

**Anaerobic digestion coupled with pyrolysis
for sewage sludge treatment to enhance
energy recovery**

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Abstract

Sewage sludge (SS), a byproduct of wastewater treatment processes, poses significant challenges in terms of disposal and management due to its high organic content, pathogenic microorganisms, and potential for environmental pollution. Anaerobic digestion (AD) is commonly used in SS treatment, but concerns are growing about the environmental pollution caused by digested sludge (DS). Pyrolysis can serve as a promising process applicable to DS-to-energy conversion. Integrating AD and pyrolysis has emerged as a promising solution to address these issues, enabling both resource recovery and waste minimization. This study aims to systematically investigate the interactions between AD and pyrolysis, with a particular focus on how these two processes influence one another from three key perspectives.

This study first examined how AD altered sludge characteristics and influenced the subsequent pyrolysis behavior, which involved the different impacts of mesophilic and thermophilic AD on subsequent pyrolysis. The pyrolysis characteristics, kinetics, and thermodynamics of raw mixed sludge (RS) and DS from both mesophilic and thermophilic AD were investigated using thermogravimetric analysis (TGA) and three kinetic models (Starink, Flynn–Wall–Ozawa (FWO), Kissinger–Akahira–Sunose (KAS)). TGA results indicated that thermophilic AD reduced the weight loss from 71.2-75.6% for RS to 55.0-59.1% for DS, more considerably than mesophilic AD (reducing from 70.6-75.2% to 61.3-69.5%), indicating higher organic matter removal. Kinetic analysis showed that the activation energy (E) of RS and DS pyrolysis ranged from 128.9 to 399.9 kJ/mol and 117.3 to 470.4 kJ/mol, respectively. Mesophilic AD generally increased the E , while thermophilic AD did not markedly alter E at low conversion rates (0.2-0.5) but increased it at high conversion rates (0.6-0.8), changing from 203.7-399.9 kJ/mol for RS to 248.9-470.4 kJ/mol for DS. Thermodynamic analysis showed that mesophilic AD generally enhanced pyrolysis favorability and reactivity but impeded activated complex formation. Thermophilic AD enhanced favorability and favored complex formation at low conversion rates but hindered it at high conversion rates. Reactivity varied with thermophilic AD extent, with excessive AD reducing reactivity.

The evolved gas analysis via pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS) confirmed that AD, on the one hand, decomposed biodegradable organics into low-molecular-weight organics, retained them in the DS, and facilitated subsequent pyrolysis. On the other hand, AD could accumulate large bio-refractory molecules, rendering DS less prone to pyrolytic decomposition. This effect of thermophilic AD was more significant than that of mesophilic AD. Given more favorable change was observed in kinetics and thermodynamics at lower conversion rates, low-temperature pyrolysis might be a promising strategy for DS treatment.

During the pyrolysis of DS, bio-oil, biochar, and syngas are generated as products. The bio-oil typically separates into two phases: an organic phase and an aqueous phase. The former has the potential to serve as a renewable alternative to heavy fuel oil in the market, while the aqueous phase, known as aqueous pyrolytic liquid (APL) has a complex composition including toxic organics and low calorific value, generally being regarded as a byproduct. The unutilized organic compounds in APL represent energy loss. However, APL also contains a high concentration of acetic acid making it a potential substrate for AD. Thus, this study also assessed the impact and toxicity of low-temperature pyrolysis-derived APL on AD performance and stability. It assessed the characteristics and toxicity of DS-derived APL on AD and determined its rate-limiting step. DS was pyrolyzed at multiple temperatures (350–650°C) and moisture levels (0–40.4%), resulting in APLs with varying AD toxicities. APL 350°C-0% showed the least toxicity, whereas APL 650°C-40.4% exhibited the greatest toxicity. Glucose (GL) and sodium acetate (SA) were introduced to elucidate the rate-limiting steps. SA, but not GL, enhanced APL conversion to CH₄. And volatile fatty acid lack was observed in treatments without SA addition. This suggested that acidification was the primary rate-limiting step. This finding was confirmed using the modified Gompertz model: SA considerably improved the maximum methane production rate, whereas GL did not.

Finally, this study attempted to mitigate the AD inhibition caused by APL through co-AD with sludge and investigated the feasibility of fully introducing APL generated at different pyrolysis temperatures (350, 500, and 650°C) into AD within the AD-pyrolysis system, assuming all DS is subjected to pyrolysis, to improve overall system

energy recovery. Batch co-AD of APL and sludge was conducted based on the actual APL yield to select the APL preparation temperatures (350, 500, or 650°C). The results showed that APL prepared at 350°C (APL350) and 500°C (APL500) had the lowest and highest toxicity, respectively. Although inhibition caused by APL was eventually overcome, there was not significantly higher CH₄ production from the APL addition reactors was observed, suggesting the negligible APL conversion to methane. A long-term (178 days) continuous co-AD was then conducted using APL350 (least toxic) and APL500 (most toxic) to enhance microbial adaptation and improve APL conversion. The results demonstrated that APL500 exerted a longer inhibition (2 months) than APL350 (one month), but eventually, the inhibition could be overcome with both the lowest and highest toxicity APL addition reactors showing significantly higher methane production. This reveals that APL was successfully converted to methane, with a conversion ratio over 73%. It could be attributed to microbial adaptation to the APL. The addition of APL significantly favored genera like *Candidatus Cloacimonas*, *Anaerolineaceae ADurb. Bin120*, *Anaerolineaceae uncultured*, and *Proteiniphilum* that synergistically degraded complex organics. The archaeal community also shifted with *Methanosarcina* dominating due to its metabolic flexibility, rapid growth rate and strong tolerance. Energy flow analysis showed that reintroducing APL into AD recovered over 60% of its energy as biogas, improving the overall energy recovery ratio in the integrated AD-pyrolysis system to over 93%. This route leverages existing AD infrastructure, reducing disposal costs and potential environmental impacts associated with APL while enhancing energy recovery.

This study offers a potential pathway for sustainable SS management by highlighting the interactions between AD and pyrolysis processes. The proposed circular integration strategy not only reduces the environmental issues associated with DS but also enhances resource recovery in the form of biogas.

Keywords: sewage sludge, anaerobic digestion, pyrolysis, aqueous pyrolytic liquid, methane production

List of Abbreviations

AD	Anaerobic Digestion
APL	Aqueous Pyrolytic Liquid
APHA	American Public Health Association
COD	Chemical Oxygen Demand
DCM	Dichloromethane
DDS	Dewatered Digested Sludge
DS	Digested Sludge
DTG	Derivative Thermogravimetry
ERR	Energy Recovery Ratio
EPA	Environmental Protection Agency of the United States
FWO	Flynn–Wall–Ozawa
GC-MS	Gas Chromatography-Mass Spectrometry
HHV	Higher Heating Value
HPLC	High Performance Liquid Chromatography
KAS	Kissinger–Akahira–Sunose
LCA	Life Cycle Assessment
LHV	Lower Heating Value
Micro-GC	Micro Gas Chromatography
MSW	Municipal Solid Waste
OLR	Organic Loading Rate
Py-GC/MS	Pyrolysis–Gas Chromatography/Mass Spectrometry
RS	Raw mixed sludge
SRT	Solid Retention Time
SS	Sewage Sludge
TC	Total Carbon
TOC	Total Organic Carbon
TS	Total Solid
TG	Thermogravimetry
VFA	Volatile Fatty Acid
VS	Volatile Solid
WWTPs	Wastewater Treatment Plants

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Chapter 1 Introduction

1.1 Background

Wastewater treatment plays a vital role in public health, flood prevention, and environmental protection. The activated sludge method is extensively employed worldwide among various treatment technologies, generating significant quantities of sewage sludge (SS) as a by-product. SS is primarily sourced from the primary and secondary sedimentation tanks of wastewater treatment plants (WWTPs), contains toxic substances such as pathogenic bacteria, parasites, heavy metals, and persistent organic pollutants, which pose ecological and health risks [1], [2], and the volume of sludge generated continues to rise with the expansion of WWTP capacity. Its growing volume further exacerbates the urgency for effective management strategies to mitigate its environmental and health impacts, thus managing and safely disposing of SS have attracted significant global attention.

Alongside the growing concerns regarding SS management, the world is also facing an escalating energy demand, driven primarily by the economic growth of developing countries. The International Energy Agency (IEA) projects that global energy demand will increase by nearly 25% by 2040, with most of this growth occurring in emerging economies [3]. Fossil fuel resources, particularly oil, remain a limited energy source, projected to last approximately 46 years at current consumption rates, with 77% of reserves concentrated in Organization of the Petroleum Exporting Countries (OPEC) member nations. This reliance on fossil fuels is particularly critical for countries like Japan. And Japan's primary energy consumption per unit of GDP is also relatively lower compared to other developed countries: 7.8 for Japan, versus 13.0 for the United States (US), 10.1 for Singapore, and 11.5 for South Korea [4].

The energy-intensive nature of wastewater treatment emphasizes the necessity for optimization and renewable resource integration within WWTPs. In 2021, the electricity consumption of WWTPs in Japan consumed approximately 72 billion kWh, about 1.42% of Japan's domestic electricity consumption (5080 TWh), making WWTPs one of the most energy-demanding public-sector projects [5]. Given Japan's

heavy reliance on imported fossil fuels, reducing energy consumption in WWTPs is increasingly urgent to enhance energy security.

Harnessing the energy potential of SS offers a promising pathway to address these concerns. SS is enriched with organic matter making it a potential renewable energy, especially since it is continuously generated as a by-product of wastewater treatment processes, ensuring a stable supply with predictable quantity and quality. Given its generation within WWTPs, SS represents a densely localized biomass that eliminates the need for additional energy expenditures related to collection and transportation. Additionally, SS is largely produced in urban areas where energy demand is typically concentrated, further supporting its potential as a biomass resource for local energy recovery initiatives.

Utilizing the energy contained in SS could provide several advantages. Firstly, energy recovery from SS can directly reduce the energy demand of WWTPs, enabling more sustainable wastewater treatment operations. Secondly, cost savings can be achieved by lowering maintenance expenses, particularly through reductions in electricity usage. Thirdly, SS-to-energy technologies can contribute to the reduction of greenhouse gas emissions, thereby supporting national and international climate goals. Additionally, anaerobic digestion (AD) processes for SS management can significantly reduce the volume of waste, alleviating the burden on disposal infrastructure. Lastly, SS valorization aligns with the principles of a circular economy, contributing to a more sustainable society by closing the loop between wastewater treatment and renewable energy production.

Considering these benefits, enhancing SS resource utilization within WWTPs is a viable strategy to achieve energy efficiency and sustainability. By integrating energy recovery processes into wastewater treatment systems, SS can be transformed from a disposal challenge into a valuable energy source, mitigating environmental impacts and supporting the transition toward renewable energy sources. Consequently, research and development in SS energy recovery technologies are essential to realizing a sustainable and circular wastewater treatment strategy.

1.2 Sewage sludge production, characteristics and energy potential

Figure 1.1 illustrates the conventional wastewater treatment process using the activated sludge method and identifies the key points where sludge is generated. Based on the treatment stage, sludge can be classified into primary sludge, secondary sludge, tertiary sludge, and mixed sludge, which is a combination of primary and secondary sludge.

Primary sludge is generated during the primary treatment stage, where raw wastewater undergoes sedimentation to remove settleable solids. This sludge predominantly consists of organic material, grit, and debris that settle to the bottom of the primary clarifiers. It is characterized by a high organic content and is relatively easy to dewater compared to other types of sludge.

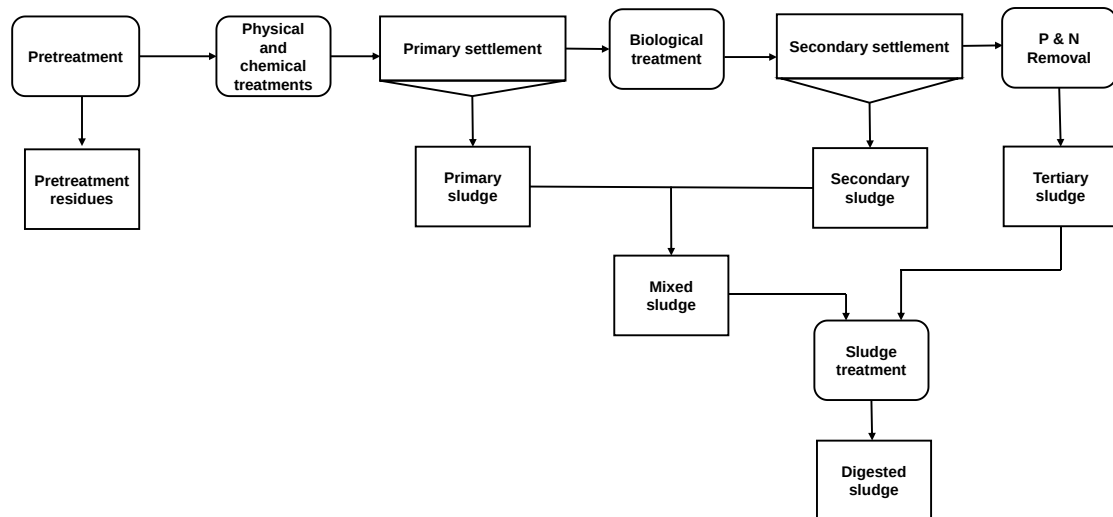


Figure 1.1 The conventional wastewater treatment process using the activated sludge method and sludge generation (Adopted from [1]).

Secondary sludge, also referred to as biological sludge, is produced during the secondary treatment stage, where dissolved and suspended organic matter is removed through biological processes involving microorganisms. This sludge primarily consists of microbial biomass, dead cells, and residual organic material. It is generally more difficult to dewater compared to the primary sludge due to its finer particle size and higher water retention capacity. Secondary sludge is often more nutrient-rich, containing higher levels of nitrogen and phosphorus, which makes it suitable for agricultural applications after proper stabilization.

Tertiary sludge arises from advanced wastewater treatment processes aimed at

removing specific contaminants that are not adequately treated in primary and secondary stages. These processes may include chemical precipitation, filtration, or adsorption for the removal of residual nutrients, heavy metals, or micropollutants. Tertiary sludge often contains high concentrations of chemical additives, such as coagulants or adsorbents, and may also include residual fine particles or other targeted contaminants. Its composition is more variable and complex, often requiring specialized treatment or disposal strategies to mitigate potential environmental impacts.

Each type of sludge exhibits distinct physical, chemical, and biological properties that influence its behavior during handling, treatment, and potential reuse. Primary sludge, with its high organic content, is a key contributor to biogas production during AD. Secondary sludge, rich in microbial biomass and nutrients, requires advanced stabilization and dewatering due to its high-water retention capacity. In contrast, tertiary sludge, often enriched with chemicals and targeted contaminants, demands specialized treatment to minimize its environmental impact.

1.2.1 Sludge production

Globally, approximately 45 million tons (based on dried solid) of SS is generated annually [6]. As shown in Table 1.1, this production varies widely by region, with China, Japan, Europe Union (EU), and the US being major contributors.

Table 1.1 Sludge production from different countries/regions.

Country/Region	Amount (tons dried solid)	Year	Reference
EU	8.67 million	2018	[7]
China	17.4 million	2023	[8]
Japan	2.35 million	2022	[5]
US	3.76 million	2022	[9]
Australia	0.372 million	2023	[10]

In 2022, China generated approximately 17.4 million tons of dried sludge, while Japan produced 2.35 million tons, and the US reported 3.76 million tons. In EU, about 10 million tons of dry sludge were generated in 2018, as documented by EurEau [7].

Additional details on sludge production in individual EU countries are presented in Figure 1.2, with France, Poland, and the Netherlands ranking as the top three producers. This variation reflects differences in population size, industrial activity, wastewater treatment practices, and environmental regulations.

SS production is expected to rise in response to stricter regulatory requirements, rapid urbanization, economic growth, and population increases in developing countries. As wastewater treatment facilities expand their coverage and upgrade treatment processes to meet more stringent environmental standards, the SS production will inevitably grow. For instance, the growing urban population in Asia is driving increased wastewater treatment, particularly in countries like China and India. Similarly, in developed regions such as Europe and North America, stringent policies such as the EU's Waste Framework Directive [11] and the sewage sludge regulations of the Environmental Protection Agency (EPA) in the US [12] necessitate the sustainable treatment and disposal of increasing amounts of sludge.

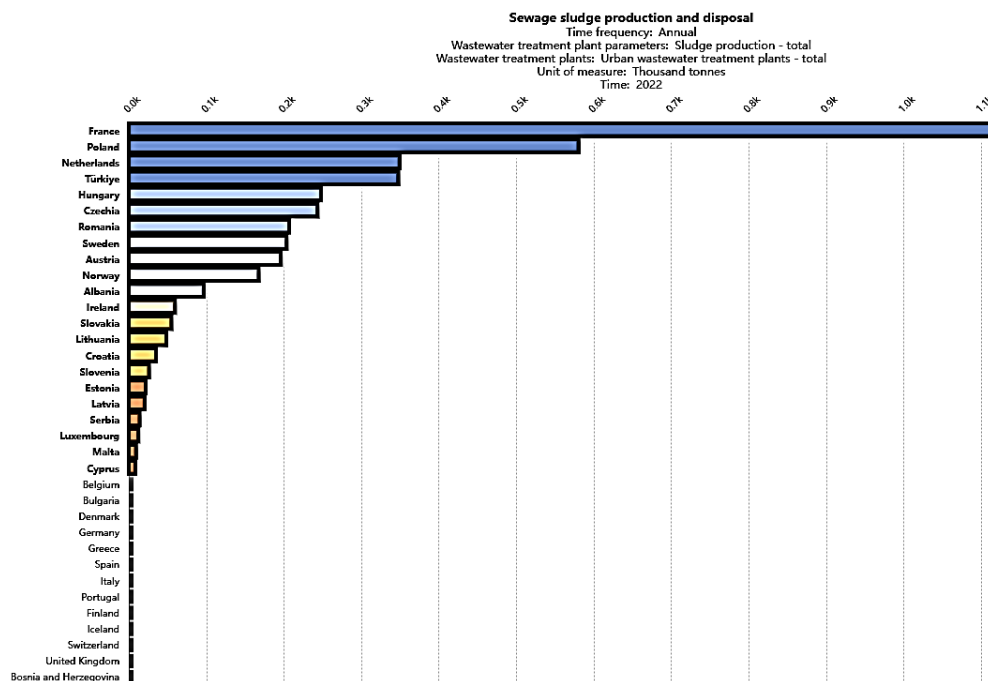


Figure 1.2 sludge production from each country in Europe (Adopted from [13]).

1.2.2 Characteristics of sewage sludge

The composition of SS can be divided into six categories [14]: (1) approximately 60% of its dry weight consists of non-toxic organic carbon, (2) nutrient compounds,

primarily those containing phosphorus and nitrogen, (3) various inorganic and organic toxic pollutants, including heavy metals such as lead (Pb), zinc (Zn), mercury (Hg), cadmium (Cd), chromium (Cr), copper (Cu), arsenic (As), and nickel (Ni), as well as pesticides, dioxins, nonylphenols, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), (4) microorganisms and pathogens, including protozoa, viruses, and bacteria, along with other parasites such as tapeworms, nematodes, and flukes, which can pose significant health risks if inadequately treated. (5) inorganic compounds, such as aluminates, magnesium, calcium, and silicates, (6) water, with its content varying from low levels to over 95%. These components are present in sludge as a mixture, making the treatment and material recycling difficult. Table 1.2 shows the characteristics of primary sludge and secondary sludge.

Table 1.2 Characteristics of primary and secondary sludge (Adopted from [15]).

Sludge type	P	S	P	S	S
TS (wt.%)	5–9	0.8–1.2	27.58	25.36	0.83–12
VS (wt.%)	60–80	59–68	60–80	59–68	30.88
pH	5–8	6.5–8	/	/	5–8
Fats (wt.%)	7–35	5–12	/	/	/
Protein (wt.%)	20–30	33–41	2–30	32–41	15–41
Alkalinity (mg/L as CaCO ₃)	500–1,500	580–1,100	/	/	/
Cellulose (wt.%)	8–15	7–9.7	/	/	/
Silica (wt.%)	15–20	/	/	/	10–20
Organic acids (mg/L as acetate)	200–2,000	1,100– 1,700	/	/	200–2,000
Energy content (kJ/kg TS)	23,000– 29,000	19,000– 23,000	23,000– 29,000	19,000– 23,000	/
Reference	[16]		[17]		[18]

P: primary sludge, S: secondary sludge; TS: total solids; VS: volatile solids

One of the defining features of sludge is its high moisture content, typically ranging

between 95% and 99%. Such high-water content significantly affects its handling, treatment, and potential energy recovery. For instance, this moisture not only increases the volume and weight of the sludge, complicating transportation and storage, but also reduces its calorific value, making direct energy recovery inefficient without prior dewatering or drying. Moisture removal is therefore a critical step to enhance the feasibility of subsequent treatment or energy recovery processes. Techniques such as thickening, mechanical dewatering (e.g., centrifugation, belt press, or filter press) and thermal drying are commonly employed to reduce moisture content to levels more suitable for treatment, such as AD, incineration, pyrolysis or gasification. Effective dewatering can also minimize the volume of sludge, reducing disposal costs and environmental impacts.

Table 1.3 Proximate and ultimate analysis of sewage sludge.

Proximate analysis (wt.%, db)									
Moisture	/	6.1	1.05	2.37	8.50	/	/	6.5	
Volatiles Matter	42.19	53.0	47.92	45.47	50.80	43.05	21.71	46.9	82.30
Ash	54.69	38.4	45.51	45.81	43.30	50.64	76.48	41.3	10.90
Fixed	3.12	8.6	5.52	6.35	/	6.32	1.81	5.4	6.80
Ultimate analysis (wt.%, daf)									
C	38.40	31.1	32.8	24.63	30.10	49.94	48.88	27.8	55.10
H	5.31	4.2	10.2	3.32	4.12	8.37	8.14	4.4	8.60
N	4.15	3.3	5.4	2.96	3.79	9.08	6.30	4.0	6.60
S	2.69	1.1	1.7	1.06	0.85	1.33	1.01	0.8	0.60
O	49.44	24.3	24.5	19.85	17.84	31.28	35.67	/	29.10
Higher Heating									
Value (HHV, MJ/kg)	6.32	13.9	/	11.14	13.34	/	/	12.0	/
Reference	[19]	[20]	[21]	[22]	[23]	[24]		[25]	[26]

Table 1.3 presents the proximate and ultimate analysis of SS. This implied the richness of organic matter in sludge. SS is characterized by its significant variability in both proximate and ultimate analysis due to differences in wastewater composition, treatment processes, and operational conditions. The proximate analysis reveals a high content of volatile matter, typically ranging from 20% to 80% (dry basis), which underscores the organic richness of SS. Carbon content varies between 27.8% and 55.1%, further supporting its potential as a feedstock for energy recovery processes.

However, the high ash content of SS, which could reach up to 76.48%, poses challenges. It can significantly reduce process efficiency by causing operational issues such as slagging and fouling in reactors. Additionally, high ash content dilutes the calorific value of sludge, making it less efficient as fuel. Oxygen content, typically between 19% and 31% by weight, also contributes to these challenges, as it reduces the HHV of the biofuels derived. For instance, bio-oil produced from SS with high oxygen and ash content tends to exhibit lower calorific value, necessitating further upgrading to enhance its quality.

Despite these challenges, SS exhibits sufficient nitrogen and hydrogen (H_2) content to enable its use in nutrient recovery and H_2 -rich gas production. The presence of sulfur, ranging from 0.6% to 2.69%, may necessitate mitigation measures to control emissions during thermal processes.

1.2.3 Energy recovery potential from sewage sludge

As illustrated in the sludge characteristics, SS is rich in organic matter, rendering it a potential renewable energy source. SS organic content can be related to the geologic location, season, the process used in WWTPs and economic level, etc. For instance, the organic content ranges from 30-50% in China (due to combined sewer systems), but can reach over 80% in Japan. This high organic matter contributes significantly to the calorific value of SS, positioning it as a viable alternative energy source. Table 1.4 presents the comparison of SS with coal and other biomass according to the value provided in previous research.

While the HHV of SS (11.10–22.10 MJ/kg, average 16.05 MJ/kg) is lower than coal (e.g., bituminous coal at 25.40–33.15 MJ/kg), it is comparable to low-rank coals such

as lignite (11.80–21.90 MJ/kg) and biomass like sawdust (18.13–20.40 MJ/kg) or olive stone (17.54 MJ/kg). These findings demonstrate SS's potential as a competitive energy resource. SS calorific value can be influenced by organic content. For instance, raw and activated sludge exhibit calorific values of 23–29 MJ/kg and 16–23 MJ/kg, respectively.

Table 1.4 Calorific values of SS, coal and biomass (Adopted from [27]).

Fuel	HHV, dry basis (MJ/kg)
Bituminous coal	25.40–33.15 (29.39) *
Sub-bituminous coal	20.10–29.00 (26.07)
Lignite	11.80–21.90 (19.19)
Sawdust	18.13–20.40 (19.14)
Olive stone	17.54
Wood pellet	18.30–19.60 (19.07)
Walnut shell	17.18
Hazelnut shell	18.40
Hazelnut husk	17.14
Chinese fir	18.38
Camphor	18.40
Switchgrass	19.60
Soybean stalk	17.50
Wheat straw	16.00
Sugarcane bagasse	17.05
Cardoon	17.33
Miscanthus	18.73
Paulownia	19.58
Willow	21.20
Poplar	19.39
Leucaena leucocephala	12.76
Rice straw	14.95–15.86 (14.80)
Sewage sludge	11.10–22.10 (16.05)

* The data in “()” represents the average value

This potential has already been partially realized in some regions. In the EU, for instance, SS contributed approximately 1.4 million tons of oil-equivalent biogas in 2016, accounting for 8.7% of the total biogas production from all sources [28].

In 2022, Japan produced 2.35 million tons of SS (mass based on dried solids), comprising approximately 80% organic matter. The energy utilization ratio of sludge in Japan reached 26% in 2022, which refers to the percentage of sludge used for biogas generation or solid fuel (Figure 1.3). Converting this entirely into energy would yield

4.51×10^{11} kJ [5], equivalent to 16.4 million tons of coal. This recovered energy would represent approximately 0.5% of Japan's annual renewable energy consumption (87PJ) [29], emphasizing its significance as a supplementary energy source.

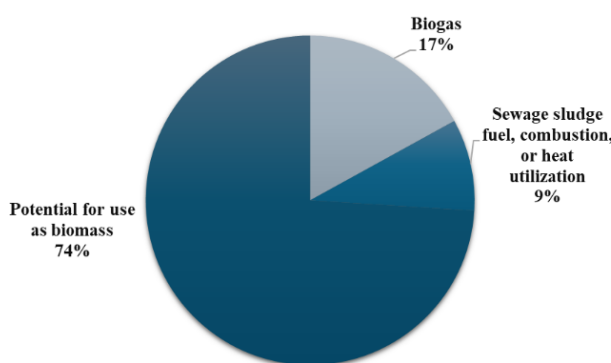


Figure 1.3 The sludge energy utilization percentage in Japan in 2022 [29].

On a global scale, approximately 45 million tons of dried SS is generated annually. Assuming an average organic content of 50%, and using the average calorific value of 16.05 MJ/kg, the total energy recoverable from global sludge production would be approximately 7.22×10^{17} J. This energy is equivalent to approximately 17.2 million tons of coal (assuming 29.39 MJ/kg for bituminous coal) or 17.9 billion m³ of natural gas (assuming 40 MJ/m³).

In regions where sludge production is particularly high, like China, Japan, the US, and the EU, integrating energy recovery into existing wastewater treatment systems could significantly benefit from integrating energy recovery into their sludge treatment practices, and reduce dependency on fossil fuels and energy import.

1.3 Anaerobic digestion-based bioenergy production from sewage sludge

1.3.1 Overview

AD is a widely adopted biochemical process for SS treatment, offering dual benefits of waste reduction and renewable energy production in the form of biogas [30]. Since the early 20th century, AD has been extensively applied for processing suspended solids and organic waste, efficiently converting approximately 40–60% of biodegradable organic matter into a mixture containing 60–65% methane (CH₄), 30–35% carbon dioxide (CO₂), and trace amounts of nitrogen, H₂, hydrogen sulfide (H₂S), and water [6].

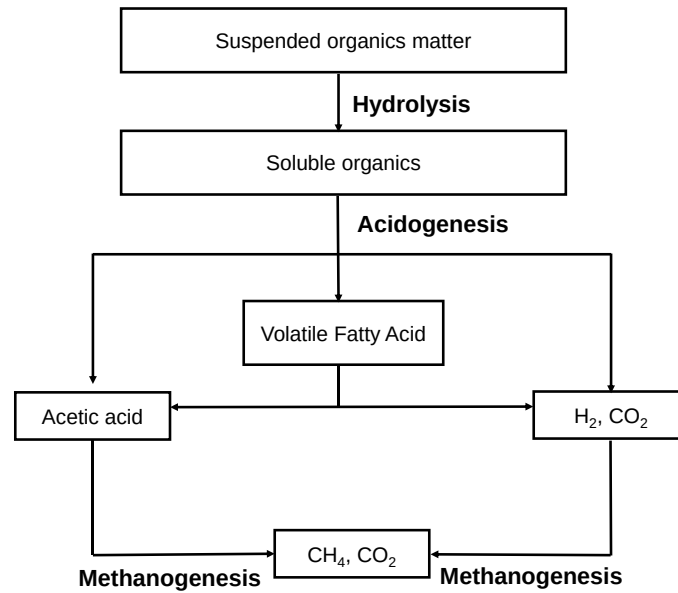


Figure 1.4 Subsequent steps in the anaerobic digestion process [31].

AD comprises four biochemical stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These sequential reactions are facilitated by a complex consortium of microorganisms, each playing a specific role in the degradation and conversion of organic matter (Figure 1.4).

(a) Hydrolysis: This initial stage involves the breakdown of complex organic polymers, such as proteins, polysaccharides, and lipids, into soluble monomers, such as amino acids, simple sugars, and long-chain fatty acids, with the assistance of extracellular enzymes. Hydrolytic bacteria such as *Clostridium*, *Bacteroides*, and *Fibrobacter* are key contributors in this phase [32].

(b) Acidogenesis: The hydrolysis products are further fermented by acidogenic bacteria into volatile fatty acids (VFAs), alcohols, ammonia, CO₂, and H₂. Bacteria such as *Lactobacillus*, *Pseudomonas*, and *Desulfovibrio* are prominent acidogens responsible for this transformation [33].

(c) Acetogenesis: During this thermodynamically sensitive phase, higher VFAs (e.g., propionic and butyric acids) are converted into acetic acid, H₂, and CO₂ by acetogenic bacteria, including *Syntrophobacter* and *Pelotomaculum*. The conversion process is energetically favorable only under low concentrations of reaction products, such as acetic acid (<10⁻¹ mol/L) and H₂ (<10⁻⁴ atm) [34].

(d) Methanogenesis: In the final stage, methanogenic archaea metabolize acetic acid and H₂/CO₂ mixtures to produce CH₄. Approximately 70% of CH₄ is generated through

acetate cleavage by organisms such as *Methanosarcina*, while the remaining 30% arises from hydrogenotrophic methanogenesis mediated by *Methanobacterium* and *Methanobrevibacter* [35].

Some researchers also divide AD into three stages: hydrolysis, acidogenesis, and methanogenesis, where the second and third stages are combined under the broader term acidogenesis.

The extent to which AD reduces SS is typically measured as a decrease in VS and TS content. According to the American Public Health Association (APHA) standard method [36], VS refers to the amount of organic matter that can be lost when heated to 550°C. Meanwhile, TS is defined as the residual material remaining in a container after evaporation at 105°C. Generally, VS is the biodegradable content of sludge, while TS is the combination of biodegradable and nonbiodegradable material. VS can be reduced by 35–60% [37], while TS generally decreases by 28–30%, depending on operational parameters and pretreatment methods [38].

Compared to other non-biological methods, AD requires significantly longer reaction times. Under mesophilic conditions, waste treatment typically requires a retention time of 15 to 30 days, and for large-scale operations primarily aimed at waste stabilization, the retention time can exceed 35 days [39]. Thermophilic digestion, operating at 50–55 °C, has gained attention for its potential benefits over mesophilic systems, including enhanced VS degradation, higher biogas yields, reduced foaming, and better dewaterability. Research by Appels et al. highlights the feasibility of sludge digestion at reduced solid retention times (SRT) [31]. However, shortening the SRT may result in less effective VS breakdown, thereby increasing the quantity of residual sludges that require further treatment.

Nonetheless, AD systems generally demand significantly lower capital investment compared to thermal treatment methods such as incineration. For instance, the cost of establishing an incineration facility can be at least three times greater than that of an AD system [40]. This economic advantage largely explains why AD is widely adopted in wastewater treatment plants for sludge management.

1.3.2 Limitations in sludge anaerobic digestion

AD fails to completely inactivate pathogens and does not completely remove heavy metals from sludge; pathogens can be found in surface water when digested sludge (DS) is used on agricultural land [41]. This poses a risk to public health and the environment.

Table 1.5 Basic characteristics of different sludge types [32].

Parameters	P	A	M	T
VSS/SS*	0.77 ± 0.07	0.75 ± 0.05	0.60 ± 0.02	0.67 ± 0.11
Proteins (mg/g SS)	140 ± 6	346 ± 111	248 ± 12	155 ± 62
Polysaccharides (mg/g SS)	198 ± 93	101 ± 35	70 ± 5	78 ± 10
Humics (mg/g SS)	80 ± 55	58 ± 35	112 ± 108	188 ± 92
Total EPS (mg/g SS)	75 ± 5	130 ± 65	78 ± 49	41 ± 9
EPS proteins (mg/g SS)	33 ± 9	76 ± 32	40 ± 7	20 ± 12
EPS polysaccharides (mg/g SS)	5.0 ± 2.3	11.9 ± 4.5	6.5 ± 2.0	5.9 ± 1.3
EPS humics (mg/g SS)	36 ± 4	42 ± 2	31 ± 4	15 ± 2
Fe (mg/L)	212 ± 17	202 ± 53	158 ± 91	-
Ca (mg/L)	235 ± 215	362 ± 575	801 ± 792	139 ± 167
EPS surface charge (meq/g SS)	0.18	0.24	0.33	0.23 ± 0.05
Zeta-potential (mV)	-35	-29.6 ± 8.5	-30.7 ± 10.5	-26.5 ± 4.4
Sludge surface charge (meq/g SS)	0.21	0.13	0.17 ± 0.12	0.17 ± 0.18
Floc size (μm)	53	125 ± 109	51 ± 21	57 ± 11
Shear sensitivity kSS	0.125	0.062 ± 0.019	0.244 ± 0.016	0.418 ± 0.337
Surface area CS ($\text{m}^2/\text{g SS}$)	21.8	15.6 ± 8.6	66.3	-

*SS represents “suspended solid” in this table. VSS represents “volatile suspended solid”.

P: Primary sludge; A: Activated sludge; M: Mesophilic; T: Thermophilic; EPS: Extracellular polymeric substances

Additionally, SS digestion is inherently difficult due to the complex composition of sludge flocs, which comprise microbial aggregates, filamentous bacteria, organic and inorganic particulates, extracellular polymeric substances (EPS), and a substantial amount of water. Among these components, EPS, which originates from microbial activity (e.g., secretion and cell lysis) or the adsorption of organic substances in wastewater (e.g., cellulose and humic acids), is a key factor contributing to the organic fraction of sludge. EPS primarily consists of proteins (PN), polysaccharides (PS), nucleic acids, humic substances, and lipids (Table 1.5). Based on its spatial distribution within the sludge flocs, EPS is classified into three categories: soluble EPS (S-EPS), loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS). S-EPS is dispersed uniformly in the water phase, LB-EPS extends from TB-EPS with a porous and easily

dispersed structure, whereas TB-EPS adheres closely to the bacterial cells on the interior of the flocs.

These three-dimensional, gel-like, negatively charged biopolymers significantly influence the physicochemical properties of sludge. EPS provides a protective barrier that shields microbial cells from rupture and lysis, thereby maintaining the structural integrity, strength, flocculation ability, and biodegradability of the sludge matrix. Furthermore, microbial cells are encased by a rigid cell wall composed of peptidoglycan chains cross-linked by peptides, which presents both physical and chemical resistance to degradation. As a result, SS, characterized by its high EPS and cellular content, exhibits a rigid structure that is inherently resistant to hydrolysis and digestion. This explains why conventional mesophilic anaerobic digesters, even with long hydraulic retention times of 20–30 days, often achieve relatively low VS destruction efficiencies of only 30–50% [32].

Thus, investigating downstream processes to recover energy from DS and mitigate potential pollution is essential. Furthermore, only approximately 50–60% of organic matter can be mitigated by AD, leaving some persistent (or non-biodegradable) organic compounds and pathogens in the DS, which poses environmental and health risks but still holds potential for energy production [42].

Therefore, AD cannot fully or effectively mineralize the organic matter present in sludge. This leaves a considerable fraction of persistent or non-biodegradable organic matter (approximately 40–50%) in the DS. Additionally, new biomass (primarily anaerobic bacteria) is generated during the AD process, further contributing to the residual sludge. As a result, large quantities of DS are produced. Such residual organics offer significant potential for energy recovery, as highlighted by [42]. While the organic content in the DS still holds promise for energy recovery, it remains recalcitrant to biological degradation.

Given these limitations, further research into downstream processes to recover energy from DS and mitigate associated environmental risks is essential. These efforts could complement AD and contribute to more sustainable sludge management practices.

1.4 Thermochemical conversion as a downstream treatment method for digested sludge

Thermochemical processing is an effective method for addressing key challenges

associated with DS management, including volume reduction, pathogen elimination, energy recovery, and the production of valuable byproducts. This technology is particularly promising for extracting energy from biologically resistant DS, as it rapidly decomposes nearly all organic matter—biodegradable or otherwise—through controlled heating and oxidation, which provides distinct advantages over biochemical processes [27]. The primary thermochemical pathways include pyrolysis, gasification, and combustion—each yields specific products and has unique application potentials, as summarized in Figure 1.5 [42].

