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Effect of Calcium Ions on Dewaterability of Enzymatic-Enhanced Anaerobic Digestion Sludge

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Abstract Waste-activated sludge (WAS) solubilized remarkably after enzymatic-enhanced anaerobic digestion, but its dewaterability was deteriorated. In this study, a novel method was performed to improve the dewaterability of enzymatic-enhanced anaerobic digestion sludge by adding $CaCl_2$ (0.01~1.00 g/g total sludge). The capillary suction time (CST), moisture content, and filtrate turbidity were employed to characterize the dewaterability of WAS, and the possible mechanisms involved were clarified. The results showed the dewaterability did not worsen when $CaCl_2$ was added before sludge digestion, and the CST, moisture content, and filtrate turbidity were notably reduced with the increase of $CaCl_2$ dosage. It also shown that calcium ions played an important role in the bioflocculation of digested sludge by neutralizing negative charges on the surface of sludge. In addition, soluble protein initially lowered a little and then observably improved with the addition of $CaCl_2$, while soluble carbohydrate was reduced sharply first and then bounced back afterwards. The interactions between calcium ions and the biopolymer further enhanced the dewatering of sludge through bridging of colloidal particles together.

Keywords Dewaterability · Enzyme · Anaerobic digestion · $CaCl_2$ · Waste-activated sludge (WAS)

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Introduction

Anaerobic digestion is an attractive technology for sludge reduction and stabilization [1–2], during which hydrolysis is believed to be the rate-limiting step. In order to accelerate anaerobic digestion, various alternative methods have been proposed to improve the rate of sludge hydrolysis, including thermal [3], thermochemical [4], ultrasonic [5], and enzymatic methods [6]. In our previous studies [6–7], the anaerobic hydrolysis of waste-activated sludge (WAS) could be enhanced by additional enzymes (protease and amylase).

Anaerobic digestion can reduce the overall mass of WAS, but anaerobic-stabilized sludge still requires a reduction in volume and decreases subsequent treatment cost by dewatering [8]. However, WAS after anaerobic digestion has been found to typically have poor dewatering characteristics [9], which reduced some of the benefits of this process. Some researchers thought that the deterioration of sludge dewaterability was owed to the changes in the composition of sludge extracellular polymer (ECP) or extracellular polymeric substances (EPS) [10]. Houghton et al. [9] investigated the relationship between the quantity and composition of ECP and the dewaterability of sludge samples, prior to and after a digestion process at full-scale municipal sewage treatment works, and found that digested sludge was generally more difficult to dewater than raw sludge. A reduction in the protein to carbohydrate ratio of the sludge ECP also was consistent with the digestion process. Shao et al. [10] indicated the increase of soluble protein, soluble protein/polysaccharide in the soluble EPS (slime), resulted in the deterioration of sludge dewaterability during hydrolysis and acidification processes.

Considering the colloidal nature of particles and the gel-like structure of a flocculated system, sludge dewatering has become the bottleneck in the operation of municipal wastewater treatment plants (WWTPs) [11]. In order to overcome these problems, various alternative methods have been proposed to improve the dewatering characteristics of sludge, including the addition of Fenton's reagent [12], microwave conditioning [13], ultrasonication [14], and electrolysis [15]. Divalent cations, such as calcium ions, could interact with functional groups of flocs and bridge negatively charged cells or biopolymers, thus leading to a promotion in sludge flocculation, settling, and dewatering [16–17]. In addition, sludge exhibits colloid properties with negative charges. Calcium ions can improve the sludge dewaterability by compressing the double layer of sludge colloids and interact with exposed binding sites to form a strengthened matrix [18–19]. Bruus et al. [20] thought that calcium associated with exopolymers could form an alginate-like gel, which constituted the backbone of the sludge floc. The extraction of calcium ions will lead to an increase in the number of small particles and subsequently an increase in the specific resistance to filtration (SRF).

