waste composition after mechanical separation (before the composting unit of the plant). Li et al. (2016) reported that organics was the most abundant component, which accounted for 64.5 % of the ms-OFMSW. Organics and plastics were defined as the major fractions for the ms-OFMSW composition in the study of Miezah et al. (2015), with 67 % and 19 %, respectively.

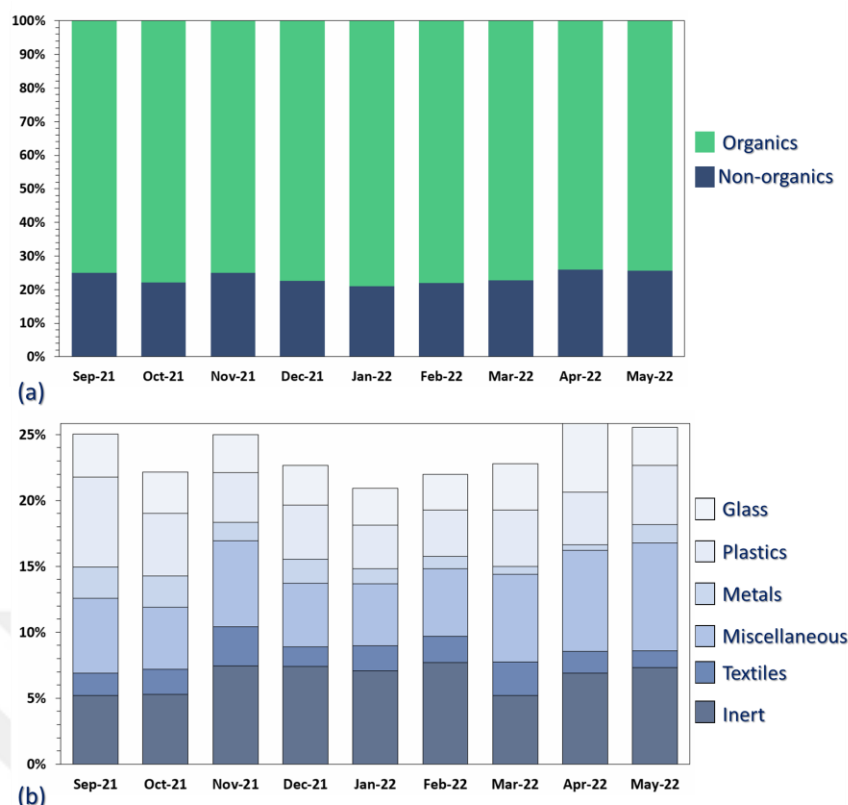


Figure 3.4 : The waste composition of ms-OFMSW a) Organics and non-organics; b) Sub-categories of non-organics.

Table 3.1 : The recyclable and non-recyclable waste distribution of OFMSW.

Waste distribution	Unit	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22
Recyclable	% w/w	12.49	10.23	8.05	8.95	7.25	7.15	8.38	9.65	8.79
Non-recyclable	% w/w	12.56	11.91	16.94	13.72	13.69	14.83	14.41	16.21	16.76

3.3.2 The physicochemical characteristics

The average values for physicochemical properties of the ms-OFMSW, as well as for the OFMSW were given in the Table 3.2. The pH of the ms-OFMSW and the OFMSW were 6.7 ± 0.5 and 6.8 ± 0.1 , respectively and the results were in line with the literature (Campuzano and González-Martínez, 2016; Cesaro et al., 2016). In the study of Castaldi et al. (2009) and Nzihou (2020) the pH of the OFMSW was reported to be in the ranges of 5.0 to 8.0. The VS/TS ratio was calculated as 70.7 ± 7.7 % for the ms-OFMSW and as 82.8 ± 6.1 % for the OFMSW. The increase in the VS/TS % was

related to the removal of non-organics from ms-OFMSW. In addition, a slight decrease in TS and VS content (9 % and 2 % decrease in TS and VS, respectively) of the ms-OFMSW was observed for the ms-OFMSW. The average VS/TS ratio for the OFMSW was in the range (61 – 95 %) reported by Campuzano and González-Martínez (2016). Allegue et al., (2020) reported that pre-sorted MSW has an 81.2 % VS/TS ratio which is consistent with the results obtained for the OFMSW.

The average values for elemental composition of the ms-OFMSW, as well as for the OFMSW were given in Table 3.3. C content was determined as 34.5 ± 3.4 % and 29.8 ± 0.8 % for the ms-OFMSW and for the OFMSW, respectively. Mironov et al. (2021) reported that the C content was occupying 36.0 ± 3.2 % of the ms-OFMSW which was consistent of the findings of this present study for the ms-OFMSW. While Komilis et al. (2012) found higher (40.5 ± 12 %) C for the OFMSW. They also found higher C/N (28.9 ± 15 %) than that of the calculated in this study (17.7 ± 3.3). The average O content was 46.9 ± 7.6 % and 58.5 ± 2.1 % for the ms-OFMSW and for the OFMSW, respectively and was higher than the reported values. O content for the OFMSW was ranging from 22.5 to 39.2 % by Nzihou (2020). Sailer et al. (2021) reported that elemental composition of the OFMSW for the C, N, H, S, and O were 42.9 – 49.8 %, 2.3 – 3.4 %, 5.6 – 6.7 %, 0.2 – 0.4 %, and 38.9 – 44 %, respectively.

Table 3.2 : The physicochemical characteristics for the ms-OFMSW and OFMSW.

Parameter	Unit	ms-OFMSW	OFMSW
		(Average $\pm$ Standard Deviation)	(Average $\pm$ Standard Deviation)
pH	-	6.7 ± 0.5	6.8 ± 0.1
TS	%	46.6 ± 4.8	37.2 ± 5.9
VS	%	33.2 ± 6.0	31.1 ± 6.7
VS/TS	-	70.7 ± 7.7	82.8 ± 6.1
Conductivity	mS/cm	6.1 ± 0.6	7.0 ± 0.8
Upper Calorific Value	J/g	$14,920 \pm 648$	9803 ± 565

Table 3.3 : The elemental composition for the ms-OFMSW and OFMSW.

Parameter	Unit	ms-OFMSW	OFMSW
		(Average $\pm$ Standard Deviation)	(Average $\pm$ Standard Deviation)
C	%	34.5 $\pm$ 3.4	29.8 $\pm$ 0.8
H	%	3.5 $\pm$ 0.2	3.1 $\pm$ 0.1
N	%	2.9 $\pm$ 0.4	1.7 $\pm$ 0.3
O	%	46.9 $\pm$ 7.6	58.5 $\pm$ 2.1
S	%	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1
C/N	-	11.9 $\pm$ 1.4	17.7 $\pm$ 3.3
Chemical Formula	-	C ₉₀ H ₁₁₁ N ₆ O ₉₂ S	C ₉₃ H ₁₁₆ N ₅ O ₁₃₆ S

The upper calorific value decreased from 14,920 $\pm$ 648 J/g for ms-OFMSW to 9803 $\pm$ 565 J/g for OFMSW due to removal of high calorific value components such as plastics. Also, these values are comparatively higher than the reported values (6401 J/g) for the mixed MSW in the study of Cheela et al. (2021). The empirical formula was derived from the elemental composition for the OFMSW: C₉₆H₁₂₁N₅O₁₄₂S. Following the empirical formula, chemical formula was calculated as C₁₀₀H₁₂₆N₅O₁₄₈S for the OFMSW. After removing the waste's S content, Komilis et al. (2012) calculated the chemical formula of the OFMSW as C₃₂NH₅₆O₁₆. Browne and Murphy (2013) and Fongsatitkul et al. (2010) reported the chemical formulas of the ms-OFMSW as C_{16.4}H₂₉O_{9.8}N and C₂₅H_{42.5}O₂₀N, respectively. The chemical and empirical formulas derived for the waste can vary due to waste composition and analysis sensitivity, making it difficult to compare values obtained from different studies.

3.3.3 Loss of environmental benefits

The following assumptions were made for calculating the environmental benefits of the AD plant, as shown in Table 3.4. The amount of each waste sub-categories was determined for waste composition study and used as input parameters. It was estimated that 13.5 tons of recyclable materials (metals, glass, and plastics) were found in the ms-OFMSW.

Table 3.4 : Input parameters for the Recyclator tool.

Input Parameters	Value (tons)
MSW ^a	300.0
ms-OFMSW ^a	150.0
Metals ^b	2.1
Glass ^b	4.9
Plastics ^b	6.5

^a: Representing the values of the full-scale AD plant.

^b: Representing the calculated values obtained from the waste composition.

