Enhancing the potential of wood waste as anaerobic digestion feedstock: a nature-based approach

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Publications

Journal papers

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Summary

The aim of this study is to explore the enhancement of methane production in the anaerobic digestion of wood waste by leveraging forest soil systems as a 'pre' treatment. A nature-based approach can provide valuable insights for managing forest residues and promoting resource recovery, making anaerobic digestion a viable wood waste management practice.

Due to the recalcitrant lignocellulosic structure of wood waste, its use as a substrate for anaerobic digestion is not commonly pursued in mainstream research. Therefore, a detailed meta-analysis was conducted to compare the anaerobic digestion potential of wood waste with other organic wastes and to demonstrate the degree of enhancement in methane production from wood waste using various pretreatment technologies. Considering that anaerobic digestion parameters can significantly impact methane production, it is essential to optimize the conditions during the anaerobic fermentation process. To achieve this, machine learning techniques were employed to analyze and fine-tune the digestion parameters specifically for wood waste. In the case of current wood waste pretreatment technologies, most are limited to the laboratory level, often overlooking labour and capital costs, rendering them impractical for operational use. For this reason, naturally decayed wood samples from forests were collected for further tests, which were classified into one of five decay classes (numbered 1-5 with increasing decay) based on a range of characteristics. Additionally, the study examined the impact of forest environmental factors on the degradation of wood waste and subsequent methane production by placing two types of wood waste in two types of vegetation zones in the forest.

The following conclusions can be drawn. Firstly, pretreatment technologies significantly enhance methane production from wood waste, making it a viable raw material for anaerobic digestion. It has been observed that employing a combination of pretreatment techniques is more effective than using a single method. In addition, the random forest algorithm can reliably predict methane yield from anaerobic digestion of wood waste. Critical factors influencing methane production include wood particle size and the substrate-to-inoculum ratio. Moreover, decayed wood samples showed a range of physicochemical properties conducive to anaerobic digestion, with decay class 3 showing the highest methane yield. Lastly, different forest environments affect the degradation of wood, although a specific treatment time is necessary to significantly impact its methane production.

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Abbreviations

AD	Anaerobic digestion
ANN	Artificial neural networks
BD	Anaerobic biodegradability
BMP	Biochemical methane potential
C/N	Carbon to nitrogen ratio
CrI	Cellulose crystallinity index
DC	Decay class
DTG	Derivative thermal gravimetry test
EMP	Experimental methane production
EPA	U.s. Environmental protection agency
FD	Fractal dimension
I/S	Inoculum to substrate
IEA	The international energy agency
L/(C+H)	Lignin content/(cellulose content + hemicellulose content)
LCH	Lignocellulose content (%)=lignin content + cellulose content + hemicellulose content
MAE	Mean absolute error
MWD	Mean weight diameter
OECD	The organisation for economic co-operation and development
ORP	Oxidation reduction potential
PMP	Predicted methane production
PRISMA	The preferred reporting items for systematic reviews and meta-analyses
PS	Particle size

R2	Coefficient of determination
REC	Regression error characteristic
RF	Random forest
RMSE	Root mean square error
SD	Standard deviation
SE	Standard error
SEC	Specific energy consumption
SVR	Support vector regression
TGA	Thermogravimetric analysis
TMP	Theoretical methane production
TOC	Total organic carbon
TS	Total solids
TTC	2,3,5-triphenyltetrazolium chloride
TTF	2,3,5-triphenyltetrazolium formazan
VS	Volatile solid
WEOC	Water extractable organic carbon

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Chapter 1 Introduction

1.1 Background

Wood plays a vital role for human beings as the most abundant source of biomass and renewable green material. In generally, almost 50% of a tree can be processed into the final product, whereas the rest is retained as wood waste (Turley et al., 2006). Wood waste, which includes leftovers from timber processing, discarded furniture, and construction debris, represents a significant portion of municipal and industrial waste streams (Souza et al., 2018). The overall amount of global wood waste is estimated to be 232.94 million m^3 in 2020 (Gao et al., 2022). However, only a limited amount of wood waste has so far been available for recycling and reuse, and an economical method has not yet been developed to make full use of wood waste. Traditionally, much of this wood waste has been landfilled, incinerated, or left to decompose naturally, which can lead to substantial methane and carbon dioxide emissions, both potent greenhouse gases (Harmon et al., 2020; Muaaz-Us-Salam et al., 2020). Given the high calorific value of wood waste, the predominant disposal scenario is to generate thermal energy from combustion (Molenda et al., 2021), but direct combustion of wood waste to produce energy or electricity is not considered to be an efficient and environmentally friendly alternative anymore (González-García and Bacenetti, 2019; Vicente et al., 2020). Particularly in small boilers or combustion chambers without emission control systems, the emissions produced could cause a lot of energy waste and serious environmental pollution (Jaworek et al., 2021; Sharma and Dasappa, 2017). With advances in technology and growing environmental awareness, the focus has shifted toward more sustainable methods of managing and utilizing this abundant resource.

As governments and organizations worldwide set stringent carbon emission targets, sustainable technologies for generating renewable energy from wood waste are receiving increasing attention (Cesprini et al., 2020; Haryanto et al., 2021). Among the plethora of options, the utilization of wood waste through anaerobic digestion (AD) presents a particularly promising avenue. AD involves microbial decomposition of organic matter under anaerobic conditions to produce biogas, which primarily consists of methane and carbon dioxide and can be used as a renewable energy source (Archana et al., 2024). The process also produces digestate, a nutrient-rich substance that can be used as a biofertilizer, further contributing to the sustainability of this approach (O'Connor et al., 2022). Considering the huge amount of wood waste generated and its frequent mixing with other organic wastes that make it difficult to be recycled, the potential of AD to transform wood waste into energy is significant (Gao et al., 2024a). It offers a dual benefit of waste reduction and energy production. By converting wood waste into biogas, not only is the volume of waste significantly reduced, but the gas produced can be used to generate electricity or heat, or can be processed into renewable natural gas and other biofuels (Gao et al., 2022). This method provides a renewable, carbon-neutral energy source that can help meet emission targets while simultaneously addressing the issue of waste disposal.

However, the high lignin content and recalcitrant crystalline cellulose structure of wood waste complicate the efficient and timely production of biogas, thereby limiting its application in AD (Li et al., 2019). To effectively utilise wood waste through AD, several challenges must be addressed. Firstly, the pretreatment of wood waste to improve the biogas potential from AD process is necessary. Pretreatment processes such as mechanical, chemical, or biological techniques are required to increase the biodegradability of wood waste (Xu et al., 2019). Secondly, the composition of wood waste can vary greatly depending on its source and prior treatment, affecting its suitability for AD. In addition, the efficiency of wood waste AD depends on various factors including the microbial consortia involved, the process conditions such as pH, temperature, and retention time, and the design of the anaerobic digester (Chew et al., 2021; Meegoda et al., 2018). Optimizing these parameters is crucial for maximizing biogas yield and making the process economically viable. Although there are many pretreatment technologies available for AD of wood waste (Ali et al., 2021b; da Silva et al., 2019), they are developed on a laboratory scale ignoring environmental impacts and economic viability. The existing pretreatment approaches are often costly, energy intensive, and potentially environmentally hazardous, which frequently precludes their application in practice.

Forests play an important role in the global carbon cycle, with plants like trees and bushes absorbing energy through photosynthesis and storing it as carbohydrates in their biomass, which is then partly released into the atmosphere with the decomposition of deadwood in the forest soil (Seibold et al., 2021). Forestry operations generate large amounts of wood wastes, including branches, treetops, and stumps, which are left in the forest soil and may increase fire risk if unmanaged (Lee and Han, 2017). Forest soil ecosystems have evolved to manage fallen timber and deadwood with efficient processes brought about by a range of organisms including fungi, bacteria, and insects. Unless harvested for application, the fate of these forest residues is to be degraded into humus as part of the forest soil. During this degradation process, the texture of the wood will gradually become soft, as the rigid and recalcitrant lignocellulosic structure is slowly destroyed, with the wood finally being completely decomposed to release all the nutrients (Petritan et al., 2023; Shorohova et al., 2021). The tough texture and recalcitrant lignocellulosic structure of wood waste limit its application in AD (Hashemi et al., 2022; Karami et al., 2022), though the degradation process tends to predispose wood waste for AD applications. Therefore, it is essential to explore the methane production potential of wood waste at different decay stages in the forest. Identifying the decay stage at which the wood waste can have the highest methane production allows for strategic collection of this material for AD application. This targeted collection optimizes the efficiency of methane production and enhances the economic viability of using wood waste as a raw material in AD applications. The study will also identify the key properties of wood waste that are beneficial for AD and provide guidance for enhancing the AD performance of wood waste.

1.2 Scope and limitations

This thesis evaluates the potential of using wood waste as a feedstock for AD and mainly investigates the use of forest soil ecosystems to naturally enhance methane yield from this waste. However, several limitations exist in this study. Firstly, the variability in wood waste composition, which depends on its source and prior treatment, may affect the generalizability of the findings. The research primarily considers laboratory-scale analyses, which may not fully capture the complexities of large-scale industrial applications. Additionally, while the study investigates the potential of natural degradation processes in forest soils, the variability in soil ecosystems and climatic conditions could limit the applicability of the results across different geographic regions. The economic feasibility of scaling up the proposed methods, including the costs associated with pretreatment technologies and potential environmental impacts, are beyond the scope of this study. Future research should address these limitations by conducting pilot-scale trials and a more comprehensive economic and environmental analysis.

1.3 Aims and research questions

The overarching objective was to explore a viable approach for enhancing the AD performance of wood waste by leveraging a nature-based degradation processes in forest soil ecosystems. This research investigated the feasibility of using wood waste as a feedstock for AD and explored whether various pretreatment methods could boost methane production. Additionally, this work examined how forest soil ecosystems contribute to the degradation of wood waste, thereby impacting methane yield. Specifically, forest soil ecosystems have evolved to efficiently manage fallen timber and deadwood by breaking down the lignocellulosic structure, and may be feasible as a technology for improving the AD performance of wood waste. This study will explore in detail the influence of forest soil systems on the basic properties of wood waste and its corresponding biochemical methane production.

The research questions of this research work are as follows:

RQ1 Can wood waste be used as a feedstock for AD?

RQ2 To what extent can pretreatment methods improve the biochemical methane potential (BMP) of wood waste?

RQ3 Can the forest soil ecosystem (nature-based system) be used as an approach for improving the AD performance of wood waste?

RQ4 What factors in the forest determine the rate of wood decay and affect its BMP?

1.4 Thesis organisation and structure

- *Chapter 1* highlights the problem that was investigated in this project, the overall aims, and the research questions.
- *Chapter 2* presents basic information on AD, reviews the types of lignocellulosic wastes that can be used in AD, and provides details on the major barriers to the application of wood waste as a feedstock for AD. In addition, it explores biological pretreatment technologies applicable to wood waste and identifies gaps between current pretreatment technologies and their practical applications. The main objectives of this thesis are further presented based on the existing gaps.
- *Chapter 3* addresses RQ 1 and RQ2, which explores the potential of wood waste as a substrate for AD through meta-analysis and investigates how various treatment technologies can increase methane production. Additionally, using machine learning techniques, it examines the impact of different AD parameters on methane yield from wood waste and constructs a model for predicting methane yield.

- *Chapter 4* addresses RQ 3, which explores the feasibility of a natural-based system as a pretreatment approach for wood waste.
- *Chapter 5* addresses RQ 4, which examines the impact of the forest soil system on the degradation of wood waste and the subsequent methane production from wood waste.
- *Chapter 6* provides a summary of the findings of the thesis and discusses future work.

Chapter 2 Literature review

2.1 Overview of anaerobic digestion

Biogas production in 2018 was around 35 million tons of oil equivalent, only a fraction of the estimated overall potential (730 million tons of oil equivalent). It is estimated that the availability of sustainable feedstocks for producing biogas is set to grow by 40% over the period to 2040, which avoids around 1000 million tons of greenhouse gas emissions (IEA, 2020). Based on studies, the primary sources of methane emissions in the United States in 2015 were livestock operations, landfills, and wastewater treatment facilities. These sources accounted for about 45% of methane emissions (measured in carbon dioxide equivalent) (EPA, 2018). AD is an approach of recovering energy from waste that diverts organic waste from landfills, produces renewable energy, and reduces greenhouse gas emissions (Costa et al., 2015). The number of AD facilities in the United States will increase from 2000 to more than 11000 if the proper type of support can be provided (EPA, 2014). Although market conditions are increasingly favorable for AD, it still faces a variety of social and economic barriers that prevent it from reaching its full potential (Linville et al., 2015; Shen et al., 2015).

2.1.1 Process mechanisms of anaerobic digestion

The AD process happens through four sequential stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Li et al., 2011). These steps are a synergistic process of diverse microbial groups, and microbial metabolic activities at different stages mutually affect each other, in close dependence on each other. In a batch reactor, all feedstocks are loaded at the same time, and all four stages of the AD process take place consecutively in the same reactor. The digestate will be emptied at the end of the given retention period or after the cessation of biogas production (Meegoda et al., 2018). Figure 2-1 depicts a simplified flow of the four AD stages.

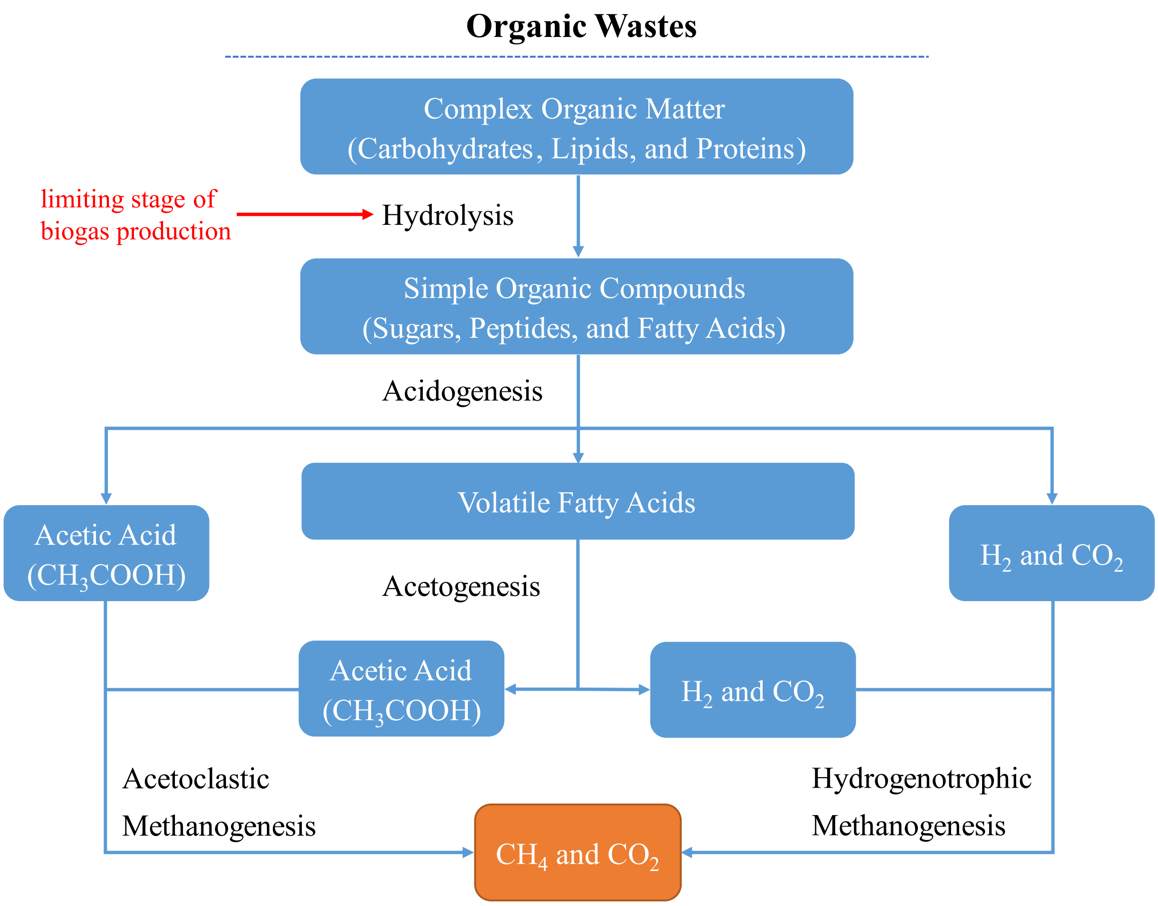

Figure 2-1. Degradation pathways during anaerobic digestion of organic wastes, modified from reference (Cheng and Brewer, 2021).

2.1.1.1 Hydrolysis

AD systems commonly encounter organic biomass containing complex polymers that cannot be utilized by microorganisms without further decomposition through hydrolysis or pretreatment (Gujer and Zehnder, 1983). Therefore, the hydrolysis process aims to convert organic macromolecules into smaller components that can then be utilized by acidogenic bacteria. During hydrolysis, hydrolyzing bacteria can secrete extracellular enzymes for converting carbohydrates, proteins, and lipids into sugars, amino acids, and long-chain fatty acids, respectively (Li et al., 2011). The hydrolysis products can diffuse across the cell membranes of acidogenic microbes after enzymatic cleavage (Lier et al., 2008). However, it is worth noting that some substrates (like lignin, hemicellulose, and cellulose) may be difficult to degrade and may be inaccessible to microbes because of the recalcitrant structure of lignocellulose.

Hydrolysis is a rate limiting step in biogas production, although previous studies have also shown that the methanogenic stage may also be a rate determining step depending on the proportion of hydrolyzed and methanogenic microbes (Luo et al., 2012; Ma et al., 2013). Owing to the importance of hydrolysis in AD kinetics, a considerable amount of attention has been focused on how to accelerate the hydrolysis process. At present, various waste pretreatment protocols are being investigated and utilized to optimize the hydrolysis process, especially in AD systems which contain large quantities of lignocellulosic waste (Kumar and Sharma, 2017). In general, the optimum temperature for microbiological hydrolysis is between 30–50 ℃, and the optimum pH is between 5–7, but no evidence has been found to suggest that hydrolytic activity increases at pH values lower than 7 (Azman, 2016).

2.1.1.2 Acidogenesis

By taking up hydrolysis products through cell membranes, acidogenic microbes have the ability to produce volatile fatty acids and other products. In acidogenesis, the reduced monomers (amino acids, peptides, long-chain fatty acids, glycerides, and sugars) are further degraded by facultative aerobes to volatile fatty acids (53–58% acetic acid, 6–13% propionic acid and 30–35% butyric acid) and other minor products (alcohols, aldehydes, hydrogen, and carbon dioxide) (Ali et al., 2018; Angelidaki et al., 2011). The concentration of these intermediates produced during acidogenesis can be affected by the conditions of the AD system. It has been reported that the concentration of volatile fatty acids fluctuated considerably in different pH conditions, and the results from different studies seem to be contradictory (Huang et al., 2015; Wu et al., 2010).

It is generally recognized that acidogenesis is faster than all other stages of AD, and the regeneration time of acidogenic bacteria is less than 36 hours compared to the other stages (Deublein, 2010). It is noteworthy that the acidification of volatile fatty acids has been widely reported as a contributor to AD failure, although the production of volatile fatty acids provides an immediate precursor to the methanogenesis stage (Akuzawa et al., 2011). Degradation of amino acids to volatile fatty acids usually occurs in pairs via the Strickland reaction, and individual amino acids may also be degraded if hydrogenotrophic bacteria are present, but the latter process is slower than the Strickland reaction. An important product of amino acid catabolism is ammonia produced by deamination, which is known to be an inhibitor of AD at a sufficiently high concentration (Kovács et al., 2013; Park et al., 2014).

2.1.1.3 Acetogenesis

After the production of acetate, parts of the raw materials have been turned into suitable substrates for acetoclastic methanogenesis (Fournier and Gogarten, 2008). Yet, the high concentration of volatile fatty acids generated cannot yet be fully utilized by methanogenic microbes. During acetogenesis, syntrophic bacteria transform previous volatile fatty acids and alcohols into acetate, carbon dioxide, and hydrogen which are substrates for the production of methane (Angelidaki et al., 2011; Cheng and Brewer, 2021). Although the acetogenesis process produces hydrogen, excessive partial pressures are detrimental to methanogenic microorganisms (Dinopoulou et al., 1988). However, hydrogen can be rapidly consumed because of the presence of hydrogenotrophic methanogens, while maintaining the hydrogen partial pressure at a level favorable for acetic acid production by generating an exergonic reaction (Stams and Plugge, 2009).

In addition, lipids undergo a proprietary acetogenesis pathway through acidogenesis and β-oxidation, where acetate is produced from glycerol in the acidogenesis pathway and from low-carbon fatty acids in the β-oxidation pathway. In view of this, it is important that only low carbon fatty acids with even numbers of carbon atoms can be degraded to acetate. The low carbon fatty acids with odd numbers of carbon atoms will degrade first to propionate (Cirne et al., 2007).

2.1.1.4 Methanogenesis

The final step is methanogenesis, where methanogenic microorganisms convert acetate, carbon dioxide, and hydrogen to generate methane, mainly consisting of acetoclastic methanogenesis (60–70%) and hydrogenotrophic methanogenesis (about 30%) (Cheng and Brewer, 2021; Izumi et al., 2010). Methanogenesis in batch reactors typically stops after about a 40-day period of operation (Meegoda et al., 2018). Methanogenic microbes belong to anaerobic archaea which is extremely sensitive to oxygen. Research revealed that within ten hours of being exposed to oxygen, 99% of *Methanococcus voltae* and *Methanococcus vannielli* cells had perished (Kiener and Leisinger, 1983). Apart from their oxygen sensitivity, methanogenic bacteria feed on a limited range of substrates. Generally, two-thirds of methane production is attributed to acetoclastic methanogenesis from acetate, and the remaining one-thirds of methane is generated from hydrogenotrophic methanogenesis. Besides of these principal routes, methanogenesis derived from methanol, methylamine, and formate has also been reported (Belay et al., 1986; Lovley and Klug, 1983).

In terms of environmental requirements for methanogenesis, methanogenic microbes typically require a higher pH than earlier stages of AD, as well as a lower redox potential, which has presented substantial problems in laboratory cultivation (Wolfe, 2011). In addition, methanogens tend to regenerate at a substantially slower rate than other bacteria in AD, ranging from 5 to 16 days (Deublein, 2010). Certainly, some methanogen strains have a faster growth rate. It has been reported that *Methanococcus maripaludis* can replicate twice in only two hours (Richards et al., 2016). Although methanogenic species may be the most sensitive microbial group in AD, recent studies have revealed that *Methanosarcina* spp. seems to be stronger and able to withstand high ammonia and pH shocks that would otherwise be harmful to other methanogenic microorganisms (De Vrieze et al., 2012).

2.1.2 Major factors affecting biogas production

The AD process is influenced by several key factors, including feedstock characteristics (Y. Chen et al., 2019; Taifouris and Martín, 2018), temperature (Krause et al., 2018a; Wei et al., 2014), pH (Guilford et al., 2019), microbial community (Mirmohamadsadeghi et al., 2021), total solids (TS) content (Pearse et al., 2018), inhibitory compounds (Barik and Murugan, 2015), etc. In general, temperatures of 10–65 °C (Khalid et al., 2011), pH of 5.0–8.5 (5.5–7.0 for hydrolysis and acidogenesis, 6.8–8.5 for methanogenesis) (Khalid et al., 2011; Park et al., 2008), carbon to nitrogen ratio (C/N) of 20–35 (Lee et al., 2009; Zhang et al., 2014) and TS content of 20–40% (Bouallagui et al., 2003) are the optimal ranges for methane yield. Temperature range can vary widely during AD, and it is divided into three categories according to the microbial activity, psychrophilic: 10–20 °C; mesophilic: 20–45 °C (usually 37 °C); and thermophilic: 50–65 °C (usually 55 °C) (Khalid et al., 2011; Yu and Fang, 2003). Compared to the effect of temperature, pH plays a more important role in AD. The microbes involved in AD, especially methanogens, are sensitive to the acid concentration in the system. The growth of methanogens could be inhibited at lower pH conditions, and the optimal pH for methanogenesis has been found to be around 7.2 (Huber et al., 1982). Improper C/N will result in the release of a large amount of ammonia nitrogen or the excessive accumulation of volatile fatty acids, which are inhibitors in AD process (Matheri et al., 2018). Therefore, an appropriate temperature, pH and C/N are needed for maintaining a stable environment in a long-term operation.

While temperature, pH, and C/N can be adjusted by controlling the operating conditions, reactor configuration, and the concentrations of N-containing additives (Kondusamy and Kalamdhad, 2014; Romero-Güiza et al., 2016; Zhang et al., 2011), feedstock characteristics have specific impacts on methane production. Feedstock characteristics include chemical composition (Wang and Barlaz, 2016), volatile solid (VS) content (Wang et al., 2013), chemical oxygen demand (Muaaz-Us-Salam et al., 2020), morphology (particle size and porosity) (Krause et al., 2018a), and nutrient content (Oh et al., 2018). These characteristics relate to biodegradability of components, VS conversion rate, hydraulic retention time, and ultimately impact BMP (Cheng and Brewer, 2021). In lignocellulosic waste, cellulose and hemicellulose are the major contributors to methane formation. They are easily degraded because of their less complicated molecular structure (Kumar et al., 2018). However, the presence of lignin limits methane production by reducing the surface area that bacteria can access through a rigid lignin-cellulose-hemicellulose matrix or high cellulose crystallinity, thereby suppressing biodegradability of other components (X.

Liu et al., 2015; Roy et al., 2020). In addition, the decomposition of lignin produces inhibitors, such as phenolic aldehydes and acids, which may also inhibit the methanogenesis process (Chen et al., 2008).

2.1.3 Feedstocks for anaerobic digestion

A variety of plant and animal biomass can be used as bioenergy resources to produce biofuel through different treatment technologies. The biofuel production can be divided into four generations according to the biomass resources: i) First-generation biofuel is producing biofuels such as ethanol, propanol, and butanol through edible biomass fermentation, including sugar-based (sugarcane and sugar beets), starch-based (wheat, corn, and barley, etc.) feedstocks or any type of vegetable oil (oilseeds); ii) Second-generation biofuels primarily utilize non-edible lignocellulosic biomass, along with agricultural and forestry residues, as well as waste biomass. This generation technology produces biomethane from plant biomass after delignification, hydrolysis; iii) Third-generation biofuel is also known as "algae fuel" as algal oil is used as feedstock to produce biofuels like biodiesel, biobutanol, and biopropanol; iv) Fourth-generation biofuel requires an advanced method in biotechnology (metabolic engineering) for biofuel production.

The feedstocks of first-generation biofuel have limited sustainability credentials, which could rise competition between food and biofuel production. As some of the inputs to food production, such as certain agricultural crops, labor, land, and other agricultural resources, are also involved in the production of first-generation biofuel. Hence the more input devoted to the production of the first-generation biofuel, the less will be available to produce certain basic foodstuffs. Indeed, the nature of this competition includes aspects of both direct and indirect competition. The latter aspect covers indirect competition among natural and agricultural resources (such as land, other agricultural resources, and the use of machinery), as well as the use of labor and capital (Datta, 2022). Third-generation biofuel also faces significant challenges. Several researchers opine that algae biofuel is an impractical choice, as growing algae inside bioreactors greatly increases the energy inputs and the cost of production. In addition, the harvesting of algae and the separation of algal oils is a difficult and energy-intensive process, which makes such biofuel production limited and uneconomical (Gomiero, 2015). Bradley et al. (2023) conducted a life cycle assessment using actual data from an operational industrial facility combining the use of a photobioreactor and a fermenter system, and found that the environmental costs of constructing the infrastructure to grow and process the algae, and generating the electricity to power the operation, far outweighed the environmental costs saved by burning the microalgae biofuel. In the fourth-generation biofuel, bioengineered plants or algae function as a carbon capture machine for the feedstock generation of biofuel (Moodley, 2021; Saha et al., 2019; Stiles et al., 2018). However, the main environmental concerns of uncontrolled development of transgenic plants relate to competition between introduced plants and native species, alteration of natural habitats, and horizontal gene transfer and toxicity (Hewett et al., 2016). It is also worth noting that cultivating a transgenic plant that can be stably applied in practical requires a significant investment in labor and funds.

Second-generation biofuel is derived from lignocellulosic wastes. Compared with food-based biofuels, second-generation biofuels use raw materials such as crop straw, rice husk, sugarcane bagasse, sawdust, and other organic wastes. Generally, they may also be referred to as “non-food biomass” and are considered an economical renewable energy source due to their abundance and absence of residual impurities including sulfur or metals (Beller et al., 2015; Havlík et al., 2011). Lignocellulosic biomass constitutes most of the easily accessible and available non-food materials in plants (Naik et al., 2010). With a sugar content of over 70%, lignocellulose is the most abundant biomass in the world and can be converted into biofuels through a variety of physicochemical, and biological processes (Peralta-Yahya et al., 2012). It has been reported that the total amount of lignocellulosic biomass available for human consumption ranges from 7 to 18 billion tons annually (Lin and Tanaka, 2006). The conversion of lignocellulosic wastes into bioenergy involves three core processes, including pretreatment, enzymatic hydrolysis, and microbial fermentation (Lin and Tanaka, 2006). Nowadays, second-generation biofuels continue to be crucial to the development of sustainable energy strategies.

Lignocellulosic wastes can be the ideal feedstocks for AD since they do not conflict with food production and do not require additional energy and cost to grow these biomasses. The most available lignocellulosic wastes are agricultural waste/by-product (crop stalks and straws), wood and branches in forests and industry and some organic fraction from municipal solid waste (wood, paper and paperboard) (Roy et al., 2020), which could emit greenhouse gas if being left on the field or transported to landfill (Covey and Megonigal, 2019;

Obulisamy et al., 2016). These kinds of resources are considered as a renewable, cost efficient and ecofriendly resource for biogas output, and thus creating a global priority. There have been numerous reports that municipal solid waste, wood waste or crop straw can be used to generate biogas (Suhartini et al., 2024; Jin et al., 2022; Olatunji et al., 2021; Rahimi et al., 2020; Y. Chen et al., 2019). However, the main hurdles in utilizing lignocellulosic wastes lie in lignin barrier, accessible surface area for enzymatic hydrolysis, and cellulose crystallinity (Millati et al., 2020; Olatunji et al., 2021; Sun et al., 2021). Thus, efficient delignification and improved digestibility of cellulose and hemicellulose in lignocellulosic wastes is usually a crucial step of pretreatment. Up to now, scholars have developed a wide range of pretreatment technologies to improve the AD performance of lignocellulosic wastes. For example, Romero-Güiza et al. (2017) found that pretreatment of wheat straw with 4 % sodium hydroxide (g/g of TS) for 5 days at 37 ℃ increased the final methane yield from 78 to 166 mL/g of VS), which was an increase of 112 % compared to the unpretreated biomass; According to Rouches et al. (2018), biogas production increased by 52% after microbial pretreatment of wheat straw with *Polyporus brumalis* BRFM985; Li et al. (2020) investigated the degradability and biogas production potential of microbial pretreated corn stover, and the results showed that microbial pretreatment accelerated the degradation rate of corn stover, improved the degradation efficiency of lignin, and increased methane production. In summary, methane production from lignocellulosic wastes through AD has a broad prospect, which not only can achieve resource recovery from wastes and reduce their negative impact on the environment, but also can help to reduce the dependence on traditional fossil fuels, cut down on greenhouse gas emissions, and promote the development of renewable energy sources.

2.1.4 Types of lignocellulosic wastes for biogas production

Lignocellulosic wastes comprise dry plant materials and therefore cover a wide range of substances, including different grasses, plant stems, trees, and residues from modern sawmills and paper mills. It can be broadly classified into virgin biomass, energy crops and waste biomass. Nearly 200 billion tons of lignocellulosic wastes are generated globally every year (Zhang, 2008), mostly low value byproduct from various industrial sectors, human activities or the natural environment such as agriculture (e.g. crop straw and stalk), municipal waste (e.g. wood, paper and cardboard) and forestry (e.g. sawmills and paper mills discards, forest management waste). All of these lignocellulosic wastes have been recognized as valuable resources by the United States Department of Energy (Samuel et al., 2010).

2.1.4.1 Municipal solid waste

Municipal solid waste mainly consists of commercial wastes, residential wastes and yard wastes generated in municipal areas in either semisolid or solid form excluding agricultural wastes and industrial hazardous wastes but including treated biomedical wastes (Rao et al., 2017). The global level of municipal solid waste generation is estimated to increase to approximately 2.2 billion tons by 2025 (Hoornweg and Bhada-Tata, 2012). Low- and middle-income regions produce most municipal solid waste, accounting for up to 90.4% of the total (Hoornweg and Bhada-Tata, 2012). The yearly municipal solid waste generation in America and China are highest in the world at 292.36 million tons (2.49 kg per capita per day) and 228.02 million tons (0.45 kg per capita per day), respectively (Figure 2-2a) (EPA, 2017a; NBS, 2019). The disposal of municipal solid waste depends on national development levels. Landfilling and thermal treatment are valued in high income countries, while composting, and open dumping still account for a large proportion in low- and lower-middle income countries (Hoornweg and Bhada-Tata, 2012). Figure 2-2b shows the data from the Organisation for Economic Co-operation and Development (OECD) (2021), U.S. Environmental Protection Agency (EPA) (2017b) and Eurostat (2021), with different countries having very different combinations of waste disposal methods based on their own development and national conditions, however globally sanitary landfilling is currently the dominant municipal solid waste disposal method.

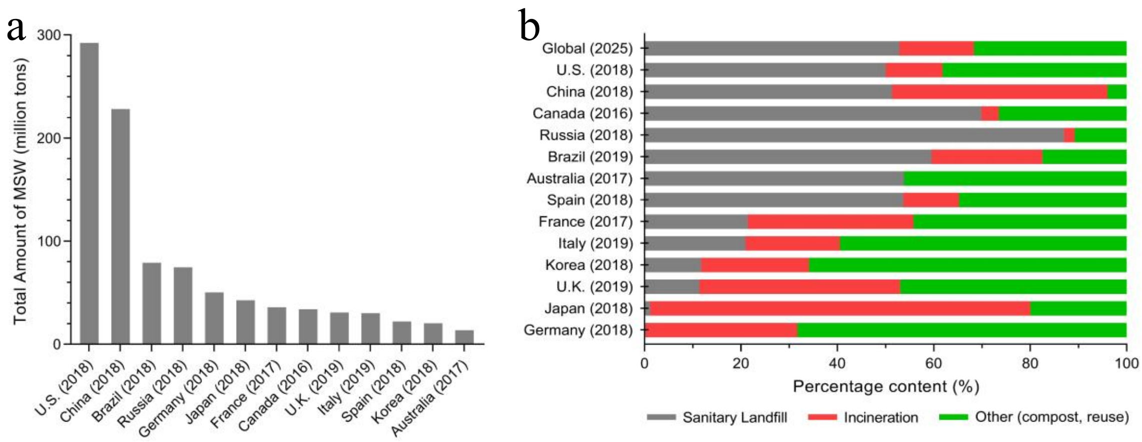

Figure 2-2. The total generation amount (a) and disposal methods (b) of municipal solid waste in different countries (EPA, 2017b, 2017a; Eurostat, 2021; NBS, 2019; OECD, 2021).

In most developing countries, municipal solid waste is not segregated at source and is transported into landfill in mixed conditions (Ferronato and Torretta, 2019). An average composition of municipal solid waste in different countries is presented in Table 2-1, showing that there exists a large percentage of lignocellulosic materials and suitable feedstocks for biogas, i.e., paper and paperboard, wood, and yard waste. Of these, wood, paper and paperboard wastes are likely to have more recycled value, since they can be reprocessed into particleboard or new cardboard (Besserer et al., 2021). However, a significant fraction of wood and paper wastes are nonrecyclable with chemical preservatives, binders or metal protectants (Dexter et al., 2019). The main destination of these lignocellulosic wastes is landfill, Table 2-2 shows lignocellulosic waste data from 1990 to 2018 in the USA (EPA, 2017b). About 15.6 million tons of wood waste is produced each year, with 71.5% landfilled. An average of 74.2 million tons of paper and cardboard waste are produced each year, with 36.9% landfilled.

Table 2-1. National average municipal solid waste composition.

Country	Organic (%)	Paper (%)	Plastic (%)	Glass (%)	Metal (%)	Other (%)
Global	46	17	10	5	4	18
Low Income	62	6	9	3	3	17
Lower Middle Income	55	10	13	4	3	15
Upper Middle Income	50	15	12	4	4	15
High Income	28	30	11	7	6	18
China	59	8	10	3	1	19
India	40	10	2	0.2	0	47.8
U.S.	39.9	23.1	12.2	4.2	8.8	11.8
Russia	40	19	14	12	4	11
Brazil	51.4	13.1	13.5	2.4	2.9	16.7
Indonesia	74	10	8	2	2	4
Nigeria	68	10	7	4	3	8
Pakistan	67	5	18	2	0	7
U.K.	46	17	10	7	5	15
Germany	14	34	22	12	5	12
Netherlands	35	26	19	4	4	12
Australia	47	23	4	7	5	13
Mexico	51	15	6	6	3	18
Portugal	34	21	11	7	4	23
Italy	44.5	19.1	8.3	12.3	2	13.8
Spain	44	18	13	9	4	12
Japan	34	34	11.8	4.3	4.7	11.2
Canada	47	15	13	2	3	20

Note: Classification according to Hoornweg and Bhada-Tata (2012). Organic: food scraps, yard waste, wood, process residues; Paper: paper scraps, cardboard, newspapers, magazines, bags, boxes, wrapping paper, telephone books, shredded paper, paper beverage cups; Plastic: bottles, packaging, containers, bags, lids, cups; Glass: bottles, broken glassware, light bulbs, colored glass; Metal: cans, foil, tins, non-hazardous aerosol cans, appliances (white goods), railings, bicycles; Other: textiles, leather, rubber, multi-laminates, e-waste, appliances, ash, other inert materials. All data are adapted from OECD (2021), EPA (2017b), Statista (2021), Ding et al. (2021), Khan et al. (2022), Millati et al. (2019) and Alfaia et al. (2017).

Table 2-2. Data on lignocellulosic composition of United States municipal solid waste from 1960 to 2018 (ten thousand tons).

Types	Management Pathway	1960	1970	1980	1990	2000	2005	2010	2015	2017	2018
Wood	Generated	303	372	701	1221	1357	1479	1571	1630	1820	1809
	Recycled	-	-	-	13	137	183	228	266	303	310
	Incinerate	-	1	15	208	229	227	231	257	288	284
	Landfill	303	371	686	1000	991	1069	1112	1107	1229	1215
Paper and paperboard	Generated	2999	4431	5516	7273	8774	8484	7131	6805	6701	6739
	Recycled	508	677	1174	2023	3756	4196	4457	4532	4417	4597
	Incinerate	-	15	86	893	973	780	474	445	449	420
	Landfill	2491	3739	4256	4357	4045	3508	2200	1828	1835	1722

Note: - means no data. Data is adapted from United States EPA (2017b).

Landfilling, if inappropriately performed or poorly operated, may contaminate the atmosphere with greenhouse gas emissions from the slow degradation of lignocellulosic waste. The presence of abundant lignin endows lignocellulosic waste with recalcitrance; it is difficult to break down through microbial action (Cragg et al., 2015). These poorly degradable fractions are typically associated with a long ‘tail’ of emissions and gradually accumulate in landfill. Furthermore, these slowly produced gases are insufficient to generate energy and difficult to capture, so the biogas (primarily methane and carbon dioxide) typically escapes into the atmosphere contributing to climate change (O’Dwyer et al., 2018).

Accelerating the degradation of lignin and subsequent methanogenesis in lignocellulosic waste is required to help to confine methane production to a shorter period of higher concentration release, thus allowing more landfill biogas to be collected as energy and preventing low emission of greenhouse gas in the long term. This can be addressed with biotechnological methods in two main ways – the application of extracellular enzymes (Schroyen et al., 2017, 2015; Ufarté et al., 2018) or enzyme-producing microorganisms (Dollhofer et al., 2018; Rahimi et al., 2020; Ranganathan et al., 2017; Sanitha et al., 2021). However, these previously mentioned studies have been carried out under laboratory level with highly controlled conditions or standardized materials, scaling-up these technologies to landfill conditions will be highly challenging and is yet to attract significant attention.

2.1.4.2 Forest residue

Forests are the largest terrestrial carbon sink and play a vital role in the global carbon cycle, where plants absorb energy through photosynthesis and store it in wood as carbon (Grassi et al., 2017; Hardersen and Zapponi, 2018). The current carbon stock in forests is estimated to be 861 billion tons, with the vast majority in soil (44%) and live biomass (42%) (Pan et al., 2011). There are two main types of wood, hardwood and softwood. Hardwoods arise from deciduous trees (e.g. oak, maple, birch) while coniferous trees (e.g. pine, spruce, juniper) produce softwoods. In 2020, global hardwood lumber production reached 2536.7 million m^3, mainly in Asia, Africa, the Americas and Europe (Table 2-3) (FAOSTAT, 2020). Global softwood production was 1375.25 million m^3, mostly in Europe and the Americas.

Table 2-3. Quantity of hardwood and softwood production and residues in 2020.

Types	Production quantity (million m^3)	Waste quantity (million m^3)	Regions
Hardwoods	995.08	102.24	Asia
	760.64	1.56	Africa
	515.78	23.49	Americas
	58.77	0.59	Central America
	134.05	5.25	Northern America
	317.33	20.05	South America
	231.87	17.57	Europe
	33.34	0.99	Oceania
	2536.7	151.05	World
Softwoods	163.83	16.83	Asia
	30.97	0.06	Africa
	555.14	25.28	Americas
	35.64	0.35	Central America
	427.83	16.77	Northern America
	91.17	5.76	South America
	571.81	43.33	Europe
	53.5	1.59	Oceania
	1375.25	81.89	World

Woody debris, comprising fallen dead trees and the remains of large branches on the forest ground, represents a large carbon pool with carbon stock ranging from 36 to 72 billion tons globally (Russell et al., 2015). Unless woody debris is harvested it will ultimately convert to lignoforms (humus forms formed by the degradation of deadwood) as a part of the soil (Tatti et al., 2018). In this process of woody debris being gradually decayed into lignoforms by decomposer communities, most of the carbon is returned to the atmosphere as methane and carbon dioxide (Covey and Megonigal, 2019). Since methane and carbon dioxide are both greenhouse gases of great concern for climate change (Saunois et al., 2020), this natural process was only recently recognized as an important sources of greenhouse gas, with estimates of carbon flux at 8.6 billion tons annually, equivalent to approximately 90% of anthropogenic emissions (Le Quéré et al., 2013).

Excepting dead wood produced by natural processes, human activities are the main source of woody debris. The harvesting of approximately 4.3 billion m^3 of wood annually (FAO, 2020) is estimated to generate 232.94 million m^3 of wood waste may be produced in the world every year, mainly in Asia and Europe (Table 2-3). Woody debris are generated during forestry operations (branches, treetop, leaves, stumps, low grade and decayed wood, slashings, sawdust) and wood processing (bark, sawdust, trimmings, planer shavings, core, screening fines), which also are classed as wood waste. These wood wastes are potential resources for bioenergy production that may have a significant impact on the profitability of the entire timber trade value chain, offsetting the negative impacts of forestry operations on ecosystem services and biodiversity (Ranius et al., 2018; Sántha and Bentsen, 2020). The added value of producing biofuel from these wood waste also comes from reducing fire risk, mitigating forest management costs, and eliminating additional emissions from degradation (Lee and Han, 2017; Nicholls et al., 2018).

Direct large-scale combustion of wood waste to generate energy or electricity is no longer considered an efficient and environmentally appropriate option. Thus, attention must be paid to develop alternative options for renewable biofuel (Amirta et al., 2016). However, the refractory nature of wood waste and the immaturity of current AD technology at the application-level limit practical examples of biogas production from wood waste. The lignin content in wood is quite high, 25–39% in softwood and 18–25% in hardwood respectively (Millati et al., 2019), which is not conducive to the degradation process of microorganisms or enzymes. To make fuller use of wood waste to generate more biogas, researchers screen for new high-efficiency lignin-degrading microorganisms (Akyol et al., 2019; Ali et al., 2017), or use the addition of other nitrogen-rich wastes, such as food waste or animal manure, to create a favorable condition for fermentation (Oh et al., 2018).

2.1.4.3 Crop straw

Agriculture wastes mainly include crop residues and livestock excreta, among which crop straw is a potentially valuable lignocellulosic waste with huge yields. As a by-product of grain production, crop straw is inevitable and its corresponding relationship with grain output is shown in the Table 2-4 (Kim and Dale, 2004; Yan et al., 2021). Based on the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT, 2022), the average annual crop straw production in the world from 2010 to 2022 can be calculated (Table 2-4). The amount of sugarcane bagasse ranked top in the world at 18575.1 million tons, followed by rice straw, corn straw, wheat straw and barley straw respectively, and the last are cotton and fiber crops. Asia is the region that produces the most food with East Asian countries like China and India major growers of crops (Nguyen and Nguyen, 2021). It is estimated that 1000 million tons of crop straw are produced yearly in China (Zhao et al., 2017), while India produces a total of 500 million tons (Kapoor et al., 2020).

Table 2-4. Quantities of crop straw reportedly by region, average 2010–2020 (million tons).

Types	**Ratio of Straw/Grain**	**Crop production**					
		World	Asia	Americas	Europe	Africa	Oceania
Rice	1.6	734.8	661.7	36.9	4.2	31.4	0.6
Barley	1.0	143.3	21.4	18.7	87.1	6.5	9.5
Corn	0.5	1038.2	325.7	520.0	114.4	77.5	0.6
Wheat	0.7	724.6	321.3	113.4	240.1	25.9	23.9
Sorghum	1.3	60.7	9.0	22.1	1.1	27.0	1.6
Oat	1.3	22.9	1.1	6.2	14.1	0.2	1.3
Beans	0.7	22.0	20.1	0.3	0.9	0.7	0.044
Tubers	2.0	6.8	3.7	0.9	0.1	1.7	0.4
Cotton	0.3	24.9	16.3	5.9	0.3	1.6	0.7
Fiber crops	0.4	0.6	0.4	0.1	0	0.048	0.0042
Sugarcane	10.0	1857.5	729.5	1001.8	0.0057	93.2	33.0

Crop straw has a low nutritional value and so only a limited amount has been traditionally used as livestock feed with the rest commonly burned in the field or sent to landfills (Gao et al., 2019). Open burning of crop straw not only produces particulate matter posing a serial health risk but also is a major cause of environmental pollution, including greenhouse gases and soil fertility destruction (Bhuvaneshwari et al., 2019; Sawlani et al., 2019). Crop straw burning varies by different countries, depending on the type of crop straw and the pattern of its management. Chen et al. (2019) claimed that Chinese farmers burned approximately 25% of crop straw, while this ratio would rise to 50% in line with FAOSTAT (FAOSTAT, 2022). China, India, United States, Brazil, Russian Federation, Indonesia, Argentina, Nigeria, Ukraine, and Thailand are the top 10 countries in terms of quantity of burned crop straw (Table 2-5). The burning of crop straw leads to inefficient utilization of agricultural waste and an increase in air pollution, which has drawn attention in various parts of the world to develop a proper plan for managing crop straw. Over the past few years, especially since 2015, different international agencies have proposed many avenues to utilize crop straw to minimize crop straw related issues (Sarkar et al., 2021).

