

## Calcium oxide enhances the anaerobic co-digestion of excess sludge and plant waste: performance and mechanism

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### ABSTRACT

The study investigates the effect of the oxidant calcium oxide (CaO) on the codigestion of excess sludge (ES) and plant waste (PW) under mesophilic anaerobic conditions to enhance methane production. The findings indicate that CaO significantly elevated methane yield in the codigestion system, with an optimum CaO addition of 6% resulting in a maximum methane production of 461 mL/g volatile solids, which is approximately 1.3 times that of the control group. Mechanistic exploration revealed that CaO facilitated the disintegration of organic matter, enhanced the release of soluble chemical oxygen demand, and increased the concentrations of soluble proteins and polysaccharides within the codigestion substrate. The presence of CaO was conducive to the generation and biological transformation of volatile fatty acids, with a notable accumulation of acetic acid, a smaller carboxylic acid within the VFAs. The proportion of acetate in the CaO-amended group increased to 32.6–36.9%. Enzymatic analysis disclosed that CaO enhanced the activity of hydrolytic and acidogenic enzymes associated with the ES and PW codigestion process but suppressed the activity of coenzyme F420. Moreover, CaO augmented the nutrient load in the fermentation liquid. The study provides an alternative scheme for the efficient resource utilization of ES and PW.

**Key words:** CaO, codigestion, excess sludge, plant waste, volatile fatty acids

### HIGHLIGHTS

- CaO effectively promotes the codigestion of ES and PW to produce methane.
- CaO can efficiently promote the dissolution and hydrolysis of organic matter in co-digested substrates.
- CaO enhances the activity of key enzymes involved in hydrolysis and acidification processes but inhibits the activity of the F420 enzyme.
- CaO increased the release of ammonia nitrogen and phosphate byproducts from codigestion.

## 1. INTRODUCTION

Anaerobic digestion is one of the crucial methods for excess sludge (ES) treatment, effectively achieving sludge reduction, harmlessness, and the recovery of energy substances such as hydrogen, volatile fatty acids (VFAs), and methane (Wang *et al.* 2019; Zhou *et al.* 2024). However, the sole anaerobic digestion of ES often encounters challenges such as carbon-to-nitrogen (C/N) imbalance and excessively high concentrations of toxic substances (e.g., heavy metals and persistent organic pollutants), which can lead to low anaerobic digestion efficiency and, in severe cases, system collapse (Hagos *et al.* 2017; Xie *et al.* 2017; Zhang *et al.* 2023a, 2023b; Fu *et al.* 2024). Therefore, the anaerobic codigestion of ES and other organic solid wastes has been extensively studied.

Plant waste (PW), a significant component of municipal solid waste, is characterized by its high carbon content and recalcitrance (Wang *et al.* 2021a). The codigestion of ES with PW can effectively balance the C/N ratio and dilute toxicants within the ES, thus enhancing the resource utilization efficiency of organic matter. This codigestion strategy not only optimizes the carbon-to-nitrogen ratio but also mitigates the inhibitory effects of toxic substances on the anaerobic digestion process (Kan *et al.* 2022; Guan *et al.* 2024). The synergistic interaction between ES and PW in the co-digestion process can improve the hydrolysis and acidification phases, leading to an increased production of VFAs and subsequently enhancing methane generation (Kan *et al.* 2021a; Wang *et al.* 2021b). This approach presents a sustainable and efficient method for the valorization

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of waste materials while also addressing the challenges associated with individual anaerobic digestion of ES and PW. Wang *et al.* (2021b) reported that when the co-digestion mixture of ES and PW was at a 1:1 ratio, the C/N ratio of the substrate was 23.5/1, and the corresponding methane yield was 176.2 mL/g, which was higher than the methane yield from the sole digestion of either ES or PW. Although co-digestion of ES and PW can effectively balance the C/N ratio, the presence of recalcitrant organic matter in the co-digestion substrate often leads to suboptimal methane production. Various pretreatment strategies have been employed to enhance the breakdown of organic matter and improve methane yields, including the use of CaO<sub>2</sub>, hydrogen peroxide, and calcium peroxide (Atelge *et al.* 2020; Kan *et al.* 2021b; Kang *et al.* 2021; Park *et al.* 2022). Calcium oxide (CaO) has garnered significant attention due to its low cost, availability, and high oxidative efficiency (Xu *et al.* 2020). Xin *et al.* (2021) reported that CaO pretreatment can significantly enhance the production of VFAs during anaerobic fermentation of sludge and the synergistic action of key microbes in CaO-assisted fermentation acidification. Furthermore, the combined pretreatment of CaO and freeze–thawing improves the recovery of VFAs and dewatering properties of anaerobically fermented waste activated sludge, with the CaO–freezing/thawing pretreatment group showing a 448.0 mg COD/g increase in VFAs production (She *et al.* 2021). Recently, Zhang *et al.* (2023a, 2023b) utilized metagenomic techniques to reveal the mechanism by which CaO improves the dry anaerobic digestion of kitchen waste, where CaO facilitated the production of dissolved organic matter and the conversion of VFAs, thus avoiding negative impacts on biogas production. However, the impact of CaO on methane production from the co-digestion of ES and PW has not been reported to date, and the underlying mechanisms involved remain unclear.

Therefore, this study focuses on the investigation of CaO-enhanced anaerobic co-digestion of ES and PW for methane production and the analysis of the associated mechanisms. Initially, different concentrations of CaO were utilized to enhance the co-digestion of ES and PW. Subsequently, the metabolic processes of key organic matter within the CaO-strengthened co-digestion of ES and PW waste were explored. Finally, the changes in key enzyme activity and digestive byproducts during co-digestion of ES and PW enhanced by CaO were analyzed. The results of this study provide a reference for the efficient resource utilization of organic matter and the expansion of the application field of CaO.

