



Innovation in valorization of cow manure: Higher hydrolysis, methane production and increased phosphorus retention using UASB technology

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ABSTRACT

Cow manure has potential to serve as a sustainable secondary fuel and phosphorus resource and cut our reliance on finite primary resources. The efficient and sustainable valorization of cow manure faces compositional challenges because of a high solids content, the lack of soluble phosphorus, fine struvite particles being the main phosphorus species and high bicarbonate concentrations. Addition of calcium could result in higher methane production and conversion of struvite to calcium phosphate. To investigate this, cow manure was digested in two up-flow anaerobic sludge blanket reactors for 456 days, one with and one without CaCl_2 addition. A positive effect of calcium addition was found for hydrolysis (29 % without calcium and 67 % with calcium), methane production ($136 \text{ L-CH}_4 \text{ kgVS}^{-1}$ without calcium and $301 \text{ L-CH}_4 \text{ kgVS}^{-1}$ with calcium) and sludge bed development. Although calcium was added in a 3:1 ratio to phosphorus, it did not result in recrystallization of struvite to calcium phosphate. Instead, it precipitated as calcium carbonate, which was further induced by additional bicarbonate production through higher hydrolysis and methane production. Still, calcium addition caused better phosphorous removal (from 38 % to 61 %), which is attributed to the enhanced sludge bed capturing and accumulating both the calcium carbonate and struvite fines. Higher methane production and improved phosphorus retention enables better valorization of cow manure. The access to resources from cow manure through this technology can contribute to the circularity of agriculture and save on finite natural resources.

1. Introduction

Natural resources for mineral phosphorus fertilizer and fossil energy are finite, geographically imbalanced and becoming scarce, which lead to governments urging the recovery and circular use of resources [1,2]. Cow manure (CM) contains energy in the form of organic matter and phosphorus, as well as other nutrients, making it an interesting alternative resource. CM is the most abundant animal manure in Europe and makes up about 75 % of all the 1.4 billion tons of animal manure produced annually [3]. In the Netherlands, the most common type of CM is a slurry (86 % [4]), which is urine and feces falling through a slatted floor into a pit below the stable for storage [5]. The longer CM remains in the pit, the more it digests by itself, releasing methane and other volatile emissions; the ammonia released during storage represents 53 % of the total ammonia emissions during manure management (44 % during manure application) [5,6].

CM is usually applied without treatment to agricultural soils for fertilization [3]. The application of animal manure is regulated by nitrogen limitations in the EU-27 area and in some countries (e.g. the Netherlands and Ireland) by phosphorus limitations [7–9]. The excess CM needs to be treated and redistributed to spare the environment and mitigate its effect on climate change [10]. In the Netherlands, the excess CM is brought from cattle farms to crop farms or frequently exported to neighboring countries. However, cattle farmers still apply artificial nitrogen fertilizer (32 % of total nitrogen fertilization [11]) to achieve desired crop and grass yields because the N:P:K ratio (mainly N:P) of CM is not ideal for fertilization. The desired N:P ratio varies between crops, but often it is ~ 8 [7,8], whereas CM has a N:P of 4. The intention of limiting manure application for environmental protection is biased as additional transport and application of artificial fertilizer are both economically and environmentally costly. Instead, the valorization of manure as energy and separated phosphorus and nitrogen fertilizers

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could effectively diminish the need for fossil fuel and finite and cost-intensive resources, while reaching sufficient and more flexible fertilization.

Anaerobic digestion of fresh CM can avoid unwanted emissions, produce biogas and potentially recover other resources such as nutrients and carbon [12]. Anaerobic digestion of CM could help achieve the goal of a 10-fold increase in the biogas production by 2030 and establishing a circular economy by 2050 in the Netherlands [13,14]. However, the economic viability is not yet convincing; therefore, only 2 % of the CM was digested in 2018 in the Netherlands [15,16]. The majority of digesters are co-digesters (>50 % animal manure), where energy crops are added to the manure to increase biogas production, whereas mono-digesters (>95 % animal manure) are a minority of mainly small units installed on farms [17]. Currently, the main advantage for farmers in installing anaerobic digesters is the income from energy production. The digestate (i.e. anaerobically digested CM) remains labeled as manure that still has the same limitation for applicability as raw manure [18]. Giving economic value and end-of-waste labels to products recovered from CM digestion would be an incentive to digest CM, recover nutrients and ultimately save on fossil fuel and finite natural resources such as phosphorus.

Improving methane production and phosphorus recovery from CM is often achieved with pre- or post-treatments for anaerobic digestion. Regarding methane production, chemical, mechanical and biological pretreatments are applied to enhance the hydrolysis of substances that are difficult and slow to hydrolyze biologically [19]. CM often contains predominantly slowly hydrolyzable organic material because of the type of animal feed and the efficient rumen digestion compared with monogastric digestions [5,20]. Regarding phosphorus recovery, in CM phosphorus is often present in fine particles that have a relatively low phosphorus concentration. Mechanical separation can separate phosphorus from the solid or liquid fraction before and after digestion, but often the concentration of phosphorus remains low [21]. Biological treatments focus on the phosphorus in solution, requiring dilution with water for biomass such as fungi to take up the phosphorus [22]. Chemical treatments can be cost-intensive, as they depend on high chemical dosing for pH adjustment in a multi-step process because of the high pH buffering capacity in CM [5,21]. One of the most studied phosphorus-recovery technology is struvite precipitation [23–25]. However, often the size of the struvite crystals limits the separation from manure solids, and co-existing cations can inhibit struvite crystallization, restricting its recovery potential [23]. Struvite contains besides phosphorus also nitrogen, which is best separately applied from phosphorus for ideal fertilization (N:P ratios). The current processes in the phosphate fertilizer industry are not based on struvite but on phosphate rock which is calcium phosphate. Another efficient route for phosphorus separation was achieved during anaerobic digestion in an up-flow anaerobic sludge blanket (UASB) reactor by calcium addition for treatment of concentrated black water (BW) [26] and pig manure [27]. The formation of calcium phosphate granules (CaP granules) allowed for simple size separation and resulted in the removal of 89 % and 74 % phosphorus from BW and pig manure, respectively, in a single and simple treatment step. The addition of calcium can be beneficial for methane production as well [28]. However, adverse effects of calcium addition on methane production are also reported [29].

This study aims to simultaneously produce methane and recover phosphorus from CM during anaerobic digestion using UASB technology with calcium addition. The main challenge is the composition of CM, which is significantly different from BW and pig manure. CM has higher solid concentrations, harder (bio)-degradable organic material, a high bicarbonate content and phosphorus present as fine struvite particles. Furthermore, the composition also can vary seasonally. We hypothesize that calcium addition can improve methane production and phosphorus removal, which would enhance valorization of CM.

2. Materials and methods

2.1. Experimental setup

Two 45 L UASB reactors with double-walled heating at 55 °C anaerobically digested CM and operated for 456 days. The CM was collected after screw-press separation at 700 µm from the pit of a dairy farm with 150 lactating cows in Grijpskerk, the Netherlands. The stable had a slatted floor with combined urine and feces collection as a slurry in the pit. Immediately after collection, the CM was manually sieved through a sieve bend with a grid size of 200 µm (Estrad sieve bend). From a mixed and cooled storage tank (4 °C), the CM was pumped every-two hours for 1 min to reach a feed flow of 800 mL day⁻¹ (peristaltic Masterflex pumps controlled by an external Siemens Logic PLC timer), corresponding to an organic loading rate (OLR) of 1 gCOD L⁻¹ d⁻¹ and a hydraulic retention time (HRT) of about 55 days (measured values in [supplementary information](#) [SI], Tables 1 and 2). The OLR of the reactors varied because the composition of the CM varied but the flow was kept the same. The composition of CM depended on the seasons throughout the operation. We separated the operation into four phases where the fluctuations in influent compositions were less (SI, Tables 1 and 2 and SI, [Fig. 1](#)). The four phases correspond with spring (phase 1), summer (phase 2), fall (phase 3), and winter and spring (phase 4).

