DETERMINING THE BIOMETHANE POTENTIAL OF PETROCHEMICAL SLUDGE AFTER PRE-TREATMENT

by
Muhammed Talha Aluç
B.Sc. in Environmental Engineering Istanbul University, 2018

Submitted to the Institute of Environmental Sciences
in partial fulfillment of the requirements for the degree of
Master of Science
in
Environmental Technology

Boğaziçi University
2024

DETERMINING THE BIOMETHANE POTENTIAL OF PETROCHEMICAL SLUDGE AFTER PRE-TREATMENT

APPROVED BY:

Prof. Dr. Burak Demirel . . . .
Thesis Advisor

Prof. Dr. Nilgün Ayman Öz . . . .

Prof. Dr. Bahar İnce . . . .

DATE OF APPROVAL: 16/01/2024

ACKNOWLEDGEMENTS

I would like to express my thanks to Prof. Dr. Burak Demirel, Prof. Dr. Bahar İnce, Prof. Dr Nilgün Ayman Öz, Dr. Asu Ziylan Yavaş, Berivan Ülger Vatansever, Filiz Ayılmaz, Elif Sena Uyal throughout my Boğaziçi University master's degree program, especially in the laboratory, thesis writing, and other processes.

I also want to express my sincere appreciation to my fiancée Şeyma Bozacı, who supported my studies throughout my academic career.

I wish success and thank my brother Abdullah Aluç, who has supported me in every aspect of my life.

ABSTRACT

DETERMINING THE BIOMETHANE POTENTIAL OF PETROCHEMICAL SLUDGE AFTER PRE-TREATMENT

Utilizing renewable energy sources has grown in importance in the modern era, particularly in light of the circular economy and sustainability ideas. Biogas is one type of renewable energy source. In addition to being a renewable energy source, biogas generation helps reduce the negative environmental and health effects of the wastes that are disposed of during anaerobic digestion operations. Especially in recent years, the increasing energy need and the use of petroleum-derived materials in energy needs constitute the thesis subject of this study. The main objective of this study was to determine the biogas production potential of pre-treated petrochemical industry wastewater sludge using batch reactors. Biogas tests were examined in two separate parts by evaluating ozone, ultrasound pre-treatment techniques and co-digestion options. In the first part, 27-day and 29-day anaerobic digestion processes were applied in the second part. Heavy metal analyses, TS, TVS, COD, pH parameters were constantly checked throughout the processes. In the first part, the highest biogas production was observed in the in parallel 1 of the second reactor with 6 mL methane /g TS_{added}. In the second part, the highest biogas production was achieved in the second parallel of the eighth reactor, with 35 mL methane /g TS_{added}. The reactor with the highest gas production was the hybrid (HYB) reactor and 65 mL biogas /g TS_{added} production was observed.

ÖZET

PETROKİMYASAL ATIKSU ARITMA ÇAMURU ÖN ARITIM İŞLEMLERİ SONRASI BİYOMETAN POTANSİYELLERİNİN BELİRLENMESİ

Yenilenebilir enerji kaynaklarının kullanılması, modern çağda, özellikle döngüsel ekonomi ve sürdürülebilirlik fikirleri ışığında önem kazanmıştır. Biyogaz yenilenebilir enerji kaynaklarından biridir. Biyogaz üretimi, yenilenebilir bir enerji kaynağı olmasının yanı sıra, anaerobik çürütme işlemleri sırasında bertaraf edilen atıkların çevre ve sağlık üzerindeki olumsuz etkilerinin azaltılmasına da yardımcı olmaktadır. Özellikle son yıllarda artan enerji ihtiyacı ve enerji ihtiyacında petrol türevi malzemelerin kullanılması bu çalışmanın tez konusunu oluşturmaktadır. Bu çalışmanın temel amacı, ön arıtılmış petrokimya endüstrisi atıksu çamurunun kesikli reaktörler kullanılarak biyogaz üretim potansiyelinin belirlenmesidir. Biyogaz testleri ozon, ultrason ön arıtma teknikleri ve birlikte sindirim seçenekleri değerlendirilerek iki ayrı bölümde incelenmiştir. Birinci bölümde 27 günlük, ikinci bölümde ise 29 günlük anaerobik çürütme işlemleri uygulanmıştır. Süreçler boyunca ağır metal analizleri, TS, TVS, COD, pH parametreleri sürekli kontrol edildi. Birinci kısımda en yüksek biyogaz üretimi 6 mL metan/g TS katkılı ikinci reaktörün paralel 1 numaralı reaktöründe gözlenmiştir. İkinci bölümde ise en yüksek biyogaz üretimi 35 mL metan/g TS ilavesi ile sekizinci reaktörün ikinci paralelinde elde edilmiştir. En yüksek gaz üretimi gerçekleşen reactor hibrit (HYB) reaktörüdür ve 65 mL biogas /g TS_{added} üretimi gözlenmiştir.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii
ABSTRACT iv
ÖZET v
TABLE OF CONTENTS vi
LIST OF FIGURES viii
LIST OF TABLES ix
LIST OF SYMBOLS/ABBREVIATIONS xi
1. INTRODUCTION 1
2. THEORETICAL BACKGROUND 4
2.1. A Brief Introduction to Anaerobic Digestion Process 4
2.1.1. Anaerobic Digestion Process 4
2.1.2. History of Anaerobic Digestion 6
2.2. Anaerobic Digestion Process Parameters 8
2.2.1 Substrates 8
2.2.2 Temperature 9
2.2.3 Mixing 10
2.2.4 pH 10
2.2.5 Oxygen 11
2.3. Inhibiting Substances 12
2.3.1 Sulfur Contents 12
2.3.2 Hydrogen Peroxide 12
2.3.3. Heavy Metal 12
2.4. Sludge Disposal Techniques 13
2.4.1. Incineration 14
2.4.2 Stabilization/solidification 14
2.4.3 Oxidation Treatment 14

2.5. Sludge Pre-treatment Techniques 15
2.5.1 Thermal Pre-treatment 17
2.5.2 Ultrasound Pre-treatment 18
2.5.3 Ozone Pre-Treatment 18
2.5.4 Acid and Alkali Pre-treatment 19
2.6. Anaerobic Digestion of Petrochemical Wastewater Sludge 20
3. METHODOLOGY 23
3.1. Anaerobic Digestion Reactors and Apparatus 23
3.1.1 Contents and Nomenclature of Anaerobic Digester Reactor 25
3.2. Seed Sludge Preparation 27
3.3. Petrochemical Wastewater Treatment Sludge Preparation 27
3.4. Pre-treatment Applications 28
3.5. Analytical Methods 28
3.6. Biochemical Methane Potential (BMP) Test 29
4. RESULT AND DISCUSSION 30
4.1. Petrochemical Wastewater Sludge and Seed Sludge Characteristics 30
4.1.1 Petrochemical Wastewater Sludge Characteristics 30
4.1.2 Seed and Feed Sludge Characteristics 34
4.2. Biogas and Methane Production 35
4.2.1 Part 1 Biogas and Methane Production 35
4.2.2 Part 2 Biogas and Methane Production 38
4.2.3 Part 1 and Part 2 Biogas Detail Results 41
4.2.4 Biogas Results and Literature Comparison 43
5. CONCLUSION 45
REFERENCES 47

LIST OF FIGURES

Figure 2.1. General outlook of anaerobic digestion 4

Figure 2.2. Biogas installed power generation capacity 7

Figure 2.3. Biogas and methane production potential comparisons in 2018 8

Figure 2.4. Relationship between temperature and methanogen bacteria 9

Figure 2.5. Effect of pH and temperature on biogas 11

Figure 2.6. Wastewater treatment facilities anaerobic digestion options in the literature 16

Figure 2.7. Treated and untreated WAS sludge biogas potential 19

Figure 3.1. Anaerobic digestion set-up 23

Figure 4.4. Part 1 cumulative reactor biogas production 36

Figure 4.5. Part 1 daily reactor methane production 37

Figure 4.6. Part 1 cumulative reactor methane production 37

Figure 4.7. Part 1 gas chromatography methane formation percentages 38

Figure 4.8. Part 2 cumulative reactor biogas production 39

Figure 4.9. Part 2 daily biogas production 39

Figure 4.10. Part 2 cumulative reactor methane production 40

Figure 4.11. Part 1 gas chromatography methane formation percentages 40

LIST OF TABLES

Table 2.1. Agricultural substrates and biogas, methane potentials 9

Table 2.2. Heavy metal inhibitory concentrations in the literature 13

Table 3.1. Reactor content volume and parameters details in Part 1. 24

Table 3.2. Reactor content volume and parameters details in Part 2. 25

Table 3.3. Reactor setup and nomenclature details. 26

Table 4.1. Total solids and total volatile solids analysis results - Part 1..30

Table 4.2. Total solids and total volatile solids analysis results - Part 2..31

Table 4.3. COD, TKN and pH analysis results - Part 1. 31

Table 4.4. COD, TKN and pH analysis results - Part 2. 32

Table 4.5. Heavy metal analysis results day 0 - Part 1 (mg/L). 32

Table 4.6. Heavy metal analysis results day 27th - Part 1 (mg/L). 33

Table 4.7. Heavy metal analysis results day 0 - Part 2 (mg/L). 33

Table 4.8. Heavy metal analysis results day 29th - Part 2 (mg/L). 33

Table 4.9. Reactor heavy metal inhibitory level. 34

Table 4.10. Characteristic features of seed and feed sludges. 35

Table 4.11. Total methane and biogas generation results - Part 1. 41

Table 4.12. Total methane and biogas generation results - Part 2. 41

Table 4.13. Thesis biogas results and literature comparison..43

LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
CH_4	Methane	
CO_2	Carbon dioxide	ton
H_2	Hydrogen	
NH_3	Ammonia	
O_3	Ozone	mL
H_2SO_4	Sulfuric Acid	
H_3PO_4	Phosphoric Acid	
NaOH	Sodium Hydroxide	
KOH	Potassium Hydroxide	
$MgOH_2$	Magnesium Hydroxide	
Fe	Iron	mg/L
Al	Aluminum	mg/L
Cd	Cadmium	mg/L
Co	Cobalt	mg/L
Cu	Copper	mg/L
Zn	Zinc	mg/L
Pb	Lead	mg/L
Ni	Nickel	mg/L
Mo	Molybdenum	mg/L
Hg	Mercury	mg/L
H_2S	Hydrogen Sulfide	

LIST OF SYMBOLS/ABBREVIATIONS

Abbreviation	**Explanation**
CO	Co-Digestion
COD	Chemical Oxygen Demand
DAF	Dissolved Flotation Unit
EPA	Environmental Protection Agency
EU	European Union
GC	Gas Chromatography
GHG	Greenhouse Gas
HRT	Hydraulic Retention Time
ICP	Inductively Coupled Plasma
IEA	International Energy Agency
İSKİ	İstanbul Su ve Kanalizasyon İdaresi
kWh	Kilowatt Hour
LCA	Life Cycle Assessment
LCFA	Long Chain Fatty Acid
RAS	Return Activated Sludge
SM	Standard Method
SRB	Sulfide Reducing Bacteria
SRT	Solid Retention Time
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TS	Total Solid
TVS	Total Volatile Solid
UASB	Upflow Anaerobic Sludge Blanket
US	Ultrasound
VDI	Verein Deutscher Ingenieure
VFA	Volatile Fatty Acid
VOC	Volatile Organic Compound
WAS	Waste Activated Sludge

1. INTRODUCTION

Energy demand is rising as the world's population expands. According to the International Energy Agency (IEA) database, both energy demand and consumption trends tend to rise globally. The detailed examinations between 2010 and 2019 show that main dependent energy sources such as coal, electricity, oil are increasing (Enerdata, 2023). As energy resources are utilized with increasing frequency, there is a natural consequence in the acceleration of CO_2 emissions stemming from global activities (BP, 2023). The differences between the sources are clear when looked at in terms of energy-related greenhouse gas emissions. For instance, oil is the most prominent source in terms of greenhouse gas intensity followed by coal (International Energy Agency, 2023a). The oil and gas sector alone has 5.1 billion tonnes of CO_2 equivalent when production, distribution and processing activities are considered. Institutions and other governmental bodies establish some scenarios and expectations for the reduction of greenhouse gas emissions (GHGs), but wars or other political situations could result in unforeseen changes in the consumption of oil and gas (IEA, 2023b; International Energy Agency, 2022). Especially Ukraine invasion affected the EU energy strategy and forced EU to take some precautions. Increased investment in renewable energy sources, the acceleration of efficient energy studies, and the diversification of energy supply channels were some of the measures made by the European Union to avoid dependence on Russian gas (European Council, 2022). For instance, following the invasion of Ukraine, the EU reduced its reliance on Russian gas supplies from 29% to 12% by 2022 (European Council, 2023). Ukraine invasion, the EU Commission and the EU's proactive stance in the war environment shows renewable energy sources importance. The EU Commission increased the 2030 target of 40% renewable energy usage rate to 45% starting in 2022 (Liao, 2023).

The utilization of renewable energy sources and current investment trends are both highly important. Globally, the capacity for renewable energy has grown significantly. The installed capacity of renewable energy increased by 107 gigawatts in 2023. Several alternative factors can be used to explain this growth. The increase in renewable energy use, especially in Europe, can also be attributed to political decisions and actions. It has led to an estimated 40% increase in European renewable energy usage capacity compared to before the Ukraine invasion (International Energy Agency, 2023b).

Energy generation from the unused wastewater is not an original phenomenon. One of the most applicable methods for generating electricity from wastewater is biogas production. The initial potential when producing electricity from wastewater is passing over the discharge limits and finding it economically feasible for the wastewater treatment facilities (Bhattacharyya & Shekdar, 2003). Especially in some wastewater facilities contains lots of pollution to the receiving environment. For instance, oil wastewater may contain hazardous residues. Additionally, it is possible to remove oil content from water by oil wastewater treatment; nevertheless, sludge complications are unavoidable. Wastewater and also oily wastewater sludge can be seen as hazardous waste as long as these wastewater sources have heavy metal formation that is listed by the Environmental Protection Agency (EPA, 2023).

One of the prominent sources of renewable energy generation techniques is bioenergy generation in wastewater recovery and treatment units. Bioenergy originates from the organic material that includes carbon-based constituents by mainly photosynthetic organisms (International Energy Agency, 2023c). Bioenergy has considerable GHG-reducing features when biomass resources are operated sustainably (Welfle et al., 2023). Bioenergy can appear in different forms. These forms are generally biopellets, bioethanol, biodiesel and finally biogas.

Biogas may occur as a natural phenomenon in nature. There can be many biogas sources such as livestock, crops, wastewater, food waste etc. but common feature of these sources are organic matter contents. With the anaerobic conditions, bacteria activities convert organic matter to biogas in a multi-step process (Da Costa Gomez, 2013). Anaerobic digestion is a mechanism that uses the organic content of the carbon bonds with microbial activity and occurs the biogas with some residues (Gómez et al., 2019). In normal circumstances biogas composition is consist of nearly 50% - 75% CH_4, 25% - 50% CO_2 and other trace elements like H_2, NH_3 and VOCs (Y. Li et al., 2019). The anaerobic digestion system eases degradability that arises in the absence of oxygen, reducing the hazardous content of the sludge. Reducing the content of hazardous substances through anaerobic digestion also facilitates the disposal of contents such as sludge. Moreover, in terms of operational and environmental issues, the neutralization and disposal of wastewater sludge can be accomplished with the help of an anaerobic digestion system (Stasinakis, 2012). Additionally, petrochemical-based hazardous waste sludge anaerobic digestion applications are particularly helpful in removing waste, lowering environmental risk, and producing products with high energy contents like methane (Kavitha et al., 2018). Methane generation is well-suited to petrochemical effluent with a high COD level (Tan et al., 2020). The four phases of anaerobic digestion are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Sarah Refai, 2016). All of the aforementioned phases also involve biological activity steps. At the end of all these processes, three main gases are produced: CH_4, H_2 and CO_2. In the first step, hydrolysis, extracellular enzymes help transform organic molecules into smaller molecules. Large molecules can be broken down and used inside the cell thanks to extracellular enzymes (Meegoda et al., 2018). The main function of the hydrolysis stage is to prepare the broken molecules for the next stage, acidogenesis (Lin et al., 2010). After the hydrolysis phase, the acidogenesis phase comes, and the molecules broken down in the hydrolysis phase are transformed into volatile fatty acids and other products through biochemical processes (Meegoda et al., 2018). Acidogenesis stages produce products including volatile fatty acids, butyrate, and propionate, which are ready to be transformed into H_2, CO_4, or acetate (Hansen & Cheong, 2019). In methanogenesis, the last stage of anaerobic digestion, the acetate produced in the previous phases finally generates methane and carbon dioxide (Ferry, 2010).

The aim of this study was to investigate and determine production of biogas potential for petrochemical wastewater treatment sludge using anaerobic digestion processes after pre-treatment. It is aimed to use pre-treatment techniques to eliminate toxicity and high metal content and to continue biogas production after the pre-treatment applied such as ozone, ultrasound and hybrid (ozone/ultrasound). Pre-treatment techniques: ozonation, ultrasound and their combined effects were examined in another study prior to this work and the most optimum pre-treatment parameters were determined for the sludge. In this work, the most optimum pre-treatment outputs of the laboratory study were used. Pre-treated petrochemical wastewater treatment sludge was used as the sole substrate in this experimental work in order to determine and evaluate biogas and methane production potential of use Biochemical Methane Potential (BMP) test.

2. THEORETICAL BACKGROUND

2.1. A Brief Introduction to Anaerobic Digestion Process

Anaerobic digestion is the term used to describe the process by which organic materials are broken down to produce CH_4, CO_2, and trace amounts of other gases. Methane produced as a result of anaerobic digestion, which is also a phenomenon in nature, is also used as a renewable energy source. As in all other biological processes, external factors and parameters are very important for the process. Factors such as substrate, temperature, mixing, oxygen, pH, etc. affect the biological processes. Intermediate and main products formed during anaerobic processes are given in Figure 2.1.

