

# Simultaneous calcium phosphate granules and methane recovery from black water

J. R. Cunha \*, T. Tervahauta\*, R. D. van der Weijden\*\*, L. Hernández Leal\*, G. Zeeman\*\*, C. J. N. Buisman\*

\* Wetsus, European Centre of Excellence for Sustainable Water Technology, P.O: Box 1113, 8900CC Leeuwarden, The Netherlands  
(E-mail: [jorgericardo.cunha@wetsus.nl](mailto:jorgericardo.cunha@wetsus.nl); [taina.tervahauta@wetsus.nl](mailto:taina.tervahauta@wetsus.nl); [luca.hernandez@wetsus.nl](mailto:luca.hernandez@wetsus.nl); [cees.buisman@wetsus.nl](mailto:cees.buisman@wetsus.nl))

\*\* Sub-department of Environmental Technology, Wageningen University, P.O. Box 17, 6700AA Wageningen, The Netherlands  
(E-mail: [renata.vanderweijden@wur.nl](mailto:renata.vanderweijden@wur.nl); [grietje.zeeman@wur.nl](mailto:grietje.zeeman@wur.nl))

## Abstract

Calcium phosphate (CaP) granules were discovered in the anaerobic treatment of vacuum collected black water (BW), using upflow anaerobic sludge blanket (UASB) technology. This allows simultaneous recovery of phosphorus (P) and methane (CH<sub>4</sub>) in the UASB reactor, and provides an alternative to the current P recovery process by struvite precipitation. After 260 days of operation, 4.4% of the total P fed was found as CaP granules (61 mgP g<sup>-1</sup> dried matter). Moreover, CaP granules had lower heavy metals content than rock phosphate. Simultaneously, 60% of the COD in BW was efficiently converted into CH<sub>4</sub> (1.9 m<sup>3</sup>CH<sub>4</sub> m<sup>-3</sup>BW at 25°C). The bicarbonate and calcium concentrations in BW showed a significant influence on the accumulation of P in the UASB reactor. Bicarbonate decreases the ion activity of free calcium, and consequently, reduces CaP precipitation and granulation. Preliminary results showed that addition of calcium enhances CaP precipitation and possibly the granulation rate, which are crucial for the process feasibility.

## Keywords

Source separated black water; phosphorus recovery; granules; methane; UASB reactor

## INTRODUCTION

Phosphate (PO<sub>4</sub>) is finite and essential for humankind, and 88% of the rock phosphate reserves are predicted to be controlled by Morocco in the early 22<sup>nd</sup> century (Cooper et al., 2011). Introduction of recycling measures and sustainable use can extend the global resource longevity beyond the 22<sup>nd</sup> century (Koppelaar and Weikard, 2013). Source separated black water (BW) contains 75% of the domestically wasted phosphorus (P), which represents 18% of the global P demand (Tervahauta et al., 2013; Schröder et al., 2010). Thus, P recovery from BW could significantly extend PO<sub>4</sub> availability. The relatively high concentration of P and organics is achieved by vacuum collection of BW (0.19 ± 0.08 gP L<sup>-1</sup> and 8 ± 2 gCOD L<sup>-1</sup>) when compared with the conventional toilet flushing system (6 and 34 L person<sup>-1</sup> d<sup>-1</sup> of water use, respectively) (Tervahauta et al., 2013). This creates attractive conditions for implementation of recovery techniques.

The existing treatment concept of BW consists of an upflow anaerobic sludge blanket (UASB) reactor for COD removal and methane (CH<sub>4</sub>) production, followed by an oxygen-limited autotrophic nitrification-denitrification (OLAND) reactor for nitrogen removal, and finally a P removal stage where magnesium oxide (MgO) is added to induce struvite precipitation (Tervahauta et al., 2013; Graaf and Hell, 2014). This concept is applied at full scale since 2008 for a community with 232 houses in Sneek, The Netherlands. At 35 °C, 78% of the organic load is converted into biogas in the UASB reactor, and 56% of the total P fed is removed from the effluent as struvite (Graaf and Hell, 2014). However, a large fraction (39%) of P accumulated within the UASB reactor since the high pH and chemical composition of BW favour P precipitation (Graaff, Temmink, et al., 2011). P accumulation increased from 39 to 51% and calcium phosphate (CaP) granules were observed after a decrease in humic substances and bicarbonate (HCO<sub>3</sub><sup>-</sup>), along with an increase in free calcium (Ca) in BW (Tervahauta, Weijden, et al., 2014). These changes in BW were due to changes in drinking water composition (Mulder et al., 2014). Higher concentrations of free Ca