One UASB reactor operated without additional calcium (R_P); the other UASB reactor received additional calcium (R_{Ca}) as calcium chloride solution ([Fig. 1](#)). The calcium addition was based on the total calcium and phosphorus content measured in CM for reaching a Ca:P molar ratio of 3 (on average 2.4 gCa L⁻¹ manure). The Ca:P molar ratio of 3 was earlier found by Cunha et al. [26] to be optimal for calcium phosphate granulation. The calcium solution was pumped directly into the reactor through a pipe with 1 cm diameter and horizontally spaced openings for distributing the calcium into the reactor. The flow of calcium solution was 8 % of the influent stream to avoid dilution and the pumping time was 30 sec every-two hours to achieve a higher flow and better distribution inside the reactor. A shared master timer controlled the pumping of the calcium solution and the feed.

Both reactors had three vertically distributed sensors measuring online the pH, redox potential, and temperature at the center of the sludge bed (SB; [Fig. 1](#)). The data were collected in a data logger and analyzed weekly to assure proper operation conditions and functioning of operation and monitoring equipment.

Both reactors had five vertically distributed SB sampling valves ([Fig. 1](#)). The SB was sampled (about 200 mL) from tap 1 to 4 and about 500 mL from tap 0 by briefly fully opening the sampling valve. Each SB sampling started on top of the reactor and went toward the bottom (tap 4 to tap 0) to avoid the sampling of lower sampling points affecting the sample collected on upper sampling points (relocation of SB).

2.2. Analysis and sampling program

The monitoring and sampling program consisted of (1) daily flow measurements of produced effluent (determined by weight, considering a density of 1 g mL⁻¹), formed biogas (measured with a gas counter from Ritter), and used calcium solution (estimated by weight, considering a density of 1 g mL⁻¹), (2) weekly compositional analysis of influent, fresh effluent and biogas and (3) (monthly planned) SB analyses on operation days 70, 98, 139, 165, 221, 229, 237, 244 and 341 as well as after stopping the reactor operation (day 456). The SB analyses became more intense for research outside this study on days 221–244 and later less frequent because of limited accessibility (Covid-19). During all sampling sessions, similar amounts of SB were sampled for R_{Ca} and R_P so that the active reactor volume was always similar and to ensure comparable reactor operation. The analyzes for influent, effluent, and SB, as well as the methodology and equipment, were as described in Schott et al. [27] and consisted of:

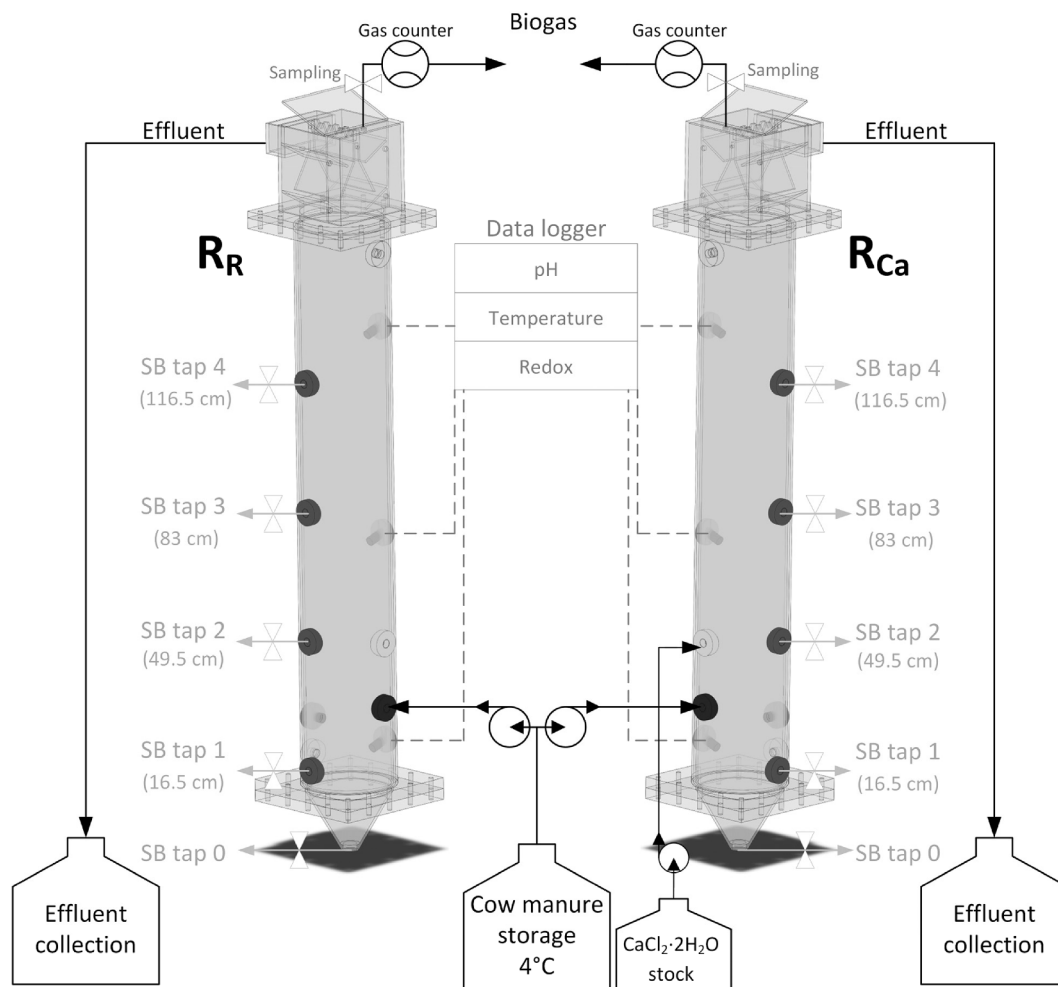


Fig. 1. Schematic representation of the setup, including the UASB reactors with heating in a double wall, a cone-shaped bottom piece for accumulating and harvesting potential phosphorus-rich particles and, on top, a gas–liquid–solid separator was placed made from polypropylene. The gas was led toward the gas exhaust through a sampling septum before going into a gas counter, the liquid toward the overflow, and solids remained in the reactor through inclined plates. pH measurements in the SB were in situ, while influent and effluent pH were measured at room temperature during weekly analysis. The SB taps show the vertically distributed SB sampling taps for SB analysis.

- pH measurements
- Gravimetric determination of total solids, fixed solids, volatile solids, total suspended solids, fixed suspended solids and volatile suspended solids (TS, FS, vS TSS, FSS and VSS, respectively)
- Determination of total carbon, total organic carbon (TOC) and inorganic carbon (IC) of paper filtered (589/1 Whatman) sample using Shimadzu TOC analyzer
- Measurements of soluble elements (i.e. phosphorus [P_{sol}], calcium [Ca_{sol}], magnesium [Mg_{sol}], sulfur [S_{sol}], iron [Fe_{sol}], sodium [Na_{sol}] and potassium [K_{sol}]) with inductively coupled plasma optic emission spectroscopy (ICP-OES; with an internal yttrium standard; Perkin Elmer Optima 5300 DV) after membrane (0.45 μm Whatman) filtration
- Measurements of anions and cations (PO_4^{3-} , SO_4^{2-} and Cl^- and Ca^{2+} , Mg^{2+} and NH_4^+ , respectively) using ion chromatography (Metrohm 930 compact IC flex) after membrane filtration
- Determination of acetic, propionic and butyric acid, which are considered the primary volatile fatty acids (VFAs) in the influent and effluent, using ion chromatography after membrane filtration
- Measurements of chemical oxygen demand (COD) as raw, paper (589/1 from Whatman) filtrate and membrane (0.45 μm Whatman) filtrate (COD_{tot}, COD_{pap}, and COD_{sol}, respectively) with Hach-Lange kits
- Quantification of total elements (P_{tot} , Ca_{tot} , Mg_{tot} , S_{tot} , Fe_{tot} , Na_{tot} and K_{tot}) using ICP-OES after microwave-induced acid digestion (Milestone Ethos Easy) for 15 min at 180 °C with 69 % HNO_3

The SB was analyzed for all parameters above on days 165 and 341. The SB samples were also analyzed with a scanning electron microscope coupled with an energy dispersive X-ray (SEM-EDX) and X-ray diffraction (XRD) as described in Schott et al. [27]. On the other days, only the solids and total elemental composition were measured.