The available information about the effective method to improve sludge dewaterability mainly focused on activated sludge and WAS. Zhou et al. [21] investigated the effects of temperatures and extracellular proteins on the dewaterability of thermophilically digested biosolids. Cheng et al. [8] discussed the relationship between sludge dewaterability and the operation factors of one-stage auto thermophilic aerobic digestion (ATAD) including digestion temperature and retention time. However, a deeper knowledge and understanding about the dewatering of themophilically digested solids is still necessary, especially about the anaerobic-digested sludge. The objectives of this study were to improve the dewaterability of enzymatic-enhanced anaerobic digestion sludge (digesting at 50 °C) by adding a conditioning chemical, $CaCl_2$, and elucidate the mechanisms in the conditioning systems from the views of zeta potential variation and biopolymer (soluble protein and carbohydrate) release.

Material and Methods

WAS and Enzymes

WAS used in this study were collected from the secondary sedimentation tank of the second municipal wastewater treatment plant in Changsha, China. Fresh sludge was rinsed twice with deionized water, further filtered through a 0.71-mm metal sieve to get rid of suspended residues and solid impurities, and then stored at 4 °C for later use. Table 1 presents the main characteristics of the WAS.

The enzymes were commercial neuter protease and α-amylase (both were purchased from Solarbio Biotechnology Ltd.). The activity of neuter protease and α-amylase was 6000 and 3700 U/g. Correspondingly, the optimal temperature of neuter protease and α-amylase was about 40 and 50–70 °C, respectively. Based on our previous study [6], 50 °C was selected as the sludge digestion temperature in a mixed-enzyme system.

Batch Experiments

The experiments were carried out in 12 identical 500-mL reactors, which are made of plexiglass and each fed 400 mL WAS. These reactors were equally divided into two groups and added 0.06 g/g mixed enzyme (the enzyme was a mixture of protease and α-amylase with a ratio of 1:3).

Group 1

$CaCl_2$ was added into six reactors at the dosages of 0, 0.01, 0.03, 0.10, 0.30, and 1.00 g/g, respectively. The reactors were sparged for 4 min by nitrogen gas to remove the oxygen and capped with rubber stoppers to maintain a strict anaerobic condition. These reactors were then placed in a water bath shaker (100 rpm) at 50 °C for 24 h.

Group 2

The other six reactors operated at a strict anaerobic condition were firstly placed in a water bath shaker (100 rpm) at 50 °C for 24 h. After that, $CaCl_2$ was added into the reactors at the dosages of 0, 0.01, 0.03, 0.10, 0.30, and 1.00 g/g, respectively.

Table 1 Basic characteristics of the WAS

Parameter	Value
pH	6.74 ± 0.15
Total chemical oxygen demand (TCOD)(mg/L)	9420 ± 250
Soluble chemical oxygen demand (SCOD) (mg/L)	150 ± 10
Total suspended solids (TSS) (mg/L)	9820 ± 200
Volatile suspended solids (VSS) (mg/L)	6850 ± 80
Total carbohydrate (mg/L)	620 ± 60
Total protein (mg/L)	5860 ± 180
Filter cake moisture content (%)	90.00 ± 0.30
CST (s)	33.4
Zeta potential (mV)	−9.44

All tests were conducted in triplicate, and the data shown in this paper were the average based on three independent experiments.

Analytical Methods

CST

Capillary suction time (CST) was measured with a CST instrument (Model 304B, Triton, UK) equipped with an 18-mm-diameter funnel and Whatman No. 17 chromatography-grade paper.

Zeta Potential

The sludge was firstly centrifuged at 5000 rpm for 5 min, and the supernatant was collected for zeta potential measurement [22]. After adjusting the sample pH to 7.0 ± 0.1 by NaOH or HCl, the measurement was conducted in a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK) at 20 °C.

Filter Cake Moisture Content

The water content (WC) of WAS was determined according to the following equation: $WC\% = (W_1 - W_2)/W_1 \times 100\ \%$.

where W_1 is the weight of the wet filter cake and W_2 is the weight of the filter cake after drying at 105 °C for 8 h until the quality is constant.