The GHG, energy, water, and landfill savings were calculated based on the input parameters for recyclables and results were given in the Table 3.5. Positive values represent the benefits of the waste stream, whereas negative values indicate the negative impact of waste stream which resulted in a decrease for the benefits or savings. Metals (399.7 GJ) and plastics (408.7 GJ) both saved almost the same amount of energy. Metals saved the most water (421.8 m³), which had the greatest positive impact. Plastics, on the other hand, had a negative impact on water savings (-73.0 m³). Due to the necessary washing procedures to prepare the recovered material for reprocessing, water consumption for plastics recycling processes was negative (NSW, 2023). In addition to energy and water savings, the recycling of metals and plastics also had a significant impact on reducing landfill space. The 117.0 m³ of landfill space saved demonstrates the environmental benefits of diverting these materials from disposal sites. This reduction in landfill waste helps to conserve natural resources and minimize pollution associated with waste decomposition.

Table 3.5 : Environmental benefits and savings from the waste streams.

Benefits/Savings	Unit	Waste Streams			
		Metals	Glass	Plastics	Total
Greenhouse benefits	tons CO ₂ eq	37.0	3.1	10.3	50.4
Energy savings	gigajoules (GJ)	399.7	33.7	408.7	842.1
Water savings	m ³	421.8	12.0	-73.0	360.8
Landfill savings	m ³	15.0	11.8	90.2	117.0

The greenhouse gas emissions associated with landfilling are primarily caused by methane (CH₄) and CO₂ present in biogas produced by anaerobic bacteria using waste as a carbon source. Nonetheless, only methane emissions contribute to global warming

because CO₂ emissions are only a partial "return" to the atmosphere of the same gas used by photosynthetic organisms to build biomass. Significant differences may exist depending on the separate collection and treatment scheme employed. Calabrò (2009) reported that source collection reduced GHG emissions by 159 kg CO₂/ton for glass and 396 kg CO₂/ton for plastics. Because composting requires more energy, CO₂ is emitted, and the author reported that food and garden waste, also known as organics, had -42 kg CO₂/ton GHG emissions. The findings of this study clearly show that separate collection and recycling reduce GHG emissions; in fact, emissions decrease as the separation rate increases. It is critical to note that, with proper disposal technology selection, MSW management can become a carbon sink.

By implementing effective waste management strategies, such as incineration with energy recovery or landfill gas capture, the carbon emissions from MSW can be significantly reduced or even turned into a net negative emission process. This highlights the importance of investing in advanced disposal technologies to maximize the potential of MSW as a valuable resource in combating climate change. Additionally, promoting public awareness and participation in recycling programs can further contribute to minimizing GHG emissions and achieving a more sustainable waste management system. Implementing source separation programs, where individuals separate recyclables from other waste at the point of disposal, can help ensure that materials are properly sorted and can be recycled more efficiently. Furthermore, investing in technological advancements such as automated sorting systems and advanced recycling facilities can increase the capacity and effectiveness of recycling processes, allowing for higher rates of material recovery and reducing the overall environmental impact of waste management.

3.4 Conclusions

In this study, waste composition and physicochemical properties of the ms-OFMSW and OFMSW were determined and compared. Organics made up the majority of the ms-OFMSW ($76.5 \pm 1.7\%$), while $9.0 \pm 1.6\%$ of the recyclable wastes escaped mechanical separation. Removing non-organics increases the VS/TS ratio by $17.8 \pm 8.8\%$, which can have a considerable impact on anaerobic digestibility of the waste.

Improved mechanical separation or source separation would boost recycling while saving energy and water. This study highlighted the need for advanced mechanical separation to increase recyclable materials in addition to improving AD performance. It is strongly advised to consider implementing advanced tools at the facilities prior to sending organics for digestion and/or, if applicable, conducting waste source separation.





4. ENHANCEMENT OF METHANE PRODUCTION BY ELECTROHYDROLYSIS PRETREATMENT FOR ANAEROBIC DIGESTION OF OFMSW²

4.1 Introduction

Municipal solid waste (MSW) generation is expected to reach 2.2 billion tons globally by 2025, with a 375.5 billion USD of management cost, which is typically composed of food waste, paper, glass, metals, plastics, textiles, yard trimmings, and so on (Negi et al. 2019). MSW characteristics show discrepancy around the world; its generation in developing countries contains a high proportion of organic waste, whereas in developed countries is more diverse, with a higher proportion of plastics and paper. As a result, effective waste management strategies should be tailored to each region's specific MSW characteristics, taking into account factors such as economic development, population density, and cultural practices, as well as promoting waste reduction and recycling, which can help reduce the environmental impact and economic cost of MSW management.

When the organic fraction of municipal solid waste (OFMSW) is not controlled, it can harm the environment, so it must be treated properly. OFMSW can be managed using anaerobic digestion (AD), composting, incineration, or landfilling. To produce a high-quality product, composting, for example, necessitates a larger area and more time, whereas incineration releases toxic emissions and requires ash residue management (Pour et al., 2021). Due to greenhouse gases (GHGs) emissions, landfilling is the least preferred option. As a result, among other treatment methods, AD has become as the

² This chapter is based on “Kabakci, Y., Kosar, S., Dogan, O., Uctug, F. G., & Arikan, O. A.”. 2024. Enhancement of methane production by electrohydrolysis pretreatment for anaerobic digestion of OFMSW. *Environmental Research*, 240, 117534. DOI: 10.1016/j.envres.2023.117534

emerging technology, producing biogas and nutrient-rich digestate. Its popularity has grown due to its ability to reduce GHGs and produce energy in a sustainable manner (Nzihou, 2020). The sale of biogas-generated electricity, heat, or fuel has the potential to generate revenue from this process (Govender et al., 2019). OFMSW is a good feedstock for AD owing to its high moisture content and biodegradability. Furthermore, AD of OFMSW can help reduce the amount of waste that ends up in landfills. AD is regarded as a cost-effective and environmentally friendly method of treating OFMSW (Mata-Alvarez, 2003). When compared to other waste treatment technologies, the advantages of AD include low energy consumption, methane (CH_4) gas production, waste stabilization, waste volume reduction, and the small footprint (Nzihou, 2020). However, AD has some drawbacks, such as the necessity of constant monitoring of crucial parameters including pH, temperature, feed rate, and the inhibitors. Furthermore, there is a requirement for post-treatment of digester effluent before being discharged to the environment if there is no beneficial use (Náthia-Neves et al., 2018).

Although AD of solid waste is a promising solution, previous research has revealed low biodegradation rates of OFMSW due to the complexity of the organic material during digestion (Logan and Visvanathan, 2019). The hydrolysis of complex organic matter to soluble particles has been shown to be the rate-limiting step in AD process (Menzel et al., 2020). Thus, pretreatment processes are frequently used to increase organic waste solubilization and improve the efficiency of anaerobic waste decomposition by breaking down complex polymeric organics into simpler molecules (Panigrahi and Dubey, 2019). Mechanical (Romero-Güiza et al., 2014), ultrasound (Rasapoor et al., 2016), thermal (Yi et al., 2014), microwave (Pecorini et al., 2016), chemical (Cesaro et al., 2019), and biological (Carrere et al., 2016) pretreatment methods have all been studied previously.

The process of solubilizing complex organic matter by breaking the bonds with flowing direct current (DC) and ions is known as electrohydrolysis (Mahmoud et al., 2010). Pretreatment steps for electrohydrolysis include ohmic heating, electrophoresis, and electro-osmosis. When electrical energy is passed through a material, resistance and heat are produced, which is referred to as ohmic heat

(Varghese et al., 2014). Particle scattering occurs in the fluid because of the uniform electric field's stimulus during electrophoresis. The velocity (v) of ionic particles changes as their mobility (m) and field strength (E) change ($V = mE$) (Mahmoud et al., 2010). The flow of liquid caused by the induction of applied potential is known as electro-osmosis. When delivered to any substance, DC causes ionization and the production of electrolyte (Veluchamy et al., 2017).

Electrohydrolysis pretreatment for AD has been applied for various substrates such as wastewater treatment sludge (Bora et al., 2020; Wang et al., 2022; Lin et al., 2023). However, limited research has been conducted on the electrohydrolysis pretreatment for OFMSW. When compared to thermal and ultrasonic pretreatment, Habarakada Liyanage and Babel (2020) found that electrohydrolysis of food waste which was collected from the Engineering Faculty canteens at Thammasat University, Thailand resulted in the highest organic solubilization. The objective of this study was to determine the effect of electrohydrolysis pretreatment on anaerobic digestibility of OFMSW with a specific purpose on decreasing the lag phase, the rate-limiting step for digestion.

4.2 Materials and Methods

4.2.1 AD plant description and sample collection

Inoculum and OFMSW samples were collected from a full-scale AD plant that serves a population of 400,000 people and receives 300 tons of mixed-collected MSW daily. The facility includes a pre-separation unit, also known as simple mechanical separation (Dehkordi et al., 2020), in which waste is screened by an 80 mm trommel. Undersize materials are sent to anaerobic digesters after being separated by a magnetic separator, which accounts for 50% of the incoming waste and is referred to hereafter as ms-OFMSW. Oversize materials are directed to a magnetic separator, which is followed by a hand sorting unit to separate recyclable materials. Figure 4.1 depicts the schematic flow of the whole treatment of MSW including the full-scale AD plant.