## 2. MATERIALS AND METHODS

### 2.1. Source and characteristics of experimental materials

The ES used in the experiment was sourced from the sludge return pipe of the secondary sedimentation tank of a certain wastewater treatment plant. This wastewater treatment plant primarily manages municipal wastewater, which is characterized by the following parameters: pH 6.5–7.2, chemical oxygen demand (COD) 150–210 mg/L, ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) 45–50 mg/L, total nitrogen 65–70 mg/L, and orthophosphate 6.4–10.0 mg/L. After collection, the ES was allowed to settle for 24 h in the laboratory to remove the supernatant. Subsequently, large particulate impurities such as stones, wooden sticks, and plastics were manually removed, and the ES was then stored in a refrigerator at 4 °C for future use. The main characteristics of the ES are presented in Table 1.

The inoculum sludge was obtained from a continuously operated anaerobic reactor in a university laboratory, which primarily treats high-concentration food wastewater and recovers biogas. After retrieval, the inoculum sludge was filtered through a 0.45- $\mu$ m mesh to remove impurities and was then set aside for use. The main characteristics of the inoculum sludge are also displayed in Table 1.

PW was collected from the garden waste of a certain park. After collection, the garden waste was manually picked to remove stones, large plastics, metal pieces, and other materials, followed by grinding in a high-speed disruptor to a particle size of 1–2.0 mm. After grinding, PW and distilled water were thoroughly mixed in a mass ratio of 2:1. The mass ratio of sludge to garden waste in the co-digestion substrate was controlled at 1:1 (based on the dry weight of total suspended solids, TSS), and the main characteristics of the co-digestion substrate are shown in Table 1.

### 2.2. Experimental setup

The experiments were conducted in five identical sequencing batch reactors (SBRs), each with a working volume of 5.0 L. The SBRs were equipped with a mechanical stirrer controlled at a speed of 500–800 revolutions per minute (rpm) during the operation. Each SBR was fitted with a 2.0 L gas collection bag at the top and a 3.0 cm diameter sludge discharge port at the bottom. A schematic diagram of the experimental equipment used in this study is presented in Fig. S1. Initially, 3.0 L of the ES and PW mixture and 2.0 L of inoculum sludge were introduced into each SBR, resulting in a substrate-to-inoculum ratio of 3/2 (by volume). Subsequently, different amounts of CaO reagent were dry-weighed and added to each

**Table 1** | Main physicochemical properties of ES and PW used in the experiment

Parameter	unit	ES	PW
pH	/	7.1 ± 0.2	6.8 ± 0.2
TSS	g/L	4.6 ± 0.3	6.8 ± 0.1
VSS	g/L	3.5 ± 0.1	5.7 ± 0.2
SCOD	mg/L	124 ± 5.6	341 ± 12.3
SPN	mg/L	85 ± 5.2	82 ± 4.1
SPS	mg/L	32 ± 0.8	158 ± 3.5
NH <sub>4</sub> <sup>+</sup> -N	mg/L	16.5 ± 0.6	17.5 ± 0.7
PO <sub>4</sub> <sup>3-</sup> -P	mg/L	8.5 ± 1.1	13 ± 1.3

reactor to achieve the desired concentrations of 2, 4, 6, and 8%. One SBR without the addition of CaO served as the control group throughout the experiment. After the addition of all materials, high-purity nitrogen gas (over 99.99% purity) was sparged into each SBR to displace oxygen and maintain anaerobic conditions. Finally, the reactors were transferred to a constant temperature water bath for mesophilic wet anaerobic digestion (35 °C). The entire experimental process lasted for 30 days, during which the biogas release, soluble COD (SCOD), VFAs, and other parameters were regularly measured to track their changes.