Filtrate Turbidity

The sludge was centrifuged at 3000 rpm for 2 min, and the turbidity of the supernatant was measured by its absorbance at 650 nm (Spectrophotometer Biochrom-WPA-S1200).

Soluble Carbohydrate and Protein

The soluble carbohydrate and protein were determined by the methods described in our previous publications [6–7].

Results and Discussion

Effect of $CaCl_2$ on Sludge Dewatering

CST

The CST value has been widely accepted and used as a significant index in measuring dewaterability of sludge, and a lower CST value symbolizes better dewaterability of sludge. Compared with the raw WAS (33.4 s), CST values climbed apparently and reached about 80.0 s after enzymatic-enhanced digestion without $CaCl_2$ added, indicating dewaterability deteriorated after digestion. This degeneration was possibly owed to the breakdown of sludge by enzyme and the rise of smaller flocs that were hard to dewater [23–24].

$CaCl_2$ could change the EPS distribution on the sludge surface which influenced CST [25]. The CST presented a notable difference at various $CaCl_2$ dosages added before and after sludge digestion (Fig. 1). It decreased sharply with increasing $CaCl_2$ dosage and then reached a plateau value, indicating a significant improvement in the dewaterability. In addition, when $CaCl_2$ was added before WAS digestion, the dewaterability did not worsen compared with the raw WAS during the anaerobic digestion process in terms of CST. As seen in Fig. 1, the addition of 0.10 g/g TS $CaCl_2$ to the sludge before digestion resulted in a fleet decrease in the CST from 83.9 to 27.6 s for enzymatic treatment, while it declined from 84.5 to 65.0 s when $CaCl_2$ was added after sludge digestion. Since CST variation was no more than 3.3 s when $CaCl_2$ dosage exceeded 0.10 g/g DS, the optimal $CaCl_2$ dosage was supposed to be 0.10 g/g DS.

The conventional dewatering characterization methods using either vacuum dewatering in a Buchner funnel or a CST apparatus may give incorrect information with regard to the choice of conditioner because they typically use small samples and thin cakes and do not consider the expression phase [26–27]. Undoubtedly, the CST decreased from 83.9 and 84.5 s to 27.6 and 65.0 s, respectively, for $CaCl_2$ added before and after enzymatic treatment, which proved, at least, $CaCl_2$ could improve the dewaterability of WAS.

Moisture Content

The dewatering efficiency of the WAS depends on not only the rate of dewatering but also the extent of dewatering. The rate of dewatering is usually measured using a CST test or SRF, whereas the extent of dewatering is usually measured by dewatered cake solids as the percent dry solids or water content [28]. Calcium ions could induce the dehydration of bacterial surfaces [29], thus leading to more water released from the sludge surface. It was evident from Fig. 2 that the sludge treated by $CaCl_2$ led to a drier filter cake at the end of the filtration stage. The filter cake moisture content of raw sludge was 90.0 %, showing a poor dewaterability, and decreased to about 83.5 % for enzymatic digestion without $CaCl_2$ added. Generally, the raw sludge floc was large in size but contained a lot of bound water. Even if the water passed through the filter cake quickly (e.g., CST was low), the water content inside the

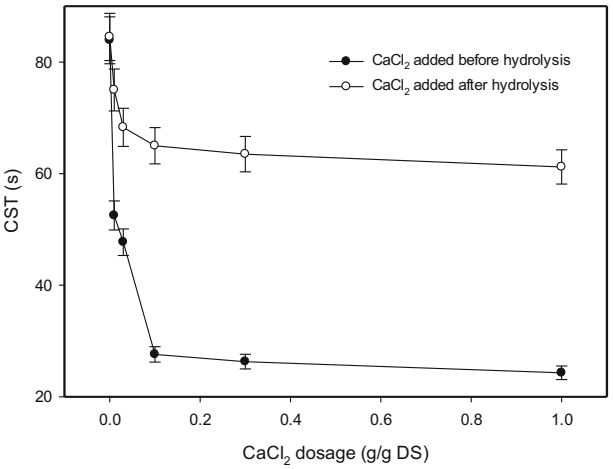

Fig. 1 CST evolution as a function of various $CaCl_2$ dosages added before and after sludge digestion

