Effects of Coal Fly Ash Pretreatment on Fibre Degradability and Enhanced Solid-State Anaerobic Digestion of Lignocellulosic Biomass

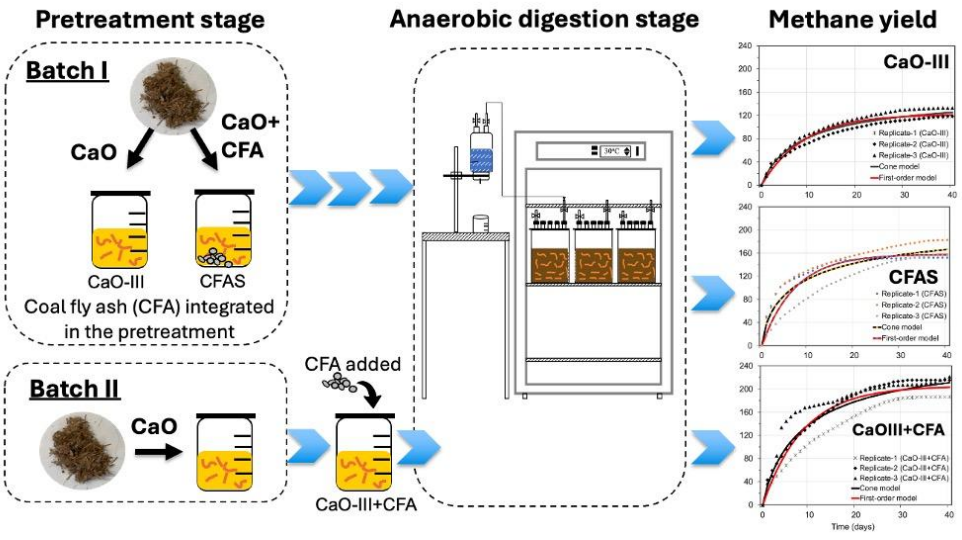
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Highlights

- Coal fly ash can be used as an alternative alkali pretreatment for anaerobic digestion.
- Significant lignin removal occurred at alkalinity ≥ ~5,000 mg $CaCO_3$/L.
- Coal fly ash increased CH_4 yield by 39% compared to CaO pretreatment.
- Improved AD performance was attributed to factors beyond alkali and trace elements.

Graphical Abstract



Abstract

Coal fly ash (CFA) is an alkali waste generated in large quantities by the power generation industry. Lignocellulosic biomass (LB) is also a significant, globally problematic waste stream. Alkali pretreatment of LB by CFA, to reduce the recalcitrance of LB before anaerobic digestion (AD), has the potential to beneficially utilise both waste streams, provide a major renewable energy source, and reduce the risks of groundwater and soil contamination associated with CFA stockpiling. Controlled, laboratory microcosm experiments were conducted to examine the effectiveness of CFA as an alkali pretreatment and alternative to calcium oxide (CaO) and sodium hydroxide (NaOH) for AD of LB. The AD experiments were conducted under solid-state conditions at 15% total solids (TS) in 1 L borosilicate glass chemostat reactors. Coal fly ash pretreatment increased methane (CH_4) yield by 93% and 39% compared to a water-pretreated control and equivalent alkalinity supplied by CaO alone (CaO pretreatment), respectively. In addition, CFA pretreatment, followed by AD, resulted in a twofold greater reduction in total fibre than CaO pretreatment, consistent with increased CH_4 yield, hydrolysis rate (27%), and volatile solids (VS) removal (29%). A consistent increase in CH_4 yield (32%) was also achieved when CFA was added after CaO pretreatment of LB, before AD. These results indicated the positive role of CFA in AD of LB extended beyond an alkali pretreatment effect.

Keywords: alkali pretreatment, anaerobic digestion, coal fly ash**,** lignocellulosic biomass

1. Introduction

The global reliance on fossil fuels and the resulting greenhouse gas emissions have raised serious concerns about their impact on climate change, underscoring the urgent need to transition toward renewable energy sources. A potential source of renewable energy is lignocellulosic biomass (LB) such as rice straw, corn stover, barley straw, sugarcane bagasse, of which approximately 21 billion tonnes is produced annually worldwide, representing an abundant global biomass resource (Gao et al., 2022). In Indonesia alone, a total of 145.5 million tonnes (Mt) of LB was generated in 2023 (USDA, 2024). Although LB is used as a cattle feed, the majority of LB remains underutilised, often disposed of by open burning after harvesting (Gao et al., 2022), causing health problems and air quality deterioration. On the other hand, LB is rich in organic matter, and the organic conversion into renewable energy (biogas) can be achieved through anaerobic digestion (AD) (Zdeb, 2021).

However, AD conversion of LB is limited by the inherent recalcitrance to anaerobic microbial degradation, thus only approximately 40% of the total solids (TS) are biodegradable (Peyrelasse et al., 2021). This is primarily attributed to the lignin matrix, which encapsulates hemicellulose and cellulose, thereby limiting microbial access to these components as carbon sources (Mansour et al. 2024). Pretreatment, therefore, plays a pivotal role in reducing the recalcitrance of LB, and one of the most effective methods identified is by alkali pretreatment. Alkali pretreatment offers several advantages over other methods, such as acid and biological pretreatments: (i) a higher delignification efficiency (Peyrelasse et al., 2021); (ii) provision of buffering capacity essential for AD due to residual alkalinity; and (iii) effectiveness at ambient temperatures (25–35^{o}C). For example, NaOH pretreatment at 35^{o}C has been reported to increase methane (CH_4) yield from rice straw by 20–80% (Sabeeh et al. 2020;

69 Mansour et al. 2024). During alkali pretreatment, ester bonds linking lignin and
70 carbohydrate complexes are hydrolysed, thus separating hemicellulose and cellulose
71 from lignin encapsulation and ultimately, biodegradability and CH_4 yield increase (Dai
72 et al., 2018; Mafa et al., 2020).

73 The high cost of chemicals used in alkali pretreatment, however, poses a
74 significant barrier to the economic viability of AD for LB, particularly in a developing
75 country setting (Egwu et al., 2021). This challenge could potentially be addressed by
76 replacing alkali chemicals with locally available, underutilised wastes possessing
77 similar alkaline characteristics. One potentially suitable material is coal fly ash (CFA),
78 which typically exhibits strong alkaline characteristics (Achternbosch et al., 2015).
79 Coal fly ash is the particulate residue carried along with the flue gas, which is captured
80 by air pollution control devices in a power station.

81 Global annual CFA production was approximately 800 Mt (Lauzurique et al.,
82 2021), while Indonesia generated around 10 Mt annually (Petrus et al. 2020). Fresh
83 CFA typically exhibits alkaline pH due to its substantial calcium oxide (CaO) content
84 (Achternbosch et al., 2015). The concentration of free CaO in CFA can range from
85 0.2% to 15% (Sutter et al., 2013; Achternbosch et al., 2015). Standard management
86 practices for CFA, such as disposal in ash ponds or landfills, pose risks of
87 environmental contamination, including the leaching of heavy metals into groundwater
88 and surface water (Prayoga and Afla, 2023). Incorporating CFA as a pretreatment
89 agent in the AD of LB could reduce CFA disposal in stockpiles and increase its
90 utilisation rate.

