INVESTIGATION OF BIOGAS PRODUCTION FROM AGRICULTURAL CROPS, CROP RESIDUES AND WASTEWATER TREATMENT PLANT SLUDGES THROUGH ANAEROBIC DIGESTION

by

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ABSTRACT

INVESTIGATION OF BIOGAS PRODUCTION FROM AGRICULTURAL CROPS, CROP RESIDUES AND WASTEWATER TREATMENT PLANT SLUDGES THROUGH ANAEROBIC DIGESTION

The context of “Water, Energy and Food Security” has become the top priority agenda worldwide with the devastating effects of the climate crisis. This urgent issue has raised concerns for environmentally-friendly and economically viable opportunities for sustainable energy production, based on the substitution of traditional energy sources with renewable sources. In line with this necessity, co-digestion of energy crops and agro-industrial wastes with wastewater sludge has emerged as a promising technique to enhance biogas production. The objective of this study is to investigate the availability and effectiveness of biomass utilization as a renewable energy source. With this purpose, the biogas production efficiencies of “co-digestion” and “mono-digestion” of wastewater sludges with different agricultural products and further effects of sludge pre-treatments on the biogas production were investigated in the study. The results demonstrated that anaerobic co-digestion of wastewater sludge and agricultural wastes proved to be more effective in terms of biogas production efficiencies compared to using single-substrate systems. Additionally, applying pre-treatment methods (ultrasonication, microwave, and pulsed electric field application) to wastewater sludges enhanced anaerobic biodegradation by accelerating the hydrolysis step and increasing the solubility of organics. Across all pre-treated reactors, increased methane yields were observed, with approximately a 33-45% increase depending on the applied techniques, compared to reactors containing unpretreated wastewater sludge. Comparative analysis of different reactor operations highlighted the potential of anaerobic co-digestion involving wastewater sludge with cloverleaf, olive/grape pomace, and maize silage as particularly promising alternatives for enhancing biogas production while simultaneously addressing the disposal challenge of these waste products.

ÖZET

ANAEROBİK ÇÜRÜTME YÖNTEMİ İLE TARIMSAL ÜRÜN VE ARTIKLAR VE ATIKSU ARITMA TESİSİ ÇAMURLARINDAN BİYOGAZ ÜRETİMİNİN ARAŞTIRILMASI

Şiddeti her sene daha da artan iklim krizinin yıkıcı etkileri ile birlikte dünya genelinde en öncelikli gündem haline gelen "Su, Enerji ve Gıda Güvenliği" konusu, geleneksel enerji kaynaklarının tarımsal atıklar gibi yenilenebilir kaynaklarla ikame edilmesine dayanan ve sürdürülebilir atık yönetimini temel alan çevreye duyarlı ve ekonomik olarak uygulanabilir yöntemlerin ivedilikle geliştirilmesi gerektiğine yönelik endişeleri de beraberinde getirmiştir. Bu konseptle, tarımsal ürün ve artıklar ve atık su arıtma çamurlarının anaerobik yöntemlerle biyogaz üretiminde birlikte kullanımı çok daha elverişli bir teknoloji haline gelmiştir. Bu tezin amacı, yenilenebilir enerji kaynağı olarak seçilen biyokütle kaynaklarının enerji üretiminde verimli kullanım alternatiflerini araştırmak ve çamur ve tarımsal artıkların direct bertaraf edilmek yerine faydalı amaçlar için yeniden kullanılacağı sürdürülebilir ve uygun maliyetli atık yönetimi çözümleri sunmaktır. Bu amaçla çalışma kapsamında, laboratuvar ölçekli reaktörlerde farklı tarım ürün ve artıkları ve atık su çamurlarının anaerobik (birlikte) çürütme yöntemi ile biyogaz üretim verimleri araştırılmış ve arıtma çamurlarına uygulanan ultrasonikasyon, mikrodalga ve vurgulu elektrik alanı gibi çeşitli ön arıtma (dezentegrasyon) tekniklerinin biyogaz üretimi üzerindeki etkileri incelenmiştir. Çalışmada, ön arıtma tekniklerinin uygulandığı tüm reaktörlerde, (uygulanan tekniklere bağlı olarak yaklaşık % 33-45 artış ile) daha yüksek metan verimleri elde edilmiştir. Çalışma sonuçları, uygulanan tüm tarımsal ürün ve artıklar içinde; atık su arıtma çamurlarının özellikle yonca yaprağı, zeytin ve üzüm (prina) atıkları ve mısır silajı ile anaerobik ortamda birlikte çürütülmesinin, ülkemizde biyogaz üretiminin iyileştirilmesinde çok daha etkili alternatifler olarak öne çıktığını ve uygulanacak bu yöntemin, çamur ve tarımsal atıkların bertarafı sorununa da sürdürülebilir bir çözüm olacağını göstermiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
CH_4	Methane	%
CO_2	Carbon Dioxide	%
COD	Chemical Oxygen Demand	mg $^{-1}$
Cl^-	Chloride	mg L^{-1}
CST	Capillary Suction Time	seconds
DD	Disintegration Degree	%
DO	Dissolved Oxygen	mg L^{-1}
DOC	Dissolved Organic Carbon	mg L^{-1}
EC	Electrical Conductivity	mS cm^{-1}
FC	Fecal Coliform	CFU (100 mL)$^{-1}$
Fe	Iron	
FS	Fecal Streptococci	CFU (100 mL)$^{-1}$
H_2O_2	Hydrogen Peroxide	
H_2SO_4	Sulfuric Acid	
HRT	Hydraulic Retention Time	days
KOH	Potassium Hydroxide	
MLSS	Mixed Liquor Suspended Solids	mg L^{-1}
MLVSS	Mixed Liquor Volatile Suspended Solids	mg L^{-1}
NaBr	Sodium Bromide	
NaCl	Sodium Chloride	
NH_4-N	Ammonium Nitrogen	mg L^{-1}
NO_2	Nitrate	mg L^{-1}
NO_3	Nitrite	mg L^{-1}
ORP	Oxidation Reduction Potential	mV
sCOD	Soluble Chemical Oxygen Demand	mg L^{-1}
SO_4^{2-}	Sulfate	mg L^{-1}
TKN	Total Kjeldahl Nitrogen	mg L^{-1}
TOC	Total Organic Carbon	mg L^{-1}
TP	Total Phosphorous	mg L^{-1}
TS	Total Solids	mg L^{-1}

VFA	Volatile Fatty Acids	mg L^{-1}
VS	Volatile Solids	mg L^{-1}
TC	Total Coliform	CFU (100 mL)$^{-1}$
T	Temperature	^{o}C

Abbreviation	**Explanation**
AD	Anaerobic Digestion
BMP	Biomethane Potential
CER	Cation Exchange Resin
CSTR	Continuously Stirred Tank Reactors
DD	Disintegration Degree
EPS	Extracellular Polymeric Substance
GC	Gas Composition
GC/MS	Gas Chromatography/Mass Spectrometry
GP	Grape Pomace
GHG	Greenhouse Gases
HRT	Hydraulic Retention Time
ISR	Inoculum to Substrate Ratio
MW	Microwave
Mtoe	Million Tons of Oil Equivalents
OLR	Organic Loading Rate
OP	Olive Pomace
PEF	Pulsed Electric Field
SD	Standard Deviation
SEM	Scanning Electron Microscopy
US	Ultrasound
UV	Ultraviolet
WAS	Wastewater Sludges
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

Substituting conventional fossil fuels derived from oil, coal, and natural gas with renewable energy alternatives stands out as one of the most crucial and urgent measures in combating the global climate crisis and mitigating extreme weather occurrences. The conventional fossil fuel-based energy sources confront mounting challenges on various environmental fronts, with perhaps the most formidable obstacle being the fulfillment of the greenhouse gas (GHG) reduction targets outlined in the Kyoto Protocol.

Numerous recent scientific studies underscore the urgent need to transition towards an economic framework characterized by circularity and low-carbon practices. Embracing renewable energy sources and implementing circular economy principles are critical steps towards achieving this goal. By transitioning to a low-carbon economy, societies create opportunities for innovation, and environmental stewardship.

On the other hand, the vast potential of renewable energy sources to fulfill the world's energy needs is becoming increasingly evident year by year. Sustainable energy generation from sources such as biomass, geothermal, solar, wind, and hydroelectric power is gaining prevalence. These renewable sources offer abundant, clean energy options. With advancing technology and the realization of economies of scale, renewable energy is becoming increasingly cost-effective and accessible, thus accelerating its adoption worldwide. This shift towards renewable energy not only addresses energy security concerns but also promotes environmental sustainability and economic development. They are expected to have a major influence on future growth in the energy industry. These sustainable solutions are anticipated to exclude conventional sources like coal and oil, highlighting the revolutionary change towards greener and more sustainable alternatives.

In recent years, Turkey has encountered challenges in meeting its expanding energy needs, primarily because of its constrained local natural resources. Approximately 75 percent of Turkey's primary energy requirements are fulfilled through imports. Energy imports surged from 2.94 million TJ in 2009 to 4.42 million TJ by 2019. Despite possessing abundant renewable energy sources like biogas, Turkey remains reliant on energy imports. Unfortunately, biogas technology and biogas plants have not been fully leveraged due to limited knowledge and technology in the country. Consequently, the number of facilities dedicated to biogas technology has yet to reach desired capacities in Turkey.

Estimates suggest that by 2050, most developed countries will rely on biomass waste to fulfill over 50% of their net energy requirements. Agricultural biomass, abundant in cellulosic raw materials crucial for biofuel production, plays a significant role in this transition, simultaneously reducing waste and addressing energy needs without jeopardizing valuable food resources (Kalak, 2023). According to the World Bioenergy Association, the collective biomass worldwide has the capacity surpassing the world's annual energy consumption by over 80 times (World Bioenergy Association, 2018).

Over the past two decades, significant research efforts have been directed towards exploring alternative energy sources derived from various waste materials. Notable among these are municipal organic waste (Rodriguez-Iglesias et al., 1998; Vieitez & Ghosh, 1999; Müller, Winter, & Strünkmann, 2004), livestock manure (Moller et al., 2004), and different types of industrial wastewaters (Kida et al., 1999; Fukuzaki & Nishio, 1997) for methane fermentation, garnering widespread attention worldwide.

The focus of much of the research on biogas has traditionally been on utilizing animal wastes as the primary raw material, predominantly sourced from livestock, such as manures (Pound et al., 1981). Recent studies have highlighted the significant untapped energy potential of biomass, particularly agricultural crops and residues, through anaerobic digestion (Nordberg et al., 1998). These agricultural byproducts are typically treated as waste before disposal, and harnessing biogas from them helps in reducing the volume of these waste materials released into the environment. Nevertheless, the utilization of biomass must not lead to competition for agricultural lands or disrupt food production, as land-use changes can result in adverse local environmental effects (Mahdavi &Vera; 2023). Moreover, cultivating energy crops requires significant amounts of water, adding to the challenge of sustainable biomass production. However, there is a growing interest in tapping into the biogas potential of agricultural residues.

In addition to the significant volumes of agricultural products and residues generated, Turkey faces a substantial and unavoidable sludge issue stemming from wastewater treatment plants (WWTP). Managing the stabilization, reuse, and minimization of these sludges—comprising a high proportion of organic and inorganic compounds—is crucial for Turkey, given its challenges with efficient waste management practices. Various conventional methods exist for sludge disposal, including incineration, landfilling, and composting, but these approaches are often difficult and costly, consuming over 50 percent of the operational budget for WWTPs (Vesilind & Spinosa, 2007).

Annually, Turkey produces over 60 million tons of agricultural wastes from vegetable-processing activities, alongside an estimated increase in wastewater treatment plant sludge production to 911 kilotons by 2040 (TURKSTAT, 2017).

Anaerobic digestion (AD) is a widely utilized process for converting organic materials into biogas through a series of sequential biochemical reactions. AD is rising as a promising technique especially for waste treatment and the production of biogas from agricultural crops and residues, such as sludge. This method offers advantages such as energy production, high-quality fertilizer yield, and disease transmission prevention. Notably, anaerobic digestion contributes to renewable energy generation by stabilizing organic waste matter, offering potential solutions for climate change mitigation and addressing high energy costs. From an environmental standpoint, methane-rich biogas serves as a renewable energy carrier, capable of being converted into heat and electricity. This helps to mitigate environmental impacts associated with the use of fossil fuels. Furthermore, it helps reduce odor and the risk of groundwater contamination from intensive livestock operations (Nie et al., 2021; Zamri et al., 2021).

However, the slow degradation of wastewater sludge poses a notable challenge for anaerobic digestion, resulting in prolonged sludge retention times within conventional digesters (Hwang & Shin, 1996; Sharmila et al., 2019).

Efforts to overcome these challenges have led to the exploration of various disintegration techniques to enhance AD efficiency. Although investigations into mechanical, chemical, and thermal disintegration techniques continue, the adoption of sludge pretreatment techniques for biogas production remains limited.

This study, encompassing five sections, aims to address these issues comprehensively. Section 2 provides the theoretical background on biogas production, anaerobic co-digestion, and sludge pretreatment techniques. Section 3 outlines the analysis methods and methodology, while Section 4 presents the results of studies on biogas production potential from selected agricultural crops, residues, and wastewater treatment plant sludges using lab-scale anaerobic digestion systems. It also examines the various pretreatment techniques effects on the digestion of sludge and biogas production, alongside exploring cost-effective waste management concepts through co-digestion of agricultural products and pre-treated sludge. Finally, Section 5 concludes with the major results and recommendations for future researches.

2. LITERATURE REVIEW

2.1. Sludge Problem

2.1.1. Wastewater Treatment Plant Sludges

Wastewater treatment plant sludges generated from water and wastewater treatment processes must undergo appropriate treatment before disposal to meet necessary environmental and health criteria. Sludges typically have a high solid content, making them suitable for transportation, storage, and land use applications. However, due to factors such as potential contamination and inability to directly recycle within the facility, proper sludge management is essential.

This need for proper sludge management is not only important in our country but also globally, as there is an increasing trend worldwide. Therefore, implementing effective treatment processes and disposal methods for wastewater treatment plant sludges is crucial to minimize environmental impact and protect public health. In Turkey, the management and disposal of wastewater sludges have become a significant problem, especially with the EU adaptation process of the country.

The sewage sludge, a semi-solid substance, is the residual liquid produced from wastewater treatment processes (Ukwatta et al., 2015; Fijalkowski et al., 2017).

There are many sources of wastewater sludges. As the source differs, the characteristics of the sludge itself also change. Typically, untreated wastewater sludge contains approximately 2.0%–8.0% total dry solids (TS), with volatile solids (VS) accounting for 60%–80% of the TS content. Additionally, it comprises various components such as grease and fats, phosphorus, nitrogen, potassium, proteins, silica, cellulose, alkalinity (expressed in mg/L as $CaCO_3$), iron, and organic acids (expressed in mg/L as Hac) (Tchobanoglous, Burton, & Stensel, 2003). The quality and quantity of the sludge directly associate with the wastewater volume being treated, the degree of the pollution, processes applied during the treatment (physical, physicochemical, biologic etc.) and the efficiency of the treatment.

Commonly, treatment plants lack the adequate treatment for the disposal of wastewater sludges. This mainly results from the deficiencies in the design or implementation of wastewater treatment plant (WWTP) or from inadequate utilization of the essential units for sludge treatment.

Consequently, sludge produced after the treatment of wastewater causes problems where it finally disposed.

Therefore, it is essential to sufficiently reduce organic matter, eliminate objectionable odors, and reduce pathogens before disposing of wastewater sludge. To achieve all these, sludge needs to be stabilized.

2.2. Sludge Stabilization

Stabilization refers to the set of operations and processes (outlined in Table 2.1) conducted on sludge to mitigate its environmental impact upon disposal. Among various methods, digestion stands out as the most commonly employed biological technique for stabilization.

Sludge stabilization refers to the process of reducing health hazards through biological, chemical, or thermal treatments, ensuring its suitability for long-term storage or appropriate handling. Stabilization involves removing pathogenic organisms and toxins, and controlling odors to enhance gas production potential. This process comprises a series of biological or chemical techniques. Common biological stabilization methods include aerobic digestion, anaerobic digestion, alkali stabilization, and composting (Tchobanoglous, Burton, & Stensel, 2003).

Table 2.1. Examples of effective biological stabilization processes of sludge (Fytili & Zabaniotou, 2008)

Process	**Description**
Sludge pasteurization	Under all circumstances, the minimum temperature requirements are 20 minutes at 70°C or 4 hours at 55°C.
Mesophilic anaerobic digestion	During primary digestion, the process requires a retention period of at least 12 days for temperatures between 35±3°C and 24 days for temperatures between 25±3°C. A subsequent stage that offers a mean retention time of at least 14 days comes after this.
Thermophilic anaerobic digestion	Seven days is the average retention period for digesting. All sludge must also mature for a long enough time at a minimum temperature of 55°C to guarantee that the compost reaction process is almost finished.
Composting	For a minimum of five days, the compost needs to be continuously kept at a temperature of at least 40°C. In addition, it needs to be heated to at least 55°C for four hours inside the pile's body during this interim. After that, a maturity period needs to be given in order to guarantee that the compost reaction process is almost finished.

2.2.1. Aerobic Digestion

This digestion method is used to reduce the amount of solid matter, including floc and microorganisms, as well as pollutants and other organic materials that constitute volatile solids. Typically, the removal of volatile solids in aerobic sludge digestion ranges between 35-50%.

During aerobic digestion, microorganisms consume nutrients (substrates) present in the environment to obtain the energy required for cellular reactions. As these nutrients are depleted, microorganisms may begin to utilize their own protoplasm (internal respiration) to sustain their metabolic processes (Tchobanoglous, Burton, & Stensel, 2003).

As a result, non-biodegradable volatile solids (also known as inert volatile solids) persist in the sludge even after aerobic digestion is completed. These non-biodegradable organic compounds remain as the final product of the aerobic digestion process.

Overall, aerobic digestion helps to stabilize the sludge, but it may not completely eliminate all inert and non-biodegradable components present in the sludge.

A process utilized to enhance the efficacy of conventional aerobic digestion is the autothermal thermophilic aerobic digestion (ATAD), which is a modification of the digestion process. ATAD is a biological stabilization method initially described by Jewell and Kabrick in 1980. It is employed for the stabilization and disinfection of urban sewage sludge and concentrated organic waste (Jewell & Kabrick, 1980).

2.2.2. Alkali Stabilization

Alkaline stabilization of sewage sludge serves as an alternative to stabilization through digestion. Lime is employed to enhance the dewatering properties of the sludge and can also be utilized for sludge stabilization purposes. In this method, lime is added to the sludge in a quantity that raises the pH value to 12 or higher. The elevated pH creates an unfavorable environment for microorganisms, resulting in inhibited decomposition, elimination of odor, and mitigation of health risks. Lime can be used in the process, or quicklime may be employed, which, due to the heat released, facilitates partial drying and an effective pasteurization process (Seyhan & Erdinçler, 2003).

2.2.3. Composting

In recent years, composting has emerged as another option for the removal and stabilization of sludge cake formed in treatment plants. The increasing demand for biosolid disposal and the limited availability of suitable sanitary landfills have prompted the accelerated development of composting as a feasible option for managing sludge.

Composting is a controlled aerobic process wherein organic matter undergoes decomposition to produce a stable product resembling humus. This method effectively reduces the volume, mass, and moisture content of waste while transforming it into a valuable soil conditioner.

During composting, the decomposition of organic matter in a controlled environment can elevate temperatures to around 70°C, potentially destroying pathogenic bacteria. Additionally, approximately 20-30% of the VS generated during composting are transformed into water and carbon dioxide (Tchobanoglous, Burton, & Stensel, 2003).

2.2.4. Anaerobic Digestion

In 1881, Mouras introduced anaerobic digestion for wastewater treatment with a septic tank (McCarty, 1982). Building upon this concept, Cameron further refined the technology with improved treatment efficiency (Chawla, 1986).

In wastewater treatment plants, anaerobic digestion stands out as one of the most effective methods for stabilizing sludge. This technique serves to treat the sludge generated from the processing of both domestic and industrial wastewaters. During anaerobic digestion, organic and inorganic components of the sludge are degraded by anaerobic microorganisms, ultimately converting them into biogas. This procedure takes place in an anaerobic environment.

Anaerobic digestion can be implemented with almost any organic material, including leftover food, sewage, animal waste, cardboard, waste paper, and grass clippings.

Anaerobic digestion consists of four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Vesilind, 1979; Parkin & Owen, 1986; Speece, 1996; Vesilind & Spinosa, 2007). Figure 2.1 illustrates the sequential steps of the anaerobic digestion process.

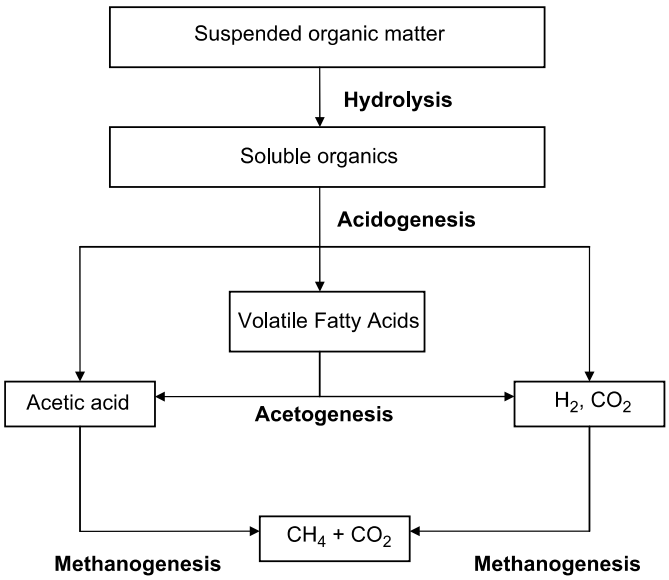

Figure 2.1. The sequential steps of the AD process.

During the initial stage of anaerobic digestion, known as hydrolysis, insoluble organic matter and high molecular weight substances are transformed into monomers, sugars, and amino acids. These components generated during hydrolysis are subsequently metabolized by acidogenic (fermentative) bacteria during acidogenesis.

In the acetogenesis phase, compounds generated in acidogenesis undergo additional metabolic processes by acetogens. This mainly leads to the generation of acetic acid, accompanied by the release of carbon dioxide and hydrogen. Methanogenesis represents the final stage in the decomposition of organic matter within anaerobic environments. It culminates in the production of methane, serving as the ultimate metabolic byproduct (Appels, Dewil, & Baeyens, 2008).

The increased need for waste energy recovery and biosolids production has led to a recent spike in interest in anaerobic sludge digestion technologies. Anaerobic digestion generates less sludge than aerobic digestion, and can offer significant economic benefits. Anaerobic digestion is essential to the management of organic waste because it improves the efficiency of waste capture and treatment, hence reducing greenhouse gas emissions linked to landfill disposal. It also makes energy recovery possible, as biogas can be converted into biofuel, used to generate heat and power, or injected into the gas system. Biogas energy stands out from other renewable sources due to its characteristics of continuous generation and the ability to be stored (DEFRA, 2011).

Anaerobic digestion has various advantages over conventional waste management technology and reduces CO_2 emissions by sixteen percent when compared to conventional sewage treatment

procedures. Smaller plants of this type can be located in towns, cutting down on haulage distances and related traffic pollution. In comparison to gasification, pyrolysis, landfilling, and incineration, anaerobic digestion produces less emissions into the air and solid waste (DEFRA, 2004).

Significant technological advances in anaerobic reactors have occurred since 1950, most notably with the creation of contemporary high-rate digesting systems made possible by effective anaerobic process mixing (McCarthy, 1982). Anaerobic digestion's main benefit is that it breaks down and stabilizes the organic materials that make up sludge, turning them into biosolids in the process. Moreover, biogas—which is made up of roughly 35% carbon dioxide and 65% methane—is the byproduct of anaerobic digestion and is a useful energy source.

The stage of hydrolysis, frequently referred to as the "rate-limiting step" in literature, plays a vital role in governing the rate of organic matter decomposition within an AD system, influenced by the sludge properties (Eastman & Ferguson, 1981).

Anaerobic digestion is capable of occurring across a range of temperature conditions. The microbial population's metabolic activities, such as the rates of hydrolysis and methane generation, are influenced by the anaerobic digester's operating temperature. Reactions occur more slowly at temperatures below 20°C, therefore psychrophilic conditions are ideal for lagoons and swamps. Certain anaerobic digester systems function within thermophilic ranges, typically between 50°C to 57°C, while others adhere to more conventional mesophilic temperature ranges, typically between 30°C to 38°C.

In anaerobic digestion processes, achieving higher stabilization degrees and shorter retention times is crucial for efficient operation. One alternative method to achieve this is through a two-stage digestion process, combining thermophilic and mesophilic stages. Recent advancements have introduced systems that utilize a combination of both temperature ranges in separate stages (Tchobanoglous, Burton, & Stensel, 2003).

At the design period of an anaerobic digestion process, there are some parameters should be considered and those parameters are:

- characteristics of the wastewater,
- volumetric organic loading rate,
- temperature,

- specific microorganism growth rate and solids retention time,
- wastewater alkalinity,
- nutrients,
- C/N ratio
- mixing, and
- biogas production.

In anaerobic digestion processes, in order to achieve higher stabilization degrees and to operate the systems in shorter HRTs, two-stage anaerobic digestion processes (thermophilic/mesophilic) are becoming an advantage. Rubio-Loza and Noyola implemented two-stage anaerobic digestion system into mixed active sludge samples (pre-sedimentation + final sedimentation) in their studies, and operated the first thermophilic stage with two short sludge retention times (2 and 3 days), followed by the mesophilic stage with 13 days and 10 days sludge retention times. The results show that, even though with short SRTs such as 2 and 3 days, the system was more effective in pathogen reduction compared to a typical one-stage anaerobic digester and the can be used for the production of class A biosolid (Rubio-Loza & Noyola, 2010).

A typical HRT for AD processes exceeds 20 days, during which organic matter degradation ranges from 25% to 60% (Nickel, Tiehm, & Neis, 1999). However, the extended time required for biological sludge hydrolysis poses a disadvantage, necessitating large digester tank volumes and hindering high rates of organic matter degradation. Additionally, foam formation during digestion and susceptibility to upsets due to toxic substances are notable drawbacks (Ganidi, Tyrrel, & Cartmell, 2009). Alkalinity addition may also be necessary, and lower temperatures can adversely affect reaction rates.

During the AD process design, several parameters must be considered, including characteristics of the wastewater, solids retention time, temperature, specific microorganism growth rate, volumetric organic loading rate, and wastewater alkalinity, nutrients, carbon-to-nitrogen ratio, mixing, toxic compounds, and biogas production.

Proper monitoring and control are essential during the operation of anaerobic digestion systems to prevent operational issues. During the design phase of an anaerobic digestion process, several parameters must be carefully considered:

- Characteristics of the wastewater
- Volumetric organic loading rate
- Temperature
- Specific microorganism growth rate and solids retention time
- Wastewater alkalinity
- Mixing
- Biogas production potential

To achieve higher stabilization degrees and operate systems with shorter hydraulic retention times (HRTs), two-stage anaerobic digestion processes (thermophilic/mesophilic) are becoming increasingly advantageous. These systems have been shown to be more effective in pathogen reduction compared to typical one-stage anaerobic digesters and can be used for producing Class A biosolids (Rubio-Loza & Noyola, 2010).

Another innovative approach is the development of advanced anaerobic digester models, such as ADM1 by the International Water Association (IWA). ADM1 is a comprehensive model that simulates the complex biochemical reactions occurring in anaerobic digesters. By accurately representing the microbial processes involved in anaerobic digestion, ADM1 allows for better process optimization and control. Researchers use ADM1 to study various factors influencing anaerobic digestion performance and to design more efficient digestion systems.

Anaerobic digestion plants have shown success in handling a variety of waste streams, including municipal, industrial, and agricultural wastes, throughout Europe. Germany and the United Kingdom are the countries with the most anaerobic digestion technology installations in wastewater treatment facilities. These facilities present a significant market potential for biogas production and make a substantial contribution to RE generation. (DEFRA, 2011).

However, in Turkey, the widespread adoption of anaerobic digestion technology, despite the country's significant potential for biogas production, has been limited. Some municipal wastewater treatment plants utilize AD system to process excess sludge from activated sludge systems as well. Nevertheless, there remains ample opportunity for growth and wider implementation of anaerobic digestion technology in Turkey.

The primary energy supply in Turkey are from various renewable energy sources mainly from hydropower, biomass, wind, solar, geothermal. Given the available potential of biomass and waste

resources in the country, particularly sludge resulting from wastewater treatment, there is a compelling case for expanding the use of anaerobic digestion and increasing biogas production. This could contribute significantly to Turkey's renewable energy goals and offer environmental and economic benefits (Yuksel et al., 2017).