directly enhance CaP precipitation and a decrease in  $\text{HCO}_3^-$  lowers the competition with  $\text{PO}_4$  for Ca ions. Furthermore, CaP precipitates are biocarriers for microbial attachment and evolve to CaP granules (Cunha et al., 2016). A biofilm forms around the CaP precipitate and creates a higher pH locally, favouring CaP growth. CaP granulation is a novel type of anaerobic granulation, and it occurred under different hydrodynamic conditions compared to biomass granulation. Commonly, biomass granulation is achieved by applying high upflow velocities ( $> 1 \text{ m h}^{-1}$ ) in the UASB reactor. This induces a selection pressure on the microbial community which stimulates biomass agglomeration and promotes the selective wash-out of disperse biomass (Hulshoff Pol et al., 2004; Liu et al., 2002). In contrast, CaP granulation occurred under much lower upflow velocity (0.01 to  $0.004 \text{ m h}^{-1}$ ) in the UASB reactor (Graaff et al., 2010). The long solids retention time (SRT) allowed the granules maturation by internal CaP crystal growth and incorporation of external inorganic particles (Cunha et al., 2016).

CaP granulation is a novel process, the recovery of P as CaP granules until now represented only 2% of the total incoming P from BW (Tervahauta, Weijden, et al., 2014). Therefore, understanding the role of the chemical composition of BW on P accumulation and CaP precipitation and granulation could be used to improve the P recovery efficiency. The potential for simultaneous recovery of methane ( $\text{CH}_4$ ) and CaP granules during the anaerobic treatment of BW was studied, using a lab scale UASB reactor. The effect of changes in soluble Ca and  $\text{HCO}_3^-$  in BW on P accumulation was assessed by correlation of these with  $\text{PO}_4$  removal, during the reactor operation. The quantitative and compositional development of suspended solids in the sludge bed were monitored over time. Finally, addition of Ca is tested in treated BW to predict the potential of P recovery as CaP granules.

## **METHODS**

### **Experimental set up**

An UASB reactor (5.1 L) fed with vacuum collected BW was started and monitored for 260 days at  $25^\circ\text{C}$ , with a HRT of 8 days. The reactor was inoculated with 2 L of sludge from the aforementioned full scale UASB reactor (Graaf and Hell, 2014). Influent and effluent were sampled one and two times per week, respectively. Sludge bed analysis at 5 cm from the bottom of the UASB reactor were performed approximately every 30 days. Approximately 100 ml of sludge were taken per sampling point. Triplicates were performed for influent, effluent and sludge analysis.

### **Physico-chemical analysis**

Total and volatile suspended solids (TSS and VSS, respectively) were quantified according the standard methods (Clesceri et al., 1998). Total and soluble COD were measured with Hach Lange kits (LCK114). Biogas composition was analysed through gas chromatography. Liquid and solid elemental analysis (P, Ca and Mg) of influent, effluent and sludge samples were performed through inductively coupled plasma – atomic emission spectroscopy (ICP-AES). An acid digestion at  $148^\circ\text{C}$  for 45 min was performed beforehand to dissolve solids.  $\text{PO}_4$  and volatile fatty acids (VFA) were quantified through ion chromatography (IC). Ammonium ( $\text{NH}_4^+$ ) was analysed using Hach Lange kits (LCK302). Soluble compounds were measured after membrane filtration ( $0.45 \mu\text{m}$ ). SRT, level of hydrolysis of solids and methanization were calculated according to Graaff et al. (2010), for the steady state period between days 55 and 260. A vertical characterization of the sludge bed at 5, 10, 20 and 30 cm high was performed at the end of the monitoring period (day 260). In this characterization, TSS, VSS and COD concentrations and P, Ca and Mg contents in TSS were measured.