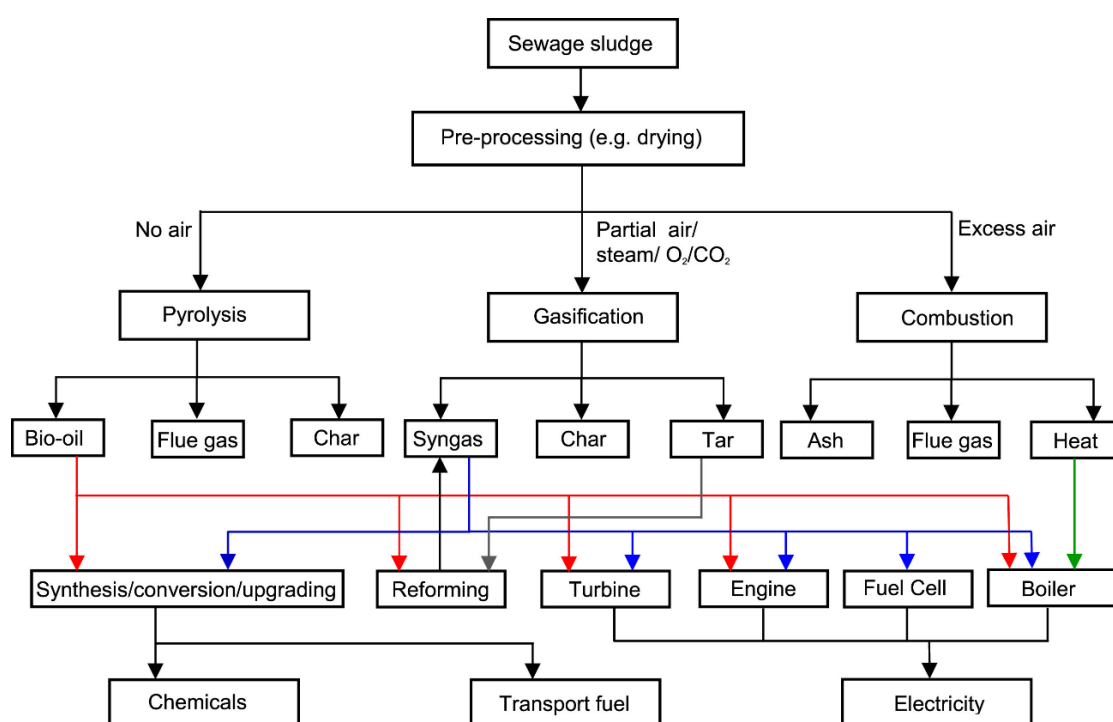


Figure 1.5 Potential applications of thermochemical conversion of sewage sludge [27].

1.4.1 Incineration

Combustion is a high-temperature thermochemical process that utilizes excess oxygen to break down carbon-based materials into CO₂ and water, releasing substantial heat along with gases. Depending on the type of input material (fuel or waste), combustion is generally categorized as conventional combustion and incineration. Conventional combustion primarily focuses on energy recovery, such as generating heat to produce steam in boilers, which can then be used for electricity generation

through steam turbines. Incineration, on the other hand, is mainly employed to minimize the volume of waste materials, particularly hazardous substances, and may or may not involve heat recovery, as is common in the treatment of municipal solid waste (MSW) and medical waste [27].

The combustion of SS generally exhibits characteristics like the combustion of solid fuels. Under excess oxygen conditions, complete oxidation of carbon and volatile compounds occurs at temperatures typically exceeding 1000°C. The combustion process of sludge can be divided into six distinct stages: drying, devolatilization and auto-gasification, combustion of volatiles, ash melting, char combustion, and ash agglomeration [42]. These stages reflect the sequential thermal and chemical transformations involved, which ensure the efficient breakdown of organic and volatile components, followed by the combustion of residual char and the stabilization of the remaining inorganic material. The primary products of sludge incineration are flue gas, slag, and fly ash [43].

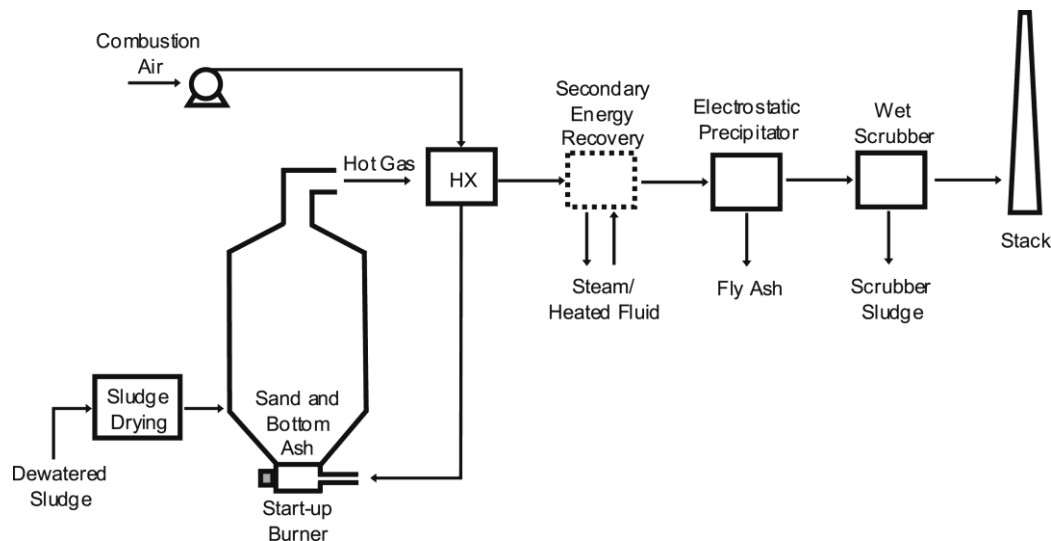


Figure 1.6 Flow diagram of a typical sewage sludge combustion process.

Figure 1.6 illustrates the process flow of a typical sludge incineration system. During incineration, gases generated within the combustion chamber exit at elevated temperatures (850°C and 1300°C). The heat generated from the incinerator can be efficiently recovered using heat exchangers, serving purposes such as preheating

combustion air, drying sludge before combustion, or producing steam for energy generation. The non-combustible byproducts too large to be carried away by flue gas, are collected at the furnace base, referred to as bottom ash. While finer inorganic materials and particulates are released with the flue gas as fly ash. To ensure compliance with emission standards, incineration plants are equipped with advanced flue gas treatment systems, including cyclone separators, wet scrubbers, electrostatic precipitators, and bag filters, which effectively minimize the release of fine particulates and harmful gases into the environment. This comprehensive process not only enhances energy recovery but also addresses environmental sustainability in sludge management.

In recent years, SS incineration has become one of the most widely adopted and accepted treatment methods globally [6]. Large-scale incinerators continue to operate, primarily for energy recovery and conversion into ash [44]. In China, SS incineration has emerged as the preferred treatment alternative due to its effectiveness in significantly reducing sludge volume and pathogens, minimizing odor, and enabling energy recovery. Shandong and Guangdong as the top two provinces in treatment capacity, handling 15.62 and 23.67 million m³/day, respectively [45]. However, the fly ash generated from both standalone sludge incineration and co-incineration with MSW is classified as hazardous waste in China and requires safe disposal. To address this, the Ministry of Environmental Protection issued a hazardous waste exemption list in 2016, in which if the fly ash from waste-to-energy plants is treated through processes such as vitrification, melting, or chemical stabilization thus meeting the requirements of the Pollution Control Standards for Domestic Waste Landfills (GB 16889-2008) [46], it can be landfilled as general solid waste.

In other countries, such as France, Denmark, Germany, Belgium, Japan, and the US, incineration also accounts for a significant portion of sludge treatment, managing approximately 20%, 24%, 14%, 15%, 55%, and 25% of the total sludge, respectively [47]. In EurEau member countries, around 27% of SS was treated via incineration in 2021, as presented in Figure 1.7.

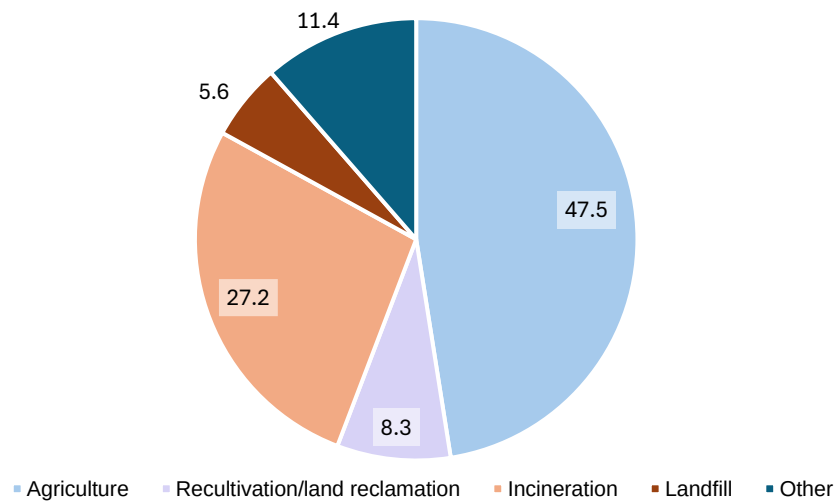


Figure 1.7 Destination of the sewage sludge production in EurEau member countries [7].

In Japan, sludge incineration is also a widely utilized treatment method, with a considerable proportion of the resulting incinerated ash being recycled as construction materials. As illustrated in Figure 1.8, the use as construction materials represented the most prevalent sludge treatment approach in 2022.

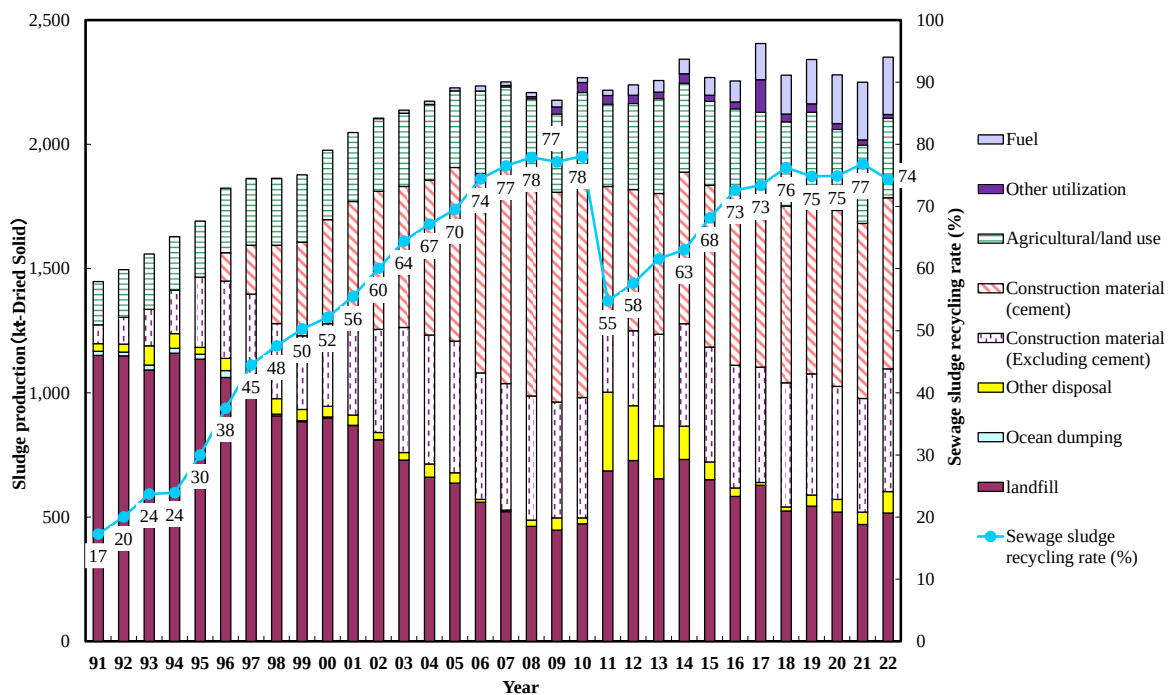


Figure 1.8 The sludge treatment methods in Japan 2022 [5].

However, the issue of greenhouse gas emissions from sludge incineration has got increasing attention. In the case of Japan, significant greenhouse gas emissions are associated with wastewater infrastructure and the energy-intensive processes involved, accounting for approximately 0.5% of the country's total emissions in the fiscal year 2010. In 2019, approximately 5.3 million tons of CO₂-equivalent greenhouse gases were emitted from the sewerage sector in Japan, accounting for approximately 0.44% of the country's total emissions in the fiscal year 2019 [48]. Specifically, as shown in Figure 1.9, CO₂ emissions from the electricity consumption of wastewater treatment facilities made up around 53% of the total in 2021, followed by nitrous oxide (N₂O) emissions from sludge incineration, which contributed about 21%.

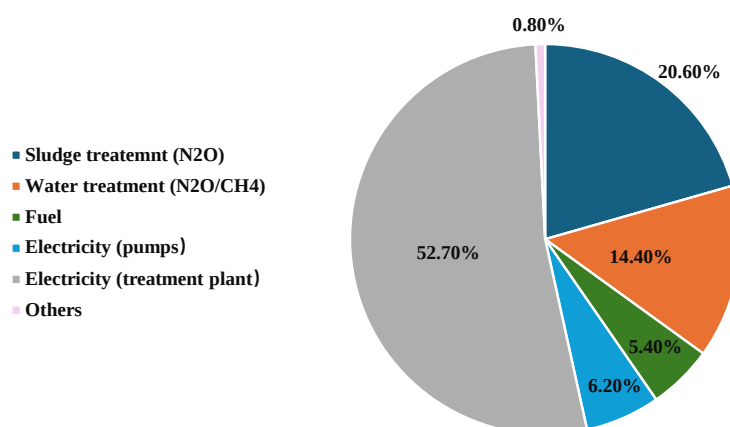


Figure 1.9 Greenhouse gas emissions associated with wastewater infrastructure in Japan, 2021 [5].

In addition to the challenges associated with greenhouse gas emissions, another limitation of sludge incineration is the release of harmful pollutants in the form of gas emissions, including CO₂, carbon monoxide (CO), CH₄, NO_x, SO_x, polycyclic aromatic hydrocarbons (PAHs), and dioxins/furans. These pollutants pose significant risks to both human health and the environment. For example, non-CH₄ volatile organic compounds (NMVOCs), NO_x, and sulfur dioxide (SO₂) contribute to air pollution, while ammonia emissions from sludge incineration can exacerbate nitrogen pollution. Although ammonia is a valuable nutrient for crops, its excessive release can lead to soil acidification and nutrient overload, which in turn cause problems such as acid rain and

eutrophication of aquatic ecosystems [49], [50]. The over-saturation of lakes, oceans, and soils can severely disrupt marine ecosystems, lead to deforestation, and impair plant health [51]. Despite the development of advanced incinerators designed to reduce air pollution and improve resource recovery, these technologies must be carefully evaluated from both economic and social perspectives, particularly in terms of their impact on air quality and global climate change.

Incineration is a widely used thermochemical process for SS treatment, which also offers the potential for energy recovery. However, its applications have primarily been focused on waste management objectives, such as reducing volume and eliminating harmful substances, rather than energy production or recovery [27].

1.4.2 Gasification

Gasification is a thermochemical process in which the carbonaceous components of the fuel are converted into combustible gases and ash in a reducing atmosphere. The primary objective of this process is the efficient production of clean combustible gases (syngas) [52], which is a mixture of CO, CH₄, carbohydrate gas (C_nH_m), and H₂. Compared to the widely used incineration technology, gasification is an attractive alternative due to its potential for energy recovery and its ability to circumvent several common issues encountered in incineration, including the need for supplementary fuels and the emission of sulfur oxides (SO_x), nitrogen oxides (NO_x), heavy metals, and particulate matter. Moreover, gasification avoids the generation of harmful compounds like dioxins and furans, which are often associated with incineration [53].

Gasification systems typically consist of four main stages: (i) drying, (ii) pyrolysis, (iii) oxidation, and (iv) reduction. In the drying stage (70–200°C), moisture is removed from SS through evaporation. The drying rate depends on factors such as the surface area of the feedstock, gas recirculation speed, relative humidity, and internal diffusion rates of water within the sludge [54], [55]. In the pyrolysis stage (350–600°C), the dried sludge undergoes thermal decomposition, producing volatile components and residual char. These volatile components and char are subsequently oxidized in the oxidation zone, where exothermic reactions generate heat that drives further drying and pyrolysis.

In the reduction or gasification zone, char undergoes partial oxidation, Boudouard reactions, steam gasification, and hydrogenation, ultimately converting into syngas. Volatile components also undergo reforming in the gas phase, influencing the composition of the produced gases.

Gasification offers the efficient production of syngas. Research has shown that when the gasification temperature is between 650–950°C, the lower heating value (LHV) of the syngas produced from SS ranges from 2.8 to 6.5 MJ/Nm³, including N₂. When N₂ is removed from the syngas, the LHV increases to 5.8–13.1 MJ/Nm³. Notably, 30–93% of the energy content (LHV) from the SS is transferred into the syngas, making it a viable energy source [56].

Despite its advantages, gasification faces certain limitations, particularly the substantial production of tar, which can be condensed in downstream equipment like compressors and gas engines. This tar production limits the technical and economic viability of the process. Char formation is another issue, as it can cause reactor blockages. To address these concerns, research has focused on using different gasifying agents and catalysts, with steam showing promise as a gasifying agent. Steam promotes H₂ production and helps reduce tar and char formation, although it requires energy input as the process is endothermic. Further exploration of steam gasification is needed to fully assess its potential to produce high-quality H₂ and minimize the production of inhibitory byproducts.

Another challenge for gasification is the high moisture content of SS, as most gasifiers require feedstocks with less than 30% moisture. Nevertheless, the energy produced from the syngas generated in the process is often sufficient to meet the energy demands of both the drying and gasification stages [23], [57]. While the flexibility of syngas utilization provides advantages, extensive gas cleaning is required to remove heavy organic pollutants, which complicates the construction and operation of SS gasification plants. Additionally, the inorganic content of SS, while aiding catalytic gasification, can increase the risk of ash deposition and heavy metal emissions, posing additional challenges for the technology [27].

1.4.3 Pyrolysis

Pyrolysis has been attracting more and more attention due to its conversion of solid waste (including non-biodegradable components) into value-added products such as biochar, bio-oil, and syngas [58], [59] and thoroughly removing pathogens. Pyrolysis has already been commercialized for bioenergy production using energy crops and biomass waste [60]. All products of the process, including the liquid phase (referred to as bio-oil), non-condensable gases (known as pyrolysis gas), and the solid fraction (biochar), possess the potential for heat and power generation. Alternatively, bio-oil can serve as a crude oil substitute for the production of transportation fuels [61], while biochar has diverse applications including being used as an adsorbent or precursor for adsorbents [62], a soil amendment [62], or as a means of carbon sequestration [63].

The pyrolysis of SS is generally categorized into three distinct stages: (i) an initial minor decomposition occurring between 200 and 300°C, primarily associated with biodegradable organic matter and microbial biomass, (ii) a major decomposition phase between 300 and 450 °C, which is attributed to organic polymers either derived from natural components in the undigested sludge or formed during biological stabilization, and (iii) a secondary minor decomposition above 450 °C, likely associated with the thermal breakdown of recalcitrant materials such as cellulose-based substances and inorganic composition [64].

Pyrolysis is primarily classified into slow and fast pyrolysis. Slow pyrolysis is characterized by a low heating rate and prolonged residence time, predominantly utilized to produce biochar or activated carbon, rather than for energy products like bio-oil or pyrolytic gas. While fast pyrolysis involves a rapid heating rate, typically around 100 °C/min, enabling the predominant production of bio-oil or pyrolytic gas [42].

Fast pyrolysis provides two potential methods for producing bioenergy from SS, with medium-temperature pyrolysis (400–550°C) being one of the most notable. This approach has garnered particular interest due to its benefits: (1) it operates at a lower temperature compared to high-temperature pyrolysis, thereby reducing energy input requirements, and (2) the bio-oil produced is highly suitable for storage and transportation. The energy-related performance of this process, as summarized from existing studies and presented in Table 1.6, demonstrates that approximately 50% of

the volatile fraction in SS can be converted into bio-oil with an energy value exceeding 30 MJ/kg, regardless of whether the feedstock has been anaerobically digested. In certain cases, the calorific value of the bio-oil is comparable to that of diesel fuel [65].

However, the bio-oil derived from SS pyrolysis typically contains a higher water content (>23%) compared to those obtained from conventional biomass [27]. This bio-oil typically separates into two phases: an aqueous phase and an organic phase. The organic phase, or bio-crude, is a complex mixture of oxygenated hydrocarbons and nitrogenous compounds, including aromatic compounds, short-chain carboxylic acids, ketones, phenols, sugars, and furan derivatives, with its composition depending on the type of biomass used [66]. It has the potential to serve as a renewable alternative to heavy fuel oil in the market [67].

Table 1.6 Energetic performances of moderate-temperature fast pyrolysis (400°C–550°C) [65].

Sludge feedstock		Pyrolysis conditions			Bio-oil product		AEE ^b (%)
Type	VS ^a (%)	Heating source	Operating mode	Temperature (°C)	Yield (%)	CV ^a (MJ/kg)	
PS ^a	84	Electric	Batch	500	42	37	67.6
WAS ^a	69	Electric	Batch	500	31	37	60.1
DS ^a	49	Electric	Batch	500	20	36	56.6
DS	47.0	Electric	Continuous	550	36.0	32.1	94.0
DS	46.6	Electric	Continuous	550	29.7	31.2	73.0
DS	38.3	Electric	Continuous	550	24.3	30.6	83.5
SS ^a	75.5	Microwave	Batch	490	40	35	/
SS	75.5	Electric	Batch	500	30	37	/

^a VS: volatile solids content; CV: calorific value; PS: primary sludge; WAS: waste activated sludge; DS: digested sludge; SS: sewage sludge.

^b AEE = apparent energy efficiency, calculated according to the ratio of the energy content of bio-oil product to the energy content of its feedstock.

The aqueous phase, known as aqueous pyrolytic liquid (APL), originates from several sources: (i) initial moisture in the SS feedstock, (ii) dehydration reactions occurring at temperatures below 600 °C, and (iii) the decomposition of oxygenated organic compounds, such as sugars and acids, at high temperatures. APL is generally regarded as a byproduct [68], which is a water-soluble liquid with a complex composition comprising over 200 organic compounds [69], including cresol, hydroxy acetaldehyde, acetol, furans, phenols, and N-containing compounds such as pyrazine

and pyridine. It also contains inorganic constituents such as $\text{NH}_3\text{-N}$, H^+ or OH^- [70], posing challenges for disposal or utilization.

The low calorific value of APL limits its direct use as a fuel, with its composition being dependent on the pyrolysis feedstock and operating temperatures. APL derived from municipal SS pyrolysis exhibits a high chemical oxygen demand (COD) of 30–500 g COD/L but currently lacks beneficial applications. If classified as waste, APL could be considered hazardous waste due to toxic organic compounds like pyridine, which may lead to environmental pollution if discharged untreated. Moreover, the unutilized organic compounds in APL represent an energy loss, the significant energy potential they hold will be wasted [71]. Therefore, developing innovative approaches to manage APL and recover its energy would be highly beneficial. Nevertheless, APL contains a high concentration of acetic acid, ranging from 25 to 166 g/L, making it a potential substrate for AD because acetic acid can be directly converted to methane [72], [73].

Furthermore, pyrolysis is an endothermic thermochemical process, meaning that part of the energy in the products results from the heat generated during the pyrolysis reaction, not just from the feedstock itself. If the total energy in the products is higher than that of the original material, the energy yield from pyrolysis is significantly influenced by the heat of the reaction. In such cases, achieving high energy output at the expense of increased energy input can reduce overall net energy production (or efficiency). As such, greater emphasis should be placed on the role of pyrolysis reaction heat when optimizing and selecting processes [71].

1.5 Advantages of integration anaerobic digestion and pyrolysis

The comparison of the thermochemical technologies for SS-to-energy conversion has been extensively explored using analytical tools such as life cycle assessment (LCA) and SWOT analysis. Research using SWOT analysis has identified pyrolysis as the most promising and advantageous process for energy recovery from SS when considering factors such as solution to the problem, greenhouse gas emission, technology maturity and legislation [74]. Compared to gasification and incineration, pyrolysis offers several 2 distinctive benefits. One of the most significant advantages is the versatile application of its products, which enhances the process's sustainability.

Pyrolysis operates at moderate temperatures under oxygen-limited conditions, reducing the release of airborne pollutants and minimizing the environmental impact. This contrasts with the higher operational temperatures and stricter equipment requirements associated with gasification and incineration. Additionally, the absence of oxygen in the pyrolysis process allows for the retention of heavy metals and essential nutrients, such as phosphorus, in the solid residues. These solid residues, primarily biochar, can be repurposed as absorbents or soil amendments, thus promoting resource recovery. The flexibility of bio-oil utilization further underscores the potential of pyrolysis. It can serve as a renewable fuel alternative or as a precursor for valuable chemical products, depending on downstream processing needs. Similarly, the gaseous byproducts of pyrolysis, which are rich in combustible compounds, can be utilized as an energy source within the process itself, enhancing energy efficiency. Collectively, these factors position pyrolysis as a near "zero-waste" system, aligning with circular economic principles and sustainable waste management practices.

Therefore, the coupled process of AD and pyrolysis has received considerable attention from researchers. Many studies have been conducted using biomass [75], sludge [76], MSW [77], and agricultural waste [78]. The integration of AD with pyrolysis holds great promise as a new technology for SS treatment [79]. Nansubuga et al. reported that a possible best-management strategy for digested high-rate activated sludge (HRAS) was energy recovery via AD followed by pyrolytic production of biochar from the dried digestate [80].

Researchers also reported that the integration of pyrolysis and AD has shown immense potential in energy production, chemical recovery, and nutrient recycling. Both are non-oxygen processes, but pyrolysis as a high-temperature thermochemical process, can handle a wide range of wastes, including non-biological materials, whereas AD is a biological process restricted to organic waste. Combining pyrolysis and AD might address their respective limitations [6].

The integration of AD and pyrolysis potentially offers a practical solution to the challenges presented by standalone AD, such as the problem of incomplete organics decomposition in AD and the pollution risk/energy loss with DS. Pyrolysis of DS has emerged as an innovative process that not only eliminates pathogens and odors but also converts DS into valuable products, such as bio-oil, pyrolysis gas, and biochar,

depending on the specific application. In addition, pyrolysis can produce aromatic carbon structures that can immobilize heavy metals, thereby reducing their availability in soil [81], lowering the risk of leaching from biochar, and reducing plant uptake of these elements. Hence, it can alleviate the negative impacts of directly applying SS to farmland.[80].

AD enhances the energy self-sufficiency of the subsequent pyrolysis step by generating biogas and residual heat, which can be utilized for drying digestate prior to pyrolysis [82]. This drying process is critical, as pyrolysis is an endothermic process with high energy demands, which is further increased by the necessary to reduce the water content of the sludge. For instance, reducing the water content from 65% to 6.5% requires approximately 4 MJ/kg of dried sludge. Utilizing residual heat from AD can effectively offset this energy demand, improving the overall energy efficiency of the integrated system [27].

Cao and Pawłowski [42] assessed the energy efficiency of AD and pyrolysis processes applied to primary and activated sludge. They evaluated three routes: route A involved the standalone application of AD to produce biogas; route B combined AD with subsequent pyrolysis of the digestate to generate bio-oil and biochar; and route C applied direct pyrolysis to sludge for the same outputs. The results showed that for SS with high VS content ($>84\%$), route A (AD standalone) produced greater energy output compared to direct pyrolysis. However, integrating pyrolysis with AD (route B) to process the residual digestate further improved the overall energy recovery efficiency. Route B not only enhanced the energy recovery from sludge but also added value to the digestate through the production of bio-oil and biochar. Moreover, the integration of AD with pyrolysis exhibited superior energy recovery efficiency for both high and low VS content sludge, surpassing the performance of both standalone processes. This highlights the potential of combined AD-pyrolysis systems in maximizing resource recovery and energy efficiency in SS management.

Li and Feng [76] conducted a comparative analysis of integrated AD and pyrolysis pathways versus standalone AD and standalone pyrolysis, focusing on life-cycle environmental impacts and energy efficiency. The study also investigated the influence of organic content in feed sludge on the performance of these three pathways. Results

indicated that AD performed the best among the three options, as it did not require energy or material inputs for thermal drying and pyrolysis. Conversely, standalone pyrolysis exhibited the lowest energy efficiency and the highest environmental burden. The integration of AD with pyrolysis demonstrated superior environmental performance and energy efficiency compared to standalone pyrolysis. This improvement is attributed to the role of AD in enhancing the conversion of sludge organics into energy, thereby reducing the organic load for pyrolysis. When the VS/TS ratio in the sludge exceeded 61%, the integrated system generated surplus energy. Additionally, a VS/TS ratio of 52% represented the energy equilibrium point, where input and output energy are balanced. Therefore, the integrated approach is particularly suitable for sludge with high organic content due to its superior energy conversion efficiency. Song et al. [82] further corroborated these findings, highlighting that biogas generated during AD could significantly improve the energy balance of the subsequent pyrolysis process. Given the high organic content of SS in Japan (80%), the integration of AD and pyrolysis presents a promising strategy for efficient energy recovery and sustainable sludge management.

Despite the promising integration of AD and pyrolysis for enhanced energy recovery and sludge management, the mutual interactions between AD and pyrolysis require further exploration to fully realize the potential of their integration. In particular, the influence of AD on pyrolysis characteristics—such as weight loss behavior, reaction kinetics, and thermodynamics—remains an area that demands deeper investigation. Furthermore, the impact of pyrolysis byproducts on AD, including its potential conversion via AD and feasibility, also warrants comprehensive investigation to clarify how pyrolysis byproducts influence the efficiency and stability of AD. As mentioned earlier, AD has been proposed as a potential strategy for managing APL, as it can convert the organic components of APL into methane-rich biogas. The presence of organic compounds such as acetic acid makes APL an attractive substrate for energy recovery in the form of biomethane. However, the application of AD for APL treatment is challenging due to the presence of toxic compounds, including phenols and nitrogen-containing organics, which can inhibit anaerobic microbial activity. A comprehensive

study of the APL toxicity to AD and the feasibility of converting APL into methane via AD is necessary to further understand the integrated system.

1.6 Research objectives and outline

This study aims to systematically investigate the mutual interactions between AD and pyrolysis, with a particular emphasis on how these two processes influence each other, focusing on three major perspectives. This study first examined how AD altered sludge characteristics and influenced the subsequent pyrolysis characteristics. Then it investigated the impact and toxicity of pyrolysis-derived byproducts (APL) on the performance and stability of AD. Finally, it evaluated the feasibility and practicality of reintroducing pyrolysis-derived APL into AD for converting to biogas and developing a circular integrated “AD-pyrolysis-AD” process, improving overall energy recovery from sludge.

The study consists of 5 chapters. The objectives and structure are detailed as follows (Figure 1.10):

Chapter 2 focuses on the effect of AD on subsequent pyrolysis characteristics. This chapter evaluated the different effects of mesophilic and thermophilic AD on the subsequent pyrolysis characteristics, kinetics, and thermodynamics. A thermogravimetric (TG) analyzer was used to pyrolyze raw and digested sludge at different heating rates to examine the pyrolysis characteristics. Then, the kinetic parameters were determined using three integral iso-conversion methods to explore the influence of AD on the pyrolysis kinetics. Also, the thermodynamic parameters were determined using the above results, clarifying the effect on the pyrolysis thermodynamics. Pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) analysis was further conducted to provide deeper insight into the mechanisms involved.

Chapter 3 investigates the effect of pyrolysis byproducts (APL) on AD. It assessed the characteristics and toxicity of DS-derived APLs to AD and determined its rate-limiting step. The toxicity of APLs prepared at various temperatures and feedstock moisture contents was assessed using an anaerobic toxicity assay. The rate-limiting step in AD was studied by adding glucose and sodium acetate as model intermediates of

hydrolysis and acidification, respectively. The determined rate-limiting step was further confirmed using the modified Gompertz model based on the estimated maximum methane production rate.

Chapter 4 further explores the feasibility and practicality of converting APL into methane through AD. The primary focus of this chapter is to mitigate the AD inhibition caused by APL through co-AD with sludge, and to determine whether APL generated at different pyrolysis temperatures (350, 500, and 650°C) within the AD-pyrolysis system, assuming all digestate is subjected to pyrolysis, can be fully reintroduced into AD. Batch co-AD of APL and sludge were conducted based on the real APL yield to select APL preparing temperatures (350, 500, or 650°C). Continuous co-AD was then conducted with the selected APLs prepared at 350°C (least toxic) and 500°C (most toxic) to enhance microbial adaption and improve APL conversion. These experiments tested the potential for fully introducing APL into the AD system and its impact on biogas production. Finally, a detailed mass and energy flow analysis was carried out to evaluate the overall energy recovery efficiency and sustainability of the integrated AD-pyrolysis-AD system.

Chapter 5 summarizes the main conclusion of this study and provides future perspectives in this field.

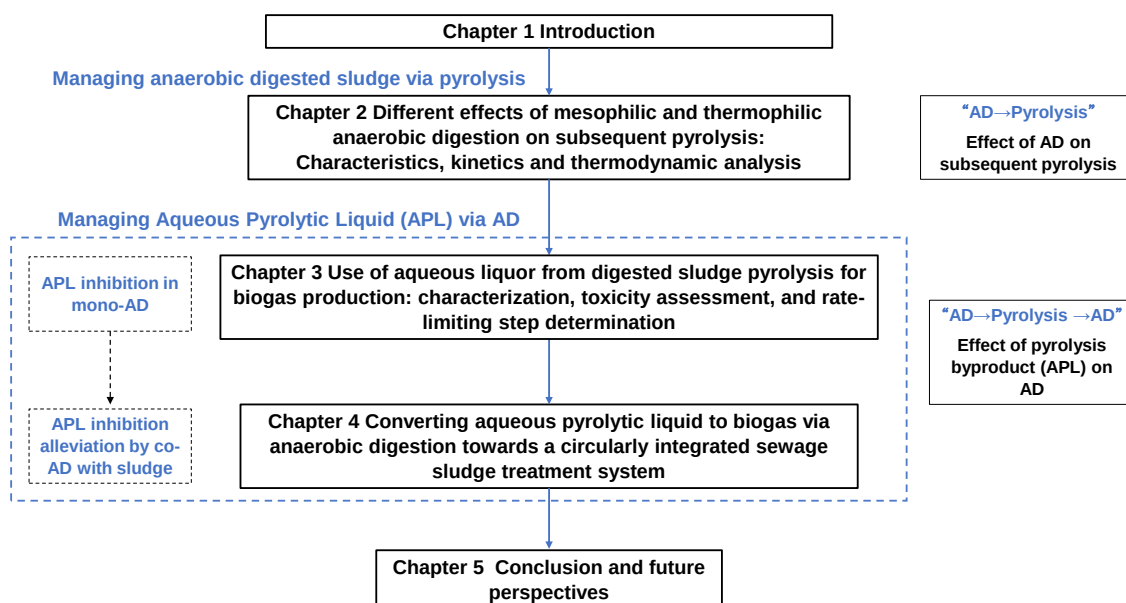


Figure 1.10 Schematic overview of the objectives and structure of this study.

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Chapter 2 Different effects of mesophilic and thermophilic anaerobic digestion on subsequent pyrolysis: Characteristics, kinetics and thermodynamic analysis

2.1 Introduction

Pyrolysis of DS has emerged as an innovative process that not only eliminates pathogens and odors but also converts DS into valuable products, such as bio-oil, pyrolysis gas, and biochar, depending on the specific application. In addition, pyrolysis can produce aromatic carbon structures that can immobilize heavy metals, thereby reducing their availability in soil [1], lowering the risk of leaching from biochar, and reducing plant uptake of these elements. Hence, it can alleviate the negative impacts of directly applying sewage sludge to farmland [2]. The combination of AD with pyrolysis holds great promise as a new technology for sewage sludge treatment [3]. Nansubuga et al. reported that a possible best-management strategy for digested HRAS was energy recovery via AD followed by pyrolytic production of biochar from the dried digestate [2]. Usually, AD proceeds at either thermophilic (50–55°C) or mesophilic (35–37°C) temperatures, where the decomposition of organic matter is enhanced under thermophilic conditions. This potentially causes a different impact on subsequent pyrolysis [4]. Kinetic and thermodynamic analyses aid understanding of the distinct effects of different AD temperatures on pyrolytic behavior and the decomposition mechanisms of organic matter [5]–[7], providing essential references for the tailored treatment of different DSs and contributing to the design of the pyrolytic process.

However, previous studies on AD coupled with pyrolysis (AD-Py) mainly explored product characteristics [8]–[11], energy recovery potential [12], [13], or product applications [14], [15], with a focus on biomass and domestic waste. To the best of our knowledge, few studies have explored the effects of AD on subsequent pyrolysis characteristics, kinetics and thermodynamics of sewage sludge, especially the different impacts of AD temperature (mesophilic and thermophilic). Li et al. investigated the effect of AD on the pyrolytic kinetics of organic solid wastes and revealed that AD increased the mean activation energy (E) [5]. Chen et al. reported that the E of sludge pyrolysis was reduced after combined AD and thermal hydrolysis [16]. Petrovič et al. [6] analyzed the kinetics and thermodynamics of two solid digestates (sewage sludge and a mixture of sewage sludge and lignocellulosic biomass). It was found that the decomposition of digestate was more complex than that of undigested sludge or the

mixture, as revealed by variations in the thermodynamic parameters. However, the different effects of mesophilic and thermophilic AD on subsequent pyrolysis have not been reported. González-Arias et al. [17] studied the pyrolytic kinetics of digestates from thermophilic and mesophilic co-AD of sewage sludge and grease, and found that samples derived from thermophilic AD exhibited a higher E than those from mesophilic AD. However, the effects on pyrolysis thermodynamics were not reported, lacking characterization to examine the mechanisms underlying these effects in detail. Hence, knowledge about the different effects of mesophilic and thermophilic AD on subsequent pyrolysis behavior, the kinetics and the thermodynamics, and the underlying mechanisms remain very limited. A study of such effects would provide a deeper understanding of sludge biochemical–thermochemical treatment and facilitate the design of subsequent pyrolysis.

This chapter aims to investigate the different effects of mesophilic and thermophilic sewage sludge AD on the subsequent pyrolysis characteristics, kinetics, and thermodynamics. A TG analyzer was used to pyrolyze the undigested and digested sludge at different heating rates to examine the pyrolysis characteristics. Then, the kinetic parameters were determined using three integral iso-conversion methods (Starink, Flynn–Wall–Ozawa (FWO), and Kissinger–Akahira–Sunose (KAS)) to explore the influence of AD on the pyrolysis kinetics. Also, the thermodynamic parameters were determined using the above results, clarifying the effect on the pyrolysis thermodynamics. Py–GC/MS analysis was further conducted to provide deeper insight into the mechanisms involved.