91 Research on the use of CFA as an alkali pretreatment for AD of recalcitrant
92 feedstocks, such as LB, is very limited. In one reported study (Xiao et al., 2020), liquors
93 extracted in deionised water from bottom ash from biomass power generation, used

for pretreatment of rice straw, increased the biogas yield by 16% compared to the control at equivalent chemical alkalinity. The increase was attributed to soluble nutrients, particularly Fe, Al, Ca, and Co, in the extract, which were present at concentrations sufficient to meet the AD requirements (Xiao et al., 2020), as these nutrients are often in limited supply in LB (Hinken et al., 2008). A similar effect may be expected with pretreatment using CFA, as CFA contains considerable concentrations of trace elements (TEs) (Lauzurique et al., 2021). The novelty of the work reported here lies in using CFA as a whole material, rather than only its extract, for alkali pretreatment. The current work also identified CFA's role as a source of TEs, as well as other potential contributions to the AD process, arising from its solid characteristics. The present study, however, was limited to identifying these potential contributions, while a detailed mechanistic investigation was beyond its scope.

The specific objectives of this study are to: (i) quantify the effects of alkali chemical and CFA pretreatment on the fibre composition of barley straw (BS) as a representative LB, both after pretreatment and AD; (ii) identify changes in functional groups of BS after pretreatment; (iii) compare AD performance, in terms of CH_4 yield, volatile solids (VS) removal and hydrolysis rate following pretreatment of BS by different alkali sources and CFA pretreatment; and (iv) identify potential supplementary benefits of CFA in relation to AD process requirements.

2. Materials and Methods

2.1. Waste feedstock preparation

Barley straw, sourced from a farm in Northamptonshire, UK, was used in the pretreatment and AD experiments, as it is a readily available and commonly used feedstock in LB pretreatment studies (Meyer et al., 2022; Álvaro et al., 2023). The

straw was dried at 105°C and homogenised by milling the bulk samples using a cross-beater (hammer) mill (Glen Creston, Retsch GmbH, Germany) equipped with a 1 mm screen. Coal fly ash was supplied in a single batch from a coal-fired power plant in the UK. Upon receipt in the laboratory, CFA sample was homogenised using a riffle sample divider to obtain representative, uniform subsamples, and to ensure consistent physicochemical characteristics during subsequent analyses and experiments. The material was then dried overnight at 105°C in a forced-air laboratory oven (Gallenkamp, UK) before use. The anaerobic microbial inoculum was obtained from fresh-digested sewage sludge sampled from a full-scale AD sewage sludge treatment plant in the UK. The inoculum was thoroughly homogenised using a kitchen hand mixer and stored in clean, sealed polyethylene bottles. The bottles were maintained in a temperature-controlled laboratory incubator (MIR 253, Sanyo, Japan) in the dark at 30°C for a maximum period of 5 d. Procedures to maintain consistent microbial activity in the inoculum source for batch AD experiments were followed (Koch et al., 2019).

2.2. Alkali pretreatment experiments

Barley straw was pretreated with three alkali pretreatment agents: CaO, NaOH, and prepared CFA to establish a comparative experimental basis under comparable alkalinity conditions. Calcium oxide, identified as the primary reactive alkaline component in fresh CFA (Achternbosch et al., 2015), was used as a reference alkali to simulate the different alkalinity conditions achievable with CFA. Three alkalinity concentrations were used: ~2,500 mg $CaCO_3$ L^{-1} (C1), ~5,000 mg $CaCO_3$ L^{-1} (C2), and 11,000 mg $CaCO_3$ L^{-1} (C3), corresponding to the addition to, and mixing with, 40 mL deionised water of 0.09, 0.16, and 0.38 g of oven-dried CaO, respectively. Sodium hydroxide pretreatment used 0.38 g of oven-dried NaOH dissolved in 40 mL of

144 deionised water (labelled S3), to provide alkalinity equivalent to the C3 treatment. For
145 pretreatment using prepared CFA (labelled CFAp), 2.5 g of prepared CFA was mixed
146 with 40 mL of deionised water. All alkali solutions were added to 5.0 g of ground BS in
147 150 mL glass beakers and manually homogenised using a laboratory spatula to
148 ensure thorough soaking. Care was taken to ensure that no residual BS remained on
149 the spatula to avoid errors in mass loss calculations before and after pretreatment.
150 Two control conditions were included, which were Untreated BS and BS treated with
151 deionised water (WT) (prepared following an equivalent pretreatment incubation
152 procedure with 40 mL deionised water added and mixed with 5.0 g ground BS, but
153 without alkali addition). The beakers were sealed with laboratory airtight film to prevent
154 evaporation and incubated at 30°C for 7 d. Upon completion of the pretreatment, BS
155 was filtered through a fritted crucible and rinsed with deionised water until the small
156 batch volume of washing liquid collected in the beaker reached a neutral pH of 6.8–
157 7.5. The pretreated BS was oven-dried at 50°C to constant weight, and the mass
158 before and after pretreatment was recorded. Dried pretreated BS was stored in sealed
159 plastic bags in a laboratory refrigerator in the dark at 4°C before fibre analyses and BS
160 characterisation.

161 An alkalinity of ~2,500 mg $CaCO_3$ L^{-1} (equivalent to 3–4%TS free CaO of the
162 total CFA mass) was tested as the minimum concentration, as this range is commonly
163 reported for CFA produced by coal-fired power plants in Indonesia (2.4–19.7%TS)
164 (Prayoga and Afla 2023). In addition, the direct effects of similar CaO concentrations
165 on BS pretreatment have not previously been reported. Whereas ~5,000 mg $CaCO_3$
166 L^{-1}, which represents a higher alkalinity found in CFA, has been reported to effectively
167 reduce recalcitrance in various LB (Sabeeh et al. 2020; Mansour et al. 2024) and was
168 tested for BS in the present study to enable comparison with literature. The highest

CaO concentration tested (~11,000 mg $CaCO_3$ L^{-1}), corresponding to the global upper limit of free CaO content in CFA (15%) (Izquierdo and Querol, 2012; Achternbosch et al., 2015), was used to assess whether increasing alkalinity is directly proportional to fibre biodegradability and CH_4 yield. Sodium hydroxide, an aggressive and highly soluble alkali (1.11 g NaOH per litre H_2O at 30°C), was included as a benchmark to assess pretreatment effectiveness, as its complete solubility ensures full alkalinity availability, unlike CaO which has limited solubility (1.14–1.16 g CaO per litre solution at 30°C), therefore expected to give a maximum alkali response.