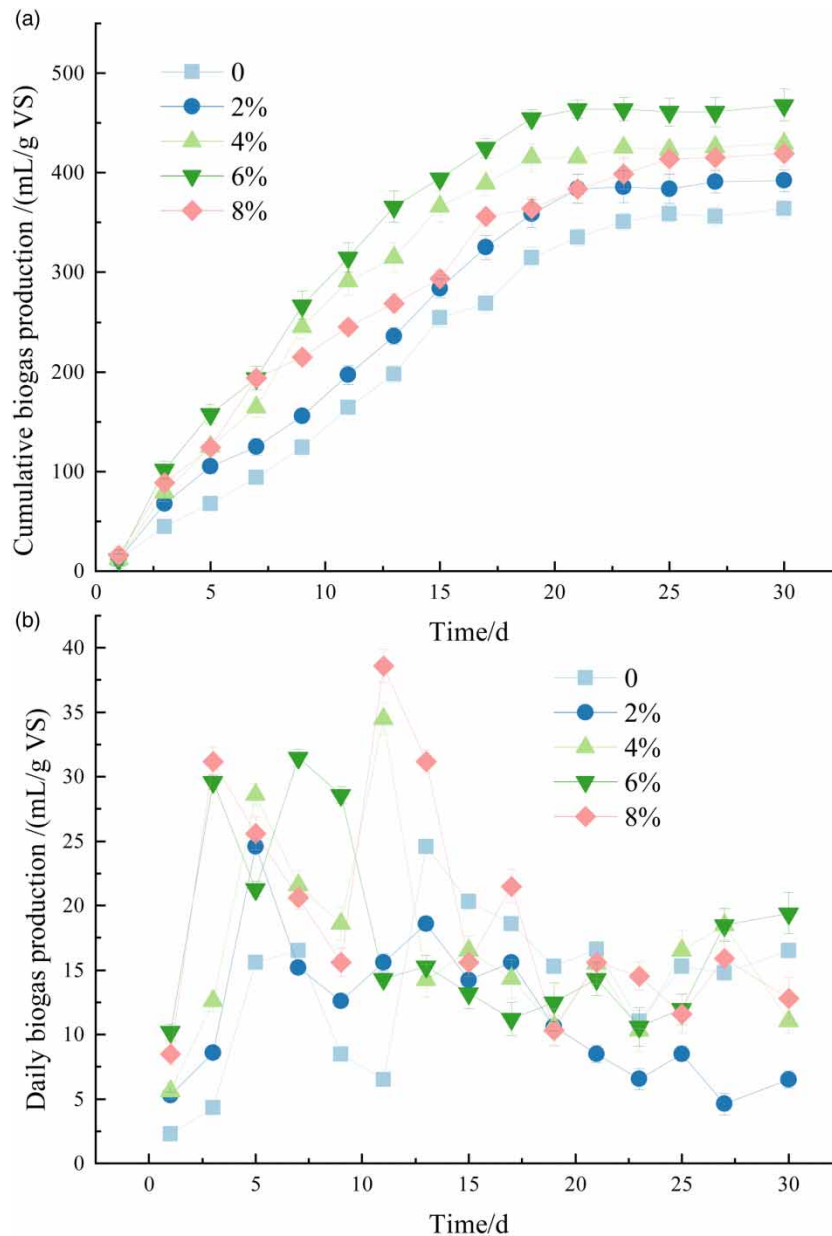
### 2.3. Analytical methods

Routine measurement indicators, such as SCOD, COD, TSS, VSS, NH<sub>4</sub><sup>+</sup>-N, and PO<sub>4</sub><sup>3-</sup>-P, were determined according to national standards (APHA 2005). VFA and biogas measurements were conducted using gas chromatography. The chromatograph used in the experiment was a GC-6890 equipped with a hydrogen flame ionization detector (FID). The specific procedure is as follows: 1 mL of filtered supernatant was taken in an Agilent gas chromatography vial, to which 0.1 mL of chromatographically pure formic acid was added to adjust the pH to ≤2.0 for acidification, and the VFA concentrations of the treated samples were determined using gas chromatography (GC-FID). Chromatographic parameters are Agilent GC-6890 gas chromatograph, a HP19095N-123 capillary column (35 m × 0.53 μm × 1 μm), and an auto-injection mode. The methane measurement procedure is as follows: anaerobic gas production was measured by the water displacement method, and the gas components were determined using the GC-FID. Chromatographic parameters are Agilent GC-7890 gas chromatograph, a HP-5-type column, a manual injection mode, a FID as the front detector and a thermal conductivity detector as the rear detector (Kan *et al.* 2023). Once the instrument was prepared, approximately 3 mL of the gas sample to be tested was quickly injected, followed by the slow and even injection of the remaining approximately 2 mL of gas. The methane content was calculated using the single-point method, with the retention time for methane being about 1.12 min and for CO<sub>2</sub> about 2.63 min.

## 3. RESULTS AND DISCUSSION

### 3.1. Influence of CaO on methane production in the co-digestion system

The effect of CaO content on the performance of sludge and waste co-digestion is depicted in Figure 1. It can be observed that methane production in all reactors initially increased rapidly before stabilizing over the fermentation period. The maximum methane production in the control group was reached at a digestion time of 25 days, with a methane yield of 359 mL/g volatile solid (VS). The presence of CaO significantly influenced methane yield. Specifically, as the CaO content increased from 2 to 6%, the maximum methane yield increased from 386 to 461 mL/g VS, which is approximately 1.3 times that of the control group. However, when the CaO concentration was further elevated to 8%, the cumulative methane production slightly decreased to 415 mL/g VS. CaO enhanced the cumulative methane production in the co-digestion process of ES and PW, with an optimal concentration of 6%, corresponding to a methane yield of 461 mL/g VS. The oxidizing properties of CaO effectively facilitated the hydrolysis of organic matter within the ES and PW co-digestion system, thereby improving the hydrolysis efficiency and promoting methane production (Zhang *et al.* 2023a, 2023b).



**Figure 1** | Effect of CaO on methane production and daily methane production in the codigestion system of ES and PW. The error bar represents the standard deviation of three measurements.

Figure 1(b) illustrates the impact of CaO exposure on the variation in daily methane production. It can be observed that CaO significantly influences the daily methane production. All groups exhibited two distinct peaks in daily biogas production approximately between 5–7 and 12–15 days, respectively. The reasons behind these two peaks are different. The first peak is attributed to the abundance of digestible substrates, while the second peak is due to the enhanced metabolic activity of methanogenic archaea. In the control group, these two peaks occurred at 7 and 13 days, with corresponding biogas yields of 16.5 and 24.6 mL/g VS, respectively. The presence of CaO increased the daily biogas production. In the CaO-amended groups, the first and second peaks of daily biogas production were approximately between 24.6 and 28.9 mL/g VS, and 31.5 and 35.9 mL/g VS, respectively, and the corresponding digestion times were advanced by 2–3 days compared to the control group. These experimental results clearly confirm that the presence of CaO is beneficial for methane production in the co-digestion of ES and PW and shortens the lag phase. The advanced biogas production and increased yields with CaO addition

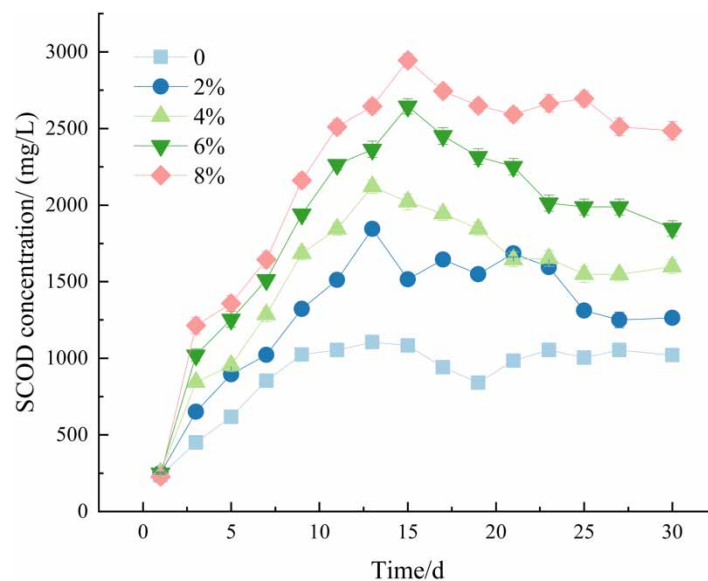
suggest that CaO may act as an effective pretreatment agent to improve the efficiency of the anaerobic digestion process. The enhanced hydrolysis and acidification phases, likely due to the oxidative nature of CaO, lead to an increased availability of substrates for methanogens, which, in turn, results in higher methane yields. The acceleration of the biogas production timeline indicates that CaO could potentially reduce the overall digestion period, offering economic and operational benefits for biogas production facilities. Further investigation into the specific mechanisms of CaO's action within the co-digestion system will provide valuable insights for optimizing the anaerobic digestion process and enhancing the valorization of organic waste streams.

