

**A Circular Economy Approach using a Dual Waste
System of Coal Fly Ash and Food Waste to Optimise
Biodegradability of Lignocellulosic Agricultural Residues
for Enhanced Methane Production in Anaerobic Digestion**

By

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Statement of Originality

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ABSTRACT

Lignocellulosic agricultural residues (LARs) represent a largely untapped resource for renewable energy production, particularly through anaerobic digestion (AD), but their recalcitrance limits biodegradability and methane (CH_4) yield. Lignin surrounds cellulose and hemicellulose, acting as a barrier to microbial hydrolytic enzymes and limiting microbial access to these compounds as carbon sources. This study investigated a novel integrated approach utilising several waste materials to maximise the AD of LARs, using barley straw (BS), coal fly ash (CFA) for alkali pretreatment and food waste (FW) for acid neutralisation. AD experiments in the solid-state system were conducted with two variations of alkalinity (CFA) and acidity (FW) to optimise process efficiency.

Alkali pretreatment using calcium oxide (CaO); as a principal alkalinity source of CFA, equivalent to $\sim 5,000 \text{ mg calcium carbonate } (\text{CaCO}_3) \text{ L}^{-1}$ was found to be optimal for pretreatment, leading to a significant change in fibre composition (e.g., 28% lignin reduction) and a 29% CH_4 yield increase, compared to water-treated BS Control. Increasing alkalinity to $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$, however, did not result in significant differences in both fibre composition and CH_4 yield. Interestingly, CFA pretreatment ($\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$) showed a 39% CH_4 yield increase compared to the equivalent alkalinity CaO pretreatment. This suggests that unique properties in CFA, particularly conductivity, stimulated microbial interaction mechanisms presumably associated with direct interspecies electron transfer (DIET) and a more efficient indirect interspecies electron transfer (IIET). High alkali-pretreated BS was effectively neutralised by 20% volatile solids (VS) fermented FW, maintaining an ideal pH for efficient CH_4 conversion.

The study concludes that integrating CFA and FW in AD of LARs reduces reliance on synthetic chemicals and potentially lowers pretreatment costs. Moreover, this approach supports circular economy principles by valorising organic and industrial waste streams, offering a sustainable solution for waste management and renewable energy production.

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ABBREVIATIONS

AD	Anaerobic digestion
ADF	Acid detergent fibre
ADL	Acid detergent lignin
Al ₂ O ₃	Aluminium oxide
aNDF	Amylase-treated neutral detergent fibre
ANOVA	Analysis of variance
ALR	Alkali loading rate
Al	Aluminium
As	Arsenic
BC	Black carbon
BS	Barley straw
BSI	British Standard Institute
C	Carbon
Ca ⁺	Calcium ions
CaCO ₃	Calcium carbonate
CaO	Calcium oxide
Ca(OH) ₂	Calcium hydroxide
CB	Carbon black
Cd	Cadmium
CFA	Coal fly ash
CHP	Combined heat and power
CH ₄	Methane
CO	Carbon monoxide
CO ₂	Carbon dioxide
Co	Cobalt
C/N	Carbon to nitrogen
Cr	Chromium
CSMY	Cumulative specific methane yield
Cu	Copper
(C ₅ H ₁₁ NO ₂ S)	Methionine
DIET	Direct interspecies electron transfer
DM	Dry matter
DNA	Deoxyribonucleic acid
d	Days
EC	Electrical conductivity

EPS	Extracellular polymeric substances
Fe	Iron
Fe ₂ O ₃	Hematite
Fe ₃ O ₄	Magnetite
FM	Fresh matter
FTIR	Fourier-transform infrared spectroscopy
FW	Food waste
GC	Gas chromatography
GHG	Greenhouse gas
GJ	Giga Joules
HCl	Hydrochloric acid
HDPE	High-density polyethylene
HMF	Hydroxyl methyl furfural
HNO ₃	Nitrite acid
H ₂	Hydrogen gas
H ₂ SO ₄	Sulfuric acid
h	hour
ICP-OES	Inductively coupled plasma optical emission spectrometry
IET	Indirect interspecies electron transfer
ISR	Inoculum to substrate ratio
K	Potassium
KOH	Potassium hydroxide
kWh	Kilowatt hour
K ₂ O	Potassium oxide
LARs	Lignocellulosic agricultural residues
LCCs	Lignin carbohydrate complexes
LOI	Loss on ignition
LSV	Linear Sweep Voltammetry
MCC	Microcrystalline cellulose
Mg	Magnesium
MgO	Magnesium oxide
Mn	Manganese
Mo	Molybdenum
Mt	Million tonnes
MWe	Megawatt electric
m/m	mass percent concentration in mass/mass
N	Nitrogen

Na	Sodium
Na ⁺	Sodium ions
NaOH	Sodium hydroxide
Na ₂ O	Sodium oxide
N ₂ O	Nitrous oxide
N/A	Not applicable
n.a	Not available
n.d	Not determined
NH ₃	Free ammonia
NH ₄ ⁺	Ammonium ions
Ni	Nickel
N ₂	Nitrogen gas
OH ⁻	Hydroxide ions
O ₂	Oxygen gas
P	Phosphorus
PAH	Polycyclic aromatic hydrocarbons
Pb	Lead
PM _{2.5}	Particulate matters
PTEs	Potentially toxic elements
PTFE	Polytetrafluoroethylene
RSD	Relative standard deviation
rRNA	ribosomal ribonucleic acid
S	Sulphur
SDG	Sustainable development goals
Si	Silica
Se	Selenium
SEM	Scanning electron microscopy
SiO ₂	Silica oxides
SLR	Solid to liquid ratio
SO ₄ ²⁻	Sulphate
SO ₂	Sulphur dioxide
STP	Standard temperature and pressure
TC	Total carbon
TCA	Tricarboxylic acid
TCD	Thermal conductivity detector
TCI	Total crystallinity index
TEs	Trace elements

TiO ₂	Titanium oxide
TN	Total nitrogen
TS	Total solids
UC	Unburned carbon
UN	United Nations
VFAs	Volatile fatty acids
VS	Volatile solids
v/v	Volume/volume
W	Tungsten
w/v	Weight/volume
XRF	X-ray Fluorescence
Zn	Zinc

1. Introduction

1.1 Research background

The issue of environmental pollution is becoming more pronounced due to rapid economic development and population growth, factors which have driven the expansion of agricultural practices and food production to support growing populations (Odejobi et al., 2023). The increase in food production has resulted in the generation of large quantities of lignocellulosic agricultural residues (LARs), which represent the largest organic carbon waste stream globally (Kukreti et al., 2024). Lignocellulosic agricultural residues are non-edible plant parts that remain in the field after crop harvesting, that include rice straw, wheat straw, sugarcane bagasse, and corn stover. Associated with this rapid economic development has been the increased demand for renewable energy sources to combat the global impacts of climate change resulting from the global consumption of fossil fuels to support industrial and economic development. The total energy consumption globally has been projected to rise from approximately 633 billion GigaJoules (GJ) in 2017 to 780 billion GJ in 2040 (EIA, 2018). In 2020, fossil fuels still supplied 80% of the total global energy demand (IEA, 2021). The combustion of fossil fuel accounted for 33,622 million tonnes (Mt) CO₂ emissions globally in 2020 (IEA, 2021), raising critical concerns about their impact on climate change and highlighting the urgent need to reduce carbon emissions and transition to renewable energy sources (Cai et al., 2021; Karthikeyan et al., 2024).

In many regions, LARs are often disposed of through open burning, which has long been considered as the simplest and most cost-effective method to decrease their volume in the field (CEC, 2014). This approach, however, is an unsustainable practice with multidimensional negative impacts (Bandgar et al., 2022; Rahmani et al., 2022; Sawasdee et al., 2023). This practice adversely affects air quality, contributes to greenhouse gas (GHG) emissions and poses serious health risks, such as respiratory problems, particularly for those living near open burning sites (Irwin, 2015; Jakhar et al., 2023; Ravindra et al., 2019). Open burning one tonne of rice straw, for example, would emit approximately 93 kg of carbon monoxide (CO), 0.48 kg of nitrous oxide (N₂O), 5.6 kg of hydrocarbons, 8.3 kg of particulate matters (PM_{2.5}), and 0.73 kg of black carbon (BC) (Cao et al., 2006; Chandra et al., 2012a; Nguyen et al., 2016). Moreover, open burning LARs also leads to dioxin emissions, which can be formed during combustion in the presence of chlorinated pesticides, and these emissions are particularly concerning, as even trace amounts of dioxins are persistent in the environment (CEC, 2014). LARs, on the other hand, hold significant potential to be managed more sustainably as a

renewable energy source (Cai et al., 2021; Bandgar et al., 2022; Li and Huang, 2024), offering a solution to mitigate the issues associated with open burning and to improve long-term energy security.

Lignocellulosic agricultural residues possess a high intrinsic energy content, estimated at equivalent to 2.8–5.5 kilowatt hour (kWh) kg^{-1} (Chandra et al., 2012a), compared to 8.5 kWh kg^{-1} for hard coal (Voegeli et al., 2014). Given a typical biogas calorific value is 6–6.5 kWh m^3 , 1 m^3 biogas (55–70% CH_4 content) is equivalent to 1.2–2.4 kg of LARs (Voegeli et al., 2014), therefore LARs are a valuable feedstock for anaerobic digestion (AD). Anaerobic digestion has several advantages and offers lower environmental impacts and energy input requirements compared to alternative energy conversion technologies, such as incineration, gasification and pyrolysis (Chandra et al., 2012b; Kainthola et al., 2020; Karthikeyan et al., 2024). While AD has been practised for decades in developed countries for clean energy production, it is also applicable to developing countries (Voegeli et al., 2014; Egwu et al., 2021; Ibarra-Esparza et al., 2023). AD can effectively valorised various organic feedstocks into high-calorific value biogas, rich in methane (CH_4), which is a critical renewable energy source (Rahmani et al., 2022). In developing countries, biogas is typically used at the household or small-scale level, while in developed countries, the primary application of biogas is for large-scale commercial electricity generation in a combined heat and power (CHP) plant (Ibarra-Esparza et al., 2023). Additionally, AD produces stabilised digestate rich in nutrients, suitable for use as agricultural fertiliser, thus supporting the circular economy through nutrient recycling (Ferdeş et al., 2023). Within the circular economy framework, converting LARs into biogas and recycling nutrients are vital strategies for decoupling economic growth from reliance on primary resource extraction (Novakovic et al., 2021).

Despite the potential for conversion of LARs to biogas production in an AD process, biogas production is often hindered by the recalcitrant characteristics of these residues, due to their structural complexity of the material, which includes cellulose, hemicellulose and lignin (Zhong et al., 2011; Mancini et al., 2018a; Kainthola et al., 2020; Karthikeyan et al., 2024). Indeed, the cellulose and hemicellulose components of LARs can potentially serve as a carbon source for anaerobic bacteria, however, their utilisation is compromised by the lignin matrix, which shields these substrates from microbial attack, representing the major barrier to the biodegradation of LARs (Antonopoulou and Gavala, 2015; Tsegaye et al., 2019). Pretreatment is therefore a main requirement for the efficient biological conversion of LARs to biogas (Novakovic et al., 2021; Peyrelasse et al., 2021). Many chemicals have been studied as pretreatment agents for chemical pretreatments, and alkali is the most popular chemical

agents because it is highly effectiveness in removing lignin (Tsegaye et al., 2019; Woiciechowski et al., 2020; Novakovic et al., 2021).

The increased cost of chemicals used for pretreatment, however, presents a challenge that can diminish the economic viability of AD operation, particularly in a developing country setting, where the economic viability of a process plant is likely to be independent of fiscal incentives that may be limited or non-existent (Egwu et al., 2021). To realise the economic feasibility of AD for LARs, especially for the application in the developing countries, it is essential to replace these expensive chemicals with locally available, low-cost alternatives materials that possess similar properties. Coal fly ash (CFA), for example, has readily available alkali components (Ahmaruzzaman, 2010; Achternbosch et al., 2015; Zierold and Odoh, 2020) and appears to be suitable candidate for this task. The annual global coal consumption was approximately 8,038 Mt in 2023 (IEA, 2023), equal to 500 Mt CFA produced (Lanzerstorfer, 2018). Some of the issues associated with CFA include the risk of leaching toxic elements and the substantial land area required for its disposal (Jain and Dwivedi, 2014; Lanzerstorfer, 2018).

Coal fly ash (CFA), however, may offer supplementary benefits to the AD process by supplying essential trace elements (TEs) necessary for the growth of anaerobic microorganisms. Its unique intrinsic properties may also create other opportunities for biogas stimulation, potentially enhancing microbial interactions through mechanism such as direct interspecies electron transfer (DIET)), similar to the effects observed with carbon-based material like activated carbon and biochar due to their conductive properties (Chen et al., 2014; C. Wang et al., 2018; S. Xu et al., 2018; König et al., 2022).

Alkali-treated LARs may contain residual alkali, which can be beneficial in buffering the AD process by controlling the decrease in pH that may occur during the acidogenesis phase of AD (Dell'Omo and La Froschia, 2018; Mansour et al., 2024). The alkali pretreatment, however, requires a high pH value of 11–12 to be achieved to break down the lignin structure (Sun et al., 2015). Such high pH conditions in the feedstock fall significantly outside the optimum range for AD (pH 5.5–6.5), thus, pH adjustment is necessary to maintain conditions within the desired range pH. This additional requirement for chemical adjustment by acid addition not only has significant implications for the overall operational costs of the AD process, but also conflicts with the circular economy principle of minimising resource use.

Food waste (FW) represents a significant global waste stream, with approximately 570 Mt generated during the consumption stage (FAO, 2021). It can rapidly acidify due to the

production of volatile fatty acids (VFAs) (Lü et al., 2015; Nilsson Påledal et al., 2018) and is considered potentially a suitable alternative waste to commercial chemical mineral acids such as hydrochloric acid (HCl) for neutralising the high pH alkali-treated of LARs. Integration of this waste stream not only contributes to the additional biogas output but also supports a circular economy by effectively utilising FW within the AD process and helps divert the waste from disposal to landfills, which remains the primary disposal method for this waste in developing countries.

The valorisation of LARs utilising CFA for pretreatment and FW for AD neutralisation could offer significant economic value and various environmental benefits. This substitution may greatly enhance the AD process performance and achieve greater economic returns, including profits for AD operators and the provision of local renewable energy resources for communities. Furthermore, environmental benefits that can result include mitigating climate change, reducing the direct impacts of open burning, reducing fertiliser consumption and supporting soil fertility, and minimising the impacts of uncontrolled waste disposal. This approach also has the potential to create sustainable benefits in developing countries, where current CFA and FW management practices can be problematic and pose serious environmental threats.

Exploitation of the task-specific properties of CFA, serving as an alkali pretreatment agent, and FW as a single acid source, to optimise biogas production from LARs in AD, is investigated for the first time in this research. Assessments of the properties and behaviour of this dual waste system to deliver potential synergistic benefits in the treatment of a third waste (LARs) in an integrated AD (LARs-CFA-FW) system to improve sustainability and efficiency of biogas production is explored.

1.2 Research aims and objectives

It is hypothesised that CFA with its alkalinity properties can serve as an alkali pretreatment agent, while FW can act as a single acid source for neutralising pretreated LARs prior to AD. This study investigates, for the first time, the properties and interactions of this dual waste system (CFA-FW) to deliver potential synergistic benefits in the treatment of a third waste (LARs) in an integrated AD system (LARs-CFA-FW), aiming to enhance sustainability and improve biogas production efficiency.

To test this hypothesis the aim of this research is to optimise the AD process for the integrated treatment of LAR by developing a dual waste system incorporating CFA and FW, to enhance

the biodegradability of LAR, to promote circular economy principles, maximise LAR as a renewable energy source, minimise chemical resource use and reduce waste outputs.

The underlying objectives in support of this overall aim (using barley straw (BS) as the representative sample for LAR) are to assess (i) the effectiveness of CFA as an alkali pretreatment substitute for inorganic alkali, (ii) the effectiveness of FW as an acid neutralising substitute for inorganic mineral acids, (iii) the supplemental benefits of the inherent physicochemical properties of CFA to the AD of LAR and biogas production, (iv) the performance of the integrated dual waste system as a process of the AD of LAR for CH₄ production, (v) to understand microbial community dynamics and identify synergistic microbial interactions in response to substrate characteristics in an integrated and optimised AD process.

The task specific objectives to achieve the overall aim are to:

1. Establish the baseline performance of alkali pretreatment and acid neutralisation steps for AD treatment of BS with commercial industrial chemicals.
2. Determine the effectiveness of alkali and CFA pretreatment on BS biodegradability determined from the changes in lignocellulosic fractions (cellulose, hemicellulose, and lignin) as indicators.
3. Establish the correlation between the extent of BS biodegradability following CFA pretreatment and CH₄ yield.
4. Determine the effectiveness of acidified, fermented FW as a neutralising agent for alkali-pretreated BS for feedstock pH control and establish the optimum addition rate to maximise the AD process.
5. Assess the extent to which TEs within CFA can facilitate/enhance the AD of BS process through comparison of the TE levels of CFA and the AD inoculum with threshold data.
6. Investigate the conductive properties of CFA and their impacts on the community structure of microbial populations
7. Quantify the combined effect of the integrated CFA, FW and BS waste AD system on CH₄ yield.
8. Propose the mechanism underpinning the microbial behaviour contributing to the enhanced CH₄ yield in the integrated AD process.

A schematic illustrating the aim and objectives of the research is presented in Figure 1.1.

Aim: to optimise the AD process for the integrated treatment of LAR by developing a dual waste system incorporating CFA and FW, to enhance the biodegradability of LAR, to promote circular economy principles, maximise LAR as a renewable energy source, minimise chemical resource use and reduce waste outputs.

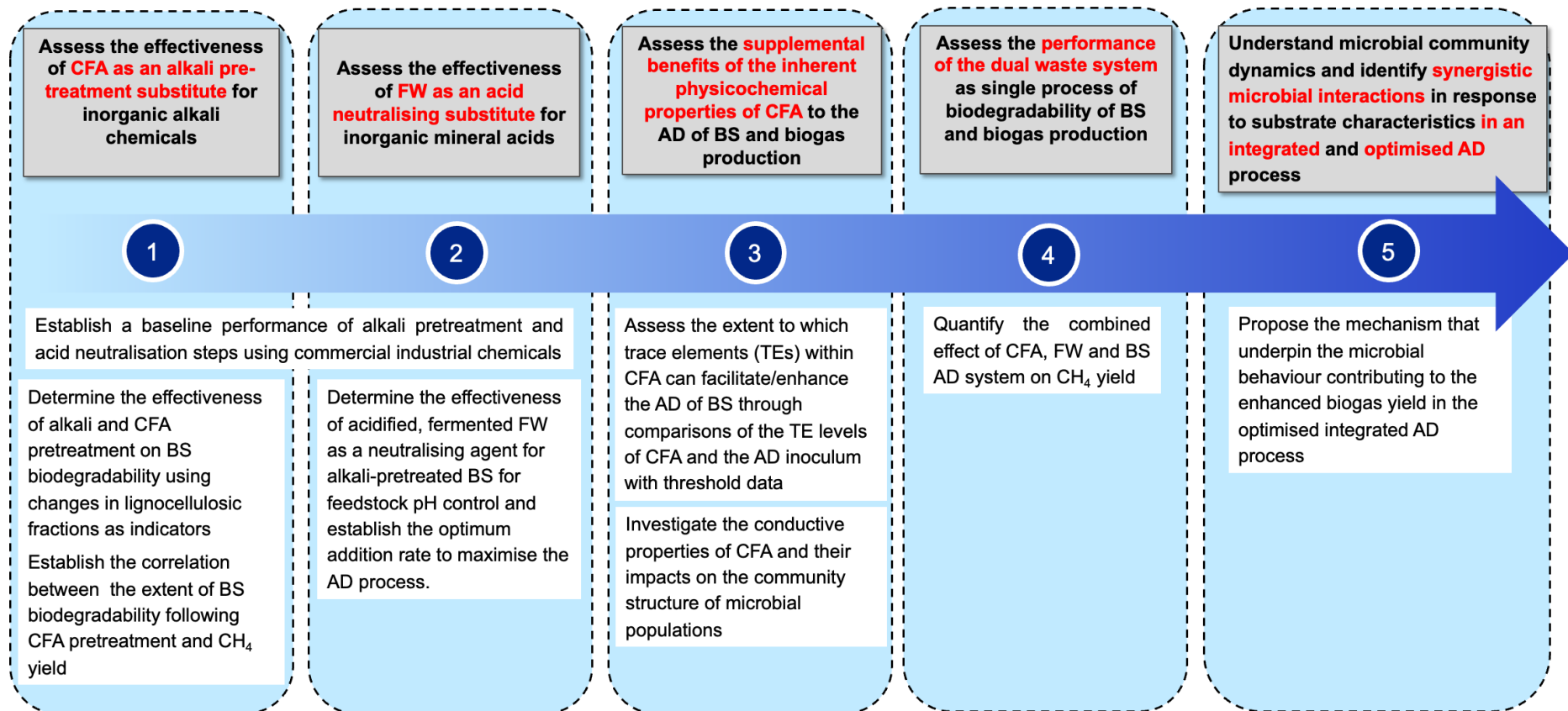


Figure 1.1 A schematic providing an overview of the research aim and objectives against the defined task specific objectives

1.3 Thesis structure

Following this introductory chapter, Chapter 1, which sets out the rationale for the research and defines the underlying aim objectives of the research, Chapter 2 presents a critical review of the literature relevant to the study. Chapter 3 describes the materials and methods used throughout the research, that include sourcing and preparation of waste feedstocks, design of the AD experimental test procedures, analytical methods used, statistical methods deployed, as well as a summary of the physicochemical properties determined for the waste feedstock. The experimental results for each phase of research are presented in Chapter 4 through 8 and Figure 1.2 provides an illustration of the experimental, developmental pathway showing the interconnectivity between the different research phases. Specifically, Chapter 4 presents results related to the effects of alkali pretreatment on fibre composition, chemical functional groups and surface morphology of BS. The focus in Chapter 5 and 6 is on the CH₄ yield in response to different alkali chemicals and CFA pretreatment monitored through solid-state AD experiments. In Chapter 7, the effectiveness of fermented FW for neutralising BS and optimising AD was assessed and the combined effect of the three wastes CFA, FW and BS in the AD system was quantified. The primary objective of Chapter 8 was to understand the potential additional benefits of CFA and the fundamental microbial mechanism that contributes to an optimised integrated AD system, extending beyond the alkalinity contribution of CFA. A general discussion is provided in Chapter 9 that draws on the findings from the experimental chapters and sets these findings in the context of current literature to demonstrate the contribution to knowledge derived from this research. Finally, conclusions and recommendations for future research are presented in Chapter 10.

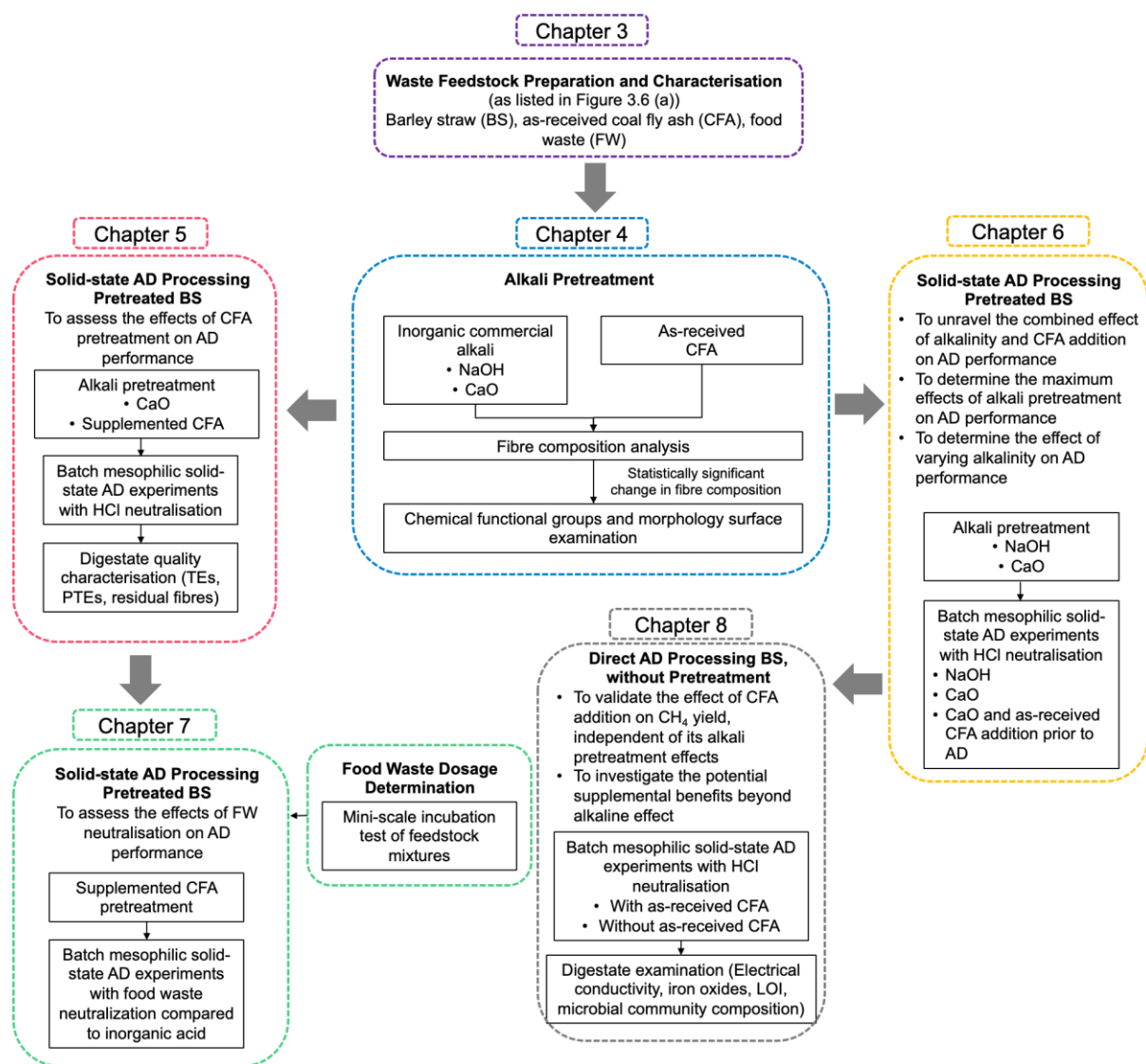


Figure 1.2 Experimental framework and research phases illustrating the research flow and connections between key concepts.

2. Literature Review

2.1 An overview of lignocellulosic agricultural residues

2.1.1 Global production and management of lignocellulosic agricultural residues

Between 2010 and 2020, the average annual global production of lignocellulosic agricultural residues (LARs) was approximately 21 billion tonnes¹, representing a substantial biomass resource (Gao et al., 2022). Figure 2.1 presents the global distribution of rice straw, sugarcane bagasse, corn stover, wheat straw and barley straw, for the year 2018.

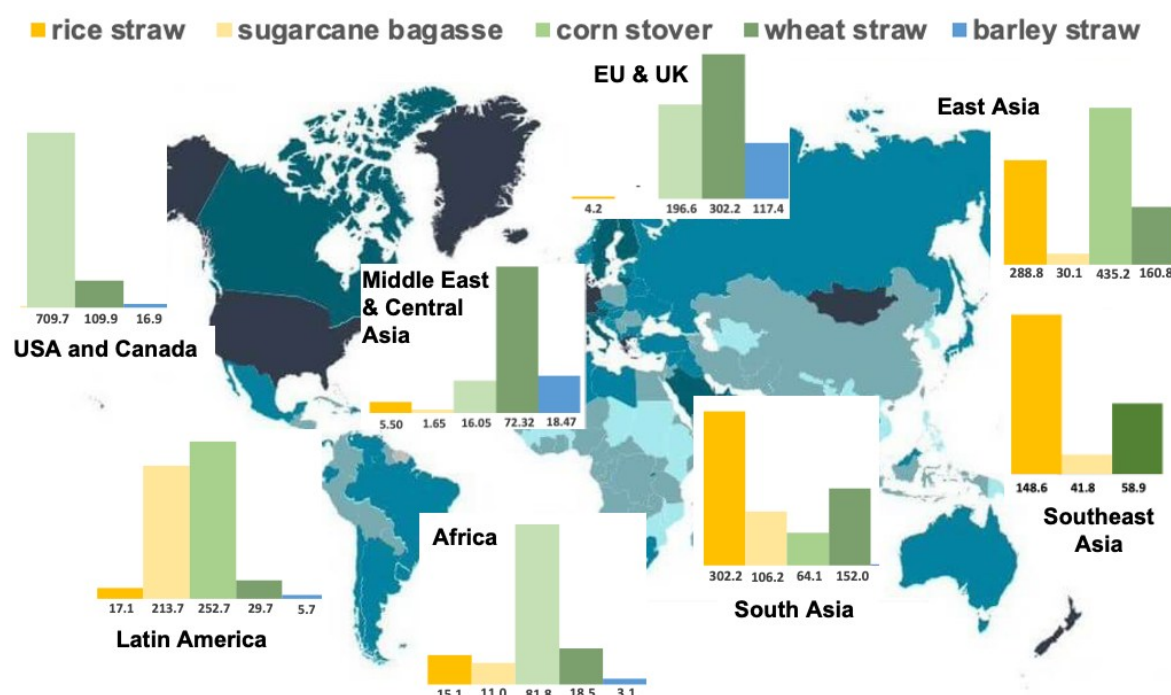


Figure 2.1 Annual global lignocellulosic agricultural residues by region (in Million tonnes) (FAO, 2014; USDA, 2019). The countries included in each region are as follows: (i) East Asia (China and Japan), (ii) South Asia (India, Pakistan, Nepal, Sri Lanka, Bangladesh), (iii) Southeast Asia (Indonesia, Philippines, Vietnam, Thailand, Cambodia), (iv) Middle East and Central Asia (Kazakhstan, Uzbekistan, Turkey, Iran and Iraq), (v) USA and Canada (USA, Canada), (vi) EU and UK (EU, Ukraine, Russia, UK), (vii) Africa (South Africa, Nigeria, Ethiopia, Egypt, Malawi, Uganda), (viii) Latin America (Brazil, Argentina, Bolivia, Chile, Mexico).

In 2018, China was the largest rice producer globally, generating approximately 275 million tonnes (Mt) of rice straw in 2018, according to USDA, (2019), followed by India, with approximately 209.2 Mt. Bangladesh and Indonesia ranked third and fourth, producing approximately 66 Mt and 53 Mt, respectively. Brazil led sugarcane bagasse production, generating approximately 192 Mt, with India and China producing 87 Mt and 30 Mt,

¹ LARs include residues from rice, barley, corn, wheat, sorghum, oat, beans, tubers, cotton, fibre crops, and sugarcane.

respectively. The US was the world largest corn producer, generating around 685 Mt of corn stover, followed by China with 435 Mt and Brazil with 142 Mt. The top wheat producer was EU, resulting in approximately 178 Mt wheat straw, with China and India also significant producers, generating 160 and 116 Mt, respectively. Barley straw production is dominated by EU countries, contributing approximately 75 Mt, followed by Russia with 22 Mt (USDA, 2019).

Lignocellulosic agricultural residues have low nutritional value, leading to only limited use as cattle feed, while the majority is often burned in fields or sent to landfills (Gao et al., 2022). The adverse effects of open burning on climate change, air quality and health were discussed in Section 1.1. The top 10 countries globally engaging in open burning of LARs, reported in 2022 were China, India, the USA, Brazil, Russia, Indonesia, Argentina, Nigeria, Ukraine and Thailand (Gao et al., 2022). Open burning also depletes the soil humus, reducing soil fertility and increasing dependence on costly artificial fertilisers (CCACoalition, 2015; Jakhar et al., 2023). Furthermore, it can cause soil contamination due to incomplete combustion, which may generate hazardous substances such as polycyclic aromatic hydrocarbons (PAH) (Jakhar et al., 2023). Polycyclic aromatic hydrocarbons are problematic because of their carcinogenic and genotoxic effects, persistence in the environment, and tendency to adsorb onto soil (Jakhar et al., 2023), potentially allowing these hazardous substances to transfer to food crops grown in affected soil.

2.1.2 Lignocellulosic agricultural residues production and management in Indonesia

Indonesia has substantial biomass resources that offer considerable potential for enhancing its energy supply. In 2015, the total biomass potential from the forestry, agriculture and plantation sectors in the country was estimated at approximately 27,586 Megawatt electric (MWe) (MEMR, 2015), a figure expected to increase in subsequent years. Of this total energy potential, LARs could contribute up to 44% (MEMR, 2015). By 2023, Indonesia produced a total of 145.5 Mt of LARs including rice straw, rice husk, corn stover, corn cob, sugarcane bagasse, and empty palm fruit bunches (USDA, 2024), which equates to an energy potential of approximately 5,371 MWe².

Unlike other LAR sources that are widely distributed across regions globally, empty palm fruit bunch is a specific type of LARs predominantly generated in Southeast Asia (Lee et al., 2020), with Indonesia being the largest producer worldwide. Rice straw, however, represents a major LAR produced in the country (USDA, 2024). Compared to other Southeast Asian countries,

² Calorific values (1,200–3,500 kcal kg⁻¹) and moisture content (12–50%) vary depending on the LAR sources (MEMR, 2015). A 26% efficiency for heat conversion to electricity was assumed (Pasek et al., 2013).

Indonesia stands as the largest producer of LARs in the region (USDA, 2024). Figure 2.2 provides a comparison of Indonesia's LAR production levels for Indonesia against those of other countries in the region.

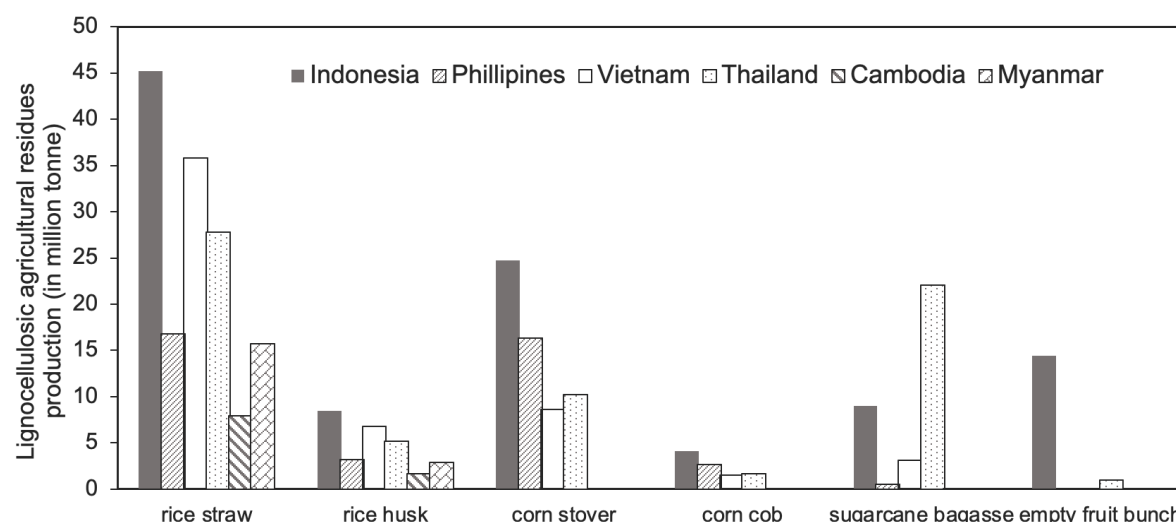


Figure 2.2 The production of lignocellulosic agricultural residues in the Southeast Asia region in 2023 (FAO, 2014; USDA, 2024).

Despite the potential and abundance of LARs, the utilisation rate of these residues, for example rice straw in Indonesia, remains low and offers scope further leverage. Currently, rice straw is primarily used as cattle feed, compost, and as a raw material for the pulp and paper industry, which accounts for 38% used of the total produced, while the remaining 62% underutilised and disposed of by open burning in the field (Purwandaru, 2013; Setiarto, 2016; Sari et al., 2021).

2.1.3 Composition and properties of lignocellulosic agricultural residues

2.1.3.1 General properties

Lignocellulosic agricultural residues are composed of 80–90% organic material, primarily consisting of cellulose, hemicellulose, and lignin (Harmsen and Bakker, 2013; Mansour et al., 2024). Cellulose is a homopolysaccharides made up of insoluble fibres of β -1,4-glucan, while hemicellulose is a heteropolysaccharides comprising non-cellulosic polysaccharides such as xylan, arabinan, mannan and glucan. Lignin, a complex polyphenolic structure, is composed of three main monomers. Minor components in LARs include proteins, oils, pectin, sugars, nitrogenous materials, chlorophyll, and ashes (Izydorczyk et al., 2024). The properties of each lignocellulosic or fibre component are detailed below.

Lignocellulosic agricultural residues typically have a low nitrogen (N) and high carbon (C) content, resulting in a high carbon-to nitrogen (C/N) ratio that can range from 51.8 to 145

(Wang et al., 2014; Yuan et al., 2014; Ajayi-Banji et al., 2020; Meyer et al., 2022). The typical N content in LARs is in the range 0.2–0.8% on a total solids (TS) basis, while the C content range is 38.45–50.5% TS (Saidur et al., 2011). The high level of organic matter in LARs is indicated by the volatile solids (VS) content, which varies between 82.5 and 90% TS (Chandra et al., 2012b; Candia-García et al., 2018), with TS varying between 89.5 and 96.1% (Ferreira et al., 2014; Mustafa et al., 2016; Kainthola et al., 2020).

2.1.3.2 Cellulose, hemicellulose, and lignin

The composition of cellulose, hemicellulose and lignin in LARs, collectively referred to as fibre in this study, varies depending on the LAR sources. Table 2.1 shows the variation in fibre composition across different LAR sources. This variability can be attributed to differences in source, variety, growth conditions and maturity levels (Van Dyk and Pletschke, 2012; Sawatdeenarunat et al., 2015; Jönsson and Martín, 2016; Izydorczyk et al., 2024).

Table 2.1 Composition of cellulose, hemicellulose and lignin (on a total solid basis) of various lignocellulosic agricultural residues (LARs) (drawn from the literature).

LAR Sources	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Barley straw	33.8–43	19.0–33.0	9.0–20.7
Corn cobs	45.0	35.0	15.0
Corn stover	35.0–41.0	16.0–30.0	10.3–17.0
Cotton stalk	31.0	11.0	30.0
Empty palm fruit bunch	40.1	23.2	15.6
Oat straw	31.0–35.0	20.0–26.0	10.0–15.0
Rice straw	38.9–44.3	24.0–33.5	5.6–20.4
Rice husk	34.4–38.3	17.4–29.3	18.3–19.2
Rye straw	38.0	36.9	17.6
Sorghum straw	32.0–35.0	24.0–27.0	15.0–21.0
Sugarcane bagasse	33.0–45.0	20.0–30.0	15.0–30.0
Sunflower stalk	31.0	15.6	29.2
Wheat straw	30.0–39.2	25.6–50.0	12.0–23.0

Sources from (Rosgaard, 2007; Chandra et al., 2012b; Adebayo and Martínez-Carrera, 2015; Sawatdeenarunat et al., 2015; Intasit et al., 2019; Meyer et al., 2022).

Cellulose typically dominates the cell wall composition of LARs, accounting for 30–60% TS of the LARs (Saidur et al., 2011; Adebayo and Martínez-Carrera, 2015; Harmsen and Bakker, 2013). Cellulose contains two primary types of linkage: (i) β - (1 $\rightarrow$ 4) - glycosidic bonds, which are covalent bonds connecting β - glucopyranose units, and (ii) hydrogen bonding, which interconnect cellulose chains to form fibrils (Harmsen and Bakker, 2013; Laftah and Wan Abdul Rahman, 2016). The latter bonding contributes to the rigid and resistant nature of

cellulose crystalline fibrous structures to hydrolysis (Harmsen and Bakker, 2013). Cellulose in LARs is present in both crystalline and amorphous forms with the amorphous cellulose being more amenable to enzymatic degradation (Kumar et al., 2009; Mittal et al., 2011).

Hemicellulose makes up a smaller fraction than cellulose, typically comprising about 20–40% TS of LARs. It is a branched and heterogenous polymer, with xylose being the primary glucose unit in hemicellulose within LARs. Its amorphous structure and shorter chain length compared to cellulose renders hemicellulose more susceptible to enzymatic hydrolysis (Harmsen and Bakker, 2013; Woiciechowski et al., 2020). The interaction between the hemicellulose and cellulose and/or lignin, however, limits the biodegradability of hemicellulose (Martins et al., 2022). Moreover, this interaction obstructs enzymes accessibility to cellulose, as hemicellulose covers cellulose fibrils. It has been reported that at least of 50% of hemicellulose should be dissolved or removed to facilitate access to cellulose (Lee et al., 2014).

Lignin constitutes 15–25% TS of LAR and is a complex aromatic polymer synthesised from phenylpropanoid units (e.g., syringil, guaiacyl, and p-hydroxyphenil). Lignin in plant tissue does not exist as an independent polymer, but it is bonded with other polymers, (cellulose and hemicellulose) forming complexes (Buranov and Mazza, 2008). Lignin has multiple functional groups, with the methoxyl group being the dominant (Li et al., 2020). Lignin serves as the primary component limiting the hydrolysis of LAR in AD, as it encapsulates the entire carbohydrate structure and reduces the surface area available for enzymatic hydrolysis (Van Dyk and Pletschke, 2012; Sambusiti et al., 2013; Dai et al., 2018; Woiciechowski et al., 2020). Sewalt et al. (1997) found that the activity of cellulases, the enzymes responsible for cellulose hydrolysis, were inhibited by up to 60% in the presence of elevated lignin concentrations. Figure 2.3 illustrates the chemical structure of cellulose, hemicellulose and lignin chains.

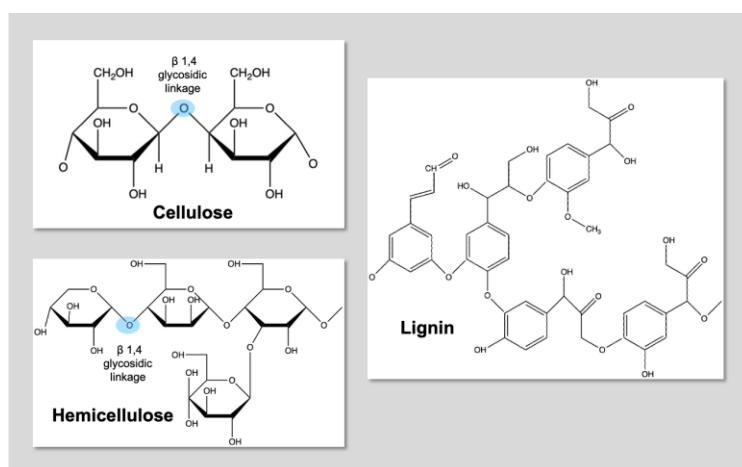


Figure 2.3 Chemical structures of cellulose, hemicellulose and lignin chains. Adopted from Amin et al., (2017).

A summary of the differences in key physicochemical features of cellulose, hemicellulose and lignin is provided in Table 2.2.

Table 2.2 Summary of differences in key physicochemical features of cellulose, hemicellulose and lignin.

	Cellulose	Hemicellulose	Lignin
Molecule structure	Contains only one sugar unit: D-glucose subunits through β -(1 $\rightarrow$ 4) glycosidic bonds	Contains several different sugar units (pentoses, hexoses ^a)	Contains phenyl-propane units ^b , methoxy groups, and noncarbohydrate polyphenolic substance
Number of glucose molecules per polymer	7,000–15,000	500–3,000	N/A
Polymer structure	Linear	Branched	Branched
Molecular weight	High (around 100,000)	Low (<30,000)	High
Dominant chemical bonds	Hydrogen	Ester	Ether and carbon-carbon
Resistance	Resistant to strong alkali, easily hydrolysed by acids	Soluble in alkali, easily hydrolysed in acids	Soluble in hot alkali, not hydrolysed by acids
Region	Two-thirds arranges in a crystalline and minor part of amorphous region	Amorphous	Amorphous
Degree of polymerisation	10–100 times higher than hemicellulose	80–200	N/A
Interaction with water	Hydrophobic and hydrophilic	Hydrophilic	Hydrophobic

^a pentoses: xylose and arabinose, hexoses: glucose and galactose, ^b p-coumaryl, coniferyl and sinapyl alcohol, N/A: not applicable. Sources from (Sun et al., 2004; Hendriks and Zeeman, 2009; Tumuluru et al., 2011; Adebayo and Martínez-Carrera, 2015; Harmsen and Bakker, 2013; Laftah and Wan Abdul Rahman, 2016).

2.1.3.3 Interconnectivity of lignocellulosic components

Several cellulose molecules form strong bonds through both intermolecular and intramolecular hydrogen bonding as well as van der Waals forces, creating cellulose microfibrils or bundles

(Leschine, 1995). These microfibrils are embedded in a matrix of other polymers (e.g., hemicellulose), and are connected by hydrogen bonding, contributing to the rigidity of the cell wall's structural backbone (Hendriks and Zeeman, 2009; Geboers et al., 2011). The space between cellulose and hemicellulose is filled with lignin, which linked through hydrogen and covalent bonding, forms a hydrophobic 3-dimensional structure of lignin-carbohydrate complexes (Lee et al., 2014; Ma et al., 2019). These complexes provide mechanical support to plant tissues and enhance resistance against insects, pathogens, and chemical degradation, making lignin the primary factor contributing to LAR recalcitrance (Woiciechowski et al., 2020). In addition, the esterified arabinofuranosyl groups within hemicellulose by aromatic acids, such as ferulic and *p*-coumaric acid, serve as cross-linkages between lignin and hemicellulose (Leschine, 1995).

In lignin-carbohydrate complexes (LCC), three main types of bonds have been identified: benzyl ester, benzyl ether, and glycosidic bonds. The reactivity of these bonds varies considerably depending on their interactions with different environments. In alkaline media, for example, ester bonds are easily hydrolysed, while benzyl ether bonds are more stable. Ester bonds are typically observed between lignin and polysaccharides (Sasmal and Mohanty, 2019); and an example of such is the ester bond connecting hemicellulose and lignin, known as ferulic acid (Faulds et al., 2008) (Figure 2.4).

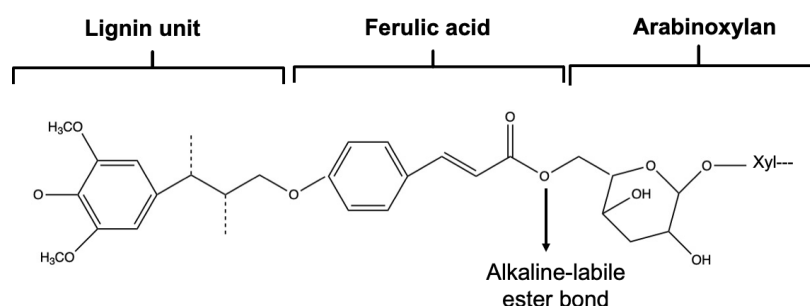


Figure 2.4 Structural linkage of lignin and hemicellulose via ester bond. Figure adopted from (Buranov and Mazza, 2008).

2.2 Circular economy and sustainability

2.2.1 Lignocellulosic agricultural residues circular economy

The principle of circular economy has been established as the transformation from the traditional linear economy principle to promote sustainability. Linear economic models, which based on extraction, production, consumption and waste generation are inherently conflicting with sustainable development, as linear economic approach leads to resource depletion, environmental quality deterioration, and high waste generation (Velenturf and Purnell, 2021).

This transformation has led to a shift in mindset from a system focused on “end of life disposal” to one aimed at “zero waste through total recycling” (Pan et al., 2021).

The current improper LARs management through open burning, inefficient use of LARs and the resulting increase in air pollution does not synergise with the principles of a circular economy, which prioritise waste prevention, reuse and recycling. The growing focus on sustainable agriculture and optimal resource utilisation, in synergy with the circular economy, has sparked interest in LARs as a valuable source for producing clean, sustainable bioenergy (Mujtaba et al., 2023). Circular economy-based LARs management not only creates opportunities to convert waste into valuable resources, but also sustain LAR materials through continuous circulation and eliminate harmful environmental and health impacts. Additionally, adopting this approach helps to reduce dependence on non-renewable resources and mitigate the environmental impacts associated with fossil fuel exploitation and combustion, which pose a huge threat to the environment, and benefits societies by providing renewable energy (Mujtaba et al., 2023).

2.2.2 Sustainable development goals (SDGs)

United Nations (UN) has set a blueprint with a total of 17 SDG with the aim to create a balance between the economic, environmental and social well-being for long-term sustainable future (Bansod et al., 2024). Some of these goals were established as responses to energy crisis, resource depletion and environmental quality deterioration (Pan et al., 2021). Circular economy is at the heart of achieving the SDG, as it promotes sustainable production and consumption through reduce-reuse-recycle practices, develops processes that keep resources in circulation and less dependency on non-renewable raw materials (Velenturf and Purnell, 2021; Izydorczyk et al., 2024). The conversion of renewable biological resources like LARs to renewable energy promotes the reuse of these residues to produce clean energy, combat climate change, and reduce both GHG emissions and fossil fuel consumption, and in doing so therefore support SDG 13 (Climate action) (Pan et al., 2021).

2.2.3 Lignocellulosic agricultural residues as alternative energy source in Indonesia

Lignocellulosic agricultural residues are known as a second-generation biofuel and are seen as a promising alternative to both fossil fuels and first-generation biofuels produced from food crops (e.g., maize). Second-generation biofuels offer several key advantages over first-generation biofuels. They are produced from non-food biomass, therefore do not compete with food crops. Cultivating crops solely for biofuel production may be unnecessary, as LARs are abundantly available, eliminating the need for additional agricultural land (Vögeli et al., 2014;

Gallegos et al., 2017). The use of LARs as second-generation biofuels utilises materials that would otherwise be discarded or left as valuable resources untapped.

Lignocellulosic agricultural residues represent abundant and low-cost sources of organic material with significant potential for conversion into various forms of biofuel, including biogas or CH₄, which can be produced through a biochemical pathway such as AD (Izydorczyk et al., 2024). In addition to their high organic content, the high availability of LARs ensures a continuous supply of biomass for biogas production. Incorporating LARs into national energy strategies can significantly support countries in achieving their sustainability targets.

Indonesia, for example, stands as the leading energy consumer in Southeast Asia yet faces a pivotal moment in its energy trajectory amidst mounting concerns over climate change, natural resource depletion, and an overreliance on unsustainable fossil fuels. In response to these challenges, the nation has set a target that at least 23% of its energy mix will be sourced from renewable sources by 2025 and 31% by 2050, respectively (MEMR, 2017). By 2023, Indonesia had transitioned 13% of its total energy supply to renewable sources, with biomass contributing 3% to the overall share of renewable energy sources (MEMR, 2023). Specifically, the National Energy General Plan or RUEN 2025 outlines a target of producing 489.8 million m³ of biogas by 2025. As of 2022, however, the actual biogas production was 45.4 million m³, revealing a significant shortfall of 444.4 million m³ that must be addressed between 2023 and 2025 (MEMR, 2022). The current biogas production strategy focuses in utilising palm oil residues, tapioca, manure, and municipal solid waste (MEMR, 2015), however, the biogas potential from LARs has yet to be fully explored and integrated into this strategy.

2.3 Biogas production from lignocellulosic agricultural residues through anaerobic digestion

2.3.1 Overview

Anaerobic digestion is a widely employed waste-to-energy technology used to convert various organic waste streams including LARs, food waste, the organic fraction of municipal solid waste, algal biomass, energy crops, sewage sludge, animal manure, into renewable bioenergy across both developed and developing countries (Chandra et al., 2012a; Voegeli et al., 2014; Ibarra-Esparza et al., 2023; Mansour et al., 2024). While AD as a waste management strategy has been implemented in developed countries for several decades, its adoption is increasingly expanding to developing countries (Ibarra-Esparza et al., 2023).

Lignocellulosic agricultural residues exhibit a large potential as a substrate in AD for generating biogas due to their high organic C content (see Section 2.1.3.1). The CH₄ potential from different sources of LARs is presented in Table 2.3.

Table 2.3 Methane potential of selected lignocellulosic agricultural residues

Lignocellulosic Agricultural Residues Source	CH ₄ Potential ^a
Corn stover	130–360 ^b
Wheat straw	120–378
Sugarcane bagasse	60–278
Rice straw	140–350 ^b
Rice husk	240
Barley straw	225–240
Empty palm fruit bunch	91
Sunflower stalks	192–297
Maize stalks	267–338
Sorghum	275–319
Ley crop silage	115

^a Value in mL g⁻¹ VS, unless stated otherwise, ^b Value in mL g⁻¹ TS. Sources from (Kaparaju et al., 2009; Brown et al., 2012; Chandra et al., 2012b; Lindmark et al., 2012; Menardo et al., 2012; Monlau et al., 2012; Sambusiti et al., 2013; Sawatdeenarunat et al., 2015; Mancini et al., 2018a; Fjörtøft et al., 2019; Lee et al., 2020; Rocamora et al., 2020).

Utilising LARs as substrates in AD offers multiple benefits, which can be summarised as follows: (a) mitigating climate change by reducing fossil fuel usage, (b) gradually transitioning from fossil fuels to renewable energy, contributing to national renewable energy targets, (c) alleviating health and air pollution impacts caused by the open burning of LARs, (d) creating a carbon-neutral cycle, where the carbon released during biogas production is absorbed by new crops, and the resulting waste residues can be reused as AD feedstock, and e) decreasing environmental footprints associated with synthetic fertiliser production and use through the production of nutrient-rich digestate.

2.3.2 Fundamental processes in anaerobic digestion and the metabolic pathways

Anaerobic digestion is the decomposition of complex organic matter by microorganisms in the absence of (or limited) oxygen to yield biogas (typically 60% CH₄, 38% carbon dioxide (CO₂) and 2% trace gases) as the main product (Franke-Whittle et al., 2014). This process involves interactions among various metabolic microorganisms through four main phases: (i) hydrolysis, (ii) acidogenesis, (iii) acetogenesis, and (iv) methanogenesis (Figure 2.5).

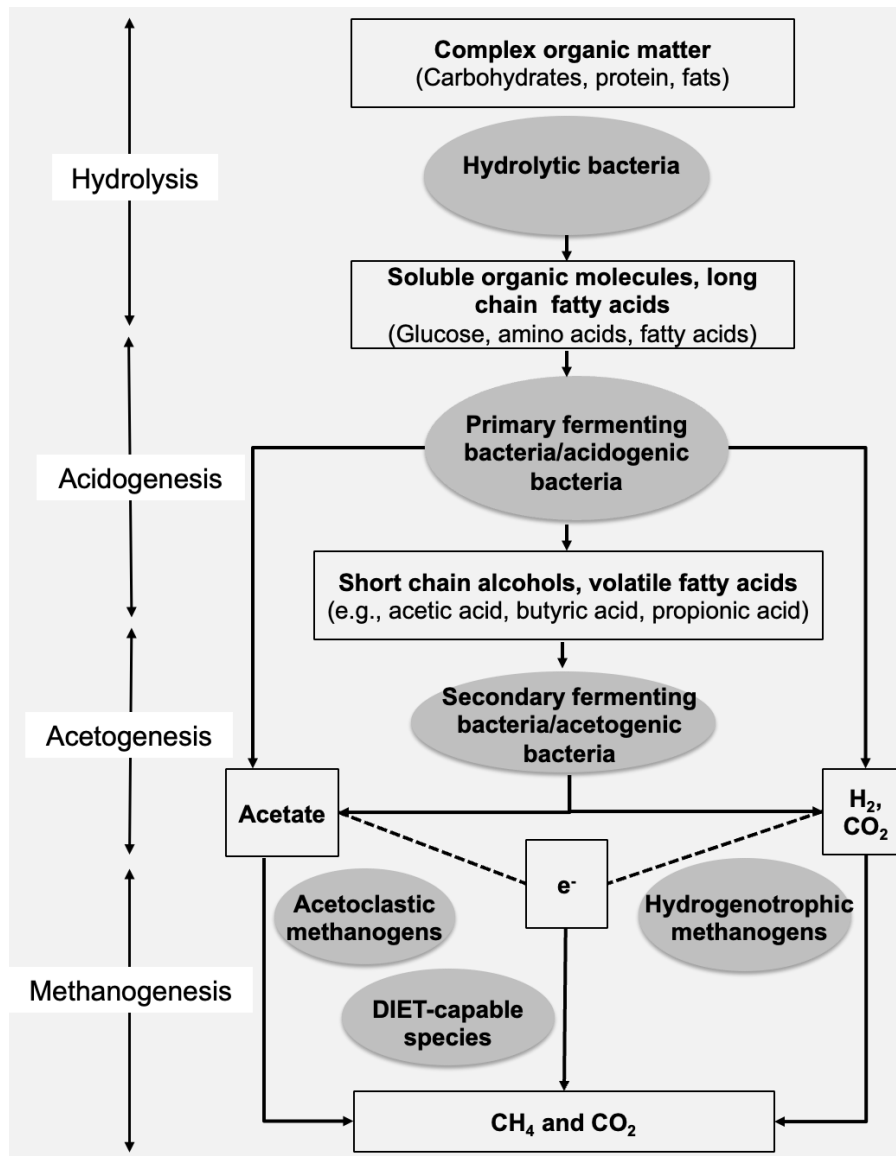


Figure 2.5 Four phases of complex organic matter break down in anaerobic digestion involving interactions among different microbial metabolic function.

During hydrolysis, hydrolytic bacteria break down complex organic matter into simpler, lower molecular weight, and soluble products, like glucose, amino acids and fatty acids. In the acidogenesis phase, primary fermenting bacteria/acidogenic bacteria utilise these products to produce short-chain alcohols, volatile fatty acids (VFAs) as the main products, along with hydrogen gas (H₂), CO₂, and acetate. The latter three products, together with C₁-compounds produced in the acidogenesis phase can be directly consumed by methanogens. However, they cannot directly consume VFAs and short chain alcohols. Instead, they rely on secondary fermenting bacteria/acetogenic bacteria to convert these substances into forms that methanogens can use (H₂, CO₂, acetate). In the final phase, two groups of microorganisms, each with different metabolic pathways, produce CH₄ and CO₂. These include: (i) acetoclastic methanogens (e.g., *Methanosarcina* and *Methanosaeta*), which utilise acetate and (ii)

hydrogenotrophic methanogens (e.g., *Methanobacterium* and *Methanospirillum*) which utilise H_2 (Angelidaki et al., 2011).

Neither secondary fermenting bacteria nor methanogens can perform their metabolic functions independently; they rely on a symbiotic partnership known as syntrophy. Methanogens depend on secondary fermenting bacteria not only for their energy sources but also to rapidly consume VFAs, as methanogens are highly susceptible to these products (Slopiecka et al., 2022). Conversely, secondary bacteria rely on hydrogenotrophic methanogens to remove H_2 to perform efficient metabolism (Xu et al., 2019). The efficiency of this syntrophic metabolism hinges on electron transfer between these two groups of microorganisms, where inefficient transfer can significantly impact the AD process (Xu et al., 2019). Electron transfer between species can occur through the diffusion of H_2 or formate as electron shuttles, a mechanism known as indirect interspecies electron transfer (IIET) (Xu et al., 2019). Propionate and butyrate conversion to CH_4 , however, can only proceed if the low H_2 partial pressure is maintained, which requires continuous H_2 consumption by hydrogenotrophic methanogens, making the efficiency of electron transfer using this mode is highly dependent on H_2 partial pressure (T. Wang et al., 2018; Xu et al., 2019).

A more efficient electron transfer mechanism, recently discovered, is direct interspecies electron transfer (DIET), which can be performed by species capable of this direct transfer (Figure 2.5). In DIET, electrons transfer between species depends on conductive protein filaments (e-pili) and membrane-bound structures c-type cytochromes (c-Cyts) distributed across microbial cell envelopes rather than through conventional diffusion of H_2 or formate as electron shuttles (Storck et al., 2016; Lovley, 2017). This mechanism is more efficient than IIET, because it does not require energy to produce H_2 , is unaffected by H_2 partial pressure, and has a faster electron transfer rate ($44.9 \times 10^{-3} \text{ e}^- \text{ cp}^{-1} \text{ s}^{-1}$) compared to IIET ($5.24 \times 10^{-3} \text{ e}^- \text{ cell pair}^{-1} \text{ s}^{-1}$) (Storck et al., 2016; Baek et al., 2018; T Wang et al., 2018).

2.3.3 Microbial degradation of lignocellulosic components under anaerobic conditions

Microbial degradation of fibre components in LARs begins with the breakdown of hemicellulose (Woiciechowski et al., 2020). Hemicellulose, which occupies the spaces between cellulose microfibrils, must be partially degraded before cellulolytic bacteria can effectively degrade cellulose (Leschine, 1995; Bali et al., 2015). Without pretreatment, 70–90% of hemicellulose can be degraded under an anaerobic environment, with the extent of degradation varying depending on LAR sources (Y Chen et al., 2020). Hemicellulose

comprises a more varied array of sugar molecules than cellulose, necessitating a greater number of enzymes for its degradation. Endo-xylanases are the primary depolymerising enzymes responsible for cleaving the xylan backbone into shorter oligosaccharides, while β -xylosidase cleaves short xylo-oligosaccharides into monosaccharide xylose. Endo-mannanase and β -mannosidase play key roles in mannan degradation. Both xylan and mannan, however, typically contain various substituents linked to the main backbone, such as arabinoses, acetyl, galactose and glucose. Removing these substituents from the backbone requires a range of ancillary enzymes, including α -L-arabinofuranosidase, α -D-glucuronidase ferulic acid esterase, and α -galactosidase, before the core enzymes can access and degrade the backbone. Lastly, ferulic acid esterases are the degradation enzymes responsible for cleaving the linkages between hemicellulose and lignin (Van Dyk and Pletschke, 2012; Azman, 2016; Lu et al., 2022).

In anaerobic environments, cellulose degradation primarily occurs extracellularly because cellulose has a crystalline nature, making it insoluble, and preventing it from being readily taken up into microbial cells (Geboers et al., 2011). In fungi, cellulose degradation involves a synergistic action of three primary enzymes, referred to as cellulases: (1) endoglucanases, which target the amorphous regions of cellulose fibres, providing access for (2) exoglucanase to break down cellobiose units from crystalline regions of cellulose, thereby reducing the degree of polymerisation and (3) β -glucosidase, which further hydrolyses cellobiose, preventing its accumulation, which could otherwise inhibit exoglucanase activity (Leschine, 1995; Van Dyk and Pletschke, 2012; Azman, 2016). The products of cellulose hydrolysis can serve as carbon and energy sources for other microbes, stimulating interactions among microorganisms in these environments (Leschine, 1995).

The mechanism by which cellulases from anaerobic bacteria hydrolyse crystalline cellulose, however, differs fundamentally from cellulose degradation by fungi. In bacteria, cellulases are primarily part of large multiprotein complexes (e.g., cellulosomes), which are attached to the cell surface (Leschine, 1995; Weimer, 2022), allowing for a more localised and concentrated degradation process. When these complexes dissociate, however, for example due to pH and temperature fluctuations, their activity on crystalline cellulose is significantly reduced (Leschine, 1995).

Lignin degradation by bacteria involves two phases. The first phase involves the depolymerisation of lignin polymers by ligninolytic enzymes such as laccase, manganese peroxidase, and lignin peroxidase into its alcohol monomer products, including coumaryl

alcohol (H-lignin)-based compounds, and methoxy group-based compounds (conypheril alcohol (G-lignin) and sinapyl alcohol (S-lignin)) (Zhao et al., 2022). These enzymes can cleave the chemical bonds interconnecting the aromatic monomers such as β -O-4 aryl ether bonds. The second phase involves cleavage of the central ring, producing aromatic intermediate products (Grgas et al., 2023). Through a series of enzymatic reactions, including cleavage, demethylation, and carboxylation involving lignin-derived aromatic substances are broken down into lower molecular weight (Zhao et al., 2022). These intermediates eventually enter the central carbon and energy metabolism through the tricarboxylic acid (TCA) cycle, where they serve as carbon sources to support microbial growth and metabolism (Lu et al., 2022; Grgas et al., 2023).

2.3.4 Key operational parameters influencing anaerobic digestion

The performance of the AD process is influenced by several operational parameters that include pH and alkalinity, VFAs, total ammonia, temperature, C/N ratio, solids content and TEs availability, which are now described.

2.3.4.1 pH and alkalinity

pH and alkalinity are critical parameters for monitoring the stability of the AD process, as the optimal pH ranges for methanogens and acidogenic bacteria differ significantly (Chandra et al., 2012b). Methanogens thrive in a pH range of 6.5 to 8.2, whereas, hydrolytic and acidogenic bacteria are more active at a lower pH typically between 5.2 and 6.5 (Y Chen et al., 2020). Acidogenic bacteria, however, can tolerate a wider pH range of 5 to 8.5 (Mao et al., 2015; Rabii et al., 2019), while pH levels outside the ideal pH range of 6.5–8.2 can severely inhibit methanogen activity. Specifically, a pH below 6.6 hinders methanogen growth, while a pH above 8.2, often indicative of excessive amount of ammonia, is also toxic to these microorganisms.

During the early stages of digestion, VFAs are produced as carbohydrates are broken down, causing an initial decrease in pH. Due to the buffering capacity of the substrate, which is often sourced from total ammonia released during proteins or urea hydrolysis in N-rich substrates, the pH can return to neutral and stabilise (Weiland, 2010; Zeshan, 2012).

In addition to pH, the increase in alkalinity reflects the substrate's adequate buffering capacity during AD (Rahmani et al., 2022). Typically, the alkalinity in a stable AD process is in the range 2,000–4,000 mg L⁻¹ (Bouallagui et al., 2009), however, depending on the substrate, previous study has reported much higher alkalinity concentrations. For example, in the AD of FW,

alkalinity in a stable AD can reach up to 13,000–25,000 mg L⁻¹ due to the high N content and the resulting high levels of ammonia (Banks et al., 2008; Climenhaga, 2008).

2.3.4.2 Volatile fatty acids

Volatile fatty acids are intermediate products formed during the acidogenesis phase. Lactic acid, acetic acid and propionic acid constitute 50–70% of all VFAs generated, while butyric acid and valeric acid are present in smaller amounts (Ye et al., 2013; Zhang et al., 2014). Among these acids, acetic and propionic acids significantly contribute to biogas production, making their concentrations useful as indicators of AD performance and offering an alternative to measuring total VFAs concentration (Zhang et al., 2014; Lee et al., 2015). VFAs, however, can inhibit the AD process, if their concentrations exceed the levels tolerable to the microorganisms. Under stable AD conditions, VFA concentrations are typically in the range 50–250 mg L⁻¹ (Ren et al., 2018). In some fully operational AD plants, VFA concentrations have been reported in the range 1,000–3,000 mg L⁻¹ without causing process instability (Angelidaki et al., 2005), however, values exceeding 3,000 mg L⁻¹ can significantly impede methanogenic activity and stop biogas production, especially if there is insufficient buffering capacity in the system (Karthikeyan and Visvanathan, 2013; Kondusamy and Kalamdhad, 2014; Odejobi et al., 2023).

With sufficient buffering capacity, even with high VFAs concentration reaching 15,000 mg L⁻¹ in the AD of FW, the biogas yields remained unaffected due to the availability of 5,000 mg L⁻¹ of ammonia (Banks et al., 2011). This highlights the importance of VFAs/alkalinity ratio as a critical parameter for assessing AD stability. A ratio <0.4 suggests a stable system, a ratio between 0.4 and 0.8 indicates prone to instability, and >0.8 indicates instability (Filer et al., 2019).

2.3.4.3 Temperature

Temperature is a critical parameter in AD processes and has a significant influence on the microbial growth rate and enzymes activity secreted by the anaerobic microorganisms (Bandgar et al., 2022; Gyadi et al., 2024). Based on the operation temperature, AD can be classified into three stages such as psychrophilic (10–20°C), mesophilic (20–40°C) and thermophilic (44–65°C). The optimal, temperature in mesophilic and thermophilic are 35°C and 55°C, respectively (Odejobi et al., 2023). Mesophilic temperature is the most common AD temperature, however, the hydrolysis and biogas generation rate are faster in thermophilic condition, as the growth rate is twice or three times that in the mesophilic conditions (Meegoda et al., 2018; Odejobi et al., 2023).