2.3. Solid-state anaerobic digestion experiments

Methane yields from alkali-pretreated, water-treated, and untreated BS were evaluated in batch solid-state AD in 1L borosilicate glass chemostat reactors with a working volume of 0.3–0.4L. For each reactor, 15.0 ± 0.1 g of ground BS (prepared following procedures in 2.1) was placed in a 1L beaker glass and subjected to 7 d pretreatment using: (i) deionised water (WTa) as the control; (ii) CaO at an alkalinity equivalent to C3 (CaO-IIIa) treatment; and (iii) prepared CFA supplemented with pure, inorganic CaO to match the alkalinity of C3 (CFAS). This supplementation in CFAS was required to represent the alkalinity functionality of prepared CFA, as its free CaO content was too small (see Section 3.1), resulting in inadequate alkalinity to change the fibre composition (see Section 3.2). Therefore, the alkalinity level was adjusted to the upper range commonly reported for CFA to enhance the sensitivity of the experimental response. Pretreatments were conducted according to the procedure described in Section 2.2. After 7 d pretreatment, pretreated BS was transferred into the chemostat AD reactors and inoculum was added at a 1:1 ratio to BS on a volatile solids (VS) basis (Koch 2019).

194 To validate the supplementary contributions of CFA beyond alkalinity effect
195 tested above, a separate batch AD experiment was performed. In this case, four
196 conditions were included: (i) BS pretreated with CaO at the same concentration as
197 CaO-IIIa, but prepared CFA was added at the end of the pretreatment period (labelled
198 as 'CaO-III+CFA'), before AD; (ii) NaOH-treated BS with NaOH concentration
199 equivalent to CaO-IIIa condition (labelled as: NaOH); (iii) CaO-treated BS at a
200 concentration equivalent to C2 (labelled as: CaO-II); and (iv) BS treated with CaO
201 concentration equivalent to C3 (labelled as: CaO-IIIb). Two controls were included,
202 which were untreated BS and water-treated BS (WTb). A blank reactor containing only
203 inoculum was included to determine the background CH_4 yield, which was subtracted
204 from the CH_4 produced in all AD batches containing BS.

205 Concentrated HCl acid (3–4 mL) was added to neutralise the pH of the
206 pretreated BS in the NaOH, CaO-IIIa, CaO-IIIb, CFAS, and CaO-III+CFA treatments,
207 where the pH was above 8. The pH of pretreated BS in CaO-II treatment was not
208 adjusted, as the pH was 7.8 ± 0.1. To achieve a final TS concentration of 15%, 50–70
209 mL of deionised water was added to CFAS and CaO-III+CFA treatments. All mixtures
210 in the reactors were thoroughly homogenised using a kitchen hand mixer and
211 incubated in a temperature-controlled incubator (MIR 253, Sanyo, Japan) in the dark
212 at 30^{o}C to simulate typical ambient conditions in Indonesia.

213 The chemostat AD reactors were connected by silicon tubing to a water
214 displacement system, comprising an inverted 500 mL high-density polyethylene
215 (HDPE) bottle filled with water. The bottle was equipped with a hypodermic needle
216 pierced through a rubber septum on the cap, allowing displaced water to drain into a
217 pre-weighed receiver bottle, representing the biogas volume produced. Before starting
218 the AD experiment, the reactors were purged with N_2 for 10 minutes. After sealing the

reactor, the oxygen (O_2) concentration was subsequently measured using a gas chromatograph (GC) (Clarus 600, Perkin Elmer, USA) to verify anaerobic conditions. Biogas samples (50 µL) were sampled weekly through the septum using a 100 µL Hamilton gas-tight syringe (Hamilton, USA) and analysed for CH_4 composition using GC (Clarus 600, Perkin Elmer, USA). The AD experiments were terminated when the cumulative biogas yield had increased to a maximum stable value, or when the daily production fell below 0.5% of the total volume for three consecutive days, which was typically within 30–40 d duration (VDI, 2016).

2.4. Methods of chemical analysis

The determination of TS and VS followed standard protocol 2540 B (APHA, 2005), while pH was measured using a pH meter calibrated with pH 4, 7, and 10 buffer solutions. The alkalinity of CaO and NaOH solutions used for pretreatment was determined by titration with 0.1 M HCl to a pH of 4.5. Specifically, for the C3 and S3 treatments, the measured alkalinity values, 11,163.1 ± 12.8 and 10,916.7 ± 164.5 mg $CaCO_3$ L^{-1}, respectively, were considered to represent an equivalent alkalinity of approximately 11,000 mg $CaCO_3$ L^{-1} and were used as the basis for comparing the pretreatment conditions. Fibre composition was determined in accordance with BS EN 16472:2006 for amylase-treated neutral detergent fibre (aNDF) (BSI, 2006), representing total fibre content, and BS EN ISO 13906:2008 for acid detergent fibre (ADF) and acid detergent lignin (ADL) (BSI, 2008). The residue remaining after the ADF procedure represents cellulose and lignin, whereas the fibrous material lost during ignition in the ADL procedure was lignin. Cellulose content was calculated as the difference between the ADF and ADL values, while hemicellulose content was determined by subtracting ADF from the total fibre fraction (aNDF). The functional

groups of treatment samples were analysed using Fourier-transform infrared spectroscopy (FTIR) (iS50 Nicolet FTIR Spectrometer, Thermo Fisher Scientific, USA) in the range of 800–4000 cm^{-1}. The total crystallinity index (TCI) value, a measure of the relative amount of the crystalline fraction of cellulosic materials, was determined from the FTIR spectra (Shafiei et al., 2014). Trace elements were quantified using an inductively coupled plasma optical emission spectrometer (ICP-OES) (AVIO 500, Perkin Elmer, USA).

2.5. Statistical analysis of data and modelling CH_4 gas yield and kinetics

The First-order and Cone models were fitted to pooled triplicate experimental data to estimate representative kinetic parameters of the AD process, including maximum specific CH_4 yield ($CSMY_o$) and hydrolysis rate constants (K_H and k) (Karki et al., 2022). The models were also fitted to individual replicate experimental data to enable statistical comparison between treatment groups. Treatment effects were evaluated using one-way ANOVA followed by Tukey's post hoc test (α = 0.05) (Shekhar Bose et al., 2021). Data modelling and statistical analysis were performed in SPSS 28.0. The equations for the First-order and Cone kinetic models are shown in Equations 1 and 2, respectively.

$$CSMY = CSMYo\ (1 - exp(-kt)) \quad (1)$$

$$CSMY = \frac{CSMYo}{1 + (K_H t)^{-n}} \quad (2)$$

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.

3. Results and discussion

3.1 Waste characteristics

The average TS contents in BS, prepared CFA, and inoculum digested sludge were 94.1%, 99.7%, and 20.1%, respectively. The average VS contents were 92.6% of TS for BS, 6.8% of TS for prepared CFA, and 68.5% of TS for the inoculum. The high TS of BS makes it suitable for solid-state AD conditions (>15%TS), offering advantages such as reduced water usage and optimised reactor volume. The average pH values were 6.4, 12.1, and 8.4 for BS, prepared CFA, and the inoculum source, respectively. The high pH of prepared CFA indicated its suitability for alkali pretreatment. Calcium oxide is present at 4.2%TS in the CFA sample, of which 0.4%TS was free CaO. Free CaO is the leachable, reactive form, whose contact with water will form hydroxide ions, thus a more suitable parameter to define CFA alkalinity than the total CaO. The CaO content was consistent with values reported for UK CFA (Sear et al., 2003), however, the free CaO was considered very small, compared to the potential maximum content of 15%TS (Izquierdo and Querol, 2012; Achternbosch et al., 2015. Prolonged storage time of the CFA sample at the power plant exposed free CaO to atmospheric CO_2, leading to $CaCO_3$ formation and reduced alkali potency (Siriruang et al., 2016). Although CFAS pretreatment required CaO supplementation due to the low CaO content of the CFA sample, this approach provided an experimental advantage by enabling the isolation of alkali-driven effects from other stimulatory chemical properties of CFA. The average cellulose, hemicellulose, and lignin contents of Untreated BS (Figure 1) were 44.3%TS, 33.1%TS, and 8.6%TS, respectively, which are consistent with values reported in the literature (Bachmann et al., 2022). In the digested sludge, these values were 19.4%TS, 4.5%TS, and 13.9%TS, respectively, which were 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).