### 3.2. Influence of CaO on the organic matter conversion characteristics in the co-digestion system

Figure 2 presents the impact of CaO content on the SCOD concentration within the co-digestion system. It can be observed that the SCOD concentration in all groups initially increased and then fluctuated downward over time. In the control group, the SCOD reached its maximum value at a digestion time of 15 days, with a maximum value of 1,084 mg/L. Between 21 and 30 days, the SCOD in the control group fluctuated within the range of 984–1,050 mg/L. The presence of CaO increased the SCOD concentration in the co-digestion system, and the extent of the increase in the SCOD concentration was closely related to the CaO content. In terms of the maximum SCOD values, as the CaO concentration increased from 2 to 8%, the maximum SCOD concentration increased from 1,844 to 2,951 mg/L, suggesting that the higher the concentration of CaO exposure, the more intense the dissolution and release process of particulate organic matter in the co-digestion system of ES and PW. The strong oxidizing ability of CaO can oxidize the release of extracellular polymeric substances (EPS) such as PS and PN from ES and promote the release of soluble sugars from PW.

The enhanced release of soluble organics due to the addition of CaO indicates a disruption of the complex organic structures within the ES and PW, making them more accessible for microbial degradation (Zhao *et al.* 2023). This leads to an accelerated hydrolysis phase, which is the rate-limiting step in anaerobic digestion. The increased availability of soluble organic substrates, such as sugars and proteins, can subsequently enhance the acidogenesis phase, resulting in higher production of VFAs and ultimately promoting the methanogenesis process.

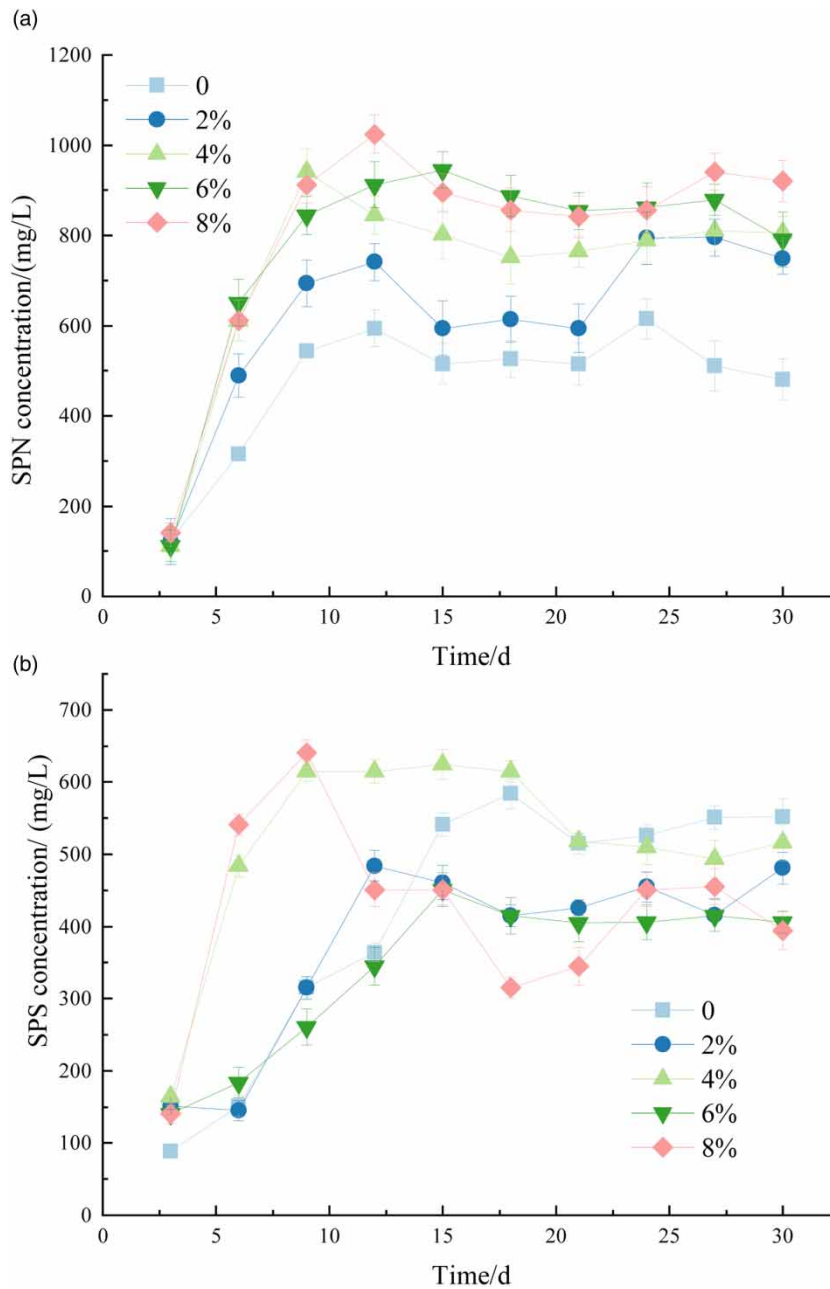
The optimization of the CaO concentration is crucial, as an excessive amount may lead to a decrease in methane production, as observed when the CaO concentration was increased to 8%. This could be attributed to potential negative effects of high CaO concentrations on the microbial community or the inhibition of certain metabolic pathways (Zhu *et al.* 2022). Therefore, the balance between the beneficial oxidative effects of CaO and its potential inhibitory effects is essential for maximizing methane production in the co-digestion process.