### **P mass balance**

The P mass balance was calculated adding up all the measured incoming total P (influent and

inoculum) and measured outgoing (accumulated, effluent and wasted). The fraction wasted consists of excess sludge and sludge bed sampling. Retention of P precipitates on top of the reactor (overflow settler) represented  $48 \pm 5\%$  of the P that leaves the reactor, therefore, it was included in the fraction effluent. The accumulated P was further divided in CaP granules, biomass and inorganic particles. The accumulated P was calculated according eq. 1, using the vertical characterization of the sludge bed on day 260. The fraction inorganic particles was calculated according to eq. 2. The fraction biomass was calculated through eq. 3. The fraction CaP granules was estimated through a separation by specific density of particles from sludge bed samples and through eq. 4. Sludge bed samples (100 ml) were taken on days 218 and 300 at two different heights of the UASB reactor (5 and 20 cm). The separation of CaP granules in these samples was carried out in an upflow column (100 x 2 cm), using filtered effluent from the UASB reactor to fluidize the sample. Here an upflow velocity of  $57 \text{ m h}^{-1}$  was applied and solids were divided in 5 fractions by density. Only granules at the densest fraction were considered as CaP granules, due to the higher specific density (inorganic content) compared to biomass and smaller inorganic particles. Elemental composition of TSS was determined for each fraction in the separation column. The procedure was performed twice for each height sampled. The P recovery as CaP granules was estimated according to the concentration and P content of CaP granules in the sludge bed after the monitoring period. The fraction non-quantifiable consists of P accumulation at the bottom of the reactor and P precipitation directly in the inlet tube, perhaps due to the low feeding flow (Rohde, 2016).

### Composition and structure of CaP granules

The morphology and elemental distribution of CaP granules were analysed with a scanning electron microscope in backscattering mode coupled with energy dispersive X-ray (SEM-EDX). The crystal phases in the granules were identified through X-ray diffraction (XRD) analysis.

### Experimental set-up for Ca addition

Precipitation of CaP species in treated BW was evaluated according the supersaturation index (SI), using the software Visual MINTEQ version 3.1 (KTH, Sweden). SI was calculated according eq. 5, considering the Davies model to calculate the activity coefficients (Mullin, 2001).  $\text{CaCl}_2$  was added in treated BW (5 L), containing  $43 \text{ mgP L}^{-1}$ , at different Ca/P molar ratios (1.5, 3.1 and 4.8) in 1 L recirculation columns. A control was run with only treated BW (0.6 Ca/P molar ratio). The formed precipitates were accumulated in the column with a hydraulic retention time (HRT) of 5.5 h. During 12 days soluble P and Ca,  $\text{HCO}_3^-$  and pH in the liquid phase were measured.

### Calculations

$$P_{\text{accumulated}} = \sum_i \left( P_{T_i} \cdot TSS_i \cdot \frac{V_s}{4} \right) \quad \text{eq. 1}$$

where  $i$  represents the four heights of the sludge bed sampled ( $i = 5, 10, 20$  and  $30$ ),  $P_T$  is the P content in TSS, TSS is the concentration of suspended solids and  $V_s$  is the sludge bed volume on day 260.

$$P_{\text{inorganic particles}} = P_{\text{accumulated}} - P_{\text{Biomass}} - P_{\text{CaP granules}} \quad \text{eq. 2}$$

$$P_{\text{Biomass}} = P_I \cdot \sum_i \left( VSS_i \cdot \frac{V_s}{4} \right) \quad \text{eq. 3}$$

where  $P_I$  is the initial P content in VSS of the inoculum ( $26 \text{ mgP gVSS}^{-1}$ ),  $VSS_i$  is the concentration of volatile suspended solids for each height of the sludge bed ( $i = 0, 10, 20$  and  $30$ ) and  $V_s$  is the sludge bed volume on day 260.