Another key advantage of thermophilic temperature is its ability to promote higher levels of pathogen destruction, which is beneficial for meeting regulatory requirements on pathogen levels in the digestate (Meegoda et al., 2018). At a temperature of 53° C, a 90% reduction in several pathogens was achieved in under an hour, compared to several days for the same pathogens at mesophilic temperatures (Meegoda et al., 2018). Thermophilic conditions, however, come with drawbacks such as a higher risk of increased ammonia toxicity, increased acidification risk, and higher investment and energy costs (Shimizu et al., 2022; Karrabi et al., 2023). Moreover, thermophilic conditions typically require a higher dosage of TEs (2.2–7.8 times), particularly iron (Fe), nickel (Ni), cobalt (Co) and zinc (Zn), compared to mesophilic conditions for glucose fermentation, due to the generally lower operational stability of thermophilic processes (Choong et al., 2016). The microbial community in AD is highly sensitive to temperature fluctuations, making it is essential to maintain a constant temperature to avoid instability in the AD processes (Bandgar et al., 2022; Odejobi et al., 2023).

2.3.4.4 Total Ammonia

The degradation of N-rich organic waste during the AD process results in the formation of total ammonia nitrogen, which exist in two principal forms: ammonium ion (NH_4^+) and free ammonia (NH_3). An NH_3 concentration below 200 mg $\text{NH}_4^+\text{-N L}^{-1}$ in AD may provide buffering by reacting with CO_2 to form ammonium carbonate, which help neutralise acidity generated by VFAs production during acidogenesis (Banks et al., 2008; Zhang et al., 2014). In contrast, the excessive levels of these N forms cause detrimental effects in AD (Banks et al., 2008; Zhang et al., 2014; Paritosh et al., 2017). Free ammonia is the most toxic form compared to NH_4^+ (Angelidaki and Ahring, 1993; Borja et al., 1996; Ren et al., 2018), where acetoclastic methanogens are the most impacted by the presence of excess NH_3 compared to the hydrogenotrophic methanogens (Angelidaki and Ahring, 1993). Free ammonia can diffuse into microbial cell membrane and disrupt key physiological processes, leading to inhibited specific enzyme functions and reduced efficiency in AD (Banks and Zhang, 2010; Zhang et al., 2014; Ren et al., 2018).

The reported NH_3 inhibition levels in the literature vary widely, due to factors such as pH, temperature, inoculum used and acclimatisation of methanogens (Angelidaki and Ahring, 1993). For example, NH_3 inhibition occurs at concentrations between 1.5 and 3.0 g N L^{-1} at pH above 7.4, while concentrations higher than 3.0 g N L^{-1} are toxic to methanogens regardless of pH (Angelidaki and Ahring, 1993). To avoid NH_3 inhibition in solid-state AD, the NH_3 concentration should be maintained below 1 g N L^{-1} (Kayhanian, 1999), consistent with the

1.5 g N L⁻¹ threshold reported by Ren et al. (2018). In full scale AD, however, it was reported that methanogens can tolerate higher NH₃ concentration, and reduced inefficiency has only been observed at NH₃ concentrations above 4 g L⁻¹ NH₃-N (Angelidaki et al., 2005). The high tolerance of methanogens to elevated NH₃ concentrations is likely due to their acclimatisation to NH₃-rich substrates in the digester (Bhattacharya and Parkin, 1989; Kayhanian, 1994; Borja et al., 1996).

Laboratory scale experiments have demonstrated that high NH₃ concentrations can induce a shift in methanogenesis pathways from acetoclastic to hydrogenotrophic methanogens, enabling the process to continue by hydrogenotrophic methanogens without instability (Borja et al., 1996; Banks et al., 2011). In terms of temperature, an NH₃ concentration of 2.5 g N L⁻¹ has no effect on the CH₄ yield at 40–55°C, but higher temperatures above 55°C resulted in reduced CH₄ yield (Angelidaki and Ahring, 1994).

The NH₃ concentration is reported to be directly proportional to temperature and pH; with an increase in temperature the NH₃ toxicity increases (Angelidaki and Ahring, 1994; Mata-Alvarez 2003; Weiland, 2010; Zhang et al., 2014; Tian et al., 2018). For example, the concentration of NH₃ increases by an order of magnitude of ten when the pH increases from 7 to 8 at the same temperature (Figure 2.6) (Fernandes et al. 2014). Significant differences in NH₃ concentrations at a single pH in the range between 7 and 10 are observed with respect to increased temperature; whilst above and below this pH range, temperature has minimal impact on the NH₃ concentration (Fernandes et al. 2014).

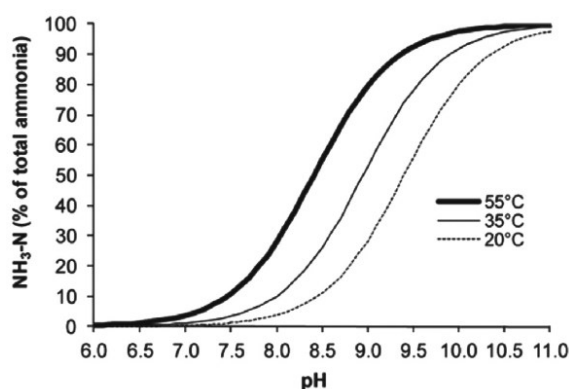


Figure 2.6 Temperature and pH influence on ammonia levels. Reproduced from Fernandes et al., (2014), with permission of the rights holder, Elsevier (the proof of permission is provided in Appendix F).

Given the interconnected effects of NH₃ inhibition and VFAs accumulation, maintaining a balance between NH₃ and VFAs production is crucial in AD. Volatile fatty acids are rapidly generated from the hydrolysis of carbohydrates, leading to a drop in pH and creating an acidic

environment. Proteinaceous compounds, which hydrolyse more slowly than carbohydrates, produce NH_3 upon breakdown, and the NH_3 can act as a buffer, neutralising the pH and restoring it to a more neutral range. If the protein content is high, however, the NH_3 produced can exceed the amount of VFAs generated, leading to NH_3 inhibition. This inhibition slows down CH_4 production and causes VFAs to build up within the system. Conversely, if the NH_3 produced is insufficient to counterbalance the VFAs production, the system will become overly acidic, leading to process failure.

2.3.4.5 Carbon-to-nitrogen (C/N) ratio

Carbon-to-nitrogen ratio reflects the proportion of organic C and TN content in any organic material. C and N are major nutrients making up bacteria cells, typically in the proportions 37–44% and 6–14% TS, respectively (Banks et al., 2010). An appropriate level of N is required by microorganisms to produce new cells as well as to degrade C of the organic material, and for this purpose, a C/N ratio of 20–30 has been reported ideal, with a ratio of 25 being the most commonly used (Chandra et al., 2012b; Mao et al., 2015; Rabii et al., 2019).

Adjusting the C/N ratio to an ideal range is crucial in AD to prevent excessive N content, which can lead to NH_3 toxicity. Substrates with excessively low C/N ratio, such as FW, are particularly susceptible to acidification due to NH_3 toxicity to methanogens (Phun et al., 2018). In contrast, a high C/N ratio indicates N deficiency and poor buffering capacity. In substrates with high C/N ratios, N is rapidly consumed by methanogens, resulting in reduced VFAs production, lower methanogenesis rate, and potentially cessation of biogas production (Kayhanian, 1999; Mao et al., 2015; Rabii et al., 2019).

The adjustment of C/N ratio through mixing different substrates (i.e., co-digestion) with contrasting C/N ratio is a common practice in AD, where for example, animal manure, sewage sludge and food waste which have low C/N ratios can be co-digested with high C/N ratios substrates such as solid waste and LARs (Haider et al., 2015; Rabii et al., 2019). Haider et al. (2015) used rice husk to balance the C/N ratio of FW, observing that lower C/N ratio around 20:1 yielded 30% more biogas compared to a C/N ratio of 35:1. In another study, Ajayi-Banji et al. (2020) co-digested corn stover with dairy manure to adjust C/N ratios to 25:1, 28:1, and 32:1. They found that a C/N ratio of 25:1 optimised VS reduction and CH_4 yield over other ratios. At higher ratios (28:1 and 32:1), insufficient NH_3 production hindered buffering against VFAs production, leading to reduced methanogenesis rates and subsequent VFAs accumulation. Similarly, Dima et al. (2020) studied the co-digestion of sugar beet root and animal manure, reporting the highest CH_4 yield was obtained at a C/N ratio of 26:1, consistent

with findings by Rodriguez et al. (2018) who identified an optimal C/N ratio of 26:1 for the complete conversion of wastepaper and macroalgae. A different outcome, however, was reported by Kainthola et al. (2020), who reported comparable biogas yield across C/N ratios of 20:1, 30:1 and 35:1 in AD of rice straw and FW.

2.3.4.6 Solids content

Anaerobic digestion can be operated either under high total TS conditions (>15%), known as dry AD or solid-state AD or under low TS conditions (<10%), referred to as wet AD (Mata-Alvarez, 2003; Brown et al., 2012; Rabii et al., 2019; Riya et al., 2021). Lignocellulosic agricultural residues typically exhibit high solids content (Section 2.1.3.1), making solid-state AD more suitable for their treatment (Karrabi et al., 2023). In solid-state AD the substrate and inoculum are homogenised outside the reactor before feeding, as mixing within the system may be limited (Rocamora et al., 2020). Compared to wet AD, solid-state AD offers advantages such as reduced water usage, simpler digestate handling, smaller reactor volumes, lower energy consumption due to less mixing requirement, and higher loading rates (Sawatdeenarunat et al., 2015; Pastor-Poquet et al., 2019; Rocamora et al., 2020; Riya et al., 2021). Solid-state AD, however, also presents drawbacks, including poor mass transfer, susceptibility to the accumulation of toxic/inhibiting components such as VFAs, NH₃ and heavy metals, when compared to wet AD (Karthikeyan and Visvanathan, 2013; Rocamora et al., 2020; Riya et al., 2021; Karrabi et al., 2023).

2.3.4.7 Trace elements

The challenge for AD of LARs is not only limited to low digestibility of some organics due to the intricate recalcitrant characteristics of fibre components (Section 2.3.5), but also the deficiency of essential trace elements (TEs) in LARs (Pobeheim et al., 2011; C. Liu et al., 2015; González-Suárez et al., 2018; Mancini et al., 2018b). Trace elements are important building blocks for methanogens, with the presence of these elements following the order: Fe >> Zn > nickel (Ni) > Copper (Cu) $\approx$ Co $\approx$ Molybdenum (Mo) > Manganese (Mn) (Choong et al., 2016; Bandgar et al., 2022). These TEs play crucial roles as co-factors of enzyme or co-enzymes involved in CH₄ biosynthesis and the growth of anaerobic bacteria (Banks et al., 2010; Demirel and Scherer, 2011; Karthikeyan and Visvanathan, 2013). Maintaining an adequate TEs concentration is necessary to prevent VFAs accumulation which can lead to severe acidification (Banks et al., 2012; Mancini et al., 2018b). While TEs at appropriate concentrations are important, excessive levels can lead to inhibition of the AD process. Table 2.4 lists the roles of essential TEs for anaerobic microorganisms and the required stimulatory TEs concentrations.

Table 2.4 The roles of trace elements essential for anaerobic digestion process

Trace Elements	Roles
Se	Formate oxidation, improve process stability
Mo	Present in enzyme formate dehydrogenase, cofactor of various flavinous enzymes
W	Component of enzyme formate dehydrogenase, which catalyses formate production by propionate oxidisers
Fe	Stimulating the formation of cytochromes and ferroxins which are vital for cell energy metabolism, increasing propionate conversion rate
Mn	Stabilising methyltransferase and redox reactions
Ni	Synthesis of cofactor F ₄₃₀ , stimulate the degradation of acetate, increasing formate dehydrogenase activity, reduce NH ₃ and sulphide toxicity
Co	Improving acetate degradation rate, boosting the acetogenesis at the initial stage of AD, serving as a cofactor in carbon monoxide dehydrogenase during the acetogenesis process
Cu	Acting as a cofactor for hydrogenases in facultative anaerobes, archaea, and <i>Clostridia</i>

Sources from (Kayhanian and Rich, 1995; Hinken et al., 2008; Banks and Zhang, 2010; Ortnier et al., 2014; Mao et al., 2015; Choong et al., 2016; Bose et al., 2021; Meng et al., 2023).

The positive effects of TEs supplementation on AD of LARs have been widely documented (Pobeheim et al., 2010; Evranos and Demirel, 2015; C. Liu et al., 2015; Mancini et al., 2018b). For example, supplementing pretreated corn stover with inorganic salts that contain Fe, Co, and Ni improved biogas yield by 28% compared to a Control group without supplementation (C. Liu et al., 2015). Similarly, increasing the Ni concentration in the AD of maize silage from 0.06 to 0.8 mg kg⁻¹ led to a 25% increase in biogas production (Pobeheim et al., 2010). Additionally, the combined supplementation of Ni, Co, Mo during the AD of maize silage enhanced the CH₄ yield by more than three-fold compared to the control reactor without supplementation (Evranos and Demirel, 2015). An insignificant increase in CH₄ yield after the individual supplementation of Co and Ni, however, was observed during the AD of pretreated rice straw (Mancini et al., 2018b), likely because the inoculum has already supplied adequate amounts of these TEs.

Supplementing TEs from external sources, such as commercially available TE solutions or salts, however, is not common in commercial practical operations due to concerns about minimising operational costs (Climenthaga, 2008). An alternative approach to address TEs deficiency is the co-digestion of LARs with other substrates (e.g., manure) that contain a broad range of TEs (Nges and Björnsson, 2012; Evranos and Demirel, 2015) or by adding low-cost materials rich in TEs, for example, incinerator and coal ashes. The effectiveness of coal or

incinerator ash as a source of TEs supplementation has been reported for the AD of various substrates, including municipal solid waste (Banks and Lo, 2003; Lo et al., 2012), paper and pulp sludge (Huiliñir et al., 2015), sewage sludge (Huiliñir et al., 2017; Montalvo et al., 2017), and winery wastewater (Lauzurique et al., 2021a).

Reports in the literature on TEs supplementation using ashes or other low-cost resources specifically for LARs as substrate in AD processes, is limited. Few studies have reported the use of low-cost resources, such as clay minerals rich in multiple TEs, to enhance the AD of maize straw (González-Suárez et al., 2018). Another example is the use of ash extracts from burned LARs, such as cocoa pods and empty palm fruit bunches, to supply TEs in the AD of cassava waste (Egwu et al., 2021).

2.3.5 Challenges for anaerobic digestion of lignocellulosic agricultural residues

The chemical interplay among fibre components makes the structure of LARs highly resistant to natural biological breakdown in AD. This makes hydrolysis is the rate limiting step during AD of LARs, thus resulting in lower gas yields and longer retention times, directly affecting the profitability of the AD operation (Janke et al., 2017). This recalcitrance is mainly linked to several factors that relate to the characteristics of the fibre components: (1) the presence of lignin, (2) the high crystallinity of cellulose, (3) the high degree of polymerisation of cellulose, (4) surface area (porosity), (5) the degree of ester bonds between lignin and carbohydrates (Van Dyk and Pletschke, 2012; Lee et al., 2014; Kim, 2018; Mustafa et al., 2018). Due to these recalcitrance characteristics, only 20–50% of the carbohydrates in LARs can be degraded in AD (Kaur et al., 2020; Peyrelasse et al., 2021). Reducing the recalcitrance of LARs is important to optimise CH₄ production in AD and ensure the economic viability of the process.

2.4 Pretreatment methods to enhance biogas production from lignocellulosic agricultural residues

2.4.1 Overview of pretreatment methods

To optimise the utilisation of carbohydrates sources in LARs efficiently and develop cost-effective, environmentally friendly, and sustainable processes for their conversion to CH₄, an effective pretreatment step is essential. Pretreatment plays a pivotal role in reducing the recalcitrance of LARs, thereby enhancing the digestibility of carbon sources present in LARs and conversion rate to CH₄ in the subsequent AD process. While pretreatment accounts for at least 20% of the total operation cost of AD of LARs (Kumar et al., 2009; Amin et al., 2017), its benefits outweigh the costs associated with no pretreatment where inefficiencies arise

through lower CH₄ yield and longer digestion retention times, that would result in higher overall costs of the AD operations (Van Dyk and Pletschke, 2012).

When selecting a pretreatment method, several factors must be considered. The ideal pretreatment should: (1) enhance glucose production while minimising carbohydrates loss, (2) be cost-effective and reduce energy consumption, and (3) minimise the production of inhibitory by-products and chemical wastes. The LARs pretreatment aims to disrupt the lignocellulosic complex, solubilise the non-cellulosic contents (lignin and hemicellulose), and preserve the carbohydrates for further valorisation. Additionally, it seeks to alter cellulose crystallinity and increase the porosity of the materials to facilitate the subsequent depolymerisation process (Bensah and Mensah, 2013; Lee et al., 2014; Sasmal and Mohanty, 2019). Previous studies have explored various pretreatment methods, including mechanical, chemical, biological and thermal processes, as well as their combinations. Table 2.5 provides an overview of the primary objectives of each pretreatment method and their associated demerits.

Table 2.5 Overview of pretreatment methods

Pretreatment Method	Objectives
Mechanical (e.g., comminution, milling, grinding)	• Increase in available specific surface area (Kaur et al. 2020; Cai et al. 2021) • Altered cellulose crystallinity and reduced degree of polymerisation (Amin et al. 2017; Cai et al. 2021) • No production of inhibitors: furfural and hydroxyl methyl furfural (HMF) (Hendriks and Zeeman 2009) • High energy requirements (Barakat et al. 2014; Cai et al. 2021) • Applied before any other pretreatment methods (Hosseini Koupaie et al. 2019; Cai et al. 2021)
Thermal (e.g., liquid hot water, extrusion, irradiation, steam-explosion)	• Increase in hemicellulose and lignin solubilisation (Hendriks and Zeeman 2009) • Avoid corrosion problems (Mustafa et al. 2018) • Formation of toxic phenolic compounds, furfural and HMF at temperature higher than 160°C (Hendriks and Zeeman 2009; Cai et al. 2021) • High in energy consumption (Mustafa et al. 2018) • Short pretreatment duration (in the order of mins) (Cai et al. 2021)
Biological (e.g., fungal)	• Increase in hemicellulose, cellulose solubilisation and lignin alteration (Zheng et al. 2014) • The lowest energy requirement among other methods (Zheng et al. 2014; Cai et al. 2021) • No chemical additions (Zheng et al. 2014) • Long pretreatment duration (10–14 d) (Zheng et al. 2014; Peyrelasse et al. 2021) • Require large space (Amin et al. 2017)

Alkali Chemical (e.g., NaOH, KOH, Ca(OH) ₂ , aqueous NH ₃)	• Increase in porosity and internal surface area by swelling (Zheng et al. 2014; Cai et al. 2021) • Decrease the degree of cellulose crystallinity and polymerisation (Zheng et al. 2014; Cai et al. 2021) • Effective delignification (Barakat et al. 2014; Peyrelasse et al. 2021) • Pretreatment time longer than acid pretreatment, requiring hours or days (Barakat et al. 2014) • High chemical costs (Kaur et al. 2020)
Chemical Acid (e.g., sulfuric acid (H ₂ SO ₄), hydrochloric acid (HCl))	• Increase hemicellulose solubilisation and lignin alteration (Hendriks and Zeeman 2009) • Potential loss of fermentable sugar (Kaur et al. 2020) • Formation of furfural and HMF (Sambusiti et al. 2013; Kaur et al. 2020; Cai et al. 2021) • Potential corrosion problems (Barakat et al. 2014; Zheng et al. 2014) • Subsequent neutralization required after acid pretreatment (Kaur et al. 2020) • High costs associated with chemical use, chemical waste handling and disposal after pretreatment (Barakat et al. 2014; Kaur et al. 2020) • Pretreatment time can be in the order of minutes (Sambusiti et al. 2013; Meegoda et al. 2018)

2.4.2 Alkali pretreatment of lignocellulosic agricultural residues

Alkali pretreatment using sodium hydroxide (NaOH), calcium hydroxide (Ca(OH)₂), potassium hydroxide (KOH) and aqueous ammonia offer several advantages over other methods, including: (i) greater delignification rate (Bensah and Mensah, 2013), despite a longer pretreatment time than an acid-based method (Sambusiti et al., 2013; Meegoda et al., 2018), (ii) twice as fast as a biological pretreatment typically of 10–14 d (Zheng et al., 2014), (iii) avoidance of downstream neutralisation or the recycling of chemical waste normally required with acid pretreatment (Amin et al., 2017; Meegoda et al., 2018), (iv) providing buffering capacity through residual alkalinity, which reduces the risk of acidification and methanogenic inhibition caused by the intrinsic production of VFAs within the process (Neves et al., 2006; Dell’Omo and La Froschia, 2018), (v) remains highly effective at ambient temperatures in enhancing fibre digestibility and CH₄ production (Oji et al., 2007; Shetty et al., 2017), and (vi) limitation of by-products that inhibit the AD process (Hendriks and Zeeman, 2009; Monlau et al., 2012; Janke et al., 2017). Acid pretreatment at high temperatures can potentially generate by-products that inhibit the AD process, such as furfural and hydroxyl methyl furfural (HMF) through the dehydration of carbohydrates (xylose, galactose, mannose and glucose) (Sambusiti et al., 2013; Amin et al., 2017; Paul and Dutta, 2018). Therefore, alkali pretreatment of LARs for AD offers significant advantages by reducing energy requirements, operating costs and process complexity.

In alkali pretreatment, the initial reactions occur upon the addition of alkaline solutions to LARs, involving solvation and saponification reactions (Hendriks and Zeeman, 2009). These reactions are the primary mechanism of alkali pretreatment, responsible for disrupting linkages in the lignin carbohydrate complexes (LCCs) network (Loow et al., 2016; Dai et al., 2018; Mafa et al., 2020). The LCCs structure shown in Figure 2.4 features ester-linked ferulic acid (see Section 2.1.3.3), serving as a susceptible point for alkali degradation. During saponification, the hydroxide ion hydrolyses these ester bonds, leading to the separation of cellulose and hemicellulose from lignin encapsulation (He et al., 2008; Dai et al., 2018; Mafa et al., 2020; Yin et al., 2021). As the protective layer lignin has been separated, the solubility of hemicellulose in alkaline solution increases, which consequently alters the fibre composition of LARs.

In addition to the saponification of the ester bonds, another mechanism during the alkali pretreatment involves the removal of acetyl and uronic acid substituents on hemicellulose (Modenbach and Nokes, 2014), and the hydrolysis of glycosidic linkages initiated by hydroxide ion attack on a carbon atom with two oxygen atoms, leads to a decrease in molecular weight (Berglund et al., 2019). Sodium hydroxide is also effective for cleaving ester and carbon-to-carbon (C-C) bonds in lignin molecules (Kim et al., 2016). The saponification reaction also influences the polymerisation degree of cellulose and impacts surface area, porosity, and crystallinity (Kim et al., 2016).

2.4.3 Influence of key parameters on alkali pretreatment

The key factors determining the efficacy of the alkali pretreatment at ambient temperature are the alkali loading rate (ALR), pretreatment duration, moisture content or solid to liquid ratio (SLR), and particle size (Zheng et al., 2009; Bensah and Mensah, 2013; You et al., 2014; Motte et al., 2015; Zhang et al., 2015), each of which are now described.

2.4.3.1 Alkali loading rate

The extent of alkali pretreatment is often quantified by the ALR, which is typically measured in terms of the weight of alkali (g) per weight of dried substrate (g) (Janke et al., 2017; Dai et al., 2018; Kumar et al., 2019; Mansour et al., 2024) or per mL volume of water used to prepare the alkaline solution (Zhang et al., 2015; Shetty et al., 2017; Noonari et al., 2019; Sabeeh et al., 2020). Table 2.6 presents the impact of different ALRs, expressed in g alkali (e.g., NaOH, Ca(OH)₂, KOH, ammonia, urea) per mL water, on total fibre reduction and CH₄ yield of different sources of LAR. Numerous studies have indicated the significant influence of ALR on the digestibility of LARs, as evidenced by the increase in fibre reduction with higher ALR (Table

2.6). Sodium hydroxide is the most extensively studied alkali for pretreatment owing to its potent basicity, high solubility, and efficacy in delignification (Bensah and Mensah, 2013; Loow et al., 2016), whereas $\text{Ca}(\text{OH})_2$ is favored for its cost-effectiveness compared to NaOH, and $\text{Ca}(\text{OH})_2$ retained in AD will not bring negative impacts to the environment (Kumar et al., 2009; Shen et al., 2019). Additionally, KOH is also a viable due to its strong basicity similar to NaOH and offers the added advantage of potentially enhancing nutrient value of the soil after pretreatment and subsequent AD (Zheng et al., 2014; Poddar et al., 2022).

Table 2.6 Effects of alkali loading rates on fibre reduction and specific CH_4 yield of different lignocellulosic agricultural residues at ambient pretreatment temperatures.

Lignocellulosic Agricultural Residues	Alkali Loading Rates (g alkali / mL water)	Fibre Reduction (% TS) Relative to Untreated LARs	Specific CH_4 Yield ^a (mL kg ⁻¹ VS)	References
Rice straw (25°C, 3 h)	NaOH 1%	20.8	398.2 (70.9)	Shetty et al. (2017)
	NaOH 2%	n.d	241.7 (48.4)	
	NaOH 3%	n.d	220.0 (35.1)	
	NaOH 5%	n.d	148.8 (-9.4)	
Rice straw (35°C, 192 h)	NaOH 0.26%	14.0	80.9 (39.8)	Dai et al. (2018)
	NaOH 0.52%	11.2	103.5 (78.9)	
	NaOH 0.78%	15.0	170.3 (194.3)	
	NaOH 1.04%	20.2	108.9 (88.1)	
Rice straw (35°C, 120 h)	NaOH 0.25%	6.5	230.0 (23.0)	Zhang et al. (2015)
	NaOH 0.5%	9.8	280.0 (46.6)	
	NaOH 1%	15.0	277.0 (45.0)	
Rice straw (37°C, 3 h)	NaOH 0.5%	5.8	402.0 (70.9)	Sabeeh et al. (2020)
	NaOH 1%	15.7	332.5 (37.7)	
	NaOH 1.5%	16.5	362.0 (49.9)	
	NaOH 2%	18.7	360.0 (49.1)	
Rice straw (25°C, 168 h)	$\text{Ca}(\text{OH})_2$ 0.1%	n.d	274.2(26.7)	Noonari et al. (2019)
	$\text{Ca}(\text{OH})_2$ 0.2%	n.d	305.0 (40.9)	
	$\text{Ca}(\text{OH})_2$ 0.3%	n.d	381.3 (76.2)	
	$\text{Ca}(\text{OH})_2$ 0.4%	n.d	346.7 (60.2)	
Rice straw (25°C, 72 h)	$\text{Ca}(\text{OH})_2$ 0.25%	0	298.0 (19.2)	Gu et al. (2015)
	$\text{Ca}(\text{OH})_2$ 0.4%	5.8	325.0 (30.0)	
	$\text{Ca}(\text{OH})_2$ 0.5%	19.5	330.0 (32.0)	
	$\text{Ca}(\text{OH})_2$ 0.6%	22.1	320.0 (28.0)	
	$\text{Ca}(\text{OH})_2$ 0.75%	28.3	298.0 (19.2)	
Rice straw (35°C, 24 h)	Ammonia 1%	26.8	831.9 ^b (118.9)	Kim et al. (2018)
	Ammonia 5%	42.9	827.3 ^b (117.7)	

Wheat straw (20°C, 24 h)	KOH 0.2%	2.3	168.0 (0.0) ^c	X. Liu et al. (2015)
	KOH 0.6%	7.2	n.d	
	KOH 1%	14.2	209.0 (22.2)	
	KOH 1.4%	19.1	234.0 (36.8)	
Wheat straw (ambient temperature ^d , 72 h)	KOH 0.17%	3.8	208.0 (13.0)	Jaffar et al. (2016)
	KOH 0.5%	12.6	210.0 (14.1)	
	KOH 1%	21.9	258.0 (40.2)	
	KOH 1.5%	25.8	217.0 (17.9)	
Wheat straw (20°C, 144 h)	Urea 1%	25.4	305.5 (45.2)	Yao et al. (2018)
	Urea 3%	36.9	273.3 (29.9)	
	Urea 5%	44.2	Failure	
Wheat straw (room temperature ^d , 24 h)	NaOH 0.4%	n.d	248.0 (12.7)	Mansour et al. (2024)
	NaOH 0.6%	n.d	260.0 (18.2)	
	NaOH 0.8%	79.5	300.0 (36.4)	
	NaOH 1.0%	n.d	285.0 (29.5)	
Wheat straw (35°C, 192 h)	NaOH 4%	42.4	n.d	Jiang et al. (2016)
	NaOH 6%	41.7	n.d	
	NaOH 8%	47.1	n.d	
Wheat straw (28°C, 8 h)	Ca(OH) ₂ 0.8%	10.3	325.0 (67.5) ^e	Kumar et al. (2019)
	Ca(OH) ₂ 1.6%	9.4	344.0 (77.3) ^e	
	Ca(OH) ₂ 2.4%	6.6	385.0 (98.4) ^e	
Wheat straw (25°C, 22 h)	NaOH 0.2%	n.d	207.9 (20.6)	Zdeb (2021)
Wheat straw (37°C, 120 h)	NaOH 0.8%	n.d	165.9 (111.6)	Chandra et al. (2012b)
Corn stover (35°C,)	NaOH 2%	16.5	225.7 (30.1)	Wei et al. (2015)
	NH ₃ 4%	21.9	228.9 (32.0)	
Corn stover (30°C, 72 h)	NaOH 0.3%	9.3	210.0 (73.4)	Zheng et al. (2009)
	NaOH 0.6%	18.0	180.0 (50.0)	
	NaOH 0.9%	19.1	200.0 (66.7)	
Corn stover (20°C, 24 h)	NaOH 1%	21.7	266.8 (0.0) ^c	Zhu et al. (2010)
	NaOH 2.5%	21.2	275.9 (0.0) ^c	
	NaOH 5%	43.4	372.4 (37.0)	
	NaOH 7.5%	66.8	Failure	
Corn stover (20°C, 12 h)	KOH 1.5%	n.d	202.7 (50.0)	Siddhu et al. (2016)
Corn stover (20°C, 72 h)	NaOH 0.3%	24.4	171.0 (14.1)	C. Liu et al. (2015)
Corn stover (20°C, 3 h)	NaOH 2%	54.6 ^f	275.0 (10.0)	You et al. (2014)
	NaOH 4%	60.8 ^f	290.0 (16.0)	
	NaOH 6%	66.9 ^f	300.0 (20.0)	

Sugarcane straw (75°C; 30 mins)	NaOH 0.35%	n.d	260.0 (0.0) ^c	Janke et al. (2017))
	NaOH 0.67%	n.d	275.0 (5.8)	
	NaOH 1.25%	n.d	291.0 (11.9)	
Sugarcane bagasse (room temperature ^d ; 96 h)	Ca(OH) ₂ 1.7%	6.6	150.0 (7.1)	Mustafa et al. (2018)
	Ca(OH) ₂ 8.5 %	11.9	175.0 (25.0)	
	Ca(OH) ₂ 11.9 %	13.3	150.0 (7.1)	

Note: n.d: not determined

^a Values in parentheses represent the percent increase in CH₄ yield compared to that in the untreated LARs, ^b mL CH₄ g⁻¹ total carbon (TC), ^c No increase compared to untreated LARs, ^d Temperature data not included, ^e Biogas yield (CH₄ content not included), ^f Lignin removal rate.

A review of the literature suggests that the smallest ALR for alkali pretreatment falls within the range of 0.1–0.25% (Table 2.6). At this loading rate, regardless of the alkali used, an insignificant reduction of the total fibre (less than 10%) is typically found for rice straw, however, a remarkable CH₄ increase by 20–40% can be achieved at this low level of ALR. In contrast, studies by X. Liu et al., (2015); Jaffar et al., (2016) reported less than 5% fibre reduction for wheat straw, with either no increase or only an insignificant increase in CH₄ yield. The limited efficacy of this ALR level in reducing the recalcitrance of LARs, has probably restricted its exploration across different sources of LARs. From the literature it is observed that alkali pretreatment of corn stover at 0.1–0.25% rate is uncommon. Only a few studies have employed alkali pretreatment alone for wheat straw at this ALR level; instead, higher ALR or high temperature pretreatment is more commonly used (Tosun et al., 1989; Tsegaye et al., 2019; Novakovic et al., 2021; Peyrelasse et al., 2021). This is likely due to the higher lignin content in wheat straw, which ranges from 12–23% TS, compared to 5–20% TS in rice straw (Rosgaard, 2007; Adebayo and Martínez-Carrera, 2015) (see Table 2.1 in Section 2.1.3.2).

For sugarcane bagasse, alkali pretreatment alone at this low ALR level is even more rare. As reported for wheat straw, more commonly studies employed high temperature alkali pretreatment to achieve significant fibre reduction and increased CH₄ yield in sugarcane bagasse (Yoon et al., 2011; Maryana et al., 2014; Talha et al., 2016; Zhu et al., 2016). Sugarcane bagasse contains the highest lignin content among rice straw, wheat straw, and corn stover, with values ranging between 15 and 30% TS (Rosgaard, 2007; Adebayo and Martínez-Carrera, 2015; Wei et al., 2015).

For NaOH-pretreated rice straw a slightly higher ALR level (0.4–0.5%) appears to be more effective, resulting in the reduction of total fibre by 10–19% TS and a significant increase in CH₄ yield by 30–70%. Elsewhere for rice straw, a consistent value of total fibre reduction

(19.5%) and CH₄ increase (32–60%) was reported with the use of different alkali (i.e., Ca(OH)₂) (Gu et al., 2015; Noonari et al., 2019). Similar results were reported for corn stover, with an 18% total fibre degradation and a 50% CH₄ increase for NaOH pretreated corn stover (Zheng et al., 2009). A moderate increase in CH₄ yield (14–18%), which is lower than that for rice straw and corn stover, was reported for pretreated wheat straw using the same loading rate (Memon and Memon, 2020; Mansour et al., 2024). This ALR level, moreover, proves inadequate for a significant increase in CH₄ yield in sugarcane straw, where the observed increase was only evident when a higher ALR of 1.25% was used.

In general, most studies agree that a significant increase in CH₄ yield for all types of LARs, excluding sugarcane bagasse, is observed when a 0.7–1.0% loading rate was used, regardless of the alkali used. Pretreatment of rice straw with NaOH, and ammonia resulted in a 38–194% increase in CH₄ yield, similar to 20–112% increase in CH₄ for wheat straw either pretreated with NaOH, Ca(OH)₂, urea or KOH. The wide range of CH₄ increase may be attributable to the lignin composition of LARs (Table 2.1) and other factors influencing the pretreatment efficiency (ALR, pretreatment duration, SLR, and particle size). An increase in CH₄ yield of 66.7% was reported for NaOH-pretreated corn stover (Zheng et al., 2009), in contrast with other results indicating no change in CH₄ yield compared to untreated corn stover (Zhu et al., 2010), likely due to differences in pretreatment duration and operating temperatures.

Although higher ALR levels often result in increased fibre degradation, it does not always lead to a corresponding rise in CH₄ yield. For instance, Yao et al. (2018) observed that wheat straw pretreated at ALRs of 3% and 5% gave higher fibre reduction compared to an ALR of 1%, yet the CH₄ yield from 3% ALR was lower than that from 1%, with the digester experiencing a failure at 5% ALR (Table 2.6). Dai et al. (2018) reported an increase in fibre reduction of rice straw with higher ALRs, but CH₄ yield decreased when the highest ALR of 1.04% was used. Similarly, Noonari et al. (2019) found that CH₄ yield increased with ALR from 0.1% to 0.3% for rice straw pretreatment but at ALR 0.4%, CH₄ yield declined. Zhang et al. (2015) found that while rice straw pretreated with 1% NaOH showed higher fibre reduction compared to 0.5%, the CH₄ yield for both ALR levels remained similar, suggesting that 0.5% was optimal. A similar optimal ALR of 0.5% was reported by Gu et al. (2015) for Ca(OH)₂ pretreatment of rice straw. A higher optimal ALR of 0.78–1.5% was reported by (Shetty et al., 2017; Dai et al., 2018; Kim et al., 2018; Yao et al., 2018) regardless of the alkali used.

Reduced CH₄ yield observed at high ALR above 1.5% may be attributed to increased alkaline ion concentrations, potentially leading to toxicity in AD. While methanogens require low

sodium (Na) concentration (100–200 mg L⁻¹) for adenosine triphosphate synthesis, sodium ions (Na⁺) concentrations exceeding 550 mg L⁻¹, inhibit anaerobic microorganism metabolism due to increased osmotic pressure and microbial dehydration (Chen et al., 2008; Shetty et al., 2017; Shen et al., 2019). Moreover, an excess of calcium ions (Ca²⁺) can lead to calcium salt precipitation, which decreases methanogenic activity (Mustafa et al., 2018). Earlier studies reported that the toxicity effect of Na, K and Ca in AD begin at concentrations of 3,000 mg L⁻¹, 12,000 mg L⁻¹ and 8,000 mg L⁻¹, respectively (Romero-Güiza et al., 2016).

A high alkali concentration at elevated temperature (e.g., 150°C) might also lead to excessive lignin degradation during pretreatment or delignification, which can subsequently result in lower CH₄ yield (Zhu et al., 2010). Under these conditions, β-O-4 bonds in lignin may break, leading to production of aromatic compounds, such as phenol, that are inhibitory to methanogens (Zhu et al., 2010; Li et al., 2023). Phenolic compounds derived from lignin degradation can deactivate or inhibit β-glucosidase, and cellulase enzymes through precipitation and irreversible binding (Poddar et al., 2022).

2.4.3.2 Solid-to-Liquid ratio

Modenbach and Nokes (2014) found that most alkali pretreatments use a solid-to-liquid ratio (SLR) of less than 10% to ensure uniform contact between lignocellulose and the chemical agent. The SLR measures the weight of dry LARs material (g) relative to the volume of water (in g or mL) (Zhao et al., 2010; Ibarra-Díaz et al., 2020). A low SLR or high water content, however, requires higher chemical consumption, increases pH neutralisation costs, demands a larger space for pretreatment area, and necessitates the removal of excess pretreatment liquor (Modenbach and Nokes, 2014), especially when solid is used, as high water content over-moisturises the substrates. The removal of excess liquor is undesirable as it contains a high fraction of soluble organic compounds that could otherwise contribute to CH₄ production.

To reduce water usage, some earlier studies implemented a higher SLR and according to Zheng et al. (2009), an SLR of 13.6% was found to be optimal, allowing the substrate to be fully saturated without excess water runoff. Mansour et al. (2024) reported that NaOH pretreatment with an SLR of 20% was slightly more effective than 10%, resulting in a 16% higher increase in CH₄ yield. Much higher SLRs, ranging from 100% to 500%, were tested in NaOH pretreatment of asparagus stover by Sun et al. (2019), who reported 135% to be optimal. It was argued elsewhere (Modenbach and Nokes, 2014) that SLRs above 15% pose challenges not seen with lower ratios. Insufficient free water in the reactor can greatly reduce pretreatment effectiveness (Kristensen et al., 2009) as water facilitates efficient mass transfer,

enabling chemicals to diffuse into the LARs (Modenbach and Nokes, 2014). Due to the wide variations in optimal SLR reported, it is difficult to pinpoint the optimal SLR for alkali pretreatment, without further studies exploring the range of SLRs mentioned in the literature.

2.4.3.3 Pretreatment duration

Optimising pretreatment duration is crucial, as excessively long pretreatment durations requires larger operational space and reduce efficiency. Like ALR, longer pretreatment durations, which often resulted in higher fibre reduction, do not always lead to proportional increases in CH₄ yield. This is evident from Song et al. (2013), who used a Box-Behnken design coupled with multiple regression analysis to determine the optimal pretreatment duration. Their analysis suggested that, at the same ALR, the CH₄ yield differences between 3, 5 and 7 d of pretreatment were insignificant, indicating 3 d as the optimal duration for NaOH pretreatment. A pretreatment time of 11 d, however, resulted in decreased CH₄ yield, likely attributed to extended exposure to alkali conditions which can lead to excessive delignification, which in turn leads to formation of inhibitory compounds like phenolics (Li et al., 2023).

The parameter, pH, was identified as a practical indicator for determining the optimal pretreatment duration, as it is easier to measure than changes in alkali concentration (Zheng et al. 2009; Yang et al. 2014). During pretreatment, the alkali agent is consumed by the LARs (Xu and Cheng, 2011), causing pH to decline. When the pH stabilises, this indicates the reaction has stopped and signals the optimal pretreatment time. Zheng et al., (2009) found that pH stabilised after 2 d of NaOH pretreatment, therefore 3 d was deemed optimal to ensure the complete chemical reaction, consistent with findings by Song et al. (2013). The optimal pretreatment duration, however, may vary with the alkali used. Yuan et al. (2014) and Yang et al. (2014) observed that pH stabilised after 5 d of ammonia pretreatment for rice straw and wheat straw, respectively, suggesting this as an optimal duration. Similarly, lime or CaO pretreatment, may need longer pretreatment duration than NaOH, due to CaO's poor solubility. Unlike NaOH which dissolved immediately upon contact with water, CaO dissolves gradually as available alkalinity is consumed by LARs (Xu and Cheng, 2011).

2.4.3.4 Particle size

Particle size of the LARs is an important factor in maximising CH₄ yields by the AD process as smaller particles offer a larger accessible surface area for chemical and biological reactions and affects the mass transfer (Menardo et al., 2012; Barakat et al., 2014; Chandra et al., 2015; Yong et al., 2015; Shen et al., 2019). Reducing particle size benefits further, the AD process by accelerating the hydrolysis rate, thereby decreasing the residence time required for the

process (Mshandete et al., 2006; Hendriks and Zeeman, 2009; Peces et al., 2015; Cai et al., 2021). Moreover, reducing particle size results in an increase in bulk density, a critical factor influencing transportation, storage costs and logistics (Liu et al., 2016).

Table 2.7 shows the impact of particle size on CH₄ yield for different LARs reported in the literature. For each LAR type each particle size measurement has a corresponding specific CH₄ yield tabled.

Table 2.7 Effect of particle size reduction of lignocellulosic agricultural residues (LARs) on specific CH₄ yield for various lignocellulosic agricultural residues (data compiled from the literature). The order of particle sizes follows the respective order of specific CH₄ yield.

LAR Sources	Particle Size (mm)	Specific CH ₄ Yield (mL g ⁻¹ VS)	References
Rice straw	0.088; 0.40; 1.0; 6.0	483 ^a ; 487 ^a ; 475 ^a ; 460 ^a	Sharma et al. (1988)
Rice straw	0.30; 0.75; 1.50	142.6; 148.1; 133.2	Chandra et al. (2015)
Rice straw	0.075; 0.150; 1.0; 20	197; 182; 161; 107	Dai et al. (2019)
Wheat straw	0.088; 0.40; 1.0; 6.0	362 ^a ; 360 ^a ; 350 ^a ; 330 ^a	Sharma et al. (1988)
Wheat straw	0.11; 0.67; 1.45	13.3; 168; 192	Motte et al. (2015)
Wheat straw	2.0; 50.0	334; 285	Menardo et al. (2012)
Wheat straw	<1.0; 30-50	239; 232	Ferreira et al. (2014)
Wheat straw	0.30; 0.75; 1.50	154.3; 199.6; 135.7	Chandra et al. (2015)
Wheat straw	2.0; 20	244.1; 179.2	Gallegos et al. (2017)
Corn stover	0.075–0.25; 0.25–1.0; 1.0–5.0; 5.0–20.0	231; 249; 229; 184	Xiao et al. (2013)
Corn silage	0.4; 0.6; 1.0; 2.0	125 ^b ; 105 ^b ; 45 ^b ; 36 ^b	Armah et al. (2019)
Barley straw	5.0; 20.0; 50.0	370; 339; 286	Menardo et al. (2012)
Maize stalk	2.0; 20.0	272; 254	Menardo et al. (2012)
Sorghum	4.0; 8.0	301.8; 281.4	Herrmann et al. (2012)
Sisal fibre	2.0; 5.0; 10.0	216; 205; 203	Mshandete et al. (2006)

^a Units in L kg⁻¹ TS, ^b Units in mL biogas d⁻¹.

Some studies have shown a modest increase in CH₄ yield (6–9%) following particle reduction from >5 mm to <5 mm (Sharma et al., 1988; Mshandete et al., 2006; Herrmann et al., 2012; Menardo et al., 2012). More significant increases of over 15%, however, have also been

reported. For instance, Dai et al. (2019) reported a 50% increase in CH₄ yield when particle size of rice straw was reduced from 20 mm to 1 mm. Similarly, Xiao et al. (2013) observed a 24% increase in CH₄ yield in corn stover with size ranging from 1–5 mm compared to the sizes ranging between 5–20 mm. Additionally, Gallegos et al. (2017) reported a 36% increase in CH₄ yield while Menardo et al. (2012) observed a 17% increase, both achieved by reducing the particle size from 20 mm to 2 mm.

Despite the advantages associated with smaller particle size, there appears to be a minimum threshold beyond which further reduction in particle size does not result in significant improvements in biogas yield (Chandra et al., 2015). For instance, Chandra et al. (2015) found that while reducing particle size from 1.5 mm to 0.75 mm led to 11–47% increase in CH₄ yield, in contrast, further reduction from 0.75 mm to 0.3 mm led to a 23% decrease in CH₄ yield. Motte observed that reducing particle size from 1.45 to 0.67 mm increased the CH₄ yield by 14%, and further reduction to 0.11 mm led to significant decrease in CH₄ yield, by 90%. Additionally, Xiao et al. (2013) reported an 8% decrease in CH₄ yield when particle size was reduced below 0.25 mm.

A review of the literature suggests that a particle size range of 0.3–2 mm is recommended for experimental AD of LARs. This agrees with Yong et al. (2015) who recommended a particle size of 0.3–1 mm, like that (of 0.5–2 mm) proposed by Barakat et al. (2014) for optimal conversion of cellulose and hemicellulose into sugar. Atelge et al. (2020) also recommended a particle size of 1–2 mm. It has been reported that cellular disruption associated with very fine particle sizes can release significant amounts of water-soluble compounds, which are readily degraded, leading to rapid VFAs production, and potentially causing acidification inhibition of the process (Motte et al., 2015). While this acidification is less likely in the context of alkali pretreatment of LARs, as the residual alkali can act as a buffer preventing the pH from dropping into the acidic range, a greater concern with reducing particle size to excessively small sizes is the high energy consumption required to mill/grind the samples to the much smaller size.

Particle reduction is a highly energy-intensive and can significantly affect the economic cost benefit of the entire AD operation (Cadoche and López, 1989; Amin et al., 2017; Rusanowska et al., 2018) as well as the net energy balance. Cadoche and López (1989) reported, for example, that a twofold reduction in particle size of LARs, including straw and corn stover, can double the process energy consumption. It is necessary, therefore, to examine the trade-off between the energy required for particle size reduction and the additional bioenergy yield obtained with a smaller particle size and higher bulk density, to determine the most appropriate

particle size for LARs by large-scale alkali pretreatment and AD. The data presented in Table 2.8 for bulk density and biogas yields were extrapolated and calculated using data from published literature (Sharma et al., 1988; Liu et al., 2016) and provide an example of the effect of reducing the particle size of wheat straw from 5 mm to 1 mm on the overall net energy balance of the AD process. The amount of possible electricity conversion from the AD was calculated using Equation 2.1 (Ayodele et al., 2018). The net energy values in Table 2.8 were calculated as the difference between the potential energy from electricity generation via AD of wheat straw and the energy consumed during milling to reduce its particle size.

$$Ep = \frac{CH_4 \times Eff \times LHV_{CH_4} \times CF}{3.6} \quad (2.1)$$

where Ep is the amount of possible electrical energy from the AD (kWh), CH_4 is the volume of CH_4 production (m^3), Eff is the electricity generation efficiency of the conversion machine (%), LHV_{CH_4} is lower heating value of CH_4 ($MJ\ m^{-3}$), CF is capacity factor, and 3.6 is conversion factor from MJ to kWh.

Table 2.8 Net energy balance comparison for anaerobic digestion of wheat straw at two particle size

Particle Size (mm)	Bulk Density (kg TS m^{-3}) ^a	Biogas Yield (mL g^{-1} TS) ^b	Substrate Fed (kg TS) ^c	Energy for Milling (kWh) ^d	CH_4 Produced (m^3) ^e	Electricity Conversion (kWh) ^f	Net Energy (kWh)
1	313.0	350.0	313.0	2.4	38.3	121.2	118.8
5	209.3	330.0	209.3	1.3	24.2	76.4	75.2

^a Values are obtained by extrapolating from the equation provided in (Liu et al., 2016); applicable to dried straw, TS: total solids

^b Source: Sharma et al. (1988)

^c Substrate mass for 1 m^3 volume

^d Specific energy consumption of the knife mill for particle reduction was interpolated from values provided by Cadoche and López, (1989) : 7.8 kWh $tonne^{-1}$ for 1 mm particles and 6.1 kWh $tonne^{-1}$ for 5 mm particles

^e Biogas produced per 1 m^3 fed, considering 70% AD efficiency (Shen et al., 2019) and CH_4 content = 50%

^f Electricity conversion from CH_4 production volume, electricity generation efficiency (Eff) from an AD plant assumed to be 36% (Voegeli et al., 2014), capacity factor (CF) = 85% (Hadidi and Omer, 2017), $LHV\ CH_4 = 37.2\ MJ\ m^{-3}$ (Ayodele et al., 2018).

Sensitivity analysis involved assessing the impact of higher specific energy consumption in hammer mills (90–130 kWh t^{-1}), aimed at achieving particle size of 1–2 mm, suitable for medium to large-scale AD plants (Dell’Omo and La Froscia, 2018). The findings indicate that

at energy consumptions of 90 and 130 kWh t⁻¹, the net energy balance changes to 93.1 kWh and 80.5 kWh, respectively, with both, however, remaining positive. This analysis shows the major potential benefits of particle size reduction on the AD of LARs, which are primarily explained by a 50% increase in bulk density and related solids loading rate that can be achieved at the finer particle size. This effect was much greater than the relatively modest increase in biogas yield with particle size reduction, and greatly outweighs the energy consumption for milling wheat straw from 5 mm to 1 mm.

2.5 Waste feedstocks with potential to enhance anaerobic digestion of lignocellulosic agricultural residues

2.5.1 Waste feedstocks as co-digestion substrates

Lignocellulosic agricultural residues are characterized by a high C/N ratio, high lignin content, poor TEs composition, all of which can be a limiting factor in the AD process (Bandgar et al., 2022; Mosadegh Ranjbar et al., 2022; Karrabi et al., 2023). Mono-digestion of LARs is often slow to initiate due to the low biodegradability of lignin, resulting in reduced CH₄ production (Mosadegh Ranjbar et al., 2022; Karrabi et al., 2023; Akbay, 2024). Anaerobic co-digestion, refer to simultaneous digestion of two or more substrates, offers a more efficient solution to these limitations harnessing synergistic effects that improve the feedstocks characteristic leading to enhanced CH₄ production. A selection of feedstocks reported as potential co-substrates for the co-digestion of LARs is now described.

2.5.1.1 Animal manure

Animal manure is a suitable co-substrate in the co-digestion of LARs, due to its multiple benefits. It provides buffering capacity by supplying sufficient carbonate, phosphate, ammonium (Ferdeş et al., 2023; Song et al., 2023). Additionally, animal manure increases a diversity of microbial consortium, as it is rich in methanogens and showed high microbial activity from the digestive tract of animals (Bandgar et al., 2022; Ferdeş et al., 2023; Song et al., 2023). Furthermore, the low C/N ratio in animal manure makes it an ideal complement for balancing the C/N ratio in LARs co-digestion (Mosadegh Ranjbar et al., 2022; Song et al., 2023). The synergistic effect in the AD processes between these substrates is shown by Elsayed et al. (2023) who reported that co-digestion of cow manure and wheat straw led to 75% increase in CH₄ yield and higher CH₄ content compared to mono-digestion of wheat straw.

2.5.1.2 Food waste

Food waste (FW) is highly biodegradable due to its high content of carbohydrate (12–74%), protein (14–18%) and lipid (4–34%) (Ferdeş et al., 2023). It is also rich in macronutrients, particularly N, which helps balance the C/N ratio during co-digestion with LARs (Song et al., 2023). Food waste is also rich in growth factors readily available to microbial populations, such as amino acids, purines, vitamins) (Ferdeş et al., 2023). Moreover, co-digestion FW with LARs provides mutual benefits by addressing common challenges in FW mono-digestion, by diluting VFAs and NH_3 , thereby reducing the risks of acidification and NH_3 toxicity (Kainthola et al., 2020; Shimizu et al., 2022).

Studies have shown the advantages of LARs co-digestion with FW. For example, co-digestion of wheat straw with FW resulted in a 18.3% increase in CH_4 yield compared to wheat straw digestion alone. Mono-digestion of wheat straw exhibited a lag phase up to 5 d, probably due to its recalcitrance, while co-digestion with FW enabled immediate biogas production, driven by the readily biodegradable nature of FW (Rahmani et al., 2022). Another study reported that co-digestion of rice straw with FW lead to a 71% increase in CH_4 yield compared to rice straw mono-digestion, attributed to the balanced the C/N ratio (Kainthola et al., 2020).

2.5.1.3 Sewage sludge

Synergistic effects due to co-digestion of LARs with sewage sludge typically arise from several factors: (i) improving the overall C/N ratio of mixtures due to the balanced nutrients provided by sewage sludge (ii) maintaining alkalinity and stabilising pH value during the AD process, aided by the total ammonia nitrogen supplied by sewage sludge, and (iii) reducing the risk of NH_3 inhibition commonly associated with mono-digestion of sewage sludge (Chen et al., 2020; Azarmanesh et al., 2023; Akbay, 2024). The typical C/N ratio of sewage sludge is 6–7 (Potdukhe et al., 2021; Li and Huang, 2024), when mixed with the LARs in specific proportions, this improves the overall C/N ratio, bringing it closer to the optimum range for AD (20–30) (see Section 2.3.4.5).

Anaerobic co-digestion of sewage sludge with rice straw has led to CH_4 yield up to twice as high as that from rice straw digestion alone (Potdukhe et al., 2021). In contrast, the lower CH_4 yield and CH_4 content observed during mono-digestion of rice straw can be explained by the slow and inefficient hydrolysis of lignocellulosic materials (Potdukhe et al., 2021). Li and Huang (2024) observed that co-digestion not only enhanced CH_4 yield by 7.2–61%, but also improved hydrolysis rate by 15%. An increase in the hydrolysis rate and CH_4 production by

17% was also reported by Chen et al. (2020) when wheat straw was co-digested with sewage sludge.

In addition to supplying various nutrients, sewage sludge is also rich in microorganisms (Akbay, 2024). Co-digestion of corn stover and sewage sludge was found to enrich the relative abundance of *Candidatus_Microthrix*, a hydrolytic microorganism, suggesting enhanced hydrolysis during the AD. The process also promoted *Bacterioidetes_vadinHA17*, a bacteria capable of degrading recalcitrant compounds such as LARs. This microbial enrichment led to more effective corn stover degradation, as also indicated by the higher VS removal observed in the co-digestion compared to mono-digestion (Li and Huang, 2024).

Sewage sludge, however, can contain pathogens and various inhibitory substances that may hinder microbial activity during AD, such as heavy metals, antibiotics, detergents, and solvents. While co-digestion with LARs, can dilute and reduce these inhibitory risks, their presence can still be a concern (Ferdeş et al., 2023).

2.5.2 Waste feedstocks with task-specific properties to improve sustainability and efficiency of the AD process

2.5.2.1 Coal fly ash

Overview

Coal accounted for approximately 30% of the world's total energy supply in 2019, representing the primary energy sources (IEA, 2021). Oil and natural gas contributed 31% and 23%, respectively, to the energy mix, followed by biomass (9.4%), nuclear (5%), hydro (2.5%) and other sources (2.2%) (IEA, 2021). The combustion of coal in coal-fired power plants generates coal ash, generally consisting of fly ash, bottom ash, and flue gas desulfurisation products. Coal fly ash (CFA) refers to particulate matter residues carried over from the combustion chamber and subsequently removed from the flue gas stream. This separation process occurs within air pollution control systems, such as electrostatic precipitators, where CFA is filtered and collected. Coal fly ash is typically grey in colour, predominantly composed of hollow-glassy spheres with diameters ranging from 0.1 μm to over 100 μm (Achternbosch et al., 2015; Zierold and Odoh, 2020).

General physicochemical properties

Coal fly ash is typically composed of approximately 60–90% amorphous glassy fraction, with silica (Si) and aluminium (Al) as the major elements and lower levels of Ca and Fe (Sear et al., 2003; Mushtaq et al., 2019; Lauzurique et al., 2021a). The pH of CFA can vary between

4.5 and 12, depending on the balance between Ca and sulphate (SO_4^{2-}). Coal fly ash with a high pH level reaching up to 12 indicates that Ca content dominates leaching over sulphur (S), with the ratio of Ca/S $\gg 1$ (Izquierdo and Querol, 2012). This agrees with Sear et al. (2003) that the pH of ash-water systems is significantly influenced by the presence of Ca^{2+} and SO_4^{2-} . Despite the wide variation in pH, most CFA globally has strong alkaline characteristic (Ahmaruzzaman, 2010; Achternbosch et al., 2015; Zierold and Odoh, 2020).

The chemical elements composition of fresh CFA varies largely influenced by the type of coal and quality, combustion and cooling conditions, and types of air pollution control systems (Kukier et al., 2003; Sear et al., 2003; Achternbosch et al., 2015; Alterary and Marei, 2021). Table 2.9 illustrates the variation in chemical composition in CFA from different coal types.

Table 2.9 Typical oxides composition of coal fly ash from different coal types

Elements (as % oxides)	Bituminous/anthracite	Sub-bituminous ^a	Lignite
SiO ₂	12.8–67.0	40.0–60.0	7.5–59.0
Al ₂ O ₃	8.0–34.9	20.0–30.0	4.1–31.5
Fe ₂ O ₃	10.0–40.0 ^b	4.0–10.0	7.2 ^c
CaO	0.2–17.1	5.0–30.0	1.4–40.0
K ₂ O	0.2–4.3	0.0–4.0	0.2–2.5
MgO	0.3–5.7	1.0–6.0	0.7–7.3
TiO ₂	1.2 ^d	-	1.5 ^d
SO ₃	0.3–14.9	0.0–2.0	1.5–36.3
Na ₂ O	0.1–5.0	0.0–2.0	0.1–5.1
P ₂ O ₅	0.3 ^d	0.9–1.2	0.5 ^d
LOI	0.0–15.0 ^b	0.0–3.0	4.5 ^e

^a All values are from (Liu et al., 2020), ^b (J. Li et al., 2018), ^c (Akar et al., 2012), ^d (Skousen et al., 2012), ^e (Qureshi et al., 2016), other values from (Achternbosch et al., 2015).

Alkaline properties of coal fly ash

The alkaline properties of CFA are primarily attributed to the presence of alkaline elements such as Ca, Na, K and magnesium (Mg) (Schramke, 1992; Achternbosch et al., 2015). The alkalinity contribution of Na, K and Mg, however, is minimal as they are tightly bound within the glass matrices of CFA, leading to low leachability (Kim, 2006; Achternbosch et al., 2015; Seki et al., 2019). During a 24 h water extraction, the leachability concentrations of Na and K were found to be 12 to 33 mg L⁻¹ and 1 to 19 mg L⁻¹, respectively (Sear et al., 2003). In contrast, Ca leaches more rapidly compared to Na, K and Mg, due to its enrichment on the particle surface and as the most water-soluble element within CFA (Izquierdo and Querol, 2012; Jones et al., 2006; Sear et al., 2003). Typically, 15 to 216 mg L⁻¹ of Ca is found in

extracted water (Sear et al., 2003), with values much higher than that of Na and K, making Ca the primary contributor to CFA alkalinity (Achternbosch et al., 2015).

Calcium in CFA originates either from calcite in feed coal or from additives introduced during combustion to capture sulphur dioxide (SO₂) and prevent its emission from being carried over in the flue gas stream (Sotiropoulos et al., 2005; Akar et al., 2012; Skousen et al., 2012; Bartoňová, 2014). Calcium in CFA is predominantly present as lime (CaO), portlandite (Ca(OH)₂), anhydrite (CaSO₄), and within glassy matrices (Schramke, 1992; Izquierdo and Querol, 2012; Achternbosch et al., 2015). The CaO content in CFA varies widely, ranging from 0.2 to 30.6% (Moreno et al., 2005; Sutter et al., 2013; Achternbosch et al., 2015), with free CaO content in CFA worldwide reaching up to 15% (Izquierdo and Querol, 2012; Achternbosch et al., 2015), and for lignite CFA, it can be as high as 25% (Izquierdo and Querol, 2012; Achternbosch et al., 2015). Free CaO is a more suitable parameter for predicting CFA alkalinity, as it readily reacts with water to form hydroxide ions, contributing to CFA alkalinity (Sear et al., 2003; Achternbosch et al., 2015).

Trace elements in coal fly ash and potential contaminant constraints

Despite the variation in the composition and properties of CFA, studies show that the leaching behaviour of various elements across CFA types follows a consistent general trend (Izquierdo and Querol, 2012). The leaching behaviour of an element depends on its distribution within the CFA particles and pH of the ash-water system. Typically, only 1–2% of the elements within CFA are found to be leachable, including Ca²⁺ and SO₄²⁻ as the predominant leachable species upon reaction with water (Sear et al., 2003; Jones et al., 2006; Izquierdo and Querol, 2012). The low leachability of most elements in CFA is due to their encapsulation within insoluble and glassy aluminosilicate matrices (Sear et al., 2003; Jones et al., 2006).

Aside from the alkaline elements present in CFA, it also contains TEs that are essential for microorganisms (e.g., Fe, Co, Ni, Mo, selenium (Se), tungsten (W)). Table 2.10 lists the typical concentrations of TEs and other minor elements contained in CFA around the world. Generally, the leachability of most TEs is limited at high pH levels (Sear et al., 2003; Izquierdo and Querol, 2012), with exceptions for Se and Mo. These elements are distributed on the particle surface and have higher leachability in the neutral and slightly alkali pH than in acidic conditions. For example, Mo reached its highest leachability at pH 6.5 (Ward et al., 2009; Izquierdo and Querol, 2012).

Table 2.10 Minor elements in coal fly ash

Minor Elements in Coal Fly Ash	mg kg ⁻¹ TS
Zn	74.0–360.0
Cu	38.0–176.3
Ni	19.6–145.0
Cd	0.4–14.9
Pb	33.6–149.8
Cr	46.0–220.1
As	0.0–33.2
Se	2.3–7.7
Mo	3.3–37.6
W	2.5–14.5
Fe	3.1–290.0
Co	17.0–77.1

Compiled from the following: (Tsiridis et al., 2006; Lopareva-Pohu et al., 2011; Shaheen et al., 2014; Damayanti, 2018; Zhao et al., 2020).

In the context of integrating CFA in AD, particularly for the land application of the AD product (digestate), some elements in CFA may impose restrictions on digestate use. Special attention is given to potentially toxic elements (PTEs) such as chromium (Cr), cadmium (Cd), copper (Cu), Zn, lead (Pb), Ni, and arsenic (As), which are significant environmental contaminants (Achternbosch et al., 2015). The threshold of these elements, except for As, are regulated by national standards that set the criteria for the safe use of digestate, for example, PAS 110 in the UK (BSI, 2014). The solubility of potentially to PTEs in water, however, is pH dependent. The digestate from AD typically has an alkaline pH, like 8.3 (Makdi et al., 2012; Akbar et al., 2021), which limits the solubility of the PTEs it contains when in contact with water, thereby reducing the likelihood of leaching and soil contamination. Additionally, PTEs in CFA tend to be concentrated in the core of CFA particles, which is not directly exposed to leaching (Sear et al., 2003; Jones et al., 2006).

The leachability of Zn, Ni and Cd was minimal around pH 7–8 but increased at pH levels both above and below this point (Akar et al., 2012). The leachability of Cr increased with pH levels above 7 but decreased at pH 11 (Akar et al., 2012). This concurs with Deonaraine et al. (2015) who found total Cr predominantly exists in an insoluble state at pH 7. Zierold and Odoh (2020) reported that the leachability of Cr also depends on its oxidation state, with hexavalent Cr being more soluble than trivalent Cr. The maximum leachability of Cu occurs under acidic conditions (pH 4–5) and decreases as the pH increases. In contrast, the leachability of Pb remains minimal, as Pb is highly immobile irrespective of the pH (Izquierdo and Querol, 2012).

2.5.2.2 Food waste

Overview

Food waste is defined as food loss occurring at the later stages of the food chain, such as at retail and consumer stages (FAO, 2011). The largest share of FW comes from consumer stage, with households contributing 61% and food service (e.g., restaurants, hotels, education establishments) contributing 26%, while 13% comes from retail (UNEP, 2021). Annual global FW generation from all stages, from agricultural production to consumer stage reaches 1.3 billion tonnes (FAO, 2019).

General physicochemical properties

Food waste is a complex mixture of organic materials with easily biodegradable components, typically comprising carbohydrates (41–62%), proteins (15–25%), and fats (13–30%) (Slopiecka et al., 2022). This energy-rich composition, coupled with its high moisture content (74–90%) (Slopiecka et al., 2022), renders FW a suitable substrate for AD (Ariunbaatar et al., 2014; Lee et al., 2015). The recoverable energy content from FW is high, ranging from 425 to 530 mL g⁻¹ VS (Banks et al., 2010; Browne and Murphy, 2013; Paritosh et al., 2017; F. Xu et al., 2018), highly influenced by the heterogenous properties of FW (Browne and Murphy, 2013). Table 2.11 shows the typical physicochemical properties of FW; the pH of FW is generally low due to its high levels of organic acids originating from the food items, such as fruit, vegetables, and dairy products.

Volatile solids typically constitute 80–90% TS in FW, indicating its biodegradable nature (Lee et al., 2015; Slopiecka et al., 2022). The VS content in FW is like that in LARs (Section 2.1.3.1), however, unlike LARs, the carbon substrates in FW are not encapsulated by recalcitrant lignin, making FW highly susceptible to microbial attack, hence hydrolysis is not the limiting step in AD of FW (Li et al., 2016). The hydrolysis rates for carbohydrates, proteins, and fats vary, with carbohydrate degrading first and most rapidly, followed by proteins and fats being the slowest (Meng et al., 2015; Slopiecka et al., 2022). The C/N ratio of FW is low, in contrast to LARs, but like LARs, FW lacks the essential TEs required by anaerobic microorganisms (F.Xu et al., 2018; Odejobi et al., 2023).

The rapid degradation of easily digestible components in FW leads to the accelerated generation of VFAs, which can potentially cause rapid acidification. The accumulation of VFAs is the most common problem causing process instability in AD of FW, leading to an acidified digester. This VFAs accumulation can be attributed to two conditions: (i) too high organic loading (Phun et al., 2018), causing acid production exceeds the buffering system capacity

and the ability of methanogens to consume VFAs and (ii) inhibition to methanogenesis due to NH_3 toxicity (Banks et al., 2010; Banks and Zhang, 2010). Food waste is high in protein, resulting in a high N content, and its decomposition in AD generates NH_3 and NH_4^+ .

Table 2.11 General physicochemical properties of food waste reported in literature

Parameter	Concentration
TS (%)	23.2–25.9
VS (% TS)	92.7
Carbohydrates (% TS)	59.0
Lipids (% TS)	19.0
Proteins (% TS)	18.1
C (% TS)	42.2–48.8
H (% TS)	6.4–7.5
O (% TS)	34.7–47.6
N (% TS)	2.7–2.9
C/N ratio	10.5–31.2
pH	3.6–5.5
Minor elements (mg kg^{-1} TS)	
Cd	<0.4
Cr	1.8
Co	<2.0
Cu	<2.0
Fe	35.1–45.7
Pb	3.6
Mn	16.3
Mo	<6.0
Ni	<1.4
Se	0.1–0.3
Zn	8.8

Data from (Banks et al., 2010; WRAP, 2010; Browne and Murphy, 2013; Suhartini et al., 2019; Tassakka et al., 2019).

2.6 Summary

Lignocellulosic agricultural residues are substantial waste streams characterised by global abundance and carbon-neutral potential, reflecting their extensive availability and underutilisation. These residues possess significant potential for conversion into biofuels, particularly biogas, through AD. The conversion process however is challenged by the inherent recalcitrance of LARs, which impede effective degradation.

To address these challenges, this research investigates the application of a dual waste system of CFA and FW, which collectively have unique properties that can enhance the AD process. Coal fly ash shows potential as an alkali pretreatment agent, which can increase the

accessibility of LARs for microbial action. Meanwhile, FW can provide a source of acid to balance pH levels of high pH pretreated LARs during the digestion process, thereby improving overall AD efficiency.

The integration of these wastes not only addresses the technical barriers associated with low biodegradability of LARs, but also aligns with circular economy principles by minimising resource use and reducing waste. By incorporating CFA and FW, this approach develops a sustainable and efficient solution for the AD treatment of LARs. This approach also enhances the overall performances processes, contributes to resource conservation and promotes the sustainable management of biomass resources.