2.3. Sludge Disintegration

The lacking of the landfill sites all around the world, the gap in the adequate application with the beneficial use of the sludge, and the high sludge treatment costs which are approximately half of the total wastewater treatment (Abbassi, Dullstein, & Rabiger, 2000), led to new approaches in the management of wastewater sludge.

Efforts to minimize sludge typically focus on reducing the organic matter content, which is crucial for the decay processes involved in treatment. Various modifications and interventions are applied to achieve this goal. These may include optimizing treatment processes to enhance organic matter removal efficiency, implementing advanced treatment technologies such as anaerobic digestion or thermal hydrolysis to maximize organic matter conversion to biogas, and promoting source control measures to reduce the introduction of organic pollutants into the wastewater stream.

By targeting sludge minimization strategies, wastewater treatment facilities can improve overall process efficiency, reduce environmental impact, and achieve cost savings in both operation and disposal. Among the other approaches, studies concerned about the reduction of the sludge (sludge minimization) gained more importance in the recent years.

These techniques are aimed at decreasing the quantity of wastewater sludge and preventing sludge production, thereby reducing the overall mass and volume at its source. Besides, there are actual scale applications of conventional digestion process modifications, which aims to increase the digestion efficiency and reduce the amount of sludge (Besze'des et al., 2009). Thus, not only minimization of the sludge that is going to be treated and decreasing the required volumes and costs, are aimed with sludge minimization, but also decreasing the amount of the sludge that is going to removed and disposed.

Sludge disintegration aims to increase the sludge's degree of stability and remove the rate-limiting step (hydrolysis) prior to digestion (Erdinçler and Vesilind, 2000; Weemaes, Grootaerd, Simoens, Huysmans, & Verstraete, 2001; Bougrier, Carrere, & Delgenes, 2005). In order to facilitate

microbial breakdown during anaerobic digestion, sewage sludge disintegration entails external pressures that alter the sludge's structural properties. The ultimate waste sludge bulk is decreased and biogas production is enhanced by this increased degradation.

In order to liberate their cell content, the applied process called cell lysate formation. This promotes faster and more effective degradation of organic substrates, which speeds up anaerobic methane fermentation and increases the production of biogas.

Disintegration can be achieved through physical, chemical, or biological means. Techniques for sludge disintegration encompass physical methods, chemical methods, mechanical methods, biological hydrolysis (enzyme treatment), and combinations of these methods (thermal and chemical treatment, where applicable) (Müller, Winter, & Strünkmann, 2004).

The disintegration process alters many characteristic properties of sludge (Müller, Winter, & Strünkmann, 2004). The sludge's floc structure breaks down, microorganisms' cell walls are broken down, and the organic materials inside the cells move into the liquid phase during disintegration. Disintegration produces less sludge than traditional digestion methods, which leads to more stable sludge and more biogas being produced (Wang et al., 2005).

Researchers have applied a number of sludge disintegration techniques on a pilot and lab basis. Sludge disintegration is mostly studied using the following methods: ultrasonic treatment; ozone oxidation; mechanical disintegration; alkali treatment; Fenton treatment; thermal treatment; and biological disintegration (enzyme usage) (Lai et al., 2001; Lehne, Müller, & Schwedes, 2001; Tiehm et al., 2001; Chang, Ying-Shih, & Lo, 2002; Lin et al., 2002; Barjenbruch & Kopplow, 2003; Nickel & Neis, 2007; Zawieja, Wolny, & Wolski, 2008; Pham et al., 2009; Biyu & Xiaofei, 2009; Xie, Liu, & Yan, 2009). The following are the primary methods used for the disintegration of sludge:

- Mechanical
    - Microwave
    - Freeze and thaw
    - Ultrasonication
- Chemical
    - Advanced oxidation by using Fenton reagent
    - Ozonation
    - Acid and alkali

- Biological
    - Enzyme treatment and also combination of mentioned techniques (Müller, 2000).

Mechanical treatment techniques such as ultrasonication, homogenization, or mechanical shearing are employed to disrupt the cell walls within the sludge. This fragmentation of these walls enhances the accessibility of organic matter to anaerobic microorganisms, thereby fostering more efficient digestion and biogas production.

The chemical treatment methods such as ozone, Fenton's reagent, chlorine, acids, and alkaline materials are utilized in wastewater treatment to facilitate the breakdown of compounds that are difficult to degrade into more easily degradable forms. These chemicals can react with organic matter, pathogens, and other contaminants present in the wastewater, transforming them into simpler and less harmful compounds.

Research studies have demonstrated the effectiveness of chemical treatments in wastewater remediation processes. By transforming complex and recalcitrant compounds into more easily degradable forms, chemical treatments improve the efficiency and performance of wastewater treatment plants, ultimately leading to better water quality and environmental protection (Tanaka et al., 1997; Sakai et al., 1997)

Through increased sludge disintegration, biogas output and digestion efficiency are expected to increase with the use of these techniques. In order to maximize these strategies' efficacy and suitability for use in wastewater treatment procedures, researchers are still investigating them.

2.3.1. Ultrasonication

Ultrasonic pretreatment is among the alternative techniques used for sludge separation. It is conducted by adjusting various parameters such as frequency, sonication power, and sonication time, which dictate the separation of the sludge. This pretreatment relies on the purification time and power and is implemented using a variable ultrasonicator. It offers advantages at low frequencies. Ultrasonic pre-treatment operates through two primary mechanisms: high frequencies chemical reactions that generate $HO_2^{\cdot}$, H, and $OH^{\cdot}$, and cavitation, which is advantageous at lower frequencies (Kim et al., 2010). Unlike chemical treatment of sludge, this mechanical process does not involve the use of additional chemicals, making it a more environmentally friendly and cost-effective option for sludge.

Ultrasonic treatment generates bubbles in the liquid phase. This step at the liquid-gas interface results in a localized increase in temperature and high pressure. This phenomenon is known as cavitation, and it can induce various effects such as mechanical stress, microstreaming, and generation of free radicals, which can contribute to the breakdown of organic matter, disruption of cell membranes, and enhancement of mass transfer processes in the treated liquid. These unique local conditions result in the formation of radicals (Bougrier et al., 2005).

Through the use of high-power ultrasound at low frequencies, sludge is vigorously and effectively disrupted during the ultrasonication process, which results in a notable release of intracellular components into the bulk liquid. Moreover, the cavitation explosion of transitory bubbles causes some insoluble particulate organic matter to change into a soluble condition. The parameters of frequency, sonication strength, and sonication time determine how well an ultrasonic pre-treatment breaks down sludge. An ultrasonicator, the settings of which may be changed according to the desired treatment duration and power, is necessary for this pre-treatment.

In conclusion, sonication density and solids concentration can affect ultrasonic treatment, which can improve WAS properties by raising sCOD concentrations. The sludge structure deteriorates more noticeably the greater the sonication strength used since it ruptures more sludge particles.

Previous studies have demonstrated the effectiveness of low ultrasonic frequencies, such as 20 kHz, in disintegrating sewage sludge (Gonze et al., 2003). The effects of ultrasonication (US) have been evaluated across a range of parameters, including initial solid concentration, power density, and treatment duration (Xie et al., 2009). Research suggests that low-intensity, long-duration treatments are more effective compared to high-intensity, short-duration treatments.

Gündüz (2009) research revealed that an increase in specific energy led to a higher degree of disintegration, with the highest degree observed at 47.4%. However, beyond 30,000 kJ/kgKM, the degree of disintegration began to decrease. The highest degree of disintegration at the maximum applied specific energy was found to be 30%. This decrease in disintegration was attributed to the high oxidation effect of radicals. Additionally, the study noted that ultrasonic pretreatment led to a reduction in the particle size of the sludge, with higher specific energy treatments resulting in more significant size reduction compared to untreated sludge.

Ultrasonic pretreatment also affects bacterial activity in digesters. Low-intensity ultrasonic treatment can enhance bacterial activity during anaerobic digestion, while higher intensity treatments

may inhibit bacterial activity in sludge (Xie et al., 2009). Studies have shown that subjecting sludge to ultrasonic pretreatment increases bacterial activity in laboratory-scale aerobic digesters (Erden et al., 2010).

Additionally, ultrasonic pretreatment has been found to affect the filterability of sludge. There exists a strong correlation between the disintegration degree and its filterability. Sludge with low degrees of disintegration may not significantly impact filterability, but as disintegration increases, filterability may deteriorate. Furthermore, ultrasonic pretreatment can elevate the temperature above 70°C, leading to a thermal conditioning effect and re-floc formation (Erden et al., 2010).

Another study was conducted to investigate the impact of ultrasonic treatment at varying energy levels on sludge disintegration, microbial activity, and sludge dewatering properties. As a result of these investigations, they observed changes in the microbial activity of the sludge and found that the dewatering characteristics of the sludge depend on the degree of sludge disintegration (Huan et al.; 2009).

In a study conducted on US disintegration, the disintegration capacity of activated sludge was examined across different solids concentrations using low ultrasonic frequency (20 kHz) while maintaining a constant size (50%) (Akın, 2008). The research found that ultrasonic intensity had an impact on the release of dissolved chemical oxygen demand and the disintegration of sludge. Specifically, it was observed that ultrasonic energy was effective in releasing proteins from the sludge.

When activated sludge with a solids content of 2% was subjected to ultrasonic energy, a high release of dissolved COD was observed. However, as the solids content increased from 4% to 6%, the release of dissolved COD decreased, respectively. The findings suggest that optimizing ultrasonic parameters and considering the characteristics of the sludge can improve the efficiency of disintegration processes in wastewater treatment applications.

2.3.2. Microwave

As a component of the electromagnetic spectrum, microwaves have wavelengths ranging from 1 mm to 300 GHz, or 300 MHz to 300 GHz in frequency. Microwave irradiation, when used as WAS disintegration, can accelerate ions and cause them to collide with other molecules. It can also cause dipoles in polar solvents to rotate and align quickly because of an oscillating electric field.

Microorganisms' secondary and tertiary protein structures are altered as a result (Banik, Bandyopadhyay, & Ganguly, 2003). Heat is produced by the rapid alignment and realignment of dipoles in materials that can absorb microwave energy, which causes changes in the vibrational energy of the molecules.

Banik et al. (2003) implanted a methanogenic bacterial strain (DSM 804) in an anaerobic microbial culture to evaluate the effect of microwaves on methane output from microorganisms. For two hours, microwaves ranging in frequency from 13.5 to 36.5 GHz were used to evaluate the properties of bacterial growth and how they affected biomethanation. According to the study, these microwaves have the potential to quicken the biomethanation process, which would raise the methane content and generate biogas more quickly. Microwaves have been shown to accelerate the growth rate of bacteria, according to growth dynamics research. 31.5 GHz was shown to be the most effective frequency for biomethanation.

The microwave pre-treatment research on WAS have concentrated on improving anaerobic digestion capacities, dewatering, and pathogen elimination because of the possibility for improved biogas generation (Eskicioglu, Kennedy, & Droste, 2008; Hong, Park, & Lee, 2004).

Zheng et al. (2009) investigated the characteristics of microwave-treated pre-sedimentation sludge and its biomethane potential. They examined various factors including microwave intensity, solids concentration and microwave temperature (ranging from 35 to 90°C).

Biomethane potential analyses were conducted on the anaerobic sludge, which was a mixture of mesophilic sludge obtained from the preliminary sedimentation tank and anaerobic digestion tank. Their findings indicated that at a temperature of 90°C, with a total solid's concentration of 4% (w/v) and at 80% microwave intensity, the dissolved chemical oxygen demand increased by 1.4 times. Furthermore, microwave radiation led to the dissolution of particulate matter.

The study concluded that microwave intensity was biologically effective at temperatures of 35, 65, and 90°C, while having no important effect on anaerobic digestion and dissolution.

Toreci et al. (2009) explored the impact of microwave (MW) pretreatment on mesophilic AD across three distinct sludge ages. Microwave pretreatment was found to conserve energy, eliminate pathogens, and accelerate the breakdown of volatile solids. The study revealed that microwave pretreatment could achieve sludge stabilization. However, it was noted that decreasing the power of

microwave (MW) pretreatment had an adverse effect on the digestion process, particularly at low sludge ages. Additionally, it was found that operating a single-stage digestion process with microwave pretreatment could hinder the performance compared to a two-stage digestion process. The optimum temperature for increasing the solubility and biogas production potential of thickened waste sludge was determined to be 175°C. Microwave pretreatment was conducted at this temperature. Interestingly, in systems with a sludge age of 20 days, it was observed that high temperature did not enhance the efficiency of the digestion process.

Some researchers have investigated the effects microwave, ultrasonic, and ultrasonic-microwave treatment on digestion. This study revealed that the ultrasonic-microwave treatment method can be used as the cost-effective technique for biogas production. In some studies, significant methane production was observed particularly after three minutes of microwave treatment and ten minutes of ultrasonic purification. These findings underscored the efficacy of combined ultrasonic-microwave treatment in enhancing biogas production from the sludge (Saifuddin & Fazlili, 2009).

2.3.3. Freeze and Thaw

Pre-treatment using freeze and thaw is a useful technique for lowering pathogens in wastewater sludges. This process mimics the natural cycle of freezing and thawing that occurs in soil, altering the characteristics of the sludge. Ice crystals form when wastewater sludge freezes in cold temperatures, leading to the separation of the phases of the sludge. The sludge's floc structure is also disturbed by the ice crystal formation, which helps to reduce microorganisms (Montusiewicz et al., 2010).

On the other hand, freeze-thaw processes need a lot of energy and time. The high operational costs associated with mechanical freezing limit the widespread application of this promising technology. Additionally, freeze and thaw pre-treatment is not suitable for use in tropical and subtropical environments where freezing temperatures are rare (Chu et al., 2001).

In industrial applications, cost of the operation is important consideration for determining feasibility of implementing freeze and thaw pre-treatment. Despite its limitations, this method remains valuable for reducing pathogens in wastewater sludges and may be suitable for use in regions with access to low-cost energy and where freezing temperatures occur naturally.

2.3.4. Chemical Disintegration

Chemical disintegration is used to transform hardly biodegradable components into more easily biodegradable ones. This is typically achieved through the application of various chemicals such as ozonation, acids, chlorine, Fenton reagent and alkali materials (Tanaka et al., 1997; Sakai et al., 1997).

Ozonation is used to break down organic compounds in the sludge into simpler, more biodegradable forms. Similarly, the Fenton reagent, which consists of hydrogen peroxide and ferrous iron, generates hydroxyl radicals that can oxidize organic matter in the sludge, facilitating its degradation. Other chemicals such as chlorine, acids, and alkalis may also be used to alter the chemical composition of the sludge, making it more amenable to biological degradation.

2.3.5. Pulsed Electric Field Application

Pulsed Electric Field (PEF) technology comprises placing a purifier between two electrodes and applying a series of short-term (10ns-20μs) high voltage (20–80 kV/cm) to the liquid in the chamber. Capacitors operating with direct current power supplies are used for this purpose, producing electric fields by rapidly discharging large amounts of stored energy. When a pulsed electric field is applied to a liquid, it creates pores on the membrane of microorganism cells, rendering them more susceptible to chemical processes and biological degradation. This process enhances the decomposition of organic matter, increases biogas production during anaerobic digestion, and shortens the process time. PEF can be applied at ambient temperature or below ambient temperature, and since it is applied in less than a second, there is minimal energy loss and heating of the substance. The key factors during PEF applications include the creation of electric fields, designing a volume suitable for uniform application of the process while ensuring minimal temperature rise within this volume, and designing electrodes to minimize electrolysis effects (Devlieghere, Vermeiren, & Debevere, 2003).

Loeffler et al. (2001) conducted a study wherein they applied an electric field to wastewater treatment sludge. They investigated the mechanical disintegration of biomass cells during the pre-treatment process in domestic sewage sludge samples subjected to high voltage. The soluble chemical substances were detected in the sludge samples subjected to the electric field. The results obtained by measuring the increase in oxygen demand indicated comparability to other mechanical digestion methods. It was noted that as the electrical energy supplied to the sludge increased from 200 W to 800 W, the degree of disintegration also increased. Furthermore, the electrical capacity, the number

of discharges (pulse or pulse), the area subjected to electrical application, and the number of pores formed were observed to increase with increasing voltage.

Choi et al. (2005) conducted a study wherein they applied pulsed electric field (PEF) technology to WAS samples and investigated its effects on biogas production during anaerobic sludge digestion. To assess the impact of PEF on the solubility of sludge from the treatment plants, an emphasis power was applied at a voltage of 19 kV and a frequency of 110 Hz. The results of the experiments indicated that sludge samples treated with PEF were 4.5 times more sensitive in terms of soluble chemical oxygen demand and exhibited 2.5 times higher biogas production compared to samples without PEF treatment. Additionally, scanning electron microscopy (SEM) images discovered that the organic content of the sludge pretreated with PEF was significantly disrupted, leading to the conclusion that PEF pretreatment caused cell destruction.

2.3.6. Ozonation

Ozone is a frequently used in sludge treatment procedures because of its capacity to break down organic polymers into smaller units. Ozone-oxidation is a process that releases cytoplasm into the bulk solution by breaking down the cell walls of microorganisms in waste activated sludge through both direct and indirect processes (Park, Ahn, & Maeng, 2002).

Two major steps of ozonation pre-treatment methods are cell disintegration and mineralization. Ozone causes the bacterial cell membrane to break down, causing the contents of the cell to leak out into the surrounding fluid and causing cell disintegration. Conversely, mineralization is the process by which soluble organic matter is oxidized to produce mineralized materials from cell walls, which in turn produces carbon dioxide.

The generation of disintegrated solids and mineralized minerals can lead to sludge reduction as a consequence of ozone pre-treatment. A soluble fraction and microparticles distributed throughout the supernatant upon thickening make up disintegrated solids. When the supernatant is circulated again, these bioavailable materials can act as a source of nutrients for microorganisms involved in the activated sludge process. More ozone oxidation can be used to get rid of the residual materials, which are meant to be mineralized materials.

With the ozone treatment, the increased solubility of these bio-substrates enhances their digestibility, making them more easily degradable in the subsequent anaerobic digestion process.

Consequently, ozone pre-treatment is utilized to increase the biodegradation efficiency of waste activated sludge.

2.3.7. Fenton Addition

The Fenton process, referred to as an advanced oxidation process, combines the oxidation impact of hydrogen peroxide with the catalytic action of iron (II) salt. While traditionally used in wastewater treatment, the Fenton process has also gained attention for its potential application in sludge digestion. Studies in recent years have demonstrated that the addition of Fenton reagents can improve the dewaterability characteristics of sludge (Neyens & Baeyens, 2003; Büyükkamacı, 2004; Dewil, Baeyens, & Neyens, 2005).

In many studies, researchers have explored the use of the Fenton process for sludge disintegration. Neyens et al. (2003) investigated the optimization of Fenton process conditions for improving dewaterability and found that optimal conditions included hydrogen peroxide and iron (II) concentrations at a pH of 3 and ambient room temperature. According to their findings, there was a 30% rise in the total solid content of the sludge cake and a 30% decrease in its volume.

Similarly, Dewil et al. (2005) applied the Fenton process under similar conditions and reported that sludge cake solid content increased from 22.5% to 40.3%. Büyükkamacı (2004) also conducted a study demonstrating that increasing concentrations of hydrogen peroxide and iron (II) led to improved dewaterability of the sludge.

These results demonstrate the Fenton process's promise as a useful technique for improving sludge's dewaterability, which will make it easier to handle and dispose of in wastewater treatment operations.

2.3.8. Biological Disintegration

Biological hydrolytic disintegration aims to enhance the degradability of organic matter by breaking down cell walls, a process facilitated by the secretion of enzymes by microorganisms (Liu & Tay, 2001; Müller, 2001). These enzymes, such as cellulases, proteases, and lipases, work to convert complex macromolecules into smaller, more accessible compounds. This enzymatic breakdown is essential for microorganisms to utilize the organic material efficiently.

Enzymatic disintegration can occur spontaneously at ambient temperatures or can be facilitated by adding specific enzymes to the system. In the enzymatic disintegration process, cell wall components are broken down by enzymes, leading to the disintegration of cells. Combining enzymatic disintegration with mechanical disintegration can significantly increase the degree of disintegration (Thomas, Jungschaffer, & Sprossler, 1993).

According to studies, adding enzymes can lower the final sludge disposal cost (Ronja, 2008). Enzymes have been found to effectively release extracellular polymeric substances (EPS), which are essential components of microbial aggregates in sludge (Dey et al., 2006).

One study investigated the bacterial hydrolysis of polymeric substances in activated sludge degradation and found that this pretreatment led to a decrease in volatile suspended solids and an increase in soluble chemical oxygen demand, indicating enhanced degradation efficiency (Wawrzynczyk et al., 2003).

All things considered; enzymatic disintegration presents a viable method for raising the effectiveness of sludge digestion procedures in wastewater treatment.

2.4. Sludge Anaerobic Co-Digestion Applications with Crops

Biogas production holds significant potential as a renewable energy source, utilizing various agricultural, industrial and domestic organic wastes. Unlike other renewable energy sources, biogas production is not limited by geographical constraints or complex technologies (Bayrakcı & Koçar, 2011).

With increasing limitations on landfilling organic waste due to new legislation, there is growing interest in co-digesting suitable organic waste with municipal sludge. Anaerobic digestion of wastewater sludges is known as one of the well-established processes. To gain a comprehensive understanding of how variations in waste input content influence the stability and efficiency of the digestion process, further research is required (Murto, Björnsson, & Mattiasson, 2004).

Turkey, as an economically developing country heavily reliant on energy imports, holds vast potential for generating energy from its agricultural resources. Among OECD countries, Turkey is ranked second in terms of projected recoverable energy potential from crop leftovers and fourth in

terms of estimated total energy potential. With a significant biomass production of over 40 million tons, Turkey has 187.4 million kWh of potential energy (Tay, 1991; Balat; 2005).

These biomass sources play a crucial role in Turkey's efforts to increase energy security, promote renewable energy utilization, and reduce dependence on fossil fuels. Through efficient utilization of these biomass resources, Turkey can enhance its energy self-sufficiency and contribute to environmental sustainability goals.

With 55.6% of its land being arable and 15% consisting of forests, Turkey has ample agricultural resources for biomass production. Figures 2.2 and 2.3 present the quantities of various agricultural products from 2005 to 2010, while Figure 2.4 illustrates the population of cattle, sheep, and goats between 2008 and 2009. These agricultural resources offer significant potential for biogas production and contribute to Turkey's energy self-sufficiency and sustainability goals.

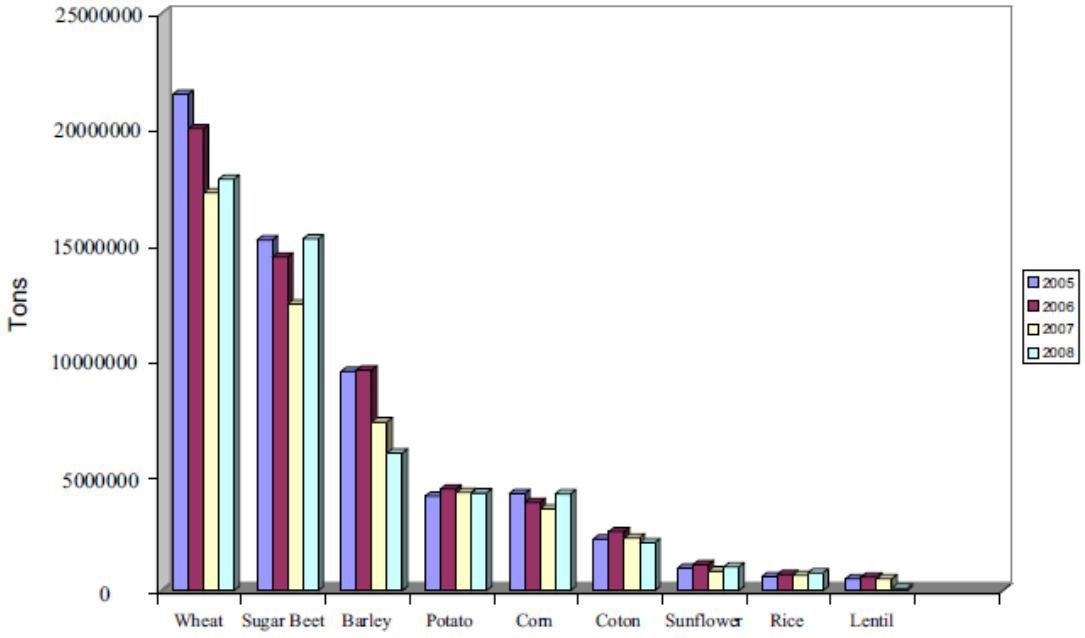

Figure 2.2. Amounts of certain energy crops from 2005 to 2008 (Bayrakcı & Koçar, 2011).

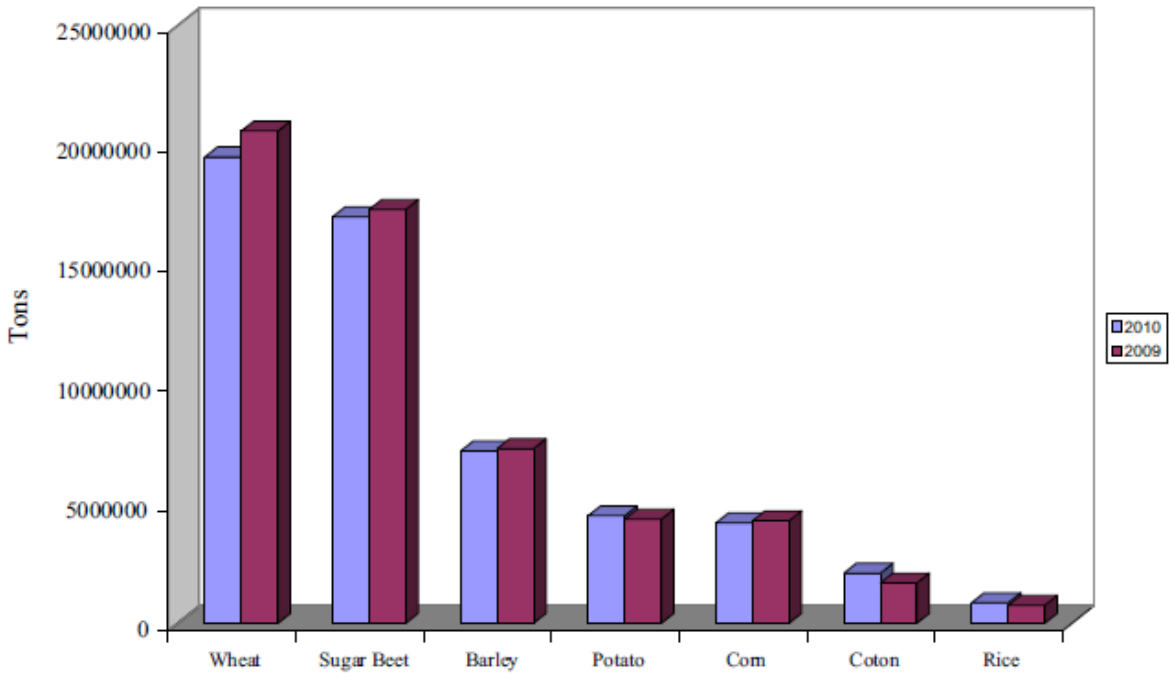

Figure 2.3. Amounts of some energy crops for the years 2009 and 2010 (Bayrakcı & Koçar, 2011).

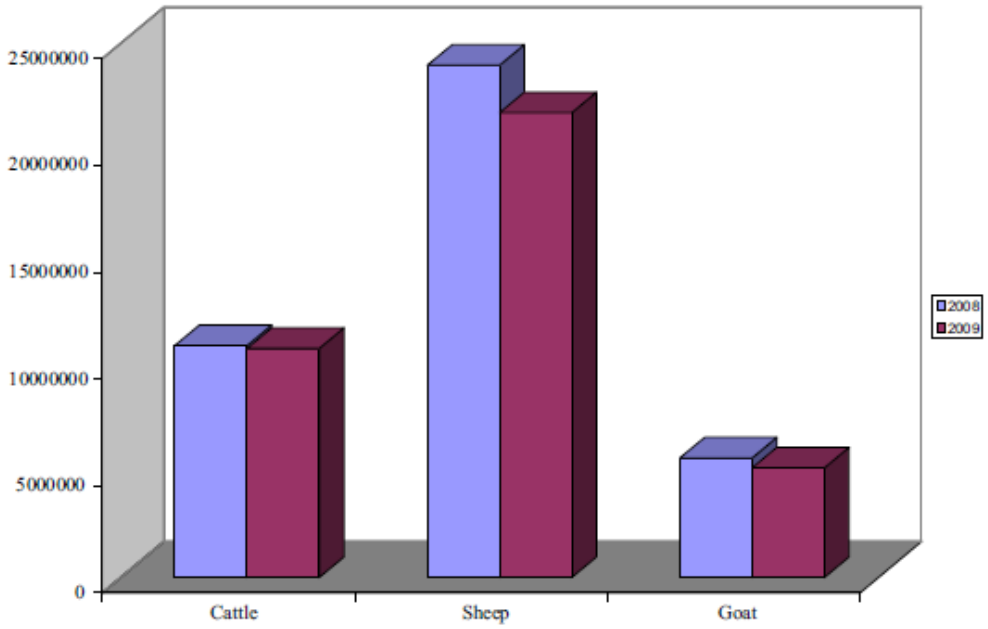

Figure 2.4. The numbers of some livestock between 2008 and 2009 (Bayrakcı & Koçar, 2011).