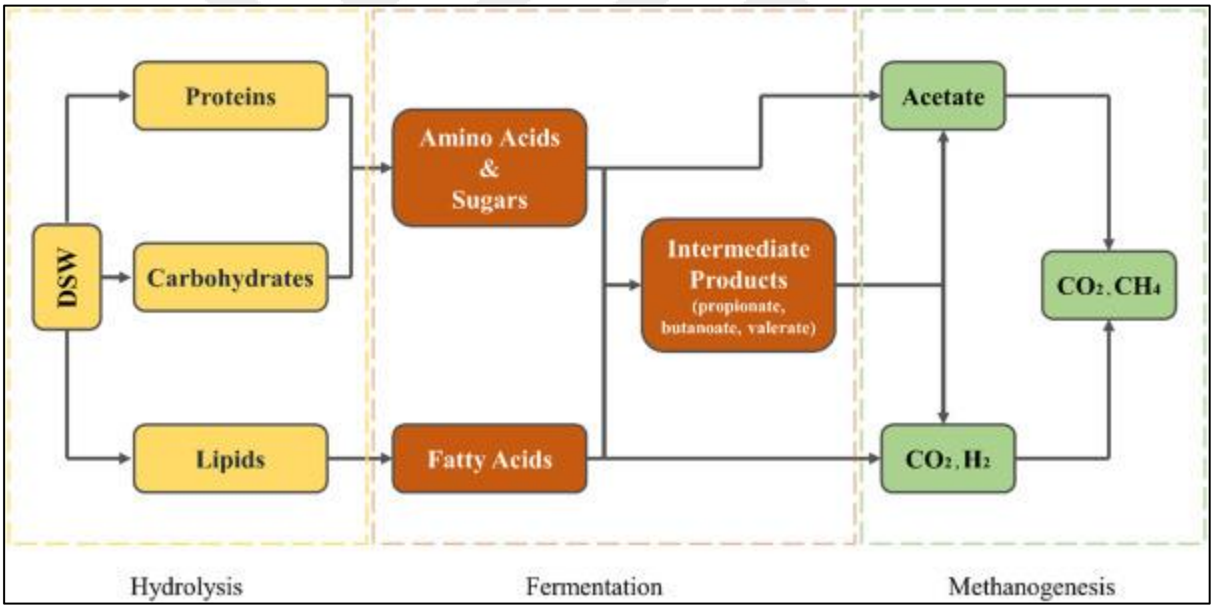

Figure 2.1. General outlook of anaerobic digestion (Masud et al., 2023).

2.1.1. Anaerobic Digestion Process

Anaerobic digestion is a process in which a series of sequential reactions take place on complicated and large fractionated organic materials. As mentioned before and as seen in Figure 2.1, anaerobic digestion is generally divided into 3 steps and investigated 4 biological phases.

2.1.1.1. Hydrolysis. The hydrolysis stage constitutes the first stage of the anaerobic digestion system. Hydrolysis can be seen as a biological pre-treatment process in anaerobic digestion for substrates (Menzel et al., 2020). Substrates with large polymer structures are reduced by extracellular enzymes to weights of less than 1000 daltons molecular weight. Depending on the substrate, the hydrolysis stage can display a variety of structures and characteristics.

Especially in wastewater treatment plants, the amount of wastewater sludge production is quite high and is constantly used in anaerobic digestion processes. Research on the hydrolysis process show different hydrolysis rates of different substrates (Ma et al., 2013). In the hydrolysis stage, partially high lipid content and long chain fatty acids negatively affect methane efficiency. For example, LCFA (Long Chain Fatty Acid) has been shown to have an inhibitory effect on microbial growth on anaerobic digestion (Wu et al., 2023; Stabnikova et al., 2005). Pre-treatment techniques are used to improve hydrolysis efficiency (Menzel et al., 2020). The problems of hydrolysis in semi-solid and liquid wastes are slow hydrolysis rate and inefficient breakdown of large molecules (Menzel et al., 2020).

Hydrolysis equation

$$(C_6H_{10}O_4)_n + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2 \tag{2.1}$$

2.1.1.2. Acidogenesis. The products generated by the first stage of hydrolysis are fermented and anaerobically broken down during the acidogenesis process. With the aid of fermentative reactions organic molecules are converted into sugar, amino acids, hydrogen, and ammonia, etc. compounds (Pavlostathis, 2011). In the acidogenesis, the products that are produced as a result of the first stage hydrolysis utilizes are fermented and anaerobically degraded. Long-chain fatty acids and alcohol-based products are turned into volatile fatty acids, H_2, and CO_2.

Acidogenesis equations

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2 \tag{2.2}$$

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O \tag{2.3}$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \tag{2.4}$$

2.1.1.3. Acetogenesis. In acetogenesis, which is the step before the final methane production, lactate, methanol and ethanol, as well as long and short chain fatty acids, except acetate, are converted into acetate, hydrogen and carbon dioxide by acetogenic bacteria. The products formed in the acetogenesis phase are finally produced as acetate (Batstone et al., 2002).

Acetogenesis equation

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 3H_2 + H^+ \quad (2.5)$$

2.1.1.4. Methanogenesis. The last step of the anaerobic digestion process which is called methanogenesis, archaea bacteria generate methane as a final product. Apart from acetate, H_2, and CO_2, which are the products for methanogenesis, methanogens help produce methylamine, methanol, and carbon monoxide (Pavlostathis, 2011). Although methane production is similar in principle to other phases, the methanogenesis stage has unique characteristics. These features are that they have low energy efficiency, consist only of archaea, and can only operate in an oxygen-free environment. Methanogen bacteria can continue their activities freshwater, seawater and extreme environments (Fenchel et al., 2012). The last academic studies indicate the beneficial aspects of methanogens (Lyu et al., 2018).

Methanogenesis equation

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2.6)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.7)$$

$$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad (2.8)$$

The equations (1,2,...,8) given above were taken from Bajpai's study in 2017 (Bajpai, 2017).

2.1.2. History of Anaerobic Digestion

Historically, the combustion potential of gases formed as a result of organic activity and decay was discovered by Jan Baptista Van Helmont in the 17th century. Within the next century, the methane content of cattle manure was identified by Sir Humphry Davy. Additionally, it is known that the first anaerobic digestion system was established in Mumbai, India, in 1859 (Lusk, 1998). The reason behind the rising popularity of the anaerobic digestion and renewable energy sources might be the energy crisises across the world. The energy crisis in the 1970s, global climate change, and the recent wars have drawn attention to the clean and sustainable features of energy use sources.

Europe uses biogas at a particularly high portion. The European Biogas Association's most recent report from 2021 states that there are 19,954 biogas and biomethane facilities in total (EBA Statistical Report, 2021). The output of biogas in Europe has expanded by over 540% during the past ten years, under rising trends. Data from the European Biogas Association indicates that, by 2050, the total gas requirement should be satisfied by 30% to 40%. Investments in technologies for biogas production are supported by this growing trend (Comesaña-Gándara et al., 2022). Just like Germany, which is Europe's pioneer in biogas use and production, other European countries are also taking steps in the development of new ways and technologies in biogas production (Achinas et al., 2017). According to IEA data, the leading countries in installed power biogas capacity are Germany, the United States, the United Kingdom, Italy and China, respectively demonstrated in Figure 2.2. The most common areas of use of annually produced biogas are energy production, cogeneration and use for heating purposes in buildings (IEA, 2020). In addition, different results are seen in global biomethane demand according to two different scenarios among global trends, stated policies scenario and sustainable development scenario. According to the Stated Policy Scenario, the global energy sector aims to be net CO_2 zero in 2050, while according to the Sustainable Development Scenario, if global CO_2 emissions remain at net zero after 2070, the world's total temperature is expected to remain at 1.8° C. As can be seen in the Figure 2.3, biogas and biomethane production, which is currently 35 Mtoe over the years, corresponds to the production of 570 Mtoe biogas and 730 Mtoe biomethane if the world's potential resources are used (IEA, 2020).

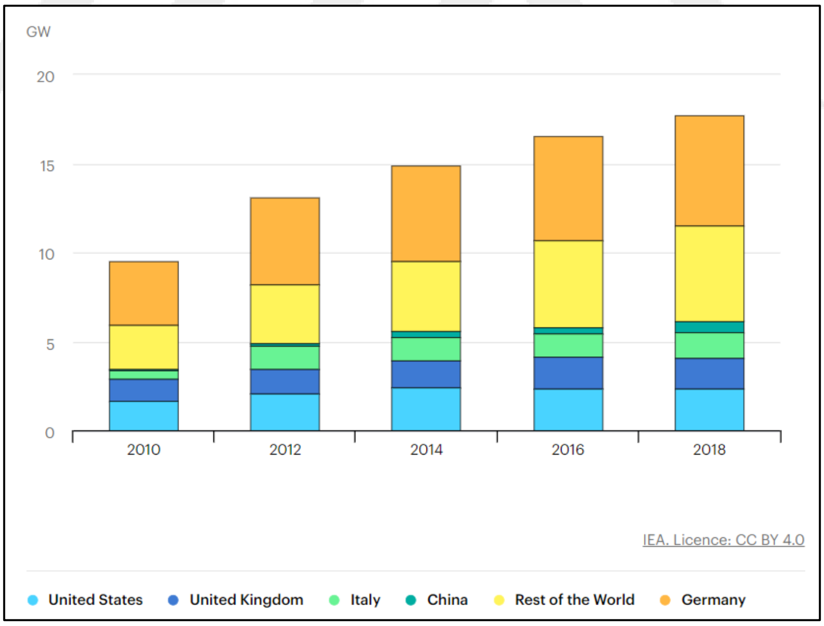

Figure 2.2. Biogas installed power generation capacity (IEA, 2020).

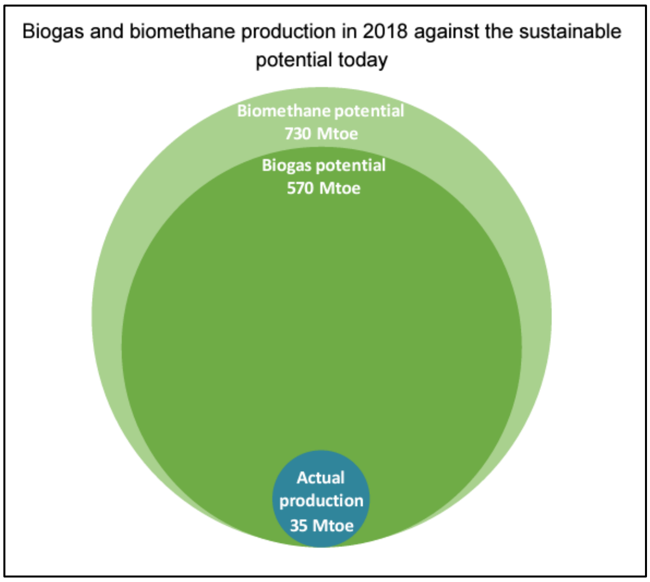

Figure 2.3. Biogas and methane production potential comparisons in 2018 (IEA, 2020).

2.2. Anaerobic Digestion Process Parameters

In the literature, there are some limit parameters such as type of substrates, temperature, mixing, pH, inhibiting substances, oxygen, sulfur content, hydrogen peroxide and heavy metal.

2.2.1. Substrates

The presence of substrate in anaerobic digestion is an initial step in the process. Anaerobic digestion uses a variety of substrate types, as can be observed from a review of the literature. Different substrates and their surface areas have distinct characteristics. For instance, it has been noted that throughout the anaerobic digestion process, the capacities for producing VFAs in food waste and agricultural wastes vary. In the anaerobic digestion mechanism, fast-growing VFA generation for food waste may result in inhibition (W. Li et al., 2017; Yang et al., 2013).

Additionally, not only the type of substrate but also its water (moisture) content is significant. Wet methods are applied for substrates with a dry matter content of less than 12-15% (Nsair et al., 2020). Substrates are classified separately according to their water content during the anaerobic digestion process. Definitions are divided into wet and dry according to their solid content (Angelonidi & Smith, 2015). The substrate/solid ratio in the wet anaerobic digestion process is thought to be less than 15%. This type of substrate is typically used in well-mixed digesters. The solids concentration of substrates used in dry processes is above 20% and can be used in most types of reactors (Abbassi-Guendouz et al., 2012). Both dry and wet systems have negative and positive aspects. Dry systems are in more use than wet systems due to their technology usage characteristics (Radwan et al., 1993; Lissens et al., 2001; Forster-Carneiro et al., 2008; Abouelenien et al., 2009). Studies in the literature summarized different methane, dry matter and biogas contents according to substrate types. The summary study is found Table 2.1.

Table 2.1. Agricultural substrates and biogas, methane potentials (Nsair et al., 2020).

Substrate	**DM %**	**oDM % (In DM)**	**Biogas Yield NL kg^{-1} FM**	**Methane Content NL kg^{-1} FM**	**Electricity Produced kWt^{-1} FM**
Pig slurry	4-19	73-86	20-35	10-21	40-71
Cattle slurry	6-11	75-82	20-30	11-19	40-61
Cattle manure	20-25	68-76	60-120	33-36	112-257
Poultry manure	34-50	60-75	130-270	70-140	257-551
Maize silage	28-39	85-98	170-230	68-120	347-469
Grass silage	15-50	70-95	102-200	46-109	208-408
Sugar beets	13-23	84-90	120-140	65-113	245-286
Olive pomace	57-90	55-86	92-147	65-104	188-300
Wheat straw	91-94	87-92	-	135-237	146-266
Corn (corn stover)	66-89	83-99	-	261-402	293-451
Rye	62-93	84-87	130	70	265

2.2.2. Temperature

As in every biological reaction, temperature is an important factor for performance in anaerobic digestion. Temperature regulation during biological reactions is a critical element. Generally, temperature ranges have been defined as Psychrophilic (5-15˚ C), Mesophilic (35-40˚ C), Thermophilic (50-55˚ C) (Bajpai, 2017). In general, the relationship between methanogenic growth character and temperature is shown in Figure 2.4. As shown in Figure 2.4, bacteria that are involved in the final synthesis of methane are more active and have a greater capacity for development at higher temperatures.

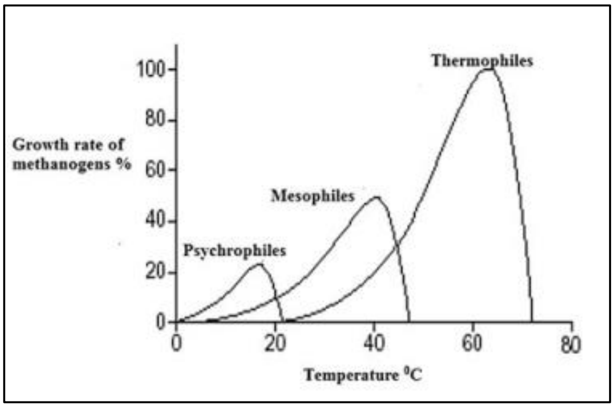

Figure 2.4. Relationship between temperature and methanogen bacteria (Bajpai, 2017).

However, one of the most important problems encountered in anaerobic digestion systems is finding the optimum temperature range for the system. The two most common temperature use ranges for anaerobic conditions are mesophilic and thermophilic conditions (Themelis, 2002). However, methane-producing bacteria can be inhibited under thermophilic conditions. Therefore, the most accurate temperature is 37˚ C (Jabłoński et al., 2015). Although biogas production has advantages such as low retention time, thermophilic processes are not often used in industrial applications because of their high heat energy content and sensitivity to toxic inhibitors (Al Seadi et al., 2008; Batstone et al., 2002).

2.2.3. Mixing

Mixing of reactors is one of the important practical parameters in anaerobic digester. In the literature different mixing types can be seen. These are continuous, intermittent and minimal mixing systems, as in different biological processes (Bajpai, 2017). Additionally, when we look at the equipment, there are mechanical, hydraulic and pneumatic mixing types (Deublein Dieter & Steinhauser A, 2011). Problems include unequal substrate distribution with enzymes and microorganisms and ineffective waste stabilization might result from inadequate reactor mixing (Karim et al., 2005; Kaparaju et al., 2008). Depending on the stage of the anaerobic digestion process, mixing may have varied outcomes. From the perspective of biogas production, it is thought that a small amount of mixing is sufficient for methanogens and acetogens (Chen et al., 1990; Bajpai, 2017). At the same time, since mixing will create a separate energy need in the reactors, the need should be analyzed well.

In Europe, mechanical mixing is the most widely utilized mixing technique when it comes to field applications. It is the most efficient mixing system (Lindmark et al., 2014). Considering the data in the literature, approximately half of the energy consumed for a digester reactor is consumed at normal operating scales. Mixing style also affects other important parameters. These parameters are HRT and SRT parameters.

2.2.4. pH

pH is a parameter that must be kept at optimum levels in order to maintain the activities of bacteria in biological systems. The reason why pH is considered important, especially in anaerobic systems, is that different types of organisms operate in the process. Various pH ranges may be necessary for various organisms (Nsair et al., 2020). For instance, the ideal pH range for acidogens is 5.5–6.5, but the pH range for methanogens is 7.8–8.2. The impacts of the intermediate products generated account for the variations in the phases' optimal pH ranges. There is an important balance element between volatile fatty acids and pH. Low pH resulting from volatile fatty acids produced in the acidogenesis and subsequent phases inhibits methanogenesis, which is the methane production stage, and has negative consequences on methane production. As a result, as seen in Figure 2.5, neutral pH ranges are seen as the most suitable management condition.

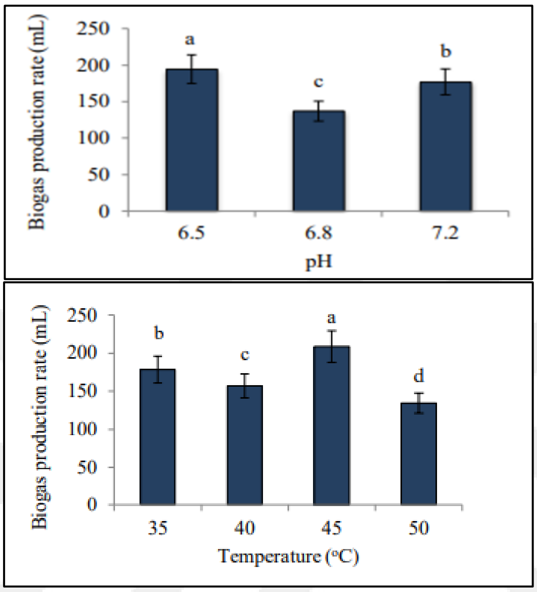

Figure 2.5. Effect of pH and temperature on biogas (Noxolo et al., 2014).