2.3. Calculations

The OLR, solid retention time (SRT), level of hydrolysis and methanization were calculated as described by Halalsheh et al. [30] and de Graaff et al. [31]. Suspended and colloidal COD (COD_{sus} and COD_{col}, respectively) and the suspended species of elements (P_{sus} , Ca_{sus} and Mg_{sus}) were determined as the difference between the total and soluble fractions, and the cumulative removal and its errors were calculated based on the daily flow data combined with the weekly measured concentrations, where the errors in the cumulative data are resulting from the analytical errors.

2.4. Geochemical modeling with PHREEQC

The geochemical modeling was executed in PHREEQC Interactive version 3.5 [32]. The model included the composition of the liquid phase and solid phase based on the measured composition of CM (SI, Tables 1–6 and SI, Fig. 1). All compounds started in solution and were allowed to form solids as “solid solutions.” The precipitates allowed in the solid solution were based on the ones observed in SEM and XRD measurements and possible polymorphs.

We used the wateq4f.dat database (version from August 21, 2012) with the tipping hurley modifications to include humate and possible interactions with calcium [33]. The database was amended with master species and reactions for acetate, propionate and butyrate from the minteq.dat database to see their effect on cation activities. We included struvite and amorphous calcium phosphate (ACP) information in the database based on the data from Ohlinger et al. [34] and Musvoto et al. [35], respectively. We removed the abiotic carbonate reduction to methane because this reduction is not observed in anaerobic digesters and only happens in the presence of catalysts or in extreme environments with high pressure and/or high temperature [36–38]. All materials, consisting of the database used (including precipitation and complexation reactions), the scripts for the different iterations and scenarios as well as resulting raw datasets are given in the PHREEQC appendix as text file to support further studies.

3. Results and discussion

3.1. The effect of calcium addition on methane production

Calcium addition resulted in 2.3 times higher methanization in R_{Ca} ($46 \pm 3 \%$) compared with R_R ($20 \pm 2 \%$) over the whole operation period based on the COD mass balance and measured methane (Fig. 2). Both reactors received CM with a carbon to nitrogen ratio of 7.2 ± 1.3 (based on TOC and NH_4^+ measurements over the entire operation period). The ammonia concentration in CM did not exceed $0.7 \text{ gNH}_3 \text{ L}^{-1}$ which is lower than the inhibiting concentration for methanogenesis of $> 1.1 \text{ gNH}_3 \text{ L}^{-1}$ [39,40]. R_{Ca} produced methane more stably and showed overall less intense and fewer fluctuations in the treatment than R_R (SI, Tables 1–6). The latter was more sensitive to the seasonal fluctuations in the manure composition (especially in phase 3 [fall]). R_{Ca} produced even more methane than conventional anaerobic digestion in continuously stirred tank reactors (CSTRs) of CM and than reported in other

studies digesting CM in CSTR [20,41] ($301 \text{ L-CH}_4 \text{ kgVS}^{-1}$ in R_{Ca} , $136 \text{ L-CH}_4 \text{ kgVS}^{-1}$ in R_R , and $200\text{--}230 \text{ L-CH}_4 \text{ kgVS}^{-1}$ conventionally [42,43]). Angelidaki and Ahning [44] reported similar methane yields ($300 \text{ L-CH}_4 \text{ kgVS}^{-1}$) as in R_{Ca} during thermophilic anaerobic digestion of CM, but in that case, the CM had a higher vS content. All the aforementioned studies and the conventional systems used CM with a VS:TS (fraction of volatile solids fed of total solids fed) of about 0.80, whereas in the current study the average VS:TS was 0.66 ± 0.02 for R_{Ca} and R_R . The lower VS:TS in the current study was due to the pre-treatment of CM with a $200 \mu\text{m}$ size separation removing mainly vS. The removed vS could have further increased methane production, but that stream was thought to find better use as soil amendment. A possible explanation for the exceptionally good methane production of R_{Ca} could be the following: (a) increased SRT and (b) higher concentration of biomass as well as respective substrates.

3.1.1. Effect of calcium addition on SRT and methanization

Adding calcium to CM treatment in a UASB reactor improved solids removal and, consequently, hydrolysis and methanization. The SRT doubled from R_R (123 days, 197 days and 224 days, in phases 2, 3 and 4, respectively; SI, Tables 1) to R_{Ca} (252 days, 406 days and 450 days in phases 2, 3 and 4, respectively; SI, Tables 1) because the calcium addition caused higher suspended and colloidal solid removal. The retention of suspended and colloidal material improved because calcium bridges between negatively charged species, stimulating the aggregation of particles [45,46]. Calcium addition also can cause precipitation of minerals, further increasing the solid concentration in the SB and consequently enlarging the SRT [26,29]. In this study, the SRT was mainly affected by the retention of suspended materials (VSS and FSS) which doubled when adding calcium.

Higher SRTs increased the contact time for hydrolysis of organic particles and consequently improved the conversion of COD_{sus} to methane in this study. More COD_{sus} was retained in R_{Ca} than in R_R (Fig. 2; $77 \pm 4 \%$ and $50 \pm 4 \%$, respectively). In R_R , the methanization is probably mostly correlated to the easier degradable fraction of removed $COD_{col\&sol}$, which represented $20 \pm 1 \%$ of the total COD entering the reactor and $49 \pm 5 \%$ of the total removed COD over the whole operation period (Fig. 2). However, in R_{Ca} , the methanization exceeded the fraction of removed $COD_{col\&sol}$, which represented $17 \pm 1 \%$ of the total COD entering the reactor and $34 \pm 4 \%$ of the total removed COD (Fig. 2). The level of hydrolysis was 29% in R_R and increased to 67% in R_{Ca} because of the calcium addition. Bahreini et al. [47] demonstrated that longer SRTs enhanced hydrolysis for waste-activated sludge (WAS) treatment. WAS is comparable with CM in (bio)degradability, especially regarding hydrolysis [48]. Previous research on WAS [49,50] showed improved hydrolysis, methanogenesis and treatment stability with increased SRTs. The SRTs were increased by increasing the recycling flow in pilot units or decreasing the feeding frequency in bench experiments. Enhancing the SRT by using the UASB technology and adding calcium avoids additional pumping for recycling while keeping a similar OLR and benefiting from improved hydrolysis and methanogenesis. Still, hydrolysis is commonly rate limiting for anaerobic digestion of particulate substrates [51], explaining the inferior methanization of COD_{sus} in R_R , conventional systems, and earlier-mentioned studies compared with R_{Ca} with the longer SRT.