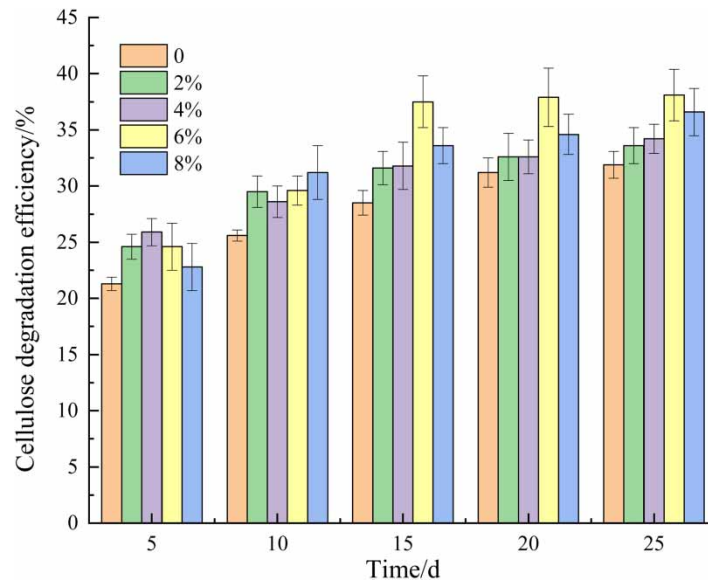
**Figure 2** | Effect of CaO on SCOD concentration in the codigestion system of ES and PW. The error bar represents the standard deviation of three measurements.



Figure 3 further demonstrates the impact of CaO on the concentrations of soluble protein (SPN) and soluble polysaccharides (SPS). During the early stages of digestion, the presence of CaO significantly increased the concentrations of SPN and SPS, as confirmed by their concentration changes depicted in Figure 3(a) and 3(b). Nevertheless, during the later stages of the digestion process, it was observed that the concentration of dissolved organic matter in certain groups with CaO present was lower compared to the control group. In terms of SPN, as the CaO concentration increased from 2 to 8%, the maximum SPN concentration rose from 741 to 1,024 mg/L, all significantly higher than the control group's 591 mg/L. For SPS, similar results were observed, with the concentrations in the CaO-exposed groups, except for the 2% CaO group, reaching 615–669 mg/L, all higher than the control group's 584 mg/L. In the subsequent stabilization phase, the SPN in the control



**Figure 3** | Effect of CaO on the concentration of SPN and SPS during the codigestion of ES and PW. The error bar represents the standard deviation of three measurements.



**Figure 4** | Effect of CaO on the efficiency of cellulose degradation in codigestion systems. The error bar represents the standard deviation of three measurements.

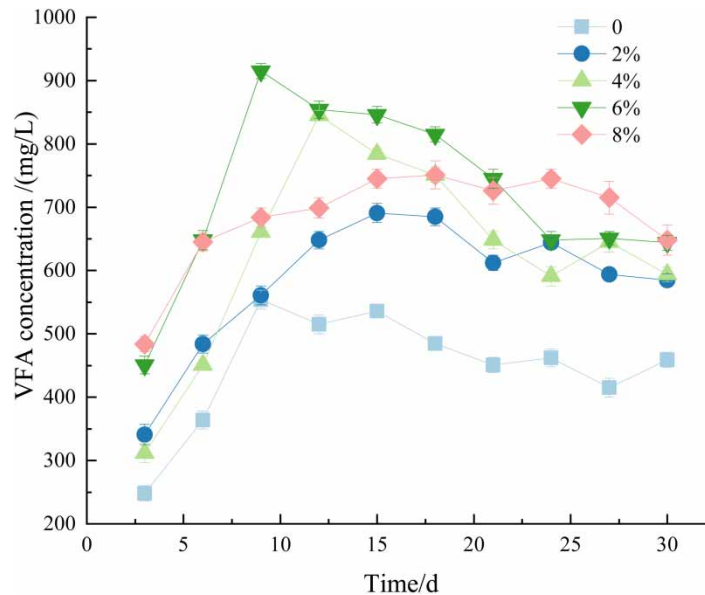
group ranged from 515 to 559 mg/L, which is still lower than the 794–951 mg/L in the CaO groups. The experimental results clearly indicate that CaO increased the concentrations of SPN and SPS in the co-digestion system of ES and PW, providing a sufficient material basis for the subsequent methanogenic process.

The main organic matter in PW is cellulose, and this work also examined the impact of CaO on the degradation efficiency of cellulose (Figure 4). It was found that the cellulose degradation efficiency in all groups showed an increasing trend over fermentation time, with no significant changes after 15 days. CaO can influence the degradation of cellulose. After 15 days, the cellulose degradation rate in the control group was about 31.9%, while, in the presence of CaO, the degradation rate increased from 33.6 to 38.1%, with the highest efficiency observed in the 6% CaO group. The mechanisms by which CaO enhances cellulose degradation can be attributed to several aspects: (1) the alkaline environment created by CaO causes cellulose to swell, which helps to improve the accessibility and surface area of cellulose, increase the contact between cellulase and cellulose molecules, and improve degradation efficiency (An *et al.* 2022). (2) The alkaline environment may enhance the activity of cellulase enzymes, as many cellulases have an optimal pH in the alkaline range. In addition, alkaline conditions facilitate the binding of cellulase to cellulose, enhancing the enzymatic efficiency (Xu *et al.* 2022). (3) Alkaline substances may chemically modify cellulose, such as through deacetylation, which reduces the crystallinity of cellulose, making it more susceptible to enzymatic hydrolysis.

The presence of CaO increases the degradation of cellulose, thereby facilitating the subsequent utilization of organic matter in the co-digestion substrate. This suggests that CaO can act as an effective agent to enhance the conversion of complex organic compounds like cellulose into simpler compounds that can be more readily used in the methanogenic process, ultimately leading to increased biogas production.