2.2.5. Oxygen

The most problematic situations that occur in anaerobic digestion processes are the complex biochemical processes and the unstable structure of anaerobic digestion processes (Krause et al., 2008). This unstable is also described as sensitive in some studies (Botheju & Bakke, 2011). For example, an unintentional small amount of oxygen entering the anaerobic digestion process may cause problems in the system. For this reason, anaerobic digestion systems must be constantly checked (Botheju & Bakke, 2011). Due to the complexity of anaerobic digestion, its requirements and stages must be constantly monitored. The microbial activity and requirements of anaerobic digestion, which has different phases, are different. For example, acetogens and methanogens maintain their activities at the highest level under the strictest anaerobic conditions. Especially the presence of methanogens, the final methane producers, in an oxygenated environment can cause inhibition and prevent methane production (Whitman et al., 2006).

2.3. Inhibiting Substances

Since biological activities are hosted by different organisms, they may be sensitive in some cases. As reviewed in Sections 2.2.2 and 2.2.5, temperature and oxygen parameters can be limiting for biological activities. For example, ingredients such as sulfur, hydrogen peroxide, and heavy metals are among the compounds and substances that affect the performance of anaerobic digestion systems (Y. Chen et al., 2008).

2.3.1. Sulfur Contents

Sulfur and sulfur-based compounds are frequently found in industrial wastewater. It is reduced to sulfur by sulfate-reducing bacteria in an oxygen-free environment (Y. Chen et al., 2008). It also raises the COD in wastewater and is harmful to sulfite methanogens and sulfate reducers. For these reasons, the methanogen process is halted. In investigations published in the literature, no specific SRB (sulphate reducing bacteria) inhibitor has been discovered (Celis-García et al., 2004).

2.3.2. Hydrogen Peroxide

Hydrogen peroxide is used to decompose anaerobic organic substances. Hydrogen peroxide damages cell walls by producing free radicals (NO· HO·) through reactions. This feature has also been found to contribute positively to methane production in some studies in the literature (Siami et al., 2020; T. Zhang et al., 2015). Although it is used as pre-treatment techniques in the literature, hydrogen peroxide generally has a toxic effect on bacteria. In anaerobic digestion processes, the activities of methanogenic bacteria are suppressed against hydrogen peroxide (Bajpai, 2017).

2.3.3. Heavy Metal

Heavy metals interfere with anaerobic processes by reacting with intracellular or extracellular enzymes at certain concentrations (Bajpai, 2017). Methane production needs to be run in optimal conditions in order to happen as efficiently as possible (Alrawashdeh et al., 2020). Low amounts of heavy metals such as Fe, Ni and Co provide the growth environment that bacteria need (Yue et al., 2007). Conversely, heavy metals such as Pb, Cu and As negatively affect anaerobic digestion processes (Nour et al., 2022).

Studies conducted in the literature emphasized that the amount of heavy metals can cause inhibitions. As stated in Table 2.2, Na, K, Ca and Al metals, which are not considered among the heavy metals, are essential metals for bacterial growth processes (Fagbohungbe et al., 2017). However, heavy metals such as Cu, Fe, Zn, Co, Ni, Cr, Mo, Pb, Cd, Hg directly affect anaerobic digestion processes. Among the metals mentioned in Table 2.2, Cd, Cr, Pb, Fe, Zn, Ni, Co, Mn metals were examined throughout the thesis studies.

Table 2.2. Heavy metal inhibitory concentrations in the literature (Nsair et al., 2020).

Metal	**Inhibitory Concentration in mg/L**	**Positive Concentration in mg/L**
Aluminum	1000-2500	-
Cadmium	36-3400	0.1-0.3
Calcium	300-8000	100-1035
Chromium	27-2500	0.1-15
Cobalt	35-950	0.03-19
Copper	12.5-1000	0-10
Iron	-	0.3-4000
Lead	67.2-8000	0.2
Magnesium	750-4000	0-720
Mercury	125	-
Molybdenum	1000	0-0.1
Nickel	35-1600	0.03-27
Potassium	400-28,934	0-400
Sodium	3000-16,000	100-350
Zinc	5-1500	0-5

2.4. Sludge Disposal Techniques

The proper circumstances must be provided for anaerobic digestion systems to function effectively. Particularly toxic and persistent pollutant parameters have contributed to the research and development of pre-treatment processes. Pre-treatment procedures allow for the knowledge of valuable wastewater content, such as petroleum-based treatment sludges, in addition to concentrating on harmful and persistent pollutants. For instance, petroleum-based treatment sludges contain high hydrocarbons and they are very valuable in terms of thermal value. Understanding the calorific values of petrochemical wastes is the subject of numerous studies that have been mentioned in the literature.

2.4.1. Incineration

The incineration is the process of complete combustion of wastes with high calorific value, especially oil industry wastes, in the presence of high amounts of air and flammable materials (Johnson & Affam, 2019). The combustion process takes place in different types of incinerators. These are generally rotary kilns, fluidized beds, and liquid injection models. The most commonly used oven types are rotary kilns and fluidized beds. For example, in a rotary kiln, operational temperatures vary between 980 and 1200˚ C (Johnson & Affam, 2019). Also is quite possible to use the fluidized bed system due to its operational benefits. Operational benefits are high mixing efficiency, processability with different fuels, and high combustion efficiency (Zhou et al., 2009). High combustion efficiencies have been found in studies conducted in the literature. On the contrary, sludges with high moisture content reduce the combustion efficiency (Sankaran et al., 1998).

2.4.2. Stabilization/solidification

Stabilization and solidification, abbreviated as S/S in the literature, is the process of preventing waste from becoming harmful to the environment through physical and chemical processes. This type of waste is generally handled in accordance with local laws and regulations. Using binders and sealing them is one of the necessary steps to organize the waste (Johnson & Affam, 2019). In addition, heavy metal-containing wastes are very harmful to the environment and human health and can be prevented by stabilization/solidification processes (Yin et al., 2006). Unlike other treatment methods discussed in the literature, S/S is low-cost and effective, but in case of environmental leaks, it is possible to release high pollutants into the receiving environment.

2.4.3. Oxidation Treatment

Oxidation pre-treatment is based on the principle of oxidative breakdown of organics and the production of hydroxyl radicals. By adding reactive substances to oily sludge, the organic compounds in the sludge are reduced to carbon dioxide and water (Ferrarese et al., 2008). The aim is to transform the reduced materials into non-hazardous materials. For example, applications such as ozone, hypochlorite and ultrasound are also used to remove hazardous substances in petrochemical sludges. Ozonation and ultrasound are among the methods frequently used in the pre-treatment processes of petrochemical sludges (Anjum et al., 2016).

2.5. Sludge Pre-treatment Techniques

The primary purpose of wastewater treatment plants is to reduce the pollution and harmful effects of unusable wastewater to an acceptable level. Different authorities have different pollution and receiving environment criteria. For example, if we look at some of the chemical pollutant parameters in drinking water, while the limit value of benzene is 0.001 mg/L according to EU regulations, this value is 5 times higher in the United States. Again, while the limit chromium value in drinking water is 0.05 mg/L in EU regulations, it is 0.1 mg/L according to United States rules (Boyd R. David, 2006). The situation is different in wastewater receiving environment parameters. Industrial pollutants, which are among the major pollutants, vary according to their subject. Pollutant characteristics are not the only factors in wastewater treatment facilities; other important factors include electro-mechanical needs, resilience of the facility, advancement in technology, treatment level, and, lastly, alternatives for sludge disposal (Volschan Junior et al., 2021).

Water pollution control and management infrastructure systems are based on the use of activated sludge systems as secondary treatment technology in treatment plants. When we look at the LCA studies on wastewater treatment plants in the literature, it is emphasized that biogas production provides an environmentally friendly advantage and also benefits the disposal of wastewater treatment sludge, which is hazardous waste (Awad et al., 2019). Anaerobic digesters support biogas production to purify solid-phase waste in treatment plants.

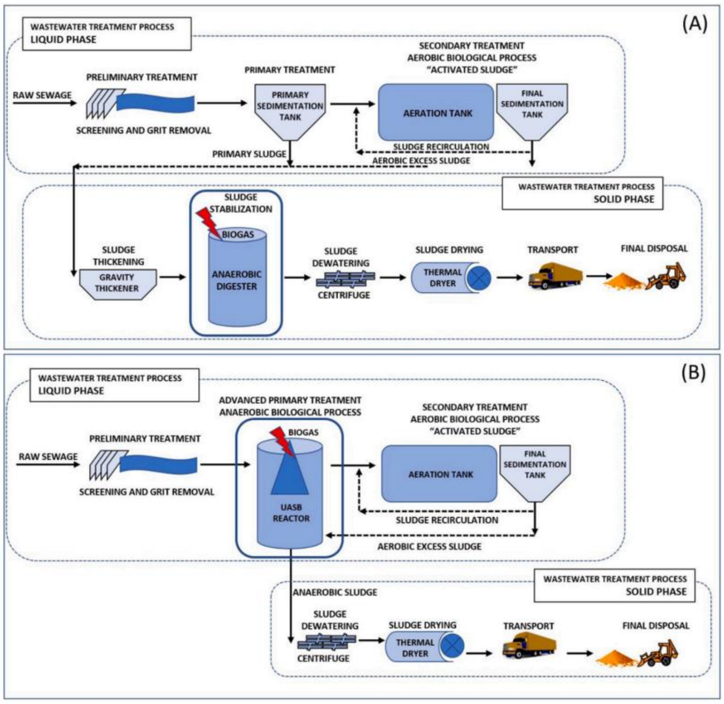

Figure 2.6. Wastewater treatment facilities anaerobic digestion options in the literature (Volschan Junior et al., 2021).

As seen in the Figure 2.6, different anaerobic digestion options are offered in options A and B (Volschan Junior et al., 2021). There are anaerobic digestion phases in different orientations in wastewater treatment plants. For example, in Figure 2.6. A, biogas production is aimed to stabilize the sludge coming out of the settling tanks. In Figure 2.6. B, biogas production is aimed with the UASB reactor after the wastewater inlet and pre-treatment step.

Anaerobic digestion processes are frequently used for the stabilization of primary and secondary treatment sludge in wastewater treatment plants. In addition, stabilization of wastewater treatment sludge reduces the organic matter rate along with methane production and is advantageous for the disposal of sludge (Andreoli et al., 2007; Volschan Junior et al., 2021). An additional benefit of anaerobic digestion, as discussed in other sections, is the production of methane as an energy source. The negative aspects are high technology investment, investment costs, qualified personnel, maintenance costs, etc. can be listed as (Anukam et al., 2019; Volschan Junior et al., 2021). The efficiency of anaerobic digestion is parallel to maximum biogas production. Therefore, the amount of biogas must be constantly controlled to understand the efficiency of anaerobic digestion.

Chemical oxygen demand is an important parameter for biogas production in wastewater treatment sludge. Methane production can be calculated based on the COD loading rate and the COD removed. (0.35 Nm^3 CH_4/kg COD). The methane content in biogas determines the calorific value of the total gas (Junior 2020). If it is assumed that there is 60% methane in 1 m^3, this corresponds to approximately 6 kWh electrical energy production (Biogas World, 2023). In order to continuously use electrical energy effectively, CO_2, H_2S, water and other elements must be separated from the system and this process is called purification (Das et al., 2022).

2.5.1. Thermal Pre-treatment

The primary purpose of thermal pre-treatment technology is the dewatering of sludge. Thermal pre-treatment process varies depending on the temperature value. Above 100˚ C is classified as high temperature pre-treatment, and below 100˚ C is classified as low temperature pre-treatment (Pilli et al., 2015). In thermal processes are dependent on constant heating. For example, when literature studies are summarized, high thermal pre-treatments are more successful in biogas production than low temperature pre-treatments. However, field applications might be more costly or require larger investments (Borges & Chernicharo, 2009).

2.5.1.1. Low Temperature Thermal Pre-treatment. The low temperature thermal pre-treatment option eliminates the high energy requirements and also prevents the formation of hazardous compounds. Low temperatures can increase biodegradability for some groups of microorganisms. Its duration is longer compared to high-temperature pre-treatment processes (Ferrer et al., 2008). In the studies on sludge in the literature, 60˚ C and 1 hour thermal pre-treatment increases the disintegration process of the sludge by revealing the enzymes in the sludge (Yan et al., 2008).

2.5.1.2. High Temperature Thermal Pre-treatment. Pre-thermal treatment at high temperatures aims to reduce high retention times and increase methane production by increasing the hydrolysis of sludge (Volschan Junior et al., 2021). High and constantly increasing temperatures facilitate the digestion of targeted substrates into small pieces by breaking down their cell walls (Kalogo et al., 2008). It has been determined that high temperatures not only increase biodegradability but also parameters such as viscosity and pathogen can be improved environmentally. The amount of temperature and exposure time are important parameters in thermal pre-treatment processes (Devos et al., 2021; Sapkaite et al., 2017). Disadvantages can be listed as odor formation and corrosion problems in equipment (Pilli et al., 2015).

2.5.2. Ultrasound Pre-treatment

The purpose of ultrasonic pre-treatment is to increase mass and heat transfer in reactions with sound waves, helping to form intermediate products and break down substances (He et al., 2017; Volschan Junior et al., 2021). During the ultrasound process, severe physical changes create microbubbles, and eventually cavitation is formed (Neumann et al., 2016). Emerging cavitation destroys the cell walls and accelerates the hydrolysis phase (Braguglia et al., 2011). In the ultrasound process, both anaerobic digestion is improved, and the sludge is dehydrated better. In addition, ultrasonic waves cause temperature increase, hydromechanical shear forces, and formation of radicals such as ·OH, ·O, ·H, etc. (Neumann et al., 2016). Just like in thermal pre-treatments, the energy given to water in ultrasound is one of the important parameters. For example, the application of low frequencies (20-40 kHz) increases methane efficiency for anaerobic digestion by facilitating mechanical effects (by not breaking down entire cells) (Z. L. Zhang et al., 2013). The negative aspect of large-scale applications of ultrasound is high energy consumption.

2.5.3. Ozone Pre-Treatment

Ozone is a frequently used and commercial pre-treatment process among the chemical pre-treatment processes. In a mechanical way, ozone produces hydroxy radicals. Hydroxy radicals interact with organic and inorganic substances (Al Momani et al., 2011). Especially in the long-chain carbon bonds, ozone affects these bonds. Breaking long-chain carbon bonds prevents the hydrolysis of solid materials, hinders the formation of excess biomass, and contributes positively to biogas production (Yasui & Shibata, 1994). The relationship between methane yield and ozone is highly dependent on the dose amount. High doses of ozone may have the effect of reducing methane efficiency (Yeom et al., 2002). Additionally, adding excessive ozone to the system causes organic matter become non-functional in anaerobic digestion processes (Volschan Junior et al., 2021). For this reason, the most optimum ozone dosing to be applied in anaerobic digesters is given in the literature as 0.1 g O_3 g^{-1} COD, 0.2 g O_3 g^{-1} TSS, 0.15 g O_3 g^{-1} TS (Bougrier et al., 2007; Weemaes et al., 2000; Yeom et al., 2002) The ozonation process may cause physicochemical changes in the area it affects. Low pHs can be observed following the ozonation process (Choudhury et al., 2022; Kianmehr et al., 2010).

Efficiency in ozone application depends on mass transfer and reaction kinetics. Both the production and transfer of ozone to sludge are quite expensive compared to other pre-treatment processes, and since ozone is unstable, it must be produced on-site in field applications (Tyagi & Lo, 2011).

As can be seen in Figure 2.7, in the study conducted in the literature, the biogas production of WAS (oil refinery-based waste activated sludge) in both ozonated and non-ozonated sludge samples at low TS and COD concentrations was examined. The amount of ozone given for WAS is 0.05 g O_3/g TS (Haak et al., 2016).

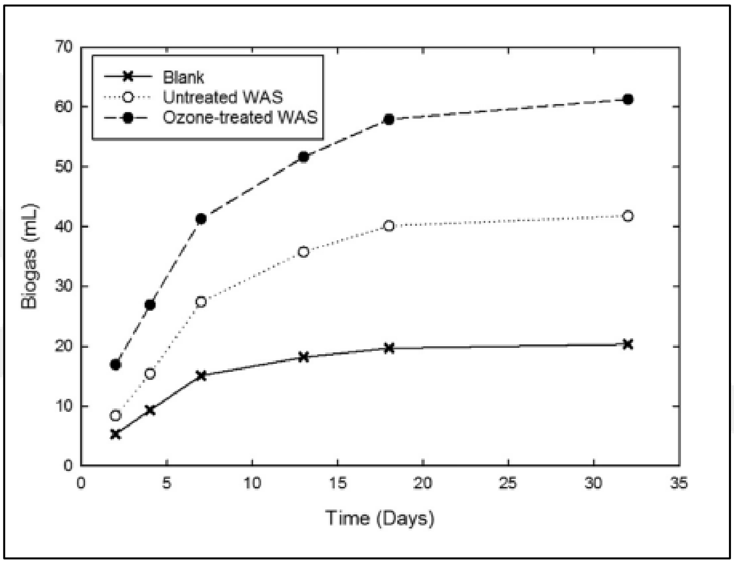

Figure 2.7. Treated and untreated WAS sludge biogas potential (Haak et al., 2016).

2.5.4. Acid and Alkali Pre-treatment

Acid and alkaline pre-treatment techniques may be preferred because they are easy to apply. At the same time, this pre-treatment technique has positive effects on methane efficiency (Volschan Junior et al., 2021). Chemicals such as HCl, H_2SO_4, H_3PO_4 or NaOH, KOH, $MgOH_2$ eliminate the need for high temperatures in pre-treatment applications and contribute positively to biodegradation at average operating temperatures (H. Li et al., 2012; Zhen et al., 2017). Acid and alkali pre-treatment techniques may affect different substrate types. For example, acids are used for lignocellulosic biomass. Alkaline substances are used for solubilization of sludge (Jin et al., 2016). One thing to be careful about when applying acid and alkaline substances for pre-treatment is the inhibition in biological treatments. To prevent inhibition, acid and alkali consumption should be reduced and used in an optimized manner (H. Li et al., 2012).