3.1.2. Effect of biomass and substrate accumulation on methanization

The SB developed faster (higher VSS enrichment) and had enhanced concentrations of biomass and substrates (higher VSS concentration in SB) when calcium was added, resulting in higher conversion rates and methane production. Calcium addition improved the VSS removal in R_{Ca} ($75 \pm 7 \%$) compared with R_R ($61 \pm 5 \%$), helping the faster SB development. The SB of R_{Ca} also had a higher VSS concentration ($55.9 \pm 1.4 \text{ gVSS L}^{-1}_{\text{reactor}}$) than R_R ($37.7 \text{ gVSS L}^{-1}_{\text{reactor}}$) at tap 1 during phase 1, demonstrating a denser SB with higher proximity. The concentration of easily biodegradable VFA

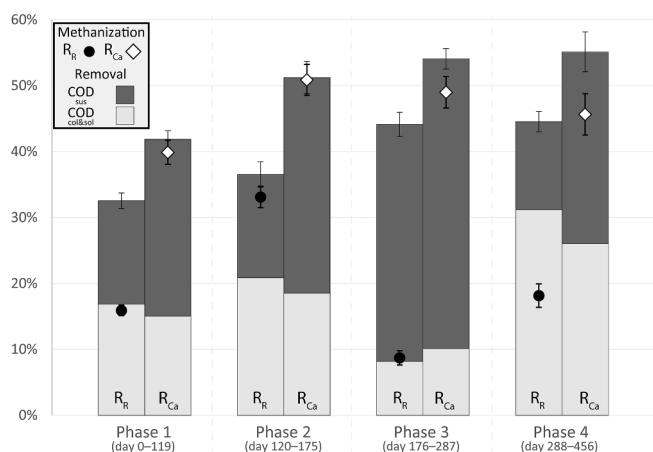


Fig. 2. The cumulative methanization as scatter plot combined with stacked columns for COD removal made of COD_{sus} and $COD_{col\&sol}$ for the reactor without (R_R) and with calcium addition (R_{Ca}) over the four operation phases. The methanization and removal values were based on cumulative in- and outflows. The error bars indicate analytical error percentages based on the standard deviations of influent and effluent measurements in duplicate.

was higher in the SB and in the effluent of R_R than in R_{Ca} (Fig. 3a; on average 1.83 gVFA L^{-1} and 0.25 gVFA L^{-1} , respectively). Acetate was present in the SB and effluent of both reactors, but more for R_R than R_{Ca} (Fig. 3b; on average $0.55 \text{ gAcetate L}^{-1}$ and $0.23 \text{ gAcetate L}^{-1}$, respectively), indicating improved methanogenesis in R_{Ca} and limited methanogenesis in R_R . Propionate made up most of the short-chain VFA in the SB and effluent of R_R but barely occurred in R_{Ca} (Fig. 3c, $1.28 \text{ gPropionate L}^{-1}$ and $0.06 \text{ gPropionate L}^{-1}$, respectively), indicating limited acetogenesis in R_R [52]. Still, the high alkalinity (as $14 \text{ gCaCO}_3 \text{ L}^{-1}$) primarily in the form of bicarbonate in CM (SI, Table 3 and 4) prevented a drop in pH in both reactors (SI, Table 5 and 6). Previous studies also reported enhanced substrate conversions when calcium was added to anaerobic treatment [53,54]. The calcium-enhanced biomass retention and biomass proximity were previously found to be key for improved conversions by fermentative bacteria. Calcium could stimulate biofilm formation, which enhances retention and interactions of microorganisms [55]. In the case of propionate to acetate conversion, the proximity of symbiotic biomass is important to enhance the interspecies hydrogen transfer, which is produced by acetogens and consumed by methanogens [56]. If this symbiosis does not work due to lack of proximity then propionate accumulates [57] because of mass transfer limitations. This would especially concern SBs of UASB reactors treating CM with low mixing. However, enhanced mixing could wash out biomass and other solids, possibly leading to lower treatment performance. Peces et al. [58] observed decreasing propionate degradation at shorter HRTs in stirred reactors, possibly resulting from washout of acetogens or a change of degradation pathways. While HRT is not a limiting factor in the reactors of the current study, the retention of acetogens capable of converting propionate would be higher in R_{Ca} .

3.2. The relation between calcium and organic loading for methane production

Increased methane production with calcium addition had been reported in previous studies, but often the calcium concentration that was applied in this study (on average 2.4 gCa L^{-1}) apparently already inhibited methane production in other studies [29,57,59,60]. Up to a certain concentration, calcium has a beneficial effect on the structure of the extracellular polymeric substance (EPS) matrix around microorganisms, thus improving mass transport [61]. Calcium can also enhance hydrolytic processes by improving enzyme activity or helping stabilize fermentation and anaerobic digestion [62]. When in excess, calcium can complex and precipitate with the substrates, increasing mass transfer limitations and slowing down biodegradation [28]. Calcium could also form inorganic precipitates and consequently limit the mass transport in

the sludge, leading to slower degradation [63,64]. When comparing studies, the calcium concentration seemingly needs to be normalized for feed/substrate. The synthetic wastewater used in Chen et al. [57] had 2 gCOD L^{-1} and methane production slowed down with 0.4 gCa L^{-1} . Yu et al. [29] found that 0.3 gCa L^{-1} was the optimal amount of calcium addition for 4 gCOD L^{-1} in synthetic wastewater for UASB reactors. Above 0.3 gCa L^{-1} , the degradation of COD decreased. In this study, the CM contained on average 62 gCOD L^{-1} , and COD degradation and methane production increased even at 2.4 gCa L^{-1} . When comparing the calcium to COD concentration ratios (Ca:COD), the calcium added in R_{Ca} (Ca:COD of 0.04) was 5 and 2 times less than in Chen et al. [57] (Ca:COD of 0.2) and Yu et al. [29] (Ca:COD of 0.08), respectively. Although the Ca:COD value could indicate how calcium addition affects methane production, the calcium concentration as a parameter alone cannot fully predict the effect of adding calcium.

To our knowledge, the impact of the Ca:COD on methane production and/or anaerobic digestion had not been compared before between studies; yet, it could be essential for achieving optimum calcium addition effects. Ahn et al. [28], Dang et al. [65], and Zhou et al. [66] studied the effect of addition of calcium while monitoring methane production. They all found the highest methane production at a Ca:COD between 0.03 and 0.05 (Fig. 4a). At slightly lower or slightly higher Ca:COD (0–0.03 and 0.05–0.08), the effect of calcium was less significant, while the methane production decreased at Ca:COD > 0.08. It is striking that the greatest benefit for methane production occurred at similar Ca:COD in the three studies even though the added calcium concentration (Fig. 4b) and the feed for anaerobic digestion were significantly different. Ahn et al. [28] used pig manure, Dang et al. [65] used municipal solid waste leachate and Zhou et al. [66] used bagasse. In the current study, the Ca:COD was 0.04 with calcium addition and 0.02 without calcium addition, and the methanization increased by a factor of 2.3 by adding calcium. The reactor with calcium addition falls within the optimum (Fig. 4b, green area) deduced from Fig. 4a, affirming the beneficial effect of calcium at Ca:COD between 0.03 and 0.05. In Cunha et al. [26], the reactor without calcium (Ca:COD of 0.02) and with calcium addition (Ca:COD of 0.055) had similar methane production, and calcium addition had no significant effect on the methane production from BW. Both Ca:CODs were in ranges where other studies have not found a significant effect of calcium addition on methane production (Fig. 4b, white area). From Ca:COD 0.05–0.08, the negative effects of calcium addition, as previously described, probably counterbalanced the positive effects of calcium addition. The negative effects were predominant in almost all studies when the Ca:COD exceeded 0.08 during the treatment, when calcification of sludge occurred or as a result of direct calcium toxicity.