3. General Materials and Methods

3.1. Waste feedstocks source and preparation

The following waste feedstocks, barley straw, food waste, coal fly ash and digested sludge were used throughout the research. The details of their sources and preparation, as appropriate are described below, and the physicochemical properties of these waste feedstocks are determined and discussed later in this chapter.

3.1.1 Barley straw

Barley straw (BS), selected as a representative LAR to investigate the effect of coal fly ash (CFA) pretreatment on AD of this substrate, was sourced from a farm in Northamptonshire, UK, and delivered in batches (2 kg) every 6-8 months for the duration of the research. The material was dried at ambient temperature in the field to a moisture content below 12% and taken to the laboratory where it was milled using a cross-beater (hammer) mill (Glen Creston, Retsch GmbH, Germany) equipped with 1 mm screen to homogenise the bulk BS as a step towards sample preparation for fibre compositional analysis (aNDF, ADF and ADL determination) (BSI, 2008, 2006). The dried, milled BS sample is shown in Figure 3.1. The milled homogenised BS was used in the solid-state AD process and throughout analytical experiments to correlate the response of fibre compositional analysis with AD performance. From an experimental standpoint, smaller particle sizes enable maximum microbial activity and gas yield (Xiao et al., 2013; Chandra et al., 2015; Motte et al., 2015; Armah et al., 2019) while also reducing the experimental error through improved homogeneity of the material.



Figure 3.1 Prepared barley straw used in the study

The milled BS was stored, for subsequent use, in sealed polyethylene containers at 4°C, to prevent possible hydrolysis. Subsamples for experimental investigations were prepared by drawing equal portions from each container prior to feeding into the riffle sample divider, where the sample was divided into two equal portions. The dividing process was repeated using the

combined portions from the initial division; a precautionary step taken to minimise the risk of variation in sample properties between containers, to ensure that the subsamples are representative of the entire batch.

3.1.2 Food waste

Selected food stuffs were purchased from a supermarket to prepare a controlled composition feedstock, representative of FW in Indonesia based on details in work by Saputri et al. (2018). This approach was preferred to collecting FW from an outlet to minimise heterogeneity between experiments and avoid systematic errors that may be introduced with outlet-derived FW samples (Ariunbaatar et al., 2014). For the purposes of this research some simplification of the composition of the FW fractions was made. Table 3.1 provides detail of the FW composition drawn from the work of Saputri et al. (2018) and the simplified, prepared FW composition used in the current research.

Table 3.1 Composition of laboratory prepared food waste

Food components	% Fresh matter fraction	
	Values from Saputri et al. (2018)	Simplified composition in this study
Rice/noodles	45.0	57.7 ^a
Tubers and roots	0.5	N/A
Fruit and vegetables	19.9	33.2 ^b
Cereal and legumes	6.1	N/A
Meat and poultry	4.9	N/A
Fish and seafood	0.1	N/A
Egg	2.1	N/A
Milk and soup	21.3	N/A
Plant-based protein	N/A	9.1 ^c

N/A: not applicable.

^a Representing 45% of the total mass from Rice/noodles fraction + 12.7% of the total mass represented by the Milk and soup fraction.

^b Representing 26.5% of the total mass from Fruit and vegetables, Tubers and roots and Cereal and legumes fractions + 6.7% of the total mass represented by the Milk and soup fraction.

^c Representing 7.1% of the total mass from Meat and poultry, Fish and seafood and Egg fractions + 2% of the total mass represented by the Milk and soup fraction.

The primary components of the modified FW composition included the following components:

- Rice/noodles (cooked rice and cooked noodles) with a total weight of 1.7 kg
- Fruit and vegetables (mix of carrots, broccoli stalks, banana) with a total weight of 1 kg, and
- Plant-based protein (tofu) weighed to 0.3 kg.

The “Milk and soup” component listed in Table 3.1 as a single component was not included as a separate fraction but distributed proportionally based on weight fraction among the three major fractions of the modified FW composition.

The FW sample was prepared by blending the different food types together in a food processor to homogenise the material to form a mashed composition (Figure 3.2). Homogenised FW was transferred to sealed polyethylene bags, a vacuum was applied to provide anaerobic conditions, and the bags were incubated in a temperature-controlled incubator (MIR 253, Sanyo, Japan) at 30°C for 7 d; conditions designed to encourage microbiological anaerobic fermentation reactions to ferment the material and reduce the pH of the FW to around 3.7. The experimental design to optimise the FW incubation period for fermentation is described in Section 7.2.2. Following incubation, the fermented FW was divided into 250 g fractions with each fraction placed in a separate polyethylene bag and stored in the freezer at -18°C. A couple of days prior to use, the frozen samples were removed from the freezer and allowed to defrost at 4°C.

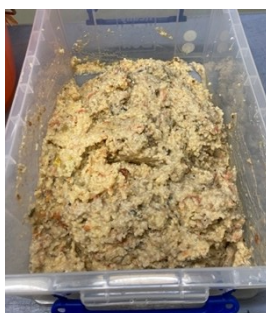


Figure 3.2 Laboratory prepared food waste

3.1.3 Coal fly ash

Coal fly ash was supplied from a coal-fired power plant in the UK. The as-received CFA samples (Figure 3.3) were collected from an ash collection bag in the power plant and stored in a sealed container to prevent leakage during transport and storage. The containers were labelled with a sample description and stored at room temperature until use. Prior to use the CFA was subsampled using a riffle sample divider to obtain representative samples and dried overnight at 105°C in an oven for subsequent use.



Figure 3.3 As-received coal fly ash from power plant

3.1.4 Digested sludge

Fresh digested sludge (Figure 3.4) for AD inoculation was obtained from the dewatering unit of two full-scale AD sewage sludge treatment plants. Digested sludge is recommended as the anaerobic microbiological inoculum source for use in AD experiments as it provides a broad and active anaerobic microbial culture able to degrade different types of organic substrates (Holliger et al., 2016; Raposo et al., 2011). In the AD plant, the sludge was digested for 12-15 d at mesophilic temperatures (37–40° C) before transfer to the dewatering unit (centrifuge). The digested sludge, referred to as inoculum, was collected from the centrifuge and transferred to polyethylene bottles of 500 or 1000 mL capacity. The containers were tightly sealed (with screw caps) and placed in a wheeled cool box for transport to the laboratory.



Figure 3.4 Digested sludge from sewage sludge anaerobic plant

Upon arrival at the laboratory, the sludge from all polyethylene collection bottles was transferred to a large bucket where the sludge was homogenised by mixing thoroughly using an electric hand blender; and a subsample of homogenised sludge was taken for TS, VS and pH analysis on the day of collection (see Section 3.3.2 and 3.3.3). The inoculum did not contain coarse inert materials therefore further preparation steps such as sieving were not necessary. Homogenised inoculum was stored in clean sealed polyethylene bottles in the incubator maintained at 30° C for a maximum period of 5 d, to follow procedures required to maintain consistent microbial activity in the inoculum source for batch AD experiments (Holliger et al., 2016; Koch et al., 2019).

3.2. Solid-state anaerobic digestion experimental procedures

3.2.1 Overview

Solid-state, air-tight and static microcosm AD reactors were constructed to quantify the effects of pretreatment and fermented FW neutralisation on the CH₄ yield of BS and fibre digestibility during the AD process. A solid-state system was selected due to its compatibility with feedstock containing over 15% TS, like LARs, and its benefits in reducing water usage, avoiding solid-liquid digestate separation, and optimising reactor volume and process efficiency (Riya et al., 2021; Rocamora et al., 2020) (see Section 2.3.4.6).

3.2.2 Experimental apparatus

Twelve solid-state AD reactors, made from borosilicate glass, each in 1 L in volume with a working volume capacity 0.3–0.4 L, were incubated in a temperature-controlled incubator (refer to Section 3.1.2 for the incubator type) at 30° C to simulate typical ambient conditions in Indonesia. Each reactor was equipped with five access ports in the reactor's glass lid: two for biogas sampling via a thick gas-tight butyl rubber septum, one connected to a water displacement system by silicon tubing (Saint-Gobain, France) with 5 mm internal diameter for biogas volume measurement, and two for feedstock feeding and nitrogen gas (N₂) flushing. The N₂ port was sealed with a screwcap adapter 19/26 joint and connected with a silicon tubing equipped with a metal clamp, which was kept closed when not in use. The feedstock feeding port was sealed with a glass stopper.

The water displacement system consisted of an inverted 500 mL high density polyethylene (HDPE) bottle (displacement bottle) filled with water, connected to the reactor by silicon tubing. A hypodermic needle was pierced through a rubber septum on the bottle lid to allow displaced water to drain into a pre-weighed receiver bottle, representing the biogas volume produced. The connection tube from the reactor to the water displacement system had a metal clamp that was closed during bottle refilling. The bottle was refilled by returning it to an upright position with the lid on top and opening the lid and filling with water. Refill frequency depended on gas production: 2–3 times per day during the initial high production phase and once daily as biogas productions slowed down. The schematic of the reactors and water displacement system along with close-up images of the sealant arrangements on the reactor's lid are shown in Figure 3.5.

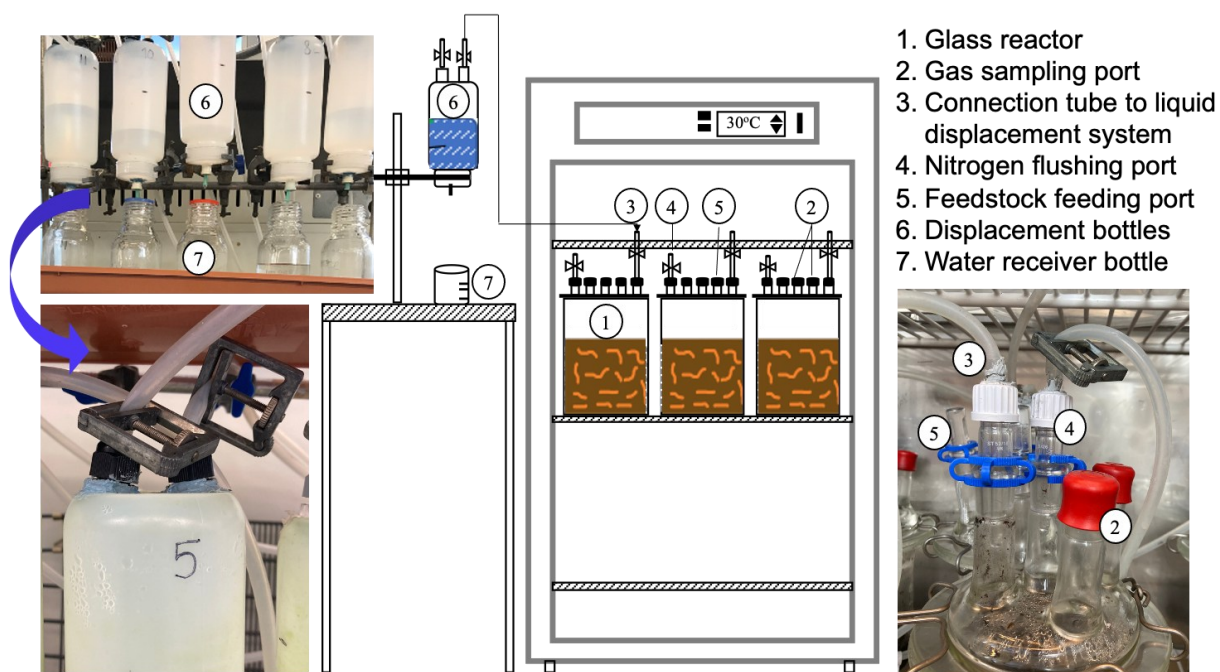


Figure 3.5 Schematic of the apparatus for batch solid-state mesophilic anaerobic digestion experiment fitted to a water displacement system to quantify CH₄ volume and the close-up picture of access ports in gas tight lid and water displacement bottle.

3.2.3 Operational procedure

The solid-state AD experiment was operated in batch mode with an inoculum-to-substrate ratio (ISR) of 1 on a VS basis, following recommendations for slowly biodegradable substrates like LARs (Holliger et al., 2016; Koch et al., 2019). After loading the feedstock and sealing the reactor's lid and four ports, the reactors were purged with N₂ for 10 minutes to establish an anaerobic environment, verified by an oxygen gas (O₂) analysis using a gas analyser (GA 2000 Plus, Geotech, UK). Reactors were manually shaken daily to release trapped gas or inhibitory substances as recommended by Angelidaki et al. (2009). The volume of biogas production is equivalent to the amount of water collected in the pre-weighed receiver bottle, which was weighed daily. Biogas samples (50 µL) were sampled weekly through the rubber septum using a 100 µL Hamilton gas-tight syringe (Hamilton, USA) for composition analysis by Gas Chromatography (GC) (See Section 3.3.13). The experiment concluded when the biogas curve plateaued or the daily biogas rate was below 0.5% of the total volume for three consecutive days, typically within 30–40 d (VDI, 2016).

3.2.4 Leakage test

Leakage is the main factor that can introduce significant errors in AD experiment (Holliger et al., 2016; Hafner and Astals, 2019). The AD apparatus was checked for leaks before each batch run. After sealing the reactors and connecting them to the water displacement system,

any constant water drops from the displacement bottle indicated a leak. Nitrogen gas was supplied to the reactor, and leak detection was performed simultaneously by applying a liquid leak detector (Swagelok, USA) around the joints to observe if froth formation occurred.

3.2.5 Methane data correction and reporting

The CH₄ data were reported as the cumulative specific methane yield (CSMY), calculated by subtracting the background CH₄ production value derived from the Blank test (see Section 3.2.6) from the total CH₄ production of the sample, then correcting to standard temperature (273.15 K) and pressure (101.33 kPa) (STP) conditions. Cumulative specific methane yield is defined as the total volume of CH₄ produced during the digestion period per unit of substrate initially added (mL CH₄ g⁻¹ VS). The correction to the experimental CH₄ data was performed using Equation 3.1 and 3.2 (Angelidaki et al., 2009; Holliger et al., 2016; Filer et al., 2019).

$$V_{STP} = V_{gas} \times \frac{(P_{gas} - P_{vap})}{P_{STP}} \times \left(\frac{T_{STP}}{T_{gas}} \right) \quad (3.1)$$

$$P_{vap} = 10^{8.1962 - \left(\frac{1730.63}{T_{gas} - 39.724} \right)} \quad (3.2)$$

where:

V_{STP} is the CH₄ volume corrected to standard temperature (T_{STP}) and pressure (P_{STP}), P_{gas} is the pressure of the experimental CH₄ (kPa), V_{gas} is the experimental CH₄ volume (mL), T_{gas} is the experimental CH₄ temperature (K), P_{vap} is the water vapour pressure (kPa).

3.2.6 Blank test and positive control

A blank test with inoculum alone was conducted to determine background CH₄ gas production, which was subtracted from the CH₄ produced during BS digestion. A quality assurance test, also serving as a positive control, used standard substrate reference: microcrystalline cellulose (MCC) (Thermo Fisher Scientific, UK) to assess the inoculum activity, and verify the reliability of the AD apparatus (Holliger et al., 2016; VDI, 2016). Microcrystalline cellulose was used for its complete biodegradability and ability to achieve 100% conversion without fermenting too quickly (VDI, 2016). The accuracy and reliability of the experimental apparatus in Section 3.2.2 was validated if the experimental CH₄ yield reached 80–85% of the theoretical value (364–415 mL CH₄ g⁻¹ VS) (Holliger et al., 2016; VDI, 2016). The AD experiments with MCC were conducted at a mesophilic incubation temperature (37°C), matching the inoculum's source (see Section 3.1.4). Approximately 9 ± 0.5 g MCC was mixed with 100 mL water and

inoculum to achieve an ISR of 2:1, as this is a suitable ratio for easily degradable substrates like MCC (Koch et al., 2019; Raposo et al., 2011).

The CSMY from AD of MCC was 274 mL CH₄ g⁻¹ VS, which was 75% of the theoretical CH₄ yield (the CSMY curve profiles of experimental data replicates and modelled data are presented in Appendix A, Figure A.1). This result indicated the reliability of the AD experimental apparatus and the suitability of the inoculum, which provided an adequate microbial consortium for effective AD process. The marginal difference (of CSMY at 75% of the theoretical CH₄ yield) from the 80–85% yield requirement was probably associated with the nature of the solid-state AD system including less effective mass transfer compared to wet AD systems (Karrabi et al., 2023; Karthikeyan and Visvanathan, 2013; Riya et al., 2021; Rocamora et al., 2020) as well as the absence of a mixing mechanism, such as mechanical mixer or biogas recirculation.

3.2.7 Replicates

The solid-state AD experiments were performed in triplicate for each treatment condition applied to BS, including blanks and positive control, to enable statistical analysis of the collected data.

3.3. Analytical methods

3.3.1 Overview

Figure 3.6 shows a schematic summary of the systematic application of the different analytical methods applied in the research. These included procedures to characterise the physicochemical properties of waste feedstocks (Figure 3.6 (a)) and the inputs and outputs of the experimental pretreatment and AD processes (Figure 3.6 (b)). All analytical experiments were performed in triplicate.

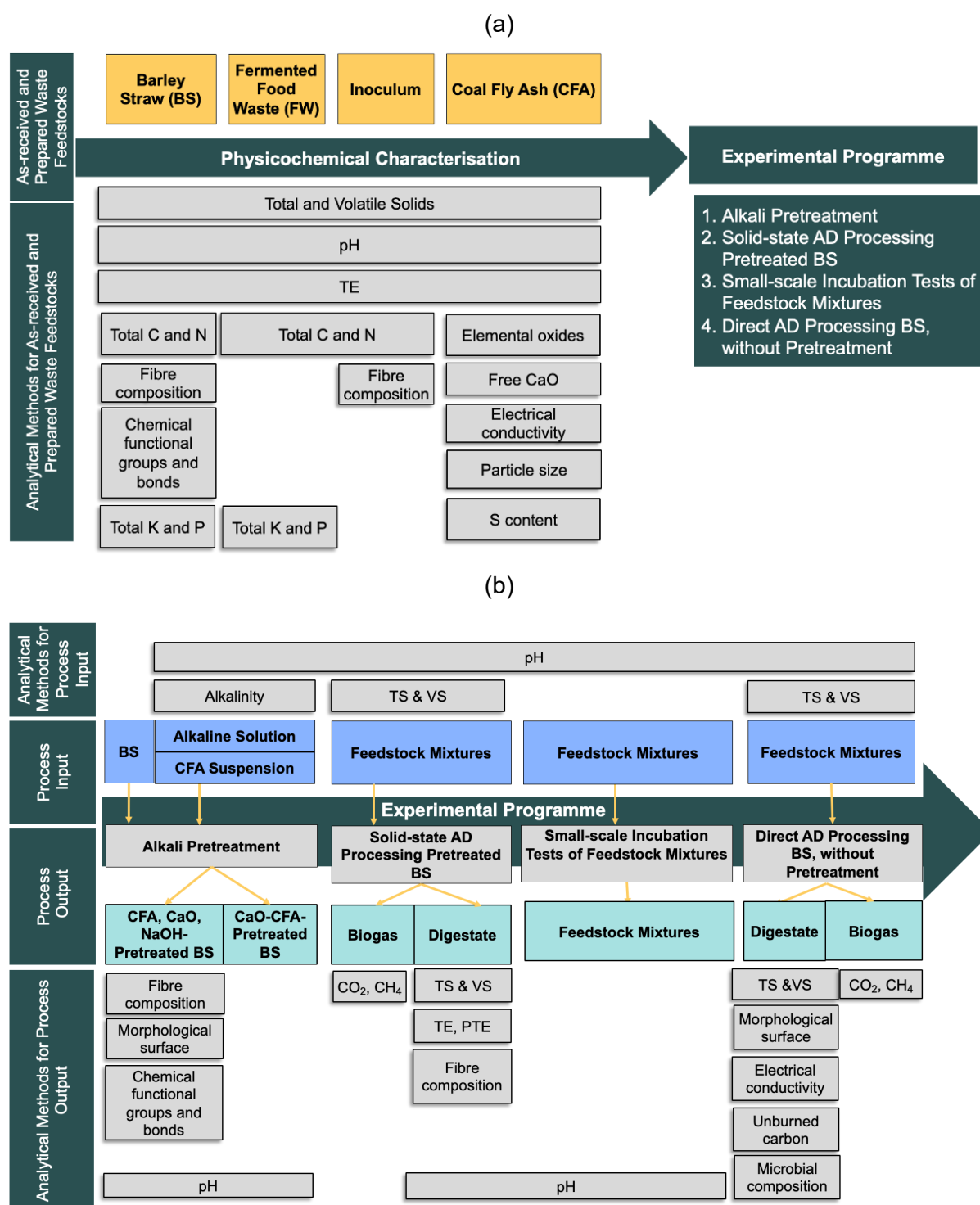


Figure 3.6 (a) Physicochemical characterisation of as-received and prepared waste feedstocks used in the research and (b) analytical methods applied to inputs and outputs of the experimental procedures.

3.3.2 Total solids (TS) and volatile solids (VS)

Total and volatile solids were determined using a gravimetric moisture determination method by adding 10–20 g of homogenised sample to a ceramic crucible and drying the sample in an oven (Gallenkamp, UK) at 105° C for overnight, followed by igniting the dried samples at 550°

C in a muffle furnace for 2 h (Gallenkamp, UK), according to standard methods 2540 B (APHA, 2005).

3.3.3 pH

For solid samples, the pH was measured according to BS EN 15933:2012 (BSI, 2012). A suspension was prepared by mixing 10–20 g of the sample with deionised water at a 1:5 (w/v) ratio and shaken using a reciprocating shaker (Laboshake, Gerhardt, Germany) for 1 h. The pH of the suspension was measured immediately using a calibrated pH meter (Jenway 3540, Appleton Woods Ltd, UK) using buffering solutions at pH 4, 7, and 10. For liquid samples, pH was measured directly on the solution according to the standard method (APHA, 2017) using the same pH meter calibrated with the same buffer solutions.

3.3.4 Particle size distribution

Particle size distribution was determined on dried as-received CFA samples using sieve analysis in accordance with BS EN 933-2:2020 (BSI, 2020). Sieves with aperture sizes of 1000, 710, 500, 355, 250, 180, 125, 90, 63, 45, and 20 μm . The pre-weighed sieves were stacked in decreasing size from top to bottom to facilitate sampling distribution, such that, for example, 300 g of oven-dried CFA was added to the top sieve, and the sieves were shaken for 30 mins using an automatic sieve shaker (Octagon Digital, Endecotts, UK) to distribute the sample fractions.

3.3.5 Fourier transform infrared spectroscopy (FTIR) and determination of total crystallinity index (TCI)

Fourier transform infrared spectroscopy spectra of untreated and pretreated BS were recorded using an iS50 Nicolet FTIR Spectrometer (Thermo Fisher Scientific, USA) fitted with a diamond window, over a wavenumber range of 4000–800 cm^{-1} . The FTIR spectra of three reference materials: (1) microcrystalline cellulose (Thermo Fisher Scientific, UK), (2) xylan from corn core (TCI, USA), and (3) lignin, alkali (Sigma Aldrich, US), were also recorded to identify the characteristic absorption bands of cellulose, hemicellulose, and lignin, respectively, and to determine the presence of these components and their functional groups in the BS sample. The spectra were manually corrected using Omnic software (Thermo Fisher Scientific, USA) and presented as absorbance values. The crystallinity index was estimated using the ratio of absorbance at 1375 cm^{-1} with 2900 cm^{-1} (A_{1375}/A_{2900}) (Nelson and O'Connor, 1964).

3.3.6 Scanning electron microscopy (SEM)

Scanning electron microscopy was used to examine the morphological changes on the BS surface before and after alkali pretreatments and to evaluate microbial attachment to CFA in the AD reactor. Samples were freeze-dried using a benchtop tray cell freeze dryer (Edwards Modulyo, Edwards Vacuum, UK) overnight prior to analysis. They were then mounted on aluminium stubs using carbon stickers and imaged using a tabletop microscope (TM4000 Plus, Hitachi, Japan).

3.3.7 Carbon and nitrogen elemental analysis

The total nitrogen (TN) content in BS, fermented FW and inoculum, as well as sulphur (S) in as-received CFA were determined using a FlashSmart organic elemental analyser (Thermo Fisher Scientific, Germany). Samples were freeze-dried (refer to Section 3.3.6 for the type of freeze dryer) to prevent organic elements loss. Prior to analysis, the sample particle size was reduced and homogenised using a laboratory disc mill (Siebtechnik Tema GmbH, Germany). Homogenised and dried samples of 0.2–0.3 g were weighed into a universal soft silver capsule (75 μ L volume). Certified reference material (Methionine ($C_5H_{11}NO_2S$)) (Thermo Fisher Scientific, Germany) was included as a Control before analysing 12 samples including BS, fermented FW and inoculum, to ensure the accuracy of the analytical procedures. For the organic C determination, samples of BS, fermented FW and inoculum, each weighing approximately 250 g fresh matter (FM), were sent to NRM Laboratories (Coopers Bridge, Braziers Lane, Bracknell RG42 6NS). The samples were shipped frozen, packed in an insulated polystyrene box with ice packs via same-day delivery post service. Organic C analysis followed the BS EN 15936:2012 standard, using dry combustion (Wilkinson, 2021).

3.3.8 Macro and minor elements

Trace elements (TEs) and potentially toxic elements (PTEs) were extracted from BS, CFA, inoculum and selected digestates using aqua regia in a microwave-assisted acid digestion process following ISO 12914 (ISO, 2012). Macronutrients potassium (K) and phosphorus (P) were extracted from BS and fermented FW. Aqua regia was used for its effectiveness in extracting metals bound to the crystalline and amorphous phases of CFA (Thanh et al., 2016; Zhao et al., 2018). Samples were freeze-dried, homogenised using a laboratory disc mill, and a 0.2–0.5 g of each sample was weighed into a pressure resistant polytetrafluoroethylene (PTFE) digestion vessel (50 mL capacity). Concentrated HCl (36% mass percent concentration (m/m), 6 mL), and nitric acid (HNO_3) (65% m/m, 2 mL), were then carefully added to the sample, and the vessel, sealed with a screw cap equipped with safety disc and release valve, was gently swirled. The samples were heated and digested in a microwave

digestion system (Multiwave 3000, Anton Paar GmbH, Germany) following a microwave programme according to a programme setting established by Wilson (2022). The programme involved ramping the temperature stepwise to 95°C in 3 mins (held for 5 mins), then to 120°C in 3 mins (held for 5 minutes), and finally to 130°C in 3 mins (held for 2 mins). After cooling at room temperature, the digestion vessels were carefully vented under a fume hood, and the samples were quantitatively transferred and made to volume with deionised water to 50 mL volumetric flasks. The diluted samples were filtered using a syringe filter through 0.45 µm cellulose nitrate membranes (25 mm in diameter) (Whatman, UK) into sample tubes.

Trace elements and major nutrients were quantified using an inductively coupled plasma optical emission spectrometer (ICP-OES) (AVIO 500, Perkin Elmer, USA). Calibration standards were prepared by mixing three different standards: multi-element 1000 mg L⁻¹ standard (Merck, Darmstadt, Germany), phosphorus standard (Sigma Aldrich, USA) and selenium standard (BDH, UAE) in 2M HNO₃ to match the acidity of the digested sample matrices. The calibration standards ranged from 0.1 to 2 mg L⁻¹ for minor elements and from 2 to 100 mg L⁻¹, for macro elements (Fe, K and P). Each batch of microwave acid digestions (16 samples) included a duplicate of method blanks, a blank spike, and a matrix spike as part of the quality control of the method performance to ensure the precision, accuracy, and sensitivity of the method due to the effect of the sample matrix as recommended in EPA 6010D (USEPA, 2018). All glassware and plasticware, including digestion vessels were soaked in dilute HNO₃ (10 %v/v) overnight, and thoroughly rinsed with deionised water, and dried in a laboratory drying oven before use.

3.3.9 Elemental oxides

Major and minor elemental oxides in pre-ignited CFA (refer to Section 3.3.11 for the ignition procedures) were measured using a Zetium X-ray Fluorescence (XRF) spectrometer (Malvern Panalytical, UK) according to ASTM D4326-04 (ASTM, 2004).

3.3.10 Free calcium oxide

Free CaO in CFA was determined following BS EN 451-1:2017 (BSI, 2017). A 20 g subsample of dried CFA was ground until it completely passed through a 63 µm sieve. From this, a 1 g sample was taken and reacted with 12 mL butanoic acid, 3-oxo-ethyl ester and 80 mL butan-2-ol in a round-bottomed flask and refluxed for 3 h on a heating mantle. The flask was connected to a spiral condenser, with a borosilicate glass joint filled with granulated NaOH to prevent reaction between the contents in the flask and atmospheric CO₂. After refluxing the mixture, the contents were filtered using a qualitative filter paper (Whatman, USA) placed in a

funnel and washed using 50 mL propan-2-ol. A few drops of bromophenol blue indicator were added to the filtrate, which was then titrated with 0.1M HCl until the colour changed to yellow. Dried reagent-grade CaO (purity >96% (Scientific Lab Supplies)) was included as a Control to verify the procedure's accuracy.

3.3.11 Loss on ignition

The loss on ignition (LOI) was measured to determine the unburned carbon (UC) in as-received CFA samples (Wang et al., 2008), following procedure outlined in (ASTM, 2019). About 1 g samples were dried in an oven at 100° C for overnight and then ignited in the furnace at 950°C for 6 h. The difference of weight before and after combustion was used to calculate LOI. The LOI measurement for particle-sized CFA fractions was conducted at the Solid and Hazardous Waste Laboratory, Institut Teknologi Bandung, Indonesia, using the same procedure applied to the as-received CFA sample.

3.3.12 Fibre composition analysis

The cellulose and lignin content of both prepared and alkali-pretreated BS, sludge and digestate were determined according to BS EN ISO 13906:2008 (BSI, 2008) for acid detergent fibre (ADF) and acid detergent lignin (ADL), and total fibre was quantified in accordance with BS EN 16472:2006 (BSI, 2006) for amylase-treated neutral detergent fibre (aNDF). The lignin fraction was represented by the fibrous material lost during ignition following ADL, while the residue following ADF determination primarily consisted of cellulose and lignin. Cellulose content was calculated by subtracting the value of ADL from ADF, and hemicellulose content was obtained by subtracting ADF from the fractions of total fibre (aNDF).

For aNDF analysis, 1 ± 0.001 g of dried sample (see Section 3.1.1 for BS sample preparation) was weighed into a 500 mL round-bottomed flask and treated with neutral detergent solution to remove nonfibrous carbohydrates, proteins and lipids. The sample was refluxed for 1 h on a heating mantle, with a condenser attached to the flask, and after 5 mins, 2 mL of amylase solution (Novozymes Termamyl α -amylase) was added to remove starch. After 1 h, the sample was quantitatively transferred to a 50 mL fritted disc crucible (P2; pore size of 40 μ m to 100 μ m) placed on a Buchner funnel, and boiling water was added until the crucible was half full. After adding 2 mL of amylase solution, the sample was reacted for at least 60 s. The fritted disc allowed complete drainage of water in $180 \text{ s} \pm 60 \text{ s}$. Vacuum was applied to remove the amylase solution, followed by repeated separate soaking in boiling water and acetone to ensure removal of detergent solution and soluble matter from the crucible after 60 s reaction,

before boiling water was added again to soak the sample until it drained away without vacuum. The crucible was dried overnight at 105°C in a laboratory oven. The aNDF fraction was calculated using Equation 3.3. For quality control, a duplicate method blank was measured at the beginning and end of fibre composition analysis. This quality control procedure was performed twice each for the experiments detailed in Chapter 4 and Chapter 5.

$$aNDF (\%DM) = \frac{m_3 - m_2 - mb}{m_1} \times 100 \quad (3.3)$$

where m_3 is the mass of the sample after extraction and drying (g), m_2 is the mass of the dried crucible, m_1 is the mass of the sample tested (g), mb is the mass of the blank crucible after extraction and drying subtracted by the mass of blank dried crucible.

For ADF, the dried sample was reacted with acid detergent containing 0.5 M sulphuric acid (H_2SO_4) to remove acid-labile organic matter, then refluxed for 60 mins using the same technique as for aNDF determination. The sample was quantitatively transferred to a fritted disc crucible and soaked in boiling water for 180 ± 60 s, after which the water was drained. These steps were repeated with acetone, and after draining, the sample was dried at 105°C overnight until the weight was constant. Acid detergent lignin was measured by reacting the cooled, dried ADF sample with 12 M H_2SO_4 for 3 h, followed by washing with boiling water until neutral pH of water drained from the bottom of crucible was achieved to a litmus paper. The crucible was securely held in place by a rubber adapter positioned between the crucible and the flask, enabling pH measurement to be conducted on a smaller batch of the drained water volumes for more precise pH changes. The crucible was dried overnight and ignited at $525^\circ C \pm 15^\circ C$. Blanks for both ADF and ADL determination were included every 10 samples, and data obtained from the blanks were used in calculations. Acid detergent fibre and ADL were calculated using Equations 3.4 and 3.5, respectively.

$$ADF (\%DM) = \frac{m_2}{m_1} \times 100 \quad (3.4)$$

$$ADL (\%DM) = \frac{m_3 - m_4}{m_1} \times 100 \quad (3.5)$$

where m_1 is the mass of the dried sample (g), m_2 is the mass of the sample after extraction and drying (g), m_3 is the mass of the sample after 12M H_2SO_4 extraction and drying (g), m_4 is the mass of the residue after ignition (g).

3.3.13 Biogas composition

Biogas composition was analysed using a GC (Clarus 600 Gas Chromatograph, (Perkin Elmer, USA), fitted with a fused silica capillary column (Carboxen-1010 PLOT) and thermal conductivity detector (TCD). The injector and detector temperatures were set at 200°C and 230°C, respectively. The oven temperature was ramped from 35°C (held for 8 mins) to 120°C (held for 3 mins) and then to 220°C held for 1 min. Helium was used as the carrier gas at a flow rate of 3 mL min⁻¹. The method was based on the product information from the GC column supplier (Supelco, 2003) with slight modification which included holding the oven temperature at specific points to ensure peak appearance. Calibration was done using standard gases containing CH₄, CO₂ and N₂.

3.4. Statistical methods and CH₄ gas modelling

The statistical significance of the pretreatment effects on various parameters such as fibre composition, mass loss, VS removal, CH₄ yield, kinetic parameters across multiple treatment groups, was assessed using one-way analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. Post hoc Tukey's test was subsequently applied to identify specific groups showing significant differences from others. For comparisons between two groups, a two-tailed paired t-test with $\alpha = 0.05$ was used.

To estimate kinetic parameters such as maximum specific CH₄ yield ($CSMY_o$) and hydrolysis rates constants (K_H and k) from the experimental CH₄ data, two kinetic models were used: the First-order and Cone kinetic models. The First-order kinetic model is commonly used, with the assumption that hydrolysis determines the behaviour of the entire process or when hydrolysis is the rate-limiting step in the AD of substrates like LARs (Bolado-Rodríguez et al., 2016; Karki et al., 2022; Rakić et al., 2023). The Cone model is an empirical model designed to assess CH₄ production from substrates in the presence of rumen microbes and estimate CH₄ production dynamics through a shape factor (n) (Karki et al., 2022). The equation for the Cone and First-order kinetic models are shown in Equation 3.6 and 3.7, respectively.

$$CSMY = \frac{CSMY_o}{1 + (K_H t)^{-n}} \quad (3.6)$$

$$CSMY = CSMY_o (1 - e^{-kt}) \quad (3.7)$$

where $CSMY$ is the cumulative specific CH_4 yield at time t , $CSMY_o$ is the maximum CH_4 yield potential ($mL\ CH_4\ g^{-1}\ VS$), k and K_H are the hydrolysis constant (d^{-1}) derived from First-order kinetic and Cone kinetic model, respectively, n is the shape factor and e is the exp (1) = 2.718.

To statistically compare $CSMY_o$ and hydrolysis rates across treatment groups, both models were fitted to each individual replicate of experimental CH_4 data within the same treatment group. Additionally, both models were fitted to the pooled triplicate experimental CH_4 data to estimate a single representative $CSMY_o$ and the hydrolysis rates from each treatment group. The model fitting employed a least squares methodology (Cano and Nielfa, 2014) in statistical software SPSS 28.0, with a significance level set at $\alpha = 0.05$.

This CH_4 gas modelling was conducted following the exclusion of outlier from the triplicate experimental CH_4 data, if the relative standard deviation (RSD) for the population, calculated as the standard deviation divided by the mean exceeded 10%, in accordance with the method outlined by Holliger et al. (2016). The final $CSMY_o$ and hydrolysis rates were selected based on the model that provided the best fit to the experimental CH_4 data. It is important to note that the variation between the model-estimated $CSMY_o$ and experimental $CSMY$ ($CSMY_{exp}$) should not exceed 5% (Bose et al., 2021).

3.5. Physicochemical properties of waste feedstocks

The physicochemical properties of milled BS, as-received CFA, inoculum and fermented FW are shown in Table 3.2. Barley straw contains 86% TS of total fibre, consisting of 44.3% TS cellulose, 33.1% TS hemicellulose, and 8.6% TS lignin. Cellulose and hemicellulose contents are 4–10% above the typical ranges of 34–42.5% TS and 19–30% TS, respectively; in contrast, lignin content falls within the reported 7.8–15% TS in the literature (Dinuuccio et al., 2010; Iroba et al., 2013; Raud et al., 2021; Rosgaard, 2007; Saidur et al., 2011). In contrast to BS, inoculum contains a low total fibre which accounts for 38% TS – comprising cellulose, hemicellulose and lignin on a TS basis of 19.4%, 4.5%, and 13.9%, respectively. These contents are in good agreement with typical values reported for digested sewage sludge ranging from 2–30%, 5–10% and 10–30%, respectively (Xiao et al., 2020; Krueger, 2021; Liu and Smith, 2022).

Table 3.2 General physicochemical properties of barley straw, coal fly ash, inoculum and fermented food waste.

Parameter	Barley Straw	Coal Fly Ash	Inoculum	Fermented Food Waste
pH	6.4 ± 0.2	12.1 ± 0.2	8.4 ± 0.1	3.6 ± 0.1
TS (%)	94.1 ± 1.7	99.7 ± 0.0	20.1 ± 1.2	21.5 ± 4.0
VS (% TS)	92.6 ± 2.9	6.8 ± 0.1	68.5 ± 5.3	96.7 ± 2.8
Organic carbon (% TS) ^a	40.3 ± 0.9	N/A	30.2 ± 2.2	45.7 ± 0.5
Total nitrogen (% TS)	0.4 ± 0.1	N/A	4.4 ± 0.9	2.1 ± 0.1
Sulphur (% TS)	N/A	0.03 ± 0.0	N/A	N/A
C/N ratio	115.1	N/A	6.9	21.8
Cellulose (% TS)	44.3 ± 4.4	N/A	19.4 ± 0.8	N/A
Hemicellulose (% TS)	33.1 ± 4.5	N/A	4.5 ± 0.5	N/A
Lignin (% TS)	8.6 ± 0.4	N/A	13.9 ± 1.2	N/A

TS: Total solids, VS: Volatile solids, C/N: carbon-to-nitrogen, N/A: not applicable. Values are presented in mean ± standard deviation (n=3). ^aOrganic carbon was determined in duplicate by an external laboratory.

Barley straw showed high levels of TS and VS, constituting 94.1% of the fresh matter (FM) and 92.6% TS, respectively. These values align with other studies reported 90.5-94.5% TS and 81.8-94.3% VS (Dinuccio et al., 2010; Zareibezini and Yaparla, 2013; Bachmann et al., 2022). The TS content of BS is higher than the typical 20% TS for solid-state AD, while the TS in the inoculum and fermented FW were slightly below this threshold, allowing the AD experiment to operate under solid-state conditions. The high pH of CFA (12.1) indicated its suitability for alkali pretreatment, contrasting to the low pH of fermented FW (3.6) confirmed for the successful acidification achieved during its preparation.

Barley straw is found to be deficient in TN content (0.4%), which is contrast with its high organic C content of 40.3%. Both the C and N content in BS are slightly lower than the reported values of 0.6% N and 43–49% C (Saidur et al., 2011; Marin-Batista et al., 2021; Raud et al., 2021; Meyer et al., 2022), resulting in a high C/N ratio of 115.1. These differences from the values reported in the literature can be attributed to factors such as the variety of BS and the difference in growth conditions (Van Dyk and Pletschke, 2012; Sawatdeenarunat et al., 2015; Jönsson and Martín, 2016). The C/N ratio of BS, however, is consistent with the reported range of 80–145 (Meyer et al., 2022), contrasting with the much lower C/N ratio of 7.0 detected in the inoculum. The TN content in the inoculum is 4.4%, consistent with reported values ranging between 4.1 and 6.9% (Facchin et al., 2013; Mancini et al., 2018a; Lallement et al., 2021).

The organic C and TN content of the laboratory-prepared fermented FW are consistent with reported values for university canteen waste in Indonesia (42.23–50.8% C and 2.1–2.71% N) (Suhartini et al., 2019; Tassakka et al., 2019; Zhen et al., 2020). The high C/N ratio of BS would be a limiting factor in AD, where the optimal C/N range is 20–30 (Wang et al., 2014; Mao et al., 2015) (see Section 2.3.4.5), however, amending BS with inoculum at the applied ISR and FW at specific rate, may correct this imbalance.

Many studies have conducted elemental analysis for FW from Indonesia (Suhartini et al., 2019; Tassakka et al., 2019), however, data reported in the literature on P and K content are scarce. The P and K content in the analysed FW are 1,344 mg kg⁻¹ and 2,916 mg kg⁻¹, respectively, which falls within the reported range of 350–1,800 mg kg⁻¹ and 2,000–3,300 mg kg⁻¹ (WRAP, 2010). For BS, the K content is 10,196 mg kg⁻¹, falling within the reported range of 9,000–11,000 mg kg⁻¹ for BS grown in England (Withers, 1991), however, the P content, measured by 472.7 mg kg⁻¹, is lower than the 700 mg kg⁻¹ reported by Withers (1991).

Table 3.3 shows the total concentration of essential TEs (Fe, Mn, Mo, Se, Ni, Zn and Cu) and PTEs (Co, Cr, Cd, Pb, as well as Cu and Zn when present in excess of AD requirements) in BS, CFA and inoculum. The TEs in BS are found to be low relative to the TEs requirements by anaerobic microorganisms (presented in Table 5.1), as reported in the literature (Hinken et al., 2008; Liu et al., 2015).

Table 3.3 Total concentration of macro and micro elements in waste feedstocks

Metals (mg kg ⁻¹ TS)	Barley Straw	Coal Fly Ash	Inoculum
Fe	64.1 ± 6.9	29,185.0 ^a	17,141.3 ± 915.4
Mn	20.4 ± 2.4	284.3 ± 13.3	279.7 ± 102.3
Ni	2.3 ± 0.4	17.9 ± 2.2	61.7 ± 36.3
Mo	0.6 ± 0.0	22.5 ± 0.5	12.8 ± 0.1
Zn	12.4 ± 2.5	74.0 ± 11.3	496.5 ± 90.8
Se	1.7 ± 0.4	7.2 ± 0.6	4.8 ± 1.8
Co	1.3 ± 0.8	6.8 ± 0.5	2.7 ± 1.3
Cu	8.2 ± 0.6	20.5 ± 1.8	397.8 ± 4.6
Cr	3.9 ± 1.9	22.0 ± 0.5	78.0 ± 4.9
Cd	0.2 ± 0.0	2.9 ± 0.3	31.0 ± 0.5
Pb	2.7 ± 0.8	9.4 ± 0.7	24.1 ± 1.7

Data are expressed as mean values (n=3) ± standard deviation, otherwise stated. ^a Fe determination was conducted in an external laboratory (NRM laboratories) using one sample.

The TEs concentration of inoculum in this study are comparable with the reported values in the literature (Fytili and Zabaniotou 2008; Facchin et al. 2013; Liu et al. 2015). Likewise, the values obtained for CFA fall within the range reported for a typical UK CFA (Sear et al., 2003). The deficiency of TEs in BS can likely be compensated by the amendment of CFA, which has high levels of TEs, in the AD process to reach a certain level for optimum CH₄ formation.

Table 3.4 presents the oxide composition of the as-received CFA samples, including the free CaO content. Silica (SiO₂) and aluminium oxides (Al₂O₃) are the predominant components, accounting for 50.6% and 22.4%, respectively. Calcium oxide (CaO), which is primarily responsible for the alkaline properties of CFA (Achternbosch et al., 2015; Schramke, 1992) (see Section 2.5.2.1), make up 4.2% of the as-received CFA sample.

Table 3.4 Major oxides and free calcium oxides in coal fly ash

Elemental oxide (% LOI)	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	K ₂ O	MgO	TiO ₂	SO ₃	Na ₂ O	P ₂ O ₅	Free CaO
Total content	50.6	22.4	7.9	4.2	2.6	1.6	0.9	0.9	0.8	0.6	0.3

LOI: Loss on ignition

The range of major oxides and free CaO presented here are consistent with values reported for UK CFA (Sear et al., 2003). Only free CaO, however, is leachable and reactive with water to form hydroxide (OH⁻) ions, contributing to CFA alkalinity (Sear et al., 2003; Achternbosch et al., 2015). Analysis shows that approximately 0.3% TS of the CFA is free CaO. This relatively low level, compared to the potential maximum content of 15% TS (Izquierdo and Querol, 2012; Achternbosch et al. 2015), could be explained due to CFA's storage time at the power plant. Indeed, free CaO is highly reactive and quickly reacts to form calcium carbonate (CaCO₃) through carbonation when exposed to atmospheric CO₂ (Siriruang et al., 2016).

4. Investigation of Alkali Pretreatment Conditions for Barley Straw

4.1 Introduction

Lignocellulosic agricultural residues (LARs) are composed of 80–90% of fibres comprising cellulose, hemicellulose and lignin (Harmsen and Bakker, 2013; Mansour et al., 2024). These fibre components are highly resistant to biological conversion during anaerobic digestion (AD) (Mancini et al., 2018a), with only 40–50% of LARs feedstock typically converted to biogas (Kaur et al., 2020; Peyrelasse et al., 2021). This resistance is largely due to lignin, which acts as a barrier, blocking microbial access to carbon sources – cellulose and hemicellulose (Lee et al., 2014; Antonopoulou and Gavala, 2015) and the complex cross-linked structure among fibre components (Roy et al., 2020). The recalcitrance of the LARs can be reduced, hence the digestibility is increased, by various pretreatment methods, including mechanical, chemical, biological, thermal, as well as their combinations (Ibarra-Díaz et al., 2020; Roy et al., 2020; Karthikeyan et al., 2024). Among these methods, alkali pretreatment stands out as an efficient method, and its effects on the fibre composition and structural changes of LARs is critical to enhancing their digestibility (Ibarra-Díaz et al., 2020).

Alkali pretreatment of LARs for AD is demonstrably effective, for example in rice straw, a minimum 20% increase in CH₄ yield has been reported (reported in Table 2.6). Alkali pretreatment disrupts the chemical bonds between hemicellulose and lignin through a reaction known as saponification, as these bonds otherwise hinder enzymatic access to cellulose (He et al., 2009; Sambusiti et al., 2013; Xiao et al., 2013; Yang et al., 2014; Michalska and Bizukoj, 2015; Shetty et al., 2017). This reaction leads to breakdown and solubilisation of lignin and hemicellulose, exposing cellulose and increasing the surface area of LARs (Martins et al., 2022; Karthikeyan et al., 2024). The enhanced surface area improves accessibility to enzymes and microbes, facilitating more efficient hydrolysis (Ibarra-Díaz et al., 2020; Novakovic et al., 2021).

The combined effects of alkali pretreatment, including the saponification reaction and hemicellulose and lignin solubilisation, alter the cellulose structure from a highly ordered, crystalline structure to a more disordered, amorphous state, which is more susceptible to hydrolysis as the less ordered structure allows enzyme to more easily penetrate and break down the cellulose chains into monosaccharides (Modenbach and Nokes, 2014; Mafa et al., 2020). By improving the accessibility of cellulose, pretreatment allows for more efficient conversion of organic matter into biogas, optimising the energy output of AD processes.

Alkali pretreatment also has the additional benefit of providing sufficient alkalinity necessary for pH control of the AD process, thereby reducing the risk of acidification and methanogenic inhibition resulting from the intrinsic production of volatile fatty acids (VFAs) within the process (He et al., 2009; Sahito and Mahar, 2014; Dell'Omo and La Froschia, 2018; Mansour et al., 2024). In practice, however, alkali pretreatment using inorganic commercial alkali can be cost prohibitive to the AD of LARs (Kumar et al., 2009; Amin et al., 2017). The substitution of commercial alkali in the pretreatment of LARs with alkaline industrial waste could be a viable alternative. This approach could significantly reduce operational costs for AD and provide greater economic and environmental benefits. This is particularly beneficial for economically developing countries where industrial waste management is often problematic and poses serious environmental threats.

Examples of potentially suitable alkaline industrial waste is coal fly ash (CFA), as the majority of CFA available worldwide has alkaline properties (Ahmaruzzaman, 2010; Achternbosch et al., 2015; Zierold and Odoh, 2020) (see Section 2.5.2.1). Coal fly ash is the residual collected particulate matter from flue gas generated by coal burning in a power plant boiler, presenting both a challenge and an opportunity. Coal fly ash has interesting chemical properties, including a high alkali pH, when fresh, due to the significant calcium oxide (CaO) content (Achternbosch et al., 2015). The content of free CaO in CFA is reported to vary widely from 0.2 to 15% (Moreno et al., 2005; Sutter et al., 2013; Achternbosch et al., 2015), depending on combusted coal type, combustion process, type of air pollution control, extent of weathering and the age of the ash (Achternbosch et al., 2015; Seki et al., 2019). Standard management routes for CFA, however, include disposal in ponds and to landfill, which poses a risk of environmental contamination, including groundwater and surface water contamination (Damayanti, 2018; Prayoga and Afla, 2023).

In Indonesia, of 600 Mt of coal produced in 2019, 138 Mt were utilised domestically (MEMR, 2023), generating around 10 Mt of CFA (ACS, 2016; Zakariya, 2022). By 2023, domestic coal use had increased to 212 Mt (MEMR, 2023), with approximately 121 Mt supplied to power plants. This rise has led to an inevitable increase in CFA production, which is expected to grow in the coming years unless a significant transition from coal-based electricity to renewable energy sources occurs.

The beneficial use of CFA in Indonesia includes its application in concrete mixtures, road base construction, cement, soil improvement, tetrapods for abrasion protection, and as additive in inorganic fertiliser (Akmalaputri, 2021; Trianto, 2022). The combined utilisation of CFA and bottom ash in 2022, however, amounted to only 2.1 Mt (Trianto, 2022), yet still relatively low

compared to the total coal ash produced. Given the high generation rate, incorporating CFA in the AD of LARs as a pretreatment agent could potentially reduce the environmental burden of CFA being stockpiled and increase its utilisation.

The specific objective of the work described in this chapter is to determine the effects of alkali chemicals and CFA pretreatments on the fibre composition, using (i) pure inorganic sodium hydroxide (NaOH), as the most aggressive and effective alkali (Zheng et al., 2014; Bachmann et al., 2022), to determine the maximum potential effects of alkali pretreatment, serving as a benchmark comparison with CFA or CaO pretreatment, (ii) pure inorganic CaO, as a primary compound responsible for the alkali properties of CFA, to determine the effective minimum and optimal alkalinity required, considering the wide variability in CaO content in CFA, (iii) as-received CFA, to determine the effect of alkalinity of CFA alone, and separately, (iv) deionised water, to determine whether water-only can significantly impact fibre composition. The investigation proceeded to examine surface morphology and functional groups, the latter to analyse the effects of pretreatment at the molecular level, focusing on treatment conditions that resulted in significant changes to fibre composition.

4.2 Materials and methods

4.2.1 Waste feedstocks

Waste feedstocks used in the pretreatment process were milled prepared barley straw (BS) and as-received CFA, mixed with fresh inoculum for AD experiments. The origin and preparation of each material was described previously in Section 3.1.1, 3.1.3, and 3.1.4, respectively.

4.2.2 Experimental design of alkali pretreatment

The alkali pretreatment experiments consisted of the following sets of experimental conditions:

- (a) Control: untreated BS and pretreatment using deionised water, labelled as “WT”
- (b) treatment conditions: (i) pretreatment using NaOH with an alkalinity concentration equivalent to $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$, labelled as “S3”, (ii) three CaO pretreatments with alkalinity concentrations equivalent to $\sim 2,500 \text{ mg CaCO}_3 \text{ L}^{-1}$, labelled as “C1”, $\sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$, labelled as “C2” and $11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$, labelled as “C3”, and (iii) as-received CFA pretreatment, labelled as “CFA”, using a CFA:BS ratio of 1:2, to achieve maximum alkalinity from as-received CFA whilst at the same time maximising the amount of BS.

Under all experimental conditions 5 g TS of BS and 40 mL deionised water were used. For S3, 0.375 g of pure, oven-dried NaOH was dissolved in deionised water by manual stirring

using a laboratory spatula. The C1, C2, and C3 were prepared by mixing different amounts of pure, oven-dried CaO: 0.09 g, 0.16 g, 0.375 g, respectively, with 40 mL deionised water in a glass beaker, equivalent to 0.2%, 0.4%, 0.9% alkali loading rate (ALR). The mixtures were then stirred with a magnetic stirrer for 2 mins to ensure uniform mixing for the C1, C2 and C3 treatment conditions. Barley straw was added separately to the NaOH and CaO chemical solutions, and to deionised water only for the WT treatment condition. For the CFA treatment condition, 2.5 g of CFA was manually stirred with deionised water in a 150 mL beaker using a laboratory spatula; and BS was added to the mixture. The BS in all treatment conditions was mixed with the solution or CFA suspension using a laboratory spatula to ensure thorough soaking. Any remaining BS on the spatula was either gently scraped off using another clean spatula and transferred back into the beaker or the spatula was lightly tapped against the edge of the beaker to ensure complete transfer of residual BS.

The beakers were covered with Parafilm, sealed with a rubber band to prevent evaporation, and incubated at 30°C for 7 d (see Section 3.1.2 for the incubator type). Before the pretreatment experiments, an evaporation test was conducted in triplicate. For the evaporation test, a 150 mL beaker was filled with 40 mL deionised water, sealed and incubated under the same conditions as those used for the pretreatment experiments to determine potential evaporation that could affect the treatment conditions. The weight of the beaker with water was recorded before and after incubation. During the pretreatment period, the contents of the beaker in CFA, C1, C2 and C3 were mixed manually once a day using a laboratory spatula to resuspend CaO that may have settled, to improve contact between CaO particles with water and BS. Care was taken to ensure that no BS was left on the spatula and all residual BS was transferred back into the beaker.

After the pretreatment, the solid fraction of BS was separated from the liquid fraction, by carefully transferring the BS to a dried and preweighed fritted disc crucible (see Section 3.3.12 for the type of crucible), ensuring none of the BS sample was lost during the transfer. The crucible was placed on top of a 400 mL beaker using a filter plate, as shown in Figure 4.1.

The beaker was rinsed repeatedly with deionised water to ensure complete transfer of the BS to the crucible. The pretreated BS was thoroughly washed with deionised water until the small batch volume of washing liquid collected in the beaker reached a neutral pH of 7.0–7.5 (see Section 3.3.3 for pH measurement procedure), and no visible CaO and CFA remained in the BS for C1, C2, C3 and CFA treatments. Untreated prepared BS was rinsed with the equivalent volume of water to account for any potential mass loss lost during the washing step.

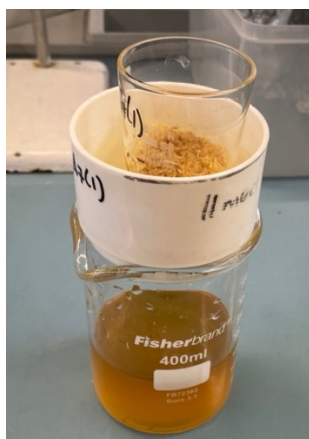


Figure 4.1 Arrangement of laboratory apparatus for washing pretreated barley straw.

To account for any residual CFA in the crucible after washing, another Control experiment was conducted with the same amount of CFA as used in the CFA treatment, but without BS, and the equivalent rinsing steps were applied. This control measurement was used to adjust for CFA residue mass when calculating mass loss during pretreatment due to dissolution or chemical degradation. The washed BS was dried at 50–55 °C in a laboratory oven (see Section 3.3.2 for details on the oven) until a constant weight was reached, and the final weight was recorded.

4.2.3 Analytical methods

The alkalinity concentration of CFA suspension, prepared at the ratio to BS as described in Section 4.2.2, was determined using acid titration with 0.1 M HCl, following EPA-NERL Method 310.1 (USEPA, 1978). pH measurements were conducted according to the procedure outlined in Section 3.3.3. Fibre composition analysis, including aNDF, ADF and ADL, were conducted according to procedures outlined in Section 3.3.12. The functional groups or bonds of both the Controls and treated BS samples were analysed using fourier-transform infrared spectroscopy (FTIR) in the range of 800–4000 cm^{-1} (as described in Section 3.3.5) and surface morphology of both Controls and treated samples of BS were obtained via Scanning electron microscopy (SEM), following the procedure detailed in Section 3.3.6. The total crystallinity index (TCI) value, a measure of the relative amount of the crystalline fraction of cellulosic materials, for each of the alkali-pretreated (C2, C3 and S3) and WT conditions were determined from the FTIR spectra according to the procedure outlined in Section 3.3.5.

4.3 Results

4.3.1 Mass loss

Figure 4.2 shows the mass loss as the first level major effect of pretreatment. The WT treatment showed a 2.2% mass loss, which was not statistically significant ($P > 0.05$) compared to untreated BS. In contrast, the positive Control S3 showed a significant ($P = 0.01$) mass loss of 20.3%, compared to the untreated BS and WT. Despite having equivalent alkalinity concentration, the mass loss after C3 (15.8%) was significantly ($P < 0.001$) lower compared to S3. C3, however, gave a significantly ($P = 0.04$) greater mass loss compared to that obtained using a lower magnitude of alkalinity used in C2 as well as to untreated BS and WT ($P < 0.001$). A significant ($P < 0.001$) mass loss was observed in C2 compared to C1, with losses of 10.6% and 2.2%, respectively. The alkalinity concentration in C1 was not adequate to remove significant loss ($P > 0.05$) in the mass of BS, compared to untreated BS Control. The mass loss in C1 was comparable with the 3.0% mass loss achieved under the CFA treatment, despite lower alkalinity ($\sim 1,500 \text{ mg CaCO}_3 \text{ L}^{-1}$) of CFA suspension. The mass loss in the CFA treatment was not significantly ($P > 0.05$) changed compared to untreated Control treatment, as expected.

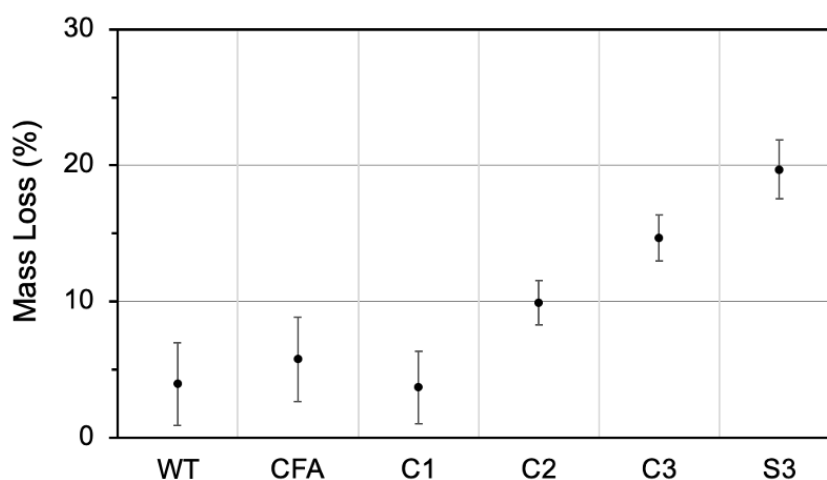


Figure 4.2. Mass loss ($n=3$) of barley straw (BS) during pretreatment by deionised water (WT), coal fly ash (CFA, CFA:BS dry mass ratio=1:2, supplying $\sim 1,500 \text{ mg CaCO}_3 \text{ L}^{-1}$), CaO $\sim 2,500 \text{ mg CaCO}_3 \text{ L}^{-1}$ (C1), CaO $\sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (C2), CaO $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (C3) and NaOH $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (S3) compared to untreated, prepared, dried BS. Error bars represent standard deviation.

4.3.2 Fibre composition

The fraction of cellulose, hemicellulose and lignin in BS after pretreatment is presented in Figure 4.3 (a), with the percent reduction of each individual component given in Figure 4.3 (b) and (c). For the untreated BS, the fraction of each fibre component is shown in Table 3.2.

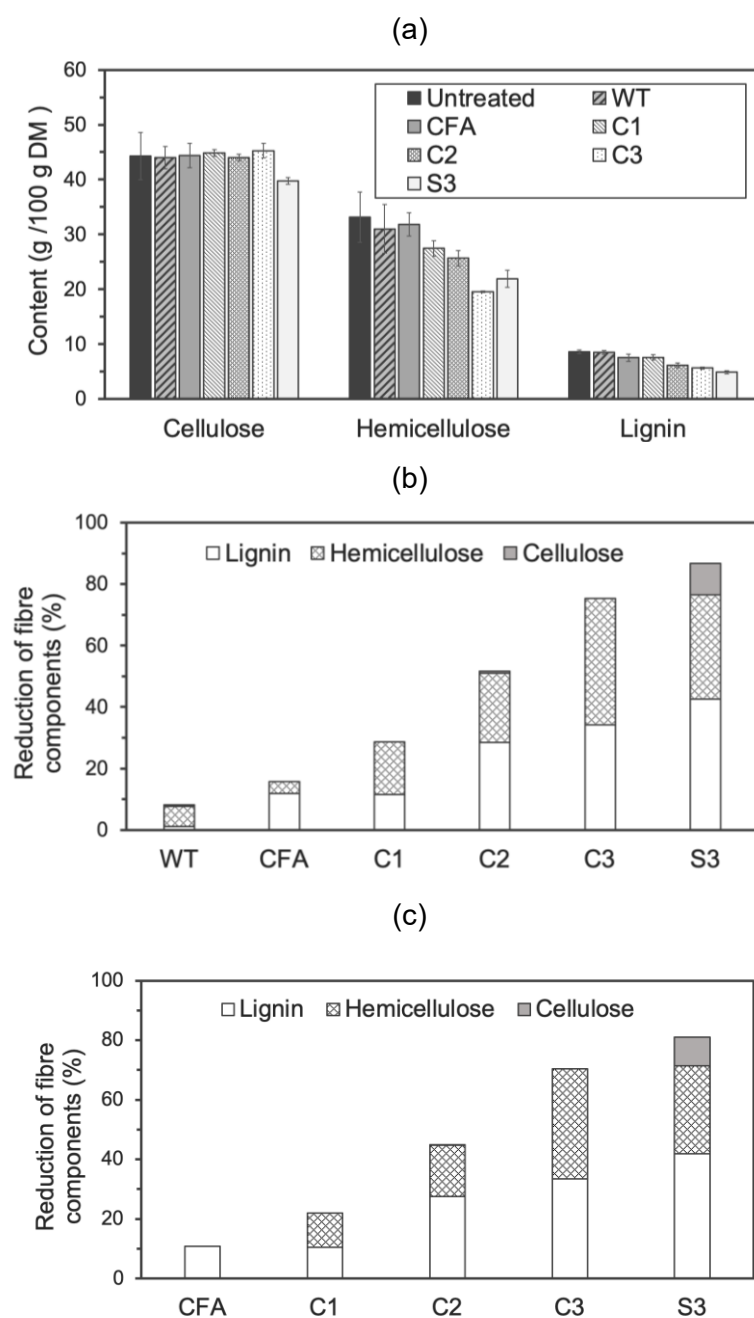


Figure 4.3 (a) Cellulose, hemicellulose and lignin composition in gram (n=3) per 100 g original, prepared barley straw (BS) sample on a total solid basis. Error bars represent standard deviation. (b) Percent reduction in the average cellulose, hemicellulose and lignin contents after pretreatment by deionised water (WT), coal fly ash (CFA, CFA:BS dry mass ratio=1:2, supplying ~1500 mg $\text{CaCO}_3 \text{ L}^{-1}$), CaO ~2,500 mg $\text{CaCO}_3 \text{ L}^{-1}$ (C1), CaO ~5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ (C2), CaO ~11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ (C3) and NaOH ~11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ (S3), relative to untreated BS and (c) Similar to (b), relative to WT treatment.

Under all treatment conditions, except S3, the cellulose content remained unaltered within the range of 44–45% TS (Figure 4.3 (a)). Hemicellulose and lignin content in untreated BS were 44.3% TS and 8.6% TS, respectively. WT did not significantly ($P > 0.05$) affect hemicellulose and lignin composition of BS, shown by only a slightly reduction (1.1–6.5%) in these components (Figure 4.3 (b)), resulting in the final contents of 31% TS for hemicellulose and

8.5% TS for lignin (Figure 4.3 (a)). S3 gave a significant ($P < 0.001$) lower lignin content (4.9% TS), compared to both Controls and had a highest lignin reduction (42–42.6%) among the treatment conditions (Figure 4.3 (b) and (c)). Similarly, the hemicellulose content in S3 (21.9% TS) was significantly ($P < 0.001$) lower compared to both untreated Control and WT, with reductions of 41% and 37%, respectively.

Both hemicellulose and lignin contents in S3 were not significantly different ($P > 0.05$) compared to that in C3, despite the lignin content in S3 being 12.7% lower. S3 also decreased the cellulose content by 10.2%, 9.6%, and 5.5% compared to untreated BS, WT and C3, respectively, resulting in a final content of 39.8% TS, however, this cellulose reduction was not significant ($P > 0.05$).

The hemicellulose and lignin content on a TS basis of BS in C3 was 19.6% and 5.6%, respectively. C3 gave a significant ($P < 0.001$) reduction in lignin, compared to the untreated BS and WT, showing the second largest reduction of 34.3 and 33.5%, respectively. Hemicellulose content in C3 significantly ($P < 0.001$) decreased by 41% and 37% compared to the untreated BS and WT, respectively. C3 achieved a greater magnitude of effect on hemicellulose and lignin compared to the lower alkalinity used in C2, albeit the difference was not significant ($P > 0.05$), indicated by reductions of 23.7% in hemicellulose and 8.2% in lignin.

Pretreated BS in C2 contained 25.7% TS hemicellulose and 6.1% TS lignin. The hemicellulose content in S2 was significantly ($P = 0.016$) different from the untreated BS, showing a reduction of 28.4%. Compared to WT, however, the reduction was not significant ($P > 0.05$), measured by 22.6%. Likewise, the lignin content was significantly ($P < 0.001$) lower, showing a reduction of 28.4% and 27.6%, compared to the untreated BS and WT, respectively. Compared to the lower alkalinity used in C1, the lignin content in C2 was significantly ($P = 0.005$) lower, with C1 showing 7.6% TS lignin content. In contrast, the C2 treatment did not significantly ($P > 0.05$) decrease hemicellulose content.

The treatment condition C1, being the weakest alkalinity concentration, C1 did not show significant ($P > 0.05$) difference in hemicellulose and lignin content, compared to both the untreated BS and WT. Hemicellulose and lignin content in C1 were 27.5% TS and 7.6% TS, respectively, which were 17% and 11.5% lower than that in the untreated BS, and 11.4% and 10.4% lower, compared to WT. The alkalinity under CFA treatment was even lower than in C1, as expected, with the reduction of lignin by 11% compared to WT was not significantly ($P > 0.05$) different, while hemicellulose remained unchanged under this treatment condition, with final contents of 31.8% and 7.6%, respectively.

4.3.3 Effect of alkali pretreatment on the chemical structure of barley straw

The FTIR spectra of cellulose, hemicellulose and lignin were measured in the reference materials (see Section 3.3.5 for the reference materials used) and presented in Figure 4.4. The spectra show the characteristics of cellulose and hemicellulose are almost similar, due to their polysaccharide structure, resulting in many overlapping vibrational modes. In contrast, the FTIR spectra of lignin show distinct characteristics, reflecting its fundamental different chemical structure as a complex aromatic polymer. A summary of the corresponding functional groups associated with the detected peaks in the three components is presented in Table 4.1.

