ENERGY PRODUCTION FROM YARD WASTES VIA ONE-STAGE AND TWO-STAGE ANAEROBIC DIGESTION AND INVESTIGATION OF PRETREATMENT EFFECT

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ABSTRACT

ENERGY PRODUCTION FROM YARD WASTES VIA ONE-STAGE AND TWO-STAGE ANAEROBIC DIGESTION AND INVESTIGATION OF PRETREATMENT EFFECT

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Energy production from lignocellulosic biomass is receiving ever-increasing interest. This thesis study investigated the energy production from yard wastes, which is an abundant lignocellulosic substrate, via one-stage anaerobic digestion (OSAD) and two-stage anaerobic digestion (TSAD) of yard waste. The effect of pretreatment options (alkaline (AP), thermal (TP) and ultrasound (UP)) and initial solids concentration (2%, 5% and 8% total solids, TS) on hydrogen and methane production yields in OSAD and TSAD were researched. Solubility increase was obtained via AP, TP and UP by 43%, 51% and 17%, respectively. OSAD batch experiments showed that the highest CH_4 yield was obtained with AP-yard waste at 5% TS as 313 mL CH_4/g VS. AP was the best pretreatment option among others for all three TS% options studied in terms of CH_4 yields. Considering the TSAD experiments, the highest H_2 yield was obtained with AP-yard waste at 2% TS (30.5 mL H_2/g VS) in dark fermentation stage. AP was found again as the best pretreatment option for all TS contents studied in terms of H_2 yields. In

methanogenesis stage of TSAD, the obtained yields were very low. The highest methane yield was obtained with TP-yard waste at 2% TS (40 mL CH_4/g VS). TP-yard waste gained the highest methane yield in all three TS contents. OSAD of yard waste provided 11 MJ/kg VS energy recovery. Yet only 2 MJ/kg VS could be achieved via TSAD of yard waste. It can be concluded in this study that it is possible to achieve energy from yard wastes whether it is pretreated or not when initial TS content is 2%. For higher TS contents such as 5%, AP improves the energy yield. TSAD is not sufficient to apply for yard wastes even if it is pretreated with AP, TP and UP. This was mainly attributed to the potential inhibitory by-products produced after either pretreatments applied and/or dark fermentation.

Keywords: Anaerobic digestion, dark fermentation, yard waste, pretreatment, solids concentration

ÖZ

BAHÇE ATIKLARINDAN TEK-AŞAMALI VE İKİ-AŞAMALI ANAEROBİK ÇÜRÜTME İLE ENERJİ ÜRETİMİ VE ÖN İŞLEM ETKİSİNİN ARAŞTIRILMASI

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Lignoselülozik biokütleden enerji üretimi giderek artan bir ilgi görmektedir. Bu tez çalışmasında, yaygın olarak bulunan lignoselülozik biyokütle bahçe atıklarından tek-aşamalı anaerobik çürütme (TAAÇ) ve iki-aşamalı anaerobik çürütme (İAAÇ) aracılığıyla enerji üretimi araştırılmıştır. Ön arıtma seçeneklerinin (alkali (AÖ), termal (TÖ) ve ultrason (UÖ)) ve başlangıç katı konsantrasyonunun (%2, 5 ve 8 toplam katı madde, TKM) hidrojen ve metan üretim verimleri üzerindeki etkisi TAAÇ ve İAAÇ deneylerinde araştırılmıştır. Çözünürlük artışı AÖ, TÖ ve UÖ için sırasıyla %43, 51 ve 17 oranlarında elde edilmiştir. TAAÇ kesikli reaktör deneyleri göstermiştir ki, en yüksek CH4 verimi, 313 mL CH_4/g UKM ile %5 TKM'de AÖ-bahçe atığıyla elde edilmiştir. AÖ, CH_4 verimi açısından diğer ön arıtımlar arasında, çalışılan tüm % TKM miktarları için en uygun ön arıtma seçeneği olmuştur. İAAÇ deneyleri göz önüne alındığında, en yüksek H_2 verimi, karanlık fermantasyon aşamasında %2 TKM 'de (30,5 mL H_2/g UKM) AÖ bahçe atığı ile elde edilmiştir. AÖ, H_2 verimleri açısından tüm % TKM miktarları için tekrar en

iyi ön işlem seçeneği olarak bulunmuştur. İAAÇ'nin metanojenez aşamasında elde edilen verimler çok düşüktür. En yüksek metan verimi, %2 TKM'de (40 mL CH_4/g UKM) TÖ-bahçe atığı ile elde edildi. TÖ-bahçe atığı, üç TKM miktarı için en yüksek metan verimini elde etmiştir. Bahçe atıkları ile gerçekleştirilen TAAÇ işlemi 11 MJ/kg UKM enerji kazanımı sağlamıştır. Fakat İAAÇ ile bahçe atığından sadece 2 MJ/kg UKM elde edilebilmiştir. Bu çalışmada başlangıç TKM içeriği %2 olduğunda ön işlem görmüş olsun veya olmasın, bahçe atıklarından enerji elde etmenin mümkün olduğu sonucuna varılabilir. %5 gibi daha yüksek TKM içerikleri için AÖ enerji verimini arttırmaktadır. İAAÇ , AÖ, TÖ ve UÖ ile işlem görmüş olsa bile bahçe atıkları için yeterli değildir. Bu durum başlıca uygulanan ön işlemler ve/veya karanlık fermantasyondan sonra üretilen potansiyel inhibitör yan ürünlere ilişkilendirilmiştir

Anahtar Kelimeler: Anaerobik çürütme, karanlık fermentasyon, bahçe atığı, ön işlem, katı konsantrasyonu

To my wonderful family

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ABBREVIATIONS

AP: Alkaline Pretreatment

BM : Basal Medium

Btu : British Thermal Unit

COD: Chemical Oxygen Demand

$_{S}$COD: Soluble Chemical Oxygen Demand

NP: Non-Pretreatment

OSAD: One-Stage Anaerobic Digestion

pH_2 : Partial pressure of hydrogen

S/I: Substrate/Inoculum

SMA: Specific Methanogenic Activity

TAN: Total Ammonia Nitrogen

TP: Thermal Pretreatment

TS: Total Solids

TSAD: Two-Stage Anaerobic Digestion

UP: Ultrasound Pretreatment

VFA: Volatile Fatty Acid

VS: Volatile Solids

w/w: Weight by weight

w/v: Weight by volume

CHAPTER 1

INTRODUCTION

Global warming, energy crisis and inconsistent fossil fuel prices are the main challenges that world has been facing. Energy Information Administration report shows that world energy need will grow by 44% from 472 quadrillion British thermal units (Btu) to 678 quadrillion Btu between 2006-2030 (IEO, 2018). According to the fact that world oil production will peak between 2030 and 2050, renewable energy technologies have been continuously developing.

Biomass is known as any biological substance gained from natural or human activities and promoted as an encouraging renewable energy alternative. Lignocellulosic biomass like grasses, branches, agricultural and forestry residues are generated renewably, which contributes to the development of a sustainable fuel industry. Moreover, greenhouse gas emission will be eliminated while producing biomass energy through the balance between absorbed (biomass growth) and emitted (biomass energy consumption) carbon dioxide (Spatari et al., 2005). Besides, since biomass is easy to achieve and locally available, development of biomass-based energy sector will be beneficial for community in terms of creating job opportunities, while enhancing sustainable government (Lin and Tanaka, 2006). Lastly, utilizing biomass resources such as yard waste, forestry waste and agricultural waste will provide beneficial way to dispose organic waste while generating energy.

Yard waste, as a lignocellulosic substance, is composed of three major components; cellulose, hemicellulose and lignin. In addition to these constituents, phenolic compounds, minerals and acetyl groups are also present in small amounts (Chen, 2017). Lignocellulosic substrates typically contain 30-70% cellulose, 15-

30% hemicellulose and 10-25% lignin, showing that cellulose has the biggest fraction (Akobi, 2016). Lignin abundance in a substrate results in resistance to microbial and enzymatic reduction (Monlau et al., 2013).

In Turkey, yard wastes under the title of biodegradable waste, which constitute approximately 70% of the urban solid waste category, cannot satisfy the potential returns in terms of quantity and energy (Ministry of Environment and Forestry, 2019). Although the exact amount of yard waste is unknown, when forest-derived branches, grass, leaves etc. are taken into consideration, it is expected that yard wastes will be generated at high rates, which indicates that Turkey has a rich biomass source in terms of this waste. Yard waste applications in anaerobic processes have been supported not only for its abundance but also its contribution to reduce greenhouse gas emission, encourage regional economies, providing reliable biomass energy sources and limit the competition with food sources (Coyle, 2011).

Biomass-based anaerobic digestion provides impressive possibilities for proper degradation of organic wastes while gaining energy (Murphy et al., 2011). Anaerobic digestion process is a very popular option especially for lignocellulosic material, since it enables degrading and stabilizing complex organic substances resulting in a valuable biogas, methane, that can be replaced with fossil fuels (Dahunsi, 2019).

Hydrogen, on the other hand, has been classified as “energy carrier” due to its high energy content (122 kJ/g Lower Heating Value (LHV) (Lay et al., 1999), and 143GJ/g Higher Heating Value (HHV) (Boyles, 1984), which is the highest amount of all fuel types that can be converted to electricity. Hydrogen is a clean energy since the end product of its combustion is water. Therefore, it is presented as an effective option in the researches for solutions to global warming and increasing pollution problems. Dark fermentation is one of the biological H_2 production processes (Bundhoo, 2019). Two-stage anaerobic digestion systems, where dark fermentation process is followed by methanogenesis, is a good option to improve

energy production from biomass (Hans and Kumar, 2019). Indeed for substrates such as agricultural residues, yard wastes, industrial and municipal wastes, it has been shown that energy production is improved in two-stage anaerobic digestion systems compared to one-stage anaerobic digestion systems (Chuang et al., 2011). However, when it comes to lignocellulosic wastes, especially yard wastes, the literature reviews on two-stage anaerobic digestion is limited (Akobi et al., 2016; Chuang et al., 2011; Pakarinen et al., 2009; Mshandete et al., 2008). It should be noted that, H_2 energy via dark fermentation in two-stage anaerobic digestions has been preferred in recent years for H_2 having widespread industrial use, generating no harmful by-products after combustion, having higher energy capacity (Ghimire et al., 2015) and due to its potential to be used for biohythane (fuel) production which is more advantageous than each of its components, sole methane or sole hydrogen (O-Thong et al., 2016). Therefore, it is worth it investigating the two-stage anaerobic digestion and dark fermentative hydrogen potential of yard wastes. However, there are some drawbacks of dark fermentative hydrogen production such as homoacetogenesis activity, end-product inhibition, etc., which results in decreased hydrogen yields. These drawbacks remain to be solved.

Mentioned resistance of lignocellulosic substances to biodegradation has been tried to overcome with pretreatment options and to increase the accessibility of hemicellulose and cellulose for hydrolytic enzymes for fermentable sugar production. An effective pretreatment should include improving sugar production with microbial degradation, avoid carbohydrate consumption, eliminate the production of inhibitory compounds and being economical (Sun and Cheng, 2002). Among many types of pretreatment options, alkaline, thermal and ultrasound pretreatment are found advantageous for yard waste according to their efficiencies in solubilisation enhancement and biogas yield increase (Chandra et al, 2012; Benabdallah El-Hadj et al., 2007; Hashemi et al., 2019).

Alkaline pretreatment is a chemical method, which breaks cross bonds between hemicellulose, cellulose and lignin, and meanwhile provides increased porosity and internal surface area, reduced crystallinity and polymerization of carbohydrates

(Sun and Cheng, 2002). Its main effect is the lignin hydrolysis, improving the reactivity of rest of the polysaccharides. Also, it reduces the enzyme inhibition caused by their binding to lignin, resulting in better enzymatic cellulose degradation (Carvalho et al., 2016). Thermal pretreatment includes temperature and pressure applications, which separates liquid organic portion of the material from solid organic portion and breaks up the cell structure of the remaining organic solids (Mata-Alvarez et al., 2000). Applying thermal pretreatment to lignocellulosic substrates will hydrolyze hemicellulose, remove lignin and extractives, and enhance cellulose digestibility (Garrote et al., 1999). Ultrasound pretreatment is a relatively new application for lignocellulosic substrates (Bozkir et al, 2018). This procedure aims to disintegrate and destruct the substrate, by immersion of substrate in a hypertonic liquid or water to which ultrasound pretreatment is applied (Bozkir et al., 2018).

Pretreatment of lignocellulosic substrates such as yard wastes before anaerobic digestion might improve the energy production from organic matter for improving its solubility. On the other hand, pretreatment might lead to inhibitory compounds production which might decrease the anaerobic treatability and in turn energy production via one-stage and two-stage anaerobic digestion. In addition, initial total solids (TS) content is also a critical parameter affecting the efficiency of anaerobic digestion. Therefore, determining the optimum solids content is a crucial step to keep this procedure efficient. Higher initial solids concentrations enables to work in smaller reactor volumes, decrease the energy need for heating and reduce material handling issues. However, after a certain point, TS content becomes inhibitory for digestion and causes declines in the efficiency (Forster-Carneiro et al., 2008).

Considering the above mentioned points, the objective of this thesis study was set as to investigate the energy potential of yard wastes which is produced in high amounts but has not been discovered as a biomass source in Turkey, with one-stage anaerobic digestion (OSAD) and two-stage anaerobic digestion (TSAD) processes. Specific objectives are listed below:

- ✓ To apply different pretreatment alternatives at different initial TS contents in order to investigate the solubilisation of yard wastes and its efficiency.
- ✓ To determine the methane production potential per unit mass via OSAD of yard waste.
- ✓ To process yard waste in TSAD consisting of dark fermentation and methanogenesis stages and to determine hydrogen and methane production potential per unit mass.
- ✓ Higher solubilisation efficiency does not always mean higher methane/hydrogen production. Therefore, all yard wastes pretreated with different methods were subjected to one- and two-stage anaerobic digestion application (Figure 1.1). By this way, it was aimed to investigate the effect of pretreatment type on OSAD and TSAD, i.e., on methane and hydrogen yields.
- ✓ To investigate the effect of initial TS content on different pretreatment types applied and methane and hydrogen yields.
- ✓ To compare the total energy production per unit mass of OSAD and TSAD.

According to the aforementioned aims, three experimental steps were carried out in a stepwise fashion as shown in Figure 1.1.

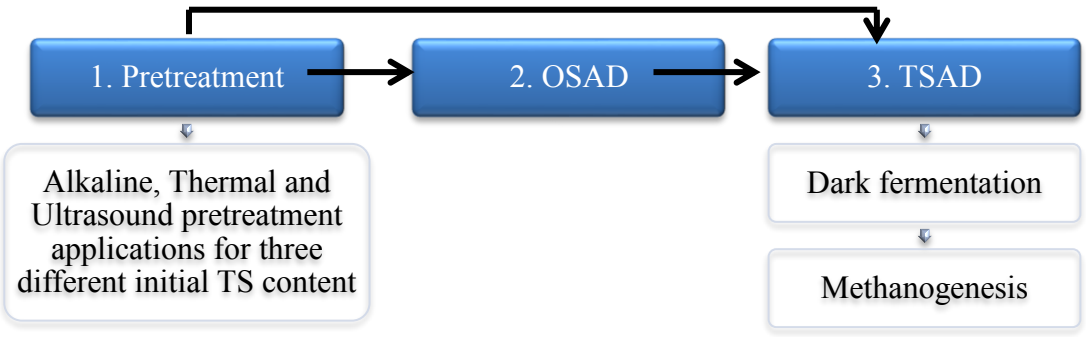

Figure 1.1 Schematic representation of the experimental steps

CHAPTER 2

LITERATURE REVIEW

2.1 Energy Need in the World and In Turkey

When the last two decades are examined, global programs, which are working on alternative energy, are seen to get interested in new and sustainable energy sources since global economy cannot depend on fossil fuels anymore (De Bhowmick et al, 2018). In 2017, renewable sources gave the highest growth rate compared to other energy sources and met nearly quarter of the total energy need. Main reasons for searching new sustainable sources are atmospheric CO_2 accumulation, decreasing fossil fuel sources, rural economic development and increasing energy demand. It is projected that, global population will reach 8.5 billion in 2030 and 9.7 billion in 2050. Correspondingly, energy need is expected to increase. As a matter of fact, energy consumption was 575 quadrillion Btu (British Thermal Unit) in 2015 and it is estimated that this amount will reach 633 quadrillion Btu in 2030 and 736 quadrillion Btu in 2040 (IEA, 2018). This energy gap should be met with sustainable energy sources.

Dependence on petroleum based materials for energy generation has been increasing continuously, although their fossil sources even came to an end. Lifetime of common fossil fuels are given in Table 2.1. Since this problem of petroleum raised the awareness, responsible ones has to develop alternative sources of energy (Isikgor and Becer, 2015).

Turkey is also affected by global changes. Rapidly increasing population, urbanization, variety in consumption habits and economic actions raise the pressure on environmental issues and natural resources. According to sustainable growth

targets, “Green Growth” has grab attention lately. Petroleum and natural gas export for primary energy demand supply of Turkey maintains external dependency. Developing alternative political actions for decreasing this dependency will provide positive impact on growth and current deficit (Ministry of Development, 2013). In this regard, for Turkey’s proper economic development, every possible local source should be considered for energy production. Especially renewable energy sources should be prioritized to meet primary energy need and generate electricity in regard of sustainable development. Fortunately, sources like biomass, geothermal and solar power are considered as primary energy target within “Energy Generation Program Based On Renewable Energy Sources”, which takes place in 10^{th}. Development Plan (Ministry of Development, 2013).

Table 2.1 Estimated Predicted lifetime for fossil fuels (Shanmugam, 2014)

Fossil Fuel	Annual Consumption rate	Estimated lifetime (years)
Coal	3×10^9 metric tons	>120
Natural Gas	2×10^{12} m^3	120
Oil	2×10^9 barrels	60

Turkey meets more than 60% of its energy needs with imports and this ratio is increasing each passing day (Ozturk and Bascetincelik, 2006). The increase in energy requirements, the costs of energy services, climate change, air pollution and the problems in the area of safe energy supply lead countries to search for more reliable and effective renewable energy sources. For the same reasons, it is very important for Turkey to meet its energy need through renewable domestic energy sources.

2.2 Renewable Energy Sources

Renewable energy preference is apparently increasing due to innovative technologies, issues about energy security and attempts to reduce traditional energy resources’ environmental impacts. In this regard, bioenergy has a substantial

contribution in enhancing renewable options. Bioenergy is projected to be the fourth biggest energy source globally, and its greenhouse gas free equivalent of fossil fuels because of its widespread and practicable characteristics (Mao et al., 2015).

Biomass is one of the most important renewable bioenergy sources. Biomass energy is composed of wastes (agricultural residues), grasses, algae, algae varieties, animal wastes and domestic organic wastes of specially grown energy plants, crops (wheat, corn, etc.). Biomass, which is the origin of plants and living organisms, can also be defined as the total mass of living organisms belonging to a species or a community of various species within a certain period of time (General Directorate of Renewable Energy, 2019). Yard wastes, agricultural wastes, municipal solid waste, organic industrial wastes, manure and biogas are common examples used as bioenergy resource. Anaerobic digestion of lignocellulosic substances such as yard wastes is one of the hot topics lately since it meets global energy needs providing environmental benefits (Mao et al., 2015).

2.3 Yard Waste as Biomass Energy Source

Yard waste, which is known as a high potential biosource, mainly consists of grass clippings, leaves, cut flowers, potted plants, weeds, bushes, branches and twigs (Hong Kong Ministry of Environment, 2019) In recent years, using yard wastes as biomass resource came to forefront, and compared to energy crop, they have more advantages. For instance, grass, which is one of the common yard waste types, is beneficial because it consumes less water compared to other crops, can grow on every type of land and it does not occupy present agricultural area like energy crops (Rinehart, 2006, 2006; Smyth et al., 2009). Unlike energy crops, grass grows naturally and it does not require special irrigation, fertilizing and grafting. On the other hand, energy crops need energy due to agricultural processes originating nearly 50% from fertilizer production, 22% from machinery, 15% from

transportation fuel and 13% from pesticide production (Weiland and Wellinger, 2019).

Yard waste, as a lignocellulosic substance, is composed of three major components; cellulose, hemicellulose and lignin. In addition to these constituents, phenolic compounds, minerals and acetyl groups are also present in small amounts (Chen, 2017). Lignocellulosic substrates typically contain 30-70% cellulose, 15-30% hemicellulose and 10-25% lignin, showing that cellulose has the biggest fraction (Akobi, 2016).

Cellulose, as the main structural polymer in the plant cell wall, is formed by linear chains with glucose molecules that generates a strong fibrous complex. In most of the cases, cellulose is insoluble because it has hydrogen bonding both inside and outside the molecule which makes strong connections to glucose units (Lübken et al., 2007). Hemicellulose consists of short branched chains of arabinose, xylose and hexoses. It attaches to cellulose chains and generates microfibrils which makes cell wall stronger. Hemicellulose is cross-linked with lignin that makes microbial degradation more difficult (Ladisch et al., 1983). Hemicellulose can be easily hydrolyzed compared to cellulose due to hydrophilic structure (Horn et al., 2012). Lignin is composed of three-dimensional phenylpropanoid molecules and behaves as a cell binder that enables strength to the plant (Rubin, 2008). Since lignin has a cross-linked hydrophobic polymer composition, it is hard to degrade anaerobically. Lignin abundance in a substrate results in resistance to microbial and enzymatic reduction (Monlau et al., 2013). Some examples of compositions of lignocellulosic materials are given below in Table 2.2.

Table 2.2 Biochemical compositions of several lignocellulosic substrates

Substrate	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Softwood	42	27	28	Mishra (1995)
Hardwood	45	30	20	
Wheat straw	36	27	11	
Grass	25-40	35-50	10-30	Sun and Cheng (2002)
Leaves	15-20	80-85	0	
Switch grass	45	31.4	12	
Poplar wood	44.5	22.5	19.5	Monlau et al. (2013)
Barley straw	37.5	25.3	16	

Anaerobic digestion of lignocellulosic compounds is problematic due to their complex structure and lignin content (Hendriks and Zeeman, 2009). These physical conditions of lignocellulosic compounds cause lower energy yields (Yang et al., 2015). Difficulties in biodegradation of lignocellulosic compounds have been trying to solve by pretreatment applications.

2.4 Pretreatment of lignocellulosic Materials

The complex structure of lignocellulosic materials, due to the lignin load and crystallinity, makes microbial digestion problematic (Hendriks and Zeeman, 2009). This incomplete fermentation of microorganisms with lignocellulosic material causes low biogas yield, in some cases even 10% of the theoretical methane yield (Yang et al., 2015). In order to eliminate this lignocellulosic recalcitrance and enhance anaerobic digestion, many types of pretreatments can be used. Applied pretreatment option can affect physicochemical properties of the material, which are mainly molecular size, surface accessibility, particle size and pore size distribution (Hendriks and Zeeman, 2009). Besides, some pretreatment options may affect chemical configuration of the material, which enables cellulose and lignin become soluble (Johnson and Elander, 2009).

Since the main aim of pretreatment is to increase cellulose availability for hydrolysis of the lignocellulosic substrates, various pretreatment applications aims

to alter the physical and chemical characteristics of this biomass and enhance hydrolysis rates. Lignin and hemicellulose removal is achieved while cellulose crystallinity increased (Chandra et al., 2012). For this reason, general expectation is to see decrease in lignin and hemicellulose content while increase in cellulose amount after pretreatment. However, this is not the certain view for all cases. In a study conducted with rice straw, increase in lignin content was observed from 16.3 to 17.3 %, after thermal pretreatment (Wang et al., 2018). Ultrasound assisted lime pretreatment with areca nut husk showed that hemicellulose content of raw substrate, 28%, increased to 35% at the end of the procedure (Sasmal et al., 2012).

While cellulose availability is increasing, by-product formation may also be observed. While uncovering the cellulose for microbial degradation, pretreatment often undergoes side reactions resulting in lignocellulose-derived by-products, which are inhibitory for biological processes. Inhibition issues become more serious when by-products accumulates in the media (Jönsson and Martín, 2016). Pretreatments aim to remove hemicellulose and/or lignin to achieve optimum results. However, these goals might affect other factors. For instance, higher lignin and/or hemicellulose solubilisation leads to degradation of solubilized parts due to the severe conditions they subject to (Jönsson and Martín, 2016).

Main by-products formed in pretreatment of lignocellulosic substrates are furans, organic acids and phenolic compounds (Hahn-Hägerdal and Palmqvist, 2000). Furan formation is observed when monomeric sugars are present with high temperature and low pH (Kabel et al., 2007). Furan production not only inhibits the microbial system, but also causes large losses in sugar yields (Pol et al., 2014). Since hemicellulose involves non-sugar portion in its structure, acetyl and uronic acid groups can be found in its composition. When polymers in lignocellulosic substrates are hydrolyzed, these acids can be released and become inhibitory in the system (Sun et al., 2004). Main inhibitory organic acids are acetic acid, formic acid, levulinic acid and lactic acid (Larsson et al., 1999). There are many types of phenolic compounds formed during pretreatment processes. Phenol, p-cresol and potentially other phenolic compounds are able to ruin cell structure of

microorganisms and inhibit the hydrolysis procedure (Sun et al., 2004). Furans reduces specific growth rate and cell productivity (Modig et al., 2002). Mentioned organic acids are able to harm cell wall and cell membrane by decreasing intracellular pH and hence causing cell death (Pampulha and Loureiro-Dias, 1989). Phenolic compound accumulation can increase the fluidity of cell membranes and inhibit enzymatic activity (Zhang et al., 2012).

In order to reduce the inhibition effect during fermentation, an additional upstream process can be added to remove formed by-products (Pol et al., 2014). Moreover, a pre-fermentation conducted with inhibitor consuming microorganisms can be also used to get rid of obtained by-products from pretreated lignocellulosic substrate (Koopman et al., 2010).

The ultimate aim of applying pretreatment process is to modify the composition of the substrate to overcome hydrolysis problems and hence improve solubilisation rate and get higher yields from cellulose and hemicellulose (Taherzadeh and Karimi, 2008). An effective pretreatment should improve sugar production with microbial degradation, avoid carbohydrate consumption, eliminate the production of inhibitory compounds and be economical (Sun and Cheng, 2002).

2.4.1 Common pretreatment methods for lignin destruction

Pretreatment methods for lignocellulosic substrates can be divided into five; physical, chemical, physico-chemical, biological and combined pretreatment (Kumari and Singh, 2018). Common processes applied are given in Figure 2.1.

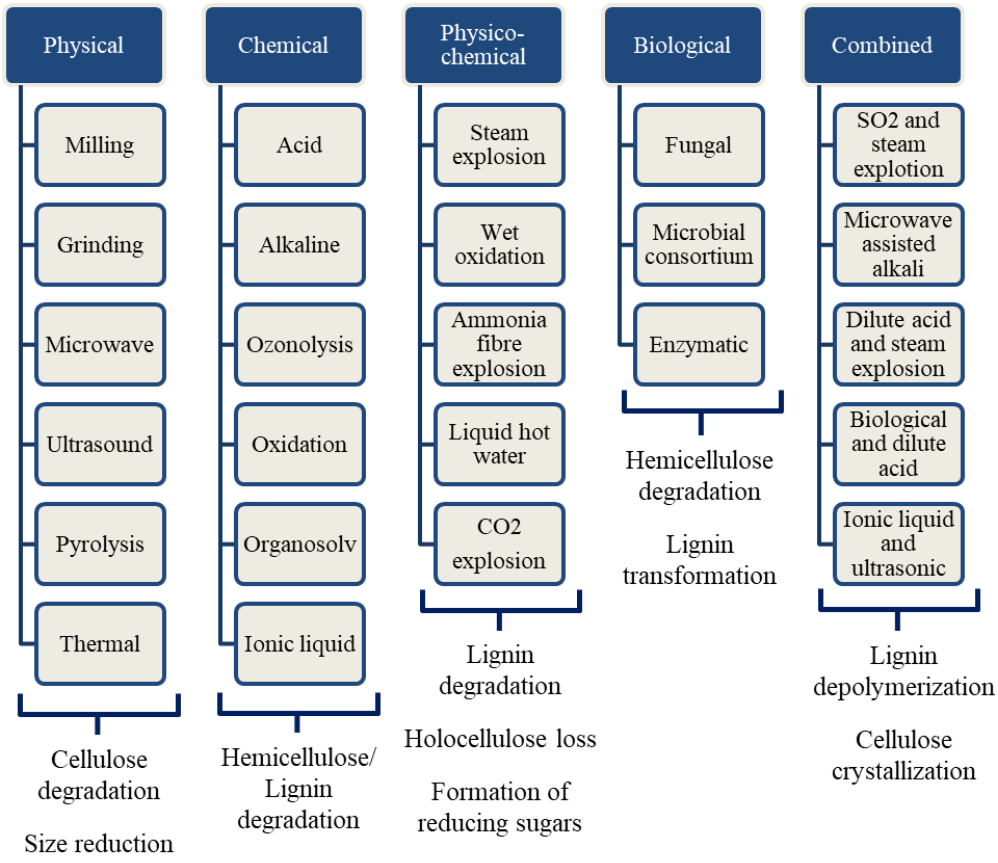

Figure 2.1 Common pretreatment processes for lignocellulosic substrate (Kumar and Sharma, 2017; Kumari and Singh, 2018; Mohapatra et al., 2017)

Physical pretreatment approaches mainly aim to reduce particle size and increase surface area of the substrate to enhance solubilisation of lignocellulosic material. Common physical pretreatment options are milling, grinding, microwave, ultrasound, pyrolysis and thermal pretreatment. Some of these options are not effective enough alone and applied as a combination with another pretreatment in literature studies (Kumari and Singh, 2018). Chemical pretreatment approaches provide disintegration of lignocellulose with chemical utilization. Hemicellulose and lignin removal enable gaining higher glucose yields (Zhu et al., 2010). Typical chemical pretreatments for lignocellulosic substrate are acid, alkaline, ozonolysis, oxidation, organosolv and ionic liquid pretreatments (Kumar and Sharma, 2017). Physico-chemical pretreatment methods provide lignocellulose degradation in

terms of oxidation and thermal pretreatment combinations (Kucharska et al., 2018). Physico-chemical pretreatment involves steam explosion, wet oxidation, ammonia fibre explosion, liquid hot water and CO_2 explosion (Kumar and Sharma, 2017; Sivagurunathan et al., 2017). Biological pretreatment methods employ microbial or fungal organisms to break down lignin. This option is expensive since microorganism purchase is costly and process needs long reaction time (W. Zhu et al., 2010). Common biological pretreatment methods are fungal, microbial consortium and enzymatic pretreatments (Kumari and Singh, 2018). Lastly, combined pretreatment options include thermochemical and thermo-physical applications. Enhanced lignin removal and solubilisation increase are intended. Main examples are SO_2 and steam explosion, microwave assisted alkali, dilute acid and steam explosion, biological and dilute acid, and ionic liquid and ultrasonic pretreatments (Kumar and Sharma, 2017; Kumari and Singh, 2018).