### 3.3. Influence of CaO on VFA production and composition in the co-digestion system

VFAs play a multifaceted role in anaerobic digestion. They are not only the substrates for methane production but also key indicators for assessing and controlling the stability of the anaerobic digestion process. By monitoring the concentration of VFAs and the VFA/alkalinity ratio, operators can adjust process parameters in a timely manner to ensure the efficient and stable operation of the anaerobic digestion system. The impact of CaO on VFA production in the co-digestion system is shown in Figure 5, where it can be observed that VFA production in all groups initially increased, then decreased, and finally stabilized over the fermentation period. The content of CaO can affect the yield and composition of VFAs. The maximum VFA value in the control group was 554 mg/L, which appeared at a fermentation time of 9 days, consistent with our previously reported values (Wang *et al.* 2021a). When CaO was added to the co-digestion system, the production of VFAs



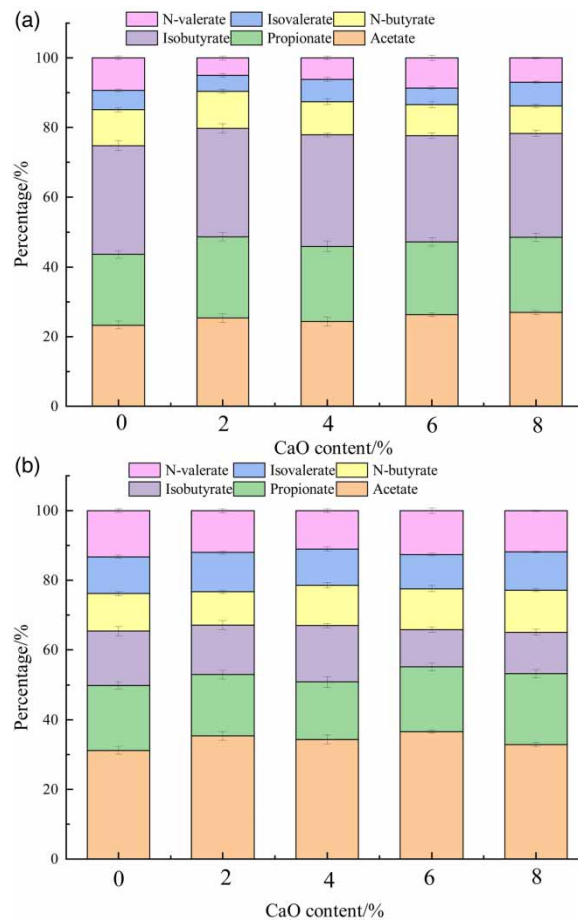
**Figure 5** | Effect of CaO on the accumulation of VFA in the codigestive system of ES and PW. The error bar represents the standard deviation of three measurements.

increased to varying degrees. Overall, as the CaO content increased from 2 to 6%, the maximum VFA production increased from 691 to 915 mg/L. However, when the CaO content was further increased to 8%, the maximum VFA production slightly decreased to 699 mg/L, but it remained higher than that of the control group. The maximum VFA production in the CaO-amended groups was all higher than that of the control group, indicating that the presence of CaO is beneficial for the accumulation of VFAs during the anaerobic co-digestion of ES and PW. The production of VFAs depends on the hydrolysis and acidification of organic matter. As previously confirmed, CaO can effectively promote the dissolution of organic matter in the co-digestion system of ES and PW, thereby providing favorable conditions for the hydrolysis process (Wang *et al.* 2021b). The disintegration effect of CaO on ES and PW promotes the release of hydrolytic enzymes, including cellulase and protease, increasing the contact between hydrolytic enzymes and substrates, and thus enhancing VFA production. The time required for VFAs to reach their maximum accumulation value is generally 9–12 days, and the presence of CaO does not significantly affect the time required for VFAs to reach their maximum value.

The findings suggest that CaO acts as a catalyst in the hydrolysis and acidification stages of anaerobic digestion by increasing the availability of organic substrates for microbial fermentation. This leads to an enhanced production of VFAs, which are essential for subsequent methanogenic activity. The optimal dosage of CaO is crucial, as it maximizes the conversion of organic matter to VFAs without causing inhibition to the microbial community. Understanding the influence of CaO on VFA production provides valuable insights for optimizing the anaerobic co-digestion process and enhancing the bioconversion of organic waste into valuable products like methane.

In the experiment, VFAs mainly included carboxylic acids ranging from C2 to C5. CaO also influenced the proportion of individual VFA components. Figure 6 illustrates the changes in the proportion of individual VFA components over time for each group. On day 5, the proportion of butyrate, including isobutyrate and n-butyrate, was the highest in all groups, maintaining a range of 37.7–41.8%. This was followed by acetate and propionate, with proportions of 23.3–26.9% and 20.3–23.9%, respectively. Previous research has found that a fermentation system with a high proportion of butyrate is indicative of a butyric-type fermentation. As fermentation progresses, the proportion of butyrate decreases, and the proportion of acetate increases, with the presence of CaO affecting the proportions of acetate and propionate. At 15 days, the proportion of acetate in the control group was 31.2%, while in the CaO-exposed groups, the proportion of acetate increased to 32.6–36.9%, with the highest proportion observed in the 6% CaO group. Additionally, the proportion of propionate in the control group was 18.6%, and except for the 8% CaO group, the percentage of propionate exceeded that of the control group, with all other groups showing a lower proportion of propionate than the control group. As the digestion process continued, the proportion of valerate gradually decreased, which is related to the degradation of larger molecular VFAs. The proportion of valerate in the