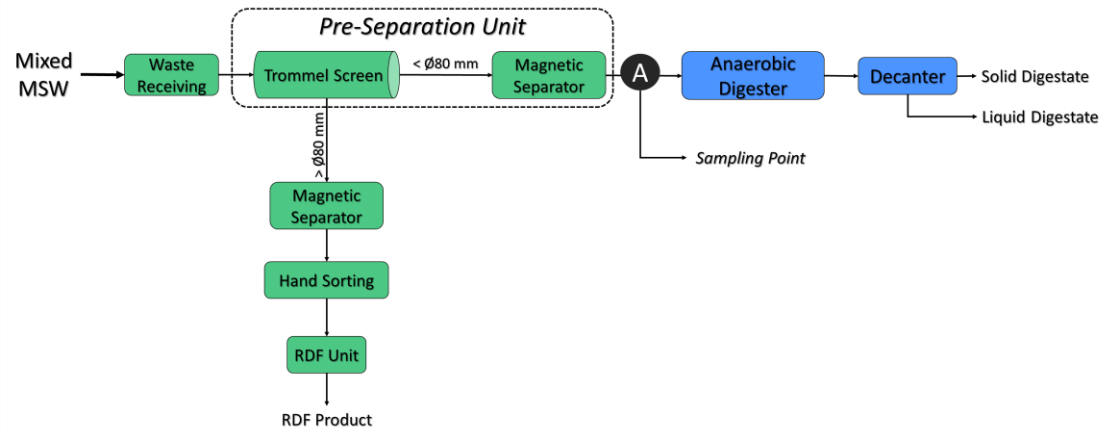


Figure 4.1 : The schematic flow of the whole treatment of MSW.

The ms-OFMSW samples were taken from the conveyor belt before the anaerobic digester (sampling point). After mechanical separation, 48.6 kg of ms-OFMSW composite samples (2 kg per hour) were collected. To preserve the waste's characteristics, samples were brought to the laboratory within 3 hours. These samples were then hand-sorted in the laboratory to obtain organics (referred to hereafter as OFMSW).

4.2.2 Physicochemical characteristics of inoculum and OFMSW

The pH of inoculum was measured as 6.9 ± 0.1 while the solids content was measured as $23.8 \pm 1.9\%$ and $15.2 \pm 1.9\%$ for TS and VS, respectively.

The physicochemical characteristics of the OFMSW were presented in Table 4.1. The pH was 6.1 ± 0.1 and it was consistent with the values (5.0 to 8.0) reported by Nzihou (2020). The VS/TS ratio of the OFMSW was $40.5 \pm 2.5\%$. In the study of Li et al. (2016) VS/TS ratio for OFMSW was in between 39-61% which was in line with the findings of this study. The C/N ratio of the OFMSW in this study was 15.6 ± 1.1 , which falls within the recommended range of 11-21 for efficient AD (Chow et al., 2020). Maintaining an appropriate C/N ratio is crucial for optimal microbial activity and biogas production in AD systems (Salangsang et al., 2022). In the study of Paritosh et al. (2018), the C and N elemental compositions of the OFMSW were 37.6 – 51.3% and 1.5 - 3.8% respectively. Values for OFMSW can change substantially

depending on several factors such as geography, climate, economy, eating and social habits, etc. (Kaza et al., 2018).

Table 4.1 : Physicochemical characteristics of OFMSW*.

Parameter	Unit	OFMSW (Average $\pm$ Standard Deviation)
pH	-	6.1 ± 0.1
TS	%	44.8 ± 1.8
VS	%	18.5 ± 0.6
VS/TS	%	40.5 ± 2.5
Conductivity	mS/cm	6.7 ± 0.4
Upper Calorific Value	cal/g	3682 ± 100
Elemental Composition		
Carbon (C)	%	36.5 ± 0.5
Hydrogen (H)	%	1.5 ± 0.4
Nitrogen (N)	%	2.4 ± 0.2
Sulphur (S)	%	0.8 ± 0.1
Oxygen (O)	%	32.6 ± 2.1
C/N	-	15.6 ± 1.1

*All the analysis were on wet base except upper calorific value and elemental analysis.

4.2.3 Electrohydrolysis experimental setup

Electrohydrolysis pretreatment was applied to OFMSW samples at 20 volts as suggested by Habarakada Liyanage and Babel (2020) for 30 and 60 minutes (referred to hereafter as 30-min. treated, and 60-min. treated) by using a custom-made electrohydrolysis setup as shown in Figure 4.2. The setup consists of a closed, cylindrical reactor with a 1-liter capacity that has a cathode in the inner (D: 4 mm) and an anode in the outer (D: 2mm) parts which are separated by 4 cm from each other and a DC power supply.

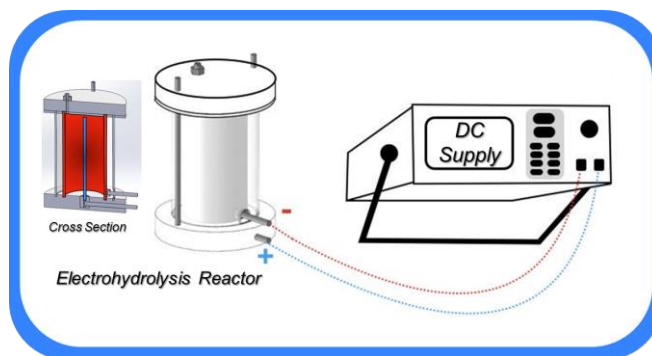


Figure 4.2 : The schematic view of the electrohydrolysis reactor configuration.

DC was delivered to the sample that was kept in the reactor with the aid of electrodes made up of stainless steel. The sample (600 g) was placed inside the reactor and then supplied with electricity. An external ammeter was used to monitor the current and applied voltage continuously. This setup allowed the electrohydrolysis to occur between the anode and cathode, with the Plexiglas layer protecting the reactor from any potential damage. The use of an external ammeter ensured the precise control and monitoring of the process.

4.2.4 BMP Batch Tests

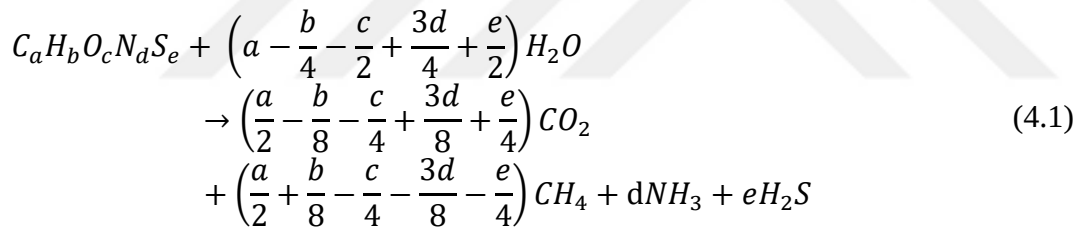
4.2.5 BMP experimental setup

The BMP test was carried out in duplicates using an automated methane potential test system ECHO ER12 D6691 (Echo Instruments, Slovenia). The total working volume of the inoculum and substrate mixture was 400 mL. Inoculum source (Gu et al., 2014; Koch et al., 2017) and substrate to inoculum (S:I) ratio have significant impact on the AD process (Demichelis et al., 2022). The inoculum was incubated for 10 days as recommended by Demichelis et al. (2022) before using for the BMP experiment to facilitate the increase of the S:I ratio and allow more OFMSW to be treated with the same volume of the AD reactor while achieving higher biogas yield and CH₄ content. S:I ratio affects AD because a good S:I ratio can balance the microorganisms in the inoculum and facilitate the most important step hydrolysis, (Li et al., 2022). In this study, 1:2 S:I ratio was used based on the amount of VS. BMP tests were performed and followed with the protocol proposed by Angelidaki et al. (2009). To ensure the

accuracy and reliability of the test results, cellulose microcrystalline (Sigma Aldrich, USA) was used as a positive control. The test lasted 30 days and the temperature was kept under thermophilic (55°C) conditions which is the same with the full-scale AD plant. The volume of CH₄ produced during the test period was monitored online and used to calculate the BMP. Nitrogen gas was purged to the bottles for 2-3 min to maintain anaerobic conditions following the addition of macronutrient (phosphate buffer) and micronutrients (0.25 mg/L FeCl₃·7H₂O; 27.5 g/L, CaCl₂·2H₂O; 22.5 g/L, MgSO₄·7H₂O; 0.05 mg/L, NiCl₂·2H₂O; 0.05 mg/L, Co(NO₃)₂·6H₂O).