Among these pretreatment options, alkaline, thermal and ultrasound pretreatment are selected for yard waste mix in this study, due to their reliable literature results with lignocellulosic substrates.The detailed information fr these three pretreatment methods are given in Sections 2.4.1.1, 2.4.1.2 and 2.4.1.3.

2.4.1.1 Alkaline Pretreatment

Alkaline pretreatment is a chemical method, which breaks cross bonds between hemicellulose, cellulose and lignin, that provides increased porosity and internal surface area, reduced crystallinity and polymerization of carbohydrates (Sun and Cheng, 2002). Inhibitors avoiding cellulose accessibility for enzyme saccharification, which are acetyl groups, uronic acid sub-solutions and lignin are eliminated with alkaline pretreatment (Kumari and Singh, 2018). The main effect of this process is lignin removal, hence it improves the reactivity of the rest of the polysaccharides. Moreover, it reduces the enzyme inhibition originated from their binding to lignin, resulting better enzymatic cellulose degradation (Carvalho et al., 2016). Several drawbacks of alkaline pretreatment are long residence time need,

and neutralization of processed substrate (Wan et al., 2011). The advantages and disadvantages of alkaline pretreatment is summarized in Table 2.3.

Commonly used chemicals for alkaline pretreatment are sodium hydroxide, calcium hydroxide, ammonia and potassium hydroxide (Kumari and Singh, 2018). Sodium hydroxide is the most preferred alkaline chemical for pretreatment. According to studies, sodium hydroxide is more effective than not pretreated lignocellulosic substrate for dark fermentation, anaerobic digestion, ethanol and buthanol production. (Battista et al., 2016; Chandra et al., 2012b; Gonçalves et al., 2014).

There are various examples of alkaline pretreatment of lignocellulosic substrate in the literature. NaOH-pretreated hardwood solubility increased from 14% to 55% and lignin content decreased from 24-55% to 20%. Compared with non-pretreated sample, alkaline-pretreated corn stover provided 37% methane yield increase, leading to 372.4 L/kg VS (Zhu et al., 2010). Alkaline pretreatment on wheat straw studies showed that 100% methane yield increase is achievable (Pavlostathis and Gossett, 1985). In another study, 4-10% NaOH is applied to rice straw, which resulted in 3-58% methane increase (He et al., 2009). 2 % NaOH pretreatment was applied to Kans grass and 70% lignin reduction was presented (Kataria and Ghosh, 2014). Another study conducted with Napier grass showed similar results, lignin reduction up to 73%, with alkaline degradation (Sanni et al., 2018). In a different study, alkaline-pretreated birch provided 84% methane yield increase in anaerobic digestion (Mirahmadi et al., 2010).

Table 2.3 Advantages and disadvantages of selected pretreatment options (Carrere et al., 2016; Kumar et al., 2009; Rodriguez et al., 2017)

	Advantages	Disadvantages
Alkaline	• Increase digestibility of cellulose and solubilize lignin efficiently • Reduce lignin amount • Ambient temperature • Low energy demand • Low inhibitory compound formation	• Chemical addition needed • Corrosion • Long residence time required
Thermal	• Scalability • High methane yield advancement	• High heat demand • Recalcitrant compounds formation risk
Ultrasound	• Applicable at atmospheric pressure and ambient temperature	• Substrate complexity decreases pretreatment performance

Based on the evaluated studies, alkaline treatment has been widely used and found as an effective option to produce biomass energy from lignocellulosic substrates. Extreme use of NaOH will inhibit anaerobic process, mainly methanogenic microorganisms, also causes water pollution problems (Chandra et al., 2012).

2.4.1.2 Thermal Pretreatment

Thermal pretreatment is a physical pretreatment method including temperature and pressure, which separates liquid organic portion of the material from solid organic portion and breaks up the cell structure of the remaining organic solids (Mata-Alvarez et al., 2000). Applying thermal pretreatment to lignocellulosic substrates will hydrolyze hemicellulose, remove lignin and extractives, and enhance cellulose digestibility (Garrote et al., 1999). High temperature and pressure will enable translating critical water present in liquid phase to raw biomass material, hence will break down material structure (Xu, 2009).

Thermal pretreatment is a very promising alternative for biomass conversion. Compared to other technologies, energy recovery from biomass to fuel is higher,

reaching values up to 80% (Toor et al., 2011). Thermal pretreatment of lignocellulosic substrates gain prominence due to improved bioethanol and biogas production (Table 2.3). Under high pressure and temperature, water can pass through biomass, solubilize cellulose, remove hemicellulose and part of lignin (Chandra et al., 2012a). Thermal pretreatment removes only a small portion of lignin, which is acid soluble part, and it does not change the structure of lignin by repolymerization by cellulose fibers. Hence lignin extraction is not applicable from hydrothermally processes materials (Aita and Kim, 2010). Main advantages of thermal pretreatment are requiring any chemicals and any corrosion resistant materials for hydrolysis reactors. Reducing feedstock size is an expensive step in commercial treatment plants. However, thermal pretreatment does not need size reduction and it only needs trace amounts of chemicals to neutralize end products, which are generated relatively less compared to other pretreatment options (Taherzadeh and Karimi, 2008).

In a study, thermal pretreated (at 200℃, 10 min) rice straw provided 222% methane production increase, compared to raw substrate (Chandra et al., 2012). Another study showed that thermally pretreated grass can provide methane yield up to 198 mL CH_4/g VS (Li et al., 2012). Thermal pretreatment of fresh grass samples provided high glucose yields by 35%, which indicates 77% cellulose convertibility (Morten et al., 2014). Another study conducted with barley and wheat straw showed that thermally pretreated lignocellulosic substrate can enhance biogas yield more than 60% (Menardo et al., 2012). In a performed study with safflower which is thermally pretreated at 120℃ for 1 hour methane yield increased from 96.5 N mL/g VS (not pretreated substrate) to 191.4 N mL/g VS ,which is 83.6% of the theoretical methane yield (Hashemi et al., 2019).

As it is seen from the literature studies, thermal pretreatment of lignocellulosic substrate is an environmentally friendly option to eliminate lignin. The properties such as being a cost effective process for not consuming chemicals and facing

corrosion problems, and changing the structure of the feedstock make thermal pretreatment a common approach.

2.4.1.3 Ultrasound Pretreatment

Ultrasound pretreatment is a relatively new application as a physical pretreatment for lignocellulosic substrates. This procedure aims to disintegrate and destruct the substrate, by immersion of substrate in a hypertonic liquid or water to which ultrasound pretreatment is applied (Bozkir et al, 2018). This process consists of complex mechanisms, which are shearing, chemical reactions with radicals, combustion and pyrolysis (Gogate, 2002). Monolithic cavitation provides physical and chemical changes in the pretreated substrate. Physical changes are originated from broken down cavitation bubbles and an elevated conversion of chemical structure due to generated free radicals. Both physical and chemical modifications cause cell wall disruption. Cavitation enables this cell wall disruption which provides increased surface area, decreased polymerization degree and enhanced biodegradability of lignocellulosic substrate (Kumari and Singh, 2018).

As mentioned above, ultrasound pretreatment is a new emerging technology which is a promising alternative for lignocellulosic substrates. Ultrasound pretreatment produces radicals and improve mass transfer by streaming the media with the help of initial energy input (Bussemaker and Zhang, 2013). Mechanism of ultrasound pretreatment involves ultrasonic waves, which generates pressure differences in the solution for physical (mechanoacoustic) and chemical (sonochemical) process enhancements. Mentioned pressure waves travel through the medium, which involves high pressure (compression) and low pressure (rarefaction) regions. The pressure movement can expand the liquid molecules and generate bubbles. While ultrasonic waves travel through the media, these bubbles expand and bond with compression and rarefraction regions, respectively, resulting more liquid drawing into the molecules. When these bubbles are collapsed, radical formation is

observed by dissociation of produced molecules around bubbles (Bussemaker and Zhang, 2013).

When ultrasound pretreatment is applied to lignocellulosic materials, sonochemical and mechanoacoustic effects which will affect the chemical and physical characteristic of these substrates is observed (Hendriks and Zeeman, 2009). The mechanoacoustic affects can change the surface structure of lignocellulosic biomass and the sonochemical generation of oxidizing radicals can cause chemical attack to lignocellulosic substances. Oxidative process may lead to inhibitory by-product formation which causes delays in hydrolysis of the substrate (Bussemaker and Zhang, 2013).

Ultrasound pretreatment process is affected by four parameters namely, ultrasonic frequency, specific energy, time interval and substrate characteristics (Benabdallah et al., 2007; Bougrier et al., 2005). Cell disintegration corresponds to inlet energy. Higher frequencies enables radical's oxidation, and lower frequencies provide pressure waves. Literature studies show that 20-40 kHz is the optimal frequency range to gain proper mechanical forces (Bougrier et al., 2005).

Main advantage of ultrasound pretreatment is that, it is applicable at atmospheric pressure and can be worked without temperature arrangement (Bozkir et al., 2018) (Table 2.3). Moreover, since additional chemical agent addition is not necessary, effluent volume increase is prevented. When the complexity of substrate increases, radical performance of ultrasound pretreatment decreases (Benabdallah El-Hadj et al., 2007).

As a disadvantage, by-product formation, mentioned in Section 2.4, is an issue may be faced during ultrasound pretreatment. In a study conducted with carnauba palm leaves, macauba pulp/shell and pine nut shell, these substrates were processed via sequential pretreatment including microwave and ultrasound processes. Ultrasonic cleaning bath was used in ultrasound pretreatment for 15 minutes and at the end, furan production was observed (Lacerda et al., 2015). In another study, ultrasound pretreatment of aquatic plants (macrophytes) was investigated for similar durations.

By-products, mainly furfural and acetic acid, were determined at high levels (4.2 and 18.3 mg/L, respectively) (Kist et al., 2018). Hence it can be said that, applied pretreatment duration in this study (15 min) is long enough to form inhibitory by-products.

In comparison to other pretreatment options, ultrasound pretreatment gained attention since it is an efficient physical disintegration way for organic substrates due to its performance, proper operational and technical stability and environmentally friendly approach (Tyagi et al., 2014). Ultrasound pretreatment has been commonly preferred as a disintegration option for organic wastes prior to anaerobic digestion (Pilli et al., 2011). In a study conducted with food and yard wastes, ultrasonic process enhanced solubilisation by 159%, compared to not pretreated substrate (Bundhoo, 2017). Another study with ultrasound pretreated sugarcane bagasse showed that lignin and hemicellulose solubilisation increased up to 90% (Sun et al., 2004). Ultrasound pretreatment of corn starch slurry for 40 seconds increased sugar yield by 5-6 times compared to untreated sample (Montalbo-Lomboy et al., 2010).

2.5 Anaerobic Digestion

Anaerobic digestion is the conversion of different types of organic substrates such as industrial wastes, animal manures, food residues, agricultural wastes etc. in a biological pathway containing no free molecular oxygen. At the end of this biological process, degraded organic material provides soluble nutrients, new cells and bioenergy via biogas which includes methane (CH_4) and carbon dioxide (CO_2) mostly (Romano and Zhang, 2008). Biogas is composed of 60-70% methane and 30-40% carbon dioxide .

Anaerobic treatment has been applied for a long time, starting from sludge degradation. Eventually, its technology is evolved and numerous organic substances are adapted to this process. Anaerobic digestion process is a very

popular option for especially lignocellulosic material, since it enables degrading and stabilizing complex organic substances resulting in a valuable biogas that can be replaced with fossil fuels (Dahunsi, 2019). Residue grass (Bedoić et al., 2019), wheatgrass (Silva and Dionisi, 2019), yard waste (Li et al., 2014), wheat straw (Bolado-Rodríguez et al., 2016), softwood spruce (Mohsenzadeh et al., 2012), safflower (Hashemi et al., 2019), aquatic weeds (Sinbuathong, 2019) can be presented among many different options used in anaerobic digestion studies.

2.5.1 Anaerobic Digestion Stages and Benefits

In anaerobic degradation process, complex organic matters are consumed and decomposed to its monomers by microorganisms with no oxygen in that environment. This biochemistry is shown in Equation 2-1 (Speece, 1983).

$$\underset{matter}{Organic} + H_2O \rightarrow CH_4 + CO_2 + NH_3 + H_2S + New\ cells \qquad \text{(Equation 2-1)}$$

Stated process consists of four major steps namely, hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 2.2). In all these stages, different types of microorganisms are responsible for decomposition of complex organic material into smaller materials for the following step.

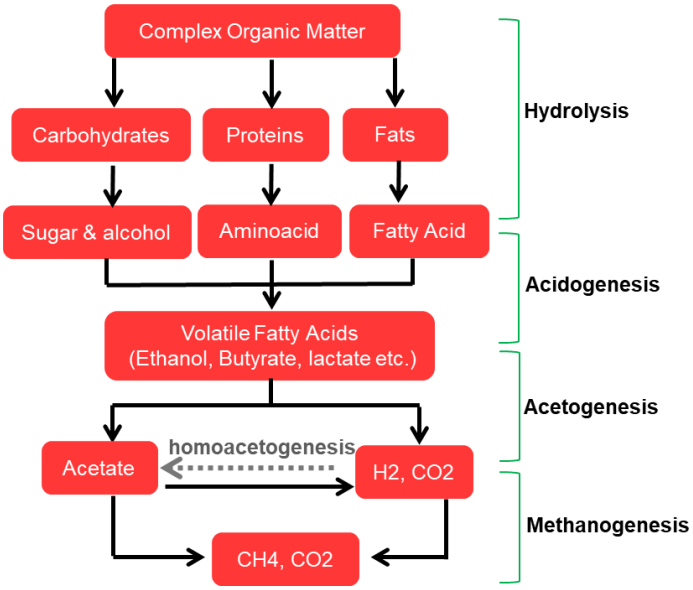

Figure 2.2 Anaerobic digestion steps

The beginning of anaerobic digestion is bacterial hydrolysis and solubilisation of complex organic matter. Hydrolysis stage enables decomposition of insoluble complex organics, which are primarily lipids, polysaccharides and proteins to smaller organics like fatty acids, monosaccharides and amino acids. This first stage is crucial for anaerobic microbial activity since complex organics cannot be consumed directly for fermentation. Hydrolysis step is the rate-limiting stage of anaerobic digestion, which causes lipid and protein to solubilize in a couple of days and incomplete decomposition lignin and lignocellulose (Equation 2-2) (Boontian, 2014). The hydrolysis of lignin is shown in Equation 2-2.

$$C_{24}H_{40}O_{20}: H_2O + 3\,H_2O \rightarrow 4C_6H_{12}O_6 \qquad \text{(Equation 2-2)}$$

The second stage of anaerobic digestion is acidogenesis. Created monomers (polysaccharides, amino acids and fatty acids) from hydrolysis are converted into hydrogen, carbon dioxide, ammonia and Volatile Fatty Acids (VFAs) by acidogenic bacteria (Figure 2.2). Main end products in this step are butyric acid, propionic acid, acetic acid, ethanol, H_2 and CO_2. Among these end products, hydrogen, carbon dioxide and acetic acid will be the substrates of methanogens in the fourth stage (Figure 2.2). Main reactions for acidogenesis are shown in Equations 2-3 (ethanol formation), 2-4 (propionic acid) and 2-5 (acetic acid formation).

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad \text{(Equation 2-3)}$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2OOH + 2H_2O \qquad \text{(Equation 2-4)}$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \qquad \text{(Equation 2-5)}$$

The next step is acetogenesis, which includes converting higher organic compounds to hydrogen, carbon dioxide and acetate and a small portion of ammonia. In this stage, homoacetogenic organisms produce acetic acid from hydrogen and carbon dioxide (Figure 2.2). Acetogens are sensitive to

environmental conditions and they are slowly growing organisms. Moreover, production of acetate will be affected by hydrogen partial pressure increased at certain amounts (Boontian, 2014; Parawira, 2014).

The fourth and the final step of anaerobic digestion is methanogenesis. Utilization of carbon dioxide, hydrogen and acetic acid is performed in order to produce methane and carbon dioxide, under strict anaerobic conditions (Equations 2-6 and 2-7) (Parawira, 2014).

$$CH_3COOH \rightarrow CH_4 + CO_2$$ (Equation 2-6)

Carbon dioxide reduction is as follows:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$ (Equation 2-7)

Anaerobic digestion is a well-known process and successfully adapted in many biological treatment fields due to its advantages in terms of economy and feasibility, such as;

- ✓ Short start-up time
- ✓ Low investment
- ✓ Well known and developed technology
- ✓ Minimized sludge production
- ✓ Reduced emission in terms of greenhouse gas
- ✓ Production of a potential fuel source, methane
- ✓ Easy to operate and carry out maintenance due to its simplicity and flexibility
- ✓ Less requirement for nutrition and chemical addition
- ✓ Degradation ability of aerobically non-biodegradable substances
- ✓ No aeration or other types of additional energy consumptions

- ✓ Adaptable for higher organic loading rates, hence easy to save space (Awad et al., 2015; Speece, 1996)

2.5.2 Factors Affecting Anaerobic Digestion

Operational factors should be satisfied for anaerobic digestion in order to maintain effective and high performance process. Among many others, the parameters affecting the anaerobic digestion, especially the batch digestion systems, such as pH, solids content, substrate/inoculum ratio, nutrients and temperature are discussed in the following sections.

2.5.2.1 pH

Anaerobic digestion strictly depends on pH, since hydrolytic enzyme activity of microorganisms is affected by this parameter. Commonly, biomethanation process is working properly at neutral pH. Methanogenic organisms prefer 6.5-8.2 and acidogenic bacteria efficiently work between 4-6.5 pH ranges (Mccarty, 1964). Below stated pH levels, methane production rate will decrease and inhibitory acid acclimations might be experienced. Therefore, pH should not be allowed to decrease below 6.5 for the sake of methanogens (Awad et al., 2015). In order to prevent pH changes in an anaerobic digester, alkalinity addition, which measures buffering capacity, is necessary. Alkalinity concentrations between 2000-4000 mg/L (as $CaCO_3$) to maintain desired pH range are required.

2.5.2.2 Solid (Substrate) Concentration

Solids concentration corresponds to the available portion of substrate in a unit volume of the digester, mainly operating solid wastes. This parameter can be also described as total solids, and crucial factors for anaerobic digestion such as pH, temperature and microbial performance might be affected by total solid content in

case of exceeding proper ranges (Boontian, 2014). Three major anaerobic digestion processes based on total solids content have been used commonly, which are conventional wet (≤10 % total solids), high solids (10-20 % total solids) and modern dry (≥20% total solids) options (Yi et al., 2014).

2.5.2.3 Substrate/Inoculum (S/I) Ratio

In batch reactors, substrate to inoculum (S/I) ratio (g COD/g VS) has a vital role for efficient biodegradability and methane generation potential (Neves et al., 2004). Inoculum provides all needed microbial communities to operate a digester and generally obtained from another anaerobic digester effluent. Too low S/I ratio may not trigger enzymes responsible for biodegradation and too high S/I ratio may cause inhibitory environment for microbial activity (Prashanth et al., 2014). Optimum S/I ratio is specific for selected substrate. Each type of feed generate different amounts of VFAs and ammonium which are a concern in terms of buffer capacity (Lesteur et al., 2014). Therefore, S/I ratios vary in the literature. For batch anaerobic systems processed with woody and herbaceous substrates and municipal wastes, S/I range of 0.5-1 gives optimum methane yields (Chynoweth et al., 1993). The optimum S/I ratio for anaerobic digestion of kitchen waste is given as 0.43-2 (Neves et al., 2004). For S/I ratio above 4.0, anaerobic digestion with wheat straw performed low methane yields (Hashimoto, 1989).

2.5.2.4 Macro- and Micro-nutrients

Anaerobic digestion requires essential nutrients in order to obtain proper microbial growth, otherwise microorganism's working efficiency will be negatively affected (Parawira, 2014). In addition to the feed which is carbon source; nitrogen, sulfur and phosphorus should be provided as macro-nutrients in the system. Trace elements which are mainly selenium, iron, cobalt, nickel, tungsten and

molybdenum should be also present in order to enhance microbial growth. Nutrient addition may increase methanogenic microorganisms' growth rate (Speece, 1996) .

2.5.2.5 Temperature

Process temperature strongly affects methanogenic activity in anaerobic digestion processes. Methane forming microorganisms are inhibited when the temperature is low. For this reason, there are two optimum temperature ranges that many methane formers properly work namely, mesophilic (30-35 ℃) and thermophilic (50-60 ℃) ranges. In addition to common ranges, with the help of revisions in reactor configurations, lower temperature range, called psychrophilic (< 20 ºC), is also available to perform methanogenic activity (Connaughton et al., 2006). Compared to thermophilic, long retention time is required for physicophilic and mesophilic conditions and pathogen removal is not successful (Parawira, 2014).

2.5.3 Determination of Biogas Potential

Biogas potential is generally expressed as the volume of cumulative biogas production divided by gram volatile solids (VS) of substrate in the reactor. Potential of produced biogas can be determined by either theoretical (via stochiometric approach) or experimental means.

Methane and carbon dioxide production in anaerobic digestion can be theoretically calculated according to Buswell's formula (Buswell and Mueller, 1952). Substrate's stoichiometric properties should be known in order to apply the formula given below in Equation 2-8.

$$C_aH_bO_c + \left(a - \frac{b}{4} - \frac{c}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right) CO_2 \text{(Equation 2-8)}$$

It is assumed that all of the generated energy is diverted to methane formation and consumed energy for microbial growth is neglected.

In general, theoretically calculated methane amount is higher than experimentally measured one. Inhibitory conditions or biodegradability problems may cause this potential difference. In order to gain reliable degradation results in the perspective of selected feed, experimental applications are necessary. Biochemical methane potential (BMP) test enables estimating the methane production potential for a significant substrate of concern by experimental inferences (Speece, 1983). In BMP tests, specific amount of substrate is mixed with microbial source (i.e. inoculum) in small batch serum bottles under anaerobic conditions. After optimum conditions are satisfied, biogas production is measured to obtain cumulative methane generation which indicates the experimental potential of the selected source. BMP of a substrate can be found using Equation 2-9. Theoretically, anaerobic digestion of 1 g COD anaerobically digested results in 395 mL CH_4 at 35℃. Comparing that value to BMP of the substrate gives information about the anaerobic digestion potential of the substrate.

$$BMP = \frac{Maximum\ Cumulative\ Methane\ Gas\ (mL)}{g\ COD\ removed} \qquad \text{(Equation 2-9)}$$

Specific methanogenic activity test (SMA) is performed with a substrate that has a known composition, like glucose, sucrose, phenol, methanol, acetic acid or acetate. Main aim of SMA is to investigate the activity of the selected sludge(Hussain and Dubey, 2015). Operating a known amount of substrate with a certain amount of the sludge provides proper estimations for initial solid concentration of reactors (Hussain and Dubey, 2015).

2.6 Dark Fermentation

2.6.1 Future fuel: Hydrogen energy

Hydrogen, also called the fuel of the future, has high energy content (122 kJ/g), which is at most 2.75 times higher than other hydrocarbons and it is one of the

most environmentally-friendly alternatives. Hydrogen, as a clean carbon-free fuel, generates only water as a by-product after combustion. Therefore, it is presented as an effective option in the search for solutions to global warming and increasing pollution problems. Compared to methane produced by anaerobic digestion, hydrogen energy has been preferred in recent years due to its widespread industrial use, generating no harmful by-products after combustion, and its higher energy capacity (Ghimire et al., 2015).

Hydrogen can be generated by electrolysis of water, thermal applications or biological processes. Globally, the most common method to produce hydrogen is steam reforming (Norskov and Christensen, 2006). According to reports in 2009, hydrogen was produced more than 1 billion m^3/day, being 48% from natural gas, 30% from oil, 18% from coal and 4% from water electrolysis (Mohan and Pandey, 2013). However, in this process, 96% of the H_2 source is still fossil fuel.

Generating hydrogen from biological ways enables handling many types of renewable waste sources (Levin et al., 2004). Microbial production of hydrogen can be practiced with photosynthetic and chemosynthetic bacteria. Photosynthetic bacteria generate hydrogen by photosynthesis or photolysis of water which are the traditional and energy-demanding options to gain hydrogen. On the other hand, chemosynthetic bacteria generate hydrogen with less energy requirement, by dark fermentation which is more environmentally friendly compared to photosynthetic processes (Ginkel and Logan, 2005). In addition, the H_2 production via dark fermentation occurs at a higher rate compared to its photosynthetic counterpart (Mohan et al, 2011). More importantly, dark fermentation does not require sterile conditions and can be conducted with mixed cultures unlike photofermentation (Mohan et al., 2011). Hence, hydrogen production with dark fermentation provides more feasible approach.

2.6.2 Dark fermentation Stages and Benefits

Dark fermentation is the conversion of organic matter into hydrogen gas, VFA and carbon dioxide by fermentation of anaerobic bacteria. This process is a form of anaerobic digestion, covers hydrolysis and acidogenesis stages of anaerobic digestion (Figure 2.2) (Ghimire et al., 2015). Dark fermentation is actually a part of anaerobic digestion, where methane formation is inhibited. After acitogenesis and acetogenesis step, microbial activity is inhibited not to produce methane (Figure 2.2). The main VFAs produced after dark fermentation are acetate and butyrate (Hawkes et al., 2007). From one mole of glucose, 4 moles of H_2 (544 mL H_2 /g hexose, 25℃) is produced with acetic acid fermentation and 2 moles of H_2 (272 mL H_2/g hexose, 25 ℃) with butyric acid fermentation, shown in Equation 2-5 (Section 2.5.1) and Equation 2-10, respectively (Ghimire et al., 2015).

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \qquad \text{(Equation 2-10)}$$

Many heterotrophic bacteria are capable of dark fermentation. They are mainly spore forming *Clostridium* species, facultative *Enterobacter* species, *Bacillus* species, *Thermoanaerobacterium* species and anaerobic acidogenic (Jo et al., 2008).

As mentioned in Section 2.5.1, dark fermentation has some advantages over other biological H_2 production methods. Compared to light intensive biological options, dark fermentation has a higher H_2 production rate (Levin et al., 2004). The external energy need is less because only mixing is applied. It is able to generate hydrogen continuously without light. Many different types of organic substrates are degradable in dark fermentation process. Besides, it provides beneficial end products like acetic acid, lactic acid and butyric acid (Kotay and Das, 2008). Simply, organic substrate treatment and H_2 production will be achieved in a suitable way.

2.6.3 Factors Affecting Dark Fermentation

Dark fermentation, especially with mixed cultures, depends on environmental aspects and operational parameters .The parameters of concern, especially in batch dark fermentation studies are, pH, temperature, substrate concentration and H_2 partial pressure (Li and Fang, 2007). The substrate type and its pretreatment method, enrichment types, microbial consortium source and reactor arrangements are also affect dark fermentative hydrogen production.

2.6.3.1 pH

pH is one of the crucial parameters in dark fermentation, because it affects directly microbial enzyme activity. It is agreed upon hydrogen studies that pH is the fundamental parameter and affects hydrogen production stages and end products (Craven, 1988). Since each enzyme works at a specific pH, it is important to regulate optimum value, not only for gaining high hydrogen yields with mixed cultures, but also designate end products and microbial community structure (Van Ginkel et al., 2001).

pH below 6 inhibits methanogenic activity for both thermophilic and mesophilic cases. However, inhibition of homoacetogens which degrade H_2 and CO_2 and produce acetic acid (Figure 2.2), starts at a pH of 5.5 under thermophilic conditions (Luo et al., 2011). The optimum pH range for hydrogen production with dark fermentation is given as 4.5-9.0 (Khanal et al., 2004; Lee et al., 2002). This wide range of optimum pH might be due to the substrate type studied, inoculum source or selected organic loading rate (Wang and Wan, 2009).

The optimum pH value for hydrogen production by dark fermentation from crop residues is 7.0 (Guo et al., 2010). In a study conducted with yard waste, dark fermentation was investigated in mesophilic environment (35℃) at pH 7.2 (Abreu et al., 2016). Similar literature results for lignocellulosic substrates are presented in Table 2.4.

Table 2.4 Reactor pH conditions for dark fermentation with lignocellulosic substrates

Substrate	pH range	Optimum pH	Reference
Yard Waste	_	7.2	Abreu et al. (2016)
Grass Silage	4-6	6.0	Karlsson et al. (2008)
Poplar leaves	4-7	7.0	Cui et al. (2010)
Wheat Straw	4-9	7.0	Fan et al. (2006)
Cassava Stillage	_	7.0	Bundhoo (2019)
Rice Straw	_	6.5	Chen et al.(2012)

2.6.3.2 Temperature

Temperature is an essential process parameter since it affects microbial metabolisms in mixed flora and hydrogen yield (Li and Fang, 2007). Temperature has an influence on transformation rate of end products in dark fermentation and complementary to economic improvements. Mixed anaerobic bacteria is very sensitive to temperature and their optimum process temperature was found to be 35 ℃ for methanogenesis (Zhang and Shen, 2006). Like other parameters, the type of substrate plays a role in determining the ideal temperature. Temperature increase within a proper range (20 to 70℃) will increase the hydrogen yield. However, too high temperature will decrease hydrogen production ability of hydrogenotrophic microorganisms (Wang and Wan, 2008).

Increase in temperature also prevents homoacetogenic activity which will prevent the consumption of H_2 produced via dark fermentation (Niel et al., 2002). Even higher temperatures improve dark fermentative hydrogen yield, providing thermophilic conditions in a reactor needs a high amount of external energy, which is not sustainable (Saady, 2013).

Some of the research studies investigate the effect of temperature on dark fermentation are listed in Table 2.5. Since lignocellulosic substrates have complex structure and their operational conditions are variable, there is no specified optimum temperature for hydrogen production (Ware and Power, 2017). Li et al.

(2007) presented that 73 studies out of 101 in hydrogen production were operated in mesophilic temperatures. Applied temperature range mostly appears as 25-40 ℃, which is mesophilic, to maintain economic feasibility.

Table 2.5 Temperature conditions for dark fermentation with lignocellulosic substrates

Substrate	Optimum T℃	Reference
Grass silage	35	Pakarinen et al. (2009)
Wheat straw	36	Fan et al. (2006)
Grass	35	Cui and Shen (2012)
Grass	35	
Rice straw	55	Chen et al. (2012)
Wheat straw	70	Mishra (1995)

2.6.3.3 Substrate Concentration

Especially in batch processes, substrate concentration should be determined properly. High concentrations of substrate will enable energy efficient process, but might cause inhibitions. When by-product limits are exceeded in hydrogen production, microbial activity is affected (Jung et al., 2011).