The age and the level of calcification of sludge influences the sludge

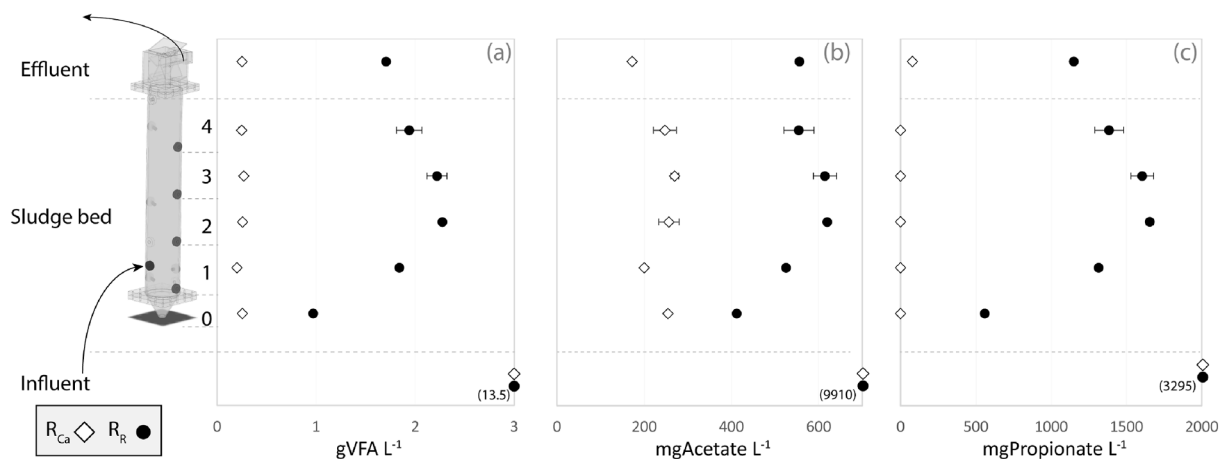


Fig. 3. The vertical distribution of (a) VFA (acetate, propionate and butyrate), (b) acetate and (c) propionate in the SB of the UASB reactors R_R as reference and R_{Ca} with calcium addition together with the influent (bottom) and effluent (top) concentrations at operation day 165 (phase 2).

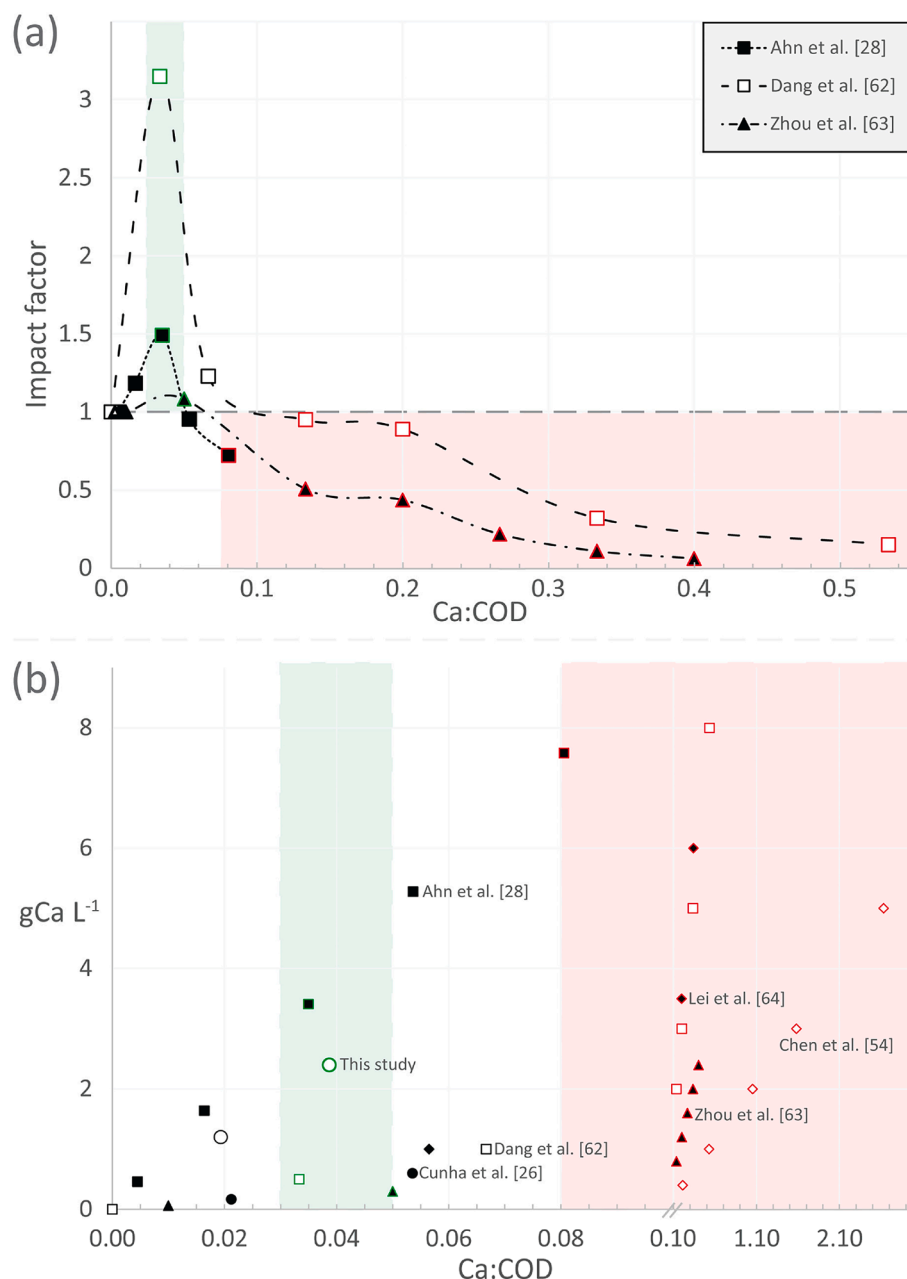


Fig. 4. (a) The relative impact factor of calcium addition on methane production based on the reference experiments conducted in each study over the Ca:COD mass ratio and (b) the calcium concentration used in this study (\circ), Ahn et al. [28] (\blacksquare), Cunha et al. [26] (\bullet), Chen et al. [54] (\diamond), Dang et al. [62] (\square), Lei et al. [64] (\blacklozenge), and Zhou et al. [63] (\blacktriangle) against the Ca:COD. In (a) and (b), the zones and marker outlines highlight the positive impact of calcium in green, no significant impact in the white area and black outline, and negative impact in red.

response to added calcium even at optimum Ca:COD. Chen et al. [54] found that sludge with a higher level of calcification was more sensitive to calcium addition than non-calcified, fresh sludge, which could benefit from higher Ca:COD. Similarly, Yu et al. [29] reported that the specific methanogenic activity (SMA) of fresh sludge benefitted from a Ca:COD of 0.2 during the first 30 days. However, continuous calcium accumulation at the bottom of the SB in all the UASB reactors tested resulted in a decrease of SMA over time. Even at optimum Ca:COD value of 0.038, the positive effect of adding calcium became detrimental after 90 days. Calcification over time at the bottom of the reactors was the cause for the reduction in SMA, and the speed of calcification depended on the calcium concentration. Still, timely removal of calcified sludge from the bottom could prevent deterioration of the local SMA. For instance, growing and harvesting CaP granules by size separation could prevent the reduction in local SMA, prolonging the beneficial effect of calcium addition during anaerobic digestion and simultaneously recovering phosphorus.

3.3. The effect of calcium addition on phosphorus removal and speciation

Calcium addition enhanced the total phosphorus removal from 38 % (R_R) to 61 % (R_{Ca}) over the entire operation time. The phosphorus was almost only present in suspended form (>90 %), and its removal was highly correlated with the removal of suspended solids. Higher solids removal went along with higher phosphorus removal (Fig. 5). The removal of suspended phosphorus (R_R 43 \pm 3 % and R_{Ca} 70 \pm 3 % over the whole operation) contributed the most to the total phosphorus removal and increased by calcium addition. Therefore, the UASB technology, which allows a different HRT and SRT, is essential to enable phosphorus removal.