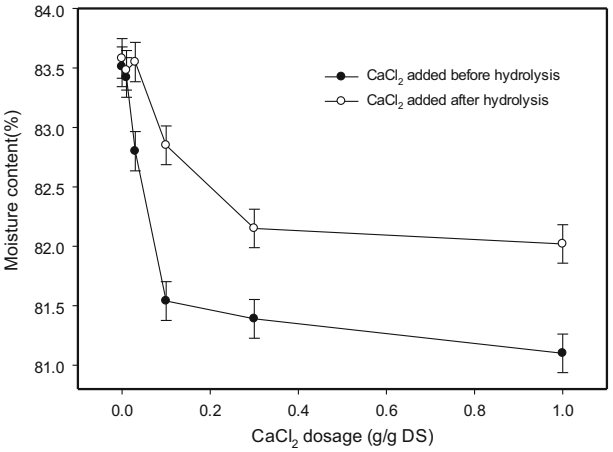

Fig. 2 Moisture content evolution as a function of $CaCl_2$ dosages added before and after sludge digestion

small pores and capillaries, as well as water bound inside the floc matrix, might remain high (e.g., moisture content was high).

As shown in Fig. 2, moisture content decreased sharply with the increase of $CaCl_2$ dosage, indicating better dewatering performance was achieved, and it was better when $CaCl_2$ was added before sludge digestion. For example, the moisture content decreased from 83.5 and 83.6 % to 81.5 and 82.8 %, respectively, for $CaCl_2$ added before and after sludge digestion as it climbed to 0.10 g/g TS. Further increase of $CaCl_2$ brought little benefit to the dewatering extent.

Filtrate Turbidity

During enzymatic-enhanced digestion of WAS, the polymeric structure tended to break down, thus leading to an increase of fine particles into the liquid phase. Meanwhile, the small colloid particles wrapped by EPS were also released into the solution, which finally tended to higher increase of the supernatant turbidity. This behavior was observed by the increase of filtrate turbidity from 0.05 to 0.198 for enzymatic treatment without $CaCl_2$ added. However, the absorbance gradually declined with the addition of $CaCl_2$ and reached to 0.130 and 0.162 for $CaCl_2$ added before and after WAS digestion at a $CaCl_2$ dosage of 0.10 g/g TS (Fig. 3). The reason for filtrate turbidity decrease might be that calcium ions compressed the double layer of sludge colloids and interacted with exposed binding sites to form a strengthened matrix [18–19]; thus, less sludge flocs could be released from sludge colloids after centrifuging.

Possible Mechanisms of Sludge Conditioning by $CaCl_2$

Morphological Character of the Sludge

The morphological character of the sludge was observed with a microscope (CX40RF200, Olympus Optical Co. Ltd., Japan), and it is shown in Fig. 4. The floc matrix that embodied a great many of cross-linked thin fibers collapsed, and the particles were rather dispersed

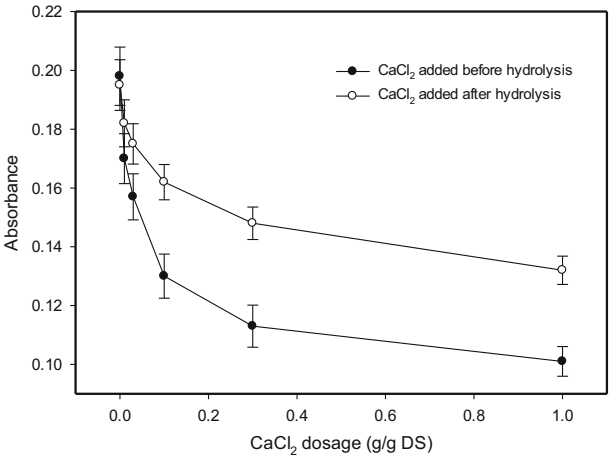

Fig. 3 Filtrate turbidity evolution as a function of $CaCl_2$ dosages added before and after sludge digestion

and smaller after enzymatic digestion (Fig. 4a, b). After the addition of $CaCl_2$, the sludge particles aggregated and formed large flocs linked with strong fibers as shown in Fig. 4c, d, which was in accordance with that large flocs often denote good settleability and dewaterability. Compared with Fig. 4c, d, the flocs were smaller when $CaCl_2$ was added