Not only substrate inhibition, but also substrate limitation will reduce hydrogen production efficiency (Fan et al., 2004). A study conducted with beer lees showed that cumulative hydrogen yield reached its maximum value when the substrate concentration is between 5-20 g/L and further increase in concentration decreased hydrogen yield gradually (Fan et al., 2004). Another study conducted with varying sucrose amounts (0.5-44.8 g COD/L) showed that, conversion efficiency decreases when substrate concentration in the reactor increases (Ginkel et al., 2001). A similar H_2 yield inhibition beyond 44.8 g COD/L was observed with sucrose in a study where an initial COD range of 10-60 g/L was applied in a continuously stirred tank reactor (CSTR). In this study, initial concentration of 30 gCOD/L supported the highest hydrogen yield of 1.09 mole H_2/ mole $hexose_{added}$ (Kim et al., 2006).

Literature studies tend to increase hydrogen yield with higher initial substrate concentrations. Improved hydrogen yields are obtained with faster reaction rates and smaller volumes of reactors requiring smaller heating energy. According to literature studies, VFAs presence interferes with hydrogentrophic dark fermentation, either due to undissociated acids or hydrogen degradation by homoacetogens (Wainaina et al., 2019). Moreover, pretreatment applications may produce some by-products such as furfural which inhibits hydrogen producing bacteria (Cui et al., 2010). Meantime, increase in initial COD concentration will lead to decrease in hydrogen yield (Ezeji et al., 2004; Fan et al., 2004) and hence reduce the substrate cost. H_2 partial pressure is also an important parameter to investigate for inhibitory situations, mentioned in Section 2.6.3.4. Thus, further investigations should be done in order to identify the optimum solids concentration to improve hydrogen yield and reduce feedstock cost.

2.6.3.4 H_2 Partial Pressure

Partial pressure of hydrogen (pH_2) in the reactor headspace is a critical parameter in the hydrogen production from organic substrates (Guo et al., 2010). Increased pH_2 inhibits hydrogen-producing microorganisms and makes this process thermodynamically improper. High pH_2 support homoacetogenesis, which is simply conversion of present carbon dioxide and hydrogen to acetate, hence reactor performance might decrease. As pH_2 in the liquid phase increases originated from biological processes, both hydrogen yield will be influenced and solventogenesis will be observed due to the changes in metabolic pathways. Solventogenesis cause accumulation of alcohols, resulting decrease in hydrogen production performance (Tunçay, 2015). As H_2 increase in the system, hydrogen production decreases and metabolic pathway tends to produce more reduced materials like butane, alanine, ethanol, lactate and acetone. However, when the process temperature increases, pH_2 affects hydrogen production less (Tamagnini et al., 2002). Acetogenesis can

also consume produced hydrogen, which is related to the concentration change in carbon dioxide pressure (pCO_2) which might affect the system (Park et al., 2005).

Most common approaches to decrease headspace and medium pH_2 and pCO_2 are continuous or intermittent sparging with an inert gas (Kim et al., 2006), agitation (Chou et al., 2008) and CO_2 sequestration and hydrogen permeable membrane application (Liang et al., 2002). These approaches are able to increase hydrogen production rate in two or even four folds (Chou et al., 2008).

In order to enhance hydrogen production performance, pH_2 should be decreased in the reactor headspace (Park et al., 2005). There are various approaches in the literature to inhibit hydrogen accumulation in the headspace. As stated above, purging with an inert gas such as N_2, used to remove H_2 gas from the headspace. 80% of hydrogen production increase was obtained with purging N_2 to the headspace (Mizuno et al., 2000). The main drawback of purging is diluting produced hydrogen gas in the system, which causes an unfeasible fermentation process (Logan et al., 2002). Continuous or semi-continuous operations has higher hydrogen yield results compared to batch reactors. The reason might be the increase in pH_2 in batch systems. Another study investigated the hydrogen pressure release with respect to total hydrogen production by decreasing headspace pressure (Logan et al., 2002). Respirometric process, which includes releasing the headspace gas continuously with a bubble-measuring tool, is found to be more effective than intermittent process, which involves removing headspace gas with syringe regularly, also called oven method. Respirometric method enables keeping headspace pressure in the proper level. Respirometric method was found to increase hydrogen production by 43% compared to intermittent option (Logan et al., 2002).

Homoacetogenesis is still a serious challenge to overcome. Researches achieved to increase hydrogen yield by decreasing pH_2. It is suggested that CO_2 scavenging is the easiest method to avoid homoacetogenesis. However, complete scavenging of CO_2 from headspace for high-rate hydrogen production is not achievable and

adjusting a chemical scavenger in a continuous system is difficult. pH_2 and pCO_2 effects on hydrogen production from dark fermentation needs further examination (Saady, 2013).

2.7 One-stage and Two-stage Anaerobic Systems

Conventional anaerobic digestion which covers the four sequential steps given in Figure 2.2 (Section 2.5.1) is also called one-stage anaerobic digestion (OSAD). Two-stage anaerobic digestion (TSAD) is cumulatively producing hydrogen by dark fermentation, followed by methane production via methanogenesis (Pakarinen et al., 2008).

In OSAD, hydrogen cannot be observed since it is degraded immediately to produce CO_2 and CH_4 (Li and Li, 2019). However, H_2 can be generated individually by maintaining the proper engineering conditions, for example through dark dermentation of TSAD. Lately, hydrogen production through dark fermentation gained interest. The primary drawback of dark fermentative hydrogen production is low energy recovery. Reason is that, fermentative hydrogen production process is not able to reduce organic substrate totally. COD removal is below 20%, which gives yield amount between 10-20% (Das and Veziroğlu, 2001). Main portion of energy of substrate is present as VFA after dark fermentation. So, these VFAs need to be utilized further to gain maximum energy recovery (Hans and Kumar, 2019). In order to convert all organic acids generated during dark fermentation further to another form of energy and increase overall energy recovery, TSAD process is suggested (Li and Li, 2019; Pakarinen et al., 2008).

Hydrogen energy has been a promising alternative due to its high energy density 142 kj/kg compared to other fuels (Wang et al., 2016). Its transportation is easier and no greenhouse gas emission occurs after combustion (Ntaikou et al., 2010). CH_4 has the second highest energy content (55kj/kg) after H_2 (Pakarinen et al., 2009). A schematic description of TSAD is shown in Figure 2.3, below.

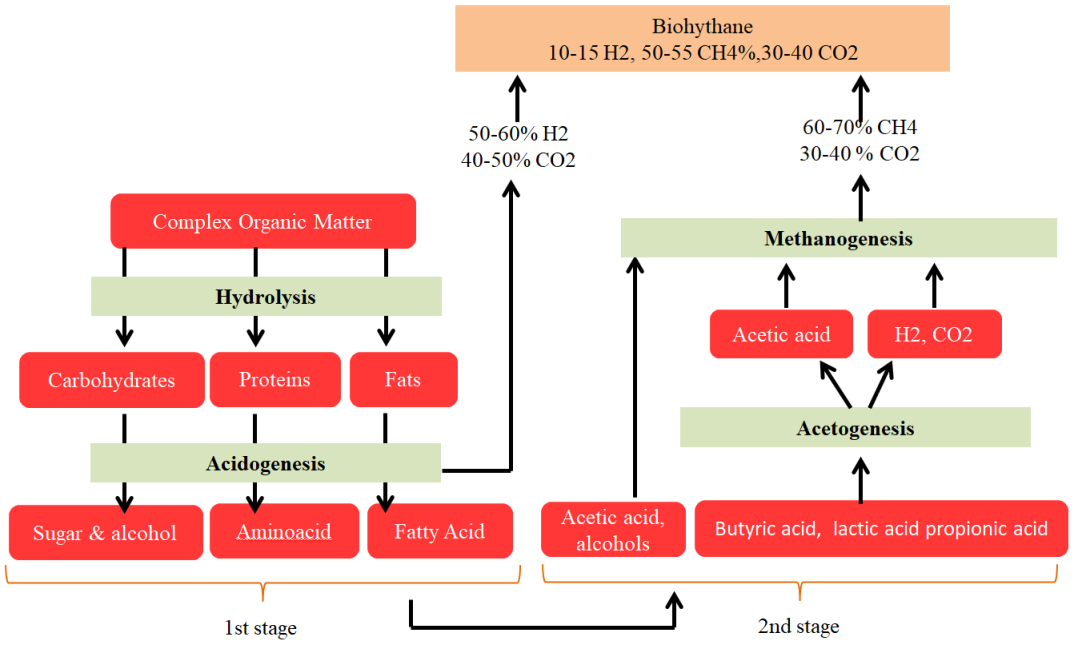

Figure 2.3 TSAD process steps, adapted from O-Thong et al. (2016)

With the help of TSAD design for co-production of H_2 and CO_2 together, an efficient procedure will be achieved, alternative to their separate production. Individual acidogenic and methanogenic processes in anaerobic digestion enhances stability of mixed cultures in the inoculum and enhanced system management (Demirel and Yenigün, 2002). The aim of TSAD is both proper waste degradation and more energy gain from the substrate. In OSAD process, many higher organic acids like butyric acid, lactic acid, propionic acid, ketones and alcohols are formed by acidogens during fermentation. After all, in a well operated system, these organic products are converted into methane gas (Cooney et al., 2007). On the other hand, in TSAD process, the end products from acidification stage using lignocellulosic substrate are beneficial for anaerobic digestion with VFA which can reach up to 25.9 gCOD/L (Nasr et al., 2011; Pavan et al., 2000). In another study, with TSAD, 13% methane yield increase compared to OSAD , which provides 0.31 mL/gCOD removal with lignocellulosic material (Viñas et al., 1993). Moreover,

another study showed that 10% overall yield increase (for H_2 and CH_4) is achieved with TSAD process with olive mill solid residue (Rincón et al., 2009).

In addition to the advantages such as improved energy yields and higher degradation efficiencies, one other advantage can be stated as the formation of biohythane. Biohythane is defined as a fuel composed of H_2 (5-20%) and CH_4 (80-95%) (Liu et al., 2013). TSAD systems receive high interest lately for their being a potential source of biological hythane (biohythane) production system (Mota and Zaiat, 2018). Biohythane is advantageous compared to sole H_2 because COD removal efficiencies can be achieved (Mota and Zaiat, 2018). Biohythane is also advantageous compared to sole CH_4 because higher yields can be achieved and burning hythane is cleaner than methane alone (Kumari and Das, 2019). For being a combination of both H_2 and CH_4, it has the advantages of both energy fuels, thus it is worth investigating it and TSAD systems.

Although TSAD systems provide working at higher loading rates, enhance process stability and flexibility, and have the advantages mentioned above, there are few units constructed on the field. Complexity and feasibility of building and operating TSAD systems are still a contradiction against their yield and production rate enhancements (Rapport, 2008).

2.8 The Anaerobic Digestion of Yard Waste

In recent years, the use of grass as a solid fuel in biogas production and combustion has been seriously considered (Olabi, 2010; Rodriguez et al., 2017). Energy recovery from anaerobic digestion with yard wastes, which is hardly biodegradable than this lignocellulosic substrate, i.e. grass, is generally studied in single-stage laboratory-scale systems. In Germany, 30-40% of the biogas plants, on average 8% by mass and in some cases up to 50%, use yard waste as co-substrate (Rodriguez et al., 2017). However, in Turkey's National Waste Management Action Plan, the use of yard waste in the composting unit, being mixed with solid waste generated after

anaerobic digestion, is presented among the integrated waste management models (Ministry of Environment and Forestry, 2019). In other words, the use of yard wastes in biogas plants is not foreseen. This may be due to the low biogas potential of yard waste due to its lignocellulosic structure (Bedoić et al., 2019). However, as mentioned in Section 2.7, considering the amount of energy consumed during the production of energy crops for bioenergy production, since yard wastes are produced without any energy consumption. Thus they become much more advantageous compared to energy plants and agricultural residues, even if biogas energy is low in life cycle perspective. Yard waste applications have been supported not only for its abundance but also its contribution to reduce greenhouse gas emission, encourage regional economies, providing reliable biomass energy sources and limit the competition with food sources (Coyle, 2011). Some methane yield results obtained from anaerobic digestion with lignocellulosic substrates are given in Table 2.6.

Table 2.6 Literature data examples for methane yields of lignocellulosic substrates

Substrate	Methane Yield (mL CH_4 /g VS)	References
Yard waste	209	Owens et al. (1993)
Grass	230	Lehtomaki et al. (2004)
Grass	300	Lehtomaki et al. (2008)
Switch grass	125	Guiot et al. (2009)
Wheatgrass	160	Romano et al. (2009)
Barley straw	229	Dinuccio et al. (2010)
Leaves	100	Bharathiraja et al. (2016)
Leaves/trimmings	118	Bharathiraja et al. (2016)
Grass	320	Dahunsi (2019)

As seen in Table 2.6, grass has a high methane potential range between 230-320 mL/g VS. Yard wastes on the other hand, results in relatively lower yields, 118-209 mL /g VS, after their anaerobic digestion, which is still quite promising. Yet, it should be noted that some of the data presented in Table 2.6 reveals the yields of yard wastes and other lignocellulosic wastes after their pretreatment. Thus, the methane yield values obtained are expected to be lower.

Various complex organic substrates consisting of proteins, carbohydrates and lipids are available to be used in hydrogen production as feedstock as well. Yet, substrates, which has high amounts of carbohydrates are more preferable due to their higher degradation rates compared to lipids and proteins. Substrates which are rich in carbohydrates are able to produce higher biogas composition than lipids and proteins (Okamoto et al., 2000), as expected.

Lignocellulosic substrates are promoted among other biomass sources due to their global abundance and lower cost. Promising hydrogen yield values such as 2.24 mol H_2/mol hexose, have been obtained with hydrogen production of lignocellulosic substrates (Cheng et al., 2011). Several hydrogen yield results from dark fermentation of lignocellulosic substrates are presented in Table 2.7. Since dark fermentative hydrogen production studies are limited with yard waste mix, similar lignocellulosic substrates are given.

Table 2.7 Hydrogen yields obtained from lignocellulosic substrates in the literature

Substrate	T(°C)	Yield (mL H_2/g VS)	Reference
Poplar leaves	35	15-45[a]	Cui et al. (2010)
Grass Silage	35	6	Karlsson et al. (2008)
Grass Silage	70	16	Karlsson et al. (2008)
Leaves	70	10.	Ivanova et al. (2009)
Grass	35	72	Cui and Shen (2012)
Cassava stillage	60	68	Luo et al. (2010)
Grass silage	70	16	Pakarinen et al. (2008)
Garden waste	70	98.	Abreu et al. (2016)
Wheat straw	35	68	Fan et al. (2006)
Maize leaves	35	17	Ivanova et al. (2009)
Sweet sorghum	35	30.5	Ivanova et al. (2009)
Ryegrass	35	73	Kyazze et al. (2008)
Fodder turnip	35	188	Monlau et al. (2013)

[a]mL/g TS

As mentioned in Section 2.7, hydrogen and methane production via TSAD process using organic wastes is one of the hot topics in anaerobic digestion studies due to its higher energy recovery outcomes and being a cleaner energy source (Thong et

al., 2016). There are various organic waste types used in TSAD processes such as wheat straw, food waste and organic solid waste (Xie et al., 2014). Related literature yield results are given in Table 2.8.

Table 2.8 Yield results of TSAD studies obtained from similar lignocellulosic substrates[a]

Substrate	H_2 Yield (mL H_2/g VS)	CH_4 Yield (mL CH_4/g VS)	[a]Reference
Wheat bran	18.9	243.5	Corneli et al. (2016)
Corn Stalk	80	227	Guo et al. (2014)
Water hyacinth	7.5	93	Chuang et al. (2011)
Poplar wood extrusion	40	299	Akobi et al. (2016)
Sweet Sorghum	10.7	107	Antonopoulou et al.(2008)
Cornstalk	7.8 17.3	13.1 11.8	Lu et al. (2009)
Napier grass	27.7	170	Prapinagsorn et al. (2018)

[a]All studies performed in batch reactors

As seen in Table 2.8, yard wastes which are the mixture of grass and yard trimmings are not specifically investigated for their H_2 production and/or H_2/CH_4 potential in TSAD systems. Lignocellulosic wastes such as forestry wastes and yard wastes should be also used in energy production in anaerobic digestion systems rather than being sent to landfills or left on sites as waste. This initiative might be a solution for the environmental problems occurring at landfills. Indeed, the information on the yard waste mix's CH_4/H_2 yield is very limited.

CHAPTER 3

MATERIALS AND METHODS

In this chapter, information about seed sludge and collected yard wastes, details on applied pretreatment options, the experimental batch set-up designs for one-stage and two-stage anaerobic digestion processes, their operational conditions and analytical methods are covered.

3.1 Seed Sludge

Seed sludge applied as inoculum in all experiments was obtained from the effluent lines of the anaerobic digesters in Ankara Central Domestic Wastewater Treatment Plant. Seed sludge characteristics are given in Table 3.1. In OSAD experiments and second-stage (i.e. methanogenesis step) of TSAD experiments, seed sludge was used without any pretreatment. In dark fermentation (i.e. first-stage of TSAD experiments), in order to eliminate methanogens and put acidogenic hydrogen producers forefront (Guo et al., 2010), heat treatment was applied. With this method, hydrogenothrophic methanogens are inhibited and hydrogen producers like *Clostridium* are promoted (Kim et al., 2006). For this aim, seed sludge was heat-treated at 105 ℃ for one hour (Ozkan et al., 2010). TS and VS content of the seed sludge (pretreated and not pretreated) used in expeiment are given in Table 3.2.

Table 3.1 Seed sludge characteristics

Parameter	Value
TS (g/L)	6.60 ±0.2
VS (g/L)	3.37 ±0.1
VS (%) (%TS)	51.13 ±1.2
pH	7.6
COD (g/L)	6790±140
sCOD (g/L)	320±211
Total N (mg N/L)	522±2
TAN (mg N/L)[a]	242±5
Total P (mg P /L)	65.5±1

[a]TAN: Total Ammonia Nitrogen: NH^{+}_{4}-N+NH_3-N

Table 3.2 TS and VS values of seed sludge used in each experiment

Experiments	Concentration (mg/L)	
	TS	VS
One-Stage Without Pretreatment	6600±189	3370±132
One-Stage With Alkaline Pretreatment	6600±189	3370±132
One-Stage With Thermal Pretreatment	34800±283	10000±549
One-Stage With Ultrasound Pretreatment	34800±283	10000±549
Two-Stage Without Pretreatment	24050±328	10683±425
Two-stage With Alkaline Pretreatment	24050±328	10683±425
Two-Stage With Thermal Pretreatment	24050±328	10683±425
Two-Stage With Ultrasound Pretreatment	24050±328	10683±425

3.2 Basal Medium

Macro- and micro-nutrients necessary for an optimal microbial growth were added into the reactors via basal medium. Composition and concentrations of the Basal medium in OSAD reactors and methanogenesis step of TSAD reactors are as follows : NH_4Cl (1200 mg/L), $MgSO_4$.7H2O (400 mg/L), KCl (400 mg/L), Na_2S.9H2O (300 mg/L), $CaCl_2.2H_2O$ (50 mg/L), $(NH_4)2HPO_4$ (80 mg/L), $FeCl_2.4H_2O$ (40 mg/L), $CoCl_2.6H_2O$ (10 mg/L), KI (10 mg/L), $(NaPO_3)_6$ (10 mg/L), $MnCl_2.4H_2O$ (0.5 mg/L), NH_4VO_3 (0,5 mg/L), $CuCl_2$.$2H_2O$ (0.5 mg/L),

$ZnCl_2$ (0.5 mg/L), $AlCl_3.6H_2O$ (0.5 mg/L), $Na_2M_oO_4.2H_2O$ (0.5 mg/L), H_3BO_3 (0.5 mg/L), $NiCl_2.6H_2O$ (0.5 mg/L), $Na_2WO_4.2H_2O$ (0.5 mg/L), Na_2SeO_3 (0.5 mg/L), Cysteine (10 mg/L) and $NaHCO_3$ (6000 mg/L) (Speece, 1996).

Composition and concentrations of basal medium used in dark fermentation experiments are as follows: $MgSO_4.7H_2O$ (400 mg/L), $FeCl_2.4H_2O$(40 mg/L), KH_2PO_4 (400 mg/L), K_2HPO_4 (400 mg/L), Cysteine (10 mg/L) and NH_4Cl (400 mg/L) (Tunçay, 2015).

3.3 Substrate Characteristics

Yard wastes, which were already sundried were obtained from METU Campus within the duties of METU Directorate of Construction and Technical Works. Proper amounts of grass and yard trimmings were provided from this collection zone in September 2018. Collected samples are shown in Figure 3.1. First of all, foreign objects like plastic, branch and metal were sorted. Then, grass and yard trimming wastes were dried at 40℃ for 24 hours in order to reduce moisture content to less than 10% (Lin et al., 2014). Size of yard wastes was reduced with food blender (Sinbo SHB 3042, Turkey), ground in hammer mill and then screened from 20 mesh screen sieve (W.S. Tyler Incorporated, Ohio, USA). After that, grass and yard trimming wastes were mixed with 1:1 ratio (w/w), sealed in plastic bags and stored at -20℃ in order to prevent biological activity prior to the use in this study. Before usage, yard waste mix were thawed at room temperature (Prapinagsorn et al., 2018). The properties of yard waste are shown in Table 3.3.

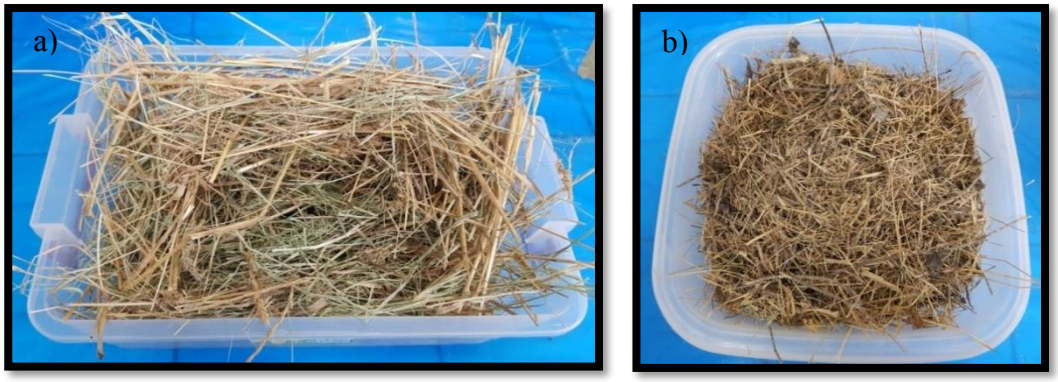

Figure 3.1 Sun dried a) yard trimmings, and b) grass clippings

Table 3.3 Characteristics of yard waste mix used in the experiments

Parameter	Value
TS (g/g)	0.93±0.01
TS (%)	93±0.70
VS (g/g)	0.81±0.01
VS (%TS)	0.87±0.30
COD (g/g)	1.17±0.23
sCOD (mg/L)	0.07±0.01
TN (g/g)	0.01±0.01
TN (%)	0.33±0.85
TAN (g/kg)[a]	0.60±00
Total P (g/kg)	0.39±0.03
pH	6.4
Cellulose (%)	49±2
Hemicellulose (%)	19±5
Lignin (%)	23±2

[a]TAN: Total Ammonia Nitrogen: NH^{+}_{4}-N+NH_3-N

3.4 Pretreatment of Yard Waste Mix

In order to define a proper pretreatment alternative for yard waste mix (50% grass and 50% yard trimmings), commonly applied options for yard wastes were examined, and alkaline pretreatment, thermal pretreatment and ultrasound

pretreatment were selected to study in this thesis work due to their ability to reduce lignin content, increase solubility and enhance microbial degradation with lignocellulosic materials (Zheng et al., 2014; Morten et al., 2014; Bundhoo et al., 2017; Rafieeniaet al., 2018).

Yard waste mix initial TS% was adjusted to 2, 5 and 8% in every pretreatment set-up. Beakers with 300 mL effective volume were used in each option. After pretreatment, obtained mixes were not filtered and used as the carbon source in the following OSAD and TSAD experiments. Before used in the experiments, fair amount of pretreated mixes were saved for compositional analysis, which are TS, VS, soluble lignin, hemicellulose and cellulose, TP, TKN, COD and sCOD. These analyses were conducted in duplicates.

Alkaline Pretreatment: For alkaline pretreatment (AP), 1 gram of dried and sieved (with 20 mesh screen) yard waste mix was treated with 20 mL 2% (w/v) NaOH solution (Ozkan et al, 2011). pH was fixed at 12. This mix was heated at 45℃ for 30 minutes on heater. At the end of the procedure, pH values of pretreated yard waste mix was brought to neutral level with HCl (Ozkan et al., 2011).

Thermal Pretreatment: For thermal pretreatment (TP), 1 gram of dried and sieved yard waste mix was solubilized in 10 mL distilled water and heated at 120℃ under 1.5 atm pressure in autoclave for 30 minutes (Ozkan et al., 2011; Li et al., 2014).

Ultrasound Pretreatment: Ultrasound pretreatment (UP) was done with an ultrasound bath (Sartorius Labsonic-P). The maximum power input of this sonicator was 400 W and sonication frequency was 24 kHz. 1 gram of dried and sieved yard waste mix was mixed with 40 mL distilled water. Without any temperature arrangement, sonication was applied for 15 minutes. (Tiehm et al., 2001; Apul and Sanin, 2010).

3.5 Experimental Procedure

In one-stage and two-stage anaerobic digestion experiments, it was aimed to investigate the effect of different pretreatment methods and different initial TS contents (2%, 5%, 8%) on methane and/or hydrogen yields, in other words energy yield. The reason for performing the different initial solid percentages is to determine the optimum initial amount of yard waste for effective anaerobic digestion and biogas production.

3.5.1 One-Stage Anaerobic Digestion (OSAD) Experiments

For OSAD experiments, batch reactors with a total volume of 110 mL and effective volume of 60 mL were used. Experiments were performed with four different batch set-ups, and each set-up differed in the type of yard waste used, i.e. either non-pretreated or one of the pretreated yard waste (AP-, TP- or UP-yard waste). Each set-up contained control, blank and test reactors. In each set, all three initial TS contents were studied. In other words, blank and test reactors were conducted with three different initial TS%. All test reactors contained basal medium (BM) (Section 3.2), one of the four yard waste mix type (NP-, AP-, TP- or UP-yard waste) with certain TS amounts (2%, 5% or 8%) and seed sludge. Control reactors contained all (seed+BM), but not the yard waste mix; while blank reactors contained all (yard waste+BM), but not the seed sludge. Reactor contents are given in Appendix A. S/I was set as 1. pH values were brought to neutral level with HCl and (or) NaOH to maintain microbial activities at optimum conditions. Lastly, after sealing with rubber stoppers, in order to provide anaerobic environment, all reactors were flushed with 100% N_2 gas for 5 minutes, then placed on a shaker with 175 rpm in a hot room at 35±2°C. Daily biogas production and gas composition analyses were performed during incubation period in order to determine the methane production potential of the reactors.

3.5.2 Two-stage Anaerobic Digestion (TSAD) Experiments

TSAD experiments are composed of two steps, each containing 4 batch reactor set-ups. The first-stage of TSAD experiments cover the dark fermentation batch experiments, while second-stage TSAD experiments cover the methanogenesis stage batch experiments, where the influent is the effluent of the previous stage, i.e. dark fermentation. An additional Mini TSAD experiment was also conducted In order to recheck obtained cumulative hydrogen and methane yield results with AP and TP-yard wastes at 2% solids concentration (Appendix G).

3.5.2.1 First Stage of TSAD: Dark Fermentation Experiments

In the first-stage of TSAD experiments, batch reactors with a total volume of 110 mL and effective volume of 60 mL were used. Similar to OSAD experiments, dark fermentation experiments were also performed with four different batch set-ups. Each set-up differed in the type of yard waste used, i.e. either non-pretreated or one of the pretreated yard waste (AP-, TP- or UP-yard waste). Each set-up contained control, blank and test reactors. In each set, all three initial TS contents were studied.In other words, blank and test reactors were conducted with three different initial TS%. All test reactors contained basal medium (BM) (Section 3.2), one of the four yard waste mix type (NP-, AP-, TP- or UP-yard waste) with certain TS amounts (2%, 5% or 8%) and seed sludge. Control reactors contained all (seed+BM), but not the yard waste mix; while blank reactors contained all (yard waste+BM), but not the seed sludge. Seed sludge was initially heat pretreated for 1 hour at 105 ℃.

The initial pH values in the reactors were adjusted to 7 with HCl and/or NaOH to mimic the suitable conditions for dark fermentation, which were determined in a preliminary study (Appendix B). The optimum pH range for hydrogen production with dark fermentation is given as 4.5 to 9 (Khanal et al., 2004; Lee et al., 2002). Research studies defend the opinion that hydrogen production needs acidic pH

conditions, yet, optimum range still depends on the substrate. Applied pH values for hydrogen production from lignocellulosic compounds are given in Table 3.4. As seen in Table 3.4, optimum pH can range between 4.5-7.2. Dye to this uncertainty, the preliminary dark fermentative hydrogen production study was conducted with yard wastes at initial pH values of 5.5 and 7. S/I values of 4 and 8 were also investigated at pH 7. The results showed that the highest production was obtained at pH 7 and S/I of 4 (Appendix B, Figure B-1). Accordingly, as mentioned before, initial pH values of the reactors were set to 7. S/I was set to 4.

Table 3.4 Applied pH values for hydrogen production from lignocellulosic substrates

Substrate	pH	Reference
Grass silage	6.0	Pakarinen et al.(2009)
Napier grass	6.0	Prapinagsorn et al.(2018)
Yard waste	7.2	Abreu et al.(2016)
Grass	7.0	Cui and Shen(2012)
Sugarcane bagasse	5.5	Pattra et al.(2008)
Rice slurry	4.5	Fang et al.(2006)
Wheat bran	5.0	Noike and Mizuno(2000)

Lastly, after sealing with rubber stoppers, reactors were flushed with 100% N_2 gas for 5 minutes, placed on a shaker with 175 rpm in a hot room at 35±2°C. Daily biogas production and gas composition analyses were performed during incubation period in order to determine the hydrogen production potential of the reactors.