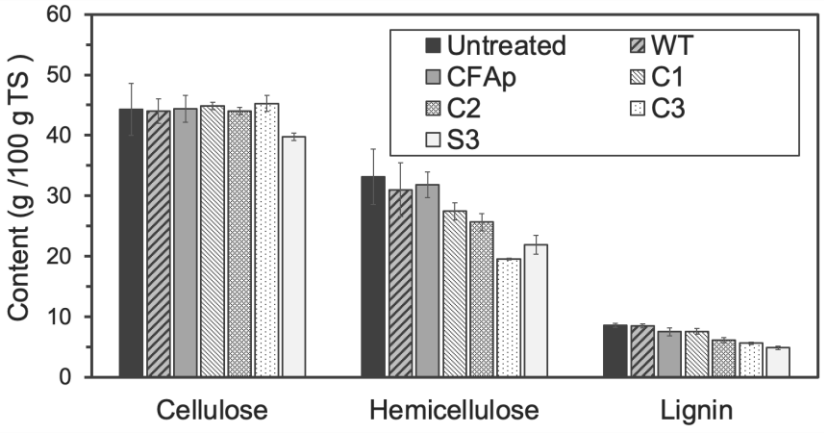


Figure 1. Mean cellulose, hemicellulose and lignin composition of untreated barley straw and pretreated barley straw (g per 100 g original, prepared barley straw (BS) sample on a TS basis) following pretreatment with: (i) deionised water (WT), (ii) prepared coal fly ash sample (CFAp), (iii) CaO ~2,500 mg $CaCO_3$ L^{-1} (C1), (iv) CaO ~5,000 mg $CaCO_3$ L^{-1} (C2), (v) CaO ~11,000 mg $CaCO_3$ L^{-1} (C3) and (vi) NaOH ~11,000 mg $CaCO_3$ L^{-1} (S3). Error bars represent the standard deviation (n=3).

3.2 Mass loss and fibre composition

The WT control treatment showed a small, albeit not significant ($P > 0.05$) mass loss of 2.2% (Figure S1), compared to Untreated BS, which may be attributed to the removal of water-soluble components. In contrast, S3 showed a significant ($P = 0.01$) mass loss of approximately 20.3%, compared to both Untreated BS, and WT. Although C3 had an equivalent alkalinity concentration to S3, the overall mass loss was significantly smaller (15.8%, $P < 0.001$). The reduced mass loss observed with CaO compared to NaOH pretreatment aligned with Bolado-Rodríguez et al. (2016), who reported greater mass loss in switchgrass after 7 d of NaOH pretreatment (29%) than with CaO (20%). The mass loss observed in C3 and S3 (15%–20%) was comparable to the value of 14% reported by Liu et al., (2015) for KOH pretreatment of wheat straw at a similar alkalinity level. C3, however, gave a significantly ($P = 0.04$) greater mass

loss than C2 and was also significantly different from Untreated BS and WT (P < 0.001). The mass loss observed during pretreatment at the alkalinity values supplied in C2, C3, and S3 (>10%) may be attributed to bond cleavage and structural alterations in fibre components, leading to depolymerisation and the release of water-soluble compounds (Martins et al., 2022). A statistically significant difference (P < 0.001) in mass loss was observed between C2 (10.6%) and C1 (2.2%). The mass loss in C2 was consistent with the 9% loss reported in the literature under similar alkalinity conditions (Liu et al., 2015). C1 showed no significant mass loss (P > 0.05), compared to CFAp, which was equivalent to 3% compared to the initial substrate mass in this condition, and which, similarly, was not significantly different from Untreated BS.

Mass loss across all experimental treatments was consistent with the overall reduction in fibre content, and was in decreasing order: S3>C3>C2>C1≈CFAp≈WT. Cellulose, hemicellulose, and lignin contents were determined separately and normalised to the original mass of prepared BS. Cellulose content was not affected by pretreatment (42–46% TS) under all conditions except S3, where it decreased to 38.3% TS (P > 0.05) (Figure 1). The results for cellulose content in most treatments, therefore, indicated that cellulose was conserved during alkali pretreatment. This was consistent with the primary mechanism of alkali pretreatment, which targets the disruption of the lignin structure, cleavage of lignin carbohydrate complexes (LCCs), and partial solubilisation of hemicellulose, rather than the degradation of glycosidic bonds that constitute the cellulose backbone (Kim, 2018; Zdeb, 2021). However, the potential accessibility of cellulose to enzymatic and microbial degradation can increase when hemicellulose and lignin are solubilised, resulting in greater cellulose degradability during subsequent AD (Barman et al., 2020).

338 WT control treatment did not significantly (P > 0.05) affect hemicellulose and
339 lignin, compared to Untreated BS, showing 5–12% reduction (resulting in the final
340 contents of 32.4%TS for hemicellulose and 8.1%TS for lignin. These minimal changes
341 aligned with the negligible mass loss observed in WT. Similar results were reported by
342 Kumar et al. (2019), and showed that water pretreatment of wheat straw reduced
343 hemicellulose by only 3%, and cellulose and lignin by <1%, while Sambusiti et al.
344 (2013) observed no changes at 40^{o}C, but noted a significant hemicellulose reduction
345 at 100^{o}C.
346 In S3, the final lignin content was 4.9%TS, corresponding to significant (P <
347 0.001) reductions of 43% and 40% relative to Untreated BS and WT, respectively,
348 representing the largest overall lignin reduction among all treatments. Hemicellulose
349 content in S3 was 22.4%TS, and was significantly (P < 0.001) smaller compared to
350 Untreated BS and WT, representing reductions of 39% and 31%, respectively.
351 Although hemicellulose and lignin contents in S3 were not significantly (P > 0.05)
352 different from C3, the overall mass loss in S3 was significantly higher, probably due to
353 total fibre loss, as lignin and cellulose contents in S3 were 11% and 16% smaller,
354 respectively, relative to C3. The smaller lignin content in S3 compared with C3 was in
355 good agreement with the literature, where NaOH achieved greater lignin removal than
356 CaO–24% more in (Mafa et al. 2020), and 36% with NaOH versus 30% with CaO in
357 Monlau et al. (2012). The statistically insignificant difference in lignin and
358 hemicellulose content between S3 and C3 suggests that, despite the lower solubility
359 of CaO compared to NaOH, a 7 d pretreatment duration allowed sufficient dissolution
360 of CaO to effectively disrupt hemicellulose and lignin. The use of the same 7 d duration
361 for both C3 and S3 conditions ensured a fair comparison, although previous studies

362 reported that NaOH is more reactive than CaO and can act more rapidly, requiring
363 only 3 d for complete reaction (Song et al., 2013).