Table 2-5. Top 10 countries of crop straw burning in the world in 2019 (FAOSTAT, 2022).

Countries	**Biomass burned (million tons)**	**CH_4 emission (kilotons)**	**N_2O emission (kilotons)**
China	68.2	184.2	4.8
India	48.1	129.9	3.4
USA	39.8	107.3	2.8
Brazil	25.9	69.8	1.8
Russian	13.6	36.8	1.0
Indonesia	11.8	31.9	0.8
Argentina	10.1	27.2	0.7
Nigeria	9.8	26.6	0.7
Ukraine	7.7	20.9	0.5
Thailand	7.5	20.2	0.5

A core sustainable development goal is the transition to a circular economy, which involves minimizing resource inputs and waste outputs within a closed-loop system pioneering wastes as secondary resources (Ghisellini et al., 2016; Kirchherr et al., 2017). Use of crop straw as material to generate biogas through AD is in line with achieving a circular economy. The biogas production rate of main crop straw residues is shown in Table 2-6 (Kim and Dale, 2004; Yan et al., 2021). As a clean renewable energy, biogas can alleviate energy shortages and minimize air pollution risk from the improper management of crop straw. There is little biogas production from agricultural waste currently, although the supply of raw crop straw is plentiful. In India, only 2.07 billion m^3 biogas are currently produced per year, though there is the potential for 29–48 billion m^3 each year based on straw volume (Mittal et al., 2018). The biogas industry of China is considered to have great potential, owing to tremendous amount of crop straw. Nevertheless, the ratio of actual biogas production to total biogas potential is only 6.17% (Chang et al., 2014). The biogas potential of crop straw is still underexplored due to an imperfect supply chain and viable business models, lack of simple pre-treatment technologies, insufficient short-term returns, and shortage of advanced technology (Kapoor et al., 2020). Many small-scale biogas plants have been operating for decades, although large-scale technically advanced biogas plants are uncommon and a recent development (Igliński et al., 2020). The priority currently is to improve the biogas potential from crop straw, which could help to eliminate air pollution threats and develop clean energy.

Table 2-6. Dry biomass ratio and biogas production rate of crop straw.

Types of crop straw	**Dry matter (%)**	**Carbohydrates (%)**	**Biogas yield (m^3/kg of dry biomass)**
Rice straw	88	49.33	0.43
Barley straw	81	70.00	0.48
Corn straw	78.5	58.29	0.46
Wheat straw	90.1	54.00	0.45
Sorghum straw	88	61.00	0.41
Oat straw	89.1	59.10	0.40
Beans straw	80	54.48	0.40
Sugarcane bagasse	71	67.15	0.43

2.1.5 Anaerobic digestion performance of different lignocellulosic wastes

The chemical composition of the feedstock and digestion parameters have a large impact on methane production. To explore the connection in detail, a mesophilic digestion (about 37 °C) with the following parameters fixed, initial pH of AD (about 7), AD time (30–50 d), inoculum was selected, which were the commonly used conditions in AD of lignocellulosic waste. There is a highly positive correlation between methane and biogas yield (Figures 2-3a and 2-3b). Additionally, lignin content and lignin content to holocellulose content ratio show a moderate negative correlation with methane yield, revealing that the presence of lignin could limit AD of lignocellulosic wastes (Figure 2-3a). Among lignocellulosic wastes, crop straws and plant residue like peel and reed, have the highest methane yield of 161.2 and 191.8 mL/g VS, respectively. The methane yield of mixed wood wastes, yard wastes and leaves are 121.8 and 65.9 mL/g VS, respectively. However, co-digestion could optimize the chemical composition of feedstock and C/N, finally improving methane yield (236.7 mL/g VS) (Figure 2-3c).

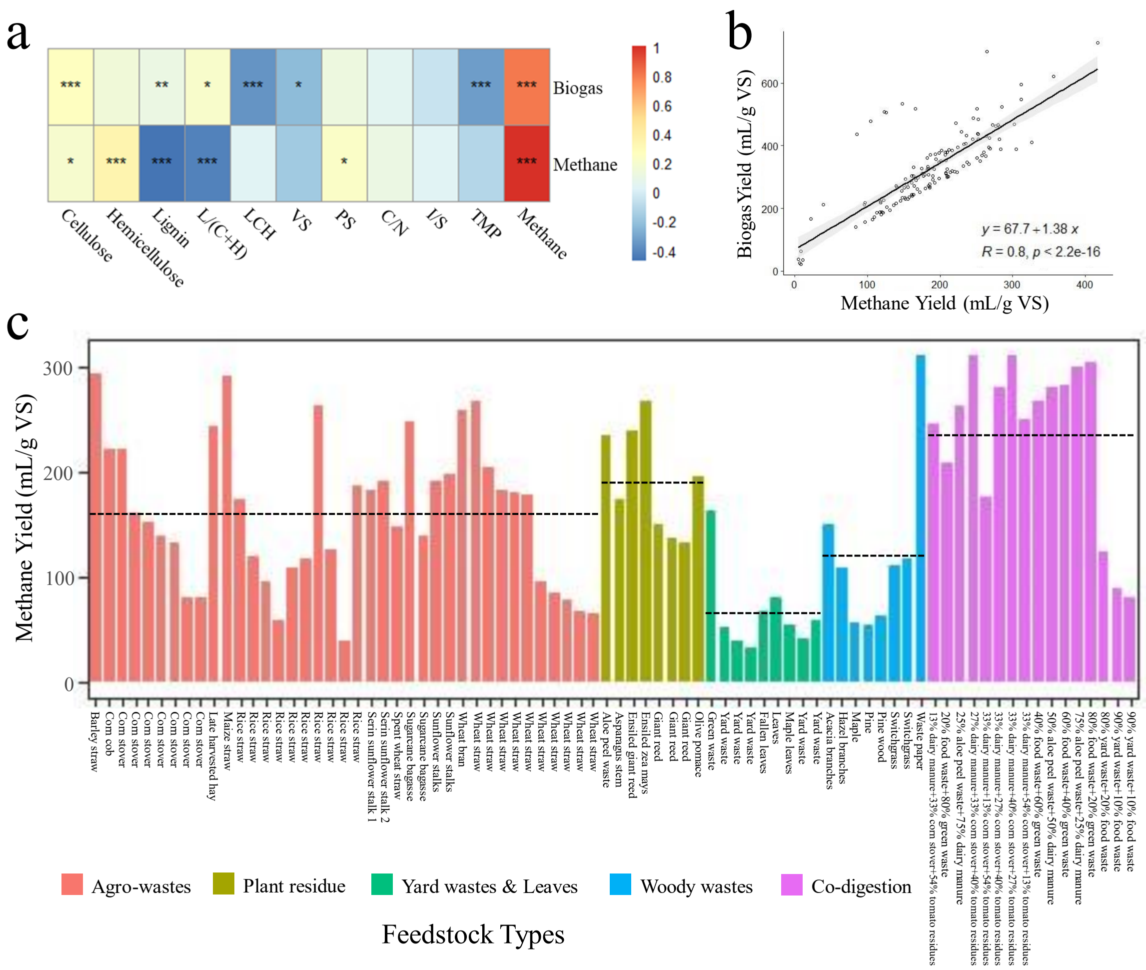

Figure 2-3. (a) The correlation between parameters and product yield. (b) Curve fitting between methane and biogas yield. (c) Methane yield of different lignocellulose types (delete data containing pretreatment process). All figures are plotted from the data in Table A-1. Lignocellulosic waste types are distinguished by color. Black dashed lines represent the average value for each waste type. L/(C+H): lignin content/(cellulose content + hemicellulose content); LCH: lignocellulose content (%)=lignin content + cellulose content + hemicellulose content; VS: volatile solid; PS: particle size; I/S: inoculum to substrate; TMP: theoretical methane potential (mL/g VS) were calculated according to the lignocellulose content (Kim et al., 2015).

At different C/N and inoculum to substrate ratio, the fitting curves of feedstock lignocellulose composition and methane production show that cellulose and hemicellulose are positively correlated with methane production overall (Figure 2-4). Generally, lignin limits methane production; however, under certain conditions, the utilization of lignin in lignocellulosic wastes can be enhanced. For example, through the co-digestion with other nitrogen-rich wastes to achieve the best digestion C/N of materials. Under standard temperature and pressure conditions, the methane yield potential of lignin (727 mL/g VS) is much higher than that of cellulose (415 mL/g VS) and hemicellulose (424 mL/g VS) (Chen et al., 2014). At the recommended C/N (25–30), some studies used sodium hydroxide solution for pretreatment (Gao et al., 2022). Alkali pretreatment is considered as an effective method for maximizing degradation of complex materials, in breaking ester bonds between lignin and other compounds along with preventing hemicellulose fragmentation (Gunes et al., 2019). The availability of lignin components was improved by alkali pretreatment, and thus the methane yield tended to increase with lignin content (Figure 2-4c), which is consistent with the results of previous research (Mu et al., 2020). Methane yield showed a negative correlation with lignin content, whatever the variation of inoculum to substrate ratio (Figure 2-4f). Yin et al. (2000) reported lignin inhibits the utilization of substrate (acetate) by bacteria in AD sludge, leading to lower methane production, and this inhibition effect enhanced with the increase of lignin content. Moreover, this inhibition could not be compensated by adding more acetate in the early stage. In combination with microbiome studies in sludge, this situation may be due to the fact that the main microbial components in sludge do not have the ability to biodegrade lignin (Lu et al., 2020; Ozbayram et al., 2018), therefore the availability of substrate decreases as lignin content increases.

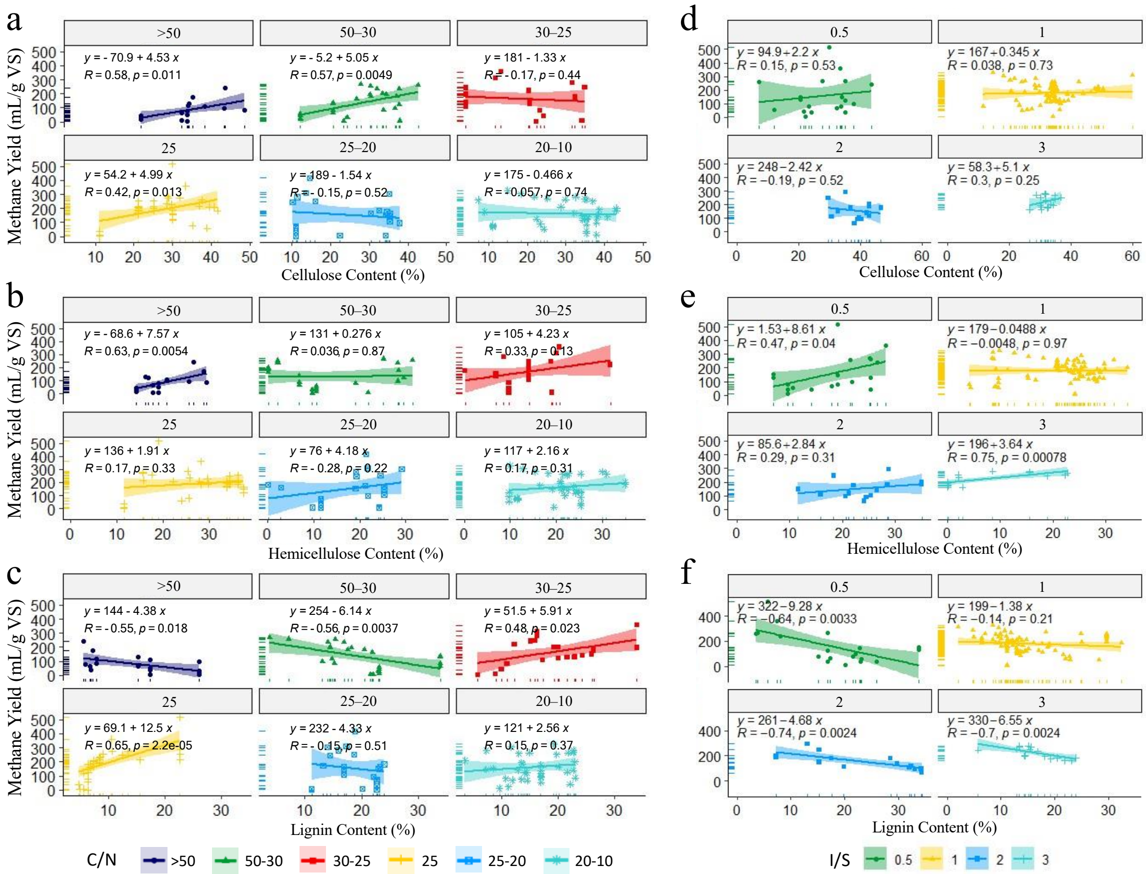

Figure 2-4. Curve fitting between methane yield and feedstock lignocellulose composition under different C/N and I/S. a and d: Cellulose content (%); b and e: Hemicellulose content (%); c and f: Lignin content (%). All figures are plotted from the data in Table A-1. C/N: carbon to nitrogen ratio; I/S: inoculum to substrate ratio.

2.2 The generation of wood waste

Globally, a large amount of wood waste is generated annually, which is not fully utilized due to the absence of a well-developed management system. It has been reported that Hong Kong generates about 1000 tons of wood waste per day (Hossain and Poon, 2018). A total of approximately 30 million tons of wood waste is created in Brazil every year, of which the wood industry accounts for 91% of the total, followed by the waste generated by urban environments and civil constructions, which account for 6% and 3%, respectively (Tuoto, 2009). The volume of wood waste in Europe in 2007 was estimated to be approximately 33 million tons, with significant differences between countries: approximately 55 to 60 kg per person per year in Eastern and Southern countries, respectively, up to 75 kg per person per year for Western countries, and 110 kg per person per year in Northern countries (Mantau et al., 2010). The management of such a huge amount of wood waste has emerged as a serious problem in the world. Wood waste can be used as a raw material for the pulp industry, in panel production, as well as in the production of heat, electricity, and bioenergy through different process technologies. Overall, wood waste can be used in two main ways: material manufacturing and energy production (Faraca et al., 2019). The replacement of original raw materials by wood waste can reduce environmental impacts and extraction, transportation, and disposal (e.g., incineration or transportation to landfills) costs. Recycling the wood residues can reduce the environmental and social burden by decreasing the amount of material, money, and energy required in the production process compared to the direct use of raw wood materials (Kim and Song, 2014). Currently, the main reutilization of wood waste as a raw material is the particleboard industry. For example, Azambuja et al. (2018) reported the blending of construction and demolition wood waste into the internal layers of medium-density particleboards. In Europe, particleboard consumption in 2019 was about 37 million m^3 (Besserer et al., 2021). The percentage of recycled wood waste in particleboard can vary among countries or regions. This proportion is about 100% in Italy; about 50% in Belgium, UK, and Denmark; between 15 and 30% in Germany, France, and Spain; and 0% in Switzerland (Vis et al., 2016). In addition, medium-density particleboards have excellent compatibility and can contain up to 100% wood waste (Faraca et al., 2019).

According to the investigation, wood waste was treated in Europe in the following order: (1) disposal (landfill or incineration) accounted for 37%, (2) material recovery (mainly particleboard) accounted for 33%, and (3) energy recovery (thermal energy production or biofuel generation) accounted for 30%. In UK and the Eastern and Southern countries, wood waste is mostly landfilled and buried, while recycling is more dominant in the Northern and Western countries, mainly material recovery in Italy and France, and energy recovery in Germany, Finland, and Sweden (Mantau et al., 2010). Hossain and Poon (2018) comparatively evaluated wood waste management strategies and potential utilizations by a life cycle assessment approach and revealed that biofuel from wood waste for energy recovery had minimal environmental impacts compared to the production of particleboard and wood-cement composite. In energy recovery, the generation of energy (electricity) through incineration has been proven to have a high negative impact on the environment and has been gradually discouraged (Mayer et al., 2021; Sagastume Gutiérrez et al., 2020; Wang et al., 2012). Some scholars have suggested that AD may be very promising in the utilization of wood waste, since AD can convert waste into bioenergy, and the remaining digestate can be used as fertilizer after only simple composting (Kubiak et al., 2023). Liang et al. (2017) compared two scenarios of cellulosic ethanol and biomethane production from wood waste through a life cycle assessment, which showed that biomethane production from wood waste has relatively high net energy gain and low environmental impacts, including acidification, global warming, eutrophication, and photochemical ozone formation.

2.3 The recalcitrance of wood waste

An extensive range of protein-rich or fat-rich wastes are the usual target for AD, however, less digestible lignocellulosic components are rarely exploited due to low energy extraction efficiency. Lignocellulosic biomass, especially wood waste, consists of mainly lignin, cellulose and hemicellulose, and the structure of these individual components and their combined form hinder their application in AD. Barriers arise from the interconnection between cellulose, hemicellulose, and lignin, forming a complex and undegradable lignocellulosic matrix (Zhang et al., 2019).

Cellulose is an unbranched biopolymer of β-1,4 glucan, whereas hemicellulose is a heterogeneous polymer of various sugars. The glucose chains in cellulose do not exist independently and tend to produce three-dimensional microfibrils with a high degree of polymerization through Van der Waals interactions and hydrogen bonds (Himmel et al., 2007). Each glucose unit is hydrogen-bonded with two intra-chains and two or three inter-chains. These hydrogen bonds give cellulose crystallinity, which makes it structurally stable and tightly packed.

The recalcitrance of lignin has been a major obstruction for the utilization of wood waste. Lignin requires high temperatures and high acidity to be dissolved and considered as the most stubborn component in lignocellulose (Grabber, 2005). It has been widely believed that the higher the lignin content, the more recalcitrant the biomass is. Lignin is structurally composed of three hydroxycinnamyl alcohol monomers, including coniferyl, *p*-coumaryl and sinapyl alcohol, with a variety of ethers and C-C bonds (Bugg et al., 2011). Once incorporated into the lignin polymer, these substituents are distinguished by aromatic ring structures and called guaiacyl, *p*-hydroxyphenyl and syringyl substituents. Besides the content, variation in the quantity of these components has a significant impact on delignification chemistry and therefore on biomass decomposition. Guaiac lignin is reported to be more likely to C-C cross-link at C-5 position, which cannot be hydrolyzed by acids or bases, leading to their ability to prevent fiber swelling and enzyme accessibility (Brandt et al., 2013). The lignin crust has been identified as a challenge in the hydrolysis of wood waste because it limits the accessible surface area of polysaccharide hydrolases to substrates. Lignin cross-links with cellulose and hemicellulose to forms a 'glue-like' structure (Figure 2-5), which effectively prevents microorganisms and enzymes from attacking easily degradable parts, thereby further limiting the biogas potential of wood waste (Pan et al., 2005). Lignin also is a source of compounds, vanillic acid and syringyl aldehyde, which could inhibit hydrolases and digestion organisms (Berlin et al., 2006). In summary, the recalcitrance of wood waste is influenced by several factors, i.e., lignin barrier, cellulose crystallinity, and accessible surface area.

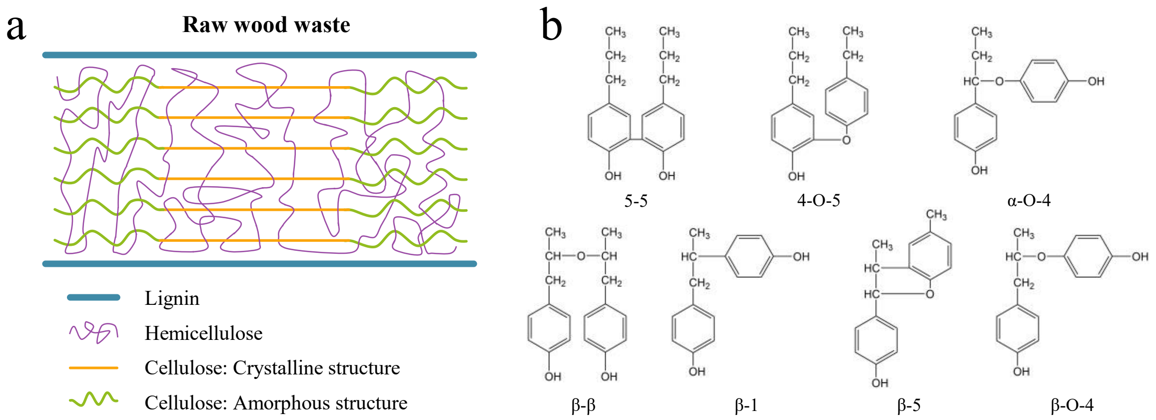

Figure 2-5. (a) Spatial arrangement of lignin, cellulose, and hemicellulose in wood waste, modified from reference (Millati et al., 2020). (b) Different types of linkages within lignin molecule and between lignin and other components, modified from reference (Khan and Ahring, 2019; Sun et al., 2022).

2.3.1 Chemical composition of wood waste

The main components of wood waste are composed primarily of lignin, cellulose, hemicellulose (Luostarinen and Hakkarainen, 2019). As shown in Table A-2, the lignin content of wood waste is typically between 20% and 30% and can be as high as about 35% in some wood types. The high lignin content of wood waste can also be observed by comparing it with other lignocellulosic wastes (Figure 2-6). The lignocellulose composition of wood or wood products, like oriented strand board, particleboard, plywood and medium density fiberboard, are similar, with a lignin content between 25% and 40% (average of about 28.8%). In contrast, the cellulose content in paper and paperboard is up to 68.6%, and crop straw is high in hemicellulose with an average value of 27.6% (Figure 2-6). Both paper, paperboard and crop straw have relatively low levels of lignin (approximately 10%), and the lignin content varies widely among the different types of crop straw, ranging from a minimum content of 5.2% in maize straw to a maximum content of 26.7% of wheat straw.

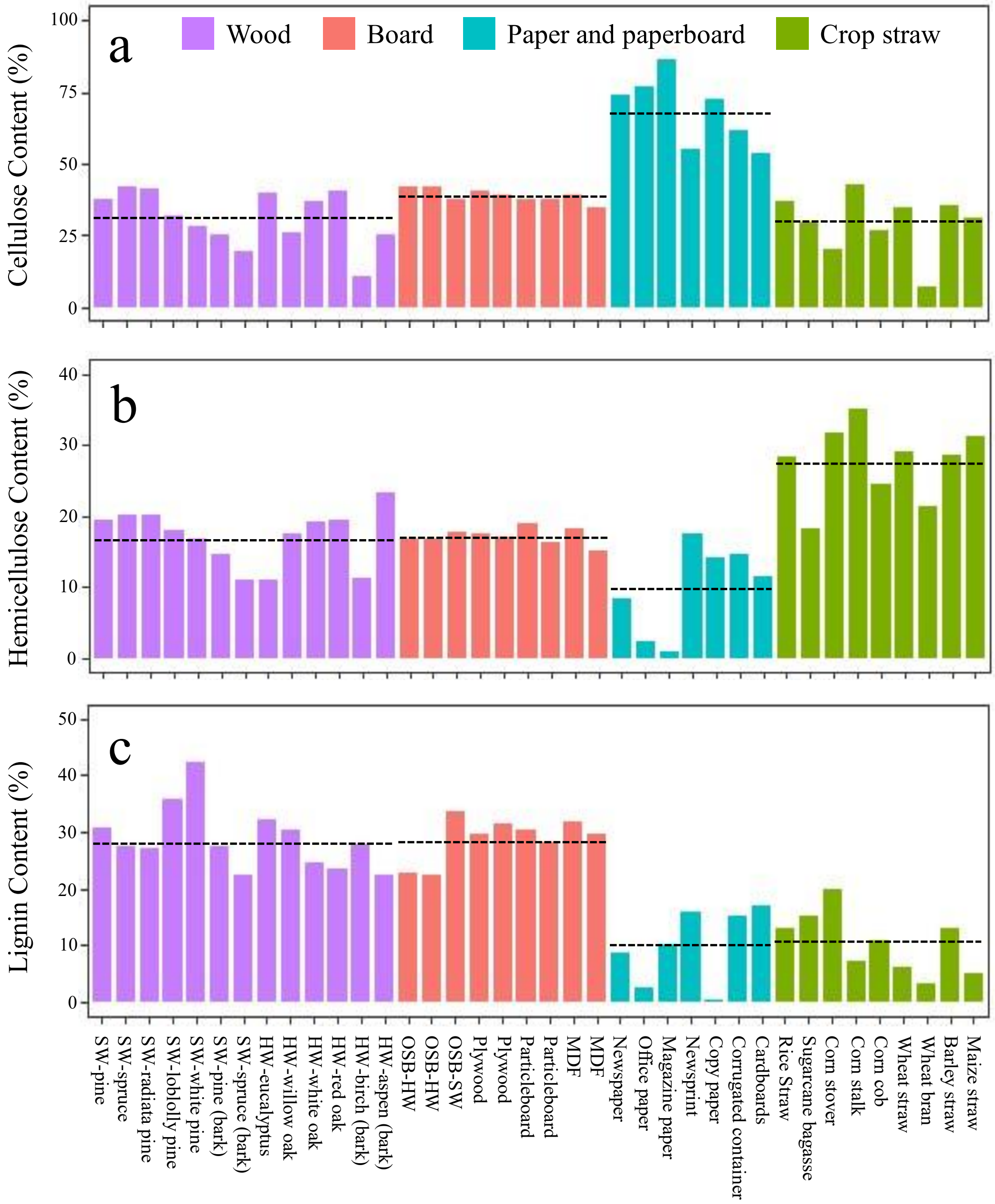

Figure 2-6. The chemical composition of wood waste and other lignocellulosic wastes plotted from the data in Table A-2. a: Cellulose content (%); b: Hemicellulose content (%); c: Lignin content (%). Lignocellulosic waste types are distinguished by color. Black dashed lines represent the average value for each waste type. SW: softwood; HW: hardwood; OSB: oriented strand board; MDF: medium density fiberboard.

2.3.2 Linkages between lignin, cellulose, and hemicellulose

Cellulose is a homogeneous long-chain polymer composed of repeating D-glucose units linked by β-1,4 glycosidic bonds. These glucose monomers are present in the pyranose of a cellulose chain with six-carbon rings, and two pyranoses being connected to each other by acetal linkages (Kalia et al., 2011). Hemicellulose is a heterogeneous polysaccharide composed of arabinose, xylose, glucose, galactose, mannose, and sugar acids. These monomers are bonded to each other through glycosidic and fructose ether linkages, forming a branched polymer structure (Roy et al., 2020; Saha, 2003). In the lignocellulose composition of wood waste, lignin occupies the free space between cellulose and hemicellulose and cross-links with cellulose and hemicellulose to form a rigid structure (Figure 2-5a). Lignin has a helical structure formed by the polymerization of three phenylpropane monomer units, which are connected by ether and carbon-carbon linkages (Fernández-Rodríguez et al., 2017; Gosselink et al., 2010). In addition, cellulose, hemicellulose, and lignin are interconnected to form numerous intrapolymer and interpolymer cross-linkages, mainly including hydrogen, ether, and ester linkages. Table 2-7 lists the different intrapolymer and interpolymer linkages (Harmsen et al., 2010), Figure 2-5b shows the linkages within lignin molecule and between lignin and other components.

Table 2-7. The cross-linkages among cellulose, hemicellulose, and lignin.

Cross-linkages	Types of bonds	Components
Intrapolymer	Ether	Lignin, hemicellulose, cellulose
	Ester	Hemicellulose
	Hydrogen	Cellulose
	Carbon to carbon	Lignin
Interpolymer	Hydrogen	Cellulose-hemicellulose
	Ether	Lignin-cellulose
	Ester	Lignin-hemicellulose

2.3.3 Available pretreatments for wood waste

Considering the recalcitrance of wood waste in AD, the process of biogas production from wood waste generally involves pretreatment. There are many pretreatment technologies for wood waste, which can be divided into physical pretreatment, chemical pretreatment, and biological pretreatment (Bhatia et al., 2017). The purpose of all pretreatment technologies is to disintegrate lignin, cellulose, and hemicellulose as completely as possible, producing smaller fragments that are easily accessible to enzymatic hydrolysis or other biorefining processes for higher yields of added-value products (Figure 2-7). In an AD plant, the cost of the pretreatment process typically exceeds 40% of budget (Sindhu et al., 2016). Although pretreatment technologies have been studied for many years and continuously improved, each method still suffers from obvious pitfalls in practice. For example, physical pretreatments such as grinding, steam explosion, ultrasound, microwave or thermal are energy-intensive and not cost efficient. Similarly, the application of chemicals like alkalis, acids or ionic liquids in pretreatment is faster but will generate wastewater and toxic substances that require extra financial expenses for chemicals recycling. In contrast, the biological pretreatment, despite being a comparatively slower process, is a cost-effective technique that requires low energy input and is relatively free of hazardous chemicals (Sharma et al., 2019). However, the effect of biological pretreatment methods is currently not ideal due to limited technology. For example, the rapid and profitable production of cellulase has not yet been achieved. Nonetheless, scholars are increasingly interested in applying microorganisms or enzymes for pretreatment, with continuous attempts to screen suitable microbial communities with diverse enzymatic components and efficient hydrolysis activities.

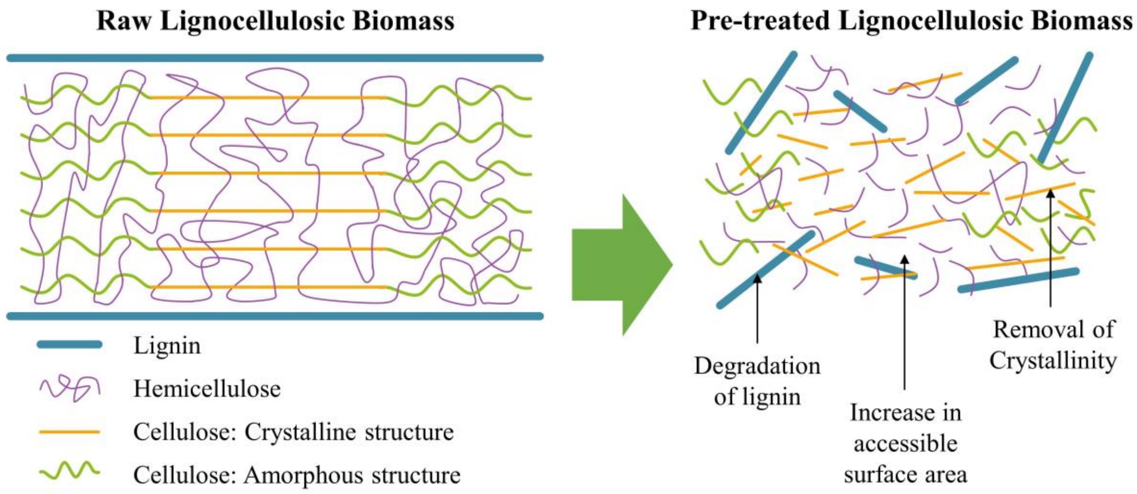

Figure 2-7. Pretreatment goals to overcome lignocellulose recalcitrant.

2.4 Economic aspects of biogas yield from wood waste

AD producing biogas is one promising option for wood waste management and valorization, as it contributes to the entire circular economy chain. It is a convenient and generally cost-effective technology that satisfies efficient disposal of waste and energy production, with possible resources recovery from digestion residues. Figure 2-8 shows the pathways of biogas production from various wood wastes and its application. Part of the organic matter in industrial biogas plants remains in the solid phase of the digestate, which can be then separated and used as fertilizer (Monlau et al., 2015). Biogas generally refers to a gas mixture consisting mainly of methane (55–65%), carbon dioxide (30–35%) and other trace gases, like hydrogen sulfide (Noorollahi et al., 2015). Biogas from AD could be combusted directly for cooking or used for power generation, which emits less greenhouse gas than fossil fuels (Agostini et al., 2017). However, the presence of carbon dioxide in biogas limits its calorific value due to its incombustibility, thus limiting its applicability and transportability. In addition, trace amounts of hydrogen sulfide could corrode equipment such as generators and diesel engines. Therefore, biogas needs to be upgraded to be used as a vehicle fuel (Neshat et al., 2017), and the upgrading process generates a highly concentrated carbon dioxide stream leading to carbon dioxide capture costs as low as $20 per ton (Koornneef et al., 2013). Carbon prices strengthen the economic case for biogas consumption, facilitating AD of wood waste, and providing rural communities with an additional source of income.

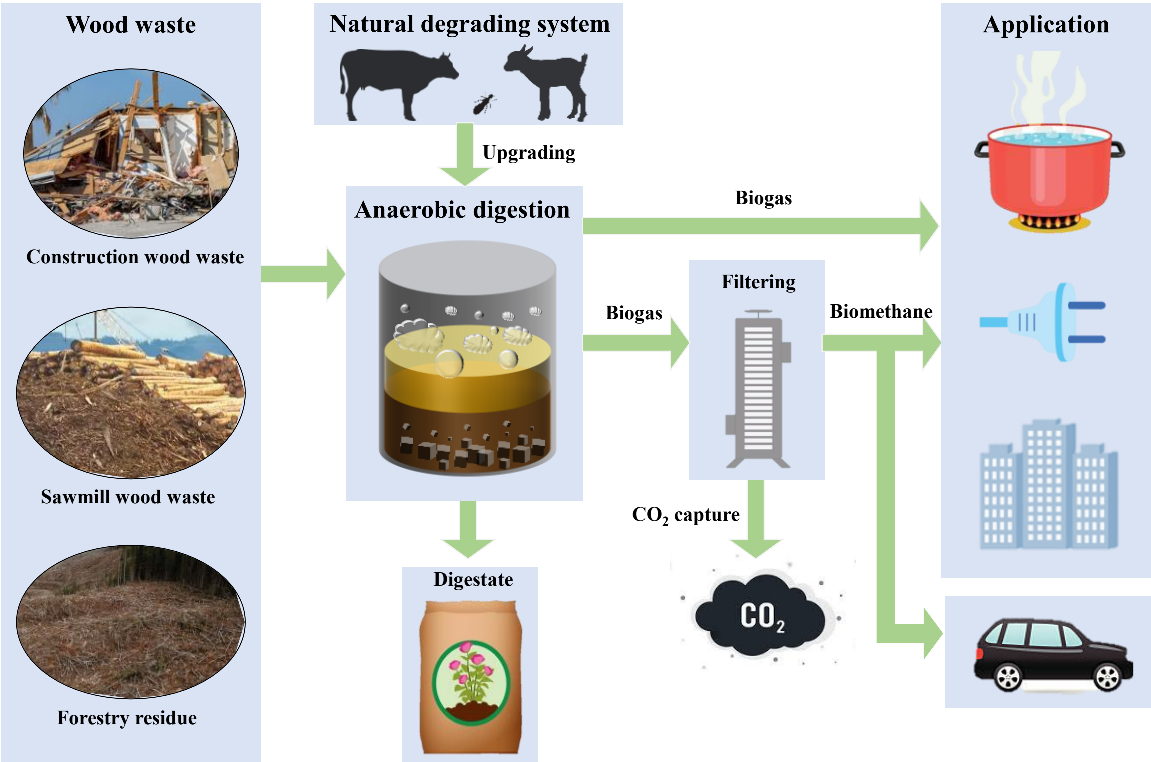

Figure 2-8. Schematic diagram of biogas production pathways from different kinds of wood wastes.

A total of 430 biogas plants worldwide were registered with the International Energy Agency (IEA) by the end of 2015. According to EPA statistics, biogas usage will reach 14 EJ in 2050, which plays an important role in how the global energy sector can reach net-zero emissions by 2050. In addition, household and village digesters in rural areas will provide nearly 500 million households with renewable energy and clean cooking by 2030 (IEA, 2020). Biogas is a consolidated market with a positive outlook, and with global policies leaning toward sustainable new energy, investment in low-carbon gases such as biogas and biomethane will rise to 14 billion dollars by 2040 (IRENA, 2023). For import-dependent countries, investment in biomethane supplies can replace the need for fuel imports. For example, China and India both have extensive biomethane potential, a large portion of which can be obtained at relatively low cost (Kapoor et al., 2020; Zhao et al., 2017). If biomethane replaces natural gas needs, the two countries would save tens of billions in import bills each year, which could help offset the cost of developing a domestic biomethane industry. Currently, about 30 million tons of oil equivalent of biomethane can be developed at a lower cost than natural gas. Methane contributes significantly to the greenhouse effect, and if policies recognize the value of avoiding methane emissions from the decomposition of feedstocks, larger biomethane production will be cost-competitive.

In the economic chain of wood waste manipulation, many factors determine management costs, in which collection and transportation to processing facilities can be important factors. The mixture of different grades of wood waste and the irregular shape of the wood waste greatly increases the cost and difficulty of its management. Disposal cost for grade A wood waste is much lower than for other grades because there is less work involved in the recycling process. The statistical report of the company, Commercial Recycling (2021), showed that the disposal costs for wood waste in 2021 were: £80 per ton for grade A; £130 per ton for mixed grades A, B and C; and £320 per ton for hazardous waste wood (plus £50 for hazardous waste consignment notes). For forest residues, there is currently no price on the open market due to uncommon collection. Only an estimated price could be provided, ranging from £18 to £50 per oven dry tons (NNFCC, 2013). In general, the costs mainly depend on these criteria: transport distance, storage and drying, type and size of machinery used, steepness of the terrain, and labor costs (NNFCC, 2014). The collection and transportation of wood waste to the AD plant could be economically achieved by introducing appropriate mechanization hardware and practices, in the form of larger and more efficient baling, handling, and transport equipment will result in lower costs. On the other hand, with industrialization and scale-up, AD plants could be set up at the source of wood waste (regions with high concentrations of forest residues) to significantly reduce transportation costs.

Since wood waste occurs mostly in a mixture of different grades, a management approach that can handle these mixtures simultaneously becomes particularly important. Ghaly et al. (2011) found that composting was effective in reducing the contaminated biomass of creosote treated wood while generating valuable biofertilizer. Covino et al. (2016) showed that co-composting is a viable and extremely effective method of decontamination and detoxification for creosote treated wood, mainly due to implicated microorganisms belonging to *Firmicutes* (Bacilli), Actinobacteria, and Saccharomycetales. In addition, AD is more environmentally friendly compared to aerobic processes and also produces bioenergy (Murphy and Power, 2006). The application of wood for AD is not limited by its grade, which is helpful in the management of the current situation where multiple grades of wood waste are mixed and can result in significant savings in waste collection costs. For contaminated wood waste, many scholars have researched and developed relative pretreatment techniques to make it more applicable to AD. For example, Ali et al. (2021a) constructed a novel bacterial population SST-4, including *Acinetobacter calcoaceticus*, *Shewanella putrefaciens*, *Bacillus cereus*, and *Novosphingobium taihuense*, were able to degrade both lignocellulose and creosote phenolic compounds from the contaminated wood with a significant increase in methane production by 84%; Ali et al. (2020) screened two microbial consortia, CS-5 and BC-4, from decomposing wood chips and found that they could remove more than 69% and

77% of total chlorophenols from contaminated catalpa sawdust, respectively, and that the combination of both microbiota increased cumulative methane production by 64%. There are two main aspects that limit the utilization of wood waste for AD. The first is the low methane production from raw wood waste. As the analysis in section 2.1.5 showed wood waste has the lowest methane production potential among all lignocellulosic wastes, which resulted in the underappreciation of wood waste as a feedstock for AD. Up to now, a range of pretreatment technologies have been developed to enhance the AD potential of wood waste. Chemical pretreatment technologies including acids (Mirmohamadsadeghi et al., 2016), alkalis (Mohsenzadeh et al., 2012), and other chemical solutions (Hashemi et al., 2022), and physical pretreatment technologies including autoclave (Eom et al., 2019), hydrothermal (Charnnok et al., 2020), and ultrasonic (Karami et al., 2022) have been developed to increase methane production from wood waste by approximately 10% to 150%. Compared to physical and chemical pretreatments, biological pretreatment is a cost-effective technology that requires only low energy inputs (Sharma et al., 2019). For instance, Kavosh et al. (2022) mixed aerobic sludge and pinewood chips directly, utilizing a variety of bacteria in the sludge for pretreatment, which resulted in a 7.3-fold increase in methane production; The cumulative methane production of albizia chips increased 3.7-fold after pretreatment with *Ceriporiopsis subvermispora* at 28 °C for 7 days (Ge et al., 2015). The other limitation is the lack of economical pretreatment technologies that can be practically applied to AD plants. The cost of the pretreatment technology exceeds 40% of the total budget for the entire AD process (Sindhu et al., 2016). The existing pretreatment technologies available for wood waste are only at laboratory level and do not have the capacity for large-scale practical application. In the case of biological pretreatment, when scaled up to factory scale, a huge number of enzymes and bacteria need to be used for pretreatment, and the labor and equipment costs required cannot be ignored. Yet, the scale of factory will bring economies of scale, which means better management and application of large equipment with competitive advantages and cost reductions (Bhatt and Tao, 2020). Through an economic evaluation of methane production from forest residue using different treatment technologies, Kabir et al. (2015) found that the capital investments to operate an AD plant that processes 20,000 tons of forest residue per year could be recovered within eight years. Overall, biogas from wood waste have great economic potential in promoting clean energy transition and achieving sustainable development goals, if suitable pretreatment methods are found.

2.5 Forest soil ecosystem as a pretreatment approach

Forests play an important role in the global carbon cycle, with plants like trees and bushes absorbing energy through photosynthesis and storing it as carbon in wood, which is then partly released into the atmosphere with the decomposition of deadwood in the forest soil (Seibold et al., 2021). Trees continue to serve an important ecological function in the forest after their death, with the decomposition of their debris allowing nutrients stored in dead tissue to be utilized by other organisms. Wood waste, including forest residues and deadwoods, can have multiple negative impacts on forest ecosystems, threatening the health and stability of the environment. On the one hand, these wood waste can act as fuel for wildfires, increasing the probability of forest wildfires, thereby causing significant damage to ecosystems, and potentially threatening the lives of humans and wildlife (Page-Dumroese et al., 2017). The changes in vegetation structure and fuel load composition in forests are closely related to fire hazards, with the accumulation of wood waste exacerbating the frequency and severity of forest fires, leading to an expected increase in fire risk (Pinto et al., 2022). On the other hand, the decomposition of organic matter in these wood waste releases greenhouse gases, which have a negative impact on climate change (Harmon et al., 2020; Lenhart et al., 2012; Osone et al., 2016). The death of trees in forests leads to the formation of coarse woody debris, which can be a source of carbon dioxide flux to the atmosphere in addition to methane efflux (Mukhortova et al., 2021). Warner et al. (2017) found that the carbon dioxide release rate was about 4.23 μmol m^{-2} s^{-1} with the degradation of coarse woody debris in an upland temperate forest, with large variations depending on the decay status of wood. Covey et al. (2016) revealed that the methane abundance in deadwood was significantly higher than the methane concentration in the atmosphere, approximately 24 times the ambient concentration. Kipping et al. (2022) investigated the greenhouse gas emissions from 793 deadwood blocks of 13 different tree species and found that the average emissions of carbon dioxide and methane from deadwood in forests were about 7500 and 7.2 nmol g^{-1} d^{-1}, respectively.

Many scholars have proposed collecting these wood materials and transporting them to biomass power plant for bioenergy generation (Chitawo et al., 2018; Gustavsson et al., 2015; Sahoo et al., 2019; Tian et al., 2023). The study showed that the cost of wood waste recovery operation from the forest was approximately $30 per dry ton, excluding the cost of transporting the material to the bioenergy facilities (collection to trucks only). Combined with transport costs, the total cost of harvesting and transporting this biomass will exceed the local market value of biomass (i.e., up to $50 per dry ton) (Bisson et al., 2016). Therefore, these wood waste, consisting of treetops, branches, worthless round logs, stumps, coarse wood debris form deadwood, are typically eliminated by slash pile burns to decrease the risk of wildfires and to clear growing space for tree regeneration (Creech et al., 2012). However, high fuel loads from burning can lead to localized changes in both abiotic and biotic site conditions, and intense heat over long durations results in the death of soil biota and soil seed banks (Korb et al., 2004). Additionally, the burning can have a significant adverse impact on air quality.

A processing technology that fully harnesses the value of these wood waste aligns with the principles of sustainable development and enhances the overall environmental benefits. The wood waste in the forest will be gradually degraded, eventually releasing all the nutrients to become part of the soil (Petritan et al., 2023). During this natural degradation process, the stubborn lignocellulosic structure of the wood waste is broken down, making it an increasingly suitable raw material for AD. This process, occurring without the need for additional intervention, allows for the effective use of decayed wood waste as AD feedstock. By strategically collecting wood waste at its optimal stage of decay, the efficiency of methane production can be maximized, enhancing the overall performance of bioenergy production. Additionally, this approach supports sustainable forest management by reducing the potential fire risk associated with unmanaged wood waste and contributing to the nutrient cycle in forest ecosystems. Leveraging the natural decay process of wood waste for AD feedstock aligns with sustainable development goals, improves the effectiveness of bioenergy production from high calorific value wood waste, and promotes better forest management practices. This integrated approach can play a significant role in advancing renewable energy technologies and supporting environmental sustainability.

2.6 Summary and research gaps identified

This chapter systematically discusses the potential of AD in wood waste management, including the generation and quantity of wood waste, the AD performance of wood waste, and the available biological pretreatment technologies to enhance methane production from wood waste. Based on the above literature review, the major research gaps can be summarized as follows:

- The enormous stock of lignocellulosic wastes are ideal AD feedstocks, as they do not conflict with food production and these biomasses do not require additional cultivation energy and costs. Among them, wood waste generates less methane from AD because of its high lignin content. Although scholars have developed many pretreatment technologies that can enhance methane production from wood waste, landfilling and incineration are still very common treatments for wood waste, while AD is often ignored. No systematic evaluation of the practicality of wood waste as an AD feedstock has been found in the literatures, as well as a lack of exploration into the degree of enhancement of methane production from wood waste by different pretreatment technologies.