3.5.2.2 Second-stage of TSAD: Methanogenesis Experiments

In the second-stage of TSAD experiments, dark fermentation reactors were filtered and these filtrates were used as substrate in methanogenesis stage. Batch reactors, which have total volume of 50 mL and effective volume of 30 mL were used. Proper amount of seed sludge and BM were added in order to arrange S/I as 1 in the reactors. The pH values were brought to neutral level (7-7.5) with HCl and (or)

NaOH to maintain microbial activities at optimum conditions. Lastly, after sealing with rubber stopper, reactors were flushed with 100% N_2 gas for 5 minutes, placed on a shaker with 175 rpm in a hot room at 35±2°C. Daily biogas production and gas composition analysis were performed during incubation period in order to determine the methane production potential of the reactors.

3.6 Analytical Methods

Solids Content

TS and VS content of yard waste mix and sludge were determined according to Standard Methods (2540 B, 2540 E) (APHA, 2005). Suspended Solids (SS) and Volatile Suspended Solids (VSS) analysess were conducted according to Standard Methods (2540 D) (APHA, 2005).

Chemical Oxygen Demand (COD)

COD content of yard waste and seed sludge was determined according to EPA approved digestion method (COD range of 0-1500 mg/L), heating was applied by Aqualytic AL 38 heater Spectrophotometric analysis was then conducted with PC Multidirect Spectrophotometer.

Soluble Chemical Oxygen Demand (sCOD)

For the determination of sCOD of raw yard waste,1 g sample was initially mixed in 20 mL distilled water for an hour, then filtered through 0.45 µm pore size glass fiber filters (Millipore). For pretreatment and characterization studies, samples were directly filtered through 0.45 µm pore size glass fiber filters. Filtered samples were then analyzed for their sCOD content by EPA approved digestion method (COD range of 0-1500 mg/L) heating was applied by Aqualytic AL 38 heater Spectrophotometric analysis was conducted with PC Multidirect Spectrophotometer.

Total Kjeldahl Nitrogen (TKN)

TKN of yard waste mix and seed sludge samples was determined according to Standard Methods (4500-Norg B) (APHA, 2005).

pH

Before pH analysis, 10 mL distilled water was add to 1 g raw yard waste mix sample, covered and stirred for 5 minutes. Later on, substrate was allowed to stand for 1 hour, which provides suspended substrate to settle out from the suspension (EPA-Method 9045D). pH values of seed sludge samples were directly measured from main source. pH was determined using pH meter (Mettler Toledo 33111). Calibration of the pH meter was done with pH 4, pH 7 and pH 10 buffer solutions.

Total Phosphorus (TP)

TP values of yard waste mix and seed sludge samples were determined according to Standard Methods (4500-P B and 4500-P E) (APHA, 2005).

Lignin, Cellulose and Hemicellulose

Lignin content of raw and pretreated yard waste mix samples were determined according to two step acid hydrolysis method generated by National Renewable Energy Laboratory (Sluiter et al., 2011). Cellulose and hemicellulose contents of non-pretreated and pretreated yard waste mix were measured with Neutral Detergent Fiber and Acid Detergent Fiber analysis, respectively (Goering and Van Soest, 1970).

Biogas Production

Water displacement device was used in order to determine biogas production from reactors. This device contains a 500 mL water reservoir connected to 50 mL burette. At the end of this system, there is a fine needle, which is connected with plastic tubing to the burette. This needle was inserted through the rubber stoppers of the reactors into the headspace, in order to measure the biogas produced amd captured in the headspace.

Biogas Composition

Biogas compositions were periodically investigated with a gas chromatograph (GC) (Thermo Electron Co.) equipped with a thermal conductivity detector (TCD). Injected sample biogas was separated as H_2, CO_2, O_2, CH_4 and N_2 by using serially connected columns (CP- Moliseve 5A and CP- Porabond Q) at a fixed oven temperature of 45 ºC. Helium was used as carrier gas at 100 kPa constant pressure. The injector, detector and oven temperatures were set to 50 ºC, 80 ºC and 35ºC, respectively. GC calibration was done immediately after every change in the device, such as; septum change, gas tube change, colon adaptation, conditioning or after a power failure. Calibration curves used for GC analysis are given in Appendix C.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter covers the results and discussion of the three experimental steps, namely, pretreatment studies, one-stage anaerobic digestion (OSAD) experiments and two-stage anaerobic digestion (TSAD) experiments.

4.1 The Results of Pretreatment Studies

For the pretreatment step, three different pretreatment alternatives were applied to raw yard waste mix, and their characterization experiments were conducted. The physical and chemical compositions of yard waste mix before and after each pretreatment are shown in Table 4.1. As seen in Table 4.1, all characterization results for raw yard waste are similar with earlier literature studies conducted with lignocellulosic substrates (Apendix D, Graph D.1). The raw yard waste mix used in this thesis study was found to have a VS content of 81 mg/g which is in the rage given for yard waste as 24-97 mg/g. Moreover, according to literature results, similar lignocellulosic materials have a sCOD range as 0.12-0.23 g/g (Appendix D, Table D.1). The raw yard waste mix used in this thesis study was found to have a celloluse, hemicellulose and lignin content as 20%, 19% and 26%, respectively (Table 4.1) which is in the rage given for similar lignocellulosic materials for celloluse, hemicellulose and lignin as 15-40%, 22.5-85% and 10-30%, respectively (Table 2.2). Used yard waste mix in this study is closer to the upper range limit in terms of lignin content. On the other hand, cellulose and hemicellulose contents are comperatively lower in the mentioned ranges of literature studies. Since cellulose and hemicellulose are the two compounds needed for microbial degradation in lignocellulosic materials, their availability should be increased.

To enhance the availability of cellulose and solubilisation are main aims of these pretreatments applications. Especially for yard waste, lignocellulosic structure is difficult to be degraded in anaerobic digestion, which can also be explained by low solubility results such as 0.13 mg/g (Khor et al., 2018; Antonopoulou et al., 2008). To eliminate the lignin and increase the availability of hemicellulose and cellulose and increase the porosity of the substrate are also driving forces of pretreatment applications (Kumari and Singh, 2018).

Solubilisation efficiency can be defined as the difference between the ratio of sCOD/COD of pretreated and non-pretreated samples (Appendix E). According to calculations, solubilisation efficiencies were found as and 86%, 88% and almost 65% for AP, TP and UP, respectively. It can be said that, alkaline and thermal pretreatments have similar and much higher solubilisation effect compared to ultrasound pretreatment. In the study conducted with organic fraction of municipal solid waste (OFMSW) including yard waste achieved 11.5% solubilisation efficiency with AP (López et al., 2008). Another study with garden waste showed that AP enhances solubility efficiency by 20%, while, TP achieved 153% of solubility enhancement with same substrate (Arici et al., 2013). Shetty et al. (2017) studied AP-rice straw which provided 80% solubilisation efficiency increase. UP increased solubilisation efficiency as 13% for olive mill effluent (Oz and Uzun, 2015). So it can be said that, solubilisation efficiency depends on the substrate, applied technique and the conditions of that technique. However, when the yard waste is considered, solubility ratios are comparable with similar literature studies which has a range as 20-153%.

Table 4.1 Physical and chemical compositions of yard waste samples[a]

Parameters	NP	AP	TP	UP
TS (g/g)	0.93±0.1	0.98±0.1	0.66±0.1	0.94±0.1
TS (%)	93.00±0.7	98.67±0.1	66.19±1.2	94.06±4.2
VS (g/g)	0.81±0.1	0.53±0.1	0.41±0.1	0.80±0.1
VS (%TS)	87±0.50	52.4±0.3	62.0±4.4	85±3.2
COD (g/g)	1.17±0.2	0.85±0.1	0.33±0.1	0.67±0.1
sCOD (g/g)	0.07±0.1	0.36±0.1	0.17±0.1	0.11±0.1
sCOD/tCOD	0.06 ±0.1	0.43±0.1	0.51±0.1	0.17±0.1
TN (mg/g)	4.42±2.4	5.48±1.1	5.72±1.6	5.19±0.1
TN (%)	0.44±0.1	0.55±0.1	0.57±0.1	0.52±0.1
TAN (mg/g)	0.53±0.4	1.83±0.4	2.14±0.3	2.06±0.3
TAN (%)	0.06±0.1	0.18±0.1	0.24±0.1	0.20±0.1
TAN/TN (%)	12	33.3	37.5	39.7
Total P (mg/g)	0.39±0.1	0.73±0.2	2.17±0.1	1.20±0.3
pH	6.40	7.84	7.96	7.82
Cellulose (%)	20.1	53.11	43.83	59.36
Hemicellulose (%)	19	4.4	13.7	17.28
Lignin (%)	26.5±0.4	17.2±0.6	29.17±1.2	27.36±1

[a] NP: Non-Pretreatment, AP: Alkali Pretreatment, TP: Thermal Pretreatment, UP: Ultrasound Pretreatment.

In lignocellulosic materials, like yard waste, cellulose is surrounded by hemicellulose and lignin, which creates recalcitrant structure avoiding microbial and enzymatic attacks (Himmel et al., 2007). In this sense, reducing lignin and hemicellulose, and increasing soluble cellulose amount is beneficial for anaerobic digestion. Microbial activity, hence methane yield will be affected positively unless there is inhibitory compound production. Cellulose amount of NP-yard waste mix, which is 20%, increased after AP, TP and UP to 53% , 44% and 60%, respectively (Table 4.1). Lignocellulosic compositions of pretreated yard wastes have similar results with literature (Section 2.3, Table 2.2). Enhanced cellulose availability is one of the indicators of pretreatment accomplishments. Hemicellulose content decreased from 19% for NP-yard waste to 4.5%, 14% and 17% after AP, TP and UP, respectively, which is also expected. On the other hand, lignin content of NP-yard waste, which is 26.5%, were measured as 17%, 29% and 27% after AP, TP

and UP applications, respectively. AP provided lignin elimination properly. But TP and UP did not affect the kept their lignin content of yard waste; they even resulted in increase in lignin content. Lignin increase observed after thermal and ultrasound pretreatments can be explained with the rapid degradation of hemicellulose and formation of pseudo-lignin compounds generated from monomeric sugars (Li, et al.,2014; Sambusiti et al., 2013).

VS% of yard waste mix decreased by as 29%, 18% and 2% after AP, TP and UP applications, respectively. AP application resulted in the highest lignin destruction. This was as expected for AP is known to be more effective for lignin degradation (Carrere et al., 2016; Kumar, 2009; Rodriguez et al., 2017). Several lignocellulosic substrates decreased in VS contents after pretreatment. In the study of pulp and paper sludge, after AP, 24% VS reduction was achieved (Lin et al., 2009). In another study, 26% VS reduction was observed after TP of olive mill solid waste (Rincón et al., 2013).

In terms of TAN contents, AP, TP and UP increased their TAN contents as 12%, 18% and 14%, compared to raw yard waste, respectively. TAN concentration is one of the crucial parameters in anaerobic digestion, which is inhibitory above 1500 mg/L (Angelidaki and Ahring, 1993). For this reason, experimental calculations were determined in order to ensure the safe ranges for inhibitory compounds.

As a result, AP and TP have the highest solubilisation efficiency with similar results as 86% and 88% respectively. Yet, TAN concentration increases gave similar results (12-18%) for all three pretreatment options. The highest cellulose availability was observed for UP as almost 60%. Highest VS% reduction was observed with AP as 29%. Similarly, AP also had the highest efficiency in terms of lignin destruction. Considering the changes in VS, lignin, hemicellulose, sCOD/COD ratio, and even cellulose values of yard waste after pretreatments, AP application was found as the one resulting in the highest solubilization. Enhanced solubilisation capacity is expected to increase anaerobic digestion performance in

the following digestion experiments. Despite of that, all three pretreatment options were studied in OSAD and TSAD processes. Because, solubilisation efficiency might be a strong indicator of yield increase in anaerobic digestion, however, cases like by-product accumulation should be also taken into consideration. Potential by-products might inhibit anaerobic digestion process (Koyama et al., 2017).

4.2 The Results of OSAD Experiments

OSAD experiments were conducted in order to investigate the methane production potential of non-pretreated and pretreated-yard waste mixes. It was also aimed to determine the optimum pretreatment type and initial TS content resulting in the highest methane (i.e. energy) yield. Three different pretreatment options in addition to non-pretreated yard waste were studied in OSAD. The reason not to define the best pretreatment option resulting in the highest solubility in pretreatment study is that, solubilisation efficiency enhancement or increase in cellulose availability are not the certain indicators for higher energy yields.

Batch reactors were operated until biogas production ceased. So, biogas production was examined for 60 days at 35±2℃. Cumulative methane production and yield results are given for each pretreatment option in the following sections. The values presented in figures and tables are the average values of the duplicates.

4.2.1 Methane Production Potential of OSAD Experiments

Average methane yields obtained in OSAD experiments are presented in Table 4.2. For all three initial TS contents (2%, 5% and 8%); NP-test reactors achieved 74-267 mL CH_4/g VS, AP-test reactors achieved 231-313 mL CH_4/g VS, TP-test reactors achieved 180-250 mL CH_4/g VS and UP-test reactors achieved 69-85 mL CH_4/g VS methane yield at the end of the incubation period. All pretreated yard waste types were investigated with respect to different initial TS contents and AP application seems to result in the highest methane yield for each TS content

studied. Results revealed that the highest methane yield was achieved with AP-test reactor (313 mL CH4/g VS) at 5% TS content.

For 2% TS content, only AP-test reactor showed 23 mL/g VS (i.e. 9%) yield increase compared to NP counterpart, which is not a significant amount to apply pretreatment in anaerobic digestion. For this reason, digesting yard waste mix without pretreatment option for OSAD is feasible for 2% TS content. AP-test reactors had a significant yield difference among other options by 313 mL/g VS at 5% TS, which enables to apply in OSAD process. TP procedure is not a feasible option as AP, although it increased yield amounts compared to NP-test reactors. On the other hand, UP option could not led to positive effect on yield for OSAD.

5% TS AP-test reactor produced nearly 80% of the theoretical methane yield (395 mL CH_4/g VS), which is a significant energy amount and higher than many literature results with lignocellulosic substrate (Table 4.3). It should be noted that literature studies in Table 4.3 were mainly conducted with single lignocellulosic materials and mostly without any pretreatment application. OSAD with grass, water hyacinth and wood gave methane yield values of 14-392 mL CH_4/g VS in the literature (Chynoweth et al., 2002; Chynoweth et al., 1993; Dinuccio et al, 2010; Lehtomäki, 2006; Pakarinen et al., 2008). This study comparatively achieved high energy yield results with yard waste mix (i.e. grass and yard trimming mix) more than many of these experiments such as alkali-pretreated grass (Pakarinen et al., 2008), in spite of having more complex structure (Table 4.3).

Table 4.2 Average methane yields observed in OSAD experiments

Yard waste type	TS %	Methane Yield (mL CH_4/g VS)	Achieved % Theoretical yield	Methane Yield (mL CH_4/g VS) - Blank	Yield Increase Compared to NP Test reactor (%)
NP	2	267	67	140	_
	5	148	37	1	_
	8	74	19	0	_
AP	2	290	73	0	9
	5	313	79	0	111
	8	231	58	4	212
TP	2	250	63	0	-6
	5	180	45	0	22
	8	184	46	0	149
UP	2	69	17	0	-74
	5	85	21	0	-43
	8	81	20	0	9

Table 4.3 Methane yield comparison between OSAD set and related literature

Substrate	Pretreatment option	CH_4 Yield (mL CH_4 /g VS)	Reference[a]
Turf Grass	_	247	Chynoweth et al.(1993)
water hyacinth	_	196	
Napier grass	_	231	
Grass	Alkaline	299	Pakarinen et al. (2009)
Grass	-	231	Lehtomäki (2006)
Grass	-	300	
Tall fescue	-	330	
Clover	-	290	
Grass	-	128-392	
Napier grass	-	194-340	Chynoweth et al. (2002)
Wood	-	14-320	
Grass	-	16-390	
Giant knotweed	-	170	Lehtomäki et al. (2008)
Barley straw	-	229	Dinuccio et al. (2010)
Yard waste	Alkaline	313	This study, OSAD Set

[a]All studies were performed in batch reactors

Since the aim of pretreatment applications is to enhance anaerobic biodegradability and methane yield of yard waste mix, comparing pretreated substrates with raw substrate is crucial in this study. Compared to NP-yard waste, AP application increased methane yield for 2%, 5% and 8% initial TS content by 9%, 111% and 212%, respectively (Table 4.2). In TP application, methane yield decreased by 6% for 2% TS, but increased by 22% and 149% for 5% and 8% TS, respectively. Considering the high methane yield of 2% NP-test reactor (267 mL CH_4/g VS), TP-test reactors were not able to reach greater yields. In UP application, methane yield decreased by 74% and 43% for 2% and 5% TS, respectively,while increased by 9% for 8% TS. The low methane yield values obtained in UP application indicates that applied pretreatment option is not appropriate for the studied yard waste.

Methane yields lower than that of blank reactors is quite interesting. As seen in Table 4.2, 2% NP-blank reactor which has no seed represented remarkable amount of methane (140 mL CH_4/g VS) during digestion. The methane yields are even more than 50% of the NP-test reactor with similar TS% content. This situation might be due to "intrinsic microorganisms" within yard waste. Yard waste might naturally contain these microorganisms in itself. These microorganisms might have been cultivated in the yard waste since yard wastes seemed to be placed in the collection field for a long period of time. Yard wastes were obtained from a field where they were gathered and stacked upon top of each other. During collection from this field, it was observed that yard wastes created wet zones in itself, which might be a sign of anaerobic digestion process. Intrinsic microorganisms are capable of producing biogas without any seed sludge presence. As seen in Table 4.2, these microorganisms lost their methane production capability when they were exposed to pretreatment processes and have solids concentration more than 2% in the reactors. Apparently, options studied are inhibiting the potential intrinsic microorganisms.

According to the results given in Table 4.2, as solids percentage increased, methane yields generally decreased. Since high solid contents cause inhibition problems in anaerobic digestion, this drop was expected (Boontian, 2014).

Average cumulative methane yield results with respect to pretreatment applications are shown in Figure 4.1. For NP-yard waste mix (Figure 4.1a), 2% TS is the best option by 267 mL CH_4/g VS. As the solids concentration increased, reaction rates decreased and also methane yield decreased. Among blank reactors, 2% blank reactor showed significant yield amount (140 mL CH_4/g VS), which is even higher than 5% and 8% TS NP-test reactors. This methane production with raw substrate affects the comparison with pretreated yard waste results, since non-pretreated reactors were expected to present lower production of methane. Methane production started at the beginning of the incubation for 2% TS NP-test reactor. Yet, 5% and 8% NP-test reactors started to produce methane after 10^{th} day. This difference in acclimation time might be due to the solubilisation ease of 2% solids concentration. 5% and 8% solids concentrations might have created substrate accumulation in the reactor, which might slow down the degradation efficiency and delay methane production. Since microorganisms had difficulty in degrading yard waste (due to the lack of hydrolyzing media), adaptation period was needed at higher solids concentrations. Consequently, OSAD of NP-yard waste mix is only feasible for 2% solids concentration.

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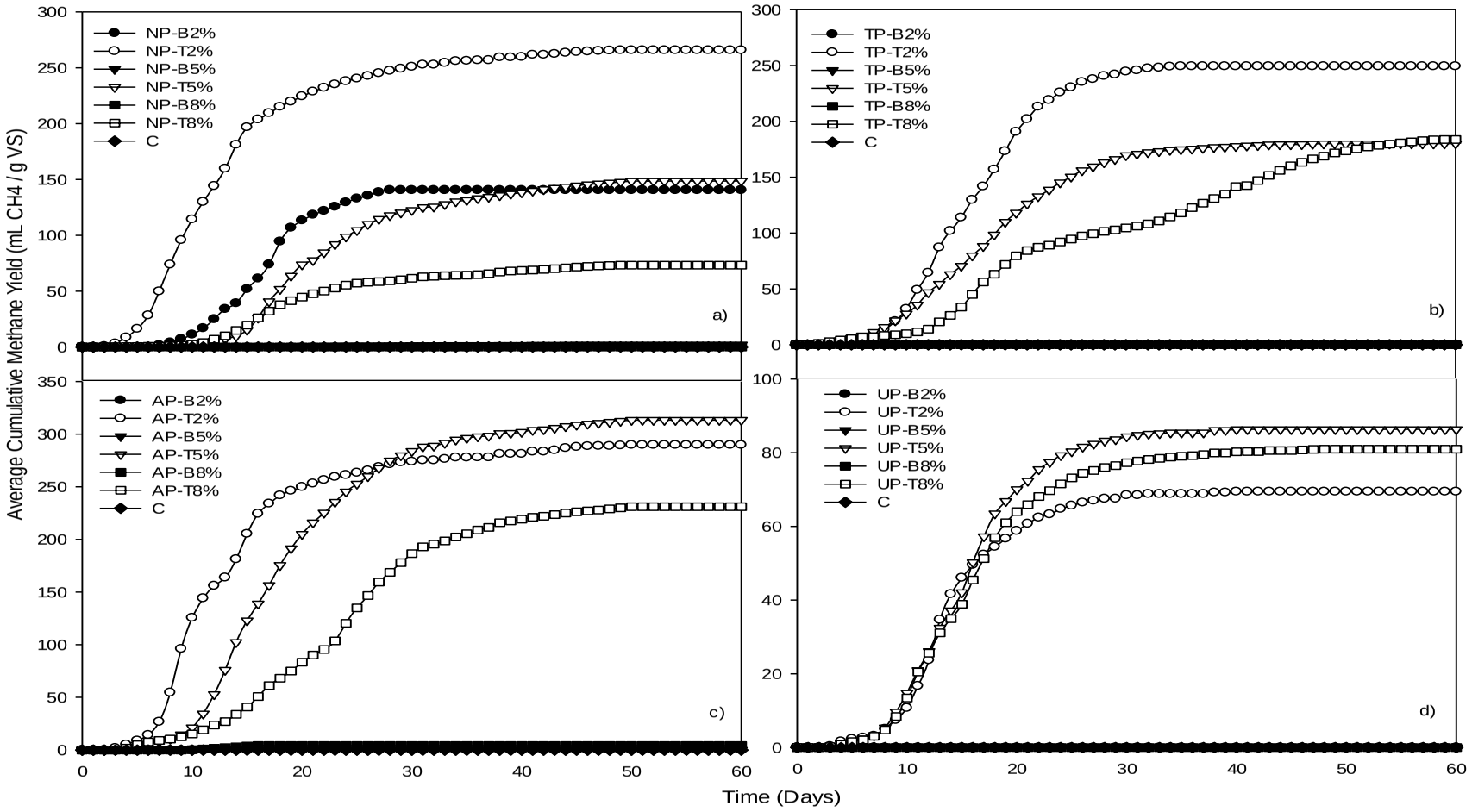

Figure 4.1 Average cumulative CH_4 yield results with respect to pretreatment applications for a) NP, b) TP, c) AP and d) UP reactors

For AP-yard waste mix, 5% TS is the best option with a yield of 313 mL CH_4/g VS. Besides, 2% AP-test reactor provided a very closer yield result by 290 mL CH_4/g VS. Like NP-yard waste mix, as the solids concentration increased, reaction rate decreased and hence acclimation time delayed due to this higher solids loading. Solids concentration increase affected AP-yard waste in a positive way up to 5%. For this reason, OSAD of AP-yard waste mix is feasible for both 2% and 5% solids concentration. However, in an economical perspective, at 5% TS content, yield increase of 8% compared to 2% NP-test reactor, is not an encouraging amount to apply additional pretreatment and waste chemicals in OSAD process.

For TP-yard waste mix, 2% TS is the best option by 250 mL CH_4/g VS. As the solids concentration increases, reaction rate decreases, acclimation time increases and also methane yield decreases. OSAD of TP-yard waste mix is feasible for 2% solids concentration for an evaluation in itself. However, compared to AP, TP application would not result in high yields and preferred for OSAD application.

According to yield results (Table 4.2) UP process was not efficient at all, because obtained yield range in UP was even lower than NP-test yield results. The reason for not being a proper pretreatment option for yard waste mix might be that acclimation of by-products of UP affected anaerobic digestion efficiency. For all three initial solids concentrations, UP-test reactors reacted in the same way. All UP-test reactors had same reaction rate, same acclimation time and similar yields. Even at the lowest TS content (2%), these by-products might have been toxic enough to inhibit the system. Another reason might be that,the used device might not be efficient enough for UP. It was planned to apply UP procedure via ultrasound probe. However, due to technical problems occured with this device, ultrasound bath, which is known to be less effective than probe, was used.

Investigated methane yield result graphs were redrawn with respect to initial TS concentrations individually (Figure 4.2). By this way, all pretreatment options were compared for the same TS content. As seen in Figure 4.2, for 2% TS content, AP-

test reactor produced the highest cumulative methane yield by 290 mL CH_4/g VS. 2% NP-test reactor followed by 267 mL CH_4/g VS. TP test reactor has a similar yield result by 250 mL CH_4/g VS and lastly UP-test reactor achieved 69 mL CH_4/g VS. Among blank reactors, only 2% NP-blank reactor produced significant amount of methane, which presents a yield as 140 mL CH_4/g VS.

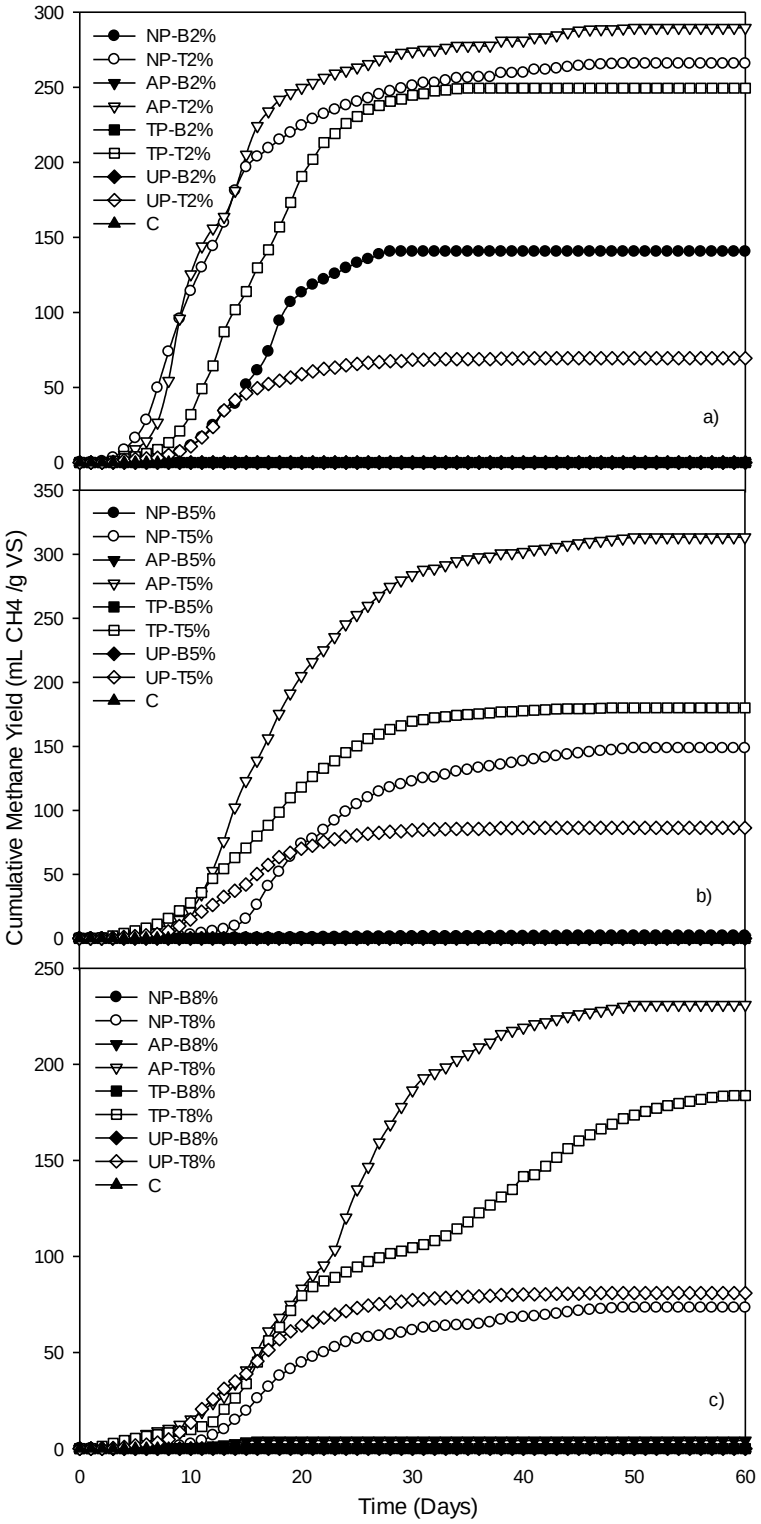

Figure 4.2 Average Cumulative CH_4Yields for a) 2% TS, b) 5% TS, c) 8% TS

Since there was enough media for microbial degradation at 2% solids concentration, all pretreated and non-pretreated reactors started methane production in less than 10 days (Figure 4.2). When these data is examined in detail, it is seen that NP-test reactor was able to start methane production before the reactors with pretreated yard waste. The reason might be that microorganisms needed additional time to adapt anaerobic environment with pretreated substrates due to their chemical/physical treatment history, i.e. potential by-products. Methane production lasted in approximately 30 days for UP- and TP-test reactors and 35 days for AP- and NP-test reactors.

With respect to 2% solids content, the optimum pretreatment option was found as AP-yard waste mix. UP-test reactor had comparatively low methane production. Solubility has been increased with ultrasound bath process (Table 4.1), but reactor's reaction in OSAD was lower than expected as discussed before. However, since AP-, TP- and NP-test reactors presented similar yield results, OSAD of pretreated yard waste at 2% TS is not feasible. Considering the significant methane production in NP-yard waste, the pretreatment effect cannot be seen distinctively on methane yield. It should be also noted that 2% NP-blank reactors did not present similar yield amounts as duplicates (Figure 4.3). The duplicate reactors displayed yields of 182 and 101 mL CH_4/g VS. 2% NP-blank reactors had been previously conducted due to this difference observed in the parallel reactors once again; yet similar results had been obtained (data not shown). This observation was not obtained in test reactor and only limited to NP-blank reactors of 2% TS where significant gas production was observed. This was attributed to non-homogeneous structure of yard waste mix. Despite the mechanical grinding and sieved to 20 mesh, apparently the well-homogeneous structure could not be achieved. Even one small organic piece difference might affect the initial organic content of the reactor. Pretreatment, on the other hand, improves the homogenity of the substrate; thus, duplicates had very similar yields in pretreated blank and test reactors. Despite the difference observed in 2% NP-blank duplicates, both reactors produced significant

amount of methane. Therefore, the average values were shown in tables and figures.