364 In C3, hemicellulose and lignin contents were 18.5%TS and 5.5%TS,
365 respectively, representing reductions of 50% and 36% (P < 0.001) compared to
366 Untreated BS, and 43% and 33% (P < 0.001) compared to WT. The higher
367 hemicellulose reduction rate reflects the greater susceptibility of hemicellulose to alkali
368 pretreatment due to its amorphous, branched structure, compared to the hydrophobic,
369 three-dimensional lignin matrix (Barman et al., 2020). The results were consistent with
370 reported ranges in the decrease in hemicellulose and lignin of 18%–60% and 20%–
371 66%, respectively, following alkali pretreatment of LB (Dai et al., 2018; Kumar et al.,
372 2019).

373 C3 achieved greater reductions in hemicellulose (27%) and lignin (11%) than
374 C2, although the differences were not statistically significant (P > 0.05), suggesting
375 that alkalinity ~5,000 mg $CaCO_3$ L^{-1} appeared to provide favourable alkalinity
376 conditions under the conditions investigated, as further increase to ~11,000 mg $CaCO_3$
377 L^{-1} did not result in significant additional improvements.

378 In C2, hemicellulose content was 25.5%TS, corresponding to a reduction of
379 31% (P = 0.016) compared to Untreated BS, whereas the reduction relative to WT was
380 smaller (21%) and not statistically significant (P > 0.05). The lignin content in C2 was
381 6.2%TS, representing significant (P < 0.001) reductions of 28% and 24% compared to
382 Untreated BS and WT, respectively. These reduction levels fell within reported ranges
383 in the decrease in hemicellulose and lignin of 11–39% and 8–28%, respectively (Gu
384 et al., 2015; Dai et al., 2018). Compared to C1, the lignin content in C2 was significantly
385 (P = 0.005) smaller, with C1 containing 6.9%TS lignin. In contrast, hemicellulose

386 content in C2 condition was not significantly (P > 0.05) different compared to C1
387 (27.5%TS).

388 Hemicellulose and lignin contents in C1 were not statistically significant (P >
389 0.05), which the reduction equals to 17% for hemicellulose and 11% for lignin
390 compared to Untreated BS, and 11% and 10% compared to WT. The results indicated
391 that an alkalinity level of ~5,000 mg $CaCO_3$ L^{-1} (as in C2) was the minimum alkalinity
392 required for effective reduction of recalcitrant characteristics in BS under the
393 experimental conditions investigated, consistent with those reported in the literature
394 (Gu et al., 2015; Noonari et al., 2019).

395 The CFAp treatment, which provided the lowest alkalinity, resulted in no
396 significant (P > 0.05) changes in fibre composition. Nevertheless, lignin content was
397 reduced by 11% compared to WT, similar to C1, likely due to the relatively small
398 difference in alkalinity concentration between CFAp (~1,500 mg $CaCO_3$ L^{-1}) and C1
399 (~2,500 mg $CaCO_3$ L^{-1}). The limited effect observed under CFAp condition was
400 attributed to the low alkalinity of the prepared CFA due to the low free CaO content of
401 the sample (Section 3.1). Using fresh CFA is therefore crucial, as long exposure to
402 atmospheric CO_2 and carbonation of CaO reduces its reactivity (Siriruang et al., 2016).
403 The similar trends observed between CFAp and C1 suggested that the fibre changes
404 observed under C2 and C3 may reflect the effects achievable during CFA pretreatment
405 if sufficient alkalinity was present.

406

407 **3.3 Chemical structures**

408 C1 and CFAp treatments were excluded from FTIR characterisation due to their
409 insufficient alkalinity to cause significant changes in fibre composition. Since C2 and
410 C3 treatments used pure CaO as the alkali agent, which is the primary reactive alkaline

component in CFA, the FTIR results obtained from these treatments may provide insight into the functional group changes that could occur during CFA pretreatment under higher alkalinity conditions. A reduction in FTIR absorbance was observed in the 1730–1740 cm^{-1} region, with intensity decreasing in the order S3<C3<C2 (Figure S2). This region is associated with the cleavage of ester and ether bonds linking lignin to carbohydrate complexes–the primary target of alkali pretreatment (Mafa et al., 2020). This cleavage leads to hemicellulose solubilisation and lignin depolymerisation, as evidenced by reduced hemicellulose and lignin contents under C2, C3, and S3 treatment conditions (Figure 1).

Further evidence of hemicellulose degradation was shown by decreased absorbance in the 1730–1740 cm^{-1} region, with significantly lower absorbance observed for C3 and S3 compared to C2, corresponding to acetyl and uronic groups in hemicellulose (Bachmann et al., 2022; Pancholi et al., 2023). Moreover, the reduction in absorbance was observed in the 1140–1170 cm^{-1}, 1000–1100 cm^{-1} and 890 cm^{-1} regions, which are assigned to glycosidic bond cleavage within hemicellulose (Md Salim et al., 2021; Pancholi et al., 2023), with the reduction was more pronounced in C3 and S3 than in C2, consistent with the compositional results. No discernible difference in absorbance was observed between C3 and S3, consistent with their comparable hemicellulose contents (Figure 1).

The decreased FTIR absorbance in the 1215–1260 cm^{-1}, and 1500–1600 cm^{-1} regions was observed under C2, C3, and S3 conditions, supporting the fibre composition result. These regions are associated with ether bond cleavage, which is a major linkage in lignin (Grgas et al. 2023), and aromatic ring disruption (Md Salim et al., 2021; Pancholi et al., 2023), respectively. This cleavage renders lignin more susceptible to further depolymerisation into lower molecular weight compounds (e.g.,

phenolic acids, aldehydes, and simple aromatics), which can subsequently serve as microbial carbon sources (Grgas et al., 2023).

In contrast to hemicellulose and lignin, cellulose remained preserved across treatments, except for S3 (Figure 1). Although cellulose content remained unchanged in C2 and C3, FTIR spectra showed structural alterations in the 3200–3500 cm^{-1} region. The greater absorbance reduction in S3, compared to WT–relative to that seen in C3–was likely attributed to changes in cellulose, supported by a 16% cellulose reduction and greater mass loss in S3 than C3, indicating cellulose solubilisation, albeit marginally. Changes in this region are associated with disruption of hydroxyl–related hydrogen (H) bonding, which affects cellulose crystallinity and water insolubility (Hermosilla et al., 2018).

The effect of alkali pretreatment on cellulose crystallinity was further reflected in the TCI values, with a smaller TCI value indicating a reduced cellulose crystallinity. The TCI of WT (0.4) was comparable to literature values for wheat straw (0.5) (Hermosilla et al., 2018), while S3 showed the smallest TCI value (0.2; ~50% lower than WT), followed by C3 (0.2) and C2 (0.3). The smaller TCI value in S3 is attributed to the higher reactivity of NaOH and prolonged incubation (7d) (Bali et al., 2015), which facilitates partial dissolution of amorphous cellulose, leading to reduced cellulose content observed in the fibre analysis.