2.2 Materials and methods

2.2.1 Materials

Sludge samples before and after AD were collected from two mesophilic digestion plants and two thermophilic digestion plants. The raw mixed sludge (RS) and DS obtained under mesophilic conditions were from the H WWTP in Osaka Prefecture, Japan, and the U WWTP in Kyoto Prefecture, Japan, respectively, both operate at an AD temperature of 36°C. These samples were labeled H RS, H DS, U RS, and U DS, respectively. The RS and DS obtained under thermophilic conditions were collected from the N and S WWTPs in Osaka Prefecture, Japan, both operate at an AD temperature of 55°C. These samples were named N RS, N DS, S RS, and S DS, respectively. The selected WWTPs, located in the Kansai region, a major metropolitan

area of Japan, represented typical residential wastewater treatment facilities employing the activated sludge method. All samples were dried in an oven at $105 \pm 1^\circ\text{C}$ for 24 h and then ground and homogenized to a particle size < 0.5 mm for subsequent analysis. The characteristics of these samples are presented in Table 2.1. The VS removal ratios during AD are given by Eq. (2-1):

$$VS \text{ removal ratio (\%)} = \frac{VS_{\text{input}} \times TS_{\text{input}} \times Mass_{\text{input}} - VS_{\text{output}} \times TS_{\text{output}} \times Mass_{\text{output}}}{VS_{\text{output}} \times TS_{\text{input}} \times Mass_{\text{input}}} \quad (2-1)$$

where TS_{input} and TS_{output} are the TS in the sludge input to and output from the AD reactor, respectively, %; VS_{input} and VS_{output} represent the VS content in the sludge input to and output from the AD reactor, respectively, TS%; and $Mass_{\text{input}}$ and $Mass_{\text{output}}$ are the sludge weights (kg) input to and output from the AD reactor, respectively.

Table 2.1 Characteristics of the sludge samples

AD type	Sam- ples name	TS ^a (%)	VS ^b (%TS)	Ash ^c (%TS)	VS removal ratio by AD (%)	Ultimate analysis (%TS)		
						C ^g	H ^g	N ^g
Mesophilic	H RS ^e	3.19±0.01	86.03±0.02 ^f	13.97±0.02	52.19	40.54±0.15	6.47±0.03	5.75±0.03
	H DS	1.75±0.01	75.11±0.11	24.89±0.11		36.77±0.88	6.27±0.18	6.07±0.08
	U RS	2.87±0.02	79.72±0.07	20.28±0.07	27.07	40.18±0.09	6.29±0.14	5.86±0.22
	U DS ^f	2.23±0.01	75.13±0.21	24.87±0.21		36.26±0.44	6.11±0.11	5.43±0.15
Thermophilic ^c	N RS	4.33±0.01	84.30±0.10	15.70±0.56	61.92	40.87±0.50	6.47±0.10	4.82±0.17
	N DS	2.17±0.01	65.35±0.56	34.65±0.56		32.50±0.39	5.71±0.10	5.13±0.05
	S RS	3.78±0.01	78.86±0.23	21.14±0.23	57.38	38.20±0.39	6.16±0.26	4.11±0.16
	S DS	1.97±0.06	64.76±0.38	35.24±0.38		31.98±0.19	5.37±0.05	4.12±0.08

^a TS: Total solids

^b VS: Volatile solids

^c Calculated from $100 - VS$ (%TS)

^d RS: Raw mixed sludge

^e DS: Digested sludge

^f Values are expressed as mean $\pm$ standard deviation, with standard deviations derived from triplicate measurements of a single sample.

^g C, H and N represent carbon, hydrogen and nitrogen, respectively.

2.2.2 TG analysis

The weight loss during pyrolysis of the samples was derived via TG analysis using a dedicated thermal analyzer (Thermo-plus EVO2, TG8120; Rigaku Corporation, Tokyo, Japan). Samples (5–10 mg) were weighed, placed in alumina containers, and loaded into the TG device. The samples were heated from ambient temperature to 800°C at heating rates of 4, 8, 12, 16, and 20°C/min under a 100 mL/min flow of N₂ (N₂-Zero-A; Sumitomo Seika Chemicals, Osaka, Japan). This TG analysis was duplicated for each condition. The weight loss curves at different heating rates were used for the subsequent kinetic and thermodynamic analyses.

2.2.3 Kinetic analyses

Commonly used kinetic models include integral and differential models. The integral methods yield accurate kinetic predictions of non-isothermal decomposition and are less affected by experimental errors than other methods [18]. Given the complexity of pyrolysis, it is challenging to obtain the precise kinetic parameters of a reaction using a single kinetic method. The various kinetic methods corroborate each other, affording more dependable outcomes [19]. Consequently, three integral iso-conversion methods, the Starink, FWO, and KAS methods, were employed when determining the kinetic parameters. The calculation of E using these three methods has been documented previously in studies on MSW [20], biomass [21], and sewage sludge [22].

Pyrolysis can be regarded as a cracking reaction that can be expressed as:



where X(s), Y(s), and Z(g) represent solid reactants, solid residue, and gas products, respectively. The condensable components in gas products Z(g) can be converted to liquid products by condensation. Assuming that there is no temperature difference within the sample and that the temperature is equal to the set temperature, Eq. (2-2) can be expressed as:

$$d\alpha/dt = kf(\alpha) \quad (2-3)$$

The expression for f(α) is:

$$f(\alpha) = (1-\alpha)^n \quad (2-4)$$

where α is the conversion rate, which can be written as $\alpha = (m_0 - m_t)/(m_0 - m_r)$. m_0 is the initial weight (mg) of the sample before pyrolysis; m_t is the instantaneous weight (mg) of the sample at time t during pyrolysis; m_r is the final weight (mg) of the sample after pyrolysis; and n is the reaction order.

According to the Arrhenius law, the reaction rate k can be expressed as:

$$k = Ae^{-\frac{E}{RT}} \quad (2-5)$$

where A is the pre-exponential factor (min^{-1}); E is the activation energy of the reaction (J/mol); T is the temperature (K); and R is the universal gas constant [$8.314 \text{ J}/(\text{mol} \cdot \text{K})$]. When the rate of temperature rise β is written as $\beta = dT/dt$, Eq. (2-5) can be reduced to:

$$d\alpha/dT = \frac{A}{\beta} e^{-\frac{E}{RT}} (1 - \alpha)^n \quad (2-6)$$

If $f(\alpha) = (1 - \alpha)^n$, the initial conditions are $\alpha = 0$ and $T = T_0$. Defining $\mu = \frac{E}{RT}$, the integral form of the conversion rate can be expressed as:

$$G(\alpha) = \int_0^\alpha \frac{d\alpha}{f(\alpha)} = \frac{A}{\beta} \int_{T_0}^T e^{-\frac{E}{RT}} dT = \frac{AE}{\beta R} \int_0^\infty u^{-2} e^{-u} du = \frac{AE}{\beta R} p(u) \quad (2-7)$$

where $p(u)$ is defined as $\int_0^\infty u^{-2} e^{-u} du$. Eq. (2-7) can be solved by different methods depending on the function $p(u)$.

2.2.3.1 The FWO method

The FWO method is a model-free method based on the following equation [23]:

$$\ln \beta = \ln \left(\frac{AE}{RG(\alpha)} \right) - \frac{E}{RT} \quad (2-8)$$

By plotting $\ln \beta$ versus $1/T$, E can be calculated from the slope ($-\frac{E}{R}$) of the obtained straight line.

2.2.3.2 The Starink method

As $\mu = \frac{E}{RT}$, an approximate solution of Eq. (2-7) can be expressed as [24], [25]:

$$\ln \frac{\beta}{T^{1.92}} = -1.0008 \frac{E}{RT} + \ln \frac{AR^{0.92}}{G(\alpha)E^{0.92}} - 0.312 \quad (2-9)$$

This is the basic equation of the Starink method. $\ln \frac{\beta}{T^{1.92}}$ is plotted against $1/T$ to obtain a straight line with a slope of $-1.0008 \frac{E}{RT}$, from which the E values at different α can be determined.

2.2.3.3 The KAS method

The basic equation of the KAS method is derived from Eq. (2-7) by taking the logarithm [25]:

$$\ln \frac{\beta}{T^2} = \ln \left(\frac{AR}{EG(\alpha)} \right) - \frac{E}{RT} \quad (2-10)$$

Thus, the plot $\ln \frac{\beta}{T^2}$ versus $1/T$ is a straight line, the slope of which ($\frac{E}{RT}$) can be used to determine E .

By plotting $\ln \beta$, $\ln \frac{\beta}{T^{1.92}}$, $\ln \frac{\beta}{T^2}$ versus $1/T$ for a specified α at heating rates of 4, 8, 12, 16, and 20°C/min, E can be determined from the slope of each regression line.

2.2.4 Thermodynamic analysis method

The pre-exponential factor (A), the variations in enthalpy (ΔH°), the Gibbs free energy (ΔG°), and the entropy (ΔS°) between the original reactants and the activated complexes were calculated using the following equations [24], [26]:

$$A = \beta \cdot E \cdot e^{\frac{E}{RT_m}} / (R \cdot T_m^2) \quad (2-11)$$

$$\Delta H^\circ = E - RT \quad (2-12)$$

$$\Delta G^\circ = E + R \cdot T_m \cdot \ln \left(\frac{K_B \cdot T_m}{h \cdot A} \right) \quad (2-13)$$

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T_m} \quad (2-14)$$

where T_m represents the temperature corresponding to the maximum decomposition rate, K; K_B is Boltzmann's constant, 1.381×10^{-23} J/K; and h is Planck's constant, 6.626×10^{-34} J.

2.2.5 Evolved gas analysis by Py-GC/MS

Evolved gas analysis was conducted using a Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) with a Multi-Shot Pyrolyzer (EGA/PY-3030 D; Frontier Laboratories Ltd., Fukushima, Japan) coupled with a GC/MS (QP2020; Shimadzu

Corp., Kyoto, Japan). Samples (0.2–0.3 mg) were weighed and placed in the Py-GC/MS. After the pyrolyzer was heated to 800°C over 0.2 min, the sample was dropped into the pyrolyzer and flash pyrolyzed. The interface temperature was set to 300°C. The evolved gas was then introduced into a GC that had an Ultra ALLOY-5 column with a length of 30 m, a membrane thickness of 0.25 μm , and an inner diameter of 0.25 mm. The column temperature was initially set at 50°C and held for 2 min, followed by a gradual increase to 300°C at a heating rate of 10°C/min and a hold time of 25 min. Helium (He-Zero-U; Sumitomo Seika Chemicals, Osaka, Japan) was used as the carrier gas flowing at a rate of 54.1 mL/min. The sample was injected in the split injection mode with a split ratio of 50:1. The separated evolved gas was then introduced to the MS. The interface temperature and ion source temperature were 300°C and 250°C, respectively. The analyses were performed in the scan mode with a scan range of 10–700 m/z.

2.2.6 Analysis method

TS, VS, carbon, hydrogen and nitrogen contents were measured using the APHA standard method [27]. In detail, TS was determined by drying the sample at $105 \pm 2^\circ\text{C}$ for 24 h. VS was measured using dried samples combusted in a muffle furnace at $550 \pm 25^\circ\text{C}$ for 1 h. Ash content (%TS) was calculated as $100 - \text{VS (\%TS)}$. The carbon, hydrogen and nitrogen contents of dried samples were measured using a C, H, and N analyzer (JM 10 Micro Corder; J-Science Lab Co., Ltd., Kyoto, Japan). Each measurement was conducted in triplicate.

2.3. Results and discussion

2.3.1 Pyrolysis characteristics at different heating rates

Derivative thermogravimetry (DTG) is the first derivative of the TG curve [7]. The TG and DTG curves of RS and DS at different heating rates are shown in Figure 2.1; the corresponding characteristic pyrolysis parameters are listed in Table 2.2. The weight loss during pyrolysis could be divided into three stages, as follows.

In Stage I (30–170°C, Figure 2.1), the low weight loss was attributable to the small amount of bound moisture remaining during drying and low-boiling-point components, such as micromolecular hydrocarbons [28]. The samples were dried at 105°C, but there was still residual bound water in the sludge that was difficult to evaporate [29].

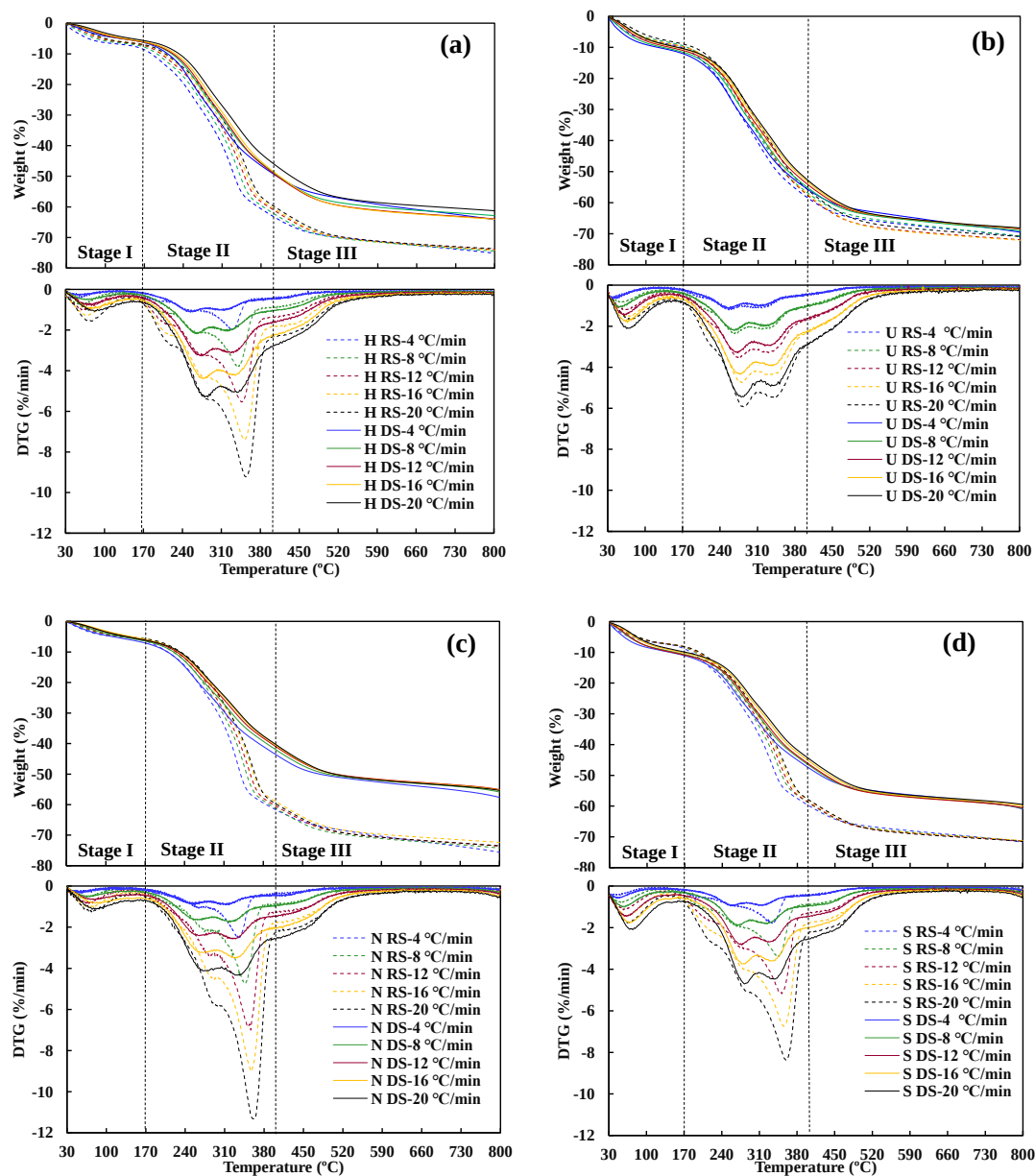


Figure 2.1 The TG and DTG curves of (a) H RS and H DS, (b) U RS and U DS, (c) N RS and N DS, and (d) S RS and S DS at different heating rates (4, 8, 12, 16, 20 °C/min)

In Stage II (170–400°C), significant weight loss occurred. There were two distinct peaks on the DTG curves that overlapped significantly. The first peak corresponded to the primary decomposition of organic matter in sludge; large molecular-weight compounds, such as hemicelluloses and cellulose, were cracked into smaller compounds [30]. This may correspond to depolymerization of biodegradable substances [31], [32]. The second peak corresponded to the secondary decomposition of organic matter [31], [33] and/or the degradation of certain recalcitrant biodegradable

organics, such as lignin [30]. After this stage, most biodegradable organic matter and some non-biodegradable organic matter could be volatilized [34].

Stage III ($> 400^{\circ}\text{C}$) was associated with the decomposition of the inorganic components, such as calcium carbonate [31]. These results agreed with those of previous studies [35], [36], but the temperature range of each stage was slightly different, which may be attributable to the heterogeneity of the sludge samples.

The weight loss percentage decreased after both mesophilic and thermophilic AD (Figure 2.1). This can be attributed to AD biodegradation of some organic matter in the sludge, reducing the relative contents of thermochemically decomposable components. Thermophilic AD reduced the weight loss percentage more significantly than mesophilic AD because the former proceeded at a higher temperature. This is consistent with the VS removal ratio results in Table 2.1. The enrichment of macromolecular organic materials, such as lignocellulose and inorganic matter, in the DS might also contribute to this result. Lignocellulose may come from paper scraps, vegetable residues, and toilet paper entrained in sewage pipes [37]. Previous studies reported that lignin in Japanese sewage sludge constituted 17.5–23.7% of the suspended solid [38]. Lignin was more recalcitrant during AD compared to lipid and protein, thus exhibiting a much lower degradation efficiency [38]; lignin is therefore enriched in DS.

The TG and DTG curves of samples from U WWTP exhibited minimal changes after AD [Figure 2.1(b)], suggesting that the extent of AD of U WWTP was not as profound as that of H WWTP, potentially implying suboptimal performance. The AD system at U WWTP uses a solid-liquid separation process after the AD reaction tank. However, the solid-liquid separation is not very efficient; some of the DS is returned as water that reaches the primary sedimentation tank, and DS is re-introduced into the AD system as input sludge. In other words, some DS is circulated in the WWTP (personal communication from the management staff of U WWTP). This reduced the difference between U RS and U DS. This observation was consistent with the VS removal ratio in Table 2.1. Herein, the extent of AD based on the VS removal ratio can be expressed as: $N > S > H > U$. These differences in the extent of AD could lead to variations in the DS compositions, changing the subsequent pyrolysis behavior.

The pyrolysis characteristic parameters at stage II for mesophilic AD samples and thermophilic AD samples are presented in Table 2.2.

Table 2.2 Pyrolysis characteristic parameters at stage II for mesophilic AD samples (H RS, H DS, U RS and U DS) and thermophilic AD samples (N RS, N DS, S RS and S DS)

Heating rate (°C/min)	Sample	T _{i1} ^a (°C)	T _{m1} ^b (°C)	T _{f1} ^c (°C)	DTG _{m1} ^d (%/min)	T _{i2} ^a (°C)	T _{m2} ^b (°C)	T _{f2} ^c (°C)	DTG _{m2} ^d (%/min)	Total weight loss (%)
4	H RS	122	252	281	-1.07	281	330	359	-1.97	-75.2
8		128	266	288	-2.11	288	339	376	-3.82	-74.5
12		134	275	299	-3.21	299	346	384	-5.53	-74.0
16		140	278	302	-4.28	302	351	389	-7.38	-74.2
20		144	283	304	-5.39	304	354	400	-9.22	-73.7
4	H DS	145	253	283	-1.09	283	315	364	-0.982	-64.2
8		145	264	286	-2.18	286	322	365	-1.99	-62.9
12		148	272	298	-3.25	298	331	377	-3.08	-63.9
16		154	277	306	-4.38	306	336	394	-4.20	-64.0
20		160	281	308	-5.27	308	338	395	-5.07	-61.3
4	U RS	132	255	278	-1.18	278	326	357	-1.05	-70.9
8		141	268	294	-2.31	294	331	367	-2.06	-70.7
12		144	273	303	-3.06	303	344	376	-3.16	-70.6
16		148	279	306	-4.12	306	342	385	-4.30	-71.3
20		151	282	309	-5.90	309	343	392	-5.42	-70.9
4	U DS	131	256	284	-1.09	284	323	352	-0.942	-69.5
8		141	266	293	-2.21	293	331	368	-1.91	-69.0
12		145	273	301	-3.29	301	336	378	-2.90	-68.4

Heating rate (°C/min)	Sample	T ₁₁ ^a (°C)	T _{m1} ^b (°C)	T _{f1} ^c (°C)	DTG _{m1} ^d (%/min)	T ₁₂ ^a (°C)	T _{m2} ^b (°C)	T _{f2} ^c (°C)	DTG _{m2} ^d (%/min)	Total weight loss (%)
16	UDS	148	277	304	-4.33	304	341	385	-3.86	-68.2
20		152	280	315	-5.45	315	343	393	-4.87	-68.1
4	N RS	141	258	282	-1.03	282	334	367	-2.51	-75.6
8		145	275	289	-2.16	289	345	379	-4.68	-74.2
12		147.	282	298	-3.30	298	354	389	-6.73	-73.6
16		152	288	301	-4.42	301	357	391	-9.02	-72.4
20		159	291	305	-5.78	305	363	403	-11.33	-73.5
4	N DS	145	251	283	-0.875	283	312	354	-0.839	-57.6
8		15	257	291	-1.64	291	323	360	-1.69	-55.9
12		159	265	294	-2.40	294	329	374	-2.56	-55.0
16		16	266	299	-3.24	299	331	379	-3.47	-55.2
20		166	272	303	-4.17	303	335	388	-4.47	-55.6
4	S RS	130	262	289	-0.940	289	334	362	-1.79	-71.7
8		13	270	286	-1.94	286	344	378	-3.40	-71.4
12		135	277	287	-2.97	287	352	389	-5.12	-71.4
16		138	286	294	-4.05	294	355	393	-6.74	-71.2
20		142	290	300	-5.11	300	360	402	-8.37	-71.5
4	S DS	140	260	286	-0.930	286	318	358	-0.908	-60.9
8		148	267	295	-1.88	295	325	363	-1.81	-60.7

Heating rate (°C/min)	Sample	T _{i1} ^a (°C)	T _{m1} ^b (°C)	T _{f1} ^c (°C)	DTG _{m1} ^d (%/min)	T _{i2} ^a (°C)	T _{m2} ^b (°C)	T _{f2} ^c (°C)	DTG _{m2} ^d (%/min)	Total weight loss (%)
12		153	276	301	-2.78	301	332	375	-2.66	-60.5
16	S DS	159	279	304	-3.74	304	337	383	-3.58	-59.9
20		163	282	309	-4.70	309	339	392	-4.47	-59.1

T_{i1}, T_{i2}: the initial temperature of the first and second DTG peaks, respectively, of stage II.

T_{m1}, T_{m2}: the peak temperatures of the first and the second DTG peaks, respectively, of stage II.

T_{f1}, T_{f2}: the final temperatures of the first and the second DTG peaks, respectively, of stage II.

DTG_{m1}, DTG_{m2}: the corresponding maximum weight loss rates at the first and second peaks, respectively, of stage II.

T_{m1} and T_{m2} denote the peak temperatures corresponding to the first and the second DTG peaks, respectively, of Stage II, as detailed in Table 2.2. For thermophilic AD, T_{m1} for the S samples remained nearly unchanged, whereas T_{m2} shifted toward lower temperatures. This shift suggested that AD rendered the substances more susceptible to thermal decomposition. For the N WWTP samples, both peak temperatures shifted lower temperatures. This may be attributable to the different extents of AD, as discussed above. N WWTP exhibited greater extents of AD, thus converting a wider range of organics that decomposed over both the first and second peak ranges in Stage II into smaller molecules that were more likely to undergo thermal degradation [39]. The lower extent of AD in S may significantly affect only organics that decompose in the range of the second peak. In terms of mesophilic DS, the T_{m1} of H WWTP samples was similar to that of S WWTP samples, attributable to the similar extents of AD of H and S WWTP. However, for the U WWTP samples, both peak positions remained almost unchanged, reflecting the suboptimal digestion performance, in turn resulting in mere organic decomposition during AD.

2.3.2 Pyrolysis kinetic analyses

The TG data were used to determine the kinetic parameters using equations (2)–(4). The E values corresponding to each α were determined by averaging the results obtained from the three methods. To ensure reliability, the calculations were conducted

twice using data from two sets of TG measurements. Both sets of calculations are shown in the supplementary file. The plotting lines used to determine E are shown in Figures 2.2–2.9. The values of R^2 and the calculated E values can be found in Tables 2.3–2.10.

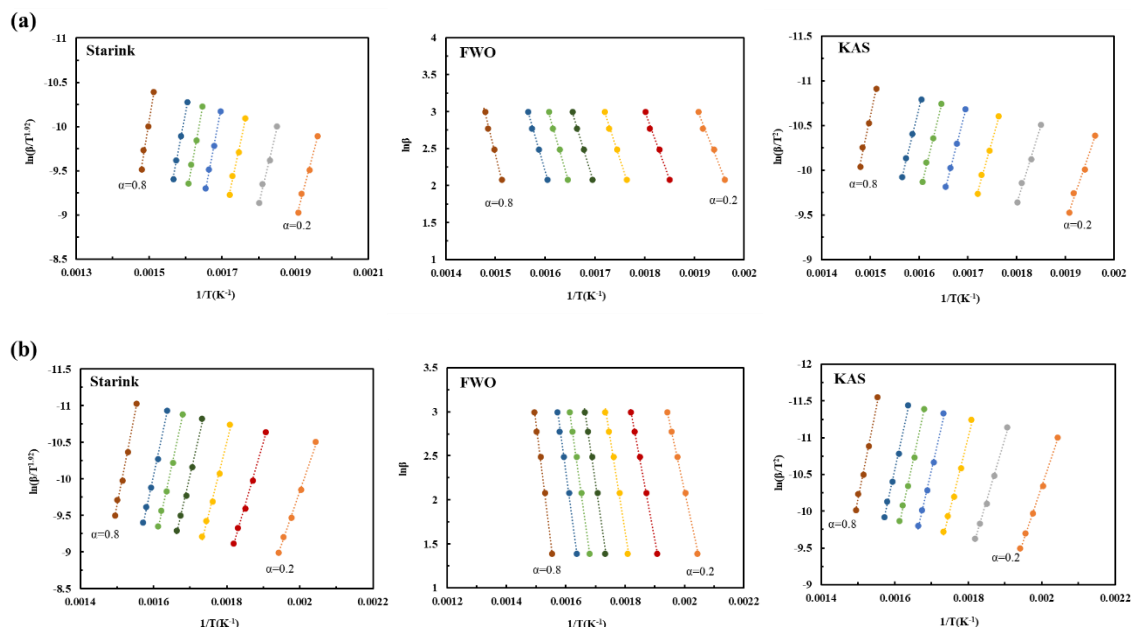


Figure 2.2 Linear plot for determining activation energy of H RS: (a) first and (b) second set of calculation.

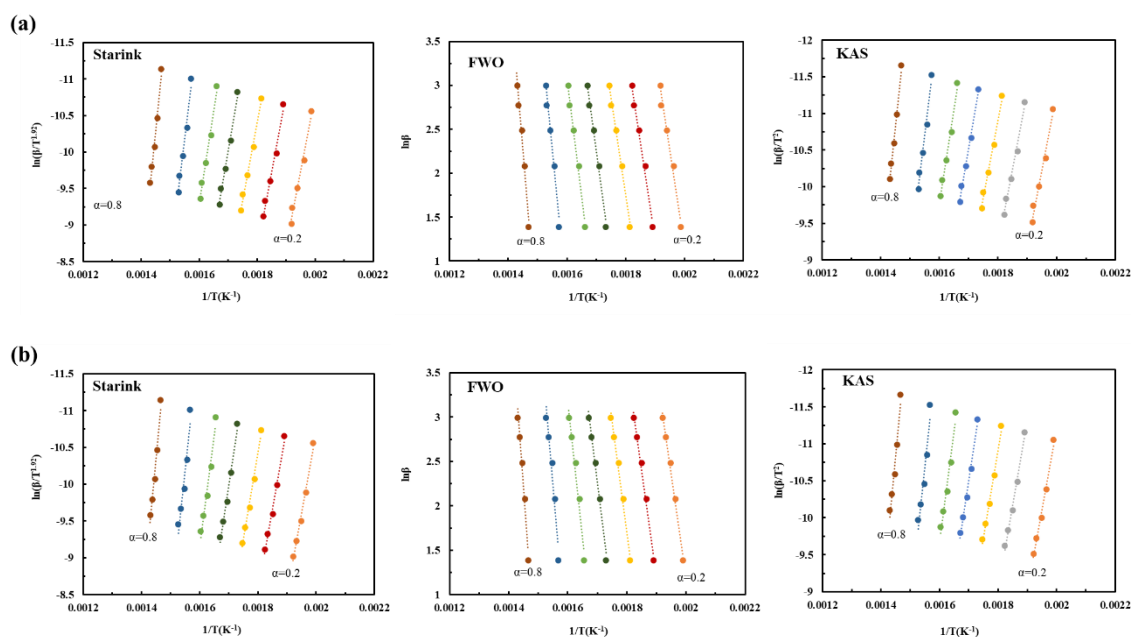


Figure 2.3 Linear plot for determining activation energy of H DS: (a) first and (b) second set of calculation.

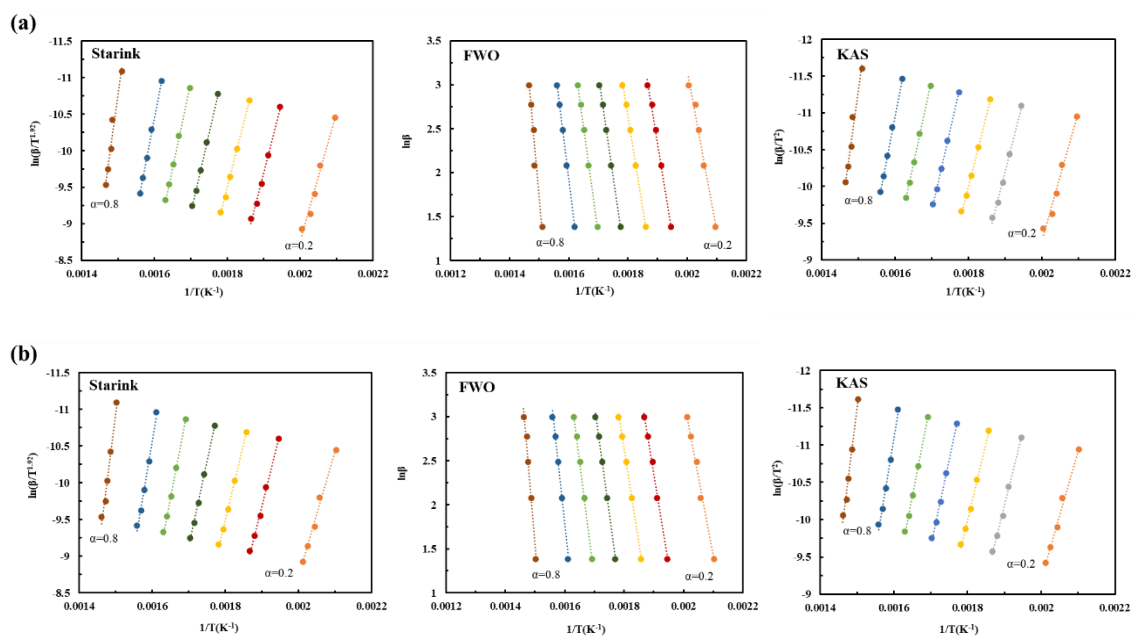


Figure 2.4 Linear plot for determining activation energy of U RS: (a) first and (b) second set of calculation.

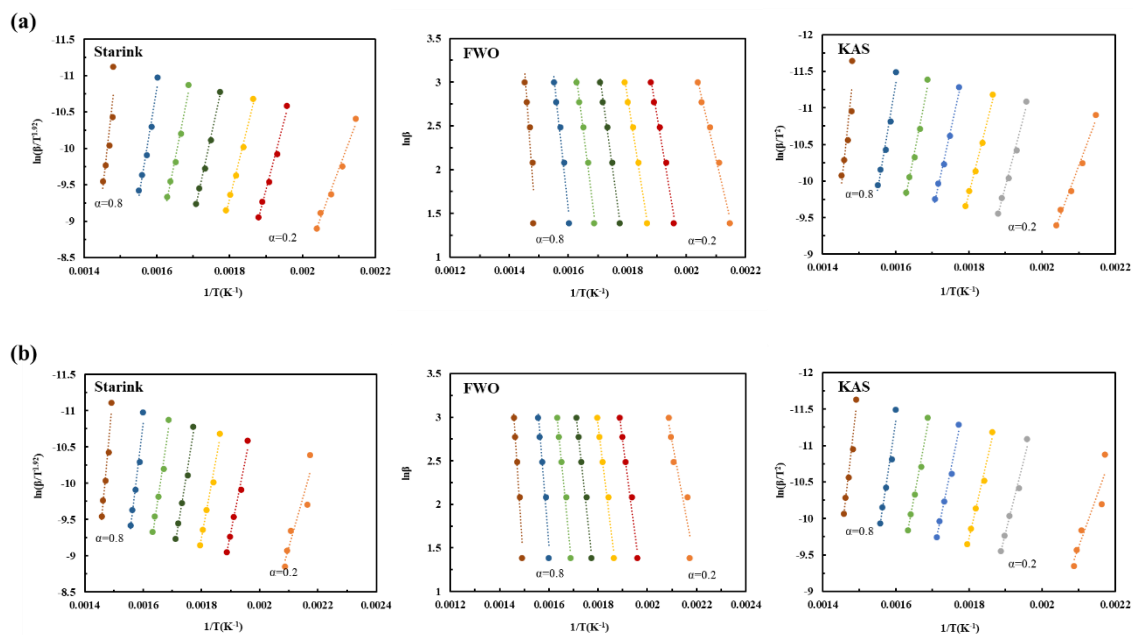


Figure 2.5 Linear plot for determining activation energy of U DS: (a) first and (b) second set of calculation.

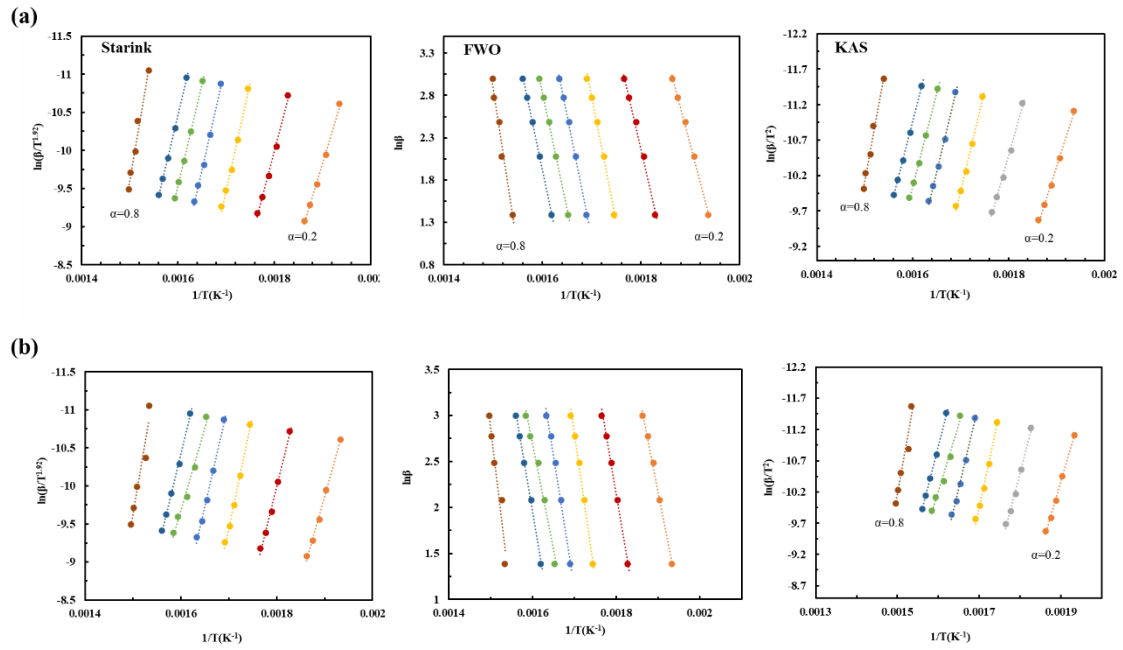


Figure 2.6 Linear plot for determining activation energy of N RS: (a) first and (b) second set of calculation.

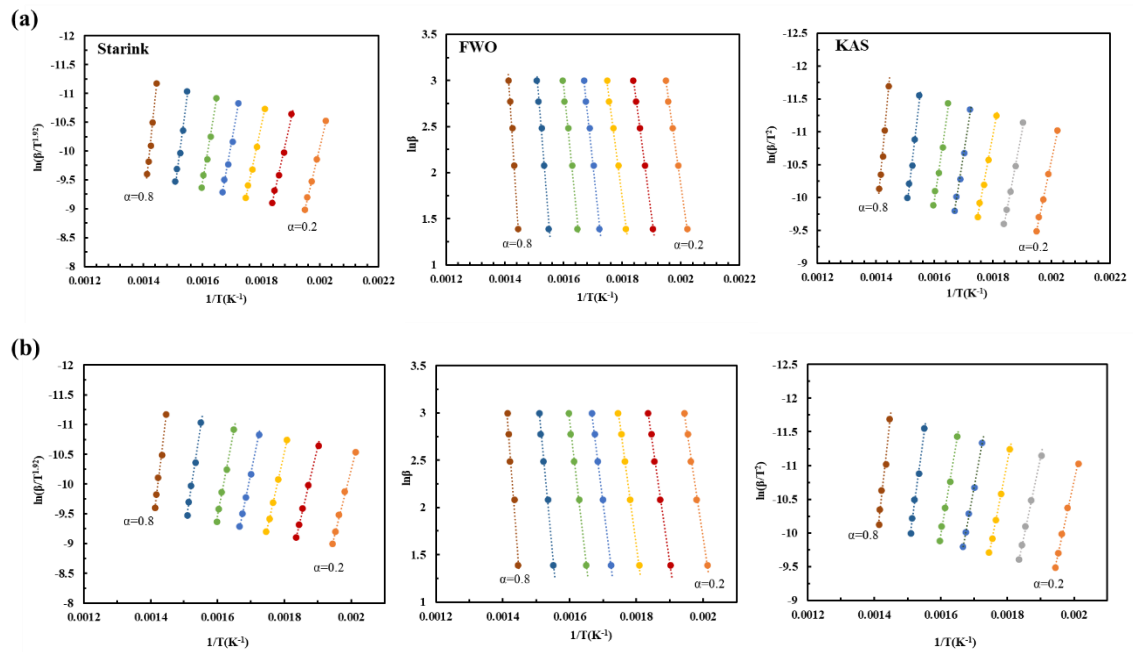


Figure 2.7 Linear plot for determining activation energy of N DS: (a) first and (b) second set of calculation.