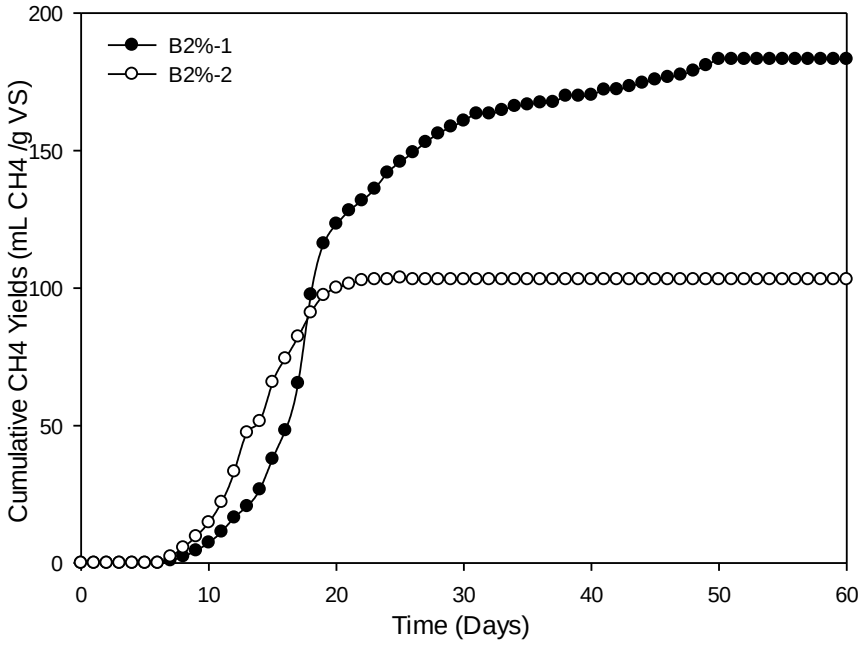

Figure 4.3 Cumulative methane yield results of 2% NP-blank reactors

For 5% TS content, AP-test reactor produced the highest cumulative methane yield with 313 mL CH4/g VS (Figure 4.2). 5% TP-test reactor followed by 180 mL CH4/g VS. NP-test reactor has a similar yield result by 148 mL CH4/g VS and lastly UP-test reactor achieved 85 mL CH4/g VS. Any of the blank reactors were able to produce methane. In this case, effect of pretreatment on energy yield can be clearly seen. NP-test reactor was still able to produce methane, but not as efficient as in 2% TS. Hence, AP- and TP-yard waste mix results distinguished from NP-yard waste in 5% solids content. The reason for not observing methane production in 5% test reactors can be explained with higher TS content. After a certain amount of solids concentration in the reactors, intrinsic microorganisms might be inhibited by high organic load.

Methane production started in less than 10 days for AP- and TP-test reactors (Figure 4.2). UP-yard waste mix needed approximately 10 days and NP-yard waste mix needed 15 days to start methane production. When these data are examined, it can be seen that NP-yard waste needed longer acclimation period compared to pretreated substrates. With respect to 5% solids content, the optimum pretreatment option is AP-yard waste mix. TP-test reactors presented higher yield result than NP-test reactors, but the difference is not distinctive enough. UP-test reactors had comparatively low yield.

For 8% TS content, AP-test reactor produced the highest cumulative methane yield as 231 mL CH_4/g VS (Figure 4.2). 8% TP-test reactor followed with 184 mL CH_4/g VS. UP-test reactor has a yield of 81 mL CH_4/g VS and lastly NP-test reactor presented 74 mL CH_4/g VS. Only AP-blank reactor was able to produce methane, which can be counted as negligible (4 mL CH_4/g VS). In 8% solids concentration case, effect of pretreatment on energy yield can be clearly seen for all three options. AP- and TP-yard waste mix reactors are well ahead from NP-test reactor. In only 8% TS concentration, UP-test reactor was able to produce more methane yield than NP-blank reactor. Like in 5% TS reactors, methane production did not observed in 8% TS blank reactors due to the solids inhibition effect on intrinsic microorganisms. As a result, with respect to 8% solids content, the optimum pretreatment option is AP-yard waste mix. TP-test reactor also presented higher yield result than NP-test reactor. UP-test reactor's yield result is not distinctive enough.

As seen in Figure 4.2, in 8% TP-test reactor, a decrease in the rate was observed between the 20^{th} and almost 35^{th} of the incubation period. This rate decrease might have originated from a by-product accumulation. The hydrolysis of this chemical may need acclimation, which might be reflected as a "lag phase". A similar case is observed with long chain fatty acids (LCFAs) accumulation (Angelidaki and Ahring, 1993). In anaerobic fermentation process, fats are primarily degraded to glycerol and LCFAs. Glycerol is converted to acetate, VFA and alcohols by acidogenic bacteria, on the other hand, LCFAs are transformed into acetate and

hydrogen by acetogenic and methanogenic microorganisms. The hydrolysis rate of fats depend on chemical and physical structure of LCFAs. Slower degradation of fats cause “lag phase”, which can be understood from the stabilization of methane production for a while. In other words, slower degradation of this by-products might have resulted in a rate-limiting step in anaerobic digestion . On the other hand, AP-, UP- and NP-test reactors did not show this lag phase. The reason might be that this product might not have been produced in other test reactors and/or did not reached critical levels. Another reason of this rate decrease might be the TAN accumulation during anaerobic digestion. TAN concentration is one of the crucial parameters in anaerobic digestion, which is inhibitory above 1500 mg/L (Angelidaki and Ahring, 1993). The highest TAN concentration (around 1200 mg/L) was obtained in 8% TP-test reactors (Section 4.2.2, Figure 4.4). This value close to inhibitory levels might have affected and slowed down the methanogenesis. The initial and final characteristics of the reactors’ content were analyzed and results were discussed in Section 4.2.2.

4.2.2 The Results of Characterization Studies for OSAD Experiments

In order to monitor anaerobic treatability in batch reactors of OSAD, main parameters were analyzed for the initial and final concentrations of reactors. Studied parameters are VS, TS, pH, COD, TP, TKN and TAN (Appendix F). According to these analyses, efficiency of anaerobic digestion were speculated. In tables and figures, capital B represents blank reactors and capital T represents test reactors.

The difference between initial and final VS concentrations expresses the digested (stabilized) waste amount in anaerobic processes. Methane production during anaerobic digestion is linked to destroyed organic matter (VS) (Labatut and Gooch, 2012). As seen in Table 4.4, NP reactors achieved VS reduction between 21-31%, AP reactors between 56-75%, TP reactors between 19-67% and UP reactors between 12-70% (Table 4.4). Literature studies for lignocellulosic substrates

present a similar range for VS reduction. A study conducted with crop residues achieved VS reduction in a range of 24-34% (Hills and Roberts, 1981). Another study with wheat grass showed a decrease in VS content as 46% (Romano et al., 2009). Anaerobic digestion with alkali pretreated corn stover achieved VS reduction between 13-38% (Zhu et al., 2010). For UP-yard waste, VS degradation increase reflected in methane yield in direct proportion. However, for NP, AP and TP substrates, as stabilized VS amount increases, decreased methane yields were observed. The reason might be that at higher solids concentrations might be affecting anaerobic system repetively, which decreases methane yield.

Table 4.4 Average VS reduction and yield results of OSAD test reactors

Reactors	VS Reduction (%)	sCOD Removal (%)	Yield (mL/g VS)	Yield Increase (%)
NP- 2%	-24	-19	267	_
NP- 5%	21	-142	148	_
NP- 8%	31	-178	74	_
AP- 2%	75	11	290	9
AP- 5%	26	46	313	111
AP- 8%	31	40	231	212
TP- 2%	56	-98	250	-6
TP- 5%	67	-8	180	22
TP- 8%	19	12	184	149
UP- 2%	-12	-358	69	-74
UP- 5%	60	-28	85	-43
UP- 8%	70	-31	81	9

COD represents the organic amount present in the substrate. The efficiency of anaerobic digestion can be evaluated with degraded COD amount, which also shows consumed organic portion (Meegoda et al., 2018). sCOD amounts mostly increased at the end of OSAD process (Table 4.5). Temperature (35℃) and constant mixing might have helped to increase solubility of yard waste during 60 days of incubation. This situation avoids calculating organic degradation properly with respect to sCOD removal. In a case where organic portions cannot be

monitored properly, it is more reliable to discuss organic degradation with VS reduction. Increase in sCOD at the end of OSAD should not mislead, because all test reactors properly operated and produced methane which is indicator of organic matter degradation (Table 4.2).

One of the reasons for observing lag phases in cumulative methane yields might be originated from instant increases in sCOD. High organic sCOD content, such as increase in by-product might have stopped microbial activity for a while. Then after acclimation, methane production continued (Table 4.2).

Table 4.5 Average COD results of OSAD test reactors

	tCOD(mg/L)	sCOD(mg/L)	sCOD/tCOD	sCOD Removal %
NP-2% (t=0)	22488	863	0.04	-19
NP-2% (t=60)	25621	1031	0.04	
NP-5% (t=0)	42323	2163	0.05	-142
NP-5% (t=60)	49320	5231	0.11	
NP-8% (t=0)	60147	2550	0.04	-178
NP-8% (t=60)	51953	7091	0.14	
AP-2% (t=0)	27925	2825	0.10	11
AP-2% (t=60)	18288	2505	0.14	
AP-5% (t=0)	49230	7025	0.14	46
AP-5% (t=60)	37000	3781	0.10	
AP-8% (t=0)	49827	9813	0.20	40
AP-8% (t=60)	57660	5900	0.11	
TP-2% (t=0)	43975	1225	0.03	-98
TP-2% (t=60)	42734	2428	0.06	
TP-5% (t=0)	63900	4025	0.06	-8
TP-5% (t=60)	57825	4373	0.08	
TP-8% (t=0)	85650	5550	0.06	12
TP-8% (t=60)	85140	4862	0.06	
UP-2% (t=0)	31875	575	0.02	-358
UP-2% (t=60)	25772	2634	0.11	
UP-5% (t=0)	37238	1420	0.04	-28
UP-5% (t=60)	30690	1822	0.06	
UP-8% (t=0)	87550	1700	0.02	-31
UP-8% (t=60)	41120	2226	0.05	

Another parameter that influences methanogenic activity is ammonia nitrogen (TAN). The initial TAN concentrations in the test reactors were in the range of

140-210 mg/L for NP, 157-227 mg/L for AP, 805-1172 mg/L for TP and 1155-1382 mg/L for UP reactors (Figure 4.4 and Appendix F). Effluent TAN concentration ranges were 209-355 mg/L for NP, 116-163 mg/L for AP, 890-1230 mg/L for TP, and 879-1064 mg/L for UP reactors. Anaerobic digestion provides additional ammonia dissolution by decomposition of organic nitrogen in reactors. Hence slight TAN increase is expected at the end of the process (Demirer and Chen, 2004). High ammonia concentrations may affect digester efficiency by acclimation. A wide range for TAN inhibition has been presented in the literature. In a critical review, McCarty and McKenney (1961) presented that TAN concentration above 150 mg/L is inhibitory for bacterial growth. In another study, effective digestion periods were achieved at higher concentrations up to 345 mg/L TAN (Ripley et al., 1985). TAN concentration is inhibitory above 1500 mg/L (Angelidaki and Ahring, 1993). These values are marked on Figure 4.4. TAN inhibition in anaerobic digestion is generally indicated by decreasing methane production rates, lowered methane yields and increased concentrations of end products like VFA (Calli et al., 2005). It should be noted, considering the initial TAN concentrations in all test reactors, that UP and TP applications increases the TAN concentration while AP did not result in TAN release. Apparently, AP application does not degrade the organic nitrogen. Hence, as seen in Figure 4.4, NP- and AP-test reactors' initial and final TAN concentrations are in the safe range (<400 mg TAN/L),but TP and UP reactors experienced much higher TAN concentrations, closer to the inhibition limit (Figure 4.4). These high TAN concentrations may be the reason of obtaining lower methane yield results in TP- and UP- test reactors compared to AP-test reactors (Table 4.2). UP and TP applications seemed to increase initial TAN concentrations, which might have affected negatively the intrinsic microorganisms and decreased the methane yields.

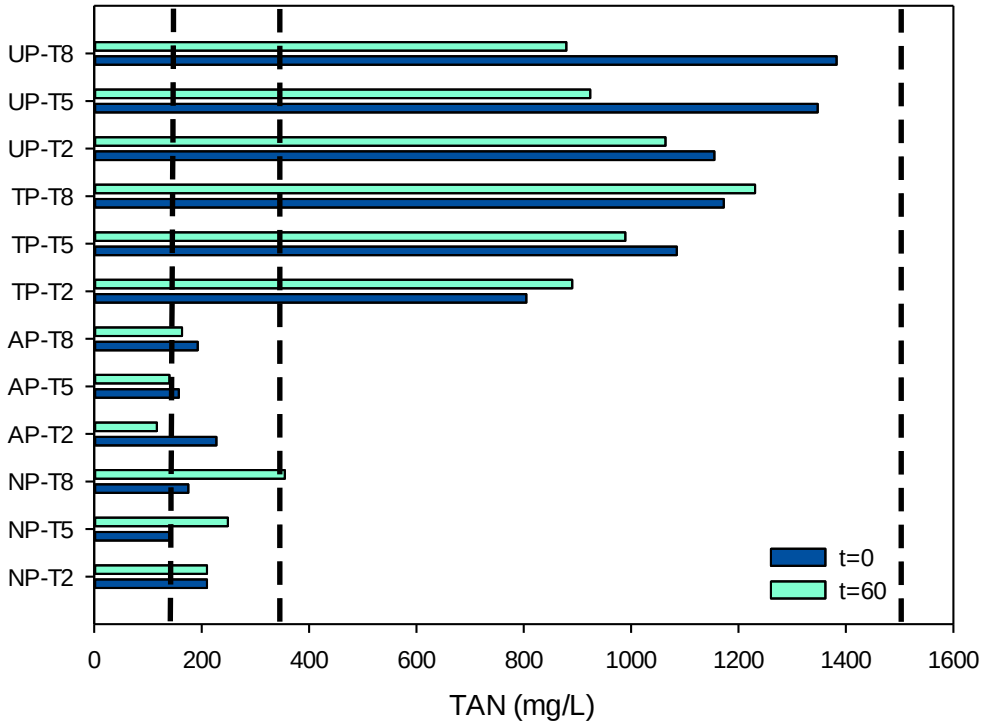

Figure 4.4 Initial and final TAN Concentrations of OSAD Test Reactors

pH is another crucial parameter influencing anaerobic processes. In a well operated anaerobic digestion system, pH is expected to increase slightly at the end, since microorganisms generate alkalinity as they consume protein rich organic substances (Labatut and Gooch, 2012). In this sense, for all NP-, AP-, TP- and UP-test reactors slight increase in pH (7.01 to 7.79) was observed (Appendix F). On the other hand, pH drop was observed in NP-, TP- and UP-blank reactors, being significant in 5% and 8% TS contents (Figure 4.7). This might be due to the acidic conditions obtained via VFA accumulation after the degradation of soluble portion. This acidic pH (i.e. 5.5) is also thought to be reason of observing almost no methane production in TP- and UP-blank reactors. Moreover, observing pH drops down to 5.5 in blank reactors is the proof of having microbial activity (i.e. intrinsic microorganisms) in NP-yard waste mix.

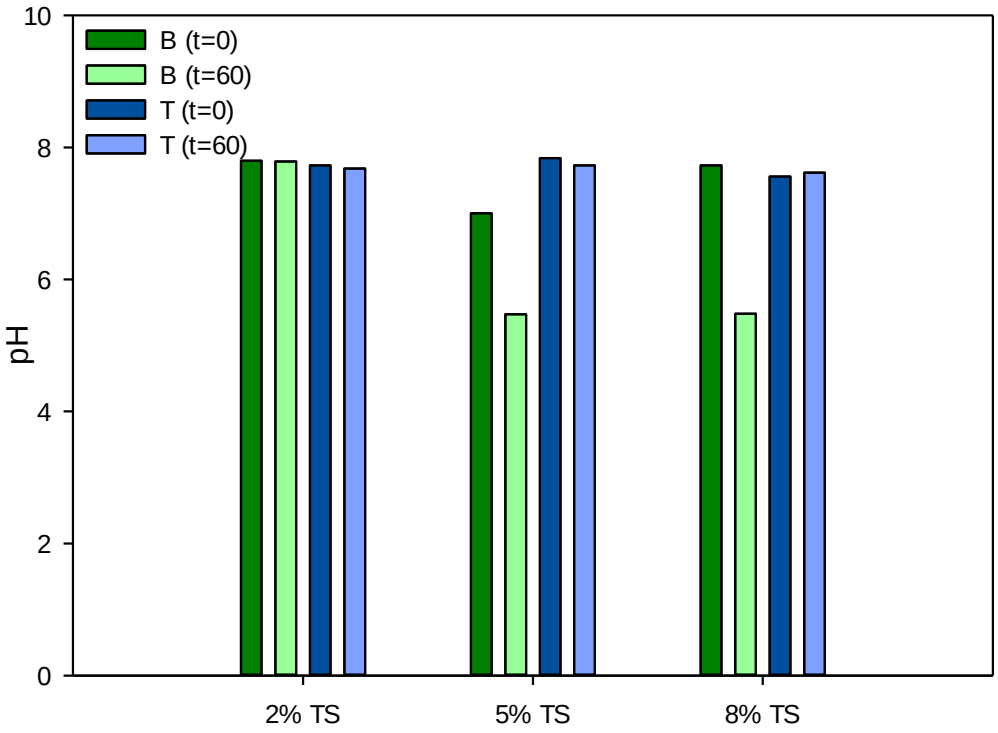

Figure 4.5 pH changes in NP-Blank (B) and NP-Test (T) Reactors

4.3 The Results of Two-stage Anaerobic Digestion (TSAD) Experiments

TSAD batch reactor studies were conducted in order to investigate the effects of pretreatment and initial TS content of yard waste mix on hydrogen and methane production. It was aimed to define the best pretreatment option and optimum solids content for yard waste mix leading to the highest H_2 and CH_4 (i.e. energy) production. Regardless of solubilisation enhancement and cellulose availability increase, and considering the facts that pretreatmentsmight end up with inhibitoey substrates, DF experiments were performed with three pretreated yard wastes, namaly, AP-yard waste, TP-yard waste, UP-yard waste and also with non pretreated yard waste (NP-yard waste).

Batch reactors were operated at 35±2 ℃ for 12 days for hydrogen production as the first-stage and 20 days for methane production as the second-stage. After DF stage, reactor effluents were filtered, and methanogenesis stage's batch reactors were fed with these filtrates. According to the results, an additional Mini TSAD experiment was also conducted in order to check the reliability of obtained data. Cumulative hydrogen and methane production results are given for each pretreatment option in following sections. The values presented in figures and tables are the average values of the replicas.

4.3.1 The Results of Dark Fermentation Experiments of TSAD Set-up

4.3.1.1 Hydrogen production Potential in Dark Fermentation Experiments

The Results of Dark Fermentation (DF) experiments as H_2 yields are presented in Table 4.6. The results revealed that, NP-test reactors achieved 5-8 mL H_2/g VS, AP-test reactors 10-30.5 mL H_2/g VS, TP test reactors 3-10 mL H_2/g VS and UP-test reactors 4.5-12.5 mL H_2/g VS hydrogen yields at the end of the incubation. AP application resulted in the highest H_2 yields among others for each TS content studied. For all yard waste mix types, 2% TS gave the highest hydrogen yield. As seen in Table 4.6, as solids percentage increased, hydrogen yields generally decreased. Since high solid contents cause inhibition problems in dark fermentation, like in anaerobic digestion, this drop was expected (Boontian, 2014).

H_2 yields obtained were very low. Theoretical hydrogen yield could not be achieved (Table 4.6), because obtaining high hydrogen yields is still a problematic issue (Wang and Wan, 2009). Literature studies conducted with similar lignocellulosic substrates are shown in Table 4.7.

It should be noted that literature studies were mainly conducted with single lignocellulosic materials and mostly without any pretreatment application . DF with

grass, leaves, wheat strar, rice straw and sweet sorghum gave hydrogen yield results between 5-68 mL H_2/g VS in the literature (Chen et al., 2012; Cui and Shen, 2012; Cui et al., 2010; Fan et al., 2006; Ivanova et al., 2009; Karlsson et al., 2008; Kyazze et al., 2008; Li and Chen, 2007; Pakarinen et al., 2008). This study achieved comperative yield results with yard waste mix (i.e. grass and yard trimming mix), although having more complex structure.

Table 4.6 Average hydrogen yields in TSAD experiments

	TS %	H_2 Yield			Achieved % theoretical yield	H_2 Yield-Blank (mL H2/ g VS)	Yield increase (%) with respect to NP-test
		mL H_2/g VS	mL H_2/ mmol glucose[a]	mol H_2 /mol glucose			
NP	2	8.32	1.11	0.04	1.10	4.60	-
	5	2.11	0.68	0.03	0.67	3.97	-
	8	1.65	0.22	0.01	0.21	2.58	-
AP	2	30.51	3.58	0.14	3.54	5.77	266
	5	9.92	1.17	0.05	1.15	4.66	370
	8	11.45	1.34	0.05	1.33	1.27	594
TP	2	10.23	4.46	0.18	4.41	1.38	23
	5	5.16	2.25	0.09	2.23	1.50	144
	8	2.89	1.26	0.05	1.25	1.23	75
UP	2	12.57	2.09	0.08	2.01	3.93	51
	5	4.54	0.75	0.03	0.74	0.32	115
	8	6.87	1.14	0.05	1.13	0.28	316

[a]1 g glucose=1.066 g COD, R=0.8205 L.atm/mol.K

Table 4.7 Maximum hydrogen yield obtained in the related literature

Substrates	Pretreatment Option	H_2 Yield (mL H_2/g VS)	Reference[a]
Corn stover	Steam Explosion	68	Li and Chen (2007)
Wheat straw	Acid (HCl)	68	Fan et al. (2006)
Poplar leaves	-	15 - 45	Cui et al. (2010)
Rice straw	-	14	Chen et al. (2012)
Grass	-	5	Cui and Shen (2012)
Grass Silage	-	6	Karlsson et al. (2008)
Grass silage	-	16	Pakarinen et al. (2008)
Maize leaves	-	17	Ivanova et al. (2009)
Ryegrass	-	73	Kyazze et al. (2008)
Sweet sorghum	-	30.5	Ivanova et al. (2009)
Leaves	-	10	
Yard waste mix	Alkaline	30.5	This Study, TSAD

[a]All studies were performed in batch reactors

Since the aim of pretreatment applications is to enhance anaerobic biodegradability and energy yield of yard waste mix, comparing pretreated substrates with raw substrate is crucial. In terms of hydrogen yield, AP application was found to increase hydrogen yield compared to NP-test reactors by 266%, 370% and 594%, for 2%, 5% and 8% TS contents, respectively (Table 4.6).

Almost all blank reactors produced hydrogen yet, in small amounts. Their yield range is 2.5-4 mL H_2/ g VS for NP, 1-5 mL H_2/ g VS for AP, 1-1.5 mL H_2/ g VS for TP and 0-4 mL H_2/ g VS for TP (Table 4.6). This situation proofs that, yard waste already contains intrinsic microorganisms and these organisms are capable of producing hydrogen. Moreover, it is observed from Table 4.6 that they are not totally inactivated after pretreatment options, like in OSAD.

It should be noted that, the information on dark fermentation of yard waste mix is limited. Nevertheless, as mentioned previously, the obtained yield data were compared with similar lignocellulosic wastes. Despite being in accordance with literature data in terms of obtained H_2 yields for both pretreated and non-pretreated lignocellulosic wastes, the values are quite low. Therefore, in order to recheck

obtained yield results, an additional "mini TSAD set-up" was conducted with AP- and TP-yard wastes at 2% solids concentration (Appendix G). The reason for choosing these options is that, alkaline and thermal pretreatments gave high hydrogen yields in DF set and provided efficient methane, which would be beneficial for methanogenesis stage later on. In this mini TSAD set, effective volume and initial thus sCOD amounts were increased to enhance anaerobic digestion. Unfortunately, the mini TSAD also produced similar hydrogen yields for AP (34 mL H_2/g VS) and increased hydrogen yield result for TP (41 mL H_2/g VS). Modifications in reactors' set-up (sCOD increased from 30 mg to 160 mg) did not improve hydrogen yield, hence it can be said that ther esults of the main DF set-up are reliable for yard waste mix in selected conditions.

In order to compare TS% effect on hydrogen production potential of pretreated and raw yard waste mix, three different solids concentrations are presented separately. For 2% TS content, AP-test reactor produced the highest cumulative hydrogen yield as 31.5 mL H2/g VS (Table 4.6). 2% UP-test reactor comes as the second with 12.5 mL H_2/g VS. TP-test reactor has a similar yield result as 10 mL H_2/g VS and lastly NP-test reactor achieved 8 mL H_2/g VS. Among blank reactors, all pretreated and non-pretreated yard waste blank reactors produced small amounts of hydrogen. 2% AP-test reactor started to produce hydrogen on the first day. For other substrate options hydrogen production started on the 2nd day. According to these results, the optimum pretreatment option for yard waste at 2% TS is AP-yard waste.

Figure 4.7 indicates that the daily H_2 % change in the headspace of AP- and UP-test reactors with 2% initial TS contents. Instead of average values, the data of each duplicates is given in Figure 4.7. Both AP- and UP-test reactors displaced fluctuations in H_2 content in the headspace.

For AP-test reactors, on the 4th and 8th days and for UP reactors, on the 3rd and 6th days hydrogen production stopped (Figure 4.7). The reason might be that, after hydrogen becomes inhibitory at 60% in the headspace, there were no hydrogen

measurement on the following day (Alshiyab et al., 2008). Yet, this inhibition seems to be reversible, because afterwards, may be alter the acclimation of the system, hydrogen production continues. Observing no hydrogen content in the headspace after the day when a certain portion was measured, might be a sign for homoacetogenic activity. In other words, it is likely that the H_2 content in the headspace was consumed, maybe via homoacetogenesis, where H_2 together with CO_2 is converted to acetic acid. This decrease in the H_2 headspace content was not attributed to the leakage from the stoppers of the reactor. Because, it was verified using a manometer that, unless the headspace is vacuumed or purged with an inert gas, the possibility to lose all produced hydrogen in a closed system is very low. In addition, if there was a leakage, all other gas contents would have reached to zero, which is not the case as shown in Appendix H.

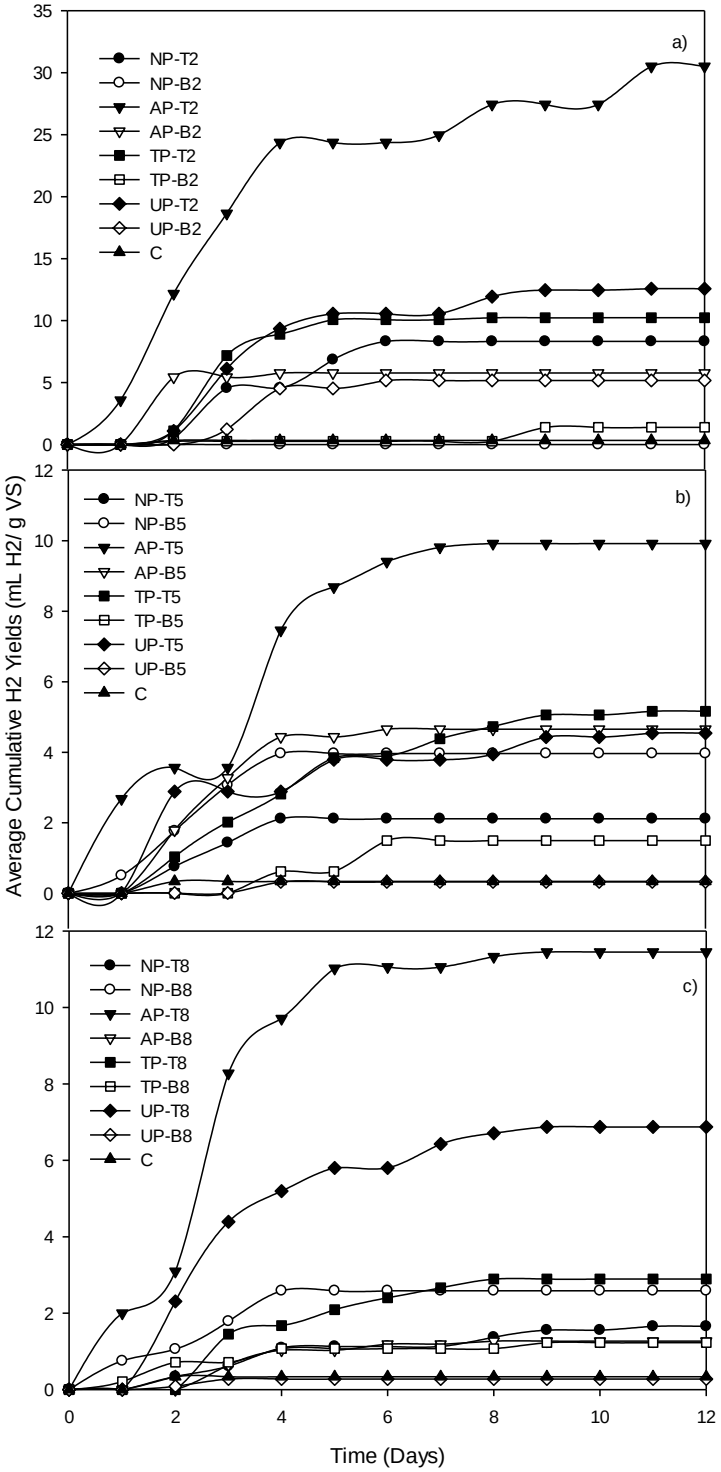

Figure 4.6 Average cumulative H_2 yields for a) 2% TS, b)5% TS c) 8% TS content for test reactors in DF

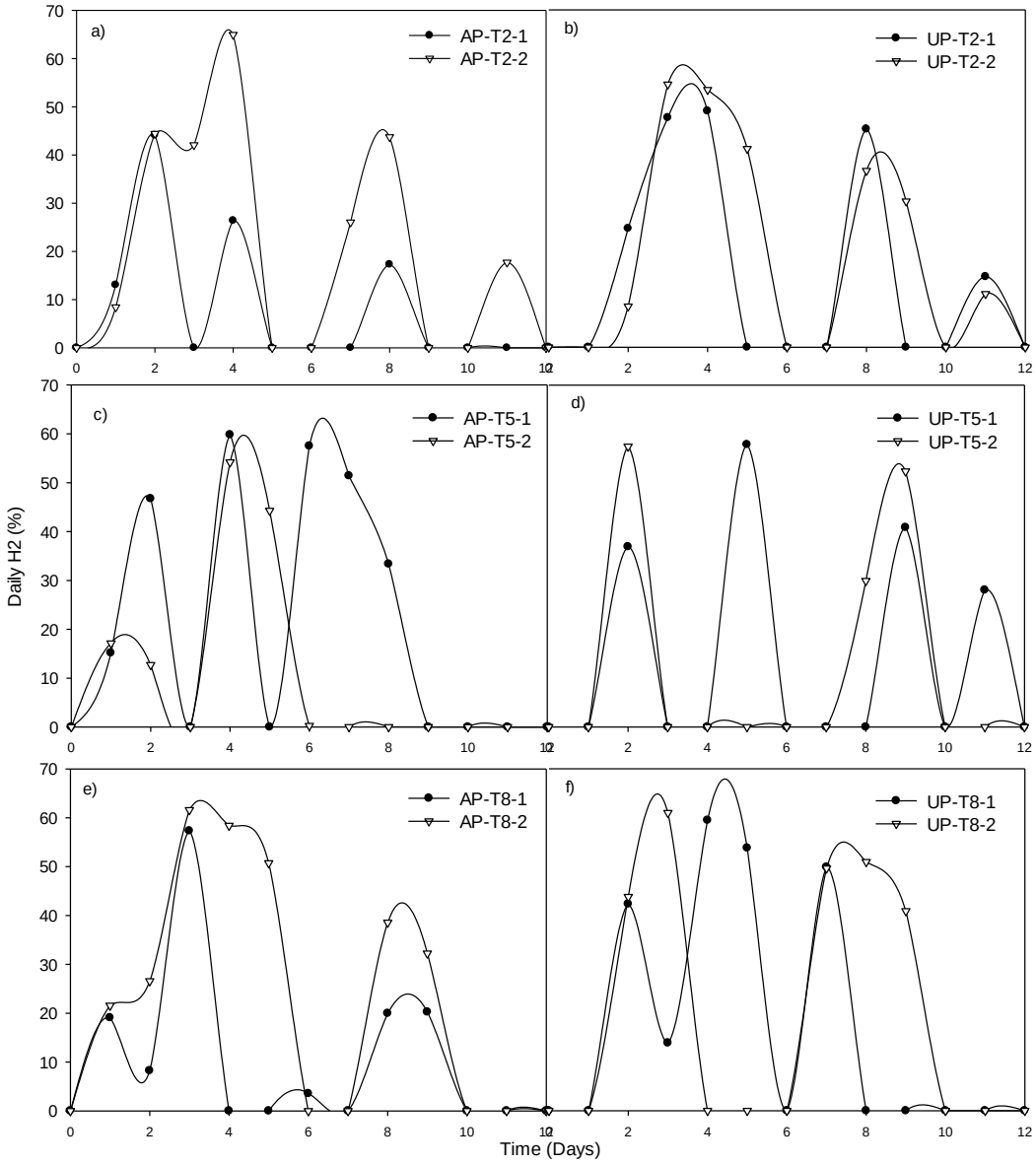

Figure 4.7 Daily H_2% content in the headspace of 2% TS containing test reactors of AP-yard waste and UP-yard waste a) 2% TS AP, b) 2% TS UP, c) 5%TS AP, d) 5% TS UP, e) 8% TS AP, e) 8% TS UP (1 and 2 in the legends refer to the duplicate reactors)

For 5% TS content, AP test reactor produced the highest cumulative hydrogen yield with 10 mL H_2/g VS (Figure 4.6). 5% TP-test reactor comes as the second with 5 mL H_2/g VS. UP-test reactor has a similar yield result as 4.5 mL H_2/g VS and lastly NP-test reactor achieved 2 mL H_2/g VS. Among blank reactors, all

pretreated and non-pretreated yard waste blank reactors produced small amounts of hydrogen.