Calcium addition likely induced aggregation and cementation of small phosphorus-containing particles (<125 μ m) originating from CM and enhanced the retention of phosphorus-rich solids in R_{Ca} [68,69]. Possibly, phosphorus-rich particles aggregated for size ranges below 0.4 mm (the lower threshold for particle size analysis in this study), which was seemingly significant in improving phosphorus retention in R_{Ca} . The

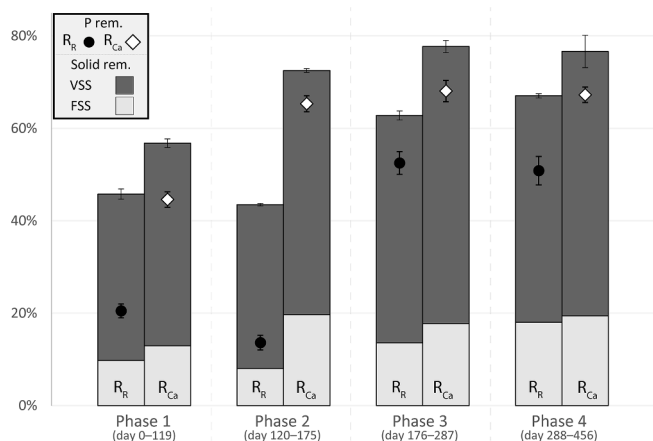


Fig. 5. Phosphorus removal as scatter plot combined with stacked columns for TSS removal made of FSS and VSS removal for R_R and R_{Ca} over four operational phases. The removal values were based on cumulative in- and outflows. The error bars indicate analytical error percentages based on the standard deviations of influent and effluent measurements in duplicate.

phosphorus-rich particles were identified as struvite particles by XRD measurements (SI, Figs. 14 and 15), which explains why phosphorus remained as small particles. Struvite barely forms crystals $>120 \mu\text{m}$ because the crystals have a smooth surface and a negative zeta potential [70,71]. Barzee et al. [72] reported that the majority of phosphorus remains in small particles ($0.45\text{--}75 \mu\text{m}$) even after CM was digested, which would be the case for the digestion in R_R without calcium addition. With calcium addition, a possible co-precipitation cemented the phosphorus-rich particles and allowed for removal in R_{Ca} .

The phosphorus and other solids accumulated mainly at the bottom of both reactors (Fig. 6a and b). The particles that accumulated in R_{Ca} had a lower VSS content, and a gradient of increasing VSS content from bottom to top was found in both reactors (Fig. 6c). These findings were in line with our previous research on BW and pig manure [26,27]. In this study, however, the gradient of phosphorus and calcium concentration in the SB was considerably less pronounced in both reactors, especially in R_{Ca} , compared with previous studies (tap $0.7.7 \pm 0.1 \text{ gCa L}^{-1}$ and tap $1.8.2 \pm 0.2 \text{ gCa L}^{-1}$, SI, Fig. 6). It seemed that the phosphorus, calcium and solids content at the bottom of the SB reached its maximum capacity and, therefore, also a lot of solids were present at tap 1 sampling day 165 (Fig. 6, phase 2). During a later sampling of R_{Ca} on operation day 340 (phase 4), the gradient was more pronounced (Tap $0.9.0 \pm 0.7 \text{ gCa L}^{-1}$ and tap $1.7.9 \pm 0.1 \text{ gCa L}^{-1}$). The settling of particles and accumulation

of phosphorus at the bottom of the SB might be considerably slower with CM than with BW and pig manure. According to studies investigating the physical properties of different wastewater types, the viscosity of CM was higher than the viscosity of pig manure and BW [73,74]. Possibly the higher viscosity caused slower settling in the SB when treating CM than when treating pig manure and BW. The particle size remained at $96\% < 0.4 \text{ mm}$ in R_R and R_{Ca} . The smaller particles combined with a higher viscosity compared with pig manure and BW can cause a lower settleability during CM treatment and, consequently, results in a less pronounced phosphorus gradient.

The phosphorus in the CM fed to the reactors was mainly in the form of struvite, and the bulk of retained phosphorus was also present as struvite in the SBs of both reactors. In both reactors, the molar ratio of magnesium to phosphorus (Mg:P; on average 1.2 in R_{Ca} and 1.3 in R_R on sampling days 165 and 340) based on the measured elemental composition was sufficient to form struvite (struvite Mg:P 1). The presence of struvite was observed with SEM (Fig. 7) and confirmed by XRD (SI, Figs. 14–16). Calcium addition in R_{Ca} caused the occurrence of two broadened peaks in the XRD spectra at theta angle 27.5 , 29 and 30.5 , which could not be identified and might indicate the presence of amorphous precipitates (SI, Fig. 15). Le Corre et al. [70] described that struvite formation becomes inhibited when calcium ions were added to the solution. When enough calcium ions were added to struvite-containing streams, struvite started to dissolve, and calcium phosphate precipitated [75]. Still, contrary to our hypothesis, struvite remained the dominant phosphorus species even with calcium addition in R_{Ca} and calcium to phosphorus (Ca:P) molar ratios up to 4 in the solids of R_{Ca} . Calcium phosphate did precipitate at the cost of struvite when calcium was added in pig manure and BW treatment [26,27]. However, in the current study, struvite crystals were incorporated into a heterogeneous cluster of mostly calcium carbonate and possibly a little calcium phosphate (Fig. 7b). Güngör et al. [76] found that most phosphorus was present as dicalcium phosphate anhydrous (57 %), and the rest was struvite in raw CM. After digestion, struvite was the dominant phosphorus solid (78 %), and the more stable calcium phosphate phase, hydroxyapatite, was the other solid present [76]. However, in raw CMs and digestates, previous studies have reported and predicted different dominant phosphorus species, mainly consisting of either calcium- or magnesium phosphates [77–80]. Precipitation of phosphorus with either calcium or magnesium seems partly dependent on their abundance but also on their availability and ion activity, as shown in this study when struvite prevailed even though additional calcium was supplied.

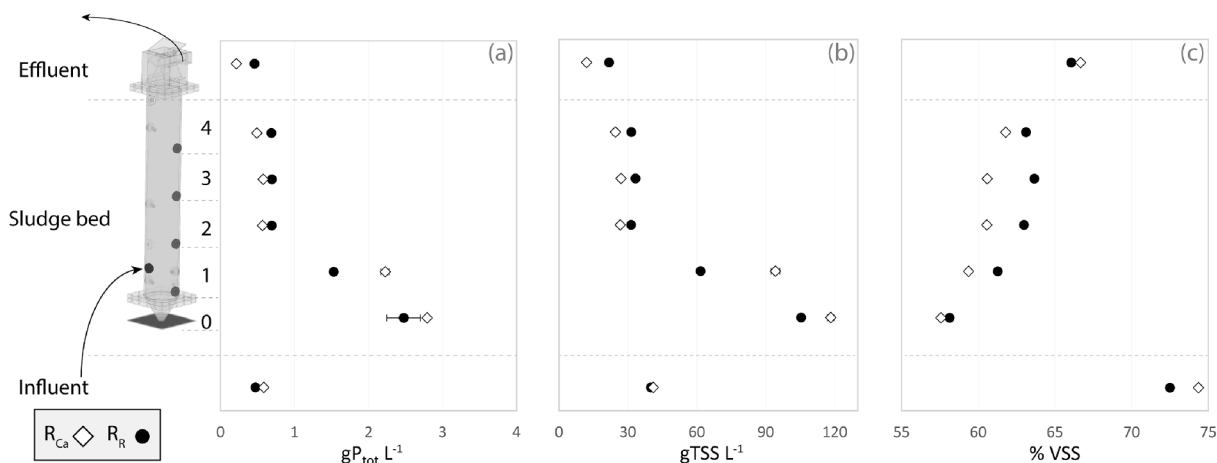


Fig. 6. The vertical distribution of (a) total phosphorus concentration (P_{tot}), (b) TSS concentration and (c) the VSS content in the SB of the UASB reactors R_R as a reference and R_{Ca} with calcium addition together with the influent (bottom) and effluent (top) concentration at operation day 165 (phase 2).