Indeed, given Turkey's status as an agricultural country with significant agricultural production wastage (Table 2.2), there is a substantial amount of agricultural waste generated. These agricultural wastes present challenges for traditional disposal methods such as landfilling or incineration, primarily due to their high moisture content and organic composition. However, these wastes can serve as an excellent feedstock for anaerobic digestion.

Table 2.2. Turkey's potential biomass (Balat, 2005).

Substrated	**Potential annually (Million tons)**	**Energy content (Mtoe)**
Agro-industry residuals	10	3.0
Animal wastes	7	1.5
Total	17	4.5

Researchers are looking into the possible advantages of mono- or co-digesting animal and agricultural wastes, also known as biomass, in response to a number of problems. This entails the individual or combined use of wet or dry procedures for the digestion of energy crops, crop wastes, and other materials. Controlled anaerobic digestion of animal manures has been shown to improve nutrient recycling in the agriculture industry while reducing greenhouse gas emissions, nitrogen outflow, and odor from manure management in addition to producing renewable energy (Lehtomaki, Huttunen, & Rintala, 2007).

The anaerobic digestion of cereals such as wheat, oat straw, and barley waste is being explored due to their significant contribution to overall agricultural production. Figure 2.5 illustrates the quantities of some agricultural products produced in Turkey between 2009 and 2010.

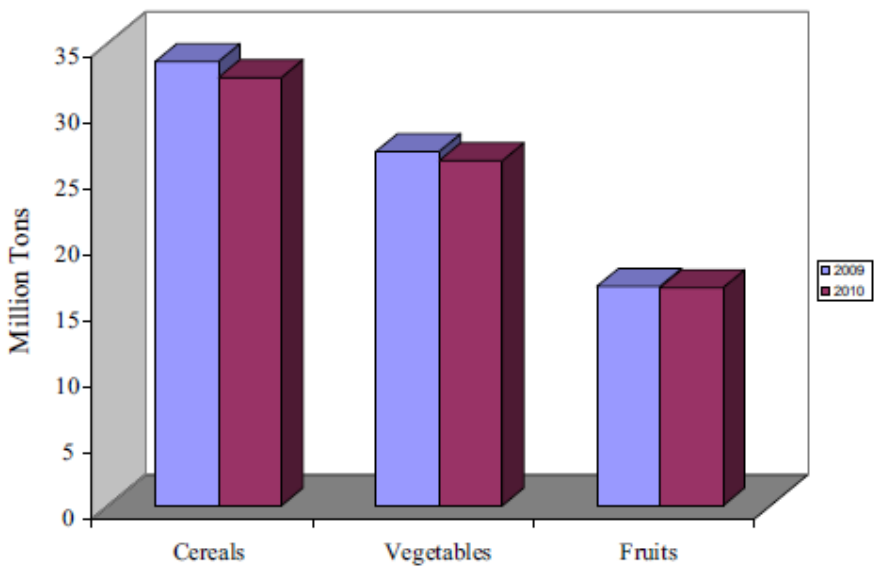

Figure 2.5. The cereals, vegetables and fruits production (Bayrakcı & Koçar, 2011).

Pohl et al. (2012) were investigated the mono digestion of wheat straw under two different solid retention times (SRTs), specifically 14 and 21 days, resulting in methane formation potentials of 38% and 50% of the substrate, respectively. The findings suggest that anaerobic digestion could be a viable

option for full-scale applications. However, optimal performance may require the addition of trace elements such as calcium (Ca), sodium (Na), among others.

Although much of the research on wheat straw digestion has focused on co-digestion with other agricultural or animal wastes, Lehtomaki and Wang conducted notable studies. In one study on manure, grass silage and oat straw using a continuous stirred tank reactor under mesophilic conditions. Methane production increased by up to 58% with the addition of 30% grass silage to the manure, while the increase was lower (16%) with oat straw addition. In another study, dairy and chicken manure were co-digested with wheat straw in batch mode, 30/1 was found to be optimal C/N ratio due to stable pH levels and low concentrations of total ammonium nitrogen.

Some researchers examined the effects of pre-treatments on barley waste and wheat straws, respectively. Alkaline hydrolysis pre-treatment of barley waste, when co-digested with kitchen waste, increased methane production. However, this pre-treatment led to a decrease in total and volatile solids reduction. Another pre-treatment method involves mechanical processing to achieve varying particle sizes ranging from 0.1 cm to 5.0 cm, with studies showing that smaller particles tend to result in increased biogas production values (Menardo et al., 2012; Neves et al., 2006).

The total solid contents of the wastes used in anaerobic digestion processes in several studies is presented in Table 2.3.

Table 2.3. Total solid contents of the wastes used in anaerobic digestion processes in some studies.

Waste	**TS %**	**Reference**
Cow manure	6.6	Lehtomaki et al. (2007)
Dairy manure	14.4	Wang et al. (2012)
Chicken manure	6.93	Wang et al. (2012)
Oat straw	63.5	Lehtomaki et al. (2007)
Grass silage	25.9	Lehtomaki et al. (2007)
Barley waste	21.4	Neves et al. (2006)

Grass silage, renowned for its rich content of digestible organic matter, proves to be an excellent substrate for anaerobic digestion. When combined with manure, this co-digestion approach can significantly enhance biogas yield. This is attributed to several factors: (i) maintaining an optimal pH for methane-producing bacteria, (ii) mitigating the inhibition caused by free ammonia/ammonium, and (iii) improving the C/N ratio (Xie, Liu, & Yan, 2012).

Different organic loading rates (OLRs) and ratios of dried grass silage (DGS) to pig manure were implemented in the study. OLRs of 1, 1.5, 2, and 3 kg VS/m^3-day were applied. Results indicated that higher OLRs led to an 88% increase in volumetric methane yield, albeit with a 38% decrease in specific methane yield. Interestingly, variations in the VS percentages of DGS did not significantly affect digestion efficiency. Thus, the study underscores the importance of OLR as a crucial parameter over the substrate ratio of silage to manure.

In Thamsiriroj's consecutive studies (2011 and 2012), the grass silage single digestion in a two-stage CSTR was investigated to determine the optimal operational period under varying OLRs. The findings suggested that prolonged application of grass silage, such as over 350 days, led to the accumulation of lactic acid, reduction of acetic acid, pH decline, decreased methane production, reduced solids destruction, and ultimately, an increase in dry solids content.

Cavinato's study is notable for investigating the co-digestion of cow dung, maize, fruit waste, and bread within a Continuous Stirred-Tank Reactor (CSTR) system, encompassing both pilot-scale and full-scale operations (Table 2.4).

Table 2.4. Characteristics of the wastes co-digested (Cavinato et al. 2010).

Substrate	**TS, gTS/kgw.w.**	**VS, %**
Solid Manure	361	84
Liquid Manure	97	46
Maize	351	93
Fruit	219	96
Bread	828	90

In Maranon's study (2012), cattle manure was co-digested with food waste and a small percentage of sewage sludge. The study investigated both 36°C and 55°C conditions, with lower methane yields observed under thermophilic conditions.

Additionally, ultrasonication was applied to the digestate mixture, with a parallel analysis conducted on a non-pre-treated sample. The findings showed that sonication's energy consumption was not offset by ultrasound's impact on biogas yield.

Tekin and Dalgıç (2000) investigated the olive pomace. Two digesters were used to implement batchwise and semi-continuous anaerobic digestion operations in an oven with temperature control. There were three different ratios of olive pomace to water slurry used: 5%, 10%, and 15% total solids. Lower concentrations of the substrate slurry (5% total solids) were associated with higher production

rates and methane compositions, suggesting that olive pomace is not quickly biodegradable. In batchwise fermentation, the resistance to digestion increased as the total solids concentration increased. During semi-continuous digestion, the maximum methane composition was achieved at 10% total solids and 80% by volume with a 20-day HRT.

In summarizing the anaerobic operations involving various agricultural and animal wastes, it is concluded that mesophilic conditions are more conducive to efficient biogas production, particularly with OLR ranging from 1.0 to around 2.6 kg of VS/m^3-day for continuous systems. Co-digestion of waste mixtures, rather than mono-digestion, consistently resulted in higher methane production. However, optimal ratios for different substrates need further investigation in co-digestion processes.

Many studies have identified pH as a challenge primarily because agricultural wastes typically have low buffering capacities. Co-digestion with sewage sludge, which has a high buffering capacity, emerges as a potential solution to this challenge.

Encouraging the co-digestion of agro-wastes with WAS is vital for both the beneficial utilization of organic wastes and the efficient treatment of wastewater sludges, leading to significant energy gains. Additionally, certain mechanical, physical, or thermal pre-treatments have shown promise in increasing biomass production within shorter timeframes.

3. MATERIALS AND METHODS

3.1. Substrates

Anaerobically digested sludge from full-scale anaerobic digesters, operating under mesophilic conditions, at a significant yeast industry in Turkey was used as an inoculant for each reactor in the study. This inoculum sludge was utilized to seed batch reactors, enhancing the rate of anaerobic degradation and methane production.

Cloverleaf, bagasse, maize silage, wheat, nutshell, potato, grass, barley, and manure are the crop and agricultural residue types that were chosen for anaerobic digestion as appropriate energy crops due to their high yield and capacity to generate biogas.

However, due to their lack of sufficient nutrients for bacterial development, early research showed that AD of just these materials was not particularly effective. The optimal C/N ratio for AD process is typically considered to be between twenty and thirty (Parkin & Owen, 1986). Sludge from wastewater treatment plants typically exhibits a lower C/N ratio, typically ranging between 6 and 16 (Tchobanoglous, Burton, & Stensel, 2003). Therefore, to significantly enhance the efficiency of co-digestion, the study also investigated the inclusion of sewage sludge alongside these agricultural residues.

The sludge samples used in the study were from the return activated sludge stream of the Paşaköy wastewater treatment facility of the İstanbul Metropolitan Municipality.

The characteristics of the inoculum and sewage sludge samples used in this investigation are listed in Table 3.1.

3.2. Operational Set-up

In the study, multiple batch reactors were simultaneously conducted in the laboratory to investigate anaerobic digestion. The substrates were subjected to digestion in 2.5 L bottles, each with an active volume of 1600 mL. The temperature of these anaerobic reactor bottles was kept at 37°C for 40 days. Throughout the duration of the experiment, the temperature was maintained and regulated

by an automatic heat controller to ensure consistency. The overall design and operational details of the setups are shown in Figures 3.1 and 3.2.

Table 3.1. Characteristics of the sludge samples used in the research.

Parameter	**Unit**	**Inoculum**	**Paşaköy WWTP**
TS	%	3.83	2.09
TS	mg/L	38,331	20,992
VS	mg/L	25,360	12,385
VS/TS	-	0.66	0.59
MLSS	mg/L	37,250	15,480
MLVSS	mg/L	24,000	9,570
MLVSS/MLSS	-	0.70	0.62
COD	mg/L	38,470	16,750
sCOD	mg/L	5,154	927
TKN	mg/L	870	980
NH_4^+	mg/L	294.00	51.75
TP	mg/L	430	410
PO_4^{-3}-P	mg/L	1310	1260
SO_4^{-2}	mg/L	110	5
Cl^-	mg/L	602	71
Capillary Suction Time (CST)	sec	>1000	51.3
Alkalinity (as $CaCO_3$)	mg/L	5302.5	1155
pH	-	7.3	6.72
Conductivity	mS/cm	20.10	3.02
Salinity	‰	14.0	1.8
Total Coliform	kob/100mL	1*106	1.4x107
Fecal Coliform	kob/100mL	5*105	4.7x106
Fecal Streptococ	kob/100mL	1*105	1x106
Salmonella	kob/100ml	8*104	5x105

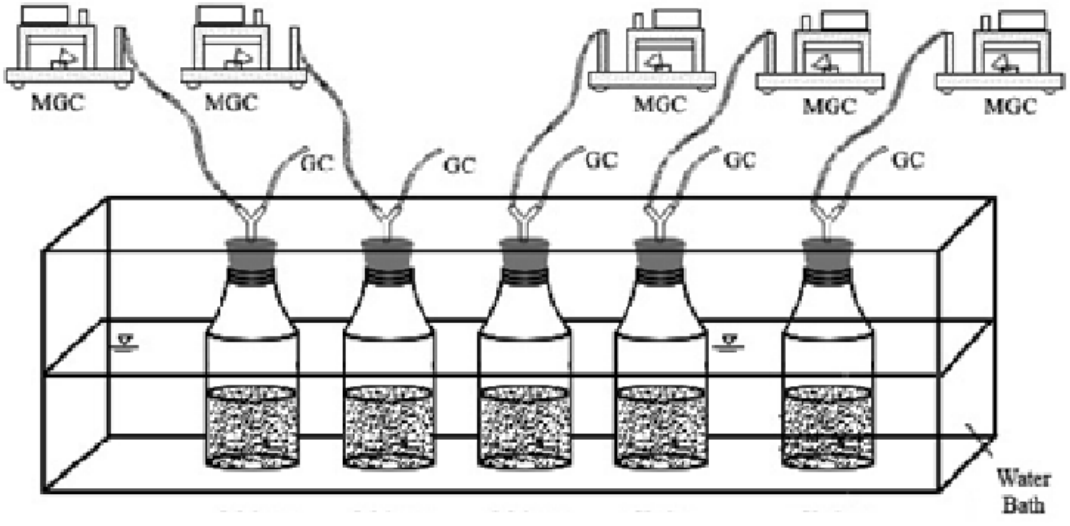

Figure 3.1. System configuration for anaerobic digestion.



Figure 3.2. The laboratory scale system for anaerobic digestion.

The top of the bottles had a V-shaped gas collection port installed. One port hole was used to collect gas samples for composition analysis, while the other opening was used to attach a MiliGascounter® (MGC) to measure the generation of biogas. CH_4 and CO_2, which were measured by HP 6850 gas chromatograph, were the main gases analyzed for composition. The helium used was at a flowing rate of 2 mL/min as the carrier.

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3.3. Sampling and Analytical Methods

Regular analysis was conducted on the biogas produced in the reactors for both quantity and composition. The gas volume was measured using a Miligascounter® type MGC 1 (Ritter, Germany). Throughout the investigation, gas volume was recorded on a daily basis, and weekly analyses of the gas composition were conducted.

The following formulas ((Equation 3.1) were used to adjust the biogas yield and methane yield values to standard temperature and pressure:

$$B = \mathrm{A} * \frac{273{,}15}{\mathrm{T}+273{,}15} * \left(1 - \frac{p}{101{,}325}\right) \qquad (3.1)$$

where,

A = biogas yield and methane yield values calculated according to results of experiments

B = the values corrected at standard conditions

T = temperature at which the reactors were operated (37 °C)

P = water vapor partial pressure at 37 °C = 6,27 kPa

The mixtures of pomaces and sludges were subjected to analysis for various parameters as outlined in Table 3.2. These analyses were performed following the guidelines outlined in the Standard Methods for the Examination of Water and Wastewaters.

Table 3.2. The analyses carried out during the study.

Parameters	**Crop**	**Sludge**	**Mixture of crop + sludge before digestion**	**Mixture of crop + sludge after digestion**	**Biogas**	**Frequency**
TS	x	x	x	x		once
VS	x	x	x	x		once
pH			x	x		once
Alkalinity			x	x		once
TKN			x	x		once
Total P			x	x		once
VFA			x	x		once
NH_4^+- N			x	x		once
PO_4^{3-}			x	x		once
Microbial Analyses			x	x		once
sCOD			x	x		once
Gas Volume					x	daily
Gas Content					x	once/week

Bandelin-Sonopuls HD 3400 Ultrasonicator was used to ultrasonically disintegrate wastewater sludges (Figure 3.3). The components of this ultrasonic homogenizer are the booster horn, ultrasonic converter (UW 3400), probe (VS200T), and generator (GM 3400). The electricity that is received (up to 60 Hz) is transformed into high frequency power at 20 kHz by the generator. This high-frequency power is transformed to ultrasound by the ultrasonic converter, which subsequently turns it into mechanical energy. An effective and reliable ultrasonic transducer system helps in this process. As a result, the probe's tip vibrates at 20 kHz, and the sonicated sample receives a high-power density transmission of these vibrations

The power (acoustical) can be determined using numerous approaches, including measurements of electrical power input, acoustic intensity, or calorimetric and chemical dosimetry methods. These techniques can help quantify the energy being delivered to the system, which in turn influences the efficiency and effectiveness of ultrasonication processes. Calorimetry was used in this work in the 400-cc sludge sample.

Furthermore, the sludge samples underwent a thermal pre-treatment of microwave disintegration by the utilization of the Berghoff Speedwave MWS-3+ Digestion System (Figure 3.4). There are twelve Teflon containers in this system, each holding sixty millilitres. It runs on a 230 V power source, 50–60 Hz frequency, and 1450 W of output power. The device can monitor pressures between 0 and 150 bars and has a maximum temperature of 300°C.

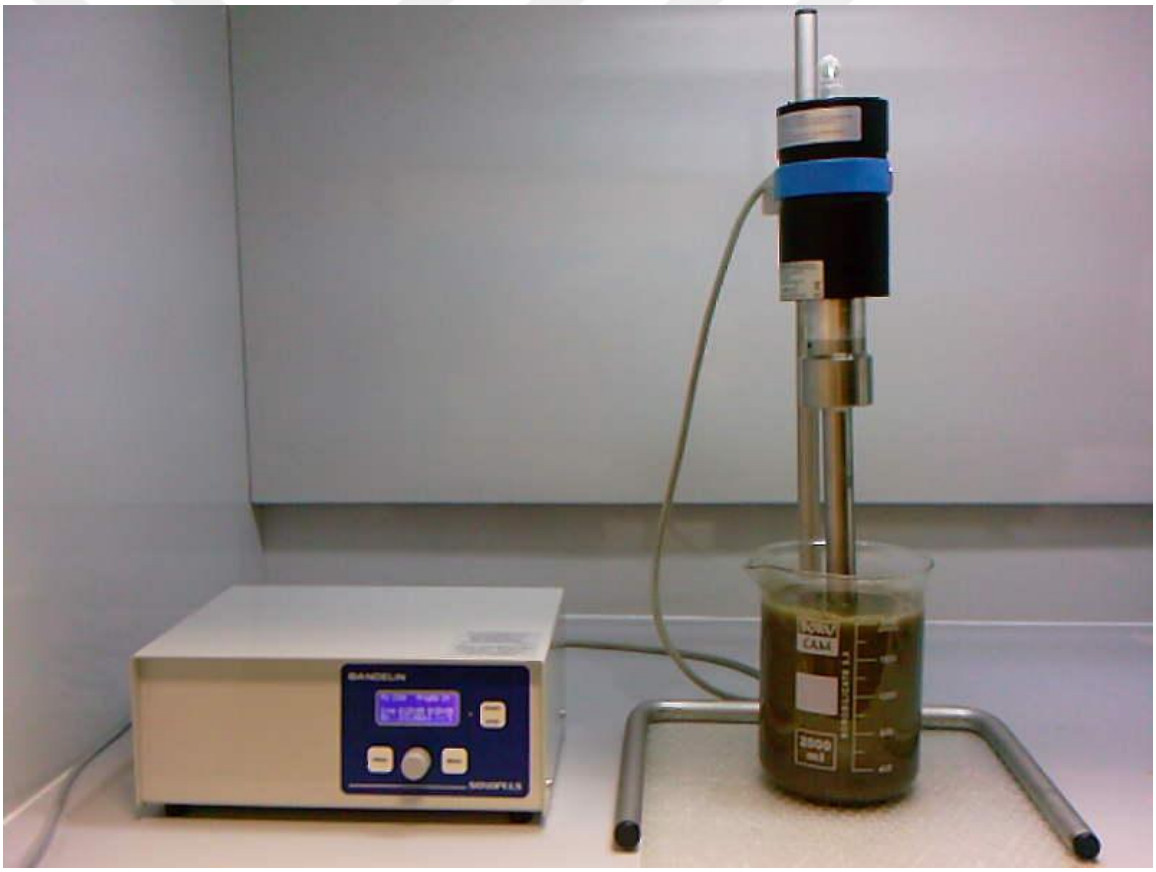

Figure 3.3. Bandelin-Sonopuls HD 3400 Ultrasonicator.



Figure 3.4. Berghof MWS-3 microwave heating unit.

Pulsed Electric Field (PEF) application is an alternative disintegration method for sewage sludge pre-treatment which is basically consists of applying high voltages (10-50 kV/cm) with short times (10 ns-20µs) to a liquid material, placed in a small banker between two electrodes. Electrodes are made of inert materials. The system of PEF is shown in Figure 3.5.

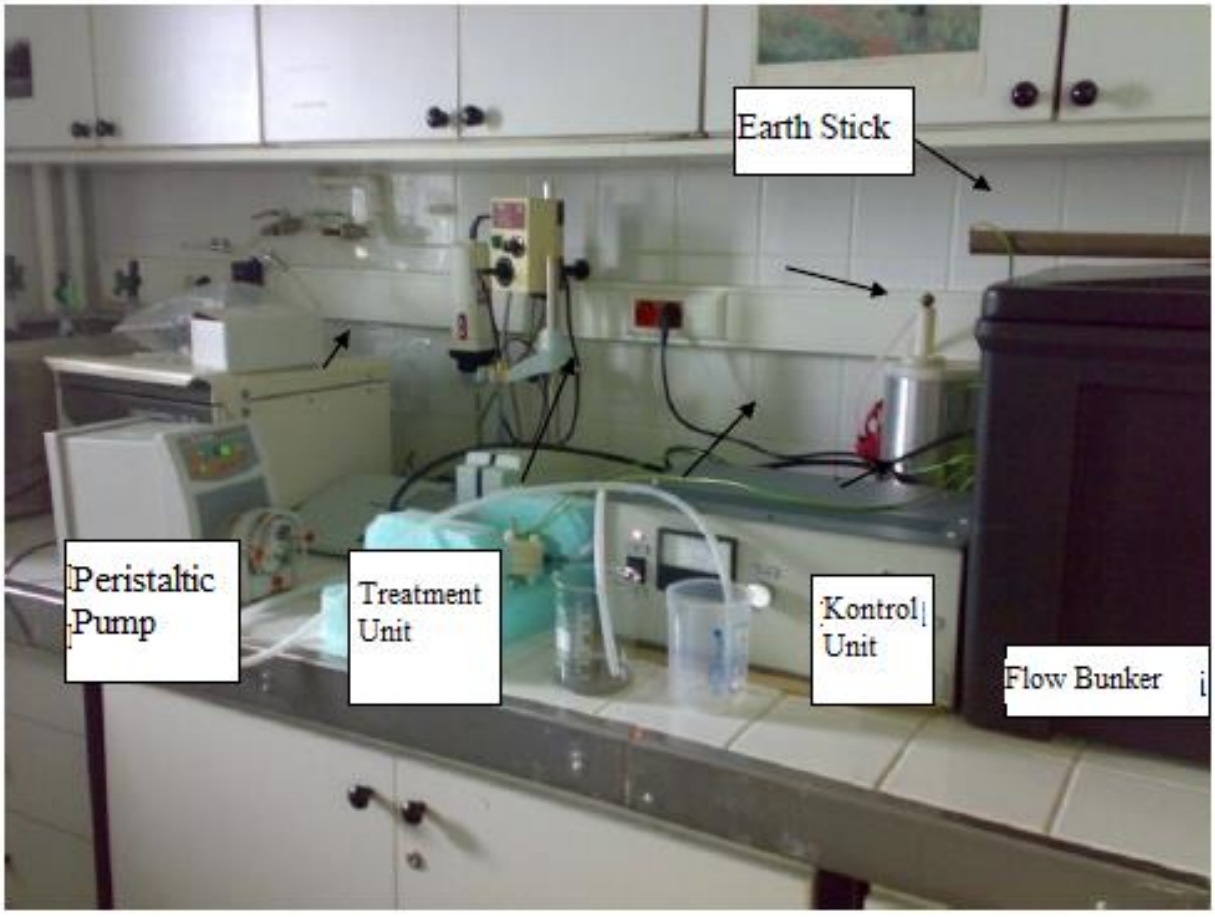

Figure 3.5. Pulsed Electric Field (PEF) application.

4. RESULTS AND DISCUSSION

4.1. Total Solid Content of the Reactors

Tekin and Dalgıç's study examined the production of biogas from olive pomace, with particular attention to the reactors' total solid (TS) content. Anaerobic conditions were maintained during batchwise and semi-continuous digestion operations using two digesters kept in a temperature-controlled oven. The temperature was at a constant level of 37±0.5 ºC throughout the study. For the runs, 5%, 10%, and 15% TS concentrations of olive pomace were combined with water slurry (Tekin & Dalgıç, 2000).

The study found that during the digestion of olive pomace, methane generation exhibited the usual curve features. Methane production grew dramatically following a 10-day lag phase, reaching a stationary phase by the 25th day. The study found that the non-biodegradable nature of olive pomace was indicated by greater production rates and methane compositions that correlated with lower concentrations of substrate slurry (5% TS). The effectiveness of the digestion process may be hindered due to the existence of insoluble chemicals that are challenging to degrade, the study found that a longer HRT of over 20 days was necessary for the digester to attain complete biodegradation.

Prior to defining the reactor loading rates, the first study assessed the impact of various TS contents on the digesting mixture containing olive pomace (OP), grape pomace (GP), and anaerobic sludge (inoculum) based on cumulative gas production. In 2.5 L bottles with an active volume of 1600 ml and TS contents of 6.5%, 7%, 8%, and 10%, the substrates and inoculum were first digested. The ISR was adjusted to 1:1 during the 40-day digesting phase. The anaerobic reactors' temperature was 37 °C. The investigation found that at a 6.5% TS concentration the greatest biogas generation was achieved, as shown in Figure 4.1.

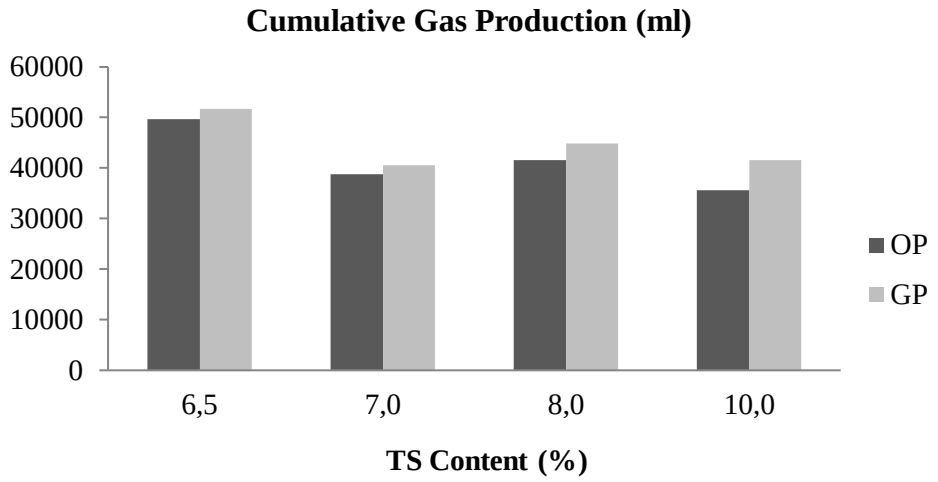

Figure 4.1. The effects of varying TS content on biogas production during anaerobic digestion.

4.1.1. Preliminary Analysis

Before the anaerobic digestion trials, the TS (%) and VS (%) contents of grape and olive samples were measured to assess the organic matter content and determine the quantities of substrates needed for the reactors.

The investigations revealed that olive pomace contained 49.75% TS, of which 75.16% were volatile solids. In contrast, grape pomace had a lower solid content, with 40.76% total solids, of which 88.52% were volatile.

Approximately 30 g samples were analyzed to determine the percentages of solids concentrations in the seeding sludge. The total solid content of the wastewater sludge was around 4.51% in both setups.

4.1.2. Set of Reactors

Anaerobic granular sludge was used as the seeding material and olive pomace as the substrate in the reactors used for the first batch of the investigation. In the second batch of the investigation, anaerobic sludge (granular) was used as the seed and grape pomace from a winemaking process as the substrate in the reactors.