2.6. Anaerobic Digestion of Petrochemical Wastewater Sludge

When petrochemical wastewater treatment sludge anaerobic digestion studies are examined in the literature, it is seen that there are not many studies. This situation causes insufficient data and interpretation difficulties in comparing laboratory results with literature results.

There are different results and focal point in research on petrochemical wastewater treatment sludge and biogas production. For example, in the study conducted by Laura Haak et al. (Haak et al., 2016), anaerobic digestion potentials were examined by treating dissolved air flotation unit (DAF) and activated sludge samples from an oil refinery together. Toxicity analysis, which is one of the crucial features of petrochemical wastewater treatment sludges, is also a subject of this study. Toxicity analysis, which is one of the crucial features of petrochemical wastewater treatment sludges, is also a subject of this study. Ozone pre-treatment techniques are also one of the subjects in examining biogas potential. In the studies carried out, the samples were taken from an unnamed refinery. In order to perform the characterization tests in the most accurate way, the samples were homogenized in industrial mixers. The collected DAF and WAS samples were pre-treated with ozone. In the study, 0.05g O_3/g TS was loaded for DAF sludge and WAS samples. The reason for choosing this low dose is that it is at a level that will not disrupt biological activity. The dosed ozone flow is 0.5 liters per minute. After the ozonation process, the samples were kept at room temperature for 1 hour to ensure stabilization. Inoculum were added to the test setups to support biological activity. Inoculum samples were taken from the anaerobic digestion unit of the municipal wastewater facility operated under mesophilic conditions. Additionally, toxicity tests were performed to detect inhibition in the study. It did not show any significant incubation in ozonated and non-ozonated sample samples. In the analysis, it was determined that the DAF sample contained excess VS and COD. It was determined that the amounts of VS and COD in WAS samples were much less than in DAF samples. A decrease in TS, VS, COD parameters was observed in DAF and WAS samples after ozone pre-treatment. If we look at biogas measurements, biodegradability increased and COD removal was observed in WAS pre-treated with ozone. It is thought that COD removal and increased biodegradability accelerate the hydrolysis stage in the anaerobic digestion process. The same applies to DAF samples. In summary, while 160L/kg COD_{added} biogas was produced for ozonated WAS samples, 80L/kg COD_{added} biogas was produced for non-ozonated WAS samples. For DAF samples, 100 liters of biogas were produced per kilogram of COD, with 0.5% inclusion added.

Again, in the study conducted by Wang et al. (Wang et al., 2016) with oil industry waste sludge samples, the optimization study with two-phase anaerobic digestion processes was summarized. In two-phase anaerobic digestion, 4 different processes (hydrolysis, acidogenesis, acetogenesis, methanogenesis) are processed in different reactors. The samples in the study were taken from a petrochemical wastewater treatment plant in Beijing, China. In the study, TS, VS, TN, COD, alkalinity, dissolved protein, fat content, etc. were measured on the samples. The pH of the inoculums used in the reactors is between 6.0 and 6.5. The reason why anaerobic digestion processes were included in the study is that both sludge stabilization and methane production are possible in anaerobic digestion (Kiyasudeen et al., 2016; Stasinakis, 2012). The single-phase anaerobic digestion process, which includes all stages, was preferred in the two-phase anaerobic digestion study due to the high waiting time and neutral pH requirements. Execution of separate phases helps to produce more effective methane by increasing hydrolysis and acidogenic performance (Demirel & Yenigün, 2002). HRT, temperature and pH, which are other important parameters in anaerobic digestion processes, were observed to be continuous during the study. At the same time, the performances of different pre-treatment techniques were also observed in the study; These are thermal, ultrasonic and alkaline pre-treatment techniques. If we look at the results of the studies, we see that the mentioned pre-treatment processes were applied in both phases. A connection has been established between the application of pre-treatment techniques and VS removal. It has been determined that the most successful pre-treatment technique is the ultrasonic thermal technique. It has been determined that pH 6.5, 55˚C and 2-day HRT conditions are the best operating range in reactor applications (hydrolytic-acidogenic). Under these conditions, the formation of 1801.5 ± 44 mg/L VFA was observed. In the study, VFA formation was examined in the HRT, pH and temperature axes.

In the literature, the pre-treatment techniques of Siddique et al. were examined in a separate study. The study focused in detail on the effects of anaerobic co-digestion and pre-treatment options on petrochemical wastewater treatment of activated sludge. Before starting the anaerobic digestion process, ultrasound and microwave options were tested on the samples (Siddique et al., 2017). The inoculum sample in the reactors was taken from recycled activated sludge from another full-scale anaerobic digestion plant. TS and VS values were examined in both inoculum samples and petrochemical wastewater treatment sludge samples. As a result of the analysis, the TS rate of the sludge samples taken from the facility was 59.89 mg/L and 76.26 mg/L in the petrochemical wastewater treatment plant samples. Biogas studies were carried out in a total of 14 different batch digesters for 31 days. The inoculum and sample mixture ratio was determined as 1:1. In the ultrasound process, it was pre-processed at 20 kHz and 60% amplitude settings for 15 to 30 minutes. Microwave was used to apply thermal pre-treatment. The most suitable temperature and working time are between 155˚ C - 185° C and 30-60 minutes, respectively. Considering their methane production potential, D7, D6 and D5 reactors showed the best performance, respectively. D1 reactor was considered as control, D2, D3 and D4 reactors were evaluated as co-digestion option with petrochemical wastewater and waste activated sludge, and no pre-treatment process was applied. The reason why D5, D6, D7 reactors are successful in methane production is the evaluation of ultrasound and temperature applications, which are pre-treatment. While the highest value produced by anaerobic co-digestion was 0.22 L CH_4/g VS_{added}, this value was 20 percent lower than the degree from samples alone. In reactors with pre-treatment, this rate is 25%-53% higher than in reactors without pre-treatment. The highest methane production potential is 0.47 L CH_4/g VS_{added}.

3. METHODOLOGY

3.1. Anaerobic Digestion Reactors and Apparatus

Anaerobic digestion analyzes are performed with laboratory test equipment. Anaerobic digestion systems consist of batch reactors and biogas gas counting is performed. As shown in Figure 3.1, 8 1-liter borosilicate amber bottles and 8 Milligascounters were used in the reactor and setups. Plastic-based hoses were used to establish the gas connection between the borosilicate bottles and the Milligascounter (MGC, Ritter, Bochum, Germany), as can be seen in Figure 3.1. Anaerobic digestion, by its nature, must be free of oxygen, so the bottles are covered with a liquid seal to ensure that they are airtight. Similar to the test setup shown in Figure 3.1, another separate setup was set up for the second trial sets. The purpose of creating test setups is to measure biogas potential in petrochemical wastewater treatment sludge.

Figure 3.1. Anaerobic digestion set-up.

In the first test setup, R1 seed, R2 ozone, R3 ultrasound and R4 hybrid (ozone and ultrasound) reactors were installed. The reason for installing redundant mechanisms is to detect possible errors that may occur in the reactors. In the second trial set, R5 seed, R6 untreated waste sludge, R7 co-digestion and R8 hybrid mechanisms were tested. R4, R8 hybrid R1, R5 seed test reactors were used again in two sets. The aim is to consider biogas potential from hybrid and seed type mechanisms. In all test setups, biogas measurements were monitored for 28 days at 2-3 day intervals.

Table 3.1. Reactor content volume and parameters details in Part 1.

Reactor Number	Reactor Content	Volume of the content	Day 0 Process Parameters	Day 27th Process Parameters	Biogas Measurement Frequency
R1	Seed	250 ml	TS, TVS, pH, COD, TKN, Heavy Metal Analysis	TS, TVS, pH, COD, TKN, Heavy Metal Analysis	Five consecutive days GC measurement and the rest once every two days GC measurement + Once every two days miligas measurement
R2	Ozone + Seed Sludge	500 ml (250 ml Seed Sludge + 250 ml Ozone Treated Sludge)			
R3	Ultrasound + Seed	500 ml (250ml Seed Sludge + 250 ml Ultrasound Treated Sludge)			
R4	Hybrid (Ultrasound Treated Sludge + Ozon Treated Sludge + Seed)	500 ml (250 ml Seed Sludge + 125 ml Ultrasound Treated Sludge + 125 ml ozone treated sludge)			

Table 3.2. Reactor content volume and parameters details in Part 2.

Reactor Number	Reactor Content	Volume of the content	Day 0 Process Parameters	Day 27th Process Parameters	Biogas Measurement Frequency
R5	Seed	250 ml	TS, TVS, pH, COD, TKN, Heavy Metal Analysis	TS, TVS, pH, COD, TKN, Heavy Metal Analysis	Five consecutive days GC measurement and the rest once every two days GC measurement + Once every two days miligas measurement
R6	Untreated Sludge + Seed	500 ml (250 ml Seed Sludge + 250 ml Untreated Sludge)			
R7	Untreated Sludge + Feed Sludge + Seed Sludge	500 ml (250 ml Seed Sludge + 125 ml Feed Sludge + 125 ml Untreated Sludge)			
R8	Hybrid (Ultrasound Treated Sludge + Ozon Treated Sludge + Seed)	500 ml (250 ml Seed Sludge + 125 ml Ultrasound Treated Sludge + 125 ml ozone treated sludge)			

Experimental results were collected from a total of 16 different reactors at 2-month intervals. Except for R1 and R5 mechanisms, all test devices contain a total of 500 ml of sample. The hot room was fixed at 37.5˚ C to keep the experimental setups under constant conditions and mesophilic conditions. The hot room is constantly monitored and temperature adjustment is ensured. Mixing is carried out before measurement and frequency of the measurement is specified in Table 3.1, and Table 3.2. The purpose of this process is to fully mix the substrate and microorganisms in the devices and to accelerate the reactions.

3.1.1. Contents and Nomenclature of Anaerobic Digester Reactor

In summary, R1 and R5 reactors were tested with a 250 ml seed sludge sample taken from İSKİ Ambarlı Wastewater Treatment Plants. The R2 reactor in Part 1 contains 250 ml of ozone pre-treated petrochemical wastewater treatment sludge and 250 ml of seed sludge sample. R3 and R4 reactors in Part 1, seed sludge and pre-treated petrochemical wastewater treatment sludge rates were determined as 250 ml each. In the R3 reactor, there is petrochemical wastewater treatment sludge that has been subjected to ultrasound treatment. Finally, in the R4 and R8 reactors, mechanisms were established to test the petrochemical wastewater treatment sludge subjected to the combined pre-treatment process.

In Part 2, R6 untreated wastewater treatment sludge was used. The aim here is to clearly understand the effects of pre-treatment processes on biogas production potential. The R7 reactor, unlike all test setups, aims to explain the relationship between biogas production potential and codigestion. In the R7 reactor, feed sludge and untreated petrochemical wastewater treatment sludge taken from İSKİ Ambarlı Wastewater Treatment Facilities were used. Details of pre-treatment procedures and relevant dose amounts are explained in 3.4 Pre-treatment Application section. Different nomenclatures were used in the reactors to make it easier to follow while testing studies were carried out in the laboratory. Table 3.3, shows these nomenclature details.

Table 3.3. Reactor setup and nomenclature details.

Parts	**Reactor No**	**Laboratory Parallel Reactor Nomenclature**
Part 1	R1	Seed 1
		Seed 2
	R2	O_3-1
		O_3-2
	R3	US-1
		US-2
	R4	HYB-1
		HYB-2
Part 2	R5	Seed-1
		Seed-2
	R6	TP-1
		TP-2
	R7	CO-1
		CO-2
	R8	HYB-1
		HYB-2

3.2. Seed Sludge Preparation

The seed sludge, which is constantly used in the test setups, was taken from İSKİ Ambarlı Wastewater Treatment Plant digestion return activated sludge. The purpose of using inoculum samples is to increase efficiency by providing efficient substrates for microorganisms in the anaerobic digestion process and contribute to methane formation. A total of 10 L seed samples for the first test set, and a total of 5 L seed and 5 L feed samples for the second set were taken from İSKİ Ambarlı Wastewater Treatment facilities. Samples were taken from the facility by expert staff, paying attention to health and safety rules. Samples were stored in PET product-derived storage containers. Experimental setups were set up and testing procedures were initiated within 24 hours after the samples were taken. When samples were not used externally, they were kept stable in a dark and 4˚ C cold room. Inoculum samples are waited until they reach room temperature before being used in the reactors. At the same time, it was ensured that complete mixing was achieved. Total solids, volatile solids and COD tests were performed before the setups were set up for seed samples. Bringing the temperatures to room temperature, mixing and 4˚ C storage conditions were also applied to all mechanisms R1-R8.

The first 8 different mechanisms (R1-R4 with a parallel mechanism) were installed. The second experimental set was established with 8 different mechanisms (R5-R8 with a parallel mechanism). The purpose of establishing two different experimental sets is to measure different biogas potentials in different samples written in Table 3.1, and Table 3.2.

3.3. Petrochemical Wastewater Treatment Sludge Preparation

The samples used for the thesis research were from a Turkish industrial company that operated a refinery and petrochemical plant. Samples were taken from the wastewater treatment plant with RAS (return activated sludge) line. A 20 L sample was provided during each process for pre-treatment studies and characteristic properties. The samples supplied were processed with the storage conditions and usage conditions mentioned in 3.1.1. section. Since the water content of the supplied sample was high, it was used by concentrating it in 1/2 ratio.

3.4. Pre-treatment Applications

The ultrasound, ozone and hybrid pre-treatments mentioned in the R2, R3, R4 and R8 reactors were implemented. For example, the ozone-purified sample in the R2 reactor was applied for 5 minutes with an ozone flow rate of 100 L/h, thus reaching the optimum purification degree in total. The most optimum dose amount is 0.080 g O_3/g TS. Again, the ultrasound pre-treatment process in the R3 reactor was applied for 15 minutes, 20 kHz, 90% amplitude, and the highest purification efficiency was 1.25 W/mL. The hybrid pre-treatment process used in R4 and R8 reactors was carried out by combining ultrasound and ozone studies. In the evaluations of the pre-treatment processes carried out by Elif Sena Uyal (Elif Sena Uyal, 2024) , it was decided that the most appropriate treatment efficiency combination would be first ultrasound treatment followed by ozone treatment. The pre-treatment criteria applied in the R2 and R3 mechanisms were also applied in the hybrid selection. Petrochemical treatment sludge was first applied for 15 minutes at 20 kHz, 90% amplitude, and then ozone pre-treatment was applied at an amount of 0.080 g O_3/g TS. The reason for the hybrid study was that in the study conducted by Elif Sena Uyal, (Elif Sena Uyal, 2024) the highest efficiency was obtained with the combined effect.

The pre-treatment process was applied to the test setups at the same time on the same day that the setup was established. It is especially important to carry out the process quickly so that the efficiency of the ozonation process is not lost.

3.5. Analytical Methods

External factors and the characteristics of the substrate and inoculum samples used are very important in examining biogas production methods. Analysis methods were carried out in Boğaziçi University Environmental Sciences Laboratories throughout all laboratory studies. Although the experimental studies differed in terms of the pre-treatment techniques carried out by Elif Sena Uyal, (Elif Sena Uyal, 2024) the studies were conducted by selecting parameters that are vital for biogas potential. The parameters chosen for biogas experiments are from literature studies and are traceable parameters suitable for biogas. Total solid (TS), total volatile solid (TVS), pH, Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen TKN, Heavy Metal analyzes were performed. In the analyses, TS, TVS, COD and heavy metal analyzes were run in parallel. All analyzes were performed in accordance with Standard Methods (Standart Methods, 2018).

Daily biogas measurements used in biogas studies are performed at the frequency shown in Table 3.1, and Table 3.2. Measurements in gas chromatography were made with an Agilent Technologies brand 6850 model measuring device. The purpose of using gas chromatography is to separate the components in the gas mixture and measure their volumes. The gases targeted to be measured were determined as CH_4, CO_2, N_2. The device constantly uses helium gas and was used between 4-5 Bar in accordance with the manufacturer's recommendations. Operating conditions in the device, the oven temperature is fixed at 150˚ C.

3.6. Biochemical Methane Potential (BMP) Test

Experimental setup and other operational arrangements for BMP test are described in Section 3.1. VDI 4630 (VDI, 2016) standard was used to perform the BMP tests. Again, the purpose of establishing the first part experimental setup mentioned in Table 3.1, and Table 3.2. is to detect the biogas production differences between ozone, ultrasound and hybrid reactors. In the second part, the aim is to investigate the potential of petrochemical wastewater treatment sludge by mixing the untreated sludge samples with the feed sludge taken from İSKİ Ambarlı Wastewater Treatment Facilities, unlike the first part. Hybrid implementation is included in both parts. The reason why hybrid applications are repeated is the high pre-treatment efficiency.

After the characterization studies were completed, the sample volumes to be placed in the reactors were determined in parallel with the total solids ratio. In line with the VDI 4630 standard, the ratio of inoculum and sample was determined as 1:1. Separate seed reactors were established to determine the effect of inoculum added to the reactors.

One of the important criteria for conducting biogas tests is the establishment of a completely oxygen-free closed reactor system. The points mentioned in Section 3.2 were applied and completely anaerobic conditions were created with pure nitrogen gas before the closed reactors were left in the temperature controlled room for BMP tests.

4. RESULT AND DISCUSSION

4.1. Petrochemical Wastewater Sludge and Seed Sludge Characteristics

Sludge samples are supplied from the refinery operator company at a rate of 20 L per week. Before being used, the samples are kept in a cool chamber at 4˚C. During the process, it is anticipated to return to ambient temperature.

4.1.1. Petrochemical Wastewater Sludge Characteristics

Analyzes of petrochemical wastewater treatment sludge were carried out before and after the installation of the reactors. TS, TVS, COD, pH, TKN and removal rates were studied.

Table 4.1. Total solids and total volatile solids analysis results - Part 1.

		Part 1 Day 0		**Part 1 Day 27th**	
Reactor Contend	Reactor	TS (g/L)	TVS (g/L)	TS (g/L)	TVS (g/L)
Seed	R1	120.5	48.3	52.9	19.3
O_3	R2	27.8	23.0	34.9	16.3
US	R3	28.0	23.0	33.2	14.6
HYB	R4	28.4	23.8	33.0	14.6

In tests performed with 25 ml sample samples, while a decrease in total solids and volatile solids is expected, an increase of 25% to 16% in the TS parameter is observed in R2, R3 and R4 reactors. Strikingly, a decrease in the TVS parameter was observed in all reactors as can be seen in the Table 4.1.