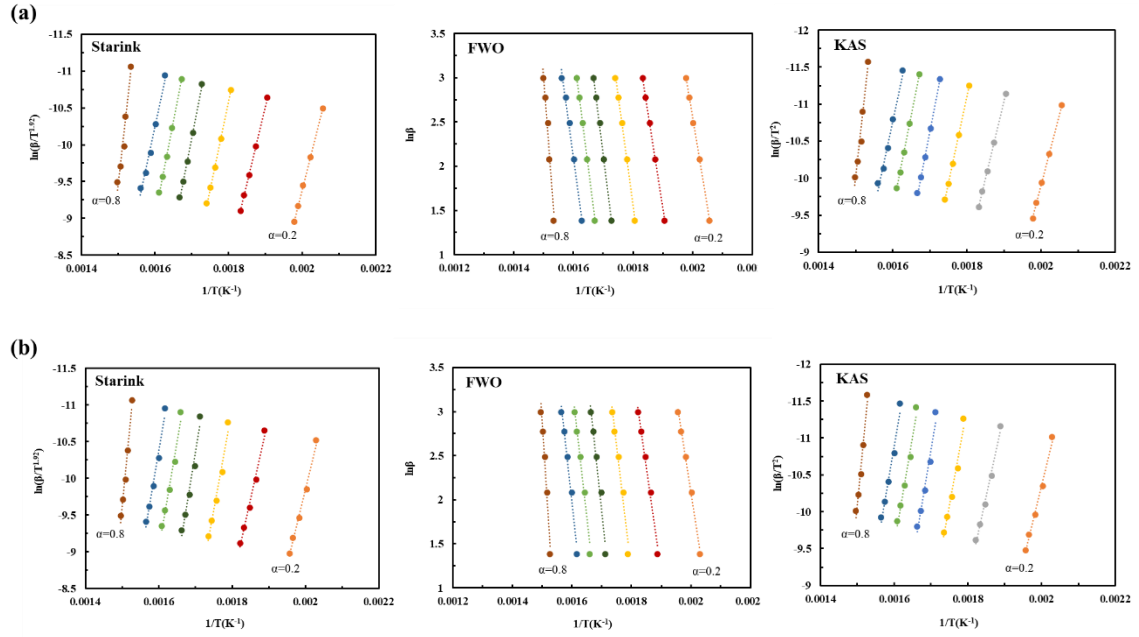


Figure 2.8 Linear plot for determining activation energy of S RS: (a) first and (b) second set of calculation.

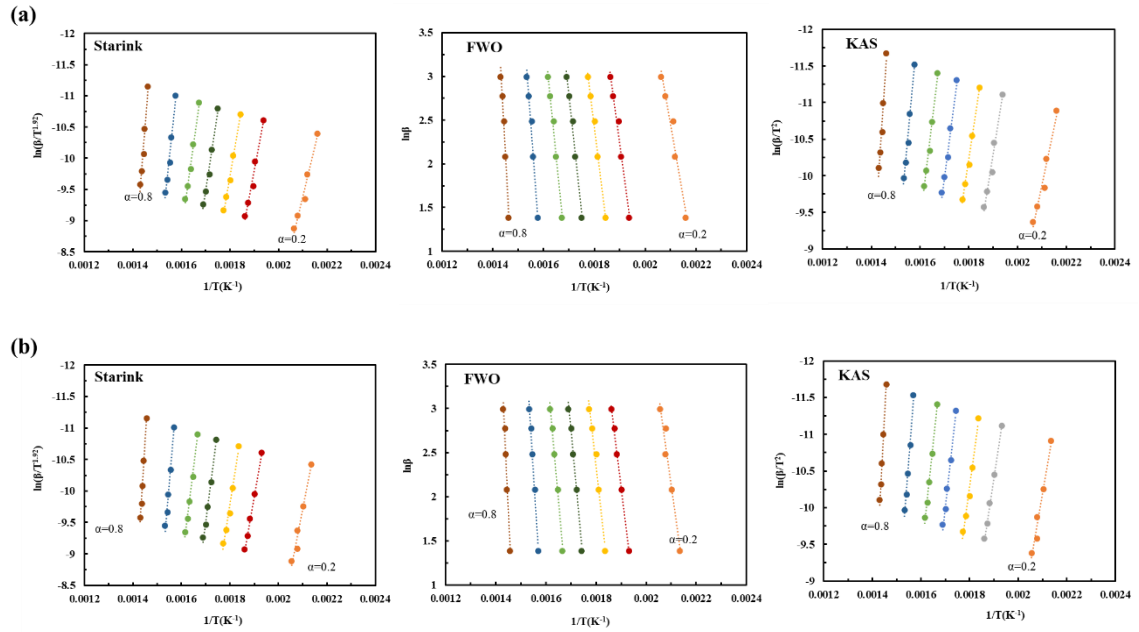


Figure 2.9 Linear plot for determining activation energy of S DS: (a) first and (b) second set of calculation.

Table 2.3 The average E determined by the three methods at different conversions for H RS.

α	Starink				FWO				KAS			
	E-H RS		E-H RS		E-H RS		E-H RS		E-H RS		E-H RS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	131	0.988	122	0.997	139	0.989	130	0.997	131	0.988	121	0.997
0.3	144	0.992	142	0.996	153	0.993	151	0.997	144	0.992	142	0.996
0.4	163	0.995	165	0.993	172	0.996	174	0.993	162	0.995	165	0.993
0.5	176	0.999	183	0.995	186	0.999	192	0.995	176	0.999	183	0.995
0.6	186	0.999	189	0.998	196	0.999	199	0.998	185	0.999	189	0.998
0.7	184	0.998	192	0.996	194	0.998	202	0.997	183	0.998	192	0.996
0.8	211	0.992	213	0.995	221	0.993	223	0.995	210	0.992	212	0.995

^a1 and 2 represent the first and second set of calculations, respectively.

Table 2.4 The average E determined by three methods at different conversions for H DS.

α	Starink				FWO				KAS			
	E-H DS		E-H DS		E-H DS		E-H DS		E-H DS		E-H DS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	171	0.975	185	0.988	179	0.977	193	0.989	171	0.975	184	0.988
0.3	175	0.983	189	0.986	183	0.985	197	0.988	174	0.983	188	0.986
0.4	176	0.989	197	0.988	185	0.990	206	0.989	176	0.989	196	0.988
0.5	196	0.986	216	0.983	206	0.987	225	0.985	196	0.986	215	0.983
0.6	217	0.984	249	0.967	227	0.985	258	0.969	217	0.984	248	0.967
0.7	274	0.976	306	0.939	285	0.974	316	0.942	274	0.976	306	0.938
0.8	321	0.985	355	0.970	332	0.986	366	0.972	320	0.985	355	0.970

^a1 and 2 represent the first and second set of calculations, respectively.

Table 2.5 The average E determined by three methods at different conversions for U RS.

α	Starink				FWO				KAS			
	E-U RS		E-U RS		E-U RS		E-U RS		E-U RS		E-U RS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	146	0.977	142	0.989	153	0.979	150	0.990	145	0.977	142	0.989
0.3	164	0.994	165	0.995	172	0.994	174	0.996	164	0.994	165	0.995
0.4	164	0.998	170	0.998	172	0.998	179	0.998	163	0.998	170	0.998
0.5	181	0.998	193	0.997	190	0.998	202	0.998	181	0.998	193	0.997
0.6	193	0.999	211	0.999	202	0.999	221	0.999	192	0.999	211	0.999
0.7	216	0.998	247	0.993	226	0.998	257	0.993	216	0.998	246	0.993
0.8	298	0.963	329	0.984	309	0.965	340	0.985	298	0.962	329	0.984

^a1 and 2 represent the first and second set of calculations, respectively.

Table 2.6 The average E determined by three methods at different conversions for U DS.

α	Starink				FWO				KAS			
	E-U DS		E-U DS		E-U DS		E-U DS		E-U DS		E-U DS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	112	0.983	118	0.873	119	0.985	125	0.886	111	0.983	118	0.872
0.3	159	0.986	169	0.979	167	0.987	178	0.981	158	0.986	169	0.979
0.4	167	0.993	181	0.984	175	0.994	190	0.985	166	0.993	180	0.984
0.5	190	0.991	204	0.986	199	0.992	213	0.987	190	0.991	203	0.986
0.6	212	0.987	225	0.985	221	0.988	234	0.986	211	0.987	224	0.985
0.7	247	0.973	278	0.964	257	0.975	288	0.967	246	0.973	277	0.964
0.8	380	0.840	361	0.960	391	0.847	372	0.962	379	0.839	361	0.959

^a1 and 2 represent the first and second set of calculations, respectively.

Table 2.7 The average E determined by three methods at different conversions for N RS.

α	Starink				FWO				KAS			
	E- N RS		E- N RS		E- N RS		E- N RS		E- N RS		E- N RS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	174	0.996	185	0.995	183	0.996	194	0.995	174	0.996	185	0.995
0.3	201	0.992	213	0.992	210	0.993	222	0.992	201	0.992	212	0.992
0.4	236	0.992	250	0.991	245	0.993	260	0.992	235	0.992	250	0.991
0.5	232	0.996	231	0.990	242	0.996	241	0.991	232	0.996	231	0.990
0.6	221	0.999	180	0.978	231	0.999	190	0.980	221	0.999	179	0.998
0.7	222	0.999	215	0.998	232	0.999	225	0.998	2211	0.999	215	0.998
0.8	310	0.978	306	0.932	321	0.980	317	0.936	310	0.978	306	0.932

^a1 and 2 represent the first and second set of calculations, respectively.

Table 2.8 The average E determined by three methods at different conversions for N DS.

α	Starink				FWO				KAS			
	E- N DS		E- N DS		E- N DS		E- N DS		E- N DS		E- N DS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	175	0.998	186	0.997	184	0.998	194	0.997	175	0.998	185	0.997
0.3	192	0.996	193	0.999	201	0.996	201	0.999	192	0.996	192	0.999
0.4	196	0.996	207	1.00	205	0.997	216	1.00	196	0.996	206	1.00
0.5	234	0.992	223	0.999	244	0.993	232	0.999	234	0.992	223	0.999
0.6	249	0.988	243	0.997	259	0.989	253	0.997	249	0.988	242	0.997
0.7	316	0.986	300	0.992	327	0.987	311	0.993	316	0.986	300	0.992
0.8	407	0.982	380	0.978	418	0.983	392	0.980	407	0.982	380	0.978

^a1 and 2 represent the first and second set of calculations, respectively.

Table 2.9 The average E determined by three methods at different conversions for S RS.

α	Starink				FWO				KAS			
	E- S RS		E- S RS		E- S RS		E- S RS		E- S RS		E- S RS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	162	1.00	172	0.991	170	1.00	170	1.00	162	1.00	162	1.00
0.3	175	1.00	190	0.987	183	1.00	183	1.00	174	1.00	174	1.00
0.4	196	0.999	231	0.972	205	0.999	205	0.999	196	0.999	196	0.999
0.5	213	0.998	252	0.967	223	0.998	223	0.998	213	0.998	213	0.998
0.6	211	0.999	243	0.979	221	0.999	221	0.999	210	0.999	210	0.999
0.7	196	0.986	244	0.977	206	0.987	206	0.987	195	0.986	195	0.986
0.8	369	0.958	428	0.980	379	0.960	379	0.960	368	0.958	368	0.958

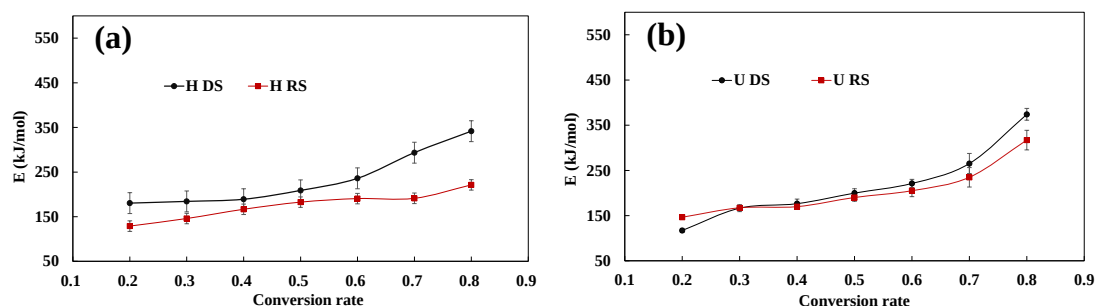
^a1 and 2 represent the first and second set of calculations, respectively.

Table 2.10 The average E determined by three methods at different conversions for S DS.

α	Starink				FWO				KAS			
	E- S DS		E- S DS		E- S DS		E- S DS		E- S DS		E- S DS	
	1 ^a	R ²	2 ^a	R ²	1	R ²	2	R ²	1	R ²	2	R ²
	(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)		(kJ/mol)	
0.2	131	0.961	162	0.964	139	0.965	170	0.967	131	0.961	162	0.964
0.3	172	0.977	182	0.997	180	0.979	191	0.997	171	0.977	182	0.997
0.4	185	0.987	205	0.974	194	0.988	213	0.976	185	0.986	204	0.974
0.5	215	0.983	242	0.985	224	0.984	252	0.986	214	0.983	242	0.985
0.6	232	0.988	259	0.988	242	0.989	269	0.989	232	0.988	259	0.988
0.7	305	0.973	414	0.981	315	0.974	378	0.982	305	0.972	368	0.981
0.8	432	0.968	502	0.985	443	0.969	513	0.986	432	0.968	501	0.985

^a1 and 2 represent the first and second set of calculations, respectively.

The E values reported here are the averages of the two determinations, as depicted in Figure 2.10, with the error bars indicating the uncertainties associated with the two sets of determinations.



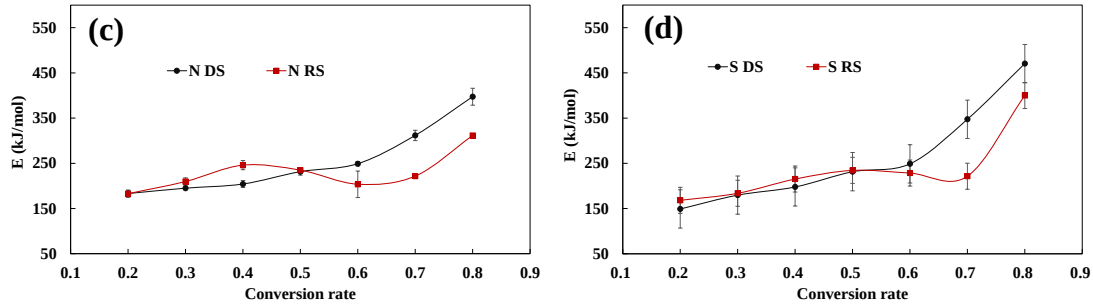


Figure 2.10 Differences in activation energies before and after AD according to the conversion rate: (a) H RS and H DS, (b) U RS and U DS, (c) N RS and N DS, and (d) S RS and S DS.

The pyrolysis E values of all samples varied with α ; a similar result was reported in a previous study [5], implying there was a complex reaction during pyrolysis [40]. The E of RS pyrolysis ranged from 128.9 to 399.9 kJ/mol, comparable to the results of Scott et al. (about 125–375 kJ/mol) [41] but higher than those of Bi et al. (105.2–207.9 kJ/mol) [26] and Naqvi et al. (10.6–306.2 kJ/mol) [35]. This disparity may be attributable to variations in the sources of the sludge samples. The E of DS pyrolysis ranged from 117.3 to 470.4 kJ/mol, slightly higher than the values reported in the literature (about 20–336 kJ/mol) [5]; this might be explained by differences in the AD operation conditions and sources of sludge.

Pyrolysis behavior varied according to the extent of AD. As previously mentioned, the extent of AD followed the order $N > S > H > U$. For the N and S WWTP (thermophilic AD) samples that underwent more extensive AD, AD did not greatly affect E at low conversion rates (0.2–0.5) but significantly increased E at higher conversion rates (0.6–0.8) [Figure 2.10 (c) and (d)]. The small change in E at low conversion rates may be explained by the fact that although AD can consume small molecules by transforming them into biogas, AD can also degrade larger molecules into smaller molecules, such as volatile fatty acids, which remain in the DS [5], thus compensating for the consumed small molecules. This means that the level of small molecules in sludge remains relatively unchanged. The increased E at high conversion rates after AD could be attributable to an increased ash content (Table 2.1) and enrichment of recalcitrant biodegradable organics with higher E values, such as lignin [20].

For samples treated under mesophilic conditions, AD raised the E at all conversion rates during H WWTP sludge pyrolysis [Figure 2.10 (a)]. Even at a low conversion rate

(0.2), H DS pyrolysis exhibited a higher E than H RS pyrolysis. This difference (compared to thermophilic AD) may be attributable to less thorough organic matter degradation during mesophilic AD, yielding fewer low-molecular-weight organics [4]. The content was further reduced on efficient conversion into biogas, thus raising the E of H DS pyrolysis even at low conversion rates. This was confirmed by the TG curve. At 220–250°C, corresponding to a conversion rate of 0.2, H DS showed less weight loss than H RS [Figure 2.1(a)], indicating fewer volatilizable organics in H DS at this conversion rate.

During the pyrolysis of U WWTP samples, the E values before and after AD remained quite similar across all conversion rates, in line with the small changes in VS content and the TG/DTG curves. The decrease in E when $\alpha = 0.2$ may be attributable to the decomposition of biodegradable molecules into small molecules (such as volatile fatty acid [5]), with inefficient conversion to biogas, resulting in the persistence of small organic molecules in U DS. This is in line with the fact that the weight loss percentage of U DS slightly exceeded that of U RS from 190–225°C (corresponding to $\alpha = 0.2$) on the TG curve [Figure 2.1 (b)]. The slight increase in the E at high conversion rates might result from the enrichment of recalcitrant biodegradable organics and ash [5].

Generally, the extent of AD affected the sludge pyrolysis E. Adjustment of AD based on practical considerations might optimize subsequent pyrolysis. In particular, all samples exhibited lower E values at low conversion rates (low temperatures), and thermophilic AD ensured that the E did not change much at these low conversion rates, implying the promising suitability for low-temperature pyrolysis of DS to produce biochar, as suggested by Nansubuga et al. [2]. Guo et al. [42] studied the impact of the number of AD pre-treatment days on biomass gasification and suggested that appropriate AD pre-treatment enhanced gasification efficiency by increasing the porosity and looseness of the biomass structure, thereby improving gasification reaction efficiency. This observation could potentially also apply to sludge, because sludge contains a substantial number of lignocellulosic fibers from kitchen waste and toilet paper. Especially in Japan, all toilet paper is flushed and enters WWTPs. An appropriate extent of AD might contribute to the enhancement of lignocellulose porosity and looseness, thereby enhancing the efficiency of subsequent pyrolysis. Further research is essential to determine the extent of AD that optimizes subsequent thermal treatment.

2.3.3 Thermodynamic analysis

The average E values were determined by the three methods under each conversion rate. T_m at which the maximum mass loss rates occurred at a heating rate of 12 K/min (Table 2.11) were used to calculate the pre-exponential factor (A), enthalpy change (ΔH°), Gibbs free energy (ΔG°), and entropy change (ΔS°). The results are shown in Table 2.11.

Table 2.11 A, ΔH° , ΔG° and ΔS° of RS and DS pyrolysis at each conversion rate at a heating rate of 12 K/min.

AD type	Sample	α	A (s ⁻¹)	ΔH° (kJ/mol)	ΔG° (kJ/mol)	ΔS° (J/(mol·K))
Mesophilic AD	H RS	0.2	7.36×10^7	124	191	-108
		0.3	2.26×10^9	141	190	-80.2
		0.4	1.45×10^{11}	162	190	-45.6
		0.5	3.61×10^{12}	178	189	-18.9
		0.6	1.77×10^{13}	186	189	-5.70
		0.7	1.98×10^{13}	186	189	-4.74
		0.8	8.09×10^{15}	216	188	45.2
	H DS	0.2	3.43×10^{14}	176	165	20.0
		0.3	8.20×10^{14}	180	165	27.3
		0.4	2.43×10^{15}	185	165	36.3
		0.5	2.09×10^{17}	204	164	73.3
		0.6	9.16×10^{19}	231	164	124
		0.7	3.72×10^{25}	289	163	231
		0.8	1.72×10^{30}	337	162	321
	U RS	0.2	2.74×10^9	141	190	-78.6
		0.3	1.82×10^{11}	162	189	-43.7
		0.4	3.03×10^{11}	165	189	-39.5
		0.5	1.76×10^{13}	185	188	-5.73
		0.6	3.54×10^{14}	200	188	19.2

AD type	Sample	α	A (s ⁻¹)	ΔH° (kJ/mol)	ΔG° (kJ/mol)	ΔS° (J/(mol·K))
Mesophilic AD	U RS	0.7	1.32×10^{17}	230	187	68.5
		0.8	1.65×10^{24}	312	186	204
	U DS	0.2	1.87×10^8	113	167	-99.9
		0.3	1.41×10^{13}	162	166	-6.55
		0.4	1.32×10^{14}	172	165	12.1
		0.5	2.59×10^{16}	195	165	56.0
		0.6	3.16×10^{18}	217	164	95.9
		0.7	6.23×10^{22}	261	164	178
		0.8	2.23×10^{33}	370	162	380
		0.2	2.24×10^{12}	178	192	-23.0
	N RS	0.3	4.50×10^{14}	204	191	21.1
		0.4	5.57×10^{17}	241	190	80.3
		0.5	6.35×10^{16}	230	191	62.3
		0.6	1.39×10^{14}	198	191	11.4
		0.7	4.70×10^{15}	216	191	40.6
		0.8	2.09×10^{23}	306	189	187
		0.2	1.12×10^{13}	178	184	-9.25
Thermophilic AD	N DS	0.3	1.28×10^{14}	190	183	11.0
		0.4	8.42×10^{14}	199	183	26.7
		0.5	2.26×10^{17}	227	183	73.1
		0.6	8.06×10^{18}	244	182	103
		0.7	2.71×10^{24}	307	181	209
		0.8	9.12×10^{31}	392	180	353
	S RS	0.2	1.38×10^{11}	163	192	-46.1
		0.3	3.01×10^{12}	179	191	-20.5
		0.4	1.48×10^{15}	210	191	31.0
		0.5	6.43×10^{16}	229	190	62.4

AD type	Sample	α	A (s ⁻¹)	ΔH° (kJ/mol)	ΔG° (kJ/mol)	ΔS° (J/(mol·K))
Thermophilic AD	S RS	0.6	2.04×10^{16}	223	190	52.8
		0.7	5.13×10^{15}	216	190	41.4
		0.8	7.29×10^{30}	395	187	331
	S DS	0.2	2.25×10^{11}	145	167	-41.0
		0.3	2.21×10^{14}	175	166	16.3
		0.4	1.26×10^{16}	193	166	50.0
		0.5	2.40×10^{19}	227	165	113
		0.6	1.17×10^{21}	244	165	145
		0.7	3.92×10^{30}	343	163	327
		0.8	2.73×10^{42}	466	162	554

A represents the collision frequency of reactant molecules and the likelihood of effective collisions, indicating the potential for a reaction. A higher A typically signifies more frequent molecular collisions. DS exhibited the increased A compared with RS, suggesting that AD enhanced collision frequency and reaction potential. However, while a larger A indicates a higher reaction potential, the ease of the pyrolysis reaction also depends on E. Even with a higher A, a high E can hinder the reaction, counteracting the effect of A. Therefore, both A and E must be considered together to accurately assess the reaction's feasibility.

ΔH° represents the energy difference between the initial reactants and the activated complex. The smaller the ΔH° , the easier the activated complex forms [43]. Generally, the ΔH° of all samples followed a pattern similar to that of E, similar results were reported in previous research [35]. A slight difference of about 4.5-5.2 kJ/mol was observed between the E and ΔH values, revealing the likelihood of pyrolysis reactions occurring. This can be attributed to the small energetic barrier to achieving product formation, aligning with previous findings [44]. ΔH° ranged from 113 to 466 kJ/mol, higher than the previous study (47-242 kJ/mol) [44], probably due to the different sources of sludge. Mesophilic AD improved the ΔH° , implying that forming the activated complex became more challenging. However, during U WWTP sample

pyrolysis at a conversion rate 0.2, this became easier given the remaining small molecules. Regarding thermophilic AD, the ΔH° of S WWTP samples during pyrolysis exhibited a similar trend to that of N WWTP samples. The AD did not change the ΔH° much at lower conversion rates (0.2–0.5) but increased ΔH° at higher conversion rates (0.6–0.8). This pattern suggests that thermophilic AD ensured that the formation of activated complexes did not change much at lower conversion rates but rendered it challenging at higher conversion rates. These results can be attributed to the same reason that caused the change in E.

ΔG° allows an overall evaluation of the favorability of transition from the initial to the activated state [35]. A lower ΔG° value corresponds to an increased favorability for a reaction to occur [45]. After either mesophilic or thermophilic AD, there was a consistent reduction in ΔG° across all conversion rates, indicating enhancement of the favorability of the reaction.

ΔS° indicates the closeness of a system to thermodynamic equilibrium. Reactants with higher ΔS° values require less time to form activated complexes because they are distant from their own thermodynamic equilibrium, reducing the reaction times and increasing system reactivity [46]. Most samples displayed both negative and positive ΔS° values, akin to sludge [44], castor residue [47], and rice husk [48]. Negative values imply increased arrangement in the activated complex than in initial reagents, while positive values suggest the opposite. ΔS° ranged from -108 to 554 J/mol, wider than that in previous research (-193–143 J/mol), indicating a complex process in this research [44]. After mesophilic AD, ΔS° demonstrated a pattern like those of E and ΔH° . DS pyrolysis displayed a higher ΔS° than that of RS pyrolysis at all conversion rates, except for the U WWTP samples at $\alpha = 0.2$. This suggested that mesophilic AD generally enhanced the reactivity of sludge pyrolysis. After thermophilic AD, the ΔS° for S WWTP sample pyrolysis increased at all conversion rates, signifying enhanced reactivity, similar to the H WWTP samples. This might be due to similar AD extent leading to comparable structures in DS, such as potentially larger surface areas and more porous structures [49], which could facilitate the gas release thus increasing reactivity. However, that of N WWTP sample pyrolysis decreased slightly at low conversion rates after AD, indicating reduced reactivity, but increased at high conversion rates, implying improved reactivity, in line with the changes in E. This could be due to excessive AD causing structural collapse thus a denser structure in the samples [42]. It might be associated with minor disorder changes at the beginning of N

DS pyrolysis. With pyrolysis proceeding, the enriched non-biodegradable organics in N DS might gradually convert into gas and solid products, releasing more gas than N RS [10] and enhancing reactivity.

Generally, mesophilic AD seems to enhance the favorability and reactivity of subsequent pyrolysis, potentially rendering the overall sludge-to-energy conversion process more efficient. Thermophilic AD made the formation of activated complexes challenging at higher conversion rates (higher temperatures) but rendered it easier at lower conversion rates. The ΔS° results indicate that the reactivity may vary with the extent of thermophilic AD. Given the E results, low-temperature pyrolysis seems to be a promising strategy for treating DS. In practical terms, these insights will guide the optimization of AD conditions to maximize the efficiency of subsequent pyrolysis, contributing to more sustainable and energy-efficient waste management practices especially when considering the different temperature regions.

2.3.4 Evolved gas analysis by Py–GC/MS

AD may exert dual effects on pyrolysis. On the one hand, AD can transform certain organic compounds into smaller molecules that decompose at lower temperatures (corresponding to lower conversion rates in this study) [39], requiring less energy and exhibiting lower E [5]. The small molecules are susceptible to decomposition by pyrolysis, increasing the levels of smaller molecules in pyrolytic evolved gas [39], [50]. Conversely, AD enriches larger bio-refractory molecules. These recalcitrant substances are less likely to undergo thermal decomposition, typically requiring higher temperatures (corresponding to higher conversion rates) thus causing higher activation energy [5] and more larger molecules in the pyrolytic evolved gas [39]. Figure 2.11 depicts the molecular weight distributions of compounds in pyrolytically evolved gas.

For H WWTP samples, the relative contents of compounds with molecular weight around 40 and 100 g/mol decreased after mesophilic AD, whereas the contents of those with higher molecular weights (around 56 and 150 g/mol) increased. This suggested a reduction in the relative content of small molecules due to efficient conversion into biogas and an increase in that of relatively larger bio-refractory molecules (i.e., protein-like, lignin-like and aromatic groups [51]) in DS, enhancing the levels of large molecular organics in the evolved gas. This further explains the kinetic results. For sludge from U WWTP, the compound distributions in the evolved gas remained relatively unchanged across all molecular weights. Only a slight shift toward a lower

molecular weight was observed around 70 g/mol, with a slight content increase within the range of 140–150 g/mol. This is consistent with the trivial effects of AD on the E of U WWTP samples.

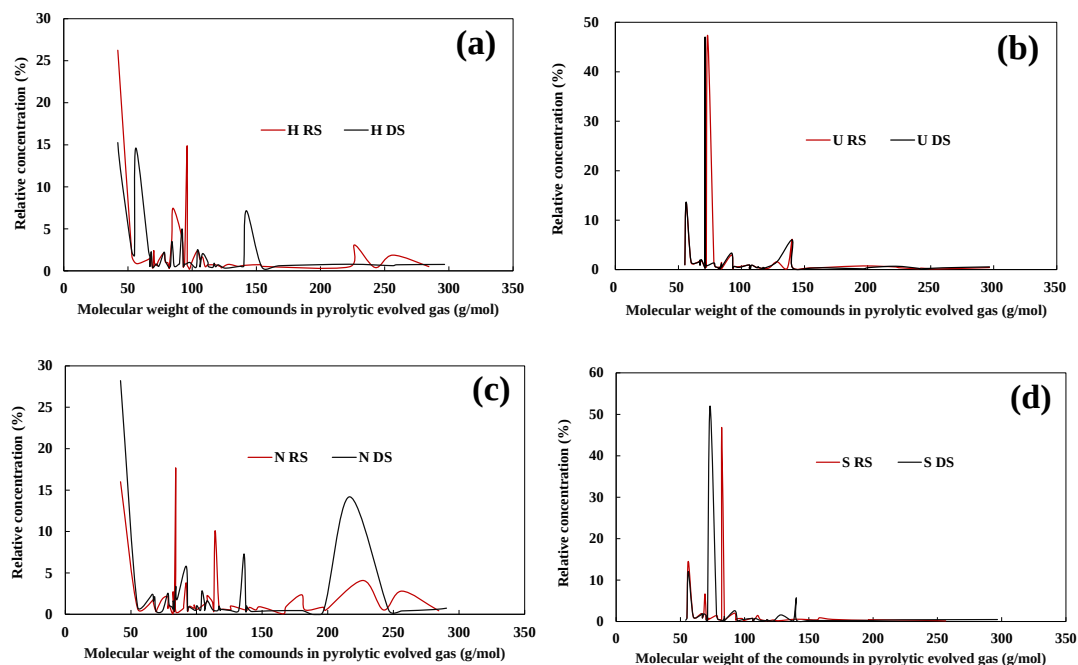


Figure 2.11 The molecular weight distributions of compounds in pyrolytically evolved gas: (a) H RS and H DS, (b) U RS and U DS, (c) N RS and N DS, and (d) S RS and S DS

The total relative contents of molecules < 100 g/mol after AD in both N and S WWTP samples did not change considerably. For example, although a sharp decrease in the 80 g/mol molecular-weight content was apparent after AD of S WWTP samples, a significant increase was observed in the lower-molecular-weight (70 g/mol) content. For N WWTP samples, a considerable decrease was found in 80 g/mol content, but the 50 g/mol content increased. This explains why E did not change much at low conversion rates, because certain organics may be decomposed into smaller molecules during AD and thus remain in the DS, compensating for the small molecules consumed by AD. The content distribution of molecules > 100 g/mol shifted towards larger molecules after thermophilic AD for both N and S WWTP samples. For example, the contents were distributed at the 120, 170–190, 200–250, and 250–290 g/mol molecular weights before AD of N WWTP samples. After AD, there was a significant decrease in the 120 g/mol molecular-weight content, accompanied by considerable increases in the higher-molecular-weight (135 and 200–250 g/mol) contents. This indicated that large

molecules were enriched in the DS. These patterns were in line with the kinetic results; thermophilic AD was associated with insignificant change in the E at low conversion rates given the insignificant variations in the levels of smaller molecules but increased the E at high conversion rates because of the accumulation of larger molecules. These results suggest that adjustment of the AD extent based on practical considerations might maximize the number of small molecules remaining in DS, thus optimizing subsequent pyrolysis.

2.4. Conclusion

The distinct effects of mesophilic and thermophilic AD on subsequent pyrolysis of sewage sludge were investigated. Both mesophilic and thermophilic AD reduced the weight loss percentages on TG analysis, with thermophilic AD having a more pronounced effect. Mesophilic AD typically increased E, whereas thermophilic AD ensured that E did not change much at low conversion rates but increased at high conversion rates. Some favorable changes in thermodynamic parameters were also observed at lower conversion rates, suggesting low-temperature pyrolysis as a promising strategy for DS treatment. Py-GC/MS results further indicated that the above results can be attributed to the dual effects of AD on organic degradation. AD degraded biodegradable organics into easily decomposable smaller molecules and enriched larger resistant molecules. These effects were more marked for thermophilic AD. Furthermore, the relative contents of smaller molecules in DS were influenced by the extent of AD. Hence, an ideal extent of AD may increase the content of small molecules, facilitating subsequent pyrolysis. Further research is essential to determine the extent of AD that optimizes subsequent thermal treatment.

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Chapter 3 Use of aqueous liquor from digested sludge pyrolysis for biogas production: characterization, toxicity assessment, and rate-limiting step determination

3.1 Introduction

The feasibility of biogas production through AD of APL has been explored by several studies. AD is considered a promising technique for detoxification and energy recovery from woody biomass or MSW-derived APL, but the pyrolysis temperature, moisture content in pyrolysis feedstock, and dosage influence the toxic effects of APL on methanogenesis [1]–[7]. Yang et al. [2] investigated the toxicity of APL prepared from the organic fraction of MSW under various pyrolysis conditions. It was found that both pyrolysis temperature and feedstock moisture content affected APL toxicity, such that higher temperature and lower moisture content resulted in increased toxicity. Additionally, the study assessed the toxicity of MSW-derived APLs through AD experiments using DS as the inoculum with a nutrient medium composed of broth, yeast extract, and glucose. This was not examined solely in the context of SS, which is not expected to directly contribute to the “AD-Pyrolysis-AD” closed cycle described below.

DS, rich in proteins and carbohydrates, has a different composition with woody biomass (lignocellulose) [7] or MSW (biomass, plastic, paper, food waste, etc.) [2], which may affect the composition and toxicity of APL, posing potential challenges for its use in AD. Seyedi et al. [8] studied the AD feasibility of sludge-derived APL. However, APL was prepared under only one pyrolysis condition (800°C, dried sample), and the rate-limiting step in AD of APL was not clarified. The specific AD step inhibited by APL remains unclear. To our knowledge, research concerning the toxicity assessment and rate-limiting step determination in AD of DS-derived APL has not been reported. Such studies could clarify the feasibility of AD as a potential option for DS-derived APL utilization and conversion. This would support an “AD-Pyrolysis-AD” closed cycle in accordance with the concept of a circular economy and thereby promoting the integration of biological and thermochemical processes for effective treatment of SS.

This chapter aims to assess the characteristics and toxicity of DS-derived APLs to AD and determine its rate-limiting step. The toxicity of APLs prepared at various temperatures and feedstock moisture contents was assessed using an anaerobic toxicity

assay. The rate-limiting step in AD was studied by adding glucose and sodium acetate as model intermediates of hydrolysis and acidification, respectively. The determined rate-limiting step was further confirmed using the modified Gompertz model and volatile fatty acid content.

3.2 Materials and methods

3.2.1 Materials

DS and DDS were obtained from A WWTP in Osaka Prefecture, Japan. The AD temperature was 36°C. DS was used as an inoculum for AD, and dewatered digested sludge (DDS) was used as feedstock for pyrolysis to prepare APL, as the high moisture content of DS makes it impractical for pyrolysis directly. All samples were stored in the refrigerator at 4°C until use. The characteristics of the samples are presented in Table 3.1.

Table 3.1 Characteristics of samples

Sample	TS (%) ^a	VS (TS%) ^b	Ash (TS%) ^c	Ultimate analysis (TS%)		
				C ^g	H ^g	N ^g
DS ^d	1.28 ± 0.01 ^f	73.4 ± 0.1	24.9 ± 0.1	36.8 ± 0.9	6.27 ± 0.18	6.07 ± 0.08
DDS ^e	16.7 ± 0.1	75.9 ± 0.1	24.1 ± 0.1	38.2 ± 0.4	6.41 ± 0.01	6.20 ± 0.07

^a TS: Total solids

^b VS: Volatile solids

^c Calculated from 100 – VS (%TS)

^d DS: Digested sewage sludge

^e DDS: Dewatered digested sewage sludge

^f Values are expressed as mean ± standard deviation, with standard deviations derived from triplicate measurements of a single sample.

^g C, H and N represent carbon, hydrogen and nitrogen, respectively.