Granular sludge and pomaces were stored at 4°C in a cold chamber and then brought to room temperature before digestion. Based on the determined TS contents of the reactors, the weights of the crops and seed sludge to be added were calculated.

A sample calculation for the loading condition of the reactor R1 (having ISR: 3/1) of the first set-up is presented below as an example (Equations 4.1 and 4.2):

TS of olive pomace: 49.75 %
TS of seed sludge: 4.521 %
TS of the blend: 6.5 %

$$\text{Weight of the crops added to the reactor} = \left[\frac{1600*6.5*100}{(1/3+1)*49.75*100}\right] = 157{,}170 \text{ g} \quad (4.1)$$

$$\text{Weight of sludge added to the reactor} = \left[\frac{1600*(6{,}5-(\frac{6{,}5}{0{,}33+1})*100}{4{,}521*100}\right] = 570{,}847 \text{ g} \quad (4.2)$$

Four distinct inoculum (seed sludge) to substrate (crop) ratios were used for the batch studies for grape and olive pomaces, as well as a combination of seed sludge and pomaces. The ratios under investigation were as 1, 1.5, 2, and 3.

The pomaces were blended into shreds. After adding the homogenized mixture to the reactors, the mixture was diluted to a final volume of 1600 mL.

Control reactors, which included inoculum sludge without any crop, were used as a baseline with a definite total solid (TS) content. During both sets of experiments, R1 reactor was operated as the control reactor.

According to preliminary research, the pH range of 7.0–8.5 is effective for methane generation (McKendry, 2002). In order to create ideal circumstances for the growth of methanogens, 1N KOH and H_2SO_4 were used to raise the pH of the reactors to about 7.5. The bottles were then sealed with stoppers, and every connection was coated with silicon to assure gas tightness. Subsequently, the reactors underwent nitrogen gas purge for a 5 minute to remove O_2 from the reactors.

4.2. Sludge Disintegration and Optimization Studies

In efforts to decrease the total sludge generated and improve the degree of sludge stabilization, different methods of mechanical, thermal, and chemical disintegration were applied to the sludge samples.

Müller & Pelletier (1998) proposed a method to calculate the disintegration degree of sludge after various treatment methods, including mechanical, thermal, and chemical disintegration. The disintegration degree (DD) of the used pre-treatment methods is calculated using the formula by Bougrier et al., 2005.

This formula calculates the reduction in total solid content of the sludge after treatment as a % of the original TS content. A higher disintegration degree indicates a greater reduction in total solid content and thus a higher degree of sludge stabilization.

4.2.1. Optimization Studies

In the optimization studies, various parameters related to the applied disintegration techniques were systematically adjusted and analyzed to enhance the efficacy and effectiveness of the anaerobic digestion method. These parameters may include the intensity and duration of mechanical, thermal, or chemical disintegration methods, as well as the combination of multiple techniques.

4.2.1.1. Optimization of ultrasonic disintegration. In the study, the sewage sludge samples were subjected to various power levels (60, 100, 200, 300 W) to determine the most appropriate power and amplitude values for ultrasonic disintegration. This approach allows researchers to assess the effects of different energy inputs on sludge disintegration efficiency and optimize the process parameters for maximum effectiveness. By systematically varying power levels and analysing the results, researchers can identify the optimal conditions that result in enhanced sludge disintegration while minimizing energy consumption and process costs related with the ultrasonication. The sewage sludge was first brought to 20±2°C and then placed into a 400 mL beaker.

After optimization studies, it was revealed that 200 W and a 70% amplitude was the most effective ultrasonication combination for a given input of energy, 10,000 kJ/kg TS. This indicates that at this SE input level, 200 W and a 70% amplitude yielded the best results in terms of sludge

disintegration efficiency. Table 4.1 lists the disintegration degrees that correspond to this ideal scenario.

Table 4.1. DD (%) obtained with the optimum value, 10000 kJ/kg TS.

Specific Energy, kJ (kg TS)$^{-1}$	**Ultrasonication Value**	**Ultrasonic Power**	**Sewage Sludge of Paşaköy WWTP**
10000	Disintegration Degree (%)	60	6.3
		100	15.6
		200	17.80
		300	26.4
	Soluble COD (mg/L)	60	702
		100	1506
		200	2850
		300	2790

According to those obtained values, sonication time of the sewage sludge calculated for the chosen specific energy and TS content of the WAS are given in Table 4.2.

Table 4.2. Sonication times applied according to the determined specific energies.

TS (mg/L)	**Sludge SE (kJ/kg TS)**	**Duration of US treatment (seconds)**
10200	5000	202
	10000	375
	15000	593
	25000	860
	50000	1840

In Table 4.3, the sCOD values and disintegration degrees of ultrasonicated sewage sludges with different SEs are presented.

Table 4.3. sCOD values and DDs calculated after the ultrasonication for different SE

Sludge SE Calculated (kJ/kg TS)	**sCOD concentration (mg/L)**	**Disintegration Degree (%)**
-	135	
5000	1650	15.54
10000	1690	16.6
15000	2006	19.36
25000	3064	30.35
50000	7063	82.16

In Tables 4.4 and 4.5, the results of rheological characteristics of the disintegrated sewage sludge are provided. These analyses offer valuable insights into the rheological characteristics of the sludge following the applied treatment process, helping to assess the effectiveness of the treatment method and its impact on sludge characteristics.

Table 4.4. CST and viscosity analysis for different specific energies.

Analysis	**Specific Energy (kJ/kg TS)**	**Results**
CST (sec)	5000	1295.6
	15000	1449.5
	50000	1652.2
Viscosity (Mpas)	5000	28.4
	15000	22.8
	50000	18

Table 4.5. The distribution of particle sizes for different SE Values.

Sludge SE (kJ/kg TS)	**Surface Weighted Mean D[3,2]**	**Volume Weighted Mean D[4,3]**	**d (0.1) µm**	**d (0.5) µm**	**d (0.9) µm**
Raw Sludge	36,200	83,931	18,863	65,037	143,369
5000	21,100	42,521	18,560	36,676	71,045
15000	6,153	32,707	2,457	17,303	54,722
50000	3,429	38,844	1,156	10,677	103,632

The Gaussian curve obtained for distribution of particle sizes is given in Figure 4.2.

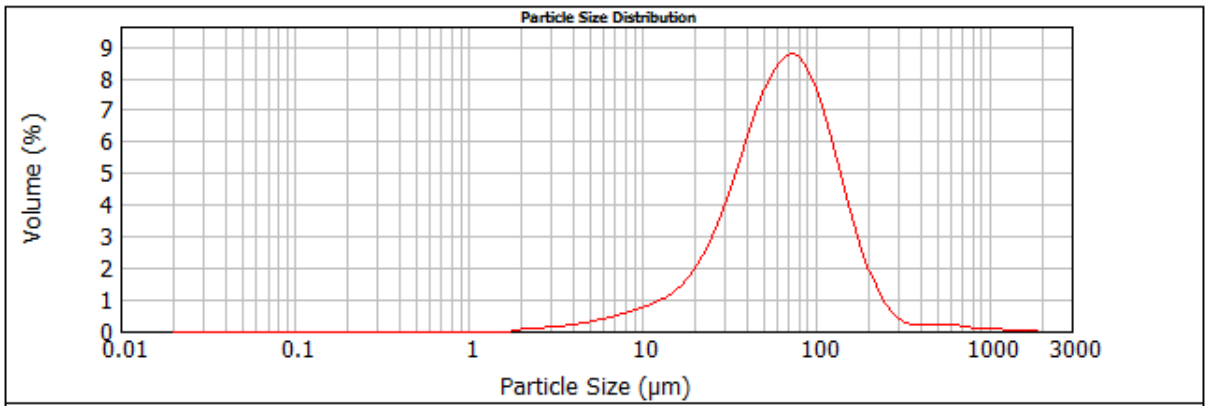

Figure 4.2. The Gausssian Curve obtained during the particle size analysis.

4.2.1.1. Optimization of microwave disintegration. In the analysis, sewage sludge was disintegrated using microwave pre-treatment under 30 bar pressures. Teflon tubes with a maximum volume of 60 mL were used, with 40 mL of sludge placed in each tube. Different temperatures (100, 150, 175, and 190°C) and durations (10 and 20 minutes) were tested.

After conducting the experiments, it was determined that the optimum conditions for microwave disintegration were a temperature of 175°C and a duration of 10 minutes. (Table 4.6). Table 4.7 and Table 4.8 shows the results of the disintegration with microwave.

Table 4.6. Different temperature values and durations applied to sewage sludge during MW disintegration.

Temperature (ºC)	**Time (min)**
100	10
	20
150	10
	20
175	10
	20
190	10
	20

Table 4.7. sCOD values obtained with different conditions applied during MW disintegration.

Conditions		**sCOD of the sewage sludge (mg/L)**
No disintegration		122,0
100 ºC	10 min	1,125
100 ºC	20 min	2,219
150 ºC	10 min	3,248
150 ºC	20 min	3,498
175 ºC	10 min	4,100
175 ºC	20 min	3,810
190 ºC	10 min	3,415
190 ºC	20 min	2,562

Table 4.8. Disintegration degrees obtained with different conditions applied during MW disintegration.

Conditions		**DD of the sewage sludge (%)**
100 ºC	10 min	10.40
100 ºC	20 min	21.75
150 ºC	10 min	32.42
150 ºC	20 min	35.01
175 ºC	10 min	41.25
175 ºC	20 min	38.25
190 ºC	10 min	34.15
190 ºC	20 min	25.30

In addition to the disintegration experiments, viscosity, CST, and particle size analyses were conducted on the disintegrated sewage sludge samples. In Table 4.9, the results of CST and viscosity analysis of the disintegrated sewage sludge are presented. Table 4.10 displays the results of particle size analysis of the disintegrated sewage sludge.

CST measures the time it takes for a given volume of liquid to be drawn into a capillary tube under a standardized pressure and provided insights into the effectiveness of sludge treatment processes. Measuring viscosity helped to assess the flow behaviour of the disintegrated sludge and provide insights into its handling and processing characteristics.

Particle size helped to assess the effectiveness of the disintegration process in breaking down larger particles into smaller ones.

By conducting these additional analyses, a comprehensive understanding on the physical properties of WAS could achieved, which can inform decision-making regarding sludge treatment and disposal methods.

Table 4.9. CST and viscosity analysis for different conditions.

Analysis	**Specific Energy (kJ/kg TS)**	**Results**
CST (sec)	175 ºC, 10 min	436.6
	175 ºC, 20 min	479.1
Viscosity (Mpas)	175 ºC, 10 min	31.2
	175 ºC, 20 min	22.8

Table 4.10. The size distribution of particles under varying conditions.

Sample	**Surface Weighted Mean D[3,2]**	**Volume Weighted Mean D[4,3]**	**d (0.1) µm**	**d (0.5) µm**	**d (0.9) µm**
Raw Sludge	36,400	81,931	18,763	63,037	145,369
175 ºC (10 min)	86,094	173,504	50,102	147,851	331,816
175 ºC (20 min)	67,027	145,140	38,547	112,010	261,114

4.2.1.2. Optimization of alkali disintegration. The optimization studies for alkali treatment included altering the pH of the samples from 10 to 12 by introducing 2 N NaOH solution, and then maintaining the samples at these respective pH levels for durations of 2, 6, 12, and 24 hours. The analysis was performed using 400 mL volume of the samples.

The quantities of NaOH required to achieve the desired pH values are provided in Table 4.11, while Table 4.12 presents the sCOD values of the alkali-disintegrated sludges alongside their corresponding disintegration degrees.

Table 4.11. Alkali treatment conditions.

pH Values of the raw sludge	Alkali pH Values	Alkali Treatment Time (hours)	NaOH Added (mL)
6.62	10	2	2.5
6.74	11		3.4
6.62	12		5.9
6.62	10	6	2.6
6.61	11		3.9
6.65	12		6.3
6.62	10	12	2.4
6.63	11		3.3
6.64	12		6.2
6.63	10	24	2.5
6.6	11		3.5
6.59	12		6.3

Table 4.12. sCOD values and disintegration degrees after different alkali treatment conditions.

pH values	Alkali Treatment Time (hours)	sCOD (mg/L)	DD (%)
6.51	0	360	-
10	2	792	4.25
11		1618	12.97
12		3256	30.26
10	6	818	4.52
11		1852	15.44
12		3990	38.01
10	12	921	5.61
11		1905	15.99
12		4088	39.05
10	24	677	3.04
11		2192	19.03
12		4117	39.35

Additionally, CST (Consistency) and viscosity measurements were performed, as shown in Table 4.13. Additionally, the disintegration samples' particle sizes were analyzed, and the results are shown in Table 4.14.

Table 4.13. CST and viscosity analysis for different alkali conditions.

pH values	Alkali Treatment Time (hours)	CST (sec)	Viscosity (Mpas)	
			cP	%
6.51	0	17.6	8	0.4
10	2	162.4	32	1.6
11		461.5	30	1.5
12		992.5	36	1.8
10	6	181.4	40	2
11		661.1	32	1.6
12		1200.4	34	1.7
10	12	151.4	36	1.8
11		590.3	32	1.6
12		1402.2	30	1.5
10	24	142.6	38	1.9
11		617.1	34	1.7
12		1669.6	32	1.6

Table 4.14. Particle size distributions with the changed alkali conditions.

pH values	Alkali Treatment Time (hours)	Particle Size				
		d (0.1)	d (0.5)	d (0.9)	D [3.2]	D [4,3]
6.51	0	19.265	56.782	151.734	35.793	72.045
10	2	17.351	56.192	132.949	32.972	70.213
11		17.578	57.204	135.624	33.2	73.921
12		16.386	52.786	142.217	29.586	75.342
10	6	15.597	49.19	122.35	29.691	66.627
11		16.677	48.84	115.664	28.211	60.383
12		15.956	51.071	130.765	28.21	67.134
10	12	15.625	50.115	125.714	29.167	67.256
11		16.712	49.088	121.548	30.125	69.213
12		16.121	51.116	131.116	29.687	70.256
10	24	16.917	52.877	130.162	32.139	74.939
11		17.354	54.489	165.577	28.02	63.652
12		15.985	50.47	128.901	34.902	83.573

4.2.1.3. Optimization of pulsed electrical field disintegration. Pulsed electrical field (PEF) is another disintegration method used in the study. The effects of PEF disintegration are observed by applying electric fields to samples with varying intensities at different pH levels. Various pH values are achieved by adding 0.5 N NaOH. The efficiency of this method is demonstrated in Table 4.15, which presents the sCOD values and calculated disintegration degrees.

Table 4.15. sCOD values and disintegration degrees of PEF disintegration.

Intensity of the PEF (kV/cm)	Time (sec)	sCOD (mg/L)	Increase of sCOD (%)	DD (%)
0	0	471.6	0	0
20	3.5	540.8	14.7	1.8
20	4.5	580.4	23.1	2.9
20	6.0	596.9	26.6	3.3
20	7.5	633.2	34.3	4.3
20	9.0	652.9	38.4	4.8
25	3.0	577.1	22.4	2.8
25	4.5	633.2	34.3	4.3
25	6.0	686.0	45.4	5.7
25	7.5	715.6	51.7	6.5
25	9.0	771.0	63.6	7.9
30	3.0	620.7	31.5	4.0
30	4.5	685.9	45.4	5.7
30	6.0	784.8	66.4	8.3
30	7.5	827.7	75.5	9.5
30	9.0	880.5	86.7	10.9

4.3.The Effects of Pre-Treatment Methods on Biogas Production Enhancement

4.3.1. The Application of Ultrasonication and the PEF Pretreatment Techniques

The primary objective of using pre-treatment methods (sludge disintegration) on waste activated sludge was to facilitate the release of cell contents from microorganisms present in the sludge. This process disrupts the cell walls, enhancing the performance of anaerobic digestion (AD).

The liberated cell contents, known as cell lysate, can accelerate anaerobic methane fermentation by promoting the better degradation of organic substrates. Pre-treatment improves the bioavailability of sludge, thereby optimizing the digestion process to increase methane production.

In this study, ultrasonication and pulsed electric field pre-treatment methods were applied, resulting in the disintegration of wastewater sludge. As a consequence, there was a reduction in the levels of solid contents within the sludge, along with changes in particle size distribution, and an increase in soluble chemical oxygen demand observed in the sludge supernatant. (Table 4.16).

Table 4.16. Changes in the particle size of the wastewater sludge at different disintegration conditions.

	Surface weighted mean D[3.2]	**Volume weighted mean D[4.3]**	**d (0.1)**	**d (0.5)**	**d (0.9)**
Raw SS	41.262	96.354	22.642	66.908	160.610
SS with US disintegration (15 min)	7.776	51.235	3.701	24.415	138.392
SS with US disintegration (30 min)	5.117	43.515	1.935	19.320	92.458
SS with PEF disintegration	33.329	85.384	17.777	52.056	126.476

Before implementing disintegration methods, the particle size distribution of sludge samples ranged from 22.642 to 160.610 µm, with a median particle size of 66.908 µm. Upon applying PEF and ultrasound disintegration to the wastewater sludge, significant reductions in particle size were observed. The median particle size decreased to 52.056 µm with PEF, and to 24.415 µm and 19.320 µm with 15 and 30 minutes of ultrasonication, respectively.

The reactors TS, VS and sCOD levels at initial and final stages are shown in Figures 4.3, 4.4, and 4.5. The removal efficiencies of TS in the reactors ranged from 15% to 33%. The reactor including wastewater sludge disintegrated for 30 minutes with ultrasonic treatment displayed the highest total solids (TS) removal efficiency at 33%. Conversely, the control reactor, which contained untreated waste activated sludge (WAS) samples, demonstrated the lowest removal efficiency at 14%.

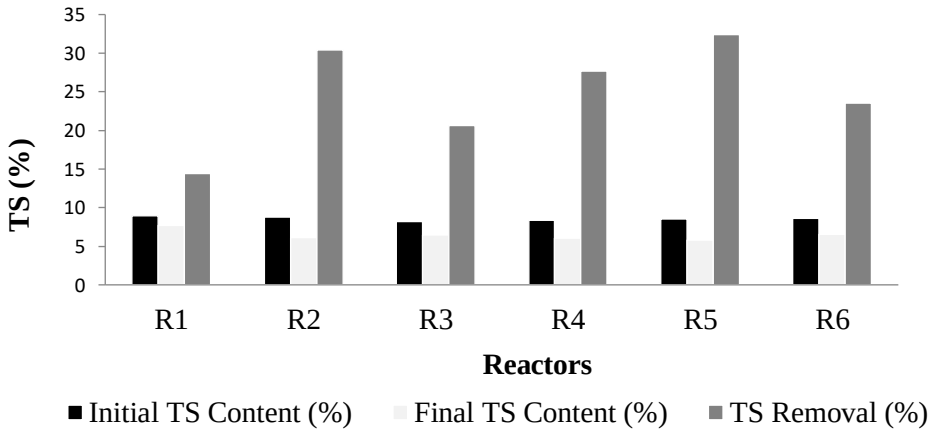

Figure 4.3. The concentrations of total solids and the corresponding removal rates in the reactors.

The VS removal efficiencies in the reactors ranged from 12% to 18%, as illustrated in Figure 4.4. The reactor with the maximum VS removal efficiency was the reactor with 30-minute ultrasonically disintegrated wastewater sludge, while the reactor with a combination of mechanically pretreated sludge and untreated waste activated sludge (WAS) had the lowest removal efficiency.

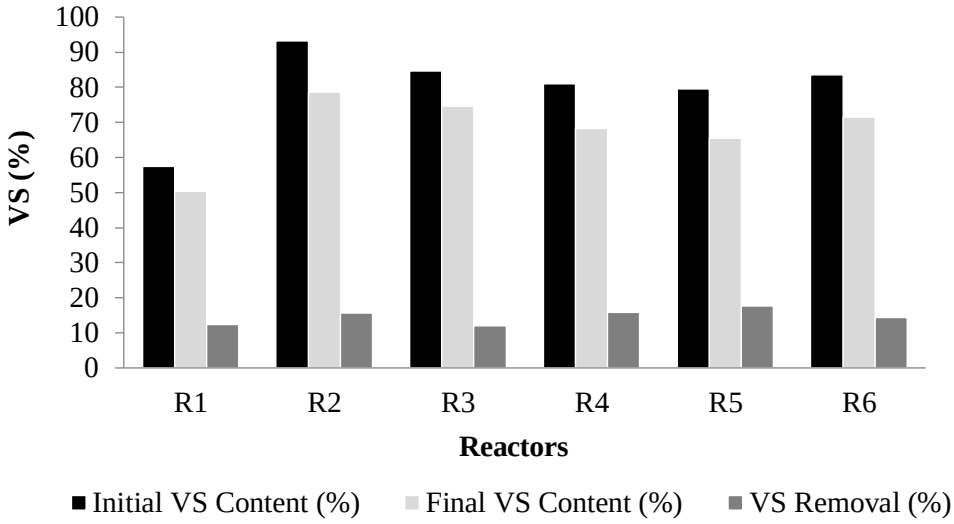

Figure 4.4. VS concentrations and the obtained removal rates in the reactors.

The elimination of soluble chemical oxygen demand (sCOD) within the reactors ranged from 76% to 85%, as illustrated in Figure 4.5. The obtained findings showed that the sCOD removal was significantly impacted by the disintegration techniques used. Compared to the 76% sCOD removal in the control reactor R1, which contained non-disintegrated wastewater sludge, the sCOD removal efficiencies increased to 85% in reactor R5 and 82% in reactor R6, both of which contained disintegrated wastewater sludge.

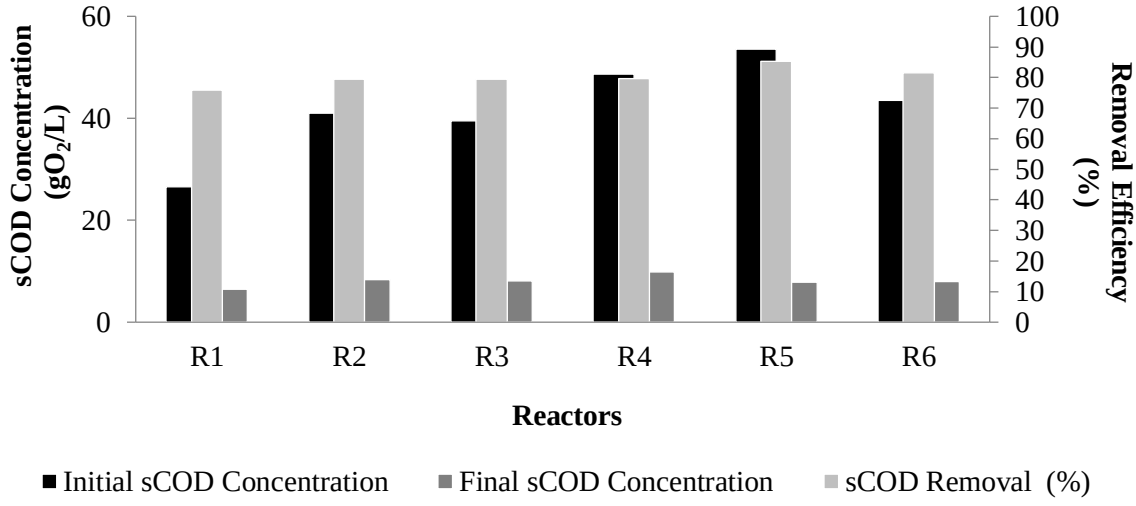

Figure 4.5. sCOD content and removal of the reactors.

In reactor R5, which contained 30-minute ultrasonicated wastewater sludge, 68.01% was the maximum methane content. The methane concentrations in the reactors ranged from 61% to 68%. Figure 4.6 presents the daily-recorded biogas productions.

Reactors R5 and R4 were found to have maximum cumulative biogas productions of 6030.08 mL and 5895.58 mL, respectively. On the other hand, after 20 days of digestion, reactor R1's biogas volume for the non-disintegrated wastewater sludge was only 1534.24 mL. The synergistic impact of co-digestion with maize silage and the 30-minute ultrasonication of the sludge can be credited for the greater biogas output in reactor R5.

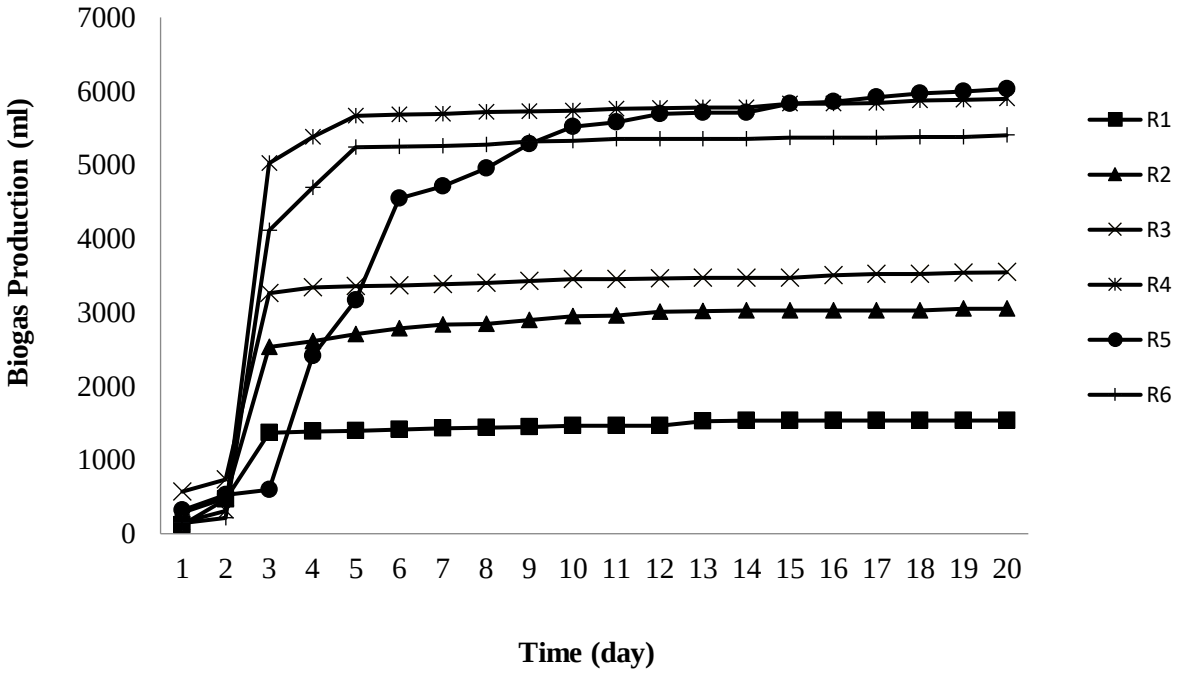

Figure 4.6. The total biogas production in the reactors.

4.3.2. The Study on the Ultrasonication and Microwave Pre-Treatment Techniques

This phase of the research focused on investigating the impact of microwave and ultrasonic pre-treatment on AD process. The inoculum sludge utilized in the study was obtained from the active anaerobic digesters at a yeast factory. Additionally, sludge samples were from the Paşaköy WWTP.

The anaerobic digesters comprised eight identical reactors, each possessing a working volume of 1600 mL. The contents of these reactors are specified in Table 4.17.

Table 4.17. The content of the reactors.

Reactors	Content
R1	I + WAS
R2	I + WAS + Ultrasonic 200W 70% Amplitude
R3	I + WAS + Ultrasonic 200W 70% Amplitude
R4	I + WAS + Microwave

The pH and ORP (oxidation-reduction potential) of the reactors are illustrated in Figure 4.7 and 4.8, respectively. The increase in the pH values at the end of the digestion, however, did not inhibit biological activity, indicating the resilience of the system to maintain favourable conditions for microbial activity throughout the digestion process.