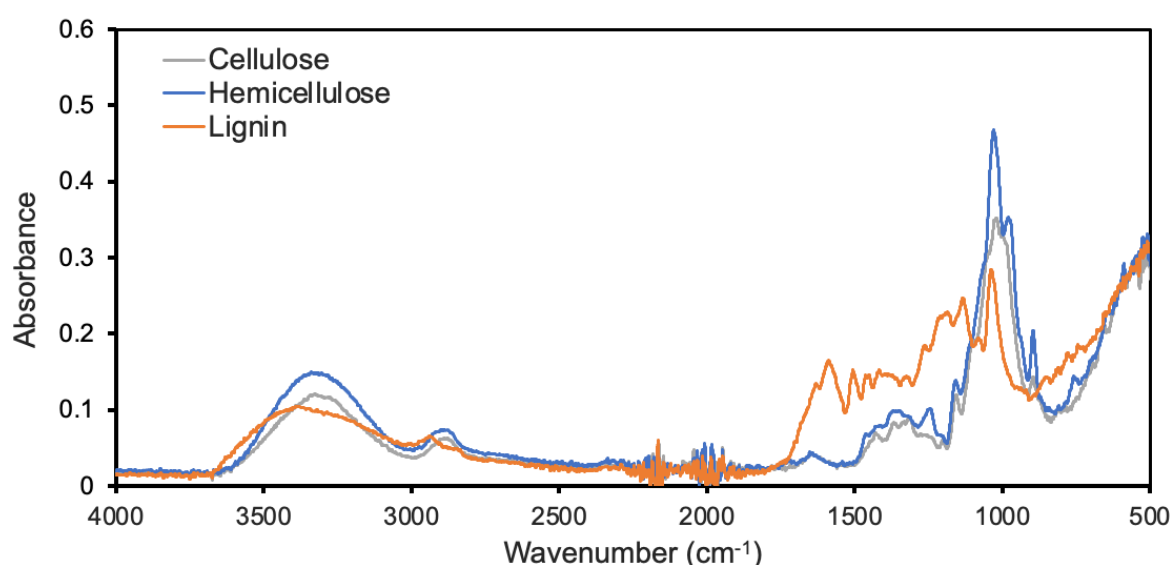


Figure 4.4 Characteristics of FTIR spectra of cellulose, hemicellulose and lignin obtained from the reference materials: microcrystalline cellulose, xylan, and lignin, respectively.

The FTIR spectra of BS before and after pretreatment are shown in Figure 4.5 (a)-(c). The similarity of the spectral pattern of BS to those of the three reference materials shown in Figure 4.4 suggests that BS shares similar structural features and functional groups of cellulose, hemicellulose and lignin. The absorbance intensities of the peaks were largely comparable between the untreated BS and WT (Figure 4.5 (a)), with the only notable difference appearing in the 1000–1100 cm⁻¹ range. In this wavenumber region, C2 showed significant changes compared to WT (Figure 4.5 (b)), while other regions exhibited less pronounced differences. C3 treatment, characterised by higher alkalinity, had more substantial impacts compared to WT, than C2, as indicated by the higher overall reduction in absorbance intensities across the S3 spectrum (Figure 4.5 (b)). Figure 4.5 (c) shows no significant differences in absorbance intensities between C3 and S3, except for slight variations in 1000–1100 cm⁻¹, 2800–2900 cm⁻¹, and 3200–3500 cm⁻¹ wavenumbers.

Table 4.1 Absorption band and the assigned chemical functional groups of cellulose, hemicellulose and lignin.

Absorbance	Fibre Components	Functional Groups/Chemical Bonds	Reference
890	Cellulose, hemicellulose	C-O-C stretching of glycosidic bonds between sugar	(Haque et al., 2012; Pancholi et al., 2023)
1000–1100	Cellulose, hemicellulose	C-O-C asymmetric stretching vibrations in the β -1,4-glycosidic bonds	(Haque et al., 2012; Braga and Poletto, 2020; Md Salim et al., 2021; Pancholi et al., 2023)
1140–1170	Lignin Cellulose, hemicellulose	Guaiacyl units C-O-C stretching vibrations of the glycosidic bonds	(Boeriu et al., 2004) (Bachmann et al., 2022)
1215–1260	Lignin	Ether bonds, guaiacyl rings	(Boeriu et al., 2004; Md Salim et al., 2021; Pancholi et al., 2023)
1370–1450	Cellulose, hemicellulose, lignin	C-H groups: methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2$) group	(Haque et al., 2012; Shafiei et al., 2014; Braga and Poletto, 2020; Pancholi et al., 2023)
1500–1600	Lignin	Aromatic skeletal vibrations, specifically the C-C stretching in the lignin aromatic skeletal structures	(Shafiei et al., 2014; Bachmann et al., 2022; Pancholi et al., 2023)
1730–1740	Hemicellulose, lignin	Ester linkages between lignin and hemicellulose Acetyl and uronic ester groups of hemicellulose	(Haque et al., 2012; Bachmann et al., 2022; Pancholi et al., 2023)
2800–2900	Cellulose, hemicellulose	C-H stretching vibrations of methyl, methylene and methoxyl groups	(Haque et al., 2012; Braga and Poletto, 2020; Pancholi et al., 2023)
3200–3500	Cellulose, hemicellulose, lignin	Hydroxyl (OH) groups	(Braga and Poletto, 2020; Bachmann et al., 2022; Pancholi et al., 2023)

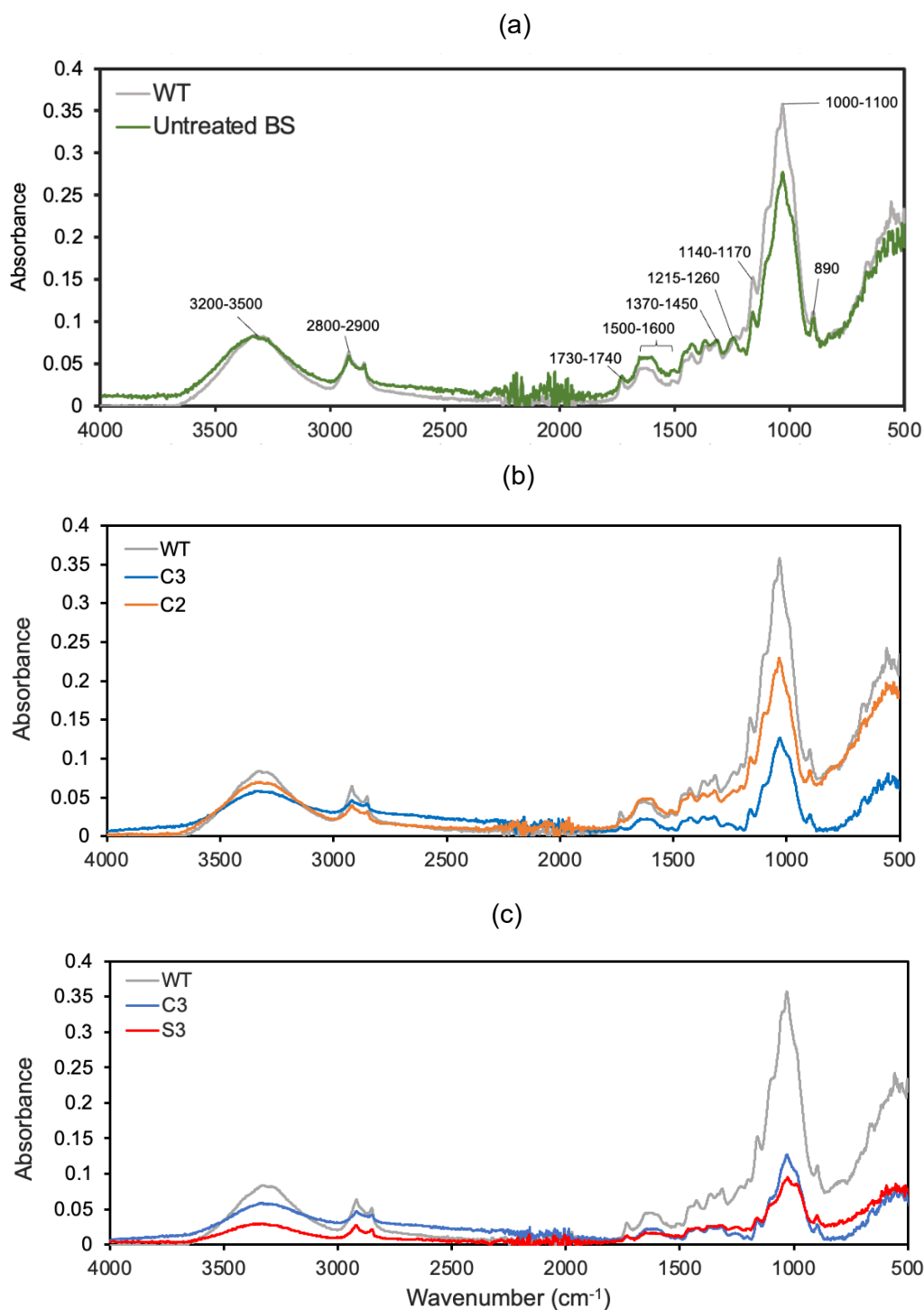


Figure 4.5 FTIR spectra of cellulose, hemicellulose, and lignin in barley straw (BS) with experimental treatments: (a) pretreated with deionised water (WT) compared to untreated BS, (b) BS pretreated with ~5,000 mg CaCO₃ L⁻¹ (C2) and ~11,000 mg CaCO₃ L⁻¹ (C3) compared to WT, (c) BS pretreated with ~11,000 mg CaCO₃ L⁻¹ using NaOH (S3) compared to C3 and WT treatments after 7 d incubation at 30°C.

The total crystallinity index (TCI) value for each of the of alkali-pretreated conditions (C2, C3 and S3) was compared to that for WT treatment and the results are presented in Table 4.2. S3 reduced the TCI by nearly half compared to the WT value (0.41). Similarly, C3 also

significantly decreased the TCI though the reduction was 16% less than that of S3, resulting in a TCI of 0.25. The magnitude of effect on TCI decreased with lower alkalinity, as seen in C2, which had a TCI of 0.27.

Table 4.2 Total crystallinity index (TCI) of barley straw (BS) with experimental treatments: (i) pretreated with deionised water (WT), (ii) pretreated with $\sim 5,000 \text{ mg L}^{-1} \text{ CaCO}_3$ (C2) and (iii) $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (C3), and (iv) with $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ using NaOH.

Absorbance at Related Wavenumber	Treatment Conditions			
	WT	S3	C3	C2
A_{1375}	0.33	0.11	0.10	0.12
A_{2900}	0.80	0.49	0.39	0.45
TCI	0.41	0.22	0.25	0.27

4.3.4 Effect of alkali pretreatment on morphological surface

Figure 4.6 shows SEM images of untreated BS and BS under WT, C2 and C3 treatment conditions. The structure of untreated BS (Figure 4.6 (a)) was smooth and compact compared to BS surface in WT that shows a patchy and uneven wavy surface texture (Figure 4.6 (b)). The surface of untreated BS was smooth due to the coating of cellulose by lignin (Barman et al., 2020). The slight wavy texture in WT probably because water cause the fibres to swell, hence the surface appeared to have a rougher texture although remained intact with regular orientation of fibres like the untreated BS. C2 treatment opened the fibre bundle and cell wall, causing the dense structure of BS to crack, as pores gradually appeared on BS surface (Figure 4.6 (c)). Increasing the alkalinity concentration to the highest level, C3, removed more of the fibre coating or lignin shielding to reveal the internal structure (Figure 4.6 (d)).

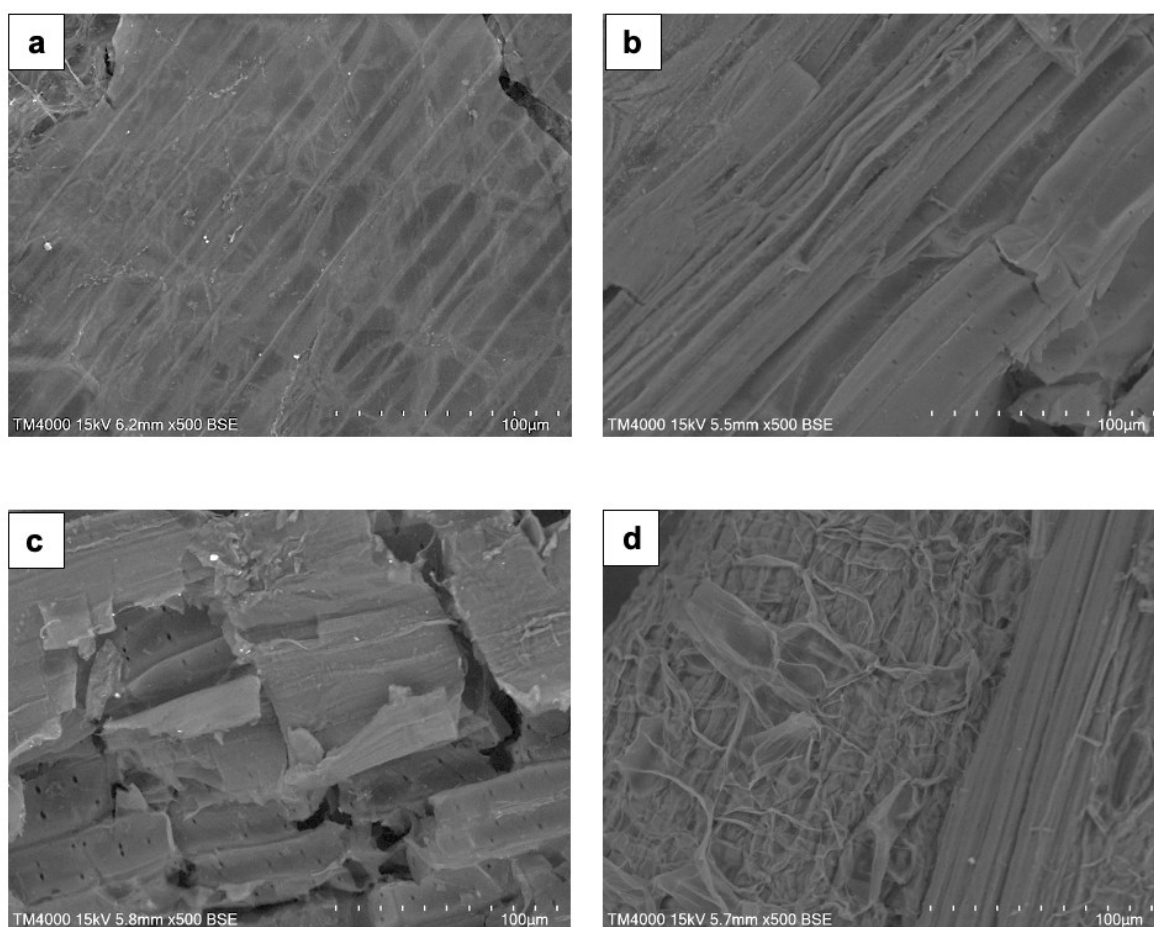


Figure 4.6 Scanning electron microscopy images at a magnification of 500x of barley straw surface following various types of pretreatment: (a) untreated, (b) water-treated (WT), (c) C2:CaO ~5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ and (d) C3: CaO~11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$. Scale bar represent 100 μm .

4.4 Discussion

The main purpose of pretreatment of LARs with alkali agents for AD is to remove lignin and its linkages with cellulose and hemicellulose to increase their accessibility for enzymatic hydrolysis. Pretreatment also aims to decrease cellulose crystallinity and increase the porosity and surface area of LARs to improve the utilisation efficacy during AD (Ibarra-Díaz et al., 2020; Mafa et al., 2020; Karthikeyan et al., 2024). In this chapter, the effectiveness of alkali pretreatment using as-received CFA and increasing alkalinity concentrations of CaO, the principal source of alkalinity in CFA, were examined, compared to NaOH, a highly aggressive alkalinity source, supplied at the maximum alkalinity level achievable by CFA, and water, as control treatments. The quantitative changes in the properties of BS substrate following pretreatment were assessed by quantifying changes in fibre composition and chemical functional groups and surface morphology.

According to the literature, an alkalinity at $\sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (equivalent to 0.4% ALR) has been shown to effectively reduce recalcitrant characteristics in a various LARs, such as rice straw, corn stover, and wheat straw, resulting in a 10–19% reduction in total fibre (of cellulose, hemicellulose and lignin) and a 30–70% increase in CH_4 yield (see Section 2.4.3.1). This alkalinity level, was tested here for BS, as it is possible that this level of alkalinity may be found in typical sources of available CFA and serve as a baseline for effective reduction of recalcitrant characteristics, allowing direct comparison and validation of results with those reported in the literature, while optimising BS pretreatment conditions. A lower alkalinity level of $\sim 2,500 \text{ mg CaCO}_3 \text{ L}^{-1}$ (equivalent to 0.2% ALR), has been tested elsewhere on rice straw and showed a 5.8 to 19.7% reduction in total fibre content (see Section 2.4.3.1); no tests at $\sim 2,500 \text{ mg CaCO}_3 \text{ L}^{-1}$, however, have been reported on BS, and this level of alkalinity was therefore selected in this work as the minimum level to evaluate its effectiveness in pretreatment. Testing at higher alkalinity, such as $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$, or equivalent to 0.9% ALR, is necessary to determine whether further reduction of recalcitrant characteristics and increase in CH_4 yield are achievable, providing the optimal balance between alkalinity and effective pretreatment. This higher alkalinity level corresponds to the potential maximum free CaO content found in CFA, around 15 % (Izquierdo and Querol, 2012; Achternbosch et al., 2015).

For molecular-level chemical characterisation, FTIR was used to identify the functional groups of the components, validating the results of the fibre composition analysis. As expected, the FTIR spectra of the untreated BS showed characteristic that aligned with those detected in the reference materials, confirming the presence of cellulose, hemicellulose and lignin in the prepared BS. Reference material also serves as a useful baseline for more accurate interpretation of absorption band assignments, complementing the information available in the literature. The FTIR spectra of BS shown in Figure 4.5 has an identical pattern reported for BS and other LAR sources (Haque et al., 2012; Mafa et al., 2020; Bachmann et al., 2022; Na et al., 2022). Fourier-transform infrared spectroscopy analysis and SEM imaging were conducted for all treatment conditions, except for C1 and pretreatment with as-received CFA, as the alkalinity were insufficient to cause significant changes in fibre composition (see Section 4.3.2).

WT did not significantly alter the fibre composition, although a slight reduction in hemicellulose content was observed (Figure 4.3 (a)). This is consistent with the FTIR spectra, which showed a marginal decrease in intensity in the $1000\text{--}1100 \text{ cm}^{-1}$ range, associated with glycosidic bonds (Table 4.1), a key component of the polysaccharide backbone in cellulose and hemicellulose. Although the overall fibre composition remained largely unchanged, SEM

images (Figure 4.6 (a) and (b)) show a slight change in the BS surface between WT and untreated BS. The surface smoothness in WT decreased slightly, suggesting potential penetration of water molecules, however, this effect was inadequate to significantly reduce hemicellulose content. The initially flat, smooth surface of untreated BS became uneven in WT, and this may be due to the removal of water-soluble contaminants (Wu et al., 2019; Suharyatun et al., 2021), which was consistent with the observed 2.2% mass loss between WT and untreated BS (Figure 4.2), albeit not significant.

A review of the literature showed studies determining the effects of water pretreatment on the fibre composition and molecular structure of BS compared to the untreated BS to be rare, therefore comparisons were made with other LARs source. The results from this study concur with the findings reported by Kumar et al. (2019) who observed that water pretreatment did not significantly change the fibre composition of wheat straw compared to untreated straw. Consistent with the results in the present study, Kumar et al. (2019) reported that hemicellulose showed the largest reduction, albeit minor (3.5% reduction), while cellulose and lignin reductions were less than 1%. Sambusiti et al. (2013) also observed no change in the fibre composition of wheat straw treated with tap water compared to the untreated straw, despite using a higher water temperature (40°C) than the present study. They found that a significant reduction in hemicellulose occurred at water temperatures of 100°C. Liu et al. (2015) similarly reported no difference in fibre composition between untreated wheat straw and water-treated wheat straw.

The results from the S3 treatment represent the maximum alkaline effects on fibre composition, with reductions in hemicellulose and lignin comparable to those achieved with C3. This suggests that despite the lower solubility of CaO (1.142–1.162 g CaO per litre solution at 30°C) (Miller and Witt, 1929) compared to NaOH (1,110 g NaOH per litre H₂O at 30°C), a 7 d pretreatment duration was sufficient for CaO to dissolve gradually and significantly reduce hemicellulose and lignin. The same pretreatment duration (of 7 d) was used for both C3 and S3 treatments, to allow for a direct comparison between the two conditions, despite 3 d being reported to be sufficient for a complete reaction with NaOH (Song et al., 2013), which was consistent with (Bensah and Mensah, 2013) who reported that NaOH requires a shorter reaction period compared to CaO.

In S3, the reduction of hemicellulose was like that of lignin, whereas in C3 and C2, the reduction of hemicellulose was higher compared to lignin. This difference between S3 and C3 in lignin reduction may be attributed to the highly reactive and soluble nature of NaOH. The higher reduction of hemicellulose compared to lignin, can be explained by the fact that

hemicellulose is more susceptible to alkali pretreatment due to its amorphous and branched structure, which breaks down more easily than the hydrophobic, three-dimensional lignin matrix. Hemicellulose interacts with cellulose, forming a physical barrier to enzyme action (Modenbach and Nokes, 2014; Barman et al., 2020). Consequently, effective solubilisation of hemicellulose can enhance the digestibility of cellulose, exposing more cellulose to further degradation in the AD of LARs (Barman et al., 2020).

The reduction rates obtained for C3 and S3 were consistent with 18.5–60% for hemicellulose and 20–66% reduction for lignin (Zhang et al., 2015; Shetty et al., 2017; Dai et al., 2018; Kumar et al., 2019). The hemicellulose and lignin reduction for C2 fell within the reported range of 11–39% and 8–28%, respectively (Gu et al., 2015; Zhang et al., 2015; Dai et al., 2018). Comparable results with those reported in the literature were also obtained for the hemicellulose and lignin reductions in C1, which were within the reported 11–16% and 13–20%, respectively (Zhang et al., 2015; Dai et al., 2018; Guan et al., 2018). As expected, reduction rates decreased with lower alkalinity concentrations.

The fibre composition results are consistent with those reported in the literature, indicating that $\sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ is the minimum alkalinity required for effective reduction of recalcitrant characteristics in BS (Gu et al., 2015; Zhang et al., 2015). This level of alkalinity is also considered optimal, as increasing it to $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ did not significantly increase the digestibility of hemicellulose and lignin (see Section 4.3.2). The alkalinity provided by the as-received CFA ($\sim 1,500 \text{ mg CaCO}_3 \text{ L}^{-1}$) was below this threshold due to the low free CaO content in the as-received sample (Table 3.4). It is crucial, therefore, to preserve the freshness of CFA by minimising its exposure to atmospheric CO_2 , to prevent the reaction between CaO and CO_2 .

Hemicellulose is a carbohydrate polymer composed of various sugar units connected by glycosidic linkages (Modenbach and Nokes, 2014; Harmsen and Bakker, 2013). Alkali pretreatment conditions C2, C3 and S3 appeared to cleave these linkages, as evidenced by the reduced absorbance intensity in the range of $1000\text{--}1100 \text{ cm}^{-1}$ (Figure 4.5 (b) and (c)). However, a similar pattern of absorbance band was also observed in the cellulose reference material within this wavenumber range, which is also associated with the glycosidic bonds in cellulose. This overlap makes it challenging to determine whether the affected component is cellulose or hemicellulose. Nevertheless, the fibre composition analysis showed a significant reduction in hemicellulose content across all treatment conditions, supporting the interpretation that the reduced intensity primarily reflects changes in hemicellulose rather than

cellulose. This assumption is consistent with Modenbach and Nokes, (2014), who noted that glycosidic bonds in cellulose remain stable at temperatures $<100^{\circ}\text{C}$ and ALR $< 4\%$.

The break down of glycosidic bonds in hemicellulose polymers was also reflected by the reduced peak intensity detected in the FTIR spectra in the $1140\text{--}1170\text{ cm}^{-1}$ range and at 890 cm^{-1} . Both S3 and C3 achieved greater hemicellulose reduction compared to C2 based on fibre composition analysis, which was consistent with the lower intensities of FTIR spectra detected in this wavenumber range. There was no difference in the absorbance intensity between C3 and S3, validating the similar reductions in hemicellulose observed in fibre composition analysis. Furthermore, hydroxide ions from the alkali pretreatment react with the ester bonds linking lignin to hemicellulose in the lignin-carbohydrate complex (LCC) network (Modenbach and Nokes, 2014), leading to bond cleavage under the C2, C3 and S3 treatment conditions and the separation of hemicellulose and lignin. This is indicated by the lower peak intensity in the FTIR $1730\text{--}1740\text{ cm}^{-1}$ range (Figure 4.5 (b) and (c)), with intensity following the order: $\text{S3} < \text{C3} < \text{C2}$. This wavenumber range is also associated with different functional groups in hemicellulose, specifically acetyl and uronic groups, thus, the lower intensity detected in C3 and S3 suggests a reduction in these groups, while the effect was less pronounced in C2.

The FTIR spectra in Figure 4.5 (c) show that both C3 and S3 affected the hydroxyl groups, as indicated by the reduced peak intensity in the $3200\text{--}3500\text{ cm}^{-1}$ range compared to WT. The C2 treatment may have also impacted these groups, but to a lesser extent than C3, as reflected by only a slight decrease in intensity relative to WT (Figure 4.5 (b)). The disruptions in the hydroxyl groups may be related to the function of hydrogen (H) bonds (Harmsen and Bakker, 2015). These bonds can serve as: (i) intrapolymer connections, linking the hydroxyl groups of glucose with oxygen atoms on the same or adjacent chains (Harmsen and Bakker, 2015; Hermosilla et al., 2018; Roy et al., 2020), which are the major bonds found in cellulose (Roy et al., 2020), and (ii) interpolymer connections, linking cellulose-hemicellulose, lignin-cellulose, or lignin-hemicellulose (Roy et al., 2020). The existence of H bonds in cellulose strongly contributes to the water insolubility and the formation of strong crystalline structures, thereby increasing the recalcitrance of the material (Harmsen and Bakker, 2015; Hermosilla et al., 2018).

The reduction in intensity for C3 and S3 treatments suggests disruption of intrapolymer and interpolymer H bonds. While it is challenging to pinpoint whether the affected H bonds are assigned to cellulose, hemicellulose, or lignin, as all three fibre components from the reference materials show a characteristic band in $3200\text{--}3500\text{ cm}^{-1}$ wavenumber range (Figure 4.4), the

greater reduction in intensity in S3 compared to C3 indicates a possible breakdown of H bonds associated with lignin and cellulose. This was consistent with the observed decrease in cellulose and the higher lignin reduction in S3, as shown by the fibre composition analysis, albeit the reductions in both components were not significant (Figure 4.3 (a)). During alkali pretreatment, H bonds are cleaved through the substitution of H atoms with Ca or Na cations, leading to the separation of the components (Harmsen and Bakker, 2015; Dai et al., 2018). This process increases the solubility and accessibility of cellulose to hydrolytic enzymes (Harmsen and Bakker, 2015; Hermosilla et al., 2018). The reduction in cellulose in S3 may also be explained by the lower intensity in the 2800–2900 cm^{-1} range (Figure 4.5 (c)), which is associated with methyl and methylene groups in cellulose (Table 4.1).

The breakdown of H bonds in cellulose affects its crystallinity, making it more amorphous and consequently more dissolvable, hence more easily accessible by hydrolytic enzymes (Kumar et al., 2009; Mittal et al., 2011; Bali et al., 2015). The possible H bonds breakdown in cellulose was consistent with the smaller TCI value calculated (Table 4.2) for S3, C3 and C2 compared to WT which indicate that both CaO concentrations and NaOH pretreatments disrupted the crystalline structure of cellulose. Based on the TCI values, S3 had the strongest effect on altering the structure of cellulose from crystalline to amorphous form, which was consistent with the intensities detected in the FTIR spectra and the fibre composition results, probably because NaOH is more reactive, and the 7 d reaction time may have facilitated partial dissolution of amorphous cellulose. This assumption was consistent with Bali et al. (2015), who reported that high-severity conditions, including extended incubation times, can disrupt H bonds and lead to the dissolution of amorphous cellulose. The obtained TCI range presented in Table 4.2 was in the same magnitude as the reported TCI of 0.52 for wheat straw (Hermosilla et al., 2018).

The reduction in lignin content observed under C3 and S3 conditions (Figure 4.3 (b)) was consistent with changes in functional groups, as indicated by FTIR spectra in the 1500–1600 cm^{-1} and 1215–1260 cm^{-1} ranges (Figure 4.5 (b) and (c)). S3 resulted in a 17% higher reduction in lignin compared to C3, albeit the difference was not statistically significant, and this was in good agreement with 24% higher lignin reduction with NaOH compared to CaO pretreatment (Mafa et al., 2020). Higher lignin reduction for NaOH compared to CaO pretreatment was also reported by Monlau et al. (2012), showing 36% and 30% reductions, respectively.

While the reduction in peak intensity in the 1215–1260 cm^{-1} ranges was evident for C2, it was less pronounced to that observed for C3 and S3 (Figure 4.5 (b) and (c)). The decrease in

intensities suggests disruptions in the aromatic structures of lignin, possibly due to partial removal, modification, or degradation during alkali pretreatment. The affected structures include carbon-carbon (C-C) and ether bonds (Boeriu et al., 2004; Shafiei et al., 2014; Salim et al., 2021; Bachmann et al., 2022; Pancholi et al., 2023), which are major types of linkages in the lignin structure (Harmsen and Bakker, 2015; Grgas et al., 2023). The cleavage of ether bonds in alkali conditions resulted in separation of the aromatic rings, which can undergo further reactions, making them more amenable to complete degradation (Harmsen and Bakker, 2015).

Partial lignin reduction detected in C2 may be related to the presence of cracks in the surface structure (Figure 4.6 (c)) compared to the smoother surface of the untreated and WT conditions, as seen in SEM images (Figure 4.6 (a) and (b)). As lignin is removed, cracks and pores appear, increasing the surface area, facilitating microbial adhesion and enabling hydrolytic enzymes to penetrate the internal structure (Shang et al., 2019; Barman et al., 2020). Additionally, these surface structure changes observed in the SEM image may also be linked to the breakdown of ester bonds between lignin and hemicellulose, as this bond cleavage is believed to cause swelling of the pretreated LARs, which also increases porosity and internal surface area (Jiang et al., 2017). The greater extent of lignin reduction and ether bond cleavage detected in C3, as indicated by the fibre composition (Figure 4.3 (c)) and FTIR spectra (Figure 4.5 (b)), was consistent with the SEM image showing that most of the protective lignin layer has been removed, exposing the internal structure, which is probably cellulose (Figure 4.6 (c)), thereby providing more access for microbes to cellulose compared to C2.

The cleavages of different linkages and alterations in the structure of fibre components resulted in depolymerisation and the formation of simpler, water-soluble products (Martins et al., 2022; Na et al., 2022), therefore were potentially rinsed away during washing and solid-liquid separation steps following pretreatment (see Section 4.2.2). This removal contributes to the significant mass loss observed under alkali treatment conditions (C2, C3 and S3) (Figure 4.2). The significant mass loss observed in S3, compared to C3, correlates with the higher solubilisation of lignin and cellulose during pretreatment. Similar results were reported by Rodrigues et al. (2016), showing greater mass loss after 7 d of NaOH compared to CaO pretreatment of switchgrass. The mass loss observed with CaO (20%) and NaOH pretreatment (29%) in their study was higher than the values obtained in the current investigation. However, other studies reported similar results. The mass loss achieved in S3 and C3 were comparable to the 17.6% reported for KOH pretreatment of wheat straw at the

same ALR (0.9%). Similarly, C2 gave a mass loss consistent with the 8.5% loss reported in the literature under similar alkalinity (X. Liu et al., 2015).

Sodium hydroxide pretreatment was more effective than CaO at equivalent alkalinity concentrations in reducing the recalcitrance of BS in agreement with Rodrigues et al. (2016) and Mafa et al. (2020). Both treatments removed lignin, altered cellulose crystallinity, and solubilised hemicellulose, which were consistent with the effects reported by Rodrigues et al. (2016) but NaOH achieved a higher degree of lignin removal and H bonds cleavage, which lead to the dissolution of cellulose, although both effects were not significantly different compared from those observed in CaO pretreatment. This difference may be explained because CaO has relatively low solubility in water compared to NaOH, however, the only marginal difference detected in this study could be due to the 7 d pretreatment duration, which allowed sufficient time for the CaO reaction. Despite the cellulose not being dissolved with CaO pretreatment, its altered cellulose structure renders it more biodegradable than the original crystalline matrix thus enhancing its utilisation efficiency by the microbes during the AD process. Despite the difference, the results of this study demonstrate the practicable benefits and effectiveness of CaO as a potential alkali pretreatment method for LARs, making it a promising method when considering the use of alkaline industrial waste, such as CFA.

In this chapter, CaO pretreatments at 5,000 and 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ have been shown to change fibre composition, surface morphology and functional groups, the effect of these pretreatments on increasing biodegradability, using CH_4 yield as an indicator, is discussed further in Chapter 6. The focus of Chapter 5, however, is on the effect of CFA pretreatment, with alkalinity adjusted by CaO, on CH_4 yield, with specific emphasis on whether the integration of CFA behaves similarly to CaO pretreatment in reducing recalcitrance and increasing biodegradability of BS.

5. Effects of Coal Fly Ash Pretreatment on Fibre Compositions and Enhanced Solid-State Anaerobic Digestion of Barley Straw

5.1 Introduction

Lignocellulosic agricultural residues (LARs) have emerged as a promising feedstock for bioenergy conversion. Anaerobic digestion (AD) offers a viable method for transforming the organic constituents of LARs into methane (CH_4), primarily due to its high adaptability to various organic substrates and low energy requirements (Zdeb, 2021). The challenge in recovering CH_4 from LARs in AD, however, is related to the presence of recalcitrant lignin which hinders the hydrolysis of cellulose and hemicellulose resulting in reduced biogas yield potential, extended retention times (60–90 d) and low organic loading rates ($1\text{--}3 \text{ kg VS m}^{-3} \text{ d}^{-1}$) compared to $6\text{--}15 \text{ kg VS m}^{-3} \text{ d}^{-1}$ for manure (Verma and Themelis, 2002; Weiland, 2006; Zdeb, 2021). Alkali pretreatment, which uses inorganic alkali, such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium oxide (CaO), or ammonia is currently the predominant method for pretreatment of LARs prior to AD (Zdeb, 2021; Sudalyandi and Jeyakumar, 2022), due its proven effectiveness in destroying lignin and has been extensively evaluated in laboratory-scale experiments (Abudi et al., 2016; Khalid et al., 2019; Kumar et al., 2019; Shetty et al., 2017).

The influence of alkali pretreatment of LARs on the biogas production under mesophilic conditions has been widely documented. Previous studies have reported significant increases in CH_4 yield ranging from 19 to 194%, following NaOH pretreatment of rice straw at temperatures between 25 and 37°C (Zhang et al., 2015; Janke et al., 2017; Shetty et al., 2017; Dai et al., 2018) (see Table 2.6 in Section 2.4.3.1). These variations in yield have been primarily attributed to differences in ALR or alkalinity concentration.

In Chapter 4, the alkali pretreatment of BS was investigated using as-received coal fly ash (CFA) and CaO with alkalinity concentrations designed to simulate the potential alkalinity reaction observed in CFA. The results of this investigation indicated that both tested alkalinity concentrations ($\sim 5,000$ and $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$), equivalent to 7% and 15% CaO content in CFA, respectively, under the experimental conditions trialled, led to significant changes in the micro-surface structure, fibre compositions, and cellulose crystallinity of the BS compared to treatment with deionised water alone. These changes, hence, suggested that the recalcitrance of BS to AD has been reduced, with lignin reductions of 28% and 34% achieved following $\sim 5,000$ and $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ CaO pretreatment, respectively, compared untreated BS. The effects of the reduced recalcitrant and increased digestibility due to CaO

alkali pretreatment on hydrolysis rates, VS removal and CH₄ yield in solid-state AD, however, have not been assessed.

Calcium oxide pretreatments showed a reduction in cellulose crystallinity degree (see Section 4.3.3), despite not solubilising cellulose under the experimental conditions used, as no reduction during fibre composition analysis (see Section 4.3.2). This reduction in cellulose crystallinity suggests increased accessibility of hydrolytic enzymes to cellulose (Kim et al., 2018), which facilitates easier cellulose degradation during subsequent AD (Kumar et al., 2009). While many studies only focus on changes in fibre composition after alkali pretreatment, assessing the impact of AD on the residual fibre compositions in the digestate of pretreated BS is essential for comprehensive evaluations.

In addition to its alkalinity properties, CFA has complex chemical properties that may potentially contribute positively to the AD of BS through interaction unrelated to pretreatment. For example, trace elements (TEs) essential for anaerobic microorganisms are present in considerable concentrations in CFA, suggesting that CFA could serve as a source of TEs (Huiliñir et al., 2015; Montalvo et al., 2018; Lauzurique et al., 2021a), potentially eliminating the need for external TE sources. Coal fly ash, however, also contains several potentially toxic elements (PTEs), which may influence the digestate quality as the product of the AD. While digestate holds potential as a valuable fertiliser in agricultural applications, the presence of PTEs raises environmental concerns. This underscores the importance of assessing PTEs concentration in the digestate produced through AD involving CFA. This characterization provides crucial insights into environmental impact and the feasibility for digestate utilization, particularly considering stricter environmental regulations and growing concerns regarding the health effects of exposure to toxic elements.

To address the research need described above, the present study aims to quantify the effects of CFA pretreatment on the CH₄ yield from barley straw (BS) in solid-state AD. The specific objectives in this chapter are: (1) to determine the potential supplementary contribution of CFA to the AD process, (2) to quantify the effects of CFA pretreatment on AD of BS based on fibre mass balance and CH₄ yield, and (3) to assess the effects of CFA on the composition of TEs and PTEs in the residual digestate in relation to the AD process requirements and meeting end product standards for land application. The findings of this research potentially contribute to optimising pretreatment strategies by employing an inexpensive material with alkali properties to enhance the digestibility of LARs and increase CH₄ yield in AD. Moreover, in a broader context of waste management, integrating CFA in the AD of BS enhances the

utilisation rate of CFA, promoting a more sustainable approach to the treatment and disposal for these waste streams.

5.2 Materials and methods

5.2.1 Waste feedstocks

The waste feedstocks used were milled BS and as-received CFA. Digested sewage sludge cake was used as the AD inoculum. The source and preparation of each material is described in Section 3.1.1, 3.1.3, and 3.1.4, respectively, with their general physicochemical properties presented in Table 3.2 (Section 3.5).

5.2.2 Alkali pretreatment of barley straw

Milled BS was pretreated using as-received CFA, which was supplemented with reagent-grade CaO (Scientific Lab Supplies) to increase its alkalinity as it was insufficient to cause significant changes in the fibre composition (see Section 4.3.2). The low alkalinity was likely related to the decreased free CaO content in the CFA due to weathering during storage at the power plant source (see Section 3.5). The alkalinity was increased to approximately 11,000 mg CaCO₃ L⁻¹, equivalent to a 15% free CaO content, which represents the maximum content commonly observed in CFAs worldwide, as described in (Achternbosch et al., 2015).

Milled BS (15 ± 0.5 g) was individually weighed and placed into nine 400 mL glass beakers. The first three beakers were filled with a mixture of as-received CFA and CaO; prepared by mixing 7.5 g of as-received CFA with 50 mL of water in a borosilicate glass bottle fitted with a screw cap; the bottle was placed in a shaker for 24 h to leach the soluble components from the CFA into the water. CaO (1.125 g) was then added to the CFA suspension and shaken manually. The next three beakers were filled with a CaO solution; prepared by mixing 1.125 g CaO with 50 mL of deionised water (equivalent to 11,000 mg CaCO₃ L⁻¹). The last three beakers were filled with 120 mL of deionised water for use as a Control. To ensure complete transfer of the suspension/solution from the first and second treatment conditions, an additional 75 mL of deionised water was used to rinse all the materials from the bottles. All the beakers were placed in the incubator set at a constant temperature of 30°C for 7 d to undergo the pretreatment process. Further detailed pretreatment conditions are outlined in Section 4.2.2.

5.2.3 Batch experiments of solid-state anaerobic digestion

Solid-state AD experiments were conducted on pretreated BS under the conditions described above in Section 5.2.2, including BS pretreated using: (1) deionised water as the Control (WT),

(2) CaO (CaO-III), and (3) CFA supplemented with CaO (CFAS). One blank condition involved inoculum only to assess the background CH₄ present in the inoculum (refer to Appendix A, Figure A.2 for the residual CH₄ production from the inoculum used in this chapter). The CaO-III and CFAS conditions were neutralised using 3-4 mL of concentrated HCl acid solution. Under the three treatment conditions (WT, CaO-III and CFAS) inoculum was added to achieve an ISR of 1 (VS basis), following the procedure outlined in Section 3.2.3. Deionised water (50 mL) was added to CFAS to adjust the TS content to approximately 15% (see Section 3.2.1), making it comparable with the TS content in WT and CaO-III treatment conditions. Complete homogenisation of the mixture was achieved using a hand-held kitchen mixer. Two subsample fractions of approximately 20 g on a FM basis, of the feedstock mixture, were taken from each reactor for the measurement of solids (TS and VS) and pH. This subsampling resulted in a final VS weight of 11–12 g of BS loaded into the reactors for the three treatment conditions. The general operating protocols of the solid-state AD experiments were detailed in Section 3.2.2 and 3.2.3.

5.2.4 Mass balance of fibre components during the pretreatment and after anaerobic digestion

The mass balance of fibre components (cellulose, hemicellulose and lignin) was calculated to determine how they were affected by high alkalinity pretreatment (CaO-III and CFAS) and after the AD of these pretreated materials, in comparison to the WT Control. The mass balance assessment for each fibre component consisted of two parts: the mass balance during pretreatment (Equation 5.1), and during the AD (Equation 5.2). The residual fibre fraction in the digested BS was calculated by subtracting the residual masses of the fibres in the digestate from those in the digested inoculum obtained from the blank Control reactor (Equation 5.3). The mass balance during the pretreatment step was calculated by applying the proportions of cellulose, hemicellulose and lignin removed, as presented in Figure 4.3 (c), and appropriately adjusting for the quantity of BS used in the pretreatment step prior to solid-state AD experiment. The following equations were used to calculate the mass balance (g) of each fibre component:

$$\text{Pretreatment step: } fib_{BSin} = fib_{BSsol} + fib_{BSout} \quad (5.1)$$

$$\text{Anaerobic digestion step: } fib_{BSout} + fib_I = fib_{deg} + fib_{dig} \quad (5.2)$$

$$fib_{dig} = fib_{DBS} + fib_{DI} \quad (5.3)$$

where fib_{BSin} is the mass of fibre in the BS before pretreatment, fib_{BSsol} is the mass of fibre in the BS solubilised during pretreatment, fib_{BSout} is the mass of fibre in the BS after pretreatment, entering AD, fib_I is the mass of fibre in the inoculum entering AD, fib_{deg} is the mass of fibre in the BS and inoculum degraded during AD, fib_{dig} is the residual mass of fibre in the BS and inoculum after AD or in the digestate, fib_{DBS} is the mass of fibre in the digested BS after AD, fib_{DI} is the mass of fibre in the digested inoculum after AD; units are in g VS.

5.2.5 Analytical methods

The determination of total (TS) and volatile solids (VS), and pH was conducted on the substrate mixtures from WT, CaO-III and CFAS treatments before and residual digestates after solid-state AD following the procedures outlined in Section 3.3.2 and 3.3.3. VS removal was calculated using Equation 5.4. Volatile solids removals were plotted against their corresponding mean experimental cumulative specific methane yield ($CSMY_{exp}$) to establish a correlation between VS removal and CH_4 yield.

$$\% VS\ removal = \frac{VS_{added} - VS_{final}}{VS_{added}} \quad (5.4)$$

where VS_{added} (g) is the amount of VS from BS added to each reactor at the beginning of the experiment, and VS_{final} (g) is the amount of VS from BS in the residual digestate at the end of the experiment.

Digestate residues from all treatment conditions were collected at the end of the AD experiment. Subsamples, each approximately 20 g on a fresh matter (FM) basis were taken from the triplicate reactor vessels for each treatment condition, manually mixed using a laboratory spatula and allowed to dry in an oven at 50°C until a constant weight was achieved. The fibre composition of these dried digestates was analysed following the procedure outlined in Section 3.3.12. The total contents of TEs and PTEs were measured in both WT and CFAS digestates according to the procedures described in Section 3.3.8. Total TEs and PTEs were compared with the TE requirements for the AD process documented in the literature, and with the UK digestate quality regulations outlined in PAS 110: 2014 (BSI, 2014), respectively. All chemical analyses were conducted in triplicate.

Biogas volume was measured using a water displacement method as detailed in Section 3.2.3, while CH₄ composition was analysed using gas chromatography (GC) according to the procedures set out in Section 3.3.13. The background CH₄ produced from the blank was subtracted from the total CH₄ volume measured in all experimental treatments (see Section 3.2.5).

5.2.6 Statistical analysis, CH₄ gas modelling, and kinetics

Triplicate measurements of CSMY_{exp} from the WT Control, CaO-III and CFAS treatments were normalised to standard temperature and pressure (STP) conditions (see Section 3.2.5). Methane gas modelling was conducted on the normalised experimental CH₄ data after removing outlier data, as detailed in Section 3.4. Modelling of the CH₄ yield and kinetic parameters, and determination of the CSMY_o followed the procedures described in Section 3.4. The statistical significance of differences in CSMY and kinetic parameters between the experimental treatments were determined by a one-way analysis of variance (ANOVA) (see Section 3.4).

5.3 Results

5.3.1 Trace elements and potentially toxic elements in digestate

The results of the total TEs and PTEs examination of WT and CFAS digestates are presented in Table 5.1. Most of the TE concentrations in CFAS reactor were higher than in WT, indicating that CFA was a rich source of TEs and its addition increased the TE levels in the mixture of BS-inoculum sludge. Trace elements in both digestates were supplied in adequate amounts compared to the requirements for AD (Hinken et al., 2008; Banks and Zhang, 2010; Facchin et al., 2013; Choong et al. 2016). Indeed, the concentrations of Fe and Mo in both digestates were significantly above the TEs requirement, and those of Mn and Co fell within the required range. Selenium in WT digestate was below the limit of detection, in contrast, its concentration in CFAS digestate was slightly above the required value.

In terms of PTE levels in the digestates, the concentrations of Ni, Zn and Cd exceeded the digestate quality standards set out in PAS 110 (BSI, 2014) (see Table 5.1 in Section 5.3.1), which are designed to permit unrestricted use of the digestate, and so are especially conservative, which was explained by the relatively high concentrations present in the digested sludge sample used as the anaerobic inoculum source (Table 3.3 in Section 3.5).

Table 5.1 Total concentrations of trace elements (TEs) and potentially toxic elements (PTEs) in the residual digestates of BS pretreated with deionised water (WT) and high alkalinity coal fly ash pretreatment (CFAS).

Elements (mg kg ⁻¹ TS)	WT	CFAS	Trace Elements Requirement ^a	BSI PAS 110 Limit ^c
<u>Essential TEs</u>				
Fe	10,060.0 ± 936.0	11,311.0 ± 715.0	205.0–2,674.0	N/A
Mn	142.4 ± 31.1	165.5 ± 7.6	2.0–334.0	N/A
Mo	18.9 ± 2.7	24.1 ± 2.1	1.7–7.0	N/A
Se	<LOD	2.4 ± 0.5	1.6	N/A
Co	4.0 ± 1.2	4.4 ± 0.9	2.4–9.0	N/A
<u>PTEs</u>				
Ni	35.8 ± 7.7	56.5 ± 5.5	N/A	40.0
Zn ^b	455.4 ± 4.5	336.0 ± 9.6	N/A	320.0
Cd	1.7 ± 1.2	1.9 ± 0.6	N/A	1.2
Cr	27.5 ± 1.5	31.4 ± 7.2	N/A	80.0
Cu ^b	166.3 ± 9.3	150.4 ± 20.5	N/A	160.0
Pb	66.0 ± 1.8	57.6 ± 2.4	N/A	160.0

Note:

(1) Data are expressed as mean values (n=3) ± standard deviation.

(2) <LOD: Below limit of detection: 0.2 mg kg⁻¹.

(3) N/A : not applicable.

^a TEs requirement were adopted from (Hinken et al., 2008; Banks et al., 2010; Facchin et al., 2013; Choong et al., 2016). Few of the values were converted from mg L⁻¹ to mg kg⁻¹ TS based on typical TS of 5% in liquid digestate reported by Akhilar et al. (2015).

^b Zn and Cu are also TEs but classified as PTEs due to their excessive concentrations.

^c Source: (BSI, 2014).

5.3.2 Mass balance of fibre components

The extent of fibre degradation depends on the combined effect of BS pretreatment and subsequent AD on the solubilisation and degradation of individual fibre components. The mass balance for each fibre component (cellulose, hemicellulose and lignin) was calculated in the pretreatment and AD steps to assess the lignocellulose degradability in CaO-III, CFAS and WT and the results are presented in Figure 5.1.

The initial quantities of fibres supplied in the pretreatment step were assumed to be equivalent in each condition; the mass of VS was approximately 11.5 ± 0.5 g, comprising of 5.26 g cellulose, 4.65 g hemicellulose and 1.08 g lignin. Hemicellulose was solubilised by 12% in WT during the pretreatment step, but the other components, cellulose and lignin, were largely unaffected which can be expected in this WT condition. During the AD step, 0.52 g cellulose and 2.05 g hemicellulose in the BS were degraded, equivalent to a reduction in size of these fractions of 10% and 50%, respectively.

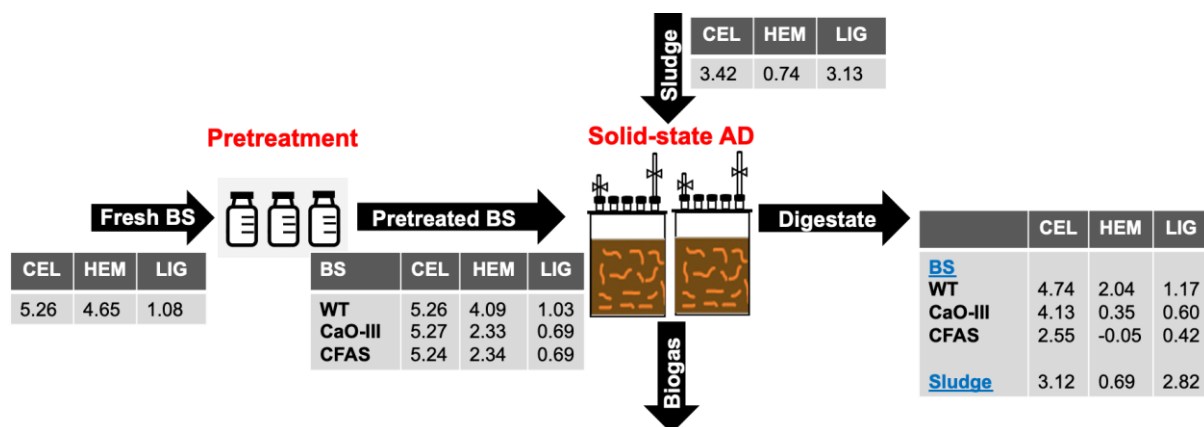


Figure 5.1 Mass balance fractions (g VS) of cellulose (CEL), hemicellulose (HEM) and lignin (LIG) in the pretreatment and solid-state anaerobic digestion for barley straw pretreated with deionised water (WT) and CaO~11,000 mg CaCO₃ L⁻¹ either with as-received CFA (CFAS) or without (CaO-III).

By contrast, the amount of lignin was unaltered, and the mass of cellulose, hemicellulose and lignin fibres remaining in the final digested BS residue after 40 d digestion were 4.74 g, 2.04 g, and 1.17 g, respectively. Whilst the procedures applied (see Section 5.2.5) attempted to minimise the extent of potential experimental variation, it is possible this variation was introduced due to the nature of the materials and system under investigation, which probably accounted for the observed difference in the final mass of lignin recorded after digestion compared to pre-digestion values in the fresh, prepared BS feedstock and after pretreatment.

High alkalinity pretreatment with CaO (CaO-III) solubilised hemicellulose and lignin and the mass of these fractions decreased by 2.32 g and 0.39 g, representing a reduction equivalent of 50% and 36%, respectively, compared to freshly prepared BS, however, cellulose was not solubilised. Hemicellulose and lignin were further degraded by AD, and so was cellulose, giving final mass residual fibres of 4.13, 0.35 and 0.60 g in the digested BS, equating to overall reduction of each fibre fraction of 85%, 13% and 22%, respectively, compared to pretreated BS, before AD.

The solubilisation rate of fibre components for CFAS were consistent with those obtained for CaO-III after the pretreatment stage, which may be expected given the equivalent alkalinity conditions. Significantly, however, pretreatment with CFA, greatly increased the digestibility of all fibre fractions compared to the equivalent alkalinity chemical treatment (CaO-III). Thus, hemicellulose was completely degraded with CFA addition (the small negative mass may be attributed to the experimental variability), and lignin and cellulose fractions were both degraded by 0.39 g and 2.69 g, respectively, compared to alkali-pretreated BS, before AD. The degradation in AD resulted in final mass residual cellulose and lignin of 2.55 and 0.42 g, respectively, while no hemicellulose remained, corresponds to 39%, 51% and 100%

reductions of lignin, cellulose and hemicellulose, respectively, compared to pretreated BS, before AD. Hemicellulose reduction was 15% higher compared to that achieved with CaO pretreatment Control, while cellulose and lignin reductions were two-fold and three-fold greater, respectively.

The total fibre (sum of cellulose, hemicellulose and lignin) reductions achieved during the AD step was 3.21 g with CaO-III and 5.3 g with CFAS treatments, corresponding to reductions of to 38.7% and 64.1%, respectively. Together, pretreatment and AD combined gave an overall reduction in the total fibre content of BS of 53.7% and 72.9% for CaO-III and CFAS, respectively, compared to 27.6% for the WT Control.

5.3.3 Effect of coal fly ash pretreatment on hydrolysis rates, volatile solids removals and CH₄ yields

The effects of CFA pretreatment on VS removal and CSMY_{exp} compared to equivalent alkalinity CaO-III pretreatment and WT Control are shown in Figure 5.2 (the triplicate values of CSMY_{exp} and VS removals are presented in Appendix C, Table C.1). The largest VS removal was detected in CFAS (57% ± 4.00), which was significantly higher, equating to 71% and 29% than that achieved by WT ($P < 0.001$) and CaO-III ($P = 0.005$) treatments, respectively.

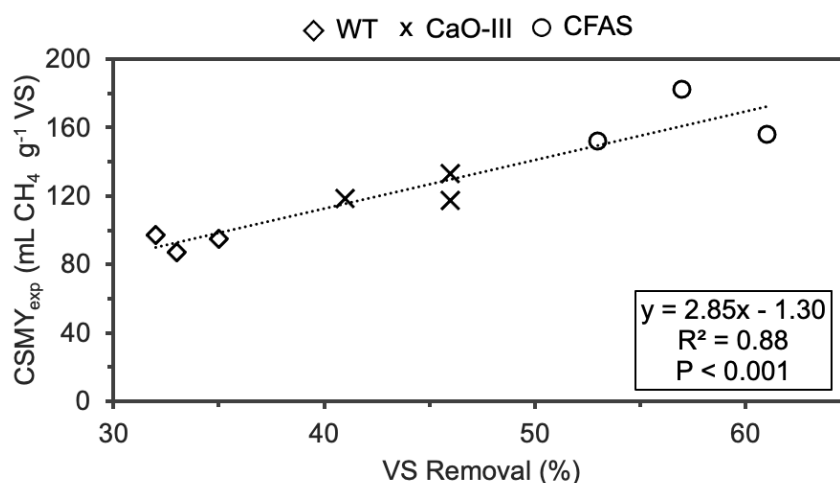
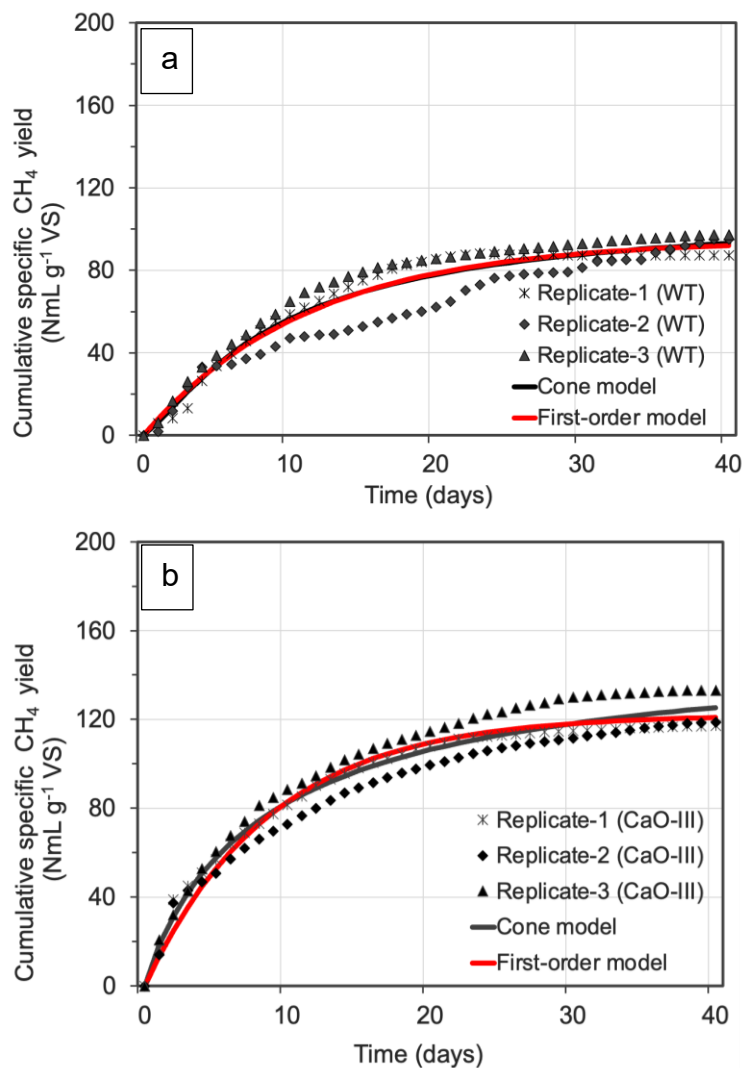


Figure 5.2 Effects of water (WT) and alkali pretreatment (CaO ~11,000 mg CaCO₃ L⁻¹) either with as-received CFA (CFAS) or without (CaO-III) on volatile solids (VS) removal and the mean experimental cumulative specific CH₄ yield (CSMY_{exp}) by solid-state anaerobic digestion of barley straw. Data for VS removal and CSMY_{exp} are presented as triplicate measurements for each treatment condition.

This was consistent with the mean CSMY_{exp} of CFAS (163.6 ± 16.6 mL CH₄ g⁻¹ VS), which significantly increased, measured by 76% and 33% compared to WT ($P < 0.001$), and CaO-III ($P = 0.01$), respectively. CaO-III gave the next highest VS removal (44.3% ± 2.89), which was significantly ($P = 0.01$) greater than the 33.3% ± 1.53 achieved in WT, consistent with the

significant ($P = 0.040$) increase in CSMY_{exp} ($122.9 \pm 8.73 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$), which was 32% higher than WT ($93.1 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$). The changes in CSMY_{exp} of all treatment conditions followed a pattern similar to the VS removal, with an R^2 equal to 0.88 indicating a very strong relationship between CSMY_{exp} and VS removal with a high level of confidence ($P < 0.001$).

Figure 5.3 shows the triplicate CSMY_{exp} and modelled CSMY for the three treatment conditions using the First-order and Cone kinetic models. All treatment conditions (WT, CaO-III and CFAS) produced CH_4 immediately, without a lag phase. Approximately half of the total CH_4 production was achieved within 4–5 d in CaO-III and CFAS treatments (Figure 5.3 (b) and (c)), while it took longer (8 d) in WT (Figure 5.3 (a)).



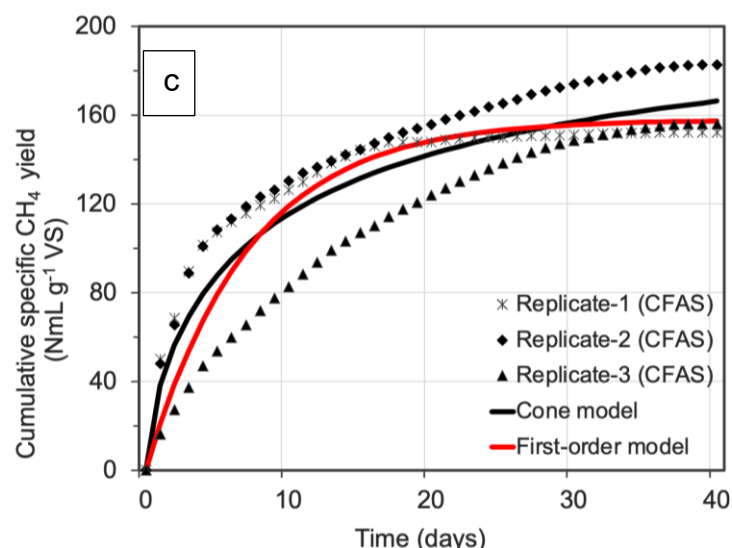


Figure 5.3 Experimental ($n=3$, measured daily) and modelled (First-order and Cone, pooled data) cumulative specific CH_4 yields (CSMY) from anaerobic digestion of barley straw, pretreated using: (a) water (WT), (b) $\text{CaO} \sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (CaO-III), and (c) identical condition to CaO-III with CFA addition (CFAS) at a BS:CFA ratio of 2:1.

The parameter for CSMY_0 obtained from the Cone and First-order kinetic models for WT, CaO-III and CFAS are presented in Table 5.2. Both First-order and Cone models provided a good fit for the experimental data profiles, indicated by the R^2 value, which was 0.91 for WT and 0.94 for CaO-III, respectively, albeit the R^2 for CFAS was slightly smaller, at 0.78 and 0.74 estimated from the Cone and First-order kinetic models, respectively.

Table 5.2 Effects of coal fly ash (CFA) pretreatment on kinetic parameters of First-order kinetic and Cone models for CSMY_0 from anaerobic digestion of barley straw; the relative error (%) between the mean CSMY_{exp} and modelled CSMY (CSMY_0 and CSMY_{40}) as well as CSMY_{40} value from the Cone model are also shown.

Treatment conditions	First-order Model			Cone Model				
	CSMY_0^a	R^{2b}	Error ^c (%)	CSMY_0	CSMY_{40}^d	n^e	R^2	Error ^c (%)
WT	94.9 c	0.914	-1.9	108.2 b	90.0 c	1.25	0.914	-3.3
CaO-III	122.2 b	0.936	0.6	155.8 ab	124.4 b	0.93	0.945	-1.2
CFAS	157.9 a	0.744	3.6	225.3 a	174.1 a	0.71	0.782	-6.4

Values in a column followed by the same letter are not significantly different at $P = 0.05$.

^a CSMY_0 : maximum cumulative specific CH_4 yield potential ($\text{mL g}^{-1} \text{VS}$) (Equation 3.6 and 3.7).

^b R^2 : coefficient of determination

^c Error (%): difference (in percentage) between the experimental (CSMY_{exp}) and CSMY_0 , except for the Cone model between CSMY_{exp} and CSMY_{40} .

^d CSMY_{40} : cumulative specific CH_4 yield at 40 d predicted from Cone model.

^e n : shape factor (Equation 3.7).

The Cone model was used to estimate $CSMY_o$ and the hydrolysis rate (K_H), as it generally provided less systematic deviation from the experimental data pattern compared to the First-order model (Figure 5.3). The $CSMY_o$ derived from the Cone model, however, was observed to deviate significantly compared to the experimental data pattern $CSMY_{exp}$ (with the difference exceeding 5%) across all three treatments. This inconsistency appeared to arise from extrapolation of the asymptote beyond the 40 d of the experimental period. The asymptote was achieved far later than the typical practical retention time used in most industrial biogas plants processing LARs (60–90 d) (Weiland, 2006) and may therefore be considered unreliable. Consequently, the $CSMY_{40}$ was also calculated for the 1–40 d data range, which was equivalent to 90.0, 124.4, 174.1 mL CH₄ g⁻¹ VS for WT, CaO-III and CFAS, respectively. These values were similar to the $CSMY_o$ determined by the First-order model (94.9, 122.2 and 157.9 mL CH₄ g⁻¹ VS), and showed a higher level of agreement (<5%) (shown in Table 5.2) when compared with the mean of $CSMY_{exp}$ (Appendix C, Table C.1), which probably provided a more reliable estimate of the ultimate CSMY from AD of pretreated BS with CFA (CFAS) and equivalent alkalinity chemical treatment (CaO-III).

The hydrolysis rate estimated from the Cone model, K_H is shown in Table 5.3 (refer to Appendix B, Table B1 for hydrolysis rates estimated from the First-order kinetic model, k).

Table 5.3 Summary of hydrolysis rate constants, K_H , estimated from the Cone model for anaerobic digestion of barley straw pretreated with: water (WT), CaO (CaO-III) and combination of CaO and CFA (CFAS).

Treatment Conditions	Replicate Values of K_H^a (d ⁻¹)			Mean K_H^b (d ⁻¹)	RSD (%)	K_H^c (d ⁻¹)
	1	2	3			
WT	0.10	0.05	0.10	0.09 ± 0.03 a	33.40	0.09 a
CaO-III	0.13	0.10	0.11	0.11 ± 0.13 a	11.70	0.11 a
CFAS	0.25	0.16	0.10	0.17 ± 0.07 a	44.70	0.14 a

Note:

(1) RSD: relative standard deviation = standard deviation x 100 / mean.

(2) Values in a column followed by the same letter are not significantly different at $P = 0.05$.

^a Hydrolysis constant estimated from individual replicate data modelling.

^b Data are expressed as mean values (n=3) ± standard deviation.

^c Hydrolysis constant estimated from pooled replicate data modelling.

The single representative K_H value (from the pooled triplicate modelling) (Table 5.3) in CFAS was 0.14 d⁻¹, which increased 27% and 55% compared to 0.11 d⁻¹ and 0.09 d⁻¹ in CaO-III and WT, respectively, albeit neither increase was not significant ($P > 0.05$). The increase in K_H

value was consistent with the significant ($P = 0.019$) increase in CSMY of CFAS compared to CaO-III, equating to 39%, and VS removal, although the latter was not significant. A significant 93% increase in CSMY ($P = 0.016$) was also observed between CFAS and WT, as expected. The K_H in CaO-III was 22% higher than WT, albeit not significant ($P > 0.05$), consistent with significantly higher VS removal and CSMY obtained by CaO-III ($P = 0.050$), equivalent to a 38% increase in CSMY compared to WT. These results indicated the positive effect of alkali pretreatment on the CH_4 yield and beyond that, the inherent properties of CFA, other than alkalinity properties that added further benefits to the CSMY.

5.4 Discussion

5.4.1 Effects of coal fly ash addition on AD requirements for trace elements

In this study, the utilisation of CFA as a more environmentally sustainable alternative to inorganic alkali, and aligned with circular economy through conservation of resources, was explored. The findings, shown in Figure 5.3 indicated a higher CSMY in CFAS compared to CaO-III, suggesting that CFA addition offers additional benefits beyond those of alkali pretreatment alone. This finding was presumed to be associated with the TEs supplementation from CFA, as suggested in the literature (Lo et al., 2009; Huiliñir et al., 2017; Montalvo et al., 2017). Attributing the positive AD response solely to the essential TEs supplied by CFA under the conditions of the experiment, however, is challenging. This challenge arises from the fact that the TEs concentration in the inoculum (Table 3.3 in Section 3.5) significantly exceeds the AD requirement (Table 5.1), suggesting that TEs are not a limiting factor for AD in the absence of CFA. The results of TEs analysis from WT and CFAS digestates (Table 5.1) supported the assumption that the enhanced CSMY observed in CFAS was not related to TEs supplementation from CFA. An alternative contributing factor may be linked to other intrinsic properties of CFA, which forms part of the study, as detailed in Chapter 8.

5.4.2 Effects of coal fly ash addition on the level of potentially toxic elements level in digestates for land application

Assessing the presence of PTEs in the digestate resulting from CFA-added AD is important to ensure its safety and suitability for land application. The results detected that Ni, Zn and Cd present in high concentration in the digestate, slightly exceeding the permissible limits defined in the BSI-PAS 110 (Table 5.1), were attributed to their higher concentration in the inoculum, which already exceeded those of CFA (See Table 3.3). The solubility of many metal elements is pH-dependent because pH affects the speciation of the metal ions and the formation of metal complexes (Koukouras et al., 2011; Izquierdo and Querol, 2012). The addition of CFA into the AD was unlikely to result in contamination of PTEs in the soil, given the low mobility

of Ni, Zn and Cd under the experimental pH conditions of the digestate ranging between 7.2 and 8. The solubility of Cu, Cr, Ni and Zn at a pH of 6 was found to be less than 1 mg kg⁻¹ total element in CFA, and this solubility decreased even further within a pH range of 7–8 (Izquierdo and Querol, 2012).