3.2.2 Pyrolysis to prepare APLs

DDS was dried to various moisture contents (0, 10.8, 25.8, and 40.4 wt.%) and ground to < 0.5 mm. These moisture contents were achieved by adjusting the drying time at 105 ± 5°C, which avoided excessive denaturation of sludge caused by complete drying. The samples were then pyrolyzed in a horizontal tube furnace at 350°C, 500°C, or 650°C. The pyrolysis tube was composed of quartz with an inner diameter of 50 mm

and a length of 1,450 mm. A quartz boat containing 20 to 30 g of sample was inserted into the right section of the tube (reaction zone), and the cap was sealed. N₂ was introduced into the tube at a flow rate of 400 mL/min to maintain an inert atmosphere. After 15 min of purging, the furnace was heated on the left side to the set temperature, and then moved to the right side to rapidly heat the samples that had been placed in the center of the furnace [9].

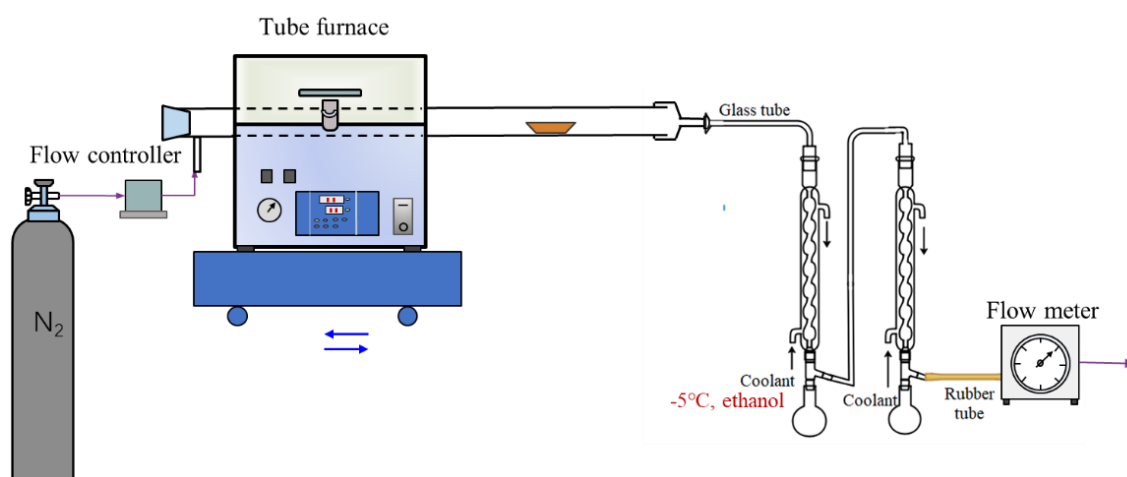


Figure 3.1 Tube furnace used for pyrolysis to prepare APLs (aqueous pyrolytic liquids).

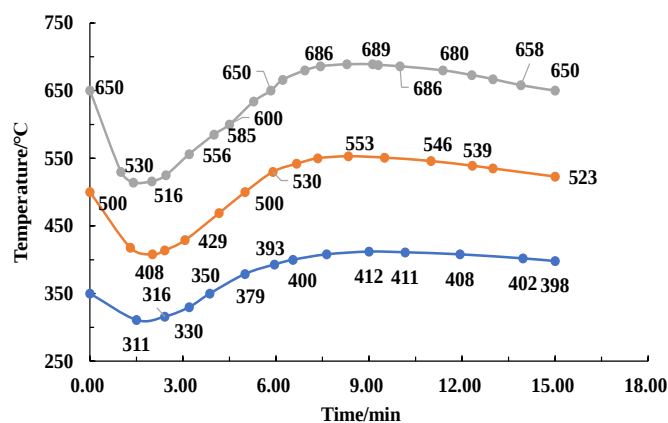


Figure 3.2 Temperature detected in the center of the furnace during pyrolysis.

Pyrolysis proceeded for 15 min; the temperature in the center of the furnace during pyrolysis is shown in Figure 3.2. The gas produced by pyrolysis passes through 2-stage condensers and a wet-type gas flow meter. After 15 min of pyrolysis, the furnace was moved back to the left side. The quartz boat was kept within the reaction zone for 30

min to cool prior to removal. The condensable fraction in the gas was cooled in the pipeline and the 2-stage condensers, both of which were washed with dichloromethane (DCM). The mixture thus obtained was processed in a rotary evaporator to constant weight (weight difference < 0.5%/h) to remove the DCM, resulting in a liquid product, which was then separated into APL (lower layer) and bio-oil (upper layer) by gravity. Both products were stored at 4°C. Pyrolysis was performed for 3 to 5 runs under each condition to acquire sufficient APL. APLs from each run under the same condition were combined into one sample for subsequent characterization and AD testing. Detailed information about each run is provided in the Appendix. The total organic carbon (TOC) and COD of the APLs were analyzed, and the composition of APLs was determined using gas chromatography-mass spectrometry (GC-MS) following the methods detailed in Section 3.2.6.

3.2.3 Anaerobic toxicity assay

An anaerobic toxicity assay was conducted in 200-mL syringe reactors, following the Hohenheim Biogas Yield Test [10] method, to assess the toxicity of APLs prepared at various temperatures and feedstock moisture contents. Each reactor contained 40 mL of inoculum sludge (DS), 20 mL of nutrient medium (NM), and 2 g/L (0.12g) of glucose. NM consisted of KH_2PO_4 (2.5 g/L), Na_2SO_4 (0.75 g/L), NH_4Cl (2.65 g/L) and cysteine (0.5 g/L). The tested APL concentration was set to $\text{VS}_{\text{inoculum}} : \text{COD}_{\text{APL}}$ of 4:1, 8:1, 16:1, and 32:1. APL was added to the required concentrations. Each experiment was conducted in triplicate to avoid accidental errors. Three reactors containing only inoculum sludge, nutrient medium, and glucose served as feed controls (no APL). The reactor setting procedure was carried out as described by Torri and Fabbri [4]. All reactors were kept at 36°C in a water bath (BT300, Yamato Scientific Co., Ltd., Tokyo, Japan) for 162 h and shaken by hand once daily for 1 min, as they could not be securely fixed during automatic shaking by water bath, risking inadequate submersion in water of the substrate in syringes. Biogas volume was read daily from the scale of the syringe. EC50 (Effective Concentration 50) refers to the APL concentration at which biogas production was inhibited by 50% relative to the feed control, which was employed to assess the toxicity of APLs. A higher EC50 value indicates lower toxicity.

3.2.4 Rate-limiting step determination in AD of APL

The AD process is commonly assumed to consist of hydrolysis, acidification, and methanogenesis [11]. To investigate which step of AD is inhibited by APL, a detailed rate-limiting step determination study was conducted with the most toxic APL at a high dosage of $VS_{\text{inoculum}}: \text{COD}_{\text{APL}} = 4:1$ in 200-mL syringe reactors following the method developed by Ma et al. [12]. For this test, 40 mL of inoculum and 0.78 mL of APL 650°C-40.4% were added to the syringes. Since hydrolysis, the initial step in AD, involves the decomposition of complex organics into simpler, soluble molecules such as glucose (GL), thus GL (Nacalai Tesque, Inc., Kyoto, Japan) was utilized as a model intermediate product from hydrolysis and added at 2 gCOD/L to syringes containing APL and inoculum. If GL enhanced CH_4 production rate, it indicates a deficiency in hydrolysis products, thus identifying hydrolysis as the rate-limiting step. In acidification, hydrolysis products are converted into organic acids, alcohols, and H_2 , etc. Therefore, sodium acetate (SA, Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) was used as a model intermediate product from acidification, being added at 2 gCOD/L to syringes containing APL and inoculum. If the CH_4 production rate is improved by SA but not by GL, this indicates a deficiency in intermediate products from acidification rather than hydrolysis, suggesting acidification as the rate-limiting step. Methanogenesis involves the conversion of organic acids and H_2 into CH_4 and CO_2 . If neither GL nor SA improves CH_4 production rate, the system does not lack intermediates from hydrolysis or acidification, determining methanogenesis converting these intermediates into methane as the rate-limiting step.

Previous research [4] indicated that APL lacks essential nutrients, particularly phosphorus, due to its negligible ash content. Thus, to investigate the effects of low nutrient levels, one treatment with inoculum, APL, and the addition of 20 mL NM (described in Section 3.2.3) was set. For comparison, there are five control groups: inoculum only, inoculum with APL, inoculum with GL, inoculum with SA, and inoculum with NM. All tests were conducted in triplicate.

The reactors were kept at 36°C in a water bath (BT300, Yamato Scientific Co., Ltd., Tokyo, Japan) until no biogas was generated and shaken by hand once daily for 1 min. The biogas volume was read every 1–3 days from the scale of the syringe, and its composition was measured using a micro gas chromatograph (Micro-GC). The organic acids content and microbial community were analyzed at the end of AD using High

Performance Liquid Chromatography (HPLC). The methods are detailed in Section 3.2.6.

3.2.5 Simulation of methane production using the modified Gompertz model

The modified Gompertz model can be expressed as Eq. (3-1) [12]:

$$P_{(\text{CH}_4)}(t) = P_{\max} \cdot \exp\{-\exp[r_m e / P_{\max}(\lambda - t) + 1]\} \quad (3-1)$$

where $P_{(\text{CH}_4)}(t)$ is cumulative methane production (mL), λ is lag time (h), $P_{\max}$ is methane production potential (mL), r_m is maximum methane production rate (mL/h), t is time (h), and e is the Napier number, 2.718281828 (constant).

For certain results, the first-stage fitting may not achieve satisfactory accuracy. In such cases, we divide the data into two segments for fitting. Assuming the segmentation starts at time t_c , the equation for the first segment fitting is as follows:

$$P_{\text{CH}_4(1)}(t) = P_{\max1} \cdot \exp\left(-\exp\left(\frac{r_{m1} \cdot e}{P_{\max1}}(\lambda_1 - t) + 1\right)\right) \quad (3-2)$$

For the second segment fitting, it follows:

$$P_{\text{CH}_4(2)}(t) = P_{\max2} \cdot \exp\left(-\exp\left(\frac{r_{m2} \cdot e}{P_{\max2}}(\lambda_2 - t) + 1\right)\right) \quad (3-3)$$

The combined fitting is as below:

$$P_{\text{CH}_4}(t) = \begin{cases} P_{\text{CH}_4(1)}(t), & t \text{ in the first phase} \\ P_{\text{CH}_4(2)}(t), & t \text{ in the second phase} \end{cases} \quad (3-4)$$

λ_1 , r_{m1} , and $P_{\max1}$ represent the lag time, maximum methane production rate and methane production potential estimated for the first stage, respectively. λ_2 , r_{m2} , and $P_{\max2}$ represent the lag time, maximum methane production rate and methane production potential estimated for the second stage, respectively.

Experimental data of $P_{\text{CH}_4}(t)$ and time (t) were used for model fitting to estimate λ , r_m , and $P_{\max}$. If the addition of an intermediate product significantly increases r_m , then the step involved in producing this intermediate product can be considered the rate-limiting step [12]. Model fitting and parameter estimation were conducted using MATLAB R2023b (MathWorks, Inc., Massachusetts, USA).

3.2.6 Analytical methods

3.2.6.1 Fundamental analysis

TS, VS, carbon (C), hydrogen (H), and nitrogen (N) contents were measured using the APHA standard method [13]. Specifically, TS was determined by drying the sample

at $105 \pm 2^\circ\text{C}$ for 24 h. VS was measured using dried samples combusted in a muffle furnace at $550 \pm 25^\circ\text{C}$ for 1 h. Ash content (%TS) was calculated as $100 - \text{VS (\%TS)}$. The C, H, and N contents of dried samples were measured using a CHN analyzer (JM 10 Micro Corder; J-Science Lab Co., Ltd., Kyoto, Japan).

The biogas composition was analyzed using a Micro-GC (Agilent 490 Micro GC; Agilent Technologies, Inc., California, USA) fitted with a Thermal Conductivity Detector (TCD). A PoraPLOT Q column, with helium as carrier gas, was operated at 40°C and 190 kPa to measure CO_2 . For CH_4 detection, a Molsieve $5\text{\AA}$ column was used with argon as the carrier gas, set at 80°C and 170 kPa.

The digestate samples were centrifuged at $3,100\times g$ for 30 min (Centrifuge, H36, Kokusan, Tokyo, Japan) and filtered with $0.45\ \mu\text{m}$ syringe filters for organic acids content measurement. Organic acids content was quantified using HPLC (L-20AD; Shimadzu Corp., Kyoto, Japan) with a CDD-10AVP conductivity detector, SIL-20AC autosampler, and CTO-10AC VP column oven. Separation was achieved on a Shim-pack SCR-102H column ($300\text{ mm} \times 8.0\text{ mm} \times 7\ \mu\text{m}$), with 5 mM perchloric acid as the mobile phase at a flow rate of 1.0 mL/min and a column temperature of 40°C . The injection volume was set to 20 μL . The Micro-GC and HPLC methods used were detailed in the work of Kim et al. [14].

3.2.6.2 APL characterization

APL samples were filtered through a $0.45\text{-}\mu\text{m}$ membrane prior to composition assessment using GC/MS and TOC analysis. For GC/MS analysis, samples were prepared by dissolving 4.5% of APL in 95.5% methanol [15]. Then, 1 μL of the mixture was injected into a GC/MS (QP2010 Plus; Shimadzu Corp., Kyoto, Japan) equipped with an SP-2560 capillary column ($100\text{ m} \times 0.20\ \mu\text{m} \times 0.25\ \mu\text{m}$) with a split ratio of 30/1 and scan range of 10 to 800 m/z. The GC oven temperature program increased from 100°C (held for 5 min) to 180°C at $4^\circ\text{C}/\text{min}$, then to 240°C at $2^\circ\text{C}/\text{min}$; it was held at 240°C for 15 min. More details are available in the work of Rizkianto et al. [16]. The GC-MS results are presented as relative contents calculated using peak area as follows: Relative content = (Peak area of target compound / Sum of peak areas of all detected compounds) $\times 100\%$. The TOC content of filtered APL samples was analyzed using a TOC analyzer (TOC-V CSH; Shimadzu Corp., Kyoto, Japan). COD was measured with COD reagent vials (1227 COD2 Mercury-free COD Reagent, HR, Hach Company, Colorado, USA). pH was measured with a portable pH meter (HM-31P;

DKK-TOA Corporation, Tokyo, Japan).

3.2.6.3 Statistical analysis

Regression analysis was employed to evaluate the effects of temperature and moisture content on the COD, TOC and toxicity of APLs. Statistical significance was determined using analysis of variance (ANOVA) and t-tests, with a significance threshold set at $p < 0.05$.

3.2.7 Microbial community analysis

Samples were collected at the end of the rate-limiting step determination experiment for microbial community analysis. DNA extraction was conducted with the Lab-Aid824s DNA Extraction Kit (Zeesan Biotech Co., Ltd., Fujian Province, China). The concentration of DNA extracts was measured using the Synergy LX reader (Agilent Technologies, Inc., California, USA) and QuantiFluor dsDNA System (Promega Corporation, Wisconsin, USA). The V4 region of 16S rRNA was amplified with the primer set 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806 R (5'-GGACTACNVGGGTWTCTAAT-3'). PCR was conducted in a 15- μ L mixture containing 7.5 μ L of TB Green Premix Ex Taq GC (2 $\times$), 0.3 μ L of each primer, 2 μ L of template DNA, and 4.9 μ L of nuclease-free water. The amplification protocol was as follows: initial activation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. An additional protocol of 95°C for 10 s followed by 65°C for 60 s, then 97°C for 1 s was also conducted. PCR was finalized at 37°C for 30 s. After purification of the PCR products, fluorescence detection was performed using the LightCycler 96 (Roche, Switzerland) instrument based on real-time PCR (intercalator method). The data were processed and analyzed using LightCycler 96 software.

3.3 Results and discussion

3.3.1 APL characteristics

Pyrolysis temperature and moisture content significantly influenced the COD of APL (Figure 3.3 (a)). When combining the two factors, the predictive model yielded an R^2 value of 0.907 ($p < 0.05$), indicating the two factors together explain over 90% of variation in COD. The COD of APL decreased with increases in both pyrolysis temperature and feedstock moisture content. Increasing temperature from 350°C to

500°C considerably affected COD at all moisture contents, but this effect weakened as temperature further increased. TOC concentration displayed an identical pattern (Figure 3.3 (b)). Both TOC and COD decreased with increasing moisture content, which could be attributed to the dilution effect on organic matter caused by higher feedstock moisture content during moisture migration into the APL. Assuming that APL produced with a feedstock moisture content of 0% entirely originated from the solids and that the APL yield from solids remained constant when the moisture content exceeded 0%, at feedstock moisture contents of 10.8%, 20.8%, and 40.4%, respectively, 65.54–75.79%, 80.48–81.91%, and 82.78–86.55% of the APL originated from moisture in the feedstock. Thus, as moisture content increased, the proportion of APL derived from water contained in the feedstock also increased. Additionally, less organic matter was available for conversion into APL in feedstock with high moisture content [2].

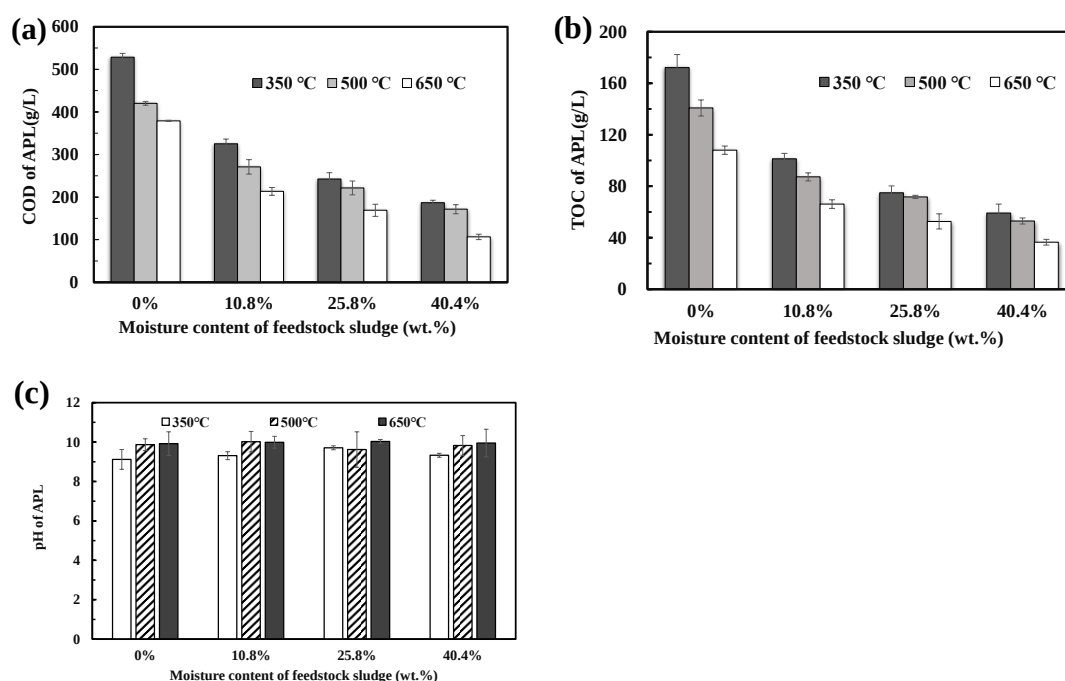


Figure 3.3 Variations in (a) COD (chemical oxygen demand), (b) TOC (total organic carbon), and (c) pH of APL (aqueous pyrolytic liquid) according to pyrolysis temperature and feedstock moisture content. Error bars represent standard deviations from triplicate measurements of a single sample.

The COD of APL in this study ranged from 106.5 to 528.5 g/L, similar to the results obtained through pyrolysis feedstock of birch bark (499 g/L) [7] and red oak (486 g/L) [17], but considerably higher than values recorded using digested cow manure and

maize (48.5–75.6 g/L) [3] and MSW (33–275 g/L) [2]. The TOC (193.4–208.4g/L) level was considerably higher than reported by Hübner and Mumme (14.5–24.7 g/L) [3]. This difference may be due to variations in feedstock, pyrolysis conditions, APL collection methods, and APL condensation temperatures between studies. The high TOC content and COD observed in this study indicate that APL is rich in organics; thus, it should be carefully added to AD to prevent organic overload. High temperature increased the pH of APL (Figure 3.3 (c)), and similar results were observed in the study by Yang et al. [2], which attributed this to the increased generation of alkali matter at higher temperatures.

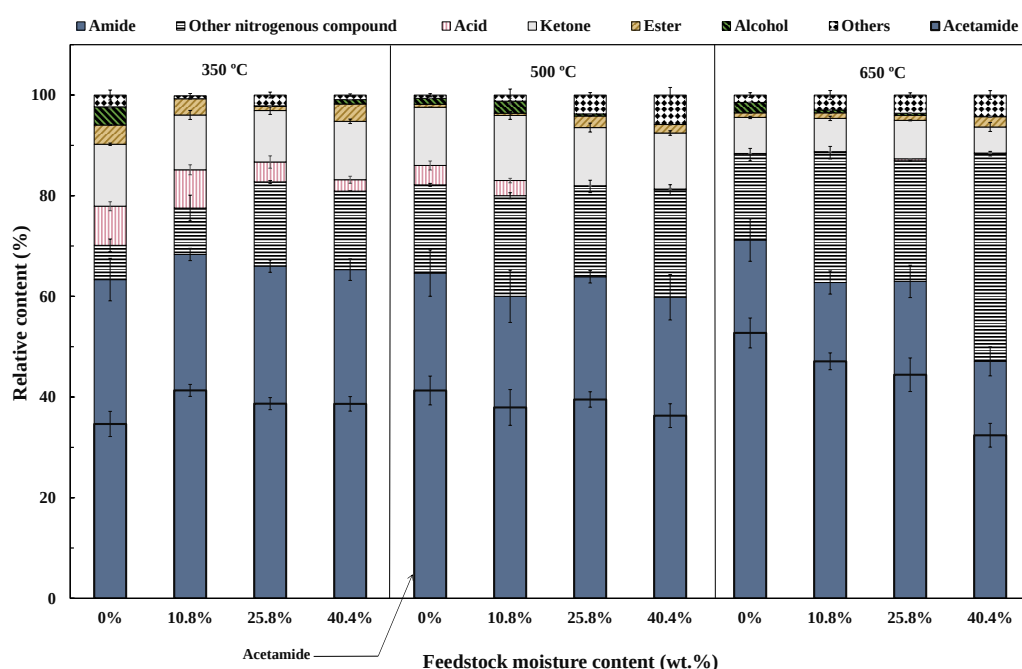


Figure 3.4 Composition of APL (aqueous pyrolytic liquid) based on GC-MS analysis, showing relative content. Error bars represent standard deviations from triplicate measurements of a single sample.

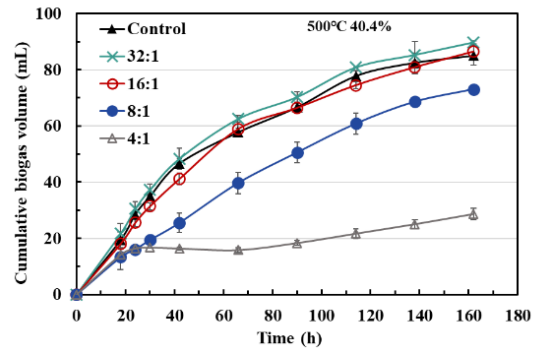
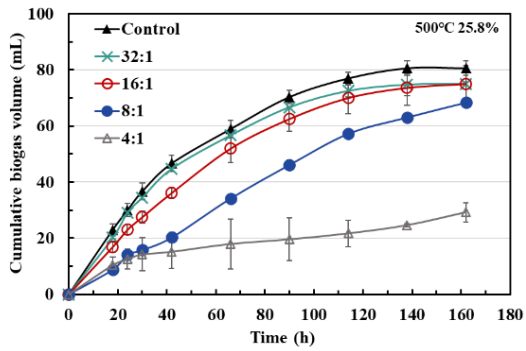
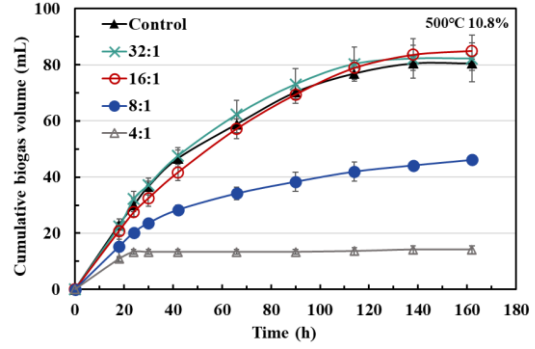
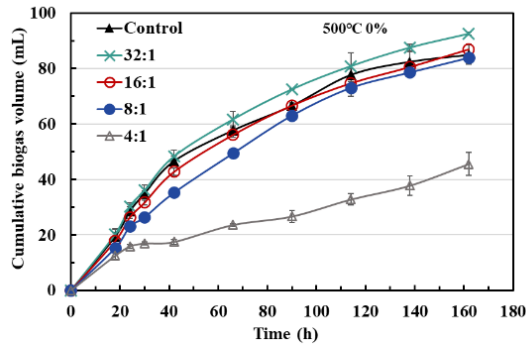
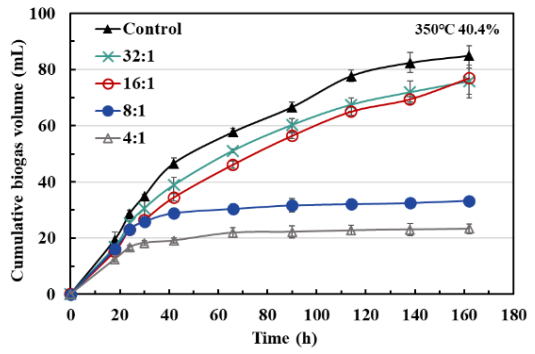
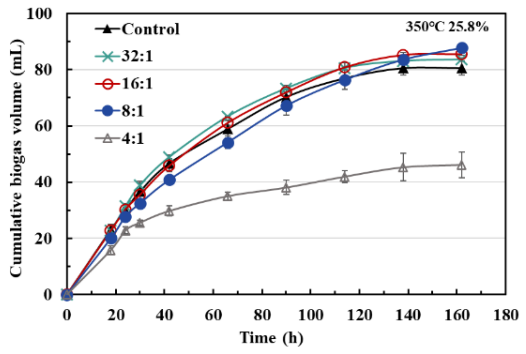
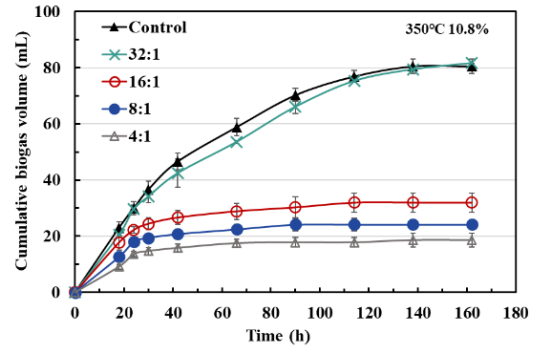
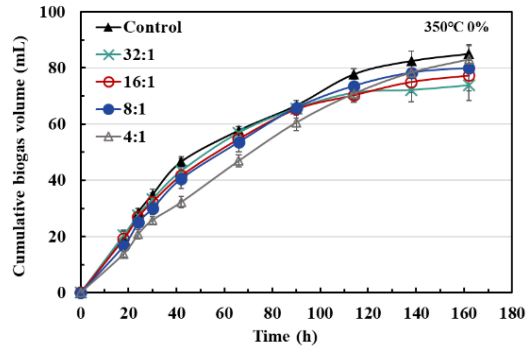
Figure 3.4 illustrates the composition of APL as analyzed by GC-MS. Amides, particularly acetamide, were dominant in APL. Acetamide constituted more than half of the detected amides, reaching relative contents as high as 30–50% of APL and considerably surpassing other components. However, a previous study by Yu et al. [18] showed that phenols were the primary APL component, followed by ketones. This difference might arise from the differing feedstocks used for APL preparation. Yu et al. utilized biomass mainly comprising lignocellulose, whereas the present study used sludge rich in proteins and carbohydrates.

Despite this discrepancy, the APL studied by Wen et al. [7] exhibited a high acetic acid concentration of 105 g/L, making it a suitable substrate for AD; acetic acid can be directly converted to methane [7], [19]. The APL used in that study was prepared via birch bark pyrolysis at 500°C and collected through ice-bath condensation. However, relatively low levels of acetic acid were observed in the present study. The highest organic acid content was obtained at the lowest temperature (350°C) and lowest moisture content (0%). As the pyrolysis temperature and moisture content increased, the organic acid content gradually decreased. At temperatures above 500°C and moisture contents above 25.82%, the organic acid content was negligible, potentially rendering APL less conducive to AD. This discrepancy compared with previous research might be attributable to differences in feedstock, pyrolysis, and condensation conditions.

The APLs produced in this study were enriched with acetamide rather than acetic acid, likely because sludge is rich in proteins and carbohydrates. During the pyrolysis process, carbohydrates can yield acetic acid [20], whereas protein degradation can produce ammonia [21]. Acetic acid can react with that ammonia through amidation and dehydration, ultimately forming acetamide. Consequently, the acetic acid content gradually decreased at high temperatures and was replaced by acetamide.

3.3.2 APL toxicity assessment via the anaerobic toxicity assay

A typical anaerobic toxicity assay result for APLs is presented in Figure 3.5. Higher dosage caused a greater reduction in biogas compared with the control group. Taking APL 350°C-10.8% as an example, the lowest addition ratio (32:1) initially showed slightly lower biogas production but eventually became comparable to the control ($p < 0.05$). This was due to higher dilution reducing APL toxicity and initial inhibition recovering through microbial adaptation. Considering all the APLs results, the results of 42h were used to determine the EC₅₀, as the pattern of biogas production for some APLs (e.g., APL 350°C-25.8%) changed due to the gradual adaptation of the microbes to the toxic conditions as described above [2].



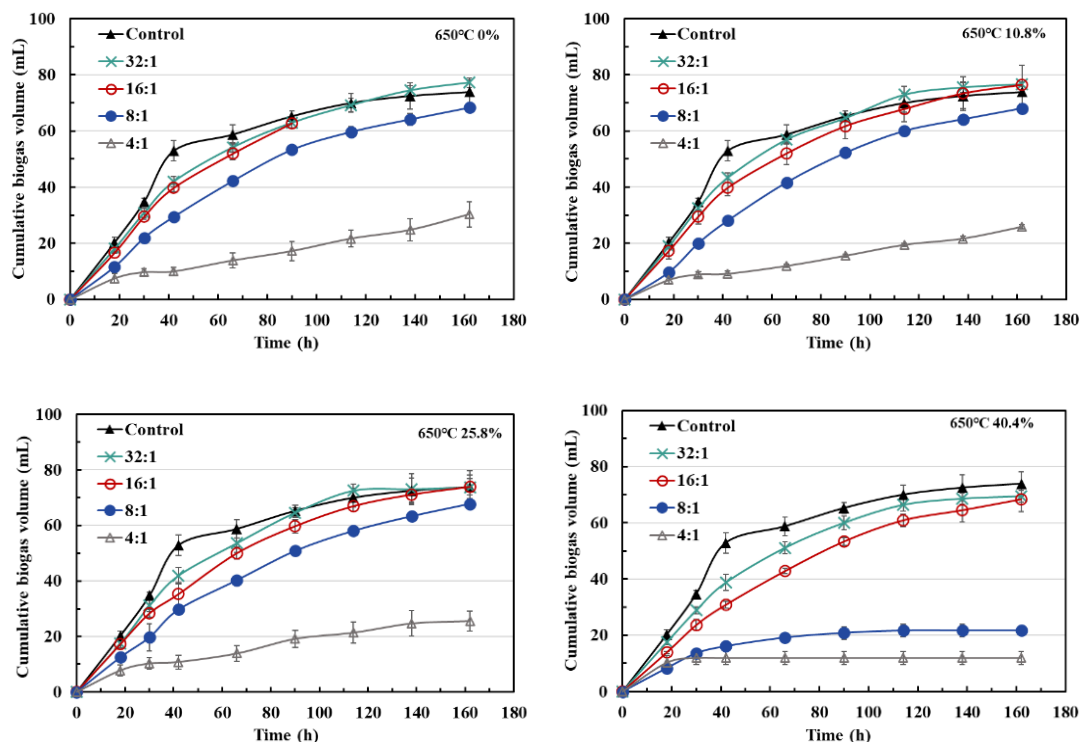


Figure 3.5 Cumulative biogas production from anaerobic digestion assay testing of APLs (aqueous pyrolytic liquids). Error bars represent standard deviations from three samples obtained from three parallel reactors, with each reactor analyzed once.

Figure 3.6 (a) illustrates the estimation method for EC₅₀ using only APL 350°C-10.8% as an example. Figure 3.6 (b) shows EC₅₀ values for all APLs.

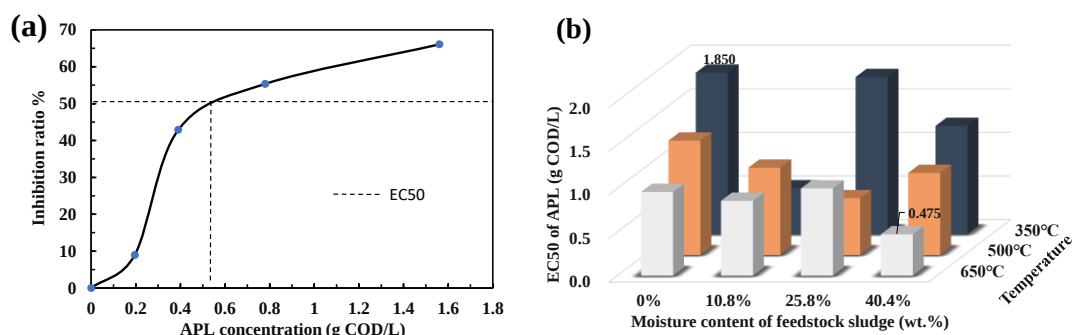


Figure 3.6 Results of (a) EC₅₀ (Effective Concentration 50) determination for APL 350°C-10.8% (aqueous pyrolytic liquid prepared at 350°C with a feedstock moisture content of 10.8%) in the anaerobic toxicity assay (error bars represent standard deviations from 3 samples obtained from 3 parallel reactors, with each reactor analyzed once), and (b) EC₅₀ values of all APL samples.

Both pyrolysis temperature and feedstock moisture content influenced the toxicity of APLs. The correlation coefficient between EC50 and temperature and moisture content were 0.26 and 0.07, respectively, indicating that temperature had a more significant influence, consistent with previous research [2]. Acetamide and organic acids in APL may enhance biogas production by serving as substrates for AD, the former can degrade into acetic acid and ammonia, and then convert into CH₄ [22]. Nitrogenous compounds, such as pyridine, may have inhibitory effects on AD. APL350-0% shows comparable biogas production to control even at a high addition amount (4:1). Also, at 650°C, increasing the moisture content from 0% to 40% reduced biogas production on the APL addition ratio of 4:1 and 8:1. This might be because the higher-biogas-production treatments showed higher levels of acids and acetamide but low nitrogenous compounds (Figure 3.4), suggesting biogas production likely resulted from the combined effects of beneficial and inhibitory components in APL, thereby impacting the toxicity. The APL with the lowest toxicity was APL 350°C-0%, whereas the sample with the highest toxicity was APL 650°C-40.4%. The former had the lowest content of nitrogenous compounds (including amides) and the highest content of organic acids, whereas the later showed the opposite results (Figure 3.4), which might explain the EC50 results. However, further research is needed to clarify the detailed relationship between APL composition and pyrolysis conditions.

3.3.3 Rate-limiting step determination in AD of APL

3.3.3.1 Methane production

The most toxic APL was APL 650°C-40.4%, as noted above; this APL was utilized to study the rate-limiting step in AD of APL. Figure 3.7 shows the trend of methane production in the rate-limiting step determination test. Treatments with easily degradable substrates, Inoculum + GL and Inoculum + SA, resulted in immediate gas production that rapidly increased and continued for approximately 200 h before ceasing. The reactor of Inoculum+ APL exhibited very low methane production that ceased at approximately 200 h, but methane was generated again beginning around 500 h, when the microorganisms had adapted to APL, until approximately 800 h. This pattern confirmed the strong inhibitory effect of APL on AD and the impracticality of APL as a sole AD substrate. The addition of GL, SA, or NM significantly improved methane production compared with Inoculum + APL ($p < 0.05$); NM exerted the weakest promotion effect, demonstrating that inhibition was not solely due to nutrient

imbalances in APL. At approximately 450 h, methane production in the Inoculum + APL + SA surpassed that in the Inoculum + SA group and was significantly higher at the end ($p < 0.05$), suggesting that SA was effective for aiding microbial adaptation to toxic conditions, and thus facilitating the conversion of APL to CH_4 . However, methane production from Inoculum + APL + GL ceased when it approached the level of Inoculum + GL, indicating that GL did not facilitate microbial adaptation and the conversion of APL to CH_4 .