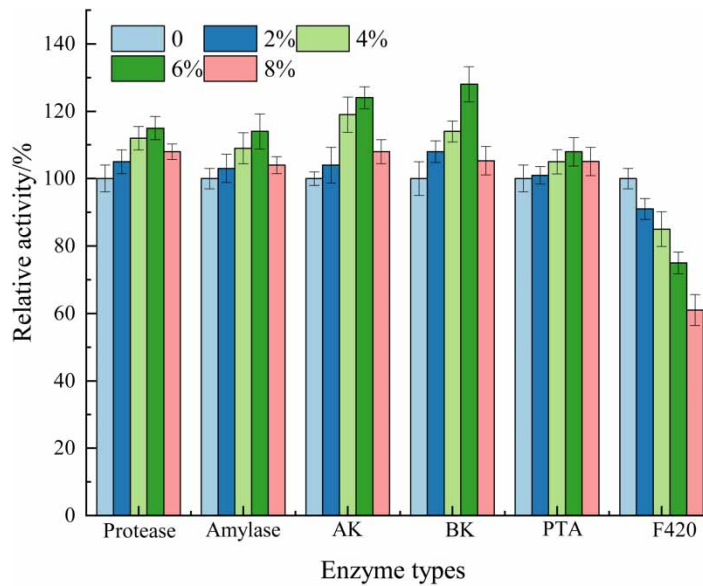
**Figure 6** | Effect of CaO on the percentage of VFA components in the codigestion of ES and PW. The error bar represents the standard deviation of three measurements.

control group was 13.2%, while in the presence of CaO, the proportion of n-valerate decreased to 11–12.6%. The analysis confirms that CaO can effectively reduce the proportion of higher molecular weight VFAs such as valerate and propionate and increase the proportion of acetate. Methanogenic archaea prefer acetate and do not utilize propionate as effectively (Zhang *et al.* 2014). The increase in the proportion of acetate in the fermentation system due to CaO is also one of the reasons for the increased methane production.

The shift in the VFA profile, particularly the increase in acetate, is significant because acetate is a preferred substrate for methanogens, which are responsible for the production of methane, the primary component of biogas. By increasing the availability of acetate, CaO enhances the efficiency of the methanogenic process, leading to higher methane yields. This underscores the strategic role of CaO in modulating the metabolic pathways within the anaerobic co-digestion system to favor the production of desired end-products, such as methane. The ability of CaO to influence the composition of VFAs suggests that it can be used to optimize the metabolic activities within the digester, potentially improving the overall performance of anaerobic digestion facilities.

### 3.4. Influence of CaO on the activity of key enzymes in co-digestion

The anaerobic co-digestion of organic matter involves the regulation of various key enzymes, and the addition of exogenous alkaline substances can modulate enzyme activity through mechanisms such as enzyme activity regulation, the promotion of intermediate product transformation, and the adsorption of inhibitors (Romero-Güiza *et al.* 2016; Li *et al.* 2019). Key enzymes related to the hydrolysis process include proteases, amylases, and cellulases for the degradation of cellulose. Enzymes related to the acidification process include acetate kinase (AK), phosphotransacetylase (PTA), and butyrate



**Figure 7** | Effect of CaO on the activity of key enzymes in the codigestion process of ES and PW. The error bar represents the standard deviation of three measurements.

kinase (BK). The key enzyme primarily associated with the methanation process is coenzyme F420. This study also investigated the impact of CaO exposure on the activity of the aforementioned key enzymes. As shown in Figure 7, the presence of CaO increased the activity of enzymes related to the hydrolysis and acidification processes. For instance, at 6% CaO, the relative activities of protease and amylase increased to 115 and 114%, respectively, while the relative activities of AK, PTA, and BK increased to 124, 128, and 108%, respectively. CaO promotes the dissolution of substrates in the co-digestion of ES and PW, thereby enhancing the full contact between hydrolytic and acidification enzymes and the substrate, facilitating enzyme activity.

Regarding the methanation process, the activity of F420 was inhibited by CaO, with a more pronounced inhibition observed with increasing concentrations of CaO exposure. Especially at 8% CaO, the relative activity of F420 was only 61%, significantly lower than that of the other groups. Despite the suppression of F420 enzyme activity by CaO exposure, the enhancement of the hydrolysis and acidification processes outweighed the inhibition of the methanation process, leading to an increase in methane production.

The findings indicate that CaO can effectively alter the enzymatic activities within the co-digestion system, favoring the hydrolysis and acidification stages that are critical for the conversion of complex organics into simpler compounds like VFAs (Zhang *et al.* 2023a, 2023b). Although the methanation process, which is the final step in methane production, was somewhat inhibited, the overall effect of CaO was to enhance methane yield by improving the efficiency of the initial stages of anaerobic digestion. This suggests that CaO could be a valuable additive in optimizing the anaerobic co-digestion process for improved biogas production.

### 3.5. Influence of CaO on reduction of organic matter and yield of digestion byproducts

In addition to producing valuable energy substances like methane, anaerobic digestion can effectively reduce the volume of the digestate, facilitating subsequent final disposal methods, such as incineration or landfilling (Zhao *et al.* 2021). The impact of CaO on VSS reduction is presented in the attachment (Fig. S2). CaO enhanced the reduction of VSS during the co-digestion process of ES and PW. Upon completion of digestion, the VSS reduction rate in the control group was 32.6%, which is roughly similar to the values reported in our previous papers (Wang *et al.* 2021a). However, the VSS reduction rate was increased to varying degrees in the CaO-exposed groups. In the CaO-exposed groups, the VSS reduction rate ranged from approximately 35.6 to 39.5%, with a trend that higher concentrations of CaO exposure led to higher VSS reduction rates. The strong oxidizing property of CaO is beneficial for the disintegration and subsequent liquefaction or gasification of organic