3.4 Mass balance of fibre components

Mass balances for cellulose, hemicellulose, and lignin were calculated on a VS basis to quantify the contribution of pretreatment in solubilising fibre and AD in degrading solubilised organic compounds under CaO-IIIa, CFAS, and WTa treatments. The solubilisation rate during pretreatment corresponds to the reduction

461 rate reported in Section 3.2. The BS fed to the AD reactors in all treatments was 11.5
462 ± 0.5 g VS, comprising 5.3 cellulose, 4.6 g hemicellulose, and 1.1 g lignin (Figure S3).
463 Hemicellulose showed the greatest overall reduction across all treatments during both
464 pretreatment and AD steps. In WT, hemicellulose decreased to 4.1 g during
465 pretreatment and to 2.1 g after AD, representing a 50% reduction. While cellulose and
466 lignin in WT were largely unaffected during pretreatment, 2.0 g of cellulose was
467 degraded during AD, leaving 4.7 g in the digestate (10% reduction), whereas lignin
468 remained unchanged at 1.1 g.

469 A more pronounced effect was observed under CaO-IIIa treatment, where the
470 pretreatment step solubilised 2.3 g hemicellulose and 0.4 g lignin, giving the final mass
471 of 2.3 g hemicellulose and 0.7 g lignin in the pretreated BS. Hemicellulose, lignin, and
472 cellulose were further degraded by AD, resulting in final mass residual fibres of 0.3 g,
473 0.6 g, and 4.1 g in the digested BS, equating to 85%, 13%, and 22% reduction during
474 AD alone. FTIR analysis confirmed that CaO pretreatment (C3) cleaved ester and
475 ether bonds within the LCCs structure (see Section 3.2), releasing hemicellulose that
476 was previously bound to lignin. This resulted in a greater fraction of hemicellulose
477 being degraded during AD under CaO-IIIa compared to WTa. This bond cleavage may
478 also explain the reduction in lignin during AD for CaO-IIIa.

479 Cellulose, however, was not solubilised during CaO-IIIa pretreatment, similar
480 to WTa. During AD, cellulose degraded from 5.3 g to 4.1 g, representing 22% reduction
481 relative to pretreated BS (before AD), compared to only 10% reduction under WTa.
482 This enhanced digestibility in the AD process was attributed to improved access to
483 cellulose following lignin and hemicellulose solubilisation during CaO-IIIa
484 pretreatment.

The degree of hemicellulose and lignin solubilisation under CFAS treatment was similar to that observed under CaO-IIIa, as expected given their equivalent alkalinity conditions. However, after 40 d of digestion, the CFAS condition resulted in significantly lower residual cellulose and lignin contents–giving the final mass of 2.5 g and 0.4 g–reduced by 38% and 30%, respectively, compared to CaO-IIIa. Additionally, hemicellulose was completely degraded under CFAS, compared to 85% degradation under CaO-III. Total fibre (sum of cellulose, hemicellulose, and lignin) reduction during AD was 39% for CaO-IIIa and 64% for CFAS. When combining pretreatment and AD, the overall fibre reduction reached 54% for CaO-IIIa and 73% for CFAS, compared to only 28% for WTa. These results suggested that the presence of CFA may have stimulated the growth or activity of fibre-degrading anaerobic microorganisms, thereby enhancing fibre degradation.

3.5 Effects of coal fly ash pretreatment on AD performance

A comprehensive interpretation of the effects of CFA pretreatment, on the anaerobic degradation dynamics of BS was conducted by integrating results for VS removal, kinetic parameters (maximum CH_4 yield potential ($CSMY_o$), and hydrolysis rate. The hydrolysis rate and $CSMY_o$ values estimated from First-order and Cone kinetic models as well as the mean experimental CSMY ($CSMY_{exp}$) for all treatment conditions (WTa, CaO-IIIa, CFAS, WTb, NaOH, CaO-II, CaO-IIIb, and CaO-III+CFA), were presented in Table 1. The mean VS removals were 33.3% ± 1.5, 44.3% ± 2.9, 57.0% ± 4.0, 31.0% ± 1.0, 60.3% ± 3.0, 40.7% ± 9.2, 45.3% ± 3.2, and 60.0% ± 6.0, respectively.

Both models provided a good fit to pooled triplicate experimental data, as indicated by R^2 values ranging from 0.74 to 0.94 for the First-order model and 0.78 to

0.96 for the Cone model. The Cone model was used to estimate $CSMY_o$ and the hydrolysis rate, as it generally showed less systematic deviation from the $CSMY_{exp}$ data pattern than the First-order model (Figure 2). In addition, the Cone model fitted well with individual replicate experimental data, as indicated by R^2 values ranging from 0.93 to 0.99 (Table 1). The $CSMY_o$ derived from the Cone model, however, deviated significantly from the $CSMY_{exp}$ (with the difference exceeding 5%) across all treatment conditions. This inconsistency appeared to arise from the extrapolation of the asymptote beyond the 40 d of the experimental period, even far later than the typical retention time used in most industrial biogas plants processing LB (60–90 d) (Weiland, 2006), therefore considered unreliable. Consequently, the $CSMY_{40}$ was calculated for the 1–40 d data range using the Cone model, where these values were similar to the $CSMY_o$ determined by the First-order model (Table 1), which provided a more reliable estimate of the ultimate CSMY from AD of pretreated BS.

The CSMY in WTa and WTb (90.0–109.2 mL g^{-1} VS) were comparable to CH_4 yields reported in literature for LB. Studies on the AD of wheat and rice straw have reported comparable values in the range of 80–130 mL CH_4 g^{-1} VS (Brown et al., 2012; Riya et al., 2021). The CSMY of CaO-IIIa and CaO-IIIb (124.4–160.2 mL g^{-1} VS) were comparable to the biogas yield of 200 mL g^{-1} VS (CH_4 composition not specified) reported following pretreatment for BS with equivalent alkalinity (Bachmann et al., 2022).

Table 1. Kinetic parameters ($CSMY_o$, K_H) of First-order kinetic and Cone models from anaerobic digestion of barley straw following coal fly ash pretreatment; $CSMY_{40}$ values from the Cone model and $CSMY_{exp}$ are also shown.