- Despite numerous publications on AD of wood waste, encompassing various types and characteristics of wood waste as well as different AD conditions, a predictive model for final methane production from wood waste based on these parameters has not been developed. Accurate predictions help to adjust process conditions to maximize biomass resource utilization and reduce waste during energy production. In practical AD plants, the ability to predict methane production while simultaneously investigating the impact of digestion conditions can optimize the AD process and enhance bioenergy production.
- Pretreatment plays a critical role in AD of wood waste to facilitate the breakdown of lignocellulosic components and enhance methane production. However, the existing pretreatment approaches for wood waste are considered economically prohibitive. To address this challenge, exploring natural pretreatment systems outlined in section 2.6 becomes a promising avenue. By utilizing the inherent capabilities of natural environments (e.g., enzymatic degradation or the activities of xylophagous animals and microbial), these systems have the potential to significantly reduce the economic burden associated with pretreatment while maintaining or even increasing the efficiency of methane production. The forests constitute the largest natural system and contain substantial quantities of wood waste resulting from logging operations or tree mortality, and these forest residues are degraded to lignoforms on forest soils through a complex series of processes. Given that the recalcitrant structure of wood waste is disrupted during this process, it is significant to understand the dynamics of this process and its impact on methane production.

- While the utilization of forest soil systems as an approach for enhancing methane production from wood waste holds promise, understanding the mechanisms underlying wood waste decay and its subsequent impact on methane production is paramount to maximizing the effectiveness of this approach. Despite recognition of the potential of forest soil system, a comprehensive understanding of the processes governing wood waste decay and its influence on methane production has yet to be developed. Numerous aspects warrant further investigation, including changes in the properties of wood waste during decay in forest soil and the subsequent implications of these alterations on methane production. In addition, environmental conditions in forests vary greatly, which can also affect wood degradation. It is important to explore the factors that play a major role in wood degradation.

Addressing these knowledge gaps is essential for unlocking the full potential of forest-based improvement strategies and advancing the sustainable utilization of wood waste for bioenergy production.

Chapter 3 The potential of wood waste in anaerobic digestion

3.1 Introduction

To date, no systematic analysis has been published to discover the potential of wood waste as feedstocks in AD. Meta-analysis, a scientific statistical method, is ideally suited for bridging the gap between traditional literature review and quantitative analysis (Terrer et al., 2021). By analyzing a large amount of data, more dependable conclusions can be drawn (Maaz et al., 2021). Thus, systematic employment of meta-analysis allows for the acquisition of reliable conclusions. Ma et al. (2020) compared methane yields among mono-digestion and co-digestion by meta-analysis and determined that relevant factors, including co-substrate type, C/N, substrate pH, organic loading rate, and hydraulic retention time, contributed to methane yields; Zhou et al. (2021) obtained a preliminary and more complete anaerobic co-digestion execution strategy in terms of co-substrate selection, mixing ratio, and synergistic evaluation through meta-analysis. The overall effect of digestion conditions and the basic properties of substrates on methane production is considered to be substantial, but there have not yet been systematic studies to optimize the operations associated with AD of wood waste.

Furthermore, machine learning (ML) has gained increased interest in the broad field of environmental science and engineering, and offers the capacity to solve complex relationships and regression tasks by processing and learning from large and multidimensional data (Zhong et al., 2021). Several ML models have been developed to monitor and classify pollutants in environments, which can automatically count and identify pollutants from pictures (Lorenzo-Navarro et al., 2021; Yurtsever and Yurtsever, 2019). Moreover, ML models have been widely applied to waste-to-energy systems in recent years, including AD (Yildirim and Ozkaya, 2023; Zhang et al., 2023b), pyrolysis (Zhu et al., 2019a), hydrothermal carbonization (Li et al., 2021b), and gasification (Elmaz et al., 2020; Li et al., 2021a). For example, Yildirim and Ozkaya (2023) used five ML algorithms to predict the biogas production based on operational parameters collected from a real-scale AD plant and found that the random forest (RF) model had the highest prediction accuracy; Zhang et al. (2023a) revealed the function of biochar in AD by tree-based machine learning; Wang et al. (2020) applied several machine learning algorithms to the regression and classification models of AD performance, identifying the decisive operational parameters of AD and predicting methane production from these parameters. However, an exhaustive literature review revealed that most of the ML work has focused on biogas prediction from the AD systems with different operational conditions, and no studies have been conducted to address the effects of wood waste as a substrate on biogas production. Therefore, the effects of wood waste as a substrate should be analyzed along with the operational parameters of the AD system, so that a comprehensive consideration of the influencing factors can provide a foundation for the improvement of biogas production in different AD plants. Additionally, the ever-increasing number of publications related to various wood wastes with different AD systems can contribute to the development of ML models to explain the complex effects of wood wastes on biogas production based on the physicochemical properties of wood wastes and the AD operational parameters (Chen et al., 2021; Wei et al., 2019). There is therefore the potential that a refined ML model can be used to calibrate system parameters to optimize biogas output.

This chapter employs a hierarchical meta-analysis approach to analyse BMP of wood waste, including the comparison between BMP of wood waste and other organic wastes and the enhancement of different pretreatment techniques on BMP of wood waste. Then, the effects of different parameters in AD of wood waste were determined through diverse ML algorithms. This chapter covers RQ 1 and RQ2 with the following chief goals (i) investigate the BMP of wood waste and other organic wastes; (ii) identify the pretreatment techniques that significantly improve BMP of wood waste; (iii) confirm the main factors affecting methane production and optimization of AD conditions; (iv) predict methane production from substrate physicochemical characteristics and AD conditions for industrial applications.

3.2 Materials and methods

3.2.1 Literature search and data selection

The literature search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Zorzela et al., 2016), in the databases Web of Science and PubMed that combined keywords related to AD with keywords related to wood waste (cutoff date 07 December 2022). The following search terms were employed: (anaerobic *digestion OR biogas OR biomethane) and (lignocellulos* OR wood* OR forest* OR sawdust*), and searches in Web of Science were limited to the topic [title/abstract/keywords] and in PubMed to [title/abstract]. A total of 4441 articles (excluding duplicates) were initially obtained, then the title and abstract were examined and finally the full manuscripts (Figure A-1). Publications included in this meta-analysis were considered to meet the following criteria: (1) investigated wood wastes as feedstocks for AD; (2) studies with "treatment" group and "control" group, where other experimental conditions were identical; (3) included methane production in a standard format (mL/g of VS or mL/g of TS); and (4) measurable data were presented for the determination of mean value and uncertainty of methane production, as standard deviation (SD) or standard error (SE). Subsequently, 42 publications (Table A-3) were retained in the meta-analysis containing 259 groups of datasets on different pretreatment methods for wood waste, 22 groups of datasets on anaerobic co-digestion of wood waste, and 488 groups of datasets on comparing wood waste with other organic wastes in AD (Table A-4).

Moreover, publications included in the machine learning analysis were considered if they met the following criteria: (1) investigated wood wastes as the feedstocks for AD; (2) included methane production in a standard format (L kg^{-1} of VS); (3) presented measurable data for the determination of mean value and uncertainty of BMP, as SD or SE; (4) detailed lignocellulosic component of wood wastes; and (5) presented details on inoculum, volume, temperature and the ratio of inoculum to substrate in AD. To form a qualified dataset, nine input variables including wood types, inoculum types, volume (ml), temperature (℃), particle size (mm), ratio of inoculum to substrate (based on VS), cellulose content (%), hemicellulose content (%), lignin content (%), and digestion time (d) were chosen, in which wood types and inoculum types were represented as categorical objects. The accumulated methane production (L kg^{-1} of VS) during AD was selected as the output variable. To avoid bias introduced by imputation, publications lacking any of the above information were not considered. Under these criteria, 1179 groups of datasets were collected from 19 publications for the machine learning analysis (Table A-5). The values of numerical objects were extracted manually using the WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/, Version 4.6).

3.2.2 Meta-analysis

Three essential results for the methane production were extracted from the screened papers: the mean (M), SD, and the number of replicates (n). If SD was not presented directly in the paper, the SE was used to calculate it according to Equation (3-1):

$$SD = SE \times \sqrt{n} \tag{3-1}$$

A natural log-transformed response ratio (ln RR) is used as a metric to estimate the magnitude of the treatment effect, the log response ratio and its variance are calculated as Equations (3-2 to 3-4):

$$\ln \mathrm{RR} = \ln\,(X_T/X_C) = \ln X_T - \ln X_C \tag{3-2}$$

$$V_{\ln \mathrm{RR}} = {S_P}^2\left(\frac{1}{n_T {X_T}^2} + \frac{1}{n_C {X_C}^2}\right) \tag{3-3}$$

$$S_P = \sqrt{\frac{(n_T - 1){S_T}^2 + (n_C - 1){S_C}^2}{n_T + n_C - 2}} \tag{3-4}$$

where X_i, S_i, and n_i denote the mean, standard deviation, and number of replicates, respectively. The subindices *T* and *C* refer to treatment and control variables, respectively;

S_P is the pooled standard deviation and X includes varieties of different indicators that affect methane production.

A subgroup meta-analysis was conducted to compare the BMP of wood waste with other organic wastes and to explore the impact of different pretreatment methods on BMP of wood waste. The types of wood waste were classified into hardwood and softwood; the pretreatment methods included biological techniques, chemical techniques, physical techniques, anaerobic co-digestion techniques, and combination of multiple methods; other organic wastes included crop straw, municipal solid waste, wild plant, and yard waste. Meta-analysis was conducted using the "metafor" package and "forestplot" package, implemented in R version 4.1.3 (https://www.r-project.org/). This chapter used and modified the codes from Zhang et al. (2020), and detailed description of the codes are available in the repository: https://github.com/pablogalaviz/Micro-Plastics-Meta-Analysis.git.

3.2.3 Implementation of machine learning models

Three types of supervised ML models, namely support vector regression (SVR) (Wang et al., 2023), RF (Long et al., 2021; Pei et al., 2022), and artificial neural networks (ANN) (Alejo et al., 2018), were selected to simulate the complex effects of AD parameters on BMP of wood waste, as all three have been found to be accurate in predicting methane production (Andrade Cruz et al., 2022). Before applying the collected data to these ML models, the data needs to be preprocessed. Since this experimentally based dataset had no missing or erroneous values, the numerical data was standardized from the beginning without taking any steps to improve the data quality. Standardization of the dataset is a key requirement for ML models to eliminate the potential for errors during the training process caused by differences in the scale of the input features (Zhu et al., 2021). Subsequently, the two individual categorical input variables, wood types and inoculum types, were given numerical attributes through the single encoder. This encoder can convert the value of each category into separate columns and assign the value of 0 or 1 to each column, and this process enables the application of categorized data as unordered numerical values to ML algorithms. Since machine learning models can only process real number inputs, data transformation is necessary (Hancock and Khoshgoftaar, 2020). In this study, the StandardScaler function from the scikit-learn library was used to standardize the data. Before building each ML model, the dataset was randomly split into a training dataset (80%) and a test dataset (20%). Figure A-2 shows the flowchart of the proposed framework.

3.2.3.1 Random Forest

RF is a supervised learning model that aggregates multiple decision trees generated from bagging of the training dataset to obtain predictions (Leng et al., 2022). Compared to a single decision tree, it offers improved accuracy and robustness. As shown in Figure A-3, the RF schematic diagram illustrates that the RF model produces results from multiple decision trees that are independent of each other. The dataset samples are initially divided into multiple groups to create sub-datasets. Multiple decision trees are trained on each assigned sub-dataset separately and grown as much as possible according to the bootstrap replication of the training data. The output of each leaf node is the average of all the labelled values in the node (Zhu et al., 2019b). Therefore, for regression problems, RF takes the average of predictions from each decision tree as the final integrated prediction result. For the prediction of the i^{th} sample, the prediction result of RF can be expressed as Equation (3-5):

$$Y_i = \frac{1}{N} \times \sum_{j=1}^{N} f_j(X_i) \tag{3-5}$$

where Y_i is the predicted value of the i^{th} sample, N is the number of the decision trees, $f_j(X_i)$ is the predicted value of the j^{th} tree for the input feature X_i.

To achieve optimal performance in the RF model, it is essential to tune key hyperparameters such as the number of decision trees (n_estimators) and the depth of each tree (max_depth). While increasing n_estimators and max_depth generally improves model performance by enhancing its ability to learn from the training data, it also introduces the risk of overfitting and demands more computational resources. Specifically, the number of trees (n_estimators) was tested across the values 100, 150, 200, 300, and 500, with a final value of 150 selected. For max_depth, values from 10 to 20 were tested, and 13 was chosen. Additionally, other hyperparameters such as min_impurity_decrease, min_samples_leaf, min_samples_split, and random_state were also optimized. The tuned values are as follows: min_impurity_decrease was set to 0, min_samples_leaf to 1, min_samples_split to 2, and random_state to 1 (Table A-6).

3.2.3.2 Support Vector Regression

SVR is a supervised ML algorithm that is an extension of support vector machines used for regression problems. It uses kernel functions projecting the input data and features to construct an optimal hyperplane in a high-dimensional hyperspace. The aim of mapping a hyperplane is predicting the targets with the minimal empirical risk, and the data located on the boundary or closest to the hyperplane are called support vectors (Were et al., 2015). When training the SVR model, it calculates the prediction error of each example, represented as the loss function L_ε (Equation (3-6)). This is one of the key mechanisms of the ML, and by minimising the loss function, the model learns more accurate mapping relationships. Thus, for a training set containing N samples, the empirical risk R_{emp} (Equation (3-7)) is the average or sum of the L_ε over the training dataset. Therefore, by minimizing the empirical risk R_{emp}, the optimal estimation function, which represents the hyperplane in the SVR hyperspace, will be determined as follows:

$$L_\varepsilon = \begin{cases} 0 & if\ \left|y_i - f(x_i)\right| \le \varepsilon \\ \left|y_i - f(x_i)\right| - \varepsilon & otherwise \end{cases} \tag{3-6}$$

$$R_{emp} = \frac{1}{N}\sum_{i=1}^{N} L_\varepsilon\left(y_i - f(x_i)\right) \tag{3-7}$$

where y_i is the actual value of the ith sample and $f(x_i)$ is the predicted value from the SVR. ε is a constant parameter defined as the epsilon-insensitive tube (default = 0.1), which limits the acceptable range of the error between the predicted target and the real target value.

$$f(x) = \sum_{i=1}^{N}(\alpha_i - \alpha_i^*)K\left(x_i, x_j\right) + b \tag{3-8}$$

where α refers to the Lagrange multiplier for determining the weight of each data point in the model. $K\left(x_i, x_j\right)$ is the kernel function and the b is a constant parameter.

In the training process, models often perform well on the training dataset but tend to perform poorly when exposed to new, unseen data. Cross-validation is a widely used technique in ML to enhance model generalization by splitting the training dataset into different datasets. Moreover, it allows the limited available data to be reused, improving the accuracy of the model (Benfenati et al., 2007). In this study, five-fold cross-validation was applied for SVR with the following five steps: Step 1: The training dataset is randomly divided into 5 subsets using non-repetitive sampling; Step 2: 4 of the 5 subsets are used for model training, and the remaining subset is used for testing; Step 3: This process is repeated 5 times, ensuring each subset is used as a test set once; Step 4: The evaluation metrics for each of the 5 models are recorded; Step 5: The average error across the five test results is calculated as the cross-validation error. Importantly, each step, including model and feature selection, is performed independently within a single fold. A more detailed explanation of five-fold cross-validation can be found in Cui et al. (2024).

3.2.3.3 Artificial Neural Network (ANN)

ANNs, which are recognized as an effective ML model, usually consist of a large number of densely connected processing core nodes (called neurons), and these neurons are frequently grouped into several layers (Chandrasekaran et al., 2021). ANNs must be composed of at least three layers: an input layer transferring data to a hidden layer(s) but not performing any computation; one or more hidden layers processing the data and forwarding the results to an output layer; and an output layer presenting the final result of all the data to an external user (Guo and Uhrig, 1992). Weighted connections connect each neuron in a layer to each neuron in the layer above it. Multilayer feedforward perceptron is another name for this type of ANN structure (Göktepe et al., 2005). Determining the number of hidden layers is a key challenge when developing an ANN as it relies on the complexity of the pattern recognition task. ANNs that include one or two hidden layers are commonly used to solve a variety of problems (Sonmez et al., 2006). Moreover, the number of neurons in the hidden layer is another important factor when developing an ANN model, and the number of neurons is determined by the complexity of the input-output link. When this relationship becomes more complex, more hidden neurons should be employed. However, scholars have argued that excessive usage of hidden neurons is believed to lead to overfitting (Heaton, 2017), which in turn affects the predictive ability of the ANN model, and consequently the generalization ability. In practice, the quantity of hidden layers and the number of neurons in the hidden layers are determined by the collected dataset and its features (Cui et al., 2024).

The training of a multi-layer feed-forward perceptron can be regarded as an unconstrained optimal problem with an overall error function that decreases as a function of the synaptic weighting of the network. The synaptic weight values of the multi-layer feed-forward perceptron are iteratively changed in order to produce the desired behavior using a training dataset consisting of input-output vectors. This process is generally performed in two steps using a backpropagation learning algorithm. In the first stage, data is transferred to the ANN through the input layer to generate the output. In the second phase, the difference between the target and generated values is transmitted from the output layer to the previous layers, and the weights of the connections are varied to minimize the transmission error. At the end of the training phase, the proper learned weights of each neuron are stored in the memory of the ANN. A separate unused dataset is provided to the ANN, which uses the stored learned weights to make predictions during testing. Finally, the expected and predicted values are compared (Erzin et al., 2008).

3.2.3.4 Model accuracy evaluation metrics

Once these models are built, their generalization performance on the testing dataset is evaluated using three metrics: the root mean square error (RMSE), mean absolute error (MAE), and the coefficient of determination (R^2). The RMSE and MAE is a measure of the prediction error of the model and how well the model fits the observations, Equations (3-9 and 3-10). Therefore, the smaller the RMSE and MAE, the better the model performs. R^2 (Equation (3-11)) is a statistic that measures the superiority of the model over a simple average model (Y=X) and shows how well the model explains the variance of the predicted values. The R^2 ranges from 0 to 1, and the closer to 1, the better the model performs.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y_i})^2} \tag{3-9}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|y_i - \hat{y_i}| \tag{3-10}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \tag{3-11}$$

where y_i is the real target value of the i^{th} sample, $\hat{y_i}$ is the predicted target value, and $\bar{y}$ is the average of the actual value of the target in all samples (n).

3.3 Results and discussion

3.3.1 Overview of anaerobic digestion on wood waste

Initially, 42 research articles were identified from the literature search results which experimentally investigated BMP of wood waste. The number of publications referencing AD has increased annually, with significant growth since 2006. Although AD has received increasing interest, there are few publications exploring AD of wood waste, with the number basically stable at four per year since the first publication in 2010 (Figure 3-1a). The search results about AD were divided into two categories. The first is ethanol fermentation, where the main feedstocks investigated are crop straws (agricultural wastes); the other part is where the main feedstocks are manure, food waste, and sludge (Figure 3-1b). Whilst AD requires microorganisms to metabolise organic matter into useful products the complex structure of lignocellulosic wastes contains bonds and functional groups that make their degradation very difficult (Gao et al., 2022). In contrast, the organic matters of manure, food waste, and sludge are more amenable to AD as subsequently have become a greater focus of research (Bhatnagar et al., 2022; Deena et al., 2022).

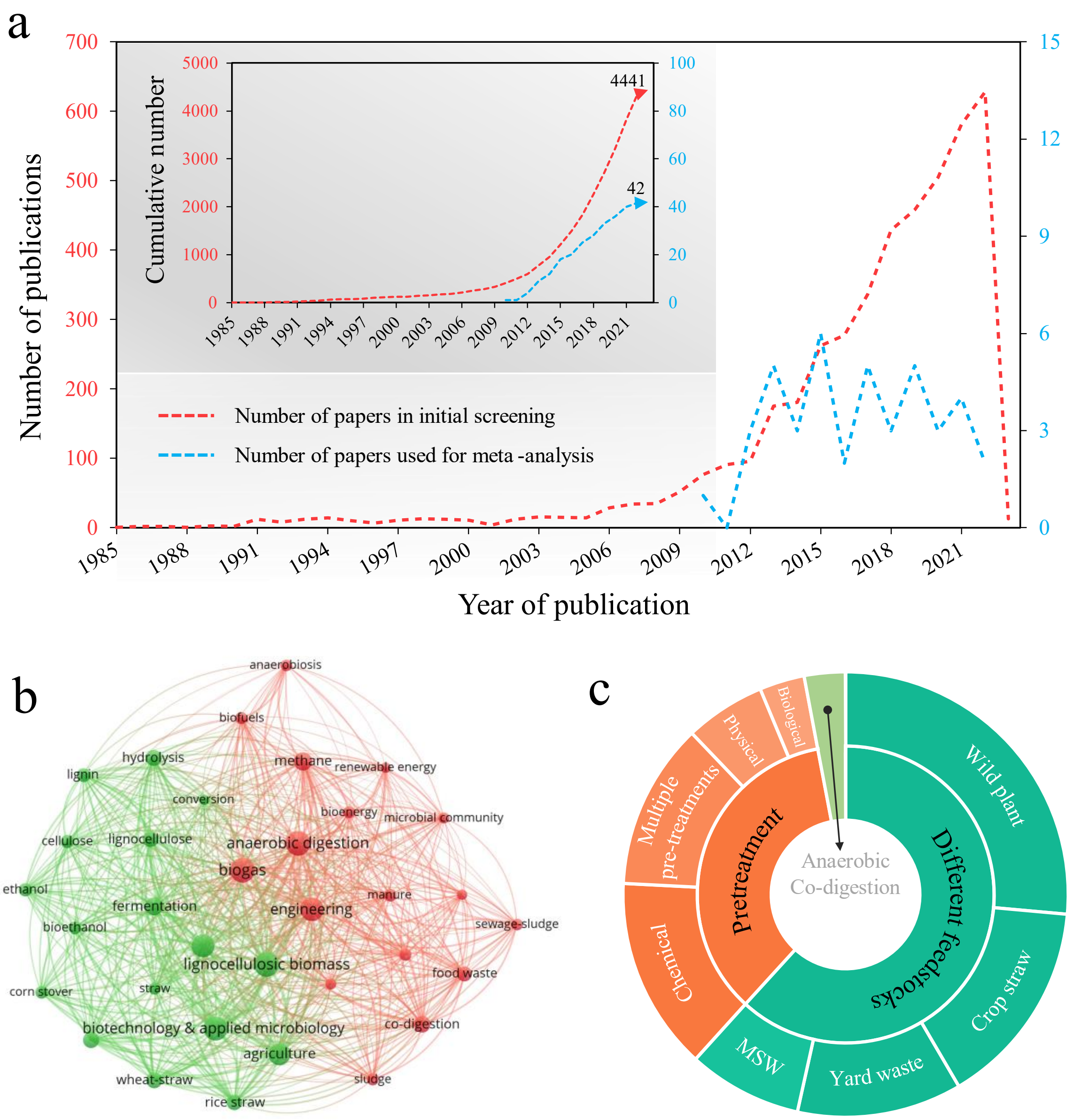

Figure 3-1. General trends in experimental research about the anaerobic digestion of wood waste. (a) Number of publications before 07 December 2022 that experimentally examined the anaerobic digestion of wood waste. (b) Keywords co-occurrence analysis, plotted by VOSviewer 1.6.18 software. (c) Respective proportion of research content (n = 769), including pretreatment techniques (n = 281) and the type of other organic waste (n = 488). MSW: Municipal solid waste.

From the selected 42 studies, a total of 769 datasets were identified and were classified by comparing different pretreatment methods and different organic wastes. Figure 3-1c shows the respective proportion of each research content, where other organic wastes include wild plant (42.73%), crop straw (24.45%), yard waste (19.38%), and municipal solid waste (13.44%). 22 groups of datasets concerned the anaerobic co-digestion of wood waste with other wastes, such as manure, food waste, crop straw, showing that anaerobic co-digestion can increase BMP by 199.23% (Table A-4). Furthermore, the maximum increase occurred when woodchips and food waste were anaerobic co-digested in a 1:1 weight ratio (Oh et al., 2018). Figure 3-2 shows wood waste had the lowest BMP compared to other organic wastes, which could be explained by the high lignin content in the wood waste (Gao et al., 2022). Yard waste consisted mainly of leaves, grass clippings, flowers, twigs, and branches, and twigs and branches account for a large part (Gunaseelan, 2016). Therefore, the BMP of yard waste was close to wood waste. Moreover, BMP also differed considerably between softwoods and hardwoods, with hardwoods being more productive. In the subsequent meta-analysis, hardwoods and softwoods are considered separately.

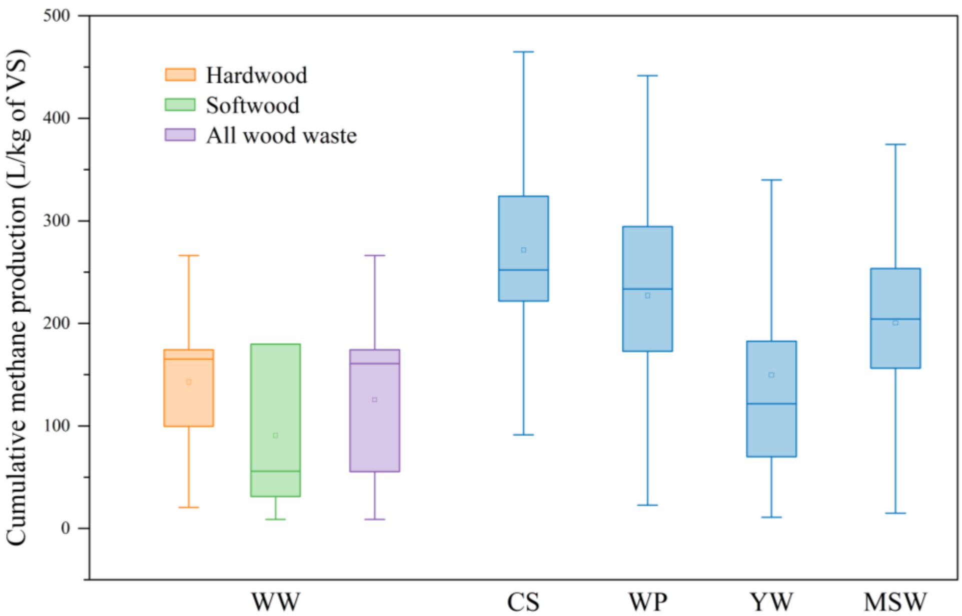

Figure 3-2. The cumulative methane production of wood waste and other organic wastes. WW: wood waste; CS: crop straw; WP: wild plant; YW: yard waste; MSW: municipal solid waste.

3.3.2 Biochemical methane potential comparison for organic wastes

For studies considering AD of different organic wastes, the feedstocks involved mainly lignocellulosic wastes, which were classified according to source and variety as wood waste, crop straw, wild plant, yard waste, and the organic fraction of municipal solid waste (Table A-4). Of these, the first four (wood waste, crop straw, wild plant, and yard waste) are all considered lignocellulosic wastes, while municipal solid waste are highly nonhomogeneous mixture generated from residential, commercial, and industrial sectors (Adhikari et al., 2018). Wild plant refers to the natural herbaceous phytomass that grows in the wild without any human intervention (Triolo et al., 2012). These wastes could partly overlap because of their complexity, for example, municipal solid waste consists of yard trimmings (garden cuttings), which is also divided into yard waste (Funk et al., 2020; Gunaseelan, 2016). In Figure 3-3, the response ratio of other organic wastes compared to wood waste is presented. Summarizing across the organic waste types, wood waste had the lowest BMP. In particular, crop straw BMP was 132% higher than wood waste, wild plant BMP was 181% higher, yard waste BMP was 89% higher, and municipal solid waste BMP was 134% higher. The summary effect size for other organic wastes in comparison to wood waste was 2.22 [95% CI: 1.82, 2.72] ($p < 0.001$). This means that on average the BMP of other organic wastes was 122% higher than that of wood waste. These results were primarily due to the chemical composition of the organic wastes. The cellulose, hemicellulose, and lignin content of crop straw was 40.67%, 16.87% and 21.76% respectively, in addition to 3.12% water soluble carbohydrate and 4.65% crude protein (X. Wang et al., 2020); The chemical composition of wild plant in terms of cellulose, hemicellulose and lignin was 45.37%, 34.33% and 15.11% respectively (Triolo et al., 2012); The cellulose, hemicellulose, and lignin content of yard waste was 39.65%, 29.35% and 23.91% respectively (Panigrahi and Dubey, 2019); In municipal solid waste, kitchen waste contained 6–16% degradable holocellulose, 31–41% sugars, 17–22% protein, and 14–25% fat, and the degradable paper had 72–94% degradable holocellulose (Zhang et al., 2022). However, wood waste consisted of 31.07% cellulose, 17.12% hemicellulose, and a high lignin content (28.82%) (Gao et al., 2022). Lignin is the major component of the recalcitrant fraction of lignocellulosic waste and an important factor limiting their BMP (Gonzalez-Estrella et al., 2017; Khan and Ahring, 2019). The overall improvement in BMP of hardwood waste was 83% compared to softwood waste with summary effect size of 1.83 [0.96, 3.49] ($p = 0.0669$) (Figure 3-3). Furthermore, a similar trend was observed when other wastes were compared to hardwood and softwood respectively. This could be explained by the high content of polysaccharides in hardwood and the lower lignin content (Wang and Barlaz, 2016). On the other hand, the hardwood xylan had a higher degree of deacetylation, making them more susceptible to degradation (Ekstrand et al., 2020).

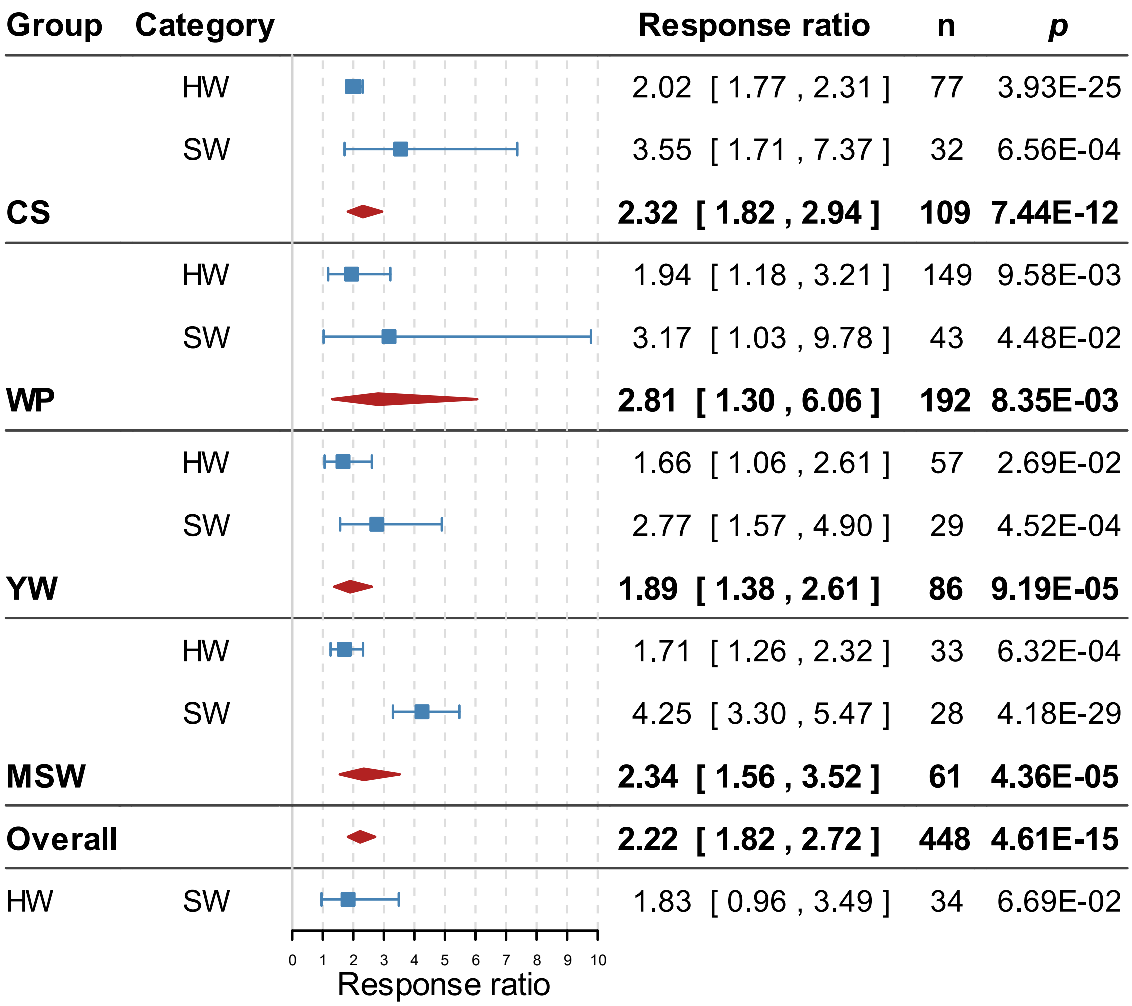

Figure 3-3. The response ratio of methane production from other organic wastes compared to wood waste. The blue square symbols show mean effect size with error bars representing 95% confidence interval, and the red diamond represents the summary effect. A ratio > 1 indicates that the methane production from the treatment is higher than that from the control group, and specifically, a response ratio of 2.22 indicates that the treatment group produces 122% higher methane compared to the control group. n refers to sample size, and *p* means the *p*-value of the Q test with p <0.05 indicating a significant difference. HW: hardwood; SW: softwood; CS: crop straw; WP: wild plant; YW: yard waste; MSW: municipal solid waste.

To investigate the impact pretreatment has on BMP, the dataset was divided into two parts, with and without pretreatment, for analysis. In the absence of pretreatment for substrates, the overall BMP of other organic wastes was 146% higher than wood waste with summary effect size of 2.46 [1.89, 3.19] ($p < 0.001$). However, this gap was reduced to 99% with summary effect size of 1.99 [1.59, 2.48] ($p < 0.001$) under the application of pretreatment techniques (Figure 3-4). The results showed that wood waste specifically had better pretreatment potential — pretreatment increased its BMP by a greater amount — than for other organic wastes. Pretreatment techniques can change the chemical structure of lignin, making it more accessible to microorganisms, which greatly increased the BMP of wood waste (Yoo et al., 2020). Additionally, the BMP of municipal solid waste fluctuated considerably (Figures 3-4c and 3-4d), as the composition of municipal solid waste varied significantly among different geographical areas. For example, the municipal solid waste investigated by Krause et al. (2018b) consisted of mainly paper and paperboard, yet Pastor-Poquet et al. (2019) focused on municipal solid waste consisting of household waste, restaurant waste, and spent coffee.

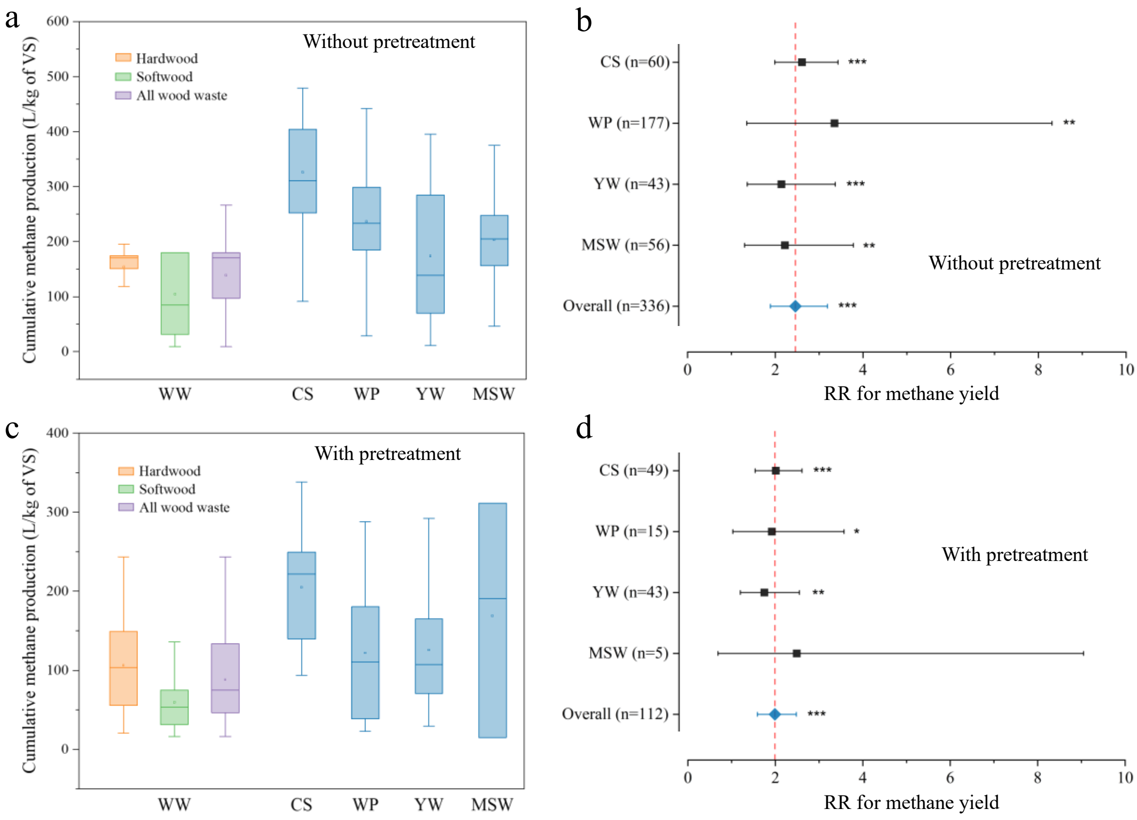

Figure 3-4. The cumulative methane production of different organic wastes (a) without pretreatment and (c) with pretreatment techniques. The response ratio of methane production from other organic wastes compared to wood waste (b) without pretreatment and (d) with pretreatment techniques. WW: wood waste; CS: crop straw; WP: wild plant; YW: yard waste; MSW: municipal solid waste. For (b) and (d), the plot shows the mean effect size (black squares and blue diamond) with error bars representing 95% confidence interval. A ratio > 1 indicates that the methane production from other wastes is higher than wood waste, and specifically, a response ratio of 2.22 indicates that other wastes produce 122% higher methane compared to wood waste. n refers to sample size and p means the p-value of the Q test, with (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

3.3.3 Impact of pretreatment techniques for wood waste

A number of studies have shown that pretreatment techniques can enhance BMP of lignocellulosic wastes by increasing the surface area of feedstock (size reduction and the wetting of biomass) and biomass decrystallization, resulting in an increase in the accessibility and biodegradability of microorganisms to the organic matter (Ali et al., 2021a; Basak et al., 2022; Raut et al., 2021). However, it is still not fully clear to what extent pretreatment techniques contribute to the BMP of wood waste and how to choose the optimal pretreatment technique for different sources and components of wood waste. The cumulative methane production of wood waste after different pretreatment techniques is shown in Figure 3-5. According to the meta-analysis depicted in Figure 3-6, the employment of pretreatment strategies significantly improved the BMP of wood waste by 113% (n=250), with an overall effect size of 2.13 [1.68, 2.70] ($p < 0.001$). Furthermore, the combination of multiple pretreatment techniques was more effective than a single approach, except for the combination of biological and chemical strategies (86%, n=39) which was slightly less effective than physical strategy (99%, n=43). Many studies have demonstrated that a combination of two pretreatments, like biological with chemical or physical strategies, was more useful compared to a strategy alone (Ponnusamy et al., 2019). An appropriate combined strategy would not only improve the decomposition of lignocellulosic feedstocks, but also optimize the utilization of their constituent components, all while keeping operating costs relatively low and optimising the product quality (De Bhowmick et al., 2018). The synergistic impact of combining physical pretreatment with either chemical or biological pretreatments was observed to markedly enhance the BMP, with effect size of 4.76 [1.98, 11.44] ($p < 0.001$) or 4.67 [2.10, 10.39] ($p < 0.001$) respectively (Figure 3-6). These findings imply that physical pretreatment plays a pivotal role in facilitating successful AD of wood waste, underscoring the necessity of employing multiple pretreatment strategies to maximize the BMP of woody biomass. The summary effect size of hardwood and softwood were 1.85 [1.49, 2.29]

($p < 0.001$) and 2.55 [1.60, 4.04] ($p < 0.001$), and akin to the outcomes on all wood waste, physical pretreatment and multiple pretreatment strategies were exceedingly advantageous in advancing the BMP (Figure 3-7). It is noted that in some specific cases combine pretreatment did not result in higher BMP values in comparison to single pretreatment.

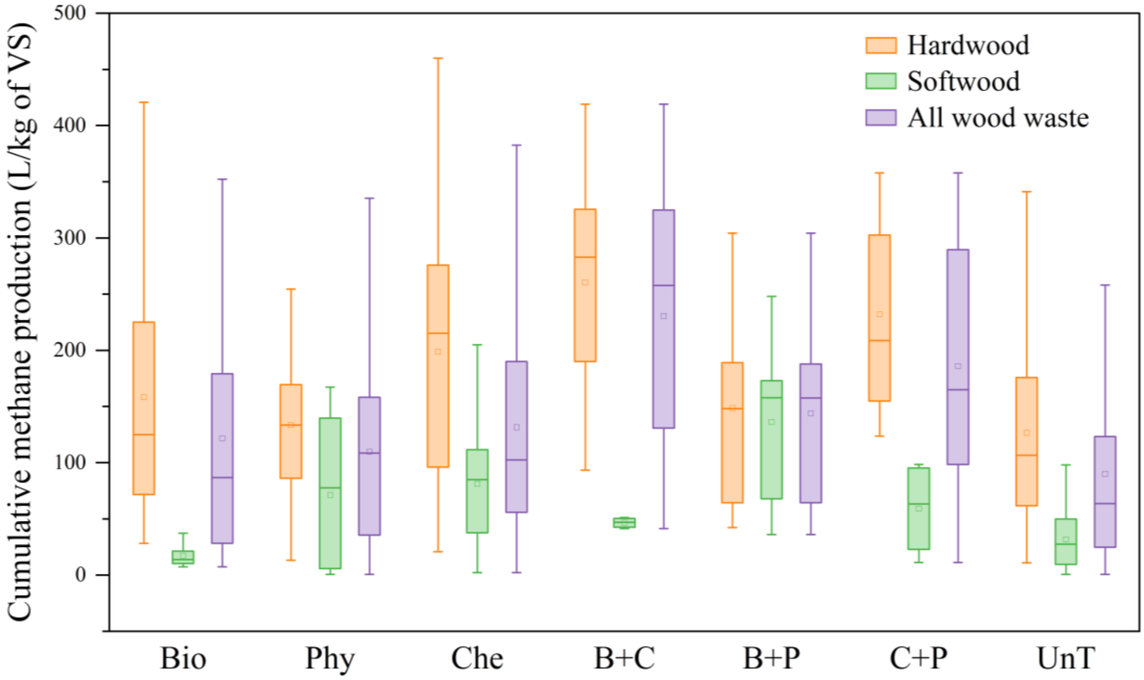

Figure 3-5. Influence of different pretreatment techniques on cumulative methane production of wood waste. Bio: biological pretreatment; Phy: physical pretreatment; Che: chemical pretreatment; B+C: biological + chemical pretreatments; B+P: biological + physical pretreatments; C+P: chemical + physical pretreatments; UnT: without pretreatment.

Group	Category	Response ratio	n	*p*
	Enzyme	1.13 [0.55 , 2.32]	9	7.39E-01
	Fungal	2.16 [0.87 , 5.34]	11	9.58E-02
	MC	2.91 [1.05 , 8.08]	3	4.09E-02
Biological		**1.84 [1.10 , 3.08]**	**23**	**1.98E-02**
	Acid	1.04 [0.83 , 1.30]	6	7.46E-01
	Alkali	1.74 [1.53 , 1.99]	32	1.17E-16
	AAS	2.40 [1.97 , 2.93]	3	4.44E-18
	Iron-based	1.04 [0.66 , 1.65]	13	8.60E-01
	NMMO	1.74 [1.47 , 2.07]	26	2.24E-10
	Organosolv	1.50 [1.06 , 2.12]	18	2.13E-02
Chemical		**1.55 [1.34 , 1.79]**	**98**	**1.99E-09**
	Autoclave	1.56 [1.07 , 2.27]	15	2.13E-02
	Hydrothermal	2.54 [0.91 , 7.09]	25	7.41E-02
	Ultrasound	1.11 [0.91 , 1.36]	3	3.09E-01
Physical		**1.99 [1.08 , 3.68]**	**43**	**2.78E-02**
	Enzyme + Iron-based	1.79 [1.52 , 2.11]	12	2.95E-12
	Fungal + Alkali	1.49 [1.12 , 1.98]	9	6.18E-03
	Fungal + Iron-based	1.49 [1.34 , 1.66]	16	2.36E-13
	MC + Alkali	3.42 [3.30 , 3.55]	2	0.00E+00
Biological + Chemical		**1.86 [1.27 , 2.72]**	**39**	**1.41E-03**
	Enzyme + Autoclave	5.17 [4.31 , 6.21]	6	7.60E-70
	Enzyme + Hydrothermal	20.1 [13.8 , 29.4]	12	2.87E-54
	Fungal + Autoclave	1.52 [0.80 , 2.88]	12	2.04E-01
	Fungal + Hydrothermal	1.78 [1.61 , 1.97]	2	1.07E-28
	MC + Hydrothermal	4.20 [4.03 , 4.38]	2	0.00E+00
Biological + Physical		**4.67 [2.1 , 10.39]**	**34**	**1.56E-04**
	CA+ Hydrothermal	10.4 [5.54 , 19.7]	4	3.89E-13
	Iron-based + Ultrasound	0.91 [0.83 , 0.99]	5	3.13E-02
	Organosolv + Hydrothermal	3.29 [1.62 , 6.66]	4	9.48E-04
Physical + Chemical		**4.76 [1.98 , 11.4]**	**13**	**4.76E-04**
Overall		**2.13 [1.68 , 2.70]**	**250**	**4.79E-10**

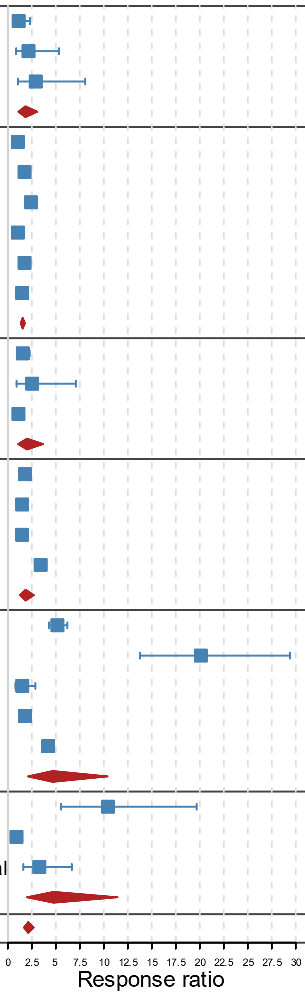

Figure 3-6. The effects of different pretreatment techniques on methane production from wood waste. For the purposes of comparison, shredding of raw materials is excluded from the scope of pretreatment. The blue square symbols show mean effect size with error bars representing 95% confidence interval, and the red diamond represents the summary effect. A ratio > 1 indicates that the methane production from the treatment is higher than that from the control group, and specifically, a response ratio of 2.13 indicates that the treatment group produces 113% higher methane compared to the control group. n refers to sample size, and *p* means the *p*-value of the Q test with *p* <0.05 indicating a significant difference. MC: microbial consortium; AAS: aqueous ammonia soaking; NMMO: N-methylmorpholine-N-oxide; CA: chemical antidote.