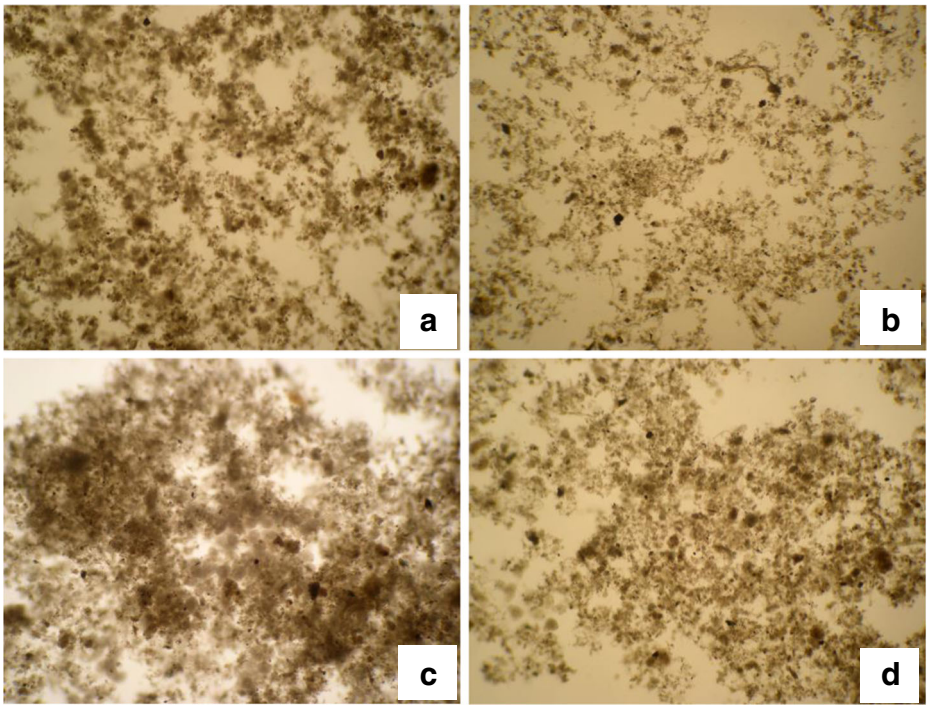

Fig. 4 Micrographs of the raw sludge (**a**), enzymatic digestion sludge (**b**), WAS treated with $CaCl_2$ added before enzymatic digestion (**c**), and WAS treated with $CaCl_2$ added after enzymatic digestion (**d**). Magnification 10 × 10

after sludge digestion (Fig. 4d), while a cloud-like strong aggregation formed and dispersed flocs almost disappeared when $CaCl_2$ was added before sludge digestion (Fig. 4c). The reason might be that sludge flocs were destroyed and became smaller after enzymatic digestion, and subsequently addition of $CaCl_2$ resulted in the formation of smaller and more compact flocs than those of simultaneous addition of $CaCl_2$ due to charge neutralization and adsorption bridge building.

Zeta Potential

The colloidal stability, which plays an important role in sludge dewatering, has a strong relation with the surface charge characterized by zeta potential [30]. The raw sludge was originally negatively charged with −9.44 mV zeta potential and decreased to −13.80 mV for the mixed-enzyme system (Fig. 5). The sludge particle has a net negative charge and forms a double layer with the counter-ion charge in the solution, thus results in an osmotic repulsion of adjacent particles and keeps a relatively stable system [31], which inhibited aggregation and caused poor settling and dewatering properties. As plenty of charges in the floc interior were inaccessible, the disintegration of flocs by enzyme and thermal treatment caused an exposure of more surfaces and then a rise in negative charges [32]; thus, the dewaterability was deteriorated.