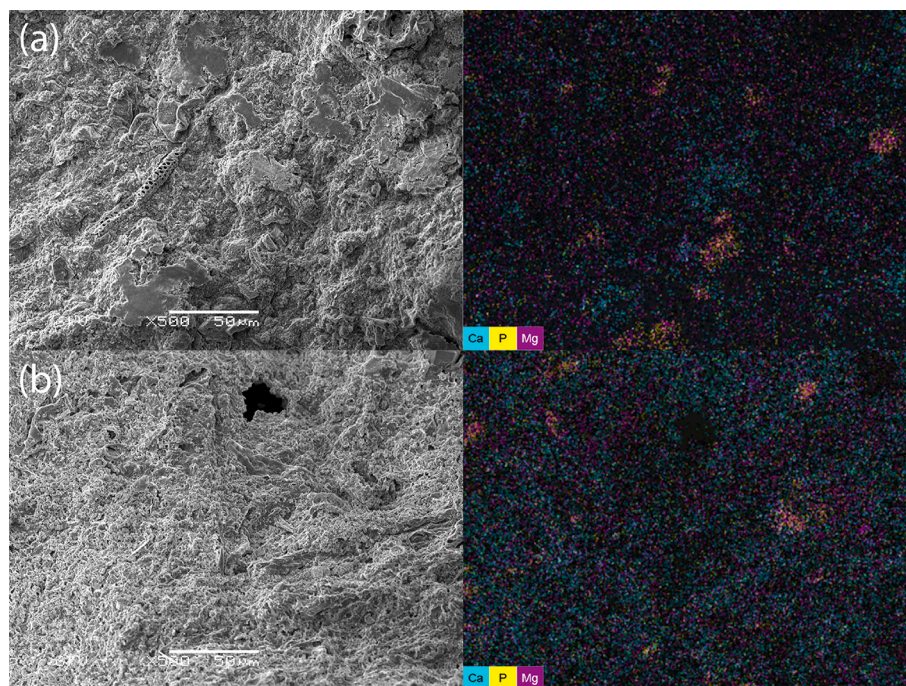


Fig. 7. SEM (left) and EDX (right) images of SB from the bottom of R_R (a) and R_{Ca} (b). In the EDX image calcium is represented as cyan, phosphorus as yellow and magnesium as purple. The overlap of phosphorus (yellow) and magnesium (purple) appears as orange and indicates the presence of struvite. The overlap of phosphorus (yellow) and calcium (cyan) appears as green and indicates the presence of calcium phosphate.

3.4. The competition for calcium is inhibiting calcium phosphate granulation

The added calcium in R_{Ca} did help phosphorus removal compared with R_R but did not clearly induce calcium phosphate precipitation or granulation as in previous studies. The relatively higher concentration of magnesium in CM compared with the other streams (SI, Fig. 1) might have caused struvite to remain and inhibit calcium phosphate precipitation and granulation. Fig. 8a shows the Ca:Mg molar ratio after calcium addition for CM (1.79), pig manure (2.91), and BW treatment (7.43). At Ca:Mg molar ratios ≥ 1 , calcium should be capable of binding more phosphate than magnesium, according to Yan and Shih [75]. Huchzermeier and Tao [81] found that calcium phosphate became the dominant phosphorus species over struvite at $\text{Ca}^{2+}:\text{Mg}^{2+}$ molar ratios of 0.5 in synthetic, filtered, anaerobically digested CM. In synthetic digestate, the orthophosphate removal as calcium phosphate was very efficient (99.9 %), but when the real, filtered digestate was tested, the precipitation dropped to insignificant percentages (0.8 %). The recipe of the synthetic digestate contained mainly calcium, magnesium, phosphorus and ammonium ions with chloride and potassium as counter ions, respectively. It is likely that the influence of organic material and its derivatives when digested, such as bicarbonate, were underestimated in the tests of Huchzermeier and Tao with synthetic digestate; this explains better recovery results in the synthetic digestate with less calcium-capturing bicarbonate than can be obtained in the real digestate with higher presence of bicarbonate. The limitation of precipitation for Huchzermeier and Tao [81] and the inhibition of struvite recrystallization to calcium phosphate in the current study could be in both cases because of anaerobic digestion resulting in high bicarbonate concentrations which contributed to the alkalinity and that can also precipitate with calcium. Calcium carbonate was previously identified as the main impurity in CaP granules from BW and pig manure treatment, but calcium phosphate was the primary precipitate [27,82]. Chen et al. [63] found calcium carbonate as the primary precipitate in granules when treating synthetic wastewater and testing the addition of calcium. Zhang et al. [83] could not qualify calcium carbonate with XRD measurements

during the treatment of BW without calcium addition but found CaP granules. Identifying the drivers for calcium carbonate formation during the treatment of waste streams would allow developing strategies to avoid its precipitation. The possible main drivers for calcium carbonate to precipitate over calcium phosphate are most likely as follows:

- The abundance of inorganic carbon in solution together with calcium ions or precursors of inorganic carbon. Cunha et al. [84] saw a negative relation between the abundance of inorganic carbon and the removal of phosphate. In Zhang et al. [83] and Chen et al. [63], it was not clear how much inorganic carbon was in the solution, but Chen et al. [63] had a higher COD concentration entering the reactor. The higher COD concentration might have caused higher production of inorganic carbon and consequently triggered calcium carbonate over calcium phosphate formation. Similarly, the higher COD conversion in R_{Ca} almost doubled the calcium carbonate precipitation (99gC in R_{Ca} and 52gC in R_R) and inhibited calcium phosphate precipitation and granulation.
- The absence of phosphate as an ion. In CM, phosphate is mainly present in suspended form as struvite (>90 %). In BW and pig manure treatment of previous studies, we saw the presence of phosphate ions (28 % and 21 %, respectively) [26,27]. The presence of phosphate ions allows calcium phosphate precipitation at lower activation energies and consequent homogeneous growth at even lower activation energy compared with a situation where phosphate was bound in a precipitate only [85]. Thus, the activation energy might have been too high to initiate the dissolution of struvite and reprecipitation of phosphate as calcium phosphate in CM. Consequently, the available calcium is bound to abundantly present inorganic carbon.

In the geochemical model (PHREEQC) presented in Fig. 8b, struvite was predicted to be the dominant phosphorus species without calcium addition in dairy (100 % struvite) and pig manure (70 % struvite) but not in BW (39 % struvite) (Ca:Mg 1.05 in CM, 1.31 in pig manure and 1.94 in BW, Fig. 8a). In Fig. 8b, the model showed the presence of ACP