Table 4.2. Total solids and total volatile solids analysis results - Part 2.

		Part 2 Day 0		Part 2 Day 29th	
Reactor Contend	Reactors	TS (g/L)	TVS (g/L)	TS (g/L)	TVS (g/L)
Seed	R5	69.9	34.6	50.8	21.7
TP	R6	44.5	25.3	34.4	17.9
CO	R7	83.5	29.0	70.7	20.0
HYB	R8	43.2	23.9	34.1	16.6

In the second part tests, unlike the first part tests, a decrease was observed in both TS and TVS. The average values of the R5-R8 reactors mentioned in Table 4.2, are reflected. The reason for giving an average value is that all analyzes were performed for each parallel reactor.

Table 4.3. COD, TKN and pH analysis results - Part 1.

		Part 1 Day 0			Part 1 Day 27th		
Reactor Contend	Reactors	COD (mg/L)	TKN (mg/L)	pH	COD (mg/L)	TKN (mg/L)	pH
Seed	R1	44811.1	2765	7.08	31685.7	2646	6.95
O_3	R2	31455.7	1911	6.86	24787.2	1886	6.76
US	R3	31067.2	1911	6.58	24363.8	1872	6.44
HYB	R4	35669.3	1883	6.67	22035.1	1869	6.53

As can be seen in Table 4.3, a decrease of approximately 25% to 40% is observed in the COD analyzes of part 1 reactors. The degradation of COD may have contributed positively to biogas production. However, the same removal rate was not found in TKN measurements. Another difference in the measurements is the slight decrease in pH. The low pH observed after biogas is a situation that can be observed in the anaerobic digestion process (Şenol et al., 2017).

Table 4.4. COD, TKN and pH analysis results - Part 2.

		Part 2 Day 0			Part 2 Day 29th		
Reactor Contend	Reactors	COD (mg/L)	TKN (mg/L)	pH	COD (mg/L)	TKN (mg/L)	pH
Seed	R5	58042	2773	7.15	25758	2663	7.01
TP	R6	38494	2136	7.11	24893	2124	7.03
CO	R7	46168	2201	7.04	27115	2208	6.96
HYB	R8	39341	2058	6.85	23675	2108	6.70

As can be seen in Table 4.4, a very high removal rate could not be achieved in the TKN parameter. There has even been an increase in some reactors. Looking at the results, TKN analyzes do not give interpretable results in examining the biogas formation potential. Additionally, no errors were observed during TKN experiments in laboratory studies.

Table 4.5. Heavy metal analysis results day 0 - Part 1 (mg/L).

	Fe	Zn	Mn	Cu	Cr	Ni	Pb	Co
Seed 1	1526.2	268.5	36.6	6.2	35.9	17.5	2.7	1.1
Seed 2	1357.5	236.0	33.6	6.2	32.0	16,0	2.4	1.0
O_3-1	725.0	172.2	17.9	2.8	17.2	8.6	1.6	0.6
O_3-2	836.2	173.5	17.9	3.6	17.1	7.9	1.4	0.6
US-1	893.1	271.6	19.1	3.7	19.8	8.9	1.2	0.5
US-2	829.3	217.2	21.6	3.6	20.3	9.7	1.1	0.7
HYB-1	733.1	149.1	16.4	2.6	14.1	7.9	0.9	0.7
HYB-2	764.3	166.0	17.2	3.3	15.8	6.9	1.3	0.6

Total metal values were measured in Part 1 and the highest concentrations were detected in Seed samples, as can be seen in Table 4.5.

Table 4.6. Heavy metal analysis results day 27th - Part 1 (mg/L).

	Fe	Zn	Mn	Cu	Cr	Ni	Pb	Co
Seed 1	1250.5	236.5	32.7	5.8	32.7	15.0	2.2	1.1
Seed 2	1036.7	207.2	26.9	4.6	25.5	12.0	2.0	0.7
O_3-1	698.6	152.8	17.7	3.8	16.2	7.6	1.2	0.6
O_3-2	708.0	147.2	17.2	3.6	15.4	7.7	1.1	0.7
US-1	600.5	167.8	17.4	3.6	15.3	7.5	1.0	0.7
US-2	718.0	152.8	17.2	3.1	15.9	7.7	1.2	0.6
HYB-1	758.6	150.9	18.7	4.1	16.3	8.5	1.3	0.6
HYB-2	744.9	240.9	17.1	3.5	15.4	8.0	0.8	0.7

Heavy metal analyses, another important parameter in biogas production, were tested both before and after reactor installations. As seen in Tables 4.5, and 4.6, Fe, Zn, Mn metals are predominantly present in the samples.

Table 4.7. Heavy metal analysis results day 0 - Part 2 (mg/L).

	Fe	Zn	Mn	Cu	Cr	Ni	Pb	Co
Seed 1	895.6	152.4	21.3	19.1	27.1	12.0	1.3	0.6
Seed 2	768.7	143.1	19.3	21.8	24.1	14.1	1.8	0.7
CO-1	1494.9	146.8	30.8	20.7	16.6	13.9	1.6	1.2
CO-2	1395.6	138.7	26.5	19.0	20.0	12.3	1.6	1.3
TP-1	1301.9	146.7	25.7	18.3	26.4	12.0	1.2	1.4
TP-2	1518.1	161.1	30.3	20.3	27.6	14.3	1.7	1.5
HYB-1	1110.0	160.5	19.3	17.6	25.0	10.8	1.0	0.8
HYB-2	929.4	305.5	18.6	20.8	21.1	9.1	1.0	1.0

Table 4.8. Heavy metal analysis results day 29th - Part 2 (mg/L).

	Fe	Zn	Mn	Cu	Cr	Ni	Pb	Co
Seed 1	557.5	109.2	12.8	12.0	20.5	8.3	0.5	0.5
Seed 2	566.9	117.4	11.7	12.0	13.6	6.7	0.5	0.7
CO-1	704.4	108.0	11.4	9.9	16.3	7.9	0.6	0.7
CO-2	597.5	97.4	11.1	10.8	12.6	8.0	0.7	0.6
TP-1	692.4	146.2	13.1	12.4	15.5	8.8	1.1	0.6
TP-2	609.9	99.9	10.5	10.5	10.4	7.7	0.7	0.4
HYB-1	533.1	114.9	12.0	11.0	12.7	7.5	1.1	0.4
HYB-2	731.2	114.9	12.0	11.9	14.0	9.0	0.7	0.4

As can be noticed from the Part 2 measurements in Table 4.7. and Table 4.8, the Cu metal concentration was found to be considerably higher compared to the Part 1 results. Other metals vary at different rates.

Table 4.9. Reactor heavy metal inhibitory level.

Heavy Metals	**Possible Inhibitory Reactors**	**Explanation**
Zn	R1-R8	The parameter is well above the limits determined in the literature.
Cu	R4-R8	Part 2 reactors are all above the limit inhibition value.
Cr	R1, R5, R6	Potential interference has been identified in inoculum sludge and untreated petrochemical wastewater treatment sludge.

Considering the heavy metal measurements and literature comparisons given in Section 2.3.3, the reactors where inhibition can be observed and heavy metal comparisons are summarized in the Table 4.9, above.

4.1.2. Seed and Feed Sludge Characteristics

The analysis studies carried out on seed sludge samples taken from İSKİ Ambarlı Facilities were also applied to other sludge samples. The characteristics of the inoculum samples taken at different times are written against the Seed 1, Seed 2 before biogas samples. COD values vary by nearly 30 percent between Seed 1 and Seed 2 samples. It is very difficult to determine the cause of the COD difference. Operation, temperature and facility operating parameters may have been different in the samples taken on 20/09/2022, 28/11/2022. In the table shown below Table 4.10, the average values of COD, TKN, TS, TVS, TKN, pH parameters in laboratory tests are taken.

Table 4.10. Characteristic features of seed and feed sludges.

	Type of Seed & Feed Sludges				
Parameters	Part 1 Seed Day 0	Part 1 Seed Day 27^{th}	Part 2 Seed Day 0	Part 2 Seed Day 29^{th}	Feed Day 0
COD (mg/L)	44811.1	31685.7	58042.4	25758.1	50103.6
TKN (mg/L)	2765.0	2646.0	2773.4	2662.8	1022.0
TS (g/L)	120.5	52.9	69.9	50.8	147.8
TVS (g/L)	48.3	19.3	34.6	21.7	33.5
pH	7.08	6.98	7.15	7.03	6.98
Fe (mg/L)	1441.9	1143.6	832.1	562.2	-
Zn (mg/L)	252.3	221.8	147.7	113.2	-
Mn (mg/L)	35.1	29.8	20.2	12.2	-
Cu (mg/L)	6.2	5.2	20.4	11.9	-
Cr (mg/L)	34.0	29.1	25.6	17.1	-
Ni (mg/L)	16.7	13.4	13.1	7.5	-
Pb (mg/L)	2.5	2.1	1.5	0.5	-
Co (mg/L)	1.1	0.9	0.6	0.6	-

4.2. Biogas and Methane Production

4.2.1. Part 1 Biogas and Methane Production

In the first part, the samples were tested for a total of 29 days to understand the biogas potential. During the analysis, biogas production was monitored using Milligascounters and gas production amounts were recorded daily and cumulatively. The Seed 2 reactor produced a maximum of 101.11 ml of gas on a daily basis and a total of 231.46 ml of gas throughout the analysis. The daily gas production of the O_3-1 reactor reached a maximum value of 115.85 ml and produced a cumulative

762.92 ml of gas during the analysis. The O_3-2 reactor produced a maximum of 102.63 ml of gas daily and a total of 248.8 ml of gas throughout the analysis. While the US 1 reactor produced a maximum of 81.12 ml of gas on a daily basis, the total gas production throughout the analysis was 561.6 ml. The daily gas production of the US 2 reactor reached a maximum of 78 ml, and this reactor produced a total of 168.48 ml of gas throughout the analysis. The daily gas production of the $Hybrid_1$-1 reactor was recorded as a maximum of 35.75 ml, and the cumulative gas production during the analysis was recorded as 156 ml. While the $Hybrid_1$-2 reactor produced a maximum of 167.48 ml of gas on a daily basis, the total gas production throughout the analysis reached 644.64 ml. Daily and cumulative gas production graphs recorded during the biogas potential analysis are given in Figure 4.4, and Figure 4.5 respectively.

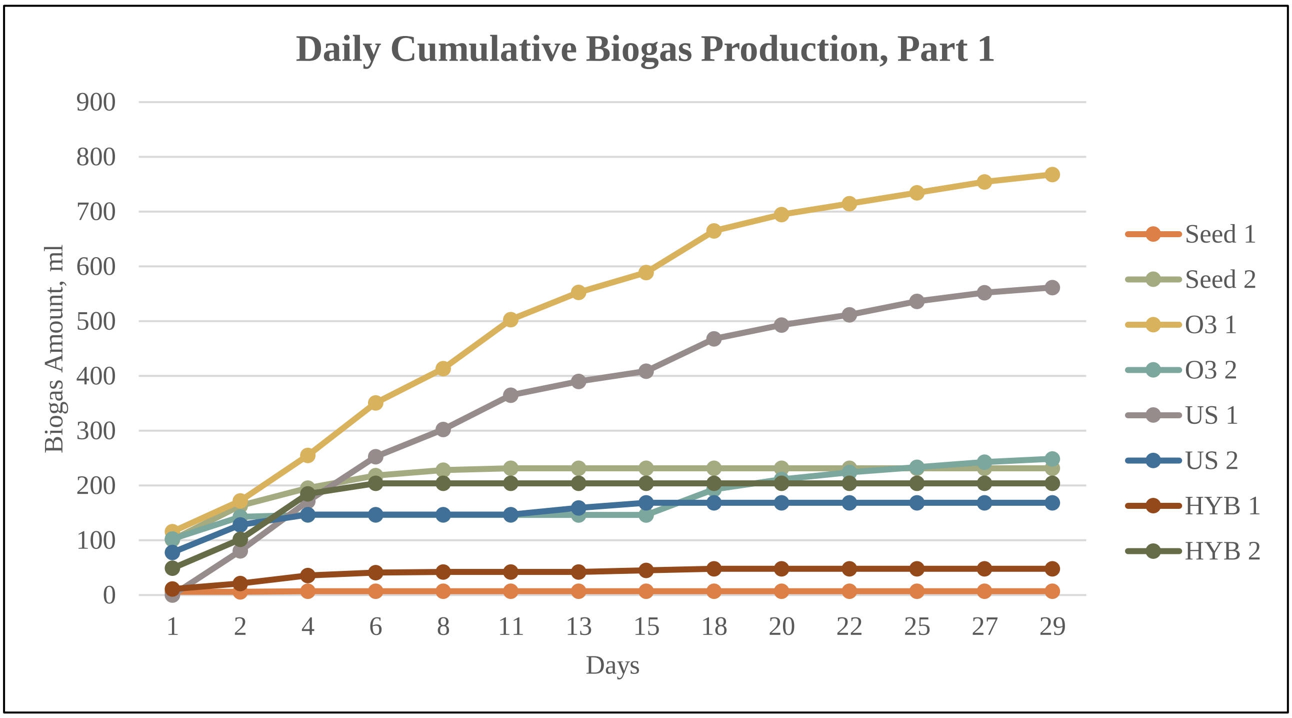

Figure 4.4. Part 1 cumulative reactor biogas production.

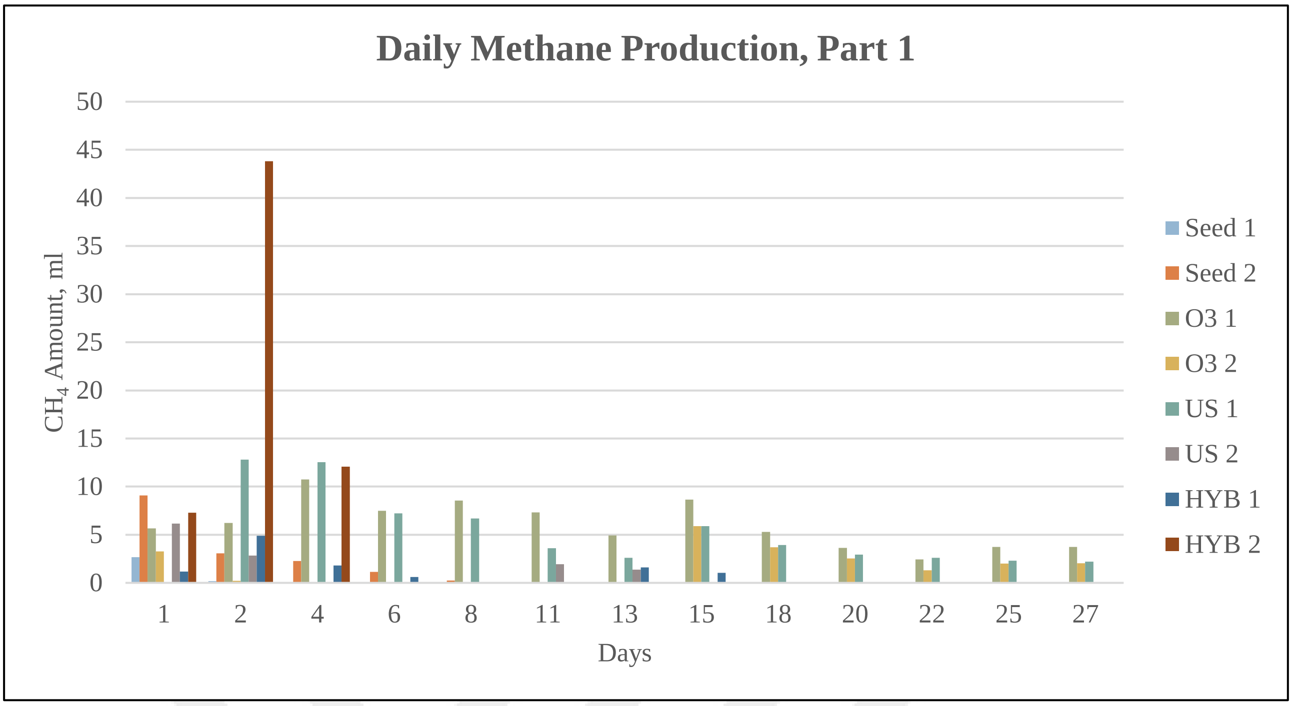

Figure 4.5. Part 1 daily reactor methane production.

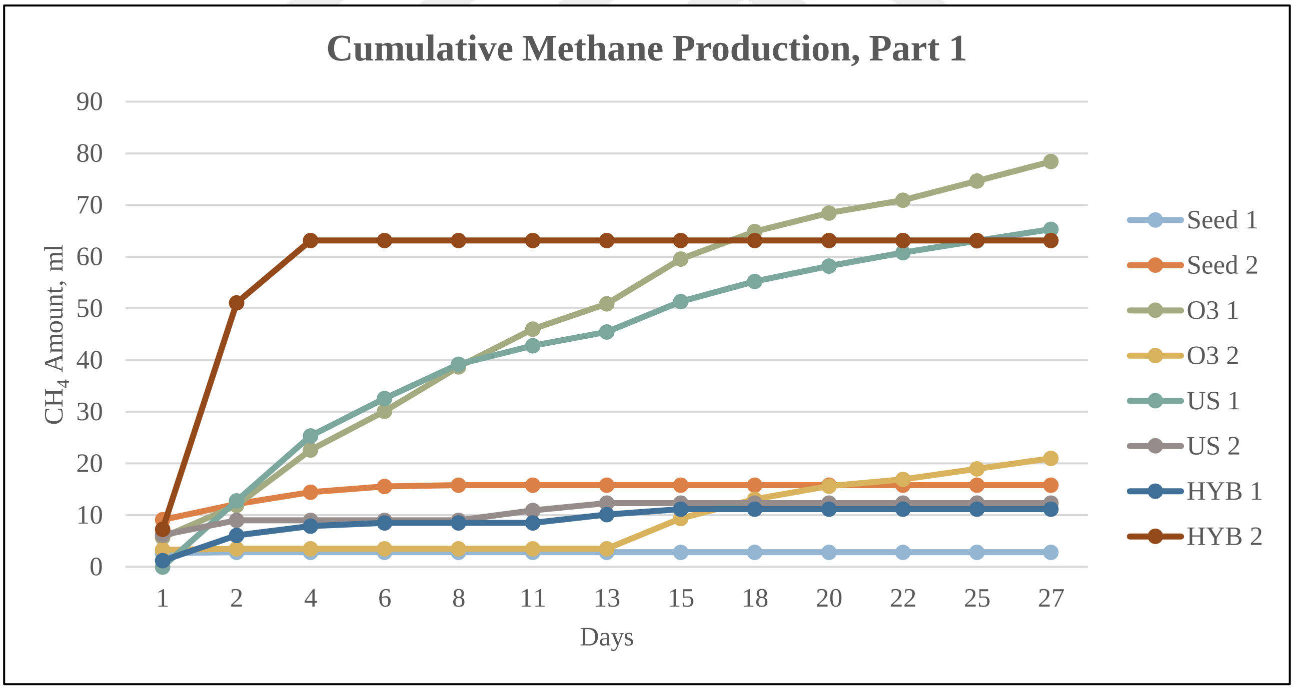

Figure 4.6. Part 1 cumulative reactor methane production.