4.2.6 Theoretical BMP calculations

A given biomass's elemental composition, chemical composition, or chemical oxygen demand (COD) can be used to calculate the theoretical BMP. The theoretical BMP value can be used to estimate the potential biogas yield of a particular biomass under specific AD conditions which is useful for designing and optimizing AD systems. Buswell and Mueller (1952) proposed the following formula for calculating the theoretical value of CH₄:



$$TBMP \left(\frac{L}{gVS} \right) = \frac{22.4 \times \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4} \right)}{12a + b + 16c + 14d + 32e} \quad (4.2)$$

Experimental BMP values for a given substrate are frequently lower than the theoretical BMP values, which can be calculated with the formula provided below (Nzihou, 2020):

$$BMP_{Experimental} = \frac{CH_4 \text{ produced, mL}}{VS_{added, g}} \quad (4.3)$$

The difference between theoretical and experimental BMP is due to the substrate containing non-biodegradable or barely biodegradable substances such as lignin or because a portion of the substrate is used to synthesize bacterial biomass (Nzihou, 2020).

4.2.7 BMP kinetics and model fitting

The modified Gompertz model which is a function that expresses cell density in terms of exponential growth rates and lag phase (λ) duration during bacterial growth periods (Kafle and Chen, 2016; Nzihou, 2020) was used in this study. Model is based on the hypothesis that the rate of CH₄ production in a batch digester corresponds to the growth rate of methanogenic bacteria. The model equation was given below and frequently employed in the simulation of CH₄ accumulation because it has been found to be a good empirical non-linear regression model (Equation 4.4):

$$P(t) = P_0 \times \exp \left\{ -\exp \left[\frac{R_{max} \cdot x \exp(1)}{P_0} (\lambda - t) + 1 \right] \right\} \quad (4.4)$$

P_t = Modelled cumulative CH₄ yield (mL CH₄/g VS)

P_0 = Modelled highest cumulative CH₄ yield (mL CH₄/g VS)

$R_{max.}$ = Maximum methane production rate (mL CH₄/g VS.day),

λ = lag phase (day),

t = time (day).

The lag phase denotes the shortest amount of time needed to produce biogas or for bacteria to adapt to their environment. The maximum CH₄ production rate ($R_{max.}$), which is a measure of the specific growth rate of methanogenic bacteria, indicates how much CH₄ is produced per unit of time.

The Solver is an MS-Excel® add-in tool, which allows users to find the best values for the kinetic equation constants to fit the experimental data, was used in this study. The kinetic parameters can be determined with greater accuracy and efficiency using this tool. In the modified Gompertz model, the constants in the solver tool were CH₄ production potential (P_0), maximum CH₄ production rate ($R_{max.}$), and lag phase. The

solver function iterates through the constant values to minimize the root-mean-square error between the two graphs. Based on the iterations, the optimal constant values were identified as the model's outputs. A graph can be created using the simulated dataset and the experimental values of cumulative CH₄ yield (Khadka et al., 2022).

The Online Biogas App (OBA), an interface from the R biogas package, was also used to estimate the BMP of the OFMSW in addition to MS-Excel® add-in tool. In order to calculate BMP values based on input data, the OBA employs a number of models, such as the first-order kinetic model and the modified Gompertz model to verify the BMP estimates' accuracy (Hafner et al., 2018).

4.2.8 Analytical experiments

TS and VS were determined according to the Standard Methods 2540G (APHA, 2017). Using a multimeter, pH and conductivity were measured (Mettler Toledo - S400). The upper calorific value was determined using an isoperibol oxygen bomb calorimeter according to the ASTM standard procedure, (Parr 6200) (ASTM D5865/D5865M-19, 2019). The samples were weighed manually using a press to weigh 0.5 g of pellets in order to prepare them for upper calorific measurement. The samples' C, H, N, and S contents were determined using an elemental analyzer (Elementar Vario Macro Cube) (ASTM D5291-21, 2010). For elemental analysis, samples were dried for three hours at 103±2°C and ground to a size of 0.6 mm. The following equation was used to determine the samples' oxygen content (ASTM D3176-159, 2016):

$$\begin{aligned} \text{Total Oxygen Weight \%} &= 100 - (\text{Total C Weight \%} \\ &+ \text{Total Hydrogen Weight \%} \\ &+ \text{Total Nitrogen Weight \%} + \text{Total Sulfur Weight \%} \\ &+ \text{Total Ash Weight \%}) \end{aligned} \quad (4.5)$$

The chemical formula for the OFMSW was calculated using the methodology described by Komilis et al. (2012).

4.3 Results and Discussion

4.3.1 BMP batch tests results

The electrohydrolysis pretreatment was applied to OFMSW and BMP tests were carried out for untreated and treated OFMSW samples for 30 days. The untreated, 30-min. treated, and 60-min. treated sample produced 480.0, 437.9 and 452.0 mL/gVS_{added} biogas, respectively (Figure 4.3b). The CH₄ content of biogas was measured as 47, 53 and 55% for the untreated and treated samples (30-min. treated and 60-min. treated), respectively (Figure 4.3a). The AD process has the potential to generate biogas with 50-70% CH₄, 25-40% CO₂, and trace gases (1-5%), which makes it a source of clean energy (Atelge et al., 2020). Our results were consistent with the reported ranges of CH₄ and CO₂ for OFMSW by Atelge et al. (2020).

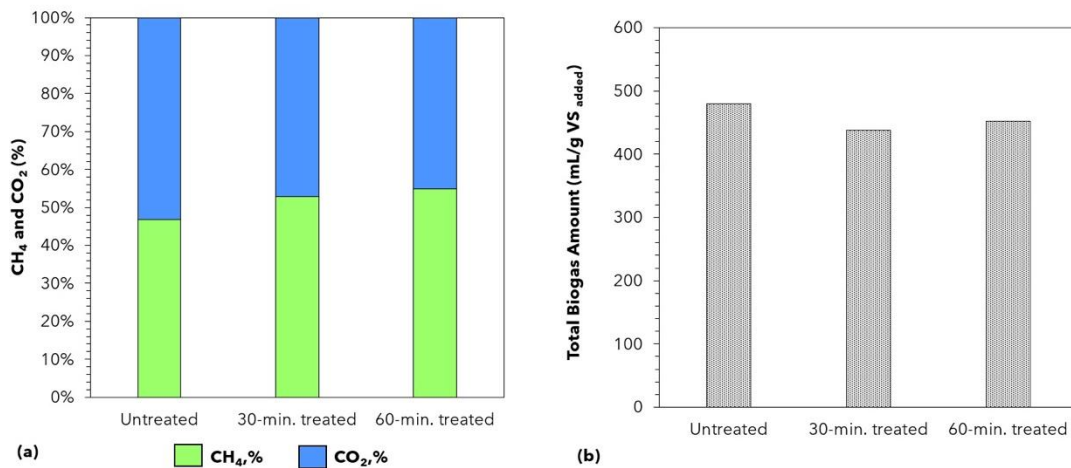


Figure 4.3 : (a) CH₄ and CO₂ % and (b) Total biogas amount for untreated, 30-min. treated, and 60-min. treated OFMSW samples.

The untreated OFMSW sample yielded a total CH₄ of 225 ± 2 mL CH₄/g VS_{added} and it was in agreement with the study of Bala et al. (2019). Zamri et al. (2021) reported that the BMP of the OFMSW ranged between 177 to 550 mL CH₄/g VS_{added}. The CH₄ production increased to 231 ± 4 and 248 ± 7 mL CH₄/g VS_{added} for 30-min. treated and 60-min. treated OFMSW samples, respectively, following the electrohydrolysis. CH₄ production increased 3% and 10% for 30-min. treated and 60-min. treated OFMSW samples, respectively. According to Kainthola et al. (2019), 60 minutes of

electrohydrolysis pretreatment of rice straw at 25 volts increased the total CH₄ yield by 42.4% compared to the untreated sample. Authors also reported that 25 volts was the best voltage for electrohydrolysis pretreatment because higher voltages resulted in lower CH₄ yields for organic matter degradation. These results point to electrohydrolysis pretreatment as a potential technique for enhancing biodegradability and yielding higher CH₄ production.

4.3.2 Kinetics and model fitting

Kinetics parameters were estimated by using the formula provided for modified Gompertz model and the results were shown in Table 4.2. The estimated parameters demonstrate the microbial population's delayed response to environmental change and its subsequent adaptation in the system. Based on the value of λ , which ranges from 0 to 9.90 days, the likelihood of an organic conversion can be predicted. A higher λ value denotes an irreversible process inhibition and a low rate of degradation. In this study, the λ value was similar for the 30-min. treated (5.6 days) and 60-min. treated (5.9 days) OFMSW samples. On the other hand, the 30-min. treated OFMSW sample showed 22.1 mL CH₄/g VS_{added}.day CH₄ production rate (R_{max}) which was higher than the 60-min. treated (20.7 mL CH₄/g VS_{added}.day) sample. The model's validity was assessed further by comparing the experimental findings with the expected values. R^2 values were found to be 0.887 and 0.913 for the 30-min. treated and 60-min. treated OFMSW samples, respectively. In other studies, using various mathematical models, similar results were also reported for the electrohydrolysis of rice straw (Kainthola et al., 2019).

Table 4.2 : Parameters and goodness of fit received from the evaluated modified Gompertz model.