Treatment conditions	$CSMY_{exp}$[a]	First-order Model		Cone Model					
		$CSMY_o$[b]	R^2[c]	$CSMY_o$	$CSMY_{40}$[d]	K_H[e]	R^2[f]	K_H[g]	R^2[h]
Batch I									
WTa	93.1 ± 5.2	94.9 c	0.91	108.2 b	90.0 c	0.09 a	0.91	0.09 ± 0.03 a	0.99
CaO-IIIa	122.9 ± 8.7	122.2 b	0.94	155.8 ab	124.4 b	0.11 a	0.94	0.11 ± 0.13 a	0.99
CFAS	163.6 ± 16.6	157.9 a	0.74	225.3 a	174.1 a	0.14 a	0.78	0.17 ± 0.07 a	0.99
Batch II									
WTb	108.9 ± 2.4	107.3 d	0.91	158.0 b	109.2 d	0.07 a	0.95	0.09 ± 0.03 a	0.93–0.99
NaOH	179.8 ± 7.9	174.5 c	0.92	180.4 b	177.3 c	0.35 b	0.93	0.34 ± 0.08 b	0.98–0.99
CaO-II	139.7 ± 5.1	133.4 b	0.87	192.8 b	140.7 b	0.11 a	0.92	0.13 ± 0.03 a	0.99
CaO-IIIb	160.4 ± 6.5	153.8 b	0.94	198.4 b	160.2 b	0.12 a	0.96	0.14 ± 0.02 a	0.98–0.99
CaO-III+CFA	207.6 ± 18.7	205.1 a	0.83	266.9 a	211.1 a	0.11 a	0.85	0.14 ± 0.11 a	0.97–0.99

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

[a] $CSMY_{exp}$: mean experimental specific CH_4 yield from triplicate data. Data are expressed as mean ± SD (n=3).

[b] $CSMY_o$: maximum cumulative specific CH_4 yield potential (mL g^{-1} VS) (Equation 1 and 2).

[c] R^2: coefficient of determination for the First-order model fitted to pooled triplicate data.

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

[e] K_H: single, representative hydrolysis constant value estimated by fitting the Cone model to pooled triplicate data (Equation 2).

[f] R^2: coefficient of determination for the Cone model fitted to pooled triplicate data.

[g] K_H: hydrolysis constant estimated by fitting the Cone model to individual replicate data (Equation 2). Data are expressed as mean ± SD (n=3).

[h] R^2: coefficient of determination for the Cone model fitted to individual triplicate data

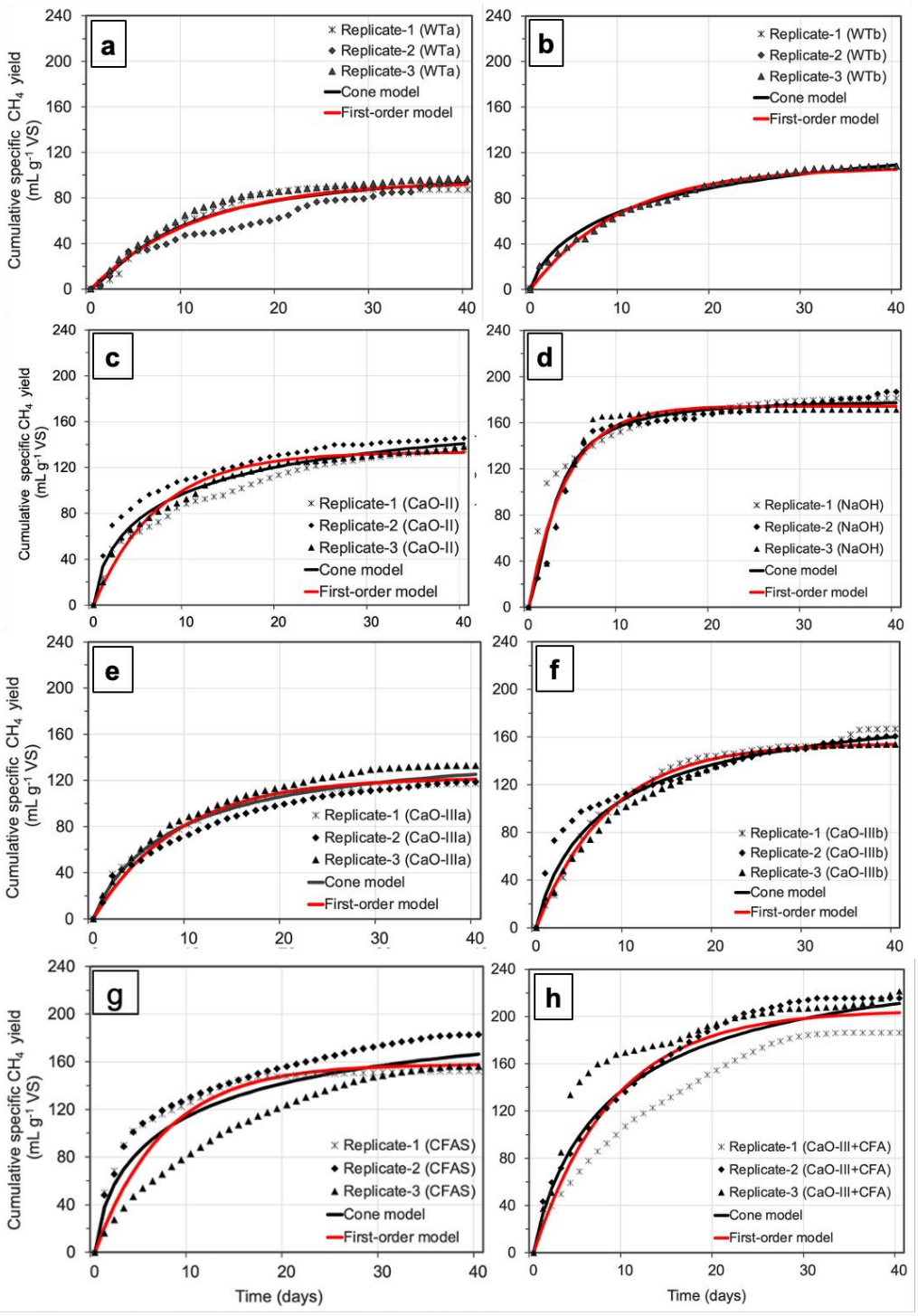


Figure 2. Experimental (n=3, dashed lines, measured daily) and modelled (First-order and Cone models, solid lines) cumulative specific CH_4 yields (CSMY) from anaerobic digestion of barley straw pretreated using: (i) water (WTa, WTb); (ii) CaO (~5,000 mg $CaCO_3$ L^{-1}; CaO-II); (iii) CaO (~11,000 mg $CaCO_3$ L^{-1}; CaO-IIIa, CaO-IIIb); (iv) NaOH (~11,000 mg $CaCO_3$ L^{-1}; NaOH); (v) CaO as in CaO-IIIa with prepared CFA incorporated during pretreatment (CFAS); and (vi) CaO as in CaO-IIIa with prepared CFA added after pretreatment, before AD (CaO-III+CFA).