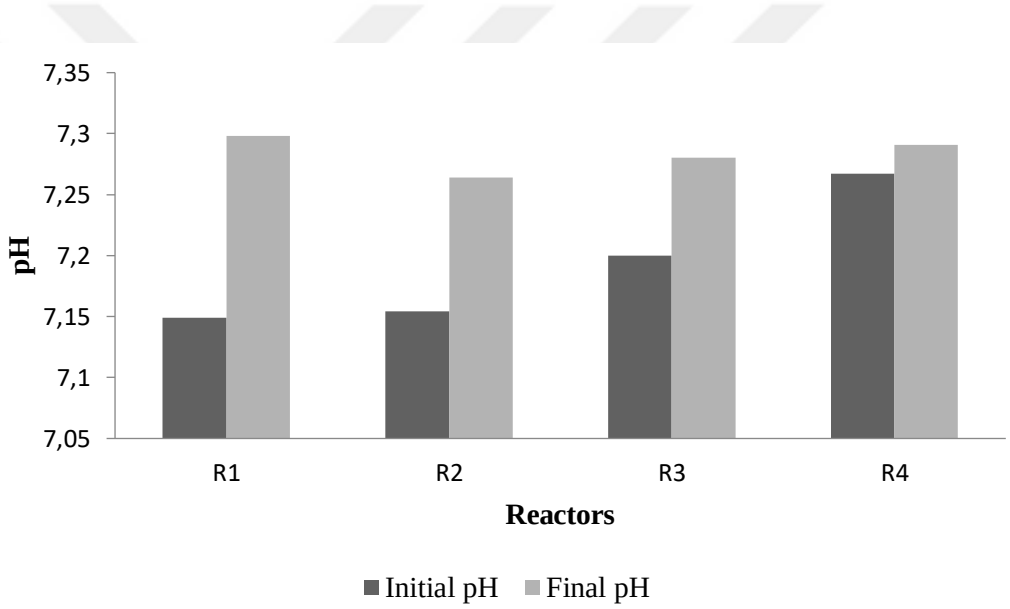

Figure 4.7. The initial and final pH values recorded for the reactors.

The ORP values of the reactors ranged between -291.6 and -361.6 during the start-up period. At the end of the AD period, all ORP values decreased, indicating the establishment of anaerobic conditions within the reactors. This decline in ORP values further confirms the progression towards favourable anaerobic conditions necessary for efficient digestion processes.

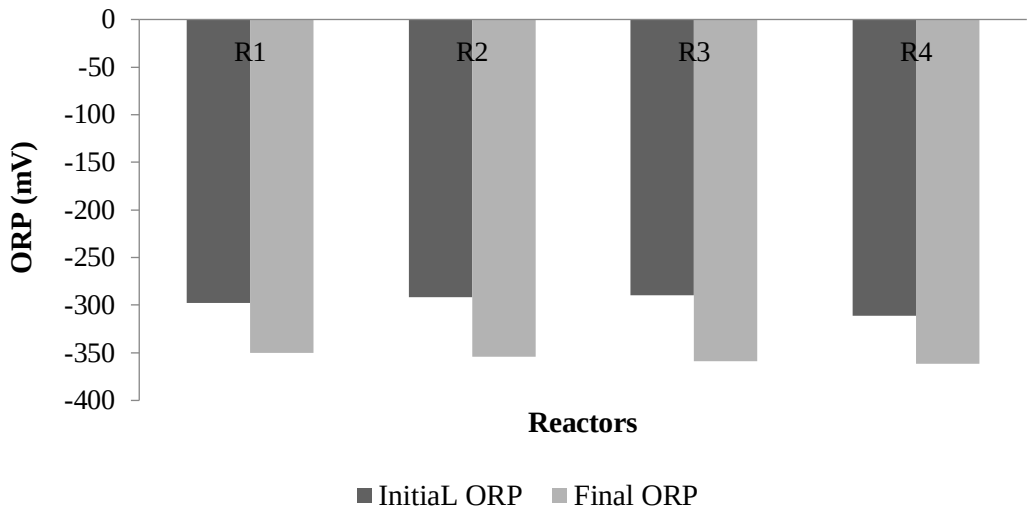

Figure 4.8. Initial and final ORP values recorded for the reactors.

The conductivity and salinity findings of the reactors are presented in Figure 4.9 and 4.10, respectively. Throughout the anaerobic digestion process, By the end of digestion, it was seen that the conductivity of samples had increased. The conductivity values of the reactors during the start-up period ranged between 7.84 and 8.03 mV, while they ranged between 14.64 and 15.21 mV at the end of the digestion phase. This increase in conductivity suggests changes in the composition of the solution as the digestion progresses, possibly due to the release of ions and organic compounds during the decomposition of organic matter.

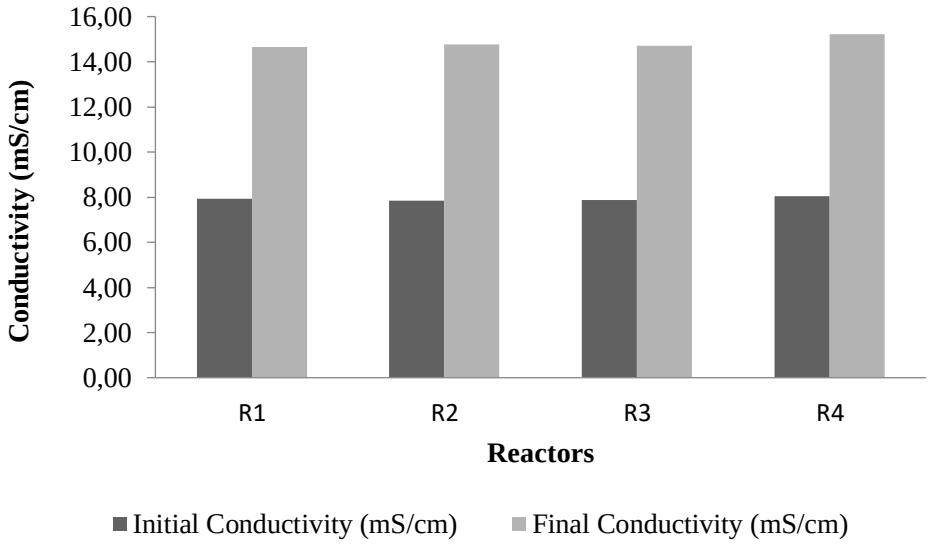

Figure 4.9. Initial and final conductivity values of the reactors.

The salinity of the reactors is reported as 5.1 and 5.2 parts per thousand (‰). Comparing these salinity measurements with the conductivity results, a similar pattern is observed between them.

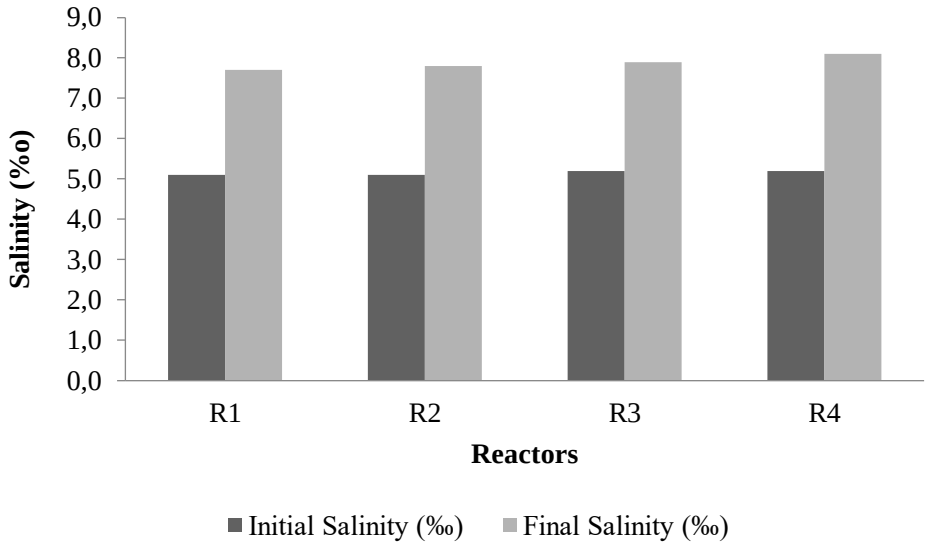

Figure 4.10. Initial and final salinity of the reactors.

TS and VS contents are shown in Figure 4.11. All reactors had TS removal efficiencies between 12% and 18%. Reactors R1, R2, R3, and R4 in particular showed TS removal efficiencies of 13%, 16%, 17%, and 18%, in that order. Remarkably, the reactor that received a microwave pre-treatment achieved the maximum TS removal efficiency of 18%.

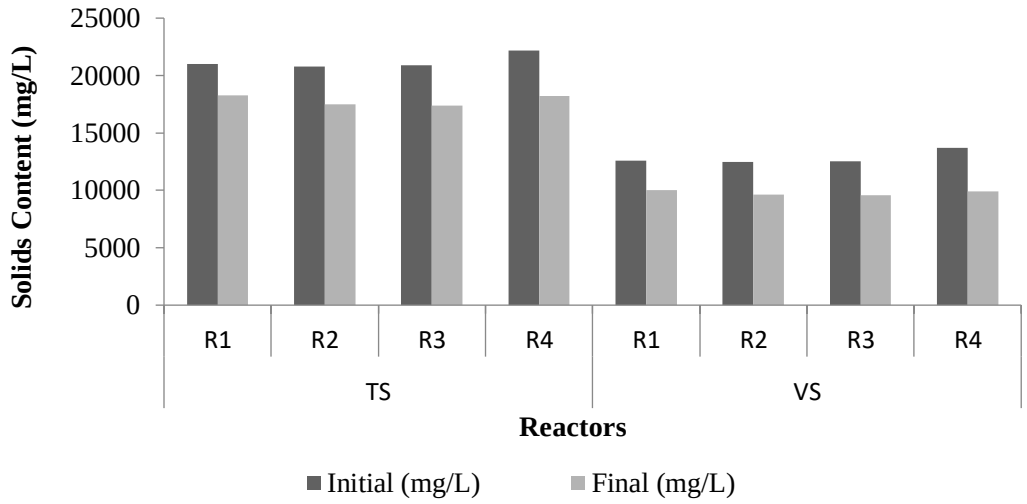

Figure 4.11. TS and VS contents and removal percentages of reactors.

The removal efficiencies of VS in the reactors varied between 20% and 27%. Reactors R1, R2, R3, and R4 exhibited VS removal efficiencies of 20%, 22%, 23%, and 27%, respectively. Reactor R4, subjected to microwave pre-treatment, attained the highest volatile solids (VS) removal efficiency of 27%.

Figure 4.12 represents the initial and final chemical oxygen demand (COD) levels of the reactors. The COD removal efficiencies across the reactors ranged from 8% to 25%. Reactor R4, which having microwave pre-treated WAS, exhibited the highest removal efficiency at 25%, whereas reactor R1, containing only the inoculum, demonstrated the lowest removal efficiency at 8%.

The sCOD removals in the reactors ranged from 3% to 34% (Figure 4.13). Reactor R4, which had microwave-pre-treated WAS, had the highest removal effectiveness (34%), while reactor R1, which had simply the inoculum, had the lowest removal efficiency (3%).

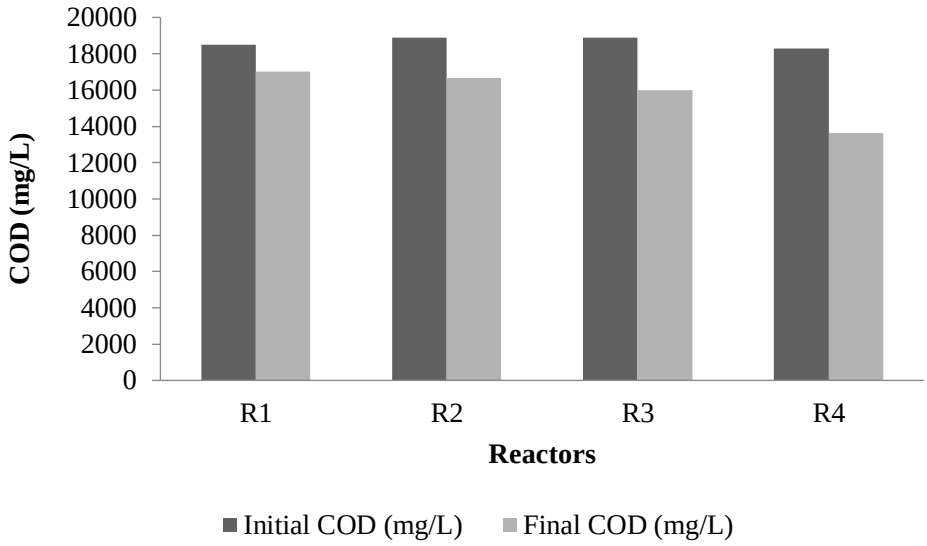

Figure 4.12. COD concentration and removal efficiencies of the reactors.

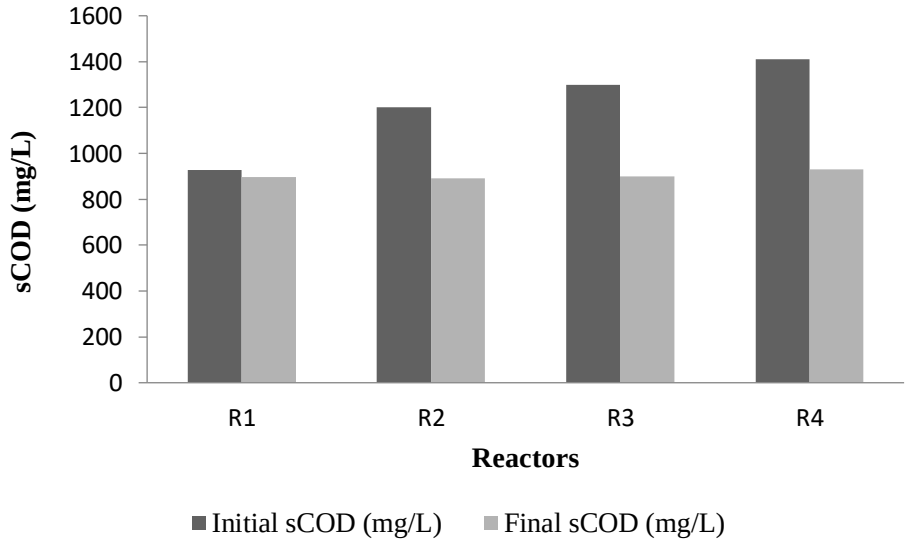

Figure 4.13. sCOD concentration and removal efficiencies of the reactors.

DOC concentrations of the reactors are given in Figure 4.14. It is evident that all DOC concentrations decreased during anaerobic digestion. Reactor R2, which including sludge having ultrasonic pre-treatment at 200W and 70% amplitude, exhibited the highest DOC removal efficiency.

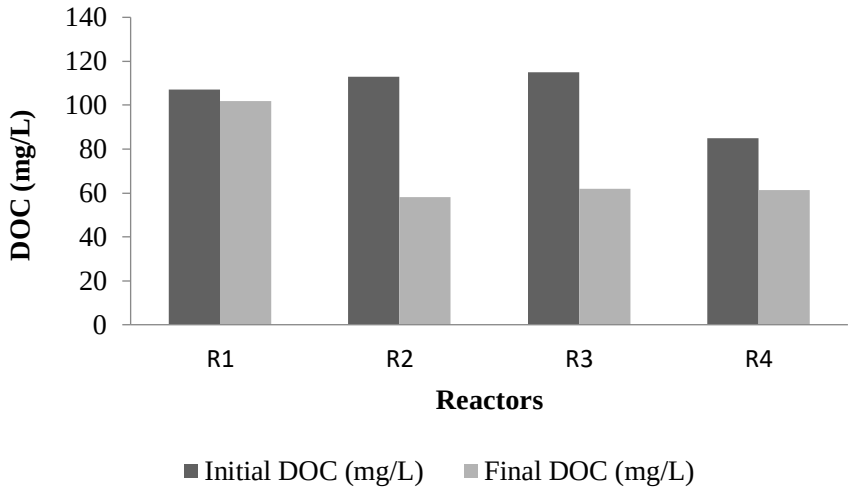

Figure 4.14. Initial and final DOC concentrations of the reactors.

The alkalinity concentrations ranged between 1940 and 2310 mg $CaCO_3$/L, while at the conclusion of digestion, the alkalinity concentrations increased and ranged between 3040 and 3150 mg $CaCO_3$/L (Figure 4.15). This increase in alkalinity indicates the production of alkaline compounds during the AD process.

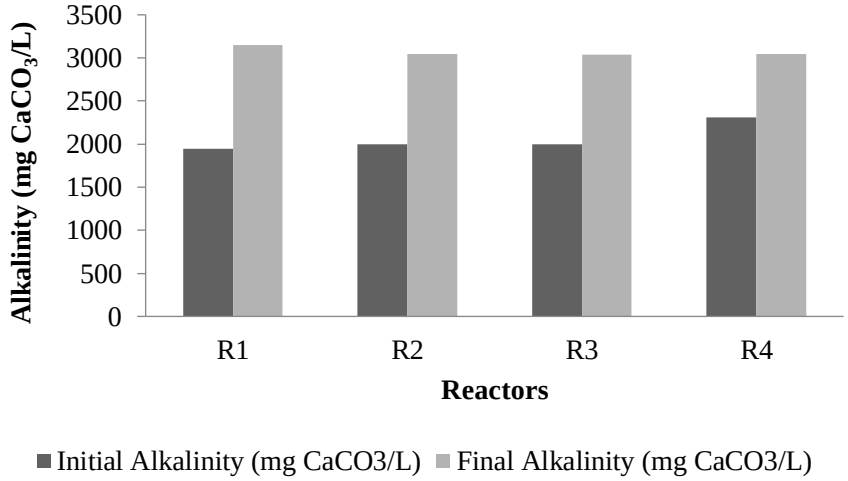

Figure 4.15. Initial and final alkalinity concentrations of the reactors.

The concentrations of ammonia, Total Kjeldahl Nitrogen (TKN), and Total Phosphorous (TP) in the reactors are provided in Table 4.18. It was observed that the level of ammonia in the reactors increased, while the TKN level decreased. All pre-treatment methods exhibited a similar effect in

increasing the concentration of ammonia. However, in terms of reducing TKN levels, ultrasonic pre-treatment was more effective compared to microwave pre-treatment.

Additionally, the TP content of the reactors increased during digestion. This increase indicates the liberation of phosphorous compounds during the breakdown of organic matter.

Table 4.18. Initial and final ammonia, TKN and TP concentrations of the reactors.

Reactors	Ammonia (NH_4^+)		Total Kjeldahl Nitrogen (TKN)		Total Phosphorous (TP)	
	NH_4^+ (mg/L) Initial	NH_4^+ (mg/L) Final	TKN (mg/L) Initial	TKN (mg/L) Final	TP (mg/L) Initial	TP (mg/L) Final
R1	256	472	1350	930	710	2300
R2	322	508	1170	840	500	2850
R3	330	520	1180	850	500	2750
R4	330	526	1140	1200	480	2500

As seen in Table 4.19; sulphate concentrations decreased over time in reactors R1 and R4, whereas sulphate concentrations remained unchanged in reactors R2 and R3, which underwent ultrasonication pre-treatment. Conversely, chloride concentrations increased over time in all reactors. Reactor R4 exhibited the most significant increase in chloride concentration.

Table 4.19. The concentrations of sulphate and chloride in the reactors.

Reactors	Sulphate (mg/L)		Chloride (mg/L)	
	Initial	Final	Initial	Final
R1	30	20	142	177
R2	15	15	283	290
R3	15	15	280	300
R4	20	10	177	319

The microbiology results of the study are given in Table 4.20.

In this study, reactor R4, with sludge pre-treated with microwave, exhibited the highest methane content at 72%, whereas the control reactor, containing solely inoculum and non-pretreated sludge, displayed the lowest methane content at 65%. Daily biogas production from the reactors was recorded over a 40-day period, and the cumulative gas production of the system is represented in Figure 4.16.

Table 4.20. Microbiology results of the reactors.

Reactors	Total Coliform Initial [kob/100mL]	Total Coliform Final [kob/100mL]	Fecal Coliform Initial [kob/100mL]	Fecal Coliform Final [kob/100mL]	Fecal Streptococ Initial [kob/100mL]	Fecal Streptococ Final [kob/100mL]
R1	$5.1x10^7$	$3.3x10^6$	$2.5x10^6$	$1x10^4$	$1x10^2$	≤10
R2	$6.7x10^7$	$3.9x10^6$	$1x10^6$	$5x10^4$	$3x10^2$	≤10
R3	$6.3x10^7$	$3.7x10^6$	$2.6x10^6$	$5x10^4$	$3x10^2$	≤10
R4	$5.3x10^7$	$3.8x10^6$	$7.3x10^6$	$4x10^4$	$2x10^2$	≤10

The biogas volume for reactor R1, containing only inoculum and non-pre-treated sewage sludge, was 480 mL after the digestion period when compared to R4 having 17,000 mL cumulative biogas productions.

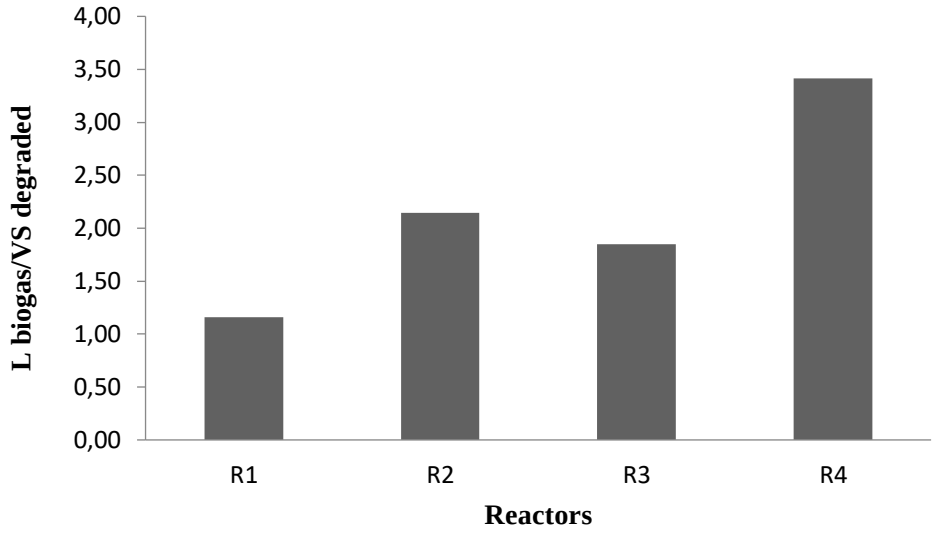

Figure 4.16. The total biogas production in the reactors.

4.4. Co-Digestion of Wastewater Sludges and Agricultural Wastes

4.4.1. 1st Set of Reactors for the Co-digestion Studies

The materials were subjected to anaerobic digestion for a period of 40 days at mesophilic temperatures (37 °C). Each reactor had a capacity of 2500 mL, with an effective working volume of 1600 mL.

A set of 16 equivalent co-digestion reactors were run simultaneously. The inoculum constituted 33% of the total mass of the reactor contents. In every reactor, the initial total solid (TS)

concentrations were standardized to 6.5% by weight. Information regarding the contents of each reactor is provided in Table 4.21.

4.4.1.1. Applied disintegration techniques. In this research, disintegration methods as ultrasonication and PEF were employed on samples of WAS. The objective was to speed up the hydrolysis phase. The degree of disintegration resulting was evaluated using Müller and Pelletier's approach (1998).

Table 4.21. The reactor contents for the co-digestion studies.

Reactors	Content
R1	I
R2	I + WAS
R3	I + WAS + Cloverleaf
R4	I + WAS + Wheat
R5	I + WAS + Olive Bagasse
R6	I + WAS + Maize Silage
R7	I + WAS + Barley Silage
R8	I + WAS + Manure

Before implementing pulsed electric field (PEF) for sludge, its efficacy and performance were evaluated across a range of electric field intensities up to 30 kV/cm. Several hydraulic retention times and pH conditions spanning from 3.0 to 11 were also investigated. The results demonstrated that the highest enhancement in the soluble chemical oxygen demand (sCOD) content of the sludge sample, approximately 827.7%, occurred when utilizing a pulsed electric field intensity of 30 kV/cm, at a pH level of 7, with a HRT of 9.0 seconds.

Following this, PEF treatment was administered to the wastewater sludge sample at a pH of 7.0, utilizing an intensity of 30 kV/cm. To attain a pH of 7.0 in the samples, 0.5 N H_2SO_4 was introduced. The soluble chemical oxygen demand (sCOD) values and disintegration degrees resulting from PEF disintegration are outlined in Table 4.22.

To assess the impact of ultrasonication on sludge characteristics, a series of batch experiments were carried out utilizing a HD 3400 Bandelin-Sonopuls ultrasonicator. Initially, the ultrasonicator was calibrated to determine the optimal operating conditions, including power output, amplitude, etc., for various sample volumes (400 mL, 500 mL, 1000 mL, and 2000 mL). After conducting the ultrasonication optimization trials, it was determined that an amplitude of 65% and a supplied power of 200W were optimal parameters. The sonication was applied to the sludge samples at a consistent frequency of 20 kHz. The duration of sonication for the WAS samples was computed based on the

selected specific energy and the total solid content of the sludge. The findings of these experiments are detailed in Table 4.23.

Table 4.22. sCOD values and disintegration degrees of PEF disintegration.

Intensity of the PEF (kV/cm)	Time (sec)	sCOD (mg/L)	Increase of sCOD (%)	DD (%)
0	0	471.6	0	0
20	3.5	540.8	14.7	1.8
20	4.5	580.4	23.1	2.9
20	6.0	596.9	26.6	3.3
20	7.5	633.2	34.3	4.3
20	9.0	652.9	38.4	4.8
25	3.0	577.1	22.4	2.8
25	4.5	633.2	34.3	4.3
25	6.0	686.0	45.4	5.7
25	7.5	715.6	51.7	6.5
25	9.0	771.0	63.6	7.9
30	3.0	620.7	31.5	4.0
30	4.5	685.9	45.4	5.7
30	6.0	784.8	66.4	8.3
30	7.0	827.7	75.5	9.5

Table 4.23. Sonication times applied to WAS samples according to the determined specific energies.

TS (mg/L)	Specific Energies (kJ/kg TS)	Sonication Time (sec.)
20900	5000	200
	10000	375
	15000	600
	25000	900
	50000	1800

In each ultrasonication experiment, 400 cc of sludge was transferred into a glass beaker and subjected to ultrasonic disintegration within a temperature-controlled environment. The final temperatures of the reactors were maintained below 18°C. The sludge samples underwent sonication for two different durations: 15 and 30 minutes. Subsequently, Reactors 4 and 5 were filled with 200 mL of ultrasonically disintegrated wastewater sludge.

4.4.1.2. Results and discussions for the 1st set of reactors. The performance of the anaerobic digester is influenced by pH and alkalinity levels. Over the digestion period, findings suggest a potentially well-balanced anaerobic digestion process.

The influence of anaerobic co-digestion involving agricultural biomass products and WAS on degradability was evaluated by analysing total and volatile solid removal efficiencies, as well as biogas and methane production. Figures 4.17 and 4.18 present the initial and final total solid (TS) and volatile solid (VS) contents of the reactors, respectively.

The TS removal efficiencies of the reactors varied from 51% to 74%. The co-digestion of cloverleaf and WAS produced the maximum TS removal effectiveness of 74%, whereas the second reactor, which included solely wastewater sludges, produced the lowest removal efficiency of 51%. It is revealed that incorporating agricultural biomass as a co-substrate resulted in an increase in the total solid (TS) removal efficiencies of the reactors by 22% to 55%, in contrast to single sludge digestion.

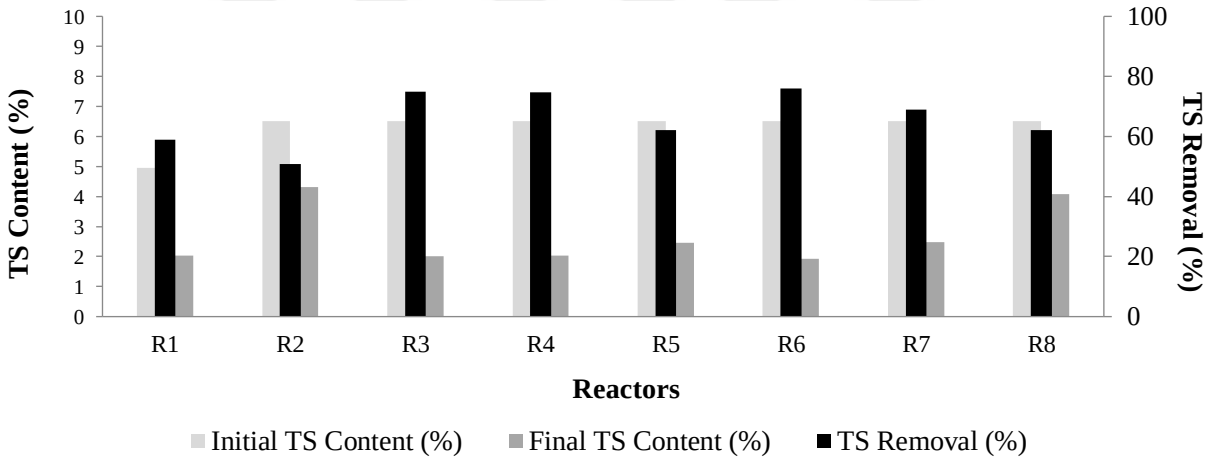

Figure 4.17. TS concentrations and the obtained removal rates in the reactors.