AP 5% test reactor started to produce hydrogen on the first day. Others started hydrogen production on the 2nd day (Figure 4.6). According to results, the optimum pretreatment option for yard waste at 5% is AP. For AP reactors, on the 4th and 8th days, for UP reactors, on the 3rd and 6th days, hydrogen production stopped (Figure 4.7). The reason might be the inhibition limit of headspace hydrogen pressure, mentioned above.

Daily hydrogen production has fluctuations for AP and UP test reactors (For AP reactors, on the 2nd and 5th days, for UP reactors, on the 5th and 8th days, hydrogen production stopped (Figure 4.7). Inhibitory hydrogen concentrations in the headspace might cause these kind of fluctuations. After the system acclimates itself, hydrogen production continues.

For 8% TS content, AP test reactor produced the highest cumulative hydrogen yield with 11 mL H_2/g VS (Figure 4.6). 8% UP-test reactor comes as the second with 7 mL H_2/g VS. TP has a yield result with 3 mL H_2/g VS and NP has a yield result with 1.5 mL H_2/g VS. Among blank reactors, all pretreated and non-pretreated yard waste blank reactors produced small amounts of hydrogen.

AP 8% test reactor started to produce hydrogen on the first day. Other substrate options started hydrogen production on the 2nd day (Figure 4.6). According to results, the optimum pretreatment option for 8% TS is AP-yard waste .

Cumulative hydrogen production has fluctuations for AP and UP test reactors (Figure 4.6). For AP reactors, on the 2nd and 5th days, for UP reactors, on the 5th and 8th days, hydrogen production stopped. Inhibitory hydrogen concentrations in the headspace might cause these kind of fluctuations. After the system acclimates itself, hydrogen production continues.

As an overall conclusion, low solid concentrations gave higher hydrogen yields for pretreated yard waste mixes. Hydrogen production in blank reactors did not stop

after pretreatment applications but decreased by the increase in solids concentrations. Observed hydrogen productions in blank reactors were originated from intrinsic microorganisms in raw substrate. Increasing solids concentration did not make hydrogen generation startup difficult, because hydrogen production is favored at high solids concentrations. Lag phases observed in all TS percentages, which may be originated from VFA and/or ammonia accumulation (Section 4.3.2). Increasing initial solids concentration from 2% to 5%, caused yield decreased for NP-, AP-, TP- and UP-test reactors by 38%, 67.5% and 50% and 54%, respectively (Figure 4.6). Lastly, fluctuations in headspace hydrogen content (Figure 4.7) which caused inhibition which might be inhibitory above 60%, might have affected hydrogen production and resulted in decreased H_2 yields.

Consequently, AP is the only pretreatment option giving distinguished yield results, compared to other options. Lower initial solids concentrations enable to apply all pretreatment options efficiently, but at higher TS%, gaining feasible yield results becomes harder. In this sense, it can be said that, despite the very low yields obtained, AP-yard waste was found as the best option for dark fermentative hydrogen production

Hydrogen production performance of the reactors were redrawn with respect to pretreatment options studied (Figure 4.8). In this way, it was aimed to analyze/compare the rate and acclimation periods for each yard waste type at different initial TS content.

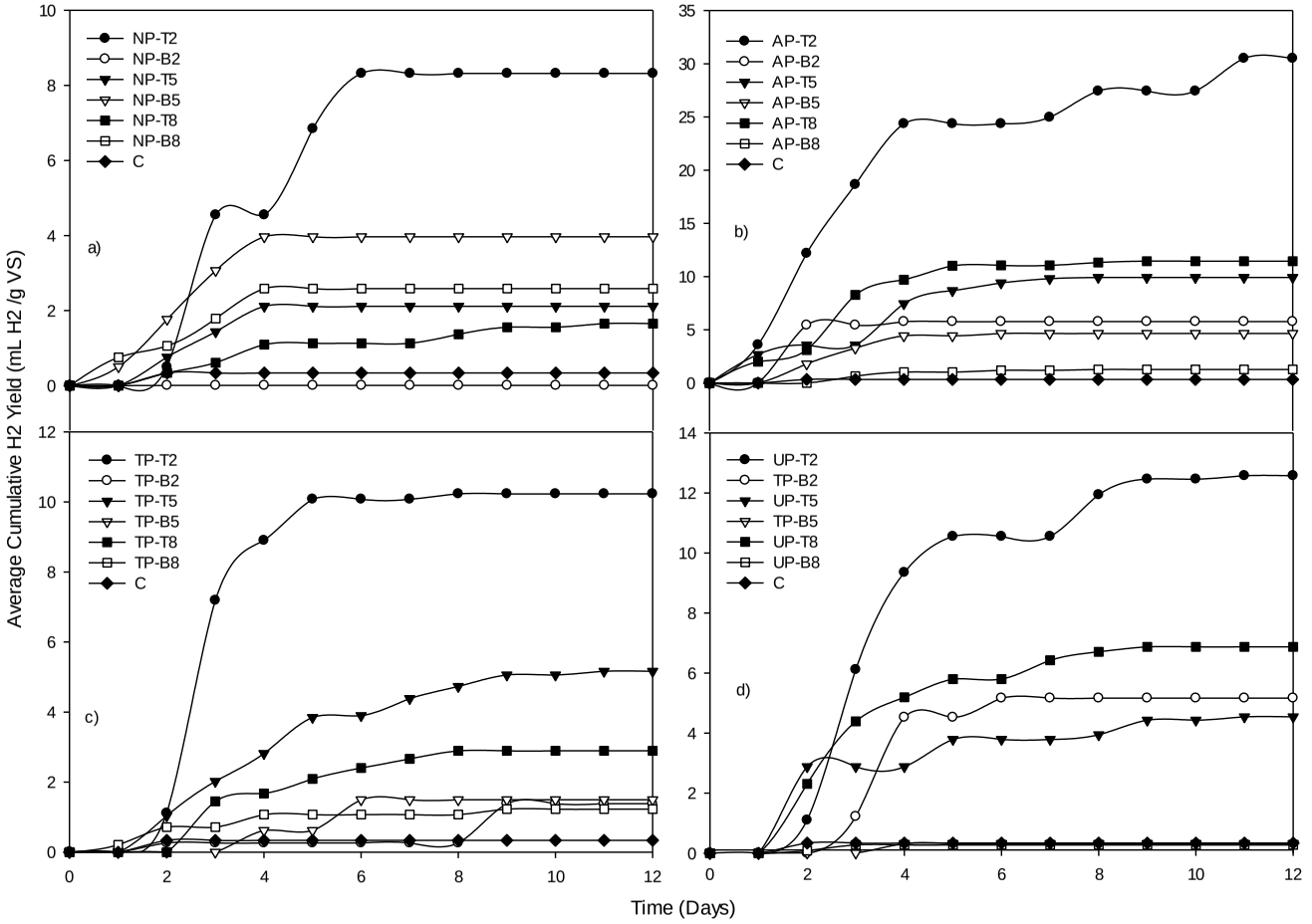

Figure 4.8 Cumulative H_2 Productions in Pretreatment Perspective for a) NP, b) AP, c) TP and d) UP

For NP-yard waste mix, 2% TS is the best option with 8 mL H_2/g VS. As the solids concentration increased, reaction rate decreased, hence hydrogen yield decreased. Blank reactors, reacted different solids contents with similar amounts. Since NP test and blank reactors produced comparable yields, especially for 5% and 8% TS contents, applying NP-yard waste to DF with inoculation is not feasible. On the other hand, since presented NP yield results are very low, even fractions make much difference in comparison. There is no need to use additional seed sludge, when it is achievable to get similar hydrogen yield results with blank reactor configuration. Consequently, TSAD of NP-yard waste mix is only feasible for 2% solids concentration.

For AP-yard waste mix, 2% TS is the best option with 30.5 mL H_2/g VS. 5% and 8% TS concentrations provided closer yield results as 10 and 11.5 mL H_2/g VS. As the solids concentration increases,reaction rate decreased, hence hydrogen yield mainly decreased. Solids concentration increase affected AP-yard waste in a negative way up to 5%. For this reason, TSAD of AP-yard waste mix is feasible for 2% solids concentration.

For TP-yard waste mix, 2% TS is the best option with 10 mL H_2/g VS. 5% and 8% TS concentrations provided yield results as 5 and 3 mL H_2/g VS. As the solids concentration increases,dur to the reaction rate decrese, hydrogen yield decreased. TSAD of TP-yard waste mix is feasible for 2% solids concentration.

For UP-yard waste mix, 2% TS is the best option with 12.5 mL H_2/g VS. 5% and 8% TS concentrations provided yield results as 4.5 and 7 mL H_2/g VS. As the solids concentration increases, hydrogen yield mainly decreases due to the reaction rate decrese. Variation in initial solids concentration did not make any difference for the initiation of hydrogen production. TSAD of TP-yard waste mix is feasible for 2% solids concentrationTSAD of UP-yard waste mix is feasible for 2% solids concentration.

In all test reactors, methane production was observed (Figure 4.9). This indicates that heat treatment of inoculum was not effective enough to inhibit methanogens.

As seen in Figure 4.9, methane production starts on 4th day of the incubation period in NP- and AP-test reactors on 3rd day in TP- and UP-test reactors, respectively. It is likely that methanogens in the test reactors acclimated after a while and started to produve methane. It should be noted that, there are two sources of microorganisms in the effective volume, one from the seed and the other from the yard waste as intrinsic microorganisms. Among those, one of them (i.e., seed) was pretreated to favor hydrogen producers. Thus, intrinsic microorganisms, coming from yard waste mix, might also contributed methane generation, in addition to hydrogen. Indeed, methane production was alsoobserved in blank reactors of NP and TP (Figure 4.9). Especially AP-test reactors produced significant amounts of methane, which should be considered in the cumulative energy gain calculations for TSAD set-up.

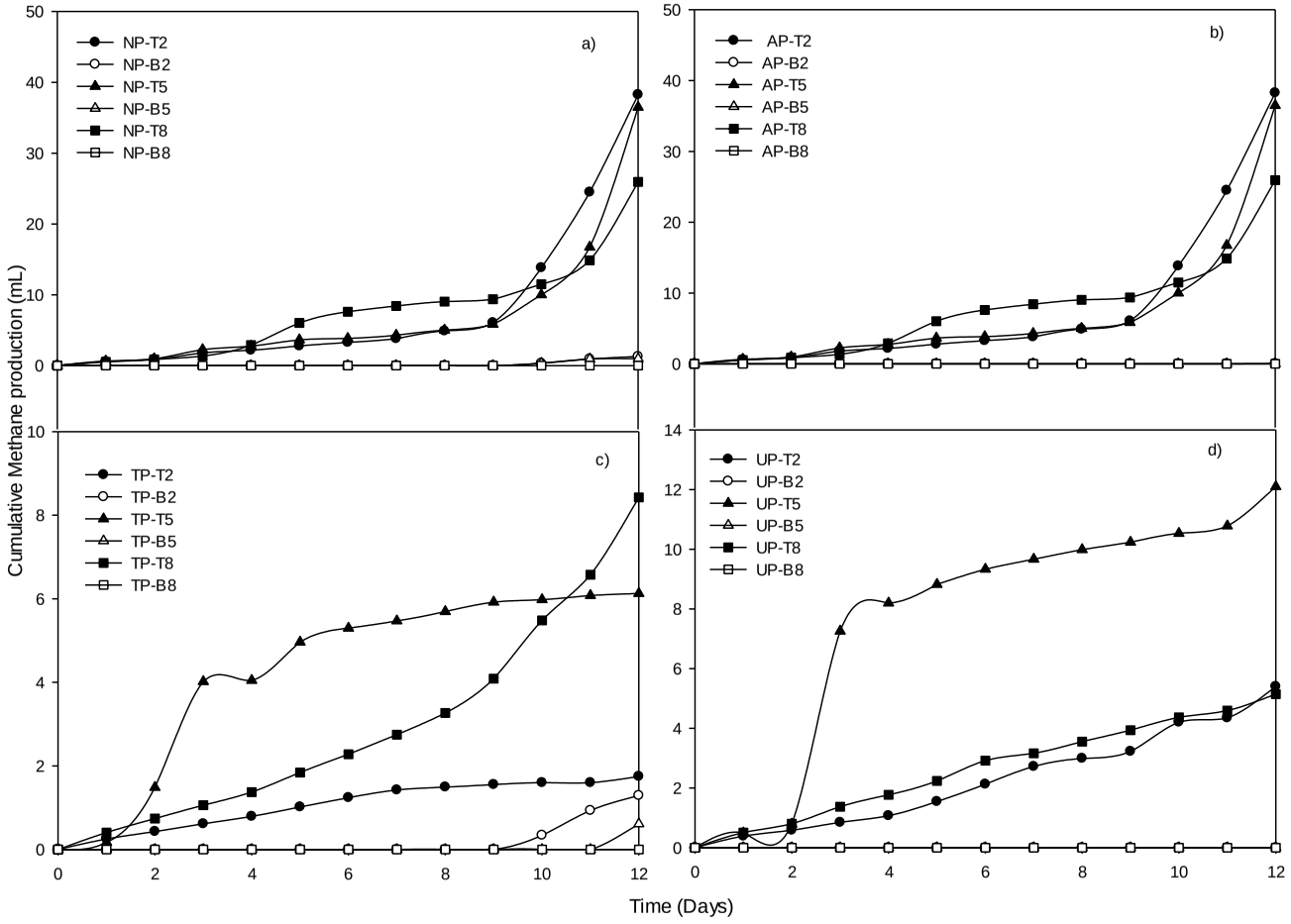

Figure 4.9 Average Cumulative CH_4 production in DF for a) NP-yard waste, b) TP-yard waste, c) AP-yard waste and d) UP-yard waste

4.3.1.2 The Results of the Initial and Final Reactor Characterization of DF experiments

In order to monitor anaerobic treatability in batch reactors of DF, main parameters were evaluated for the initial and final concentrations of reactors. Studied parameters are VS, TS, pH, COD, TP, TKN and TAN (Appendix I). According to these analysis, efficiency of anaerobic digestion and possible inhibitory developments were investigated. In tables and figures, capital B represents blank reactors and capital T represents test reactors.

The difference between initial and final VS concentrations expresses the digested (stabilized) waste amount in anaerobic processes. Methane production during anaerobic digestion is linked to the destroyed organic matter (VS) (Labatut and Gooch, 2012)

As seen in Table 4.8, hydrogen yield is mainly related with organic degradation for all reactors. High hydrogen yields suggest that, pretreatment options had significant effect on hydrolysis (Table 4.1, Section 4.1) and reflected this effect on VS reduction. NP-test reactors achieved VS reduction between 18-25.5%, AP-test reactors between 33-48%, TP-test reactors between 26.5-43% and UP-test reactors between 12-35% (Table 4.8). For AP- and UP-yard waste, VS degradation increase directly reflected in hydrogen yield. However, for NP- and TP- yard waste, as stabilized VS amount increases, decreased hydrogen yields were observed. The reason might be that at higher solids concentrations, VS degradation became inhibitory for anaerobic system, which resulted in decreased hydrogen yields. Literature studies for lignocellulosic substrates present a similar range for VS reduction.

Table 4.8 Average VS reduction, COD removal and yield tesults of DF test reactors

Reactors	VS Reduction (%)	Yield (mL H_2 /g VS)
NP- 2%	24.10	8.32
NP- 5%	17.97	2.11
NP- 8%	25.56	1.65
AP- 2%	34.16	30.51
AP- 5%	47.87	9.92
AP- 8%	32.83	11.45
TP- 2%	33.30	10.23
TP- 5%	26.61	5.16
TP- 8%	43.87	2.89
UP- 2%	30.76	12.57
UP- 5%	12.43	4.54
UP- 8%	28.97	6.87

COD represents the organic matter contained in the substrate. Process efficiency of DF can be evaluated according to removed COD amount (Meegoda et al., 2018). COD results of the initial and final concentrations of DF test reactors are given in Table 4.9. sCOD consumption was observed in all reactors, which also shows consumed organic portion. Unlike OSAD reactors, there were no sCOD increase after DF process. The reason might be that, 12 days of incubation was not enough to increase present soluble organics in the reactors.

NP-,AP-, TP- and UP-test reactors had a sCOD removal range of 21.5-26%, 32-60%, 21-28% and 20.5-32.5%, respectively (Table 4.9). Generally, COD removal in fermentative hydrogen production is less than 20% (Das and Veziroğlu, 2001). Higher removal efficiencies obtained despite of the low hydrogen yields can be explained with methane production in DF stage.

Cumulative methane yields obtained in DF experiments are shown in Table 4.10. According to these data, considerable amount of methane was observed, as mentioned previously. Mentioned organic reduction (i.e. sCOD removal or VS reduction) which is more than expected, is linked from this additional methane generation in DF reactors. Higher sCOD removal observed in AP-test reactors were

attributed to their higher methane production in these reactors (Figure 4.9 and Table 4.10)

Table 4.9 Average COD Results of DF Test reactors

	COD(mg/L)	sCOD(mg/L)	sCOD/tCOD	sCOD Removal %
NP-2% (t=0)	22135	4369	0.20	26.1
NP-2% (t=12)	22197	3231	0.15	
NP-5% (t=0)	54812	61740	1.13	28.2
NP-5% (t=12)	50147	44277	0.88	
NP-8% (t=0)	90463	19978	0.22	21
NP-8% (t=12)	99475	15775	0.16	
AP-2% (t=0)	19399	7823	0.40	32
AP-2% (t=12)	19453	5313	0.27	
AP-5% (t=0)	49569	10547	0.21	50
AP-5% (t=12)	40287	5169	0.13	
AP-8% (t=0)	71123	18941	0.41	39.3
AP-8% (t=12)	70464	11498	0.16	
TP-2% (t=0)	7891	3887	0.49	34.7
TP-2% (t=12)	7034	2538	0.36	
TP-5% (t=0)	19870	10027	0.50	18.2
TP-5% (t=12)	20489	8196	0.40	
TP-8% (t=0)	36813	19079	0.52	27.5
TP-8% (t=12)	37417	13832	0.37	
UP-2% (t=0)	14516	3174	0.22	27.1
UP-2% (t=12)	15537	2503	0.16	
UP-5% (t=0)	36850	5079	0.14	23.6
UP-5% (t=12)	35489	3880	0.11	
UP-8% (t=0)	59518	16741	0.28	31.6
UP-8% (t=12)	60341	11436	0.19	

Table 4.10 Average hydrogen and methane yields obtained in DF

	% TS	H_2 (mL H_2 /g VS)	CH_4 (mL CH_4 /g VS)
NP	2	8.32	38.01
	5	2.11	2.37
	8	1.65	1.35
AP	2	30.51	37.33
	5	9.92	14.19
	8	11.45	6.30
TP	2	10.23	1.65
	5	5.16	2.30
	8	2.89	2.00
UP	2	12.57	4.94
	5	4.54	4.36
	8	6.87	1.14

Another parameter that affects DF is TAN. The initial TAN concentrations in the test reactors was in the range of 97-298 mg/L for NP, 79-202 mg/L for AP, 890-1043 mg/L for TP and 1016-1378 mg/L for UP reactors (Appendix I). Effluent TAN ranges are recorded as 429-1008 mg/L for NP, 196-254 mg/L for AP, 902-1364 mg/L for TP, and 1174-1478 mg/L for UP reactors (Figure 4.10). Anaerobic digestion provides additional ammonia dissolution by decomposition of organic nitrogen in reactors. Hence TAN increase is expected at the end of the process (Demirer and Chen, 2004). High ammonia concentrations may affect digester efficiency by inhibition. As mentioned before, a wide range of TAN inhibitory levels has been presented in the literature, such as 150 mg/L (McCarty and McKenney, 1961), 345 mg/L (Ripley et al., 1985) and above 1500 mg/L (Angelidaki and Ahring, 1993). These values are marked on Figure 4.10. In this regard, AP-, and NP-test reactors initial and final TAN concentrations are in the safe range, however, TP- and UP-test reactors had much higher TAN concentrations, closer to the inhibition level (Figure 4.10). These high concentrations may be the reason of obtaining lower hydrogen yield results than expected (Section 4.3.1.1, Table 4.6).

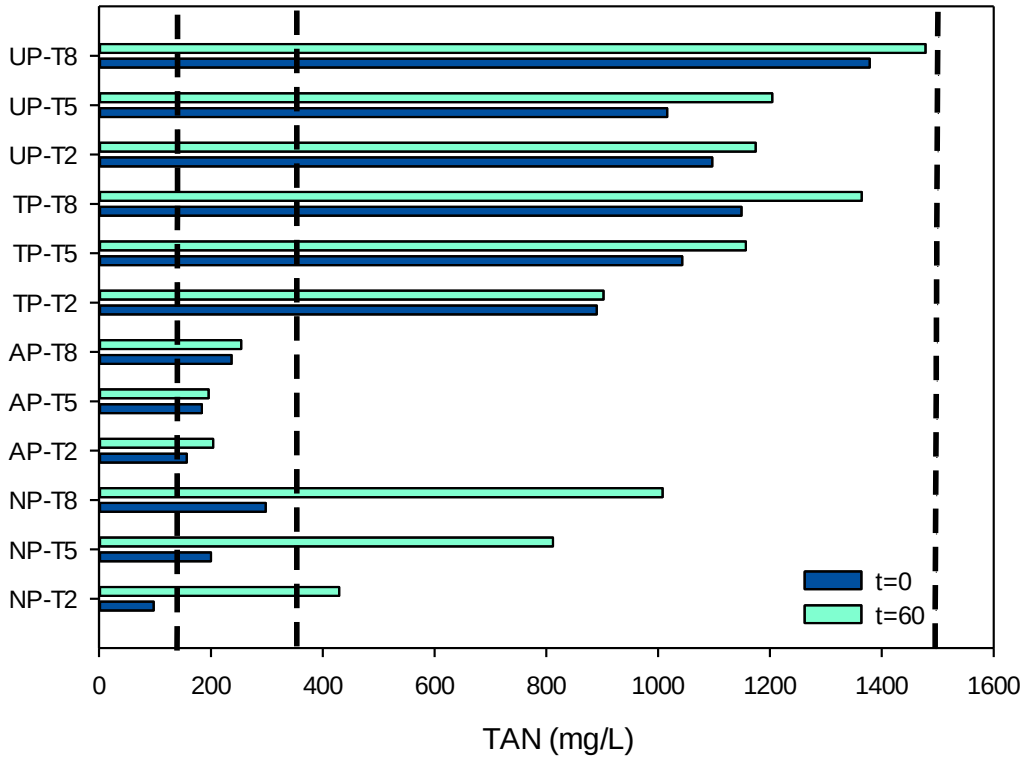

Figure 4.10 Initial and final TAN results of DF test reactors

Another important parameter for DF is pH. There is no specific optimum pH range for DF, due to the diverse substrate, inoculum or culture conditions. However, it can be said that pH range for DF of lignocellulosic wastes range in 4-6 (Yuan, 2017). In this sense, for all NP, AP, TP and UP test reactors increase in pH was observed. All test and blank reactors of DF set had a final pH range between 5.5-6 wich is within the levels of dark fermentation range. Therefore, the low H_2 yields observed were not attributed to the low pH levels (Appendix I).

Since reactors were operated as batch, pH changes were not monitored during digestion period. On the other hand, daily biogas composition analyses were conducted, which provides daily CH_4, CO_2 and H_2 headspace contents. However, predictions on pH drop can be done via CO_2 consumption and carbonate equilibria.

Acetogenic bacteria are able to reduce CO_2 and H_2 as carbon source and produce acetate (Garcia-Gonzalez and De Wever, 2018). *Rather than only dissolution of CO_2 in water, its consumption for acetic* acid formation may lead to pH drop during digestion period. According to daily gas composition data (Appendix H), CO_2 consumption was observed, which *might indicate homoacetogenesis and* and cause decrease in H_2 yield.

4.3.2 The Results of Methanogenesis Experiments of TSAD Set-up

4.3.2.1 The Methane Production Potential

At the end of the first-stage of TSAD (i.e. DF), reactor effluents were filtered, and methanogenesis reactors were fed with these filtrates. Biogas production was examined at 35±2 ℃ for 20 days. As mentioned previousy, additional mini TSAD experiment involving both DF and methanogenesis stepswas also conducted, in order to check the reliability of obtained data.

The methane production yield results are given in Table 4.11. As seen in Table 4.11, all pretreatment applications improved the anaerobic digestion of NP-yard waste, TP being the most effective one. Despite of this improvement, it is seen that the yield values are quite low for all pretreatment and initial TS contents studied. The methane yield range obtained in NP-, AP-, TP- and UP-test reactors are 0.7-3 mL CH4 /g VS, 8.5-11 mL CH4 /g VS, 30-40 mL CH4 /g VS and 12.5-19.5 mL CH_4 /g VS, respectively. None of the blank or control reactors were produced methane in this stage. For all substrate options, 2% TS is the optimum solids concentration in terms of methane yield.

As seen in Table 4.11, as solids percentage increased, methane yields slightly decreased. Since high solid contents cause inhibition problems in anaerobic digestion, this drop was expected. However, as mentioned previously, obtained yield results are very low, compared to theoretical methane yield (395 mL CH_4/g

VS). This inefficient methanogenic activity might be originated from several situations. First of all, initial organic load might be insufficient for anaerobic digestion, because only the soluble portion of DF effluent was added to the second-stage. As mentioned in OSAD results, with the help of long incubation period at 35±2℃ and constant mixing, undissolved yard waste mix continued being solubilized in the reactors and increased available readily degradable substrate for microorganisms (Section 4.2.2, Table 4.5). In DF stage of TSAD case, however, not only hydrogen but also significant amounts of methane was produced, hence COD removal was more than expected. Despite the high initial sCOD concentrations in the reactors (Appendix K), the remaining biodegradable soluble portion of organics might be insufficient for methanogenic activity in the second-stage. In this sense, mini TSAD set-up was conducted via increasing reactor volume and in turn initial COD amount. Similar results were obtained with this improved experiment namely, almost 4 and 37 ml CH_4/g VS for AP- and TP-test reactors, respectively (Appendix G). This indicates that much of organic load increase did not have a significant effect on methane yields. Secondly, excess VFA concentration accumulated from dark fermentation period may be the reason for low yield results in TSAD. Thirdly, it might be due to low activity of seed sludge. Thus, seed sludge was checked if it had a sufficient activity or not to speculate on the low CH_4 yields via Specific Methanogenic Activity (SMA) assays (Appendix J). SMA results showed almost 65% activity with respect to the theoretical methane production. Therefore, it can be concluded that the activity of seed sludge is not that high, yet might be sufficient enough for acetic acid degradation. However, when the seed sludge was exposed to the effluent of DF stage, it might have been affected more. After pretreatment and even dark fermentation stage, some by-products that are inhibitory to the methanogenesis might have been produced. These products might be high TAN concentrations (Section 4.3.2.2, Figure 4.11) and/or some other by-products produced through pretreatment (Gottumukkala et al., 2019)

Table 4.11 Average methane yields obtained in methanogenesis experiments

	TS %	Methane yield (mL CH_4/g VS)	Achieved % theoretical yield	Ave yield increase compared to NP-test reactor (%)[a]
NP	2	3.00	0.75	_
	5	2.89	0.73	_
	8	0.68	0.17	_
AP	2	11.40	2.88	280
	5	9.77	2.47	238
	8	8.52	2.15	1153
TP	2	39.94	10.11	1231
	5	33.10	8.37	1045
	8	29.92	7.57	4300
UP	2	19.51	4.94	550
	5	12.47	3.15	331.5
	8	13.05	3.30	1819

[a]Ave: Average

Methane yields of TSAD experiments from literature studies conducted with similar lignocellulosic substrates are shown inTable 4.12. Methane yield was usually expected to increase in TSAD process, because DF is able to degrade the COD below 20%, which gives yield amounts between 10-20% (Das and Veziroğlu, 2001). Additional methanogenesis step is expected to increase overall energy recovery. However, in the literature there are some examples for obtaining low methane yields after dark fermentation stage as observed in this thesis study. In a study conducted with cornstalk, methane yield result was 13 mL CH_4/g TS (Lu et al., 2009). Another TSAD study with sweet sorghum showed that 29 mL CH_4/g TS was observed (Antonopoulou et al., 2008). These low yield amounts were attribured to the lignocellulosic structure of the mentioned substrates, which disables microbial degradation and hence anaerobic efficiency (Rafieenia et al., 2018).