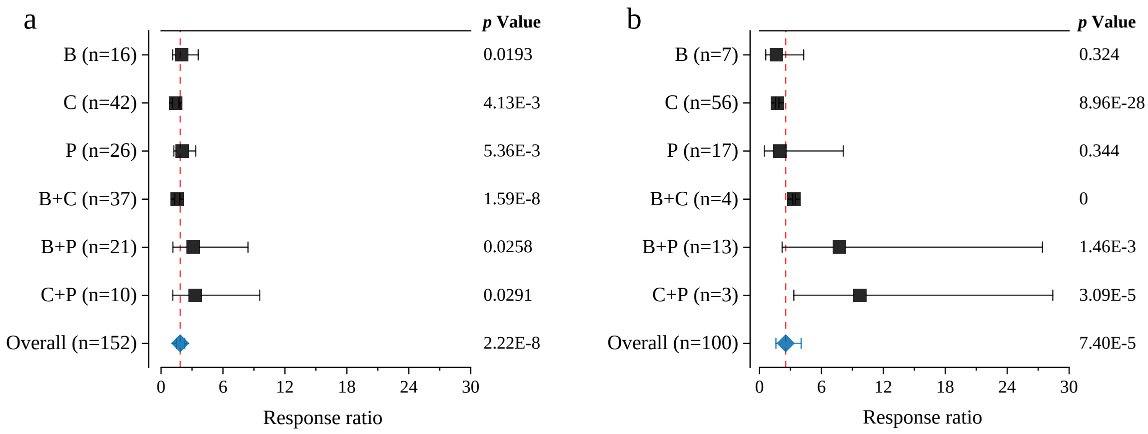

Figure 3-7. The effects of different pretreatment techniques on methane production of (a) hardwood waste and (b) softwood waste. B: biological; C: chemical; P: physical; B+C: biological + chemical; B+P: biological + physical; C+P: chemical + physical. The plot shows the mean value (black squares and blue diamond) with error bars representing 95% confidence interval. A ratio > 1 indicates that the methane production from the treatment is higher than that from the control group.

The largest increase in the methane yield for wood waste was observed after the combination of biological and physical pretreatments (Table 3-1). Hydrothermal treatment together with cellulolytic enzyme was the method with highest increased BMP (3074.2%) when compared to untreated wood waste (Matsakas et al., 2015). The process of hydrothermal treatment has been shown to be an effective means of augmenting the solubility of biomass, thereby creating a more conducive environment for enzymatic activity (Posmanik et al., 2017). Additionally, an important factor contributing to the significant improvements in BMP observed in these studies was the markedly low levels of methane production from untreated wood, close to 5 ml/g of VS (Matsakas et al., 2015). Despite the considerable gains in BMP that can be achieved with these pretreatment strategies, it is imperative that a thorough investigation is carried out into the energy consumption and materials associated with these techniques, in order to surpass the cost-benefit threshold in practical applications. Physical techniques tend to be energy intensive, whereas chemical techniques could result in the production of environmentally hazardous substances, and have their own environmental footprints (Sharma et al., 2019). By contrast, biological techniques, while being a comparatively slower process, are typically economical approaches that require minimal energy input and are largely devoid of hazardous chemicals (Gao et al., 2022). Indeed, among individual pretreatment techniques, biological pretreatment exhibited the most substantial increase in BMP relative to physical and chemical pretreatment, registering an impressive 713% growth (Table 3-1). Yet, when it comes to wood waste, the research on biological pretreatment and the identification of microbial consortium involved in wood degradation is still in its infancy.

Table 3-1. Growth rate of the pretreatment techniques for wood waste under specific pretreatment configuration.

Pretreatment	**Average growth rate in methane production (L/kg of VS)**			**Maximum growth rate in methane production**	
	$\bar{X}_{Control}$	$\bar{X}_{Treated}$	$\bar{X}_{Increase}$ **(%)**	**Specific pretreatment configuration**	**Maximum growth rate (%)**
Biological					
Enzyme	56.1	66.2	51.7 (n=9)	30 FPU/g cellulolytic enzyme at 50 °C for 12 h (Matsakas et al., 2015)	185.4
Fungal	108.0	178.0	178.7 (n=12)	*Ceriporiopsis subvermispora* at 28 °C for 7 days (Ge et al., 2015)	265.5
MC	44.7	81.1	286.7 (n=3)	Aerobic sludge pretreatment at 37 °C and 90 rpm for 10 d (Karami et al., 2022)	713.0
Chemical					
Acid	26.4	27.6	6.5 (n=6)	85% phosphoric acid at 60 °C for 45 min (Mirmohamadsadeghi et al., 2016)	39.8

Alkali	80.5	153.1	101.9 (n=36)	NaOH at -15 °C for 16 h (Mohsenzadeh et al., 2012)	556.8
AAS	30.8	76.9	150.5 (n=3)	AAS at 22 °C for 3 d (Antonopoulou et al., 2015)	176.9
Iron-based	237.5	297.9	27.3 (n=13)	0.5 mM Fe(II) and H_2O_2 (Hashemi et al., 2022)	49.0
NMMO	45.2	85.8	90.1 (n=20)	75% NMMO for 15 h (Kabir et al., 2013)	298.2
Organosolv	54.9	78.4	65.6 (n=18)	Ethanol extractives (Tajmirriahi et al., 2021)	319.6
Physical					
Autoclave	79.4	104.0	90.6 (n=15)	Steam explosion at 20 bar for 10 min (Eom et al., 2019)	669.7
Hydrothermal	42.7	109.8	36.3 (n=17)	Hydrothermal at 170–210 °C for 30 min (Charnnok et al., 2020)	258.4
Ultrasound	118.8	117.6	12.0 (n=3)	Ultrasonic at 40 KHz and 40 °C for 30 min (Karami et al., 2022)	35.7
Biological + Chemical					
Fungal + Iron-based	190.7	279.3	54.8 (n=16)	*Pleurotus ostreatus* at room temperature for six weeks + Fe(III) and H_2O_2 (Hashemi et al., 2022)	136.8
Enzyme + Iron-based	190.7	326.1	88.2 (n=12)	0.5 mM Fe(II) and H_2O_2 + Enzyme at 50 °C for 96 h (Hashemi et al., 2022)	155.1
Fungal + Alkali	95.4	139.6	46.4 (n=8)	*Abortiporus biennis* at 27 °C for 30 d + NaOH at 80 °C for 24 h (Alexandropoulou et al., 2017)	115.0
MC + Alkali	13.6	46.0	237.3 (n=2)	NaOH at room temperature for 10 min + MC (Karami et al., 2022)	245.2
Biological + Physical					
Enzyme + Autoclave	10.9	56.5	418.5 (n=6)	Steam explosion at 16 bar for 10 min + 30 FPU/g enzyme (Eom et al., 2019)	491.4
Enzyme + Hydrothermal	8.2	167.9	2069.5 (n=8)	Hydrothermal at 210–215 °C for 5 min + 30 FPU/g cellulolytic enzyme at 50 °C for 12 h (Matsakas et al., 2015)	3074.2
Fungal + Autoclave	106.8	159.8	68.0 (n=12)	Steam explosion at 210 °C for 10 min + 2% *Caldicellulosiruptor bescii* culture (v/v) (Mulat et al., 2018)	143.2

Fungal + Hydrothermal	28.9	51.8	79.3 (n=2)	Hydrolysis + *Petronet alfa* (Baghbanzadeh et al., 2021)	88.6
MC + Hydrothermal	9.6	40.3	319.9 (n=2)	Liquid hot water + MC (Karami et al., 2022)	320.9
Physical + Chemical					
CA + Hydrothermal	10.6	108.5	972.5 (n=4)	Hydrothermal at 210–215 °C for 5 min + Sodium dithionite as a chemical antidote (Matsakas et al., 2015)	1677.1
Iron-based + Ultrasound	341.3	310.3	3.0 (n=5)	0.001 M Fe(III) + 0.001 M H_2O_2 + 2 h ultrasonication duration (Lamb et al., 2019)	4.9
Organosolv + Hydrothermal	57.2	159.6	194.1 (n=4)	Ethanol organosolv + Hydrothermal at 170–210 °C for 30 min (Charnnok et al., 2020)	376.3

MC: Microbial consortium; AAS: Aqueous ammonia soaking; NMMO: N-methylmorpholine-N-oxide; CA: Chemical antidote.

3.3.4 Methane yield predicted by machine learning models

3.3.4.1 Description of the collected datasets

The characteristics of all the variables used for ML are shown in Table A-5, while the data distribution is shown in Table A-7. For pretreated wood materials, the properties of materials after pretreatment were provided for analysis. The inoculum could be divided into sewage sludge and effluent from anaerobic digestion of manure, both of which were common types of high-nitrogen inocula (high nitrogen) to balance the typically high C/N of feedstock and enhance the digestion performance (Karrabi et al., 2023); Wood types were divided into hardwood and softwood according to the previous meta-analysis results. Although the data collected primarily employed the mesophilic conditions (30–40 °C), the reaction progressed more rapidly under thermophilic conditions, resulting in higher biogas production rates (Bowen et al., 2013). The AD of wood waste lacked data on thermophilic conditions.

Meanwhile, the data on digestion volumes was also incomplete, as the volumes in the dataset were obtained from lab-scale.

To further reveal insights into the collected datasets, linear correlations between pairwise variables were analysed using Pearson correlation coefficient. As shown in Figure 3-8, there was a weak positive correlation between inoculum types and lignin content with methane production. Many parameters, like temperature and I/S ratio, did not present a linear correlation with methane production, while these parameters have been proved to influence the methane production of AD (Nie et al., 2021; Yao et al., 2013). Therefore, further internal relationships between these variables should be discovered based on big data and non-linear methods.

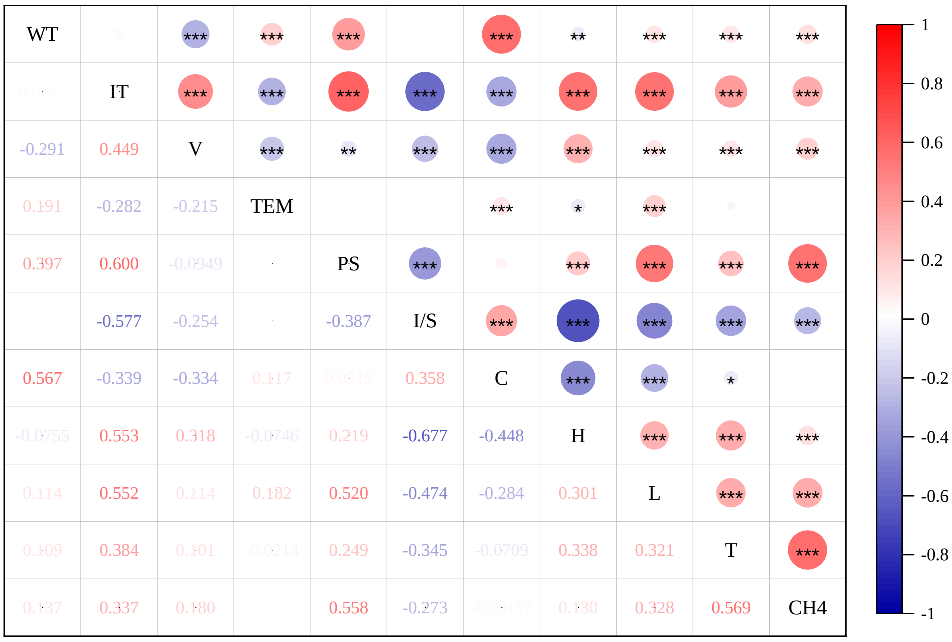

Figure 3-8. Pearson correlation between pairwise variables. WT: wood types; IT: inoculum types; V: volume (mL); Tem: temperature (℃); PS: particle size (mm); I/S: ratio of inoculum to substrate (based on VS); C: cellulose content (%); H: hemicellulose content (%); L: lignin content (%); T: digestion time (d); CH4: methane production (L/kg of VS). (*) p<0.05; (**) p<0.01; (***) p<0.001.

3.3.4.2 Methane production predicted by machine learning algorithms

The collected data (except for the methane production data) were processed as input variables for the ML models and the methane production values were considered as output variables. After the parameter optimization process (Figure 3-9 and Figure 3-10; Table A-6 and Table 3-2), all three models showed good fitting results, where RF (R^2=0.9643, RMSE=15.52) was followed by ANN (R^2=0.9640, RMSE=16.30) and SVR (R^2=0.9451, RMSE=20.92) (Table 3-3 and Figures 3-11a to 3-11c). This could be explained by the selection of major parameters affecting the methane yield from AD and by a greater number of data compared to the publications (Wang et al., 2023; Xu et al., 2022; Zhang et al., 2023a). To visualize better the results, regression error characteristic (REC) curves were used to estimate the error in an absolute deviation form of all ML models (Figure 3-11d). The REC curve represents the cumulative distribution function of the error, with a smaller area over the curve denoting greater accuracy. As shown in Figure 3-11d, RF had the highest prediction accuracy among the three ML algorithms. RF is an ensemble learning method that constructs a multitude of decision trees and combines their outputs to improve the accuracy and stability of predictions (Bagherzadeh et al., 2021; Zhou et al., 2019). Meanwhile, RF shows superior performance on high-dimensional, large, and noisy data, while avoiding overfitting problems (You et al., 2017). Long et al. (2021) used six ML algorithms to predict methane yield by combining genomic data with corresponding operational parameters and found that RF achieved the most accurate predictions both when using only operating parameters as input variables and when combining these parameters with genomic data. RF exhibited advantages of high generalizability and swift convergence when applied to AD data, which aligns with the results of this chapter. In general, the optimal RF model can reliably and precisely forecast and guide practical AD experiments.

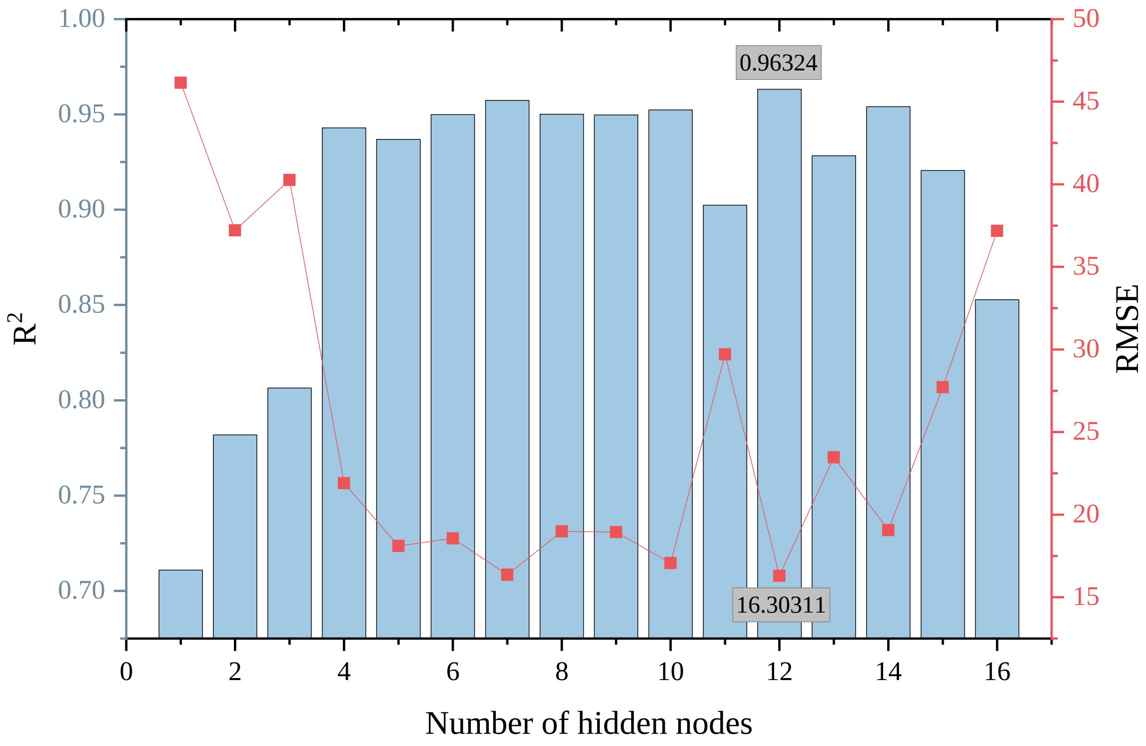

Figure 3-9. Performance of the artificial neural networks (ANN) model with different number of nodes in the hidden layer.

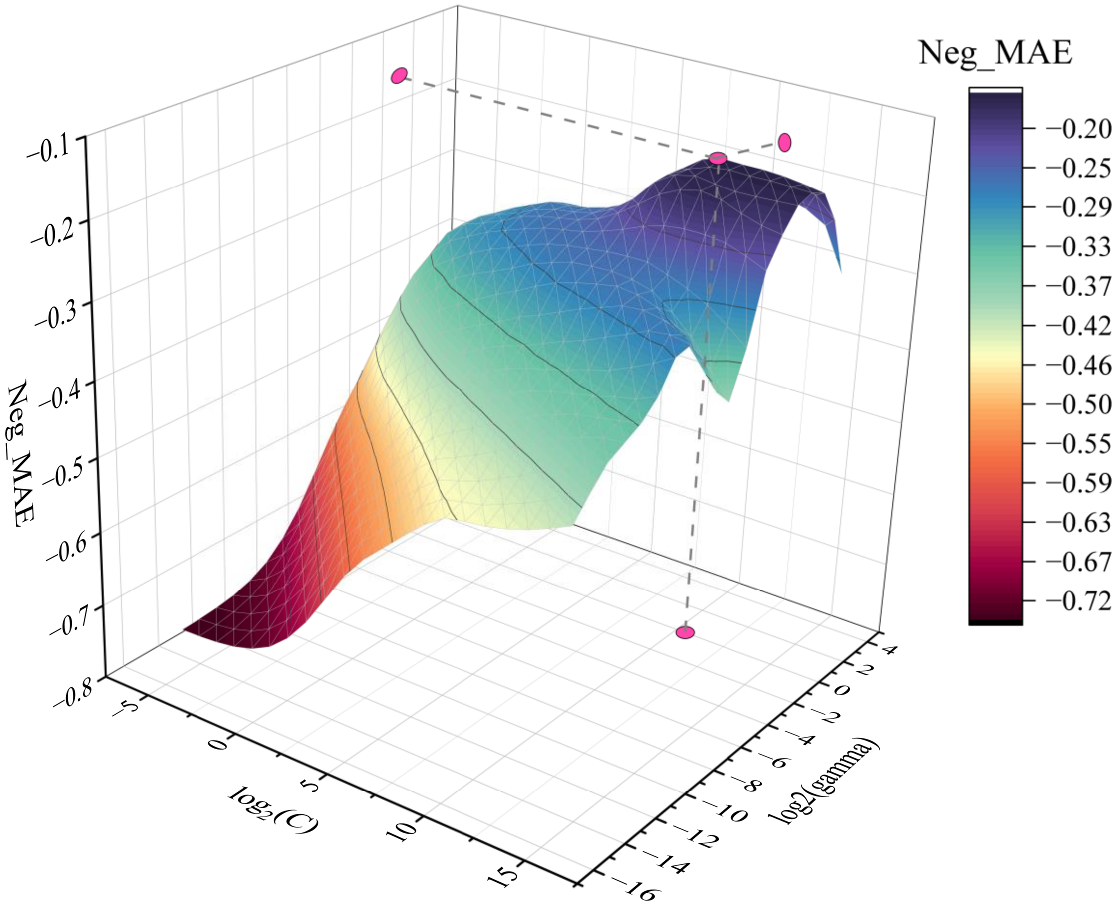

Figure 3-10. Cross validation-based Grid search of the support vector regression (SVR) model.

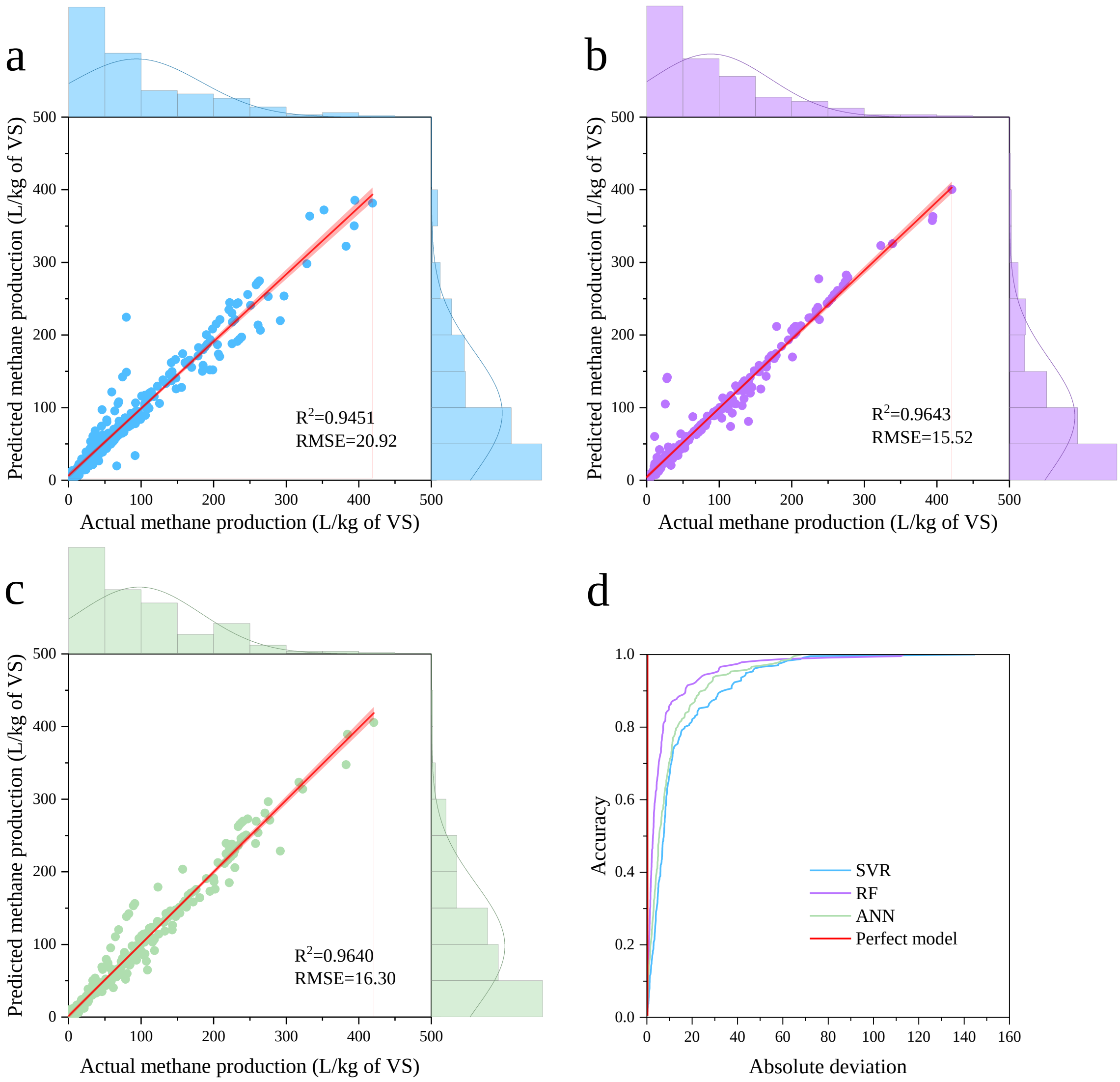

Figure 3-11. Performance of the testing datasets of (a) support vector regression (SVR), (b) random forest (RF), and (c) artificial neural networks (ANN). (d) The regression error characteristic curves of three machine learning models.

Table 3-2. Selection of kernel function in the support vector regression (SVR) model.

Kernel function	Statistical parameters		
	R^2	RMSE	MAE
Linear SVR	0.711	47.710	35.986
Poly SVR	0.840	35.570	23.963
RBF SVR	**0.880**	**30.711**	**20.940**
Sigmoid SVR	-116.450	962.421	516.736

Table 3-3. Performance of machine learning models on predicting methane production.

ML models	R^2	RMSE	MAE	STDEV
SVR	0.9451	20.9235	12.7287	86.6033
RF	0.9643	15.5247	6.9357	80.4058
ANN	0.9640	16.3031	9.9939	85.4359

SVR: support vector regression; RF: random forest; ANN: artificial neural network; RMSE: root mean square error; MAE: mean absolute error; STDEV: standard deviation.

To weigh the impact of various factors on methane production, RF was employed to assess feature importance and the results are illustrated in Figure 3-12. The two most important factors were digestion time (40.5%) and particle size (25.8%). Firstly, the digestion time exhibits a close association with methane production. Specifically, as time elapses, the availability of organic matter to microorganisms in an AD system increases, thereby leading to an escalation in cumulative methane production. In batch reactors, the optimal digestion duration is about 30 days (Dai et al., 2019). Secondly, particle size plays an important role in AD by affecting the surface area of substrate. Dai et al. (2019) confirmed this view and illustrated that the reduction in particle size had a facilitative effect on methane production. For lignocellulose composition, the lignin content of the substrate had a more significant effect on AD compared to cellulose and hemicellulose (Figure 3-12). This was consistent with the established situation where lignin was the main obstacle to breakdown of lignocellulosic wastes (Khan and Ahring, 2019). It is worth noting that the temperature did not have an important effect, which could be explained by the data collected in this chapter mostly adopting similar temperatures (30–40 °C). Therefore, thermophilic conditions could be future studies for the AD of wood waste. Overall, the outputs of the RF model could identify important factors influencing the AD system. Moreover, as the dataset is expanded and additional variables are incorporated, the outcomes have the potential to become even more representative.

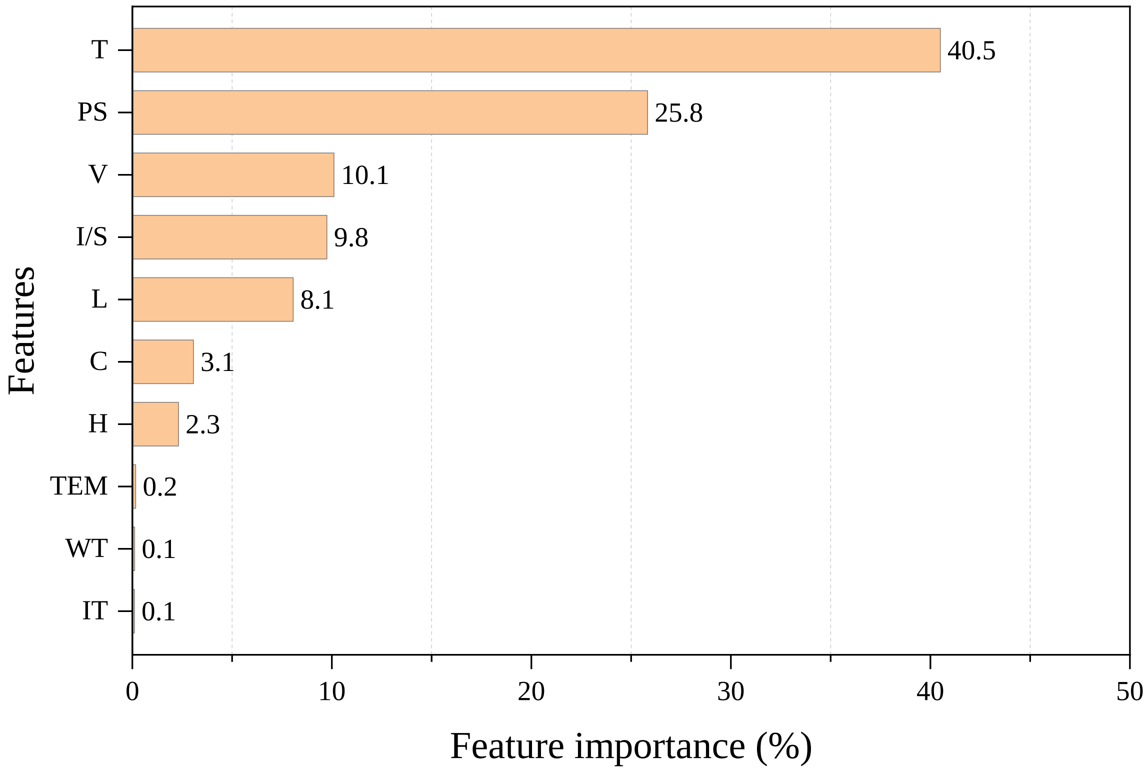

Figure 3-12. Feature importance of each variable based on random forest. WT: wood types; IT: inoculum types; V: volume (mL); Tem: temperature (℃); PS: particle size (mm); I/S: ratio of inoculum to substrate (based on VS); C: cellulose content (%); H: hemicellulose content (%); L: lignin content (%); T: digestion time (d).

3.3.5 Limitations and future perspectives

The production of cleaner energy based on AD as an alternative to fossil fuel has drawn increasing attention. Among the feedstocks for AD, lignocellulosic wastes and especially wood waste are less effective in methane production than other organic wastes (Gao et al., 2022). Therefore, the data related to wood waste and AD are scarce and scattered in the literature. In this chapter, methane production data from wood waste under different

AD systems was collected for meta-analysis, and the involved mutual variables were selected for ML, with the aim of providing a systematic insight into the potential for methane production from AD of wood waste. The meta-analysis showed that wood waste had a lower BMP than other organic wastes but had good pretreatment potential, while all three types of ML models accurately predicted methane production using the digestion parameters after a certain period of training. The existing limitations and future perspectives are summarized as follows.

The results of this chapter have several limitations due to the quality and quantity of data collected from publications. Firstly, very few studies have evaluated methane production from the AD of wood waste. In addition, most of these data were obtained from laboratory experiments, where feedstocks consisting of a single wood material were added to the AD in the experimental design. For example, the volume of AD system in several studies was as low as 60 mL (Eom et al., 2019), which is far less than the practical situation. The temperature collected in the dataset was only mesophilic, with thermophilic conditions often present in AD plants not fully represented. Therefore, some uncertainty exists when extrapolating the results of this chapter to practical AD of wood waste. Secondly, since the vast majority of studies were on a laboratory scale, the pretreatment techniques did not take into account the energy and material consumption and economics of practical applications. In contrast to chemical and physical pretreatment approaches, biological approaches can be more eco-friendly technique with low capital and operating costs (De Bhowmick et al., 2018). Unfortunately, there is a lack of biological approaches, especially natural biodelignification systems, that are as rapid and effective as physical and chemical approaches. Thirdly, for machine learning, the data distribution of some features was inconsistent owing to a variety of variations in experimental goals, methodologies, and conditions. Many publications cannot provide the data on the ten variables (wood types, inoculum types, volume (mL), temperature (℃), particle size (mm), ratio of inoculum to substrate (based on VS), cellulose content (%), hemicellulose content (%), lignin content (%), digestion time (d)) selected to form the ML dataset. In addition, the elemental composition of feedstock, generally missing in the publications, is also an important parameter for predicting methane production (Wang et al., 2023; Zhang et al., 2023a). These situations limited the scale of the dataset available in this chapter.

The optimization of AD is a complex issue that depends on multiple factors and cannot be directly and accurately measured (Gao et al., 2024b). To reduce the complexity of the experiments, a single wood type was commonly used. Therefore, due to the limited number of publications and data that can be extracted, it is difficult to systematically evaluate the effect of mixed wood types on AD and the performance of anaerobic co-digestion between wood waste and other organic wastes. In addition, current studies are focused on exploring the improvement of methane production from wood waste by different pretreatment technologies, while few studies have reported the impact of pretreatment on the microbial composition. Among other lignocellulosic wastes, pretreatment techniques have been demonstrated to alter the microbial composition, especially functional microbes, that plays an important role in AD processes (Ge et al., 2022; Kang et al., 2021; Raut et al., 2021).

Therefore, future research should focus on the construction of a comprehensive database that includes studies with microbiome data under uniform experimental conditions and similar experimental methodologies.

3.4 Conclusions

This chapter addressed RQ1 and RQ2. Specifically, the meta-analysis results showed a 122% lower BMP for wood waste compared to other organic wastes, but this gap could be mitigated to 99% when pretreatment techniques were considered. Further analysis on different pretreatment techniques showed that the employment of pretreatment methods significantly improved the BMP of wood waste by 113% and the combination of multiple pretreatment techniques was more effective than a single approach. This result proves that wood waste can be used as a substrate for AD, but it requires the assistance of pretreatment techniques. Moreover, three ML algorithms were applied to predict methane production based on ten selected variables involved in the literature. The optimal algorithm was RF with the R^2=0.9643 and the RMSE value of 15.52 L/kg of VS in the testing dataset. Feature importance analysis revealed that digestion time and particle size presented the highest importance. This model will help optimize the parameters in wood waste AD and enhance methane production.

Chapter 4 A nature-based approach to enhance the anaerobic digestion application of wood waste

4.1 Introduction

In forest ecosystems, dead trees play a crucial ecological role by decomposing and releasing stored nutrients (Seibold et al., 2021). The decomposition process, involving fungi, bacteria, and arthropods, spans several years and creates a complex community (Tarasov et al., 2018; Yoon et al., 2023). Due to the complexity of the natural wood degradation process in forest ecosystems, it is difficult to simulate and apply this system as a pretreatment method for woody biomass under experimental conditions. Notably, large quantities of forest residues are generated during timber harvesting and from forest maintenance treatments such as cleaning and thinning (Pergola et al., 2022). It is estimated that there are approximately 230,000 hectares of plantation forests in Queensland, with up to 600,000 tons of forest residues generated by logging annually (Garvie et al., 2021). Kurvits et al. (2020) investigated the quantity of logging residues at four forest sites in Southeast Estonia from 2013 to 2014 and found that forest residues reached up to a dry weight of 29 tons per hectare. These forest residues are gradually degraded into humus, becoming part of the forest soil. Throughout this process, the tough lignocellulose structure of the wood is slowly breaks down (texture gradually softens) until the wood is fully decomposed, releasing all its nutrients (Petritan et al., 2023; Shorohova et al., 2021). Interestingly, the increased accessibility and biodegradability of the lignocellulosic structure tends to make wood waste more suitable for AD (Hashemi et al., 2021). In addition, the softened texture of the material allows them to be shredded more easily, which can also save the energy required to pre-process the material prior to AD (Naimi et al., 2013).

This chapter responds to RQ 3. It is hypothesized that exposing woody wastes to forest ecosystems for a period of time is sufficient to enhance its digestibility as part of a pretreatment process prior to AD. In addition, the collection and valorization of such residues can help to mitigate forest management costs, reducing fire risk and additional emissions from degradation (Lee and Han, 2017; Molenda et al., 2021; Nicholls et al., 2018). The objectives of this study were to determine the differences in physicochemical composition and the methane production potential between wood samples at different stages of decay from forest environments. Identifying the decay stage at which the wood waste can have the highest methane production allows for strategic collection of this material, enhancing the economic viability of using wood waste as a raw material in AD applications.

4.2 Materials and methods

4.2.1 Experimental materials

The wood samples were collected from two sites, with Site 1 being an unmanaged seminatural forest in Cardiff, UK (51°31'4"N, 3°14'46"W) and Site 2 being a large managed forest in Cardiff, UK (51°32'25"N, 3°14'58"W). At each site, at least 15 dead fallen logs were sampled, each of which was allocated to a particular *decay class* (DC), determined based on visual and mechanical inspections (Table 4-1), with DC1 showing minimal signs of decay and DC5 showing highly advanced levels of decay (Tatti et al., 2018). Considering the external appearance and the species of the surrounding trees, the wood samples from Site 1 were probably hardwood silver birch (*Betula pendula*), expressed here as Birch. The sampling location at Site 2 consists primarily of European ash (*Fraxinus excelsior*), expressed in this paper as Ash, confirmed from site records and by examining the surrounding tree morphology. Site records were obtained from DataMapWales (https://datamap.gov.wales/maps/new#/) using the "NRW Woodland sub-compartment data" layer on May 31, 2023. Wood samples were dried and then shredded using a Fritsch 55743 rotary knife mill with a 2 mm screen and finally stored at 4 °C until further analysis.

Table 4-1. The five-decay class system used for the description of woody debris, modified from Tatti et al. (2018).

Characteristics	**Decay class**				
	DC1	**DC2**	**DC3**	**DC4**	**DC5**
Bark	bark normally intact	loose bark	usually without bark	absent	absent
Texture	intact	intact to partly soft	wood of outer layers of stem fairly soft, core still hard	small soft portions of the log easily discernible	soft and powdery (very few portions of the log remain coherent)
Knife test	knife blade penetrates a few millimeters	knife blade penetrates max 2 cm	knife blade penetrates 2-5 cm	knife blade penetrates all the way	breaks up easily by hand
Color of wood	original color	original color	original color to faded	light brown to reddish brown or yellowish	faded to light yellow or grey, or red brown to dark brown

4.2.2 Experimental procedure

The AD experimental apparatus, consisting of multiple 1L bioreactors in a temperature-controlled water bath with gas collection facility, was supplied by Anaero Technology UK, described in more detail by Muaaz-Us-Salam et al. (2020). Sewage sludge digestate from an AD reactor was used as an anaerobic inoculum. Prior to AD testing, the sludge was incubated at 35 ℃ for 3 days and shaken manually twice a day to ensure homogenization. For every experiment, fresh sludge was sampled from the same AD reactor. Each bioreactor was filled with 700 mL of inoculum with a headspace volume of 300 mL. Then a certain mass of wood samples at different DC was added, the blank group did not have any wood sample added (only 700 mL inoculum). The weight of wood samples in each reactor was calculated to ensure the ratio of decaying wood samples to inoculum was 1:4 based on VS levels. All bioreactors were incubated at 35 ℃ for 35 days with continuous stirring at 45 rpm. Liquid samples (5 mL) were taken during the AD process using sterile pipettes and transferred to 15 mL sterile containers for the measurement of some parameters during the AD process. Biogas produced from each reactor was collected during the experiment in 5 L Tedlar gas bags and analyzed for methane content (see section 4.2.3.4). The AD experiments were performed in triplicate for each wood sample and in duplicate for the blank group (only inoculum).

Since the hardness of the wood samples varies among different decay classes, which resulted in their different particle sizes after grinding process. To further investigate the effect of particle size on the performance of AD, the AD experiments were conducted with the same particle size birch samples. In combination with the results of AD and particle size analyses of all birch samples, DC1 and DC3 samples were selected to be analyzed at 0.5–1 mm. The detailed AD process was the same as the process described above.

4.2.3 Analytical methods

4.2.3.1 Specific energy consumption for grinding

The effect of DC on specific energy consumption (SEC) during shredding (comminution) was determined. A Fritsch 55743 rotary knife mill was used, equipped with a 2 mm screen and a 2100 W motor. For all samples, a pre-weighed 50 grams wood block (dry matter) was placed in the rotary knife mill for 60 seconds, and then the wood collected in the tray was weighed. The SEC was calculated according to Equation (4-1) as presented in previous literature (Miao et al., 2011; Moiceanu et al., 2019).

$$\mathrm{SEC} = \frac{P \times T}{m} \tag{4-1}$$

where SEC is total specific energy consumption for grinding a unit of dry matter (MJ/kg of dry matter); P is the power (kW) of the milling machine while grinding wood samples; T is the total time (h) of grinding operations; m is dry matter mass (kg) of wood samples collected in the tray.

4.2.3.2 Analysis of particle size distribution

Particle size distribution was measured using sieving methods. Specifically, wood samples were put into a Fritsch 55743 rotary knife mill equipped with a 2 mm screen and operated for 1 min. The collected sample was passed through a tower of differently sized sieves. The sieve stack contained stainless steel sieves (diameter 200 mm) with mesh sizes of 2000, 1000, 500, 250, 125, and 75 μm. The sieving steps were performed on a vibratory shaker (Matest A060-01) for 30 min to ensure adequate separation of the samples. Samples were finally sieved into seven fractions (<75, 75–125, 125–250, 250–500, 500–1000, 1000–2000, and >2000 μm), and the proportion of samples in each particle size class was calculated based on the weight.

The mean weight diameter (MWD) and fractal dimension (FD) can be applied to further characterize the particle size of the sample (Rabot et al., 2018; Zhang et al., 2021). MWD is the sum of the weighted mean diameters of all size classes, whilst FD is a comprehensive indicator of sample composition and textural homogeneity. The MWD of dry-sieved samples was calculated using Equation (4-2):

$$\text{MWD} = \sum_{i=1}^{n} \frac{r_i + r_{i+1}}{2} \times m_i \tag{4-2}$$

where r_i is the aperture of the *i*th sieve, m_i is the proportion of sample weight remaining on the *i*th sieve, and n is the number of sieves. The FD was calculated using Equation (4-3):

$$\frac{M(r < R_i)}{M_r} = \left(\frac{R_i}{R_{max}}\right)^{3-FD} \tag{4-3}$$

where $M(r < R_i)$ is the cumulative sample mass with a radius smaller than R_i, M_r is the total sample mass with a radius smaller than R_{max}, R_i is the radius of each dimensional fraction, R_{max} is the maximum radius, and FD is the fractal dimension of the sample.

4.2.3.3 Physicochemical features of solid samples

TS and VS of the wood samples were measured following the Standard Methods 2540 protocol (Rice et al., 2017). The ultimate analysis (carbon, hydrogen, nitrogen, and oxygen content) was measured using an elemental analyzer (Flash Smart, Thermo Fisher Scientific

Co., USA). The cellulose, hemicellulose, and lignin contents were determined by thermogravimetric analysis (Díez et al., 2020; Rego et al., 2019), and the detailed approach is shown in the section 4.2.3.6. The total organic carbon (TOC) of wood samples were measured with a TOC-VCPH (Shimadzu, Kyoto, Japan) following the manufacturer's instructions. The potassium hydrogen phthalate was used as a standard for measuring the total carbon content, and sodium hydrogen carbonate and sodium carbonate (anhydrous) were used as a standard for measuring the inorganic carbon content. The calibration curves of the total carbon and inorganic carbon content are shown in Figure A-4. Wood bulk density for different DC samples was measured using the water displacement method, following Edelmann et al. (2023). Dried wood pieces, approximately 3 cm in length and width and 5 cm in height, were weighed using an analytical balance. To prevent water absorption, the surface of each sample was coated with a thin layer of paraffin. The paraffin-coated samples were then fully submerged in a graduated cylinder containing water, and the volume displacement was recorded. The difference in water volume before and after submersion represented the wood block's volume, which was then used to calculate its bulk density. The analysis of water extractable organic carbon (WEOC) was modified from Mo et al., (2022). Specifically, a total of 5 g of shredded wood was added to 30 mL of Milli-Q water and shaken at 200 rpm for 2 h at room temperature. The supernatant was subsequently filtered through sterile 0.45 μm filters and stored in the dark at 4 °C prior to further analyses. Finally, the WEOC concentrations were measured by a TOC-VCPH (Shimadzu, Kyoto, Japan) following the manufacturer's instructions.

The crystallinities of all decaying wood samples were measured using a X'Pert^3 MRD XL Materials Research X-ray Diffraction System (Malvern Panalytical, Malvern, UK) equipped with CuKα radiation. Scans were obtained from $2\theta = 10–40°$ with step size of 0.02 at 0.6 s per step. The cellulose crystallinity index (CrI) of the samples can be calculated according to Equation (4-4):

$$\mathrm{CrI} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \tag{4-4}$$

where I_{002} is intensity of diffraction from 002 plane at $2\theta = 22°$ and I_{am} is the intensity of background measured at $2\theta = 18°$ (Kumar et al., 2019; Xiaoying Liu et al., 2015).

4.2.3.4 Physicochemical features of liquid and gas samples

TS and VS of the inoculum were measured following the standard methods (Rice et al., 2017). The inoculum was dried and subjected to ultimate analysis with an elemental analyzer (Flash Smart, Thermo Fisher Scientific Co., USA). The pH and oxidation reduction potential (ORP) of inoculum and liquids samples collected during AD experiments were measured by a freshly calibrated pH probe (Mettler-Toledo, Switzerland) and a freshly calibrated ORP probe with an Ag/AgCl electrode (Mettler-Toledo, Switzerland). All 5 mL liquid samples obtained during AD experiments were filtered through sterile 0.45 μm filters. Subsequently, a 2 mL filtered sample was added to 18 mL Milli-Q water (10 times dilution) and mixed thoroughly for TOC measurement.

The biogas production in each bioreactor was determined by gas flow meters incorporated within the AD apparatus combined with data logging equipment, and the methane content of the biogas was determined using a portable biogas analyzer (RASI 700 BIO, Eurotron Instruments UK ltd, Germany). The instrument was calibrated using a series of standard gases with concentration gradients (labeled methane concentration) prior to testing the methane content in the samples. The calibration curve is shown in Figure A-5.