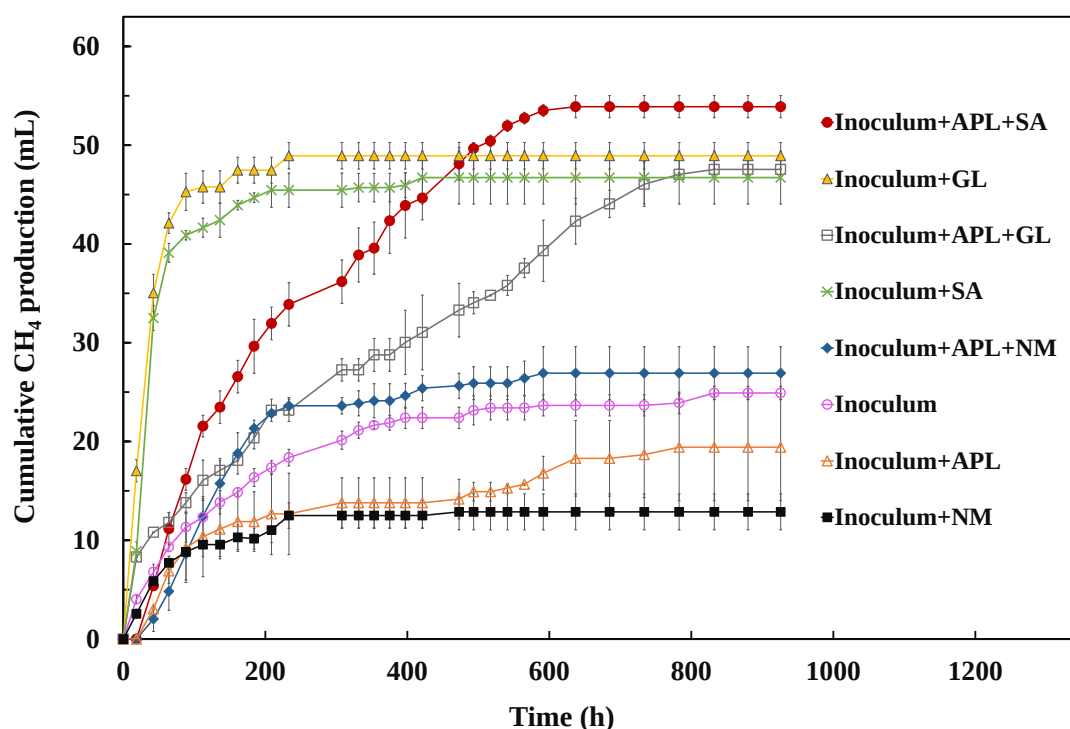


Figure 3.7 Cumulative methane production during the rate-limiting step determination in anaerobic digestion of APL 650°C-40.4%, which refers to aqueous pyrolytic liquid prepared at 650°C with a feedstock moisture content of 40.4%. Error bars represent standard deviations from 3 samples obtained from 3 parallel reactors, with each reactor analyzed once. (SA: sodium acetate; GL: glucose; NM: nutrient medium)

Additionally, Inoculum + APL + SA and Inoculum + APL exhibited a similar 20-h lag phase, whereas Inoculum + APL + GL did not. This suggested that GL eliminated the initial 20-hour lag phase compared to Inoculum + APL, whereas SA did not. Given that GL is a hydrolysis product, its presence eliminated the lag phase, suggesting that the system lacked hydrolysis products during the initial 20 h, impeding methanogenesis. However, methanogenesis commenced after 20 h in Inoculum + APL + SA and Inoculum + APL, indicating that hydrolyzing microorganisms quickly adapted to APL

and hydrolysis could commence. Furthermore, Inoculum + APL + SA demonstrated a higher CH₄ production rate (with a steeper slope in Figure 3.7) after 20 h, reaching maximum CH₄ production earlier than Inoculum + APL + GL. This implied that SA, the acidification product, might enhance the CH₄ production rate. Therefore, AD might be primarily limited by the acidification step, which could lead to a lack of organic acids in the non-SA-addition treatments.

3.3.3.2 Organic acids concentration

Figure 3.8 shows the organic acids content in each treatment, providing further evidence for the inhibition of the acidification step.

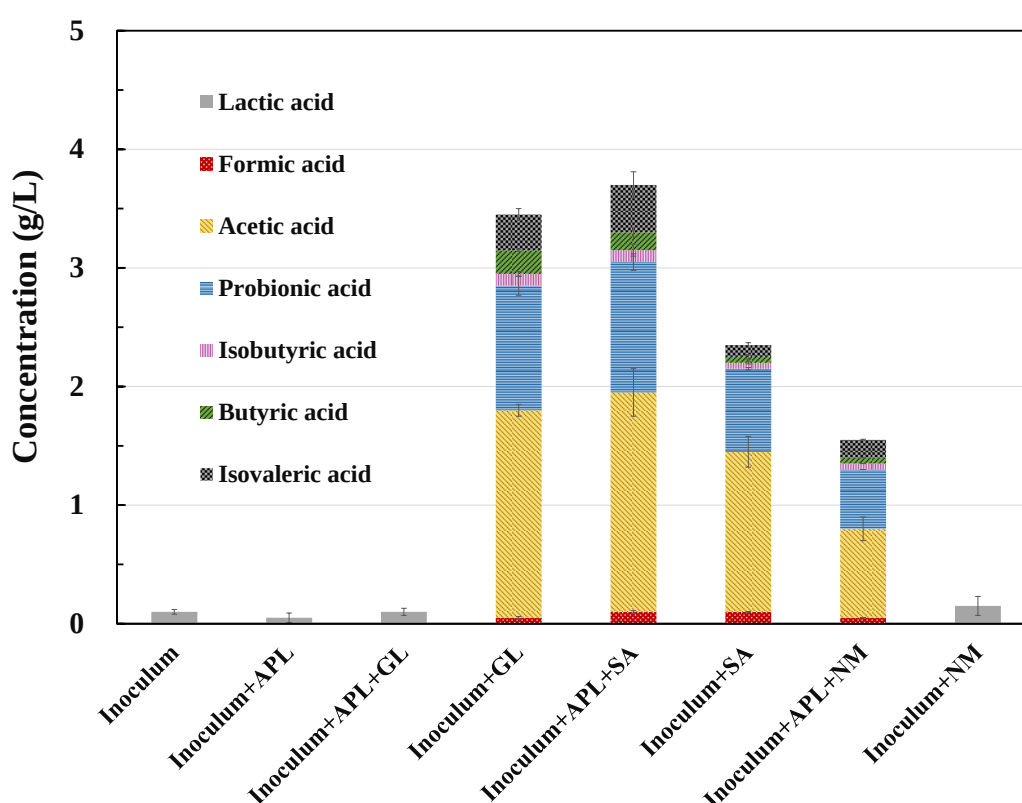


Figure 3.8 Results of organic acids analysis at the end of the rate-limiting step determination test using APL 650°C-40.4%, which refers to aqueous pyrolytic liquid prepared at 650°C with a feedstock moisture content of 40.4%. Error bars represent standard deviations from 3 samples obtained from 3 parallel reactors, with each reactor analyzed once. (SA: sodium acetate; GL: glucose; NM: nutrient medium)

Inoculum + APL + GL exhibited significantly lower organic acids content than Inoculum + GL ($p < 0.05$), demonstrating that APL inhibited organic acids generation. Moreover, Inoculum + APL + GL exhibited organic acids content comparable to

Inoculum + APL ($p < 0.05$), suggesting that GL addition did not facilitate organic acids generation. The addition of NM may enhance acidogenesis by supplying essential elements to acidogenic microbes thus alleviating the inhibitory effect, as illustrated by the significantly higher organic acids content of Inoculum + APL + NM compared with Inoculum + APL ($p < 0.05$), but the improvement was limited. Inoculum + APL + SA, Inoculum + SA, and Inoculum + GL with high methane production also had high final organic acids levels; however, methane production stopped in these treatments, likely due to excessive organic acids accumulation inhibiting methanogenesis, implying the probable GL and SA overloading. Despite this, the high final organic acids concentrations suggest that the system initially produced sufficient organic acids to support methane production. Conversely, Inoculum + APL and Inoculum + APL + GL with lower methane production than Inoculum + APL + SA also demonstrated lower final organic acids levels, implying that methane production ceased probably due to insufficient organic acids. These results indicate a lack of organic acids in the non-SA-adding treatments, suggesting that acidification might be the main rate-limiting step.

Furthermore, in organic acids of Inoculum + APL + SA, acetic acid may originate from the added SA and/or the decomposition of APL, and organic acids besides acetic acid may result from APL. Therefore, SA likely promoted the degradation of APL, which was also evident from the higher CH_4 production of the Inoculum + APL + SA than the Inoculum + SA. This is likely due to SA could also serve as a carbon or energy source for some acidifying microbial [23], enhancing their activity and APL conversion.

3.3.3.3 Microbial community analysis

To further elucidate the impact of APL on AD, digests were collected at the end of the experiment for microbial community analysis. Figure 3.9 shows the relative abundances of archaea and bacteria at the genus level with relative abundances above 2%; genera with lower relative abundances were included in “others”.

All samples were dominated by *Blastocatellia* with an abundance of 13–15% across all samples. *Blastocatellia* is a class in the phylum Acidobacteria [24] that was also found in the AD of wood vinegar wastewater from pyrolysis [25]. Its dominance can be attributed to the capability to degrade and detoxify complex organics [26], [27] and thrive in harsh conditions [28]. *Gallicola* and *Clostridium_sensu_stricto_13* are genera within the phylum *Firmicutes* that play significant roles in AD due to their hydrolytic and acidifying capabilities [29]. One family of *Firmicutes*, *Clostridiaceae*, which

includes *Clostridium_sensu_stricto_13*, can produce organic acids and alcohols from carbohydrates or peptones. This family was detected as a major taxon in a previous study investigating AD of APL derived from sludge [19]. Both *Gallicola* and *Clostridium_sensu_stricto_13* exhibited low abundances (below 5%) in the present study; this discrepancy is likely due to differences in sludge source and AD operation conditions. *Desulfatiglans* are sulfate-reducing microorganisms, among which *Desulfatiglans anilini* can degrade aniline under strictly anoxic sulfate-reducing conditions [30], which may facilitate the degradation of complex organic compounds in APL.

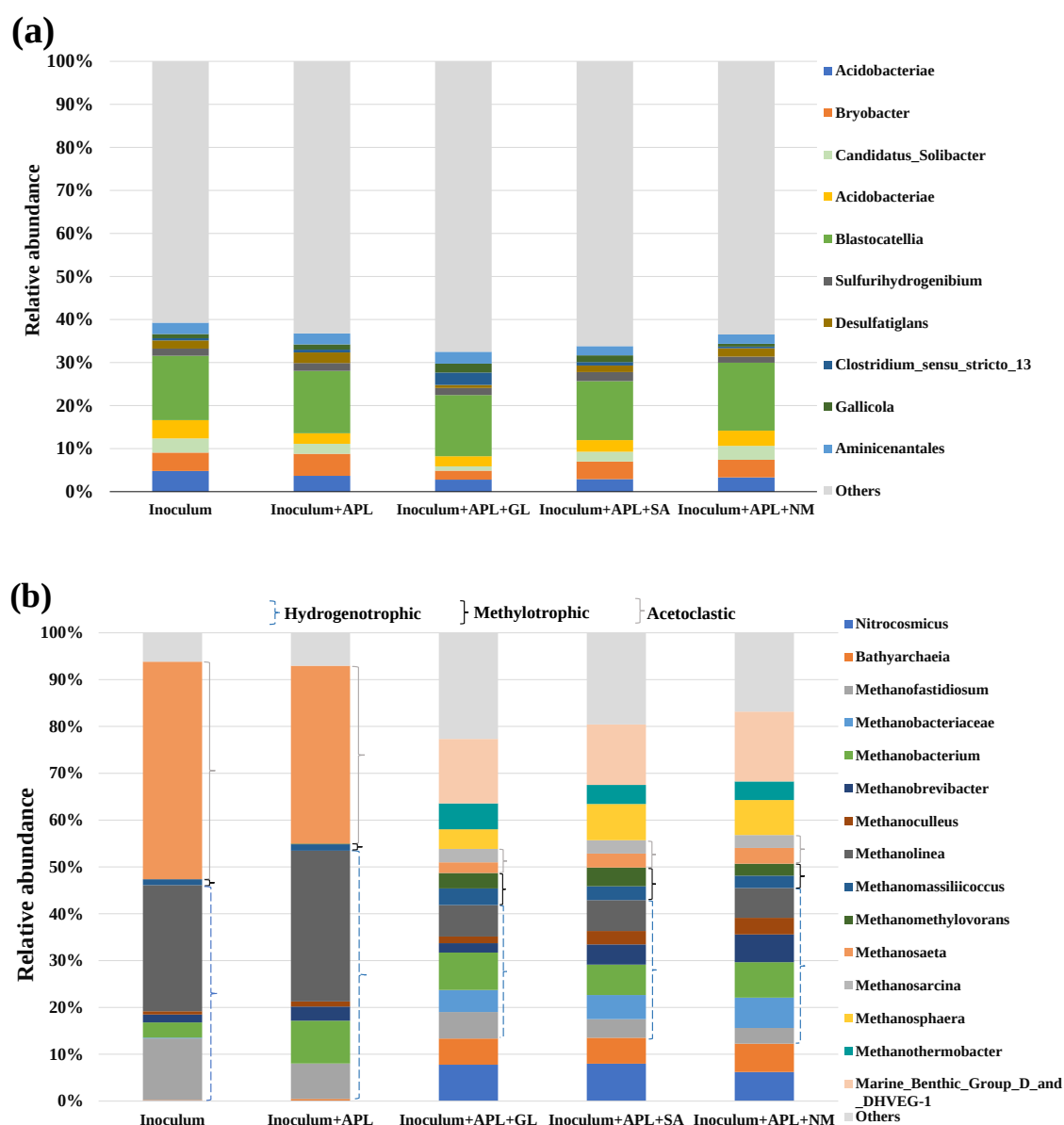


Figure 3.9 Relative genus-level abundances of (a) bacterial (> 2%) and (b) archaeal communities at the end of AD

The bacterial community did not considerably change with the addition of various additives. Although GL addition improved methane production, production was delayed compared with SA addition or Inoculum + GL, and the bacterial community did not substantially change. This similarity might have arisen because bacteria often preferentially degrade GL to produce organic acids, but the presence of APL prevented the degradation of GL. Thus, the bacteria may initially attempt to degrade APL, but its toxicity hinders immediate conversion to methane and requires an adaptation period. Thus, a delay was observed in methanogenesis for Inoculum + APL + GL compared with Inoculum + GL. No change was apparent upon NM addition, suggesting that a lack of nutrients is not the dominant inhibitory mechanism. Because SA can be directly converted into methane through methanogenesis, bacteria may be not strongly affected by SA. Therefore, no significant change in the bacterial community was observed.

As depicted in Figure 3.9 (b), both Inoculum and Inoculum + APL exhibited simple community compositions dominated by *Methanosaeta*, *Methanolinea*, *Methanobacterium*, and *Methanofastidiosum*. Among these genera, *Methanosaeta* undertakes acetoclastic methanogenesis, whereas *Methanolinea* and *Methanobacterium* are hydrogenotrophic methanogens utilizing H₂ and CO₂ [31]. *Methanofastidiosum* is associated with methylotrophic methanogenesis [32]. In Inoculum + APL, the abundances of *Methanolinea* and *Methanobacterium* increased, whereas that of *Methanosaeta* decreased compared with Inoculum. This difference suggests a reduction in acetoclastic methanogenesis, likely attributable to the reduced availability of organic acids in the APL addition treatment.

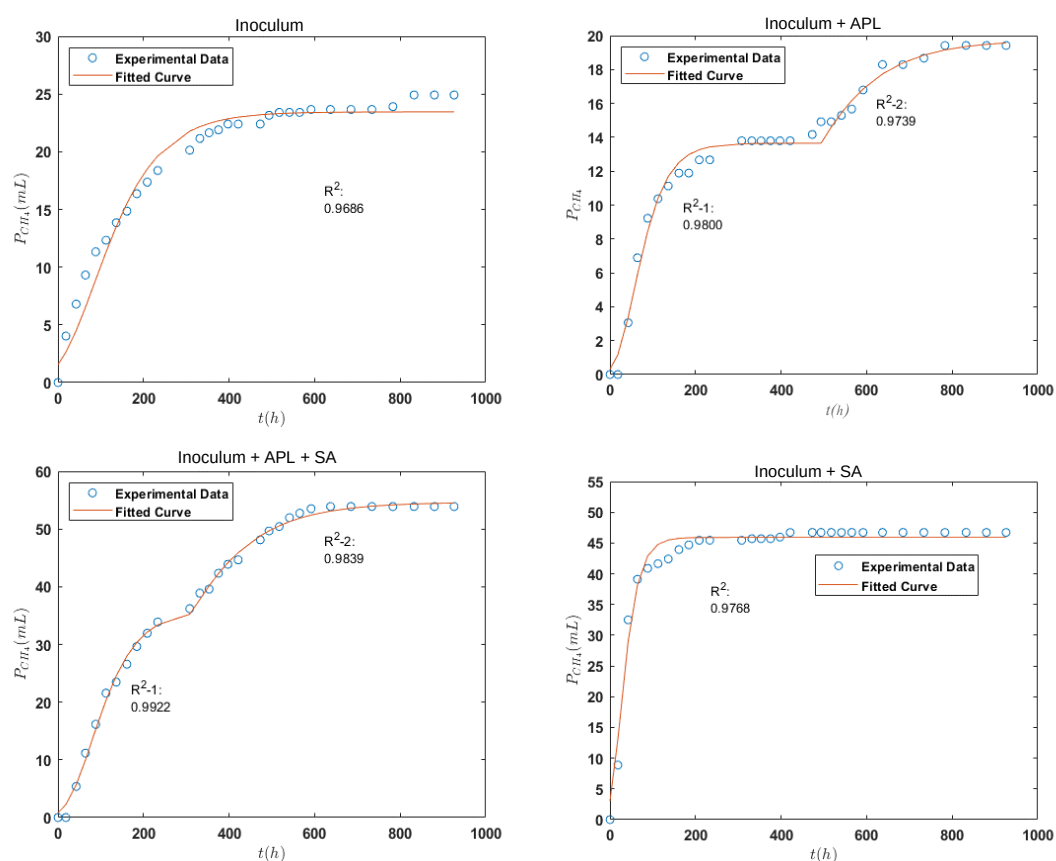
Furthermore, Inoculum + APL + GL, Inoculum + APL + SA and Inoculum + APL + NM showed more diverse sets of methanogenesis genera, indicating increased richness within each type of methanogenesis and improved archaeal diversity compared with Inoculum and Inoculum + APL. Specifically, the relative abundance of hydrogenotrophic methanogens was reduced from 53.0% (Inoculum + APL) to 34.0% (Inoculum + APL + GL), 33.5% (Inoculum + APL + SA), or 37.2% (Inoculum + APL + NM). Acetoclastic methanogens decreased from 38% (Inoculum + APL) to approximately 5%, whereas the abundance of methylotrophic methanogens substantially increased from negligible levels to 11.0% (Inoculum + APL + GL), 14.7% (Inoculum + APL + SA), and 12.7% (Inoculum + APL + NM), respectively. The addition of SA produced the highest abundance of methylotrophic methanogens, possibly indicating the degradation of certain organic compounds in APL, such as

acetamide, N-methyl-, butanamide, 3-methyl-acetamide, and N, N-dimethyl-compounds. The degradation of these organic compounds might lead to the production of substances such as methanol and methylamine, increasing their abundance. This finding suggests that SA addition might facilitate microbial degradation of APL.

Based on these results, additions of SA, GL, and NM all increased the diversity of methane-generating archaea, potentially enhancing community resilience and allowing survival in harsher conditions. This explains the improvement of methane production associated with SA, GL and NM addition.

3.3.3.4 Fitting of the modified Gompertz model

A good fit for the Gompertz model was obtained, with $R^2 > 0.92$ (Figure 3.10). As $P_{\max}$ illustrates, all treatments showed values very close to the experimental results. Thus, the model can properly represent experimental data. The estimated parameters are listed in Table 3.2.



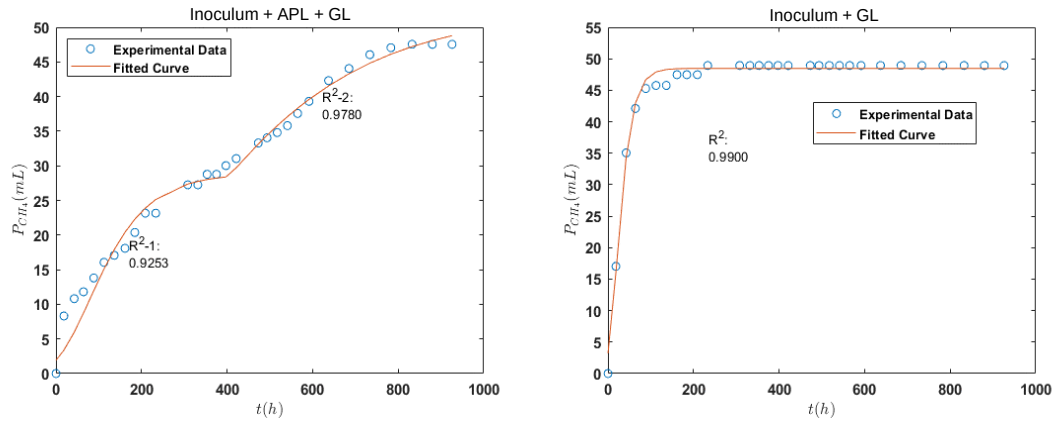


Figure 3.10 Simulation of methane production using the modified Gompertz model. (SA: sodium acetate; GL: glucose; NM: nutrient medium)

Table 3.2 Estimated parameters from the fitting of the modified Gompertz model for various intermediate additions

Substrate	λ (h)	r_m (mL/h)	$P_{\max}$ (mL)
Inoculum	4.01×10^{-9}	0.101	23.5
Inoculum + APL	14.2 (λ_1^a)	0.118 (r_{m1}^a)	13.7 ($P_{\max1}^a$)
	264 (λ_2^b)	0.0631 (r_{m2}^b)	19.7 ($P_{\max2}^b$)
Inoculum + APL+ SA	19.6 (λ_1)	0.223 (r_{m1})	36.0 ($P_{\max1}$)
	94.1 (λ_2)	0.170 (r_{m2})	54.6 ($P_{\max2}$)
Inoculum + APL+ GL	0.531 (λ_1)	0.136 (r_{m1})	28.9 ($P_{\max1}$)
	43.6 (λ_2)	0.0803 (r_{m2})	52.3 ($P_{\max2}$)
Inoculum + GL	0	0.868	48.5
Inoculum + SA	0	0.709	45.9

^a λ_1 , r_{m1} , and $P_{\max1}$ represent the lag time, maximum methane production rate and methane production potential estimated for the first stage, respectively.

^b λ_2 , r_{m2} , and $P_{\max2}$ represent the lag time, maximum methane production rate and methane production potential estimated for the second stage, respectively.

During the fitting process, λ for Inoculum + GL and Inoculum + SA were set to 0 h because GL and SA are highly degradable substrates that can immediately generate methane. The inoculum-only treatment exhibited a short lag phase of 4.01×10^{-9} h, but after addition of APL to the inoculum, the lag phase extended to 14.2 h for the first

stage and 264 h for the second stage. The first stage likely mainly involved the remained substrate degradation in the inoculum, and the presence of APL prolonged the lag phase. The second stage may be mainly associated with APL degradation, and the extended lag phase indicates APL toxicity. When SA was added to Inoculum + APL, the resulting Inoculum + APL + SA treatment exhibited a first-stage lag phase of 19.6 h, comparable to that of Inoculum + APL. In contrast, GL addition significantly shortened the lag phase to 0.531 h for the first stage, consistent with the experimental results. These findings suggest that some hydrolyzing microbes did not adapt to APL during the initial period but could adapt very quickly (as indicated in Section 3.3.1), thus hydrolysis was not considered the primary rate-limiting step.

Inoculum + GL (0.868 mL/h) and Inoculum + SA (0.709 mL/h) exhibited the highest r_m value due to the easy conversion of GL and SA into methane. Inoculum + APL+ GL and Inoculum + APL + SA displayed lower r_m values than Inoculum + GL and Inoculum + SA, respectively, indicating that APL reduced the maximum methane production rate due to its toxicity. Comparison of Inoculum + APL + SA to Inoculum + APL showed that r_m increased from 0.118 mL/h to 0.223 mL/h in the first stage, nearly doubling, and from 0.0631 mL/h to 0.170 mL/h in the second stage, nearly tripling. However, the r_m values of Inoculum + APL and Inoculum + APL + GL were very similar in both stages, with minimal obvious improvement. This finding suggests that GL addition did not increase the maximum methane production rate much, but SA addition could substantially improve it, confirming that acidification is the rate-limiting step in the AD of digested-sludge-derived APL.

3.4 Conclusion

APL 350°C-0% and APL 650°C-40.4%, derived from DS, exhibited the lowest and highest toxicity to AD, respectively. Acidification was identified as the rate-limiting step in AD of APL, as indicated by methane production, organic acids contents, archaeal communities, and maximum CH₄ production rates estimated with the modified Gompertz model. Insights gained from this study provide a foundation for applying AD to the conversion of APL to energy, thereby promoting the integration of biological and thermochemical processes for the effective treatment of sludge.

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Chapter 4 Converting aqueous pyrolytic liquid to biogas via anaerobic digestion towards a circularly integrated sewage sludge treatment system

4.1 Introduction

In the previous chapter, the toxicity of APL on AD was explored, revealing that APL exhibits strong toxicity during the AD process. This toxicity was found to be influenced by several factors, including the moisture content of the sludge and the pyrolysis temperature. During the sole AD of APL, the acidification step was significantly limited, indicating the substantial negative impact of APL on microbial activity.

To mitigate APL toxicity, several pretreatments have been explored to remove toxic compounds and enhance APL's anaerobic degradability, such as air stripping [1], biochar addition [2], nutrient addition [3], partial oxidation with Fenton's reagent or sodium hypochlorite as well as overliming and activated carbon adsorption [4]. Air stripping reduced high $\text{NH}_3\text{-N}$ concentrations in APL derived from wastewater solids, but inhibition persisted [1]. Both biochar and nutrient addition were shown to improve methane yield [2], [3]. Continuous digestion using the overlimed pretreated APL increased biogas production when improving APL loading but overloading eventually stopped the biogas generation [4]. One study also investigated feedstock pretreatment, rather than the APL, where wood biomass was treated with 10% acetic acid at 90 °C for 10 minutes before pyrolysis to reduce the alkaline content. This reduced inhibitor levels in the APL and increased methane production [5].

However, these methods require extra chemical or operation steps, increasing operational costs. Reintroducing DS-derived APL into anaerobic digesters and co-digesting (co-AD) it with sewage sludge may be a more economical solution. This approach utilizes existing AD infrastructure, reducing costs and environmental impact while improving energy recovery. Co-AD with sludge can dilute toxic substances in APL and provide anaerobic microorganisms with more nutrients and buffering capacity to anaerobic microorganisms, which may alleviate toxicity [6]. Furthermore, microbial

adaptation can enhance the ability to utilize APL and tolerate its stress over time, improving growth, survival, and degradation of APL [5]. This adaptation may eliminate the need for pre-treatment, offering a more cost-effective solution.

Studies on co-AD of APL and other substrates have been conducted. Yue et al. [7] found that APL inhibited methane production in batch AD (26 days) of cow manure without char but added char increased methane by 57.5–133.7%. Yu et al. [8] reported that APL increased methane at high dilution rates (50x, 100x) but inhibited it at 5x dilution in swine manure AD. Since APL was sourced from corn stalks and not digested swine manure, an independent closed-loop AD-pyrolysis-AD system was not formed. Neither study explored long-term microbial adaptation without additives or APL dilution, providing limited insights into the long-term microbial adaptation to APL.

Some studies have investigated long-term co-AD of APL. Hamalainen et al. [9] conducted 221 days of continuous co-digestion of sludge-derived APL with sludge but lacked microbial analysis. Seyedi et al. [1] performed over 500 days using APL from wastewater solids and synthetic primary sludge, with the APL load gradually increasing to 0.50 gCOD/Lr-d. However, due to the changing APL loading, a stable methane production improvement contributed by APL was not observed. Additionally, the APL dosage did not reflect actual yields. If the APL dosage is set according to the actual yield and maintained at a constant level, microbial adaptation could be potentially enhanced, leading to improved conversion of APL into biogas. This would offer more insight into real-world applications. Therefore, conducting long-term co-AD based on the actual yield of APL is crucial to examine microbial adaptation and establish a practical circular integrated AD-pyrolysis system, providing valuable insight into future AD management of DS-derived APL.

This chapter explores the feasibility and practicality of converting APL into methane through AD. The primary focus is to mitigate the APL inhibition through co-AD with sludge, and to determine whether APL produced at different pyrolysis temperatures (350, 500, and 650°C) within the AD-pyrolysis system can be fully reintroduced into

AD, assuming all digestate is subjected to pyrolysis. Batch co-AD of APL and mixed sludge was carried out to select pyrolysis temperature for APL preparation. The selected APL was then subjected to continuous co-AD to enhance microbial adaptation and APL conversion. And the microbial shifting was analyzed during the co-AD to clarify the beneficial strains.

4.2 Materials and methods

4.2.1 Materials

The RS (used as the substrate for AD), DS (used as the inoculum for AD), and DDS (used as feedstock for pyrolysis to prepare APL) were obtained from A WWTP, Osaka Prefecture, Japan. The AD temperature is 36 °C. All samples were stored in the refrigerator at 4 °C before use. The characteristics of the above samples are shown in Table 4.1.

Table 4.1 Characteristics of the samples

Sample	TS (%) ^a	VS (TS%) ^b	Ash (TS%) ^c
RS ^d	3.61±0.10 ^g	81.8±0.0	18.2±0.0
DS ^e	2.17±0.00	71.3±0.2	28.7±0.2
DDS ^f	15.3±0.1	74.3±0.1	25.7±0.1

^a TS: Total solids

^b VS: Volatile solids

^c Calculated from 100 – VS (%TS)

^d RS: Raw mixed sludge

^e DS: Digested sewage sludge

^f DDS: Dewatered digested sewage sludge

^g Values are expressed as mean ± standard deviation, with standard deviations derived from triplicate measurements of a single sample.

4.2.2 Pyrolysis to prepare APLs

Based on the sludge drying performance in Japan and Germany, the actual moisture content in the sludge varies by 15% (Germany, PYREG project) [10], 30.7% (Mitsubishi) [11], 27% (Mitsubishi) [12], and 10-50% (National Institute for Land and

Infrastructure Management) [13]. Considering these variations comprehensively, a moisture content of 25.8% closely resembles the real situation. Consequently, the DDS dried to a moisture content of 25.8% and then grounded to less than 0.5mm for pyrolysis. A tube furnace was utilized for pyrolysis to prepare APL, as detailed in Section 3.2.2. The temperatures used were 350°C, 500°C, and 650°C, respectively. The produced gas passed through two-stage -5°C condensers, achieved using ethanol. Condensable components cooled down in the condensers and pipelines. After pyrolysis, the pipelines and condenser were washed with DCM to obtain a mixture of APL, bio-oil, and DCM. DCM was removed in a 43°C rotary evaporator. Subsequently, the APL (lower phase) could be easily separated from the bio-oil (upper phase) by gravity. The obtained APLs were stored at 4°C, named APL350, APL500, and APL650, respectively. Pyrolysis was performed multi-runs under each condition to acquire sufficient APLs. APLs from each run under the same condition were combined into one sample for subsequent characterization and AD testing.

4.2.3 Batch co-AD of APL and SS

Batch AD was conducted in 500 mL syringes to select the APL for continuous AD. Each reactor received 50 g of inoculum and 100 g of SS. The APL was added according to the real yield from the pyrolysis experiment. During pyrolysis, the yields of APLs were 29.57%, 31.89%, and 29.63% at 350°C, 500°C, and 650°C, respectively. For 100 g of mixed sludge, the final APL generated from integrated AD and pyrolysis was 0.852 g, 0.919 g, and 5.84 g at 350°C, 500°C, and 650°C, respectively. Thus, the addition amount of APL was set as SS: APL of 100:0.852, 100:0.919, and 100:0.854 for APL350, APL500, and APL650, respectively. Three reactors without APL addition were designated as controls.

The reactor setup procedure followed that of Torri and Fabbri [2]. All reactors were maintained at 36°C and 80 rpm in a water bath (BT300, Yamato Scientific Co., Ltd., Tokyo, Japan) for 65 days. Biogas volume was recorded daily by reading from the

syringe scale, and its composition was measured using a Micro-GC. The VFA content was measured every 1-2 weeks using HPLC. Micro GC and HPLC's detailed methods are the same as those in Section 3.2.6.

4.2.4 Semi-continuous co-AD of APL and SS

The APL used for the semi-continuous co-AD process included the most toxic APL obtained at 500°C (APL500) and the least toxic APL at 350°C (APL350), as identified from both batch co-AD tests and anaerobic toxicity assay in Chapter 3. The experiment was carried out in three 3L reactors at 36°C, each with a working volume of 2L, and stirred using mechanical agitation at 120 rpm (Figure 4.1).

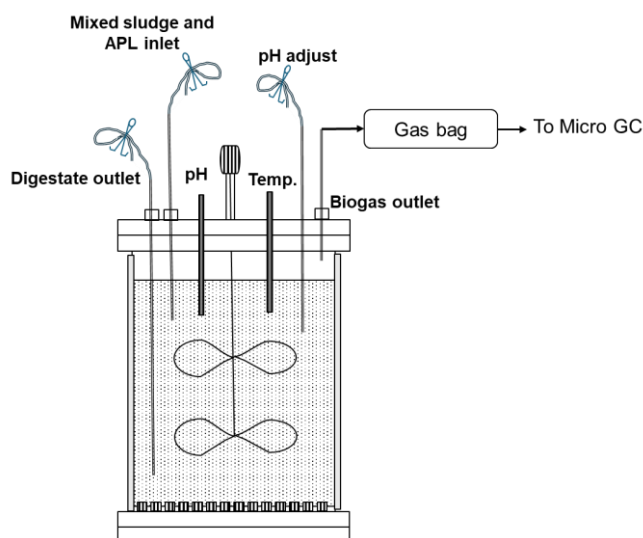


Figure 4.1 The anaerobic digester used for semi-continuous experiment

One reactor served as the control, while the other two were designated for APL350 and APL500 addition, referred to as the “Control,” “APL350 reactor,” and “APL500 reactor,” respectively. The reactors gradually started over 14 days, with the organic loading rate (OLR) increasing from 0.33 to the target value of 0.97 g VS/L/d (1.31 g COD/L/d). Following this initial phase, the reactors were operated until steady-state conditions were reached. APL addition began on day 38.5, and the reactors continued to operate until methane production stabilized in all reactors, totaling 178 days, with a SRT of 30 days.

Every 3.5 days, 233 mL and 235 mL of digestate were removed from the control and APL addition reactors, respectively. The control reactor was replenished with 233 mL of mixed sludge, while the APL350 and APL500 reactors received 233 mL of mixed sludge supplemented with 2.12 mL of APL350 and 2.42 mL of APL500, respectively based on the real APL yields. The OLRs of the control and APL reactors were 1.31 and 1.51 gCOD/L/d. The pH was continuously monitored using a real-time recording system and maintained between 6.8 and 7.2 by adjusting with 2mol/L NaOH or HCl solutions. During the experiment, biogas was collected in gas bags and measured twice weekly for volume (using a 500 mL syringe) and composition (via Micro GC). The digestate was measured twice weekly for TS, VS, and VFA content (via HPLC). The methods are detailed in Section 3.2.6.

The APL conversion ratio at the stable stage of continuous co-AD was defined as Eq. (1):

$$\text{APL conversion ratio (\%)} = \frac{\text{Actual } P_{\text{CH}_4, \text{APL}}}{\text{Theoretical } P_{\text{CH}_4, \text{APL(COD)}}} \times 100\% \quad (4-1)$$

where *Actual* $P_{\text{CH}_4, \text{APL}}$ represents the average actual CH_4 production contributed by APL at the stable stage (day 125 to day 178), mL/d. *Theoretical* $P_{\text{CH}_4, \text{APL(COD)}}$ represents the theoretical CH_4 production from APL based on its COD (0.35 L CH_4 /g COD [25]), mL/d.

4.2.5 Carbon balance during semi-continuous co-AD

The input C includes C from both the feeding sludge and APL. Output C consists of C converted into CH_4 and CO_2 and the C present in the digestates. To measure the C content in the input sludge and digestate, both were centrifuged (Centrifuge, H36, Kokusan, Tokyo, Japan) at $3,100 \times g$ for 30 min. The supernatant was filtered through a $0.45 \mu\text{m}$ membrane filter, and total carbon (TC) concentrations were measured using a TOC analyzer. The precipitate was dried at 105°C for 24 h, ground to $<0.5 \text{ mm}$, and analyzed for C content by a CHN analyzer. The C content was the sum of the C in the liquid and solid phases. The TC content in APLs was also determined using a TOC

analyzer after filtration with 0.45 µm membrane filter. C converted to biogas was calculated based on biogas volume. Details of the CHN and TOC analyzer methods are given in section 3.2.6.

The input C, output C and C remaining in the reactor were calculated as follows:

$$\text{Input C (g)} = \text{Input } C_{\text{mixed sludge}} \text{ (g)} + \text{Input } C_{\text{APL}} \text{ (g)} \quad (4-2)$$

$$\text{Input } C_{\text{mixed sludge}} \text{ (g)} = 233 \text{ mL} \times \text{TS\%} \times C_{\text{mixed sludge solid}} \text{ (\%)} + 233 \text{ mL} \times (1-\text{TS\%}) \times \text{TC}_{\text{mixed sludge liquid}} \text{ (g/L)} \times 10^{-3} \quad (4-3)$$

$$\text{Input } C_{\text{APL}} \text{ (g)} = V_{\text{APL}} \text{ (mL)} \times \text{TC}_{\text{APL}} \text{ (g/L)} \times 10^{-3} \quad (4-4)$$

$$\text{Output C (g)} = \text{Output } C_{\text{digestate}} \text{ (g)} + \text{Output } C_{\text{biogas}} \text{ (g)} \quad (4-5)$$

$$\text{Output } C_{\text{digestate}} \text{ (g)} = 233 \text{ mL} \times \text{TS\%} \times C_{\text{digestate solid}} \text{ (\%)} + 233 \text{ mL} \times (1-\text{TS\%}) \times \text{TC}_{\text{digestate liquid}} \text{ (g/L)} \times 10^{-3} \quad (4-6)$$

$$\text{Output } C_{\text{biogas}} \text{ (g)} = V_{\text{biogas}} \text{ (L)} / 22.4 \text{ (L/mol)} \times 14 \text{ (g/mol)} \quad (4-7)$$

$$\text{C in the reactor (g)} = (2000-233) \text{ mL} \times \text{TS\%} \times C_{\text{digestate solid}} \text{ (\%)} + (2000-233) \text{ mL} \times (1-\text{TS\%}) \times \text{TC}_{\text{digestate liquid}} \text{ (g/L)} \times 10^{-3} \quad (4-8)$$

where Input $C_{\text{mixed sludge}}$ (g) refers to the input C contributed by the mixed sludge; $C_{\text{mixed sludge solid}}$ (%) is the C content in the mixed sludge solid; $\text{TC}_{\text{mixed sludge liquid}}$ (g/L) represents the TC concentrations in the mixed sludge liquid; Input C_{APL} (g) means the input carbon contributed by APL, V_{APL} (mL) is the APL addition volume every 3.5 days, TC_{APL} (g/L) represents the TC concentration in APL. Output $C_{\text{digestate}}$ (g) is the output C with the digestate; $C_{\text{digestate solid}}$ (%) represents the C content in the digestate solid; $\text{TC}_{\text{digestate liquid}}$ (g/L) is the TC content in the digestate liquid. Output C_{biogas} (g) is the output C with biogas, V_{biogas} (L) is the biogas production volume at standard condition (273.15 K, 1 atm) every 3.5 days.