However, after the addition of $CaCl_2$, the negative charge of the sludge was neutralized by the positive charge emerging on the surface of the sludge [33]. Figure 5 shows the zeta potential of the sludge supernatant as a function of $CaCl_2$ dosage. It could be found that the zeta potential increased sharply with the addition of $CaCl_2$ within 0.10 g/g DS and then rose gently and tended to an isoelectric point with further increase of the dosage. The soluble biopolymers with high affinity for Ca^{2+} were integrated into the flocs, consolidating the flocs' inner interaction to form compacted flocs, which was consistent with the increase of zeta potential induced by $CaCl_2$ in Fig. 5. The zeta potential climbed sharply from −13.80 and −13.70 mV to −9.02 and −9.50 mV, respectively, for $CaCl_2$ added before and after WAS digestion as it elevated to 0.10 g/g DS.

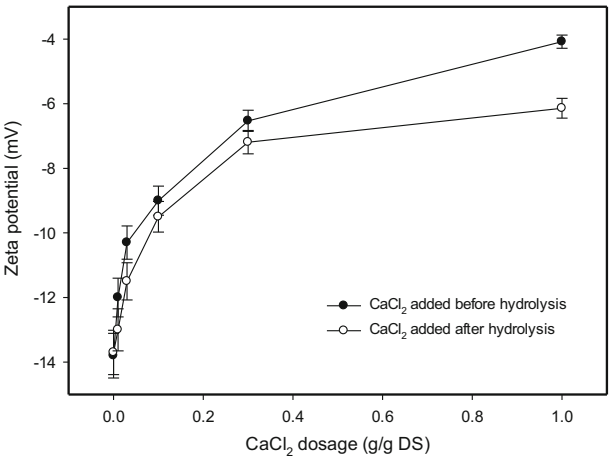

Fig. 5 Zeta potential evolution as a function of $CaCl_2$ dosage added before and after sludge digestion

Compared with the −9.44-mV zeta potential for the raw sludge, the dewaterability was not deteriorated during the anaerobic digestion process when $CaCl_2$ was added before WAS digestion (−9.02 mV). The positive charges continually neutralized the negative charges on the surface of the sludge during the whole WAS digestion process when $CaCl_2$ was simultaneously added; thus, the contact between positive and negative charges was more complete. In addition, as plenty of charges in the floc interior were inaccessible, the disintegration of flocs by enzyme treatment could cause more exposure of surfaces and more negatively charged functional groups; thus, simultaneous addition of $CaCl_2$ contributed to higher increase of zeta potential. The increase of zeta potential was beneficial to destroy sludge particle stability; thus, sludge particles congregated with each other and formed large flocs to settle, leading to an improvement in bioflocculation [34], which supported the decrease of filtrate turbidity in Fig. 3.

Biopolymer in Solution

Sludge dewaterability was significantly deteriorated after anaerobic digestion due to the disruption of EPS floc structure and integrity [23]. But in the presence of $CaCl_2$, as mentioned above, better dewaterability was observed. As Neyens et al. [24] pointed out, biopolymer or EPS in sludge could not only help form sludge flocs but also cause such flocs to wrap more water and suspension solids. Ye et al. [35] also suggested that protein and carbohydrate in the liquid entrap the water which aids in the retention of water and significantly contributes to the water-binding capacity of the sludge floc matrix. Thus, the decomposition of these components would destroy their binding with water and set the water free, resulting in a better dewaterability.