Sample	$R_{max.}$ (mL CH ₄ /g VS _{added} .day)	λ (day)	CH ₄ Production, mL CH ₄ /gVS _{added}		
			Experimenta l	P ₀	P _t
Untreated	30.1	9.9	225.1	232. 8	232. 0
30-min. treated	22.1	5.6	231.2	241. 6	239. 6
60-min. treated	20.7	5.9	248.2	266. 2	260. 8

The theoretical CH₄ potential was calculated as 341.4 mL CH₄ /g VS_{added} and the chemical formula determined as C₁₄₄H₆₉N₈O₉₇S for the untreated OFMSW sample. The results showed that 66% of the theoretical CH₄ was efficiently yielded by the experimental study for the untreated OFMSW sample. The results of this study demonstrated a strong relationship between experimental and predicted values. The experimental outcomes matched those predicted by the modified Gompertz model and shown in Figure 4.4. The 60-min. treated OFMSW sample recorded the highest total CH₄ production.

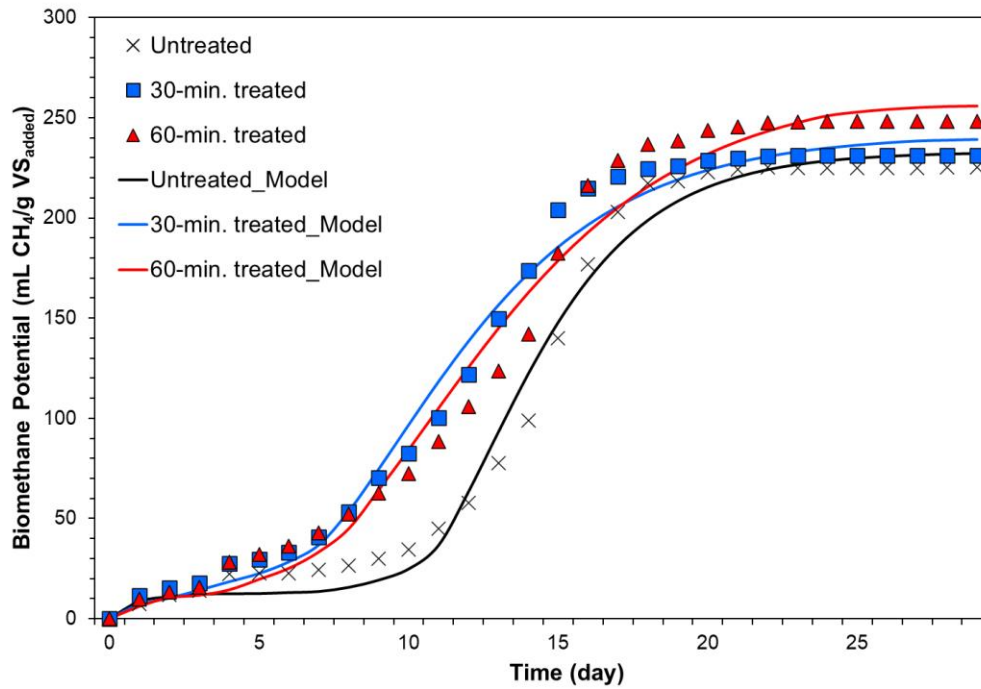


Figure 4.4 : Modified Gompertz model fitting for cumulative CH₄ yield by electrohydrolysis pretreatment for the untreated, 30-min. treated, and 60-min. treated OFMSW samples.

4.4 Conclusions

In this study, electrohydrolysis pretreatment was investigated to enhance the anaerobic digestibility of OFMSW. For this purpose, samples were pretreated with electrohydrolysis for 30 and 60 minutes. The results showed that the time required for hydrolysis, also known as the "lag phase", was reduced up to 43% by electrohydrolysis pretreatment. The cumulative CH₄ production of the OFMSW was increased up to 10% by electrohydrolysis pretreatment. Electrohydrolysis may prove to be a valuable pretreatment method for decreasing the lag phase and as well as increasing the potential for CH₄ production for OFMSW.



5. EFFECT OF DIRECT ENZYME ADDITION ON THE ANAEROBIC DIGESTION OF OFMSW³

5.1 Introduction

The organic fraction of municipal solid waste (OFMSW) is made up of proteins, starchy, fatty, and cellulosic materials. It is difficult to convert OFMSW into soluble fractions, and its management is an important part of long-term waste management practices. OFMSW provides a renewable, abundant, and low-cost source of raw materials for the production of a variety of value-added products such as biofuels, bioplastics, bio-pesticides, organic acids, other chemicals (i.e., acetone and butanol, glycerol), and enzymes (i.e., lipase, amylase, and pectin) (Tyagi et al., 2018). It is important to note that as income levels rise, the percentage of organic matter in waste tends to decrease. In low- and middle-income countries, food and green waste account for more than 50 % of the total waste generated (Kaza et al., 2018). Conversely, in high-income countries, the amount of organic waste is comparable in absolute terms, but due to higher amounts of packaging waste and other nonorganic waste, the fraction of organic waste is around 32% (Kaza et al., 2018).

Common methods of OFMSW treatment include composting, incineration, and landfilling, each with its unique set of challenges and considerations. Composting, for example, requires a larger area and more time to produce a high-quality product, whereas toxic emissions and ash residue management are required for incineration. Landfilling is no longer a viable waste management option in many countries in Europe, and around the world due to environmental concerns such as enormous land use, leachate production, greenhouse gas (GHG) emissions, and so on. The application of AD to organic wastes has grown in popularity among policymakers owing to its credibility as a reliable technology (Cecchi et al., 2011). Under the New Green Deal,

³ This chapter is based on “Kabakci, Y., Kosar, S., Dogan, O., Uctug, F. G., & Arikan, O. A.”. 2024. Effect of direct enzyme addition on the anaerobic digestion of OFMSW. *Sustainable Chemistry and Pharmacy*, 37, 101415. DOI: 10.1016/j.scp.2023.101415

the European Union (EU) aims to achieve carbon neutrality by 2050 (EUR-Lex-52019DC0640-EN-EUR-Lex, 2023). Bioenergy production, which accounts for 70 % of total renewable energy supply, is a cornerstone of the EU climate strategy (IRENA, 2020). Anaerobic digestion (AD) of organic waste could play a critical role in meeting the key target of 32 % renewable energy by 2030 (Rossi et al., 2022). AD offers low energy consumption, methane (CH₄) gas production, waste stabilization, waste volume reduction, and a small footprint (Nzihou, 2020). It has the potential to generate revenue through the sale of biogas-generated electricity, heat, or fuel (Arthurson, 2009). Additionally, AD of OFMSW can help reduce the amount of waste that ends up in landfills, thus extending their lifespan. However, the use of AD for OFMSW, on the other hand, is still limited. The high moisture content and biodegradability of OFMSW make it an ideal feedstock for AD, rendering it a cost-effective and environmentally friendly method for treating this fraction of municipal solid waste (MSW).

Despite the fact that AD is a promising technology, previous research has revealed that OFMSW biodegradation rates are low due to the complexity of the organic material during digestion (Logan and Visvanathan, 2019). The hydrolysis of complex organic matter into soluble particles has been identified as the rate-limiting step in the AD process (Menzel et al., 2020). In order to increase the efficiency of anaerobic waste decomposition, pretreatment is frequently used (Panigrahi and Dubey, 2019). Previous research has looked into mechanical (Romero-Güiza et al., 2014), ultrasound (Rasapoor et al., 2016), thermal (Yi et al., 2014), microwave (Pecorini et al., 2016), chemical (Cesaro et al., 2019), and biological (Carrere et al., 2016) pretreatment methods applied to OFMSW. The superiority of biological pretreatment over thermochemically treated substrates has been demonstrated by an increase in AD performance (Paritosh et al., 2018).

Given the heterogeneous and inconsistent composition of OFMSW, enzymatic hydrolysis is particularly challenging. Enzyme cocktails (mixtures of different types of enzymes) that are capable of degrading complex substrates like carbohydrates (cellulase, hemicellulose, pectinase), lipids (lipase, lipolytic acyl hydrolase, lipoxxygenase), and proteins (protease) have been used to address this issue (Mlaik et al., 2020). Yin et al. (2016) showed that substrate hydrolysis can be improved by promoting the microbial growth via using commercially available enzymes.

Ariunbaatar et al. (2014) reported that enzyme treatment is likely to be the most preferred method when energy requirements, economic costs, and environmental impact are considered.