4.2.3.5 Calculation and prediction of methane production

The theoretical methane production (TMP) of wood samples was calculated from the elemental composition (expressed in molar fractions) using the Buswell formulae (Lübken et al., 2010), Equations (4-5) and (4-6):

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right) CO_2 + cNH_3 \tag{4-5}$$

$$\text{TMP} = 22.4 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right) / (12n + a + 16b + 14c) \tag{4-6}$$

The anaerobic biodegradability (BD) of wood samples was calculated according to Equation (4-7):

$$\text{BD (\%)} = \textit{Experimental methane production (EMP)}/\textit{TMP} \times 100 \tag{4-7}$$

The experimental biogas production was fitted using a modified Gompertz kinetic model, which is one of the most commonly employed models in the literature for fitting biogas production (Isha et al., 2021). The final biogas production was calculated based on the best-fit Gompertz model and then multiplied by the methane content to obtain the predicted methane production (PMP). The kinetic model is shown in Equation (4-8):

$$M = P_b \times exp\left\{-exp\left[\frac{R_m \times e}{P_b}(\lambda - t) + 1\right]\right\} \quad (4\text{-}8)$$

where M is the biogas production (mL/g of VS) relative to the time t (d); P_b is the maximum biogas potential of the substrate (mL/g of VS); R_m is the maximum biogas production rate (mL/g of VS.d), λ is the lag phase time taken for biogas production (1/d), e is Euler's number which is taken here as 2.7183.

Furthermore, there was no well-developed model available to predict the methane production from AD of wood waste. Chapter 3 generated a machine learning model (RF) with good predictive performance, which was applied in this chapter to predict methane production from all decayed wood samples.

4.2.3.6 Thermogravimetric analysis for the wood samples

Preparation of Materials

Each wood sample was crushed in a mill by a high-speed pulverizer and passed through a sieve with an aperture of 90 μm. The wood samples were then placed in a 100 ℃ blast oven for 2 hours to eliminate moisture. The fraction of extractives in the dried biomass was determined by using a Dionex® Accelerated Solvent Extractor (ASE100) with 95% ethanol as extraction solvent.

This sample size was used to minimize the heat transfer resistance and mass transfer diffusion effects. The heat transfer effect in the thermogravimetric analysis (TGA) was discussed by comparing the derivative thermal gravimetry test (DTG) curves of different sample masses under the same experimental conditions. The DTG curve did not change when the sample mass was reduced from 8.0 mg to 6.0 and 4.0 mg, indicating that a sample mass of 8.0 mg was sufficient to eliminate the mass transfer limitation. The maximum Biot number (Bi) of a single wood sample particle could be calculated as Equation (4-9):

$$\mathrm{Bi} = \frac{hd}{\lambda} = \frac{10\ {}^{W}/_{(m^2K)} * 90 \cdot 10^{-6}\ m}{0.04\ {}^{W}/_{(mK)}} = 0.0225 < 0.1 \tag{4-9}$$

where h is the convective heat transfer coefficient, and the convective heat transfer coefficient of natural convection ranges from 1 to 10 W/(m^2K); d is the diameter of the wood sample particle, 90 µm; λ is the thermal conductivity of the wood sample, which varies from 0.04 to 0.4 W/(mK) based on wood type, density, and moisture content. Therefore, the maximum value of the calculated Bi for the wood sample particles was less than 0.1, indicating that the internal temperature of the particles is uniform and closely follows the temperature rise set by the instrument. This results in minimal temperature gradients within the particles during pyrolysis, reducing the influence of heat and mass transfer factors in the experiments.

Pyrolysis Test Conditions

TGA was performed using a Mettler Toledo analyzer (TGA/SDTA 851e). The pyrolysis was carried out under a nitrogen atmosphere with a flow rate of 50 mL/min. The heating rate was 10 °C/min. The heating temperature started at 30 °C to a final temperature of 1000 °C and maintained at 1000 °C for 1 h. The sample weight was 8.0 mg. To reduce temperature-related errors, the equipment used was calibrated across the entire temperature range. In addition, the actual sample temperature was used directly to solve the kinetic equations and to calculate the actual sample heating rate.

Gauss Peak Fitting

The thermochemical decomposition of biomass can be represented by three main kinetics corresponding to the degradation of hemicellulose, cellulose, and lignin, respectively. In addition, water is present as a fourth non-structural component in moist biomass samples. For the estimation of the lignocellulosic content, the DTG experimental curve was treated with deconvolution using the symmetric Gaussian type curves (Castells et al., 2021; Mu et al., 2022; Rego et al., 2019). Moreover, there might be more than one curve required to fit each fraction because of the complexity of the components, and Table A-8 shows the minimum number of pseudo-components to quantify each fraction (Díez et al., 2020).

The multiple peak fit tool in Origin 2021 was used to separate the four components' reaction rate curves from the total DTG curve. The pyrolysis peak temperatures of the four components were set to be consistent with the literatures (Díez et al., 2020; Mu et al., 2022). The correlation coefficients R^2 of the relevant results are generally larger than 0.95, indicating that the fitting results are accurate and credible. Finally, the lignin content of wood samples was measured based on Muaaz-Us-Salam et al. (2020) and this was used to validate the calculated resulted by TGA curves. Specifically, 0.25 g of dried wood sample was weighed into a 25 ml conical flask, added to 3.75 ml of a 95% sulfuric acid solution, and shaken for 2 hours at room temperature. Then, 140 ml of deionized water was added to the obtained solution and the mixture was refluxed in a round-bottomed flask for 4 hours. The residue was collected on Whatman® quantitative filter paper No. 42 (2.5 μm) and washed three times with deionized water. The residue (insoluble lignin) was dried at 110°C for 1 h and its weight was measured on an analytical balance, from which the percentage lignin was calculated.

4.2.4 Statistical analysis

All experiments were conducted with three technical replicates, unless otherwise specified. In this paper, statistical analyses were done using Origin 2021. All values are presented as the mean ± s.d., unless otherwise specified. Statistical significance was assessed using the two-tailed Student's t-test or One-way ANOVA test with significance at a p value of 0.05. Moreover, The Pearson correlation coefficients (r) between the variables were also calculated. The strength of the correlation was described by the absolute value of r (0.00–0.19 very weak; 0.20–0.39 weak; 0.40–0.59 moderate; 0.60–0.79 strong; 0.80–1.0 very strong).

4.3 Results and discussion

4.3.1 Energy consumption and particle size analysis

Generally, mechanical pretreatment is considered as the most important and promising preliminary step for handling and converting biomass into bioenergy before proceeding to the next process (Kamarludin et al., 2014). Without a sufficiently small particle size or large relative surface area, the organic matter in the substrate cannot be utilized by microorganisms to produce biogas during AD. For example, 2 cm wood cubes in digested sewage sludge produced approximately the same amount of biogas as blanks with only sludge (Gao et al., 2023; Muaaz-Us-Salam et al., 2020). As shown in Figure 4-1, the SEC gradually decreased as DC increased. Figure 4-1a shows that the SEC of Birch decreased from 10.56 to 2.92 MJ/kg of dry matter for DC1 to DC5, while Ash correspondingly dropped from 9.44 to 2.60 MJ/kg of dry matter (Figure 4-1b). In addition, the decrease rate of SEC for both wood samples gradually became slower with increased DC, showing no statistical difference between wood samples from DC3 to DC5. These results imply that the higher the DC, the more energy can be saved in reducing the particle size of these wood samples for utilization in AD. It is worth noting that the criteria for these DC include their hardness, with DC5 being extremely soft due to its woody structure has been essentially destroyed (Tatti et al., 2018). During the sampling, it was found that DC4 and DC5 can be broken even with slight force by hand. Therefore, wood samples with high DC may not require physical pre-processing.

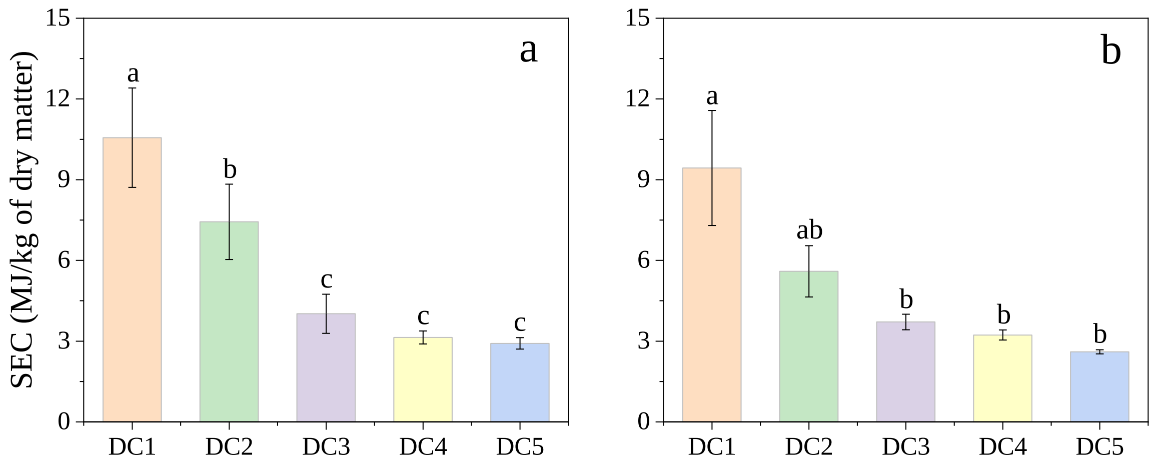

Figure 4-1. Specific energy consumption (SEC) for grinding wood samples from five decay classes, (a) Birch; (b) Ash. Different lowercase letters above each column indicate significant differences (columns with different letters are statistically significantly different from one another).

Reducing the particle size of substrates is a competitive option for increasing methane production from AD as it releases more organic matter and cell compounds, and directly increases the microbially accessible surface area, thus improving biodegradability (Dai et al., 2019). After grinding the samples, the particle size distribution was analyzed (Table 4-2). The samples of DC1 and DC2 had the highest proportion in the 1–2 mm particle size class, and the proportion decreased as the particle size class increased. The proportion of the fine particle size class gradually increased from DC1 to DC5, with the proportion of 0.5–1 mm particle size class being the largest in DC4 and DC5. In addition, the MWD analysis also showed the particle size of wood samples reduced gradually from DC1 to DC5. Specifically, the MWD of Birch decreased from 0.93 to 0.57 mm for DC1 to DC5, while Ash correspondingly dropped from 1.10 to 0.78 mm (Table 4-2). Reduced particle size can significantly facilitate hydrolysis and acidification processes, resulting in increased volatile fatty acid content and VS degradation (Luo et al., 2021). Liu et al. (2017) reported the effects of particle size of two forest residues on methane production through AD batch experiments, and the results showed that methane yield improved when the substrate particle size was reduced from 4 mm to 1 mm. A similar pattern has been demonstrated in other lignocellulosic wastes, such as rice straw, where methane production improved with a decrease in substrate particle size (Dai et al., 2019; Ji et al., 2022).

Table 4-2. The particle size distribution, MWD, and FD of wood samples after grinding by the knife mill.

Wood type	Decay class	Proportion of wood mass in particle size class (%)							MWD (mm)	FD
		>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	0.125–0.25 mm	0.075–0.125 mm	<0.075mm		
Birch	DC1	2.12 ± 0.35	41.95 ± 5.60	30.31 ± 0.36	14.71 ± 2.91	6.82 ± 1.31	2.27 ± 0.59	1.83 ± 0.78	0.93 ± 0.07	1.85 ± 0.13
	DC2	0.31 ± 0.01	29.70 ± 0.89	26.80 ± 3.09	23.32 ± 3.81	9.59 ± 1.54	5.86 ± 0.66	4.42 ± 0.57	0.76 ± 0.00	2.15 ± 0.01
	DC3	0	21.27 ± 1.73	23.94 ± 3.02	19.32 ± 0.18	22.69 ± 1.92	7.92 ± 3.12	4.85 ± 3.73	0.62 ± 0.05	2.22 ± 0.19
	DC4	0	23.52 ± 0.64	27.72 ± 0.53	18.20 ± 2.83	15.89 ± 2.70	9.49 ± 1.27	5.18 ± 0.02	0.67 ± 0.02	2.26 ± 0.02
	DC5	0	16.75 ± 1.03	24.89 ± 4.54	23.66 ± 6.02	17.78 ± 1.57	9.92 ± 0.04	7.01 ± 1.08	0.57 ± 0.03	2.34 ± 0.03
Ash	DC1	1.51 ± 0.14	56.41 ± 2.55	28.49 ± 1.82	8.03 ± 0.53	3.48 ± 0.26	1.32 ± 0.10	0.76 ± 0.11	1.10 ± 0.03	1.57 ± 0.03
	DC2	0.75 ± 0.12	52.80 ± 0.45	29.39 ± 0.01	9.78 ± 0.28	4.12 ± 0.22	1.72 ± 0.02	1.45 ± 0.10	1.06 ± 0.01	1.74 ± 0.02
	DC3	0.14 ± 0.04	36.21 ± 0.97	29.62 ± 2.07	19.81 ± 1.02	9.78 ± 0.33	2.64 ± 0.02	1.80 ± 0.18	0.86 ± 0.00	1.90 ± 0.02
	DC4	0	32.78 ± 6.26	37.83 ± 0.58	17.31 ± 3.93	7.16 ± 1.21	3.09 ± 0.24	1.84 ± 0.78	0.86 ± 0.10	1.88 ± 0.14
	DC5	0	29.68 ± 2.63	31.63 ± 1.96	19.88 ± 0.38	10.69 ± 2.56	5.27 ± 0.21	2.86 ± 0.48	0.78 ± 0.04	2.06 ± 0.02

MWD: mean weight diameter (mm), FD: fractal dimension.

Fractal theory has been applied to quantitatively assess the basic morphology of the substrate, which is a potential indicator reflecting the AD efficiency (Wang et al., 2016). In this chapter, the FD of different DC samples were statistically different ($p<0.05$) after grinding under the same conditions. As shown in Table 4-2, the FD average of both wood samples at DC1 was significantly lower than DC5 ($p<0.05$), while there were no significant differences in FD values between DC3, DC4 and DC5 ($p>0.05$). As the FD increases, the complexity of particle morphology grows, accompanied by an increase in surface area (Dai et al., 2019). This enhanced surface area facilitates greater interaction between particles and the surrounding medium, thereby accelerating particle diffusion. This effect is particularly pronounced for smaller particles or when the spacing between particles is relatively large, as the increased surface area further boosts diffusion efficiency (Lai et al., 2021). Therefore, the DC5 samples may have enhanced mobility in the AD system compared to the DC1 samples, which facilitates its full utilization by microbes. Moreover, the wood samples after DC3 showed no significant difference, suggesting that this class (DC3) may achieve optimal conditions for sample crushing.

4.3.2 Physicochemical features analysis of decaying wood samples

The possibility of using these wood samples as a suitable substrate for AD was primarily verified through various tests, prior to conducting the biomethane potential experiments. As shown in Table 4-3, TS content decreased with DC, while the VS content did not change much. Samples with a high DC have a looser texture, which allows them to easily retain more water. Compared to Ash, in general Birch had a smaller TS content and a much larger drop from DC1 (59.47%) to DC5 (18.39%). For lignocellulosic biomass, the nitrogen content is a key factor limiting their AD performance (Song et al., 2024). A low level of nitrogen can lead to nitrogen limitation, which prevents the microorganisms from fully utilizing the carbon source, thus reducing the production of methane (Piątek et al., 2016). The total carbon in all five DC were nearly the same, but the nitrogen content was higher in the high DC samples. Therefore, the C/N of DC5 was much lower than that of DC1. It has been reported that the optimal C/N for maximal methane production is between 20 and 30 (Kumar et al., 2021). Although the C/N of DC5 samples was also higher than the optimal value, it is easier to achieve the superior system by mixing them with sludge (low C/N). During AD, TOC can be biodegraded in hydrolysis, acidification and methanation steps to produce biogas (Provenzano et al., 2014). Furthermore, WEOC is a critical contributor in these processes, as microbial metabolism occurs in the water-soluble phase (Xing et al., 2012). Therefore, the significantly higher TOC (Figure 4-2a and 4-2b) and WEOC (Figure 4-2c and 4-2d) in high DC samples indicate a better potential for methane production from AD. It is noteworthy that the WEOC did not consistently increase with decay level, showing a tendency of first increasing and then decreasing. The DC3 samples of Birch had the highest WEOC (Figure 4-2c), and the DC4 samples of Ash had the highest WEOC (Figure 4-2d). This might be due to the release of organic matter from the forest residues in the presence of insects and microorganisms, leading to an increase in WEOC content at the beginning of decay process. When it reaches the final stages of decomposition, there is no more available organic matter to be released, and the previously released organic matter is utilized by other organisms or enters the soil, leading to a decrease in the WEOC content.

Table 4-3. Properties of decaying wood samples and inoculum.

Properties	Birch					Ash					Inoculum
	DC1	DC2	DC3	DC4	DC5	DC1	DC2	DC3	DC4	DC5	
pH value	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	8.41 ± 0.36
Total solid (%)	59.47 ± 15.12	34.90 ± 3.81	26.30 ± 6.44	22.36 ± 10.16	18.39 ± 6.80	90.73 ± 1.79	87.81 ± 0.03	81.39 ± 5.70	80.84 ± 9.06	77.19 ± 6.35	8.14 ± 0.05
Volatile solid (%)	99.31 ± 0.60	99.08 ± 0.61	96.95 ± 0.40	97.28 ± 1.52	96.61 ± 0.73	98.05 ± 0.80	98.77 ± 0.11	98.31 ± 0.79	95.74 ± 2.96	97.25 ± 0.08	69.09 ± 0.11
Ultimate analysis											
Carbon (%)	47.16 ± 0.29	48.46 ± 0.87	47.56 ± 1.00	47.62 ± 1.45	50.55 ± 0.77	47.60 ± 0.40	47.19 ± 0.12	47.96 ± 0.04	48.41 ± 0.38	47.59 ± 0.40	31.80 ± 0.83
Nitrogen (%)	0.33 ± 0.04	0.84 ± 0.18	0.77 ± 0.19	1.18 ± 0.24	1.19 ± 0.10	0.13 ± 0.00	0.20 ± 0.10	1.40 ± 0.28	1.90 ± 0.08	1.77 ± 0.35	5.40 ± 0.08
Hydrogen (%)	5.76 ± 0.15	5.52 ± 0.16	5.53 ± 0.20	5.54 ± 0.22	5.45 ± 0.16	5.47 ± 0.14	5.62 ± 0.07	5.67 ± 0.06	5.77 ± 0.05	5.73 ± 0.01	5.07 ± 0.25
Oxygen (%)	44.76 ± 0.71	43.49 ± 1.06	44.04 ± 0.77	43.96 ± 1.27	41.26 ± 0.18	46.82 ± 0.52	46.98 ± 0.14	44.97 ± 0.19	43.92 ± 0.41	44.92 ± 0.63	36.20 ± 0.08
C/N ratio	146.73 ± 17.62	60.43 ± 12.92	65.82 ± 16.24	42.47 ± 10.46	42.92 ± 3.93	465.76 ± 104.05	302.11 ± 146.92	35.80 ± 7.32	25.49 ± 0.86	27.84 ± 4.91	5.89 ± 0.07
Biochemical analysis											
Extraction (%)	7.18 ± 0.59	5.06 ± 0.59	4.24 ± 0.75	3.94 ± 0.13	3.67 ± 1.31	8.47 ± 0.97	8.14 ± 0.45	6.77 ± 0.12	6.78 ± 0.65	3.90 ± 0.19	ND
Hemicelluloses (%)	23.37 ± 0.61	19.07 ± 1.04	22.41 ± 2.28	22.37 ± 0.60	17.94 ± 0.42	22.46 ± 2.19	22.01 ± 0.48	20.44 ± 0.05	22.45 ± 1.19	27.25 ± 0.72	ND
Cellulose (%)	34.29 ± 2.38	28.51 ± 1.09	27.09 ± 1.02	24.83 ± 1.50	16.24 ± 1.33	38.64 ± 1.17	34.15 ± 0.75	31.03 ± 1.38	28.35 ± 0.77	19.26 ± 0.72	ND
Lignin (%)	23.67 ± 2.07	34.85 ± 2.30	35.78 ± 2.70	35.63 ± 0.48	49.17 ± 1.90	26.88 ± 0.47	31.19 ± 1.04	38.33 ± 1.19	38.86 ± 0.35	44.61 ± 1.26	ND

ND: not determined. The % content of total solid was calculated based on wet mass; others were based on dry mass.

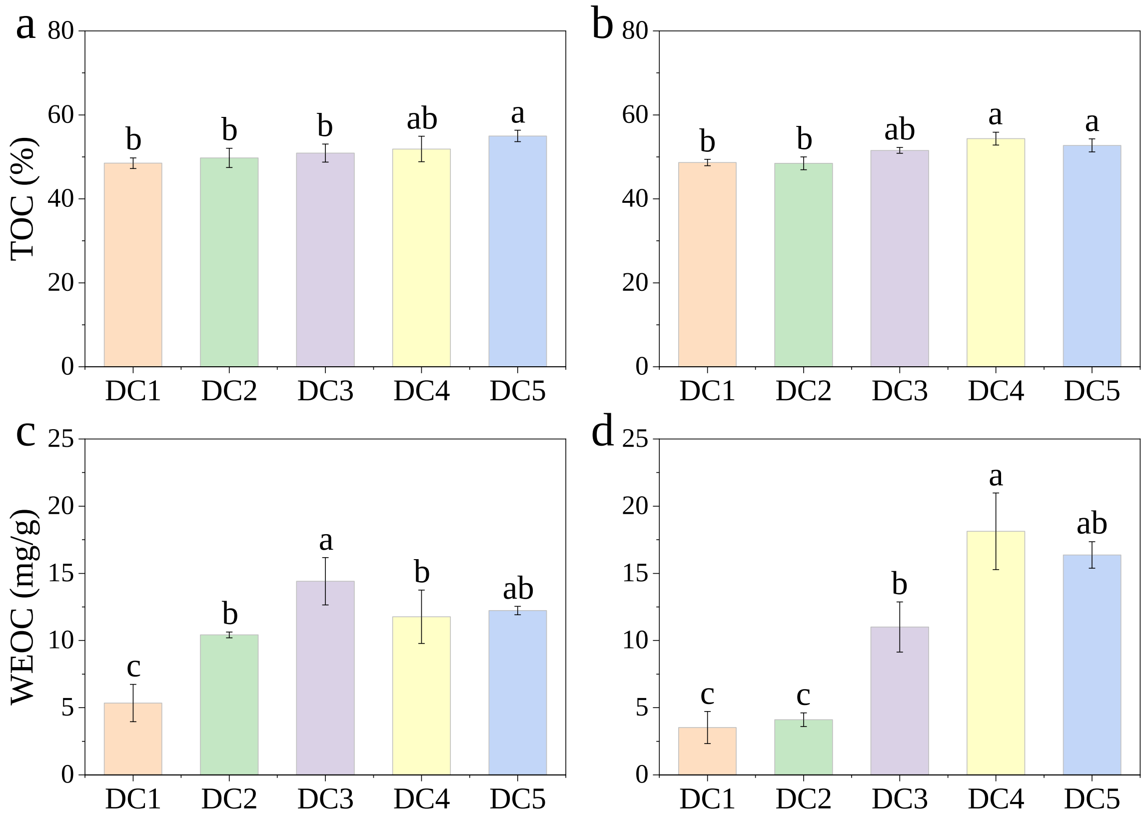

Figure 4-2. Different categories of organic carbon from five decay classes wood samples. The total organic carbon (TOC) content of (a) Birch and (b) Ash, and water extractable organic carbon (WEOC) of (c) Birch and (d) Ash. Different lowercase letters above columns indicate a difference at a 0.05 level.

The lignocellulose composition is an important factor that affects the AD performance of forest residues. Figure 4-3 and Figure 4-4 show the thermogravimetric experimental data and derivative thermogravimetric curves fitting results using the Gaussian model, and the calculated lignocellulose composition of all wood samples are provided in Table 4-3. In both types of wood samples, the cellulose content decreased with DC, in contrast with a gradually increased lignin content. The hemicellulose content did not vary much among the five DC samples, and two types of wood samples showed different tendencies. With an increase in DC, Birch presented an overall decrease, while Ash first decreased and then increased. The significantly increased lignin content of higher DC may be due to the reduction of other components such as cellulose, indicating that the forest soil system was not effective in removing lignin of forest residues, and it became proportionally more significant as a component as decay occurred. However, the decay has permitted biological access to, and degradation of, cellulose and hemicellulose, suggesting that a certain amount of decay and breakdown may be advantageous as a pretreatment method for AD. Similar effects have been observed in other studies, such as the application of chemicals (Mohsenzadeh et al., 2012; Salehian et al., 2013), hydrothermal (Karami et al., 2022) and steam explosion (Eom et al., 2019; Mulat et al., 2018) leading to a reduction in the cellulose content and an increase in the lignin content of wood waste.

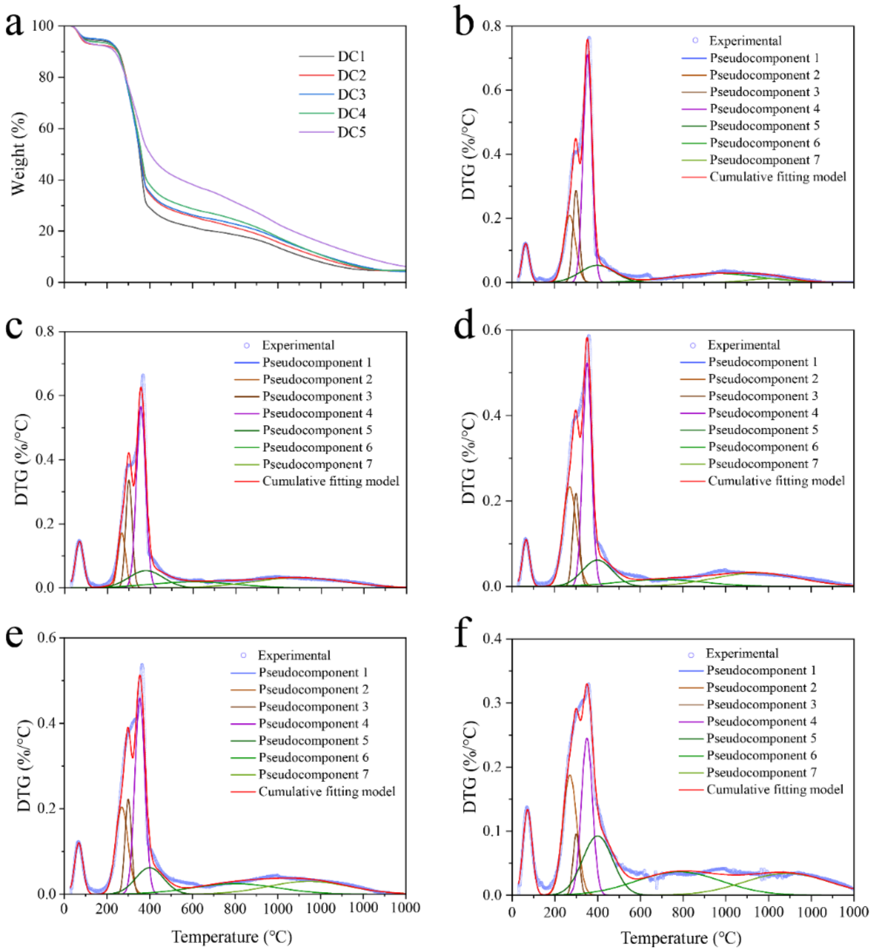

Figure 4-3. (a) TGA curves comparison of Birch samples from five decay classes. The gauss peak fitted experimental DTG curves, (b) DC1; (c) DC2; (d) DC3; (e) DC4; (f) DC5.

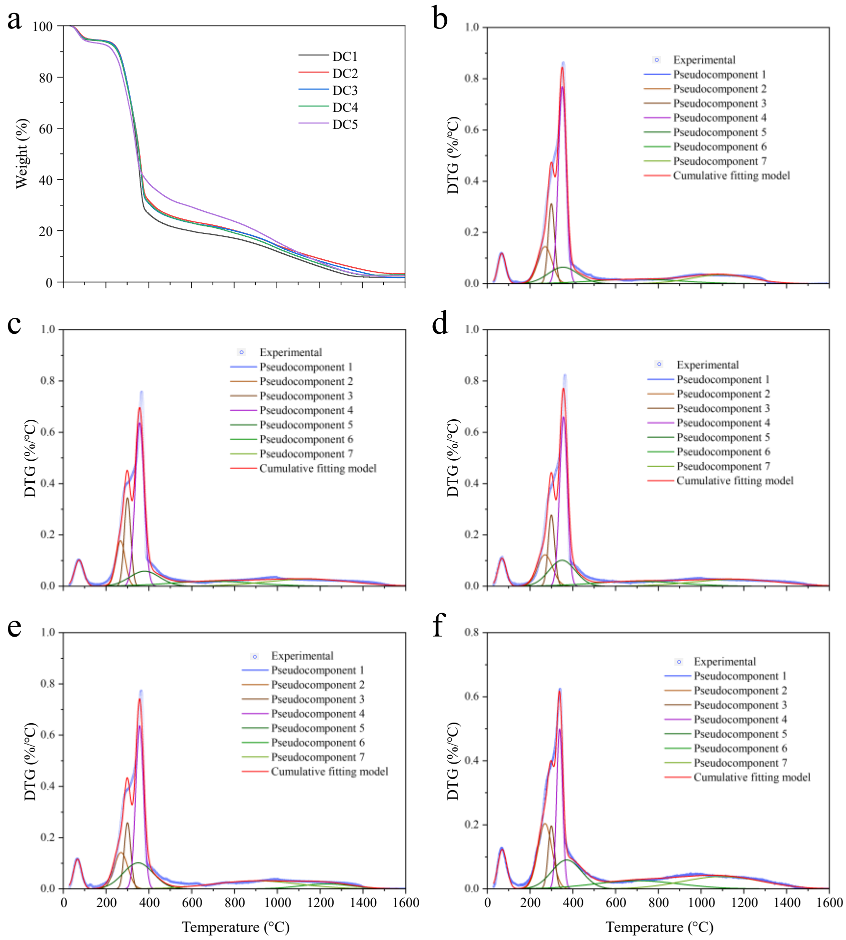

Figure 4-4. (a) TGA curves comparison of Ash wood samples from five decay classes. The gauss peak fitted experimental DTG curves, (b) DC1; (c) DC2; (d) DC3; (e) DC4; (f) DC5.

Due to the presence of crystalline cellulose in biomass samples, the 2θ value of X-ray diffraction shows a sharp peak between 18° and 22° (Awoyale and Lokhat, 2021). Cellulose crystallinity reflects the proportion of cellulose crystalline regions, and the CrI of the substrate determines its biodegradability during AD. The CrI of Birch DC1 samples was 35.43%, close to the value of raw pine wood; and the CrI of Ash DC1 samples (41.61%) was close to that of untreated acacias (Darmawan et al., 2016). It indicated that the degradation of DC1 samples was quite small, and its CR nearly approached that of fresh wood. The CrI of DC5 samples was much lower than that of DC1 samples, with a value of 16.47% in Birch (Figure 4-5a) and 26.43% in Ash (Figure 4-5b). As mentioned above, this may be due to the different composition in different DC samples. The content of soluble matter and amorphous cellulose in lignocellulosic biomass is higher than that of crystalline cellulose, which can result in a lower CrI (D' Silva et al., 2022). The hydrolysis of amorphous cellulose by cellulase was found to be about 30 times faster than that of crystalline cellulose (Zhu et al., 2011). The low CrI value in DC5 samples meant that their cellulose crystalline region was destroyed, leaving more cellulose (amorphous cellulose) available for microbial hydrolysis. Moreover, it was found that corn straw pretreatment with hydrogen-nanobubble water (He et al., 2022) or a pure bacteria system (Xu et al., 2018) also reduced the CrI of substrate and enhanced the methane production from AD of corn straw. Therefore, the forest soil system could degrade the cellulose crystallinity, allowing the forest residues to decompose more easily by AD.

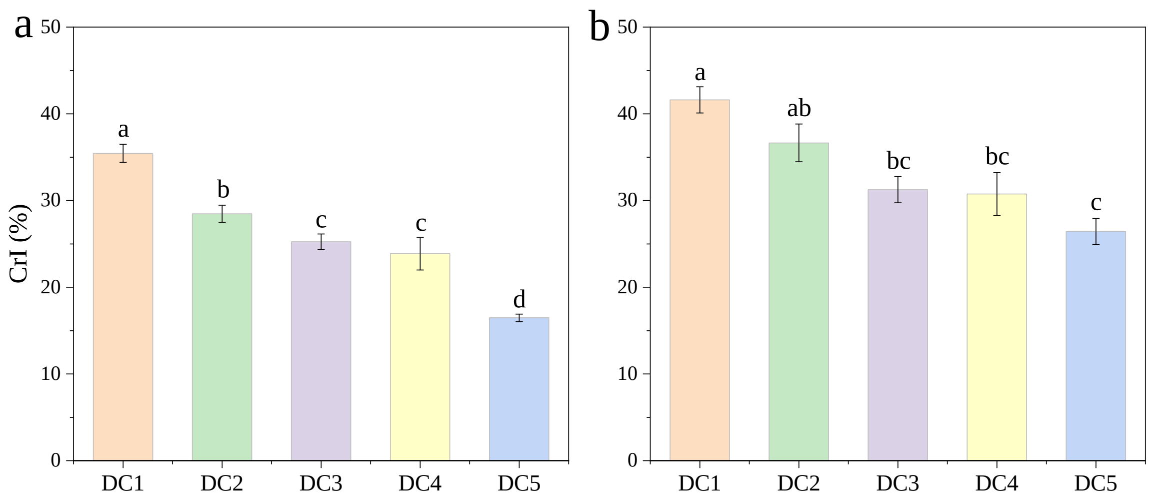

Figure 4-5. The Crystallinity Index (CrI) of (a) Birch and (b) Ash. Different lowercase letters above columns indicate a difference at a 0.05 level.

4.3.3 Effect of five decay classes on anaerobic digestion performance

The patterns of daily biogas yield were shown in Figure 4-6a and 4-6b. As higher DC samples had lower MWD (Table 4-2), resulting in a larger surface area, they would be more susceptible to hydrolysis and acidification compared to DC1 samples, producing more feedstock for methanogen utilization. To further explore the effects of particle size on biogas production, the Birch DC1 and DC3 samples with same particle size were selected for a separate AD experiment. Although the biogas yield of Birch DC3 samples was still considerably higher than that of DC1 samples, the gap between these samples at the same particle size was smaller than the previous (Figure 4-7). It was also noted that all DC samples showed high daily biogas yield in the initial few days. During the early stages of AD, the feedstock is mainly hydrolyzed and acidified, and the primary contributors to the biogas are WEOC from the wood samples and the residual organic matter in the sludge.

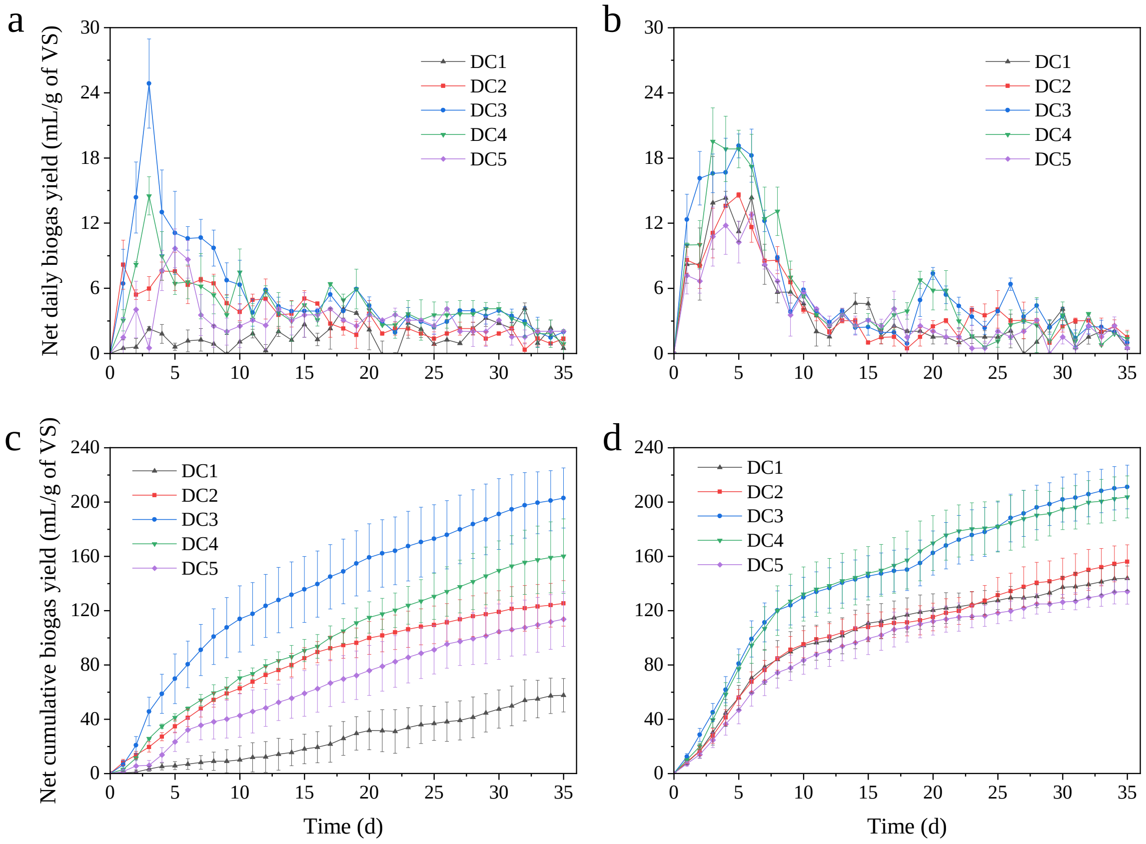

Figure 4-6. Effect of different decay classes of wood samples on the biogas yield. Daily biogas yield of (a) Birch and (b) Ash, and net cumulative biogas yield of (c) Birch and (d) Ash.

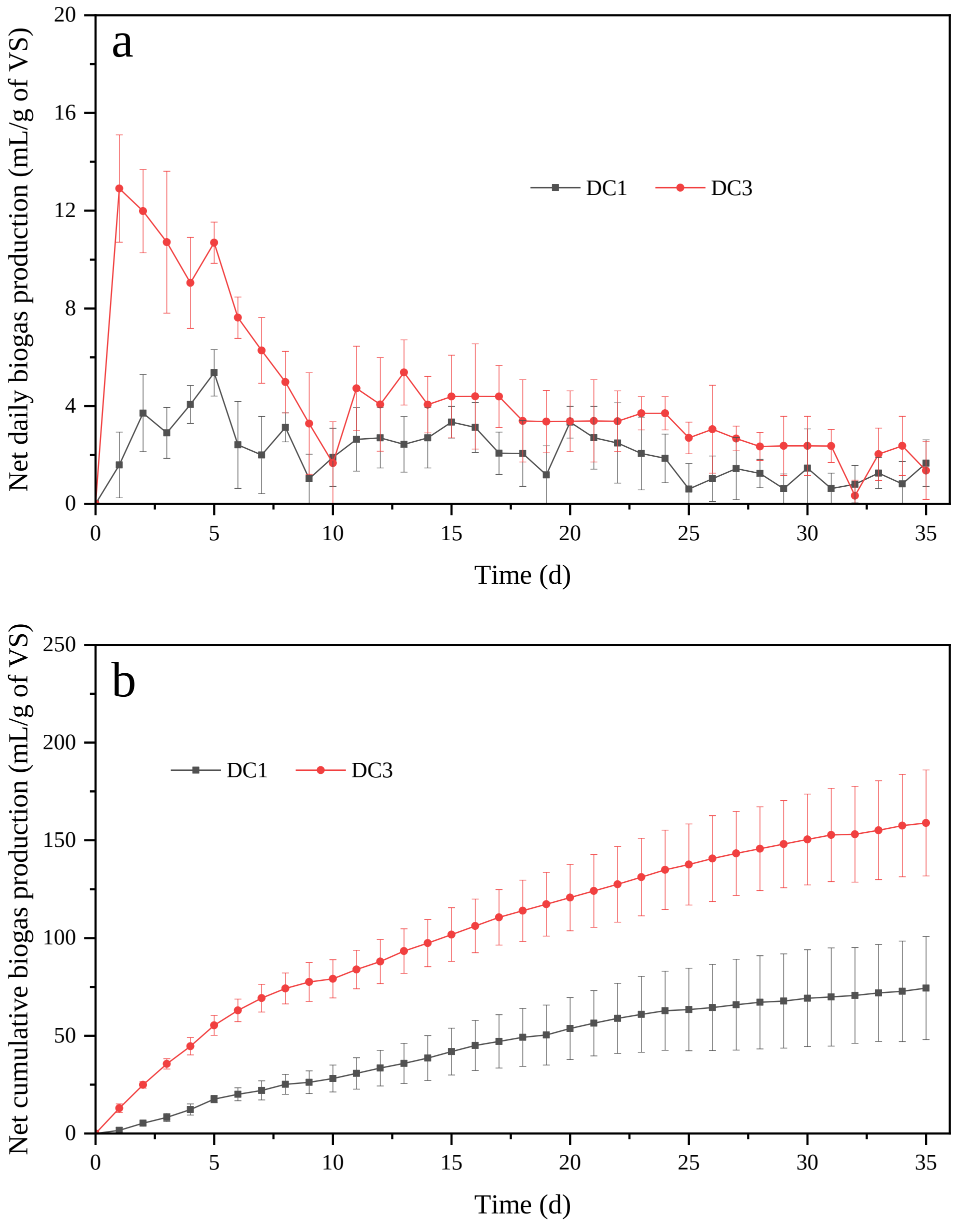

Figure 4-7. The biogas production of Birch DC1 and DC3 with 0.5–1 mm particle size, (a) daily biogas production and (b) cumulative biogas production.

As shown in Figure 4-6c and 4-6d, the net cumulative biogas yield varied significantly among different DC samples. These curves showed a rapid increase in biogas within the first 10 days, reaching about 50% of the total output. The final biogas yield increased from DC1 to DC3 (Birch) and DC3/4 (Ash) before decreasing at higher DC. For Birch, the biogas yield of DC3 samples was 3.52 times higher than that of DC1 samples, but this value narrowed down to 2.14 at the same particle size (Figure 4-7). This result suggested that decayed wood, besides increasing biogas production by being more prone to small particle sizes, had physicochemical properties that were more favourable for microbial utilization in AD system. Figure 4-8 illustrates the effect of DC on pH, ORP and TOC during AD. The initial phase of AD, which mainly involves the process of substrate hydrolysis and acidification, leads to a continuous accumulation of volatile fatty acids (He et al., 2022). The results showed that the pH decreased continuously after the AD started and reached the lowest value on the 5th day. The redox potential of the AD system can affect the microbial growth activities, and low redox potential means more strict anaerobic conditions and stronger reduction (Xu et al., 2014). Methane production requires the consumption of reducible substances. The breakdown of organic matter caused an initial increase in TOC content of the liquid phase, which then declined as methanogens utilized these materials to produce methane. The Pearson correlation analyses also revealed that these parameters were correlated with biogas yields (Figure 4-9).

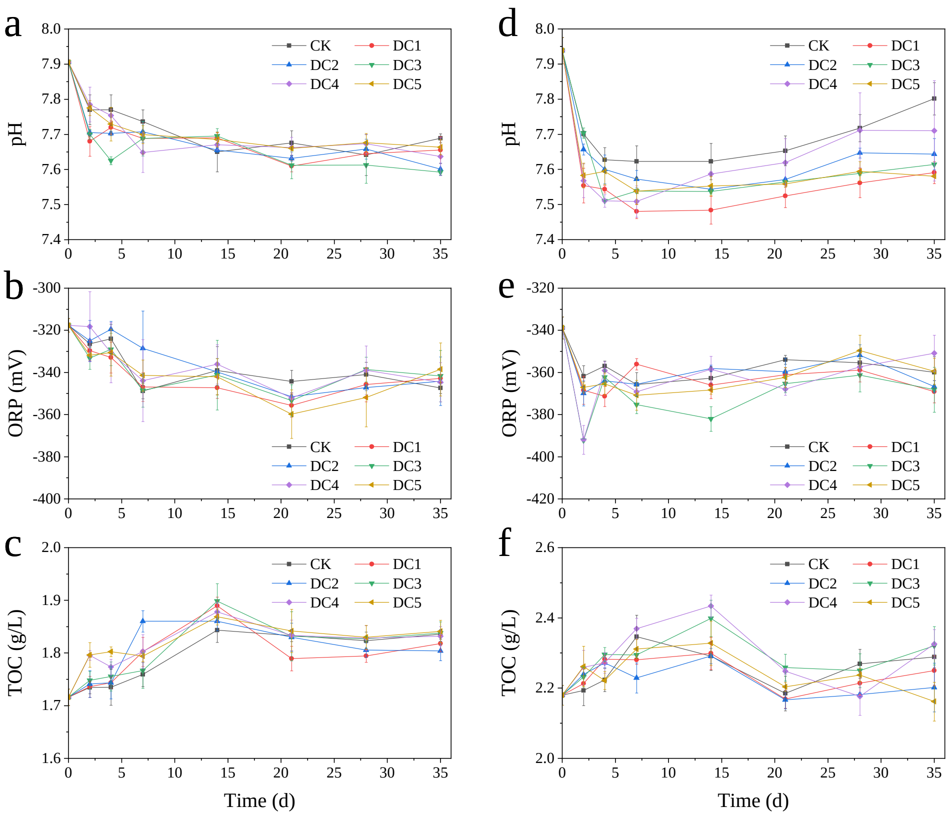

Figure 4-8. Effect of different decay classes samples on the parameters during AD, (a–c) Birch; (e–f) Ash. CK: blank group (only inoculum). ORP: oxidation reduction potential; TOC: total organic carbon.