552 The K_H in CaO-IIIa, (0.11 d^{-1}) was 22% higher than WTa (0.09 d^{-1}) (Table 1),
553 albeit not significant ($P > 0.05$), consistent with significantly higher VS removal (33%)
554 ($P = 0.01$) and CSMY (38%) ($P = 0.050$) The K_H in both CaO-II (0.11 d^{-1}) and CaO-IIIb
555 (0.12 d^{-1}) increased by 57% and 71%, respectively, compared to that of WTb (0.07 d^{-1}),
556 even though it was not significant ($P > 0.05$). These increases in K_H were consistent
557 with the significant increase in both CSMY and VS removal, with CaO-II and CaO-IIIb
558 showing 29% ($P = 0.046$) and 47% ($P < 0.001$) higher CSMY, and 31% and 46%
559 greater VS removal, respectively, compared to WTb. The magnitude of CSMY and VS
560 removal increased by 14% and 11% ($P > 0.05$), respectively, with the higher alkalinity
561 concentration, however, K_H between CaO-II and CaO-IIIb were comparable. The
562 increase in K_H values between water-pretreated BS (WTa and WTb) and CaO-
563 pretreated BS (CaO-II, CaO-IIIa, and CaO-IIIb), suggesting that the degradation rate
564 of organic compounds in BS was accelerated due to fibre solubilisation under CaO
565 pretreatment (Figure 1), which ultimately increased CSMY. These results aligned with
566 a previous study reporting a positive correlation between fibre solubilisation and
567 hydrolysis rate (Monlau et al., 2012). The obtained K_H for both water- and CaO-
568 pretreated BS fell within the reported values for untreated (0.06 to 0.11 d^{-1}) (Brown et
569 al., 2012; Bolado-Rodríguez et al., 2016) and alkali-pretreated LB (0.11–0.13 d^{-1})
570 (Noonari et al., 2019).

571 Although CaO pretreatment significantly improved CSMY compared to water-
572 treated BS, it was less effective than NaOH. NaOH pretreatment, which supplied an
573 equivalent alkalinity of ~11,000 mg $CaCO_3$ L^{-1}, similar to CaO-IIIa and CaO-IIIb, was
574 expected to be the most aggressive and reactive alkali pretreatment (Bali et al., 2015;
575 Bachmann et al., 2022). Indeed, this was reflected in the highest CSMY achieved
576 among the alkali pretreatment conditions without CFA addition in Batch II. NaOH

pretreatment increased CSMY by 11% compared to CaO-IIIb, albeit not significantly (P > 0.05), however, the VS removal was significantly (P = 0.039) higher in NaOH pretreatment, with a 33% increase. This maximum CSMY with NaOH pretreatment, however, was achieved approximately 10 d earlier than with CaO-IIIb (Figure 2 (f)), and K_H of NaOH pretreatment (0.35 d^{-1}) (Table 1) further supported its hydrolysis kinetics, being nearly three-fold higher (P = 0.022).

Notably, CSMY in the CFA-III+CaO condition was higher than both CaO-IIIb 32% (P < 0.001), and even than the maximum alkalinity effect achieved by NaOH pretreatment 19% (P < 0.001). The results were consistent with that achieved under CFAS, with CSMY was 39% higher (P = 0.019) than CaO-IIIa, suggesting CFA addition provides benefits beyond alkali supplementation alone. The enhanced CSMY observed in the CFAS and CFA-III+CaO treatments is unlikely to be explained by dilution of volatile fatty acids (VFAs) resulting from the additional 40–70 mL of deionised water added to these treatments. This interpretation is supported by the pH profile of the CaO treatment, which remained within the range generally considered favourable for methanogenesis (pH 5.9–7.2) during the early stage of AD, indicating no evidence of acidification under alkali pretreatment conditions. Furthermore, the CFAS and CFA-III+CaO treatments showed immediate and continuous CH_4 production without an observable lag phase (Figure 2 g-h), suggesting no evidence of acidification-induced inhibition or excessive VFA accumulation.

The enhanced CSMY was also unlikely to be attributable to trace element (TEs) supplementation from the prepared CFA. This was because TE concentrations in the inoculum, as indicated in WTa digestate, already exceeded the requirements for AD, indicating that TEs were not a limiting factor for AD in the absence of CFA. Specifically, the concentrations of Fe in both digestates (WTa: 10,060.0 ± 936.0 mg kg^{-1} TS, CFAS:

11,311.0 ± 715.0 mg kg^{-1} TS) and Mo (WTa: 18.9 ± 2.7 mg kg^{-1} TS, CFAS: 24.1 ± 2.1 mg kg^{-1} TS) exceeded the TEs requirement (Fe: 205.0–2,674.0 mg kg^{-1} TS; Mo: 1.7–7.0 mg kg^{-1} TS), while Mn (WTa: 142.4 ± 31.1 mg kg^{-1} TS, CFAS: 165.5 ± 7.6 mg kg^{-1} TS) and Co (WTa: 4.0 ± 1.2 mg kg^{-1} TS, CFAS: 4.4 ± 0.9 mg kg^{-1} TS) fell within the required range (Mn: 2.0–334.0 mg kg^{-1} TS; Co: 2.4–9.0 mg kg^{-1} TS) (Hinken et al., 2008; Banks et al., 2010; Facchin et al., 2013; Choong et al., 2016). Selenium was below the detection limit in WTa digestate but was present in CFAS (2.4 ± 0.5 mg kg^{-1} TS), slightly above the minimum threshold (1.6 mg kg^{-1} TS).

The VS removal in CaO-III+CFA increased significantly by 33% (P = 0.044) compared to CaO-IIIb, despite the representative K_H value being comparable. This result was consistent with 29% (P = 0.005), greater VS removal achieved under CFAS than CaO-IIIa, and a 27% higher K_H value (0.14 d^{-1} vs. 0.11 d^{-1}), albeit not statistically significant (P > 0.05). The higher VS removal and K_H in CFAS was consistent with a twofold greater reduction in total fibre during the AD step compared to CaO-IIIa (Section 3.4), highlighting the enhanced breakdown of organic matter with CFA addition. 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 improved AD performance. The specific properties of CFA contributing to the improvement of AD performance, irrespective of the pretreatment effects, warrant further investigation in future studies.

4. Conclusions

To increase the digestibility and CH_4 yield of LB during AD, LB pretreatment is required, however, the scalability of this approach has been constrained by the use of chemicals and associated operational costs. In the present study, the combined

effects of CFA pretreatment and AD resulted in a 73% reduction in total fibre, compared to 54% for CaO pretreatment and 28% for water pretreatment. The observed fibre degradation was associated with cleavage of ester bonds in LCCs, disruption of acetyl and uronic groups in hemicellulose, disruption of hydrogen bonds in lignin or the LCCs network, breakdown of glycosidic bonds, disruption of aromatic lignin structures, and transformation of crystalline regions into more amorphous structures. These compositional and structural changes increased CSMY, VS removal, and hydrolysis rate by 93%, 71%, and 55%, respectively, compared to the water-pretreated control. The results indicated that CFA has the potential to serve as an alternative to alkali chemicals in the AD of LB, although a minimum alkalinity of approximately 5,000 mg $CaCO_3$ L^{-1} was required for effective pretreatment. Beyond its alkalinity effect, CFA consistently increased CSMY when used either in the pretreatment or added after pretreatment before the AD. Both approaches increased CSMY by 39% and 32%, respectively, relative to the CaO pretreatment control. The results suggested that the positive effects of CFA pretreatment were not solely attributable to alkalinity or TE supplementation, and that other physicochemical properties of CFA, may also contributed to the improved AD performance. Further investigation is therefore warranted to clarify the mechanism involved.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT in order to enhance the clarity and readability of the text. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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E-supplementary data of this work can be found in the online version of this article.

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