Enzyme can be employed in four different ways: 1) Dose to AD directly; 2) Pretreated and dosed to AD; 3) Dose to AD in two phases; and 4) Dose to AD via recirculated leachate (Brémond et al., 2018). Enzyme addition has been investigated for different biomass including source-sorted MSW (Rintala and Ahring, 1994), sludge (Rajin et al. 2018, Villa et al. (2022), silage, straw, and animal manure (Weide et al., 2020). Determining of optimum enzyme dose is critical for enzyme application. Rajin et al. (2018) examined substrate and enzyme type to increase anaerobic digestion and determined that 0.06 g amylase/g dry sludge was the optimal dose. Villa et al. (2022), on the other hand, demonstrated the effect of enzyme dose to sludge by showing 14 % and 10 % higher biogas production when enzyme added in bulk or gradually, respectively. Dubrovskis et al. (2019) showed that 0.5 mL alpha amylase enzyme addition to birch leaves pellets increased the average CH₄ content by up to 23 %. To the best of our knowledge, no previous studies on the addition of the alpha amylase enzyme, obtained from *Aspergillus oryzae*, to OFMSW are available. The main aim of this study was to determine the effect of alpha amylase enzyme on the anaerobic biodegradability of OFMSW to maximize organic matter solubilization. For this purpose, BMP experiment was conducted by adding alpha amylase enzyme to OFMSW at different concentrations to determine the optimum dose under thermophilic conditions.

5.2 Materials and Methods

5.2.1 MSW plant description and sampling

A full-scale MSW plant that serves a population of 400,000 people and receives 300 tons of mixed-collected MSW daily was used to collect inoculum and OFMSW samples. A pre-separation unit, also known as simple mechanical separation (Dehkordi et al., 2020), is included in the facility, where waste is screened by an 80 mm trommel. After being filtered by a magnetic separator, undersized materials are transferred to anaerobic digesters, accounting for 50 % of the incoming waste and referred to as ms-OFMSW. Oversize materials, on the other hand, are directed to a magnetic separator,

which is then followed by a hand sorting unit to separate recyclable materials. The schematic flow of the full-scale MSW plant treatment line is depicted in Figure 5.1.

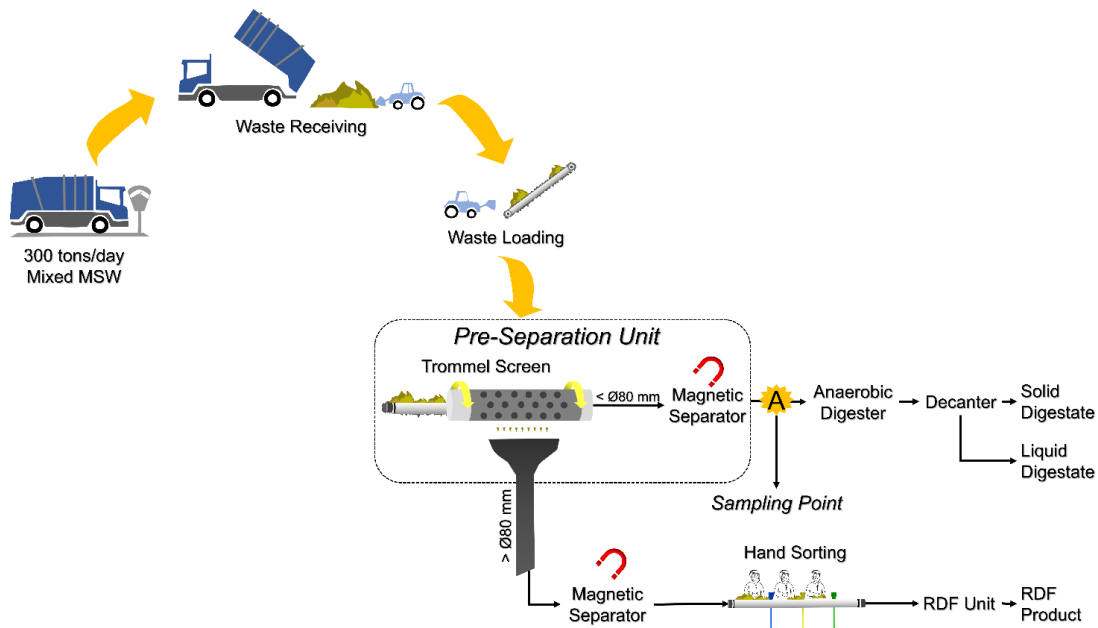


Figure 5.1 : The schematic flow of the full-scale MSW plant.

The ms-OFMSW samples were taken from the conveyor belt before the anaerobic digester (sampling point). 48 kg of ms-OFMSW composite samples (2 kg per hour) were collected after mechanical separation. To preserve the characteristics of the waste, samples were delivered to the laboratory within 2-4 hours. The organics in these samples—henceforth referred to as OFMSW—were then manually separated in the laboratory.

5.2.2 Inoculum and OFMSW characteristics

OFMSW samples were ground with a laboratory blender to the optimum particle size range of 1-3 mm for AD, and then stored at 4 °C to maintain methanogenic activity, as suggested by Astals et al. (2020). pH and volatile solids (VS) of inoculum were measured as 6.7 ± 0.2 and 11.9 ± 1.2 %, respectively. The physicochemical properties of the OFMSW were given in Table 5.1. The VS/total solids (VS/TS) ratio of OFMSW was measured as 45.9 ± 3.1 %. In the study of Li et al. (2016) VS/TS ratio ranged between 39 and 61%, which was consistent with our findings. Carbon (C), oxygen (O)

and hydrogen (H) are the typical components in OFMSW (Tyagi et al., 2018). In this study, C, O, and H were measured as $38.4 \pm 4.5\%$, $35.2 \pm 1.2\%$ and $3.8 \pm 1.4\%$, respectively. The C/N ratio for the OFMSW was determined as 16.0 ± 0.4 , which was consistent with the values (varied between 11 and 21 for organic substrates) reported by Campuzano and González-Martnez (2016).

Table 5.1 : The physicochemical characteristics of the OFMSW.

Parameter	Unit	OFMSW (Average $\pm$ Standard Deviation)
pH	-	6.2 ± 0.1
TS	%	42.3 ± 1.2
VS	%	19.4 ± 1.1
VS/TS	%	45.9 ± 3.1
Conductivity	mS/cm	6.2 ± 0.1
Upper Calorific Value	cal/g	3445 ± 125
Elemental Composition		
Carbon (C)	%	38.4 ± 4.5
Hydrogen (H)	%	3.8 ± 1.4
Nitrogen (N)	%	2.4 ± 1.1
Sulphur (S)	%	0.9 ± 0.2
Oxygen (O)	%	35.2 ± 1.2
C/N	-	16.0 ± 0.4

5.2.3 The BMP tests

5.2.4 The BMP setup and experimental procedure

The automated BMP system ECHO ER12 D6691 (Echo Instruments, Slovenia) was used to perform the test in duplicates. A validation test was performed with microcrystalline before applying the test to OFMSW to be sure about the accuracy of the test system (Fig. S1). Total working volume of the reactors were 400 mL. Before being used in the BMP experiment, the inoculum was incubated for 10 days as suggested by Demichelis et al. (2022) in order to decrease the ISR ratio and enable more OFMSW to be treated with the same volume of the AD reactor while achieving a higher biogas yield and CH₄ content. In this study, the ISR was 2 based on the amount of added VS. Batch BMP tests were carried out in accordance with the protocols proposed by Angelidaki et al. (2009) and Holliger et al. (2020). The test lasted 30 days under thermophilic (55°C) conditions to provide the same as it was in the full-scale AD plant. During the test period, the amount of CH₄ and CO₂ produced was followed online. At the start of the experimental setup, macronutrients (phosphate buffer) and micronutrients (0.25 mg/L FeCl₃·7H₂O; 27.5 g/L, CaCl₂·2H₂O; 22.5 g/L, MgSO₄·7H₂O; 0.05 mg/L, NiCl₂·2H₂O; 0.05 mg/L, Co(NO₃)₂·6H₂O) were added to

the reactors. To ensure the accuracy and reliability of the test results, cellulose microcrystalline (Sigma Aldrich, USA) was used as a positive control.

5.2.5 Enzyme addition

The powdered form of the *Aspergillus oryzae* derived amylase enzyme, which had a specific activity of 30 U/mg (Sigma Aldrich CAS 9001-19-8), was used in this study as direct dosing, as recommended by Brémond et al. (2018). Using direct dosing of the amylase enzyme simplifies the process and might reduce the costs associated with enzyme preparation and highly skilled staff requirements. Enzyme doses applied to OFMSW were 0.15, 0.3, 0.5, 1.0, 1.5 and 3.0 mg/gVS_{added} (referred to hereafter as untreated, 0.15-Enz.treated, 0.3-Enz.treated, 0.5-Enz.treated, 1.0-Enz.treated, 1.5-Enz.treated, and 3.0-Enz.treated).

5.2.6 The kinetics and model fitting for batch BMP tests

The modified Gompertz model, which is a function that expresses cell density in terms of exponential growth rates and lag phase duration during bacterial growth periods (Nzihou, 2020) was applied in this study. The model was developed on the assumption that the rate of CH₄ production in a batch digester corresponds to the rate of growth of methanogenic bacteria (Eq. 1). Because it is a good empirical non-linear regression model, the model equation is frequently used in the simulation of CH₄ accumulation.

$$B(t) = B_0 \cdot \exp \left\{ -\exp \left[\frac{R_{max.} \cdot \exp}{B_0} (\lambda - t) + 1 \right] \right\} \quad (5.1)$$

B_t = Modelled cumulative CH₄ yield (mL CH₄/g VS)

B_0 = Modelled highest cumulative CH₄ yield (mL CH₄/g VS)

$R_{max.}$ = Maximum CH₄ production rate (mL CH₄/g VS.day),

λ = lag phase (day),

t = time (day).