$$P_{\text{CaP granules}} = \sum_b \left( P_{T_b} \cdot f_b \cdot TSS_b \cdot V_{S_b} \right) \quad \text{eq. 4}$$

where  $b$  represents each height of the sludge bed sampled for particle separation (5 and 20 cm),  $P_{T_b}$

is the P content in TSS from the densest fraction (CaP granules) of each height,  $f_b$  is the percentage of TSS from the sludge sample as CaP granules,  $TSS_b$  is the concentration of TSS in the sludge sample and  $V_{sb}$  is the volume fraction of the sludge bed for each height sampled (1 and 1.3 L, respectively).

$$SI_y = \frac{IAP_y}{K_{sp_y}} \quad \text{eq. 5}$$

where SI is the supersaturation index of a certain specie y, IAP is the ion activity product of the elements in y and  $K_{sp}$  is the solubility product constant of y. For  $SI > 1$  y is supersaturated, for  $SI < 1$  y is under saturation and for  $SI = 1$  y is in apparent equilibrium.

## RESULTS AND DISCUSSION

### UASB reactor performance

The UASB reactor performance was based on total COD removal and  $CH_4$  production. More than 80% of the total COD loading was removed with a methanization rate of  $65 \pm 26\%$  (Table 1). Approximately 91% of the suspended COD ( $COD_{ss}$ ) was removed with a hydrolysis level of  $65 \pm 34\%$  (fraction of  $COD_{ss}$  converted into  $CH_4$ ). This resulted in a solids retention time (SRT) of 316 days. The high deviations in Table 1 are a consequence of chemical changes in BW over time. The effluent was nearly free of solids ( $< 0.5 \text{ gCOD}_{ss} \text{ L}^{-1}$ ). Sludge discharge stabilized after 55 days of operation at  $0.5 \pm 0.1 \text{ gVSS d}^{-1}$  ( $0.6 \pm 0.2 \text{ gTSS d}^{-1}$ ). The concentration of volatile fatty acids (VFA) in the effluent was always below  $0.05 \text{ g L}^{-1}$ . Influent and effluent pH and concentration of  $NH_4^+$  was respectively  $7.9 \pm 0.4$  and  $7.4 \pm 0.2$  and  $0.8 \pm 0.1$  and  $0.7 \pm 0.1 \text{ g L}^{-1}$ . The  $CH_4$  production was  $1.9 \text{ m}^3 \text{ m}^{-3}\text{BW}$  at  $25^\circ\text{C}$ . The high deviations are due to compositional changes of BW during the monitoring period. Similar treatment performance was observed by Graaff et al. (2010).

**Table 1.** Key performance parameters of BW anaerobic treatment using a UASB reactor. Mean total and soluble OLR, total and suspended COD removal rates, methanization and level of hydrolysis of solids for the operational period between days 55 and 260.