Koukouzas et al. (2011) and Zhao et al. (2020) suggested that the PTEs present in CFA do not pose any significant leaching risk. Zhao et al. (2020) reported that the typical concentrations of Cu, Zn, Cd and Pb are unlikely to have an adverse impact on groundwater, except under extreme pH conditions below 5, a pH that is not typical of normal environmental conditions. Koukouzas et al. (2011) observed that even under a worst-case scenario of extremely acidic conditions with a pH of 2.88, no element leached beyond the maximum permissible limits, except for Cr. Based on the above findings, the present study suggests that the application of digestates containing CFA under the experimental conditions used here is presumably environmentally safe for land application.

5.4.3 Effects of coal fly ash pretreatment on fibre composition in residual digestates

The highest overall digestibility among fibre components in BS was recorded for hemicellulose, regardless of treatment conditions (Figure 5.1), with half of the hemicellulose in WT lost due to degradation during AD, owing to its inherent biodegradability. Fibre degradation was also observed in the digested sludge used as inoculum during 40 d digestion period, with 6.7% VS hemicellulose and 9.9% VS of lignin lost. These degradation rates were higher than the reported range of 1.0–5.2% VS for hemicellulose and 0.9–4.8% VS for lignin in sewage sludge during mesophilic AD (Liu and Smith, 2022). The 8.6% VS degradation of cellulose observed here was consistent with the reported range of 6–16% (Liu and Smith, 2022).

The enhanced digestibility of cellulose and hemicellulose under CaO-III and CFAS treatments was evident from the significant fraction loss detected during AD (Figure 5.1). In WT, only 10% of cellulose was lost during AD compared to 21% and 51% loss in CaO-III and CFAS, respectively. The loss of cellulose fraction was minimal due to the preserved crystalline structure in the absence of alkali pretreatment, resulting in a very slow degradation rate (Ma et al., 2019). In addition, the cross-linkages of lignin and the carbohydrate complexes remained insoluble, impeding microbial degradation (Leschine, 1995; Du et al., 2019). In contrast, alkali pretreatment in CaO-III and CFAS altered the highly ordered crystalline regions of cellulose into a less-ordered, amorphous form, which is more digestible, and can be rapidly degraded during the initial stages of digestion (Mittal et al., 2011; Bali et al., 2015).

The greater loss observed in the hemicellulose compared to the cellulose fraction was attributed to hemicellulose's non crystalline nature, making it more susceptible to hydrolysis (Wanwu Li et al., 2018). Additionally, hemicellulose is typically located on the outer surface of fibres and in interfibrillar spaces, which serves as an additional physical barrier that restricts cellulose accessibility. Consequently, hemicellulose becomes the preferred substrate for microorganisms during the AD (Bali et al., 2015; Ma et al., 2019). Following the degradation of hemicellulose, cellulolytic bacteria can initiate the degradation of cellulose within the plant cell walls (Hendriks and Zeeman, 2009; Chen et al., 2021).

Of the three fibre components, lignin is the most resistant substance in the AD process due to its insolubility, high molecular weight, highly branched, and non-uniform structure (Monlau et al., 2012; Shafiei et al., 2014). Consequently, final mass residual lignin in the digested BS in WT did not exhibit a significant difference from the initial amount fed to the AD. Despite lignin being solubilised by 34% in CaO-III and CFAS during pretreatment step, no further significant degradation was observed during AD step for CaO-III. In contrast, the effect of alkali pretreatment on lignin was more pronounced with the involvement of CFA (CFAS treatment). It was hypothesised that the presence of CFA stimulated anaerobic microorganism in degrading more fibre components, as demonstrated by a lower mass of cellulose and lignin content, reduced by 38% and 30% lower, respectively, in the final residual digested BS after 40 d digestion, compared to the CaO-III treatment, with complete hemicellulose degradation observed in CFAS. Cumulatively, the total fibre degradation during the combined pretreatment and AD steps was 36% higher with CFA compared to that achieved in CaO-III.

The alkali pretreatment in CaO-III and CFAS led to a total fibre loss of 24.5%, while during the AD step, the losses were higher, with 38.7% in CaO-III and 64.1% in CFAS (see Section 5.3.2). These fibre losses during pretreatment and AD were significantly higher compared to the 5.5% and 23.4% losses, respectively, obtained in WT. The greater contribution by AD step to fibre losses in CaO-III and CFAS compared to pretreatment step aligned with the findings of Kim et al. (2018), who reported a 53% loss by AD compared to a 25% loss during NH_3 pretreatment. The cumulative fibre loss resulting from the combined effects of alkali pretreatment and AD without CFA (CaO-III) was 53.7%, which was lower than the reported cumulative loss of 78.3–83.3% of total fibre in rice straw under NH_3 pretreatment (Kim et al., 2018), while the cumulative loss for the CFAS treatment condition, equating to 72.9%, was comparable with the published value.

5.4.4 Effects of coal fly ash pretreatment on anaerobic digestion

The CSMY curve profiles for the WT condition in Figure 5.3 show a similar trend to observations made in the AD of barley and wheat straw (Marin-Batista et al., 2021; Álvaro et al., 2023). This trend is characterised by a slow degradation and longer decomposition time, as indicated visually by the gentle slope of the WT curve profile (Figure 5.3 (a)) when compared to the steeper slopes observed in the CaO-III (Figure 5.3 (b)) and CFAS CSMY curve (Figure 5.3 (c)). The slower hydrolytic metabolism occurred in WT was indeed due to the presence of larger fractions of insolubilised recalcitrant fibre compared to that in CaO-III and CFAS.

Alkali pretreatment under CaO-III and CFAS experimental conditions have demonstrated significant effectiveness in reducing the recalcitrance of BS, thereby increasing CSMY. The primary mechanisms responsible for reducing the recalcitrant degree are delignification and hemicellulose solubilisation (Kim, 2018; Zdeb, 2021). The increased CSMY in CaO-III was consistent with previous experimental results, which showed 33% lignin reduction and 41% hemicellulose solubilisation following CaO pretreatment at equivalent alkalinity level used in the experiments here (Figure 4.3 (c)). Following the primary reaction of alkali pretreatment, which breaks down ester bonds that connect hemicellulose and lignin (see Section 2.4.2), these components were separated, rendering both more susceptible to microbial degradation (see Section 2.3.3). While hemicellulose is naturally highly digestible, its association with lignin limits its digestibility (see Section 2.1.3.2), therefore, the cleavage of ester bonds led to a greater fraction of hemicellulose being degraded during AD in CaO-III compared to WT (Figure 5.1). The slight reduction in lignin content of BS in the residual digestate of CaO-III compared to its content after pretreatment, before the digestion, was also presumably associated with the cleavage of ether and C-C bonds in lignin during pretreatment (see Section 4.4), producing lower molecular weight lignin-derived products, which were eventually converted to biogas as they entered the carbon and energy metabolism cycle (Van Dyk and Pletschke, 2012; Azman, 2016; Lu et al., 2022).

Lignin reduction and hemicellulose solubilisation increased access to cellulose, providing a larger surface area for cellulolytic bacteria to conduct exocellular degradation and allowing cellulases to hydrolyse cellulose that was previously encapsulated by lignin (Shafiei et al., 2014; Noonari et al., 2019). With access to cellulose opened, the alkali reaction further disrupts the crystalline structure and breaks intramolecular hydrogen bonds within the crystalline cellulose structures, enhancing its susceptibility to enzymatic hydrolysis (Modenbach and Nokes, 2014; Mafa et al., 2020). Disrupted crystalline structure of cellulose was evident from

the reduced total crystallinity index (TCI) estimated from the FTIR spectra in previous experiments (see Section 4.3.3). The impact of this disruption on cellulose degradation during AD and the final cellulose content in the residual digestates after 40 d of AD has been discussed in Section 5.4.3.

The effects of alkali pretreatment enabled the degradation of more carbohydrates (cellulose and hemicellulose), as evidenced by a twofold increase in total fibre reduction in CaO-III relative to WT, which in turn, led to significantly higher VS removals and CSMY detected in both CaO-III and CFAS compared to WT (see Section 5.3.3). The results further showed a high level of confidence in the positive correlation between VS removal, which reflects the transformation of organic materials, and CSMY, with a low *P* value (Figure 5.2) indicating that the relationship is highly unlikely to be due to chance.

The increase in the degree of total fibre solubilisation has led to the acceleration of organic compound degradation, as evidenced by higher K_H in the CaO-III and CFAS compared to WT. This quantitative measurement, determined through K_H determination by fitting the experimental data to mathematical models, supports the earlier visual observation of slower degradation from the WT curve. These results were corroborated by an earlier study reported a positive correlation between the degree of fibre solubilisation and the hydrolysis rate (Monlau et al., 2012). The obtained K_H for the WT and CaO-III treatments fell within the reported range of 0.06 to 0.11 d⁻¹ (Brown et al., 2012; Bolado-Rodríguez et al., 2016; Lallement et al., 2021) and 0.11–0.13 d⁻¹ (Noonari et al., 2019) for untreated and alkali-pretreated LARs, respectively.

The CSMY in both WT (90 mL g⁻¹ VS) and CaO-III treatment (124.4 mL g⁻¹ VS) were comparable to CH₄ yields reported in literature for BS. Despite challenges in finding literature with matched pretreatment parameters (including alkalinity concentrations, pretreatment time), and AD operating conditions (temperature, ISR, C/N ratio, water content) as those employed in the present study, several studies offer insights. Meyer et al. (2022) reported CH₄ yields ranging from 77.2 to 84.7 mL CH₄ g VS⁻¹ for the direct AD of BS, without pretreatment, which are 10–20% smaller than the CSMY obtained here. Another study on AD of BS reported an exceptionally low CSMY of 25 mL CH₄ g⁻¹ VS (Neves et al., 2006), which is 25% of the CSMY obtained in the present study. Studies on the AD of other types of straw, such as wheat and rice straw, have reported comparable values in the range of 80–130 mL CH₄ g⁻¹ VS (Brown et al., 2012; Riya et al., 2021).

The CSMY of CaO-III, with alkalinity concentration equivalent to 0.9% alkali loading rate (ALR), was comparable to the biogas yield of 200 mL g⁻¹ VS (CH₄ composition not specified)

reported following 1% ALR NaOH pretreatment for BS (Bachmann et al., 2022). Several studies, however, have reported higher (36–100%) CSMY values for NaOH-pretreated rice straw with equivalent chemical alkalinity compared to those obtained in the present study. For example, Dai et al. (2018) reported a CSMY of 170.3 mL CH₄ g⁻¹ VS, while Zhang et al. (2015) observed a CSMY nearly twice as high compared to the CSMY obtained in the present study. Factors such as solid to liquid ratio (SLR) in the pretreatment, different LAR source and properties, and different AD operating conditions may contribute to the difference in CSMY observed between the present study and those reported in the literature.

Significant additional benefits in total fibre degradation, organic degradation rate, and kinetic parameters in AD were observed with the incorporation of CFA. The results show a significant increase in CSMY compared to CaO-III, as demonstrated by direct statistical comparisons of the means of CSMY_{exp} values (see Section 5.3.3); findings further confirmed by mathematical modelling results (see Table 5.3). This increase in CSMY corresponded with the significantly higher VS removal achieved in CFAS (see Section 5.3.3), reflected by complete hemicellulose degradation and a twofold increase in combined cellulose and lignin degradation in CFAS compared to CaO-III (see Section 5.3.2). The reductions of total fibre in CFAS during AD step was twice that of CaO-III, consistent with a higher hydrolysis rate, which indicates a more efficient breakdown of organic matter with CFA addition, suggesting there are specific properties of CFA that promote microbial activity and enhance CSMY. These specific properties were investigated further in Chapter 8, while the focus of Chapter 6 is to determine the effects of CFA addition to alkali-pretreated BS, to disentangle the effects of pretreatment from other contributions of CFA on AD performance.

6. Impacts of Alkali Pretreatment and Coal Fly Ash Addition in Enhancing Anaerobic Digestion of Barley Straw

6.1 Introduction

While extensive research has compared different alkali pretreatment methods and their impact on biogas production in the AD of different sources of LARs (reported in Table 2.6), there is a gap in exploring alternative materials capable of substituting the use of alkali. Coal fly ash (CFA) emerges as a potential waste material for this role, and its effectiveness in enhancing CH₄ yield was detailed in Chapter 5. Research utilising CFA in the alkali pretreatment remains limited, however, to the best of author's knowledge, one study has focused on this area, involving bottom ash from biomass power generation. Xiao et al. (2020) used liquors extracted from bottom ash in deionised water to pretreat rice straw and observed that an optimal dosage of 0.37% w/v resulted in a 16% increase in biogas yield compared to an equivalent chemical alkalinity Control. This increase was attributed to the supply of soluble nutrients and residual alkalinity from the bottom ash in the AD process (Xiao et al., 2020).

Coal fly ash has unique physicochemical properties, such as a high pH when fresh, attributed to its significant CaO content (Sear et al., 2003; Achternbosch et al., 2015), and high concentrations of TEs (see Section 2.5.2.1) (Huiliñir et al., 2015; Montalvo et al., 2017; Lauzurique et al., 2021a). These properties are important for AD optimisation and can be present in the extractable components of CFA, as explored in the previous study (Xiao et al., 2020). Beyond these extractable components, CFA may have other physicochemical properties that can enhance AD performance, which remain underexplored. The novelty of the present study lies in investigating the benefits of using total CFA rather than only the extractable component in the leached ash, not only as a source of alkalinity for pretreatment but also for its supplementary contributions to the AD process.

Through carefully controlled experiments conducted in Chapter 5, it became evident that the CH₄ yield following CFA pretreatment was significantly greater than could be explained by the response to alkaline conditioning alone with an equivalent alkalinity Control using CaO. This indicated that factors beyond alkalinity contribute to the observed enhancement in CH₄ yield. The experiments in the current study were unable to isolate the effect of TEs supplementation from CFA, given the inoculum sludge was rich in TEs, therefore ensuring an adequate supply (Table 3.3), and digestates without CFA contained sufficient TEs (Table 5.1), further supporting the assumption that the presence of TEs in the CFA did not significantly influence CH₄ yield.

The main aim of this study was to unravel the combined effect of alkalinity and CFA addition on CH₄ yield in the AD of BS, diminishing the influence of CFA from the alkalinity effects on fibre compositions and confirming that the positive responses were specifically attributable to CFA addition. The investigations involved CFA addition to alkali-treated BS prior to the AD process, which were achieved through solid-state AD experiments with BS, and the following experimental objectives: (1) to determine the AD response to water and alkali pretreatment using (i) pure inorganic NaOH, (ii) pure inorganic CaO, and (iii) pure inorganic CaO and CFA addition prior to AD, and (2) to determine the AD response to varying alkalinity levels in relation to the fibre composition changes detailed in Chapter 4.

6.2 Materials and methods

6.2.1 Waste feedstocks

The waste feedstocks used were milled BS, as-received CFA, and fresh digested sewage sludge cake for AD inoculum. The source and preparation of each material are described in Section 3.1.1, 3.1.3, and 3.1.4, respectively, and their general physicochemical properties are presented in Table 3.2.

6.2.2 Batch experiments of solid-state anaerobic digestion

The design of the AD experiments is presented in Figure 6.1 (see Figure 1.2 for the overall experimental programme). The solid-state AD experiments consisted of one Blank (inoculum only), two Controls groups: (1) untreated BS and (2) water pretreated BS, and three alkali pretreatment groups (with codes assigned to these treatments in parentheses): (1) one NaOH pretreatment (alkalinity concentration equivalent to ~11,000 mg CaCO₃ L⁻¹) – (NaOH), (2) CaO pretreatment using two CaO concentrations (alkalinity concentration equivalents to ~5,000 and 11,000 mg CaCO₃ L⁻¹) – (CaO-II and CaO-III), respectively, and (3) a CaO pretreatment using a CaO alkalinity concentration equivalent of ~11,000 mg CaCO₃ L⁻¹ with CFA addition prior to AD – (CaO-III+CFA). The selection of the two CaO concentrations (~5,000 and 11,000 mg CaCO₃ L⁻¹) was based on the statistically significant effects of these alkalinity levels observed on fibre composition (See Section 4.3.2). The solid-state AD experiments for the Controls and alkali-pretreated groups were conducted in triplicate.

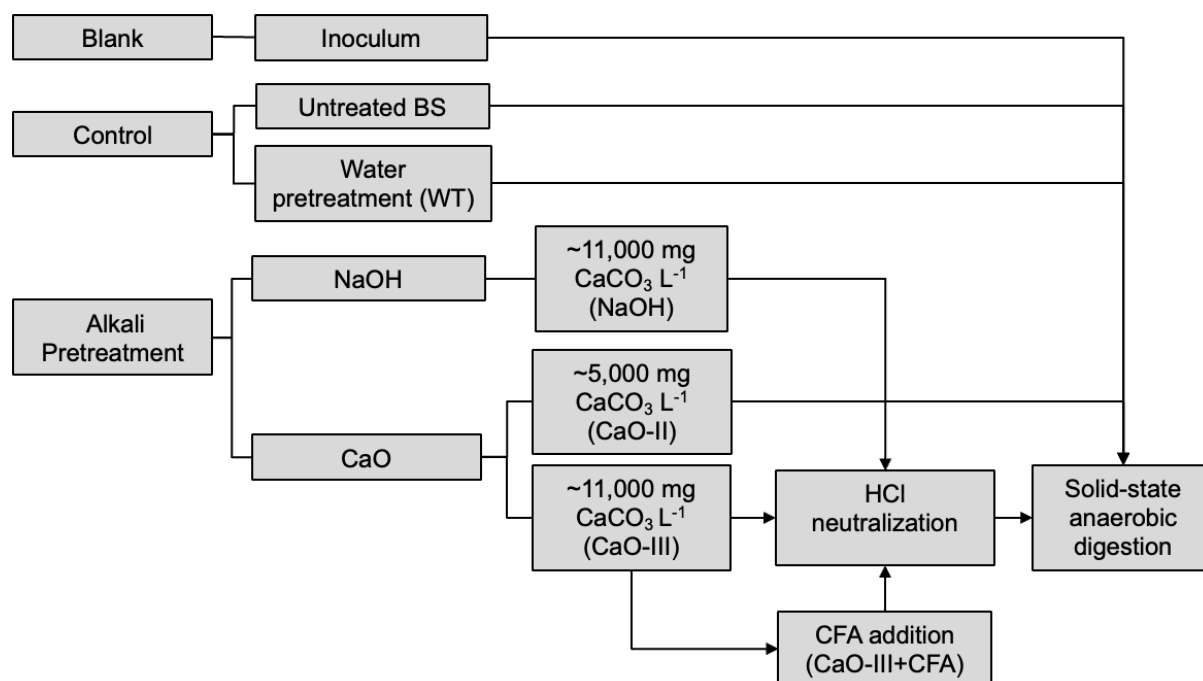


Figure 6.1 Schematic showing the design of the solid-state anaerobic digestion experiments, and the codes assigned to the experimental treatments.

The pretreatment procedures for WT, NaOH and CaO treatments followed the same procedures detailed in Section 4.2.2, with the specific quantities of BS, CaO/NaOH and water used in this experiment provided in Table 6.1. For the CaO-III+CFA condition, 7.5 g of as-received CFA was added to pretreated BS at the end of the pretreatment period. The mixtures of BS with CFA, CaO, NaOH and water were mixed thoroughly using a laboratory spatula.

Table 6.1 Experimental metrics applied for all treatments: untreated barley straw (BS), water pretreated BS (WT), pretreated barley straw with NaOH ~11,000 mg CaCO₃ L⁻¹ (NaOH), CaO~11,000 mg CaCO₃ L⁻¹ (CaO-III), CaO ~5,000 mg CaCO₃ L⁻¹ (CaO-II), and identical condition to CaO-III with CFA addition prior to anaerobic digestion (CaO-III+CFA).

Experimental Treatments	Barley Straw (g VS)	Chemical (CaO or NaOH) (g) ^a	CFA (g) ^a	Inoculum (g VS)	Water (mL) ^b	Water (mL) ^c	pH ^d
NaOH	13.0 ± 0.5	1.125	0.0	13.0 ± 0.5	125.0	0.0	7.2 ± 0.2
CaO-II	13.0 ± 0.5	0.600	0.0	13.0 ± 0.5	125.0	0.0	7.8 ± 0.1
CaO-III	13.0 ± 0.5	1.125	0.0	13.0 ± 0.5	125.0	0.0	7.7 ± 0.2
CaO-III +CFA	13.0 ± 0.5	1.125	7.5	13.0 ± 0.5	125.0	70.0	7.5 ± 0.1
Untreated BS	13.0 ± 0.5	0.0	0.0	13.0 ± 0.5	N/A	130.0	7.2 ± 0.3
WT	13.0 ± 0.5	0.0	0.0	13.0 ± 0.5	125.0	0.0	7.7 ± 0.1
Inoculum	N/A	N/A	N/A	25.0 ± 0.5	N/A	120.0	7.4 ± 0.1

Note: N/A = not applicable.

^a Mass used in the pretreatment on a total solid basis, ^b Water used in the pretreatment step, ^c Extra water added after the pretreatment step to adjust the solids content to 15% TS, ^d final pH of feedstock mixtures prior to loading into anaerobic digestion reactors.

In the untreated Control, 130 mL of deionised water was added to 15 g of dry BS to adjust the solids concentration to an approximate TS of 15%, in accordance with the operational procedures for solid-state AD experiments (see Section 3.2.1). Concentrated HCl acid (3–4 mL) was added to neutralise the pH of the pretreated BS in the NaOH, CaO-III and CaO-III+CFA treatment conditions. In the case of CaO-II treatment condition, pH adjustment was not necessary as the pH of pretreated BS under this treatment was 7.8 ± 0.09 . Inoculum was added to both Controls and pretreatment conditions to achieve an ISR of 1 (VS basis) and homogenised using a hand-held kitchen mixer. Two fractions of subsamples, approximately 20 g each, based on FM, were taken from each experimental condition for TS, VS and pH measurements. The general operating protocols of the solid-state AD experiments were detailed in Section 3.2.2 and 3.2.3.

6.2.3 Analytical methods

The determination of TS and VS was conducted on all experimental substrate mixtures before, and on residual digestates after solid-state AD experiments following the procedures outlined in Section 3.3.2. The VS of the input mixtures and the residual digestate were used to assess the effectiveness of the AD process in terms of organic matter removal rate (% VS removal), using Equation 5.4. Volatile solids removals were plotted against their corresponding CH₄ yield to establish a correlation between VS removal and CH₄ yield. Biogas volume was measured using a water displacement method (see Section 3.2.3) and CH₄ composition was measured using GC (see Section 3.3.13). The background CH₄ gas from the Blank (refer to Appendix A, Figure A.3 for the residual CH₄ production from the inoculum used in this chapter) was subtracted from the measured total CH₄ volume in both the Control and treatment groups. All analyses were conducted in triplicate.

6.2.4 Statistical analysis, CH₄ gas modelling and kinetics

Triplicate measurements of CSMY_{exp} from both Control and alkali-pretreated groups were normalised to STP conditions (see Section 3.2.5). Methane gas modelling was conducted on the normalised CSMY_{exp} values after removing outlier data, as detailed in Section 3.4. Modelling of the CH₄ yield and kinetic parameters, and determination of the CSMY_o followed the procedures described in Section 3.4. The statistical significance of differences in CSMY and kinetic parameters between the experimental treatments were determined by a one-way analysis of variance (ANOVA) (see Section 3.4).

6.3 Results

6.3.1 Effects of alkali pretreatment and CFA addition on hydrolysis rates, volatile solid removals and CH₄ yields

Figure 6.2 shows the effects of CaO-III+CFA compared to WT Control, NaOH, and the two CaO pretreatments on VS removal and the CSMY_{exp}. The triplicate CSMY_{exp} values and VS removals for each treatment condition are provided in Appendix C, Table C.2.

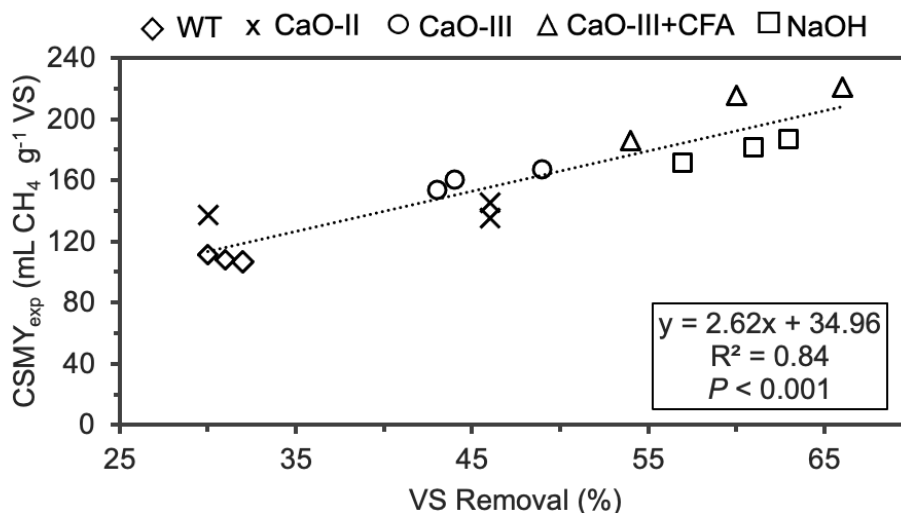


Figure 6.2 Effect of pretreatment with water (WT Control), different alkali sources and concentrations (NaOH, CaO-III, CaO-II), and a combination of alkali pretreatment and CFA addition (CaO-III+CFA) on volatile solids (VS) removal and experimental cumulative specific CH₄ yield (CSMY_{exp}) by solid-state anaerobic digestion of barley straw. Triplicate data are presented for each experimental condition.

The mean CSMY_{exp} of CaO-II was 139.7 ± 5.07 mL CH₄ g⁻¹ VS, which significantly ($P = 0.020$) increased by 28%, compared to WT Control (108.9 mL CH₄ g⁻¹ VS), and this was consistent with a 32% increase in VS removal rate of 40.7 ± 9.24 % in CaO-II compared to 31.0 ± 1.00 % in WT Control, although not statistically significant ($P > 0.05$). A higher alkalinity concentration, CaO-III significantly ($P = 0.05$) increased the VS removal rate by 46% compared to WT, to 45.3 ± 3.21 %, aligned with the significant ($P < 0.001$) increase, equating to 47% increase in CSMY_{exp} of 160.4 ± 6.49 mL CH₄ g⁻¹ VS. Neither VS removal nor CSMY_{exp} in CaO-III increased significantly ($P > 0.05$), compared to CaO-II, which equivalent to 11% and 15% increase, respectively. The significant increase in CSMY_{exp} observed in both CaO-II and CaO-III compared to the WT Control highlighted the effectiveness of CaO pretreatment in increasing the digestibility of BS.

The largest VS removal occurred with NaOH pretreatment, and interestingly, CaO-III+CFA pretreatment gave similar VS removal, equivalent to $60.3\% \pm 3.05$ and $60\% \pm 6.00$, respectively. These removal rates were significantly higher (NaOH; $P = 0.039$ and CaO-

III+CFA; $P = 0.044$), equating to 33% increase, compared to that of CaO-III. Despite both NaOH and CaO-III+CFA pretreatments showing similar VS removal rates, interestingly, the mean $CSMY_{exp}$ of CaO-III+CFA pretreatment ($207.6 \pm 18.75 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$) was significantly ($P = 0.037$) greater compared to the NaOH pretreatment ($179.8 \pm 7.91 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$). Figure 6.2 shows that the changes in $CSMY_{exp}$ of all treatment conditions followed a pattern similar to that observed for the VS removal, with an R^2 equal to 0.84 indicating a very strong relationship between $CSMY_{exp}$ and VS removal with a high level of confidence ($P < 0.001$).

Figure 6.3 shows the triplicate $CSMY_{exp}$ values and modelled $CSMY$ data for all treatment conditions using the First-order and Cone kinetic models. Apart from untreated BS Control, the triplicate $CSMY_{exp}$ values of all treatment conditions: WT control, NaOH, CaO-II, CaO-III and CaO-III+CFA generally showed good agreement, with a calculated relative standard deviation (RSD) of 2.2%, 4.4%, 3.6%, 4.0%, and 9.0%, respectively (refer to Appendix C, Table C.2 for mean and standard deviation values of $CSMY_{exp}$ replicate data used in calculating RSD), consistent with recommendation for acceptable variation in test results (Holliger et al., 2016) (see Section 3.4). The RSD for the triplicate $CSMY_{exp}$ of untreated BS was 17%, and this divergence was due to the $CSMY_{exp}$ from the third replicate significantly deviating ($81.9 \text{ mL CH}_4 \text{ g}^{-1} \text{ VS}$) from the other replicates, as shown in Figure 6.3 (a). The RSD was improved to an acceptable value of 3.19% when the third replicate experimental data was excluded, hence only two replicates were involved in the CH_4 gas modelling for the untreated BS Control.

Table 6.2 shows the parameter for $CSMY_o$ determined from the Cone and First-order kinetic models ($CSMY_o$ from each replicate used for statistical comparisons are provided in Appendix B, Table B.2). The R^2 values obtained for both models indicated good agreement was obtained with the experimental data, but the Cone model demonstrated a slightly superior fit with values in the range: 0.85–0.97 compared to 0.83–0.96 for the First-order kinetic model. The predicted $CSMY_o$ from the Cone model, however, was significantly larger than the mean $CSMY_{exp}$, with the difference exceeding 5%. This inconsistency appeared to arise from extrapolation of the asymptote that would be achieved beyond the 40 d or up to approximately 200 d, which is an uncommon retention time in large-scale AD operations for processing LARs (Weiland, 2006), and therefore should not be considered a reliable estimate.

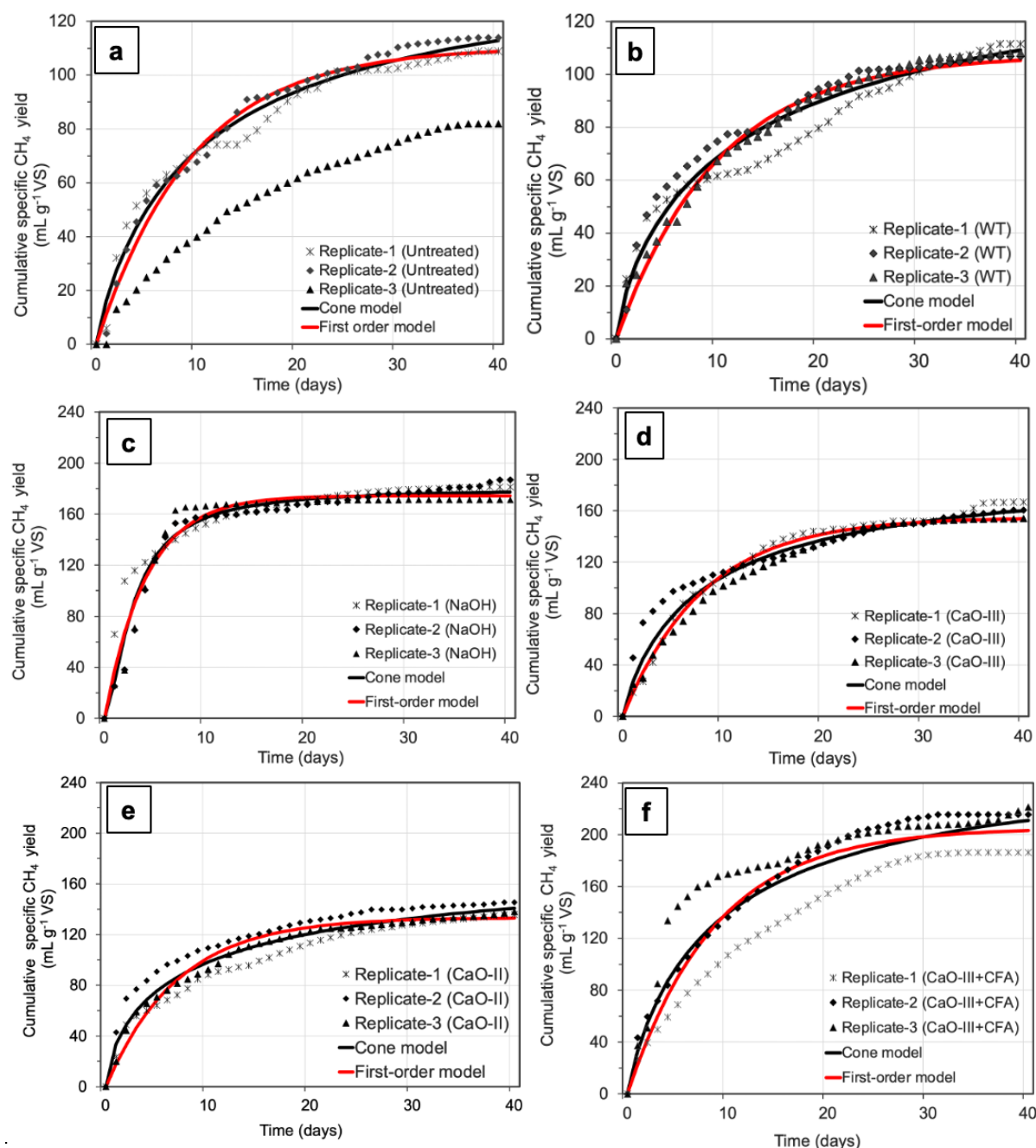


Figure 6.3 Experimental ($n=3$, measured daily) and modelled (First-order kinetic and Cone, pooled data) cumulative specific CH_4 yields (CSMY) from anaerobic digestion of barley straw with different treatment conditions: (a) untreated barley straw, (b) water pretreated barley straw (WT), (c) pretreated barley straw with $\text{NaOH} \sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (NaOH), (d) $\text{CaO} \sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (CaO-III), (e) $\text{CaO} \sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (CaO-II), and (f) identical condition to CaO-III with CFA addition (CaO-III+CFA). Replicate 3 dataset was excluded from the modelling of the untreated Control condition.

Consequently, the CSMY value was estimated by the Cone model for the 1–40 d data range (CSMY_{40}), which were equivalent to 103.2, 109.2, 177.3, 140.7, 160.2, 211.1 $\text{mL CH}_4 \text{ g}^{-1} \text{VS}$ for the untreated Control, WT Control, NaOH, CaO-II, CaO-III, and CaO-III+CFA treatment conditions, respectively. These values were similar to the CSMY_0 determined by the First-order kinetic model (102.5, 107.3, 174.5, 133.4, 153.8, 205.1 $\text{mL CH}_4 \text{ g}^{-1} \text{VS}$, respectively),

and showed a higher level of agreement (<5%) (Table 6.2) when compared with the mean $CSMY_{exp}$, reported above, which probably provided a more reliable estimate of the ultimate $CSMY$ from AD of BS, including both Control and pretreated BS with NaOH and CaO.

Table 6.2 Effects of pretreatment using deionised water (WT) and different alkali sources and dosages on kinetic parameter of the First-order kinetic and Cone models for $CSMY_o$ from anaerobic digestion of barley straw; the relative error (%) between the mean $CSMY_{exp}$ and modelled $CSMY$ ($CSMY_o$ and $CSMY_{40}$) as well as $CSMY_{40}$ value from the Cone model are also shown.

Treatment Conditions	First-order Model			Cone Model				
	$CSMY_o^a$	R^{2b}	Error (%) ^c	$CSMY_o$	$CSMY_{40}^d$	n^e	R^2	Error (%) ^c
Untreated	102.5 d	0.96	-0.9	147.5 b	103.2 d	0.89	0.97	-1.6
WT	107.3 d	0.91	1.6	158.0 b	109.2 d	0.77	0.95	-0.3
NaOH	174.5 c	0.92	2.9	180.4 b	177.3 c	1.54	0.93	1.4
CaO-II	133.4 b	0.87	4.5	192.8 b	140.7 b	0.69	0.92	-0.7
CaO-III	153.8 b	0.94	4.1	198.4 b	160.2 b	0.89	0.96	0.1
CaO-III+CFA	205.1 a	0.83	1.2	266.9 a	211.1 a	0.89	0.85	-1.7

Note: Values in a column followed by the same letter are not significantly different at $P = 0.05$.

^a $CSMY_o$: maximum cumulative specific CH_4 yield potential ($mL\ g^{-1}\ VS$) (Equation 3.6 and 3.7).

^b R^2 : coefficient of determination of the modelled CH_4 data

^c Error (%): difference (in percentage) between the experimental ($CSMY_{exp}$) and maximum cumulative specific CH_4 yield ($CSMY_o$), except for the Cone model between $CSMY_{exp}$ and $CSMY_{40}$.

^d $CSMY_{40}$: cumulative specific CH_4 yield at 40 d predicted from Cone model.

^e n : shape factor (Equation 3.6).

The $CSMY$ of WT Control was not significantly different ($P > 0.05$) compared to untreated BS Control. The hydrolysis rates of both Controls were relatively slow, only achieving 50% of the total $CSMY$ within the initial 7 d, as observed in Figure 6.3 (a) and (b), supported by the K_H obtained from the Cone model (Table 6.3) showing the same values for untreated and WT Control ($0.07\ d^{-1}$) (k obtained from the First-order kinetic model are presented in Appendix B, Table B.2). In contrast, all the alkali-pretreated conditions produced 50% of their overall $CSMY$ within 3-4 d (see Figure 6.3 (c) to (f)).

Sodium hydroxide pretreatment, supplying an alkalinity equivalent to $\sim 11,000\ mg\ CaCO_3\ L^{-1}$ as in CaO-III, was expected to be the most aggressive and reactive form of alkali pretreatment (Zheng et al., 2014; Bali et al., 2015; Bachmann et al., 2022). Indeed, this was confirmed by the highest $CSMY$ achieved among the alkali pretreatment conditions (Table 6.2). Sodium hydroxide pretreatment did not significantly ($P > 0.05$) increase the $CSMY$, despite 11% increase in $CSMY$ compared to CaO-III. This maximum $CSMY$, however, was achieved 10 d earlier with NaOH compared to CaO-III (Figure 6.3 (c)), and the K_H of NaOH treatment from

the Cone model (0.35 d^{-1}) (Table 6.3) supported this, with a value that was almost three-fold higher ($P = 0.022$).

Table 6.3 Summary of hydrolysis rate, K_H (d^{-1}) estimated from the Cone model for anaerobic digestion of untreated barley straw (BS) and BS pretreated with: water (WT), NaOH, CaO equivalent to $5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (CaO-II) and $11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (CaO-III), and identical to CaO-III with coal fly ash addition prior to AD (CaO-III+CFA).

Treatment Conditions	Replicate Values of K_H^a			Mean K_H^b	RSD (%)	K_H^c
	1	2	3			
Untreated	0.08	0.10	n.d	$0.09 \pm 0.01 \text{ a}$	16.00	0.07 a
WT	0.07	0.12	0.07	$0.09 \pm 0.03 \text{ a}$	32.00	0.07 a
NaOH	0.43	0.29	0.29	$0.34 \pm 0.08 \text{ b}$	25.00	0.35 b
CaO-II	0.09	0.15	0.15	$0.13 \pm 0.03 \text{ a}$	25.00	0.11 a
CaO-III	0.16	0.14	0.12	$0.14 \pm 0.02 \text{ a}$	16.00	0.12 a
CaO-III+CFA	0.07	0.09	0.26	$0.14 \pm 0.11 \text{ a}$	77.00	0.11 a

Note:

(1) RSD: relative standard deviation = standard deviation $\times$ 100 / mean.

(2) n.d: not determined following the exclusion of one of the CSMY_{exp} due to high deviation $>10\%$ of the population.

(3) Values in a column, followed by the same letter are not significantly different at $P = 0.05$.

^a Hydrolysis constant estimated from individual replicate data modelling.

^b Data are expressed as mean values ($n=3$) $\pm$ standard deviation.

^c Hydrolysis constant estimated from pooled replicate data modelling.

Calcium oxide pretreatments were less effective than NaOH; however, both CaO-II and CaO-III significantly increased the CSMY (Table 6.2), with the values were 29% ($P = 0.046$) and 47% ($P < 0.001$) higher, compared to WT Control. This was consistent with the 57–71% increase in K_H of both CaO-III (0.12 d^{-1}) and CaO-II (0.11 d^{-1}), compared to that of WT Control (0.07 d^{-1}) (Table 6.3), even though it was not significant ($P > 0.05$). The magnitude of CSMY increased by 14% with higher alkalinity concentration, however, the differences in CSMY and K_H between CaO-II and CaO-III were not significant ($P > 0.05$).

The addition of CFA to CaO-pretreated BS prior to AD (CaO-III+CFA condition) with the equivalent alkalinity of NaOH treatment significantly ($P < 0.001$) increased the CSMY (Table 6.2) by 19%, 32% and 93%, compared to NaOH, CaO-III and WT Control, respectively. Despite the significant increase in CSMY, the K_H of CaO-III+CFA (0.11 d^{-1}) (Table 6.3) was significantly smaller ($P = 0.023$) than that of NaOH pretreatment, while it was comparable to that of CaO-III.

6.4 Discussion

This study investigated the effects of CFA addition to the alkali-pretreated BS, prior to the AD process, specifically distinguishing these effects from those caused by alkalinity on fibre composition. This was compared to the effects of equivalent alkalinity chemical treatment using CaO and NaOH.

The effect of alkali pretreatment was visually reflected from the shape of the individual curve of triplicates $CSMY_{exp}$ for alkali-pretreated groups including the NaOH, CaO-III and CaO-II treatments that exhibit a single-stage degradation profile (Figure 6.3 (b), (c), (d), (e)), in contrast to the multi-stage profile detected in the untreated BS and WT Control (Figure 6.3 (a) and (b)). This multi-stage profile presumably arose from the separate degradation of low and high complexity substrates (Brown et al., 2012; Gomes et al., 2021). The initial stage can be attributed to the conversion of low complexity, or rapidly digestible substrates such as free sugars, oligosaccharides, organic acids, while the subsequent stage is related to fibre hydrolysis (Brown et al., 2012; Azman, 2016). In contrast to multi-stage degradation patterns observed in the untreated BS and WT Control, in the alkali-pretreated groups, the high complexity fraction, i.e., fibre, has been converted into more digestible forms, resulting in a single-stage degradation pattern.

The precision of K_H for CaO-III+CFA was notably low at 77% (Table 6.3). The complexity of the experimental conditions in CaO-III+CFA, which involved a mixture of three waste feedstocks: BS, inoculum sludge, and CFA, could introduce more sources of variability in kinetic parameters compared to simpler substrates. The variability in K_H among replicate experimental treatment conditions can range from 55–70%, as reported by Raposo et al. (2011). Under controlled experimental conditions in the present study, where the consistency of waste feedstocks quality and digestion conditions were carefully maintained, the variability in K_H observed among replicates in CaO-III+CFA treatment is probably indicative of the inherent natural variability that may occur in the operation of solid-state AD, where proper mixing can often be inadequate. In the present study, the homogenisation process in the solid-state AD experiment relied solely on external mixing of the waste feedstocks prior to transfer to the reactor. Despite consistent procedures being followed to ensure an equal degree of homogenisation of feedstocks among the reactors (see Section 6.2.2), there remains a possibility that the degree of mixing varied between reactors within the same treatment condition, resulting in a more homogenous distribution of the waste feedstock as the substrate and microorganisms in one replicate compared to the others.

As digestion progresses and enzymes are excreted while organics are degraded, the absence of internal mixing (within the reactor) means that the transport of degradable products and enzyme relies solely on natural diffusion, consequently, these components may not be uniformly distributed within the reactor (Wang et al., 2017; Paul and Chakraborty, 2019). This could account for differences in the effectiveness of substrate conversion by microorganisms across the reactor, resulting in kinetic parameters variation (Rocamora et al., 2020; Singh et al., 2020). This natural variation could also be attributed to the heterogeneity in the CFA particle size distribution, as the experiments conducted here used the as-received CFA sample without selecting specific particle sizes. Each particle size of CFA may possess different inherent properties that influence the surface attachment of microorganisms that eventually affects kinetic parameters such as the hydrolysis rate. The specific surface area, for example, increases with decreased particle size, implying that smaller particle sizes of CFA have the potential to support a greater microbial biomass through increased microbial surface attachment (Jaafar et al., 2015; Montalvo et al., 2017).

This study explored whether water pretreatment could be an alternative method to increase the digestibility of fibre and CH₄ yield in the AD of LARs. The results show that water pretreatment did not change the CSMY compared to the untreated Control, consistent with the unaltered fibre composition (see Section 4.3.2). In contrast to this, Bali et al. (2015) suggested that water pretreatment may be considered as an efficient and cost-effective pretreatment method, as the CSMY of water-pretreated wheat straw obtained from their experiments was higher than all CSMY available in the literature for the untreated wheat straw. This is possible, however, substrate quality and inoculum source can vary widely, and different compositions of substrate can lead to different results (Sawatdeenarunat et al., 2015) (Table 2.1). Therefore, controlled experiments are important to ensure a consistent quality of substrate and operating AD condition.

The results obtained from the present study were consistent with previous research, despite the limited availability of literature reporting on the effects of water pretreatment with similar pretreatment durations on CH₄ yield of BS. With the paucity of literature on BS, therefore, comparisons were made with wheat straw and sunflower stalk. Sambusiti et al. (2013) used wheat straw with lower lignin content (6.6% TS) to that of the BS used in the present study (8.6% TS) (Table 3.2). They reported that water pretreatment did not affect the CH₄ yield of wheat straw, despite using a relatively high water temperature (40°C) compared to the room temperature deionised water used in the current study. The pretreatment, however, was conducted in much shorter duration (1 h) compared to the 7 d in the present study. Similarly, Liu et al. (2015) reported no difference in CH₄ yield between water-pretreated and untreated

wheat straw, however, the lignin content of wheat straw used in their study was higher (18.4% TS) than BS used in the current study and the duration of the treatment of 24 h was also much shorter than that the 7 d applied here. Consistent results were also reported by Monlau et al. (2012), who observed that water pretreatment at temperatures 30, 55, and 80° C for 24 h did not increase CH₄ yield of sunflower stalks.

In contrast to water pretreatment, alkali pretreatment using either NaOH or CaO in the present study have been proven to significantly increase CSMY compared to both untreated and WT Controls. The CSMY in the NaOH treatment reflected the maximum response to the alkalinity applied in the present study, however the insignificant difference in CSMY between NaOH and CaO pretreatment indicates that with equivalent alkalinity concentration, CaO pretreatment is also effective to increase the digestibility of BS. NaOH pretreatment was only slightly more effective than CaO, indicated by the higher VS removal and the K_H value. The insignificant difference in CSMY between the two conditions aligns with the fibre composition results reported earlier (Section 4.3.2) which showed no significant variation in cellulose, hemicellulose and lignin content. Similar results were reported by Monlau et al. (2012), who observed no significant difference in CH₄ yield from sunflower stalks following pretreatment using Ca(OH)₂ and NaOH.

Sodium hydroxide pretreatment resulted in a significantly higher K_H compared to CaO-III (Table 6.3), consistent with its effectiveness in lignin removal, H bonds cleavage and solubilisation of amorphous cellulose, as shown in previous experiments (see Section 4.4), albeit not statistically significant. Although the CSMY between NaOH and CaO-III pretreatment showed no significant difference, the improved hydrolysis rate with NaOH could potentially shorten the overall digestion period. The increase in CSMY achieved by NaOH and CaO pretreatment (47–62%) at equivalent alkalinity concentration, compared to WT Control, fell within the reported range of 38–119% in the literature for rice straw under similar alkalinity conditions (Dai et al., 2018; Sabeeh et al., 2020; Shetty et al., 2017; Zhang et al., 2015) (see Table 2.6).

The CSMY increase observed in CaO-III and NaOH treatments corresponded with higher VS removals, as indicated by the strong linear relationship between these variables, as shown in Figure 6.2. The enhanced VS removal following alkali pretreatments suggests that complex fibres were effectively broken down into soluble compounds including starch, pectin, sugar and some inorganics (Noonari et al., 2019), therefore increasing the availability of more VS for microbial degradation during the AD process. The magnitude of the effect decreased with CaO concentration, with CaO-II treatment achieving a lower but still significant CSMY increase

compared to WT. The increase in CSMY for CaO-II was in good agreement with the reported range of 28–33.5% for equivalent alkalinity (Gu et al., 2015; Zhang et al., 2015). The higher CSMY in CaO-II compared to WT Control aligned with its VS removal rates, which fell within the 37–70% range reported for NaOH-pretreated rice straw under equivalent alkalinity conditions (Abudi et al., 2016; Shetty et al., 2017; Dai et al., 2018). Both CaO-II and CaO-III also accelerated the K_H compared to WT Control, though not significantly.

The results of the present study suggest that an alkalinity concentration of 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ is optimum, and higher concentrations will not significantly affect CSMY and VS removal. This result was consistent with Zhang et al. (2015), who reported that CSMY remained stable despite using higher alkalinity levels. They found that a 0.5% w/v NaOH loading rate, equivalent to approximately 6,000 to 6,500 mg $\text{CaCO}_3 \text{ L}^{-1}$, was optimal in their study. Similarly, Shetty et al. (2017) observed an insignificant increase in CSMY (7.6%) with increased alkalinity concentration from 5,000 to 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$. In contrast, Dai et al. (2018) reported an alkalinity concentration $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ led to a 64% increase in CSMY compared to $\sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$, which was significantly higher than that achieved in the present study. In the context of using CFA as the alkaline agent, where CaO is the primary source of alkalinity, the use of CFA with higher CaO content is advantageous. It requires smaller quantities of CFA and water to achieve similar alkalinity levels, optimising digester capacity for substrates in large-scale applications.

The CSMY of CaO-III+CFA was even greater than the maximum alkalinity effect achieved by NaOH pretreatment, which is considered as the most efficient method of alkali pretreatment. This was evidenced through direct statistical comparison of the mean CSMY_{exp} values between these two treatments. Mathematical modelling was used to comprehensively understand the data and validate observed trends, and the modelling results further validated the observation from direct statistical comparison. VS removal in CaO-III+CFA was higher than in CaO-III, however similar VS removals were observed between CaO-III+CFA and NaOH treatment conditions. Despite this, higher CSMY was achieved in CaO-III+CFA, indicating that more CH_4 was generated per gram of VS destructed in CaO-III+CFA.

The results of the present study confirmed the enhanced CSMY observed with the addition of CFA, as hypothesised in Chapter 5. Specifically, a 32% increase in CSMY was observed in CaO-III+CFA compared to CaO-III, consistent with the 39% increase achieved in the previous results presented in Chapter 5 when CFA was incorporated into the pretreatment. In addition, the CSMY increase in CaO-III+CFA compared to the equivalent chemical alkalinity Control was higher compared to the 16% increase reported in the literature when extractable bottom

ash was used as an alkali substitute in pretreatment (Xiao et al., 2020). Certainly, this suggests CFA interactions in the AD of BS were not attributed to the pretreatment effect only and that other attributes, beyond alkalinity and soluble nutrients, contribute to the observed enhancement. Before the potential for enhanced AD performance with CFA, in relation to other attributes, is explored (in Chapter 8), another important factor in the development of an integrated AD system is to investigate the role of food waste, as a neutralising agent in alkali-pretreated BS in solid-state AD, which is described in Chapter 7.

7. Fermented Food Waste for pH Neutralisation of Alkali-Pretreated Barley Straw in Solid-State Anaerobic Digestion

7.1 Introduction

Alkali pretreatment is an effective strategy to reduce the recalcitrance of fibres in LARs to improve digestibility in the AD process. Experimental results presented in Section 5.3.2 and 5.3.3 demonstrate that pretreatment with alkali CFA (supplemented by CaO) increased the solubilisation of lignin and hemicellulose, and altered cellulose crystallinity, contributing to a 38% increase in CH₄ yield, compared to the water-treated BS as the Control. To facilitate the breakdown of the complex lignin structure and the linkages between lignin and carbohydrates, however, a high pH value of 11–12 is required (Monlau et al., 2012; Sun, 2015; Jiang et al., 2017). These pH levels in the feedstock are significantly higher than the optimum pH range for hydrolysis and acidogenesis phases in AD, which lies between 5.2 and 6.5 (Dai et al., 2018; Y. Chen et al., 2020). High pH conditions can disrupt the balance of microbial populations, inhibit the growth and activities of acidogenic microorganisms, and lead to NH₃ toxicity (although this is unlikely in the AD of LARs, given the low NH₃ and TN content (0.4% TS, Table 3.2) and impede the overall process efficiency (Lindner et al., 2015; Tian et al., 2018). Lowering the pH of alkali-pretreated LARs is therefore essential to avoid potential toxicity risks to the anaerobic microbial community (Bhattacharya and Parkin, 1989) and to optimise the benefits of alkali pretreatment for CH₄ production.

The conventional approach to reducing the pH of alkali-pretreated LARs in laboratory scale experiments typically involves washing alkali-pretreated LARs with deionised water until the pH becomes neutral, before feeding the anaerobic digester (Panagiotopoulos et al., 2010; Elsayed et al., 2018; Mancini et al., 2018; Mafa et al., 2020; Sabeeh et al., 2020). In a practical, fully operational situation, however, this would require large volumes of water and would lead to the loss of valuable solubilised carbon sources in LARs, such as cellulose and hemicellulose, which contribute to CH₄ production. Alternatively, inorganic mineral acids, such as HCl acid, are commonly added before loading the substrate into the reactor (Cheng et al., 2010; Khatri et al., 2015; Zhang et al., 2015; Shetty et al., 2017; Shen et al., 2019). In large-scale commercial applications, however, the use of such acids would represent a significant cost, impacting the overall economic viability of the process, and furthermore, may not be available locally in more remote and developing regions (Atelge et al., 2020). The focus of the current research is to investigate alternative sources to mineral acids for pH neutralisation, and in this context the potential for a secondary waste stream, food waste (FW), as a neutralising agent, is explored.

Food waste generates significant amounts of volatile fatty acids (VFAs) during storage or incubation, and the pH value of the material can decrease from 6.3 to 3.7 within 7 d (Lü et al., 2015b; Nilsson Pålédal et al., 2018). Fermented FW, therefore, could represent a viable substitute to inorganic acids or post-pretreatment washing for neutralising the pH of the LARs feedstocks. Moreover, FW is also rapidly biodegradable and is a suitable substrate for AD, contributing significantly to the overall CH₄ output from the LARs AD process (Ariunbaatar et al., 2014).

Food waste is the major polluting waste stream in Indonesia, accounting for 53–60% of the total municipal solid waste (Qonitan et al., 2021). The country is the largest FW producer in the Southeast Asia region, with FW from households alone reported to reach 21 Mt annually (UNEP, 2021), predominantly from cereal and grain groups (Bappenas, 2021). Increasing the reutilisation rates of FW is therefore critically important for reducing the volume sent to landfill, which currently serves as the primary disposal method for FW. This disposal practice has several consequences, including significant GHG emissions, with FW degradation in landfill accounting for approximately 13% of GHG emissions (Al-Iraqi et al., 2023). Moreover, landfilling FW poses risks to surface and groundwater through potential leachate contamination if not properly managed (Ren et al., 2018). From a resource recovery perspective, landfilling FW results in the loss of valuable nutrients and energy that could otherwise be recovered through AD and composting.

Food waste has been utilised extensively as a single substrate or co-digested with the other substrates in AD (Banks et al. 2008; Arelli et al. 2018; Rahmani et al. 2022; Shimizu et al. 2022; Odejobi et al. 2023). The AD of FW, however, can be challenging due to the rapid release of VFAs, which can imbalance the pH of the process. Therefore, co-digestion of FW with other substrates, such as sewage sludge or animal manure can be beneficial as these provide abundant nutrients, compared to FW, and provide buffering capacity to balance the rapid generation of VFAs from FW (Arelli et al., 2018; Odejobi et al., 2023). Anaerobic co-digestion of LARs and FW is also common to optimise the high carbon-to nitrogen (C/N) ratio of LARs and dilute potentially high level ammonia from FW, as this can introduce ammonia inhibition (Álvarez et al., 2010; Brown and Li, 2013; Sawatdeenarunat et al., 2015; Koyama et al., 2017).

The use of fermented, acidified FW for neutralising alkali-pretreated LARs, however, remains limited. Previous research has used fermented FW to neutralise alkali-pretreated aquatic macrophytes, albeit also in conjunction with hydrochloric (HCl) acid (Koyama et al., 2017).

The novelty of the present study, in comparison to earlier research, lies in the sole utilisation of fermented FW as the neutralising agent for pretreated LARs. This innovative approach maximises the utilisation of acidic properties in FW because of rapid VFAs generation without the need to add to the operational costs associated with use of inorganic acid chemicals. Diverting FW from landfill disposal and integrating it with the AD of LARs, has the potential to create mutual benefits between these substrates. The acidic properties of fermented FW will aid in neutralising pretreated LARs, while LARs helps in regulating the rapid generation of VFAs and ammonia from FW, thereby minimising excessive acidification and or ammonia inhibition risk compared to FW mono-digestion.

The aim of the research described in this chapter is to quantify the relationship between fermented FW addition and the pH of alkali-pretreated BS and to establish the optimum rate of addition for stable AD conditions through solid-state AD experiments. The specific objectives are to: (1) estimate the addition rate of fermented FW based on theoretical acid-base neutralisation principles, and experimentally, determine the impact of bulk addition of fermented FW to the pretreated BS, and (2) quantify and compare the AD response (CH_4 yield, hydrolysis rates, and VS removal) to pH neutralisation of pretreated BS by fermented FW and HCl acid.

7.2 Materials and methods

7.2.1 Waste feedstocks and inoculum

Milled BS was pretreated using a high alkalinity suspension ($\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$), which was prepared from a mixture of CaO and as-received CFA. The pretreatment procedures for BS are described in Section 5.2.2. FW was prepared as a mix of ingredients using method described in Section 3.1.2. Digested sewage sludge was used as the inoculum source for the solid-state AD experiments. Details regarding the source and preparation of substrates and inoculum are described in Section 3.1. The general physicochemical properties of BS, fermented FW, CFA and inoculum are presented in Table 3.2.

7.2.2 Optimisation of food waste incubation period for pre-fermentation

Approximately 2 kg of laboratory-prepared FW (fresh matter (FM) basis) was placed in a polyethylene bag which was vacuum-sealed (refer to Section 3.1.2 for the incubation procedure). During the incubation, each day, three samples, each weighing 20 g, were removed from the bag for pH measurements following the procedure outlined in Section 3.3.3. This procedure was continued until no further pH changes were observed, indicating pH

stabilisation had been reached and fermentation optimised. After each subsampling, the bag was vacuum-sealed and incubated again.

7.2.3 Neutralisation of alkali-pretreated barley straw by fermented food waste

This section describes the experimental procedures used to determine the optimum addition rate of fermented FW for neutralising alkali-pretreated BS. A schematic flowchart illustrating the procedures followed is shown in Figure 7.1.

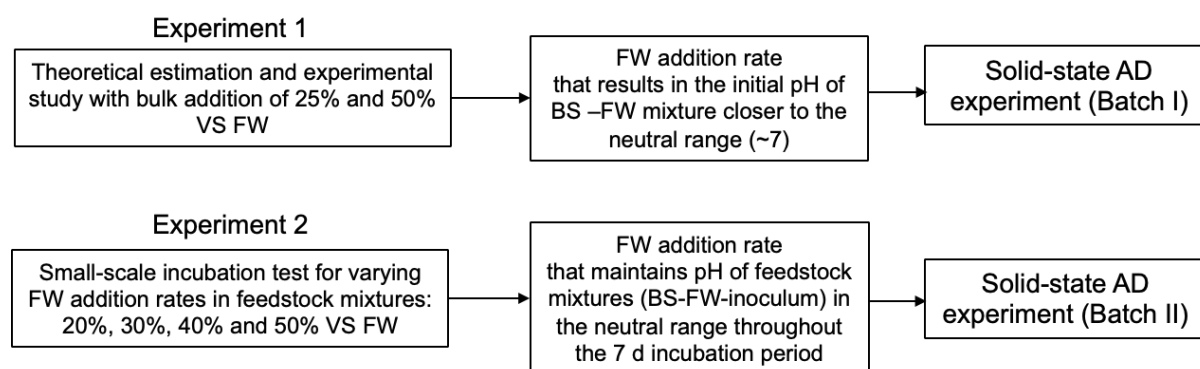
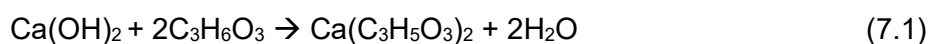


Figure 7.1 Flowchart for determining fermented food waste (FW) addition rate

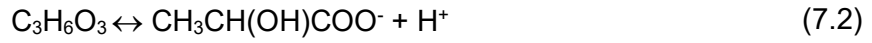
7.2.3.1 Experiment 1: Trial experiment to determine the optimum FW addition rate from bulk addition of FW to the alkali-pretreated BS

As part of the preliminary tests conducted under Experiment 1 two approaches were adopted: a theoretical estimation and an experimental study which are now described. (1) A theoretical estimation of the amount of FW to achieve optimum pH neutralising conditions was made based on the principles of acid-base neutralisation and stoichiometry, where the number of moles of H^+ must be equal to the number of moles of OH^- to achieve neutralisation. Lactic acid ($C_3H_6O_3$) was assumed to represent fermented FW, given its prevalence in FW fermentation (Wang et al., 2002; Ye et al., 2013; Lü et al., 2015). The balanced chemical equation illustrating the neutralisation reaction between the strong base $Ca(OH)_2$, derived from the reaction of CaO with water during BS pretreatment, and the weak acid $C_3H_6O_3$ is presented in Equation 7.1.



Since the experiment involves a weak acid, which partially dissociates in water, the dissociation constant (K_a) was incorporated into the estimation of the hydrogen ions $[H^+]$ concentration. The dissociation reaction of $C_3H_6O_3$ is shown in Equation 7.2. The K_a for this

reaction is expressed in Equation 7.3, and Equation 7.4 presents the relationship between K_a and pK_a .



$$K_a = \frac{[H^+][CH_3CH(OH)COO^-]}{[C_3H_6O_3]} \quad (7.3)$$

$$K_a = 10^{-pK_a} \quad (7.4)$$

where: pK_a of lactic acid = 3.86 (Lide, 2007).

(2) An experimental study was conducted by mixing 10 g of pretreated BS on a FM basis, and fermented FW, at two addition rates, namely 25% (labelled as FW-25_t) and 50% (VS based) (labelled as FW-50_t), equivalent to: 14 g and 42 g, respectively, on a FM basis. The mixtures were homogenised manually using a laboratory spatula, and the pH of the excess liquor from each mixture was measured using a pH meter (Section 3.3.3). The FW addition rate that resulted in a liquor pH of the BS-FW mixture closer to 7 was selected as input feed the solid-state AD reactor.

Solid-state AD experiments were conducted to determine the CH₄ response from high alkalinity-pretreated BS neutralised with fermented FW at a 50% VS addition (selected as the rate that resulted in a pH value closest to 7). As a benchmark, the CH₄ yield contribution from fermented FW alone; labelled as FW-only, was also studied (Details on the experimental treatment conditions for FW-50_t and FW-only for the solid-state AD experiments Batch I are given in Section 7.2.4 “Batch I”).

7.2.3.2 Experiment 2: Small-scale Incubation Tests of Feedstock Mixtures

This experiment was designed to adopt a more detailed dose response approach, to assess the interaction between BS, inoculum, and the rate of fermented FW addition on the pH dynamics. Small-scale feedstock mixtures with varying fermented FW addition rates were incubated, and pH was monitored daily. An incubation period of 7 d was a timeline chosen as this duration is representative of when changes in pH value fluctuate to the greatest extent before stabilising (Li et al., 2016).

Alkali-pretreated BS was mixed with fermented FW separately using four addition rates (VS based): 20%, 30%, 40%, and 50% of the total substrates (labelled as FW-20, FW-30, FW-40, FW-50, respectively) in 1L mixing bowls, and as a Control alkali-pretreated BS without any neutralisation either supplied from fermented FW or mineral acid addition (labelled as WN), was also included. Inoculum (digested sludge) was added to each mixture. The quantities of substrates, deionised water, and inoculum used are listed in Table 7.1.

Table 7.1 Composition of substrate mixtures, water and inoculum addition in small-scale incubation test.

Experimental Treatments	Fermented FW (% VS)	Pretreated BS (g FM)	Fermented FW (g FM)	Inoculum (g FM)	Water (mL)
WN	0.0	109.3	0.0	680.7	340.0
FW-20	20.0	87.4	92.3	680.7	320.0
FW-30	30.0	76.5	138.5	680.7	150.0
FW-40	40.0	65.6	184.6	680.7	80.0
FW-50	50.0	54.6	230.8	680.7	40.0

The combination of materials in each treatment condition were homogenised together using a kitchen hand blender and the homogenised sample was sub-divided and transferred to twenty-four 250 mL borosilicate glass Schott bottles with screw caps. Each treatment was replicated three times and pH measurements were recorded daily over a 7 d period. Each bottle contained mixtures of 33–40 g (on an FM basis). The headspace of each bottle was purged by N₂ for 1 min, and the bottles were sealed with a screw cap to provide an air-tight environment. The bottles were placed in a temperature-controlled laboratory incubator, in the dark, set at 30°C. The pH was monitored by sacrificing three bottles to provide triplicate measurements daily for each treatment condition.

The optimum fermented FW addition rate determined from this experiment, was selected to investigate the effect of this rate on CH₄ yield through solid-state AD experiments (Batch II). A Control treatment, labelled as HA, consisting of high alkalinity-pretreated BS neutralised with HCl acid was included for comparison (Details on the treatment conditions for the optimum rate and HA conditions for the solid-state AD experiments Batch II are given in Section 7.2.4 “Batch II”).

7.2.4 Batch experiments of solid-state anaerobic digestion

Using the results derived from Experiment 1 and 2, two batches solid-state AD experiments (Batch I and Batch II) were conducted sequentially in 1L reactors and in triplicate, to determine

the effect of the selected fermented FW addition rate. The experimental treatment conditions applied were described as follows:

- (a) **Batch I:** (i) **FW-50_t condition:** pretreated BS from 7 ± 0.5 g VS was mixed with equal VS weight of fermented FW, in 1L mixing bowl. To this mixtures, 20 mL deionised water was added to provide a target final TS content of approximately 15%. (ii) **FW-only condition:** a mixture contained 10 ± 0.5 g VS each of fermented FW and inoculum.
- (b) **Batch II:** (i) **FW-20 condition:** pretreated BS from 10 ± 0.5 g VS of BS was mixed with 20% VS FW that was equivalent to 3 ± 0.5 g VS of fermented FW and 40 mL deionised water (ii) **HA condition:** pretreated BS from 13 ± 0.5 g VS of BS was added with 50 mL deionised water and neutralised with 3–4 mL concentrated HCl acid.

Inoculum (digested sludge) was added to each mixture under the conditions described above to achieve an ISR of 1 (see Section 3.2.3). The water added to each mixture was to provide a target final TS content of approximately 15% (see Section 3.2.1). After homogenisation, by mixing using a kitchen hand blender, two subsample fractions, each of approximately 20 g FM of each feedstock mixture, was taken for the determination of the initial TS, VS and pH values. The general operating protocols of the solid-state AD experiments followed the procedures outlined in Section 3.2.2 and Section 3.2.3.

7.2.5 Analytical methods

The determination of pH of substrates and inoculum mixtures were analysed according to the procedures outlined in Section 3.3.3. The solids reduction in the substrate mixtures was determined by measuring TS and VS contents before and after solid-state AD, following the procedures given in Section 3.3.2. Volatile solids removal was calculated using Equation 5.4 to assess the efficiency of the AD process and substrate degradation.

The organic carbon (C) and total nitrogen (TN) content of individual materials: BS, fermented FW, and inoculum were determined following the method described in Section 3.3.7. The C/N ratio in FW-only, FW-20, FW-50 and HA treatments were calculated based on the values of the individual materials in the mixtures, using Equation 7.5.

$$C/N = \frac{FW (TS \times C) + BS (TS \times C) + I (TS \times C)}{FW (TS \times N) + BS (TS \times N) + I (TS \times N)} \quad (7.5)$$

where *FW*, *BS*, and *I* are the weight (on a fresh matter (FM) basis; in grams (g)) of food waste, barley straw, and inoculum, respectively, *TS* is the total solids (% FM), *C* is the organic carbon

content in the respective material (% TS) and N is the total nitrogen content in the respective material (% TS).

Biogas volume was measured using a water displacement method as detailed in Section 3.2.3, and CH_4 concentration in the biogas was determined by GC according to the procedures given in Section 3.3.13. The background CH_4 produced from the blank or inoculum-only reactor, was subtracted from the total CH_4 volume measured in both the HA and FW-20 treatments (see Section 3.2.5) (refer to Appendix A, Figure A.4 for the residual CH_4 production from the inoculum used in this chapter).