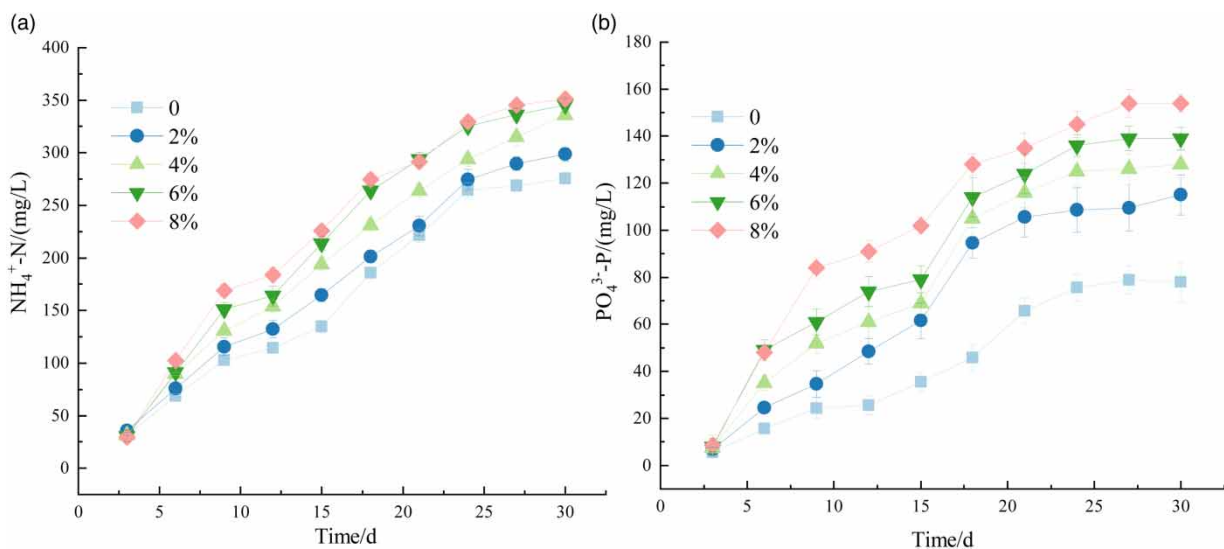
matter in ES and PW, thereby improving the VSS reduction rate. An increase in the VSS reduction rate is advantageous for the subsequent transportation and final disposal of the digested sludge, such as landfilling or incineration.

The enhancement of VSS reduction by CaO indicates that it can improve the overall efficiency of the anaerobic digestion process by not only increasing methane production but also reducing the volume of residual sludge. This dual benefit of CaO makes it a promising additive for anaerobic digestion, contributing to both energy recovery and waste minimization. The increased VSS reduction rate also implies a reduction in the environmental impact and operational costs associated with sludge disposal, making the process more sustainable and economically viable.

The anaerobic digestion process inevitably generates byproducts such as  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ , which increase the load of nutrient salts in the digestate. As shown in Figure 8, the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  in all groups initially increased and then stabilized over time, and CaO can affect the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ . At the end of co-digestion, the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  in the control group were 269 and 78 mg/L, respectively. The presence of CaO significantly increased the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  at the end of the anaerobic digestion, with the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  increasing to 298–351 and 115–154 mg/L, respectively. CaO effectively promotes the degradation of proteins and phosphorus-containing organic compounds, thereby increasing the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  in the fermentation liquid. The higher the concentration of CaO exposure, the more intense the disintegration of organic matter, resulting in higher concentrations of released  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ . It is important to note that in this study, the maximum concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  in the fermentation liquid did not reach the inhibitory thresholds. The pH variations within each group are displayed in Fig. S3. It can be observed that in the control group and the 2% CaO group, the pH initially decreased and then gradually increased over time. In the later stages of digestion, the pH was approximately 7.5–8.2. However, exposure to more than 4% CaO significantly elevated the pH within the digestion system, with a higher dose of CaO exposure leading to a more pronounced increase in pH. In the 8% CaO group, the maximum pH value rose to 9.55. The increase in pH within the CaO exposure groups was associated with the formation of  $\text{Ca}(\text{OH})_2$ .

### 3.6. Significance of CaO for the resource utilization of organic solid waste

CaO, as an oxidizing agent, has been widely used in the water treatment field for the removal of refractory organic substances and the inactivation of pathogenic microorganisms (Zou *et al.* 2009; Huang *et al.* 2018). This study further expands the application field of CaO by applying it to the co-digestion system of ES and PW to enhance the recovery of energy substances and to reveal the underlying mechanisms. CaO can effectively improve the solubilization of organic matter in the ES and PW co-digestion system, providing ample material support for the acidification and methanation processes. Although CaO inhibits the activity of coenzyme F420, the two steps closely related to methane accumulation, namely hydrolysis and acidification,



**Figure 8** | Effect of CaO on the concentration of ammonia nitrogen and phosphate during the co-digestion of ES and PW. The error bar represents the standard deviation of three measurements.

are strengthened, thereby overall increasing the accumulation of methane. The recommended dosage of CaO is 6%, corresponding to a methane yield of 461 mL/g, which is approximately 1.3 times that of the control group.

Given the stability and ease of storage of CaO, it has great potential for promotion and application in the deep treatment of sewage and sludge, especially in enhancing the efficiency of anaerobic digestion of ES and PW and promoting resource recovery. Although this paper confirms that CaO can accelerate methane production in the co-digestion of ES and PW, the potential of combining CaO with other enhancement strategies such as ultrasonication, free nitrous acid, and other pretreatment methods to further improve the resource utilization efficiency of organic matter requires further investigation.

The exploration of CaO as an additive in the anaerobic digestion process provides a valuable contribution to the field of waste management and renewable energy production. The findings suggest that CaO can serve as a cost-effective and efficient pretreatment agent to enhance the bioconversion of organic waste into biogas, offering a sustainable solution for waste treatment and energy generation. Future research should focus on optimizing the application of CaO in terms of dosage, frequency, and combination with other pretreatment methods to maximize the benefits of anaerobic digestion and to minimize any potential negative impacts on the process.

#### 4. CONCLUSION

This work reports a novel strategy for enhancing methane production from the co-digestion of ES and PW by applying CaO. The optimal content of CaO was 6%, with the maximum methane yield reaching 461 mL/g, which is approximately 1.3 times that of the control group. Mechanistic studies indicate that CaO improves the solubilization of organic matter during the co-digestion process of ES and PW and promotes the acidification process, leading to an increase in the accumulation of VFAs. CaO facilitated the accumulation of acetate in the co-digestion of ES and PW, which is conducive to the subsequent methane production process. Enzymatic analysis revealed that CaO enhanced the activity of enzymes related to the hydrolysis and acidification processes but inhibited the activity of coenzyme F420. The increase in the solubilization and hydrolysis of organic matter in the co-digestion system by CaO is the main reason for the increased methane production.

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#### DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

#### CONFLICT OF INTEREST

The authors declare there is no conflict.

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