In Figure 4.18, it can be observed that incorporating agricultural biomass into WAS as co-substrates resulted in an increase in VS removal efficiencies across all reactors, ranging from 25% to 60%. This consequently led to higher levels of biogas production. The VS removal efficiencies varied from 54% to 87%, with reactor R3 displaying the highest efficiency at 87%, while reactor R2, containing only wastewater sludge, exhibited the lowest efficiency at 54%. The VS reductions noted in this investigation align with findings from other studies. For example, Parkin and Owen reported VS reductions of 50% in WAS digestion, which closely resembles the outcomes of this study.

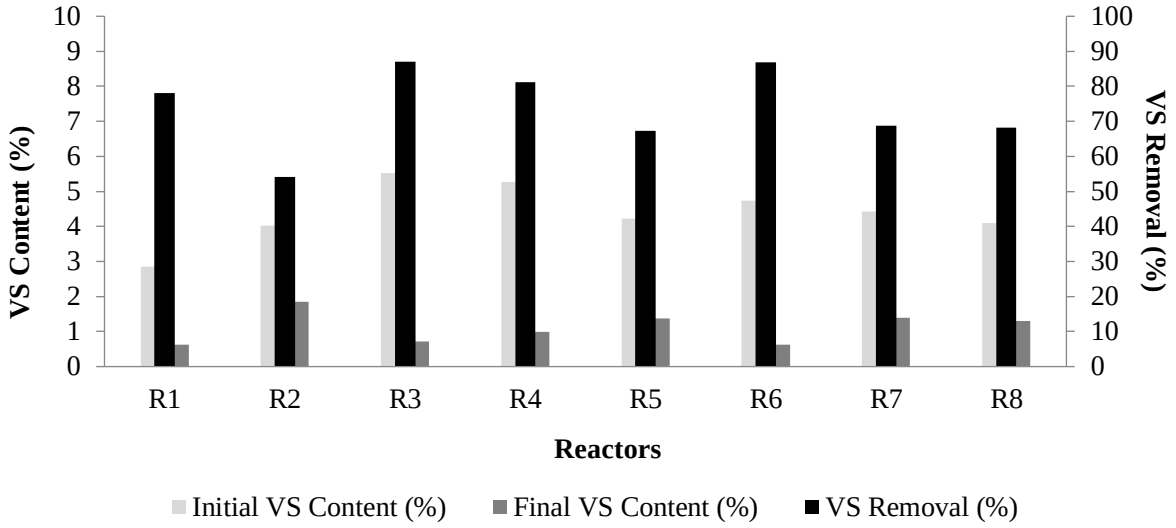

Figure 4.18. VS concentrations and the obtained removal rates in the reactors.

The reactors demonstrated sCOD removal percentages ranging from 54% to 91%, mirroring the trends observed in VS removal efficiencies. Again, the reactor containing cloverleaf and WAS attained the highest sCOD removal efficiency at 91.05%, while the lowest efficiency of 54% was observed in reactor R2, containing solely WAS samples.

Table 4.24 displays the outcomes of the microbiological analyses which indicate that the applied process effectively eliminated pathogens. Fecal coliform and fecal streptococci concentrations in the reactors were negligible in the final samples, while total coliform concentrations remained within acceptable limits.

Table 4.24. Microbiology results of the reactors.

Reactors	Total Coliform "Initial" [kob/100mL]	Total Coliform "Final" [kob/100mL]	Fecal Coliform "Initial" [kob/100mL]	Fecal Coliform "Final" [kob/100mL]	Fecal Streptococ "Initial" [kob/100mL]	Fecal Streptococ "Final" [kob/100mL]
R1	$2.1x10^6$	$1.1\ x10^2$	$2.2x10^5$	≤1	$3.2x10^4$	42
R2	$2.0x10^6$	$1.5\ x10^2$	$1.6x10^5$	≤1	$4.4x10^4$	14
R3	$2.5x10^6$	$4.1\ x10^2$	$3.8x10^5$	1.4	$1.4x10^4$	≤1
R4	$4.2x10^6$	$3.0\ x10^2$	$1.7x10^5$	2.5	$4.6x10^4$	12
R5	$2.0x10^6$	≤ 1	$1.5x10^5$	2.3	$5.4x10^4$	≤1
R6	$2.5x10^6$	$4.0\ x10^2$	$4.0x10^5$	78	$2.3x10^4$	12
R7	$1.7x10^6$	≤ 1	$1.1x10^5$	≤1	$1.7x10^4$	≤1
R8	$2.5x10^6$	$2.2\ x10^2$	$1.8x10^5$	≤1	$3.3x10^4$	≤1

In the chromatographic VFA analysis, acetic acid and propionic acid emerged as the primary components, alongside butyric, caproic, and valeric acids, within the reactors. Throughout the anaerobic digestion process, methanogens completely degraded VFAs to produce CH_4 and CO_2.

The NH_4^+-N concentrations in the reactors varied between 25 and 54 mg/L, falling within safe levels that mitigate potential ammonia inhibition, as documented in the literature. Moreover, the findings regarding VFA and methane production suggested no inhibition of methanogenic activity attributable to free ammonia nitrogen.

In this study, the highest methane contents were observed in reactors R3 and R4, containing mixtures of WAS with cloverleaves and barley (the energy crop substrate), at 59% and 58%, respectively. Cumulative biogas methane production in the reactors over a 40-day period is shown in Figure 4.19.

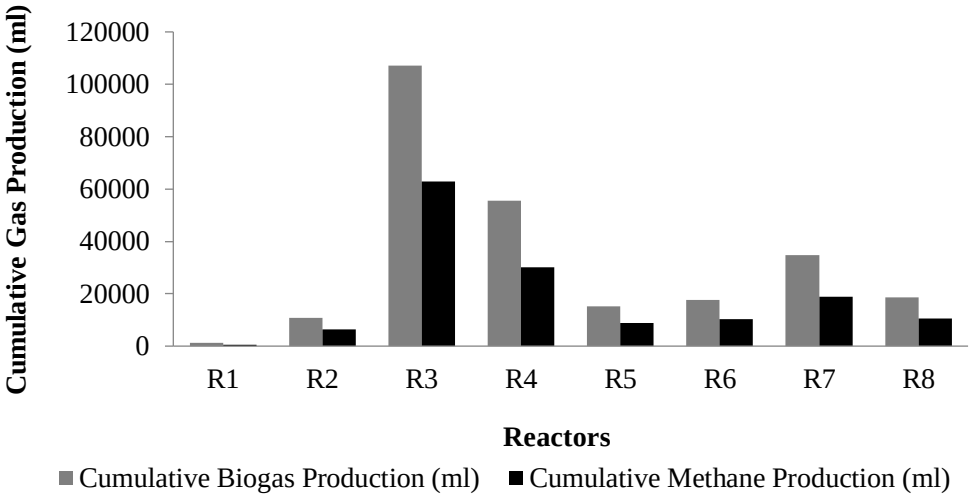

Figure 4.19. The cumulative productions of biogas and methane in the co-digestion reactors.

The overall biogas and methane yield of all the anaerobic digesters are represented in Figure 4.20. It is evident that compared to single sludge digestion, the addition of agricultural biomass to WAS as co-substrates, thereby mixing nutrient and carbon-rich wastes, led to increased biogas and methane yields. This enhancement can be attributed to achieving a more appropriate C/N ratio.

The highest cumulative biogas productions among the reactors were recorded at 107,058 mL for reactor R3, containing cloverleaf, and 55,500 mL for reactor R4, with wheat. In contrast, reactor R2, which contained only wastewater sludge, yielded around 11,000 mL of biogas at the end of the digestion. This demonstrates that in reactor R3, the cumulative biogas production was nearly 10 times

higher, with the methane yield being twice as high as that in reactor R2 containing only sludge samples.

The overall biogas and methane yield of all the anaerobic digesters are showed in Figure 4.20. It is clear that incorporating agricultural biomass into waste activated sludge (WAS) as co-substrates, thus blending nutrient and carbon-rich wastes, resulted in higher biogas and methane yields when compared to single sludge digestion. This improvement can be attributed to achieving a more appropriate C/N ratio.

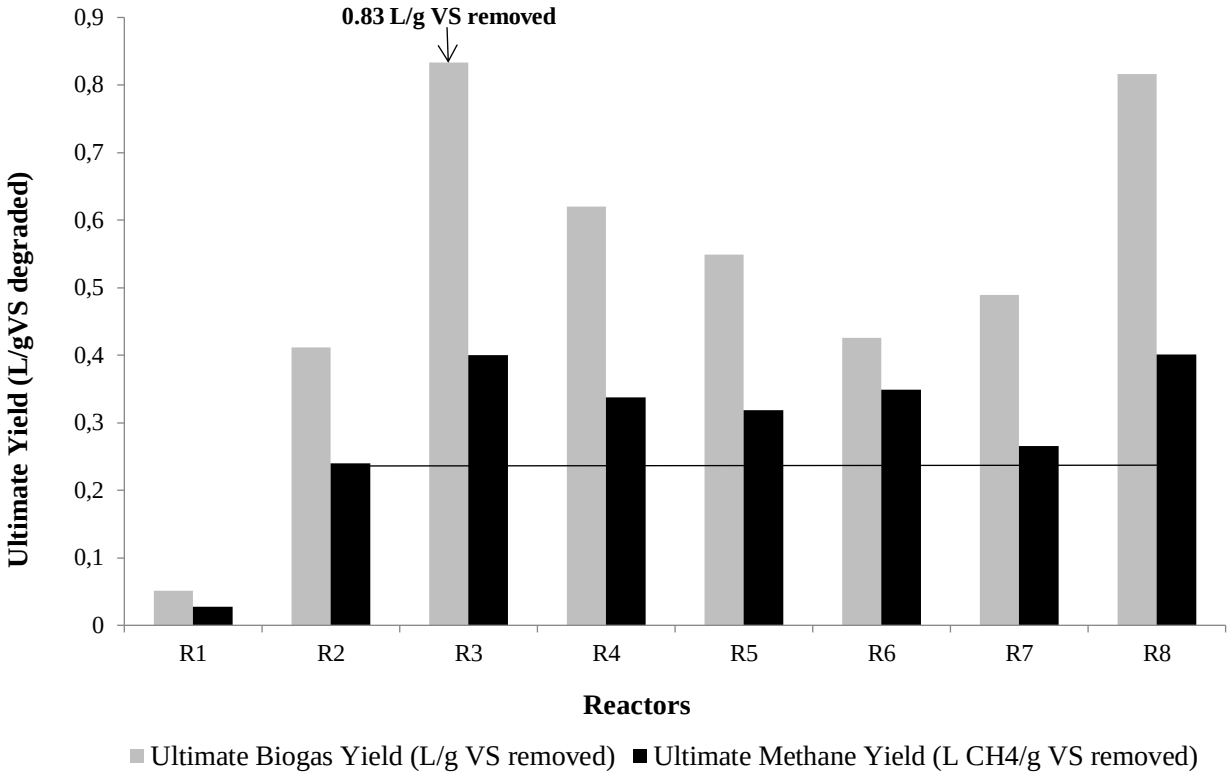

Figure 4.20. Ultimate biogas and methane yields at the reactors (L/g VS $_{removed}$).

In summary, the results given in Figure 4.20 underscore cloverleaf as the most effective substrate for co-digestion with waste activated sludge (WAS), as evidenced by cumulative gas production, biogas, and methane yields.

The highest biogas yield of 833 m^3/g VSr_{emoved} was attained during the co-digestion of sludge with cloverleaf. This indicates that, apart from cloverleaf, agricultural waste or residual materials can offer competitive alternatives to agricultural crops in terms of gas production.

Incorporating agricultural biomass, whether it's energy crops or waste, as a co-substrate was discovered to improve anaerobic digestion and enhance the efficiency of biogas/methane production. Among the chosen crop residuals, maize silage demonstrated particular effectiveness in terms of biogas production.

4.4.2. 2nd Reactor Set for the Co-digestion Studies

In this phase of the study, the objective is to identify the most effective energy crop in terms of solids reduction, organic matter removal, and biogas production. The inoculum sludge used in this study was obtained from the full-scale anaerobic digesters of a yeast factory. Wastewater treatment plant sludge samples were sourced from Paşaköy WWTP.

The chosen agricultural biomasses included both energy crops and crop residuals. Grass served as an energy crop, whereas potato peel, nutshell, and olive bagasse represented crop residuals.

Before conducting the anaerobic digestion experiments, the selected biomass samples were analysed for their total solid (TS %) and volatile solid contents (VS %). The characteristics of the selected agricultural biomass are outlined in Table 4.25.

Table 4.25. Characteristics of the selected biomass.

Biomass	**TS (%)**	**VS (%)**	**Nitrogen Weight (%)**	**Carbon Weight (%)**	**Hydrogen Weight (%)**
Nutshell	89.8	67.9	7.79	49.99	6.05
Potato Peel	23.1	21.4	6.03	44.82	6.54
Olive Bagasse	91.7	72.5	10.42	51.99	7.28
Grass	44.6	27.9	1.39	26.55	4.69

4.4.2.1. System configuration and reactor contents. The substrates were subjected to anaerobic digestion in 2500 mL reactors, each with an active working volume of 1600 mL, maintained at 37°C. The HRT was 40 days, and the temperature was held constant throughout the experiment.

Based on preliminary studies indicating that methane production occurs within the pH range of 7.0-8.5 (McKendry, 2002), the pH of the reactors was adjusted to approximately 7.5 to provide favorable conditions for bacterial growth. Subsequently, the bottles were sealed with stoppers, and a layer of silicon was applied to all connections and joints to ensure gas tightness. Nitrogen gas was purged into the reactors for 5 minutes to establish anaerobic conditions.

Twelve anaerobic reactors were run simultaneously in parallel. The initial total solid (TS) contents of the reactors were adjusted to 6.5%, and the ISR of the mixtures was set at 0.5. The blends of wastewater sludges and selected agricultural biomass were prepared based on weight (w/w) proportions. The details of the reactor contents are outlined in Table 4.26.

Table 4.26. The contents of the reactors.

Reactors	**Content**
R1	Inoculum (100%)
R2	Inoculum (33%) + WAS (67%)
R3	Inoculum (33%) + WAS (61%) + Nutshell (6%)
R4	Inoculum (33%) + WAS (41%) + Potato Peel (26%)
R5	Inoculum (33%) + WAS (61%) + Olive Bagasse (6%)
R6	Inoculum (33%) + WAS (54%) + Grass (13%)

4.4.2.2. Results and discussions for the 2nd set of reactors. The absence of VFAs, maintenance of neutral pH, and attainment of favorable alkalinity values by the end of the digestion process served as initial indicators of effective anaerobic degradation.

The pH and alkalinity values of the reactors at the start and end of the experiments are shown in Table 4.27. Acetic and propionic acids were identified as the predominant components in the chromatographic VFA analysis, with no VFAs detected at the conclusion of the experiments in any of the reactors.

Figures 4.21 and 4.22 illustrate the initial and final TS and VS contents of the reactors, respectively. The inclusion of agricultural biomass as a co-substrate had a beneficial impact on TS removal, enhancing efficiencies by 12% to 56% compared to single sludge digestion.

Figure 4.21 demonstrates that incorporating agricultural biomass into wastewater sludges as a co-substrate enhanced the removal efficiencies of volatile solids in all reactors by 0.31% to 52% compared to single sludge digestion. The VS removal efficiencies of the reactors ranged from 55% to 83% (as shown in Figure 4.22).

Table 4.27. Alkalinity and pH of the reactor contents at the first and the last day of the digestion.

Reactors	Initial pH	Final pH	Initial Alkalinity (mg $CaCO_3$/L)	Final Alkalinity (mg $CaCO_3$/L)
R1	7.59	7.69	6138.2	5626.5
R2	7.19	7.39	6138.6	2046.0
R3	7.24	7.44	3069.3	2301.7
R4	7.16	7.26	3400.5	2301.7
R5	6.89	7.02	3580.5	2557.5
R6	7.61	7.81	7161.2	3580.5

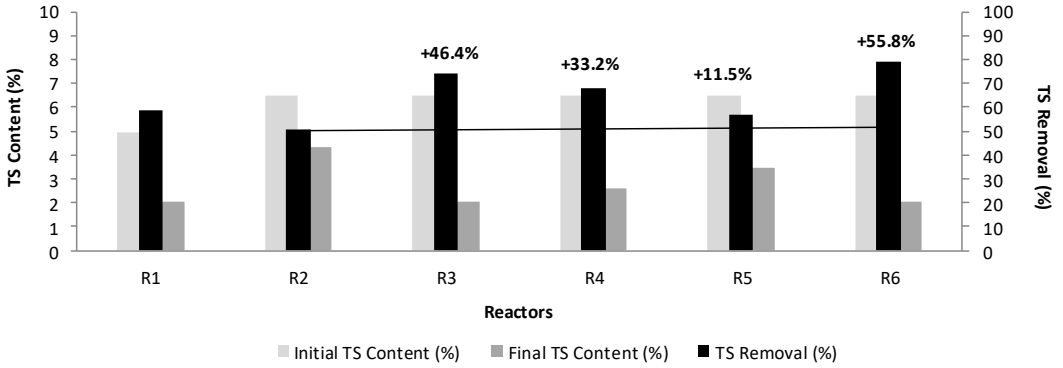

Figure 4.21. TS content and removal of the reactors.

Similar to the TS removal efficiencies, reactor R6, containing a mixture of wastewater sludge and grass, achieved the highest volatile solid removal efficiency of 83%, while reactor R2, with only wastewater sludge, exhibited the lowest removal efficiency of 55%. In the co-digested reactors, vs reductions were mostly above 66%, except for reactor R4 containing potato peels (54.37%). These findings align with values reported in the literature. Parkin and Owen observed a 50% VS reduction for wastewater sludge in their study, whereas this research recorded a removal efficiency of 54.22% (Parkin & Owen, 1986).

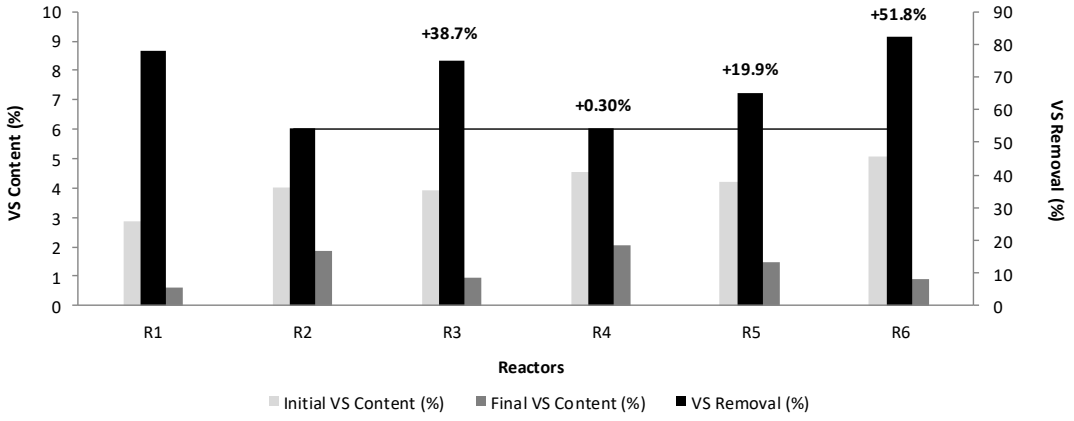

Figure 4.22. VS content and removal of the reactors.

sCOD removals in the reactors ranged from 55.40% to 89.6%. These results mirrored a very similar trend to the volatile solid (VS) removal efficiencies. Reactor R5, incorporating a blend of wastewater sludge co-digested with olive bagasse and grass, demonstrated the highest removal efficiencies of 90% for soluble chemical oxygen demand (sCOD). Following closely, reactor R6, with a similar composition, exhibited an sCOD removal efficiency of 87.17%. Conversely, reactor R2, comprising solely wastewater sludge samples, recorded the lowest sCOD removal efficiency at around 55%.

Microbiological study results, presented in Table 4.28, indicated that the mesophilic anaerobic co-digestion process in all reactors effectively stabilized the microbial populations through a reduction in their numbers. Additionally, negligible concentrations of fecal coliform and fecal Streptococci were observed at the conclusion of the anaerobic digestion process.

Table 4.28. The results of the microbiology study.

Reactors	**Total Coliform Initial [kob/100mL]**	**Total Coliform Final [kob/100mL]**	**Fecal Coliform Initial [kob/100mL]**	**Fecal Coliform Final [kob/100mL]**	**Fecal Streptococ Initial [kob/100mL]**	**Fecal Streptococ Final [kob/100mL]**
R1	$2.0x10^6$	$1.7x10^2$	$2.2x10^5$	1.8	$4.0x10^4$	68
R2	$2.0x10^6$	$1.3x10^2$	$2.0x10^5$	3.7	$3.6x10^4$	51
R3	$1.9x10^6$	$6.2\ x10^2$	$1.4x10^5$	6	$2.1x10^4$	65
R4	$2.8x10^6$	≤ 1	$1.6x10^5$	≤ 1	$2.6x10^4$	≤ 1
R5	$1.7x10^6$	$1.2x10^2$	$1.8x10^5$	1.8	$3{,}8x10^4$	24
R6	$3.9x10^6$	$1.1x10^2$	$1.6x10^5$	24	$4.7x10^4$	≤ 1

Due to the high quantities of soluble organic matter in the selected biomass, which were rapidly converted into acids, elevated levels of VFAs were observed at the beginning of the digestion process. However, by the end of digestion, VFA levels had decreased significantly and were found to be negligible. This indicates the efficient conversion of organic matter into methane, ensuring the stability and effectiveness of the anaerobic digestion process.

The methane content of the biogas produced in the reactors serves as an indicator of the performance of anaerobic digestion. The highest methane contents, reaching 58.24% and 58.10%, were observed in reactors R2, containing only wastewater sludge, and R5, containing a mixture of wastewater sludge and olive bagasse, respectively. However, reactor R3, containing a combination of wastewater sludge and nutshell, exhibited a notably lower methane content of 39.3%. This lower methane content can be attributed to the high cellulosic content of the nutshells, which limits their biodegradability.

The nutshells used in the study were unable to be ground into a powder form with the available grinder in the laboratory, leading to coarse grinding that restricted the solubility of organics in the nutshells, consequently resulting in the lower CH_4 content in the biogas obtained from reactor R3.

Additionally, methane contents of the reactors are indicative of the stability and performance of the anaerobic digesters. In this study, the highest methane content of 59% and 58% was achieved in reactors R3 and R8, respectively, which contained mixtures of wastewater sludge with cloverleaves and grass. Conversely, the lowest methane content of 39% was observed in reactor R5, which utilized nutshells as a substrate.

The cumulative biogas and methane productions in the reactors were recorded daily over a 40-day period, and the results are presented in Figure 4.23.

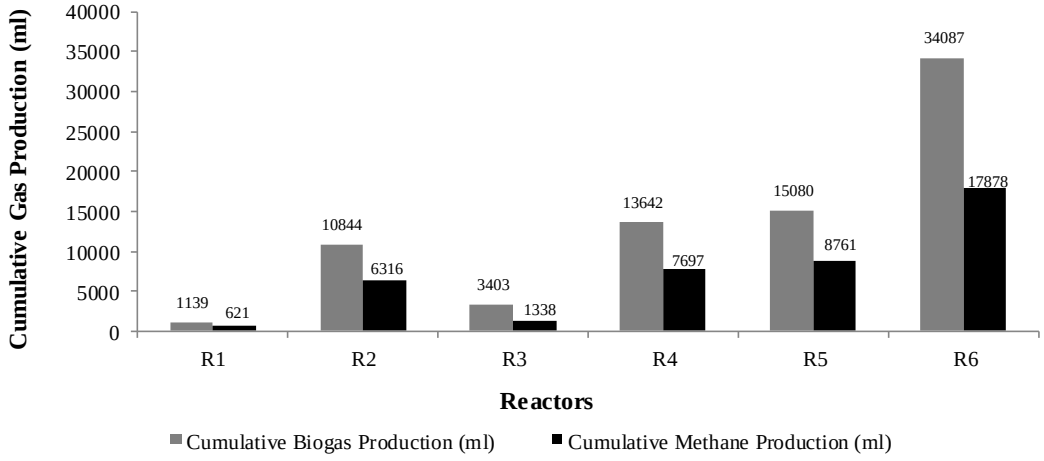

Figure 4.23. The cumulative biogas and methane productions in the reactors.

The maximum cumulative biogas productions were observed to be 34,086 mL in reactor R6, which contained grass, and 15,080 mL in reactor R5, containing olive bagasse. In contrast, reactor R2, containing only wastewater sludge, yielded a biogas volume of only 10,844 mL after 40 days of digestion. This indicates that reactor R6 achieved nearly three times higher cumulative biogas and methane productions compared to reactor R2.

The ultimate biogas and methane yields from the batch mesophilic anaerobic digesters are illustrated in Figure 4.24. It is evident from the figure that co-digestion facilitated more favorable carbon-to-nitrogen ratios (C/N) by blending nutrient and carbon-rich materials. Furthermore, the addition of grass as an energy crop and residuals to the wastewater sludges as co-substrates notably

enhanced the biogas productivity of the wastewater sludge by 26-33%, except for reactor R3, which included nutshell.

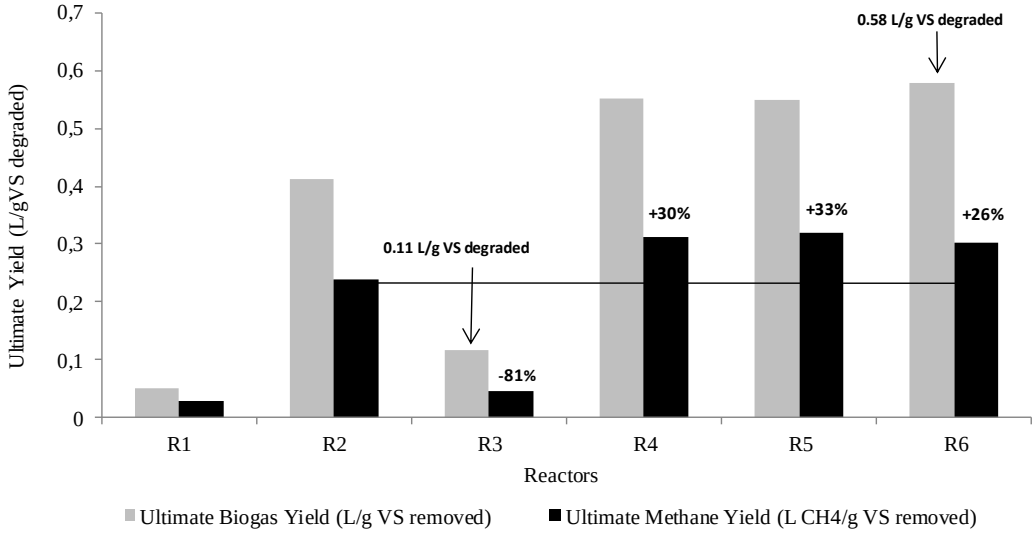

Figure 4.24. Ultimate biogas and methane yield in the reactors (L/ $gVS_{degraded}$).

Overall, grass emerges as the most successful co-substrate in terms of biogas and methane production. The highest biogas yield of 0.58 L/$gVS_{degraded}$ was achieved when sludge was co-digested with grass, compared to 0.24 L/$gVS_{degraded}$ for single sludge digestion.

Nevertheless, agricultural waste materials such as olive bagasse and potato peels showed comparable biogas and methane yields to grass. Biogas yields of 0.55 and 0.54 L/$gVS_{degraded}$ were obtained when sludge was co-digested with these waste residuals, respectively.

These results exceed some findings in similar studies. For instance, while Heiermann et al. (2002) reported a highest biogas yield of 0.987 L/$gVS_{degraded}$ using barley as an energy crop, while Amon et al. (2007) achieved biogas yields of 0.208–0.268 L/$gVS_{degraded}$ during anaerobic digestion of maize and dairy cattle manure.

Similarly, methane yields from potato wastes, which can vary depending on factors like volatile solid content and potato type, were comparable to recent studies. The methane yield of 0.31 L $CH_4/gVS_{degraded}$ from co-digestion of potato peels with sludge aligns closely with data reported by Parawira et al., who obtained a methane yield of 0.32 L $CH_4/gVS_{degraded}$ during batch digestion of potato waste with an ISR of 1.5, compared to 0.5 in this study.

4.5. Effect of Pre-treatment on the Co-digestion of WAS and AgroWaste (Maize Silage)

In this part of the study, an experimental setup aimed at studying the impact of co-digestion of sewage sludge with agro-wastes, along with pre-treatments using ultrasonic and microwave techniques. The study involves using inoculum sludge obtained from a large-scale anaerobic digester and wastewater treatment plant sludge from a specific facility.