In addition to daily and cumulative (total) gas production amounts, gas composition was also regularly monitored with a gas chromatography device. Cumulative methane production amounts are given in Figure 4.6.

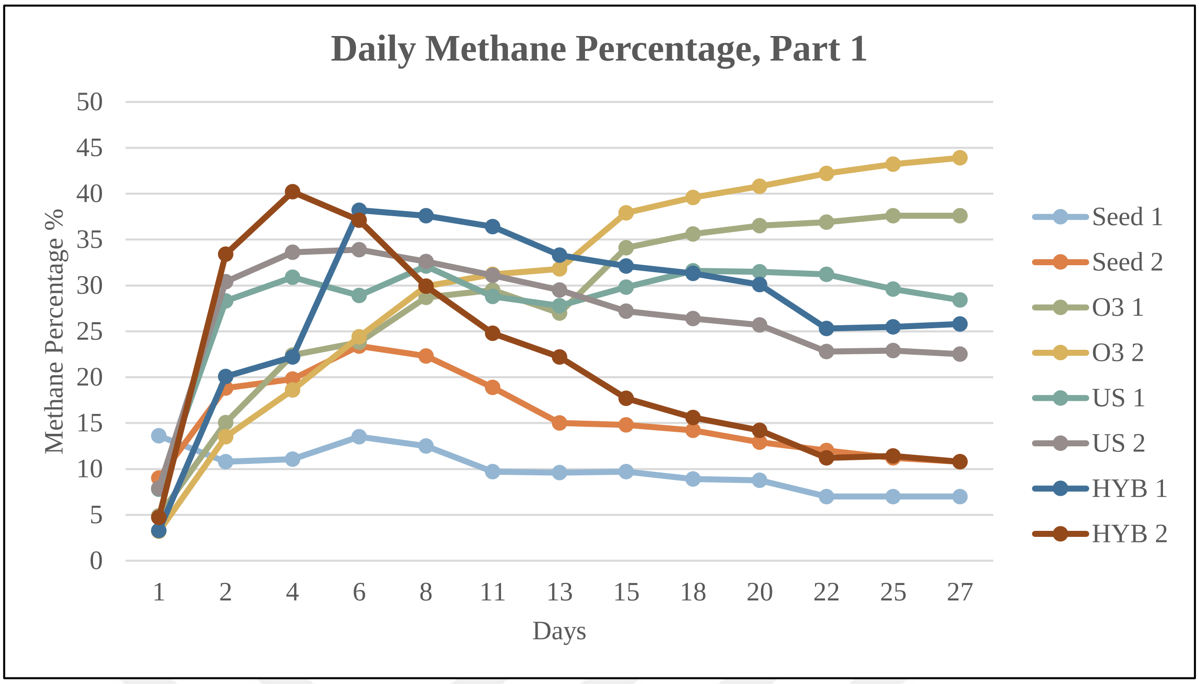

Figure 4.7. Part 1 gas chromatography methane formation percentages.

As can be seen in Figure 4.7, the methane gas percentages measured daily in the gas chromatography device are shared. The methane content of the tested gas is parallel to the cumulative methane production.

4.2.2. Part 2 Biogas and Methane Production

In the second part, studies were carried out in accordance with the mechanisms mentioned in Section 3.1 and the reactor contents were determined. The Hybrid application used in the first part biogas experiments was also repeated in the second part biogas experiments.

In the second part biogas experiments, the reactors were operated for a total of 29 days to analyze the biogas potential of the relevant samples. During the analysis, biogas production was monitored using milligas meters and gas production amounts were recorded daily and cumulatively. The seed reactor produced a maximum of 107.25 ml of gas on a daily basis and a total of 296 ml of gas throughout the analysis. The daily gas production of the CO reactor reached a maximum value of 72.82 ml and produced a cumulative 261.49 ml of gas during the analysis. While the TP reactor produced a maximum of 79.87 ml of gas on a daily basis, the total gas production throughout the analysis was 1264.88 ml. The maximum daily gas production of the Hybrid$_1$-1 reactor was recorded as 118.56 ml, and the cumulative gas production during the analysis was recorded as 2280.72 ml. While the Hybrid$_1$-2 reactor produced a maximum of 59.09 ml of gas on a daily basis, the total gas production throughout the analysis reached 516.26 ml. Daily and cumulative gas production graphs recorded during the biogas potential analysis are given in the Figure 4.8, and Figure 4.9.

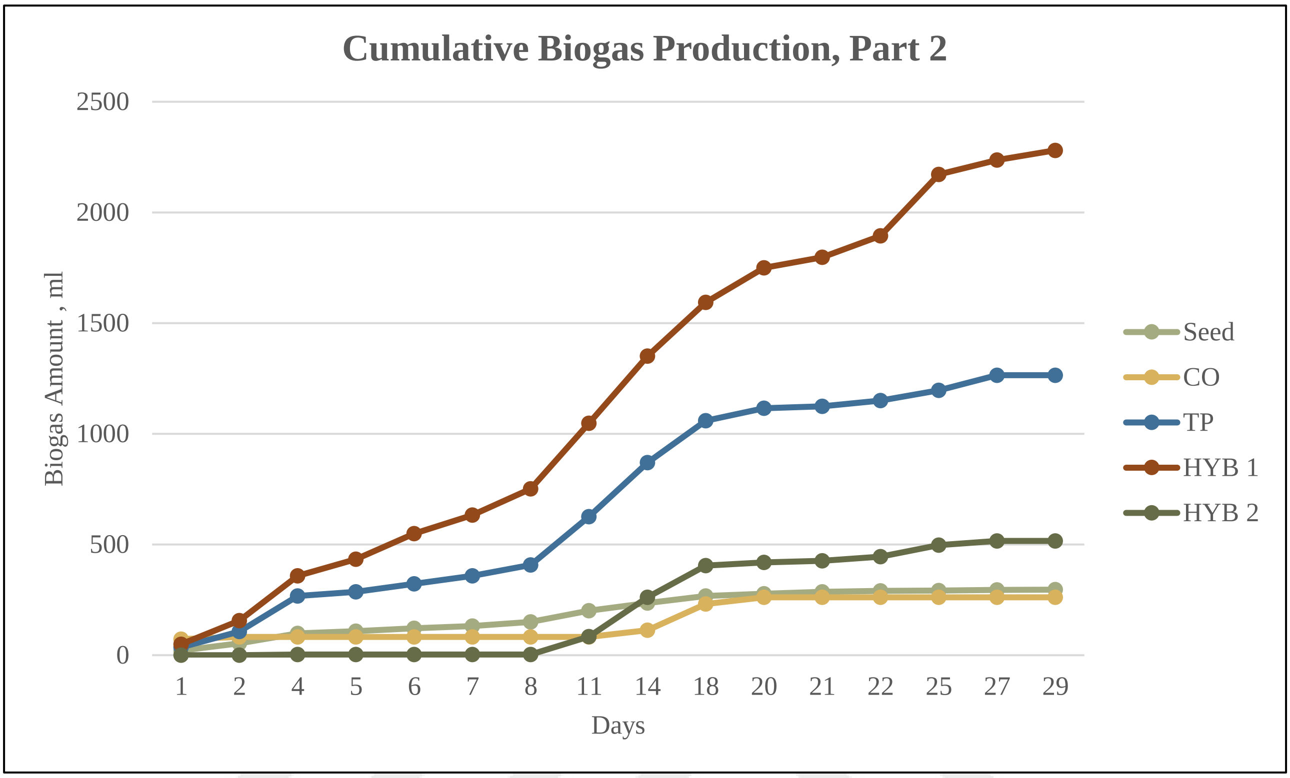

Figure 4.8. Part 2 cumulative reactor biogas production.

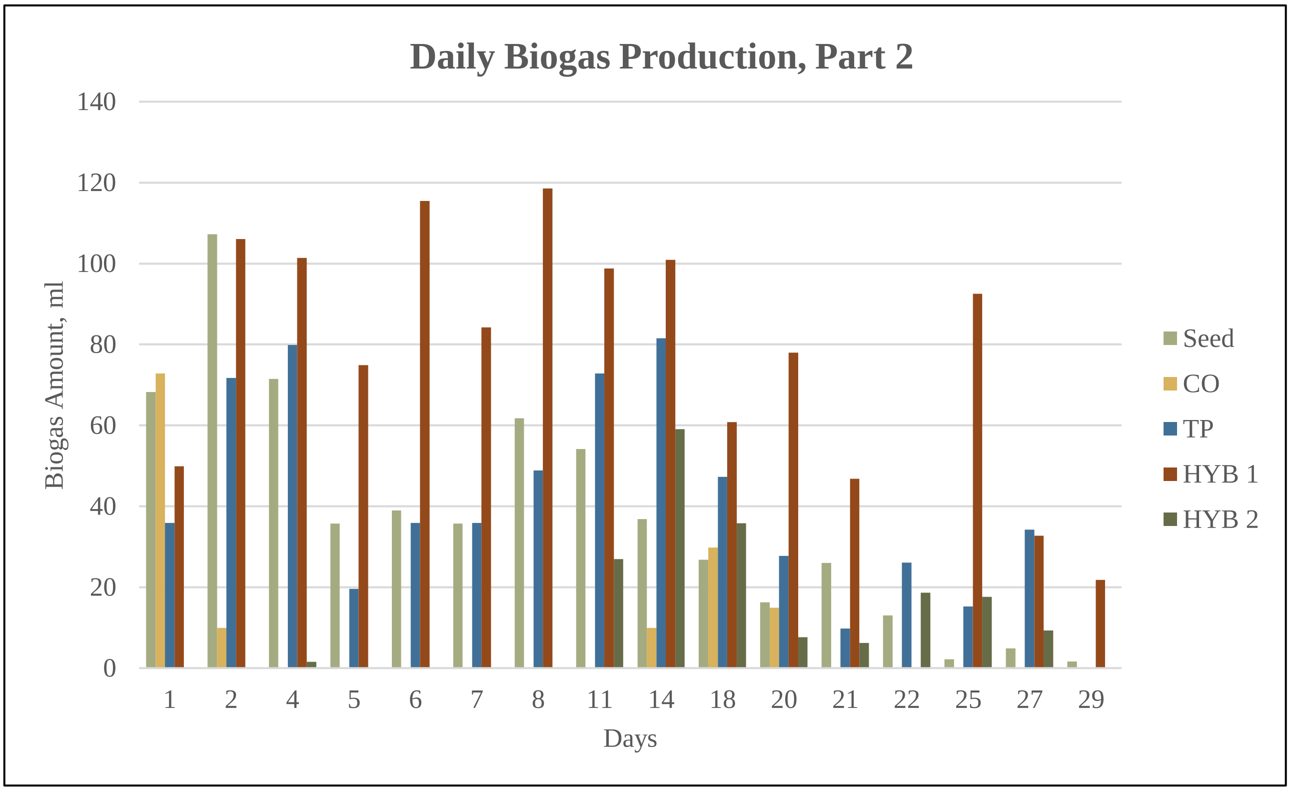

Figure 4.9. Part 2 daily biogas production.

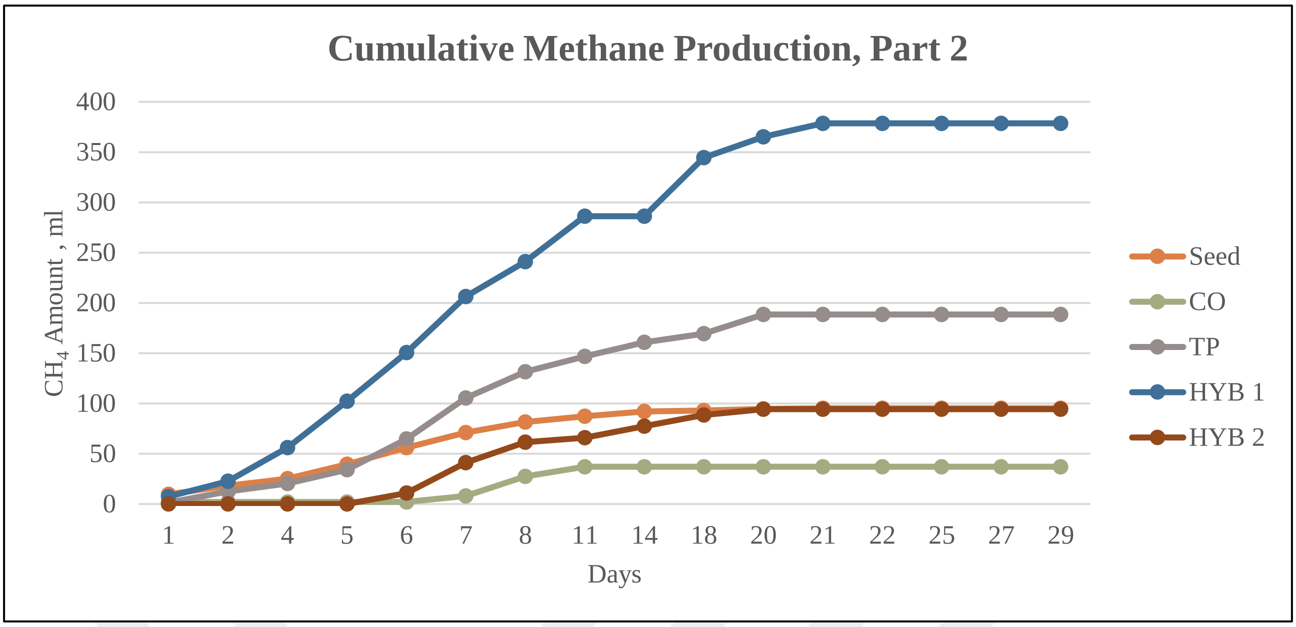

Figure 4.10. Part 2 cumulative reactor methane production.

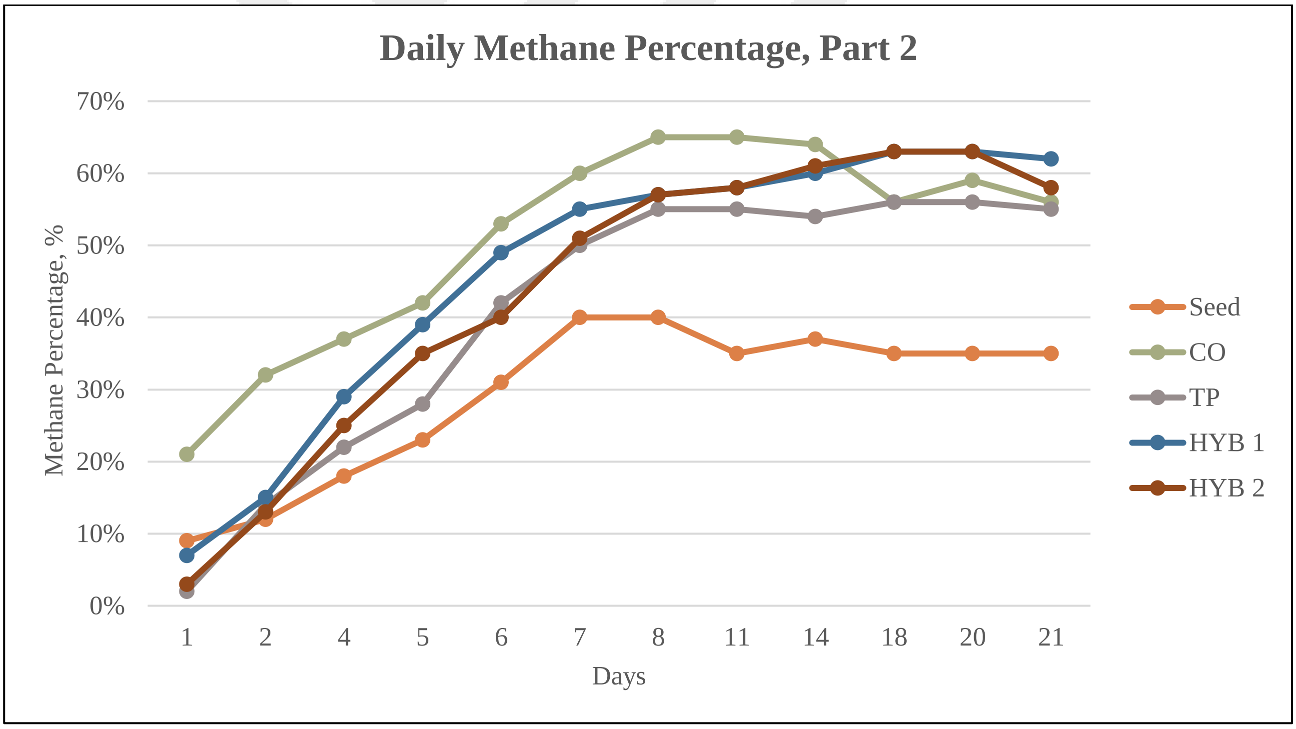

Figure 4.11. Part 1 gas chromatography methane formation percentages.

In addition to gas production amounts, gas composition was also regularly monitored with a gas chromatography device. Methane rates in the produced gas and cumulative production amounts of these gases are given in Figure 4.10, and Figure 4.11.