4.2.6 Microbial community analysis

The microbial community analysis was conducted using digestate samples collected on days 0, 63, 90, 118, and 178 from the continuous reactor. These time points correspond to key stages of the experiment: before the experiment, before the addition

of APL, during APL-induced inhibition, during the release of APL-induced inhibition, and the stable methane production stage after inhibition. The analysis method is detailed in Section 3.2.7

4.2.7 Mass and energy flow in the integrated AD-pyrolysis system

The mass and energy flow were calculated based on the yields and LHV of feedstocks and products of AD and pyrolysis. The lower heat value of sludge samples and biochar and bio-oil were calculated by Eq. (4-9) and (4-10) [14].

$$\text{HHV}(\text{MJ/kg}) = 33.5 \times \text{C} + 142.3 \times \text{H} - 15.4 \times \text{O} - 14.5 \times \text{N} \quad (4-9)$$

$$\text{LHV}(\text{MJ/kg}) = \text{HHV} - 2.44 \times 9 \times \text{H} \quad (4-10)$$

where C, H, and N were the mass percentages of carbon, hydrogen, and nitrogen obtained by the elementary analyzer.

The LHV of syngas was calculated following Eq. (4-11) [15]:

$$\text{LHV} (\text{MJ/Nm}^3) = 0.108 \times \text{H} + 0.1264 \times \text{CO} + 0.3582 \times \text{CH}_4 + 0.665 \times \text{C}_n\text{H}_m \quad (4-11)$$

where H₂, CO, CH₄, and C_nH_m were the components contents (vol%) in the gas products.

The energy recovery ratio (ERR, %) in the coupled AD and pyrolysis system was calculated following the Eq. (4-12):

$$\text{ERR} = \frac{\text{Total Energy of Products (sum of biogas, syngas, biochar and bio-oil)}}{\text{Total Energy of Mixed Sludge}} \times 100\% \quad (4-12)$$

Based on the product yield and LHV data calculated above, a mass and energy flow analysis were visualized with the SankeyMATIC online software.

4.2.8 Statistical analysis

Statistical significance was determined using analysis of variance (ANOVA) and Mann-Whitney U Test, with a significance threshold set at $p < 0.05$.

4.3 Results and discussion

4.3.1 Batch co-AD of APL and sludge

Figure 4.2 shows the cumulative methane production during batch AD. In 300 hours, the control exhibited the highest production, followed by APL350, APL650, and APL500. Both APL350 and APL650 exhibited a reduction in the methane production rate, which lasted longer; both recovered around 350 hours. APL500 showed the longest decrease in the methane production rate. Its production rate decreased around 350 hours and did not recover to around 550 hours, lasting for over 200 hours; almost double the duration of APL350 and APL650.

This trend is consistent with the toxicity results of the APL from the toxicity assessment results in Chapter 3. It was found that the toxicity of APL prepared with a feedstock moisture content of 25.8% followed the order: APL500 > APL650 > APL350. The highest toxicity at 500°C might be due to milder pyrolysis at 350°C yielding larger, less toxic molecules, while 500°C might be optimal for forming a range of toxic compounds that are not completely broken or large enough to be benign. At 650°C, high temperatures break down these compounds into simpler, less toxic, and more biodegradable molecules. This is also consistent with the APL yield results, where 500°C corresponded to the highest APL yield.

However, from about 650 hours, APL350 and APL500 exhibited methane production levels very close to the control. By around 800 hours, their production was almost the same as the control. This might be due to the inhibition release caused by the gradual microbial adaptation to the toxicity of APLs. However, APL500 did not show similar production levels until about 1000 hours, likely due to its highest toxicity causing the delay. Up to 1580 hours, all treatments showed similar final methane production levels as the control, illustrating that inhibition was eventually released in all reactors, even for the most toxic APL. From ANOVA and Mann-Whitney U Test, no significant difference was observed in the final methane production between each treatment ($p < 0.05$). The above results instructed that the inhibition from APL can be finally released

by microbial adaptation in the co-digestion process, but no higher methane production from APL-added treatments was observed, likely APL was not converted into methane.

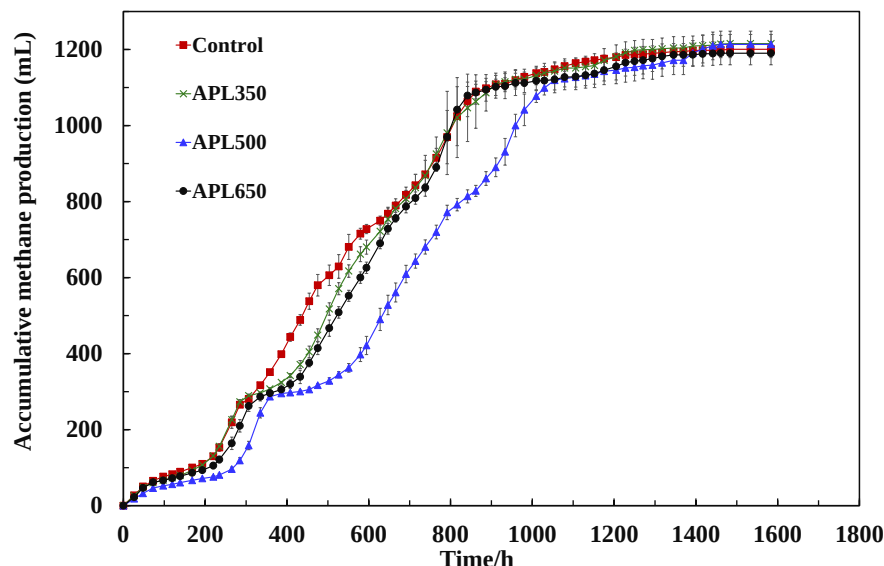


Figure 4.2 Cumulative methane production during the batch co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). Error bars represent standard deviations from 3 parallel reactors, with each reactor analyzed once. (APL350, APL500, and APL650 refer to reactors supplemented with APL produced at 350°C, 500°C, and 650°C, respectively, while Control represents reactors without APL addition)

Figure 4.3 shows the methane content in the biogas during the batch AD. The methane content gradually increased from an initial range of 15-25% to approximately 75% by the end. Initially, the control exhibited the highest methane content, but as the microbes adapted to the APLs, all reactors showed similar methane content, around 75%. This higher-than-typical methane content may be attributed to the fact that the biogas was retained in the headspace for a prolonged period during the experiment, allowing more CO₂ to be converted into methane. By the end of the experiment, all reactors exhibited similar methane content, indicating that the inhibition had been released. This finding is consistent with the methane production results.

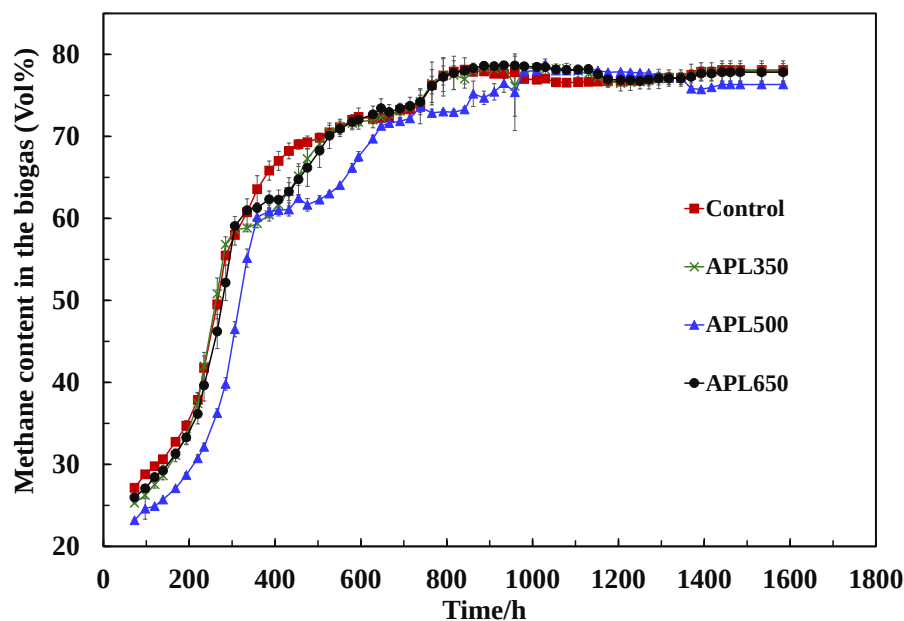


Figure 4.3 Methane content during the batch co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). Error bars represent standard deviations from 3 parallel reactors, with each reactor analyzed once. (APL350, APL500, and APL650 refer to reactors supplemented with APL produced at 350°C, 500°C, and 650°C, respectively, while Control represents reactors without APL addition)

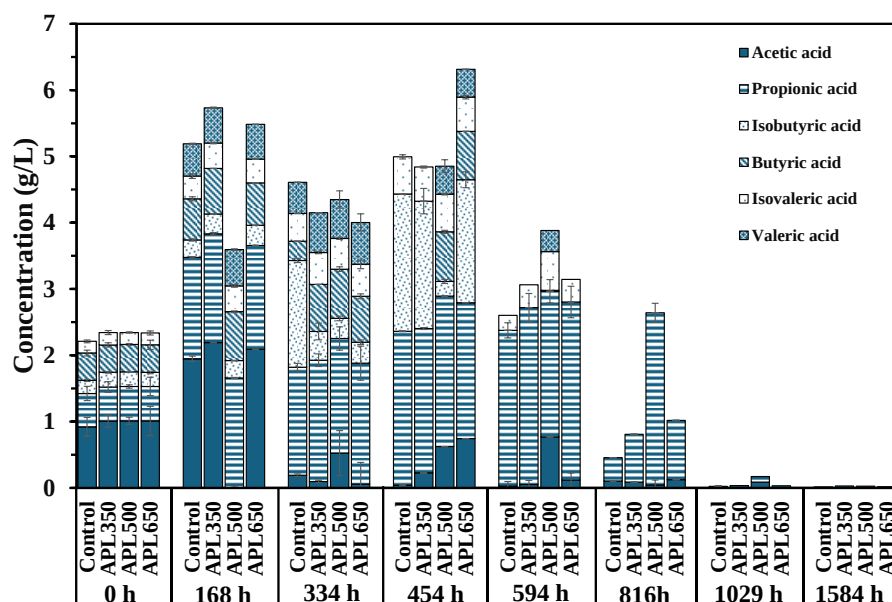


Figure 4.4 VFAs content during the batch co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). Error bars represent standard deviations from 3 parallel reactors, with each reactor analyzed once. (APL350, APL500, and APL650 refer to reactors supplemented with APL produced at 350°C, 500°C, and 650°C, respectively, while Control represents reactors without APL addition)

Figure 4.4 presents the VFA measurement results to provide deeper insights into methane production dynamics. Noticeably, the APL addition treatments had comparable VFAs levels with control, illustrating that the APL addition did not inhibit the VFAs generation. This is different from the APL solely AD results that acidification was identified as rate-limited step, probably because co-AD of APL and SS eliminated the inhibition in VFAs generation by the dilution of inhibitory substances in APL or providing anaerobic microorganisms with more nutrients and buffering capacity [6]. The inhibition in the co-digestion could be attributed to the accumulated VFAs.

Generally, all the treatments showed a trend in VFA content of “increase-decrease”. APL 500 treatment showed a relative delay than the others. As AD progressed, valeric, isovaleric, butyric and isobutyric acids could be converted to propionic and acetic acids [16]. Then propionic acid could convert to acetic acid and finally methane [6]. As AD advanced, the variety of VFAs diminished, shifting towards propionic and acetic acids, e.g., by 816 hours, only propionic and acetic acids were observed, with propionic acid being predominant, and ultimately leaving only negligible acetic acid by 1584h due to depletion of organic matter. This shift in VFA composition highlights the progressive breakdown of complex organic compounds into simpler intermediates and their eventual utilization for methane generation, consistent with the typical pathways of AD.

However, in the APL500 group, this transition process was noticeably delayed. While other treatments exhibited significant accumulation and subsequent reduction of propionic and acetic acids at 168-816 h, the APL500 treatment showed a slower rate of VFAs conversion and degradation, with prolonged persistence of higher VFAs (594-1029h). This delay suggests that the intermediates in APL500 required more time for transformation, potentially due to the higher toxicity of APL500, which could also explain the delayed methane production.

These results indicate that while anaerobic co-digestion of sludge and APL eventually overcame APL-induced inhibition, methane production in APL reactors was not significantly higher than in the control, suggesting limited APL conversion to

methane. Therefore, continuous digestion was conducted to enhance APL conversion.

4.3.2 Semi-continuous co-AD of APL and sludge

4.3.2.1 Methane production

During the startup phase, methane production was unstable due to variations in the OLR. Starting from day 28, the three reactors produced comparable methane levels and were operated under stable conditions for 10.5 days. From day 38.5, APL addition began, leading to significant methane production declines in the APL350 and APL500 reactors, as shown in Figure 4. Methane production dropped sharply from approximately 100 mL/g VS/d to below 20 mL/g VS/d within seven days, reflecting over 80% inhibition caused by APL toxicity.

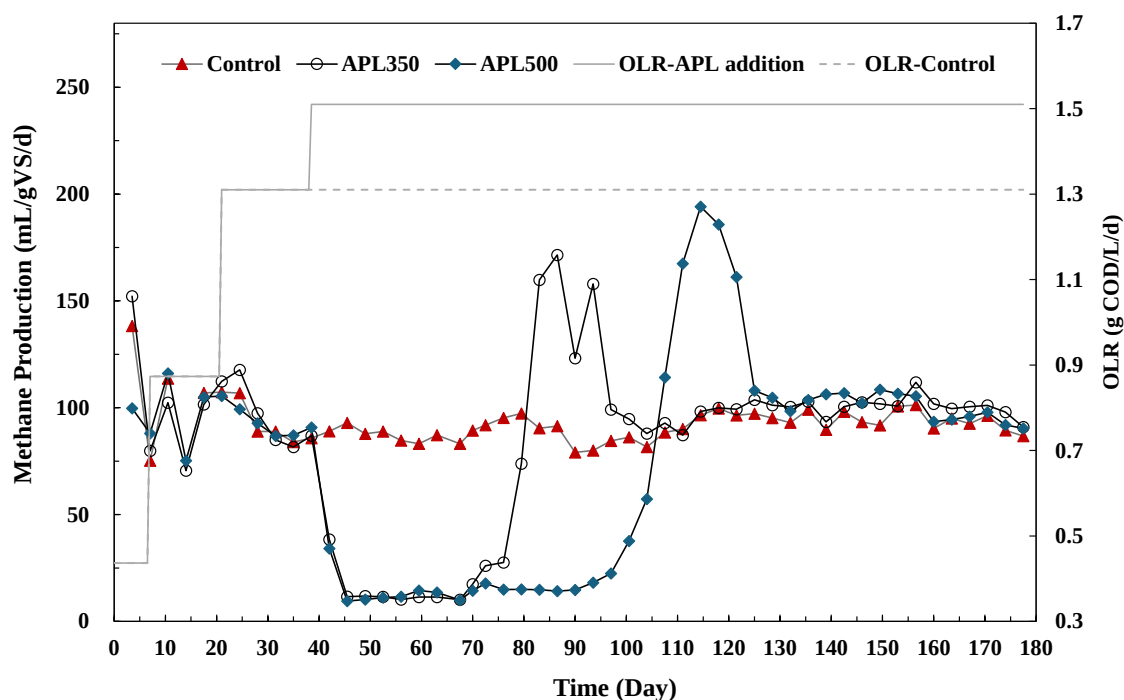


Figure 4.5 Methane production during the semi-continuous co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). (APL350 and APL500 refer to the reactor supplemented with APL produced at 350°C and 500°C, respectively, while Control represents the reactor without APL addition)

The inhibition persisted for 29 days in the APL350 reactor, with methane production beginning to recover on day 67.5, sharply increasing from day 72.5. It surpassed the control around day 80, peaking at 171 mL/g VS/d on day 86.5. Subsequently, production gradually declined to values comparable to the control between days 111 and 116.5, then exhibited higher methane production than the control from day 120 onward, indicating successful microbial adaptation and degradation of APL350. Methane production in the APL500 reactor remained inhibited until day 90, with the inhibition lasting approximately two months, indicating a stronger inhibitory effect than APL350. This aligns with the findings in the research of our previous study [13] and Section 3.1. Methane production gradually increased thereafter, suggesting microbial adaptation to APL500 toxicity also. The peak production, recorded on day 112.5, was approximately twice that of the control. This was likely due to accumulated organics, such as VFAs, being converted into methane. Afterward, as these accumulated organics were depleted, methane production decreased but remained higher than the control, likely due to the conversion of APL to methane.

From day 125 to day 178, both APL350 and APL500 reactors showed stable methane production, with average yields of 94.36 mL/gVS/d (control), 100.68 mL/gVS/d (APL350), and 100.91 mL/gVS/d (APL500), corresponding to 564.28, 602.07, and 603.45 mL/d, respectively. The methane production difference between APL350 and the control was 37.79 mL/d, and between APL500 and the control was 39.17 mL/d, reflecting the methane production from APL. Based on COD, the theoretical methane production was 51.41 mL/d for APL350 and 53.60 mL/d for APL500, yielding APL conversion ratios of 73.50% and 73.07%, respectively (Eq. (4-1)). This demonstrates that even the most inhibitory APL can be converted to over 73% methane through co-digestion with SS, highlighting the effectiveness of co-digestion in enhancing APL conversion.

4.3.2.2 TS and VS variations

The TS and VS content remained stable at approximately 2.0% and 1.5%, respectively, from the beginning until the APL addition. However, after the APL addition on day 38.5, TS and VS levels in both the APL350 and APL500 reactors gradually increased. This was due to the unconverted sludge accumulating in the reactors, consistent with the observed decrease in methane production (Figure 4.6).

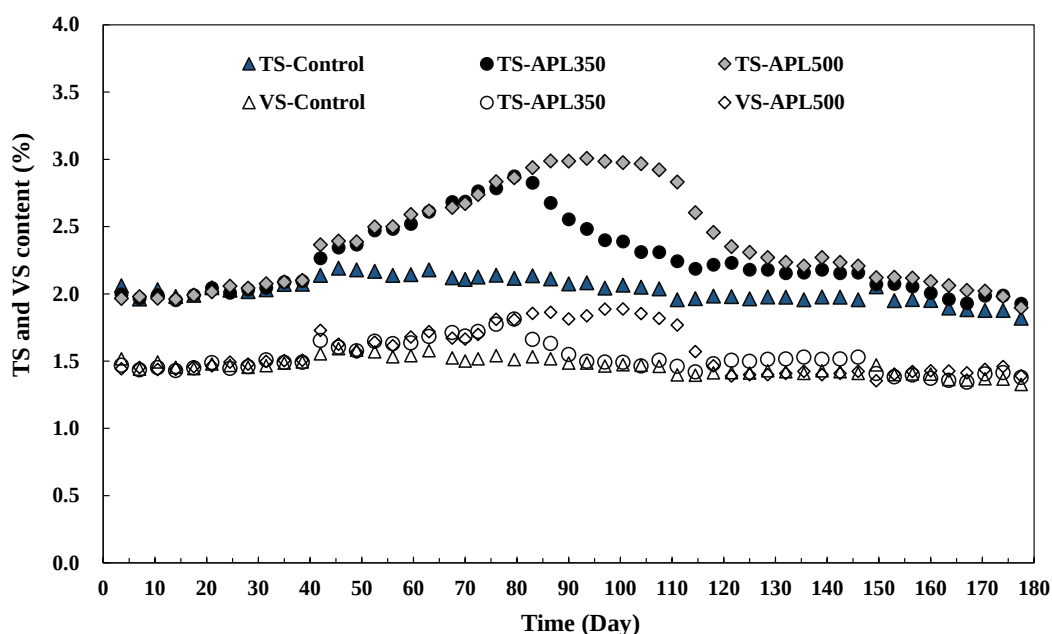


Figure 4.6 TS (total solid) and VS (volatile solid) content changes during the semi-continuous co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). (APL350 and APL500 refer to the reactor supplemented with APL produced at 350°C and 500°C, respectively, while Control represents the reactor without APL addition)

In the APL350 reactor, TS and VS increased until day 80, despite methane production recovering from day 68, suggesting organic accumulation due to input exceeding output. After day 80, TS and VS decreased as accumulated organics were converted to methane. This decrease in TS and VS further reduced the methane production from day 90 due to less organics available for methane generation. In the APL500 reactor, TS and VS rose until day 90, then stabilizing between days 90 and 115 despite increased methane production during this period. This stability reflected a balance between organic

accumulation and consumption. After day 115, TS and VS declined as accumulated organics were converted to biogas. Notably, there was a lag in the reduction of TS and VS compared to the recovery of methane production. This was likely due to methane generation in the early recovery stage being insufficient to offset the continued accumulation of organic matter in the reactor. Eventually, TS and VS levels in both reactors stabilized at values comparable to the control, aligning with steady methane production and alleviating inhibition.

4.3.2.3 VFA content variations

VFA measurements commenced on day 28, coinciding with the stabilization of methane production. Before the APL addition, VFA levels in all reactors were negligible, while methane production remained stable and high (Figure 4.7). This indicates a well-maintained balance between VFA production and conversion to methane, suggesting the reactors operate under an optimal OLR without inhibition.

Following APL addition on day 38.5, VFA began accumulating in the APL350 and APL500 reactors. In the APL350 reactor, VFA levels peaked at approximately 7.5 g/L on day 76, with acetic acid contributing the most (around 5 g/L). This significant accumulation highlights the inhibitory effect on VFA conversion to methane [18]. Starting from day 79.5, when methane production began to increase sharply, the accumulated VFA levels started to decrease as they were metabolized into methane. By day 93.5, VFA levels in the APL350 reactor had dropped to negligible amounts like the control, indicating the restoration of a balance between VFA generation and methane conversion. However, this decline in VFA led to a reduction in methane production after day 93.5, as the accumulated VFA had been largely consumed.

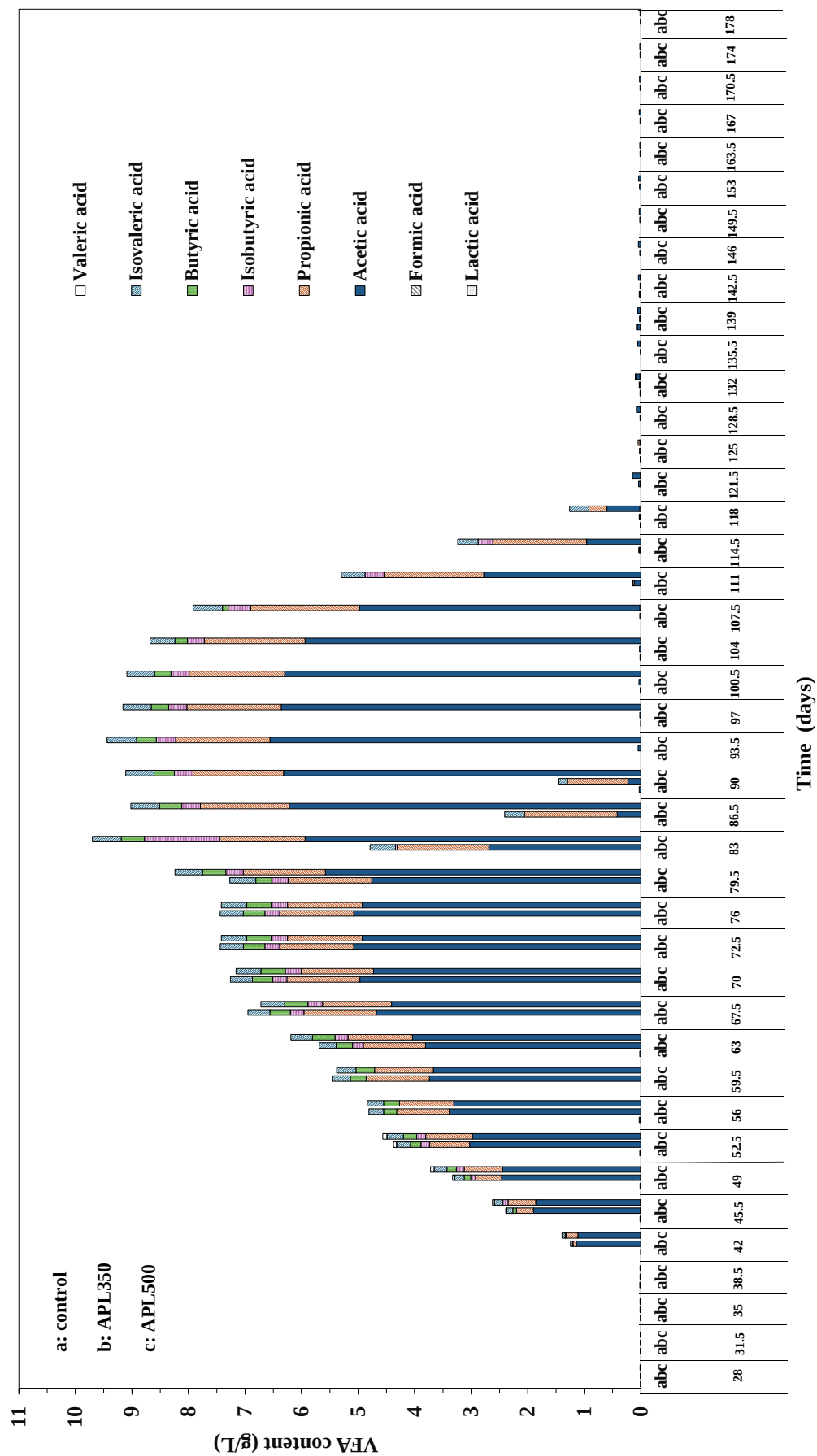


Figure 4.7 The VFA (volatile fatty acid) content during semi-continuous co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). (APL350 and APL500 refer to the reactor supplemented with APL produced at 350°C and 500°C, respectively, while Control represents the reactor without APL addition)

The VFA trend in the APL500 reactor exhibited a similar pattern but with a more pronounced accumulation and a delayed decrease. After APL addition, VFA levels continued rising until day 83, reaching a plateau of 9–10 g/L, where they stabilized until day 100.5. Such a high concentration can be toxic to methanogens. The toxicity is due to an increase in the undissociated form of the VFA. They can flow freely through the cell membrane where they dissociate and hence cause a pH reduction and a disruption of homeostasis [18]. Subsequently, VFA levels began to decline, eventually reaching negligible levels by day 115, signaling the establishment of a balance between VFA production and consumption. The prolonged VFA accumulation and delayed reduction in the APL500 reactor underscore the higher toxicity and stronger inhibition caused by APL500, aligning with the toxicity assessments discussed in Chapter 3.

4.3.2.4 pH variations

The pH in the reactors was associated with the VFA levels. The control maintained a stable pH range of 6.8–7.2 after the startup stage, so no pH adjustment was necessary. Since the addition of APL on day 38.5, the pH of APL350 and APL500 began to decrease, likely due to VFA accumulation. 2 mol/L NaOH was added daily to maintain the pH within the range of 6.8–7.2 (Figure 4.8).

For APL350, methane production sharply increased around day 80, leading to an increase in pH, likely due to VFA consumption and conversion to methane, as evidenced by the decreasing VFA levels from day 80 onward. The pH was maintained between 6.8 and 7.2 using 2 mol/L HCl. However, by day 93.5, the low VFA content indicated a good balance between VFA generation and conversion, allowing the pH to remain stable within the desired range without further adjustment, thus pH adjustment discontinued.

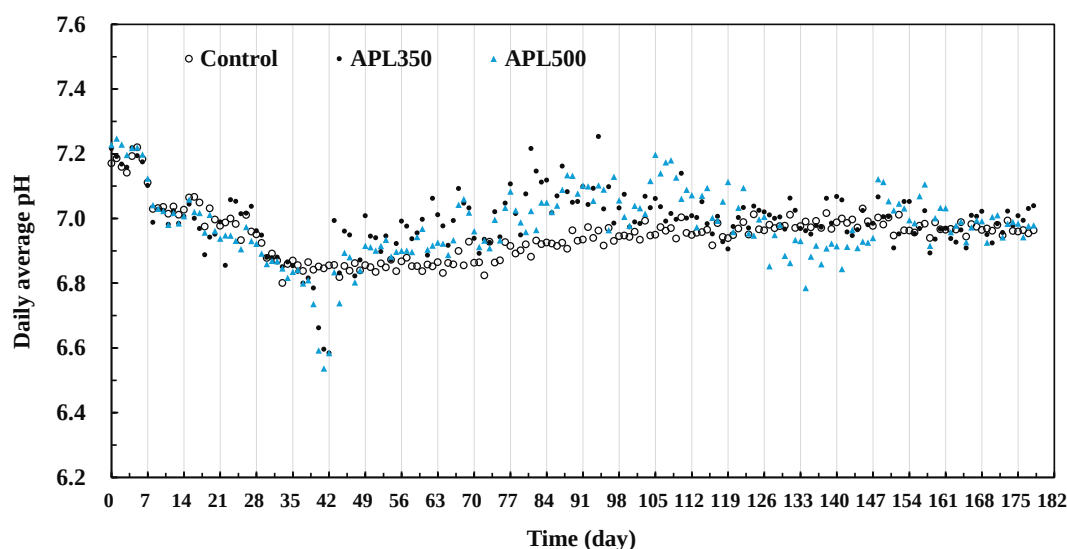


Figure 4.8 pH variation during the semi-continuous co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). (APL350 and APL500 refer to the reactor supplemented with APL produced at 350°C and 500°C, respectively, while Control represents the reactor without APL addition)

For the APL500 reactor, the pH continued to decrease until day 100.5 due to VFA accumulation after the APL addition at day 38.5, necessitating daily adjustments with 2 mol/L NaOH to maintain a pH of 6.8-7.2. Around day 109, the pH began to rise as VFA accumulation decreased, likely due to its conversion to methane, illustrated by increased methane production from day 90 to day 115. Therefore, 2 mol/L HCl was added to maintain the pH range. By day 140, the pH remained within the 6.8-7.2 range due to balanced VFA generation and consumption, as shown in Figure 4.8. Therefore, the pH adjustments were discontinued, with subsequent changes attributed to the sludge feeding.

4.3.2.5 Carbon balance during continuous AD

Figure 4.9 illustrates the carbon balance during continuous AD at representative intervals, approximately every two weeks. On day 28, when no APL was added, all three reactors displayed a well-maintained balance between input and output carbon. However, on day 42, the addition of APL disrupted the carbon balance in the APL350 and APL500 reactors, with input carbon exceeding output, particularly low in the form

of biogas, due to inhibited biogas production. This imbalance became more pronounced on days 56 and 70, reflecting severe inhibition of biogas generation and an accumulation of unconverted carbon in the reactors.

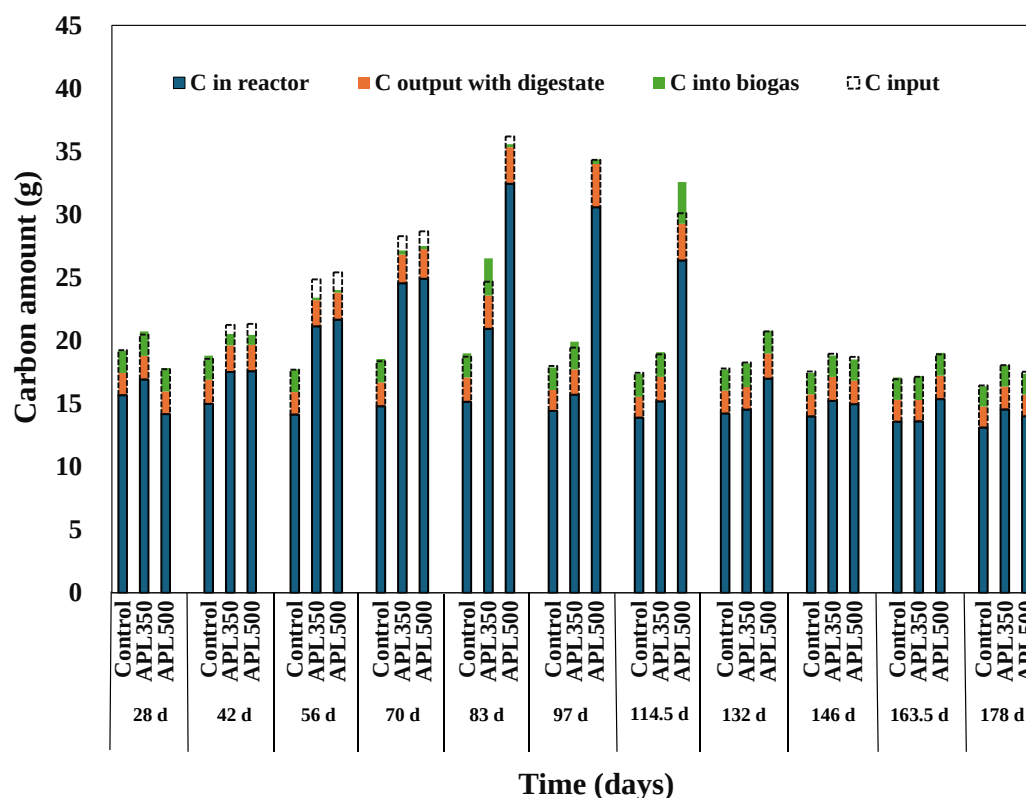


Figure 4.9 The carbon balance during the semi-continuous co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). (APL350 and APL500 refer to the reactor supplemented with APL produced at 350°C and 500°C, respectively, while Control represents the reactor without APL addition)

On day 83, when biogas generation peaked in the APL350 reactor, it exhibited higher carbon output than input, driven by a significant increase in carbon converted to biogas, leading to a reduction in the carbon remaining in the reactor. In contrast, biogas generation in the APL500 reactor remained inhibited. By day 114.5, the APL350 reactor achieved a stable carbon balance, with biogas generation also stabilizing as inhibition subsided. Meanwhile, the APL500 reactor reached peak biogas production, showing elevated carbon output, particularly in biogas, and a subsequent decline in carbon remaining in the reactor. Eventually, the APL500 reactor exhibited a pattern like the APL350 reactor, restoring the balance between input and output carbon.

4.3.2.6 Microorganism community analysis

Figure 4.10 provides the relative abundances of archaea and bacteria at the genus level with relative abundances above 2%; genera with relative abundances < 2% were included in “others”. In bacterial taxonomy, the genus name alone may not always provide sufficient information, particularly when labeled as “*uncultured*”. The family name is therefore prefixed to the genus name, separated by “;” to ensure clarity. For example, “*Anaerolineaceae;uncultured*” indicates that this genus within the family *Anaerolineaceae* is uncultured. Additionally, when the genus name is unavailable, only the family name is presented with the genus name represented by “_”. For instance, “*Camaamonadeceae;_*” signifies that the genus within the family *Camaamonadeceae* is unknown.

Initially, the inoculum exhibited a simple community structure with low diversity (day 0). After the addition of APL, the microbial community underwent changes from day 63, coinciding with the change of CH₄ production in both APL350 and APL500 reactors. These shifts reflect the selective pressures imposed by APL's complex composition and the resulting adjustments in bacterial populations.

In bacterial communities, hydrolytic bacteria such as *Aminobacterium*, its appearance of in APL reactors but absent in the control signals a shift toward better amino acid utilization and acetate, H₂, and CO₂ production [19]. The *Anaerolineaceae ADurb. Bin120* and *Anaerolineaceae uncultured* belong to the family *Anaerolineaceae*. Their enrichment indicates their adaptation to APL by efficiently degrading hydrocarbons, carbohydrates, and proteins, generating intermediates like acetate, formate, and H₂ for methanogenesis [20], [21]. The ability to ferment alkanes and degrade aniline further underscores their potential to metabolize APL's components [22]. The increased abundance of *Proteiniphilum*, which specializes in protein hydrolysis [23], supports enhanced organic matter degradation in sludge and APL.

Acidogenic bacteria, such as *Candidatus Cloacimonas* showed increased abundance, underscoring its pivotal role in APL degradation. Known to thrive in anaerobic digesters [24], it primarily uses amino acids for energy, converts formate into H₂ and CO₂, and degrades protein-rich substrates [25]. Its involvement in lipid and long-chain fatty acids

degradation [26]–[28] further underscores its adaptability to APL’s complex organic load. Similarly, the *Christensenellaceae R-7 group* increased in APL reactors, where it degraded carbohydrates and amino acids to produce acetate and butyric acid.

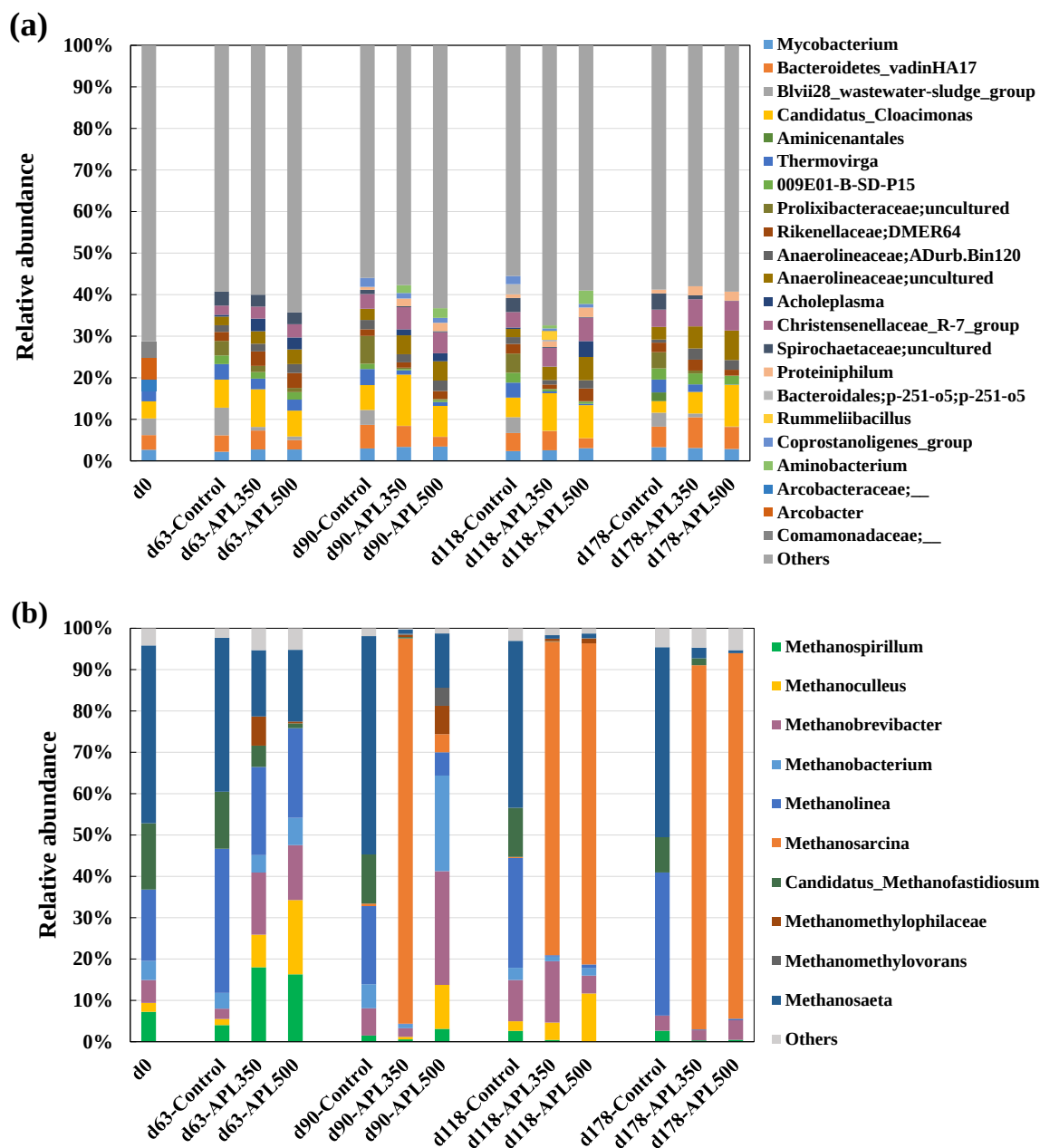


Figure 4.10 Relative genus-level abundances of (a) bacterial (> 2%) and (b) archaeal communities during the semi-continuous co-AD of APL (aqueous pyrolytic liquid) and SS (sewage sludge). (APL350 and APL500 indicate reactors supplemented with APL produced at 350°C and 500°C, respectively, while the Control represents the reactor without APL addition. 'd' denotes 'day'.)