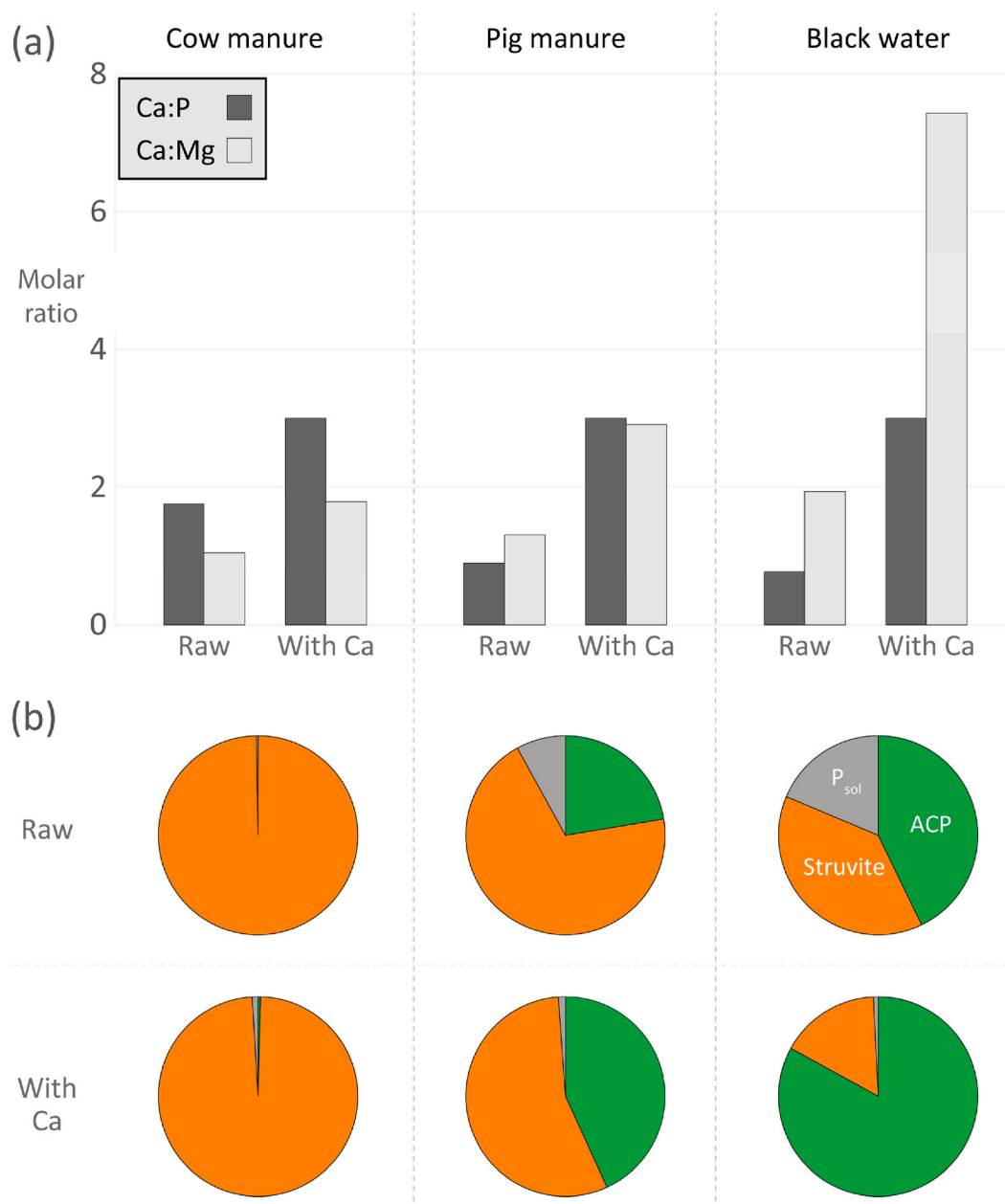


Fig. 8. (a) The molar ratios of calcium over phosphorus (Ca:P) in dark grey and calcium over magnesium (Ca:Mg) in light grey and (b) the phosphorus distribution in struvite (orange), ACP (green) and soluble phosphorus (grey) according to geochemical modeling with PHREEQC in pie charts with and without calcium addition in cow manure, pig manure and black water.

for pig manure (22 % ACP) and BW (43 % ACP) even without calcium addition. However, in CM, struvite dominantly prevailed even when calcium was added (99 % struvite), whereas in pig manure and BW, 43 % and 83 % of the phosphorus, respectively, was present as ACP. In pig manure and BW, the model showed that 56 % and 16 % of the phosphorus, respectively, remained as struvite, which was not actually observed in the SB of pig manure nor BW treatment with calcium addition [27]. This discrepancy probably comes from the model predicting the precipitates based on the concentrations in the raw waste streams but not in the SB during the treatment. In the SB, calcium accumulated over time in complexed form with other compounds (bicarbonate, EPS, organic matter) and consequently will cause supersaturation of ACP at some point [86]. The formation of ACP in granules probably led to energetically favorable surface precipitation of more ACP and recrystallization of struvite [85]. Thus, over time, the SB became rich in calcium, creating a local supersaturation where almost

all struvite became ACP [27]. A similar process could have happened in CM treatment, but possibly over a significantly longer time, as struvite is dominant in Fig. 8b. The limited ACP formation in CM treatment thus was probably related to the lower Ca:Mg ratio, but may also be caused by the relatively higher competition for calcium by bicarbonate in CM treatment than in pig manure and BW treatment.

Sommer et al. [5] also pointed to calcium carbonate as a likely precipitate when calcium was added to manure. Over the entire operation time of our reactors, the retained 330 gCa would bind 99 g of inorganic carbon in R_{Ca} compared with 52 g of inorganic carbon in R_R . Still, inorganic carbon was produced in both reactors (SI, Tables 3 and 4). However, in R_{Ca} , the inorganic carbon concentrations in the liquid phase were lower throughout the SB than in R_R (SI, Fig. 10). The CO_2 content in the biogas of R_{Ca} (phase 1, 38 %; phase 2, 38 %; phase 3, 36 %; and phase 4, 34 %; based on cumulative production data) was also lower than in the biogas of R_R (phase 1, 43 %; phase 2, 41 %; phase 3, 40 %;

and phase 4, 35 %; based on cumulative production data). The mass balance for carbon on day 340 (last SB sampling) was missing 4 % carbon in R_{Ca} and had an overestimation of 1 % in R_R (SI, Fig. 17). The mass balance agreed with double the calcium carbonate formation in the SB of R_{Ca} compared with R_R . This was qualitatively examined with SEM-EDX (Fig. 7) and XRD (SI, Figs. 14 and 15) measurements. The geochemical model PHREEQC also predicted that all calcium precipitated as either calcite, vaterite or aragonite. The combined results indicate that bicarbonate is the most significant and abundant competitor for reacting with calcium and not struvite or phosphate. A competitor that still has a beneficial effect as the formed calcium carbonate can cement struvite particles together and thus increase overall particle size and enhance sedimentation.

3.5. The potential of recovering methane and phosphorus from cow manure

The technology is easy to implement and maintain, which makes it suitable for farms. At an averagely sized farm (150 cows) in the Netherlands, the reactor unit would need to be of about 200–300 m³, depending on the farm-specific CM characteristics. Such a farm-scale reactor could produce about 37 thousand m³ of CH₄ per year based on the treatment efficiency of R_{Ca} . That is enough energy to heat a full-scale reactor to 55 °C and allow 28 dwellings to be supplied with energy for space heating. When extrapolating for the entire country of the Netherlands, about 0.5 million dwellings of the 7.9 million dwellings in the Netherlands could be heated on digested CM if all the CM would be digested (estimated based on numbers from [3]). A current waste could thus be turned into a significant contribution for energy supply.

While digesting the CM in a UASB reactor with calcium addition, the removal of phosphorus would allow for a higher application of nutrients originating from CM. Dairy farmers are commonly limited in applying the manure on their land, because of phosphorus application limit regulations. Removing 61 % phosphorus would allow for more than double the nitrogen application on the same area originating from CM. This would diminish the need for artificial nitrogen fertilizer in dairy farming and substantially reduce economic and environmental costs [11] while contributing toward a circular economy. The phosphorus in the sludge bed could be transported in higher concentrations than in untreated CM to regions where phosphorus is needed. Thus, the transportation costs and CO₂ emissions could be reduced when transporting phosphorus as SB instead of as untreated CM. The removal, recovery and reuse of phosphorus could be further improved if higher phosphorus concentrations and quantities would be present as easy to separate CaP granules.

4. Conclusions

Calcium addition increased the phosphorus removal from 38 % to 61 % and the methanization from 20 % to 46 %. Additional calcium stimulated the conversion of suspended material to methane and enhanced the SB development. Hydrolysis improved from 29 % to 67 % because of calcium addition. We found that calcium concentration alone did not describe the effect of calcium addition well enough and found an optimum of calcium addition in the range of 0.03–0.05 in Ca:COD when comparing this study and the literature. The optimum Ca:COD range demonstrated across multiple digester and substrate types suggests that the Ca:COD can generally help to optimize methane production during anaerobic digestion. More particles and phosphorus were retained with calcium addition, but phosphorus remained in the form of struvite. Struvite could prevail because the Ca:Mg molar ratio was low (1.8) compared with previous studies (2.9 in pig manure and 7.4 in BW) and because bicarbonate captured calcium. Most of the calcium precipitated as calcium carbonate, limiting the calcium availability for calcium phosphate formation and granulation. The presence of inorganic carbon and the Ca:Mg molar ratio are two indicators to predict the success of

CaP granulation, granting the possibility to identify new waste streams for CaP granulation. Still, the higher methane production and removal of phosphorus by adding calcium improves the valorization of CM. The phosphorus-rich SB and the nitrogen-rich digestate can be used separately, saving on mined and artificial fertilizer.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The [supplementary information](#) file contains most data that were collected.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2022.140294>.

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