4.2.3. Part 1 and Part 2 Biogas Detail Results

More detailed results of experiments (Part 1 and 2) are summarized in Table 4.11 and 4.12., respectively.

Table 4.11. Total methane and biogas generation results - Part 1.

Reactor	Volume of Sample (ml)	Volume of Seed (ml)	Reactor TS (%)	Reactor TVS (%)	Methane generation (ml)	Biogas generation (ml)	Methane composition (%)
Seed 1	250	250	5.9	2.2	2.8	4.5	63.2
Seed 2	250	250	5.8	2.1	15.8	31.4	50.3
O_3 1	250	250	3.6	1.6	78.4	139.7	56.1
O_3 2	250	250	3.8	1.8	21.0	36.3	57.9
US 1	250	250	3.4	1.5	65.3	114.9	56.8
US 2	250	250	3.5	1.5	12.3	26.1	47.3
HYB 1	250	250	3.4	1.5	11.2	21.8	51.3
HYB 2	250	250	3.4	1.5	63.1	112.5	56.2

Table 4.12. Total methane and biogas generation results - Part 2.

Reactor	Volume of Sample (ml)	Volume of Seed (ml)	Reactor TS (%)	Reactor TVS (%)	Methane generation (ml)	Biogas generation (ml)	Methane rate (%)
Seed 1	250	250	5.1	2.2	-	-	-
Seed 2	250	250	4.9	2.1	101.2	235.4	43.0
CO-1	250	250	7.4	2.0	56.5	85.2	66.3
CO-2	250	250	6.4	1.9	-	-	-
TP-1	250	250	3.3	1.8	-	-	-
TP-2	250	250	3.4	1.8	209.9	372.8	56.3
HYB 1	250	250	3.3	1.6	420.0	778.1	54.0
HYB 2	250	250	3.4	1.6	104.3	170.2	61.3

When we examine the Part 1 results given in Table 4.11 in detail, it can be seen that the reactors those produced the most gas (biogas, methane) were the O3-1 and US-1 reactors, respectively. Although gas production occurred in each reactor, it was impossible to talk about efficient gas production when compared to literature data.

At the same time, the results are different in Part 2 compared to Part 1. As can be seen in Table 4.12, no gas formation was observed in Seed-1, CO-2 and TP-1 reactors. This might have taken place due to a technical failure in the system or by some inhibitory factors originating from the sludge that prevented biogas formation. Potential inhibitory factors, especially in test setups, are mentioned in Table 4.10, and Section 2.3.

On the other hand, another reason for this unstable gas production can be shown as the constantly changing characterization of raw sludge samples. According to the results reported by Uyal, the basic parameters for TS, TVS, COD, pH were examined and it was found that, for instance the TS amounts of petrochemical wastewater treatment sludges supplied on 3 different dates differ from each other by approximately 30%, both increasing and decreasing (Elif Sena Uyal, 2024). Operational changes could have affected wastewater/sludge characteristics and the performance of the industrial wastewater treatment plant.

Another parameter, COD, was approximately 32% less in the second sludge compared to the first sludge sample. When we compared the first sludge sample with the third sludge sample, approximately 35% more COD value was found. In particular, this change in COD may be due to operational changes in the wastewater treatment plant and seasonal conditions. Again, a stable sample for heavy metal content could not be obtained from the wastewater treatment plant. The reasons for these changes in parameters cannot be fully determined (Elif Sena Uyal, 2024).

As a different reason, it is known that domestic and industrial wastewater are treated together in petrochemical wastewater treatment plants. These wastewaters with different contents may not have created a suitable environment for biogas production. Constant monitorable conditions are preferred in biogas production.

When the results of both parts are examined, it is seen that hybrid reactors are among the most successful reactors in biogas production. Here, it can be interpreted that ozonation and ultrasound pre-treatment processes have a positive effect on the samples. However, the more striking point in the study is that the samples named TP, which did not undergo any pre-treatment in Part 2, produced considerable amount of biogas when compared to the hybrid pre-treated samples.

4.2.4. Biogas Results and Literature Comparison

Biogas and methane yields observed as a result of Part 1 and Part 2 BMP experiments are given in Table 4.13, and the results are compared with literature values. As far as encountered, there exists a limited amount of data in literature about pre-treatment of petrochemical industry sludge and subsequent biogas production.

Table 4.13. Thesis biogas results and literature comparison.

Reactor	Biogas Yield (mL Biogas production / g TVS added)	Methane Yield (ml Methane production / g TVS added)	Reference
Ozone (O_3)*	12.13	6.81	(Haak et al., 2016)
Literature Value	NA	NA	
Ultrasound (US)*	**9.98**	**5.67**	(Siddique et al., 2017)
Literature Value	**400**	**270**	
Hybrid * (US + O_3)	**65.47**	**35.33**	NA
Literature Value	NA	NA	
Co-digestion * (CO)	5.75	3.81	(Wang et al., 2016)
Literature Value	NA	NA	
Raw Sludge (TP)*	30.41	17.12	(Haak et al., 2016)
Literature Value	NA	NA	

* Thesis Work (Uyal, 2024)

As seen in the Table 4.13, that biogas and methane yields were both lower than the values given when compared to the studies conducted in the literature.

All data summarized in Table 4.13, were created with the data obtained from both literature studies and test setups. In their study by Haak and colleagues (details are examined in Section 2.6), ozone pre-treated sewage sludge produced 160 mL of methane per gram of COD removed. In test studies, it was well below this value. This might have occurred since Haak and her colleagues treated domestic wastewater treatment sludge and petrochemical wastewater treatment sludge together. On the other hand, domestic wastewater treatment sludges have higher organic content compared to petrochemical wastewater treatment sludges. Again, Haak et al. produced 80 mL of methane per gram of COD removed in a mixture of untreated petrochemical wastewater treatment sludge and domestic wastewater. In test studies, methane production of 32.50 mL per gram of COD removed was observed (Haak et al., 2016)

In the study conducted by Wang et al., 228 mL of methane was produced per gram of COD removed. In test studies, this value was observed as 4.28 mL, well below the literature value. This may be due to the use of a controlled 2-phase anaerobic digestion system (Wang et al., 2016).

5. CONCLUSION

A series of pre-treatment methods have been determined in order to increase the biogas production potential of the sludge obtained from the petrochemical wastewater treatment plant. Sludge characterization was carried out to determine the operating parameters of these methods. Continuous monitoring of characterization is very important in terms of efficiency and follow-up of work. Characterization and pre-treatment studies were carried out by in another work by Elif Sena Uyal, which was the first stage of this project. For the characterization study carried out for pre-treatment purposes, it was determined that the TS value in the sludge samples taken at weekly intervals was lower than the literature and that densification could increase the biogas production potential. In the weekly evaluations of sludge samples concentrated in the ratio of 1:2, it was determined that the TS value was the most variable parameter that could affect the process performance during operation and required control of operating conditions.

Three different methods were used in the pre-treatment processes. These methods include ozonation, ultrasound and hybrid (ultrasound, ozone) processes. Different frequencies and power settings were tried in the ultrasound pre-treatment process, but the most optimum removal efficiency was determined to be 15 minutes, 20 kHz and 90% amplitude, and the samples to be used before biogas tests were pre-treated under these conditions.

Additionally, ozonation, another pre-treatment option, was used. Literature values in ozonation were examined and as a result of laboratory studies, it was determined that the most optimum condition was 0.080 g O_3/g TS dose at 100 L ozone flow rate per hour.

In hybrid pre-treatment studies, the sample was first exposed to optimum conditions of 15 minutes, 20 kHz and 90% amplitude ultrasound, and then was treated with ozone at 0.040 g O3/TS criteria. The aim of all pre-treatment processes was to increase the petrochemical industry wastewater sludge.

BMP (Biochemical Methane Potential) tests were carried out to evaluate the biogas production potential of the samples provided within the scope of the thesis study. When the effects of the used pre-treatment techniques (Ozone-O_3/Ultrasound-US/Hybrid) on the biogas production potential of the sludge are evaluated and compared, it appears that the hybrid process (Ozone-O_3+Ultrasound-

US) provides the highest biogas yield. It seems that the biogas yields obtained as a result of Ozone (O_3) and Ultrasound (US) processes applied individually are much lower. The biogas yields obtained as a result of the co-anaerobic digestion process (co-digestion), which is recommended as an alternative to the application of the pre-treatment technique, are also very low (No pre-treatment was applied to the wastewater treatment sludge used in this experiment and İSKİ wastewater treatment plant feed sludge was used as an additional substrate).

A comparison of the biogas yields obtained as a result of individually applied Ozone (O_3), Ultrasound (US) and Hybrid (Ozone-O_3/Ultrasound-US) pre-treatment processes with a limited number of similar studies we have come across in the current literature is presented. No study has been encountered in the literature on the effect of hybrid pre-treatment on the biogas production potential of petrochemical industry wastewater sludge. However, both Ozone (O_3), Ultrasound (US), and co-digestion biogas efficiency values obtained in this study are well below the literature values. When examined on the basis of different biogas yield calculation parameters, the hybrid pre-treatment results obtained in this study seem to be slightly closer to the literature values. Despite the application of pre-treatment processes, the reason for the low biogas production potential obtained compared to limited literature information is considered to be the complex petrochemical wastewater properties and the possible interference/inhibition caused by other process-derived organic/inorganic substances, especially various heavy metals contained in the wastewater. Recently, there have been publications in the literature regarding the potential for biogas production from petrochemical industry wastewater, but the inhibition effects that process-derived heavy metals or other organic/inorganic substances may have on biological systems should be taken into consideration. Especially when the anaerobic digestion process is examined, it is known that microorganisms that produce methane are extremely negatively affected by interferences such as heavy metals.

As a result, within the scope of this study, biogas production from petrochemical wastewater treatment sludge seemed technically possible as a result of hybrid pre-treatment (Ozone-O_3/Ultrasound-US) application, which was optimized by considering the chemical properties of wastewater treatment sludge, but it was observed that the calculated biogas and methane yields were low. Production of biogas from petrochemical sludge used in this study, despite pre-treatment methods applied, does not sound economically feasible under current conditions. Further studies can be recommended to treat the sludge using different pre-treatment techniques in order to determine whether biogas production potential from this sludge can be improved.

REFERENCES

Abbassi-Guendouz, A., Brockmann, D., Trably, E., Dumas, C., Delgenès, J. P., Steyer, J. P., & Escudié, R. (2012). Total solids content drives high solid anaerobic digestion via mass transfer limitation. *Bioresource Technology*, *111*, 55–61. https://doi.org/10.1016/j.biortech.2012.01.174

Abouelenien, F., Nakashimada, Y., & Nishio, N. (2009). Dry mesophilic fermentation of chicken manure for production of methane by repeated batch culture. *Journal of Bioscience and Bioengineering*, *107*(3), 293–295. https://doi.org/10.1016/j.jbiosc.2008.10.009

Achinas, S., Achinas, V., & Euverink, G. J. W. (2017). A Technological Overview of Biogas Production from Biowaste. *Engineering*, *3*(3), 299–307. https://doi.org/10.1016/J.ENG.2017.03.002

Al Momani, F. A., Schaefer, S., & Sievers, M. (2011). Effect of ozone pre-treatment on sludge production of aerobic digestion processes. *International Journal of Sustainable Engineering*, *4*(2), 181–189. https://doi.org/10.1080/19397038.2010.534643

Al Seadi, T., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S., & Janssen, R. (2008). *Biogas handbook*. University of Southern Denmark Esbjerg.

Alrawashdeh, K. A. B., Gul, E., Yang, Q., Yang, H., Bartocci, P., & Fantozzi, F. (2020). Effect of heavy metals in the performance of anaerobic digestion of olive mill waste. *Processes*, *8*(9). https://doi.org/10.3390/PR8091146

Andreoli, C. V. (Cleverson V., Sperling, M. von., & Fernandes, Fernando. (2007). *Sludge treatment and disposal*. IWA Publishing.

Angelonidi, E., & Smith, S. R. (2015). A comparison of wet and dry anaerobic digestion processes for the treatment of municipal solid waste and food waste. *Water and Environment Journal*, *29*(4), 549–557. https://doi.org/10.1111/wej.12130

Anjum, M., Al-Makishah, N. H., & Barakat, M. A. (2016). Wastewater sludge stabilization using pre-treatment methods. In *Process Safety and Environmental Protection* (Vol. 102, pp. 615–632). Institution of Chemical Engineers. https://doi.org/10.1016/j.psep.2016.05.022

Anukam, A., Mohammadi, A., Naqvi, M., & Granström, K. (2019). A review of the chemistry of anaerobic digestion: Methods of accelerating and optimizing process efficiency. In *Processes* (Vol. 7, Issue 8, pp. 1–19). MDPI AG. https://doi.org/10.3390/PR7080504

Awad, H., Gar Alalm, M., & El-Etriby, H. K. (2019). Environmental and cost life cycle assessment of different alternatives for improvement of wastewater treatment plants in developing countries. *Science of the Total Environment*, *660*, 57–68. https://doi.org/10.1016/j.scitotenv.2018.12.386

Bajpai, P. (2017). *Anaerobic Technology in Pulp and Paper Industry*. https://doi.org/10.1007/978-981-10-4130-3

Batstone, D. J., Angelidaki, I., & Vavilin, V. (2002). *Anaerobic digestion model No 1 (ADM1)*. https://www.researchgate.net/publication/11198259

Bhattacharyya, J. K., & Shekdar, A. V. (2003). Treatment and disposal of refinery sludges: Indian scenario. *Waste Management and Research*, *21*(3), 249–261. https://doi.org/10.1177/0734242X0302100309

Biogas World. (2023). *How much energy is in biogas?* Https://Www.Biogasworld.Com/Biogas-Faq/.

Borges, E. S. M., & Chernicharo, C. A. L. (2009). Effect of thermal treatment of anaerobic sludge on the bioavailability and biodegradability characteristics of the organic fraction. *Brazilian Journal of Chemical Engineering*, *26*(03), 469–480. www.abeq.org.br/bjche

Botheju, D., & Bakke, R. (2011). Oxygen Effects in Anaerobic Digestion-A Review. *The Open Waste Management Journal*, *4*, 1–19.

Bougrier, C., Battimelli, A., Delgenes, J. P., & Carrere, H. (2007). Combined ozone pretreatment and anaerobic digestion for the reduction of biological sludge production in wastewater treatment. *Ozone: Science and Engineering*, *29*(3), 201–206. https://doi.org/10.1080/01919510701296754

Boyd R. David. (2006). *The Water We Drink: An International Comparison of Drinking Water Standards and Guidelines*. https://davidsuzuki.org/science-learning-centre-article/the-water-we-drink-an-international-comparison-of-drinking-water-standards-and-guidelines/

BP. (2023). *BP Energy Outlook 2023 Edition*. https://www.bp.com/content/dam/bp/business-sites/en/global/corporate/pdfs/energy-economics/energy-outlook/bp-energy-outlook-2023.pdf

Braguglia, C. M., Gianico, A., & Mininni, G. (2011). Laboratory-scale ultrasound pre-treated digestion of sludge: Heat and energy balance. *Bioresource Technology*, *102*(16), 7567–7573. https://doi.org/10.1016/j.biortech.2011.05.025

Celis-García, M. L. B., Ramírez, F., Revah, S., Razo-Flores, E., & Monroy, O. (2004). Sulphide and oxygen inhibition over the anaerobic digestion of organic matter: Influence of microbial immobilization type. *Environmental Technology*, *25*(11), 1265–1275. https://doi.org/10.1080/09593332508618367

Chen, T. H., Chynoweth, P., & Biljetina, A. R. (1990). Anaerobic Digestion of Municipal Solid Waste in a Nonmixed Solids Concentrating Digestor. *Applied Biochemistry and Biotechnology*. https://doi.org/https://doi.org/10.1007/BF02920276

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: A review. In *Bioresource Technology* (Vol. 99, Issue 10, pp. 4044–4064). https://doi.org/10.1016/j.biortech.2007.01.057

Choudhury, S. P., Saha, B., Haq, I., & Kalamdhad, A. S. (2022). Use of petroleum refinery sludge for the production of biogas as an alternative energy source: A review. In *Advanced Organic Waste Management: Sustainable Practices and Approaches* (pp. 277–297). Elsevier. https://doi.org/10.1016/B978-0-323-85792-5.00021-6

Comesaña-Gándara, B., García-Depraect, O., Santos-Beneit, F., Bordel, S., Lebrero, R., & Muñoz, R. (2022). Recent trends and advances in biogas upgrading and methanotrophs-based valorization. *Chemical Engineering Journal Advances*, *11*. https://doi.org/10.1016/j.ceja.2022.100325

Da Costa Gomez, C. (2013). Biogas as an energy option. In *The Biogas Handbook: Science, Production and Applications* (pp. 1–16). Elsevier Inc. https://doi.org/10.1533/9780857097415.1

Das, J., Ravishankar, H., & Lens, P. N. L. (2022). Biological biogas purification: Recent developments, challenges and future prospects. In *Journal of Environmental Management* (Vol. 304). Academic Press. https://doi.org/10.1016/j.jenvman.2021.114198

Demirel, B., & Yenigün, O. (2002). Two-phase anaerobic digestion processes: A review. In *Journal of Chemical Technology and Biotechnology* (Vol. 77, Issue 7, pp. 743–755). https://doi.org/10.1002/jctb.630

Deublein Dieter, & Steinhauser A. (2011). *Biogas from Waste and Renewable Resources*. Wiley-VCH.

Devos, P., Haddad, M., & Carrère, H. (2021). Thermal Hydrolysis of Municipal sludge: Finding the Temperature Sweet Spot: A Review. In *Waste and Biomass Valorization* (Vol. 12, Issue 5, pp. 2187–2205). Springer Science and Business Media B.V. https://doi.org/10.1007/s12649-020-01130-1

EBA Statistical Report. (2021). https://www.europeanbiogas.eu/wp-content/uploads/2021/11/EBA-STATISTICAL-REPORT-2021-SHORT-VERSION.pdf

Elif Sena Uyal. (2024). *Ozonation, Sonication, and Their Combination for Petrochemical Wastewater Sludge Pre-treatment to Enhance Sludge Disintegration*. Boğaziçi University.