7.2.6 Statistical analysis and CH_4 gas modelling

Triplicate CSMY_{exp} data from each treatment condition: FW-20 and HA, were normalised to STP conditions (see Section 3.2.5). CH_4 gas modelling was conducted on the normalised CSMY values after removing outlier data, as detailed in Section 3.4. Modelling of the CH_4 gas yield and kinetic parameters, and determination of the CSMY_0 followed the procedures described in Section 3.4. The statistical significance of differences in kinetic parameters including final CSMY and hydrolysis rates between the experimental treatments was determined by a two-tailed paired t-test (see Section 3.4).

7.3 Results

7.3.1 Food waste fermentation

The variation in pH value of laboratory-prepared FW (see Section 3.1.2) during fermentation for a 12 d incubation period at 30°C (see Section 7.2.2) is shown in Figure 7.2. The initial pH value of the FW was 5.35, which decreased by >1 pH unit within 24 h to 4.23. No change in pH was recorded for the next 2 d period, but a further reduction to 3.78 was measured after 7 d and it decreased slightly to 3.76 and 3.7, after 9 and 12 d of incubation, respectively.

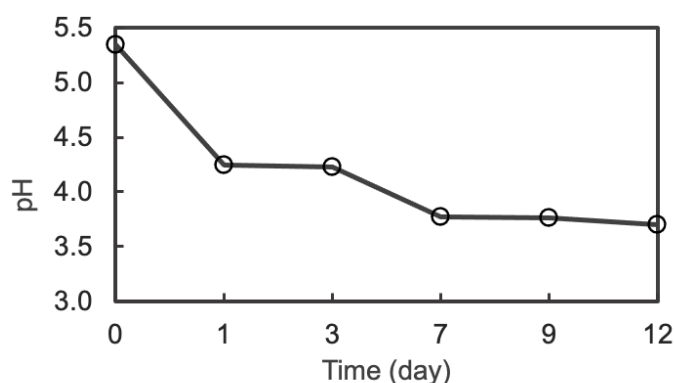


Figure 7.2 pH value of fermenting food waste (FW) during incubation for 12 d at 30°C

7.3.2 Effect of fermented food waste addition rate derived from Experiment 1 on pH and CH₄ yield of digesting alkali-pretreated barley straw

The pH values of feedstock mixtures containing alkali-pretreated BS and the addition of fermented FW with 25% (FW-25_t) and 50% (FW-50_t) separately in Experiment I (Section 7.2.3.1) are presented in Table 7.2. The theoretical estimation of the amount of FW to achieve optimum pH neutralising conditions in Experiment 1 indicated that relying on fermented FW alone to reduce the pH from 12 to neutral would require an excessive amount of fermented FW, as the major acids generated in FW fermentation are weak acids, such as lactic acid. The laboratory experiment confirmed this estimation and showed that FW-25_t treatment only reduced the pH of alkali-pretreated BS from 12.3 to 11.9 (Table 7.2). Increasing the addition rate of fermented FW to 50% of VS (FW-50_t) was considered as a practicable operational maximum amount of FW addition in AD of BS, which resulted in a modest reduction in pH from 12.3 to 9.5. The FW-50_t treatment gave a more pronounced reduction in pH of the mixture of alkali-pretreated BS compared to the FW-25_t treatment, therefore, the effects of this degree of pH reduction on the AD of pretreated BS was evaluated in the solid-state AD experiment (Batch I) (see Section 7.2.3.1).

Table 7.2 Initial pH of high alkalinity-pretreated barley straw (BS) and pH changes after addition of fermented food waste (FW) at 25% and 50% addition rates (on a volatile solid basis) to the pretreated BS (n=3).

Experimental condition	pH
Alkali-pretreated BS	12.30 ± 0.11
25% VS (FW-25 _t)	11.90 ± 0.20
50% VS (FW-50 _t)	9.50 ± 0.24

Note: pH values are expressed as mean ± standard deviation (n=3).

The solid-state AD experiments (Batch I) showed that both fermented FW-only and FW-50_t treatment conditions did not produce biogas (results not shown), probably due to excessive acidification; the pH following both treatments was 5.5 and 5.4, respectively, for FW-only and FW-50_t on Day 14 (data included in Table 7.3 for reference), which is inhibitory to methanogenesis (Mao et al., 2015; Rabii et al., 2019). Thus, the high rate of FW examined caused a significant pH imbalance that led to process failure.

7.3.3 Effect of fermented FW addition rate derived from Experiment 2 on pH of feedstock mixtures during the small-scale incubation test

The results of Experiment 2, illustrated in Figure 7.3, showed the pH decreased with incubation time and the magnitude of the response increased with the rate of fermented FW added. Despite the initial high pH of pretreated BS, like that observed in Experiment 1 (Table 7.2), the inoculum (pH 8.4, see Table 3.2) reduced the pH value of the initial mixture with pretreated BS to within the range 7.5–8.5. The initial pH of WN treatment (pretreated BS + inoculum) before incubation was 8.5. The addition of fermented FW to levels of FW-20 and FW-30, only slightly reduced the initial pH to 8.4, due to low rates of acidified, fermented FW. At higher rates of fermented FW addition, however, a more pronounced pH reduction was observed, with values ranging from 7.5 to 7.8 in FW-40 and FW-50 treatments as a direct effect of incorporating the fermented FW on the initial pH value of the mixture.

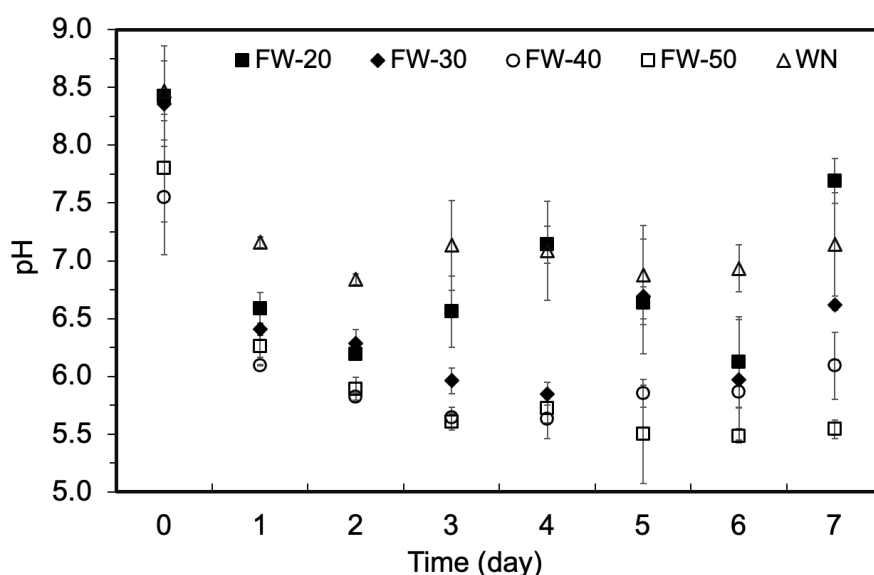


Figure 7.3 Effect of fermented food waste addition rate on the pH value of alkali-pretreated barley straw and digested sludge inoculum (ISR=1) mixtures during small-scale incubation test for 7 d at 30° C. Error bars represent the standard deviations (n=3).

Within 24 h, the pH value of WN treatment decreased to 7.2 due to the hydrolysis and acidification of pretreated BS, and thereafter remained relatively stable in the neutral range for the WN treatment with the lowest value recorded of 6.8 on Day 2. The pH levels for FW addition treatments, however, were significantly lower than WN treatment having decreased to 6.1–6.6 within 24 h of the AD process (Figure 7.3).

The results showed a general pattern of decreasing pH with increasing amount of fermented FW addition, and FW-40 and FW-50 treatments both gave the largest overall reduction to pH

5.8 on Day 2. For FW-40 the pH reduced further to 5.6 on Day 3, before rising after Day 5 to ≥ 6 . FW-50, on the other hand, continued to decrease slowly to pH 5.5 on Day 6.

FW-20 and FW-30 showed a more variable and intermediate acidification response, compared to the higher rates of FW addition. The pH decreased to 5.8 by Day 4 and subsequently increased to 6.0–6.6 for FW-30. FW-20 maintained a pH profile generally within the optimum range for CH₄ conversion between 6.0 and 7.5 and was therefore selected for further assessment of fermented FW neutralisation of alkali-pretreated BS on AD performance.

Before reporting on the effects of the optimum FW-20 application in the AD process on CSMY and VS removal in Section 7.3.5, the characteristics of substrate and inoculum mixtures in solid-state AD reactors for both batches (Batch I and II) are discussed below (in Section 7.3.4).

7.3.4 Characteristics of substrate and inoculum mixtures in solid-state anaerobic digestion reactors

The mean (n=3) pH values for the feedstock mixtures (FW-only, FW-20, FW-50_t, and HA) loaded into the 1L reactors (for Batch I and II as appropriate), and for the digestates after treatment, were measured and the results are presented in Table 7.3.

Table 7.3 pH before and after solid-state AD experiments (Batch I and Batch II) of four experimental treatment conditions: fermented food waste (FW)-only, pretreated barley straw (BS) neutralised with (i) 20% fermented FW (FW-20), (ii) 50% fermented FW (FW-50_t) and (iii) HCl acid (HA). C/N ratio of the four treatments is also shown.

Parameter	FW-only (Batch I)	FW-20 (Batch II)	FW-50 _t (Batch I)	HA (Batch II)
pH before AD	7.7 ± 0.26	8.5 ± 0.02	8.2 ± 0.17	7.5 ± 0.05
pH after AD	5.5 ± 0.06 ^a	7.6 ± 0.16 ^b	5.4 ± 0.05 ^a	7.9 ± 0.13 ^b
C/N ratio	10.2	12.1	11.3	12.8

Note: Data are expressed as mean ± standard deviation (n=3).

^a pH measured on the Day 14 due to biogas cessation.

^b pH measured on Day 40.

The addition of inoculum to FW-20 and FW-50_t lowered the pH of the pretreated BS and FW mixtures to 8.5 and 8.2, respectively, consistent with the pH value of the input feedstocks pH range of 7.6–8.2 observed in Experiment 2 (reported in Figure 7.3). The C/N ratio of the FW-only, FW-20, FW-50_t, and HA treatments were 10.2, 12.1, 11.3 and 12.8, respectively (Table

7.3) and below the optimum for AD, due to the large N supply from digested sludge inoculum (TN = 4.4% TS) (see Table 3.2).

7.3.5 Effect of fermented food waste addition rate derived from Experiment 2 on volatile solids removal and experimental CH₄ yield

Figure 7.4 shows the VS removal of BS and the mean CSMY_{exp} under both HA and FW-20 treatments. The mean CSMY_{exp} significantly ($P = 0.001$) increased by 47.2% from 156.7 ± 11.2 mL CH₄ g⁻¹ VS in the HA to 230.5 ± 12 mL CH₄ g⁻¹ VS in FW-20, due to the 73.8 mL CH₄ g⁻¹ VS contribution from fermented FW. The VS removal of BS also increased from 57 ± 0.2 % to 60.7 ± 4.7 % (refer to Appendix C, Table C.3 for replicate data of CSMY_{exp} and VS removals) with FW addition, equating to 6.5% increase, despite the increase not being significant ($P > 0.05$). This VS removal was calculated specifically for BS, after subtracting the total residual VS from the VS of FW, which was assumed to be 10% of the total. This assumption was based on typical VS removal rates for AD of FW, which range from 84–95% (Serna-Maza et al., 2014; Banks et al., 2018).

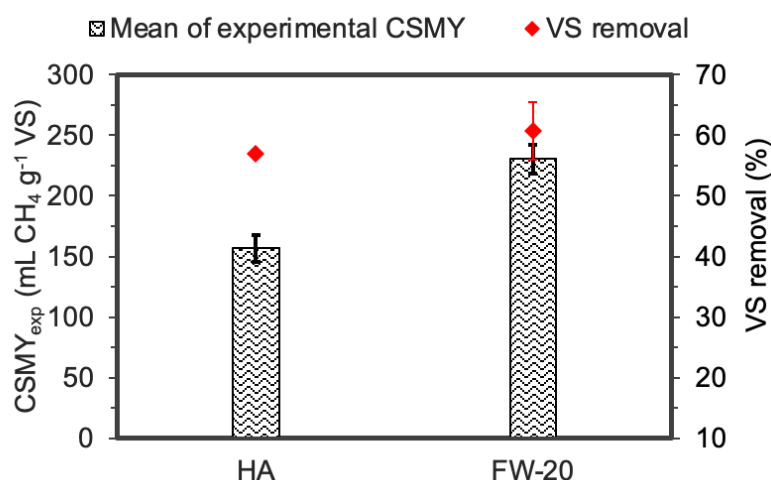


Figure 7.4 Effect of neutralisation of alkali-pretreated barley straw by 3-4 mL of concentrated hydrochloric acid (HA) and 20% VS of fermented food waste (FW-20) on volatile solids (VS) removal and mean experimental cumulative specific CH₄ yield (CSMY_{exp}) in solid state anaerobic digestion. Error bars; red and black, show the standard deviation of VS removal and CSMY_{exp}, respectively (n=3).

7.3.6 Effect of fermented food waste neutralisation on hydrolysis rates and CH₄ yields

Daily CH₄ yield from solid-state AD experiments for FW-20 and HA treatment conditions are presented in Figure 7.5. The individual replicates of daily CH₄ yield showed generally similar patterns of behaviour across HA and FW-20 treatments. The CH₄ production began immediately without a lag-phase in both treatments, with FW-20 had a 1.5-fold higher peak

production than HA, with maximum values of 40–50 mL CH₄ g⁻¹ day⁻¹ and 27–37 mL CH₄ g⁻¹ day⁻¹, respectively, on Day 1, due to the additional contribution to the CH₄ yield from fermented FW. For HA, the CH₄ yield initially increased but then declined more gradually compared to FW-20. Some fluctuations were observed in HA daily CH₄ production, particularly in Replicate 1, up to Day 15, with CH₄ production largely ceasing after Day 25. In contrast, in FW-20 replicates, particularly Replicate 1 and 2 maintained CH₄ production for a longer duration, extending up to Day 7–8 compared to HA. The CH₄ production in FW-20 generally remained low subsequently, with fluctuations reaching around 10 mL g⁻¹ VS, particularly for Replicate 3, which experienced the most rapid decrease in CH₄ yield after an initial rise at the beginning of the incubation period.

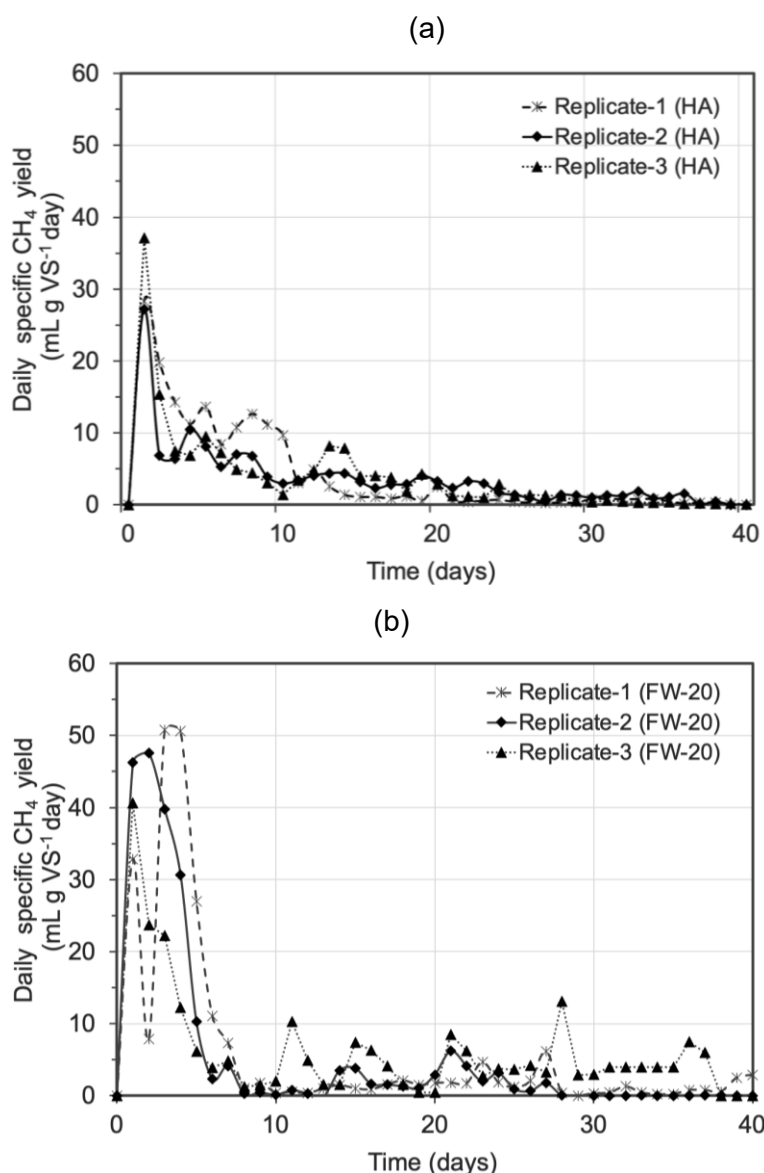


Figure 7.5 Effect of neutralisation of alkali-pretreated barley straw by (a) 3–4 mL of concentrated hydrochloric acid (HA) and (b) fermented food waste (FW) with 20% VS FW addition rate (FW-20) on daily CH₄ yield (n=3) during 40 d solid-state anaerobic digestion.

The distinct trend observed in Replicate 1 for HA (Figure 7.5 (a)) and Replicate 3 for FW-20 (Figure 7.5 (b)) may impact the hydrolysis rates (k or K_H). These deviations, however, were not considered outliers that warranted exclusion from the CH₄ gas modelling analysis. This is due to the replicates CSMY_{exp} values for the replicates for both HA (ranging from 145.0 to 167.5 mL CH₄ g⁻¹ VS) and FW-20 treatments (ranging from 217.5 to 241.1 mL CH₄ g⁻¹ VS) showing consistency, as indicated by a RSD of 7.1% and 5.2%, respectively (refer to Appendix C, Table C.3 for mean and standard deviation values of CSMY_{exp} replicate data used in calculating RSD), within the range of acceptable variation for test results (RSD < 10%) (Holliger et al., 2016) (see Section 3.4).

Experimental and modelled CSMY data are shown in Figure 7.6 (refer to Appendix B, Table B.2 for CSMY_o replicate data from the First-order kinetic and Cone models). The First-order model tends to underestimate and then overestimate CSMY over the first 40 d of digestion, in contrast, Cone model provided less systematic deviation from the experimental data pattern compared to the First-order model, particularly during Day 0-30 of the AD period. The parameter CSMY_o obtained from the Cone and First-order kinetic models is presented in Table 7.4. Both models provided a good explanation of the experimental data patterns, indicated by the R² value, which was 0.84 and 0.83, respectively, for HA, respectively, albeit the R² was slightly smaller, at 0.78 and 0.72, respectively for FW-20.

Table 7.4 Effects of HCl acid and fermented food waste neutralisation on kinetic parameters of First-order kinetic and Cone models for CSMY_o from anaerobic digestion of barley straw; the relative error (%) between the mean CSMY_{exp} and modelled CSMY (CSMY_o and CSMY₄₀) as well as CSMY₄₀ value from the Cone model are also shown.

Treatment Conditions	First-order Model			Cone Model				
	CSMY _o ^a	R ^{2b}	Error (%) ^c	CSMY _o	CSMY ₄₀ ^d	n ^e	R ²	Error (%) ^c
HA	155.6 a	0.83	0.68	193.1 a	160.0 a	0.96	0.84	-2.13
FW-20	210.6 b	0.72	8.65	285.9 b	224.7 b	0.72	0.78	2.54

Values in a column followed by the same letter are not significantly different at $P = 0.05$.

^a CSMY_o: maximum cumulative specific CH₄ yield potential (mL CH₄ g⁻¹ VS) (Equation 3.6 and 3.7).

^b R²: coefficient of determination of the modelled CH₄ data

^c Error (%): difference (in percentage) between the mean of experimental (CSMY_{exp}) and CSMY_o, except for the Cone model between CSMY_{exp} and CSMY₄₀.

^d CSMY₄₀: cumulative specific CH₄ yield at Day 40 from Cone model.

^e n: shape factor (Equation 3.6).

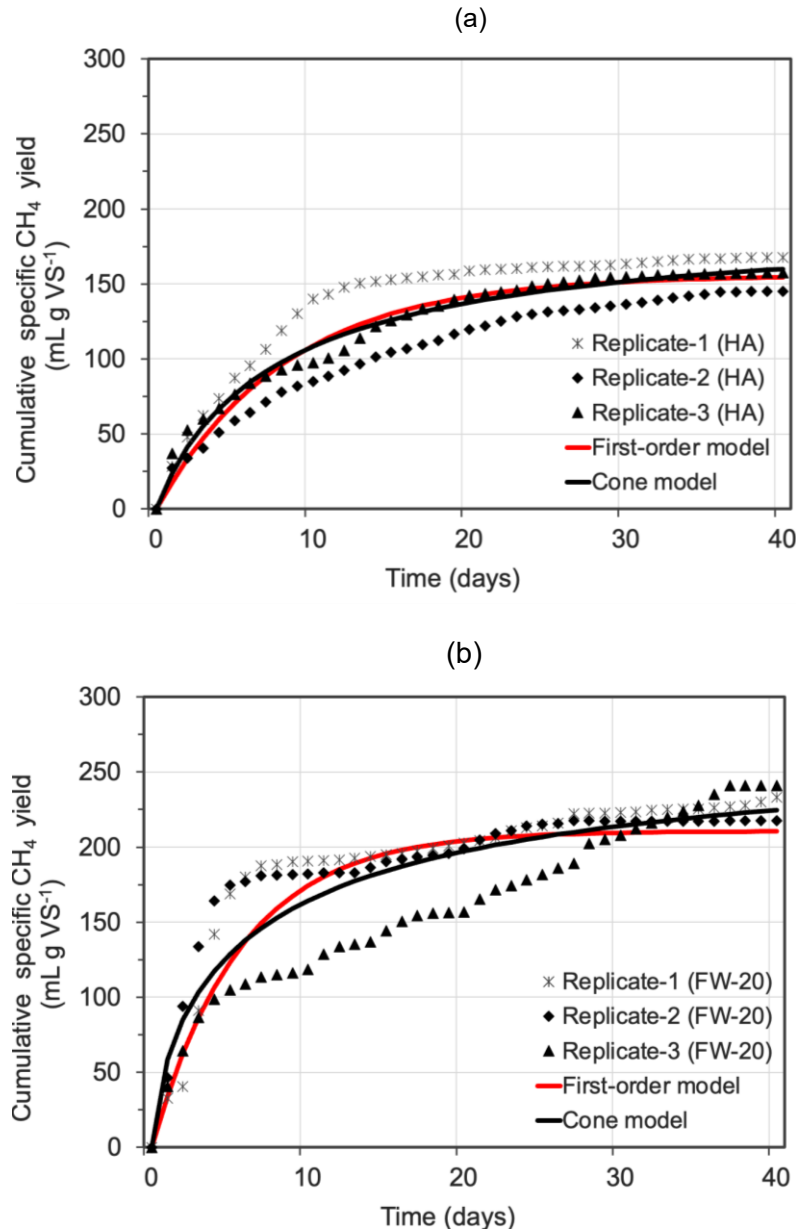


Figure 7.6 Experimental ($n=3$, measured daily) and modelled (First-order and Cone, pooled data) cumulative specific CH_4 yield (CSMY) from anaerobic digestion of barley straw neutralised with (a) HCl acid (HA) (3–4 mL of concentrated HCl added before inoculum addition) and (b) fermented food waste (FW) with 20% VS FW addition rate (FW-20).

The Cone model was used to estimate CSMY_0 and K_H as it generally provided better fit to the experimental data pattern compared to the First-order model, particularly during the experimental duration. The CSMY_0 derived from the Cone model, however, deviated significantly compared to the experimental data pattern (with the difference exceeding 5%) for both HA and FW-20 treatment conditions (see Appendix B Table B.3 for CSMY_0 replicates data). This inconsistency appeared to arise from extrapolation of the asymptote beyond the 40 d of the experimental period and may therefore be considered unreliable. Consequently, the CSMY_{40} was also calculated for the 1–40 d data range, which was equivalent to 160 $\text{mL CH}_4 \text{ g}^{-1} \text{ VS}$ for HA treatment compared to 224.7 $\text{mL CH}_4 \text{ g}^{-1} \text{ VS}$ for the FW-20. These values

were similar to the $CSMY_o$ determined by the First-order model (155.6 and 210.6 mL CH₄ g⁻¹ VS, respectively), and showed a higher level of agreement (<5%) when compared with the mean $CSMY_{exp}$ (Figure 7.4), which probably provided a more reliable estimate of the ultimate $CSMY$ from AD of pretreated BS with FW and HCl neutralisation. The $CSMY$ for FW-20 was 40.4% higher ($P < 0.001$) than for HA, due to the contribution of FW to the CH₄ yield. The difference in $CSMY$ of HA and FW-20 gave the $CSMY$ of fermented FW alone, equivalent to 64.7 mL CH₄ g⁻¹ VS.

Table 7.5 presents the hydrolysis rates K_H and k estimated using the Cone and First-order kinetic models. The Cone model fitting to individual experimental $CSMY$ data estimated K_H values ranging from 0.05–0.22 d⁻¹ for HA and from 0.03–0.43 d⁻¹ for FW-20 (Table 7.5). The First-order model, showed less variability, estimating k values between 0.09 and 0.16 d⁻¹ for HA and between 0.07 and 0.29 d⁻¹ for FW-20. The pooled replicate data modelling gave K_H values of 0.13 and 0.15 d⁻¹ as a single, representative hydrolysis rate for HA and FW-20, respectively, while the k values were 0.12 d⁻¹ for HA and 0.18 d⁻¹ for FW-20. The hydrolysis rate observed for FW-20 was in good agreement with rates reported by Zhou et al. (2014), ranging between 0.14–0.19 d⁻¹ for the co-digestion of corn stover and FW. No statistically significant differences ($P > 0.05$) were detected for K_H or k between the experimental treatments, despite increasing by 50% and 16%, respectively, in FW-20 compared to HA treatment.

Table 7.5 Summary of hydrolysis rate constants (d⁻¹) estimated from the First-order kinetic (k) and Cone models (K_H) from anaerobic digestion of barley straw with HCl acid (HA) and 20% volatile solids (VS) food waste neutralisation (FW-20); the relative standard deviation (% RSD) of the individual replicate modelled k and K_H is also shown.

Model	Treatment Conditions		Replicate Values ^a			Mean Values of K_H and k	RSD (%)	Modelled Values ^b
			1	2	3			
Cone	HA	K_H	0.22	0.05	0.08	0.12 ± 0.09 a	75.00	0.13 a
	FW-20	(d ⁻¹)	0.03	0.30	0.43	0.25 ± 0.20 a	80.00	0.15 a
First-order	HA	k	0.16	0.09	0.11	0.12 ± 0.04 a	33.00	0.12 a
	FW-20	(d ⁻¹)	0.07	0.22	0.29	0.19 ± 0.11 a	58.00	0.18 a

Note:

(1) Values after ± represent standard deviation.

(2) RSD: relative standard deviation = standard deviation x 100 / mean.

(3) Values in a column followed by the same letter are not significantly different at $P = 0.05$.

^a Hydrolysis constant estimated from individual replicate data modelling.

^b Hydrolysis constant estimated from pooled replicate data modelling.

The Cone model provided higher variability in estimating hydrolysis rates across replicates compared to the First-order model, as indicated by the higher RSD value (Table 7.5). The RSD for K_H was in the range: 75–80%, while the value for k was lower and in the range: 33–58%. Similar variability in hydrolysis rate with RSD values ranging from 55% to 70% was reported in a previous study (Raposo et al., 2011).

7.4 Discussion

7.4.1 Food waste fermentation

Prepared FW underwent incubation for preliminary fermentation and was utilised as an alternative to inorganic acids for neutralising high alkalinity-pretreated BS for solid-state AD. The observed decline in pH during FW fermentation from 5.35 to 3.70 in 12 d (Figure 7.2) was in good agreement with (Wang et al., 2002; Lü et al., 2015) for stored FW. The rapid decrease in pH within the first 24 h indicated the prompt initiation of hydrolysis (Angelidaki et al., 2011; Degueurce et al., 2019). Following hydrolysis, acidification occurred, leading to the generation of organic acids and subsequent pH reduction. These processes are facilitated by indigenous hydrolytic and acidogenic microbes naturally present within FW (e.g., lactic acid bacteria) (Degueurce et al., 2019). Wang et al. (2002) and Lü et al. (2015) observed a sharp decrease in pH during the first 2 to 3 d of FW storage, followed by relatively stable pH until the termination of the 14 d experiment. In the current study, a continuous decline in pH was observed over the initial 7 d period of incubation, after-which time it remained relatively stable. Therefore, this time period was selected to maximise FW fermentation for neutralisation of alkali-pretreated BS in the AD process and minimise potential carbon losses from the FW as a co-digestion substrate for CH_4 production (Degueurce et al., 2019).

7.4.2 Neutralisation of alkali-treated barley straw with fermented food waste

The theoretical acid-base analysis and experimental tests described in Experiment 1 (see Section 7.2.3.1) suggested that FW-25_t treatment was inadequate for effective neutralisation (see Table 7.2), consequently, the effect of this rate on CH_4 yield was not monitored in the Batch I of solid-state AD experiments. In contrast, the FW-50_t treatment was necessary to achieve the largest neutralisation effect. However, CH_4 monitoring in the Batch I of solid-state AD experiments indicated the hydrolysis and acidogenesis reaction of FW continued and the magnitude of the pH decline at FW-50_t from 8.2 to 5.4 (Table 7.3) caused major process inhibition.

A more detailed dose-response approach was developed in Experiment 2 (see Section 7.2.3.2), which provided an accurate representation of the conditions in larger scale reactors,

as it captured the dynamic interactions between waste feedstocks under the experimental AD conditions, which strongly influence the system's pH, and therefore offers a more reliable assessment of FW addition rates compared to Experiment 1. This was demonstrated by the results, as the pH of FW-50 in Experiment 2 was 5.5 (Figure 7.3), consistent with the pH observed when testing the response of FW-50_t treatment on CH₄ yield in the Batch I solid-state AD experiments (Section 7.2.3.1), which resulted in a pH of 5.4 (Table 7.3). This experimental approach also allowed for a variation in rates of FW addition to quantify the pH response. pH was a pivotal parameter of interest in this experiment, because it can serve as an indicator of VFA levels (Yong et al., 2015; Hegde and Trabold, 2019) and is critical for the stability of the AD processes (Brown and Li, 2013; Li et al., 2016; Kainthola et al., 2020), primarily due to the fact that methanogens are fastidious and highly sensitive to pH (Li et al., 2016).

The pH drop in the early days of incubation observed in the small-scale incubation test of feedstock mixtures and pH recovery in the later days (Figure 7.3) was in line with the pH profile for co-digestion of FW with grass showing a significant pH drop to 5.5 on Day 5, followed by a gradual recovery to pH of 7.5 (W. Li et al., 2018; Gandhi et al. 2022). Zeshan (2012) reported similar pH recovery, reaching 8 when ammonia was produced, which was similar to the pH observed for FW-20 on Day 7. This was also consistent with Rahmani et al. (2022) who observed pH recovery to 7.3 on Day 7 in co-digestion of wheat straw and FW. This pH recovery can be attributed to the decreased rates of VFAs production as they were progressively converted to CO₂ and CH₄ (Yong et al., 2015; Koyama et al., 2017) and increased buffering by NH₃ resulting from protein hydrolysis of FW.

The pH of WN treatment was not in the optimum range for hydrolysis and acidogenesis stages, suggesting that neutralisation was certainly required. In contrast, the 20% VS addition rate (FW-20) was sufficient to lower the pH of the system during the hydrolysis and acidogenesis stages, creating a slightly acidic environment (pH 6.1–6.7) preferred by hydrolytic and fermentative bacteria, where this pH range supports both bacterial groups in achieving faster growth rate (Hossain et al., 2022). The addition rate was balanced with the system's buffering capacity, such as from residual alkali-pretreated BS, ammonia produced from FW degradation, and the buffering capacity provided by the inoculum, allowing the pH to recover to the neutral range in the later stages, which is optimum for methanogens.

7.4.3 Interpretation of the modelled data range determined by the Cone and First-order kinetic models

The Cone model showed less systematic deviation in the modelled data range during the early 30 d of digestion (Figure 7.6) because it accounts for varying hydrolysis rates that can occur during the initial and intermediate phases of digestion. This is indicated by the shape factor (n) in the Cone model, which is affected by the curve shape (Karki et al., 2022). In contrast, the First-order model does not consider this variability. By 30 d, the impact of hydrolysis rate diminishes as the digestion progresses and the substrate becomes depleted, making the hydrolysis rates less impactful to the CH_4 production rate profile. The simplicity of the First-order kinetic model may make it less sensitive to small variations in the data, rendering it unable to capture subtle differences in the profile among replicates. This also explains the more consistent estimates of hydrolysis rates among replicates and the greater variability in K_H estimated by the Cone model (Table 7.5).

7.4.4 Effect of fermented food waste neutralisation on anaerobic digestion performance

The absence of biogas production and the recorded low pH of around 5.5 after two weeks of digestion for both FW-only and FW-50_t treatments (Table 7.3) were probably due to acid pH conditions, causing a toxic effect on methanogens within the reactor. This was supported in Experiment 2 for the FW-50 treatment, where the pH in FW-50 decreased to acid conditions (pH ~5.5) and failed to recover within 7 d of incubation (Figure 7.3). This suggests that the decrease in pH to 5.5 for FW-50 recorded at Day 14 may have been reached within 5 d, as shown for FW-50 in Figure 7.3. These technical issues, associated with the acidification and insufficient buffering capacity, are common challenges causing process instability in AD of FW, either in mono or co-digestion (Chen et al., 2008; Banks et al., 2011; Ye et al., 2013).

The initial pH in the fermented FW-only and FW-50_t reactors was close to 8 (Table 7.3), suggesting sufficient alkalinity provided by the inoculum. The acidity of the fermented FW, however, consumed the digester alkalinity to an extent that the release of NH_3 from protein degradation was insufficient to restore the pH. Methanogens, especially acetoclastic types, are particularly susceptible to low pH levels (Li et al., 2016; Hegde and Trabold, 2019). The persistently low pH suggested that the acidification rate from degradation of the substrates was more rapid than the conversion of VFAs into biogas and eventually caused methanogens to cease functioning (Bouallagui et al., 2005; Hegde and Trabold, 2019).

The C/N ratio of FW-20 was smaller than that of HA (Table 7.3), as expected, albeit slightly, as FW addition increased the total N in the overall C/N ratio of the mixture, and higher FW addition rates (FW-50_t), further lowered the C/N ratio of the mixture. Compared to FW-only, the C/N ratio in co-digestion of BS and FW, as observed in FW-20 and FW-50_t, was higher because the C from the BS contributed to the overall C content of mixture. The minor difference in the C/N ratio between FW-20 and HA is probably due to the significant N content already present in the inoculum, diminishing the impact of FW, despite its relatively high N content in FW compared to BS.

The VS removal of the FW-20 was 10.4–41.2% higher than a 40–55% VS removal reported for co-digestion of LAR and FW reported in the literature (Brown and Li, 2013; Haider et al., 2015; Rahmani et al., 2022). The CSMY in FW-20 (285.9 mL CH₄ g⁻¹ VS) was 23.4% higher than that reported in Koyama et al. (2017) for alkali-treated macrophyte neutralised with FW at similar addition rate as used in this study. FW-20 showed a 40% higher CSMY, indicating that FW indeed contributed to the CH₄ yield. This suggests that, despite the potential risk of ammonia toxicity due to the high N content in FW, FW-20 gave comparable response to HA, confirming that FW is a viable alternative to conventional inorganic acid chemicals, thus promoting overall process sustainability.

The present study has demonstrated that fermented FW can effectively serve as a neutralising agent, without compromising the stability of AD process. In addition to aiding in pH neutralisation, FW co-digestion with BS offers multiple benefits over FW mono digestion, particularly in balancing the rapid production of VFAs from FW, thus reducing the risk of acidification. In a broader context of waste utilisation and management, co-digestion allows the simultaneous treatment of two waste streams, thereby maximising resource utilisation and diverting FW from landfills. The findings of this present study offer a holistic approach to addressing multiple challenges within waste management, by closing the loops of multiple waste streams: LARs, FW and CFA to enhance LARs treatment efficiency in the AD.

8. Coal Fly Ash Stimulation to Enhance Anaerobic Digestion of Barley Straw Via Direct Interspecies Electron Transfer

8.1 Introduction

Coal fly ash (CFA) pretreatment has proven effective in enhancing CH₄ yield of BS, with a significantly greater increase than BS treated with water, and interestingly, the increase was also greater than could be explained by the response to alkali conditioning with an equivalent alkali Control treatment. This was evidenced by a 93% and 39% increase in CH₄ yield compared to water-pretreated Control and CaO pretreatment, without CFA, respectively (see Section 5.3.3). Moreover, the addition of CFA to alkali-treated BS prior to AD resulted in a 32% and 19% increase in CH₄ yield when compared to equivalent alkalinity supplied by CaO and NaOH pretreatment, respectively (see Section 6.3.1). Sodium hydroxide is widely recognised as the most potent alkali and its pretreatment has been proven to be the most effective in delignification (Zheng et al., 2014; Bali et al., 2015; Kaur et al., 2020; Bachmann et al., 2022). The higher CH₄ yield observed under CFA addition after pretreatment, even in comparison to NaOH pretreatment Control, suggests that CFA has important properties that can stimulate anaerobic microorganisms.

Huiliñir et al. (2017); Montalvo et al. (2017) and Lauzurique et al. (2021a) found that TEs supplementation by CFA had an important positive effect in the AD process. While this may be a further benefit of CFA addition, analysis of the inoculum sludge used in the current study was found to be a rich source of TEs (reported in Table 3.3), which were therefore in adequate supply, irrespective of any TEs contribution from CFA. The stimulation of the AD process observed here therefore must be associated with some other attribute of CFA. According to Lauzurique et al.(2021b); Xie et al. (2022); Song et al. (2023), the intrinsic semiconductive attributes, arising from the presence of iron oxides, in CFA, are reported to have the potential to facilitate direct interspecies electron transfer (DIET). The as-received CFA used in the current work contains 8% TS of iron oxides (Table 3.4), which may be present in the forms of magnetite (Fe₃O₄) and hematite (Fe₂O₃) (Kukier et al., 2003; Kim, 2006; Ahmaruzzaman, 2010; Koukourzas et al., 2011), and could explain the observed positive response detected in the solid-state AD experiments. Previous studies have demonstrated that Fe₃O₄ and Fe₂O₃ can enhance CH₄ production by 27–147% in AD through a conduction-based DIET mechanism (Kato et al. 2012; Cruz Viggi et al. 2014; Wang et al. 2018; Dyksma and Gallers 2022; Zhou et al. 2024), in a similar stimulatory manner to carbon-based conductive materials (Chen et al., 2014; C. Wang et al., 2018; Xu et al., 2018; König et al., 2022).

Direct interspecies electron transfer is the most recent and more effective mechanism for electron transfer compared to the earlier mechanism involving diffusion of H₂ or formate as electron shuttles, known as indirect interspecies electron transfer (IIET) (Storck et al., 2016). The electron transfer is crucial during the methanogenesis phase, as it reflects the efficiency of syntrophic interactions between secondary fermenting bacteria and archaea methanogens, in providing energy sources (Xu et al., 2019; Słopiecka et al., 2022). In DIET, the transfer of electrons between bacteria and archaea depends on the presence of biologically electrically conductive protein filaments (e-pili) and membrane-bound structures c-type cytochromes (c-Cyts) distributed across microbial cell envelopes (Malvankar et al., 2011; Zhou et al., 2024).

The presence of conductive or semiconductive materials facilitates microbial aggregates to attach to the material's surface, serving as electron conduits between electron-donating (e.g., secondary fermenting bacteria) and electron-accepting cells (e.g., archaea methanogens) (Chen et al., 2014; Martins et al., 2018). In semiconductive materials with a particle size larger than the cell size, multiple cells can directly attach to the material, enabling rapid interspecies electron exchange without relying on electron carriers like H₂ (Lovley, 2011) or requiring close physical proximity for electron transfer through biological electrical connections (Zhao et al., 2016a; Tang et al. 2021). Earlier studies indicated that with the addition of conductive and semiconductive materials to the AD process several positive effects were observed, such as reduced lag time, decreased VFAs accumulation, higher microbial growth rate, and stable operation at higher organic loading rates, all of which resulted in increased CH₄ yield (Kato et al., 2012; Li et al., 2015; Martins et al., 2018; C. Wang et al., 2018; T. Wang et al., 2018).

The presence of iron oxides in the CFA, however, is insufficient to determine whether CFA potentially facilitates DIET between syntrophic acetogens and archaea methanogens. The electrical conductivity (EC) is a critical physicochemical property of a material which can indicate the potential for accelerating syntrophic metabolism within the DIET mechanism (Martins et al., 2018; Xu et al., 2019; Tang et al., 2021). An increase in the EC of the microbial aggregates or digestate suggests the improvement in electronic connections or electron transport among syntrophic bacteria and methanogens, facilitated by either conductive pili or conductive/semiconductive materials (Baek et al., 2018; Martins et al., 2018; C. Wang et al., 2018; Xu et al., 2019; Deng et al., 2022). Increases in EC of digestate in DIET-related experiments in the presence of conductive and semiconductive materials, such as magnetite (T. Wang et al., 2018), activated carbon (Zhao et al., 2016b), and biochar (C. Wang et al., 2018) have been reported.

The aim of this study was to investigate potential supplementary benefits of CFA addition in the solid-state AD of BS in relation to the DIET mechanism. The specific objectives of the experiments were: (1) to determine the AD response to CFA addition in the direct AD of BS without pretreatment step, (2) to quantify the EC of as-received CFA, the digested sludge inoculum, and the digestates with and without CFA addition, (3) to analyse the microbial community composition under the experimental conditions, particularly focusing on the presence of microorganisms capable of performing DIET using 16S ribosomal ribonucleic acid (rRNA) gene analysis. To the best of the author's knowledge, this study is the first to experimentally investigate the role of CFA in facilitating DIET, providing novel insights on the mechanism by which CFA can enhance microbial efficiency and stability in AD, highlighting the potential for CFA to contribute to the process beyond its alkaline properties.

8.2 Materials and methods

8.2.1 Waste feedstocks

The waste feedstocks used were milled BS, as-received CFA, and fresh digested sewage sludge cake for AD inoculum. The source and preparation of each material was described in Section 3.1.1, 3.1.3, and 3.1.4, respectively, and their general physicochemical properties were presented in Table 3.2. Carbon black (CB) (Sigma Aldrich, UK; 100 mesh particle size), with a TS content of $99.3\% \pm 0.25$, was dried overnight according to the procedure in Section 3.3.2, and used as a standard material for EC measurements (Pantea et al., 2001; König et al., 2022).

8.2.2 Batch experiments of solid-state anaerobic digestion

Solid-state AD experiments were conducted in nine reactors, under three treatment conditions: (i) inoculum only or Blank, (ii) BS and inoculum referred to as 'Control', and (iii) BS and inoculum with CFA added, referred to as 'CFA-AD'. Each of the treatments was conducted in triplicate. In both the Control and CFA-AD treatments, inoculum was added at a 1:1 ratio to BS on a VS basis, resulting in each individual reactor containing (on a FM basis): 125 ± 0.1 g of inoculum, and 15 ± 0.1 g of BS. For the CFA-AD treatment, CFA was added to BS at a ratio of 1:2 (on a FM basis). Deionised water in amounts of 125 g and 195 g was added to the Control and CFA-AD treatment mixtures, respectively, to achieve a final TS concentration equivalent to 15% (see Section 3.2.1). The materials were thoroughly mixed using a kitchen hand mixer. The initial pH of the mixtures in both the Control and CFA-AD reactors was adjusted to between 6.5 and 7 by adding 1 mL of concentrated HCl.

The experimental conditions and procedures applied for the solid-state AD experiments are detailed in Section 3.2.2 and 3.2.3. Two fractions of subsample of approximately 20 g (on a FM basis) were taken from each mixture of experimental materials for the measurement of solids (TS and VS) and pH. The quantity of BS fed to the AD reactors was half of the total VS weight input to the reactor (after subsampling) and the calculated amounts of VS supplied to each reactor as BS and as inoculum are listed in Table 8.1.

Table 8.1 Volatile solids and the initial pH of feedstock mixtures

Experimental Treatments	Barley Straw (g VS)	Inoculum (g VS)	pH (before adjustment)
Control	12.00	12.00	8.50 ± 0.08
CFA-AD	12.00	12.00	8.40 ± 0.28

8.2.3 Analytical methods

The determination of TS, VS, and pH was conducted on the substrate mixtures before loading into the AD reactor vessel and on residual digestates after solid-state AD following the procedures outlined in Section 3.3.2 and 3.3.3. Volatile solids removal was calculated using Equation 5.4 (in Section 5.2.5) to assess the efficiency of the AD process and the extent of substrate degradation. The particle size distribution of the dried CFA was determined using a sieve analysis method described in Section 3.3.4. The iron oxide content of different particle sizes in the as-received CFA samples was determined using the procedures described in Section 3.3.9. Loss on ignition (LOI), representing the unburned carbon (UC) content in the as-received CFA was measured according to the procedures given in Section 3.3.11.

Biogas volume was measured using a water displacement method as detailed in Section 3.2.3, while CH₄ composition was analysed using GC according to the procedures given in Section 3.3.13. The background CH₄ produced from the Blank was subtracted from the total CH₄ volume measured in both the Control and CFA-AD treatments (see Section 3.2.5) (refer to Appendix A, Figure A.5 for the residual CH₄ production from the inoculum used in this chapter).

Digestate residues from the Control and CFA-AD treatments were collected at the end of the AD experiment. The surface morphology of the digestate was examined using SEM in accordance with procedures outlined in Section 3.3.6, specifically focusing on the bacterial attachment to the CFA surface and identifying any visible differences in the structures of biomass sludge granules with CFA addition.

8.2.4 Electrical conductivity measurement

Electrical conductivity measurements were conducted on all experimental materials, including: as-received CFA, particle-sized CFA fractions, CB as a reference material, as well as digestates from inoculum (digested inoculum), Control and CFA-AD reactors. The EC measurements were made (without the need for preparation) on digested inoculum and the digestates collected from both Control and CFA-AD reactors. The EC of the digested inoculum was determined to differentiate between the contribution of mineral in the inoculum sludge to EC and that derived from the semiconductive components present in as-received CFA samples. For EC measurements of as-received CFA and CB (% TS determined in this work for CFA and CB as $99.7\% \pm 0.01$ and $99.3\% \pm 0.25$, respectively), however preparation was required. Deionised water was added to each material, with a mass ratio of 1:3 (water:CFA and water:CB), equivalent to 22% moisture content; each sample was thoroughly mixed using a laboratory spatula to form a paste. The ratio was trialled to provide the minimum water content necessary to prepare a paste that was suitable for application to the electrode surface.

The EC measurements were conducted using Linear Sweep Voltammetry (LSV) techniques using an Autolab PGSTAT30 potentiostat/galvanostat (Metrohm, Switzerland) at room temperature (20–21°C) (Payakaniti et al., 2017). Samples for testing were uniformly applied onto the surface of the stainless-steel electrode plate (dimensions: 12 mm x 20 mm x 3 mm) with an identical plate placed on top, resulting in sample thickness of approximately 0.8–1.4 mm (Singh et al., 2017). The electrode plates were held securely in place using a cell clip 2545 and fixer (Landt Instruments, NY) and connected to the potentiostat (Figure 8.1). The electrical potential was incrementally increased from -0.3 to 0.3 V in steps of a 0.025 V, following the method of (Malvankar et al., 2011; Shrestha et al., 2014). Current responses were recorded to generate the current-potential (I-V) curve. Sample conductivity was calculated using Equation 8.1 (Malvankar et al., 2011).

$$\sigma = \frac{L}{RS} \quad (8.1)$$

where σ is the conductivity (mS m^{-1}), R is the reciprocal of the slope in the current-voltage curve (Ω), L is the thickness of the sample (m) and S is the cross-sectional area of the stainless-steel electrode (m^2).

The EC of CFA were measured for different particle size fractions of the material. Particle size fractions (of 90–125, 60–90, 45–60, and 25–45 and $<20 \mu\text{m}$) were prepared using a graded stack of laboratory sieves (see Section 3.3.4), constituting >95% of the mass of the as-

received CFA. The iron oxide content of the particle fractions and as-received CFA was measured by X-ray fluorescence (XRF) spectrometry (see Section 3.3.9).

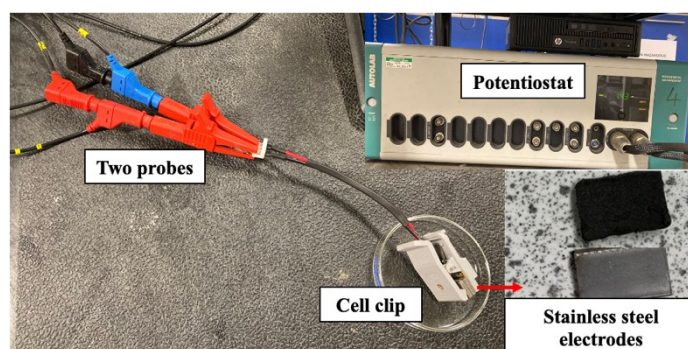


Figure 8.1 Experimental electrical conductivity measurement device

The potential effect of moisture content of the samples on EC was determined by adjusting the moisture content of the paste prepared with as-received CFA to three different moisture levels (including the initial value of 22%). It was necessary to conduct the drying process *in situ* to maintain contact between the sample and the electrode plates. This process was performed by allowing water evaporation from the samples while they were held between the electrode plates at room temperature (20–21°C) for 0, 15 and 60 minutes; thus, the initial moisture content was 22%, and this decreased to 20% and 6%, respectively, with increasing drying period. Measurements were conducted on three independent replicate samples.

8.2.5 Statistical analysis, CH₄ gas modelling and kinetics

Triplicate CSMY_{exp} measurements from each treatment: Control and CFA-AD, were normalised to STP conditions (see Section 3.2.5). CH₄ gas modelling was conducted on the normalised CSMY_{exp} values after removing outlier data, as detailed in Section 3.4. Modelling of the CH₄ yield and kinetic parameters, and determination of the CSMY_o followed the procedures described in Section 3.4. The statistical significance of differences in CSMY and kinetic parameters between the experimental treatments were determined by a two-tailed paired t-test (see Section 3.4).

8.2.6 Microbial community analysis

High throughput 16S rRNA gene sequencing was conducted on the digested inoculum as well as residual digestates of Control and CFA-AD treatments. 16S rRNA gene sequencing is the most common, cost-effective and valuable method providing information on the composition of the AD microbial community and the potential presence of microorganisms capable of performing DIET, both for bacteria and archaea (Callahan et al., 2016). The digestate samples

were collected from the reactors at the end of the experimental incubation period (40 d). Digestate samples from the three replicate reactors for each condition were combined, thoroughly mixed and divided into two 50 mL centrifuge tubes with screw caps, each containing 3.0 ± 0.5 g on an FM basis. One tube was designated for bacterial composition analysis, while the other tube was allocated for archaeal composition analysis. All tubes were frozen at -20°C after sample preparation and transported in an insulated polystyrene box containing ice packs for analysis. The samples were carried by the author to Novogene (UK) Company Ltd, 25 Science Park, Cambridge CB4 0FW, UK, for Deoxyribonucleic acid (DNA) extraction and microbial community analysis 16S rRNA gene sequencing.

Genomic DNA was extracted using a cetyl trimethylammonium bromide (CTAB)-based protocol (Novogene, 2023). The extracted DNA was subjected to 16S rRNA gene using V3-V4 region universal primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGTATCTAAT) for the bacteria (Novogene, 2023). Amplicon sequencing of the archaeal 16S V45 region was conducted using primer 519F (CAGCCGCCGCGGTAA) and 915R (GTGCTCCCCCGCCAATTCCT) (Novogene, 2023). The amplification cycling scheme consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation each at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s, with a final elongation at 72°C for 5 min (Novogene, 2023).

Sequences generated from gene sequencing of bacterial and archaeal 16S rRNA were processed and analysed using Qualitative Insights into Microbial Ecology (QIIME 2 version 2023.5) (Bolyen et al., 2019) supported with a q2-feature classifier plugin (Bokulich et al., 2018). Sequence reads underwent denoising and pair-end read joining using the Divisive Amplicon Denoising Algorithms method (DADA2) (Callahan et al., 2016). 16SrRNA gene sequences were assigned to phylogenetically consistent (>97% similarity) higher-order bacterial taxonomy groups based on a naïve Bayesian rRNA classifier and were compared to sequences deposited in the SILVA 138 database (Quast et al., 2013).

8.3 Results

8.3.1 Electrical conductivity of digested inoculum, as-received coal fly ash, and digestates

The EC values for as-received CFA, CB, digested inoculum, and experimental digestates from the Control and CFA-AD treatments are presented in Table 8.2. The EC of the as-received CFA (0.6 mS m^{-1}) was 10,000 times smaller compared to the reference CB material ($21.6 \times 10^3 \text{ mS m}^{-1}$).

Table 8.2 Electrical conductivity (in mS m⁻¹) of coal fly ash (as-received), carbon black (reference material), digested inoculum and digestates from the Control and CFA-AD treatments.

As-received CFA	Carbon Black	Digested Inoculum ^a	Control ^a	CFA-AD ^a
0.60 (0.06)	21.6 x 10 ³ (6.55 x 10 ³)	0.0049 (0.0025)	0.0057 (0.005)	0.14 (0.002)

Note: Data are expressed as mean values (n=3), parentheses indicate the standard deviation.

^a Digestate samples were collected at termination of the batch solid-state AD experiment after 40 d of incubation at 30°C.

This EC for CB was in good agreement with the reported 10 x 10³–100 x 10³ mS m⁻¹ in the literature (Pantea et al., 2003; König et al., 2022). There was no significant difference ($P > 0.05$) in the EC values between the digested inoculum and residual digestate from the Control, confirming no effect on EC arising from digested BS. In comparison, the EC of the digestate collected from the CFA-AD treatment was 25 times larger compared to the Control, and the difference was highly statistically significant ($P < 0.001$). Therefore, CFA was shown to have the potential to enhance EC of the AD environment, and the results provide evidence that CFA may support DIET mechanisms in the AD process of BS.

8.3.2 Effect of particle size on electrical conductivity and iron oxide content

The particle size distribution of CFA (Table 8.3) shows that particles ranging from 20–45 µm constitute the largest mass fraction at 42.4% TS, followed by particle smaller than 20 µm (24.6% TS). The mass fraction for the particle size range 45–63 µm at 9.7% TS, was close to that for 63–90 µm particle size (10.1% TS). At particle sizes above this range progressively smaller mass fractions were observed viz. 5.8% TS for 90–125 µm, 3.3% TS for 125–180 µm, and 1.9% TS for 180–250 µm. Particles larger than this had a mass fraction of <1% TS of the total. Generally, the mass fraction decreases with increasing particle size.

The smallest particle size fraction of as-received CFA collected, <20 µm had an EC value equivalent to 0.07 mS m⁻¹, which was comparable to the 0.06 mS m⁻¹ detected in the 20–45 µm range, representing the lowest overall EC value, compared to the other size ranges (Table 8.3). The EC increased by two orders of magnitude for the particle size range of 45–63 µm, to 3.5 mS m⁻¹, and again for particles size of 63–90 µm, to 80 mS m⁻¹, and to a maximum value recorded of 270 mS m⁻¹ for particles size range 90–125 µm. The results showed, however, that EC was negatively correlated with the iron oxide content ($r = -0.82$) and the relationship was not statistically significant ($P > 0.05$), as shown in Figure 8.2 (a). Indeed, particles with the

largest overall EC in the range: 90–125 μm contained less iron oxide than the other particle size ranges which had smaller EC. Some fractions of CFA showed high EC values, nevertheless, the overall EC of the total sample of as-received CFA was low (0.6 mS m^{-1}) as these fractions represent a small proportion of the mass of the material.

Table 8.3 Effect of particle size and moisture content on the electrical conductivity of as-received coal fly ash, and of particle size on iron oxide content and loss on ignition (LOI).

Parameter	Mass Fraction (% TS)	Electrical Conductivity of Coal Fly Ash (mS m^{-1})	Iron Oxides Content (% TS)	LOI (%)
As-received CFA	N/A	0.60 (0.06)	7.90	5.40 (0.50)
Particle size (μm)				
< 20	24.60	0.07 (0.03)	n.d	n.d
20–45	42.40	0.06 (0.02)	12.30	2.40 (0.70)
45–63	9.70	3.50 (0.50)	11.50	3.30 (0.40)
63–90	10.10	80.00 (0.01)	12.60	6.20 (0.40)
90–125	5.80	270.00 (0.13)	9.60	7.20 (0.30)
125–180	3.30	n.d	n.d	n.d
180–250	1.90	n.d	n.d	n.d
Moisture (%)				
22 (0.68)	N/A	0.64 (0.07)	N/A	N/A
20 (0.30)	N/A	0.30 (0.23)	N/A	N/A
6 (0.08)	N/A	0.26 (0.33)	N/A	N/A

Note:

(1) Data are expressed as mean values ($n=3$), parentheses indicate the standard deviation.

(2) N/A: not applicable.

(3) n.d: not determined.

In terms of LOI measurements (Table 8.3), the results show that the highest value recorded was for the largest particle size range of 90–125 μm , at 7.2%. The LOI decreased proportionally with smaller sizes, to 6.2% and 3.3% for 60–90 μm and 45–60 μm particle size ranges, respectively, and finally to a minimum value of 2.4% for 20–45 μm particle size range. The LOI for the total as-received CFA was 5.4%. The relationship between EC and LOI was strong and positive ($r=0.98$), and the relationship was statistically significant ($P < 0.05$) (Figure 8.2 (b)).

The EC declined slightly with decreasing moisture content, but this was not statistically significant at $P > 0.05$ (Table 8.3).

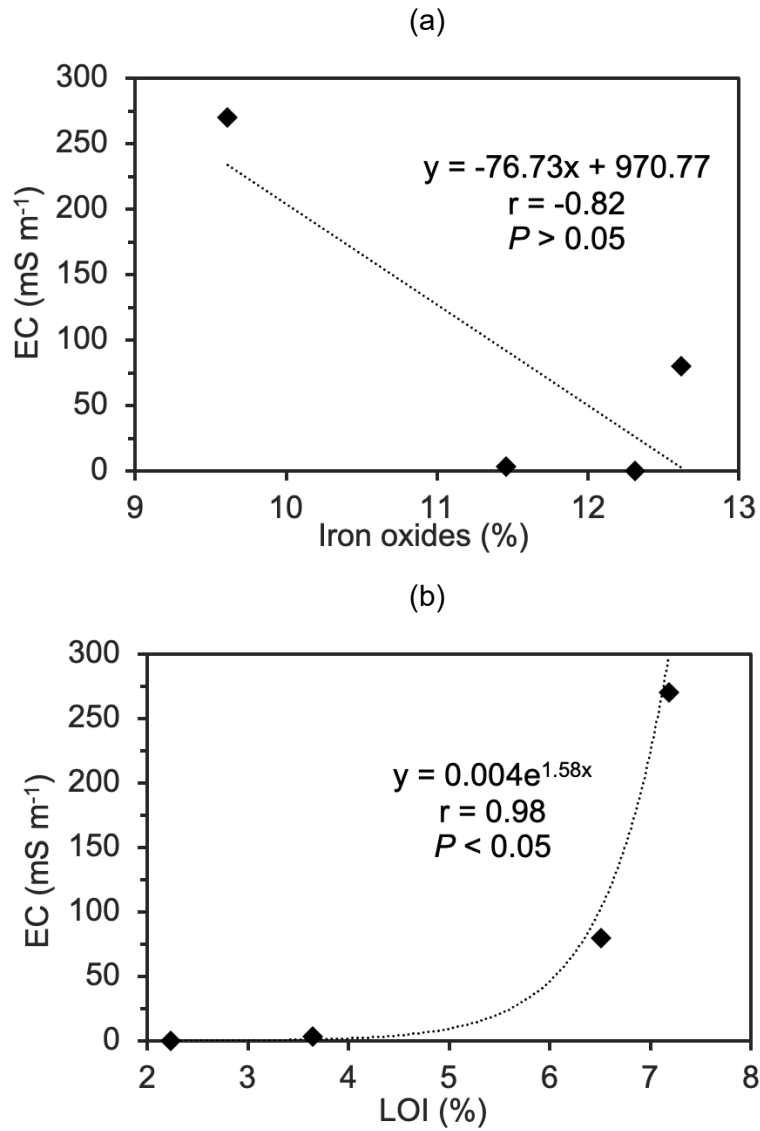


Figure 8.2 Electrical conductivity of different particle size of as-received coal fly ash (CFA) in relation to (a) iron oxides and (b) loss on ignition (LOI).

8.3.3 Effect of coal fly ash addition on volatile solids removal, hydrolysis rates and CH₄ yields

This experiment investigated the effects of direct CFA addition on the AD of BS (CFA-AD) and the removal rate of VS and CH₄ yield (Figure 8.3) (the triplicate values of CSMY_{exp} and VS removals for each treatment conditions are presented in Appendix C, Table C.4). Triplicate values of CSMY_{exp} of both Control and CFA-AD treatments generally showed good agreement, with a relative standard deviation of 10% and 10.7%, respectively, (refer to Appendix C, Table C.4 for mean and standard deviation values of CSMY_{exp} replicate data used in calculating RSD), within the range of acceptable variation in BMP test results (Holliger et al., 2016) (see Section 3.4).

The VS removal in the Control and CFA-AD treatments were 28.7 ± 7.1 % and 37.0 ± 5.6 %, respectively. CFA-AD treatment resulted in 128.0 ± 13.7 mL CH₄ g⁻¹ VS, which significantly ($P = 0.040$) increased the mean CSMY_{exp}, measured by 26%, compared to the Control (101.5 ± 10.5 mL CH₄ g⁻¹ VS), and this was consistent with a 29% increase in VS removal, although the VS response was not statistically significant ($P > 0.05$).

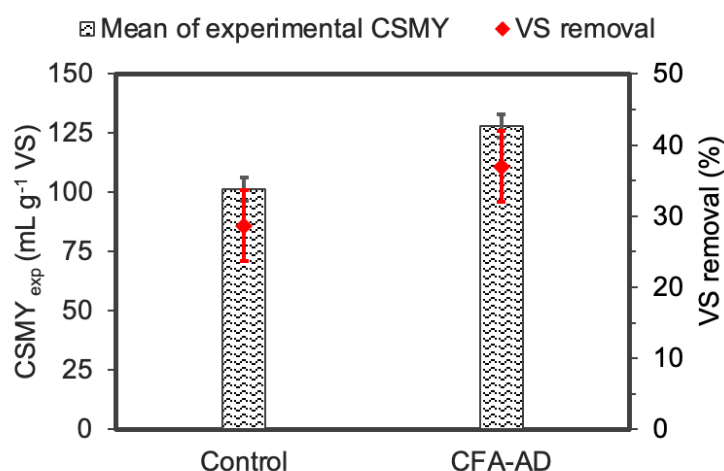


Figure 8.3 Effect of the direct addition of CFA into anaerobic digestion (CFA-AD) of barley straw on volatile solids (VS) removal and experimental cumulative specific yield (CSMY_{exp}). Error bars; red and black, show the standard deviation of VS removal and CSMY_{exp}, respectively (n=3).

Experimental and modelled CSMY data are shown in Figure 8.4. The kinetic parameters for CSMY₀ and hydrolysis rates obtained from First-order (k) and Cone kinetic models (K_H) of the AD process are presented in Table 8.4 (CSMY₀ and hydrolysis rates from each replicate used for statistical comparisons are provided in Appendix B, Table B.4). Both models provided a good description of experimental data; the Cone model demonstrated a slightly superior fit with R^2 values of 0.88 compared to 0.87 for the First-order kinetic model. The Cone model was used to estimate CSMY₀ and hydrolysis rate (K_H), as it showed less systematic deviation from the experimental data compared to the First-order model, particularly during day 0–30 d of the AD period.

The CSMY₄₀ was also calculated for the 1–40 d data range due to inconsistencies observed between the CSMY₀ derived from the Cone model and the CSMY_{exp} (refer to Section 5.3.3 for further explanation of these inconsistencies), which was equivalent to 132.3 mL g⁻¹ VS for CFA-AD, compared to 101.5 mL g⁻¹ VS for the Control. These values were similar to the CSMY₀ value determined by the First-order model (127.7 and 97.6 mL g⁻¹ VS, respectively) and showed a higher level of agreement compared to CSMY_{exp} (refer to Appendix C, Table

C.4 for the difference (errors) (in percentage) between the experimental ($CSMY_{exp}$) and $CSMY_{40}$ values), which probably provided a more reliable estimate of the ultimate CSMY from AD of BS. Thus, direct addition of CFA to AD of BS significantly ($P = 0.038$) increased the CSMY by 30% compared to the Control condition, consistent with the significant ($P < 0.1$) increase of K_H , which at 0.15, was almost twice that for the Control condition (0.08). Therefore, CFA addition to AD of BS significantly increased the CH_4 yield and improved the rate of the process, above the effects associated with alkali pretreatment of BS.

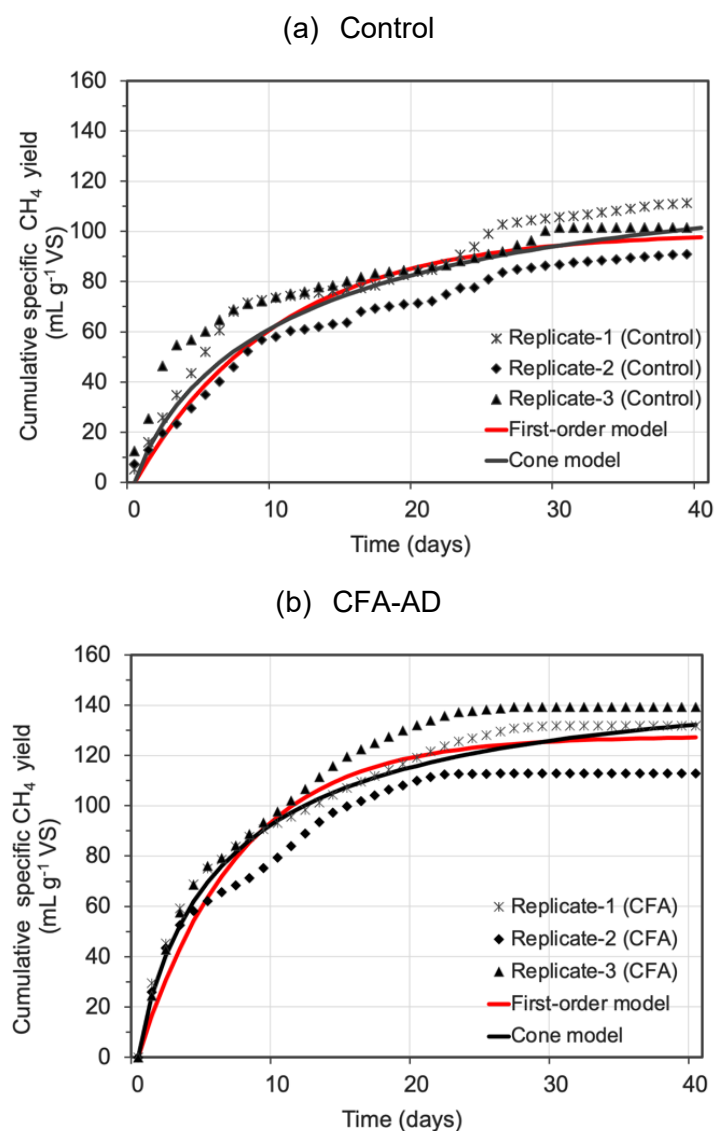


Figure 8.4 Experimental ($n=3$, daily measurements) and modelled (First-order and Cone) specific CH_4 yield from anaerobic digestion of barley straw (BS) with different treatment conditions:(a) Control and (b) CFA-AD at a BS:CFA ratio of 1:2.

Table 8.4 Effect of CFA addition on the kinetic parameters $CSMY_o$ and hydrolysis rates estimated by the First-order and Cone kinetic models from anaerobic digestion of barley straw; $CSMY_{40}$ value from the Cone model is also shown.

Treatment	First-order Model			Cone Model				
	$CSMY_o^a$	k^b	R^{2c}	$CSMY_o$	$CSMY_{40}^d$	K_H^e	n^f	R^2
Control	97.6 a	0.10 a	0.87	137.3 a	101.5 a	0.08 a	0.89	0.88
CFA-AD	127.2 b	0.14 a	0.87	157.9 b	132.3 b	0.15 a	0.90	0.88

Note: Values in a column followed by the same letter are not significantly different at $P = 0.05$.