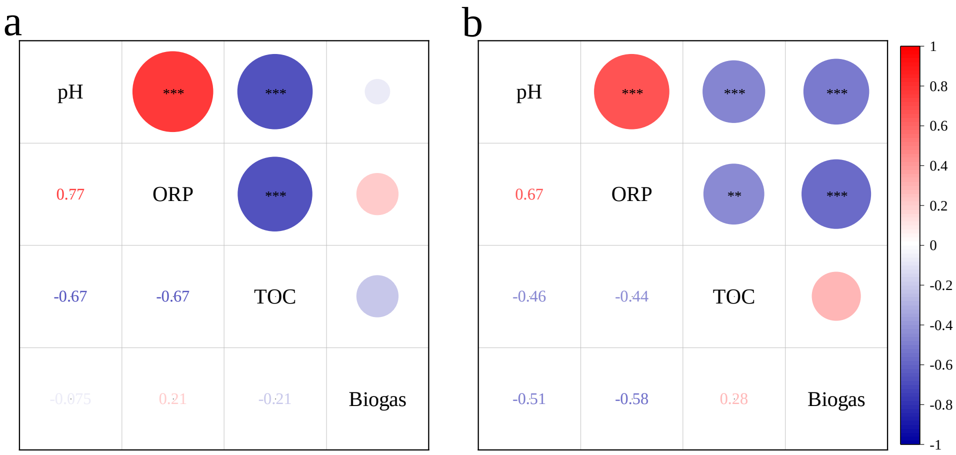

Figure 4-9. The correlation plot shows the Pearson product-moment correlation of all parameters and the biogas production for the relative days, (a) Birch; (b) Ash. ORP: oxidation reduction potential; TOC: total organic carbon. ** means $p<0.01$; *** means $p<0.001$.

Although the biogas production differed considerably between different DC, the methane content of these biogas did not vary greatly, with an overall value of around 60% (Figure 4-10). According to the final measurements, the highest values of net methane yield were found in the DC3 or DC4 samples after 35 d, with 134.76 and 142.51 mL/g VS for the Birch DC3 and Ash DC4 samples, respectively. The theoretical methane production, calculated by the elemental composition using the Buswell formula, did not differ significantly between different DC (Table 4-4). Therefore, the biodegradability index corresponded to the total biogas yield results, with DC3 samples being the highest and DC1 samples the lowest. The degradation of wood in the forest did not significantly change its elemental (carbon, hydrogen, oxygen, and nitrogen) composition (Table 4-3).

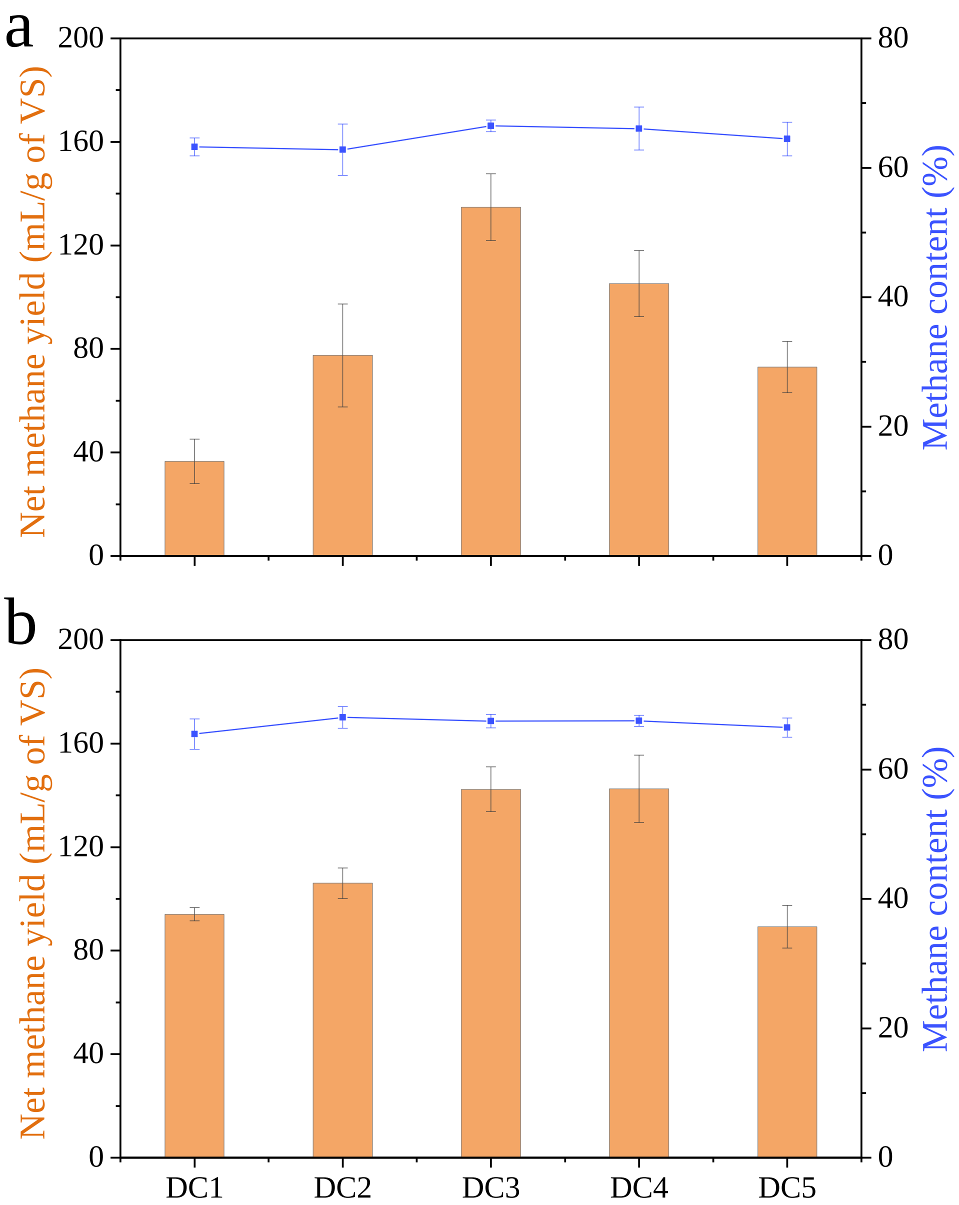

Figure 4-10. The methane content and net methane yield of (a) Birch and (b) Ash wood samples for five decay classes.

Table 4-4. Experimental and calculated results of the wood samples for methane production.

Wood type	Decay class	EMP (ml/g of VS)	Fitted results		Predicted results		TMP (ml/g of VS)	BD (%)
			Gompertz model	Error (%)	ML model	Error (%)		
Birch	DC1	36.54	36.30	0.66	58.67	60.56	451.68	8.09
	DC2	77.50	76.12	1.78	75.18	2.99	456.42	16.98
	DC3	134.76	127.7	5.24	108.87	19.21	447.66	30.10
	DC4	105.28	103.55	1.64	81.56	22.53	445.53	23.63
	DC5	73.01	71.6	1.93	78.58	7.63	476.70	15.31
Ash	DC1	94.05	84.19	10.48	86.27	8.27	432.83	21.73
	DC2	106.04	89.27	15.81	72.70	31.44	432.27	24.53
	DC3	142.35	131.94	7.31	96.20	32.42	440.57	32.31
	DC4	142.51	126.35	11.34	108.63	23.77	448.12	31.80
	DC5	89.23	80.63	9.64	77.63	13.00	435.25	20.50

EMP: Experimental methane production; PMP: Predicted methane production; TMP: Theoretical methane production; BD: Biodegradability index; ML model: machine learning model (random forest).

Prior to performing AD, it is desirable to determine the optimal parameters for maximum methane yield from woody waste. To the best of our knowledge, an approach that can predict methane yield from woody waste while addressing the issues involved in identifying the optimal digestion conditions and feedstock properties to maximize methane yield has not yet been developed. As shown in Table 4-4, the random forest model predicted biogas production well with an error rate of around 25%. The machine learning model (random forest) prediction accuracies of some samples were close to the results of the Gompertz fitting (Table 4-5), indicating that the machine learning model is instructive in practical AD with woody waste. It is worth noting that the predictions for the Birch DC1 samples were less accurate. This may be because the previously established database for creating machine learning models was not comprehensive and lacked data on this part of relatively low methane production (Gao et al., 2024a). Therefore, more experiments on AD of woody biomass are needed in the future in order to expand the database for improving the model.

Table 4-5. Kinetic parameters estimated for the Modified Gompertz model.

Wood type	Decay class	P_b (mL/g VS)	R_m (mL/g VS.d)	λ (1/d)	T_{50} (d)	T_{90} (d)	R^2
Birch	DC1	99.56	2.06	6.54	20.5	31.9	0.992
	DC2	124.97	5.81	-0.51	10.0	23.2	0.993
	DC3	197.66	9.02	-1.77	8.9	22.5	0.973
	DC4	173.93	5.60	-1.07	12.5	27.3	0.986
	DC5	126.06	4.11	0.39	13.9	28.2	0.987
Ash	DC1	133.71	8.98	-0.67	6.8	17.0	0.973
	DC2	144.25	7.58	-1.57	7.9	20.3	0.951
	DC3	202.60	9.78	-2.28	7.9	21.2	0.951
	DC4	192.64	11.80	-0.84	7.3	18.3	0.968
	DC5	125.89	7.92	-0.48	7.5	18.2	0.980

P_b is the maximum biogas potential of the substrate (mL/g VS); R_m is the maximum specific biogas production rate (mL/g VS.d); λ is the lag phase time taken for biogas production (in days); T_{50} is the half-life and is defined as the time taken (days) to produce 50% of the biogas production; T_{90} is defined as the time taken (days) to produce 90% of the biogas production. R^2 is the correlation coefficient, and a good fitting to the data is indicated by high R^2 values (above 0.95).

4.3.5 Applications and Future considerations

The present study introduces a novel nature-based approach to enhance suitability of wood waste, particularly forest residues, for AD, providing a sustainable solution to two key issues that have hampered the practical application of wood waste AD. Firstly, the low methane production from wood has been overcome with an average increase of 160% in methane yield after this nature-based approach (DC3 compared to DC1). This is comparable to other, more intensive, pretreatment technologies such as combined hydrothermal/enzymatic treatment (168% improvement) (Matsakas et al., 2015), aqueous ammonia soaking (151% improvement) (Antonopoulou et al., 2015), or combined ethanol organosolve/hydrothermal treatment (194% improvement) (Charnnok et al., 2020). Secondly, the lack of economical pretreatment technologies further hindered the practical utilization of wood waste AD. The proposed method leverages natural systems without requiring significant additional economic inputs, with the potential to be highly cost-effective as a result. By harnessing the inherent capabilities of natural forest processes, this nature-based approach offers a practical and scalable solution for enhancing the efficiency of AD operations for wood waste.

The economic viability of AD with wood waste has been thoroughly described in the literature. Teghammar et al. (2014) conducted an economic assessment of biogas production from forest residues with pretreatment enhancement, showing that an AD plant processing 50000 tons dry weight of forest residues per year is economically viable. The techno-economic assessment showed that methanol pretreatment was more financially acceptable than acetic acid and ethanol, and the capital investment for operating an AD plant treating 20,000 tons of forest residues per year could be recouped within eight years (Kabir et al., 2015). Moreover, AD was shown to be an environmentally friendly method of recovering energy from wood waste compared to other management processes through life cycle assessment analysis (Liang et al., 2017; da Costa et al., 2020; Nogueira et al., 2021). To further explore the differences between all DC samples and find the best DC for application in AD, a net profit analysis was conducted (Figure 4-11). Considering that the AD process was the same for all DC samples, the net energy output could be calculated from the electricity input of grinding the samples and the methane production. According to Wu et al. (2016), the calorific value of methane is 11.06 kWh/m^3. As shown in Fig. 6, DC3 had the highest net energy output, while DC4 also exhibited a high net energy output, indicating both have the potential to be used in AD plants. It is worth noting that does not consider the effect of subsidies or economies of scale. For fire protection reasons and energy considerations, the forest residues should not be retained in the forest. Unfortunately, the direct recycling of these forest residues also may result in the removal of minerals that would otherwise fertilize the soil and promote the future growth of trees (Grodsky et al., 2018). These results may provide guidance on specific collection times for forest residues in practice. This study proposes to collect DC3 samples, which satisfies the requirement of releasing minerals from the wood waste to maintain the forest ecology while keeping the highest methane yield.

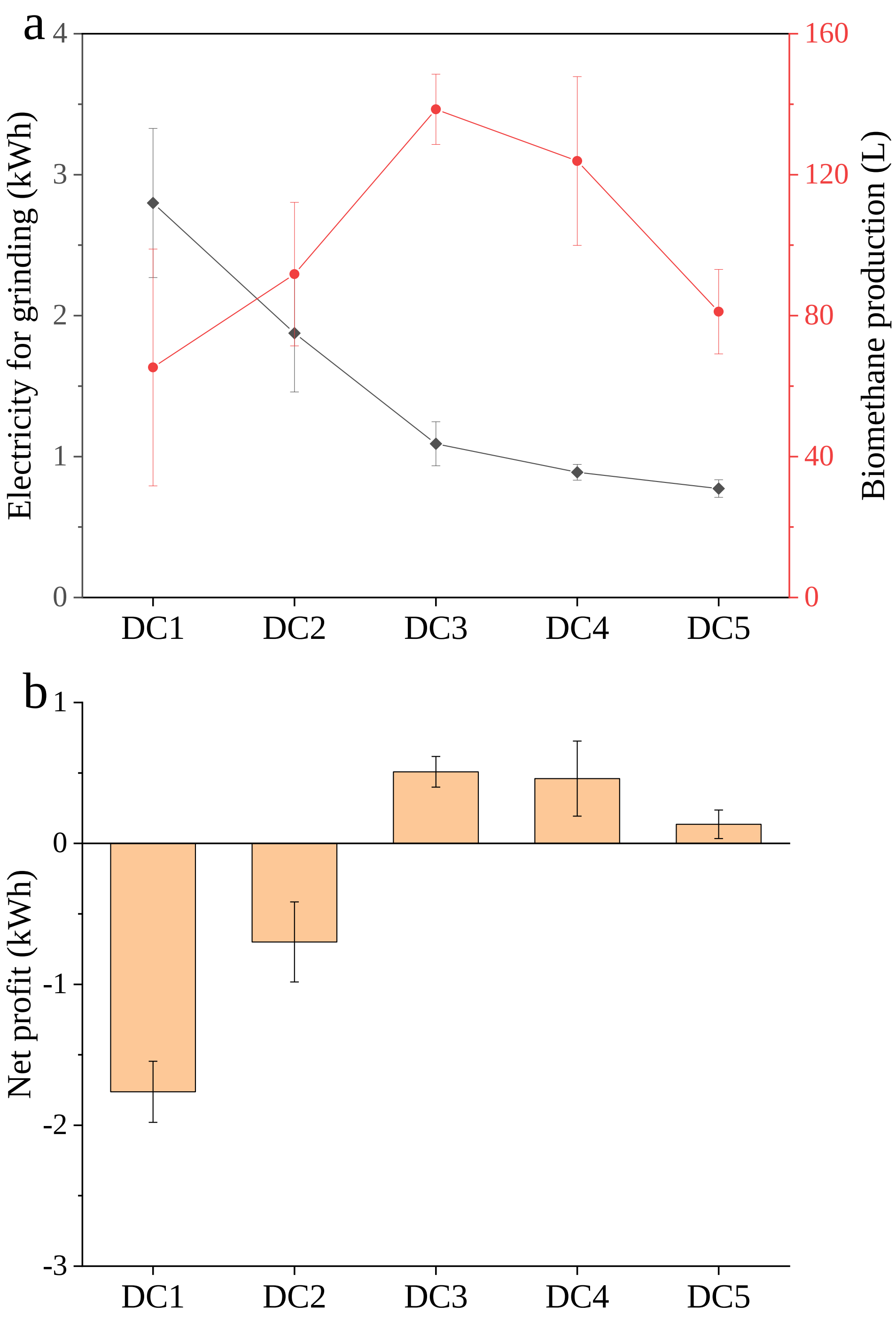

Figure 4-11. The net profit analysis of anaerobic digestion for processing 1 kg of samples from five decay classes. (a) The average electricity consumption for grinding and methane production from wood samples; (b) The net energy output, calorific value of methane production minus electricity consumption.

To better utilize this nature-based approach, more detailed experiments and analyses are still needed to bridge the following issues. Firstly, more wood samples of various species are needed to verify the generalizability of this approach, as well as to investigate the duration for forest residues to reach DC3 in different forest environments to facilitate the collection of samples. Secondly, there is a loss of mass in the degradation process of forest residues (Oberle et al., 2020; Seibold et al., 2021), so it is important to explore how mass loss varies with DC. As shown in Figure 4-12, the bulk density of wood samples decreased with increasing DC, indicating a loss in wood mass during this degradation process. If the reduction in the volume of the wood block during decay is ignored, the total methane production at each stage can be estimated using the wood's bulk density and methane yield per unit weight. Based on this calculation, the final DC3 stage sample produced 117% of the methane generated at the DC1 stage. Thirdly, anaerobic co-digestion of wood waste with other organic wastes can balance the C/N of the substrate and enhance methane production (Li et al., 2019; Oh et al., 2018). Additionally, the combination of other pretreatment technologies can improve substrate availability to microorganisms in AD (Wang et al., 2024; Zhang et al., 2017). Therefore, anaerobic co-digestion of different DC wood waste and other organic wastes and the possibility of combining this nature-based approach with other pretreatments need to be explored for maximizing energy recovery from wood wastes.

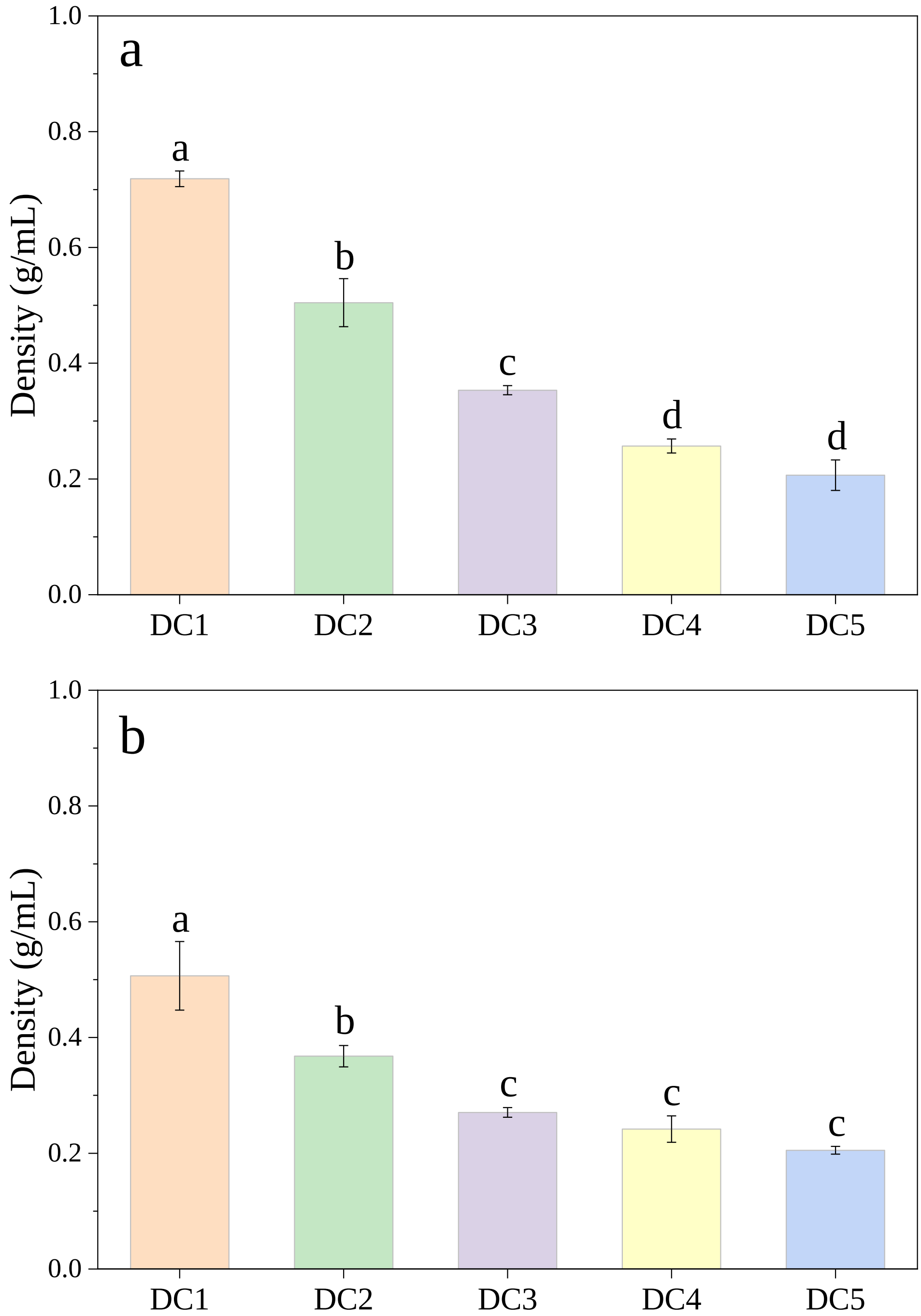

Figure 4-12. The bulk density of (a) Birch and (b) Ash wood samples for five decay classes.

4.4 Conclusion

This chapter addressed RQ3 by investigating a novel nature-based approach to promote AD application of wood waste. Based on the experimental results, the following conclusions can be drawn:

1) The texture of the forest residues was softened by processing with this nature-based approach, making them easier to be shredded. The SEC results showed that the energy required to grind the same dry weight of wood sample decreased with increasing DC. A subsequent particle size analysis showed that wood samples with higher DC had smaller average particle sizes after grinding.

2) High DC wood samples had higher WEOC, lower cellulose crystallinity, and a more suitable C/N for AD. Although the lignin content was relatively higher in high DC wood samples, the recalcitrance of their lignocellulosic structure was disrupted. These changes are expected to lead to enhanced methane production because of a breakdown of the lignocellulosic structure, increasing access to microorganisms involved in AD.

3) A preliminary test of this nature-based approach by AD experiments showed that wood samples from DC 1 had the lowest methane yield, while DC 3 samples had the highest, with a notable 160% increase compared to DC 1. In addition, the net profit analysis indicated that wood samples from DC 3 to DC 5 had a net energy output when applied in AD, with the net energy output being relatively high in DC 3 and DC 4. Therefore, these results imply that DC3 might be the optimal level of decay for forest residues collection.

This chapter is the first to introduce the concept of a nature-based approach to enhance the AD performance of wood waste, and to explore it in detail experimentally. This approach, when combined with a strategic collection method, can significantly improve the overall profitability and sustainability of bioenergy production from wood waste. Furthermore, the feasibility of more forest ecosystems in boosting the AD application of forest residues should be further explored, as this can broaden the applications of this novel nature-based approach.

Chapter 5 Impact of the nature-based approach on wood waste degradation and the biochemical methane potential

5.1 Introduction

The content of chapter four demonstrated that the decayed wood waste from forest can be effectively utilized as raw material for AD, which can generate significant amounts of methane without the need for any pretreatment. This new approach will help improve the management of wood waste and prevent the wastage of biomass energy. However, despite the potential benefits of utilizing wood waste from forest as feedstock in AD, it remains unclear how various forest conditions affect wood decay and the biochemical methane potential of wood waste. The variation in decay rates of forest wood waste reflects a combination of intrinsic and extrinsic driving factors (Zanne et al., 2015). Tree species with higher density and less nutrient content tend to decompose more slowly, while wider trunks are more resistant to decay (Hu et al., 2018). The decay rate of wood waste in forests is also influenced by surrounding environmental characteristics, such as higher soil nutrient availability, temperature, and moisture, which often accelerate decay processes (Fravolini et al., 2016; Gora et al., 2018). Even more important is the activity of decomposing organisms, including fungi, bacteria and animals, which directly influence the decay rate by interacting with the continuously changing substrate, the external environment and each other (Fukasawa, 2021; Oberle et al., 2020; Ulyshen, 2016). During the process of wood waste degradation in forests, there was a slight increase in nitrogen content, while the content of carbon and hydrogen remained relatively constant (Shorohova et al., 2021; Wojciech et al., 2019), which is the same as the findings in chapter 4. In addition, decayed wood waste has a range of properties that are favorable for utilization by microbes in the AD process, including a destroyed lignocellulosic structure and a high WEOC content. Although the decayed wood waste has been shown to increase methane production and DC3 has the highest methane yield, there is also a loss of mass and a decrease in organic matter content during the degradation of wood waste in forests, which is not conducive to its utilization as a feedstock for AD. Fraver et al. (2013) found that wood waste in the forest was slowly losing mass as it degraded, and the rate of reduction varied for different types of wood. Generally, wood blocks from diffuse porous angiosperms decompose faster than those from annular porous angiosperms and gymnosperms (Edelmann et al., 2023). Moreover, the loss of mass over time varies for the same wood species in different environments. Through field experiments on wood decomposition at 55 forest sites across 6 continents, Seibold et al. (2021) found that the deadwood decomposition rates increased with rising temperature, while high precipitation levels promoted the decomposition of deadwood in forests. It is critical to understand the impact of these factors on the decomposition of wood waste for optimizing the utilization of wood waste and maximizing bioenergy production.

This chapter aims to fill this gap by examining the effects of forests on wood degradation and subsequent methane production. By conducting field experiments and analyzing data from various forest environments and wood waste characteristics, this chapter aims to (a) elucidate the effects of different forest environments, primarily vegetation conditions, on wood waste degradation, (b) understand the changes of basic physicochemical properties of wood waste in forests over time and to further explore the relationship between these properties and methane yield, (c) determine the optimal timing of wood waste collection in different forest environments for guiding effective wood waste management practices to maximize benefits. Understanding these dynamics will inform the development of sustainable management practices for wood waste in forests, with potential implications for bioenergy production and climate change mitigation efforts.

5.2 Materials and methods

5.2.1 Experimental materials

The types of wood can be classified into hardwood and softwood. The microstructure of softwoods is relatively homogeneous and dominated by a structure known as the tracheid (Capuani et al., 2020). Hardwoods, unlike softwoods, have more complex anatomical characteristics and greater structural variation (Stagno et al., 2021). In this chapter, two types of wood which were common in UK forests, European ash (*Fraxinus excelsior*) and Japanese larch (*Larix kaempferi*), were chosen for the test. Of these, European ash (expressed as Ash) is a hardwood and Japanese larch (expressed as Larch) is a softwood. The wood samples were taken from live trees as part of forestry management and cut into uniform cubes (approximately 15 cm long, 10 cm wide, and 5 cm high) using a table saw upon arrival at the laboratory. Since bark only accounts for 10-25% dry weight of the trunk portion of a tree (Chang et al., 2020), and to ensure consistency of samples for accurate experiments, the bark was not considered in this chapter.

5.2.2 Site description and sample placement

After obtaining approval from Natural Resources Wales, two locations in the forest (51°32'25''N, 3°14'58''W) were selected for experimentation, as illustrated in Figure 5-1. Trees grown at site A are Ash (hardwood area), and trees grown at site B are Larch (softwood area). The two selected locations are both situated far from paths to prevent potential damage to the samples or the surrounding environment by forest walkers. In addition, climate conditions at the experimental sites were obtained from a website (https://www.climate.top/united-kingdom/cardiff/).

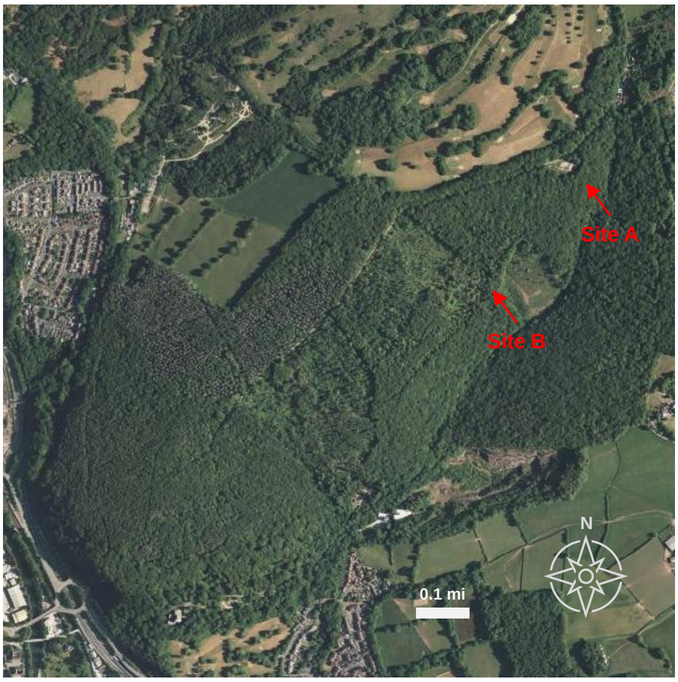

Figure 5-1. Satellite aerial view of the forest where the wood samples were placed. The red arrow indicates the sampling locations, site A (51°32'48.2" N, 3°14'17.7" W) and site B (51°32'38.8" N, 3°14'31.3" W). Image from World Map, Satellite (https://satellites.pro/).

Both experimental sites contained hardwoods and softwoods to investigate the effects of different vegetative environments on the degradation of different species of wood. As shown in Figure 5-2, the wood samples were directly laid flat on the soil surface, with softwood and hardwood samples alternately arranged. Each group consisted of six wood samples, and a total of four groups were placed. After placing the wood samples, a mesh cover was utilized to safeguard them against potential damage by large animals (e.g., squirrels and pet dogs), and secured in place with tent pegs, as shown in Figure 5-3. The purpose of the small holes was to ensure that insects and small animals had access to the wood samples. During the sample placement at the forest, approximately 0.5 kg of surface soil samples (depth less than 10 cm) were excavated from each experimental site, and four soil samples were randomly taken at each experimental site. Soil surface temperatures and soil temperatures at a depth of 10 cm were measured respectively at the experimental sites using a portable digital thermometer.

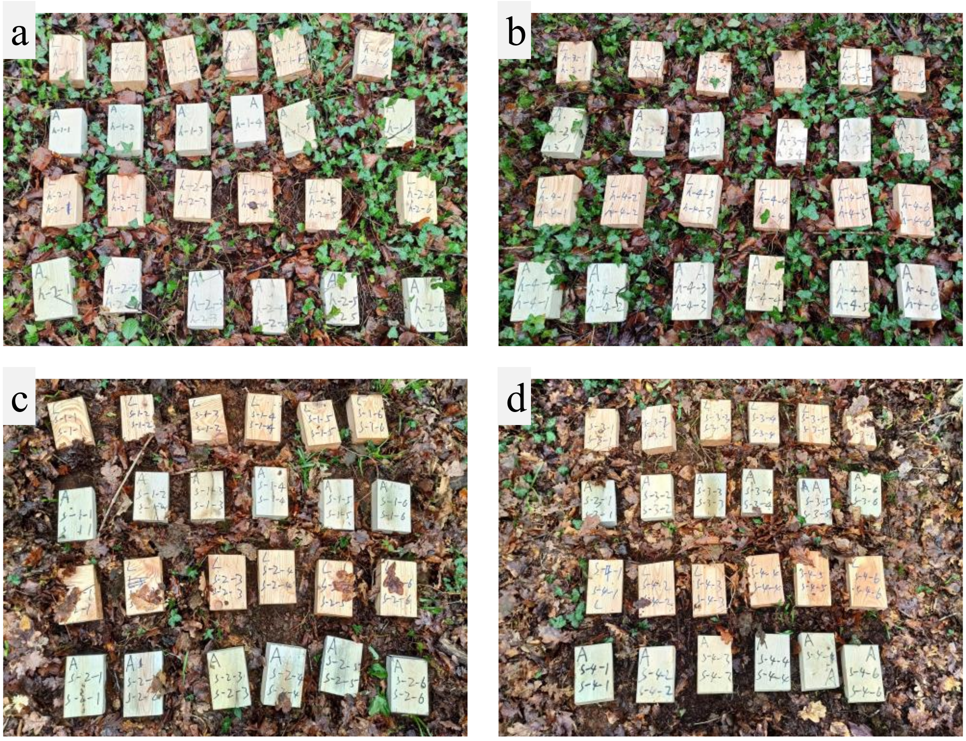

Figure 5-2. Pictures of wood samples placed in the forest. Softwood samples and hardwood samples were placed alternately at the same experimental site. (a–b) Hardwood area and (c–d) softwood area.

Figure 5-3. The final picture of the experimental site.

5.2.3 Sample recovery and preparation

Sampling was conducted every 6 months in the forest over the period February 2023 to February 2024, with 6 hardwood samples and 6 softwood samples collected at each sampling site. As a result, the 6-month samples were taken in August 2023, while the 12-month samples were collected in February 2024. Four soil samples were randomly excavated using the same method as used during sample placement. Meanwhile, Soil surface temperatures and soil temperatures at a depth of 10 cm were measured using a portable digital thermometer. All samples were wrapped in plastic bags to minimize moisture changes and transported to the laboratory.

For the collected wood samples, the soil and plant humus contaminated on the surface were removed to ensure homogeneity of the sample, and then the sample was photographed. It was also visually inspected for signs of decay, such as pitted corners, split edges, or surface pits. The wood samples were weighed, then placed in an oven at 105 °C for 2 days to dry. After drying, the samples were weighed again to calculate the moisture content. Considering the different environments of the wood portions in contact with soil and air, the dried wood was divided into three parts using a table saw into three parts as shown in Figure 5-4, including the air-oriented portion (part A), the unexposed portion (part B), and the portion in contact with soil (part C). Finally, all wood samples were shredded using a knife mill equipped with a 2mm screen and then stored at 4°C prior to further analysis.

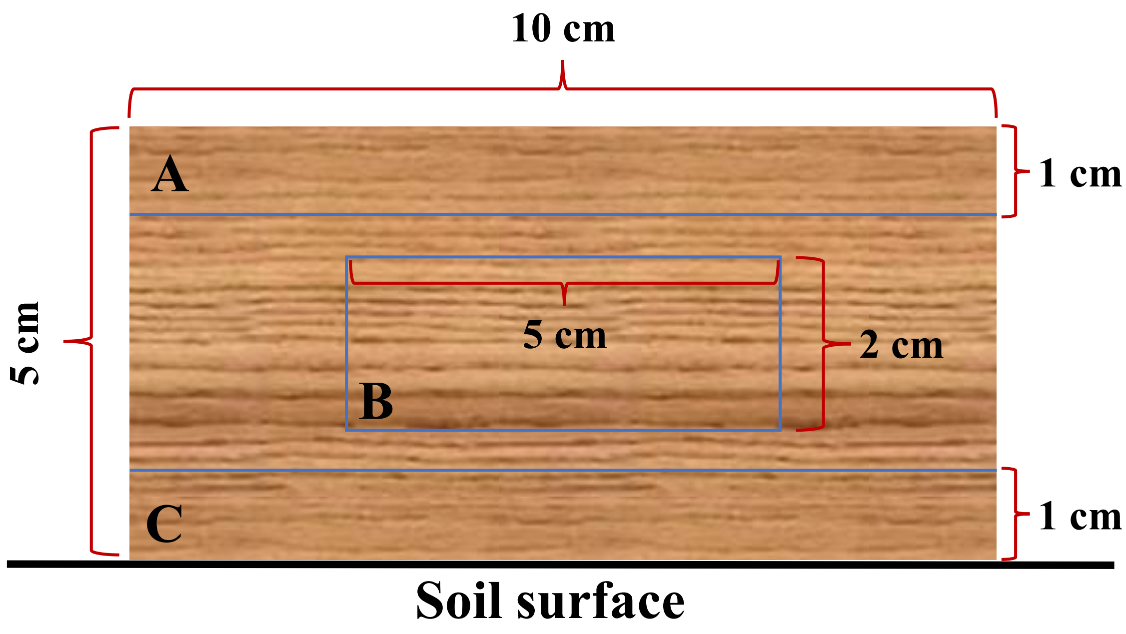

Figure 5-4. The wood samples will be divided into three parts for further testing.

5.2.4 Anaerobic digestion experimental for wood samples

AD process for wood chips was the same as in section 4.2.2. 700 mL of inoculum was added to the bioreactor, followed by the introduction of wood samples to achieve a substrate to inoculum VS ratio of 1:4. The total reaction volume in the bioreactor was 700 mL, with 300 mL of headspace. Due to testing revealing no significant differences in properties among the A, B, and C parts of the wood samples, separate AD experiments were not conducted on these three parts. Instead, the A, B, and C parts were added to the bioreactor according to their mass ratios in the original wood blocks. All bioreactors were incubated at 35 °C for 25 days, and the methane content in the collected biogas was subsequently measured.

5.2.5 Analytical methods

5.2.5.1 Soil samples

For soil samples, pH measurements were carried out immediately after transportation to the laboratory, with the method modified from Spohn and Stendahl (2024). Specifically, 10 g of wet soil was weighed into a 50 ml plastic bottle, then 25 mL of deionised water was added, and the pH was measured using a freshly calibrated pH probe (Mettler-Toledo, Switzerland). The moisture content of the soil was measured based on the following procedure: 5 g of wet soil was taken and placed in an oven at 105 °C overnight, and the dry weight of the soil was weighed. The moisture content was calculated as the difference between the initial wet weight and the final dry weight, expressed as a percentage of the wet weight. The preparation of liquid samples for measuring WEOC and element content in soil was consistent. A total of 5 g of wet soil was added to 30 mL of Milli-Q water and shaken at 200 rpm for 2 h at room temperature. The supernatant was subsequently filtered through sterile 0.45 μm filters and stored in the dark at 4 °C prior to further analyses. The WEOC concentrations were measured by a TOC-VCPH

(Shimadzu, Kyoto, Japan) following the manufacturer's instructions. Elemental analyses (Fe, Mg, K, Ca, Mn, Na, Al, Ni, Zn, Cu, Co, and Mo) were carried out using an inductively coupled plasma optical emission spectroscopy (Perkin Elmer Optima 2100).

Soil microbial activity was assessed through the measurement of dehydrogenase enzyme content (Harbottle and Al-Tabbaa, 2008). 2,3,5-Triphenyltetrazolium chloride (TTC) served as an artificial electron acceptor, which was reduced to produce 2,3,5-triphenyltetrazolium formazan (TTF, a water insoluble red dye) in the presence of dehydrogenase. One gram of wet soil was placed in a 2 ml centrifuge tube and mixed with 1.4 ml of 0.75% TTC (Merk, UK) solution containing 50 mM Trizma hydrochloride (Merk, UK). The obtained slurry was vortexed and incubated for 24 hours at 32 °C. Subsequently, the slurry was stored at -20 °C for at least 1 hour to minimize subsequent biological activity. Following this, the slurry was centrifuged at 13000 g for 4 minutes, and the aqueous supernatant was carefully removed. The resulting precipitated sample was extracted repeatedly with ethanol until no pink or red colour was visible. The total extract was then quantified by weight, and its concentration was determined using a UV/Vis spectrophotometer at 485 nm. Finally, the total amount of TTF can be calculated. To control for soil colour interference, a blank test was carried out using Trizma hydrochloride solution without TTC. The standard curves for different concentrations of TTF (VWR, UK) in ethanol are shown in Figure A-6 and were used for calibration.

5.2.5.2 Wood samples

The VS content of the wood samples was determined as follows: 2 g of dried wood samples were placed in a crucible and heated at 550 °C for at least 6 hours. The weight of the ash was measured after it cooled down to room temperature. The VS content was calculated as the difference between the initial weight and the weight of the ash. The element content (C, H, N, and O) was measured using an elemental analyzer (Flash Smart, Thermo Fisher Scientific Co., USA). The cellulose, hemicellulose, and lignin content of wood samples were measured as in section 4.2.3.3. Moreover, the detailed measurement methods for WEOC and crystallinity index were the same as described in section 4.2.3.3.

5.2.6 Statistical analysis

All experiments were conducted with three technical replicates, unless otherwise specified. Statistical analyses were done using Origin 2021 software. All values are presented as the mean ± s.d., unless otherwise specified. Statistical significance was assessed using the two-tailed Student's t-test or One-way ANOVA test with a p value of 0.05.

5.3 Results and discussion

5.3.1 Environmental conditions and basic properties of soil samples

The climate conditions at the experimental site are shown in Table 5-1. The average temperature was the highest in July (16.0 °C) and was the lowest in February (4.2 °C). The average precipitation fluctuated slightly throughout the year, ranging from 57–100 mm, and the annual precipitation was 961 mm. The relative humidity varied with the seasons and was generally low in spring and summer (around 75%) and high in autumn and winter (above 85%). It has been found that temperature, precipitation, and humidity in the forest can affect wood decomposition rate (Hu et al., 2018). It was reported that the decomposition rate of wood waste was higher under warmer forest conditions than in cooler forests (Błońska et al., 2019).Specifically, the decomposition rate of wood waste in forests increased with temperature, and the precipitation also had an effect on it (Seibold et al., 2021). The temperature at the site was low, so the decomposition rate of wood samples will be very slow. A higher humidity can stimulate microbial activity, but excessive humidity will lead to anaerobic conditions in the forest soil that reduce the rate of decomposition (Błońska and Lasota, 2017).

Table 5-1. Weather condition of experimental sites.

Month	Average maximum temperature (°C)	Average minimum temperature (°C)	Average temperature (°C)	Average precipitation (mm)	Relative humidity (%)	Average sunlight hours/Day
January	6.9	2.0	4.4	91	89	1h 44'
February	6.9	1.6	4.2	67	87	2h 41'
March	9.2	2.7	5.9	76	82	3h 58'
April	11.9	4.2	8.0	57	74	5h 38'
May	15.1	7.1	11.1	64	74	6h 21'
June	18.1	10.1	14.1	66	73	6h 54'
July	20.0	12.0	16.0	74	76	6h 09'
August	19.8	12.1	15.9	80	78	6h 01'
September	17.5	10.5	14.0	92	81	4h 40'
October	14.1	8.1	11.1	96	85	3h 21'
November	10.1	4.5	7.3	100	88	1h 56'
December	8.0	2.8	5.4	98	89	1h 30'
Annual	13.1	6.5	9.7	961	81.3	4h 15'

The soil surface temperature, soil temperature at 10 cm, and pH in the two test sites were not generally different (Table 5-2). Although the temperature was not optimal for microbial activity during the degradation of wood samples, the soil pH was close to neutral, which is a favorable condition for decomposition process (Horodecki and Jagodziński, 2019). Soil moisture in hardwood area was slightly higher than in softwood areas in 0 month and 6 months stages, while there was no difference between the two areas in 12 months stage. Soil moisture affected the activity of wood-degrading fungi in the soil, with increased moisture in the soil leading to increased fungal abundance and extracellular enzyme activity (A’Bear et al., 2014). In the process of deadwood decomposition, oxidative depolymerization of macromolecules, such as lignin, into relatively small soluble compounds that can be absorbed and utilized by microorganisms is often the limiting step in rate (Freschet et al., 2013). This process involves a variety of extracellular enzymes that catalyze oxidation reactions and has been shown to be influenced by the elemental composition of the soil (Jones et al., 2020). In this chapter, soil samples at softwood area were consistently higher in manganese than hardwood area, while the iron content was also higher in softwood area at 0 month and 6 months stage. Many studies have revealed a positive correlation between manganese concentrations and deadwood decomposition across a wide range of forest ecosystems (Berg et al., 2015; Trum et al., 2015). In addition, iron was also involved in these redox reactions, accelerating the decomposition of deadwood (Jones et al., 2020). Therefore, the softwood areas of this chapter were more favourable for wood decomposition in terms of soil properties.

Table 5-2. Basic properties of soil samples at the experimental sites.

Parameters	0 month		6 months		12 months	
	Hardwood area	Softwood area	Hardwood area	Softwood area	Hardwood area	Softwood area
Soil surface temperature (°C)	12.50 ± 0.14	12.57 ± 0.12	16.70 ± 0.16	16.53 ± 0.21	4.03 ± 0.26	4.47 ± 0.21
Soil temperature at 10 cm (°C)	9.00 ± 0.08	9.60 ± 0.16	15.33 ± 0.25	15.50 ± 0.08	6.53 ± 0.12	4.97 ± 0.17
Moisture (%)	45.30 ± 1.21	38.75 ± 0.83	34.67 ± 0.59	29.51 ± 1.76	29.79 ± 3.10	30.02 ± 0.42
pH	6.96 ± 0.02	6.58 ± 0.11	6.78 ± 0.22	6.73 ± 0.19	7.33 ± 0.02	7.43 ± 0.03
Elements (mg/kg)						
Fe	4.44 ± 0.12	7.78 ± 1.34	8.13 ± 1.70	19.73 ± 1.94	15.21 ± 1.81	11.91 ± 1.72
Mg	3.21 ± 0.34	5.72 ± 1.13	4.22 ± 0.03	7.17 ± 0.40	3.83 ± 0.44	3.88 ± 0.26
K	22.43 ± 6.42	19.90 ± 1.26	4.19 ± 0.33	14.31 ± 1.45	18.63 ± 1.49	20.46 ± 1.64
Ca	12.16 ± 5.37	9.40 ± 2.13	9.16 ± 0.56	15.95 ± 1.10	21.82 ± 2.19	23.18 ± 2.40
Mn	0.06 ± 0.02	0.27 ± 0.10	0.41 ± 0.12	0.83 ± 0.10	0.23 ± 0.01	0.31 ± 0.03
Na	13.87 ± 3.09	7.72 ± 1.59	6.10 ± 0.38	2.88 ± 0.17	81.07 ± 0.12	79.71 ± 1.17
Al	2.11 ± 0.13	3.25 ± 0.36	7.20 ± 2.83	8.86 ± 0.89	10.77 ± 1.43	4.16 ± 0.60
Ni	ND (Not detectable)	ND	ND	ND	ND	ND
Zn	ND	ND	ND	ND	ND	ND
Cu	ND	ND	ND	ND	ND	ND
Co	ND	ND	ND	ND	ND	ND
Mo	ND	ND	ND	ND	ND	ND

The temporal variation of WEOC concentrations in soil samples is shown in Figure 5-5. Soil WEOC concentrations at both experimental sites were highest at the 6 months stage, which corresponded to the summer season. Similar results have been reported that WEOC levels in soils reached peaks in the summer and autumn seasons (J. Zhang et al., 2020). The variation of WEOC concentrations over time is related to soil temperature, moisture, and vegetation (Embacher et al., 2007). Soil temperature is considered to be one of the major factors influencing WEOC, and both are generally positively correlated (J. Zhang et al., 2020). In this chapter, the impact of vegetation on soil WEOC was probably slight. The hardwood area had the highest WEOC content at 0 month stage, and softwood area had the highest WEOC content at 6 months stage, while there was no difference in WEOC between both areas at the 12 months stage. Figure 5-6 presents the soil dehydrogenase activity in soil samples from the two experimental sites at different sampling stages. On the one hand, soil samples collected after 6 months had the highest dehydrogenase activity, which was consistent with the finding that the microbial activity in soil has a positive correlation with temperature (Kim et al., 2022). On the other hand, the dehydrogenase activity in softwood area was higher than that in hardwood area at all three sampling stages, suggesting higher microbial activity in the softwood area. In general, the softwood area was more favorable for wood decomposition in terms of soil microbial activity.