Enerdata. (2023). *Global energy trends*. https://www.enerdata.net/publications/reports-presentations/world-energy-trends.html

EPA. (2023, May 29). *Defining hazardous waste: listed, characteristic and mixed radiological wastes*.

European Council. (2022, March 22). *Impact of Russia's invasion of Ukraine on the markets: EU response*.

European Council. (2023, February 7). *The EU's diversification away from Russian gas* .

Fagbohungbe, M. O., Herbert, B. M. J., Hurst, L., Ibeto, C. N., Li, H., Usmani, S. Q., & Semple, K. T. (2017). The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. In *Waste Management* (Vol. 61, pp. 236–249). Elsevier Ltd. https://doi.org/10.1016/j.wasman.2016.11.028

Fenchel, T., King, G. M., & Blackburn, T. H. (2012). Bacterial Metabolism. In *Bacterial Biogeochemistry* (pp. 1–34). Elsevier. https://doi.org/10.1016/b978-0-12-415836-8.00001-3

Ferrarese, E., Andreottola, G., & Oprea, I. A. (2008). Remediation of PAH-contaminated sediments by chemical oxidation. *Journal of Hazardous Materials*, *152*(1), 128–139. https://doi.org/10.1016/j.jhazmat.2007.06.080

Ferrer, I., Ponsá, S., Vázquez, F., & Font, X. (2008). Increasing biogas production by thermal (70 °C) sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochemical Engineering Journal*, *42*(2), 186–192. https://doi.org/10.1016/j.bej.2008.06.020

Ferry, J. G. (2010). The chemical biology of methanogenesis. *Planetary and Space Science*, *58*(14–15), 1775–1783. https://doi.org/10.1016/j.pss.2010.08.014

Gómez, D., Ramos-Suárez, J. L., Fernández, B., Muñoz, E., Tey, L., Romero-Güiza, M., & Hansen, F. (2019). Development of a modified plug-flow anaerobic digester for biogas production from animal manures. *Energies*, *12*(13). https://doi.org/10.3390/en12132628

Haak, L., Roy, R., & Pagilla, K. (2016). Toxicity and biogas production potential of refinery waste sludge for anaerobic digestion. *Chemosphere*, *144*, 1170–1176. https://doi.org/10.1016/j.chemosphere.2015.09.099

Hansen, C. L., & Cheong, D. Y. (2019). Agricultural waste management in food processing. In *Handbook of Farm, Dairy and Food Machinery Engineering* (pp. 673–716). Elsevier. https://doi.org/10.1016/B978-0-12-814803-7.00026-9

He, Z., Wang, Z., Zhao, Z., Yi, S., Mu, J., & Wang, X. (2017). Influence of ultrasound pretreatment on wood physiochemical structure. *Ultrasonics Sonochemistry*, *34*, 136–141. https://doi.org/10.1016/j.ultsonch.2016.05.035

IEA. (2020). *Outlook for biogas and Prospects for organic growth World Energy Outlook Special Report biomethane*. https://iea.blob.core.windows.net/assets/03aeb10c-c38c-4d10-bcec-de92e9ab815f/Outlook_for_biogas_and_biomethane.pdf

International Energy Agency. (2022). *Russia's War on Ukraine*.

International Energy Agency. (2023a, March 2). *Global energy-related greenhouse gas emissions*.

International Energy Agency. (2023b, June). *Renewables are at the forefront of Europe's response to the energy crisis*.

International Energy Agency. (2023c, July 11). *Bioenergy – Why is it important* .

Jabłoński, S., Rodowicz, P., & Łukaszewicz, M. (2015). Methanogenic archaea database containing physiological and biochemical characteristics. *International Journal of Systematic and Evolutionary Microbiology*, *65*, 1360–1368. https://doi.org/10.1099/ijs.0.000065

Jin, B., Wang, S., Xing, L., Li, B., & Peng, Y. (2016). Long term effect of alkali types on waste activated sludge hydrolytic acidification and microbial community at low temperature. *Bioresource Technology*, *200*, 587–597. https://doi.org/10.1016/j.biortech.2015.10.036

Johnson, O. A., & Affam, A. C. (2019). Petroleum sludge treatment and disposal: A review. *Environmental Engineering Research*, *24*(2), 191–201. https://doi.org/10.4491/EER.2018.134

Kalogo, Y., Monteith, H., & Eng, P. (2008). *State of science report: energy and resource recovery from sludge*. https://edepot.wur.nl/118980

Karim, K., Hoffmann, R., Klasson, K. T., & Al-Dahhan, M. H. (2005). Anaerobic digestion of animal waste: Effect of mode of mixing. *Water Research*, *39*(15), 3597–3606. https://doi.org/10.1016/j.watres.2005.06.019

Kavitha, S., Rajesh Banu, J., Kumar, G., Kaliappan, S., & Yeom, I. T. (2018). Profitable ultrasonic assisted microwave disintegration of sludge biomass: Modelling of biomethanation and energy parameter analysis. *Bioresource Technology*, *254*, 203–213. https://doi.org/10.1016/j.biortech.2018.01.072

Kianmehr, P., Parker, W., & Seto, P. (2010). An evaluation of protocols for characterization of ozone impacts on WAS properties and digestibility. *Bioresource Technology*, *101*(22), 8565–8572. https://doi.org/10.1016/j.biortech.2010.06.061

Kiyasudeen, K., Hakimi, M., Shlrene, Q., & Ismail, A. (2016). *Prospects of Organic Waste Management and the Significance of Earthworms*. https://doi.org/10.1007/978-3-319-24708-3

Krause, L., Diaz, N. N., Edwards, R. A., Gartemann, K. H., Krömeke, H., Neuweger, H., Pühler, A., Runte, K. J., Schlüter, A., Stoye, J., Szczepanowski, R., Tauch, A., & Goesmann, A. (2008). Taxonomic composition and gene content of a methane-producing microbial community isolated from a biogas reactor. *Journal of Biotechnology*, *136*(1–2), 91–101. https://doi.org/10.1016/j.jbiotec.2008.06.003

Li, H., Li, C., Liu, W., & Zou, S. (2012). Optimized alkaline pretreatment of sludge before anaerobic digestion. *Bioresource Technology*, *123*, 189–194. https://doi.org/10.1016/j.biortech.2012.08.017

Li, W., Loh, K.-C., Zhang, J., Tong, Y. W., & Dai, Y. (2017). *Two-stage anaerobic digestion of food waste and horticultural waste in high-solid system*. https://doi.org/10.1016/j.apenergy.2017.05.042

Li, Y., Alaimo, C. P., Kim, M., Kado, N. Y., Peppers, J., Xue, J., Wan, C., Green, P. G., Zhang, R., Jenkins, B. M., Vogel, C. F. A., Wuertz, S., Young, T. M., & Kleeman, M. J. (2019). Composition and Toxicity of Biogas Produced from Different Feedstocks in California. *Environmental Science and Technology*. https://doi.org/10.1021/acs.est.9b03003

Liao, S. (2023). The Russia–Ukraine outbreak and the value of renewable energy. *Economics Letters*, *225*. https://doi.org/10.1016/j.econlet.2023.111045

Lin, L., Yan, R., Liu, Y., & Jiang, W. (2010). *In-depth investigation of enzymatic hydrolysis of biomass wastes based on three major components: Cellulose, hemicellulose and lignin*. https://doi.org/10.1016/j.biortech.2010.05.084

Lindmark, J., Thorin, E., Bel Fdhila, R., & Dahlquist, E. (2014). Effects of mixing on the result of anaerobic digestion: Review. In *Renewable and Sustainable Energy Reviews* (Vol. 40, pp. 1030–1047). Elsevier Ltd. https://doi.org/10.1016/j.rser.2014.07.182

Lusk, P. (1998). *Methane Recovery from Animal Manures: The Current Opportunities Casebook*. http://www.doe.gov/bridge/home.html

Lyu, Z., Shao, N., Akinyemi, T., & Whitman, W. B. (2018). Methanogenesis. In *Current Biology* (Vol. 28, Issue 13, pp. R727–R732). Cell Press. https://doi.org/10.1016/j.cub.2018.05.021

Ma, J., Frear, C., Wang, Z. W., Yu, L., Zhao, Q., Li, X., & Chen, S. (2013). A simple methodology for rate-limiting step determination for anaerobic digestion of complex substrates and effect of microbial community ratio. *Bioresource Technology*, *134*, 391–395. https://doi.org/10.1016/j.biortech.2013.02.014

Masud, M. H., Rashid, M., Hossan, Md. N., & Ahmed, M. M. (2023). Domestic Waste To Energy, Technologies, Economics, and Challenges. In *Reference Module in Earth Systems and Environmental Sciences*. Elsevier. https://doi.org/10.1016/b978-0-323-93940-9.00026-8

Meegoda, J. N., Li, B., Patel, K., & Wang, L. B. (2018). A review of the processes, parameters, and optimization of anaerobic digestion. In *International Journal of Environmental Research and Public Health* (Vol. 15, Issue 10). MDPI AG. https://doi.org/10.3390/ijerph15102224

Menzel, T., Neubauer, P., & Junne, S. (2020). Role of microbial hydrolysis in anaerobic digestion. In *Energies* (Vol. 13, Issue 21). MDPI AG. https://doi.org/10.3390/en13215555

Neumann, P., Pesante, S., Venegas, M., & Vidal, G. (2016). Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. In *Reviews in Environmental Science and Biotechnology* (Vol. 15, Issue 2, pp. 173–211). Springer Netherlands. https://doi.org/10.1007/s11157-016-9396-8

Nour, H. E., Alshehri, F., Sahour, H., & El-Sorogy, A. S. (2022). Evaluation of sediment and water quality of Ismailia Canal for heavy metal contamination, Eastern Nile Delta, Egypt. *Regional Studies in Marine Science*, *56*. https://doi.org/10.1016/j.rsma.2022.102714

Noxolo, S. T., Edison, M., & Tesfagiorgis, H. B. (2014). Effect of Temperature and pH on The Anaerobic Digestion of Grass Silage. *In Proceedings of the 6th International Conference on Green Technology, Renewable Energy and Environmental Engineering, Cape Town, South Africa*, *November*, 27–28.

Nsair, A., Cinar, S. O., Alassali, A., Qdais, H. A., & Kuchta, K. (2020). Operational Parameters of Biogas Plants: A Review and Evaluation Study. In *Energies* (Vol. 13, Issue 15). MDPI AG. https://doi.org/10.3390/en13153761

organischer Stoffe Substratcharakterisierung, V. (2016). *VEREIN DEUTSCHER INGENIEURE Characterisation of the substrate, sampling, collection of material data, fermentation tests VDI 4630 VDI-RICHTLINIEN*. www.vdi.de/richtlinien

Pavlostathis, S. G. (2011). Kinetics and Modeling of Anaerobic Treatment and Biotransformation Processes. In *Comprehensive Biotechnology, Second Edition* (Vol. 6, pp. 385–397). Elsevier Inc. https://doi.org/10.1016/B978-0-08-088504-9.00385-8

Pilli, S., Yan, S., Tyagi, R. D., & Surampalli, R. Y. (2015). Thermal pretreatment of sewage sludge to enhance anaerobic digestion: A review. In *Critical Reviews in Environmental Science and Technology* (Vol. 45, Issue 6, pp. 669–702). Taylor and Francis Inc. https://doi.org/10.1080/10643389.2013.876527

Sankaran, S., Pandey, S., & Sumathy, K. (1998). Experimental investigation on waste heat recovery by refinery oil sludge incineration using fluidised-bed technique. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *33*(5), 829–845. https://doi.org/10.1080/10934529809376764

Sapkaite, I., Barrado, E., Fdz-Polanco, F., & Pérez-Elvira, S. I. (2017). Optimization of a thermal hydrolysis process for sludge pre-treatment. *Journal of Environmental Management*, *192*, 25–30. https://doi.org/10.1016/j.jenvman.2017.01.043

Sarah Refai. (2016). *Development of efficient tools for monitoring and improvement of biogas production*. http://hss.ulb.uni-bonn.de/2017/4617/4617.pdf

Şenol, H., Aşkın ELİBOL, E., Açikel, Ü., Şenol, M., Üniversitesi, G., ve Biyomühendislik Bölümü, G., Mühendisliği Bölümü, M., Üniversitesi, C., Mühendisliği Bölümü, K., Anadolu Lisesi, B., & öğretmenliği Bölümü, İ. (2017). Derleme Makalesi / Review Article Türkiye'de Biyogaz Üretimi İçin Başlıca Biyokütle Kaynakları. In *BEU Journal of Science* (Vol. 6, Issue 2).

Siami, S., Aminzadeh, B., Karimi, R., & Hallaji, S. M. (2020). Process optimization and effect of thermal, alkaline, H2O2 oxidation and combination pretreatment of sewage sludge on solubilization and anaerobic digestion. *BMC Biotechnology*, *20*(1). https://doi.org/10.1186/s12896-020-00614-1

Siddique, M. N. I., Munaim, M. S. A., & Wahid, Z. B. A. (2017). The combined effect of ultrasonic and microwave pre-treatment on bio-methane generation from co-digestion of petrochemical wastewater. *Journal of Cleaner Production*, *145*, 303–309. https://doi.org/10.1016/j.jclepro.2017.01.061

Standart Methods, Standart Methods (2018).

Stasinakis, A. S. (2012). *Review on the fate of emerging contaminants during sludge anaerobic digestion*. https://doi.org/10.1016/j.biortech.2012.06.074

Tan, Y., Zheng, C., Cai, T., Niu, C., Wang, S., Pan, Y., Lu, X., Zhen, G., Qian, G., & Zhao, Y. (2020). Anaerobic bioconversion of petrochemical wastewater to biomethane in a semi-continuous bioreactor: Biodegradability, mineralization behaviors and methane productivity. *Bioresource Technology*, *304*. https://doi.org/10.1016/j.biortech.2020.123005

Themelis, N. J. (2002). *Anaerobic digestion of biodegradable organics in municipal solid wastes*. Columbia University.

Tyagi, V. K., & Lo, S. L. (2011). Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: An up to date review. In *Reviews in Environmental Science and Biotechnology* (Vol. 10, Issue 3, pp. 215–242). https://doi.org/10.1007/s11157-011-9244-9

Volschan Junior, I., de Almeida, R., & Cammarota, M. C. (2021). A review of sludge pretreatment methods and co-digestion to boost biogas production and energy self-sufficiency in wastewater treatment plants. *Journal of Water Process Engineering*, *40*. https://doi.org/10.1016/j.jwpe.2020.101857

Wang, Q., Liang, Y., Zhao, P., Li, Q. X., Guo, S., & Chen, C. (2016). Potential and optimization of two-phase anaerobic digestion of oil refinery waste activated sludge and microbial community study. *Scientific Reports*, *6*(August), 1–10. https://doi.org/10.1038/srep38245

Weemaes, M., Grootaerd, H., Simoens, F., & Verstraete, W. (2000). Anaerobic digestion of ozonized biosolids. *Water Research*. https://doi.org/https://doi.org/10.1016/S0043-1354(99)00373-5

Whitman, W. B., Bowen, T. L., & Boone, D. R. (2006). The Methanogenic Bacteria. In *The Prokaryotes* (pp. 165–207). Springer New York. https://doi.org/10.1007/0-387-30743-5_9

Wu, L. J., Hao, Z. W., Li, X. X., Ye, F., Yang, F., & Lyu, Y. K. (2023). Excess methane production and operation stability for anaerobic digestion of oily food waste controlled by mixing intensity: Focusing on heterogeneity of long chain fatty acids. *Journal of Environmental Management*, *335*. https://doi.org/10.1016/j.jenvman.2023.117573

Yan, S., Miyanaga, K., Xing, X. H., & Tanji, Y. (2008). Succession of bacterial community and enzymatic activities of activated sludge by heat-treatment for reduction of excess sludge. *Biochemical Engineering Journal*, *39*(3), 598–603. https://doi.org/10.1016/j.bej.2007.12.002

Yasui, H., & Shibata, M. (1994). An Innovative Approach To Reduce Excess Sludge Production In The Activated Sludge Process. *Water Science Technology*. https://doi.org/https://doi.org/10.2166/wst.1994.0434

Yeom, I., Lee, K., Lee, Y., Ahn, K., & Lee, S. (2002). Effects of ozone treatment on the biodegradability of sludge from municipal wastewater treatment plants. *Water Science Technology*. https://doi.org/https://doi.org/10.2166/wst.2002.0641

Yin, C. Y., Mahmud, H. Bin, & Shaaban, M. G. (2006). Stabilization/solidification of lead-contaminated soil using cement and rice husk ash. *Journal of Hazardous Materials*, *137*(3), 1758–1764. https://doi.org/10.1016/j.jhazmat.2006.05.013

Yue, Z. B., Yu, H. Q., & Wang, Z. L. (2007). Anaerobic digestion of cattail with rumen culture in the presence of heavy metals. *Bioresource Technology*, *98*(4), 781–786. https://doi.org/10.1016/j.biortech.2006.03.017

Zhang, T., Wang, Q., Ye, L., Batstone, D., & Yuan, Z. (2015). Combined free nitrous acid and hydrogen peroxide pre-treatment of waste activated sludge enhances methane production via organic molecule breakdown. *Scientific Reports*, *5*. https://doi.org/10.1038/srep16631

Zhang, Z. L., Zhang, L., Zhou, Y. L., Chen, J. C., Liang, Y. M., & Wei, L. (2013). Pilot-scale operation of enhanced anaerobic digestion of nutrient-deficient municipal sludge by ultrasonic pretreatment and co-digestion of kitchen garbage. *Journal of Environmental Chemical Engineering*, *1*(1–2), 73–78. https://doi.org/10.1016/j.jece.2013.03.008

Zhen, G., Lu, X., Kato, H., Zhao, Y., & Li, Y. Y. (2017). Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: Current advances, full-scale application and future perspectives. In *Renewable and Sustainable Energy Reviews* (Vol. 69, pp. 559–577). Elsevier Ltd. https://doi.org/10.1016/j.rser.2016.11.187

Zhou, L., Jiang, X., & Liu, J. (2009). Characteristics of oily sludge combustion in circulating fluidized beds. *Journal of Hazardous Materials*, *170*(1), 175–179. https://doi.org/10.1016/j.jhazmat.2009.04.109