The lag phase is the shortest period of time required to produce biogas or for bacteria to adapt to their surroundings. The maximum CH₄ production rate ($R_{max.}$), which is a measure of methanogenic bacteria's specific growth rate, indicates how much CH₄ is produced per unit of time.

The Solver, an add-in tool for MS-Excel®, was employed to find the optimal values for the constants within the kinetic equation. This adjustment aimed to achieve the best

possible fit with the experimental data. The constants within the adapted Gompertz model, namely CH₄ production potential (B₀), maximum CH₄ production rate (R_{max}), and lag phase (λ), were subject to iterative optimization through the solver function and to minimize the root-mean-square error (RMSE) between the simulated and experimental values. Following multiple iterations, the most suitable constant values were identified as the outcomes of the model. To visualize the results, a graph was generated using both simulated data and the experimental cumulative CH₄ yield values (Khadka et al., 2022). Alongside the MS-Excel® add-in tool, the Online Biogas App (OBA), an interface derived from the R biogas package, was utilized to estimate the CH₄ potential of OFMSW (Hafner et al., 2018). The OBA incorporates various models (first-order kinetic model and the modified Gompertz model) to compute CH₄ based on input data.

5.2.7 Analytical measurements

The C, H, N, and S contents of the samples were determined using an elemental analyzer and the ASTM 5291-21 standard method (Elementar Vario Macro Cube) (ASTM 5291-21, 2010). Samples were dried for three hours at 103 ± 2 °C and ground to 0.6 mm in size for elemental analysis. The oxygen (O) content was determined using the equation provided below (ASTM D3176-15, 2016) where A represents the ash:

$$O = 100 - (C + H + N + S + A)(\% w/w) \quad (5.2)$$

Standard Methods 2540G were used to determine TS and VS (APHA, 2017). pH and conductivity were measured with a multimeter (Mettler Toledo - S400). The upper calorific value was determined using an isoperibol oxygen bomb calorimeter in accordance with the ASTM standard procedure (Parr 6200). (2019, ASTM D5865M-19). The OFMSW chemical formula was calculated using the methodology described by Komilis et al (2012).

5.3 Results and Discussion

5.3.1 The BMP test results

Total biogas amount for the samples were in between 405.6 and 437.1 mL/gVS_{added} and shown in Fig. 3. The highest percentage of average CH₄ (68 %) was obtained in the 0.5-Enz.treated reactor although other reactors had in between 54 - 62 % CH₄ (Fig. 3). The AD process has a high potential for producing biogas that contains 50-70 % CH₄, 25-40 % CO₂, and trace gases (1-5%), making it a renewable energy source (Atelge et al., 2020). According to Rossi et al. (2022), CH₄ and CO₂ content of biogas ranged between 60-70 % and 30-40 % for untreated OFMSW, respectively.

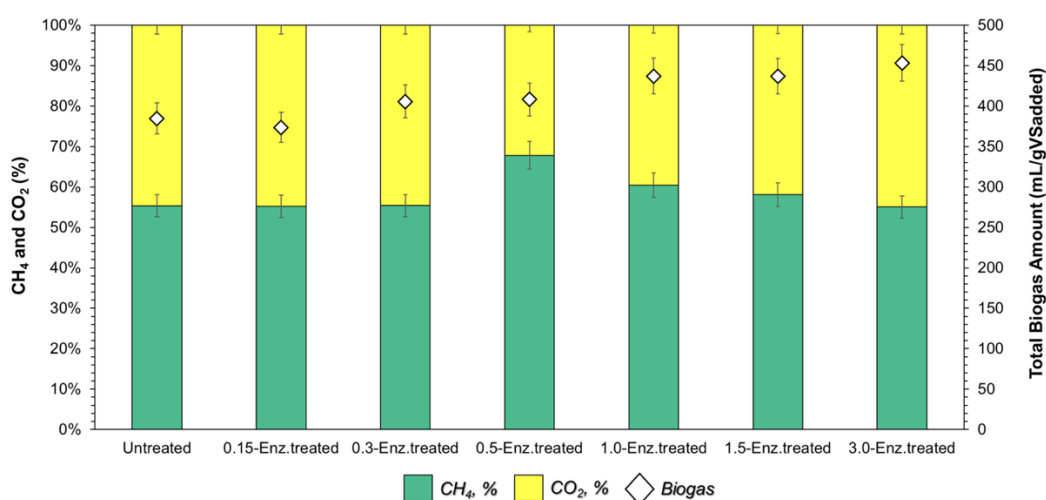


Figure 5.2 : The total biogas volume and distribution of the CH₄ and CO₂ and obtained for the untreated and enzyme treated OFMSW samples.

The untreated OFMSW sample yielded 213.0 mL CH₄/g VS_{added}. According to Zamri et al. (2021), the CH₄ of untreated OFMSW ranged from 177 to 550 mL CH₄/gVS_{added}, which were in line with our results. The CH₄ amount for the enzyme treated samples ranged between 206.2 to 276.7 mL CH₄/gVS_{added}. Enzyme treated reactors achieved 5.2 to 23.0 % higher CH₄ yield than the untreated reactor; the highest CH₄ (276.7 mL CH₄/gVS_{added}) was recorded for 0.5-Enz.treated sample as average. According to Dubrovskis et al. (2019), adding 0.5 mL alpha amylase enzyme increased the average CH₄ content by 19.9 and 22.9 % for 10 g of lucerne pellets and 10 g of birch leaves pellets, respectively. On the other hand, Mlaik et al. (2019) reported that CH₄ potential for the OFMSW (It is obtained after manual sorting of MSW by removing materials such as bones, plastics, hard ligaments, and other inorganic materials) was increased from 189 mL/gVS to 672 mL/gVS after adding 10 mL enzyme cocktail / 50 mL sample

(cocktail of β -glucosidase, α -amylase and CMCase obtained during *Aspergillus niger* fermentation). Mlaik et al. (2020) showed that adding 10 mL enzyme cocktail / 50 mL sample (an enzyme cocktail rich in β -glucosidase as a result of *Aspergillus niger* fermentation, facilitated by a novel culture medium comprising OFMSW and macroalgae) increased the CH_4 amount from 170 mL $\text{CH}_4/\text{gVS}_{\text{added}}$ (untreated OFMSW) to 500 mL $\text{CH}_4/\text{gVS}_{\text{added}}$ (enzyme treated OFMSW). Rintala and Ahring (1994) studied addition of xylanase, lipase, protease and a mixture of these in batch and in continuous experiments under thermophilic conditions using source-sorted MSW. They did not find significant increase in methane production in the batch and continuous assays due to easily-degradable characteristics of source-sorted MSW.

Our results differ from the findings of literature values. There might be several reasons for this difference, such as the characteristics of organic waste, enzyme application method, and enzyme dose. Additionally, variations in experimental conditions and the specific type of enzyme used could also contribute to the disparities in the results. It is important to further investigate these factors in order to better understand and optimize the use of enzymes for biogas enhancement in future studies.

5.3.2 Model fitting and kinetics

Kinetic parameters were predicted using the formula provided for the modified Gompertz model and shown in Table 5.2. The estimated parameters show how the microbial population adapts to environmental change over time and with some delay, as shown by the estimated parameters.

Table 5.2 : Parameters and goodness of fit obtained from the modified Gompertz model.

Sample	$R_{\text{max.}}$ (mL $\text{CH}_4/\text{gVS}_{\text{added.}}$ day)	λ lag Phase (day)	CH_4 Production, mL $\text{CH}_4/\text{gVS}_{\text{added}}$		
			Experimental	B_t	Theoretical
Untreated	14.91	3.53	213.0	212.6	402.6
0.15-Enz.treated	13.53	3.51	206.2	208.9	-
0.3-Enz.treated	15.69	3.40	224.7	224.5	-
0.5-Enz.treated	17.66	2.85	276.7	280.7	-
1.0-Enz.treated	16.55	3.15	264.3	268.0	-
1.5-Enz.treated	18.07	3.39	253.9	254.7	-
3.0-Enz.treated	16.55	3.22	249.6	250.0	-

The model's accuracy was evaluated by comparing experimental results to simulated values and R^2 values were found greater than 0.942. Based on the value of λ , the likelihood of an organic conversion can be predicted (Table 5.2 and Figure 5.3). A higher λ value denotes an irreversible process inhibition and a low rate of degradation. In this study, the λ value was in between 2.85 to 3.53 days for the untreated and enzyme treated OFMSW samples (the lowest value was achieved for 0.5-Enz.treated). 0.5-Enz.treated was found to be efficient enzyme dose when the R_{max} and λ value was considered in addition to CH_4 production (Figure 5.3). Similar to our study, Brémond et al. (2018) reported that treated OFMSW had relatively lower λ value and higher R_{max} than the untreated samples.