As shown in Fig. 6, when $CaCl_2$ was added before WAS hydrolysis, soluble protein firstly declined a little and then gradually increased and dramatically exceeded the original value,while it increased slowly initially and then rapidly went up when $CaCl_2$ was added after hydrolysis. Correspondingly, the soluble carbohydrate for $CaCl_2$ added before WAS hydrolysis firstly followed a sharp decrease trend and then gradually increased with the addition of $CaCl_2$, while it increased obviously firstly and then showed a slow increase trend when $CaCl_2$ was added after hydrolysis. The particulate organic matter within the floc structure was maintained partially by metal ion bridges; thus, the addition of cations such as Ca^{2+}, Mg^{2+}, or Fe^{3+} led to less release of protein and carbohydrate [36] (function A). In addition, the soluble biopolymers (protein and carbohydrate) integrated into the flocs have high affinity for Ca^{2+}; thus, the addition of calcium could lower their concentration in the solution [37] (function B). But when the $CaCl_2$ dosage exceeded a certain point, the biopolymers increased again (Fig. 6). As Guan et al. [19] reported, minimal soluble biopolymers were released at a bridging equilibrium point and tended to be exchanged by Ca^{2+}, thus being released into the supernatant again when the point was overrun.

The differences between simultaneous and subsequent addition of $CaCl_2$ might be that the additional cations play a different role in the simultaneous or subsequent addition of $CaCl_2$. When $CaCl_2$ was added before anaerobic digestion, function A played a dominant role, thus contributing to less release of protein and carbohydrate. The calcium could bridge with the functional groups of soluble protein and carbohydrate once they dissolved into the liquid, which conjointly reduced the release of biopolymers into the liquid. However, a large amount of biopolymers was released

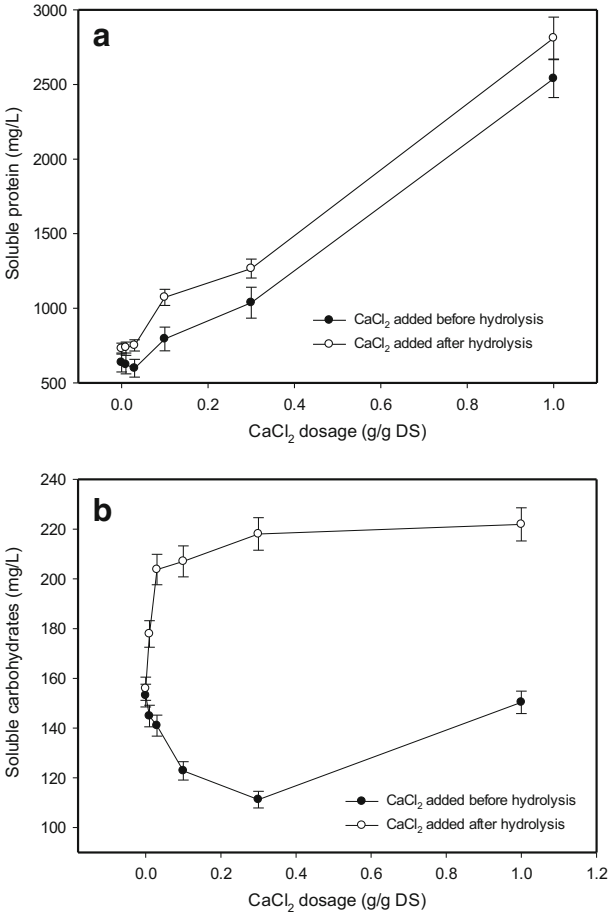

Fig. 6 Soluble protein (**a**) and carbohydrate (**b**) evolution as a function of $CaCl_2$ dosages added before and after sludge digestion

into the liquid during enzymatic-enhanced digestion of WAS. When $CaCl_2$ was added after digestion, function B played an important role.

Conclusions

Sludge dewaterability was deteriorated after enzymatic-enhanced anaerobic digestion, but it could be efficiently improved by calcium ions. When $CaCl_2$ was added before WAS digestion, the dewaterability was not deteriorated. The CST, moisture content, and filtrate turbidity were remarkably reduced with the addition of $CaCl_2$ due to the flocculation and bridging of calcium ions. The optimal $CaCl_2$ dosage was supposed to be 0.10 g/g DS. Calcium ions could alter the zeta potential via neutralizing negative surface charges, which promoted sludge dewatering. Meanwhile, the bridging between calcium ions and soluble protein decreased their concentrations in the solution and played an important role in sludge dewatering.

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