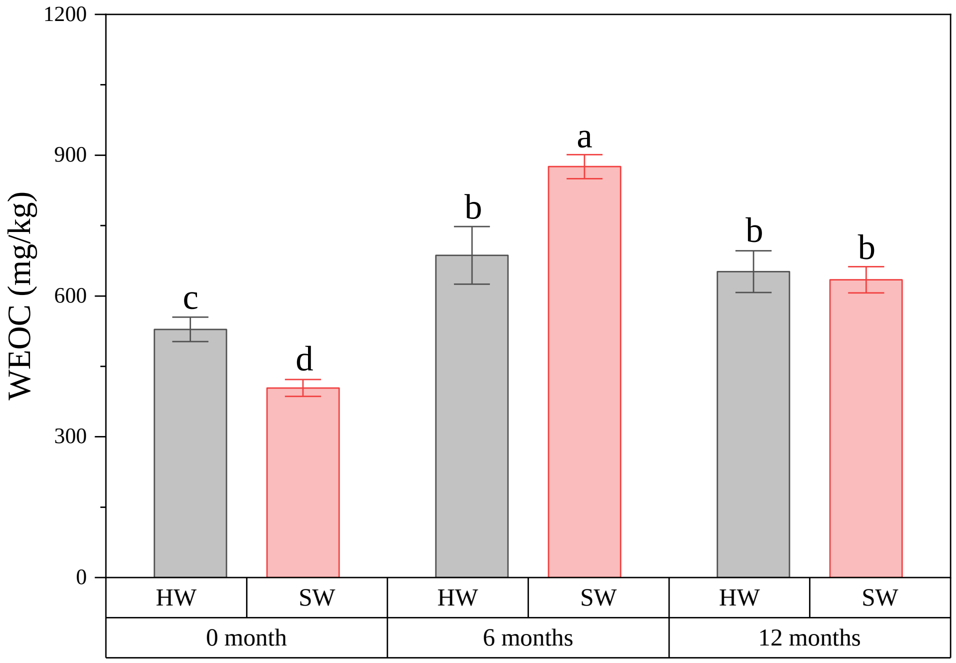

Figure 5-5. Water extractable organic carbon (WEOC) of soil samples at two experimental sites. HW: hardwood area; SW: softwood area. Different lowercase letters above columns indicate a difference at a 0.05 level.

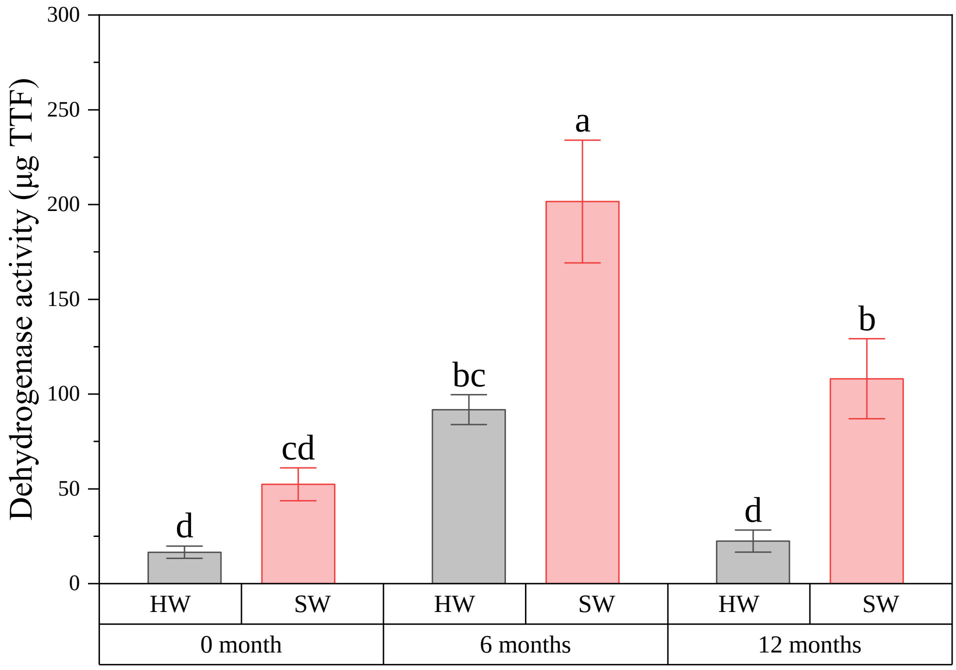

Figure 5-6. Dehydrogenase activity of soil samples at two experimental sites, measured by production and recovery of 2,3,5-triphenyltetrazolium formazan (TTF). HW: hardwood area; SW: softwood area. Different lowercase letters above columns indicate a difference at a 0.05 level.

5.3.2 Physicochemical features analysis of wood samples

Table 5-3 shows the basic properties of the raw material of wood samples. There was no significant difference in these parameters between the two types of wood. It was found that the decomposition process of wood waste in the forest is mainly determined by the wood properties, especially the nitrogen content and the diameter of the wood (Hu et al., 2018). The rate of degradation of wood waste is inversely proportional to its carbon content and directly proportional to the nitrogen content (Kahl et al., 2017). Compared to other wood species, the wood samples in this study had a relatively moderate carbon content and a relatively high nitrogen content, implying that they might have a comparatively rapid rate of decomposition (Martin et al., 2014; Martin and Thomas, 2011). Figure 5-7 shows the thermogravimetric experimental data and derivative thermogravimetric curves fitting results, and the calculated lignocellulose composition are provided in Table 5-3. Although there was no significant difference in the carbon and nitrogen content of two wood types, the Ash samples had a higher cellulose content and lower lignin content. The result indicated that Ash had higher AD potential as lignin is the main component limiting the biogas production of lignocellulosic wastes (Gao et al., 2022). However, in contrast to the results of the lignocellulose composition analysis, the WEOC content of Larch was significantly higher than that of Ash (Figure 5-8). This indicated that there was more organic matter in Larch that was released in the AD system, which can then be utilized by a range of related microbes to produce more biogas. There was no significant difference in crystallinity index between the raw samples of Ash and Larch (Figure 5-8).

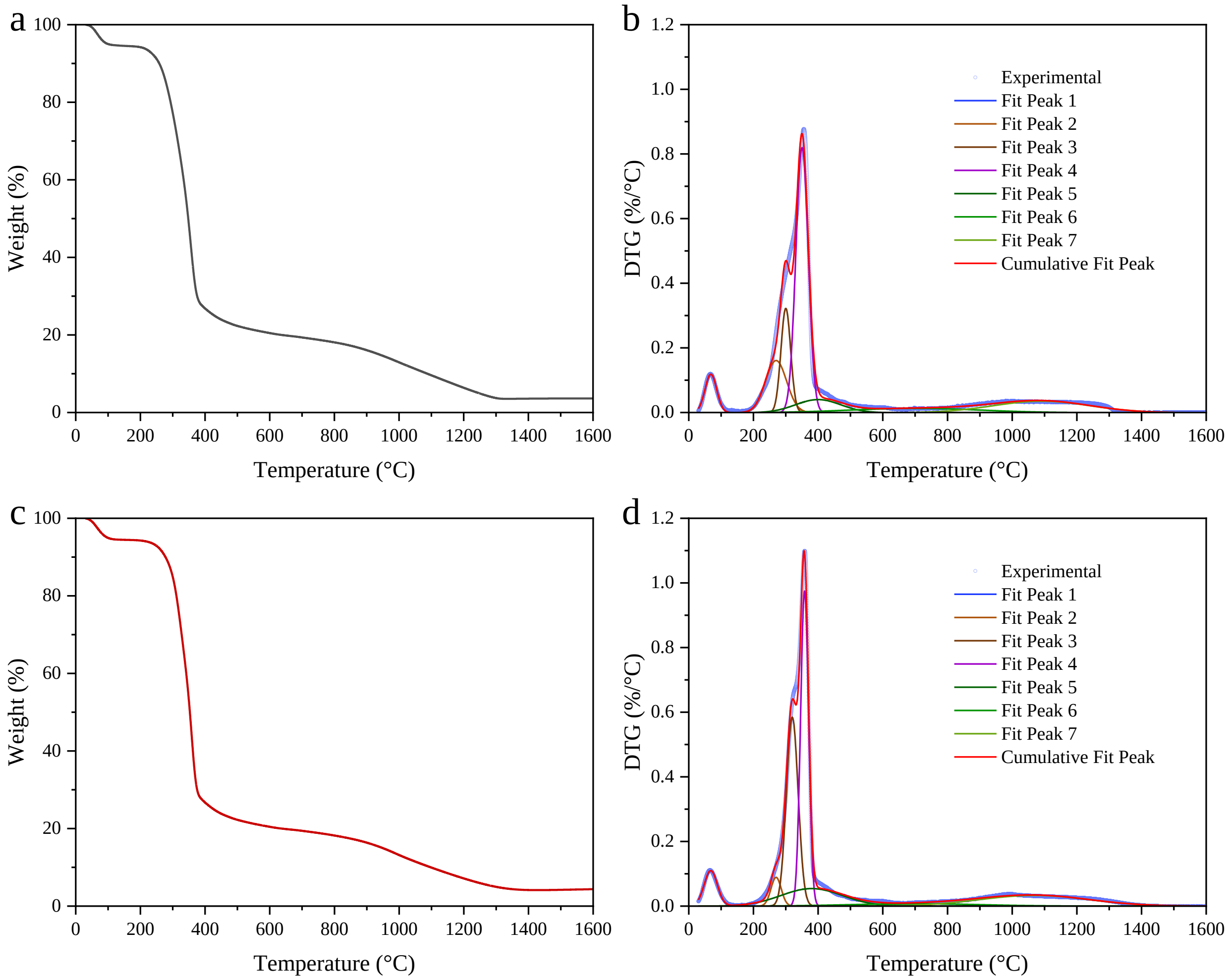

Figure 5-7. TGA curves and the gauss peak fitted experimental DTG curves of (a and b) Ash and (c and d) Larch samples.

Table 5-3. Basic properties of the raw wood samples.

Parameters	Ash	Larch
Total solid (%)	70.25 ± 0.39	60.80 ± 1.52
Volatile solid (%)	99.25 ± 0.29	99.73 ± 0.35
Ultimate analysis		
Nitrogen (%)	0.34 ± 0.03	0.33 ± 0.03
Carbon (%)	45.94 ± 0.19	46.60 ± 0.91
Hydrogen (%)	5.87 ± 0.10	5.98 ± 0.08
Oxygen (%)	47.85 ± 0.06	47.09 ± 0.98
C/N	136.21 ± 13.29	143.81 ± 9.37
Biochemical analysis		
Extraction (%)	8.11 ± 0.10	5.39 ± 0.22
Hemicelluloses (%)	25.00 ± 0.31	27.88 ± 0.25
Cellulose (%)	40.92 ± 0.73	33.08 ± 1.04
Lignin (%)	25.88 ± 0.52	30.82 ± 0.19

Note: The % content of total solid was calculated based on wet mass; others were based on dry mass.

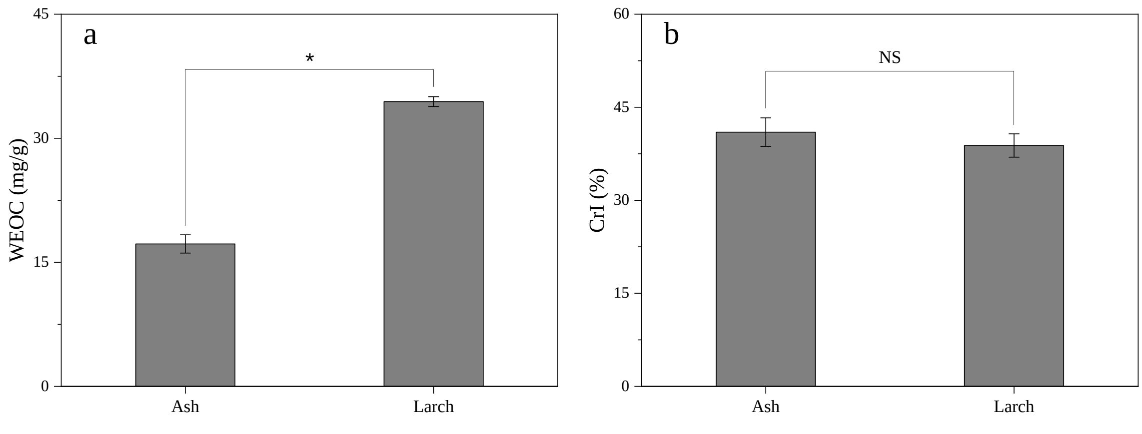

Figure 5-8. Water extractable organic carbon (WEOC) and Crystallinity Index (CrI) of raw wood samples. * indicates a difference at a 0.05 level, NS means not significant.

After placing the samples in the forest for 6 months, fungal hyphae were visible on the side of the sample in contact with the ground, and some eggs were visible on the wood samples after 12 months (Figure 5-9). However, there were no obvious pits caused by insect feeding on the wood samples were observed, and no visible softening in the texture of the samples due to degradation occurred. This might be explained by the short test period which was not enough for microorganisms and insects to fully degrade the samples. Generally, the decomposition of deadwood in the forest is a long-term process spanning several years or even many decades involving a variety of invertebrates and microorganisms that form a complex community (Hardersen and Zapponi, 2018). In addition, the volatile solid content and elemental composition of wood samples also did not change significantly compared to the initial samples after 6 and 12 months of experimental periods in the forest (Table 5-4). The mass loss of the samples is shown in Table 5-5, with an increase in mass of about 2.9% after 6 months and 4.6% after 12 months. This can be explained by the fact that the contaminants on the sample, such as soil or mycelium, are difficult to remove completely by brushing, resulting in an increase in weight.

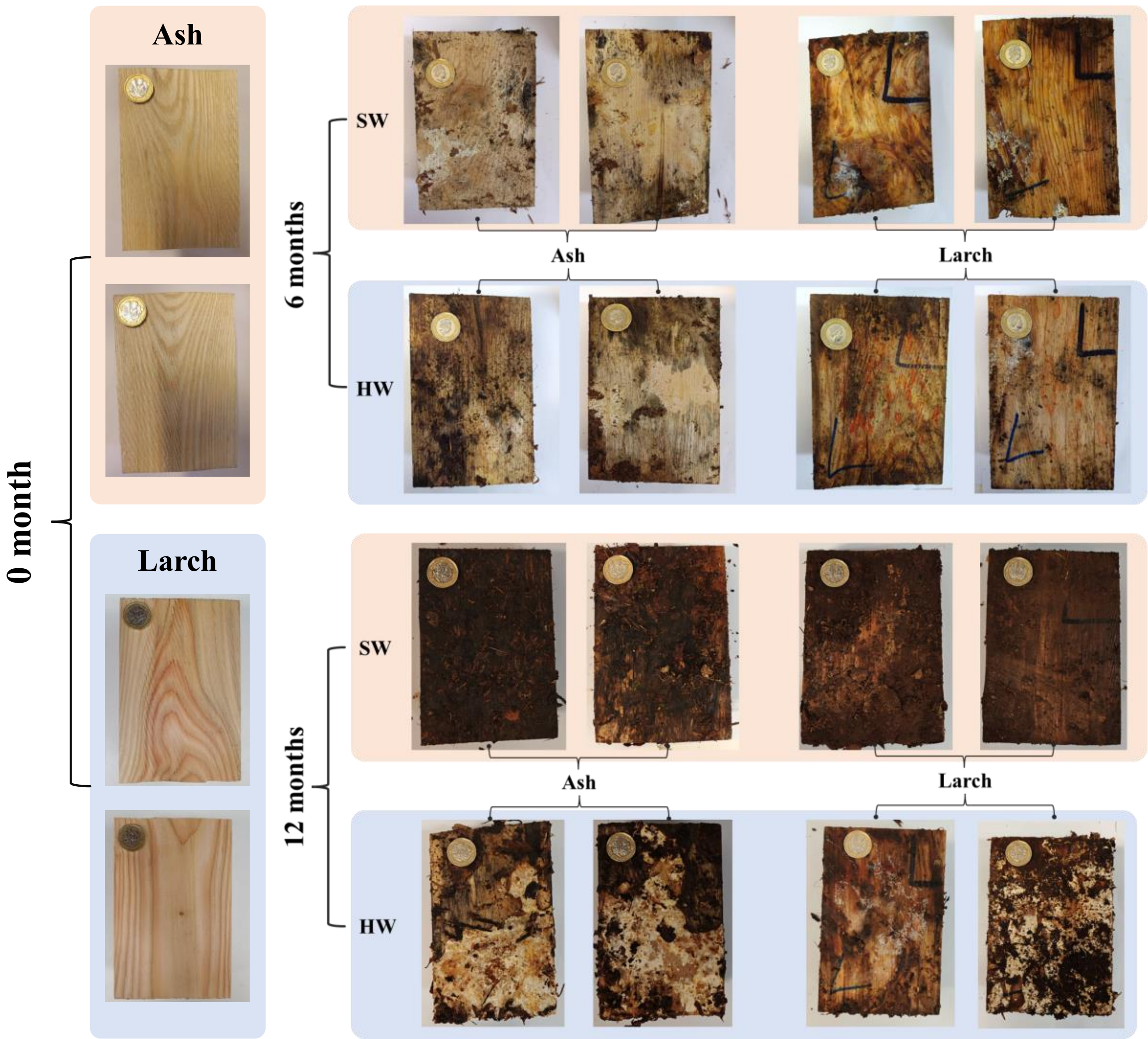

Figure 5-9. Images of the wood samples. All samples show the side in contact with the ground.

Table 5-4. Basic properties of the 6 months and 12 months stages wood samples.

Samples	Total solid (%)	Volatile solid (%)	Nitrogen (%)	Carbon (%)	Hydrogen (%)	Oxygen (%)	C/N
6-A-H-a	71.75 ± 1.50	99.32 ± 0.20	0.33 ± 0.02	47.17 ± 0.26	5.90 ± 0.05	46.59 ± 0.28	143.52 ± 9.47
6-A-H-b	71.75 ± 1.50	99.57 ± 0.22	0.38 ± 0.02	46.52 ± 0.10	5.71 ± 0.05	47.39 ± 0.17	124.25 ± 4.69
6-A-H-c	71.75 ± 1.50	99.70 ± 0.05	0.36 ± 0.04	46.84 ± 0.13	5.85 ± 0.04	46.96 ± 0.20	133.21 ± 12.78
6-A-S-a	69.38 ± 3.38	99.52 ± 0.05	0.33 ± 0.03	47.23 ± 0.09	5.87 ± 0.04	46.57 ± 0.08	144.29 ± 12.83
6-A-S-b	69.38 ± 3.38	99.52 ± 0.05	0.34 ± 0.03	47.44 ± 0.11	5.86 ± 0.01	46.37 ± 0.12	142.37 ± 10.30
6-A-S-c	69.38 ± 3.38	99.57 ± 0.05	0.38 ± 0.02	47.31 ± 0.47	5.94 ± 0.01	46.37 ± 0.46	126.41 ± 6.30
6-L-H-a	73.90 ± 1.30	99.48 ± 0.12	0.39 ± 0.02	46.90 ± 0.04	5.97 ± 0.14	46.75 ± 0.19	122.00 ± 4.66
6-L-H-b	73.90 ± 1.30	99.40 ± 0.06	0.35 ± 0.02	46.62 ± 0.09	6.07 ± 0.14	46.96 ± 0.22	135.39 ± 6.16
6-L-H-c	73.90 ± 1.30	99.63 ± 0.17	0.40 ± 0.04	46.70 ± 0.17	6.11 ± 0.00	46.79 ± 0.21	117.88 ± 11.37
6-L-S-a	79.79 ± 2.07	99.77 ± 0.11	0.37 ± 0.04	46.95 ± 0.01	6.20 ± 0.01	46.49 ± 0.03	129.82 ± 12.49
6-L-S-b	79.79 ± 2.07	99.65 ± 0.04	0.38 ± 0.07	47.67 ± 0.35	6.00 ± 0.08	45.95 ± 0.36	131.21 ± 23.67
6-L-S-c	79.79 ± 2.07	99.51 ± 0.09	0.38 ± 0.02	46.71 ± 0.12	5.96 ± 0.17	46.96 ± 0.30	124.74 ± 4.68
12-A-H-a	58.27 ± 1.02	99.48 ± 0.05	0.32 ± 0.02	46.71 ± 0.35	6.02 ± 0.01	46.96 ± 0.37	148.57 ± 5.97
12-A-H-b	58.27 ± 1.02	99.28 ± 0.29	0.32 ± 0.01	46.32 ± 0.14	5.85 ± 0.06	47.51 ± 0.21	147.07 ± 1.89
12-A-H-c	58.27 ± 1.02	99.57 ± 0.09	0.34 ± 0.03	46.74 ± 0.09	6.01 ± 0.06	46.92 ± 0.00	140.31 ± 10.73
12-A-S-a	59.91 ± 3.24	99.52 ± 0.03	0.35 ± 0.02	46.72 ± 0.05	5.94 ± 0.04	46.99 ± 0.10	133.90 ± 7.52
12-A-S-b	59.91 ± 3.24	99.40 ± 0.23	0.35 ± 0.00	47.01 ± 0.12	5.97 ± 0.04	46.68 ± 0.17	136.28 ± 1.62
12-A-S-c	59.91 ± 3.24	99.54 ± 0.09	0.39 ± 0.06	47.52 ± 0.09	5.97 ± 0.01	46.13 ± 0.02	126.02 ± 18.23
12-L-H-a	57.13 ± 1.67	99.58 ± 0.31	0.41 ± 0.02	47.13 ± 0.09	6.04 ± 0.03	46.42 ± 0.08	116.53 ± 4.10
12-L-H-b	57.13 ± 1.67	99.71 ± 0.03	0.38 ± 0.02	47.30 ± 0.06	5.94 ± 0.06	46.39 ± 0.13	126.32 ± 4.91
12-L-H-c	57.13 ± 1.67	99.61 ± 0.33	0.35 ± 0.02	47.56 ± 0.02	6.10 ± 0.06	45.98 ± 0.06	136.34 ± 7.84
12-L-S-a	57.11 ± 2.23	99.54 ± 0.16	0.33 ± 0.01	47.39 ± 0.45	6.05 ± 0.03	46.23 ± 0.41	145.88 ± 3.61
12-L-S-b	57.11 ± 2.23	99.55 ± 0.41	0.32 ± 0.03	47.92 ± 0.01	6.01 ± 0.00	45.75 ± 0.02	151.07 ± 14.20
12-L-S-c	57.11 ± 2.23	99.56 ± 0.09	0.34 ± 0.03	47.93 ± 0.01	6.14 ± 0.03	45.59 ± 0.05	142.08 ± 12.58

For sample name, 6 and 12 refer to the stage of sample collection; A and L refer to Ash and Larch; H and S refer to hardwood area and softwood area; and a, b, and c refer to parts A, B, and C of the sample, respectively. For example, 6-A-H-a is part A of Ash wood placed in hardwood area for 6 months, and 12-L-S-c is part C of Larch wood placed in softwood area for 12 months. The % content of total solid was calculated based on wet mass; others were based on dry mass.

Table 5-5. The weight loss of wood samples after 6 months and 12 months experiments.

Sample weight loss (%)	6 months	12 months
A-H	-2.59 ± 0.80	-5.12 ± 1.53
A-S	-3.06 ± 1.13	-5.20 ± 1.64
L-H	-3.67 ± 0.86	-4.33 ± 1.49
L-S	-2.46 ± 0.93	-3.91 ± 1.28

Note: The weight loss of samples was calculated based on dry mass. In the sample name, A and L refer to Ash and Larch; H and S refer to hardwood area and softwood area, respectively. For example, A-H is Ash wood placed in hardwood area, and L-S is Larch wood placed in softwood area.

The amount of WEOC in the wood samples after being placed for different time periods is shown in Figure 5-10. The WEOC content in Larch was still significantly higher than that in Ash after 6 months and 12 months periods. This was consistent with the initial WEOC levels in both wood types, but the WEOC content decreased compared to the initial wood samples. After 6 months of experiment, there was no significant difference in the WEOC content between the A, B, and C parts of the same sample. In addition, samples from the two experimental sites (hardwood areas and softwood areas) showed no significant differences. However, the differences in WEOC content between the samples were revealed after the 12 months experiment. Firstly, the WEOC content in the three parts of Larch showed significant differences, with the levels ranging from A to B to C. Secondly, Ash and Larch samples placed in the hardwood area had less WEOC content than those placed in the softwood area. Since the WEOC concentration in the soil was significantly lower than that in the wood samples, the wood samples slowly decreased in WEOC content to soil as they degraded in the forest, and the decrease rate was different in different vegetation environments. In addition, the WEOC in the samples may show a trend of gradual decrease to the soil from the bottom to the top, i.e., the fastest loss of WEOC is in the part in contact with the soil.

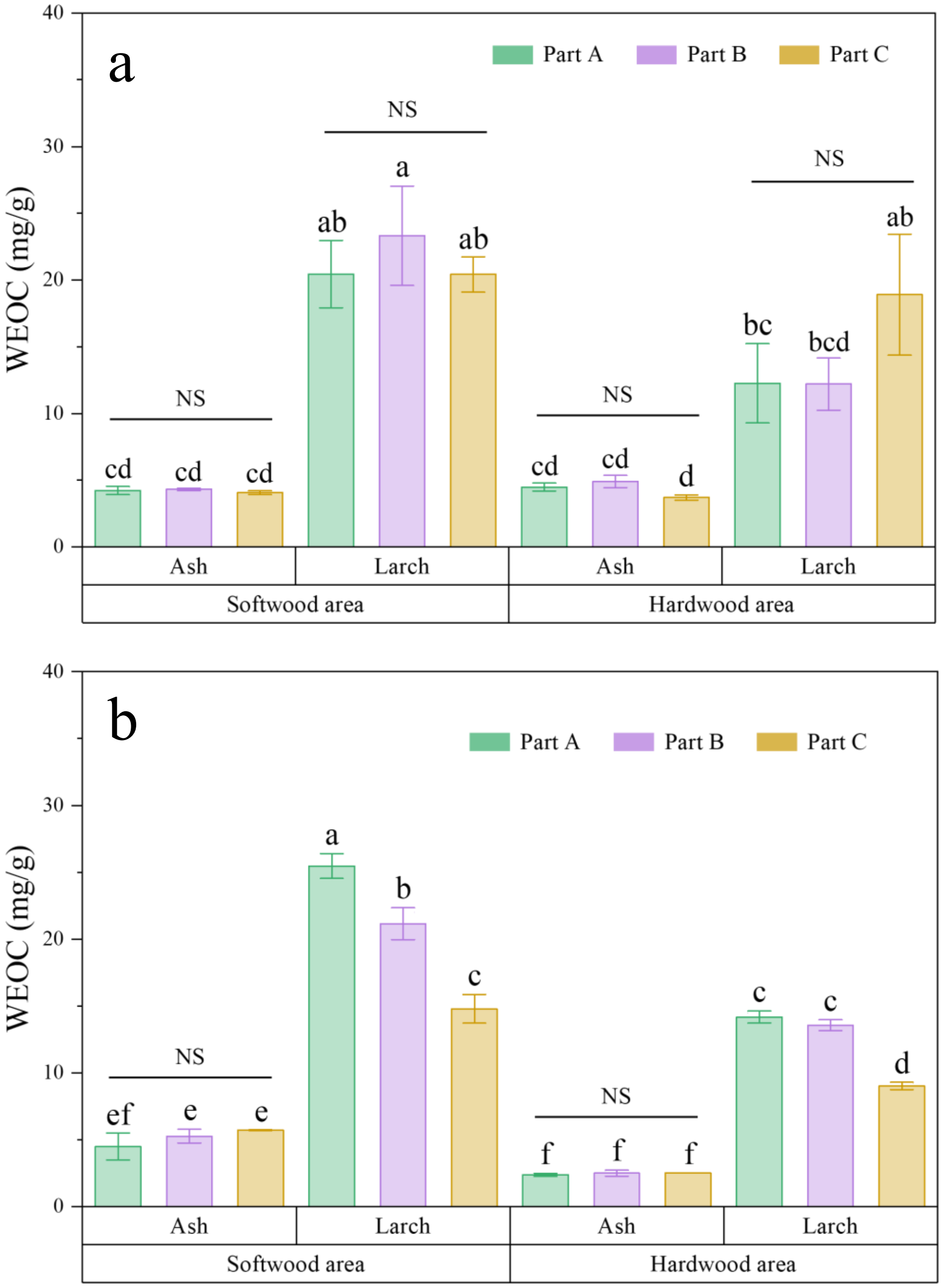

Figure 5-10. Water extractable organic carbon (WEOC) of wood samples at two experimental sites, (a) collected after 6 months and (b) collected after 12 months. Different lowercase letters above columns indicate a difference at a 0.05 level, NS means not significant.

5.3.3 Anaerobic digestion performance of wood samples

To further explore biochemical methane potential of wood samples placed in the forest for different durations, the AD experiments were carried out. Figure 5-11 shows the AD results of two types of wood raw materials, indicating that the biogas production of Ash was significantly higher than that of Larch. Lignocellulosic composition analysis showed that Ash had lower lignin content and higher cellulose content compared to Larch (Table 5-3), which could be the main factor contributing to the higher biogas yield of Ash. Generally, hardwoods have higher polysaccharide content and lower lignin content compared to softwoods, along with higher degrees of deacetylation in hardwood xylan (Ekstrand et al., 2020; Wang and Barlaz, 2016). These properties enhance the availability of Ash (hardwood) to functional microorganisms during AD. Gao et al. (2024a) compared the AD performance of hardwood and softwood wastes by meta-analysis and found that the methane production of hardwood waste was 83% higher than that of softwood waste. Although the high WEOC content of Larch is more favorable to AD (Figure 5-8a), it is not sufficient to change the effect of the properties of wood raw materials.

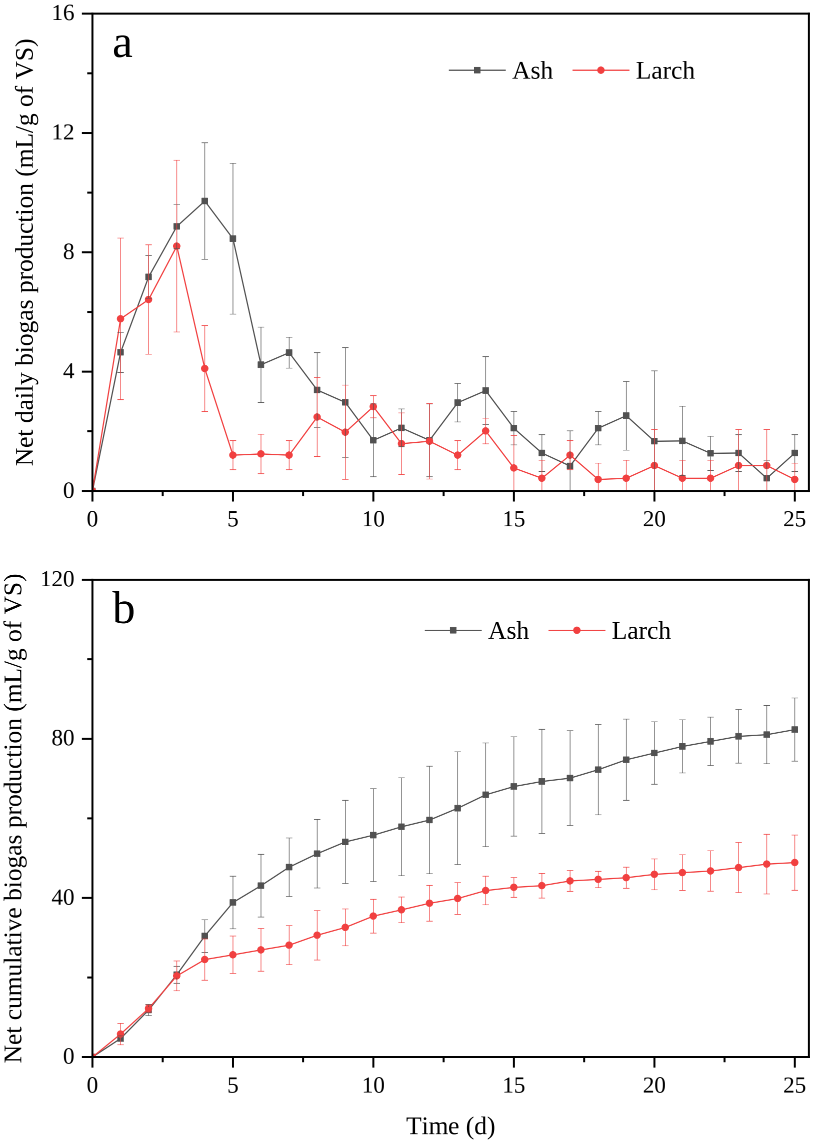

Figure 5-11. Biogas production from raw wood samples. (a) Daily biogas yield and (b) cumulative biogas yield.

Figures 5-12 and 5-13 present the AD results of wood samples which were placed in the forest for 6 and 12 months, respectively. Since the basic properties of the three parts (Part A, B, and C) of the samples were not significantly different, they were not tested separately. The results showed that there were no significant differences in biogas production between the wood samples placed in the two vegetation environments, but biogas production in Ash was still significantly higher than in Larch. In addition, the methane content of all wood samples did not differ significantly and basically ranged from 60% to 65%, with an average value of 62.2%. The final methane yields for all samples are shown in Figure 5-14.

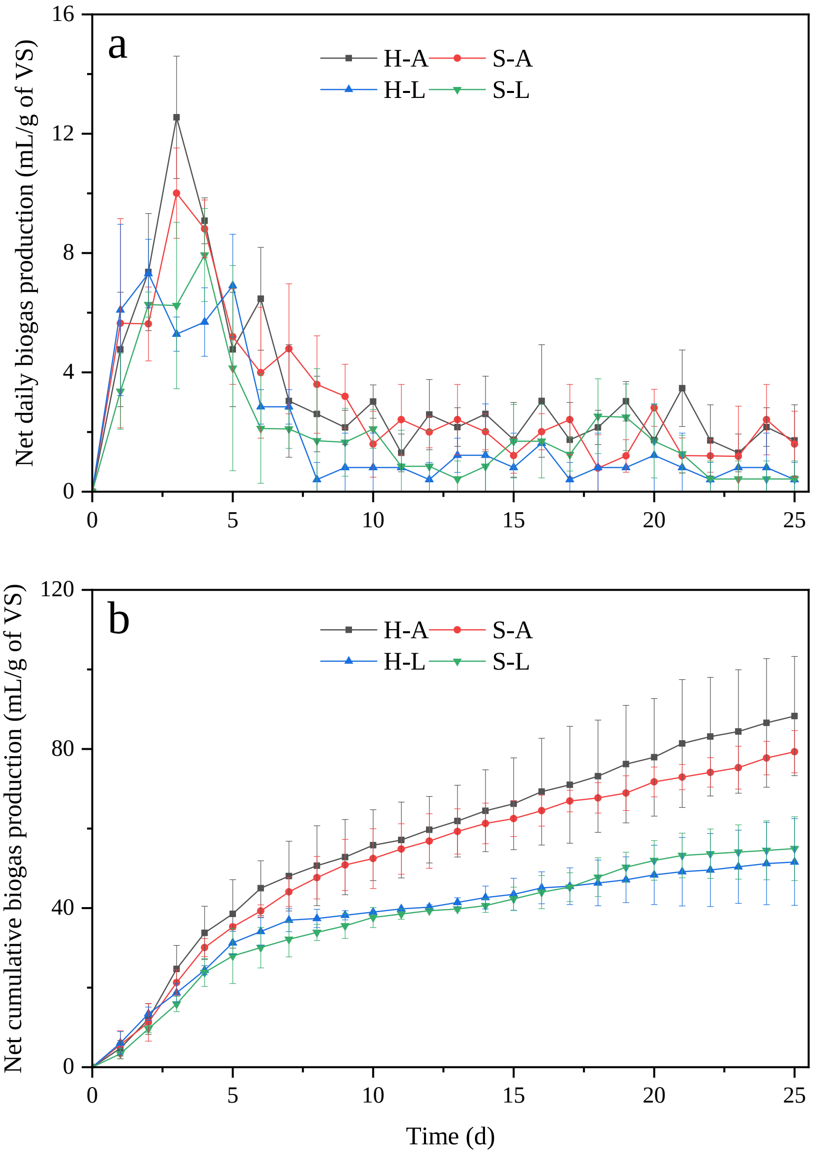

Figure 5-12. Biogas production from wood samples that were placed in the forest for 6 months. (a) Daily biogas yield and (b) cumulative biogas yield. H and S refer to hardwood area and softwood area; A and L refer to Ash and Larch, respectively. For example, H-A is Ash wood placed in hardwood area, and S-L is Larch wood placed in softwood area.

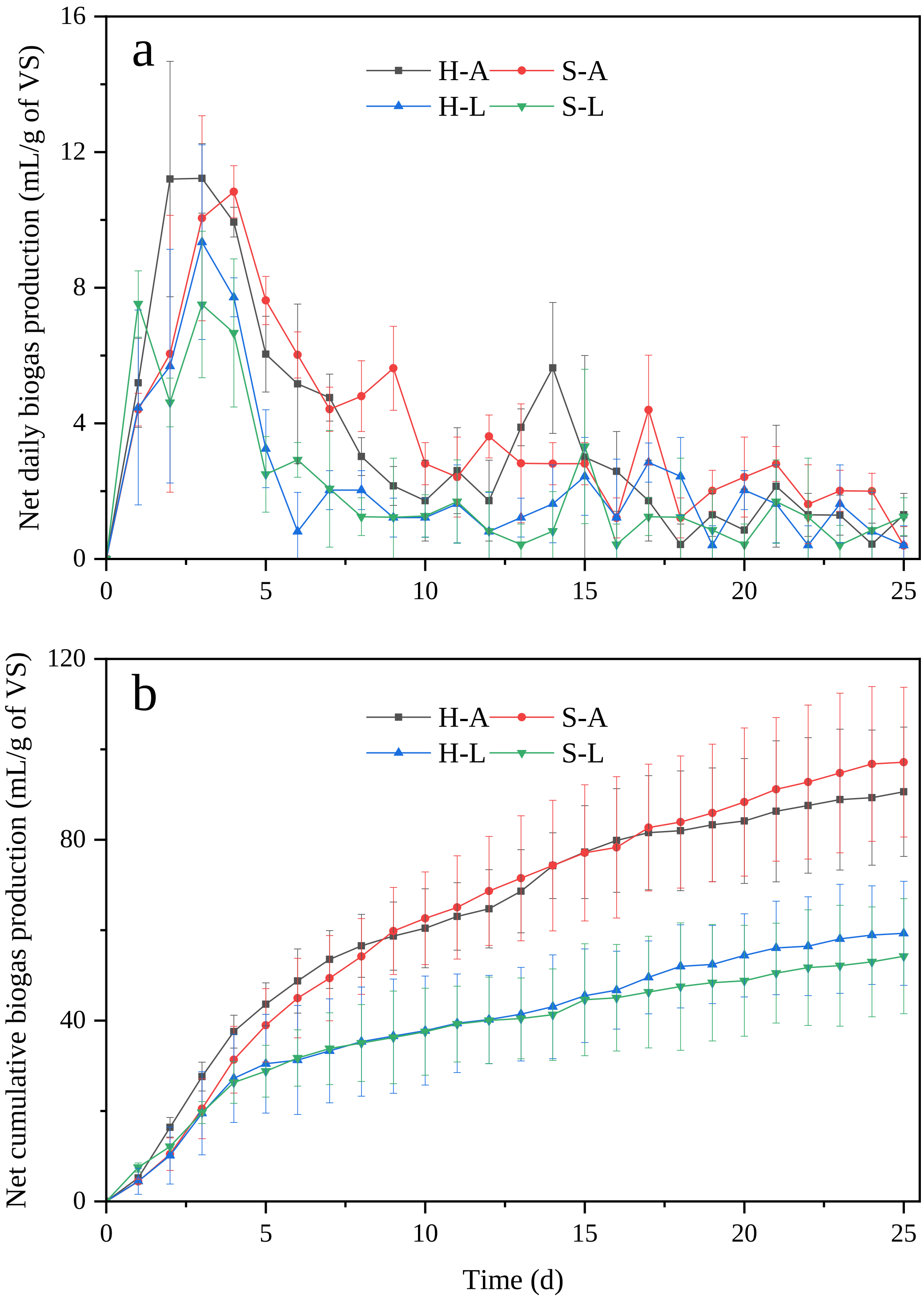

Figure 5-13. Biogas production from wood samples that were placed in the forest for 12 months. (a) Daily biogas yield and (b) cumulative biogas yield. H and S refer to hardwood area and softwood area; A and L refer to Ash and Larch, respectively. For example, H-A is Ash wood placed in hardwood area, and S-L is Larch wood placed in softwood area.

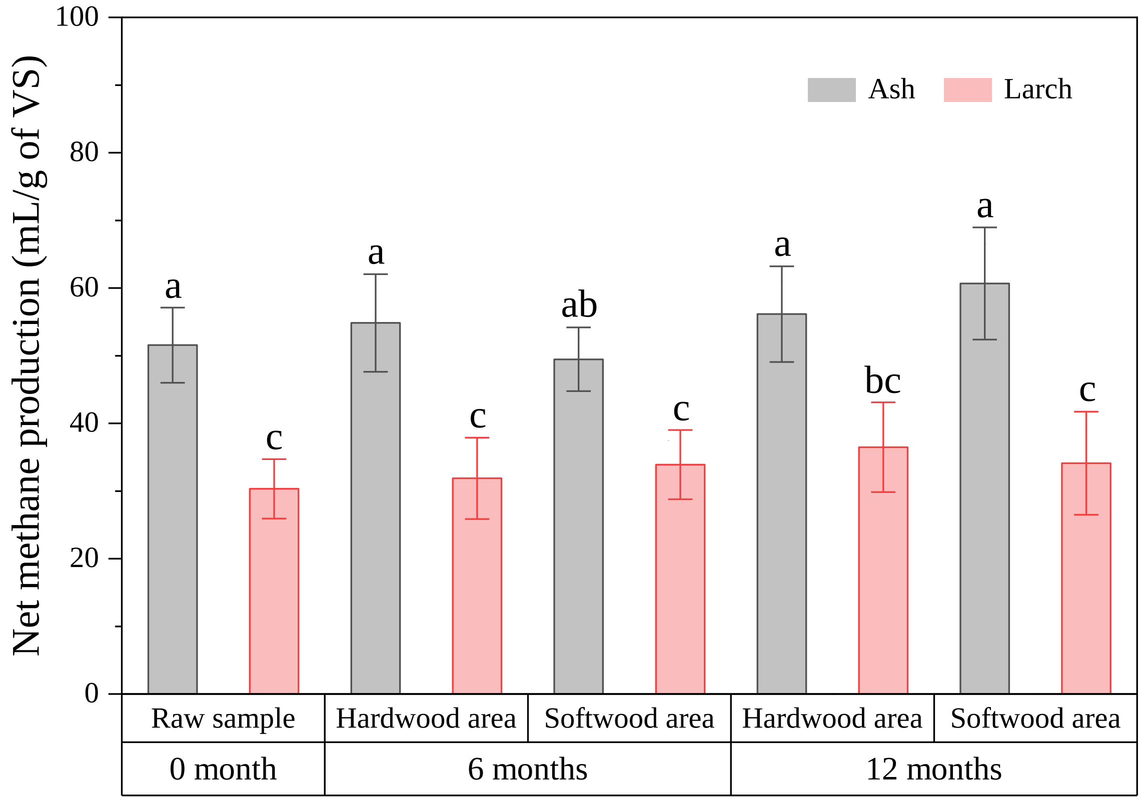

Figure 5-14. The net methane production of all wood samples.

5.4 Conclusions

This chapter aims to address RQ 4. The physicochemical properties of soils in different vegetation environments varied greatly, with the most noticeable differences being in the elemental content of the soil and soil microbial activity. Different forest environments affected the physicochemical properties of wood samples, particularly in terms of differences in WEOC content. The results showed that the softwood area, where soil conditions were more favourable for wood degradation, had a higher WEOC content than the hardwood area. Furthermore, the AD results indicated that the cumulative biogas production of wood samples treated for 12 months was higher than that of the original samples and the samples treated for 6 months, although these differences were not statistically significant. This chapter demonstrated that forest soil ecosystem can be applied to enhance methane yield of wood waste, but a sufficient length of treatment is required to achieve a desired result.

Chapter 6 Overarching discussion, conclusions and future work

6.1 Discussion

This study offers promising insights into the potential of using DC3 wood samples for AD to enhance methane production. However, several limitations must be acknowledged, and further research is essential to refine the process and address key challenges. One key limitation identified in Chapter 5 is that a one-year period of monitoring in the forest was insufficient to observe a significant effect of wood samples on methane production. To improve the understanding of this process, future research should focus on determining the optimal time required for wood blocks to reach the decay stage (DC3) under different forest conditions. Gaining a clearer understanding of these timeframes will enable better management of wood degradation for AD, allowing for more efficient methane production.

Nonetheless, practical challenges arise when attempting to track and collect wood samples over time. The decomposition rates of wood blocks vary considerably even within the same forest, making it difficult to standardize sampling protocols. In addition, large-scale removal of decaying wood presents potential ecological risks, such as the reduction of soil organic matter, which could disrupt nutrient cycles and hinder tree growth. These concerns underscore the importance of considering the broader environmental implications of wood removal.

To mitigate these risks while optimizing the efficiency of AD, a selective harvesting strategy may be necessary. Targeting areas with abundant fallen wood could help minimize ecological disruption and reduce the potential negative impacts of large-scale wood removal.

Future studies should focus on refining collection techniques, optimizing the degradation timeline, and evaluating the ecological consequences of removing decaying wood from forest ecosystems. Achieving a sustainable approach to wood waste management for AD will require careful coordination between bioenergy production efforts and forest conservation priorities, ensuring that both environmental integrity and energy goals are achieved in a balanced manner.

In summary, while this study contributes valuable insights into wood decay for AD, addressing the identified limitations through further research will be crucial for optimizing the process and ensuring its sustainability.