Additionally, maize silage samples with specified total solids (TS) and volatile solids (VS) percentages were prepared and stored before use. These samples were then ground to a specific length before being introduced into the digestion process.

The substrates were digested in 2.5 L bottles with 1600 mL active volumes, with the reactors operated at a constant temperature of 37 °C and a hydraulic retention time (HRT) of 40 days. The experimental setup involves six identical reactors, with the content and loadings detailed in Tables 4.29 and 4.30.

The provided figures (Figure 4.25 and Figure 4.26) display the pH and ORP values of the reactors, respectively. The pH values of the reactors are observed to range between 7.15 and 7.3, indicating a slightly alkaline environment.

Table 4.29. The content of the reactors.

Reactors	**Content**
R1	Inoculum+ WAS
R2	Inoculum+ WAS (50%) + MS (50%)
R3	Inoculum+ WAS (40%) + MS (60%)
R4	Inoculum+ US disintegrated WAS for 15 min (50%) + MS (50%)
R5	Inoculum+ US disintegrated WAS for 30 min (50%) + MS (50%)
R6	Inoculum+ MW disintegrated WAS (50%) + MS (50%)

Table 4.30. Reactor loading rates.

	R1	**R2**	**R3**	**R4**	**R5**	**R6**
Inoculum (g)	800.20	800.30	800.20	800.30	800.30	800.20
Sewage Sludge (g)	800.20	400.00	320.00	400.00	400.00	400.00
Maize (g)	0	400.00	480.00	400.30	400.30	400.20
Applied Ultrasound Energy (KJ)	0	0	0	146.79	264.09	0
Applied MW Temperature (ºC)	-	-	-	-	-	175
Final Volume (g)	1600.40	1600.60	1600.20	1600.40	1600.30	1600.40

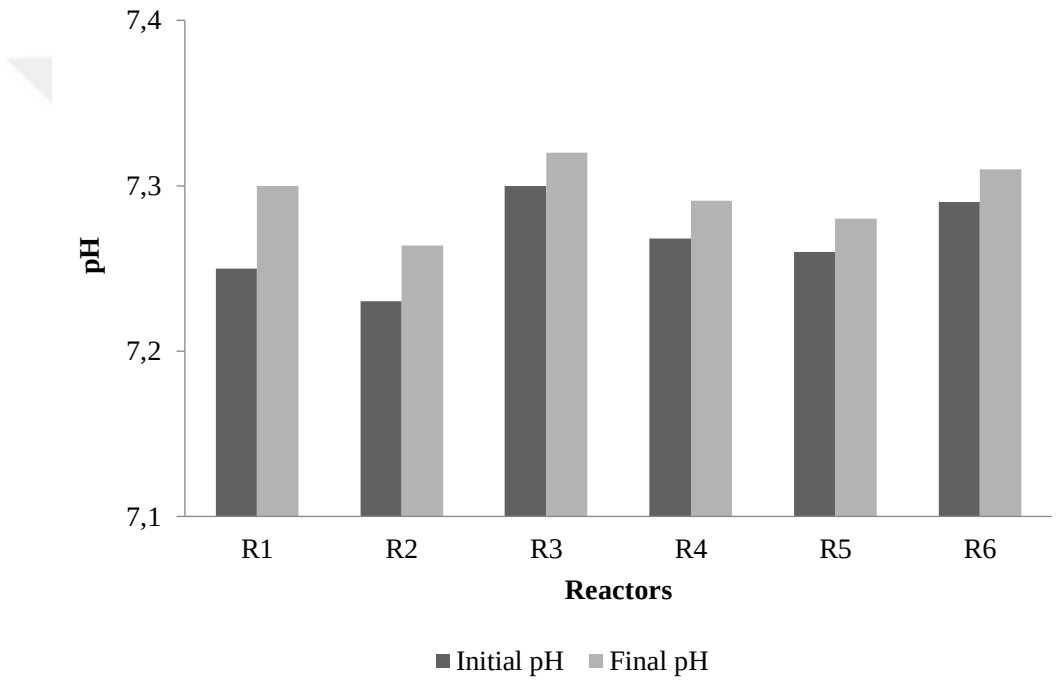

Figure 4.25. Initial and final pH of the reactors.

ORP values of the reactors range between -246.6 and -376.16 during the start-up period. Towards the end of the digestion period, a decrease in all ORP values is observed, indicating the establishment of anaerobic conditions within the reactors.

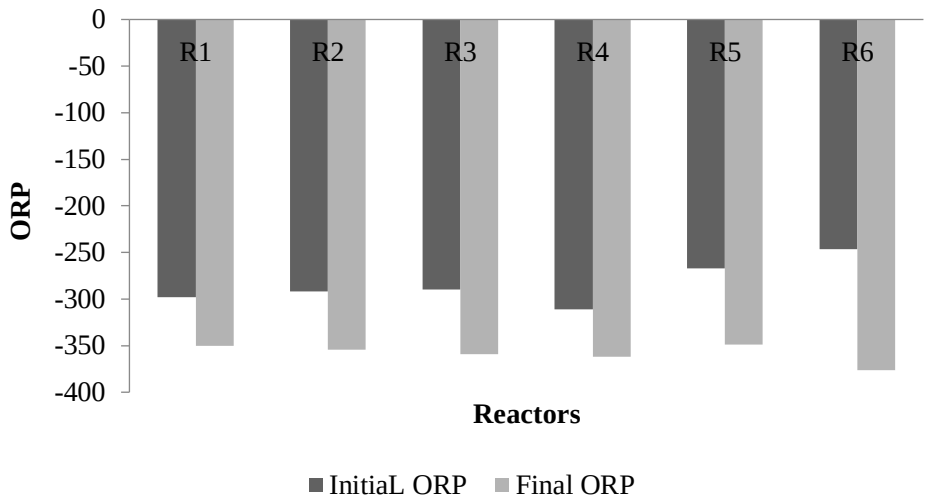

Figure 4.26. Initial and final ORP of the reactors.

Conductivity and salinity results of the reactors are given in Figure 4.27 and 4.28, respectively. The conductivity results of the reactors at the start-up period range between 7.64 and 8.06 mV, while at the end of the digestion, they range between 14.64 and 15.02 mV.

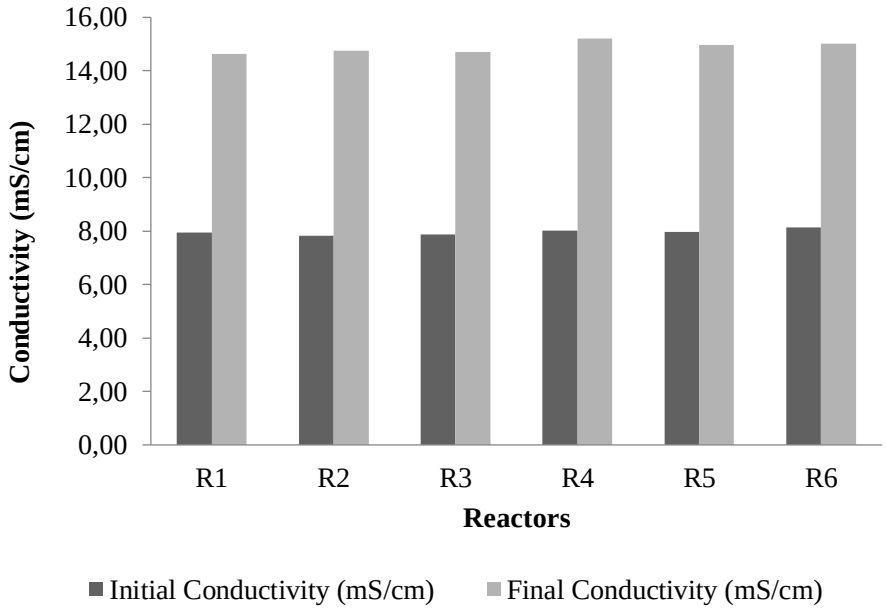

Figure 4.27. Initial and final conductivity of the reactors.

The salinity of the reactors is measured to be 5.1 and 5.2‰. When comparing the salinity with the conductivity results, a similar pattern is observed between them.

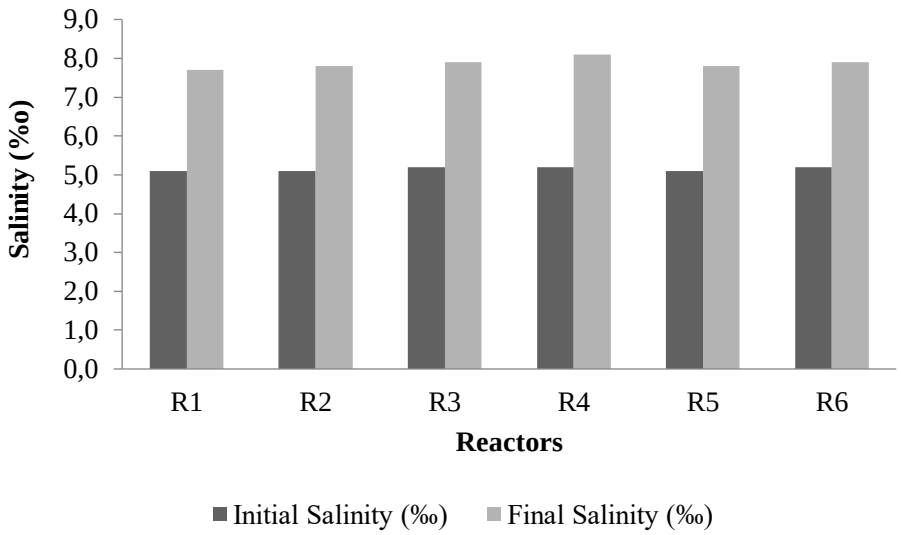

Figure 4.28. Initial and final salinity of the reactors.

The TS removal efficiencies of the reactors varied between 13% and 27%. The highest TS removal of 27% was achieved in reactor R6, which underwent a 5-minute microwave disintegration at 175°C. In contrast, the lowest removal efficiency of 13% was observed in the control reactor R1, which contained only sewage sludge. Overall, the application of microwave pre-treatment resulted in higher TS removal efficiencies compared to the untreated sewage sludge.

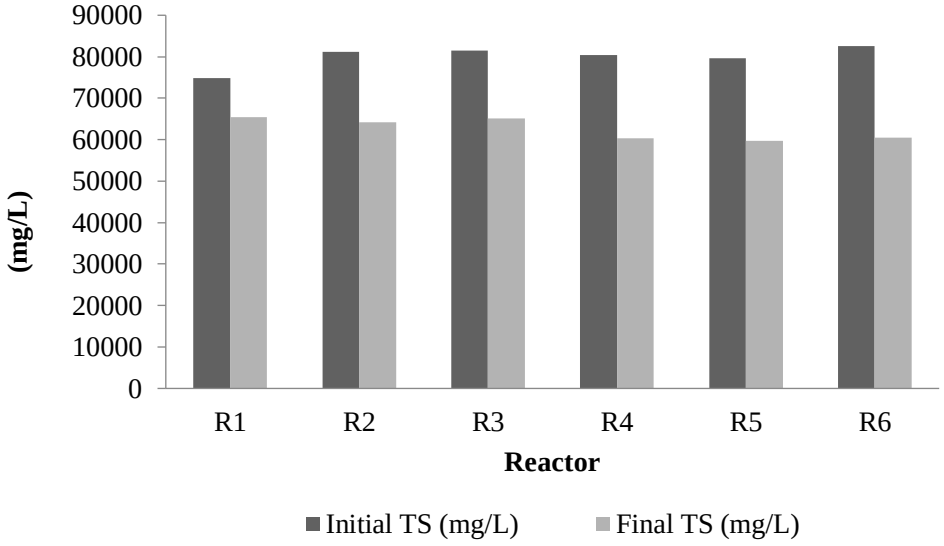

Figure 4.29. TS content of the reactors.

The VS removal efficiencies of the reactors ranged from 25% to 37%. Reactor R6, having microwave disintegration, exhibited the highest VS removal efficiency of 37%, while the lowest removal efficiency of 25% was observed in reactor R1 containing only sewage sludge. Overall, the

application of microwave pre-treatment contributed to higher VS removal efficiencies compared to the untreated sewage sludge.

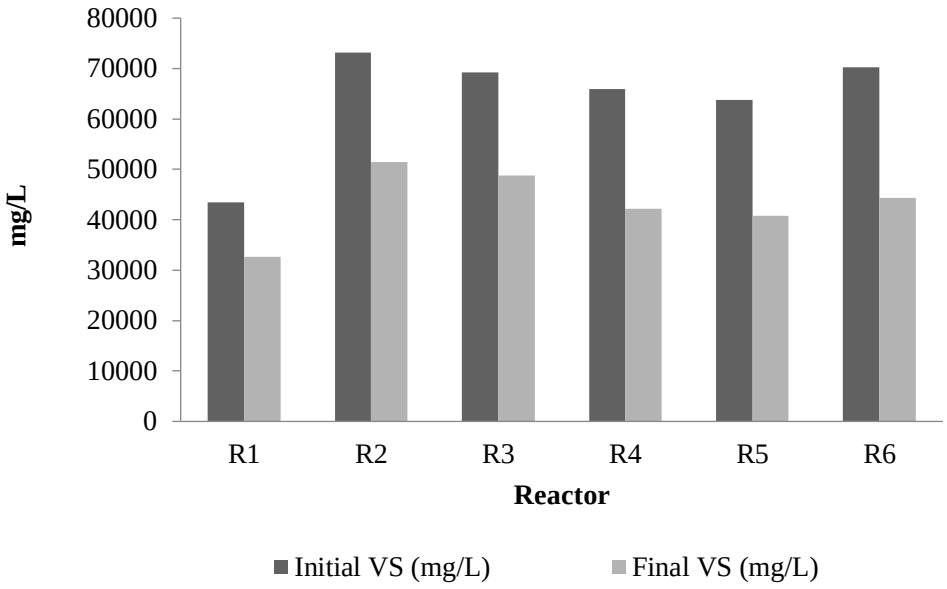

Figure 4.30. VS content of the reactors.

Figure 4.31 illustrates the initial and final COD content of the reactors. The COD removal efficiencies ranged from 24% to 44%. Reactor R6, with microwave pre-treatment, exhibited the highest removal efficiency of 44%, while the lowest removal efficiency of 24% was observed in reactor R1 containing only the inoculum. This indicates that microwave pre-treatment contributed to enhanced COD removal compared to the untreated inoculum.

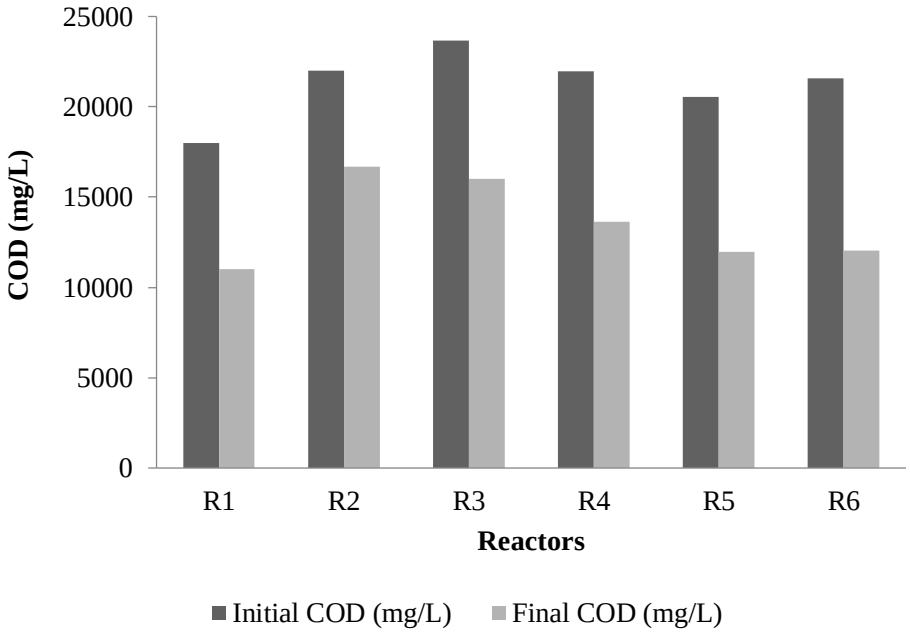

Figure 4.31. Initial and final COD contents of the reactors.

Figure 4.32 illustrates the range of sCOD removal in the reactors, which varied from 22% to 65%. The results indicate a significant improvement in sCOD removal due to the applied disintegration methods. For instance, while reactor R2, serving as the control with non-disintegrated sewage sludge and maize sludge, achieved a sCOD removal of 22%, reactors R5 and R6, subjected to ultrasonication and microwave disintegration, respectively, achieved notably higher removal efficiencies of 62% and 65%. This underscores the effectiveness of disintegration techniques in enhancing sCOD removal during anaerobic digestion.

In Figure 4.33, the decrease in DOC concentrations of the reactors with anaerobic digestion is shown. Reactor R5, subjected to 30 minutes of ultrasonic pre-treatment, exhibited the highest DOC removal, followed by reactor R6 with a 57% removal achieved through microwave disintegration. These findings demonstrate the positive impact of disintegration techniques on DOC removal during anaerobic digestion.

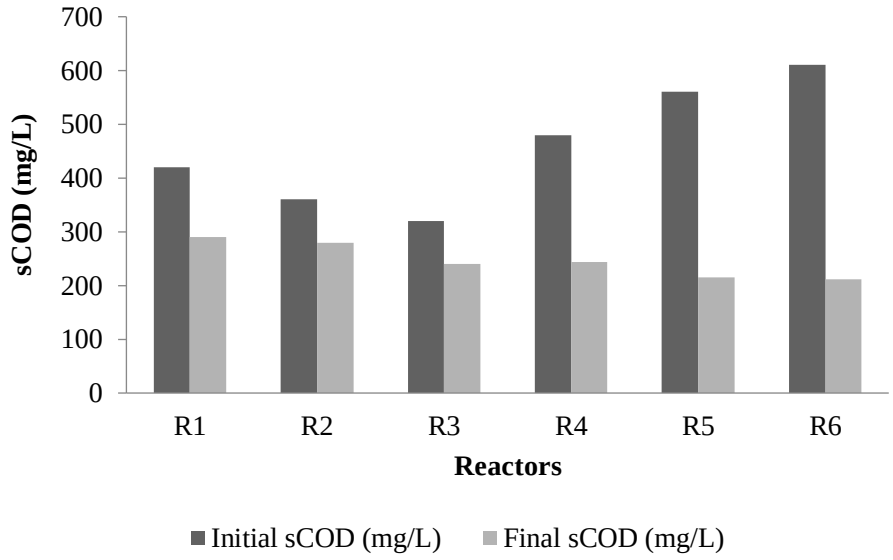

Figure 4.32. Initial and final sCOD contents of the reactors.

Figure 4.34 presents the alkalinity concentrations of the reactors, showcasing initial values ranging between 1920 and 2503 mg $CaCO_3$/L. At the conclusion of the digestion process, the alkalinity concentrations increased, with final values ranging between 3050 and 3120 mg $CaCO_3$/L.

This rise in alkalinity concentrations indicates the successful degradation of organic matter and the generation of alkaline byproducts, contributing to the buffering capacity of the system and ensuring the stability of the anaerobic digestion process.

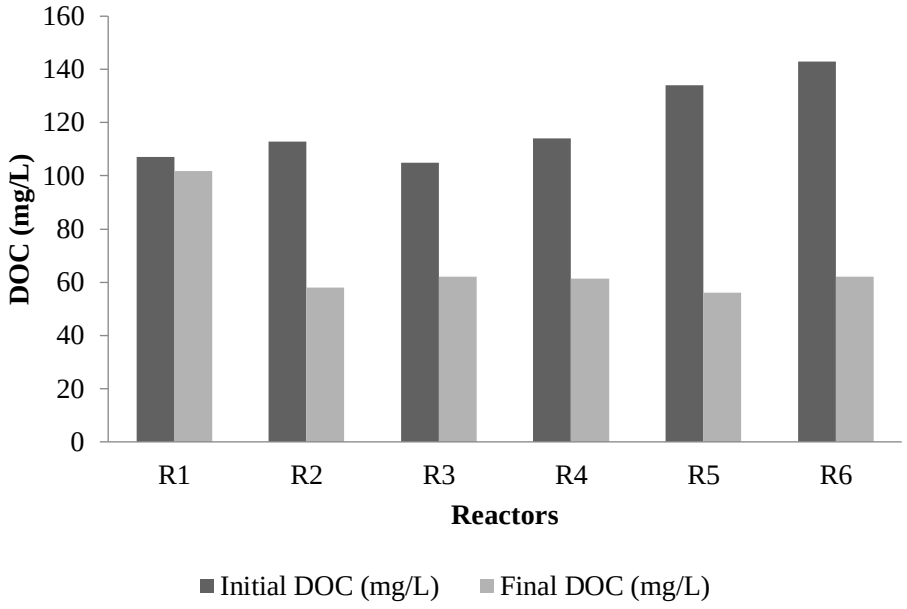

Figure 4.33. Initial and final DOC concentrations of the reactors.

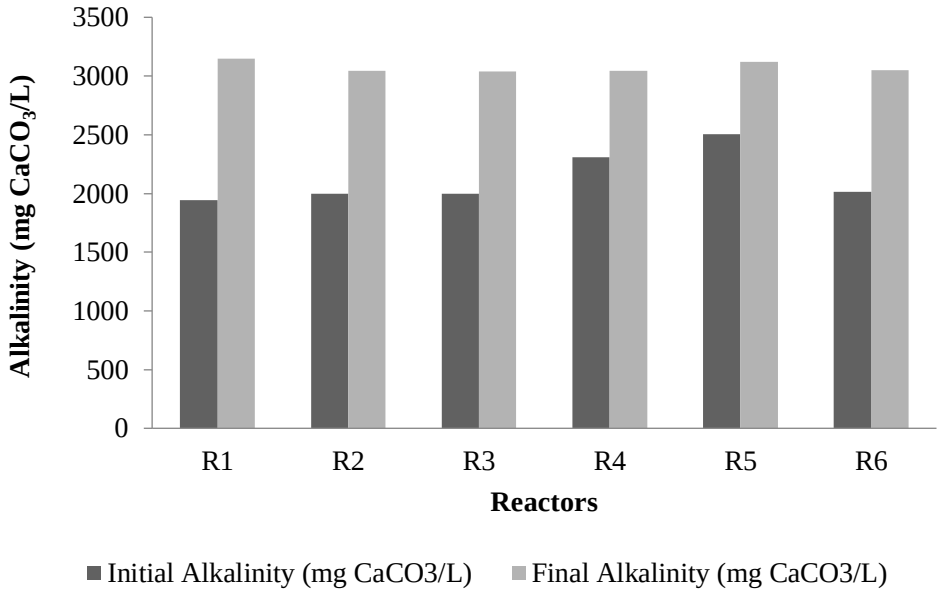

Figure 4.34. Initial and final alkalinity concentrations of the reactors.

In Table 4.31, the concentrations of ammonia, Total Kjeldahl Nitrogen (TKN), and total Phosphorous in the reactors are provided. Throughout the anaerobic digestion process, an increase in ammonia levels is observed across all reactors, with varying magnitudes. Conversely, the TKN levels decrease, indicating the breakdown and conversion of nitrogenous organic compounds into ammonia. Notably, ultrasonic pre-treatment appears to be more effective in reducing TKN levels compared to microwave pre-treatment. The total Phosphorous concentrations remain relatively stable across the reactors.

Table 4.31. Initial and final ammonia, TKN, and TP concentrations of the reactors.

Reactors	Ammonia (NH_4^+)		Total Kjeldahl Nitrogen (TKN)		Total Phosphorous (TP)	
	NH_4^+ (mg/L) Initial	NH_4^+ (mg/L) Final	TKN (mg/L) Initial	TKN (mg/L) Final	TP (mg/L) Initial	TP (mg/L) Final
R1	278	452	1250	730	810	2450
R2	305	509	1190	790	470	2860
R3	296	523	1210	810	490	2850
R4	293	516	1180	1106	490	2600
R5	306	465	1206	1009	500	2150
R6	301	510	1198	1170	560	2220

Table 4.32 provides the concentrations of sulphate and chloride in the reactors. It is observed that sulphate concentrations decrease over time in reactors R1, R4, R5, and R6, while they remain relatively constant in reactors R2 and R3. Conversely, chloride concentrations increase over time in all reactors, with the highest increase observed in reactor R5.

Table 4.32. Sulphate and chloride content of the reactors.

Reactors	Sulphate (mg/L)		Chloride (mg/L)	
	Initial	Final	Initial	Final
R1	30	20	152	179
R2	15	15	276	290
R3	15	15	274	310
R4	20	10	162	250
R5	25	10	210	302
R6	20	15	212	296

The methane contents in the reactors varied between 61% and 72%. This indicates that different treatment methods or conditions might have influenced methane production to varying degrees across the reactors. It appears that reactor R5, which contained 30-minute ultrasonicated sewage sludge, yielded the highest methane content of 72.01% among all the reactors studied. This suggests that the ultrasonication treatment had a positive impact on methane production during the anaerobic digestion process. Recording biogas production over a 40-day period allows for a comprehensive understanding of the anaerobic digestion process and the performance of the reactors over time. This longitudinal

data provides insights into the stability and efficiency of biogas production, helping to evaluate the effectiveness of the treatment methods employed in the study. In the reactors, it seems that the biogas production remained relatively stable after the 22nd day of digestion. This could indicate that the anaerobic digestion process reached a steady state or equilibrium where the biogas production rate remained fairly constant.

The significant increase in biogas production observed in reactors R5 and R6 compared to reactor R1 highlights the positive impact of co-digestion with maize silage and the application of sludge disintegration techniques such as ultrasonication and microwave treatment. This synergistic effect enhances the breakdown of organic matter and promotes methane production during anaerobic digestion. As a result, reactors R5 and R6 exhibit substantially higher cumulative biogas production, demonstrating the effectiveness of these combined treatment strategies in improving biogas yield from sewage sludge.

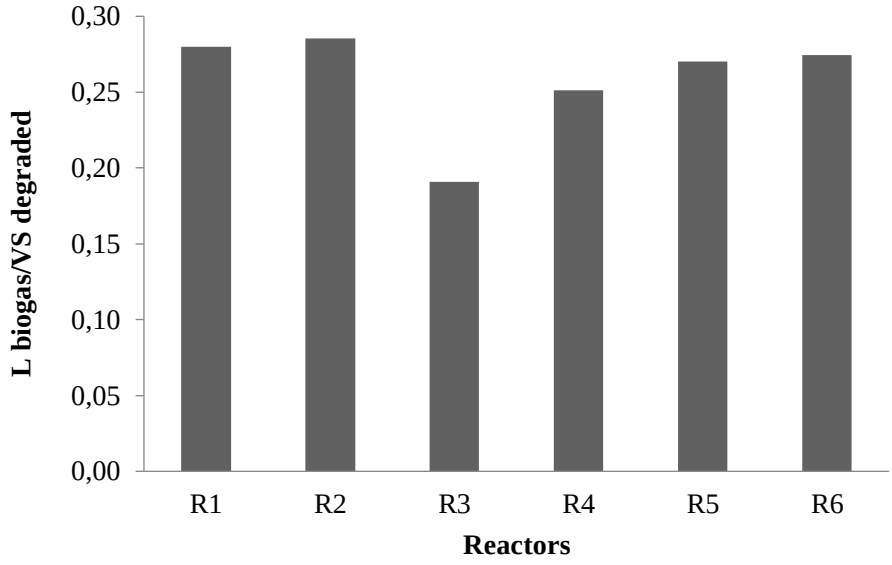

Figure 4.35. L biogas/ VS degraded of the reactors.

4.6. Ultrasound Assisted Biogas Production from Co-Digestion of Wastewater Sludges and Olive and Grape Pomaces: Comparison with Microwave Pre-Treatment

The research examines how ultrasonication and microwave pre-treatments affect biogas production when wastewater sludge is co-digested with agricultural residues, namely olive pomace (OP) and grape pomace (GP).

Table 4.33. Characteristics of sludge samples used in the study.

Parameter	**Unit**	**Inoculum**	**WAS**
TS	mg/L	45210	20992
VS	mg/L	35360	12385
MLSS	mg/L	37250	15480
MLVSS	mg/L	24000	9570
COD	mg/L	38470	16750
sCOD	mg/L	5154	927
TKN	mg/L	870	980
NH^{4+}	mg/L	294.00	51.75
Alkalinity (as $CaCO_3$)	mg/L	5302.5	1155
pH	-	7.3	6.72
Total Coliform	cfu/100mL	$2*10^6$	$1.4x10^7$
Fecal Coliform	cfu/100mL	$2.2*10^4$	$4.7x10^6$

The investigation involved co-digestion experiments for wastewater sludge (WAS) and olive pomace and grape pomace. Prior to anaerobic digestion (AD) trials, the pomaces analysed for their characterization to determine their total solids (TS) and volatile solids (VS) contents. Olive pomace has 38.20% TS, with 61.44% being volatile, while grape pomace had 40.76% TS, with 88.52% being volatile.