Table 4.12 The results of TSAD experiments with similar lignocellulosic substrates in the literature

Substrate	Pretreatment	H_2 Yield[a]	CH_4 Yield[b]	Reference[c]
Wheat bran	-	18.9	243.5	Corneli et al. (2016)
Corn Stalk	-	80	227	Guo et al. (2014)
Grass silage	Alkaline	5.64	467	Pakarinen et al. (2009)
Water hyacinth	-	7.5	93	Chuang et al. (2011)
Poplar wood	Extrusion	40	299	Akobi et al. (2016)
Sweet Sorghum	-	10.1	29.1	Antonopoulou et al. (2008)
Cornstalk	-	7.8	13.1	Lu et al. (2009)
Yard Waste	Alkaline	30.51	49	This Study

[a](mL H_2/g VS), [b](mL CH_4/g VS), [c]All studies performed in batch reactors

In OSAD process, which includes only methane production, AP was the best option. However, in TSAD process, TP distinguished in terms of methane yield. The highest methane yield, as 40 mL CH_4 and highest % theoretical yield increase as 10% was achieved with 2% TP-yard waste mix (Table 4.11). The reason might be that, AP-yard waste mix released inhibitory by-products during DF and its effluent decreased methane yield in methanogenesis step. Sodium hydroxide, the main chemical in alkaline pretreatment, needs neutralization at the end of pretreatment, which creates salts that can inhibit butanol fermentation (Gottumukkala et al., 2019). This inhibition has a major effect on dark fermentative hydrogen production. These products might be also inhibiting to methanogens which are known as the most sensitive ones among the anaerobic digestions's microorganisms.

In order to compare TS% effect on hydrogen production potential of pretreated and non-pretreated yard waste mix, three different solids concentrations are investigated. In the perspective of initial TS %, all three options (2%, 5% and 8%) were found as the best alternatives for TP-yard waste for producing the highest yield among others (Table 4.10). For 2% TS content, TP-test reactor produced the highest cumulative methane yield with 40 mL CH4/g VS. 2% UP-test reactor

followed by 20 mL CH4/g VS. AP test reactor has a yield result with 11 mL CH4/g VS and lastly NP-test reactor achieved 3 mL CH4/g VS.

For 5% TS content, TP-test reactor again produced the highest cumulative methane yield with 33 mL CH_4/g VS. UP 5% test reactor is in the second line with 12.5 mL CH_4/g VS. AP test reactor has a yield result with 10 mL CH_4/g VS and lastly NP test reactor achieved 3 mL CH_4/g VS.

For 8% TS content, TP test reactor produced the highest cumulative methane yield with 30 mL CH_4/g VS. UP 5% test reactor is in the second line with 13 mL CH_4/g VS. AP test reactor has a yield result with 8.5 mL CH_4/g VS and lastly NP test reactor achieved 0.7 mL CH_4/g VS.

Consequently, TP-yard waste is the best pretreatment option, giving comperatively higher yield results. Lower initial solids concentrations enable to apply all pretreatment options efficiently, but at higher TS%, considering the potential effects of inhibitory by-products, gaining feasible yield results becomes harder. In this sense, it can be said that, TP-yard waste is the best option for methanogenesis in TSAD process.

4.3.2.2 The Results of Characterization Study in Methanogenesis Experiments

In order to monitor anaerobic treatability in batch reactors of methanogenesis, main parameters were evaluated for the initial and final concentrations of reactors. Studied parameters are VS, TS, pH, COD, TP, TKN and TAN (Appendix K). According to these analyses, efficiency of anaerobic digestion and possible inhibitory developments were investigated. Since second-stage of TSAD process was not very efficient, in terms of biogas production and yield, slight changes in the characteristics of the reactors' content were expected. In tables and figures, capital B represents blank reactors and capital T represents test reactors.

As seen in Table 4.13, cumulative methane yield was directly related with organic degradation for all reactors. Higher yields are due to higher organic removals. However, for obtaining low CH_4 yields (Table 4.11), VS reduction efficiencies were low as expected. NP-test reactors achieved VS reduction between 0.1-0.2 %, AP reactors between 0.1-0.7 %, TP reactors between 3-5% and UP reactors between 1-4%. For all reactor, as TS content increased, stabilized VS amount decreased. Literature studies with similar lignocellulosic substrates showed that, VS reduction is in the range between 12-46% (Hills and Roberts, 1981; Romano et al., 2009; Zhu et al., 2010).

Table 4.13 Average VS reduction, COD removal and yield results of methanogenesis test reactors

Reactors	VS Reduction(%)	sCOD Removal(%)	Yield (mL CH_4 /g VS)
NP- 2%	0.23	0.63	3.00
NP- 5%	0.24	0.09	2.89
NP- 8%	0.03	0.41	0.68
AP- 2%	0.71	0.14	11.40
AP- 5%	0.32	1.02	9.77
AP- 8%	0.13	0.64	8.52
TP- 2%	4.93	6.5	39.94
TP- 5%	4.70	2.73	33.10
TP- 8%	2.92	2.69	29.92
UP- 2%	1.92	1.68	19.51
UP- 5%	4.26	0.72	12.47
UP- 8%	1.34	0.90	13.05

The efficiency of anaerobic digestion can be evaluated with degraded COD amount, which also shows consumed organic portion (Meegoda et al., 2018). Similar to the low VS removal, low removal efficiencies were also expected for sCOD (Table 4.14). As seen in Table 4.14, SCOD removal in methanogenesis stage of TSAD are in the range between 0.1-0.6% for NP, 0.1-1% for AP, 3-6.5% for TP and 1-2% for UP reactors. These are ranges are very low compared to similar literature results. In a study conducted with grass silage liquor showed that,

sCOD reduction was 86% (Abu-Dahrieh et al., 2011). Another study of TSAD of grass silage presented an organic removal as 80% (Jagadabhi et al., 2011). TSAD of sisal leaf presented 50% of organic removal efficiency (Mshandete et al., 2008). 60 % of SCOD removal was achieved with anaerobic digestion of grass silage (Jagadabhi et al., 2011). Lastly, a study of anaerobic digestion of Acacia leaf showed that, sCOD removal was 69% (Chaiyapong and Chavalparit, 2016).

Table 4.14 Average COD results of methanogenesis test reactors

	COD(mg/L)	sCOD(mg/L)	sCOD/tCOD	sCOD Removal %
NP-2% (t=0)	24191	3631	0.15	0.5
NP-2% (t=20)	24213	3611	0.15	
NP-5% (t=0)	5214	4627	0.89	0.3
NP-5% (t=20)	52007	4610	0.88	
NP-8% (t=0)	99975	16775	0.17	0.5
NP-8% (t=20)	99842	16684	0.17	
AP-2% (t=0)	19953	5613	0.28	0.4
AP-2% (t=20)	19898	5590	0.28	
AP-5% (t=0)	42287	5261	0.12	1
AP-5% (t=20)	42291	5208	0.12	
AP-8% (t=0)	73461	12498	0.17	0.7
AP-8% (t=20)	73401	12408	0.17	
TP-2% (t=0)	7046	2573	0.37	7
TP-2% (t=20)	7004	2391	0.34	
TP-5% (t=0)	24689	8496	0.34	3.3
TP-5% (t=20)	24523	8209	0.33	
TP-8% (t=0)	38617	13932	0.36	2.7
TP-8% (t=20)	38591	13547	0.35	
UP-2% (t=0)	16537	2603	016	2.8
UP-2% (t=20)	16341	2529	0.15	
UP-5% (t=0)	37489	3680	0.10	1.6
UP-5% (t=20)	37134	3619	0.10	
UP-8% (t=0)	64321	12436	0.19	1.1
UP-8% (t=20)	64194	12299	0.19	

Another parameter that influences methanogenic activity is TAN. The initial TAN concentrations in the reactors was in the range between 434-1048 mg/L for NP-, 200-268 mg/L for AP, 972-1287 mg/L for TP- and 1104-1378 mg/L for UP-test reactors (Appendix K). Effluent TAN ranges are between 453-1057 mg/L for NP-,

203-274 mg/L for AP-, 1035-1287 mg/L for TP-, and 1196-1307 mg/L for UP-test reactors (Figure 4.11). As mentioned previously, TAN concentration is inhibitory above 1500 mg/L (Angelidaki and Ahring, 1993). TAN inhibition in anaerobic digestion is generally indicated by decreasing methane production rates, lowered methane yields and increased concentrations of by-products like VFA (Calli et al., 2005). Due to the fact that UP, and TP test reactors has initial TAN concentrations close to the inhibition limit, methane production efficiency might be affected by accumulation of TAN.

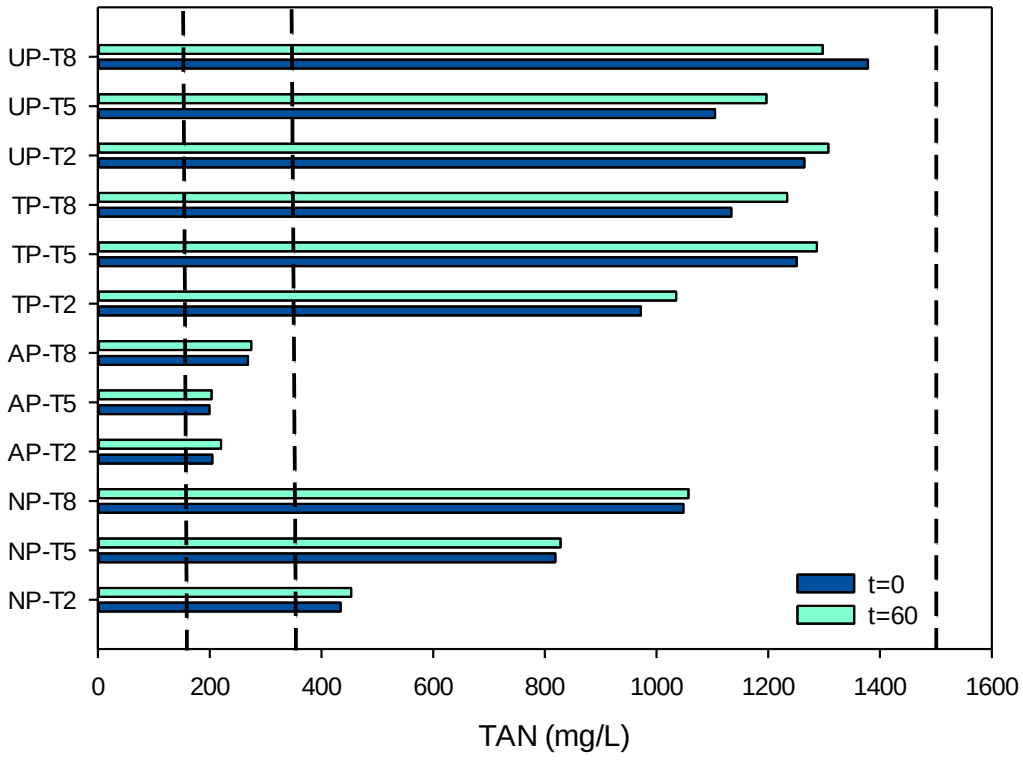

Figure 4.11 Initial and final TAN concentrations in the methanogenesis stage of TSAD test reactors

Lastly, pH is another crucial parameter influencing anaerobic processes. In a well operated anaerobic digestion system, pH is expected to increase slightly at the end,

since microorganisms generate alkalinity as they consume protein rich organic substances (Labatut and Gooch, 2012). At the end of methanogenesis step, all test and blank reactors increased their pH amounts between 7.2-7.5 (Appendix K). Therefore, the low CH_4 yields were not attributed to pH.

4.4 The Comparison of Energy Production in OSAD and TSAD Experiments

Apart from yield comparison, gained cumulative energy amount is also crucial to determine the best treatment option for conversion of yard waste into energy and make reasonable analyze between OSAD and TSAD. Since methane production was observed both in DF and methanogenesis stages of TSAD process, comparison with respect to cumulative energy amounts is also important. Overall energy recoveries of OSAD and TSAD processes are given in Table 4.15 and Table 4.16, respectively.

Table 4.15 OSAD yield and energy recovery

	% TS	Methane Yield (mL CH_4/g VS)	Energy from CH_4 (MJ/kgVS_{added})[a]
NP	2	267	9.30
	5	148	5.15
	8	74	2.58
AP	2	290	10.10
	5	313	10.90
	8	231	8.04
TP	2	250	8.70
	5	180	6.27
	8	184	6.41
UP	2	69	2.40
	5	85	2.96
	8	81	2.82

[a] Gross heating value of CH_4 is 55.58 kJ/g (Lewandowski 1999).

Table 4.16 TSAD yield and energy recovery

	TS %	Yields (mL H_2 or CH_4 /g VS)			Energy (MJ/kg VS_{added})[a]		
		1st stage, H_2	1st stage, CH_4	2nd stage, CH_4	From H_2	From CH_4	Total
NP	2	8.3	38.1	3.0	0.1	1.4	1.5
	5	5.1	2.4	2.9	0.1	0.2	0.3
	8	1.6	1.3	0.7	0.1	0.2	0.3
AP	2	30.5	37.3	11.4	0.3	1.7	2.0
	5	9.9	14.2	9.7	0.1	0.8	0.9
	8	11.4	6.3	8.5	0.1	0.5	0.6
TP	2	10.2	1.6	39.9	0.1	1.5	1.6
	5	5.2	2.3	33.1	0.1	1.3	1.4
	8	2.9	2.0	29.9	0.1	1.1	1.2
UP	2	12.6	4.9	19.5	0.2	0.7	0.9
	5	4.5	4.4	12.5	0.2	0.6	0.8
	8	6.9	1.1	13.05	0.1	0.5	0.6

[a] Gross heating values of H_2 and CH_4 are 141.8 kJ/g and 55.58 kJ/g, respectively (Lewandowski 1999).

As seen in Table 4.15, energy recovery from digestion of yard wastes is remarkably greater in OSAD process. The lowest energy amount produced in OSAD (2.58 MJ/kg VS_{added}) is even higher than the highest energy amount produced in TSAD process (2 MJ/kg VS_{added}). These results, together with the yield results reveal that, under the studied conditions and pretreatment applications, OSAD should be applied to gain energy from yard wastes. If OSAD is applied to the raw yard waste, 2% TS content should be prefered among the other initial TS contents. In other words, if 2% TS is applied in OSAD processses, considering the chemical usage and energy application in pretreatment alternatives applied, there is no need for pretreatment. However, for higher initial TS contents, pretreatment should be applied in OSAD process. InTable 4.15, it can be seen that alkaline pretreatment is the best alternative both in terms of methane yield and energy yield. 5% TS AP-test reactor gave the highest methane yield (313 mL CH_4/g VS) and the highest energy yield (11 MJ/kg VS_{added}). Alkaline pretreatment almost doubles the energy recovery from yard waste at 5% initial TS content.

In TSAD, energy yield values ranged as 0.3-1.5 MJ/kg VS_{added} for NP-yard wastes and 0.6-2 MJ/kg VS_{added} for pretreated yard wastes. The majority of the energy gained (i.e. 58% of the total energy) is due to methanogenesis stage. Nevertheless, these low energy yields well indicates the potential inhibition in TSAD system, which should be investigated in detail. Studies conducted with lignocellulosic materials showed higher energy recoveries. For example, common energy recovery from rice flour co-digested with swine manure via TSAD was determined to be 9.85 MJ/kg VS; in particular, 1.4 MJ/kg VS (corresponding 14.2% of total energy) comes from hydrogen production, while 8.45 MJ/kg VS produced from methane production (Schievano et al., 2014)

In TSAD, AP-yard waste again gave the optimum cumulative energy yields as in OSAD system. Since methane production was observed in the first stage as well, energy produced from two stages were considered in calculations. Table 4.16 shows that, 2% TS AP reactor gave the highest hydrogen yield (30.51 mL H_2/g VS) and cumulative methane yield (49 mL CH_4/g VS) and the highest energy yield from hydrogen (0.34 MJ/kg VS added) and from methane (1.70 MJ/kg VS added).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The main objective of this study is to investigate the energy potential of yard wastes which is produced in high amounts but has not been discovered as a biomass source in Turkey, with OSAD and TSAD processes. Different pretreatment methods were applied with different initial solids concentrations, in order to investigate the solubilisation of yard wastes and its efficiency.

Based on the results gained in this study, the conclusions obtained as follows;

- NP-yard waste had a sCOD/COD ratio of 6%. All pretreatment applications increased this ratio. TP-yard waste gave the highest result as 51% , meaning 45% increase in solubilization compared to NP-yard waste. AP-yard waste followed this solubilisation efficiency by 43% and UP-yard waste by 17%. Having the highest solubilisation efficiency might not be the sign for the best microbial degradation and energy yield. Pretreatment procedures might release inhibitory by-products, which affect anaerobic digestion negatively. In this sense, regardless of the solubilisation efficiency, all pretreatment options were investigated in OSAD and TSAD processes.
- Accessible cellulose amount of NP-yard waste, which is 20%, increased after AP, TP and UP applications by 53% , 44% and 60%, respectively.
- It is possible to produce energy from yard wastes either pretreated or non-pretreated. If it is non-pretreated, initial solid content becomes a decisive parameter. In OSAD experiments, for all three initial solids contents (2%, 5% and 8%), NP-test reactors achieved yield amounts between 74-267 mL CH_4/g

VS, AP-test reactors achieved 231-313 mL CH_4/g VS, TP-test reactors achieved 180-250 mL CH_4/g VS and UP-test reactors achieved 69-81 mL CH_4/g VS at the end of 60 days of incubation.

  - As TS content increased, methane yields generally decreased. AP-test reactor gained the highest methane yield as 313 mL CH_4/g VS at 5% TS content.

  - AP-test reactors obtained the highest methane yield for all three initial TS contents among other test reactors. Yield results showed that, the highest solubilisation efficiency (obtained by TP-yard waste) does not always indicate higher yields. The reason for lower methane yield results for TP- and UP-test reactors compared to AP-test reactors might have been that, during pretreatment procedures, inhibitory by-products might be formed and inhibited microbial activity during OSAD. From yield results it can be seen that, especially for UP-test reactors, inhibitory by-product formation was so effective that, even at the lowest TS content (2%) inhibition started.

- In OSAD experiments, at 2% TS content, pretreatment application is not feasible, because NP-test reactor's yield amount (267 mL CH_4/g VS) was similar to 2% AP-test reactor's yield (290 mL CH_4/g VS). 9% yield difference is not enough to waste additional chemicals for anaerobic digestion, in terms of feasibility. This unexpectedly high methane production in raw yard waste was due to intrinsic microorganisms. Observing remarkable amount of yield (140 mL CH_4/g VS) in 2% NP-blank reactor might be the proof for these microorganisms activity. Intrinsic microorganisms made AP option unnecessary at 2% TS concentration. However, at higher solids concentrations (5% and 8%) and when pretreatment was applied.

  - At 5% TS, AP application is feasible thanks to its significant yield difference (165 mL CH_4/g VS) compared to NP-test reactor. At 8% TS, again AP-test reactor is applicable in OSAD process.

- As TS concentration increased, reaction rates decreased and acclimation time increased, which is due to the inhibition resulted of high solids concentrations.

- According to characterization results of OSAD reactors, lag phases observed in cumulative yield graphs were originated from sCOD increase during incubation period. 35℃ and constant mixing for 60 days provided additional solubilisation for yard waste mix. Since effluent sCOD was higher than initial concentrations, sCOD reduction could not be observed. Organic degradation could be observed via VS reduction, which had a range between 21-70%. For TP- and UP-test reactors, TAN concentrations were closer to inhibitory limit (1500 TAN mg/L) at the end of OSAD. This might also a reason for lower yield results.

- Considering the total energy production results, it was obtained from TSAD experiments that it is not feasible to digest yard waste in TSAD systems, whether pretreatment is applied or not.

  - In the DF stage of TSAD process, for all three initial solids contents (2%, 5% and 8%), NP-test reactors achieved yield amounts between 5-8 mL H_2/g VS , AP-Test reactors achieved 10-30.5 mL H_2/g VS, TP-test reactors achieved 3-10 mL H_2/g VS and UP-test reactors achieved 4.5-12.5 mL H_2/g VS at the end of 12 days of incubation. As TS content increased, hydrogen yields decreased.

  - AP-test reactor gained the highest yield amount by 30.5 mL H_2/g VS via 2% TS content.

  - For all yard waste mix types, 2% TS gave the highest hydrogen yield. Almost all blank reactors produced hydrogen,yet in small amounts. Intrinsic microorganisms are capable of producing hydrogen. Moreover, they are not totally inactivated after pretreatment options, like in OSAD process. Hydrogen production in blank reactors were not avoided via

pretreatment applications but reduced by the increase in solids concentrations.

- In terms of hydrogen yield, the optimum pretreatment option for all initial TS contents was found as AP-yard waste.
- In DF stage of TSAD methane production was observed in all test reactors. This might be indicating that heat treatment of inoculum was not effective enough to inactivate methanogens. Besides, intrinsic microorganisms might also have contributed to methane generation, in addition to hydrogen. Since methane production was not observed simultaneously with hydrogen, it can be said that, inhibited methanogens acclimated after a while and started to produce methane.
- According to the characterization results of DF reactors, NP-test reactors had a sCOD removal range between 21.5-26%, AP-test reactors had 32-60%, TP-test reactors had 21-28% and UP-test reactors had between 20.5-32.5%. Since COD removal in fermentative hydrogen production is stated to be less than 20% (Das and Veziroğlu, 2001). Higher COD removal efficiencies obtained in this study, due to the methane production related COD consumption.
- NP-test reactors achieved VS reduction between 18-25.5%, AP-test reactors between 33-48%, TP-test reactors between 26.5-43% and UP-test reactors between 12-35%.
- AP- and NP-test reactors initial and final TAN concentrations are in safe range, however, TP- and UP-test reactors attributed much higher TAN concentrations, closer to inhibition levels. These high concentrations may be the reason of obtaining lower hydrogen yield than expected.
- In methanogenesis stage of TSAD, NP-test reactors achieved methane yields between 0.7-3 mL CH_4/g VS, AP-test achieved 8.5-11 mL CH_4/g

VS, TP-test reactors achieved 30-40 mL CH_4/g VS and UP-test reactors achieved 12.5-19.5 mL CH_4/g VS methane yield. None of the blank or control reactors produced methane in this stage. For all substrate options, 2% TS is the optimum solids concentration in terms of methane yield. TP-yard waste gave the highest methane yield for all initial TS concentrations.

- These low methane yields obtained in methanogenesis stage might be due to the inhibitory compounds and also the activity of the seed sludge. The activity of seed sludge is not that high (66% of theoretical yield) however it might be sufficient for acetic acid degradation. Yet, when this seed sludge was inoculated with DF effluent with potential inhibitory compounds, it might have been affected negatively. Lower methane yields might be related to the by-products which are inhibitory for methanogenesis were produced after pretreatment options or DF step.

- OSAD process had remarkably greater energy recoveries between 2.58-11 MJ/ kg VS_{added}, compared to TSAD process as 0.3-2 MJ/ kg VS_{added}.
- According to yield and energy recovery results, under the studied conditions and pretreatment options, OSAD should be applied to obtain sufficient energy from yard wastes. If 2% TS content will be used, pretreatment might not be necessary. However, if higher TS contents, such as 5% TS, will be applied, alkaline pretreatment is recommended. It should be noted that feasibility studies should be also performed beforehand.

According to obtained yield and energy recovery results, it was concluded that OSAD with yard waste presented high amounts compared to similar literature studies with lignocellulosic yard waste processes. On the other side, in TSAD process, overall yield and energy results were low. The most important parameter to evaluate these systems is yield results. Various recommendations can be given for the optimization of these processes. First of all, intrinsic microorganism can be

enriched and used as the main microbial source in digestion processes. Since these organisms are readily adapted to yard waste, their acclimation period might be shorter and yield results might be improved. Secondly, hybrid pretreatment systems are recommended. By-products formed during pretreatments might be eliminated via following pretreatment process (Pol et al., 2014). Sequential pretreatment might provide an optimized break down in lignocellulosic composition and improve hydrolysis efficiency during anaerobic digestion and/or increase yield results. For example hydrogen peroxide, followed by ultrasound pretreatment eliminated more lignin than individual trials, hence hydrolysis yields were the highest for this sequential system (Bussemaker and Zhang, 2013). As a third alternative, by-product analysis can be analyzed. If the characterization and concentration of by-products is determined, their elimination options can be monitored efficiently. Lastly, co-digestion of yard waste with manure is offered. Co-digestion of lignocellulosic substrates are commonly adapted on field. For example, in Germany, 30-40% of the biogas plants, on average 8% by mass and in some cases up to 50%, use yard waste as co-substrate (Rodriguez et al., 2017). The important point is that, TAN concentrations should be monitored carefully not to go beyond inhibitory limits. Since yard waste TAN concentrations observed to reach higher amounts at the end of incubation and after some of the pretreatment processes (ultrasound and thermal in this thesis study), additional TAN source (i.e. manure) should be characterized in detail.

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A. Reactor Configurations of OSAD

	Reactor	Seed Sludge (mL)	Feed (Yard Waste) (gr)	BM (mL)	Water (mL)	Effective Vol (mL)
NP	Control	45.00	-	8.60	6.40	60.00
	Blank (2%)	0.00	1.20	8.60	51.40	60.00
	Blank (5%)	0.00	3.00	8.60	51.40	60.00
	Blank (8%)	0.00	4.80	8.60	51.40	60.00
	Test (2%)	45.00	1.20	8.60	6.40	60.00
	Test (5%)	45.00	3.00	8.60	6.40	60.00
	Test (8%)	45.00	4.80	8.60	6.40	60.00
AP	Blank (2%)	0.00	1.20	8.60	51.40	60.00
	Blank (5%)	0.00	3.00	8.60	51.40	60.00
	Blank (8%)	0.00	4.80	8.60	51.40	60.00
	Test (2%)	45.00	1.20	8.60	6.40	60.00
	Test (5%)	45.00	3.00	8.60	6.40	60.00
	Test (8%)	45.00	4.80	8.60	6.40	60.00
TP	Blank (2%)	0.00	1.49	8.60	51.40	60.00
	Blank (5%)	0.00	3.75	8.60	51.40	60.00
	Blank (8%)	0.00	6.81	8.60	51.40	60.00
	Test (2%)	45.00	1.49	8.60	6.40	60.00
	Test (5%)	45.00	3.75	8.60	6.40	60.00
	Test (8%)	45.00	6.81	8.60	6.40	60.00

A. Reactor Configurations of OSAD (Continued)

	Reactor	Seed Sludge (mL)	Feed (Yard Waste) (gr)	BM (mL)	Water (mL)	Effective Vol (mL)
UP	Blank (2%)	0.00	1.27	8.60	51.40	60.00
	Blank (5%)	0.00	3.20	8.60	51.40	60.00
	Blank (8%)	0.00	5.10	8.60	51.40	60.00
	Test (2%)	45.00	1.27	8.60	6.40	60.00
	Test (5%)	45.00	3.20	8.60	6.40	60.00
	Test (8%)	45.00	5.10	8.60	6.40	60.00

B. Preliminary Study of First-stage of TSAD

Due to the uncertainty in initial pH of dark fermentation for selected yard waste mix, before main set up, pre-study with pH 5.5 and 7 was applied. In this preliminary study, batch reactors with total volume of 110 mL and effective volume of 60 mL were used. Experiments were performed with a batch set-up, including blank, test reactors of AP yard waste mix. All test reactors contained basal medium (BM), alkaline pretreated yard waste with certain TS% amount (2%) and seed sludge , while blank reactors contained all (yard waste + BM), but not the seed sludge. Seed sludge was initially heat pretreated for 1 hour at 105 ℃.

Two different initial pH adjustments were held. For acidic conditions, pH was reduced to 5.5, and two different S/I amounts were selected, which are 4 and 8. For neutral conditions, pH was adjusted to 7 and S/I value was studied as 4. pH adjustments were done with HCL and/or NaOH.

Lastly, after sealing with rubber stopper, in order to provide anaerobic environment, all reactors were flushed with 100% N_2 gas for 5 minutes, placed on a shaker with 175 rpm in a hot room at 35±2°C. Daily biogas production and gas composition analysis were performed to determine the hydrogen production potential of the reactors.

According to the obtained results shown in Figure B.1 , pH 7 and S/I 4 gave the optimum results both in terms of hydrogen yield (12.8 mL H_2/g VS) and biogas production (8 mL).