6.2 Conclusions

The overarching objective of this thesis was to explore an economically viable approach for enhancing the AD performance of wood waste by leveraging a nature-based degradation processes of forest soil ecosystems. This work aimed to investigate whether wood waste could be effectively used as a feedstock for AD, examine the potential improvements in methane yield through various pretreatment methods, and evaluate the influence of forest soil ecosystems on wood waste degradation and subsequent methane production. The aforementioned pertains to the four research questions addressed in this thesis. RQ1 and RQ2, covered in Chapter 3, demonstrated that wood waste can be utilized as a feedstock in AD with the support of pretreatment technologies. However, the current pretreatment technologies are not economically efficient and not practicable, prompting the exploration of RQ3 (Chapter 4) and RQ4 (Chapter 5). The findings in Chapter 4 and Chapter 5 indicated that the forest soil ecosystem enhanced the methane production from wood waste, which combined with strategic collection can be applied in practice as a pretreatment technology for wood waste.

Wood waste, primarily composed of lignocellulosic biomass, presents a challenging substrate for AD due to its complex structure and high lignin content. The meta-analysis conducted in Chapter 3 revealed that the BMP of wood waste is significantly lower—122% lower—than that of other organic wastes. This substantial gap underscores the inherent recalcitrance of wood waste, which poses a significant barrier to its efficient utilization in AD processes. However, the study also demonstrated that pretreatment techniques could mitigate this gap, reducing it to 99%. This finding supports the hypothesis that wood waste, albeit challenging, can be a viable feedstock for AD when subjected to appropriate pretreatment methods. Chapter 3 further delved into various pretreatment methods to enhance the BMP of wood waste. The analysis showed that pretreatment significantly improved BMP by 113%, with the combination of multiple pretreatment techniques proving more effective than single approaches. Furthermore, Chapter 3 compared the accuracy of three ML algorithms in predicting methane production from wood waste, concluding that the RF model outperformed the others. This predictive model holds significant value for optimizing AD parameters and enhancing methane production efficiency from wood waste.

Chapter 4 introduced a novel nature-based approach utilizing forest soil ecosystems to enhance wood waste degradation for AD. Experimental results confirmed the efficacy of this approach. Wood samples treated with this system exhibited improvements across several physicochemical parameters conducive to AD, such as reduced cellulose crystallinity, increased WEOC, and a more suitable C/N. In AD tests, DC1 samples yielded the lowest methane production, while DC3 samples achieved the highest, with a remarkable 160% increase compared to DC1 samples. Furthermore, DC3 exhibited the highest net energy output, suggesting that DC3 could be the optimal stage for collection and utilization in AD.

Chapter 5 investigated how various forest soil environments influence the decay process of wood waste and subsequent methane production. This chapter revealed significant differences in the physicochemical properties of soils from different vegetation types, especially in elemental content and microbial activity. These differences affected the degradation of wood waste and its performance in AD. Although forest soil ecosystems can enhance methane production, a sufficiently long treatment period is required for optimal results.

6.3 Suggestions for future work

Based on the findings derived from this thesis above, several avenues for future research can enhance the understanding and application of wood waste in AD processes:

- Given the promising results observed in chapter 4, it is crucial to explore the performance of this nature-based approach involving forest soil ecosystems on wider area to determine the generalisability of the technique. Studies should focus on different forest environmental conditions and various wood waste types, as the degradation processes are influenced by the wood species and the specific forest environment. Such research would provide valuable insights into the efficiency of this nature-based approach and provide guidance on its application. For example, the selective and appropriate placement of more wood waste in the forest areas with fast degradation rates, as well as the additional application of wood waste that is easily degradable.
- One key area for future research is the investigation of the long-term effects of this nature-based approach on the quality of wood waste. As the wood waste degrades in the forest, its mass will decrease, reducing the raw material available for AD. This change in mass needs to be considered to maximize the overall benefits. Moreover, understanding the changes in the wood waste mass at different decay classes and its BMP can offer significant opportunities for optimizing collection strategies.

- Exploring the microbial dynamics involved in wood waste degradation within forest soil ecosystems is an intriguing area for future research. Key microorganisms involved in these processes can be identified by comparing the common characteristics in forest areas where wood waste degradation occurs at a rapid rate. These microbes will be further isolated and investigated for their pretreatment effect on wood waste.
- It is crucial to explore the economic viability of this nature-based approach in practical applications at an industrial scale through a comprehensive economic analysis. This analysis would delve into the entire process of collecting wood from forests and utilizing it in AD, assessing factors such as initial collection costs, transportation expenses, processing costs, and potential revenue streams from methane production and by-products. Such an investigation is essential to determine the feasibility and profitability of utilizing wood waste degraded by forest soil systems as a raw material for AD.
- To demonstrate that the collection of wood waste from forests for AD is the optimal disposal method, a detailed life-cycle assessment can be conducted. This assessment would compare its environmental impacts with alternative utilization options, such as direct retention in forests or collection for incineration. By evaluating factors across the entire life cycle, including carbon emissions, energy consumption, and ecosystem impacts, such a study would provide comprehensive insights into the environmental sustainability of AD compared to other disposal methods. This information is crucial for making informed decisions regarding the management of wood waste to minimize environmental footprint while maximizing resource efficiency.
- Finally, addressing the economic viability of pretreatment technologies remains critical to advancing AD of wood waste. Future research should prioritise the development of cost-effective pretreatment methods to increase the BMP of wood waste while minimising operating costs. This includes exploring novel pretreatment technologies or

combinations thereof tailored specifically for woody biomass. Research efforts could focus on integrating the nature-based approach proposed in this thesis with other advanced technologies to enhance the efficiency of biomass conversion processes.

Appendix

Appendix figures

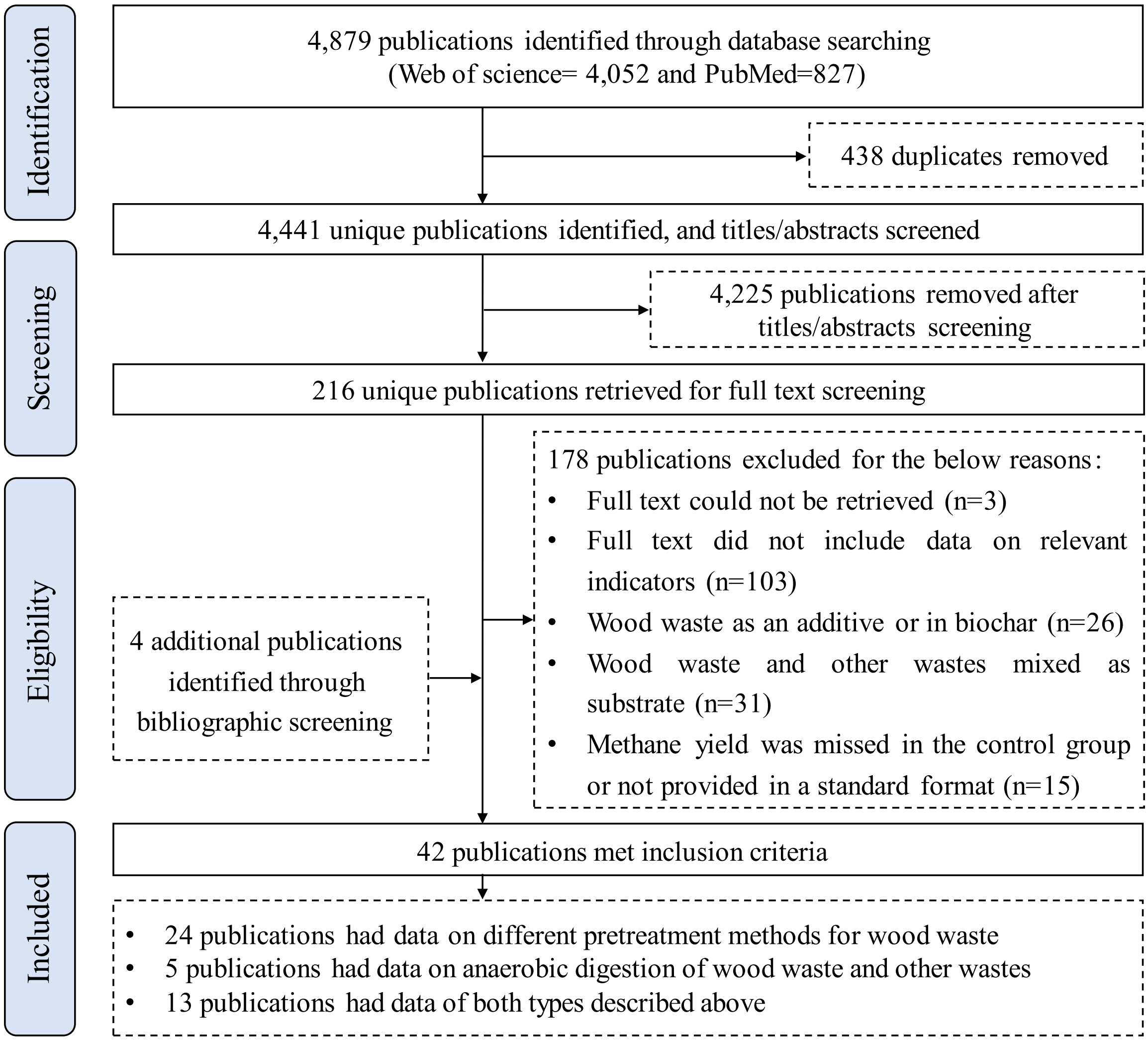

Figure A.1. The preferred reporting items for systematic reviews and meta-analyses flowchart of the publications screening process.

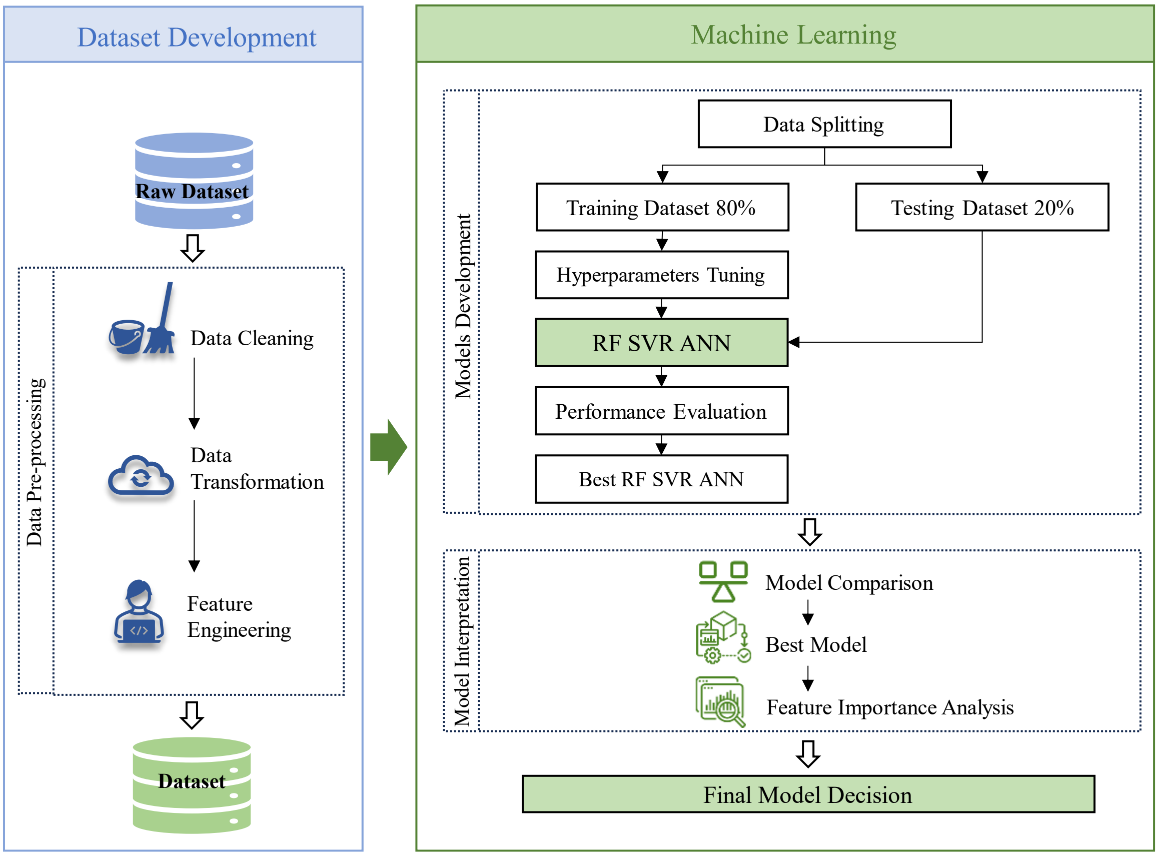

Figure A-2. Flowchart of the machine learning framework to determine the effect of wood waste on anaerobic digestion. RF: random forest; SVR: support vector regression; ANN: artificial neural network.

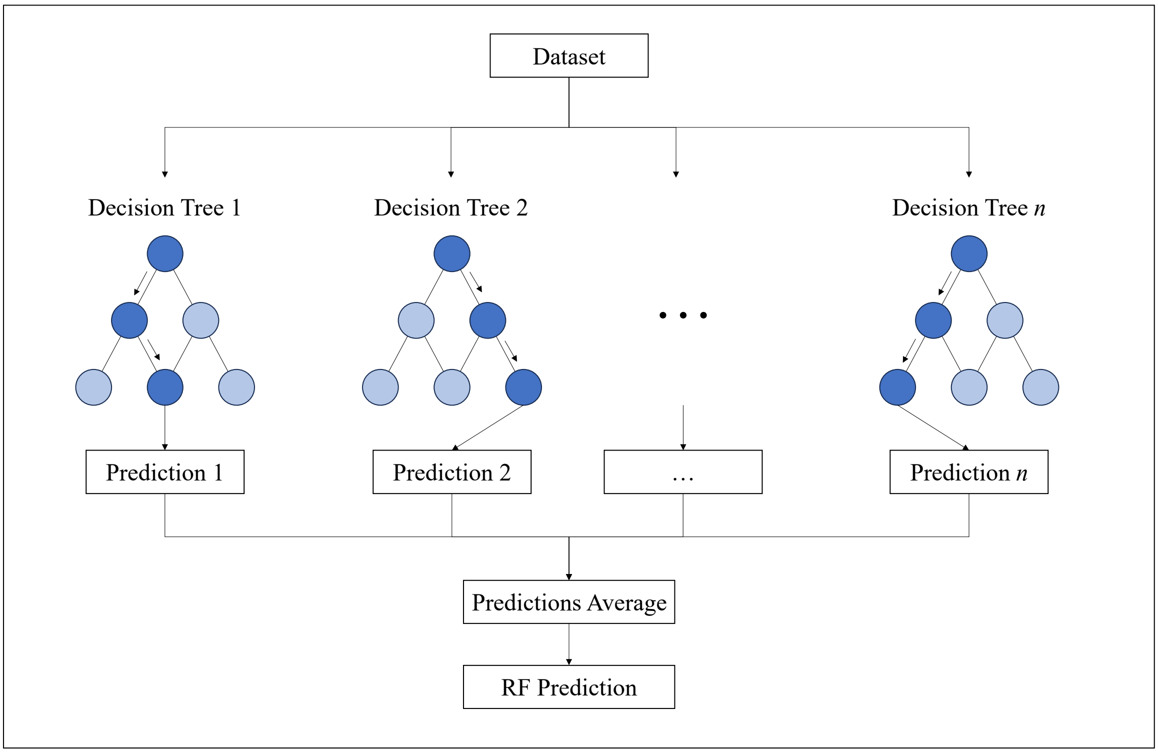

Figure A-3. Mechanism Map of the Machine Learning Random Forest (RF) model used in chapter 3.

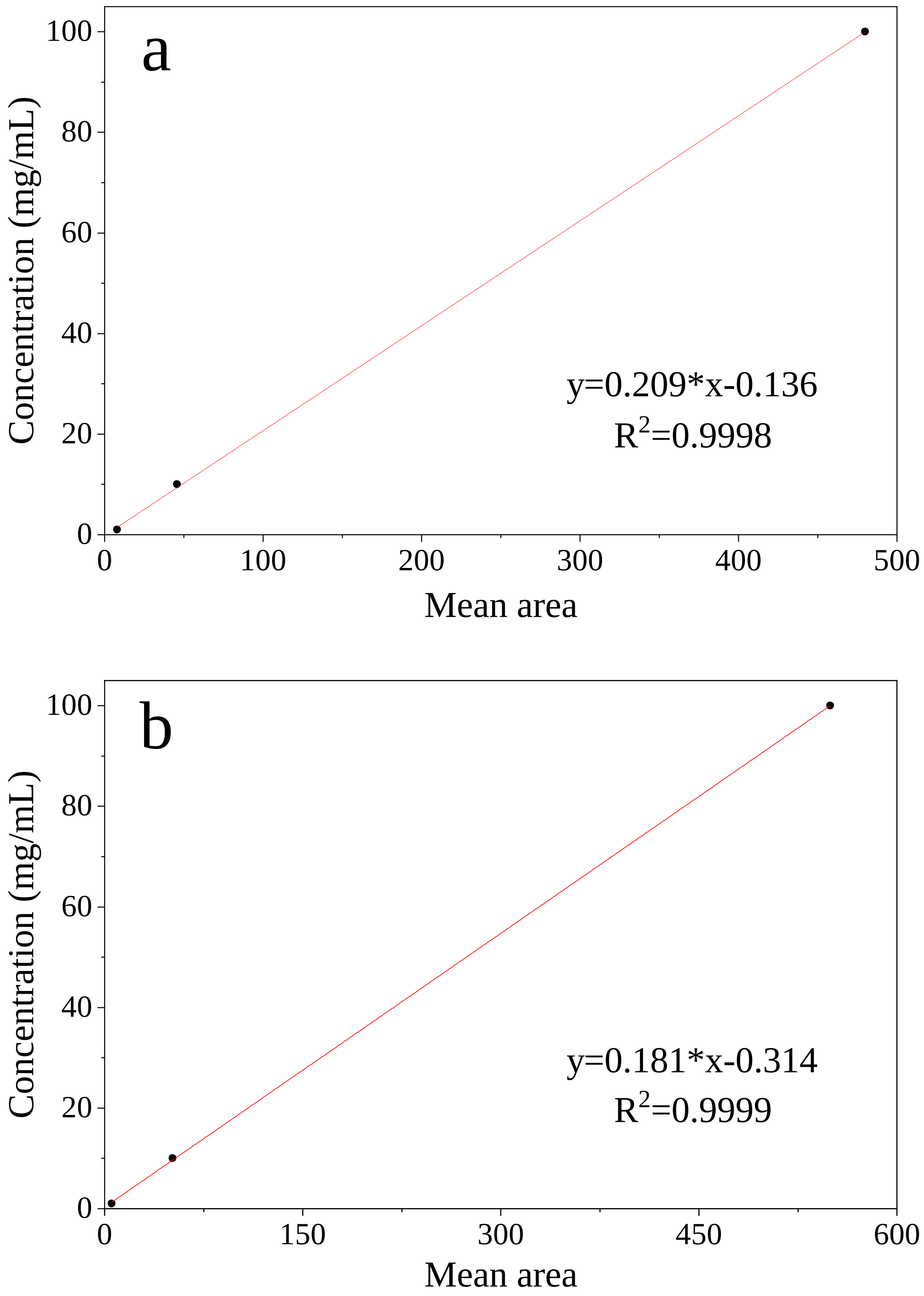

Figure A-4. The calibration curves used for testing organic carbon content in samples. (a) Total carbon content; (b) Inorganic carbon content.

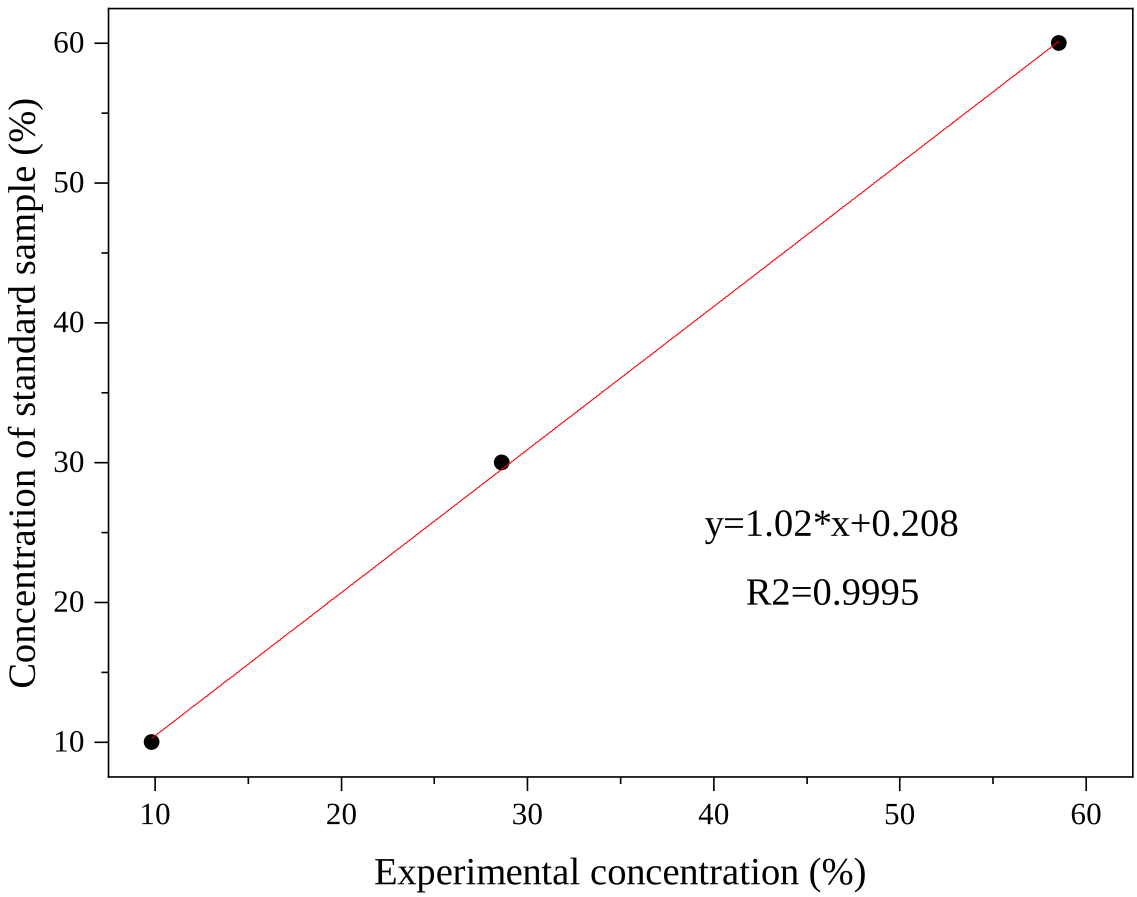

Figure A-5. The calibration curve used for testing methane content in biogas.

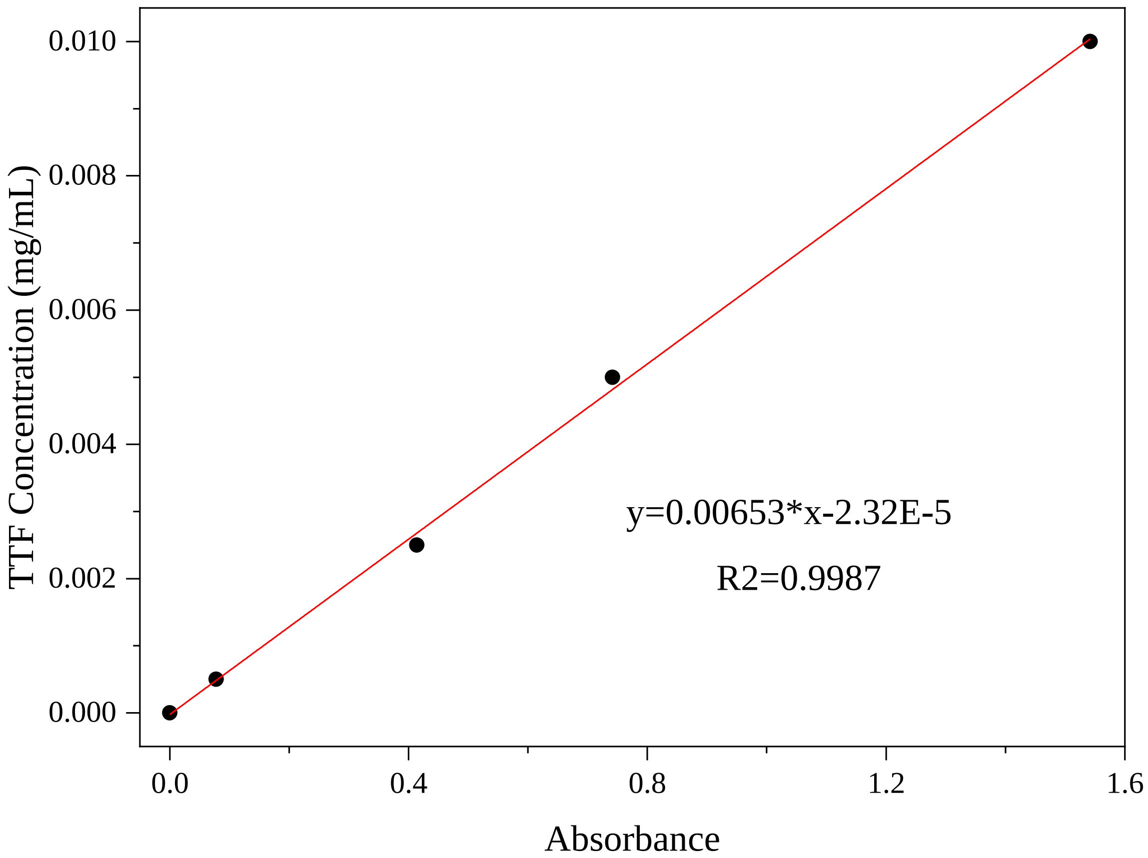

Figure A-6. The calibration curve used for testing microbial enzyme activity in soil samples.

Appendix tables

Table A-1. Anaerobic digestion feedstock type, composition, operational parameters, and product yield.

Feedstock	C	H	L	L/(C+H)	LCH	VS	PS	C/N	I/S	T	Time (day)	TMP	Biogas yield (mL/g VS)	CH4 yield (mL/g VS)	Reference
Rice Straw (dried at 40 ℃+anaerobic granular sludge)	34.3	24.2	11.2	0.19	69.7	86.3	0.11	19.4	1	37	40	326.4	222	117.9	(Gu et al., 2014)
Rice Straw (dried at 40 ℃+paper mill sludge)	34.3	24.2	11.2	0.19	69.7	86.3	0.11	24.5	1	37	40	326.4	64.1	8.5	(Gu et al., 2014)
Rice Straw (dried at 40 ℃+municipal sludge)	34.3	24.2	11.2	0.19	69.7	86.3	0.11	22.1	1	37	40	326.4	37.6	5.1	(Gu et al., 2014)
……															
……															
Wheat Straw (2% H_2O_2 (0 ℃, 60 min))	38.4	20.8	6.8	0.11	66	N.A.	20-30	84.9	N.A.	37	35	297	N.A.	108.5	(Song and Zhang, 2015)
Wheat Straw (3% H_2O_2 (0 ℃, 60 min))	34.3	16.1	6.0	0.12	56.4	N.A.	20-30	78.7	N.A.	37	35	254.2	N.A.	128.4	(Song and Zhang, 2015)
Wheat Straw (4% H_2O_2 (0 ℃, 60 min))	33.9	16.8	5.7	0.11	56.4	N.A.	20-30	80.8	N.A.	37	35	253.4	N.A.	118.7	(Song and Zhang, 2015)

C: Cellulose content (%); H: Hemicellulose content (%); L: Lignin content (%); L/(C+H): Lignin content/(Cellulose content+Hemicellulose content); LCH: Lignocellulose content (%)=Lignin content+Cellulose content+Hemicellulose content; VS: Volatile solids; PS: Particle size (mm); I/S: inoculum (sludge) to substrate; T: Temperature (℃); TMP: theoretical methane potential (mL/g VS) were calculated according to the lignocellulose content (Chen et al., 2014). The detailed data are available in the article (Gao et al., 2022).

Table A-2. Chemical composition of various lignocellulosic wastes.

Categories	Substrate types	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Volatile solids (%)	Organic carbon (%)	Reference
Wood	SW-pine	37.3	19.6	30.9	99.4	44.9	(Wang et al., 2013)
	SW-spruce	42.0	20.3	27.4	98.5	44.7	(Wang et al., 2013)
	SW-radiata pine	41.1	20.1	27.1	99.2	44.1	(Wang et al., 2011)
	SW-loblolly pine	31.8	18	35.8	98.8	48.2	(Wang and Barlaz, 2016)
	SW-white pine	27.8	16.9	42.4	99	51	(Wang and Barlaz, 2016)
	SW-pine (bark)	25.4	14.7	27.6	96.7	none	(Valentín et al., 2010)
	SW-spruce (bark)	19	11	22.6	96.1	none	(Millati et al., 2019)
	HW-eucalyptus	40.0	11.0	32.0	99.9	44.5	(Wang et al., 2011)
	HW-willow oak	26.2	17.6	30.2	96.4	43.7	(Wang and Barlaz, 2016)
	HW-white oak	36.7	19.2	24.7	99.2	44.4	(Wang et al., 2013)
	HW-red oak	40.5	19.6	23.5	99.6	44.6	(Wang et al., 2013)
	HW-birch (bark)	10.7	11.2	27.9	97.1	none	(Miranda et al., 2013)
	HW-aspen (bark)	25.4	23.4	22.6	99.6	none	(Millati et al., 2019)
Board	OSB-HW	41.6	16.8	22.7	99.1	44.2	(Wang et al., 2013)
	OSB-HW	42.1	16.8	22.5	99	45.6	(Wang et al., 2011)
	OSB-SW	37.6	17.9	33.6	98.9	43.9	(Wang et al., 2011)
	Plywood	40.7	17.6	29.5	97.5	44.7	(Wang et al., 2013)
	Plywood	38.8	17	31.4	96.4	46.9	(Wang et al., 2011)
	Particleboard	37.7	19	30.2	99.1	43.1	(Wang et al., 2013)

Categories	Substrate types	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Volatile solids (%)	Organic carbon (%)	Reference
	Particleboard	37.3	16.3	28.2	98.9	45.1	(Wang et al., 2011)
	MDF	39	18.4	31.8	99.5	43.6	(Wang et al., 2013)
	MDF	34.8	15.2	29.5	98.6	43.9	(Wang et al., 2011)
Paper and paperboard	Newspaper	74.3	8.5	8.6	none	none	(Bayard et al., 2016)
	Office paper	77.0	2.4	2.6	none	none	(Bayard et al., 2016)
	Magazine paper	86.6	1.1	10.0	none	none	(Bayard et al., 2016)
	Newsprint	54.7	17.5	16.1	91	35.7	(Wang et al., 2013)
	Copy paper	72.4	14.2	0.6	88.1	39.1	(Wang et al., 2013)
	Corrugated container	61.8	14.6	15.4	96.9	50.8	(Wang et al., 2013)
	Cardboards	53.5	11.6	17.0	none	none	(Bayard et al., 2016)
Crop straw	Rice Straw	36.7	28.3	13.1	81.5	40.1	(Mustafa et al., 2017)
	Sugarcane bagasse	29.7	18.3	15.3	96.8	34.7	(Liu et al., 2017)
	Corn stover	20.4	31.8	20	87.6	38.9	(Li et al., 2016)
	Corn stalk	43	35	7.3	93.8	46.5	(Meng et al., 2016)
	Corn cob	26.6	24.5	10.9	88.81	47.9	(Ali et al., 2018)
	Wheat straw	34.7	29.2	6.3	95.3	50.3	(Ali et al., 2018)
	Wheat bran	7.3	21.4	3.5	95.4	43.9	(Corneli et al., 2016)
	Barley straw	35.4	28.7	13.1	95.5	none	(Liu et al., 2017)
	Maize straw	30.7	31.2	5.2	91	47.2	(Ali et al., 2018)

Note: softwood (SW); hardwood (HW); oriented strand board (OSB); medium density fiberboard (MDF).

Table A-3. List of 44 publications which contributed to the methane production dataset used in the meta-analysis and machine learning analysis.

Article number	Article Title	Article Content	Year	Publication Journal	DOI
1	Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production	Papers had data on different pretreatment methods for wood waste	2010	BioResources	10.15376/biores.5.2.928-938
2	Alkali pretreatment of softwood spruce and hardwood birch by NaOH/thiourea, NaOH/urea, NaOH/urea/thiourea, and NaOH/PEG to improve ethanol and biogas production	Papers had data on different pretreatment methods for wood waste	2012	Journal of Chemical Technology and Biotechnology	10.1002/jctb.3695
3	Anaerobic digestion of poplar processing residues for methane production after alkaline treatment	Papers had data on different pretreatment methods for wood waste	2013	Bioresource Technology	10.1016/j.biortech.2012.12.160
4	Effect of the N-Methylmorpholine-N-Oxide (NMMO) Pretreatment on Anaerobic Digestion of Forest Residues	Papers had data on different pretreatment methods for wood waste	2013	BioResources	10.15376/biores.8.4.5409-5423
5	Improvement of biogas production from pine wood by alkali pretreatment	Papers had data on different pretreatment methods for wood waste	2013	Fuel	10.1016/j.fuel.2012.12.092
6	Biogas Production from N-Methylmorpholine-N-oxide (NMMO) Pretreated Forest Residues	Papers had data on different pretreatment methods for wood waste	2014	Applied Biochemistry and Biotechnology	10.1007/s12010-014-0747-z
7	Fungal Pretreatment of Albizia Chips for Enhanced Biogas Production by Solid-State Anaerobic Digestion	Papers had data on different pretreatment methods for wood waste	2015	Energy & Fuels	10.1021/ef501922t
8	Sequential parametric optimization of methane production from different sources of forest raw material	Papers had data on different pretreatment methods for wood waste	2015	Frontiers in Microbiology	10.3389/fmicb.2015.01163
9	Potential methane production of spent sawdust used in the cultivation of Gymnopilus pampeanus	Papers had data on different pretreatment methods for wood waste	2016	Journal of Environmental Chemical Engineering	10.1016/j.jece.2016.10.009
10	Effective bio-pretreatment of sawdust waste with a novel microbial consortium for enhanced biomethanation	Papers had data on different pretreatment methods for wood waste	2017	Bioresource Technology	10.1016/j.biortech.2017.03.187

11	Fungal pretreatment of willow sawdust and its combination with alkaline treatment for enhancing biogas production	Papers had data on different pretreatment methods for wood waste	2017	Journal of Environmental Management	10.1016/j.jenvman.2016.04.006
12	Fungal Pretreatment of Willow Sawdust with Abortiporus biennis for Anaerobic Digestion: Impact of an External Nitrogen Source	Papers had data on different pretreatment methods for wood waste	2017	Sustainability	10.3390/su9010130
13	Enhancing methane production from lignocellulosic biomass by combined steam-explosion pretreatment and bioaugmentation with cellulolytic bacterium Caldicellulosiruptor bescii	Papers had data on different pretreatment methods for wood waste	2018	Biotechnology for Biofuels	10.1186/s13068-018-1025-z
14	Effect of power ultrasound and Fenton reagents on the biomethane potential from steam-exploded birchwood	Papers had data on different pretreatment methods for wood waste	2019	Ultrasonics Sonochemistry	10.1016/j.ultsonch.2019.104675
15	Enhanced digestion of bio-pretreated sawdust using a novel bacterial consortium: Microbial community structure and methane-producing pathways	Papers had data on different pretreatment methods for wood waste	2019	Fuel	10.1016/j.fuel.2019.06.012
16	Enhanced enzymatic hydrolysis and methane production from rubber wood waste using steam explosion	Papers had data on different pretreatment methods for wood waste	2019	Journal of Environmental Management	10.1016/j.jenvman.2019.01.041
17	Integrated process for the production of fermentable sugar and methane from rubber wood	Papers had data on different pretreatment methods for wood waste	2020	Bioresource Technology	10.1016/j.biortech.2020.122785
18	Conversion of rubber wood waste to methane by ethanol organosolv pretreatment	Papers had data on different pretreatment methods for wood waste	2021	Biomass Conversion and Biorefinery	10.1007/s13399-020-00710-4
19	Effects of pinewood extractives on bioconversion of pinewood	Papers had data on different pretreatment methods for wood waste	2021	Fuel	10.1016/j.fuel.2020.119302
20	Enhancing hydrolysis and bio-methane generation of extruded lignocellulosic wood waste using microbial pre-treatment	Papers had data on different pretreatment methods for wood waste	2021	Renewable Energy	10.1016/j.renene.2021.01.131
21	Mesophilic aerobic digestion: An efficient and inexpensive biological pretreatment to improve biogas production from highly-recalcitrant pinewood	Papers had data on different pretreatment methods for wood waste	2022	Energy	10.1016/j.energy.2021.122361
22	Stimulating biogas production from steam-exploded birch wood using Fenton reaction and fungal pretreatment	Papers had data on different pretreatment methods for wood waste	2022	Bioresource Technology	10.1016/j.biortech.2022.128190

23	Integration of Shiitake cultivation and solid-state anaerobic digestion for utilization of woody biomass	Papers had data on different pretreatment methods for wood waste	2015	Bioresource Technology	10.1016/j.biortech.2015.01.102
24	Biogas production from food waste via anaerobic digestion with wood chips	Papers had data on different pretreatment methods for wood waste	2018	Energy & Environment	10.1177/0958305x18777234
25	Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production	Papers had data on anaerobic digestion of wood waste and other wastes	2012	Bioresource Technology	10.1016/j.biortech.2012.08.079
26	Comparison of solid-state to liquid anaerobic digestion of lignocellulosic feedstocks for biogas production	Papers had data on anaerobic digestion of wood waste and other wastes	2012	Bioresource Technology	10.1016/j.biortech.2012.08.051
27	Evaluation of the correlations between biodegradability of lignocellulosic feedstocks in anaerobic digestion process and their biochemical characteristics	Papers had data on anaerobic digestion of wood waste and other wastes	2015	Biomass & Bioenergy	10.1016/j.biombioe.2015.06.021
28	Effects of temperature and particle size on the biochemical methane potential of municipal solid waste components	Papers had data on anaerobic digestion of wood waste and other wastes	2018	Waste Management	10.1016/j.wasman.2017.11.015
29	High-solids anaerobic digestion requires a trade-off between total solids, inoculum-to-substrate ratio and ammonia inhibition	Papers had data on anaerobic digestion of wood waste and other wastes	2019	International Journal of Environmental Science and Technology	10.1007/s13762-019-02264-z
30	Alkali Pretreatment for Improvement of Biogas and Ethanol Production from Different Waste Parts of Pine Tree	Papers had data of both types described above	2013	Industrial & Engineering Chemistry Research	10.1021/ie302805c
31	Ionic liquid pretreatment to enhance the anaerobic digestion of lignocellulosic biomass	Papers had data of both types described above	2013	Bioresource Technology	10.1016/j.biortech.2013.10.026
32	Biogas production from lignocelluloses by N-methylmorpholine-N-oxide (NMMO) pretreatment: Effects of recovery and reuse of NMMO	Papers had data of both types described above	2014	Bioresource Technology	10.1016/j.biortech.2014.03.107
33	Enhanced Solid-State Biogas Production from Lignocellulosic Biomass by Organosolv Pretreatment	Papers had data of both types described above	2014	Biomed Research International	10.1155/2014/350414
34	Effect of Feedstock Components on Thermophilic Solid-State Anaerobic Digestion of Yard Trimmings	Papers had data of both types described above	2015	Energy & Fuels	10.1021/acs.energyfuels.5b00301

35	The Effect of Aqueous Ammonia Soaking Pretreatment on Methane Generation Using Different Lignocellulosic Biomasses	Papers had data of both types described above	2015	Waste and Biomass Valorization	10.1007/s12649-015-9352-9
36	Improvement of Solid-State Biogas Production from Wood by Concentrated Phosphoric Acid Pretreatment	Papers had data of both types described above	2016	BioResources	10.15376/biores.11.2.3230-3243
37	Anaerobic digestion of lignocellulosic biomasses pretreated with Ceriporiopsis subvermispora	Papers had data of both types described above	2017	Journal of Environmental Management	10.1016/j.jenvman.2017.01.075
38	Assessment of hydrothermal pretreatment of various lignocellulosic biomass with CO2 catalyst for enhanced methane and hydrogen production	Papers had data of both types described above	2017	Water Research	10.1016/j.watres.2017.04.068
39	Evaluation on the Methane Production Potential of Wood Waste Pretreated with NaOH and Co-Digested with Pig Manure	Papers had data of both types described above	2019	Catalysts	10.3390/catal9060539
40	Application of enzymatic and bacterial biodelignification systems for enhanced breakdown of model lignocellulosic wastes	Papers had data of both types described above	2020	Science of the Total Environment	10.1016/j.scitotenv.2020.138741
41	Does Acid Addition Improve Liquid Hot Water Pretreatment of Lignocellulosic Biomass towards Biohydrogen and Biogas Production?	Papers had data of both types described above	2020	Sustainability	10.3390/su12218935
42	Biorefining for olive wastes management and efficient bioenergy production	Papers had data of both types described above	2021	Energy Conversion and Management	10.1016/j.enconman.2021.114467
43	Biorefinery potential of Eucalyptus grandis to produce phenolic compounds and biogas	Papers no control group, only used for machine learning	2020	Canadian Journal of Forest Research	10.1139/cjfr-2020-0201
44	Evaluating the Influence of Temperature and Flow Rate on Biogas Production from Wood Waste via a Packed-Bed Bioreactor	Papers no control group, only used for machine learning	2021	Arabian Journal for Science and Engineering	10.1007/s13369-020-04900-0

Table A-4. List of 769 data used for the meta-analysis.

Article number	Wood type	Group	Category	Description of pretreatment	CKmean	CKsd	CKn	Tmean	Tsd	Tn
27	Corylus avellana	Compared with hardwood	Crop straw (Barley straw)	Untreated	97	2	3	278	2	3
27	Robinia pseudo-acacia	Compared with hardwood	Crop straw (Barley straw)	Untreated	151	2	3	278	2	3
37	Hazel	Compared with hardwood	Crop straw (Barley straw)	Untreated	99.72	2.16	3	273.91	6.83	3
37	Hazel	Compared with hardwood	Crop straw (Barley straw)	Autoclaved (121 ℃ 30 min)	113.5	2.15	3	253.04	10.26	3
37	Hazel	Compared with hardwood	Crop straw (Barley straw)	Autoclaved + Water	103.58	4.31	3	252.17	13.68	3
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……										
34	Woodchips	Co-digestion	Maple leaves	Wood chips:Maple leaves=74:26 based on TS	48.6	2.94	3	58.2	4.5	3
34	Woodchips	Co-digestion	Maple leaves	Wood chips:Maple leaves=49:51 based on TS	48.6	2.94	3	71.1	4.33	3
39	Hardwood (Eucalyptus)	Co-digestion	Pig manure	Pig manure:Wood chip=02:01 based on VS	175.81	17.11	3	234.88	20.2	3
39	Hardwood (Eucalyptus)	Co-digestion	Pig manure	Pig manure:Wood chip=02:01 based on VS	243.53	29.23	3	309.06	10.05	3

The detailed data are available in the article (Gao et al., 2024a).

Table A-5. List of 1179 data used for the machine learning analysis.

Article number	Wood types	Inoculum types	Volume (mL)	Temperature (℃)	Particle size (mm)	Ratio of inoculum to substrate (based on VS)	Cellulose content (%)	Hemicellulose content (%)	Lignin content (%)	Digestion time (d)	Methane production (L/kg of VS)
3	hardwood	Effluent from anaerobic digestion of manure	2000	35	12	0.028	47.7	25.6	23.6	2	12.06
3	hardwood	Effluent from anaerobic digestion of manure	2000	35	12	0.028	47.7	25.6	23.6	4	18.76
3	hardwood	Effluent from anaerobic digestion of manure	2000	35	12	0.028	47.7	25.6	23.6	6	27.32
3	hardwood	Effluent from anaerobic digestion of manure	2000	35	12	0.028	47.7	25.6	23.6	8	34.02
3	hardwood	Effluent from anaerobic digestion of manure	2000	35	12	0.028	47.7	25.6	23.6	10	39.3
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44	hardwood	sewage sludge	20000	40	1	0.5	57.79	12.8	25.28	10	157.39
44	hardwood	sewage sludge	20000	40	1	0.5	57.79	12.8	25.28	15	346.36
44	hardwood	sewage sludge	20000	40	1	0.5	57.79	12.8	25.28	20	494.76
44	hardwood	sewage sludge	20000	40	1	0.5	57.79	12.8	25.28	25	587.93
44	hardwood	sewage sludge	20000	40	1	0.5	57.79	12.8	25.28	30	603.09

The detailed data are available in the article (Gao et al., 2024a).

Table A-6. Hyperparameter selection of the random forest (RF) model.

Hyperparameters	Range	Tuned values of RF
n_estimators	(100,150,200,300,500)	150
max_depth	[10,20]	13
min_impurity_decrease	(0,0.001,0.01,0.1,0.2)	0
min_samples_leaf	(1,2,5,8,10)	1
min_samples_split	(2,5,8,10)	2
random state	[1,100]	1

Table A-7. Distribution of the 1179 datapoints used for machine learning.

Features		Unit	Abbreviation	Data range	Mean	SD
1	Wood types	-	WT	0 or 1	0.89	0.31
2	Inoculum types	-	IT	0 or 1	0.59	0.49
3	Volume	mL	V	60–20000	1526.57	1878.04
4	Temperature	°C	TEM	30–62	35.31	3.97
5	Particle size	Mm	PS	0.4–30.0	8.79	6.16
6	Ratio of inoculum to substrate	based on VS	I/S	0.01–8.63	1.15	1.54
7	Cellulose content	%	C	19.5–68.1	43.58	9.17
8	Hemicellulose content	%	H	1.8–36.7	17.32	8.52
9	Lignin content	%	L	8.1–47.3	27.34	10.05
10	Digestion time	d	T	1–70	26.95	19.27
11	Methane production	L/kg of VS	CH_4	0.06–603.09	91.22	83.07

Table A-8. Minimum number of pseudo-components for each fraction.

Component	Temperature range (°C)	Number of pseudo-components
Water	25–150	1
Hemicellulose	200–350	2
Cellulose	250–400	1
Lignin	150–1000	3

Reference

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