To improve organic solubility and accelerate the hydrolysis step, mechanical (ultrasonication) and thermal (microwave) disintegration methods were applied to the WAS samples. Microwave treatment was conducted at 190°C under 30 bar pressures for two different durations: 10 and 20 minutes.

The anaerobic co-digestion experiments were carried out in eight replicate batch reactors, each with a volume of 2.5 L, containing 1600 mL of active volume. The reactors were operated under mesophilic conditions (37°C) with a hydraulic retention time of 30 days. The inoculum to substrate ratios (ISR) in the reactors were adjusted to 1/1. The composition of the reactor contents is provided in Table 4.34.

In reactors R4 and R7, the sludge samples were subjected to ultrasonic pre-treatment utilizing the Bandelin-Sonopuls HD 3400, which operated at a frequency of 20 kHz and provided power of 200 W. The specific energy applied amounted to 10,000 kJ/kg TS.

Table 4.34. The content of the reactors.

Reactors	Content
R1	Inoculum
R2	Inoculum + WAS
R3	Inoculum + WAS + OP
R4	Inoculum + Ultrasonically Pre-treated WAS + OP
R5	Inoculum + Microwave Pre-treated WAS + OP
R6	Inoculum + WAS + GP
R7	Inoculum + Ultrasonically Pre-treated WAS + GP
R8	Inoculum + Microwave Pre-treated WAS + GP

For reactors R5 and R8, MW disintegration was employed. The sludges were subjected to microwave treatment for 10 minutes at 175°C and 30 bar pressures. This process employed a specific energy of 87,000 kJ/kgTS. These parameters were determined based on optimization studies detailed in earlier sections by the author.

After the microwave pre-treatment, the samples were immediately withdrawn from the heating source and cooled to room temperature within sealed vessels. This cooling step was to prevent the evaporation of organics before the subsequent anaerobic digestion process.

During the evaluation of results, standard statistical methods were applied, which included calculating the standard deviation for datasets with more than one duplicate data point. Furthermore, the absolute difference between primary and duplicate data points was taken into account.

4.6.1. Ultrasound optimization

Ultrasonic disintegration was applied to wastewater sludge (WAS) samples using various nominal power outputs of 60, 100, 200, and 300 W. These power outputs were determined by calorimetry to be equivalent to 42, 49.6, 101.7, and 113.3 W, respectively. The aim was to identify the most suitable conditions for disintegration.

Before ultrasonication, 400 mL of wastewater sludge samples were brought to ambient temperature (20 ± 2 °C). The heat capacity of the sludge was determined to be 4.15 J/g/°C through a calibration process of ultrasonication. This calibration process utilized the heat capacities of water (Cp_{water} = 4.186 J/g/°C) and solids in the sludge (Cp_{solids} = 1.95 J/g/°C), as described by Kim and Parker (2008), with the equation provided below:

$Cp = \text{Water content of sludge} \times Cp + \text{Water content of solids} \times Cp_{\text{sludge water solids}}$

$Cp = 0.998 \times 4.186 J/g/^{\circ} C + 0.012 \times 1.95 J/g/^{\circ} C = 4.15 J/g/^{\circ}C$

The determined heat capacity of 4.15 J/g/°C closely matched the literature value of 4.18 J/g/°C. Consequently, Cp_{sludge} was adopted as 4.18 J/g/°C. Table 4.35 presents the impact of ultrasonication at varying power levels on the breakdown and solubility of sludge solids (at 10,000 kJ/kg TS with 70% amplitude). From the experimental investigation, 200 W was identified as the optimal power level, demonstrating superior effectiveness in breaking down and dissolving organic solids within the sludge. Following this determination, sludge samples were subjected to different specific energies under the condition of 200 W.W and 70% amplitude. The resulting changes in sludge characteristics are detailed in Table 4.36.

Table 4.35. Effect of applied ultrasonication on disintegration and solubility of sludge solids

SE (kJ/kg TS)	Parameter	Ultrasonic Power	Wastewater Sludge of WWTP
10000	DD (%)	60	6.30
		100	15.60
		200	17.80
		300	26.40
	sCOD (mg/L)	60	702
		100	1506
		200	2850
		300	2790

Table 4.36. Effect of ultrasonication at different specific energies on sludge characteristics.

Specific Energy	CST (sec)	Viscosity (Mpas)	Particle Size				
			Surface Weighted Mean D[3,2]	Volume Weighted Mean D[4,3]	d (0.1) µm	d (0.5) µm	d (0.9) µm
Raw Sludge	52	34	36.400	81.931	18.763	63.037	145.369
175 ^{0}C- 10 min	437	31	86.094	173.504	50.102	147.851	331.816
175 ^{0}C- 20 min	479	23	67.027	145.140	38.547	112.010	261.114

An observation revealed that increasing the specific energy during ultrasonic pre-treatment resulted in a decrease in particle size and viscosity of sludge samples, along with an increase in the solubility of organics. Researchers such as Menardo et al. and Neves et al. have explored thermal and ultrasonic pre-treatments applied to agricultural wastes. Consistent with the findings of this study, they noted that ultrasonic pre-treatment could reduce the particle size of sludge samples to a range of 0.2 to 5 µm, leading to enhanced methane production.

It was found that increasing specific energies led to an increase in CST values, indicating a deterioration in the dewaterability of sludge samples. However, the achieved specific energy is directly proportional to the applied sonication time, and higher energies require longer sonication times. Following optimization studies, the most energy-efficient operational conditions for ultrasonication were identified as 200 W (101.7 W, actual power) and 70% amplitude, achieving a specific energy of 10,000 kJ/kg TS (5085 kJ/kg TS, calculated after calibration).

4.6.2. Optimization of microwave disintegration

In this study, microwave (MW) pre-treatment was conducted by exposing sludge samples to MW irradiation at temperatures of 100, 150, 175, and 190°C for durations of 10 and 20 minutes under 30 bar pressures. The results of these experiments are summarized in Table 4.37.

Table 4.37. Effect of MW pre-treatment on disintegration and solubility of sludge samples.

Conditions	sCOD of WAS (mg/L)	DD of WAS (%)
No disintegration	122	-
100 ^{0}C 10 min	1125	10.40
100 ^{0}C 20 min	2219	21.75
150 ^{0}C 10 min	3248	32.42
150 ^{0}C 20 min	3498	35.01
175 ^{0}C 10 min	4100	41.25
175 ^{0}C 20 min	3810	38.25
190 ^{0}C 10 min	3415	34.15
190 ^{0}C 20 min	2562	25.30

After conducting the experimental runs, it was established that the optimal operational conditions for MW disintegration, with regards to energy efficiency, were 175°C and 10 minutes (equivalent to 87,000 kJ/kgTS). The analyses of solubility, dewaterability, viscosity, and particle size of the sludge samples under this optimal condition are provided in detail in Table 4.38.

4.6.3. Anaerobic co-digestion

Initially, the study evaluated the influence of various TS contents on the cumulative biogas production from the anaerobic mono-digestion of olive pomace (OP) and grape pomace (GP). The pomace substrates and inoculum were subjected to digestion under mesophilic conditions (37°C) for a duration of 30 days in reactors with a working volume of 1600 mL within 2500 mL reactors. These reactors operated at four different TS concentrations: 6.5%, 7%, 8%, and 10%, with an initial

substrate-to-inoculum ratio (ISR) of 1:1. The results indicated that the highest biogas production occurred at a TS content of 6.5% (Figure 4.36).

Table 4.38. Effect of 10 min MW pre-treatment on sludge characteristics at 175 °C

Sample	CST (sec)	Viscosity (Mpas)	Particle Size				
			Surface Weighted Mean D[3,2]	Volume Weighted Mean D[4,3]	d (0.1) µm	d (0.5) µm	d (0.9) µm
Raw Sludge	52	34	36.400	81.931	18.763	63.037	145.369
SE=5000 kJ/kg TS	1296	28	22.100	42.821	18.360	39.676	73.045
SE=10000 kJ/kg TS	1345	24	14.240	36.648	10.478	30.820	60.472
SE=15000 kJ/kg TS	1449	23	6.133	32.407	2.657	19.303	59.722
SE=20000 kJ/kg TS	1528	20	4.620	35.480	2.074	16.264	65.453
SE=50000 kJ/kg TS	1652	18	3.419	38.744	1.176	11.677	109.639

Consequently, the TS contents of the reactors for the remainder of the study were adjusted to 6.5% in accordance with the outcomes of the olive pomace digestion study (Tekin & Dalgıç, 2000). Subsequently, the overall anaerobic co-digestion efficiency of the study was evaluated in terms of the efficiencies of organic removal and biogas production.

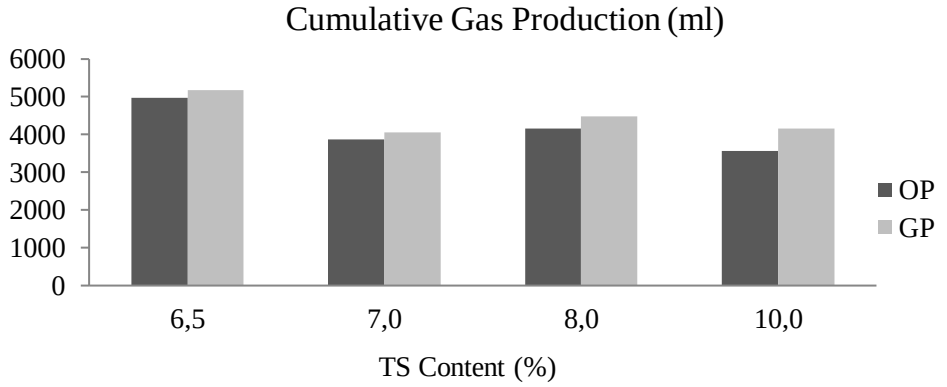

Figure 4.36. Effect of TS concentrations on the biogas production.

In each reactor, the pH was initially set around 7–7.5 to create optimal conditions for methanogenic activity. The initial and final alkalinity levels in the reactors remained within acceptable ranges, varying from 2350–3500 mg $CaCO_3$/L initially to 3000–5820 mg $CaCO_3$/L later. Due to the naturally high alkalinity levels in the agricultural substrates, the co-digestion reactors

showed higher alkalinity concentrations compared to the mono-sludge digestion reactor. Figure 4.37 illustrates the total solid (TS) and volatile solid (VS) contents as well as the removal efficiencies of the reactors.

Organic stabilization was effectively achieved as the organic content decreased in all reactors over the digestion period. The addition of co-substrates supplied diverse nutrients and balanced the C/N ratio within the reactors. Reactors containing grape pomace showed slightly higher TS and VS removal rates compared to those with olive pomace.

Monitoring the concentrations of ammonia nitrogen at the outset and during digestion is critical in anaerobic digestion processes (Bodur et al., 2024). Organic nitrogen present in WAS, olive, and grape pomaces undergoes conversion to ammonium during anaerobic degradation. High levels of ammonium nitrogen at elevated pH values can lead to the production of free ammonia nitrogen, which can inhibit methanogenic archaea significantly.

In this investigation, the final concentrations of ammonium nitrogen in the digesters ranged from 118–405 mg/L, notably below reported inhibitory thresholds in the literature (Yenigün & Demirel, 2013). Overly aggressive pre-treatment at temperatures exceeding 175°C may reduce the biodegradability of nitrogenous organic materials, potentially resulting in the formation of inhibitory levels of ammonia. However, no instances of ammonia inhibition were observed in the anaerobic digesters examined in this study.

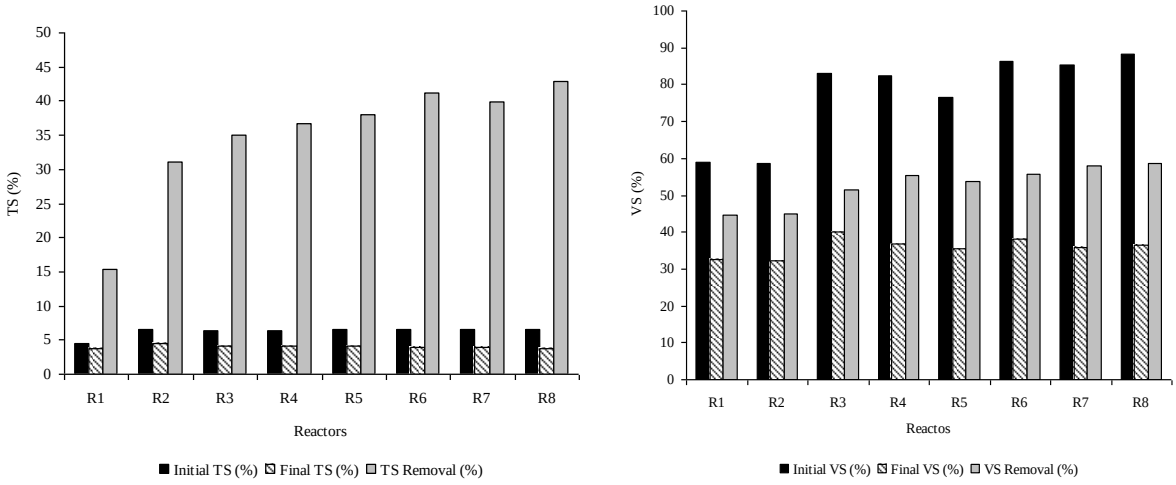

Figure 4.37. TS and VS concentrations and removal efficiencies in the reactors.

4.6.4. Biogas production

The cumulative biogas productions in the reactors are illustrated in Figure 4.38. The initial phase of the digestion period holds particular significance, as it is during this time that maximum substrate utilization usually occurs, typically within 5 to 7 days. It is evident that from the outset of the anaerobic digestion (AD) process, both sludge pre-treatment methods facilitated the hydrolysis step, leading to increased biogas production levels.

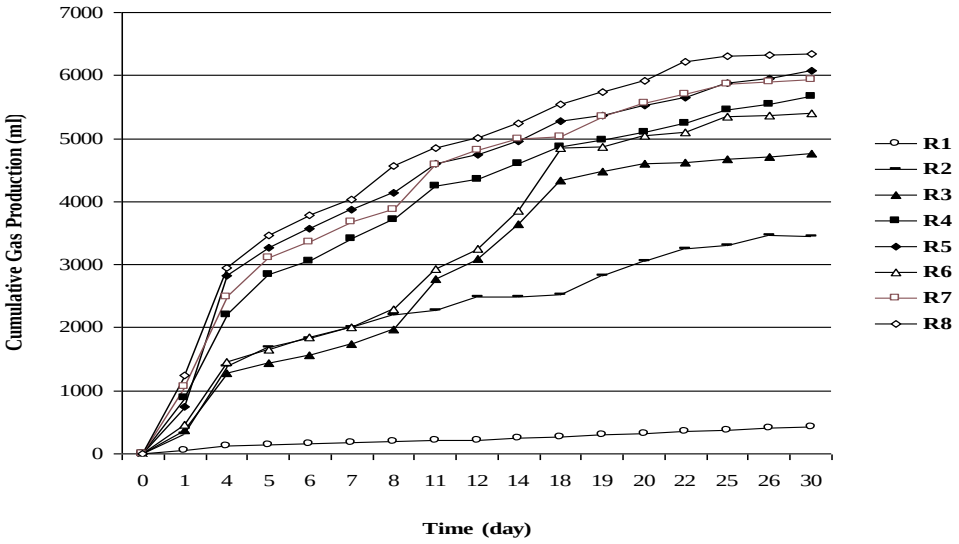

Figure 4.38. Cumulative biogas productions in the reactors.

The rapid increase in gas production can be attributed to the enhanced solubility of sludge organics. Results indicate that microwave pre-treatment, conducted at a specific energy of 87,000 kJ/kgTS, performed slightly better than ultrasonic pre-treatment, carried out at a specific energy of 10,000 kJ/kgTS, in terms of biogas production. Among the reactors, the highest cumulative biogas production was observed in reactor (R8), which contained grape pomace and microwave pre-treated waste activated sludge (WAS).

Both olive and grape pomaces were identified as suitable crop residues for biogas production through the anaerobic digestion process. It was observed that the biogas production efficiency of grape pomace was higher than that of olive pomace. ANOVA applied to evaluate the biogas production data revealed statistically significant differences between the mean values obtained in the reactors, with a confidence level of 95%. Additionally, the Tukey HSD Test identified the closest reactor pairs in terms of biogas production as pre-treatment-applied reactors: R4-R7, containing ultrasonicated waste activated sludge (WAS), and R5-R8, containing microwave-irradiated WAS.

The higher biogas production observed can be attributed to the synergistic effect of co-sludge pre-treatment and co-digestion. A recently published review by Zhen et al. (2017) reported that ultrasonication pre-treatment in sludge digestion improved biogas production by 24 to 138% in batch mesophilic anaerobic digestion (AD), while microwave pre-treatment resulted in improvements of 45–79%. This suggests that both ultrasonication and microwave pre-treatments can significantly enhance biogas production efficiency in anaerobic digestion processes (Zhen et al., 2017). Similarly, Mata-Alvarez et al. demonstrated that anaerobic co-digestion can increase CH4 production by 50–200% depending on the operating conditions and co-substrates used.

In addition to biogas production volume (in mL), the composition of biogas, particularly the methane content, is a crucial parameter in evaluating the performance of an anaerobic digestion process. As illustrated in Figure 4.39, the methane contents of the biogas produced in the reactors ranged between 54% and 65%. The highest methane composition of 65% was achieved between the 12th and 20th days of digestion in Reactors R6 and R7, followed closely by reactor R8, which reached 64%.

Figure 4.40 shows the biogas and methane yields of the reactors over time, showcasing similar trends in biogas and methane yields obtained from the co-digestion of waste activated sludge (WAS) with both grape and olive pomaces. This indicates that the co-digestion process with grape and olive pomaces has a positive effect on both overall biogas production and methane content in the produced biogas. However, the biogas production efficiency of grape pomace was found to be higher than that of olive pomace.

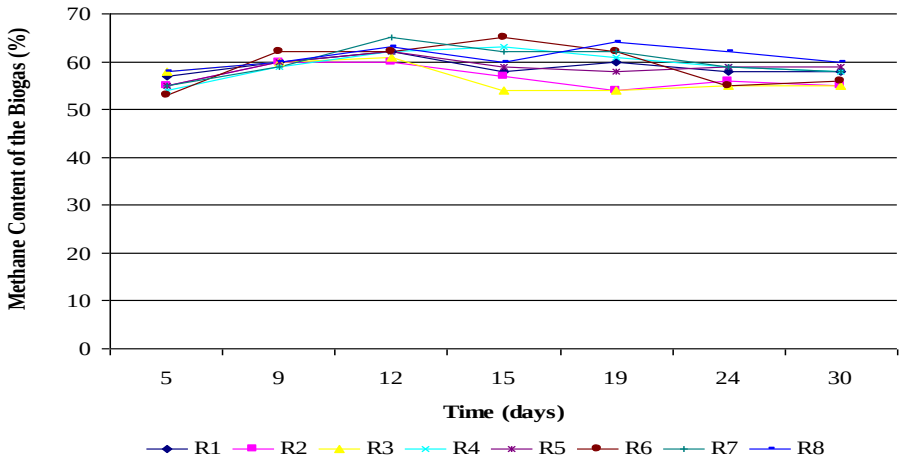

Figure 4.39. Methane contents of the biogas in the reactors.

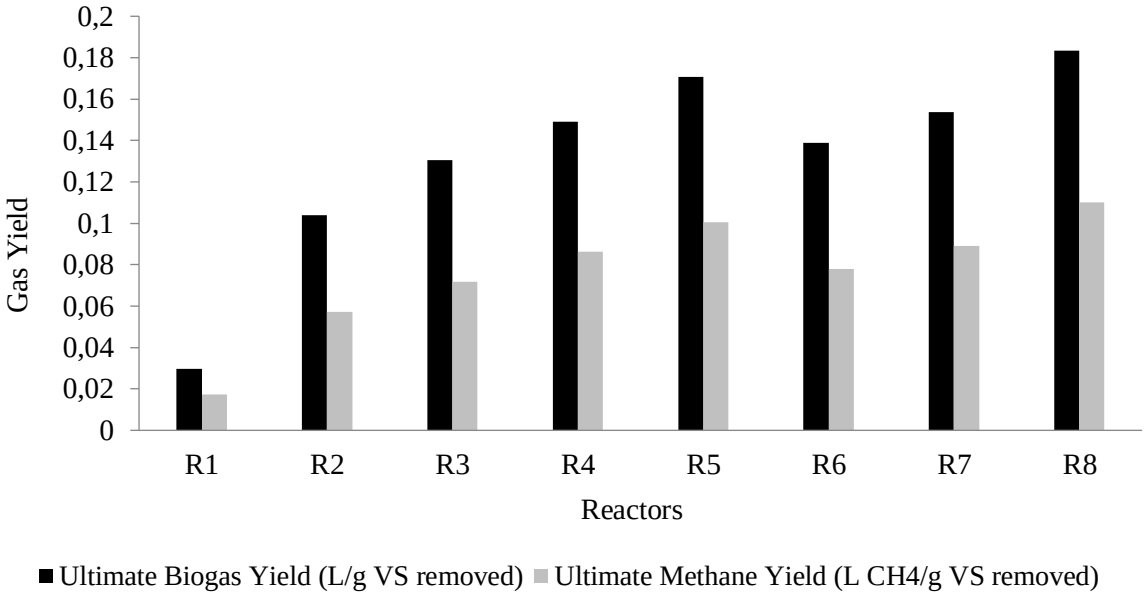

Figure 4.40. Biogas and methane yields in the reactors.

The maximum biogas yields were achieved through the co-digestion of these pomaces with microwave pre-treated waste activated sludge (WAS). When olive pomace (OP) and grape pomace (GP) were added to the WAS in the co-digestion process, the biogas yield was enhanced by 31% and 40%, respectively, due to the improved carbon-to-nitrogen (C/N) ratio of the mixtures.

Additionally, biogas yields were further increased by about 11–14% and 30–32% by applying ultrasonic and microwave pre-treatments to WAS samples prior to anaerobic digestion (AD), respectively. In this study, the maximum biogas yield of 0.18 $L/gVS_{removed}$ and methane yield of 0.11 $L/gVS_{removed}$ were obtained from the co-digestion of microwave pre-treated WAS and grape pomace in reactor R8.

Ultrasonic pre-treatment of waste activated sludge (WAS) in the co-digestion of olive pomace (OP) and grape pomace (GP) also resulted in slightly lower biogas and methane yields compared to microwave pre-treatment. However, the obtained results were quite comparable with the reported values in the literature.

Amon et al. conducted research on the anaerobic digestion of energy crops, such as maize, and dairy cattle manure, investigating their impact on biogas production. The highest biogas yields achieved in their experiments ranged between 0.108–0.268 $L/gVS_{removed}$. Similarly, Fantozzi and Buratti reported a biochemical methane potential of olive pomace as 110 NL/kgVS using a mesophilic batch reactor. These findings correspond with the outcomes of the present study, further confirming

the efficacy of the co-digestion process and the potential of utilizing crop residues for biogas production.

When comparing the effectiveness of ultrasonic (US) and microwave (MW) pre-treatments based on the applied specific energies, ultrasonication demonstrated significantly better performance than MW irradiation. Despite the specific energy applied to the sludge samples being 10,000 kJ/kgTS for ultrasonication and 87,000 kJ/kgTS for microwave pre-treatments, respectively, ultrasonication yielded comparable or even higher biogas outputs than microwave irradiation.

This discrepancy is noteworthy considering that the energy applied for microwave pre-treatment was nearly nine times higher than that for ultrasonication. However, the biogas yield achieved with microwave pre-treatment was only about 6–15% higher than that of ultrasonication. This suggests that ultrasonication may offer a more efficient and cost-effective approach for sludge pre-treatment in anaerobic digestion processes compared to microwave irradiation.

5. CONCLUSIONS AND RECOMMENDATIONS

The aim of this study was to examine the potential for biogas production using a variety of agricultural biomass sources co-digested with wastewater treatment plant sludges through the anaerobic digestion. By evaluating the co-digestion of agricultural biomass and crop residues, with WWTP sludges, the research aimed to assess the feasibility and efficiency of utilizing these biomass sources for biogas production. Through anaerobic digestion, the study aimed to elucidate the potential synergies and benefits of combining different organic substrates to enhance biogas yields while also addressing waste management challenges in the agricultural and wastewater treatment sectors.

The study findings revealed that the anaerobic co-digestion of agricultural products and waste activated sludge (WAS), including both energy crops and waste materials, emerged as a sustainable and preferable option for biogas production when compared to single-sludge digestion methods. This conclusion was supported by an observed increase in methane yields ranging from 11% to 67% in the co-digestion reactors, attributed to the improved balance of nutrients and synergistic effects between the different substrates.

The highest efficiency for biogas yield was achieved through the co-digestion of WAS and cloverleaf, reaching 0.83 $L/gVS_{removed}$. This finding underscores the potential of co-digestion strategies in maximizing biogas production and highlights the importance of diversifying feedstock sources in anaerobic digestion processes to optimize methane yields.

The conducted studies have illustrated that anaerobic digestion of agricultural wastes, particularly olive pomace (OP) and grape pomace (GP), presents a viable and convenient option for enhancing biogas production in Turkey.

An initial investigation on the influence of different total solid (TS) content on the digestion mixture comprising OP, GP, and anaerobic sludge (inoculum) indicated that the highest biogas production occurred at a 6.5% TS content level. This finding suggests that optimizing the TS content in the digestion mixture is crucial for maximizing biogas yield and highlights the importance of understanding the influence of substrate composition on anaerobic digestion performance.

By incorporating residual pomaces into the digestion process, a viable alternative for addressing the disposal challenge of these agricultural waste products, which are produced in significant

quantities in Turkey, can be achieved. This approach not only addresses waste management concerns but also contributes to sustainable energy production.

Furthermore, utilizing these crop residuals for biogas production instead of energy crops offers several environmental advantages. It diminishes the demand for dedicated energy crops, which frequently necessitate significant resources such as water, fertilizer, and land for cultivation. By repurposing agricultural by-products for biogas generation, the environmental footprint associated with conventional energy crop cultivation can be reduced, promoting resource efficiency and environmental sustainability.

However, the effectiveness of anaerobic digestion solely focusing on pomaces can be substantially improved through co-digestion with waste activated sludge (WAS). Hence, in subsequent studies, incorporating sewage sludge into pomace samples could enhance the efficiency of anaerobic digestion by attaining the optimal C/N ratio.

The study also explored the impacts of sludge pre-treatment techniques on biogas production. The biogas production potential of agricultural wastes co-digested with pre-treated waste activated sludge (WAS) through ultrasonication, pulsed electrical field application, and microwave irradiation was assessed. The findings revealed that all applied sludge disintegration techniques accelerated the rate-limiting hydrolysis step and enhanced the anaerobic biodegradability of organics in the sludge samples, consequently leading to increased cumulative biogas yield in reactors with pre-treated wastewater sludges.

Among the employed disintegration techniques, ultrasonication exhibited a superior capacity to enhance anaerobic biodegradation compared to pulsed electrical field application, particularly in terms of cumulative gas production and organic removal efficiency. Additionally, these techniques resulted in significantly higher reductions in pathogens, thereby enhancing the overall environmental sustainability and safety of the anaerobic digestion process.

The efficiency of ultrasound treatment was affected by both sonication density and the duration of sonication applied. Improved anaerobic biodegradation and methane production enhancement were particularly noticeable in reactors containing sludge samples ultrasonicated for 30 minutes. Key changes observed were a decrease in particle size and an elevation in soluble chemical oxygen demand (sCOD) concentrations in the waste activated sludge (WAS) samples. Higher sonication power led to more extensive rupture of sludge particles and complete breakdown of sludge structure.

Microwave pre-treatment of sludge similarly accelerated the rate-limiting hydrolysis phase. Nonetheless, ultrasonication emerged as a more favorable method for sludge pre-treatment when considering the applied specific energies. Even though microwave irradiation utilized nearly nine times more specific energy than ultrasonication, it resulted in only a modest 10–15% rise in biogas/methane yields. These findings emphasize the importance of adjusting sonication parameters to achieve maximum efficiency in biogas production during anaerobic digestion processes.

Overall, co-digestion of pre-treated WAS with agricultural residues represents a holistic approach to sustainable waste management and renewable and energy harnessing within the context of water-energy-food nexus. Additionally, producing biogas using crop residuals, rather than energy crops, presents an environmentally friendly solution to not only reduces greenhouse gas emissions but also promotes circular economy principles by turning waste into valuable energy resources.

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