^a $CSMY_o$: maximum cumulative specific methane yield ($\text{mL g}^{-1} \text{VS}$) (Equation 3.6 and 3.7).

^b k : hydrolysis rate constant from First-order model (d^{-1}) (Equation 3.7).

^c R^2 : coefficient of determination of the modelled CH_4 data

^d $CSMY_{40}$: cumulative specific methane yield at 40 d predicted from Cone model.

^e K_H : hydrolysis rate constant from Cone model (Equation 3.6).

^f n : shape factor (Equation 3.6).

8.3.4 SEM images of coal fly ash and barley straw digestate

The SEM image of CFA showed that the material was composed of spherical particles of varying size (Figure 8.5 (a)), as confirmed by the particle size analysis distribution (Table 8.3). Digestate from the Control (Figure 8.5 (b)) has an amorphous structure comprising the complex organic matter matrix, and the surface morphology is very similar to the sludge structure reported in Zhou et al. (2024). The digestate from CFA-AD treatment (Figure 8.5 (c)) showed spherical CFA structures in intimate contact with the organic material, however, due to the complexity and amorphous organic matter matrix, it was not possible to discern any specific cellular structures of individual bacteria cells and colonies attached to the CFA particle surface as initially anticipated. Moreover, the digestate with CFA showed gelatinous materials, which were probably extracellular polymeric substances (EPS) encapsulating the CFA particles and the organic matrix. This was not observed in the Control treatment and may indicate enhanced microbial colonisation and activity in the digestate with CFA addition. A similar sludge morphology was reported by Zhou et al. (2024) with carbon-based conductive material (carbon nanotubes), where microbial species were indiscernible due to being enclosed by large amounts of EPS.

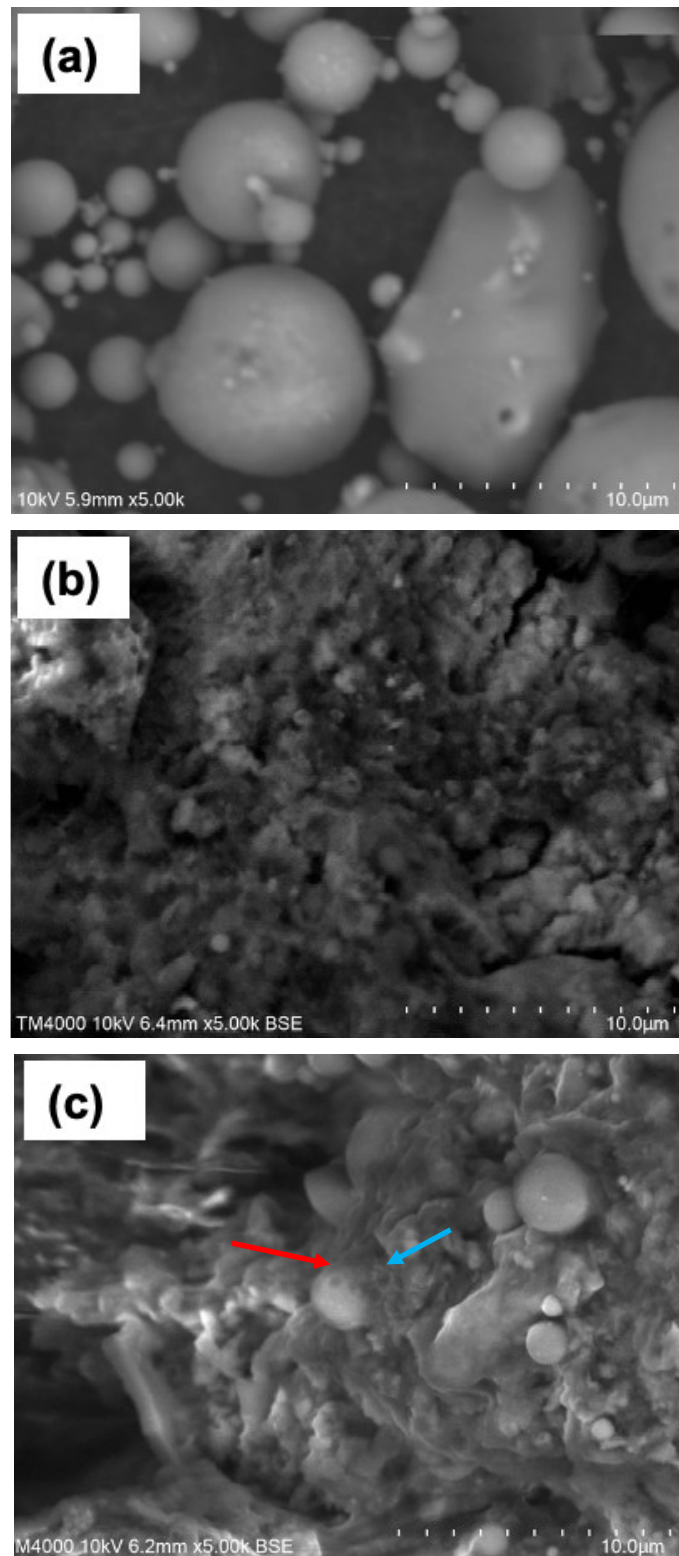


Figure 8.5 Scanning electron microscopy images of (a) as-received coal fly ash (CFA), and digestates from (b) Control, and (c) CFA addition digestion conditions (CFA-AD). The red arrow indicates CFA particles and blue arrow indicates organic matter, possibly extracellular polymeric substances (EPS). All images were at 5000x; the whole scale bar represents 10 μm .

8.3.5 Microbial community profiles

The microbial community composition of bacteria and archaea in both AD conditions (Control and CFA-AD), and in the digested inoculum are shown in Figure 8.6 (a) and 8.6 (b).

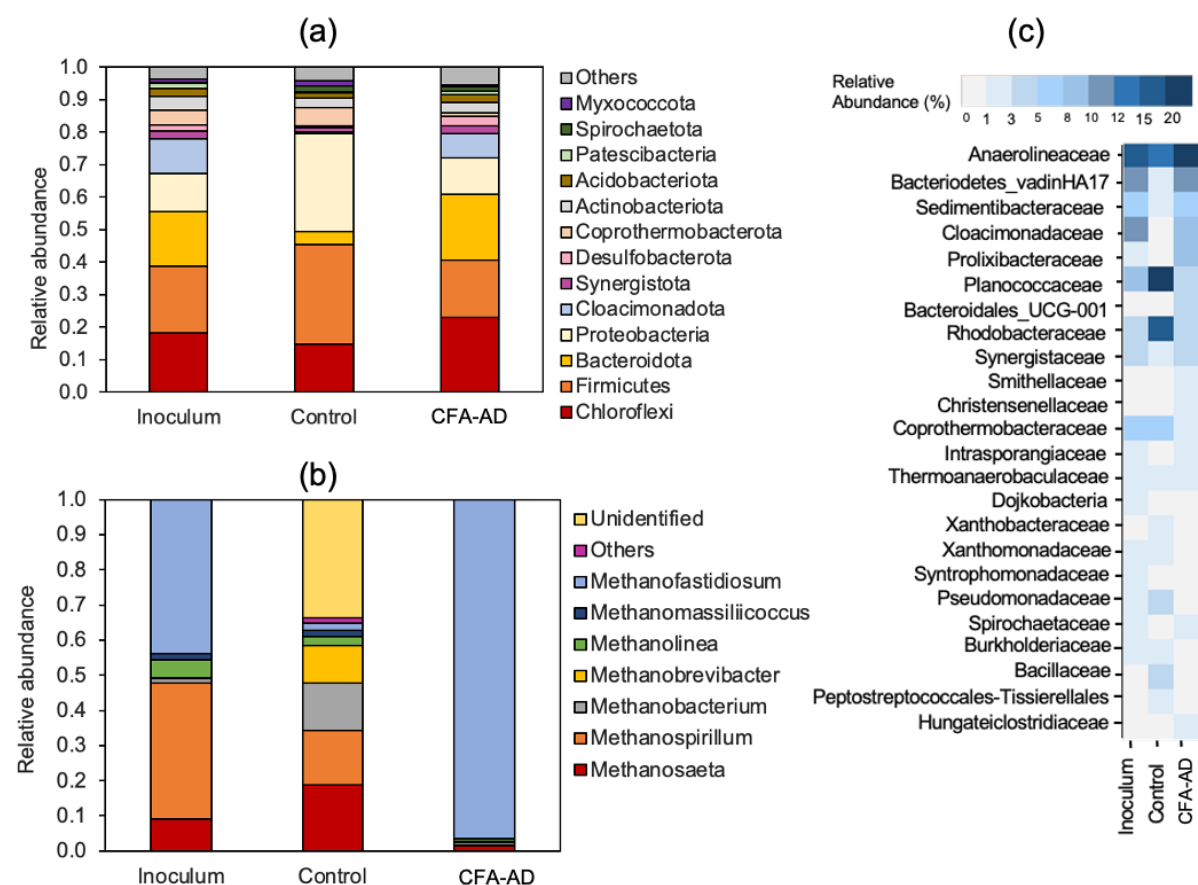


Figure 8.6 The microbial community composition for (a) bacteria at the phylum level and (b) archaea at the genus level in digested inoculum, the Control, and CFA-AD treatments with barley straw. Relative abundances 1% were grouped together as “Others”. (c) Heatmap representing the bacterial profile at the family level with >1% relative abundance. The colour scale ranges from 0 to 20% relative abundance.

The bacterial community profile is presented at phylum level. The most dominant phylum were *Firmicutes*, *Chloroflexi*, *Bacteroidota* and *Proteobacteria*, collectively constituting 65.5, 76.6 and 71.7% of the bacterial population, respectively, in the digested inoculum, the Control and CFA-AD reactors following AD of barley straw for 40 d. In order of decreasing abundance in the digested inoculum, the phyla were arranged as: *Firmicutes* (20%), *Chloroflexi* (18%), *Bacteroidota* (16%), *Proteobacteria* (11.3%) and *Cloacimonadota* (10.5%). The relative abundance of *Firmicutes* and *Proteobacteria* increased significantly in the Control to 29.4% and 29.1%, respectively, while the abundance of *Bacteroidota* decreased to 3.9% and *Chloroflexi* was slightly reduced to 14%, compared to the digested inoculum. The bacterial community profile in the CFA-AD reactor was a close match to that of the digested inoculum, with *Chloroflexi* and *Bacteroidota* being the most abundant members, equivalent to 22.8 and

20.4% of the bacterial population, respectively. *Firmicutes* decreased to 17.5% while *Proteobacteria* did not change, remaining at 11.1% relative to the digested inoculum.

To further characterise the bacterial community, a heatmap was generated at the family level illustrated in Figure 8.5 (c) with the detailed related data presented in Appendix D, Table D1. The most dominant families in the digested inoculum were *Anaerolineaceae* and *Bacterioidetes_vadinHA17* with the abundance was 17.3% and 9.7%, respectively. The abundance of these bacteria declined markedly to 12.8 and 1.2%, respectively, in the Control reactor. In contrast, however, the abundance of *Planococcaceae* and *Rhodobacteraceae* increased to 21.3 and 15%, respectively, in the Control compared 6.4% and 2.8%, respectively, in the digested inoculum. These two families represent the largest members of the community in the digested inoculum. Like the profile for digested inoculum, *Anaerolineaceae* and *Bacterioidetes_vadin HA17* were dominant in the CFA-AD reactor, with the latter enriched with *Anaerolineaceae* to 22.1% (higher than the value of 17.3% for digested inoculum), however, *Bacterioidetes_vadin HA17* slightly declined to 8.4%, compared to that in the digested inoculum. It is also important to note that the abundance of acetogenic bacteria, critical for propionate degradation, such as *Smithellaceae* (Xia et al., 2019) was enriched in CFA-AD reactor by twofold, shown by the increase from 0.7% in the digested inoculum to 1.4%. In contrast, the composition of this bacteria declined to 0.12% in the Control, relative to the digested inoculum.

The archaeal distributions presented at genus level in Figure 8.5 (b) showed that hydrogenotrophic methanogens, *Methanospirillum* and *Methanofastidiosum*, were dominant in the digested inoculum, with abundances of 37% and 42%, respectively. Well-known acetoclastic methanogens, e.g., *Methanosaeta* (Amin et al., 2017) represented a smaller fraction (8.8%). *Methanosaeta* increased to 18.2%, and *Methanospirillum* and *Methanofastidiosum* decreased significantly to 15% and 1.8%, respectively, in the Control relative to digested inoculum. By contrast, the hydrogenotrophic methanogens; *Methanobacterium* and *Methanobrevibacter* (Venkiteshwaran et al., 2015; Amin et al., 2017), increased from 1.2 to 13% and 0.2 to 10.3%, respectively, in the Control, compared to digested inoculum. A further important observation was that addition of CFA to the AD process increased the abundance of *Methanofastidiosum* by a factor of two relative to digested sludge inoculum, and it was therefore the most dominant archaea representing almost 87% of the archaea community.

8.4 Discussion

8.4.1 Electrical conductivity

The EC of as-received CFA of 0.6 mS m^{-1} , presented in Table 8.2, was comparable to the reported $0.018\text{--}0.37 \text{ mS m}^{-1}$ range for CFA (Singh et al., 2017), which is also typical of semiconductor materials ($10^{-5}\text{--}10^3 \text{ mS m}^{-1}$) (Barroso-Bogeat et al., 2014), indicating that CFA is a semiconductive material. The EC of the as-received CFA was also comparable with the values reported for biochar ($0.2\text{--}5 \text{ mS m}^{-1}$), a material that has been shown to promote DIET in many studies (Chen et al., 2014; Baek et al., 2018; König et al., 2022; Zhou et al., 2024), hence indicated that the EC for biochar is adequate for mediating electron exchange for DIET. This suggests that the conductivity of the as-received CFA is sufficient to support DIET between syntrophic partners in the AD system. The consistency between the EC of the reference material (BC) and values reported in the literature, demonstrated the confidence in EC measurement procedure.

Conductive and semiconductive material have been demonstrated to mediate cell-to-cell electron transfer, substituting biological electrical connections such as pili or biological nanowires (Kato et al., 2012). *Geobacter sulfurreducens* and *Geobacter metallireducens* were the first bacteria species identified to participate in DIET express genes encoding conductive pili, protein filaments crucial for electron transfer processes (Baek et al., 2018). The EC of as-received CFA was comparable with that of the conductive pili of *Geobacter sulfurreducens* ($0.60\text{--}4.18 \text{ mS m}^{-1}$) (Malvankar et al., 2011; Liu et al., 2020), although higher values up to 500 mS m^{-1} have also been reported (Malvankar et al., 2011), suggesting a potential link between the observed increase in CH_4 in the CFA treatment and DIET. The high variation in the EC of the *Geobacter* pili is influenced by the strain variation, growth conditions, and the abundance of structural pilin protein (PilA) (Shrestha et al., 2014).

The increase in EC in the microbial aggregates or biomass sludge serves as potential evidence supporting the establishment of DIET in the enrichments (C. Wang et al., 2018). The EC of the digested inoculum (0.0049 mS m^{-1}) (Table 8.2) was one to two orders of magnitude lower than conductivities for the reported seeding sludge ($0.08\text{--}0.7 \text{ mS m}^{-1}$) (Morita et al., 2011; Shrestha et al., 2014; C. Wang et al., 2018). There is correlation between sludge conductivity and the presence of electrogenic bacteria, such as *Geobacter* species in the sludge (Lovley, 2017). The absence of *Geobacter* species in the digested sludge used as the inoculum in the experiments here (Figure 8.6), could be a plausible reason for the low conductivity obtained in the digested inoculum used in the present study. The EC, however, was increased in CFA-AD reactor for 25 times to 0.14 mS m^{-1} (Table 8.2) compared to that of Control condition (0.0057

mS m⁻¹), consistent with the 1.6 to 82 times increase in EC observed in inoculum following the addition of conductive materials reported elsewhere (C. Wang et al., 2018; Wang et al., 2022). The results suggest that the increased EC indicate that conductive properties within as-received CFA may stimulate the growth of electrogenic bacteria in the CFA-AD reactor, potentially facilitating the DIET mechanism.

The results showed that, despite the variation in moisture content (Table 8.3 – 6%, 20%, 22%), the EC of the as-received CFA under these moisture conditions was not significantly different, indicating that moisture was not a significant factor that could lead into inconsistencies or errors in the EC measurement.

Table 8.3 shows that the EC of as-received CFA increased with particle size, however, there was no evidence to indicate that this was related to iron oxide content (Figure 8.2 (a)). Although the contribution to the EC from iron oxide, cannot be ruled out, other factors apparently influenced the EC of CFA in relation to particle size. Indeed UC, from incomplete combustion of coal is electrically conductive (Choo et al., 2014b; Tao et al., 2015). The results in Table 8.3 showed that increasing EC with particle size was explained by the greater LOI (which serves as a measure of UC content) of larger particle size fractions, further confirmed with an exponential relationship between LOI and EC, showing a strong positive and significant relationship (Figure 8.2 (b)).

Coal fly ash is a complex material and the other metal oxides in CFA, such as Al₂O₃, tin (II) oxide (SnO), tin (IV) oxide (SnO₂), titanium oxide (TiO₂), tungsten (VI) oxide (WO₃) and zinc oxide (ZnO), are also semiconductor, and their presence collectively may contribute to the EC of CFA (Barroso-Bogeat et al., 2014). Other studies indicated that semiconductive properties of CFA were also influenced by mesoporous structure (Ramos et al. 2008; Macías-García et al. 2019), chemical surface (Pantea et al., 2003) and textural properties (Sathish-Kumar et al., 2012).

8.4.2 Effect of CFA addition on hydrolysis rate, volatile solids removal and CH₄ yield

The results here have confirmed the findings presented in Chapter 5 and Chapter 6 regarding the direct effect of CFA incorporation on the AD, on increased CH₄ yield, contributing significantly beyond solely supplying alkalinity during pretreatment. The observed 30% increase in CH₄ yield (Table 8.4) with CFA addition relative to the Control was consistent with reported 33–147% increase in CH₄ production during AD involving semiconductive iron oxides, including Fe₃O₄ and Fe₂O₃ (Cruz Viggi et al., 2014; Ambuchi et al., 2018). The VS

removal also increased in the CFA-AD treatment, albeit not significant. A positive response with CFA addition was also observed in terms of the K_H value. Despite approaching the statistical significance ($P < 0.1$), the K_H for CFA-AD (of 0.15) was almost twice the Control conditions, indicating that addition of CFA had a profound effect not only on CH_4 yield and the VS removal, but also on the hydrolysis rate.

8.4.3 Changes in the bacterial and archaeal community composition

The phylum level analysis (Figure 8.6) indicated that all of the experimental AD conditions were dominated by *Firmicutes*, *Chloroflexi*, *Bacteriodota* and *Proteobacteria*. These are commonly identified bacterial phyla in AD of solid waste, sludge and wastewater and are reported to have a significant role in the hydrolysis and acidogenesis stages of AD (De Vrieze et al., 2015; R. Wang et al., 2021). Indeed, *Bacteriodota* and *Firmicutes* are hydrolytic bacteria, responsible for the breakdown of polymeric substrates, such as carbohydrates, to their respective monomers or oligomers, using amylase, cellulase, lipase, pectinase, and proteinase enzymes (Venkiteshwaran et al., 2015; Y. Wang et al., 2022). *Bacteriodota* may be enriched in anaerobic environments with low levels of VFAs, salts and NH_3 (De Vrieze et al., 2015; Amin et al., 2017). Thus, the increased abundance of this phylum in AD reactors amended with CFA relative to the Control may be explained by the capacity of CFA to act as an adsorber of VFAs and toxic compounds (Mushtaq et al., 2019; Lauzurique et al., 2021b).

The adsorption capacity of CFA, like activated carbon, is influenced by several attributes, including the UC, high surface area, pore volume, and porosity (Ge et al., 2018; Alterary and Marei, 2021), as well as environmental factors like pH and temperature. The adsorption mechanism onto CFA particles involves: (1) attraction of organic matter towards adsorption sites and formation of a monolayer on the CFA (Siyal et al., 2020), and (2) a ligand exchange mechanism, which involves the interaction of complexation, chelation, and hydrogen bonding (Dai et al., 2020).

Chloroflexi and *Proteobacteria* are major groups included in the known species of acidogenic bacteria (Venkiteshwaran et al., 2015) that exhibit distinct characteristics. *Chloroflexi* is recognised for its capacity to degrade complex organic compounds (R. Wang et al., 2021). CFA increased the enrichment of the bacterial family, *Anaerolineaceae*, which represented more than 99% of *Chloroflexi* abundance. *Anaerolineaceae* produces cellulosomes, which are enzymes involved in cellulose degradation (Ambuchi et al., 2018). This behaviour is consistent with the results of the fibre analysis reported in Section 5.3.2 showing increased degradation

of the cellulose fraction with CFA pretreatment compared to the equivalent alkalinity CaO pretreatment.

Syntrophic acetogenic bacteria play an important role in the biodegradation of acidogenesis products and VFAs that are not directly utilisable by methanogens, such as propionate, butyrate, and valerate, converting them into other forms that can be directly utilised, including acetate, H₂ and CO₂ (Venkiteshwaran et al., 2015). Among the syntrophic acetogens, *Smithellaceae* had high abundance in the presence of CFA, consistent with previous studies reporting the stimulation of *Smithellaceae* by conductive materials including Fe₃O₄, carbon nanotubes (Xia et al., 2019) and biochar (Zhao et al., 2016a). Furthermore, the presence of *Smithellaceae* is essential for maintaining stable and efficient AD operations, as certain VFAs, especially propionate, can inhibit methanogenesis at high concentrations (Venkiteshwaran et al., 2015).

The increased abundance of *Methanosaeta* in the Control condition compared to the digested sludge inoculum suggests that acetate production from the degradation of BS in AD enriched this archaea group, as acetate is the primary substrate for this group. In contrast, the abundance of *Methanosaeta* decreased in CFA-AD reactor compared to the digested sludge inoculum. Although acetate may also be present in the CFA-AD reactor like that in the Control reactor, the absence of *Methanosaeta* in CFA-AD reactor indicated that acetate is not the predominant substrate and the acetoclastic pathway is not operating here. Instead, *Candidatus methanofastidiosum* was significantly enriched in the CFA reactor, suggesting the presence of other substrates that favour the growth of this group.

8.4.4 Possible mechanisms explaining the effect of CFA addition on increased CH₄ yield from anaerobic digestion of barley straw

Drawing on evidence derived from integrating EC and microbial analysis data to identify DIET capable species, as proposed by Sitthi et al. (2022), two potential microbiological mechanisms and pathways are proposed to explain the observed increases in the rate and CH₄ yield of AD of BS with CFA addition.

The study presented here suggests that CFA supports DIET. The initial evidence for this is the presence of iron oxides (Table 3.4) and UC within as-received CFA, which the latter is represented by LOI value contained in Table 8.3, coupled with the EC value measured for as-received CFA found to be in the range comparable to that reported for the EC value of pili of *Geobacter* species (Malvankar et al., 2011; Liu et al., 2020). Iron oxides such as Fe₃O₄ and Fe₂O₃, have been extensively shown to promote DIET in AD systems, significantly increasing

organic matter degradation and CH₄ production (Kato et al., 2012; T. Wang et al., 2018; Xu et al., 2019). The as-received CFA had LOI of 5.4% and 8% of iron oxides, and its EC was comparable to that of biochar, suggesting that CFA may support promote DIET interactions between syntrophic fermenting bacteria or syntrophic acetogens and methanogens (Chen et al., 2014; Zhao et al., 2015; König et al., 2022).

Microbial analysis by 16S rRNA gene amplicon sequencing showed that addition of CFA to the AD process significantly increased the abundance of *Anaerolineaceae* (Figure 8.6), consistent with studies reporting the occurrence of DIET in the AD processes with the addition of Fe₂O₃, GAC, carbon felt and biochar (Lei et al., 2016; Ambuchi et al., 2018; Wang et al., 2021; Wang et al., 2022; Zhu et al., 2023). These bacteria possess metabolic pathways for the degradation of carbohydrates and proteins (Bose et al., 2021; R. Wang et al., 2021; Z. Wang et al., 2022), and produce cellulosomes, which are multiprotein enzyme complexes responsible for hydrolysing recalcitrant cellulose (Ambuchi et al., 2018). More importantly, they are identified as electrogenic bacteria, capable of directly transferring electrons to conductive materials through DIET (Ambuchi et al., 2018; C., Wang et al. 2018; Gustave et al., 2019; Zhu et al., 2023).

16S rRNA gene analysis also showed that CFA also markedly increased the abundance of the archaeal genus, *Methanofastidiosum*. The extracellular electron transfer capability and potential participation in DIET of these methanogens have not been studied as extensively as those of *Methanosarcina* and *Methanosaeta*, which are two genera of methanogenic archaea with a capacity to directly accept electrons by the DIET mechanism (Morita et al., 2011; Martins et al., 2018; D. Wang et al., 2018). The dominance of *Methanofastidiosum* in the CFA-AD reactors, however, combined with other evidence, suggested that they could be involved in DIET. For example, König et al. (2022) reported a positive correlation between *Methanofastidiosum* with *Geobacter* in the presence of graphene and suggested a potential syntrophic metabolism may exist between these microorganisms under DIET conditions. Furthermore, a syntrophic partnership between *Methanofastidiosum* and *Syntrophobacter* was also observed with the addition of activated carbon-added polyvinyl alcohol (PVA) beads (Sitthi et al., 2022). Another study reported the enrichment of *Methanofastidiosum*; which increased from 1% in the inoculum-only reactor to 19%, and of *Anaerolineaceae* in the presence of zero-valent iron and graphene oxide during AD of industrial wastewater (Lan et al., 2023). All instances suggested that *Methanofastidiosum* may be stimulated by the conductive environments and may play a role in DIET in conductive material-mediated AD process. Consequently, as the most enriched methanogens in the CFA-AD reactor,

Methanofastidiosum is a plausible methanogenic partner with *Anaerolineaceae* in promoting CFA-mediated DIET in the AD of BS under the conditions of the experiment.

The second potential mechanism proposed is that addition of CFA increased the efficiency of electron transfer mediated by H₂ diffusion (IIET). The 16S rRNA gene analysis indicated that CFA enhanced the abundance of *Smithellaceae*, a propionate-degrading bacteria (Zhao et al., 2016a; Xia et al., 2019), which is not a DIET-capable fermenting bacteria (Hatamoto et al., 2007; Sousa et al., 2007; Zhao et al., 2016b). The metabolism of *Smithellaceae* under normal AD conditions is challenged because the degradation of VFAs such as propionate, butyrate, and valerate is highly sensitive to H₂ concentration and is thermodynamically unfavourable (Kato et al., 2012; Nobu et al., 2020; Harirchi et al., 2022). Consequently, the process only functions when H₂ is maintained at low concentrations (1.2–2.8 Pa H₂) (Cruz Viggi et al., 2014; Nobu et al., 2020; Zhu et al., 2020). Indeed, propionate degraders are inhibited by H₂ producing bacteria in the methanogenic ecosystem when the H₂ concentration increases to 2.8 Pa (Nobu et al., 2020). The presence of CFA, however, stimulated the abundance of *Methanofastidiosum*, a factor that may play a crucial role in overcoming this thermodynamically challenging metabolic processes within the methanogenic ecosystem in AD. Propionate degradation may be maintained by *Methanofastidiosum* because this methanogen balances H₂ production by consuming H₂ from propionate oxidation resulting from the activity and abundance of H₂ producing bacteria, such as, *Bacterioidota*. While other hydrogenotrophic methanogens require a high H₂ concentration, equivalent to at least 1.7 Pa (Nobu et al., 2020), *Methanofastidiosum* can grow at very small concentrations of H₂, as low as 0.1 Pa, in the presence of methylated thiols. The exceptionally high abundance of this organism detected with CFA strongly suggests, therefore, that this metabolic pathway could also be operating in the CFA reactor.

In a complex microbial environment, such as an AD, different microorganisms may utilise different electron transfer mechanisms (DIET and IIET) simultaneously, such as the two possible scenarios proposed here. This may be possible, for instance, because CFA may stimulate the formation of extracellular polymeric substances (EPS) (Figure 8.5), to provide a stable and protective structure that may shield microbes from external disturbances, such as environmental changes or toxic substances, and create favourable niches for electron exchange either via DIET or IIET mechanism between different microbial species (Zhou et al., 2024).

In IIET, CFA may optimise electron transfer, especially through propionate degradation metabolism, while in DIET, CFA stimulated the growth of electrogenic bacteria and its archaea

methanogenic partner, facilitating a syntrophic partnership and a more efficient metabolic processes and electrons exchange. It is plausible that both mechanisms operated concomitantly, creating a dynamic and efficient system for the degradation of organic matter, increasing the hydrolysis rate and CH₄ yield in the AD of BS.

9. General Discussion

9.1 Overview of the research

This study examined, for the first time, a novel integrated approach utilising several waste materials to maximise the AD of LARs (using BS as a LARs representative), utilising CFA for alkali pretreatment, and FW for acid neutralisation. The experimental results demonstrated an increase CH_4 yield, relative to untreated BS, and pretreatment with chemical alkali reagents, and the effectiveness of FW neutralisation compared to mineral acid. The leachable components in CFA can provide beneficial alkalinity required for pretreatment of LAR, consistent with the results reported by Xiao et al. (2020) who used the extraction filtrate from a leached bottom ash generated from biomass power generation, for rice straw pretreatment (see Section 6.1). Furthermore, the bound, less-leachable components in CFA contributed to a more efficient AD process, potentially through the enhanced microbial interactions, i.e., mediation of DIET and the facilitation of more efficient ILET. This suggests that integrating total as-received CFA is more beneficial than relying solely on ash extraction filtrate, as demonstrated in the study reported by Xiao et al. (2020). This is also the first time that FW has been applied as the single acid source for pH neutralisation in AD, particularly for alkali-pretreated LAR. Promoting the use of CFA and FW in place of commercial inorganic chemicals can therefore potentially reduce the cost of large-scale pretreatment and AD of LARs operations and, at the same time, support the principles of circular economy. Indeed, these developments are particularly relevant in a developing country context, where open burning of LARs is prevalent, contributing to climate change and health issues such as respiratory disease, where disposal of CFA and FW also lead to significant impacts on human health and the environment, and where investment in energy resilience and renewable sources is required.

9.2 An integrated anaerobic digestion of barley straw with coal fly ash pretreatment and fermented food waste neutralisation

The as-received sample of CFA collected from a UK power station was exposed to weathering processes which decreased the alkali potency of the material. However, this provided several experimental advantages as the alkalinity level was adjusted with CaO, the main reactive alkali in fresh CFA, therefore it was possible to factorially separate the alkali response from other stimulatory chemical properties of CFA (presented in Chapter 5 and 6). Thus, CaO was used in a controlled experiment on alkali pretreatment to represent CFA and the alkalinity level was adjusted to achieve equivalent values, typical of CFA. The effectiveness of CaO pretreatment was assessed by quantifying changes in the fibre composition, functional groups, and surface

morphology of BS before and after pretreatment (presented in Chapter 4). The effects of CFA pretreatment on solid-state AD performance were determined by measuring the CH₄ yield, organic matter degradation rate, and hydrolysis rate (Chapter 5), and the results compared to the pretreatment conditions with both deionised water and CaO, supplied directly in chemical form, which is a well-established method with known effectiveness in altering LAR properties, to benchmark and compare CFA performance and effectiveness.

The extent of pure inorganic alkali pretreatment is typically expressed in terms of the concentration of the alkaline agent used, either in terms of the amount per unit volume of water (Shetty et al., 2017; Noonari et al., 2019; Sabeeh et al., 2020) or per unit weight of the substrate treated (Janke et al., 2017; Dai et al., 2018; Kumar et al., 2019; Mansour et al., 2024). When using waste as a substitute for alkaline agents in pretreatment, however, measuring the pH and alkalinity of the waste in water (at the proportions used for pretreatment) was considered the most practical and appropriate approach to determine the waste's reaction as an alkaline agent. This approach was used to define the alkalinity strength of as-received CFA suspension, as well as to benchmark against CaO and NaOH solutions, to identify the specific concentration of the alkalinity source in the as-received CFA sample.

Calcium oxide pretreatment at 2,500 mg CaCO₃ L⁻¹ modified the fibre composition by removing 10.4% of the lignin fraction (TS) and solubilising 11.4% of the hemicellulose component, compared to water-treated BS Control, albeit not statistically significant (discussed in Section 4.3.2). Statistically significant changes in both fibre composition, measured by a 27.6% reduction for lignin and 22.6% for hemicellulose (presented in Section 4.3.2), alongside a 29% increase in CSMY, compared to water-treated BS, to 140.7 mL CH₄ g⁻¹ VS (presented in Table 6.2), were observed at a higher alkalinity of 5,000 mg CaCO₃ L⁻¹, marking the minimum concentration for effective alkali pretreatment. The cellulose content, however, remained unaltered at 44% TS after this pretreatment. This alkalinity, equivalent to 0.4% ALR, was consistent with (Zheng et al., 2014; Gu et al., 2015; Noonari et al., 2019) who reported 0.4% as the minimum ALR required for effective pretreatment of rice straw and corn stover (see Section 2.4.3.1). The VS removal achieved at this alkalinity was 40.7% and the K_H was 0.11 d⁻¹, both values increased by 32% and 57%, respectively, compared to water-treated BS, albeit not statistically significant ($P > 0.05$). Consequently, when using CFA for alkali pretreatment of LAR, this concentration is the minimum alkalinity required for the CFA suspension.

The results showed that 5,000 mg CaCO₃ L⁻¹ alkalinity was optimal as a further increase in concentration to 11,000 mg CaCO₃ L⁻¹ had no significant effect on fibre composition

(presented in Section 4.3.2), CSMY, hydrolysis rate and VS removal (discussed in Section 6.3.1). The hemicellulose and lignin content in BS after 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ pretreatment was 19.6% TS and 5.6% TS, respectively, which was 23.7% and 8.2% lower, compared to hemicellulose and lignin content in BS after 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ pretreatment, which were 25.7% and 6.1%, respectively. The CSMY, VS removal and K_H from the AD of BS after 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ pretreatment were 160.2 mL $\text{CH}_4 \text{ g}^{-1}$ VS, 45.3% and 0.12 d^{-1} , respectively (presented in Chapter 6). The increase in CSMY compared to the AD of BS with 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ pretreatment was 14%, aligned with insignificant increases of 11% and 9% in VS removal and K_H , respectively.

While the chemical degradation of the three fibre components during the pretreatment step was expected to be similar under both CFA and CaO pretreatments, due to the equivalent alkalinity conditions, interestingly, the AD process following CFA pretreatment resulted in greater degradation of hemicellulose, lignin and cellulose, equating to 100%, 39% and 51%, respectively, compared to 85%, 13% and 21% under CaO pretreatment (Control) (see Section 5.3.2). These reductions are based on pretreated BS, before AD. Therefore, hemicellulose was completely degraded, while cellulose and lignin reduction in CFA pretreatment was twice and three times, respectively, that in CaO pretreatment. The reductions in all fibre components contributed to the enhanced AD performance, as indicated by 39% increase of CSMY from 124.4 mL $\text{CH}_4 \text{ g}^{-1}$ VS for CaO pretreatment Control to 174.1 mL $\text{CH}_4 \text{ g}^{-1}$ VS for CFA pretreatment (see Table 5.2). Additionally, VS removal increased by 29% from 44.3% to 57% (see Section 5.3.3), while the hydrolysis rate increased from 0.11 d^{-1} to 0.14 d^{-1} , equating to a 27% increase (see Table 5.3).

Irrespective of the alkalinity effect supplied by CFA pretreatment, CFA addition either to alkali-pretreated BS or to fresh BS without pretreatment showed consistent significant increases in CSMY compared to the Control condition. Specifically, the former condition gave a 32% increase in CSMY compared to the CaO pretreatment Control (presented in Table 6.2), while a 30% increase was detected for the latter condition, compared to water-treated BS without CFA addition (presented in Table 8.4). Indeed, CFA can be used directly in AD of LARs without pretreatment given its significant response in CSMY, however, the magnitude of response of CFA used in alkali pretreatment and AD was greater, measured as 93% compared to water-treated BS (presented in Table 5.2).

The significantly increased CH_4 yield observed with CFA integration was probably linked to the stimulation of microbial interactions, as the genomic sequencing analysis of the samples (Section 8.3.5) showed that CFA primarily promoted the growth of *Anaerolineaceae* and

Smithellaceae bacteria, as well as *Methanofastidiosum*, the latter accounting for 87% of the archaea population in CFA-stimulated reactor (Figure 8.5(b)), compared to the equivalent CaO treatment. *Anaerolineaceae* and *Methanofastidiosum* may participate in the DIET mechanism, while in contrast, interactions between *Smithellaceae* and *Methanofastidiosum* may promote a more efficient conversion of acidogenesis intermediate products, such as propionate, through traditional IIET mechanism. Both these possible mechanisms are discussed below in Section 9.5.4.

In an integrated waste AD system developed in the present study, FW is another waste feedstock involved with the task-specific properties to balance the high pH of CFA-pretreated BS and maintain it within the optimum range for CH₄ conversion. The optimum addition rate of 20% VS FW, determined in Section 7.3.3, effectively maintained intermediate pH levels between 6 and 7.5 (Figure 7.3). The slightly acidic environment (pH ~5.8) (Figure 7.3) created by FW supported the activity of hydrolytic and acetogenic bacteria without disrupting the system's buffering capacity. Consequently, the pH can recover to 7.7, and the degradation of proteins in FW may aid in enhancing the system's buffering capacity to maintain a neutral pH for methanogenesis. A previous study reported that buffering alkalinity of 13,900 mg L⁻¹ was supplied during the AD of FW, primarily from protein degradation, which was equivalent to ammonia concentration of 5,200 mg L⁻¹ (Banks et al., 2008). This alkalinity was sufficient to balance the high VFA concentrations of 27,400 mg L⁻¹, maintaining the pH between 7.3 and 7.7. Methane monitoring in the current study with 20% VS FW neutralisation showed performance comparable to that of inorganic mineral acid, with immediate CH₄ production starting on Day 1 and no observed lag phase and peak CH₄ production was 1.5-fold higher compared to the Control condition with mineral acid neutralisation (Figure 7.5). The alkali-pretreated BS with FW neutralisation resulted in a significantly higher CSMY, which was 40% greater, where FW addition contributed 64.7 mL CH₄ g⁻¹ VS to the total CH₄ yield of 224.7 mL CH₄ g⁻¹ VS (Table 7.3).

The experimental results consequently demonstrated that CFA pretreatment significantly improved fibre digestibility, reduced the fibre concentration in the residual digestate after AD and this was concomitantly linked to increased VS removal, hydrolysis rate and CSMY compared to both water-treated and CaO-pretreated BS, proving its effectiveness as a substitute for alkaline inorganic chemicals. Despite the risks of acidification due to the rapid degradation of FW, FW neutralisation has effectively maintained intermediate pH levels, demonstrating its efficacy as an acid source in an integrated waste system in AD for biogas production.

9.3 Relationship between fibre composition and structural changes in barley straw and CH₄ yield

Lignocellulosic agricultural residues comprise three major fibre components: cellulose, hemicellulose, and lignin. Lignin is the most resistant to biological degradation and also limits access of microorganisms to otherwise relatively degradable cellulose and hemicellulose fibres (Monlau et al., 2012). The resistance of lignin to microbial attack is mainly due to its insolubility in water and its complex, irregular chemical structure (Grgas et al., 2023). The lignin monomers, which are phenylpropane units, are formed and connected by ether and C-C bonds, which are among the most resistant bonds to degradation (Grgas et al. 2023).

The lignin, hemicellulose and cellulose compositions in pre-digested BS under experimental conditions: untreated, water-treated, and BS treated with CaO at both 5,000 mg CaCO₃ L⁻¹ and 11,000 mg CaCO₃ L⁻¹, as well as 11,000 mg CaCO₃ L⁻¹ with NaOH (presented in Section 4.3.2) were plotted against their corresponding CSMY values presented in Section 6.3.1 to create linear regression (Figure 9.1). The correlation between lignin contents and CSMY values (Figure 9.1 (a)) reported here showed a strong and significant ($R^2 = 0.97$, $P < 0.05$) negative correlation, reflecting the impact of alkali pretreatment on lignin content and subsequent CSMY, which was consistent with an earlier study which reported a similar trend between lignin and CH₄ yield, with $R^2 = 0.92$ (Monlau et al., 2012). Based on the linear regression model, the CSMY in this study decreased by approximately 18.7 mL CH₄ g⁻¹ VS per 1% increase in lignin content. Significant reduction was also observed on hemicellulose after alkali pretreatment, with its correlation with CSMY judged as moderately strong, as indicated by an R^2 value of 0.89 and $P < 0.05$ (Figure 9.1 (b)). The linear regression model showed that for every 1% decrease in hemicellulose content, CSMY increases by approximately 5.2 mL CH₄ g⁻¹ VS. The greater response of CSMY to lignin content indicates lignin poses a more significant barrier to AD than hemicellulose, as a result, the negative impact of recalcitrant lignin on CSMY is significantly larger than the positive contribution of hemicellulose solubilisation.

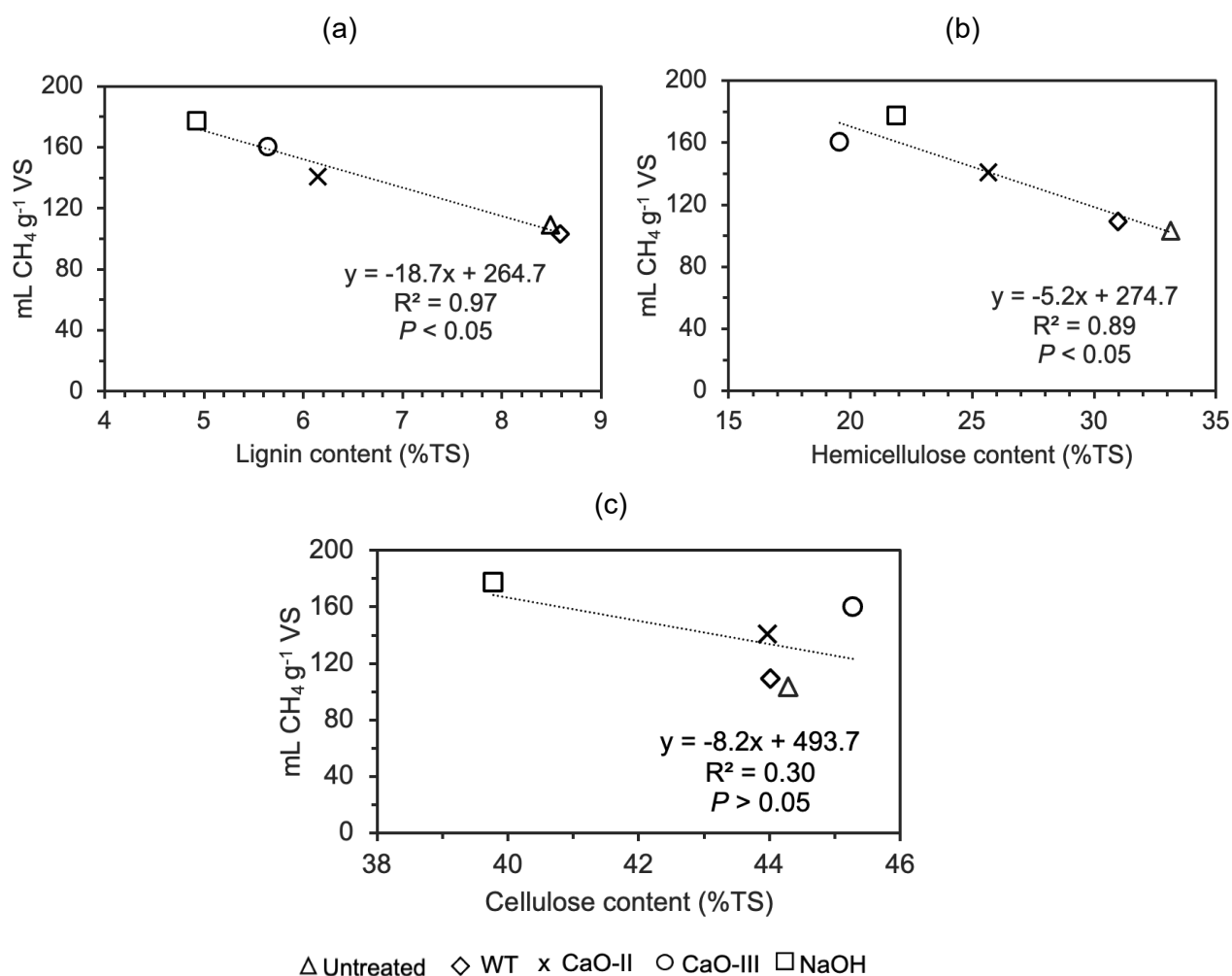


Figure 9.1 Cumulative specific CH₄ yield for anaerobic digestion of barley straw (BS) in relation to (a) lignin, (b) hemicellulose, (c) cellulose content of BS (% TS) before pretreatment (Untreated) and after pretreatment with deionised water (WT), two pretreatment alkalinity levels of CaO ~5,000 (CaO-II) and 11,000 mg CaCO₃ L⁻¹ (CaO-III) and one alkalinity level of NaOH ~11,000 mg CaCO₃ L⁻¹ (NaOH). Data presented are mean values (n=3).

Unlike lignin and hemicellulose, the cellulose content remained unchanged before and after pretreatment (Figure 4.3 (a)), as cellulose was preserved during alkali pretreatment. This was consistent with the primary target of alkali pretreatment, which is to break down the lignin structure, disrupt the LCCs, and partially solubilise hemicellulose, rather than to attack the glycosidic bonds that make up the cellulose backbone (Kim, 2018; Zdeb, 2021). Consequently, the correlation between cellulose content in BS after pretreatment, before digestion, and CSMY was not strong ($R^2 = 0.30$) and not statistically significant ($P > 0.05$) (Figure 9.1(c)).

The negative relationship between either lignin or hemicellulose and CSMY, therefore suggests that higher lignin content is unfavourable for CH₄ production, consistent with the observation that CH₄ production is strongly hindered by increasing lignin content of a

feedstock due to its recalcitrant nature and resistance to microbial degradation (Sawatdeenarunat et al., 2015). The decrease in hemicellulose content indicates an improvement in digestibility of LARs, as more fermentable sugars, such as oligosaccharides and monosaccharides, are formed (Van Dyk and Pletschke, 2012; Azman, 2016; Lu et al., 2022), which serve as readily substrate for methanogens.

The collective removal of lignin and hemicellulose after pretreatment, rather than their individual contributions, as might be implied from each profile, is therefore responsible for the overall CSMY observed in Figure 9.1. The removal of lignin and hemicellulose increases CSMY due to cellulose being more exposed, thereby facilitating greater access for hydrolytic enzymes and microbial action to degrade cellulose into fermentable sugars. In the absence of lignin removal, as observed in water-treated BS, only 10% of cellulose was degraded during AD, in contrast, CaO pretreatment increased cellulose degradation by twofold during AD (presented in Figure 5.1), which was attributed to the transformation of cellulose from a crystalline to an amorphous form. The fundamental mechanisms of lignin and hemicellulose removal are summarised below.

9.3.1 Lignin removal/delignification

Lignin removal was indicated in the reduced absorbance of FTIR spectra in the ranges of 1215–1260 cm^{-1} and 1500–1600 cm^{-1} (see Section 4.3.3) for BS after alkali pretreatments using $\sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ and $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$, compared to water-treated BS as the Control treatment. These wavenumber ranges represent the cleavage of ether bonds; a predominant bond in lignin polymer (Modenbach and Nokes, 2014) and disruptions in lignin aromatic rings, respectively (Boeriu et al., 2004; Md Salim et al., 2021; Pancholi et al., 2023). The structural changes and ether bond cleavages led to the breakdown of lignin polymer into smaller, less complex structures of lower molecular weight, that may include more water-soluble fragments, such as phenolic acids, aldehydes and simple aromatic compounds (Zhao et al., 2022; Grgas et al., 2023). Such depolymerised products are more amenable to enzymatic degradation by laccases and peroxidases, and the resulting products can ultimately serve as carbon sources for microbes (Lu et al., 2022; Grgas et al., 2023). While lignin in water-treated BS was not affected during AD, minor lignin degradation occurred, equating to 13% degradation under CaO pretreatment with $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ (presented in Figure 5.1), probably due to structural modifications of lignin that occurred during the pretreatment.

9.3.2 Hemicellulose solubilisation

A significant fraction of hemicellulose is linked to lignin through ester and ether bonds, forming lignin carbohydrate complexes (LCCs) network, which is the primary target of alkali pretreatment reaction (see Section 2.4.2) (Loow et al., 2016; Dai et al., 2018; Mafa et al., 2020). Calcium oxide pretreatment under the experimental conditions cleaves these bonds, as evidenced by the notable decrease in absorbance intensity within the 1730–1740 cm^{-1} wavenumber (Haque et al., 2012; Bachmann et al., 2022; Pancholi et al., 2023) range in the spectra of CaO-pretreated BS. The process thus separates hemicellulose from lignin, increasing the susceptibility of both components to further alkali reactions, followed by hemicellulose solubilisation and depolymerisation of lignin (discussed in Section 9.3.1). Hemicellulose solubilisation was evidenced by a decrease in absorbance intensity in the 890 cm^{-1} and 1000–1100 cm^{-1} ranges for pretreated BS compared to water-pretreated Control, with both wavenumber ranges indicating the breakdown of glycosidic bonds in hemicellulose polysaccharides (Haque et al., 2012; Braga and Poletto, 2020; Md Salim et al., 2021; Pancholi et al., 2023).

These mechanisms therefore explain the increase in hemicellulose solubility by CaO pretreatment using both $\sim 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ and $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ alkalinity, which was reflected in the reduction in hemicellulose content (see Section 9.2). The disruption of lignin-carbohydrate complexes exposed more hemicellulose fractions to microbial degradation, resulting in 85% hemicellulose degradation after $\sim 11,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ CaO pretreatment, compared to 50% degradation in water-pretreated BS during AD (see Figure 5.1).

9.4 Phenolics derived from lignin degradation and inhibition risks

The CH_4 production profiles from AD of NaOH and CaO-pretreated BS show immediate CH_4 production from the first day without any extended lag periods (see Figure 6.3 (c), (d) and (e)), which indicates limited or the absence of inhibitory substances, such as phenol release. Phenol release is a potential risk associated with alkali pretreatment (Bachmann et al., 2022), as it can occur under specific conditions of lignin degradation, such as excessive alkaline concentration or alkali loading rate (ALR) (X. Liu et al., 2015; Bolado-Rodríguez et al., 2016), high temperatures above 150° C (Čater et al., 2014; Cai et al., 2021), extended pretreatment durations (Li et al., 2023), or a combination of these factors, which limits methanogenic activity due to phenol's strong toxicity (Bolado-Rodríguez et al., 2016; Kim, 2018). The toxicity of phenolics is attributed to their aromatic properties, enabling them to disrupt cell membranes,

ultimately impairing cell growth and activity of the enzymes (Kim et al., 2018; X. Chen et al., 2020b).

When selecting pretreatment conditions, it is crucial to avoid the formation of phenolic compounds from lignin degradation, as these can hinder the effectiveness of alkali pretreatment during subsequent AD (Bensah and Mensah, 2013; Lee et al., 2014; Sasmal and Mohanty, 2019). Indeed, combined alkali pretreatment and elevated temperatures can improve the digestibility of LAR and allow for shorter pretreatment duration (Talha et al., 2016; Mustafa et al., 2018) compared to alkali pretreatment alone, however, the combined treatment may not be economically viable, due to increased heating costs and inhibitory effects of phenolics on AD (Bolado-Rodríguez et al., 2016). There are trade-offs between using single alkali pretreatment and combining it with high temperatures, that should be carefully evaluated before selecting the most efficient pretreatment method. While the combined treatment offers benefits, as mentioned above, it could lead to longer AD retention times due to the presence of phenolics as an inhibitor and higher heating costs. Conversely, single alkali pretreatment might require a longer pretreatment duration and produce fewer soluble compounds, but it could lead to shorter AD retention times and lower overall operational costs.

9.5 Implementation of integrated dual waste system in anaerobic digestion of lignocellulosic agricultural residues

Indonesia produced a total of 145.5 Mt of LARs in 2023, including rice straw, rice husk, corn stover, corn cob, sugarcane bagasse, and fruit palm bunches. Among these, rice straw, which shares a similar lignocellulosic composition with BS (used as the LARs representative in this study (see Table 2.1) accounts for 45 Mt annually. If it is assumed that CFA pretreatment of rice straw results in the same CH_4 yield found for BS in this study (174 mL g VS^{-1} , see Table 5.2), then the AD of rice straw could generate approximately 6,800 million m^3 of CH_4 , equivalent to 4,600 MWe. This amount of CH_4 production exceeds Indonesia's National Energy General Plan 2025 target of producing 489.8 million m^3 of biogas by 2025.

In 2019, Indonesia produced approximately 10 Mt of CFA. For the implementation of CFA as pretreatment agent, a minimum alkalinity of $5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ is required, corresponding to CFA with 7% free CaO added to LAR at a 1:2 ratio (on a dry matter (DM) basis)) and an 18% solid loading rate (SLR), as used in this study. If all CFA produced contains 7% free CaO, the available supply would only be sufficient to treat 50% of the rice straw. The actual usable CFA fraction for pretreatment, however, would be even lower, as pretreatment effectiveness depends on the CaO content, which varies significantly in CFA produced in Indonesia, ranging

from 2.4–19.7% (free CaO content unknown) (Damayanti 2018; Prayoga and Afla 2023). This imbalance underscores the need to explore other alkaline waste streams with sufficient alkalinity ($\geq 5,000 \text{ mg CaCO}_3 \text{ L}^{-1}$ to enable the full utilisation of LARs in an integrated AD.

The integrated dual waste system in AD of LARs in the case study area (Indonesia) can be implemented following the framework outlined in Figure 9.2. The AD plant is ideally located close to the agricultural fields to optimise the transport efficiency of LARs, considering that LARs are the primary substrate and are transported in large quantities. Consequently, locating the AD plant near the power plant, where the CFA is sourced, is not a priority. Although the efficacy of ash CaO chemistry in pretreatment of LARs for AD processes is susceptible to storage, aging, and weathering, especially due to exposure to moisture and atmospheric conditions, which reduce the alkalinity of the material (Łączny et al. 2021) – only 0.5% of CaO is lost after 36 days of storage (Łączny et al. 2021). This minimal lost explains why locating the AD plant near the CFA source is less critical than locating it closer to the agricultural field.

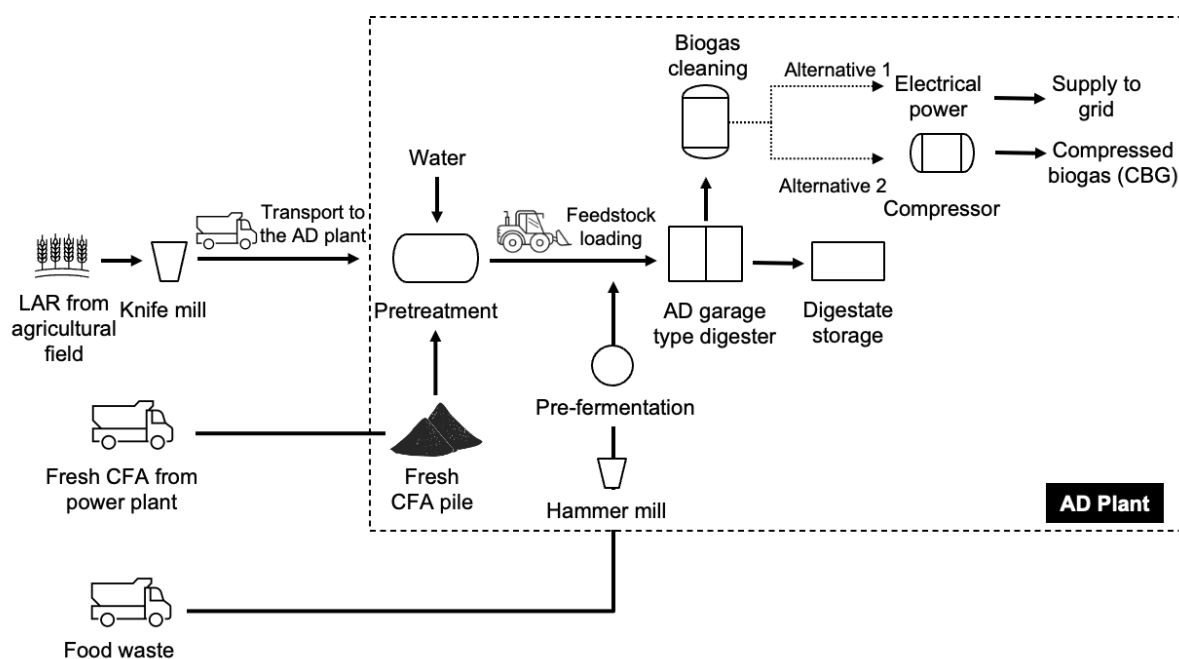


Figure 9.2 Conceptual framework diagram of the application of dual-waste system (coal fly ash (CFA) and food waste) in an integrated anaerobic digestion (AD) of lignocellulosic agricultural residues (LARs).

Whilst the reduction of particle size is important for optimal hydrolysis of carbohydrates such as cellulose and hemicellulose to glucose, it represents a critical aspect affecting the transport efficiency, as the smaller size can increase the transported volume due to reduced bulk density compared to larger sizes (see Section 2.4.3.4). This suggests that a mobile grinding machine sited on agricultural land rather than at the AD plant is preferred as it allows for both efficient

hydrolysis and improved transport logistics. The recommended particle size of LARs for laboratory experiments is 1–2 mm, whereas large AD plants typically use 30 mm (Dell’Omo and La Froschia 2018).

A feasible design for the AD of LAR is dry batch AD, such as garage-type reactors, due to the high solids of LAR (86–96%), the addition of CFA, and the minimal water used during pretreatment. While the effectiveness of CFA as an alkaline agent depends on its CaO content, the sourcing of FW as a neutralising agent for CFA-treated LARs is more flexible. This allows for a wide range of food sources, including traditional markets (fruit and vegetable waste) and source-segregated FW from canteens, restaurants, or households for access to FW and ensure a steady supply of FW for AD neutralisation. Before being fed into the AD reactor, FW is transported to the AD plant, milled, and pre-fermented for 7 d, after which it is mixed with pretreated LAR.

The potential end use identified for biogas generated from the integrated AD as shown in Figure 9.2 are biogas conversion to electrical power through generator (Alternative 1) and compression into compressed biogas (CBG) (Alternative 2). The CBG can then either be bottled into high pressure cylindrical steel tanks or direct injection into the customer’s industrial gas cylinders that are not served by national gas pipelines (Daeng 2017; Banjarnahor 2019) with target markets that can include small to medium sized industries and commercial enterprises (e.g., hotels and restaurants).

9.6 Additional stimulatory effects of coal fly ash in anaerobic digestion of barley straw

9.6.1 Coal fly ash as a trace elements supplement

The as-received CFA has an ample supply of TEs (Table 3.3), in contrast to BS, which is deficient in TEs relative to the requirements for anaerobic microorganisms (Table 5.1). This was consistent with the literature, where CFA has been well documented as a TEs supplement in the AD of various substrates (Huiliñir et al., 2015; Montalvo et al., 2018; Lauzurique et al., 2021a) (see Section 2.5.2.1). The inoculum sludge used in the experiments, however, was a rich source of TEs, which were therefore in adequate supply, making it challenging to determine the effects of TEs associated with CFA. Moreover, the TEs level in BS-inoculum mixtures, without CFA, was also above the AD requirements, suggesting that TE requirements for the AD process are met even in the absence of CFA, hence TE supplementation is not the primary factor driving enhanced CH₄ production in this context.

9.6.2 Coal fly ash as a potential adsorbent for volatile fatty acids and ammonia nitrogen

Excessive VFA and NH_3 and their imbalance are inhibitory and can impair digester stability and CH_4 yield. The microbial analysis results showed that the CFA-stimulated reactor was enriched with *Bacterioidota*, which increased from 16% in the digested inoculum to 20.4% (presented in Section 8.3.5). Several studies (De Vrieze et al., 2015; Amin et al., 2017; Harirchi et al., 2022) reported that *Bacterioidota* grows optimally in low VFA and NH_3 levels, suggesting that the levels of VFAs and NH_3 present in the reactor might be maintained at a low level by CFA. Previous studies have shown that chemically synthesised CFA can be an effective adsorbent for the removal of NH_3 , phenol and organic dyes from wastewater (Chen et al., 2018; Hosseini Asl et al., 2018), attributed to its specific surface area ($2,500\text{--}94,400\text{ cm}^2\text{ g}^{-1}$) (Schure et al., 1985; Shaheen et al., 2014), porosity and UC content (Desai and Madamwar 1994; Ahmaruzzaman 2010; Shaheen et al., 2014; Chen et al., 2018; Martins et al., 2018).

The role of CFA in this mechanism, associated with the low VFA and NH_3 levels needs validation in further investigation. Coal fly ash potential to adsorb VFAs and NH_3 , however, could be advantageous in an integrated CFA-FW-BS AD system, where VFA and NH_3 levels might be relatively high. This increase would presumably result from higher BS biodegradability; following CFA pretreatment, and FW degradation leading to more VFAs (Park et al., 2015; Ren et al., 2018), and protein degradation from FW contributing to NH_3 formation (Karthikeyan and Visvanathan, 2013; F. Xu et al., 2018). Food waste is highly biodegradable and has relatively low C/N ratio of 10.2 (Table 7.3), which may encounter various potential inhibitors including VFAs production and NH_3 from protein degradation (X. Chen et al., 2014), causing rapid conversion of carbohydrates to VFAs at an early stage of the digestion process (F. Xu et al., 2018). The continuous accumulation of VFAs decreases the pH and results in souring and eventually failure of the system in the absence of sufficient buffering capacity (Gandhi et al., 2022; Harirchi et al., 2022), similar to what was observed in mono-digestion of FW and AD of pretreated BS with overloading of FW, equal to 50% VS addition rate, which resulted in pH drop to 5.5, and the process was terminated on Day 14 (see Section 7.3.2).

The negative effect of VFAs accumulation in the AD of FW which resulted in low VS removal rate or CH_4 yield have been reported. For example, Lee et al. (2015) reported that VFAs concentration above $4,000\text{ mg L}^{-1}$ inhibited microbial activity, leading to VS removal of 56.4%, which is below their expected standard (65%). In the AD of kitchen waste, the increase in VFAs from 261 to $2,969\text{ mg L}^{-1}$ has led to the decline of CH_4 production by 75.4% (Li et al.,

2013). While NH_3 improves the buffering capacity of a reactor by neutralising organic acids and preventing inhibition by VFAs, an excessive increase in NH_3 can otherwise lead to toxicity, with methanogens being the most affected group in AD. This can lead to increase in VFAs as their conversion to CH_4 by methanogens is disrupted (Odejebi et al., 2023). The inhibition in AD of FW due to NH_3 has been reported in several previous studies. While a total ammonia concentration of up to 1.10 g L^{-1} was beneficial for increasing biogas production, a concentration of 1.9 g L^{-1} resulted in a 7.8% decrease in biogas output, and further increase of total ammonia to above 3.78 g L^{-1} severely inhibited CH_4 conversion activity (Sheng et al., 2013). A 50% decrease in methanogenic activity was reported when $\text{NH}_4\text{-N}$ concentration of $4.09\text{--}5.55 \text{ g L}^{-1}$ was present in AD of organic fractions of municipal solid waste (Mata-Alvarez, 2003).

Coal fly ash addition into an integrated AD system may help ameliorate the negative effects of VFAs. The porous structure and surface area of CFA can provide numerous adsorption sites for VFAs, which can trap and hold the VFAs (Siyal et al., 2020), which may reduce the concentrations and mitigate potential process inhibition.

9.6.3 Coal fly ash as a support for enzyme immobilisation

Previous studies indicated that immobilising enzymes, including laccase and lignin peroxidase, onto magnetic particles and silica-based support increases enzyme activity compared to the activity of free, mobile enzymes (Datta et al., 2021), for example, in degrading polyaromatic compounds, including lignin (Bautista et al., 2015; Amin et al., 2018). It was also reported that the stability of enzymes to pH and temperature was improved (Datta et al., 2021). Magnetic particles such as Fe_2O_3 and silica material are suitable support for enzyme immobilisation due to their nontoxic, biocompatible nature, high surface area and adsorption capacity (Datta et al., 2021) (X Chen et al., 2020a; Zhao et al., 2022). Given that silica oxide (SiO_2) and Fe_2O_3 are primary components of CFA, it is plausible that CFA could exhibit similar capacity in supporting enzyme immobilisation as SiO_2 and Fe_2O_3 nanoparticles. The mesoporous CFA surface may act as a suitable support for enzyme adsorption due to its pore size and high surface area (see Section 9.5.2). With pore sizes ranging from 13.66 to 32.3 nm (Zhong et al., 2014; Taufiq et al., 2018), CFA is likely suitable for the adsorption of enzymes which typically have diameters between 2 and 10 nm (Bayne et al., 2013). Adsorption-based immobilisation involves the attachment of enzymes on the exterior surface of the support based on ionic interactions, van de Waals or dipole interactions. The second possible mechanism that may be involved is covalent bonding, where a covalent bond is formed

between the chemical functional group on the support and the nucleophilic group on the enzyme (Datta et al., 2021).

Free enzymes often experience a loss of activity upon repeated use. In contrast, immobilised enzymes have been shown to retain significant activity even after nine consecutive cycles (Datta et al., 2021). In practical operational situations, biogas plants operated in a solid-state system, such as garage type digester, typically recycle around 50% of the digestate to inoculate fresh feedstock in the digester, which helps to maintain the microbial population for subsequent cycles (Yang et al., 2015). A possible advantage of immobilised enzymes on CFA in digestate recycled as inoculum is that it could further enhance the benefits of reusing the digestate across batches in AD by maintaining higher enzyme activity.

9.6.4 Potential role of coal fly ash in direct and indirect interspecies electron transfer in the AD of lignocellulosic agricultural residues

This study proposed that the DIET and ILET metabolic mechanisms occurred concomitantly in the CFA-stimulated reactor (see Section 8.4.4), responsible for increasing efficiency of AD of BS by CFA, shown schematically as Mechanism 1 and 2, respectively, in Figure 9.3. The 16S genomic sequencing results showed a dominance of *Candidatus methanofastidiosum*, accounting for 87% of the archaeal community in CFA-stimulated reactor (see Section 8.3.5). The reasons for the abundance of this group remain uncertain, however, two key factors may have strongly favoured its growth: the conductive environment created by CFA and the availability of alternative substrates, which reduce competition with other microbial groups.

Methanofastidiosum encodes both hydrogenotrophic metabolic pathway (shown by blue arrow in Figure 9.3) and the more recently identified metabolic pathway for this archaea group, namely the demethylated thiol pathway (shown by red arrow in Figure 9.3) (Nobu et al., 2020; Walter et al., 2022). Methylated thiol as one of the substrates for *Methanofastidiosum*, may be formed from the reaction between the methyl group in lignin with SO_4^{2-} . Lignin is constituted of three primary monolignols: sinapyl monolignol or syringyl monolignol, p-coumaryl alcohol and coniferyl alcohol (Buranov and Mazza 2008; Li et al. 2020). The structure of syringyl monolignol includes two methoxy groups, which each contains a methyl group (Buranov and Mazza 2008; Li et al. 2020). Whereas SO_4^{2-} may be released from sludge inoculum and CFA. The S content in the typical anaerobic sludge is 1.1% TS (Arelli et al., 2021), however, extra S can be supplied by CFA. The S content in the sample of CFA used in the experiment was 0.033 % TS (see Table 3.2), which was 50% higher than the upper range of the typical S range contents reported in UK CFA (0.003–0.02% TS) (Moreno et al., 2005). Sulphur is the one of

the most leachable elements from CFA (see Section 2.5.2.1), with the leachability in water as SO_4^{2-} ranging between 40 and 80% of the total S in CFA (Izquierdo and Querol, 2012).