In contrast, the decline in the *Blvii28 wastewater-sludge* group and near disappearance of *Spirochaetaceae* in the APL reactors suggests their sensitivity to APL-induced toxicity or VFA fluctuations.

In summary, the addition of APL favored diverse microbial genera such as *Candidatus Cloacimonas*, *Anaerolineaceae ADurb. Bin120*, *Anaerolineaceae uncultured*, and *Proteiniphilum*. These microorganisms, equipped with the enzymatic machinery to degrade complex organic substrates, exhibited synergistic activity in breaking down proteins, amino acids, hydrocarbons, lipids, and anilines. Their ability to adapt and function effectively in the challenging environment created by APL ensured their survival and dominance. Sensitive taxa like *Spirochaetaceae* declined, reflecting the selective pressures imposed by the new conditions.

Since the archaea community is less diverse than the bacterial community, it was more significantly affected by APL. Archaea classification on genus level is presented in Table 4.2.

Table 4.2 The classification of Archaea based on the genus level observed in the reactors

Hydrogenotrophic methanogens	Methylotrophic methanogens	Acetoclastic methanogens
<i>Metanospirillum</i>		
<i>Methanoculleus</i>	<i>Methanomethylovorans</i>	
<i>Methanobrevibacter</i>	<i>Methanomethylophilaceae</i>	<i>Methanosaeta</i>
<i>Methanobacterium</i>	<i>Methanolinea</i>	<i>Methanosarcina</i>
<i>Methanolinea</i>	<i>Methanosarcina</i>	<i>Candidatus_Methanofastidiosum</i>
<i>Methanosarcina</i>	<i>Candidatus_Methanofastidiosum</i>	
<i>Candidatus_Methanofastidiosum</i>		

The predominant change in archaeal community is the shift in the APL reactors methanogenic community structure towards a *Methanosarcina* replacing *Methanosaeta*

and *Methanolinea* that dominated in control (Figure 10 (b)). This shift was more rapid in APL350 reactor compared to APL500 reactor, likely due to the stronger inhibition of the latter.

The observed dominance of *Methanosarcina* can be attributed to its superior metabolic flexibility, high growth rate, and tolerance to harsh conditions, positioning it as a robust methanogen [19]. *Methanosarcina* can utilize various substrates for methanogenesis, including acetate, hydrogen, and carbon dioxide [29], as well as methanol and methylamines [30]. This allows it to efficiently metabolize various intermediates generated during the degradation of complex organic waste, such as APL. In addition, *Methanosarcina* has a significantly faster growth rate, with a doubling time of approximately 1.0-1.2 days, compared to other methanogens with minimum doubling times of 4-6 days. *Methanosarcina* exhibits a high half-saturation constant (K_s) of 200–280 mg COD L⁻¹ [31]. This enables it to dominate at higher VFA concentrations, particularly acetate (<15 g/L), as observed in APL reactors. In contrast, *Methanosaeta*, characterized by lower K_s (10-50 mg COD/L), thrives in conditions where acetate concentrations are below 3g/L [31], as in the control reactor where VFA levels remained negligible. *Methanosarcina* also exhibits remarkable tolerance to various stressors such as pH shock (0.8-1.0 units), exceeding *Methanosaeta* (<0.5 units). Furthermore, *Methanosarcina* is less affected by specific inhibitors from acetate metabolism, such as *Fluoroacetate* and *Fluoromethane*, further supporting its robust performance [32]. These explained the dominance of *Methanosaeta* in the APL addition reactors.

Methanolinea was dominant in the control reactor. It can utilize H₂ and formate for growth and methane production. It is a common genus in mesophilic sludge digesting municipal sewage [33]. *Methanolinea* grows well in acidic environments and exhibits higher tolerance to acidity than other methanogens, this might explain its dominance in the control reactor, because no pH adjustment was conducted for the control reactor, whose pH remained between 6.8 and 7.0 [34]. *Candidatus Methanofastidiosum* also

displayed metabolic versatility, utilizing substrates such as H₂, CO₂, acetate, methanol, and methylamines for methane production [35]. However, it has a negligible abundance in APL reactors, probably due to its lower tolerance to pH shock, high VFA concentrations, and APL toxicity. Nevertheless, it remained the third most dominant genus in the control reactors, underscoring its role in methane production under less inhibitory conditions.

Methanobrevibacter and *Methanoculleus* are hydrogenotrophic methanogens, exhibiting dynamic population trends in response to APL addition. Both demonstrated an initial increase during the inhibition phase (e.g., day 63 in APL350 and APL500), followed by a decline during the recovery phase as VFA levels decreased (day 90 in APL350 and day 118 in APL500). A subsequent stabilization phase was observed in APL350 where the VFA level was negligible. This suggested it may be sensitive to the VFA level.

The addition of APL significantly altered the archaeal community, favoring the dominance of *Methanosarcina* replacing *Methanosaeta* and *Methanolinea* due to its metabolic flexibility, rapid growth, and resilience. These findings provide valuable insights into microbial community dynamics under stress deducted by APL, which is crucial for optimizing inoculum to enhance APL degradation and conversion via AD.

4.3.3 Mass and energy flow in the integrated AD-pyrolysis system

The mass and energy flows were calculated based on the yields and LHV of feedstocks and products of AD and pyrolysis, as presented in Table 4.3. The SS and DS had LHVs of 15.38 MJ/kg and 13.68 MJ/kg, respectively. The decrease in LHV after digestion can be attributed to the AD process converting a portion of the organic matter into methane, thereby reducing the organic content in the sludge.

The bio-oil produced had an LHV ranging from 31.59 to 34.74 MJ/kg, which aligns closely with the literature-reported value for sludge bio-oil (33.40 MJ/kg) [37]. Biochar derived at 350°C exhibited a higher LHV (11.85 MJ/kg) than that derived at 500°C, likely because the higher pyrolysis temperature promotes the evaporation of organic compounds from the solid phase into gaseous products [38]. Similarly, APL showed LHVs of 3.90 MJ/kg and 3.57 MJ/kg at 350°C and 500°C, respectively, which can also

be explained by the preferential migration of organics into the gas phase at elevated temperatures.

Table 4.3 Yields and LHV pyrolytic products

Name	H (%)	C (%)	N (%)	O* (%)	Ash (%)	LHV (MJ/kg)	Yield (kg/kg feedstock)
SS	6.36±0.10	38.87± 0.71	5.35± 0.06	29.36± 0.88	20.06± 0.22	15.38	-
DS	5.51±0.30	35.08±0.93	5.28±0.23	25.33±1.52	28.81±0.06	13.68	-
350°C 25.8%	Bio-oil 10.76±0.10	72.05±0.02	5.10±0.07	10.40± 0.01	1.70±0.13	34.74	0.06
Biochar	3.08±0.29	34.18±3.92	5.15±0.57	16.60± 0.12	41.00± 0.11	11.85	0.46
APL	7.06±1.25	7.81±0.21	2.29±0.07	-	-	3.90 [#]	0.32
500°C 25.8%	Bio-oil 8.40±0.04	72.51±0.57	8.46±0.10	10.27±0.04	0.36 ±0.01	31.59	0.05
Biochar	1.48±0.15	31.28±3.74	4.43±0.52	9.38±0.56	53.43±0.09	10.18	0.32
APL	7.69±0.91	7.78±0.91	2.72±0.09	-	-	3.57 [#]	0.35

*O=100%-C- H- N- Ash

[#]LHV of APL: calculated from 16.1 kJ/g COD [36].

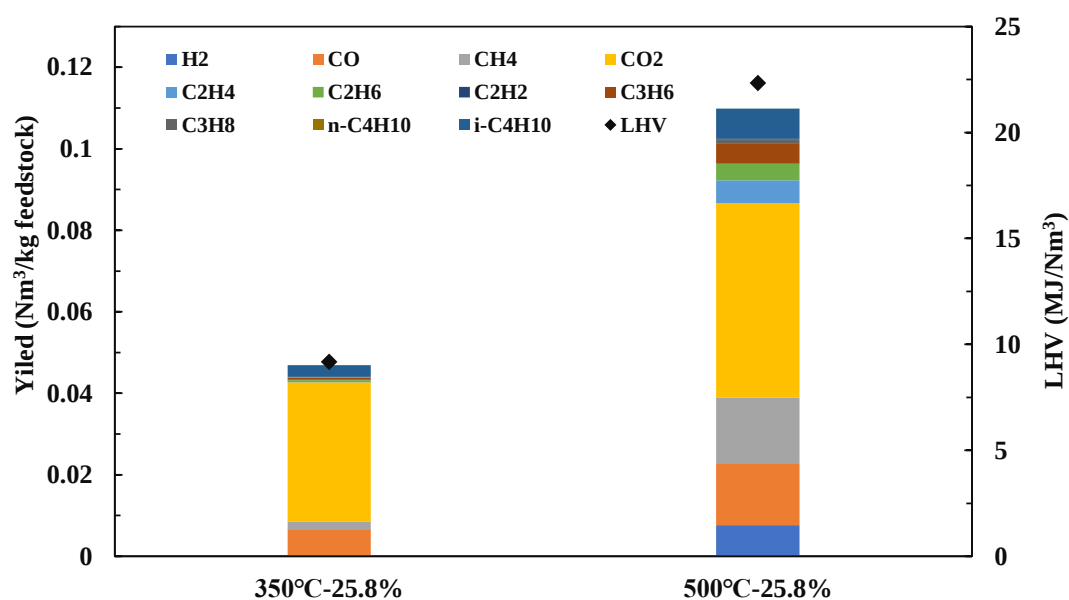


Figure 4.11 LHV and yields of pyrolytic syngas

The syngas composition and LHV are shown in Figure 4.11. CO₂ was the predominant component, likely produced through carbon oxidation, the water-gas

reaction, and the water-gas shift reaction [39]. At 500°C, higher levels of H₂, CH₄, CO, and C_nH_m were observed, attributed to the elevated temperature promoting the cracking of organic compounds, their evaporation into the gas phase, and favoring endothermic reactions [38]. Consequently, syngas produced at 500°C exhibited a significantly higher LHV (22.34 MJ/Nm³) compared to that generated at 350°C (9.18 MJ/ Nm³), owing to the increased yield of energy-rich gases.

Table 4.4 presents the CH₄ production during AD under scenarios involving the reintroduction of APL into the AD process. Methane production was calculated by averaging the data from day 125 to day 178 of stable methane output for both the control and APL addition reactors. Assuming a daily input of 1000 kg of mixed sludge into the AD reactor, according to the pyrolysis results in chapter 3, subsequent dewatering, drying, and pyrolysis would yield 8.18 kg and 9.10 kg of APL at 350°C and 500°C, respectively.

Table 4.4 Calculated daily CH₄ production and energy generation from 1000 kg of mixed sludge input into AD

AD feedstock	CH ₄ production (L/d)	LHV of CH ₄ LHV (kJ/L)	Total energy in CH ₄ (MJ/d)
Control			
1000 kg/d mixed sludge	8586.80	35.80	307.41
APL-350°C			
1000 kg/d mixed sludge + 8.18 kg/d APL350	9161.92	35.80	328.00
APL-500°C			
1000 kg/d mixed sludge + 9.10 kg/d APL500	9182.92	35.80	328.75

In the control scenario without APL reintroduction to AD, the AD reactor produced 8586.80 L/d of CH₄, equivalent to 307.41 MJ/d. When APL generated at 350°C was reintroduced, the combined sludge and APL generated 9161.92 L/d of CH₄, equivalent to 328.00 MJ/d, representing an improvement of 20.59 MJ/d over the control. Similarly, when APL derived at 500°C was reintroduced, CH₄ production increased to 9182.92 L/d, equivalent to 328.75 MJ/d, an enhancement of 21.34 MJ/d compared to the control.

Using the product yield data outlined above, a mass flow analysis was visualized with the SankeyMATIC online software. When 1000 kg of mixed sludge was fed into

the AD reactor, 16.83 kg was converted into methane, leaving 983.18 kg of DS. After dewatering, most of the DS mass (857.47 kg) was lost with the biogas slurry, underscoring the critical role of dewatering before drying. The remaining 125.70 kg of dewatered sludge was dried to a moisture content of 25.85%, resulting in 25.97 kg of dried sludge, while 99.73 kg was lost as steam during drying (Figure 4.12).

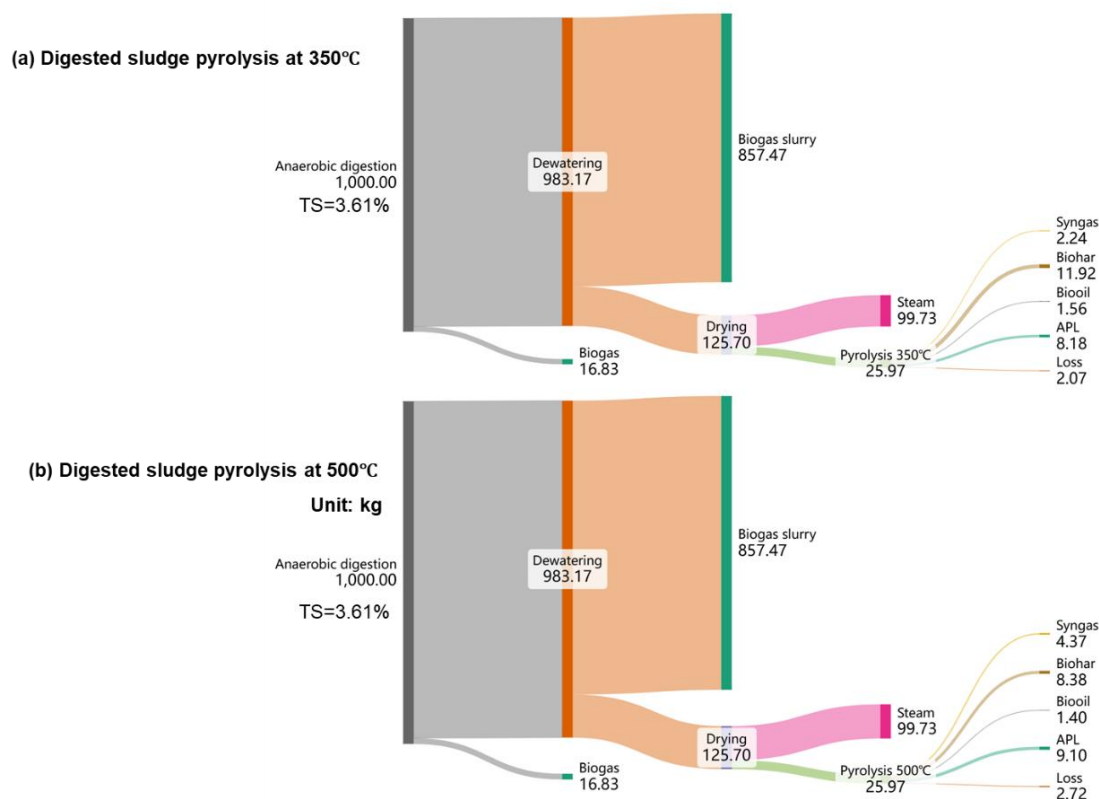


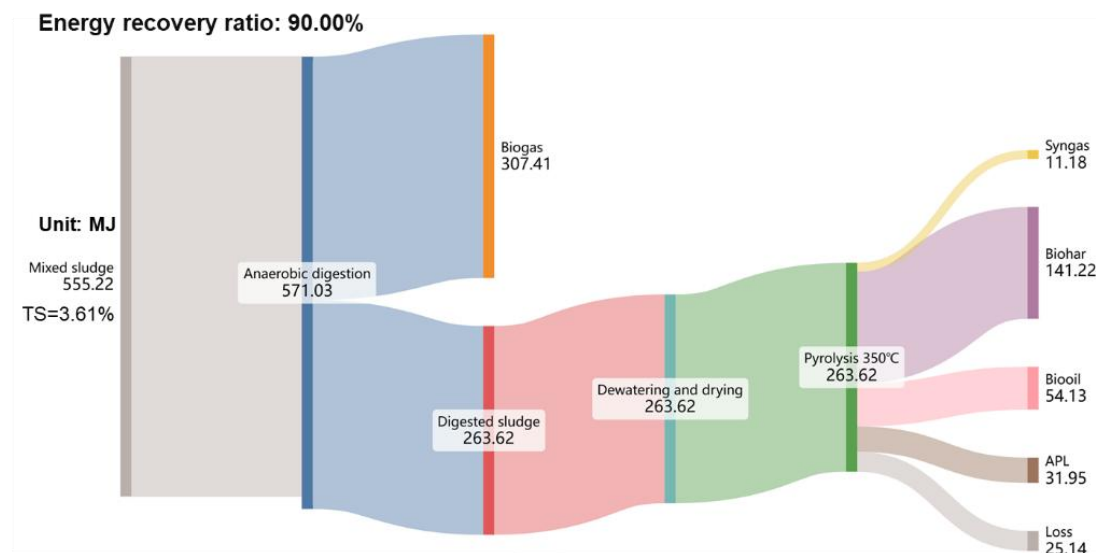
Figure 4.12 Mass flow in the anaerobic digestion-pyrolysis system

The dried sludge was subsequently pyrolyzed at 350°C and 500°C, with distinct mass distributions (Figure 4.12). At 350°C, 2.24 kg, 11.92 kg, 1.56 kg, and 8.18 kg of the dried sludge were converted into syngas, biochar, bio-oil, and APL, respectively, while 2.07 kg was lost as coke or uncollectable residues adhering to the pyrolysis tube. At 500°C, the conversions shifted to 4.37 kg of syngas, 8.38 kg of biochar, 1.40 kg of bio-oil, and 9.10 kg of APL, with 2.72 kg lost. The increased conversion to syngas and APL at higher temperatures is consistent with enhanced organic cracking at elevated temperatures.

Figure 4.13 presents the energy flow in the AD-pyrolysis system (the scenario without APL reintroductions). During AD, 555.22 MJ of energy contained in the sludge was input into the AD reactor, 307.41 MJ was converted into biogas, presenting 55.37%, and another 44.63% of the energy remained in the DS. This is consistent with the

discussion above: AD can only convert 40-60% of the organics into biogas, implying its incomplete degradation of the organics. Assuming that during the dewatering and drying, the energy contained in the DS was unchanged, then the energy of 263.62 MJ was contained in the pyrolysis feedstock.

(a) Digested sludge pyrolysis at 350°C



(b) Digested sludge pyrolysis at 500°C

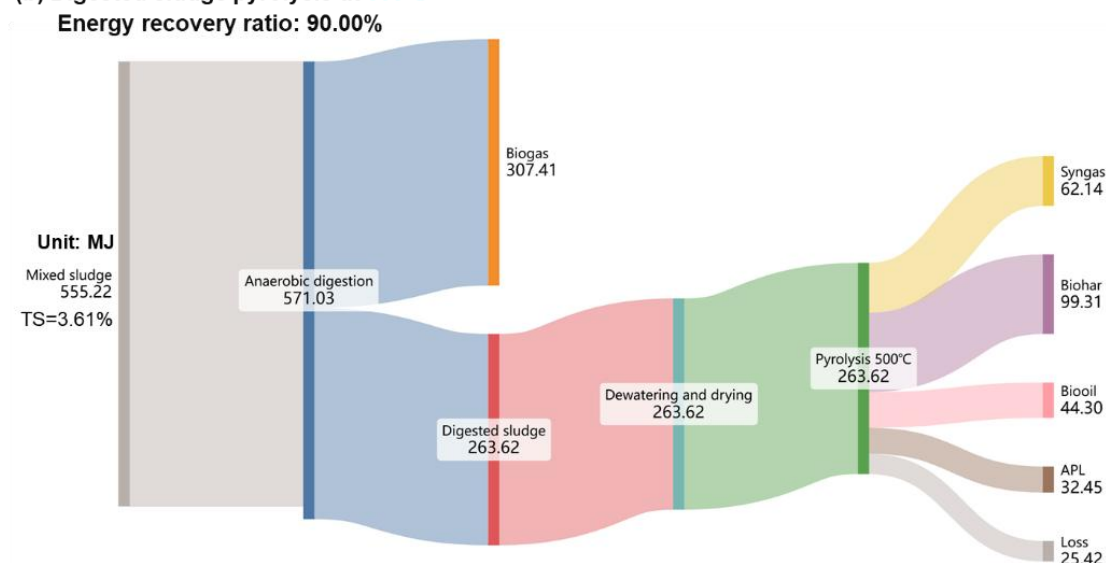


Figure 4.13 Energy flow in the anaerobic digestion-pyrolysis system

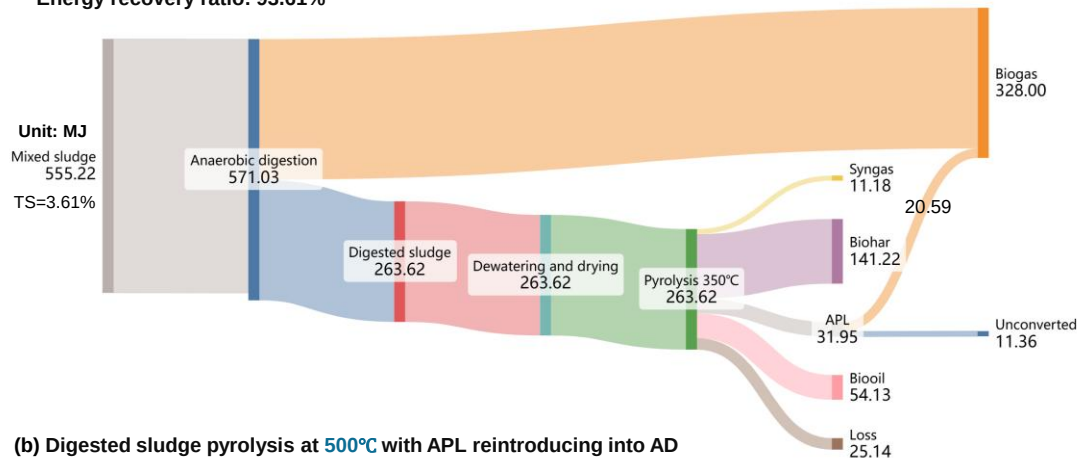
In the scenario of dried DS pyrolysis at 350°C, most energy (141.22 MJ) remained in the biochar due to the low temperature. The least energy was transferred to syngas, only 11.18 MJ. 54.13MJ of energy was transferred to bio-oil. 33.95MJ of energy was transferred into APL. If this part of energy was not recovered, it means an energy loss with APL. In addition, 25.14 MJ of energy was lost with coke or the uncollectable

products stuck in the pyrolysis tube.

In the scenario of pyrolysis at 500°C, more energy was converted to syngas (62.14MJ) but less into biochar (99.31MJ) and bio-oil (44.30%). This could be also attributed to the increasing temperature favoring the gaseous product generation. The energy converted into APL was comparable with the scenario of pyrolysis at 350°C, indicating a necessity for energy recovery from APL. The two scenarios achieved a comparable overall energy recovery of 90%.

(a) Digested sludge pyrolysis at 350°C with APL reintroducing into AD

Energy recovery ratio: 93.61%



(b) Digested sludge pyrolysis at 500°C with APL reintroducing into AD

Energy recovery ratio: 93.74%

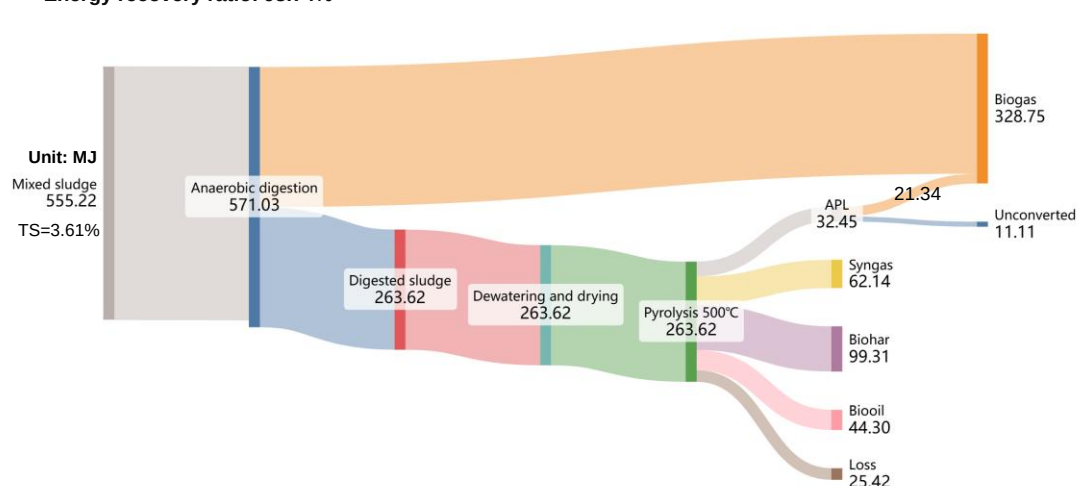


Figure 4.14 Energy flow in the anaerobic digestion-pyrolysis system with returning APL (aqueous pyrolytic liquid) to digester for biogas generation

Figure 4.14 illustrates the energy flow for scenarios involving energy recovery from APL via AD (reintroducing APL into AD). In the case of pyrolysis at 350°C, 20.59 MJ of energy was recovered in the form of biogas, representing 64.44% of the total energy

contained in the APL. This resulted in an overall energy recovery ratio of 93.61%, which marks an improvement of 3.61% compared to the control scenario. For pyrolysis at 500°C, 21.34 MJ of energy was recovered as biogas from APL, corresponding to 65.76% of the APL's total energy, with the overall energy recovery ratio reaching 93.74%, an increase of 3.74%.

These results underscore the potential of integrating APL into AD as an effective approach to enhance energy recovery from sewage sludge. While over 60% of the energy contained in APL could currently be recovered through AD, future advancements, such as the selection of optimized inoculum, could further improve this recovery rate. This strategy not only increases the overall energy recovery from sludge but also reduces costs and environmental impacts associated with APL treatment and disposal. Importantly, it leverages existing AD infrastructure, eliminating the need for specialized equipment to process APL, thereby promoting cost-effective and resource-efficient integration into existing waste management systems.

4.4 Conclusion

This chapter successfully alleviated the APL inhibition on AD by the co-AD with sludge. It investigated the feasibility of fully introducing APL generated at different pyrolysis temperatures (350, 500, and 650°C) into AD within the AD-pyrolysis system, assuming all DS is subjected to pyrolysis. Batch co-AD of APL and sludge revealed that APL prepared at 350°C and 500°C exhibited the lowest and highest toxicity, respectively. Although inhibition caused by APL was eventually overcome, the methane yield in APL reactors did not significantly surpass the control, suggesting limited APL conversion to methane.

Continuous co-AD was then conducted with APL350 and APL500 to enhance the conversion of APL into methane. APL500 exerted a longer inhibition (2 months) than APL350 (one month), but eventually, the inhibition could be overcome with both the lowest and highest toxicity APL addition reactors showing higher methane production. This could be attributed to microbial adaptation to the APL. The addition of APL significantly favored genera like *Candidatus Cloacimonas*, *Anaerolineaceae* ADurb. Bin120, *Anaerolineaceae uncultured*, and *Proteiniphilum* that synergistically degraded complex organics. The archaeal community also shifted with *Methanosarcina* dominating due to its metabolic versatility and resilience. Energy flow analysis showed

that reintroducing APL into AD recovered over 60% of its energy as biogas, improving the overall energy recovery ratio in the AD-pyrolysis integrated system to over 93%. This route leverages existing AD infrastructure, reducing disposal costs and potential environmental impacts associated with APL while enhancing energy recovery. Future studies may focus on optimizing inoculum and operational parameters to maximize APL conversion.

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Chapter 5 Conclusion and future perspectives

The integration of AD and pyrolysis has emerged as a promising solution to address the challenges associated with DS in the stand-alone AD process, enabling resource recovery and waste minimization. This study systematically investigates the interactions between AD and pyrolysis, focusing on how these two processes influence each other.

Chapter 2 revealed that AD significantly alters the characteristics of sewage sludge, affecting its subsequent pyrolysis behavior. Both mesophilic and thermophilic AD were found to impact pyrolysis, with the latter exhibiting a more pronounced effect. TGA demonstrated that thermophilic AD reduced weight loss more significantly than mesophilic AD. Kinetic analysis showed that mesophilic AD generally increased the E , while thermophilic AD did not markedly alter E at low conversion rates (0.2-0.5) but increased it at high conversion rates (0.6-0.8). Thermodynamic analysis also revealed that mesophilic AD generally enhanced pyrolysis favorability and reactivity, while thermophilic AD improved activated complex formation at lower conversion rates but hindered it at higher rates. Py-GC/MS analysis further confirmed that the different effects were due to AD's dual effect on organics decomposition. AD decomposes biodegradable organics into low-molecular-weight compounds that are favorable to subsequent pyrolysis, while it also accumulates larger, bio-refractory molecules that make the DS less prone to pyrolytic decomposition. Thermophilic AD exerted a more significant such effect than mesophilic AD. Low-temperature pyrolysis might be a promising strategy for DS treatment, as a more favorable change was observed in kinetics and thermodynamics at lower conversion rates.

Chapter 3 examined the toxicity of pyrolysis-derived APL on AD. The results revealed that APL, derived from DS pyrolyzed at various temperatures (350–650°C) and moisture contents (0–40.4%), exhibited significant toxicity to AD, with the least toxicity observed for APL 350°C-0%, and the highest toxicity observed at APL 650°C-40.4%. SA addition, rather than GL, enhanced the conversion of APL to CH₄, improved the CH₄ production rate, and improved organic acid content, indicating that acidification was the primary rate-limiting step in mono-AD of APL. The modified Gompertz model further confirmed that SA indeed improved the maximum methane production rate, while GL did not, thus acidification was identified as the rate-limiting step in the mono-AD of APL. These results highlighted the importance of mitigating

APL inhibition in AD and enhancing APL conversion to biogas.

Chapter 4 focused on mitigating the AD inhibition caused by APL through co-AD with sludge, and determining whether APL generated at different pyrolysis temperatures (350, 500, and 650°C) within the AD-pyrolysis system, assuming all digestate is subjected to pyrolysis, can be fully reintroduced into AD. The batch co-AD of APL and sludge demonstrated that while inhibition from APL was eventually overcome, methane production in the APL reactors did not significantly surpass those of the control, suggesting negligible APL conversion. Continuous co-AD using APL500 (the most toxic) and APL350 (the least toxic) showed that the former caused a longer inhibition period (2 months) compared to the latter (1 month). During inhibition, methane production reduction, VFA accumulation and pH dropping were observed. However, both APL addition reactors overcame inhibition exhibiting higher methane production than the control eventually, which is attributed to microbial adaptation. The microbial community shifted towards genera like *Candidatus Cloacimonas*, *Anaerolineaceae ADurb. Bin120*, *Anaerolineaceae uncultured*, and *Proteiniphilum*, which were associated with complex organic degradation, while *Methanosarcina* dominated the archaeal community due to its metabolic versatility, rapid growth rate and strong tolerance. Energy flow analysis showed that reintroducing APL into AD recovered >60% of its energy as biogas, increasing the overall energy recovery ratio to over 93% in the integrated AD-pyrolysis process. This demonstrates that returning APL into AD co-digesting with SS is a potential strategy for managing APL. This can reduce disposal costs and potential environmental impacts associated with APL while enhancing energy recovery.

Future research may focus on the following aspects:

- (1) Developing adsorption and pretreatment methods for enhancing APL conversion: More research is needed to develop effective adsorption or pretreatment strategies that enhance the conversion of APL in AD systems. This could involve exploring methods to mitigate APL toxicity, such as pre-treating APL or adding additives to improve its integration into AD. One promising strategy could be to introduce biochar generated during DS pyrolysis into AD to enhance both APL conversion and the overall performance of the AD process.
- (2) Enhancing microbial tolerance to APL through acclimatization or optimizing the inoculum: Improving the tolerance of AD microorganisms to APL or utilizing suitable inoculum may play a significant role in improving APL

conversion and the accelerating inhibition recovery in the co-AD with sludge. This study has demonstrated that microbial adaptation enables APL degradation during AD. Research into using composite inoculum, such as mixtures from different substrate-fed anaerobic digesters, may enhance microbial diversity, improve tolerance, and increase the capacity for degrading complex organic compounds. Additionally, adding the beneficial microbial strains identified in this study, such as *Candidatus Cloacimonas*, *Proteiniphilum*, and *Methanosarcina* could potentially help shorten the inhibition period and accelerate methane production recovering.

- (3) Assessing long-term feasibility and scalability of the integrated AD-pyrolysis system: Future studies should also focus on evaluating the long-term feasibility and scalability of integrating AD and pyrolysis in real-world applications. This might involve assessing economic viability and conducting an LCA to understand the environmental impacts of this integrated process.

By exploring these aspects, better determination of the practicality and sustainability of the integrated AD-pyrolysis process on a larger scale could be possible.

Appendix

Table S1 Sample amounts and product yields at each pyrolysis conditions for each run.

No.	Conditions	Run No.	Sample/g	Biochar/g	Yield/%	APL ^c /g	APL yield/%	Biooil/g	yield/%	Syngas + loss/g ^a	Syngas + loss ^b /%
1	350-0%	Run 1	29.9	15.6	52.2%	9.87	6.47%	33.7	22.1%	28.2	18.5%
		Run 2	30.1	16.8	51.9%						
		Run 3	30.8	16.3	54.4%						
		Run 4	30.7	16.3	53.2%						
		Run 5	30.9	16.2	52.7%						
2	350°C-10.8%	Run 1	30.8	18.9	52.7%	16.8	17.8%	13.9	14.7%	12.0	12.7%
		Run 2	32.6	16.6	58.1%						
		Run 3	31.0	8.33	53.6%						
3	350°C-25.8%	Run 1	21.0	9.96	39.8%	19.6	29.6%	7.79	11.7%	10.8	16.2%
		Run 2	23.3	9.89	42.7%						
		Run 3	22.0	6.37	44.8%						
4	350°C-40.4%	Run 1	20.2	7.02	31.5%	34.4	33.5%	15.0	14.6%	18.8	18.4%
		Run 2	21.0	6.71	33.5%						
		Run 3	20.6	7.00	32.5%						
		Run 4	20.3	7.30	34.4%						
		Run 5	20.5	14.0	35.7%						
5	500°C-0%	Run 1	32.0	13.7	43.7%	21.4	13.7%	24.0	15.4%	41.9	26.7%

		Run 2	30.9	13.6	44.4%						
		Run 3	30.5	13.9	44.5%						
		Run 4	31.5	14.2	44.2%						
		Run 5	31.8	12.4	44.6%						
6	500°C- 10.8%	Run 1	30.7	11.8	40.5%	21.5	23.8%	12.6	14.0%	19.7	21.9%
		Run 2	28.7	12.2	41.1%						
		Run 3	30.7	7.43	39.5%						
7	500°C- 25.8%	Run 1	23.0	7.00	32.3%	21.9	31.9%	6.58	9.58%	18.2	26.5%
		Run 2	21.7	7.58	32.1%						
		Run 3	24.0	4.75	31.6%						
8	500°C- 40.4%	Run 1	18.4	5.31	25.8%	14.9	38.3%	3.57	9.18%	10.3	26.6%
		Run 2	20.5	13.3	25.9%						
9	650°C-0%	Run 1	31.4	11.6	42.3%	19.1	12.6%	12.4	8.18%	57.6	38.0%
		Run 2	31.1	13.1	37.2%						
		Run 3	30.8	12.5	42.7%						
		Run 4	29.6	12.0	42.4%						
		Run 5	28.8	10.2	41.5%						
10	650°C- 10.8%	Run 1	27.8	10.0	36.7%	16.5	19.2%	6.18	7.21%	32.1	37.5%
		Run 2	29.1	10.7	34.3%						
		Run 3	28.7	6.82	37.2%						

11	650°C- 25.8%	Run 1	23.2	6.76	29.5%	20.1	29.6%	4.77	7.05%	22.8	33.7%
		Run 2	22.6	6.46	29.9%						
		Run 3	22.0	5.02	29.4%						
12	650°C- 40.4%	Run 1	21.1	5.20	23.8%	17.7	42.9%	2.16	5.24%	11.2	27.1%
		Run 2	20.2	15.6	25.8%						

^a Calculated from: Total weight of sample – Total weight of biochar – Weight of APL – Weight of biooil

^b There was some products loss during pyrolysis and products collection. The losses were due to carbon deposition on the pyrolysis tube wall and the uncollectable biooil remaining in the condenser.

^c APL: aqueous pyrolytic liquid

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