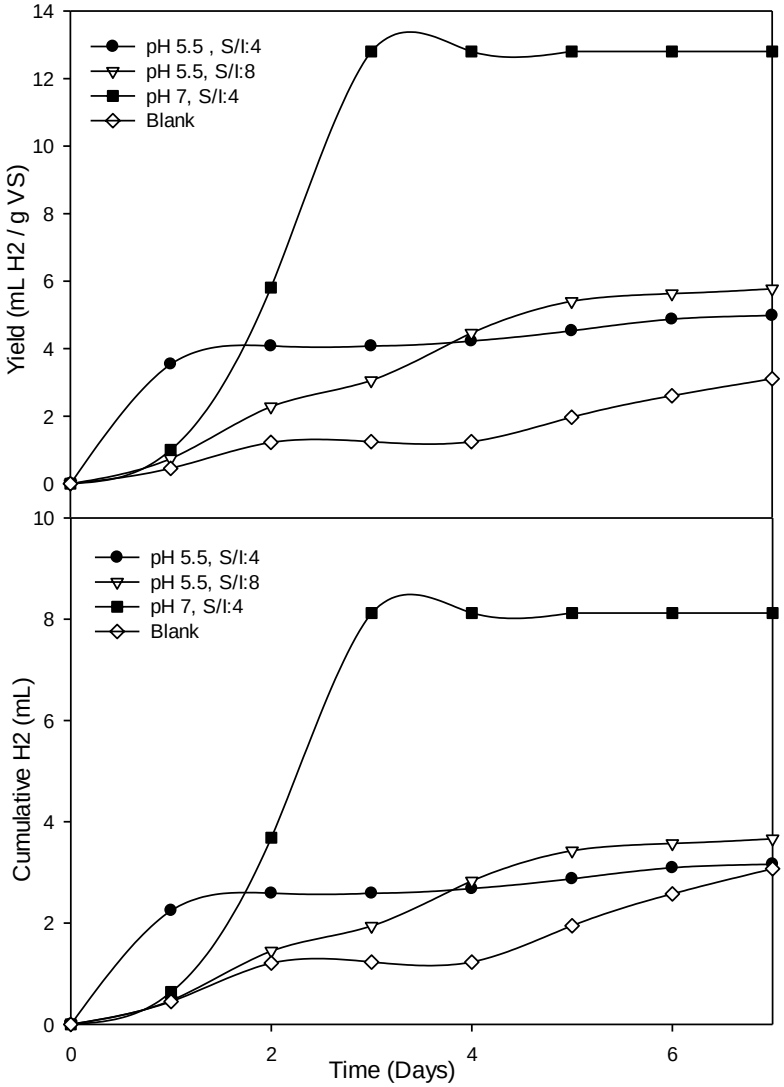

Figure B.1 Cumulative Hydrogen Production and Yield Results Obtained in Preliminary Study

C. Calibration curves used for GC analysis

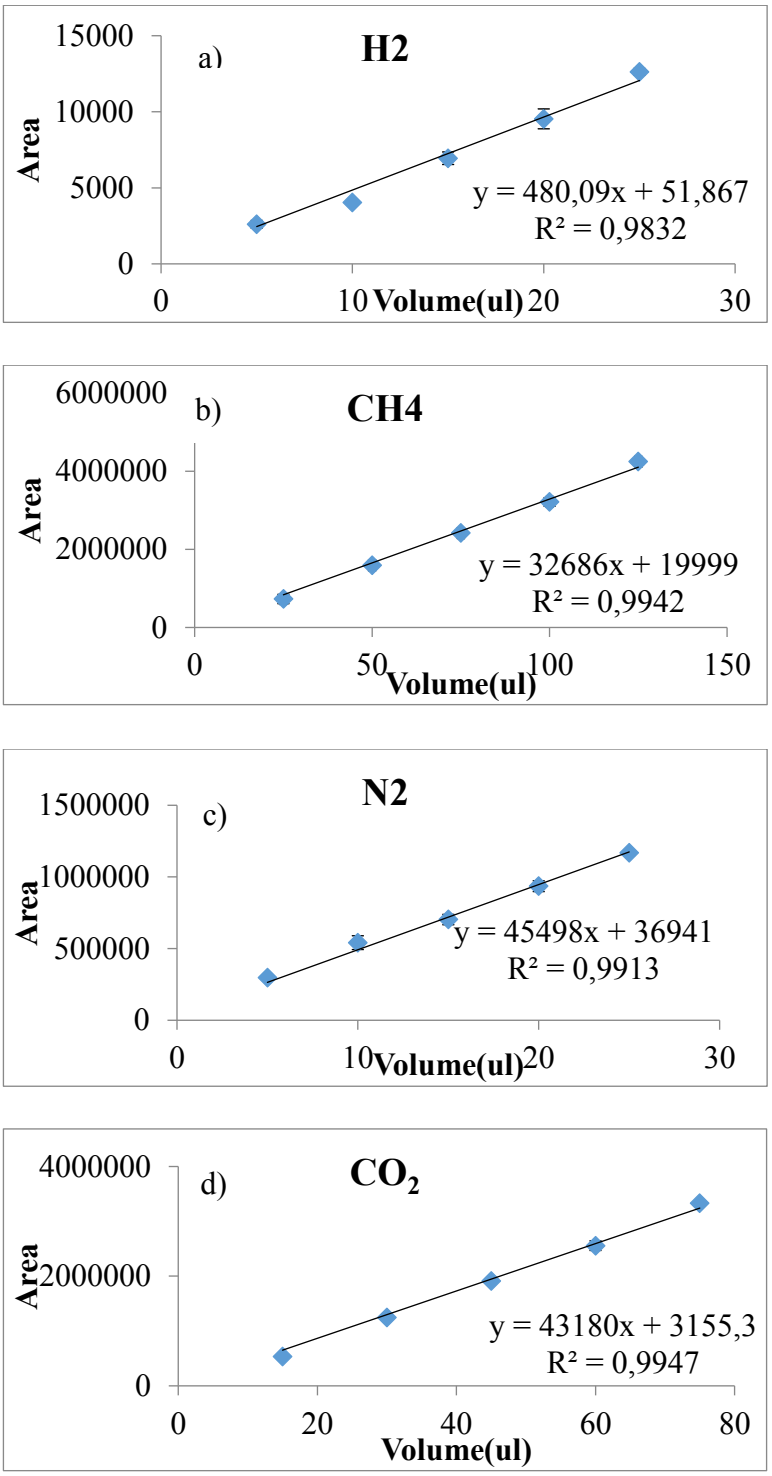

Figure C.1 Calibration Curves of a) H_2, b) CH_4, c) N_2, d) CO_2

D. Literature Characterization Results with Lignocellulosic Substances

Table D.1 Characterization Results of Lignocellulosic Samples

Sample	pH	COD	sCOD	VS	TS	TN	TAN	Total P	References
Garden waste	_	934[b]	174[b]	847[b]	914[b]	_	_	_	Abreu et al.(2016)
Grass	_	_	_	89	_	_	_	_	Yang and Wang (2018)
Leaves	6.8	_	_	85	91.5	0.90	_	_	Liew et al. (2011)
Yard waste	_	_	_	65	93	1.30	_	_	Zhang et al. (2014)
Yard waste	_	_	_	93	94	0.35	_	_	Cherosky (2012)
Green waste	_	_	_	81	90	1.04	_	_	Zou et al.,(2018)
Leaves	6.2	_	_	22.4	_	2.24	_	0.50	Gunaseelan (1988)
Grass silage	4.1	_	228[b]	24[a]	26[a]	19.6[b]	1.40[b]	_	Lehtomäki et al. (2008)
Grass	_	_	_	79_a	32.5[a]	7.65[c]	_	_	Chiumenti et al. (2018)
Grass	_	_	0.13[d]	0.24[d]	0.30[d]	_	3.30[b]	_	Khor et al. (2018)
Switch grass	_	350[e]	_	29[a]	31[a]	9900[e]	_	2300[e]	Jackowiak et al. (2011)
Wheat straw	_	1150[c]	_	819[c]	916[c]	4.85[c]	_	_	Bolado-Rodríguez et al. (2016)
Bagasse	_	_	_	97.60[a]	59[a]	1.85[a]	_	0.34[a]	Talha et al.(2016)
Sweet sorghum	7.5	18.5[f]	17.5[f]	1.87[f]	2[f]	0.01[f]		0.01[f]	Antonopoulou et al. (2008)

[a]%, [b]mg/g, [c]g/kg, [d]g/g, [e]mg/kg, [f]g/L

E. Solubilisation Efficiency Calculation

Solubilisation efficiency

$$= \frac{(\frac{sCOD}{COD})_{Pretreated} - (\frac{sCOD}{COD})_{Non-Pretreated}}{(\frac{sCOD}{COD})_{Pretreated}} * 100 \quad \text{(Equation E-1)}$$

- Calculation for AP:

$$= \frac{0.43-0.06}{0.43} * 100 = 86\%$$

- Calculation for TP:

$$= \frac{0.51-0.06}{0.51} * 100 = 88\%$$

- Calculation for UP:

$$= \frac{0.17-0.06}{0.17} * 100 = 65\%$$

F. Initial and Final Characterization Results of OSAD Reactors

	TS(mg/L)	VS(mg/L)	VS% (%TS)	pH	COD (mg/L)	sCOD (mg/L)	sCOD /Tcod	TKN(mg/L)	sTAN(mg/L)	sTAN/ TKN	TP (mg/L)
NP-B2(t=0)	7775	2625	34	7.80	4888	1525	0.31	1523	263	17	2
NP-B2(t=60)	23558	17583	75	7.79	20456	2497	0.14	1064	216	22	2
NP-B5(t=60)	57212	48425	85	5.47	51930	11803	0.23	970	227	24	7
NP-B8(t=0)	35675	29325	82	7.73	56070	5013	0.09	1348	228	17	3
NP-B8(t=60)	73275	56600	77	5.48	75485	16669	0.22	1446	338	23	12
NP-T2(t=0)	17500	8475	48	7.73	22488	863	0.04	1033	210	20	14
NP-T2(t=60)	19200	10533	55	7.68	25621	1031	0.04	1460	210	16	2
NP-T5(t=0)	54575	44900	82	7.84	42323	2163	0.05	1488	140	9	12
NP-T5(t=60)	52033	35483	68	7.73	49320	5231	0.11	1624	249	15	2
NP-T8(t=0)	89750	70150	78	7.56	60147	2550	0.04	2258	175	8	9
NP-T8(t=60)	70700	48158	68	7.62	51953	7091	0.14	1589	355	22	2
AP-B2(t=0)	13175	77000	16	7.68	9813	3250	0.33	1313	140	11	13
AP-B2(t=60)	24350	13650	55	6.70	23275	6475	0.28	1414	140	10	4
AP-B5(t=0)	38175	14850	38	7.66	26163	6288	0.24	1803	58	5	10
AP-B5(t=60)	60975	32525	53	7.25	36400	7895	0.22	756	35	5	6
AP-B8(t=0)	70275	30700	44	7.55	35865	10013	0.28	1103	210	19	6
AP-B8(t=60)	86550	44075	51	7.18	54840	10145	0.19	1379	117	8	5
AP-T2(t=0)	26075	30750	37	7.72	27925	2825	0.10	1155	228	20	3
AP-T2(t=60)	23550	7563	32	7.79	18288	2505	0.14	1397	117	9	2
AP-T5 (t=0)	59650	28500	48	7.64	49230	7025	0.14	2118	158	7	5
AP-T5(t=60)	52737	21113	40	7.72	37000	3781	0.10	819	140	17	2
AP-T8(t=0)	100325	47000	47	7.53	49827	9813	0.20	1820	193	11	15
AP-T8(t=60)	77700	32800	43	7.67	57660	5900,00	0.11	1033	163	16	2

F. Initial and Final Characterization Results of OSAD Reactors (Continued)

	TS(mg/L)	VS(mg/L)	VS% (%TS)	pH	COD (mg/L)	sCOD (mg/L)	sCOD /Tcod	TKN(mg/L)	sTAN(mg/L)	sTAN/ TKN	TP (mg/L)
TP-B2(t=0)	313875	53600	47	7.01	29925	2375	0.08	665	457	1	1
TP-B2(t=60)	22630	14430	64	6.38	25576	6160	0.24	705	423	1	2
TP-B5(t=0)	94675	72075	77	7.32	43875	3855	0.09	966	429	0	6
TP-B5(t=60)	42570	32780	77	5.77	65280	9888	0.15	1025	456	0	3
TP-B8(t=0)	92850	76925	83	7.16	104350	5035	0.05	567	392	1	7
TP-B8(t=60)	83970	65740	78	5.08	23276	4032	0.17	587	364	1	3
TP-T2(t=0)	58600	42375	68	7.30	43975	1225	0.03	1134	805	1	3
TP-T2(t=60)	34450	18650	54	7.98	42734	2428	0.03	1097	890	1	3
TP-T5(t=0)	74500	51500	69	7.24	63900	4025	0.03	1666	1085	1	6
TP-T5(t=60)	26717	16914	32	7.92	57825	4373	0.03	1384	989	1	5
TP-T8(t=0)	89925	64450	72	7.17	85650	5550	0.03	1694	1173	1	10
TP-T8(t=60)	76240	52380	69	7.43	85140	4862	0.03	1497	1231	1	7
UP-B2(t=0)	28000	21300	74	7.06	17535	1350	0.08	217	476	2	2
UP-B2(t=60)	12030	6170	50	7.87	23040	2648	0.11	197	396	2	2
UP-B5(t=0)	44925	36950	82	7.39	38575	2030	0.05	686	494	1	7
UP-B5(t=60)	28820	20230	70	6.46	14160	4256	0.30	699	512	1	5
UP-B8(t=0)	74700	63800	85	7.04	36320	1885	0.05	315	513	2	8
UP-B8(t=60)	35830	26270	73	5.95	15060	2096	0.14	409	528	1	7
UP-T2(t=0)	22475	10975	49	7.63	31875	575	0.02	1049	1155	1	3
UP-T2(t=60)	27235	12345	45	8.15	25772	2634	0.10	1128	1064	1	3
UP-T5 (t=0)	45200	45200	71	7.23	37238	1420	0.04	1769	1348	1	7
UP-T5(t=60)	32995	17795	54	8.03	30690	1822	0.06	1229	924	1	6
UP-T8(t=0)	62000	43200	70	7.39	87550	1700	0.02	1935	1383	1	11
UP-T8(t=60)	22907	13054	28	7.98	41120	2226	0.05	1237	879	1	9

NP:Non-pretreatment, AP: Alkali Pretreatment, TP: Thermal Pretreatment, UP: Ultrasound Pretreatment

G. Mini TSAD Set-up

In order to be sure about very low yield results obtained in TSAD experiments, especially in methanogenesis stage, an additional mini set-up experiment was conducted. AP- and TP-yard waste mixes were selected since AP was found as the best option for methane production in OSAD and TP was found for hydrogen production in TSAD. Like the main set, the first-stage covers the dark fermentative batch experiments, while second-stage covers the methanogenesis stage batch experiments, where the influent is the effluent of the previous stage, i.e. dark fermentation.

The main change in this trial is scaling up the size of batch reactor and increasing the initial sCOD amount. Therefore, sCOD amount in the reactor was set to 160 mg for DF. In the first-stage of TSAD experiments, batch reactors with total volume of 250 mL and effective volume of 140 mL were used. 2% TS is adapted for AP and TP reactors. S/I was arranged as 4. Seed sludge was initially heat-pretreated for 1 hour at 105 ℃. The initial pH values in the reactors were adjusted to 7 with HCl and/or NaOH to mimic the suitable conditions for dark fermentation.

In the second-stage of TSAD experiments, DF reactors were filtered and, these filtrates were used as substrate in methanogenesis stage. Again effective volume was set as 140 mL in 250 mL total volume. Proper amount of inoculum seed, BM and water were added in order to arrange S/I as 1 in the reactors. The pH values were brought to neutral level with HCl and (or) NaOH to maintain microbial activities at optimum conditions

Lastly, after sealing with rubber stoppers, in order to provide anaerobic environment, all reactors of both stages were flushed with 100% N_2 gas for 5 minutes, placed on a shaker with 175 rpm in a hot room at 35±2°C. Daily biogas production and gas composition analyses were performed to determine the hydrogen and methane production potential of the reactors.

Similar yield results were obtained in this set-up. Results are shown in Table H.1, Table H.2 and Table H.3.

Table G.1 Average hydrogen yields in the first-stage of TSAD experiments

	TS (%)	H_2 Yield-Test[a]	H_2 Yield-Blank[a]
AP	2	34.38	4.96
TP	2	42.36	4.25

[a]mL H_2/g VS

Table G.2 Average methane amounts in the first-stage of TSAD experiments

	TS(%)	CH_4 (mL)	CH_4 Yield-Test (mL CH_4/g VS)	CH_4-Blank (mL)
AP	2	46.00	73.71	0
TP	2	14.5	29.47	0

Table G.3 Average methane amounts in the second-stage of TSAD experiments

	TS (%)	CH_4 (mL)	CH_4 Yield-Test (mL CH_4/g VS)	CH_4 –Blank (mL)
AP	2	0.62	3.79	0
TP	2	5.93	36.53	0

H. Daily Headspace Gas Composition Change During DF Experiment

An example of daily headspace gas composition change for 2% TP-Test reactor is given in Figure I.1

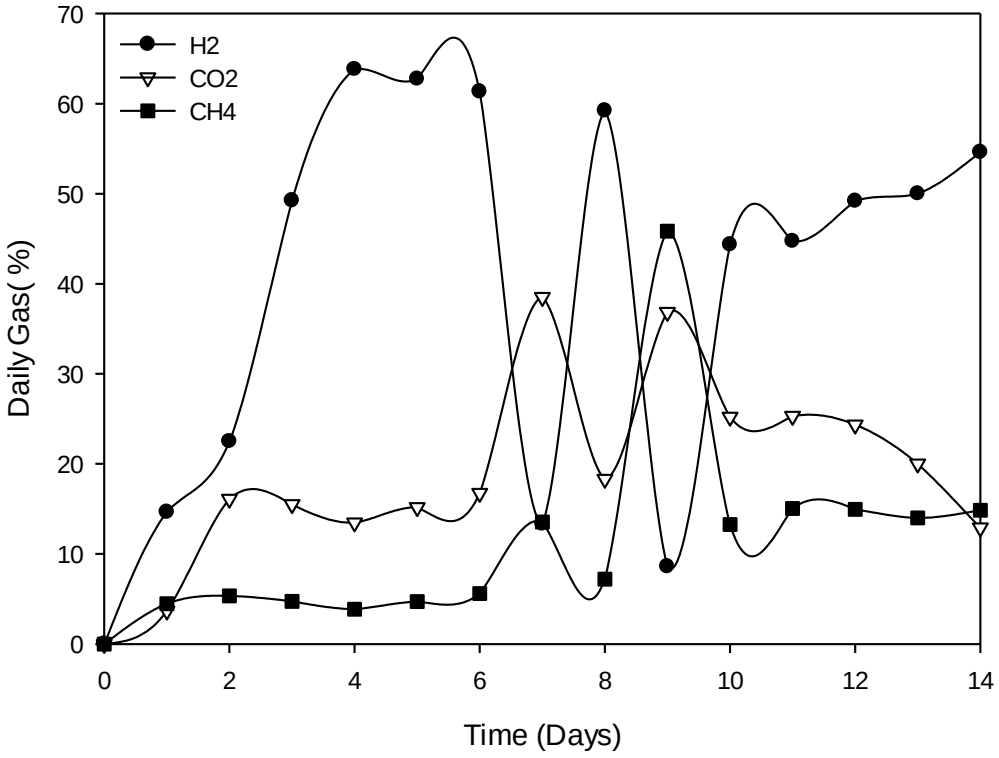

Figure I.1 Daily headspace gas composition change for 2% TP-test reactor

İ. Characterization Results of DF

	TS(mg/L)	VS(mg/L)	VS% (%TS)	pH	COD (mg/L)	sCOD (mg/L)	sCOD /tCOD	TKN(mg/L)	sTAN(mg/L)	sTAN/ TKN	TP (mg/L)
NP-B2(t=0)	19357	14631	76	7.11	20463	3037	0.15	95	60	0.64	8
NP-B2(t=12)	18745	13647	73	5.40	20156	2709	0.13	104	71	0.69	6
NP-B5(t=0)	45130	36479	81	6.90	50749	6994	0.14	234	124	0.53	20
NP-B5(t=12)	49324	32486	66	5.98	50743	6357	0.13	149	135	0.90	18
NP-B8(t=0)	70461	56237	80	7.00	87234	20421	0.23	359	247	0.69	28
NP-B8(t=12)	69521	41146	59	6.02	86117	13357	0.16	375	257	0.69	26
NP-T2(t=0)	20134	16531	82	7.90	22135	4370	0.20	135	98	0.72	10
NP-T2(t=12)	26548	12547	47	5.48	22197	3231	0.15	190	429	2.26	12
NP-T5(t=0)	47613	39200	82	7.10	54812	61741	1.13	299	200	0.67	27
NP-T5(t=12)	50318	32157	64	6.02	50147	44277	0.88	349	812	2.33	24
NP-T8(t=0)	75342	60879	81	7.06	90463	19978	0.22	401	298	0.74	36
NP-T8(t=12)	74263	45317	61	6.1	99475	15775	0.16	486	1008	2.07	29
AP-B2(t=0)	20694	10268	50	6.90	16478	5974	0.36	987	120	0.12	15
AP-B2(t=12)	21457	9547	44	5.87	15441	5310	0.34	1017	142	0.14	14
AP-B5(t=0)	41236	21697	53	6.92	40218	9497	0.24	1503	87	0.06	33
AP-B5(t=12)	45267	19874	44	6.02	37339	7254	0.19	1047	94	0.09	28
AP-B8(t=0)	72497	40697	56	6.97	62871	14648	0.23	1297	120	0.09	52
AP-B8(t=12)	76584	29157	38	6.10	58158	7727	0.13	1197	169	0.14	49
AP-T2(t=0)	22549	12874	57	7.06	19399	7823	0.40	1674	157	0.09	20
AP-T2(t=12)	26974	8476	31	5.79	19453	5313	0.27	1549	205	0.13	16
AP-T5 (t=0)	50348	27964	56	7.10	49569	10547	0.21	1984	184	0.09	40
AP-T5(t=12)	48317	14578	30	6.03	40287	5169	0.13	1958	196	0.10	29
AP-T8(t=0)	79387	30589	39	7.06	71123	18941	0.27	1746	237	0.14	58,69
AP-T8(t=12)	76284	20547	27	6.1	70464	11498	0.16	1893	254	0.13	47,66

İ. Characterization Results of DF (Continued)

	TS(mg/L)	VS(mg/L)	VS% (%TS)	pH	COD (mg/L)	sCOD (mg/L)	sCOD /tCOD	TKN(mg/L)	sTAN(mg/L)	sTAN/ TKN	TP (mg/L)
TP-B2(t=0)	12988	8463	65	6.60	6131	9988	1.63	588	351	0.60	39
TP-B2(t=12)	14856	6948	47	5.49	6438	9367	1.45	603	369	0.61	29
TP-B5(t=0)	31749	20687	65	698	17006	7935	0.47	948	413	0.44	81
TP-B5(t=12)	36457	16847	46	6.01	18348	6198	0.34	1011	456	0.45	77
TP-B8(t=0)	50124	30167	60	6.89	25694	11874	0.46	846	297	0.35	120
TP-B8(t=12)	49531	22577	46	6.23	21476	6960	0.32	824	310	0.38	101
TP-T2(t=0)	14874	9014	61	7.09	7891	3887	0.49	1097	891	0.81	41
TP-T2(t=12)	16473	6012	36	6.02	7035	2538	0.36	1284	902	0.70	48
TP-T5(t=0)	39713	22567	57	7.11	19870	10028	0.50	1578	1043	0.66	100
TP-T5(t=12)	31549	16563	52	6.03	20489	8197	0.40	1496	1157	0.77	101
TP-T8(t=0)	55188	36799	67	7.10	36814	19080	0.52	1268	1149	0.91	140
TP-T8(t=12)	57624	20655	36	6.04	37417	13832	0.37	1301	1364	1.05	129
UP-B2(t=0)	19655	15334	78	7.03	11516	2783	0.24	107	60	0.56	20
UP-B2(t=12)	20146	14147	70	6.04	11563	2630	0.23	114	64	0.56	16
UP-B5(t=0)	49623	39647	80	7.00	30874	5793	0.19	902	422	0.47	51
UP-B5(t=12)	52354	38541	74	6.23	31525	5694	0.18	951	458	0.48	49
UP-B8(t=0)	79648	64315	81	7.00	50941	9342	0.18	1059	502	0.47	84
UP-B8(t=12)	80146	63745	80	6.30	50741	9135	0.18	1201	521	0.43	87
UP-T2(t=0)	21597	17842	83	7.12	14516	3175	0.22	1135	1097	0.97	26
UP-T2(t=12)	20144	12354	61	6.04	15538	2503	0.16	1178	1175	1.00	21
UP-T5 (t=0)	51801	44129	85	7.12	36850	5079	0.14	1486	1016	0.68	65
UP-T5(t=12)	53145	38642	73	6.03	35489	3881	0.11	1397	1204	0.86	57
UP-T8(t=0)	83493	71297	85	7.14	59519	16742	0.28	1984	1378	0.69	90
UP-T8(t=12)	80146	50642	63	6.04	60341	11437	0.19	1963	1478	0.75	77

J. The Results of SMA Assays

SMA experiments were conducted with acetic acid (HAc. : CH_3COOH) of 3000 mg/LCOD concentration with 60 ml effective volume and 2935 mg VS/L of seed sludge.

The seed sludge used in OSAD and TSAD experiments was tested via SMA-1 and SMA-2 experiments, respectively. Activity results are given in Table K.1

Table K.1 SMA yield results of seed sludge

	HAc (mL)	Cumulative CH_4 yield (mL CH_4/g VS)	Theoretical CH_4 yield (mL CH_4/g VS)	Activity (%)
SMA-1	0.16	42.30	67.06	63.07
SMA-2	0.20	47.35	83.82	56.49

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K. Characterizatıon results of methanogenesis

	TS(mg/L)	VS(mg/L)	VS% (%TS)	pH	COD (mg/L)	sCOD (mg/L)	sCOD /tCOD	TKN(mg/L)	sTAN(mg/L)	sTAN/ TKN	TP (mg/L)
NP-B2(t=0)	15745	12377	79	7.00	22351	2709	0.12	104	73	0.70	6
NP-B2(t=20)	15791	12309	78	7.12	22413	2700	0.12	120	74	0.62	6
NP-B5(t=0)	46324	31486	68	7.02	51742	6657	0.13	157	148	0.94	19
NP-B5(t=20)	4639	31461	67	7.15	51674	6611	0.13	167	164	0.98	18
NP-B8(t=0)	70522	43147	61	7.17	91137	14351	0.16	397	267	0.67	25
NP-B8(t=20)	70943	43130	61	7.21	91064	14301	0.16	400	279	0.70	25
NP-T2(t=0)	22548	13548	60	7.00	24191	3631	0.15	199	434	2.18	13
NP-T2(t=20)	22534	13517	60	7.40	24214	3611	0.15	190	453	2.39	14
NP-T5(t=0)	51317	31157	61	7.22	52147	4627	0.09	350	819	2.34	22
NP-T5(t=20)	51029	31084	61	7.34	52007	4611	0.09	359	828	2.30	22
NP-T8(t=0)	71263	45322	64	7.08	99976	16775	0.17	493	1048	2.12	30
NP-T8(t=20)	71091	45311	64	7.50	99842	16684	0.17	503	1057	2.10	28
AP-B2(t=0)	22457	9648	43	7.04	16421	5110	0.31	1101	151	0.14	15
AP-B2(t=20)	22194	9610	43	7.56	16402	5059	0.31	1110	167	0.15	13
AP-B5(t=0)	46267	18874	41	7.07	39331	7358	0.19	1037	99	0.10	29
AP-B5(t=20)	46214	18807	41	7.48	39294	7300	0.19	1079	105	0.10	29
AP-B8(t=0)	79584	28157	35	7.09	59152	7826	0.13	1183	189	0.16	47
AP-B8(t=20)	79712	28094	35	7.47	59087	7806	0.13	1195	190	0.16	50
AP-T2(t=0)	29973	8976	30	7.10	19954	5614	0.28	1549	205	0.13	16
AP-T2(t=20)	29914	8913	30	7.52	19899	5590	0.28	1574	220	0.14	16
AP-T5 (t=0)	49313	18579	38	7.16	42287	5262	0.12	1998	199	0.10	28
AP-T5(t=20)	49009	18519	38	7.53	42291	5208	0.12	1895	203	0.11	27
AP-T8(t=0)	74286	21547	29	7.13	73461	12498	0.17	1853	268	0.14	49
AP-T8(t=20)	74194	21518	29	7.59	73401	12408	0.17	1874	274	0.15	50

K. Characterizatıon results of methanogenesis (Continued)

	TS(mg/L)	VS(mg/L)	VS% (%TS)	pH	COD (mg/L)	sCOD (mg/L)	sCOD /tCOD	TKN(mg/L)	sTAN(mg/L)	sTAN/ TKN	TP (mg/L)
TP-B2(t=0)	15857	7000	44	7.02	6338	9467	1.49	648	3433	5.30	29
TP-B2(t=20)	1579	6994	43	7.30	6422	9409	1.47	635	3591	5.66	28
TP-B5(t=0)	39458	17847	45	7.21	16348	6498	0.40	1141	496	0.43	80
TP-B5(t=20)	39046	17800	46	7.50	16321	6409	0.39	1205	504	0.42	74
TP-B8(t=0)	43531	24577	56	7.09	28476	6973	0.24	824	310	0.38	101
TP-B8(t=20)	43510	24094	55	7.41	29001	6963	0.24	831	313	0.38	95
TP-T2(t=0)	16672	6312	38	7.03	7046	2573	0.37	1484	972	0.66	50
TP-T2(t=20)	16745	6001	36	7.51	7004	2391	0.34	1494	1035	0.69	50
TP-T5(t=0)	34549	18563	54	6.90	24689	8497	0.34	1596	1251	0.78	99
TP-T5(t=20)	32517	17691	54	7.52	24524	8209	0.33	1600	1287	0.80	100
TP-T8(t=0)	51625	22655	44	7.00	38617	13932	0.36	1208	1134	0.94	121
TP-T8(t=20)	52734	21994	42	7.46	38591	13547	0.35	1267	1234	0.97	111
UP-B2(t=0)	22150	13147	59	7.16	13562	2434	0.18	148	62	0.42	19
UP-B2(t=20)	22436	12375	55	7.56	13622	2412	0.18	149	65	0.43	19
UP-B5(t=0)	53351	36571	69	7.09	32524	5791	0.18	982	499	0.51	44
UP-B5(t=20)	53215	35941	68	7.52	32522	5713	0.18	971	503	0.52	48
UP-B8(t=0)	89145	69746	78	7.11	51749	9413	0.18	1301	571	0.44	89
UP-B8(t=20)	89941	68912	77	7.59	52419	9409	0.18	1347	792	0.59	90
UP-T2(t=0)	22134	12354	56	7.10	16537	2603	0.16	1279	1264	0.99	24
UP-T2(t=20)	22993	12117	53	7.54	16342	2529	0.15	1266	1307	1.03	26
UP-T5 (t=0)	51144	35642	70	6.96	37489	3680	0.10	1393	1105	0.79	59
UP-T5(t=20)	52178	34122	65	7.60	37135	3619	0.10	1407	1197	0.85	60
UP-T8(t=0)	79146	51641	65	7.11	64321	12436	0.19	1867	1378	0.74	80
UP-T8(t=20)	79371	50948	64	7.42	64194	12300	0.19	1899	1297	0.68	79