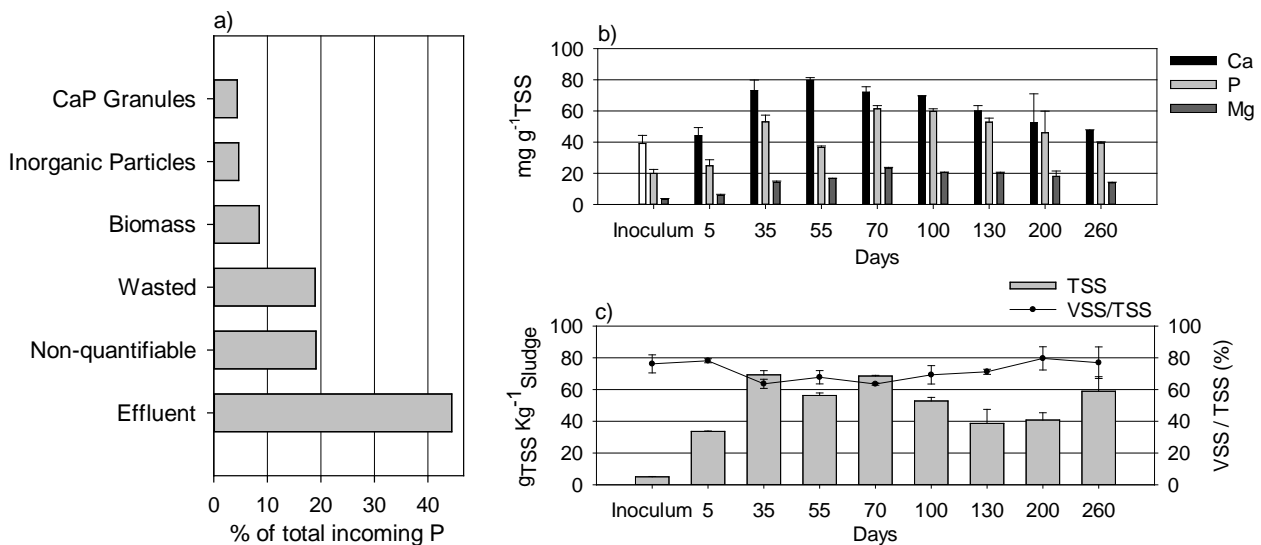
Operation time	Days	260
HRT	Days	7.8 $\pm$ 0.6
Solids loading	$\text{gTSS d}^{-1}$	2.5 $\pm$ 1.6
$OLR_{\text{Total}}$	$\text{kgCOD m}^{-3} \text{ d}^{-1}$	1.0 $\pm$ 0.3
$OLR_{\text{Soluble}}$	$\text{kgCOD m}^{-3} \text{ d}^{-1}$	0.4 $\pm$ 0.2
$COD_{\text{Total}}$ removal		87% $\pm$ 4%
$COD_{ss}$ removal		92% $\pm$ 4%
Methanization	$\text{mgCOD-CH}_4 \text{ mg}^{-1}\text{COD-BW}$	0.65 $\pm$ 0.26
Level of hydrolysis of solids		0.65 $\pm$ 0.34
SRT	Days	316

### Phosphorus removal and recovery, and granules distribution in the sludge bed

Of the total amount of P fed, 44% was found in the effluent (Figure 1a), indicating an accumulation of 56% in the reactor. Soluble P slight increase in the effluent, from  $56.9 \pm 12.3 \text{ mgP L}^{-1}$  in the BW to  $59.5 \pm 6.9 \text{ mgP L}^{-1}$ . Particulate P in BW ( $134 \pm 83 \text{ mgP L}^{-1}$ ), which represents  $67 \pm 11\%$  of the total P, was mostly retained in the reactor. The low upflow velocity ( $0.4 \text{ cm h}^{-1}$ ) in the UASB reactor eased accumulation of fine particulate P. The slight increase in soluble P was perhaps due to liberation of organic P. The P mass balance is shown in Figure 1a. Approximately 4.4% of the total P fed was present as CaP granules after 260 days of operation. Yet, sampled granules were considered as wasted sludge. The non-quantifiable P consisted of accumulation of inorganic particles at the bottom of the UASB reactor and inlet tube. EDX analysis showed that these particles

were mainly CaP and struvite with P content of approximately 13 and 21 wt% of dried matter, respectively. However, the total amount of P at the bottom and inlet tube could not be quantified. The P distribution in the sludge bed after 260 days was 25, 26 and 48% as CaP granules, inorganic particles and biomass, respectively. In time the inorganic particles can grow to granules, but further research is needed to estimate the growth rate and conditions to favour granulation.

The development of suspended solids (TSS and VSS) and P, Ca and Mg contents in the sludge bed at 5 cm from the bottom of the UASB reactor in time are shown in Figure 1b and 1c, respectively. The sludge bed height was maintained at 60% of the reactor volume. After 35 days of operation, the TSS concentration at 5 cm from the bottom of the reactor was on average  $55 \pm 12$  gTSS kg<sup>-1</sup>Sludge with an organic content (VSS) of  $72 \pm 6\%$ . This is in line with the study of Graaff et al. (2010). The P content of the sludge increased from  $20 \pm 3$  mg g<sup>-1</sup>TSS in the inoculum to  $61 \pm 2$  mg g<sup>-1</sup>TSS after 70 days. A similar increase is observed for Ca and Mg (Figure 1b), suggesting accumulation of Ca and Mg phosphates. The accumulation of these precipitates is perhaps associated with the formation of granules firstly at the bottom of the reactor. The decrease in TSS concentration and P, Ca and Mg contents after 100 days of operation is possibly a result of granulation. On day 260, the TSS concentration at 5, 10, 20 and 30 cm high in the sludge bed was  $60 \pm 9$ ,  $46 \pm 6$ ,  $37 \pm 7$  and  $41 \pm 8$  gTSS kg<sup>-1</sup>Sludge, respectively.