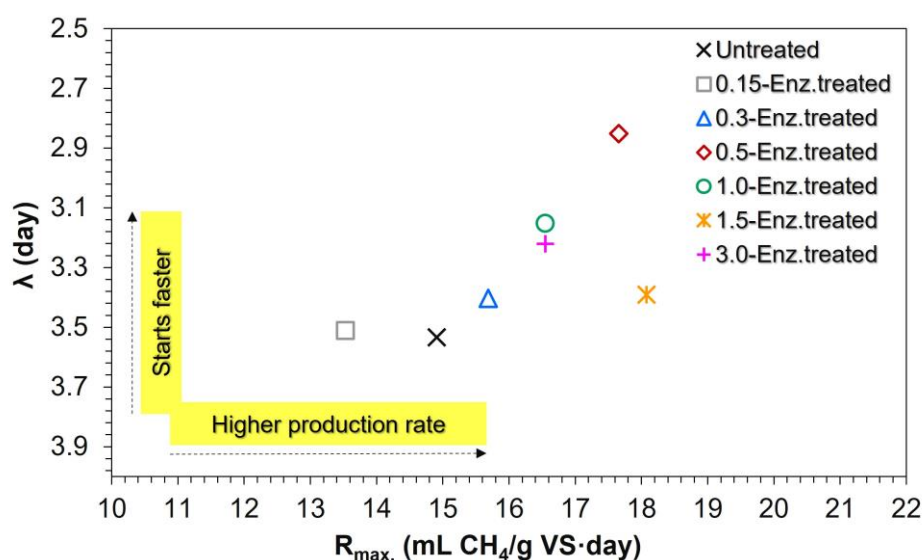


Figure 5.3 : The correlation of R_{max} vs λ obtained for the untreated and enzyme treated OFMSW samples.

The theoretical BMP was calculated as 402.6 mL CH_4 /gVS_{added} using the formula provided by Buswell and Mueller (1952) and the chemical formula was derived as $C_{139}H_{164}N_7O_{95}S$ for the OFMSW. The experimental CH_4 was yielded as 69 % of the theoretical BMP from the untreated OFMSW sample. The findings of this study revealed a strong relationship between experimental and simulated values (Figure 5.4). The experimental results were consistent with those simulated by the modified Gompertz model.

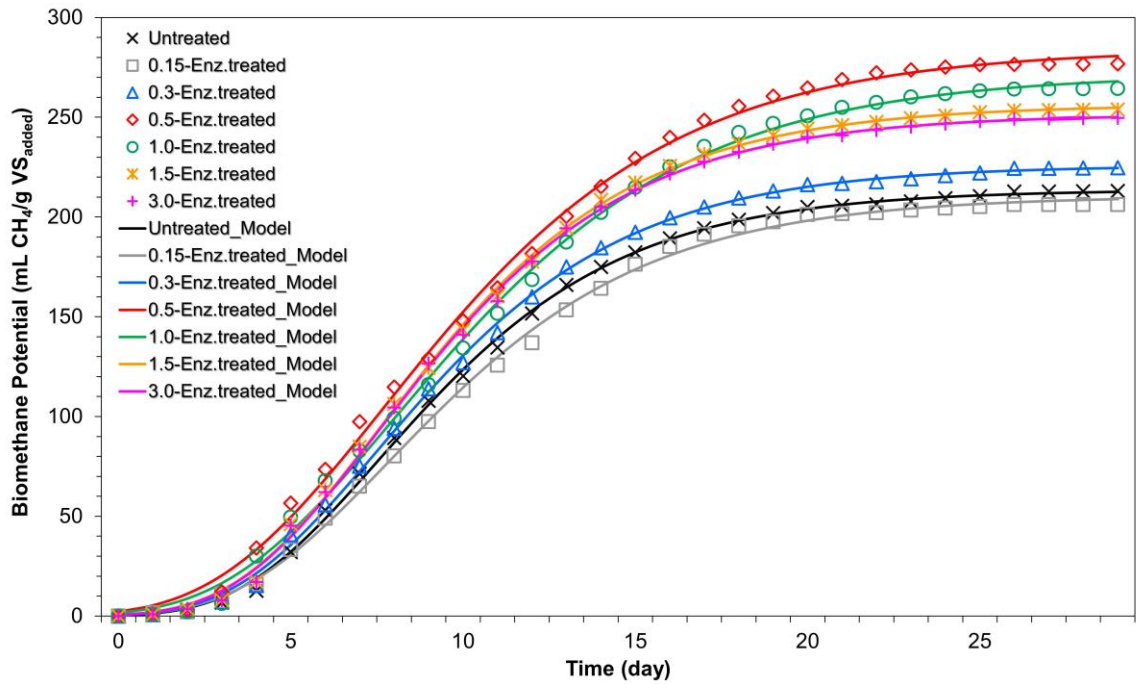


Figure 5.4 : Modified Gompertz model fitting for cumulative CH₄ yield obtained for the untreated and enzyme treated OFMSW samples.

Because of its simple working conditions, greater accessibility to the feedstock (i.e., OFMSW, sludge), and higher biomass transfer rate, enzyme treatment is an appealing choice for accelerating the degradation process. Enzymes also maintain their activity for a longer amount of time since no inhibitory chemicals are produced and can also be collected after the treatment (Dhull et al., 2022). These advantages of enzyme treatment allow its applicability for the commercial use in full scale applications. However, economic feasibility of the enzyme treatment limits the practical applications. On the other hand, Herrero Garcia et al. (2019) reported that commercial enzyme costs typically 30-65 €/day for 1 MW AD at European level which makes enzyme application feasible.

5.4 Conclusions

This study highlights the significance of treatment via direct alpha-amylase enzyme dose to increase the digestibility of the OFMSW under thermophilic conditions. According to the findings, adding 0.5 mg/g VS_{added} enzyme resulted in the highest CH₄ yield and the shortest lag phase (2.85 days). The experimental CH₄ yield and R_{max} were increased up to 17.5% (1.5-Enz.treated) and 23.0% (0.5-Enz.treated), respectively, by alpha amylase enzyme, which also decreased the lag phase to 19.3% (0.5-Enz.treated). This study showed that the biomass-to-bioenergy approach can be enhanced by

applying enzyme treatment strategies to AD for a sustainable waste management by reducing the GHGs and minimizing waste and pollution.



6. CONCLUSIONS

6.1 Overall Conclusions

The following conclusions can be derived from the studies carried out within the scope of this thesis:

- In the waste composition analysis, it was found that the majority of the ms-OFMSW consists of organics, comprising $76.5 \pm 1.7\%$, while a significant portion of recyclable wastes, accounting for $9.0 \pm 1.6\%$, evades mechanical separation. It was revealed that eliminating non-organics led to a notable rise in the VS/TS ratio by $17.8 \pm 8.8\%$, which may significantly influence the anaerobic digestibility of the waste. To enhance waste management efficiency, it is recommended to prioritize enhancing mechanical separation or implementing source separation methods, thereby boosting recycling rates while concurrently conserving energy and water resources.
- Electrohydrolysis pretreatment resulted in a significant reduction of the "lag phase" by up to 43% and concurrently increased the cumulative CH₄ production of OFMSW by up to 10%. The study identifies electrohydrolysis as a promising method for reducing the lag phase and enhancing the CH₄ production potential of OFMSW.
- In the enzyme treatment study, the effectiveness of the alpha-amylase enzyme dose is evident as the addition of 0.5 mg/VS_{added} enzyme resulted in the highest CH₄ yield and shortest lag phase (2.85 days), while experimental CH₄ yield increased by up to 17.5% (1.5-Enz.treated) and R_{max} by 23.0% (0.5-Enz.treated) with the application of alpha-amylase enzyme. The application of alpha-amylase enzyme treatment demonstrated a significant improvement in anaerobic digestion performance, notably reducing the lag phase by 19.3% in the 0.5-Enz.treated condition.

6.2 Future Perspectives

In waste-to-energy systems, various pretreatment approaches aim to maximize AD performance and sustainability. Within the scope of this thesis two different pretreatment methods were applied to OFMSW. According to the findings both methods have their respective advantages and disadvantages based on their applications. However, further research and development efforts are needed to address operational challenges, enhance treatment procedures, and scale up for AD technologies to be widely adopted as sustainable waste management practices. The complex properties of OFMSW present challenges in considering it as the sole substrate for the AD process.

While this study highlights the efficacy of electrohydrolysis pretreatment and enzyme treatment methods in enhancing AD efficiency, additional research is necessary to fully explore their potential and address remaining gaps in knowledge. The costs of enzyme pretreatment applications can be reduced by enriching the microbial environment in the anaerobic reactor or by producing the enzyme on-site from the waste. Although the life cycle assessment (LCA) method was not applied in this study due to data constraints, future studies should incorporate LCA to evaluate not only the economic outcomes but also the life cycle impacts of the pretreatment methods to improve AD.

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