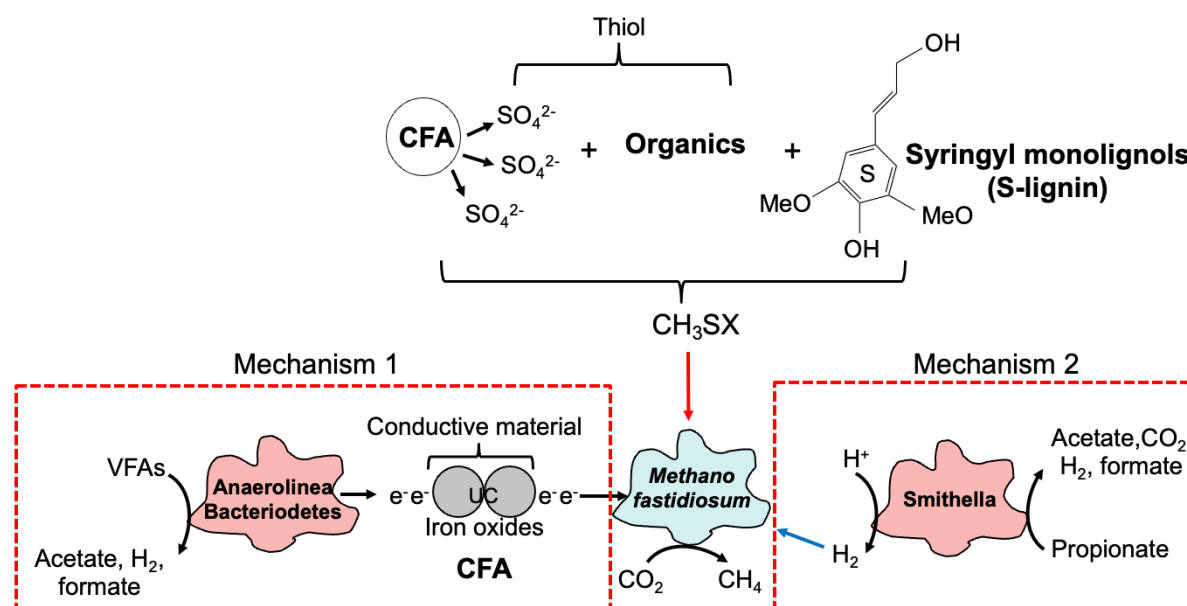


Figure 9.3 Proposed mechanisms explaining the potential effects of coal fly ash (CFA) on the anaerobic digestion of barley straw (BS). Mechanism 1 involves CFA, containing iron oxides and unburned carbon (UC), mediates direct interspecies electron transfer (DIET) between syntrophic fermenters (pink) and a methanogen partner (blue). Mechanism 2 involves syntrophic interactions utilising hydrogen (H_2) as the electron shuttle by indirect interspecies electron transfer. The red arrow represents the methylated thiol metabolic pathway, and the blue arrow represents the hydrogenotrophic metabolic pathway. “Organics” represent carbohydrates, proteins, and other organic compounds in BS. “Syringyl monolignols” are an example of lignin-based aromatic compounds containing two methoxy groups. MeO, Methoxy; *Anaerolinea*, *Anaerolineaceae*; *Smithella*, *Smithellaceae*.

This archaea group may efficiently scavenge H_2 to reduce CO_2 through IIET metabolic mechanism, which eventually maintain a low H_2 partial pressure, thermodynamically favourable for the degradation of intermediate products such as propionate and butyrate by acidogens, like *Smithellaceae*. This could be the plausible explanation for the enrichment of *Smithellaceae* from 0.7% in the digested inoculum to 1.4% in CFA-stimulated reactor (see Section 8.4.3).

The more important mechanism that may be operating, which was supported by experimental evidence is DIET. Direct interspecies electron transfer is a critical mechanism that can significantly enhance CH_4 production in AD processes (Kato et al., 2012; Li et al., 2015; Martins et al., 2018; C. Wang et al., 2018; T. Wang et al., 2018). Unlike IIET, where electrons are transferred via intermediates like H_2 or formate, DIET allows for direct electron exchange

between syntrophic microorganisms through conductive materials or microbial structures such as pili and c-Cyts (Malvankar et al., 2011; Martins et al., 2018; Tang et al., 2021). The importance of DIET in AD lies in its ability to streamline electron transfer, making the process more efficient and reducing energy losses (Lovley, 2011). This efficiency translates to faster substrate degradation and increased biogas production. Moreover, DIET fosters synergistic interactions between fermentative bacteria and methanogenic archaea, enhancing the overall stability and resilience of the microbial ecosystem.

Earlier studies have shown that the introduction of conductive materials such as biochar, magnetite and activated carbon can facilitate DIET, resulting in higher CH₄ yields. Cruz Viggi et al. (2014) observed that adding magnetite to the AD of propionate increased CH₄ production by up to 33%. Similarly, a 27% increase in CH₄ production was found when magnetite was added to high-solids sewage sludge AD (T. Wang et al., 2018), and a 35% increase was reported with hematite addition to beet sugar wastewater AD (Ambuchi et al., 2018). The addition of carbon-based conductive materials, such as activated carbon and biochar to AD fed with ethanol increased biogas production by 20% and 8%, respectively (König et al., 2022). In the present study, the direct AD of BS (without pretreatment) with CFA addition resulted in a 30.3% CH₄ yield, which aligns with the values reported in the literature. These materials provide the necessary conductive surfaces that enable direct electron flow between microbial partners, bypassing the need for intermediate compounds (Martins et al., 2018). Additionally, studies have observed shifts in microbial community compositions towards DIET capable species when such materials were added, further supporting the role of DIET in enhancing AD processes (Martins et al. 2018).

Multiple lines of evidence from the experimental results suggest that CFA may facilitate DIET in the AD of BS. Firstly, the EC measurements indicated that the addition of CFA increased the digestate conductivity of the AD system by 25 times (presented in Section 8.3.1), consistent with the reported range of a 1.6 to 82 times increase in EC observed in inoculum following the addition of conductive materials (C. Wang et al., 2018; Wang et al., 2022). This suggests that CFA addition enhanced electron transfer processes in the reactor and may facilitate more effective metabolic interactions between syntrophic partners resulting in higher conversion of organic substrates (indicated by VS) to CH₄, compared to the Control, without electron transfer mechanism. The EC of CFA of 0.6 mS m⁻¹ fell within the biochar's conductivity ranges from (0.2–5 mS m⁻¹), a conductive material that has been extensively studied for its role in promoting DIET (Chen et al., 2014; Baek et al., 2018; König et al., 2022; Zhou et al., 2024).

The results of this study showed that EC of CFA increases with UC content (see Section 8.3.2), suggesting that the conductivity of CFA provided primarily by UC, aligned with the studies by Choo et al. (2014) and Tao et al. (2015) who reported that UC plays an important role in CFA conductivity. Iron oxides possibly contribute to the CFA conductivity too, although this is apparently less important.

Microbial community analyses revealed that CFA conductivity presumably stimulated shifts in the populations of bacteria and archaea (presented in Section 8.3.5). Even though *Geobacter*, a genus of bacteria known for its ability to perform DIET (Lovley, 2017; Bose et al., 2021; Storck et al., 2016), was not detected in this study, there was a shift in bacterial community compositions towards other DIET capable bacteria (*Anaerolineaceae*) (Ambuchi et al., 2018; C. Wang et al., 2018; Gustave et al., 2019; Zhu et al., 2023) in the reactor with CFA, further supporting that DIET may be operating. The syntrophic cooperation between *Anaerolineaceae* and *Methanosaeta* in the presence of biochar have been reported in AD fed with alkanes (Liang et al., 2015) and AD of sewage sludge and kitchen waste (J. Wang et al., 2021). The archaea community in the reactor with CFA was almost 90% dominated by *Candidatus methanofastidiosum*, which is an archaea reported to be involved in syntrophic metabolism with *Geobacter*, a syntrophic bacteria known for its role in DIET, in the presence of conductive material (König et al., 2022).

Coal fly ash physical properties, including its porous surface area and adsorption capabilities offer sites for substrates localisation and accumulation, creating a favourable environment for microbial adhesion (Desai and Madamwar, 1994; Martins et al., 2018; Lauzurique et al., 2021b; Song et al., 2023). This environment may further support the attachment and growth of *Anaerolineaceae* and *Methanofastidiosum* in the medium, enhancing their interactions to support DIET. *Anaerolineaceae* is also known for its capability in producing cellulosomes, a multiprotein cellulase complex that plays critical role in cellulose degradation (see Section 2.3.3). Cellulosomes act synergistically with other enzymes to break down cellulose into simpler sugars that can be further utilised by other microorganisms in the AD process (Leschine, 1995; Weimer, 2022). The enrichment of *Anaerolineaceae* enhanced the efficiency of cellulose hydrolysis, leading to higher CH₄ yields. This was evident from the significant cellulose degradation in the AD, with the percent loss in the reactor with CFA being two-fold greater than in those treated with an equivalent alkalinity provided by CaO, without CFA addition (presented in Figure 5.1). This suggests that CFA may provide additional benefit by facilitating the establishment of a microbial community that is more effective at breaking down complex carbohydrates, leading to improved AD performance.

This investigation demonstrated an integrated waste AD system, incorporating BS, FW neutralisation and CFA pretreatment and stimulation for bioenergy and agronomic soil improver/fertiliser production, which could be suitable for implementation in developing countries, such as Indonesia. As the largest producer of rice straw and corn stover in Southeast Asia (see Figure 2.2), Indonesia generates a substantial quantity of LARs. The challenges associated with the recalcitrance of LARs for energy production and the extensive use of chemicals for pretreatment to increase LARs digestibility has been effectively addressed by integrating CFA and FW in AD of LARs, to enhance the efficiency of AD, maximise biogas output, and align with sustainability and circular economy principles, resource efficiency and environmental stewardship in waste management.

10. Conclusions and Recommendations

10.1 Conclusions

To increase the digestibility and biogas yield of LARs treated by AD, pretreatment of the feedstock is required but it represents one of the most costly steps in the AD of LARs, limiting its scalability due to high energy demand, chemical use, operational expenses, and process complexity. This study offers a novel and sustainable approach that aligns with circular economy principles addressing these challenges by integrating two major waste streams to mitigate these challenges by integrating CFA as alternative alkali source and fermented FW as acid source, for the effective pretreatment and AD process of LARs. By leveraging the inherent alkalinity of CFA and the rapid generation of VFAs contributing to the acidity in FW, this approach not only enhances overall process efficiency, but also reduces reliance on synthetic chemicals, reducing both costs and environmental impacts. The following conclusions address the research objectives set out in Chapter 1.

The first objective was to establish the baseline performance of alkali pretreatment and acid neutralisation steps for AD treatment of LARs (using BS as a case study LAR) with commercial industrial chemicals. The objective was met with the following outcomes achieved:

- The changes in fibre composition with CaO pretreatment supplying 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ of alkalinity resulted in a VS removal of 40.7%, which was 32% larger than measured for the water-treated Control, consistent with the 29% increase observed in CSMY to 140.7 mL $\text{CH}_4 \text{ g}^{-1}$ VS relative to the Control. Consistent with the effects on fibre composition, increasing alkalinity to 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ increased VS removal by 11%, to 45.3%, and CSMY by 14%, to 160.2 mL $\text{CH}_4 \text{ g}^{-1}$ VS, although the differences were not statistically significant compared to 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$. Compared to water-treated Control, CaO pretreatment at 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ increased CSMY by 47%, consistent with a 46% increase in VS removal. The results showed that BS biodegradability improved with increasing alkalinity of pretreatment, but not significantly, indicating 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ was the optimal alkalinity concentration. NaOH pretreatment had the greatest effect on CSMY at an equivalent concentration of 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ on CSMY, resulting in 177.3 mL $\text{CH}_4 \text{ g}^{-1}$ VS. The difference in CSMY compared to CaO pretreatment was 11%, but this was not statistically significant, even though the difference in VS removal was higher at 33%.
- Alkali pretreatment with a modest alkalinity of CaO, such as 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ resulted in a pH of 7.8, which is within the optimal range for the AD process, making

neutralisation unnecessary. In contrast, the pH of pretreated BS with higher alkalinity (e.g., 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$), was 12.3, therefore pretreatment with this level of alkalinity requires neutralisation to adjust the pH to its optimal range.

- The integration of fermented FW at the optimum addition rate of 20% VS to the AD of pretreated BS with CFA pretreatment at 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ increased the VS removal from 57% to 60.7% and the hydrolysis rate from 0.13 d^{-1} to 0.15 d^{-1} , although neither increase was statistically significant. This integrated AD waste system resulted in 224.7 mL $\text{CH}_4 \text{ g}^{-1}$ VS, which was 40% higher compared to the CFA-pretreated BS neutralised with mineral acid, due to an additional CH_4 yield of 64.7 mL $\text{CH}_4 \text{ g}^{-1}$ VS from FW degradation.

The second objective was to determine the effectiveness of alkali and CFA pretreatment on BS biodegradability determined from the changes in lignocellulosic fractions (cellulose, hemicellulose, and lignin) as indicators. The objective was met with the following outcomes achieved:

- The gross effect of alkali pretreatment was evident from the significant mass loss of solids (11–20%) observed with two alkalinity concentrations of CaO and one concentration of NaOH, indicating that complex fibres (cellulose, hemicellulose and lignin) were converted into soluble compounds. The mass loss measured during NaOH pretreatment was 20.4%, which was 25% higher than for CaO with equivalent alkalinity, possibly associated with the 12.7% and 5.5% greater reductions in lignin and cellulose, respectively.
- As-received CFA supplied very low alkalinity ($\sim 1,500 \text{ mg CaCO}_3 \text{ L}^{-1}$), and did not significantly affect the fibre composition. The hemicellulose and cellulose content remained unchanged compared to water-treated BS, although a slight reduction in lignin content, equivalent to 11%, was observed.
- The lowest alkalinity used in the experiments, 2,500 mg $\text{CaCO}_3 \text{ L}^{-1}$, also had no statistically significant effect on the cellulose, hemicellulose and lignin content of BS. Nevertheless, a small reduction of 11% in hemicellulose and 10% in lignin was observed compared to water-pretreatment of BS. Higher alkalinity at 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$, however, significantly reduced hemicellulose and lignin by 22.6% and 27.6%, respectively, while cellulose remained unaltered, compared to water pretreatment of BS, suggesting this was the minimum alkalinity required for effective pretreatment by CFA.

- Increasing alkalinity above 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ to 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ further reduced lignin and hemicellulose by 8.2% and 23.7%, respectively, compared to 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ alkalinity concentration, albeit not significantly. Therefore, 5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ was identified as the optimum alkali concentration for the AD pretreatment step. Compared to water pretreatment, 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ pretreatment reduced 33.5% of lignin and 37% of hemicellulose, while cellulose was unaffected.
- Pretreatment at 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ reduced lignin significantly, making greater fractions of hemicellulose and cellulose degradable during AD, equivalent to 85% and 22%, respectively, compared to 50% and 10% after water pretreatment. Lignin reduction led to greater access of hydrolytic enzymes to hemicellulose and cellulose that are encapsulated and protected from enzymic attack by lignin. This was further supported by SEM images showing the internal structure of fibre bundles of BS, that were presumably cellulose, which became exposed after 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ CaO pretreatment.
- The molecular mechanism underlying the reduction of lignin and hemicellulose by CaO alkali pretreatment was determined by the FTIR spectra showing changes in functional groups of fibre components that were attributed to: (i) cleavage of ester bonds in LCCs, leading to the separation of lignin and hemicellulose, (ii) disruption of acetyl and uronic groups in hemicellulose (both mechanisms in (i) and (ii)) were indicated by the reduced FTIR absorbance intensities in 1730–1740 cm^{-1} wavelength range for CaO and NaOH-pretreated BS compared to water-pretreatment, (iii) disruption of hydroxyl groups or hydrogen bonds within the lignin structure or the LCCs network (shown by the decreased intensities in the 3200–3500 cm^{-1} range, (iv) breakdown of glycosidic bonds in the polysaccharide backbone, suggested by reduced absorbances in the 890 cm^{-1} , 1000–1100 cm^{-1} , and 1140–1170 cm^{-1} wavelength ranges, and (v) disruption of the aromatic rings in lignin, following LCCs disruption, due to the cleavage of C-C and ether bonds, which are the primary bonds in lignin (identified from reduced intensities in the 1215–1260 cm^{-1} wavelength range), resulting in the formation of lignin degradation byproducts that are more soluble, with lower molecular weight, increasing the degradability by anaerobic microbes.
- Alkali pretreatment preserved cellulose, however, the crystalline structure of this important fibre fraction in LARs was transformed into the amorphous form, which is more susceptible to chemical and biological degradation, directly enhancing the biodegradability of BS. The degree of transformation was identified through decreasing total crystallinity index (TCI) (which is a measure of the extent of alteration in cellulose crystallinity) values in the following order: water-treated (0.41) > CaO ~

5,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ (0.27) > CaO ~ 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ (0.25) > NaOH (0.22). The breakdown of hydrogen bonds, predominant in cellulose, was also involved in the process and this was demonstrated from the reduction in FTIR intensities in the 3200–3500 cm^{-1} wavelength range observed following NaOH pretreatment. The extent of crystalline alteration, indicated by decreasing TCI, was greater for NaOH compared to CaO at equivalent alkalinity, consistent with the observed cellulose solubilisation for NaOH, which was not observed in CaO pretreatment, probably due to higher solubility of NaOH than CaO. This solubilisation, however, was small, representing 10% reduction compared to water pretreatment.

- The alteration of cellulose structure from crystalline to amorphous form also contributed to the increased digestibility in the AD process. The combined effects of CaO pretreatment at 11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$ and AD reduced the overall sum of cellulose, hemicellulose and lignin by 53.7% in the digested residue of BS, which was almost twice the decrease, equivalent to 27.6%, observed with water pretreatment.
- CaO pretreatment over 7 d demonstrated effects comparable to NaOH pretreatment, although NaOH was marginally more effective in lignin reduction and dissolution of amorphous cellulose, albeit not significantly. The lignin content in BS was 8.6% and following NaOH pretreatment was reduced to 4.9% TS, compared to 5.6% TS with CaO pretreatment at equivalent alkalinity (~11,000 mg $\text{CaCO}_3 \text{ L}^{-1}$). The hemicellulose content of BS was initially 33.1% TS and was relatively similar after both treatments, equivalent to 21.9% TS for NaOH and 19.6% TS for CaO. Barley straw initially contained 44.3% TS of cellulose, which was unaffected following CaO pretreatment, equating to 45.3% TS, while cellulose reduced to 39.8% TS with NaOH pretreatment.
- The marginal differences observed in fibre composition obtained for CaO and NaOH pretreatment indicated that CaO is effective as an alkali pretreatment of LARs for AD. Thus similar effects of alkali pretreatment by CFA would be expected at equivalent alkalinity as this is predominantly a source of CaO.
- Interestingly, however, CFA pretreatment with equivalent alkalinity (note the alkalinity of CFA was supplemented by CaO amendment), to the CaO-only Control condition, increased the degradation of the total fibre (sum of cellulose, hemicellulose, and lignin) content of BS during the AD step by 64.1% for CFA compared to 38.7% for CaO pretreatment without CFA. The combined effects of alkali pretreatment and AD steps led to a total 72.9% reduction in total fibre in CFA pretreatment, compared to 53.7% in CaO pretreatment without CFA, suggesting other specific properties of CFA,

in addition to alkalinity, promoted microbial activity and interactions that improved the efficiency of LAR degradation by AD.

The third objective was to establish the correlation between the extent of BS biodegradability following CFA pretreatment and CH₄ production. This objective was met with the following outcomes:

- Significant changes in fibre composition led to enhanced fibre biodegradability, as indicated by the increase in hydrolysis rate, which was improved by 55% after CFA pretreatment from 0.09 d⁻¹ in water pretreatment to 0.14 d⁻¹. Enhanced biodegradability of BS was also explained by higher VS removal and a significant increase in CSMY during AD of BS. CFA pretreatment equivalent at the 11,000 mg CaCO₃ L⁻¹ alkalinity rate increased VS removal by 71%, consistent with 93% increase in CSMY, compared to water-treated BS, demonstrating the effectiveness of CFA pretreatment in reducing BS recalcitrance to AD.
- The increased fibre destruction observed by CFA pretreatment compared to CaO with equivalent alkalinity was also accompanied by increases in the CSMY, hydrolysis rate and VS removal, equivalent to 39%, 27%, 29%, respectively, compared to the CaO-only Control, providing further confirmation that factors beyond alkalinity in CFA contributed to the enhanced AD performance.
- The correlation between cellulose content in the pretreated BS before digestion and CSMY was not strong ($R^2 = 0.30$) and not statistically significant ($P > 0.05$), as alkali pretreatment did not target cellulose reduction. The CSMY was, however, strongly and negatively correlated with both the lignin ($R^2 = 0.97$, $P < 0.05$) and hemicellulose ($R^2 = 0.89$, $P < 0.05$) contents. CSMY decreased incrementally by 18.7 mL CH₄ g⁻¹ VS for each 1% increase in lignin content and by 5.2 mL CH₄ g⁻¹ VS for each 1% increase in hemicellulose. The impact of lignin content on reducing CSMY was almost four times greater than hemicellulose, due to the recalcitrant properties of lignin, which strongly influenced the CSMY. Reducing lignin is therefore a critical factor in improving CSMY by alkaline pretreatment.

The fourth objective was to determine the effectiveness of acidified, fermented FW as a neutralising agent for alkali-pretreated BS for feedstock pH control and establish the optimum addition rate to maximise the AD process. In meeting this objective several key findings were:

- A 7 d fermentation period was identified as optimal to acidify FW, as no further decrease in pH was observed and the pH was stable at 3.7.

- Fermented FW effectively neutralised high alkali-pretreated BS (11,000 mg CaCO₃ L⁻¹), with an optimal addition rate of 20% VS, which maintained a pH profile generally within the ideal range for CH₄ conversion of pH 6.0–7.5. The 20% VS fermented FW addition rate provided a slightly acidic environment, which is beneficial to hydrolytic and acidogenic populations in the early stages of digestion, and may provide additional buffer capacity, avoiding significant acidification and pH drop that would be detrimental to the methanogenic community. Thus, FW is a suitable alternative to conventional inorganic acid chemicals for adjusting the pH after alkali-pretreatments.

The fifth objective was to assess the extent to which TEs within CFA can facilitate/enhance the AD of BS process through comparison of the TE levels of CFA and the AD inoculum with threshold data. The outcome was:

- The baseline TE values in both CFA and inoculum were sufficient relative to the TE requirements of anaerobic microorganisms. While the addition rates of CFA and inoculum can influence the overall TE concentrations in the final waste feedstock mixture in the integrated AD system, the TE levels measured in the digestate from the Control (without CFA) showed adequate supply and comparable to those in the digestate from the reactor with CFA pretreatment. Therefore, the enhanced CSMY observed in CFA pretreatment was not associated with TE supplementation from CFA.

The sixth objective was to investigate the conductive properties of CFA and their impact on the community structure of microbial populations. The findings in meeting this objective were:

- The conductivity of CFA was 0.6 mS m⁻¹ and was similar to other well-established conductive materials used in the AD process, such as biochar (0.2–0.5 mS m⁻¹). This is critically relevant because the response observed in the AD experiments reported here with CFA was similar to observations of the stimulating effects of other types of conductive materials used in the AD process, which have been extensively studied in relation to DIET. Indeed, the conductivity of CFA fell within the range of *Geobacter sulfurreducens* biofilm conductivities (0.6–4.18 mS m⁻¹), suggesting that CFA may similarly facilitate DIET. Therefore, a further set of experiments was conducted to examine the direct effects of CFA to the AD reactor on the AD response, without a pretreatment stage.
- The CFA-stimulated reactor referred to as direct AD of BS with CFA stimulation without a pretreatment stage, exhibited nearly 25 times higher conductivity compared to the Control reactor, without CFA, and results from the analysis of the microbial community

structure showed that the prevalence of electrochemically active bacteria, *Anaerolineaceae*, was markedly increased to 22.1% compared to the digested inoculum, 17.3%. This is critical evidence supporting the presence of DIET as these bacteria are capable of transferring electrons to conductive materials via the DIET mechanism. Moreover, the increased LARs degradation under these conditions may be explained because they also produce cellulosome–multiprotein complexes containing essential enzymes for cellulose degradation.

- The CFA-stimulated reactor also provided conditions favourable to *Candidatus methanofastidiosum* increasing the abundance to 87% compared to the Control (42%). This member of the archaea has been detected in the other studies performing DIET with *Geobacter*, therefore, the results suggested that this group potentially established a syntrophic relationship exchanging electrons with *Anaerolineaceae* by the DIET mechanism under the conditions of the experiment.
- *Methanofastidiosum* was probably enriched by CFA due to the presence of methylated thiols in CFA, which serve as an alternative substrate for this group of methanogens, reducing competition and promoting growth of the organism. Methylated thiols could be potentially formed through the reaction of lignin-based aromatic compounds with S elements released from CFA and from the digested sludge inoculum.
- The CFA-amended reactor increased the relative abundance of *Smithellaceae* from 0.7% in the digested inoculum to 1.4%. While *Smithellaceae* is not typically involved in DIET (Hatamoto et al., 2007; Sousa et al., 2007; Zhao et al., 2016b), it plays a key role in degrading intermediates from the acidogenic phase, such as propionate.
- This study is the first to explore the microbial response and interactions in a CFA-amended AD reactor system for LAR treatment in relation to the conductive properties of CFA. A previous study reported iron oxide as the primary contributor to the conductive properties of CFA, however, the results presented here show that UC in CFA is potentially a more important contributor to the conductivity of the material (shown by the strong and positive relationship between EC and LOI, with $r = 0.98$ and $P < 0.05$), and its ability to support DIET, and improve the efficiency of AD processes.

The seventh objective was to quantify the combined effect of the integrated CFA, FW and BS waste AD system on CH₄ yield. The findings from this part of the investigation, although reported above in the context of another objective met, are summarised here:

- In an integrated waste AD system developed in this study, which involved FW neutralisation for high alkalinity CFA-pretreated BS, CH₄ was produced immediately

on Day 1 without lag phase observed, with peak CH₄ production was 1.5-fold higher compared to the Control condition with mineral acid neutralisation. This integrated AD system resulted in 224.7 mL CH₄ g⁻¹ VS, which was 40% higher compared to Control. The results indicated the effective neutralisation of alkalinity from the pretreatment step and the absence of inhibition from the introduced acidity at the optimum FW addition rate identified (20% FW).

The eighth and final objective was to propose a mechanism underpinning the microbial behaviour contributing to the enhanced CH₄ yield in the integrated AD process.

- CFA-stimulated reactor significantly increased CSMY by 30%, from 101.5 mL g⁻¹ VS in the Control reactor without CFA to 132.3 mL g⁻¹ VS. Thus, the improvement in CSMY was directly linked to the presence of CFA, independent of alkali pretreatment, and was related to the effects of CFA on microbial behaviour. Additionally, the hydrolysis rate in the CFA-stimulated reactor approximately doubled, increasing from 0.08 d⁻¹ in the Control reactor to 0.15 d⁻¹, and there was also an increase of 29% in VS removal, from 28.7% to 37%. These observed effects, suggest that weathered CFA, with alkalinity lower than 5,000 mg CaCO₃ L⁻¹, for example from stockpiles, also has potential for direct supplement to AD to increase CH₄ yield from LARs.
- The CFA may enhance the degradation of LARs and increase the CH₄ yield from AD of LARs through two possible microbial mechanisms that may occur concomitantly: (1) **DIET mechanism**, by promoting the growth of *Anaerolineaceae* and *Methanofastidiosum*, which may form a mutualistic relationship for electron transfer, using CFA as an electron conduit between the two groups, and (2) **IIET mechanism**, indicated by the abundance of *Smithellaceae* and *Methanofastidiosum*. *Smithellaceae* is a propionate degrader, but propionate oxidation under standard AD conditions is energetically unfavorable unless byproducts (like H₂), are rapidly consumed by methanogens. The CFA-stimulated reactor favoured *Methanofastidiosum*, which also utilises a hydrogenotrophic metabolic pathway, which may make propionate degradation energetically feasible, with *Methanofastidiosum* scavenging H₂ and maintaining ideal conditions by lowering the partial pressure of H₂.

The results of this study support the initial hypothesis that CFA can effectively serve as an alkali pretreatment agent, while FW provides sufficient acidity to neutralise pretreated LARs prior to AD. The analysis of the integrated LAR-CFA-FW system revealed synergistic interactions that not only improved biogas production efficiency but also enhanced the sustainability of the treatment process; with the mechanisms underpinning the microbial

behaviour contributing to enhanced biogas yield in the integrated AD process explained in terms of DIET and IIET.

10.2 Recommendations for Future Research

This research has demonstrated the potential for CFA and FW in optimising the AD of BS, increasing the CH₄ yield above that possible from the effects of alkali-pretreatment on lignin degradation of BS. This raises a number of fundamental questions and practical opportunities for further research to develop and upscale the process into an operationally viable system for LARs treatment:

1. Reinforcing the evidence for DIET mechanisms in CFA-AD systems: (1) Understand the role of *Methanofastidiosum*, and the specific mechanisms underpinning the syntrophic relationship with *Anaerolineaceae* and *Smithellaceae*, by investigating the extracellular electron transfer activity in the system; (2) Elucidate the microbial pathway mechanism operating in the system by spiking H₂ in the reactor headspace above the tolerable limit for syntrophic acetogens as suggested by Cruz Viggi (2014); the DIET mechanism operates independently of H₂ concentration, thus, if DIET is operating, methanogenesis rates will remain unaltered in the presence of high concentrations of H₂ (Xia et al., 2019).

2. Determine the potential to increase CH₄ yield by other CFA physicochemical properties: Investigate the impact of other properties of CFA on CH₄ stimulation, to include UC, surface area, and porosity, characteristics that could enable CFA to act as a potential adsorbent for VFAs and NH₃. Moreover, the predominant components of CFA, such as iron oxides and silica, may provide a suitable support for enzyme immobilisation, particularly for lignin peroxidase, an essential enzyme involved in lignin degradation. A possible advantage of this immobilisation is that direct CFA addition to AD could stimulate lignin degradation compared to condition without CFA. This can be investigated by determining the fibre composition—cellulose, hemicellulose and lignin—after AD with direct CFA addition, without pretreatment step. In addition to iron oxides and UC, other oxides (Al₂O₃, SnO, SnO₂, TiO₂, WO₃ and ZnO) in CFA, and the mesoporous structure, chemical surface, and textural properties have been suggested to influence the conductive properties in CFA, and investigating these factors will provide a more comprehensive understanding of CFA's conductivity.

3. Quantify the process N balance and effects of FW on N supply to the integrated AD of LARs: In practical implementation, the inoculum material will only serve as a starter

microbial culture, with subsequent batches of LARs inoculated with digestate from the first batch. LARs are N limited, therefore the N supply from FW may also have a significant role in adjusting the C/N ratio, as well providing an acid source for neutralisation. Therefore, further work will be necessary to quantify the process N balance to ensure this major element can be adequately supplied by FW.

4. Undertake an economic viability assessment of the proposed integrated waste system utilising CFA and FW to optimise the AD of LARs: A techno-economic assessment is necessary to evaluate the commercial, operational viability of the proposed approach. This would involve analysing the costs associated with AD technology implementation, pretreatment, transportation of waste feedstocks from their sources, other operational expenses, as well as assessing the potential economic benefits, such as income from tipping fees for the CFA and FW treated in the AD plant, electricity sales, government incentives, and cost savings, and where appropriate to assess the hidden costs that can arise from environmental impacts on the local community.

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APPENDICES

Appendix A: Anaerobic Digestion of Reference Standard Material and and Blank Inoculum

A.1 Chapter 3: Cumulative specific CH_4 yield from the anaerobic digestion of microcrystalline cellulose

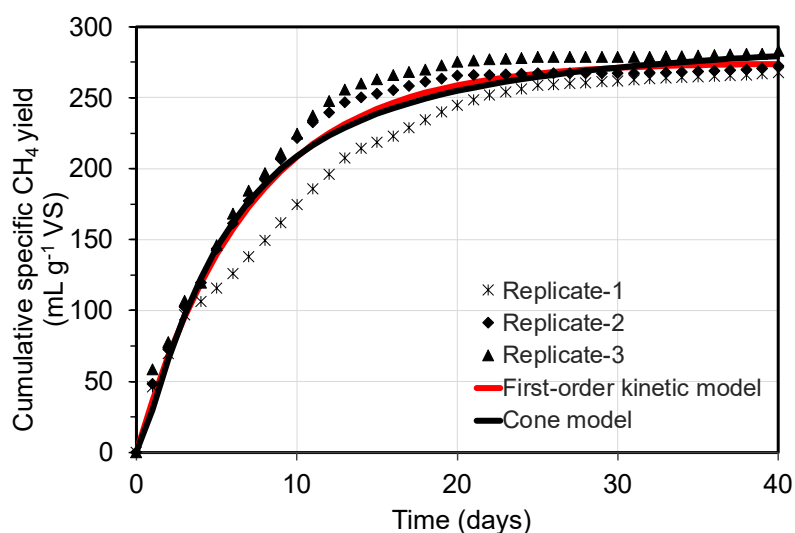


Figure A.1. Experimental ($n=3$, measured daily) and modelled (First-order and Cone kinetic model) cumulative specific CH_4 yields (CSMY) of microcrystalline cellulose during 40 d anaerobic digestion at 35°C .

A.2 Chapter 5: Residual CH_4 production from the anaerobic digestion of digested sludge as inoculum

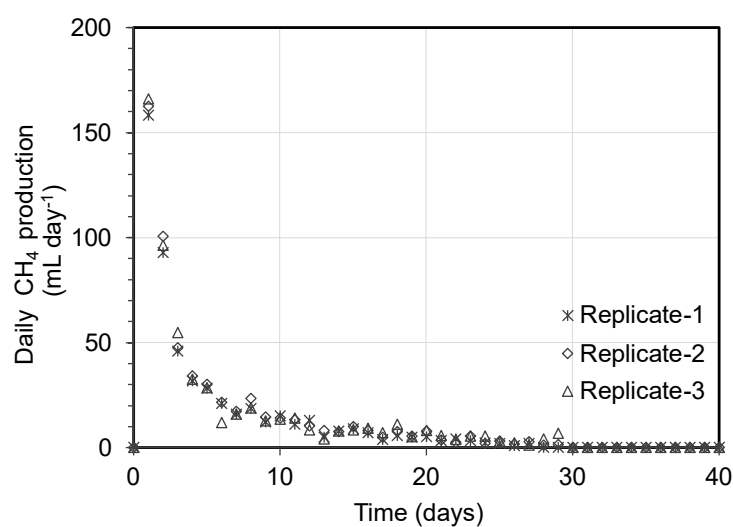


Figure A.2. Residual CH_4 production ($n=3$, measured daily) of digested sludge inoculum used in Chapter 5 monitored over 40 d digestion at 30°C .

A.3 Chapter 6: Residual CH₄ production from the anaerobic digestion of digested sludge as inoculum

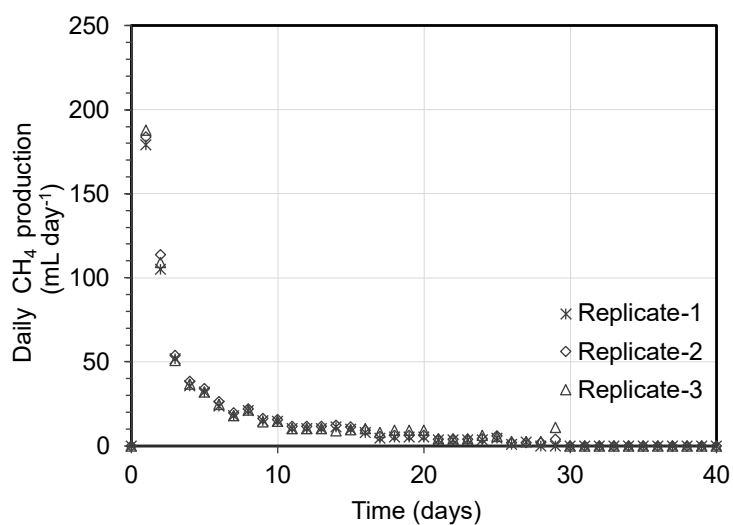


Figure A.3. Residual CH₄ production (n=3, measured daily) of digested sludge inoculum used in Chapter 6 monitored over 40 d digestion at 30° C.

A.4 Chapter 7: Residual CH₄ production from the anaerobic digestion of digested sludge as inoculum

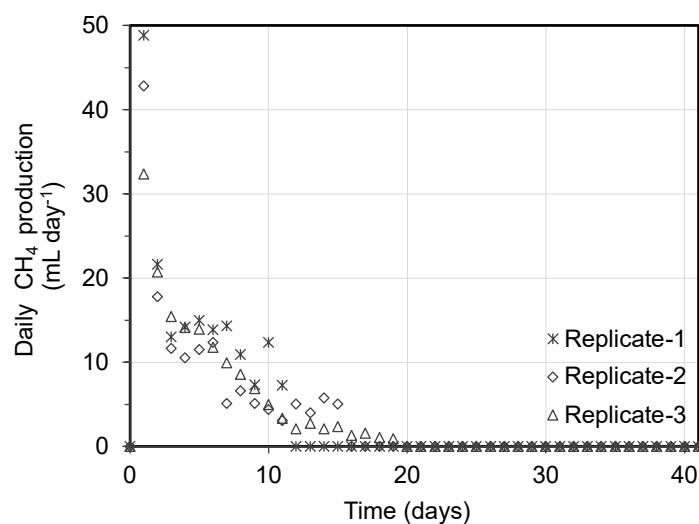


Figure A.4. Residual CH₄ production (n=3, measured daily) of digested sludge inoculum used in Chapter 7 monitored over 40 d digestion at 30° C.

A.5 Chapter 8: Residual CH₄ production from the anaerobic digestion of digested sludge as inoculum

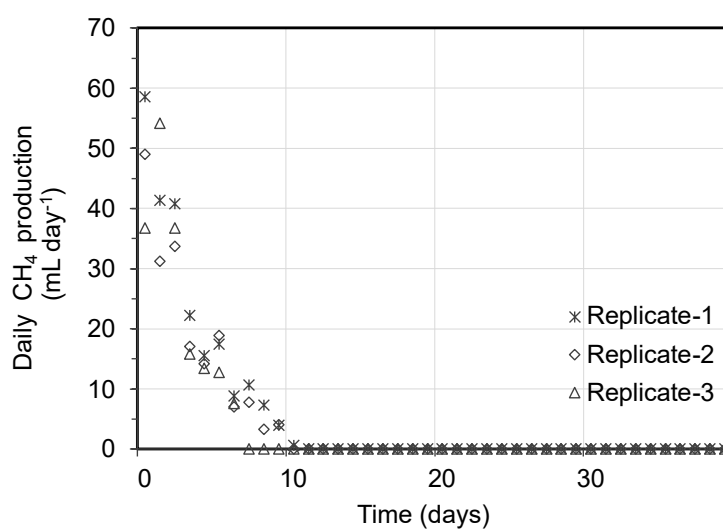


Figure A.5. Residual CH₄ production (n=3, measured daily) of digested sludge inoculum used in Chapter 8 monitored over 40 d digestion at 30° C.

Appendix B: Kinetic Parameters for Modelled CH₄ Gas Production using First-order and Cone Kinetic Models

Symbols used in this Appendix B are described below.

$CSMY_o$: maximum cumulative specific methane yield (mL g⁻¹ VS) (Equation 3.6 and 3.7).

k : hydrolysis rate constant from First-order model (d⁻¹) (Equation 3.7).

R^2 : coefficient of determination of the modelled CH₄ data

$CSMY_{40}$: cumulative specific methane yield at 40 d predicted from Cone model.

n : shape factor (Equation 3.6).

Table B.1 Kinetic parameters determined from both First-order and Cone kinetic models for anaerobic digestion of barley straw pretreated with: (i) deionised water (WT), (ii) CaO ~11,000 mg CaCO₃ L⁻¹, (iii) as-received CFA supplemented with CaO ~11,000 mg CaCO₃ L⁻¹ (CFAS).

Treatment Conditions	First-order Model			Cone Model			
	$CSMY_o$	k	R^2	$CSMY_o$	$CSMY_{40}$	n	R^2
WT (Rep-1)	92.5	0.14	0.98	93.0	87.1	1.94	0.99
WT (Rep-2)	103.9	0.05	0.97	136.0	90.6	1.00	0.97
WT (Rep-3)	98.0	0.14	0.99	106.5	92.9	1.39	0.99
CaO-III (Rep-1)	117.0	0.15	0.99	138.7	117.2	1.03	0.99
CaO-III (Rep-2)	117.1	0.07	0.98	171.4	128.9	0.80	0.99
CaO-III (Rep-3)	133.0	0.11	0.99	167.5	135.4	0.97	0.99
CFAS (Rep-1)	149.3	0.37	0.95	166.3	150.2	0.97	0.99
CFAS (Rep-2)	171.4	0.06	0.90	235.6	202.2	0.97	0.99
CFAS (Rep-3)	168.1	0.07	0.99	264.5	184.3	0.60	0.99

Table B.2 Kinetic parameters determined from both First-order and Cone kinetic models for anaerobic digestion of: (i) untreated barley straw (BS), and BS pretreated with: (ii) deionised water (WT), (iii) CaO ~5,000 mg CaCO₃ L⁻¹ (CaO-II), (iv) CaO ~11,000 mg CaCO₃ L⁻¹ (CaO-III), (v) as-received CFA supplemented with CaO ~11,000 mg CaCO₃ L⁻¹ (CFAS), (vi) NaOH ~11,000 mg CaCO₃ L⁻¹.

Treatment conditions	First-order Model			Cone Model			
	$CSMY_o$	k	R^2	$CSMY_o$	$CSMY_{40}$	n	R^2
Untreated (Rep-1)	106.1	0.11	0.94	154.4	110.1	0.77	0.98
Untreated (Rep-2)	114.7	0.10	0.98	142.7	115.6	1.02	0.99
Untreated (Rep-3)	n.d	n.d	n.d	n.d	n.d	n.d	n.d
WT (Rep-1)	109.8	0.08	0.86	152.9	105.6	0.78	0.93
WT (Rep-2)	104.0	0.13	0.95	137.9	108.4	0.82	0.98
WT (Rep-3)	111.3	0.09	0.99	153.3	111.8	0.91	0.99

NaOH (Rep-1)	173.6	0.29	0.86	205.9	183.1	0.73	0.99
NaOH (Rep-2)	176.5	0.21	0.95	178.9	177.2	1.89	0.98
NaOH (Rep-3)	168.5	0.25	0.94	167.7	167.5	2.72	0.98
CaO-III (Rep-1)	158.5	0.12	0.99	171.7	159.3	1.38	0.99
CaO-III (Rep-2)	148.2	0.17	0.83	233.7	160.4	0.56	0.98
CaO-III (Rep-3)	157.4	0.10	0.99	189.4	158.8	1.08	0.99
CaO-II (Rep-1)	131.8	0.11	0.94	182.1	135.0	0.79	0.99
CaO-II (Rep-2)	137.6	0.19	0.88	195.2	146.8	0.61	0.99
CaO-II (Rep-3)	132.5	0.14	0.97	164.5	137.5	0.91	0.99
CaO-III+CFA (Rep-1)	199.2	0.08	0.99	275.2	212.7	1.19	0.99
CaO-III+CFA (Rep-2)	220.9	0.10	0.98	295.3	225.1	0.89	0.99
CaO-III+CFA (Rep-3)	204.7	0.19	0.95	222.9	210.8	1.21	0.97

Table B.3 Kinetic parameters determined from both First-order and Cone kinetic models for anaerobic digestion of CaO-treated barley straw (BS) neutralised with: (i) inorganic mineral hydrochloric acid (HA) and (ii) 20% fermented food waste (volatile solid basis) (FW-20).

Treatment Conditions	First-order Model			Cone Model			
	$CSMY_o$	k	R^2	$CSMY_o$	$CSMY_{40}$	n	R^2
HA (Rep-1)	166.2	0.16	0.99	176.03	168.7	1.45	0.99
HA (Rep-2)	147.7	0.09	0.98	229.05	148.9	0.86	0.99
HA (Rep-3)	158.7	0.11	0.96	235.38	164.2	0.75	0.98
FW-20 (Rep-1)	240.7	0.07	0.88	445.75	227.5	0.69	0.95
FW-20 (Rep-2)	217.8	0.22	0.92	222.85	219.8	1.72	0.95
FW-20 (Rep-3)	208.2	0.29	0.89	223.42	215.5	1.16	0.95

Table B.4 Kinetic parameters determined from both First-order and Cone kinetic models for: (i) direct anaerobic digestion of barley straw (BS) without coal fly ash addition (Control) and (ii) with coal fly ash (CFA) addition at a CFA to BS ratio of 1:2 (CFA-AD).

Treatment Conditions	First-order Model			Cone Model				
	$CSMY_o$	k	R^2	$CSMY_o$	K_H	$CSMY_{40}$	n	R^2
Control (Rep-1)	110.1	0.09	0.95	152.8	0.07	110.5	0.91	0.97
Control (Rep-2)	94.2	0.08	0.99	116.6	0.08	91.4	1.10	0.99
Control (Rep-3)	96.6	0.14	0.92	130.3	0.12	101.7	0.79	0.97
CFA-AD(Rep-1)	129.8	0.14	0.95	175.5	0.13	135.9	0.77	0.99
CFA-AD (Rep-2)	113.2	0.14	0.96	137.7	0.17	117.1	0.92	0.97
CFA-AD (Rep-3)	140.0	0.13	0.98	165.5	0.16	143.9	1.02	0.99

Appendix C: Experimental Methane Yield and Volatile Solids Removal

Table C.1 Experimental cumulative specific CH₄ yield (CSMY_{exp}) and volatile solid (VS) removals (n=3) for anaerobic digestion of barley straw pretreated with: (i) deionised water (WT), (ii) CaO ~11,000 mg CaCO₃ L⁻¹, (iii) as-received CFA supplemented with CaO ~11,000 mg CaCO₃ L⁻¹ (CFAS).

Treatment Conditions	CSMY _{exp} (mL CH ₄ g ⁻¹ VS)		VS Removal (%)	
	Replicate Values	Mean	Replicate Values	Mean
WT (Rep-1)	87.2	93.1 ± 5.22	33.0	33.3 ± 1.53
WT (Rep-2)	95.0		35.0	
WT (Rep-3)	97.1		32.0	
CaO-III (Rep-1)	117.1	122.9 ± 8.73	46.0	44.3 ± 2.89
CaO-III (Rep-2)	118.7		41.0	
CaO-III (Rep-3)	133.0		46.0	
CFAS (Rep-1)	152.1	163.6 ± 16.61	53.0	57.0 ± 4.00
CFAS (Rep-2)	182.7		57.0	
CFAS (Rep-3)	156.1		61.0	

Table C.2 Experimental cumulative specific CH₄ yield (CSMY_{exp}) and volatile solid (VS) removals (n=3) for anaerobic digestion of: (i) untreated barley straw (BS), and BS pretreated with: (ii) deionised water (WT), (iii) CaO ~5,000 mg CaCO₃ L⁻¹ (CaO-II), (iv) CaO ~11,000 mg CaCO₃ L⁻¹, (v) as-received CFA supplemented with CaO ~11,000 mg CaCO₃ L⁻¹ (CFAS), (vi) NaOH ~11,000 mg CaCO₃ L⁻¹.

Treatment Conditions	CSMY _{exp} (mL CH ₄ g ⁻¹ VS)		VS Removal (%)	
	Replicate Values	Mean	Replicate Values	Mean
Untreated (Rep-1)	108.9	101.6 ± 17.23	N/A	N/A
Untreated (Rep-2)	114.0		N/A	
Untreated (Rep-3)	n.d		N/A	
WT (Rep-1)	111.6	108.9 ± 2.45	30	31.0 ± 1.00
WT (Rep-2)	106.9		32	
WT (Rep-3)	108.2		31	
NaOH (Rep-1)	181.3	179.8 ± 7.91	61	60.3 ± 3.05
NaOH (Rep-2)	186.8		63	
NaOH (Rep-3)	171.2		57	
CaO-III (Rep-1)	166.9	160.4 ± 6.49	49	45.3 ± 3.21
CaO-III (Rep-2)	160.4		44	
CaO-III (Rep-3)	153.9		43	
CaO-II (Rep-1)	135.8	139.7 ± 5.07	46	40.7 ± 9.24
CaO-II (Rep-2)	145.4		46	
CaO-II (Rep-3)	137.9		30	

Treatment Conditions	CSMY _{exp} (mL CH ₄ g ⁻¹ VS)		VS Removal (%)	
	Replicate Values	Mean	Replicate Values	Mean
CaO-III+CFA (Rep-1)	186.2	207.6 ± 18.75	60	60.0 ± 6.00
CaO-III+CFA (Rep-2)	215.4		54	
CaO-III+CFA (Rep-3)	221.2		66	

N/A: not applicable

Table C.3 Experimental cumulative specific CH₄ yield (CSMY_{exp}) and volatile solid (VS) removals (n=3) for anaerobic digestion of CaO-treated barley straw (BS) neutralised with: (i) inorganic mineral hydrochloric acid (HA) and (ii) 20% fermented food waste (volatile solid basis) (FW-20).

Treatment Conditions	CSMY _{exp} (mL CH ₄ g ⁻¹ VS)		VS Removal (%)	
	Replicate Values	Mean	Replicate Values	Mean
HA (Rep-1)	167.5	156.7 ± 11.2	57	57.0 ± 0.2
HA (Rep-2)	145.0		57	
HA (Rep-3)	157.5		57	
FW-20 (Rep-1)	241.1	230.5 ± 12.0	62	60.7 ± 4.7
FW-20 (Rep-2)	233.0		65	
FW-20 (Rep-3)	217.5		55	

Table C.4 Experimental cumulative specific CH₄ yield (CSMY_{exp}) and volatile solid (VS) removals (n=3) for: (i) direct anaerobic digestion of barley straw (BS) without coal fly ash addition (Control) and (ii) with coal fly ash (CFA) addition at a CFA to BS ratio of 1:2 (CFA-AD).

Treatment Conditions	CSMY _{exp} (mL CH ₄ g ⁻¹ VS)		Error (%)	VS Removal (%)	
	Replicate Values	Mean		Replicate Values	Mean
Control (Rep-1)	112.2	101.6 ± 10.5	0.0	35	28.7 ± 7.1
Control (Rep-2)	90.9			21	
Control (Rep-3)	101.5			30	
CFA-AD(Rep-1)	131.8	128.0 ± 13.7	-3.3	36	37.0 ± 5.6
CFA-AD (Rep-2)	112.8			32	
CFA-AD (Rep-3)	139.3			43	

Appendix D: Methane Content

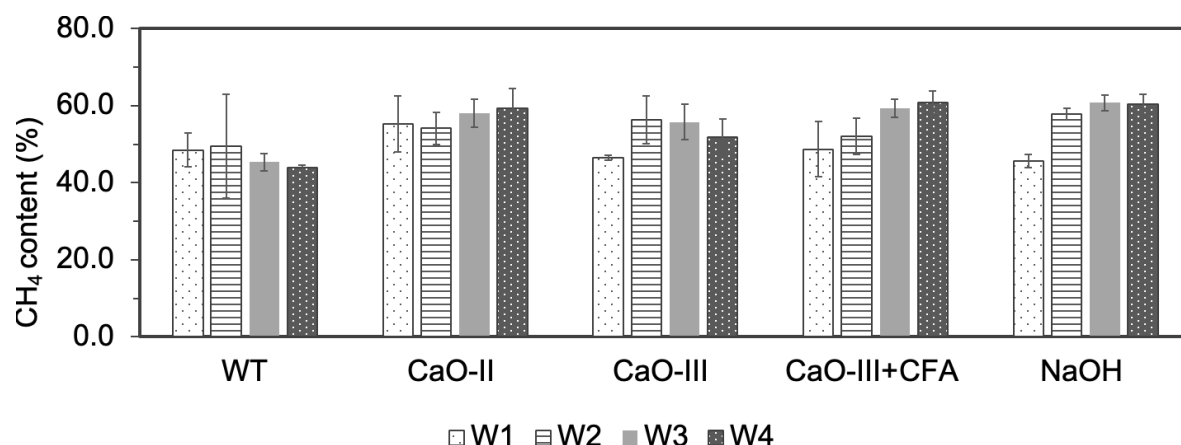


Figure C.1 Mean CH₄ content during anaerobic digestion of: (i) untreated barley straw (BS), and BS pretreated with: (ii) deionised water (WT), (iii) CaO ~5,000 mg CaCO₃ L⁻¹ (CaO-II), (iv) CaO ~11,000 mg CaCO₃ L⁻¹, (v) as-received CFA supplemented with CaO ~11,000 mg CaCO₃ L⁻¹ (CF), (vi) NaOH ~11,000 mg CaCO₃ L⁻¹, measured weekly (Week 1 (W1), Week 2 (W2), Week 3 (W3), Week 4 (W4)). Data from three replicates data are presented for each condition.

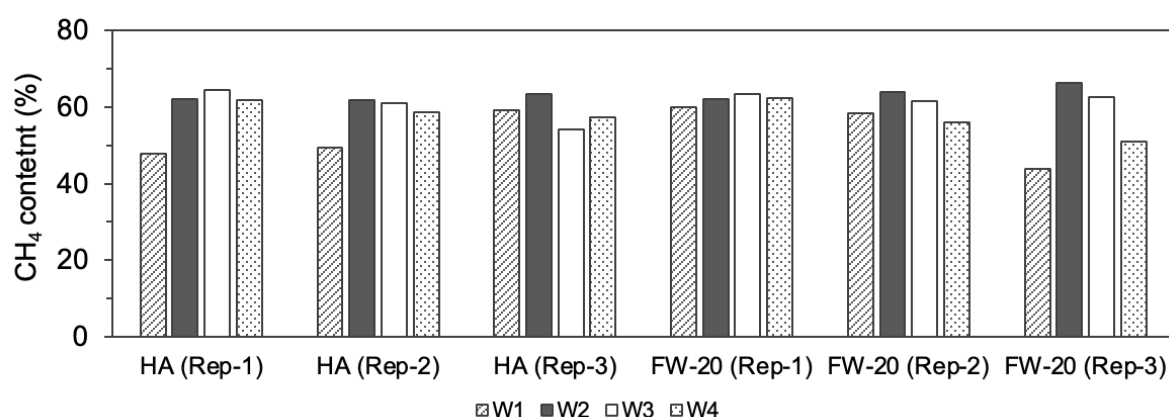


Figure C.2 Weekly monitoring ((Week 1 (W1), Week 2 (W2), Week 3 (W3), Week 4 (W4)) of CH₄ content during anaerobic digestion of CaO-treated barley straw (BS) neutralised with: (i) inorganic mineral hydrochloric acid (HA) and (ii) 20% fermented food waste (volatile solid basis) (FW-20). Data from three replicates data are presented for each condition.

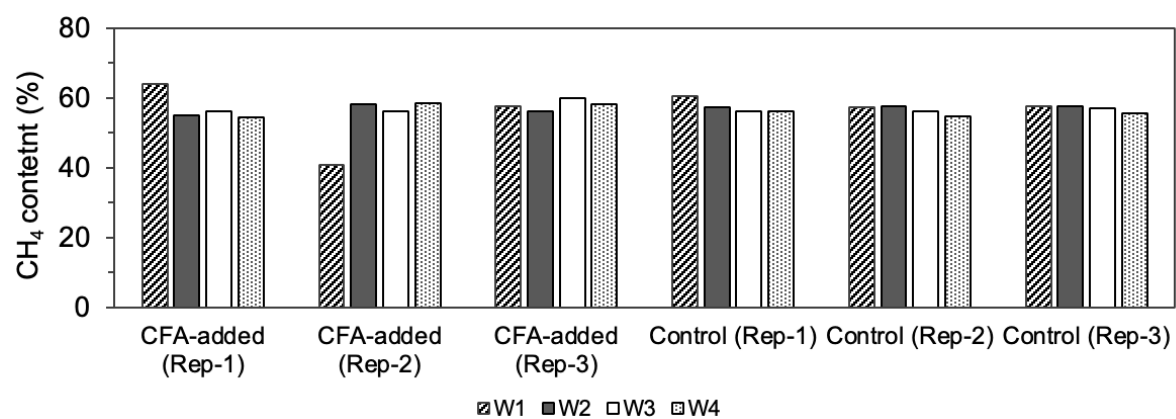


Figure C.3 Weekly monitoring ((Week 1 (W1), Week 2 (W2), Week 3 (W3), Week 4 (W4)) of CH₄ content during anaerobic digestion of barley straw (BS): (i) without coal fly ash addition (Control) and (ii) with coal fly ash (CFA) addition at a CFA to BS ratio of 1:2 (CFA-AD). Data from three replicates data are presented for each condition.

Appendix D: Microbial Community Composition

Table D.1 Relative abundance of bacterial community composition (in %) at family level derived from 16S gene sequencing in the inoculum and digestates from the Control and CFA-AD reactors after 40 d digestion.

Bacterial family	Inoculum	Control	CFA-AD
Anaerolineaceae	17.4	12.8	22.1
Bacteroidetes_vadinHA17	9.7	1.2	8.4
Sedimentibacteraceae	4.2	1.0	5.6
Cloacimonadaceae	9.7	0.3	6.3
Prolixibacteraceae	1.7	0.3	5.2
Planococcaceae	6.4	21.3	2.6
Bacteroidales_UCG-001	0.1	0.1	2.2
Rhodobacteraceae	2.8	15.0	3.7
Synergistaceae	2.3	1.5	2.6
Smithellaceae	0.7	0.1	1.4
Christensenellaceae	0.9	0.6	1.3
Coprothermobacteraceae	4.4	5.4	1.1
Intrasporangiaceae	1.7	0.2	1.1
Thermoanaerobaculaceae	1.4	0.6	0.9
Dojkabacteria	1.2	0.0	0.8
Bacteroidales_UCG-001	0.1	0.1	2.2
Coprothermobacteraceae	4.4	5.4	1.1
Xanthobacteraceae	0.4	1.4	0.5
Xanthomonadaceae	1.3	1.4	0.7
Syntrophomonadaceae	1.6	0.2	0.4
Pseudomonadaceae	1.4	2.4	0.8
Spirochaetaceae	1.2	0.1	1.7
Burkholderiaceae	1.1	1.6	0.8
Bacillaceae	0.0	2.0	0.0
Peptostreptococcales-Tissierellales	0.1	2.6	0.1
Dojkabacteria	1.2	0.0	0.8
Hungateiclostridiaceae	0.8	0.6	1.6
Others	21.8	21.7	24.0

Table D.2 Relative abundance of archaeal community composition (in %) at genus level derived from 16S gene sequencing in the inoculum and digestates from Control and CFA-AD reactors after 40 d digestion.

Archaeal Genus	Inoculum	Control	CFA-AD
Methanosaeta	8.8	18.2	1.3
Methanospirillum	36.9	15.1	0.3
Methanobacterium	1.2	13.1	0.7
Methanobrevibacter	0.2	10.3	0.3
ANME-1b	0.0	2.8	0.0
Methanolinea	5.0	2.3	0.0
Methanomassiliicoccus	1.7	1.9	0.0
Methanofastidiosum	41.9	1.8	86.9
SCGC_AAA011-D5	3.8	0.0	9.7
ANME-1a	0.0	1.6	0.0
Others	0.0	0.0	0.0
Unidentified	0.0	32.6	0.0

Appendix E: Gas Chromatography Curve Calibration

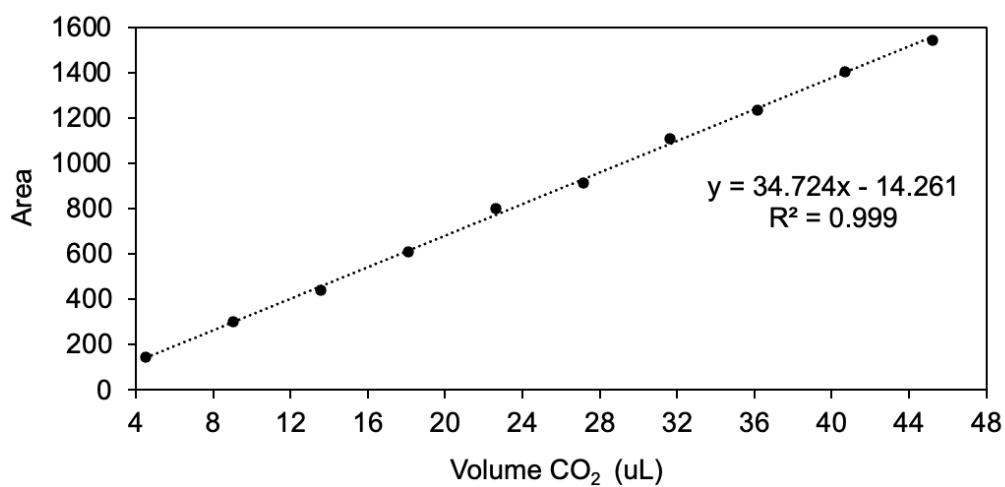


Figure E.1 Calibration curve of CO₂ for gas chromatography (2021)

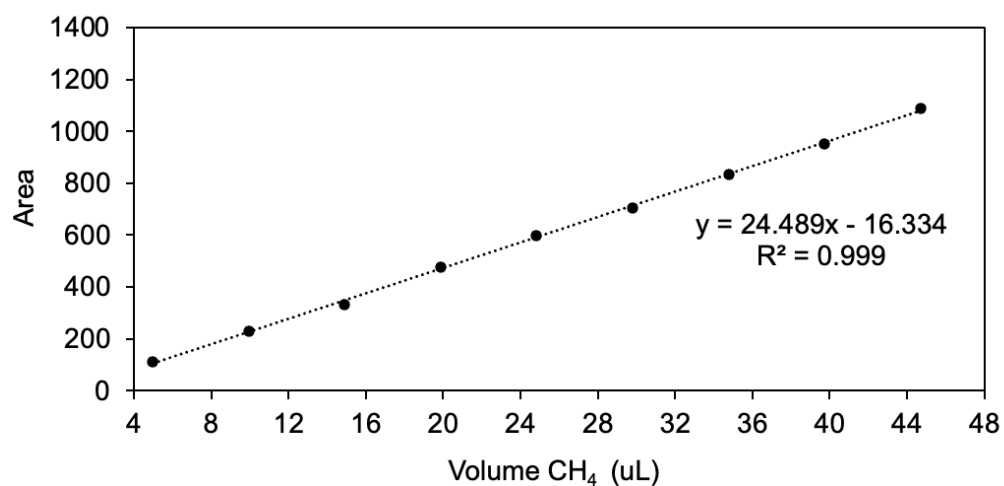


Figure E.2 Calibration curve of CH₄ for gas chromatography (2021)

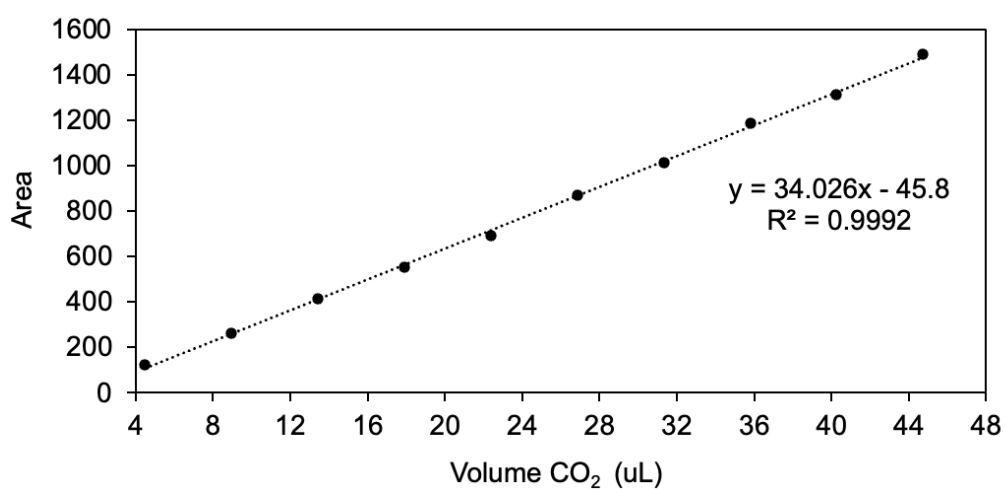


Figure E.3 Calibration curve of CO₂ for gas chromatography (2022)

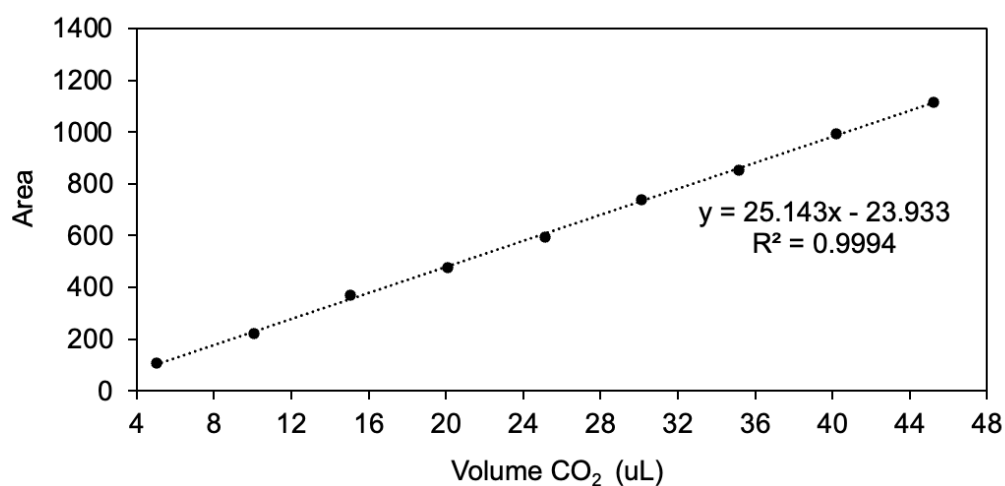


Figure E.4 Calibration curve of CH₄ for gas chromatography (2022)

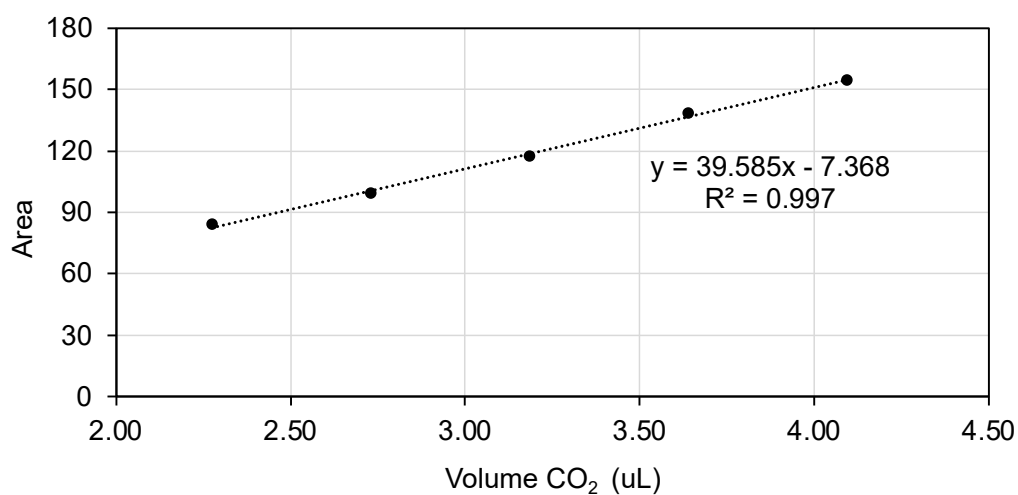


Figure E.5 Calibration curve of CO₂ for gas chromatography (2023)

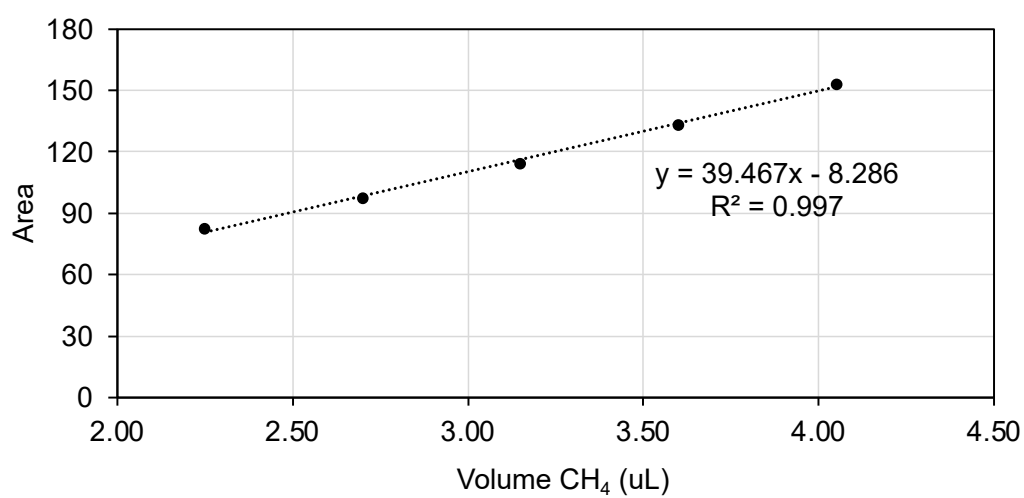


Figure E.6 Calibration curve of CH₄ for gas chromatography (2023)

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