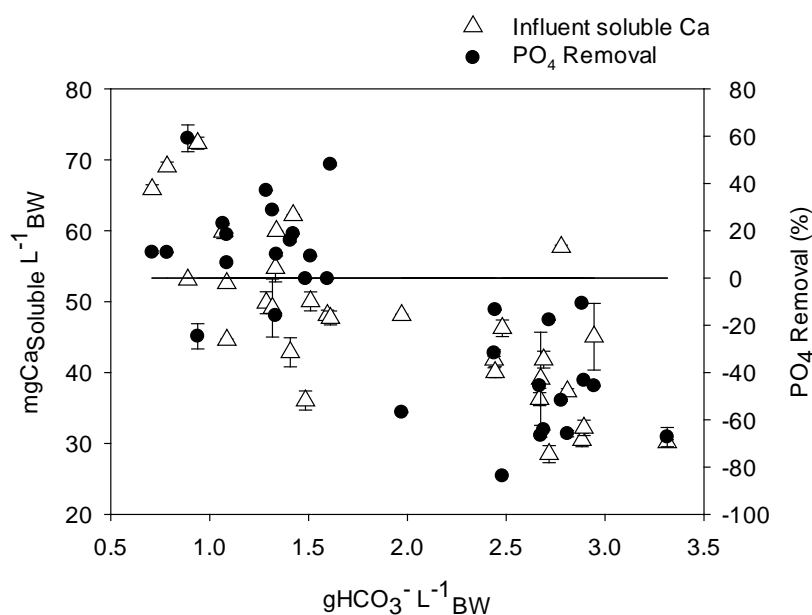
**Figure 1.** P mass balance for 260 days of operation, assuming the organic P content of biomass as  $26$  mg g<sup>-1</sup>VSS (a). Development of TSS concentration (c), P, Ca and Mg contents (b) and percentage of VSS to TSS (c), at 5 cm from the bottom of the UASB reactor over time.

### Effect of soluble Ca and HCO<sub>3</sub><sup>-</sup> in BW on PO<sub>4</sub> removal

During the operation a wide concentration range was observed for several compounds in the influent BW, which possibly affected the P accumulation in the UASB reactor due to thermodynamic changes. According to Figure 2, when HCO<sub>3</sub><sup>-</sup> is above  $2$  g L<sup>-1</sup> the soluble PO<sub>4</sub> concentration increases during the treatment, due to hydrolysis of organic P and release of PO<sub>4</sub> from already formed CaP. This is because HCO<sub>3</sub><sup>-</sup> competes with PO<sub>4</sub> for Ca ions to precipitate as calcium carbonate (CaCO<sub>3</sub>) (Cao and Harris, 2008). For values of HCO<sub>3</sub><sup>-</sup> below  $2$  g L<sup>-1</sup>, part of the hydrolysed and free PO<sub>4</sub> accumulated in the reactor. The soluble Ca increases from approximately  $30$  to  $70$  mg L<sup>-1</sup> with the decrease in HCO<sub>3</sub><sup>-</sup> from  $3$  to  $1$  g L<sup>-1</sup> (Figure 2). Free Ca is higher at low HCO<sub>3</sub><sup>-</sup> levels, enabling CaP precipitation. More than 80% of the Mg in BW is already in the solid form (not shown). Moreover, accumulation of Mg (mainly as struvite) was observed in the sludge bed, inlet tube and top settler of the UASB reactor. Thus, the presence of Mg can contribute to the P

accumulation. However, the soluble Mg in BW ( $14 \pm 9 \text{ mg L}^{-1}$ ) does not have a significant influence on  $\text{PO}_4$  removal compared to soluble Ca.

In this study and in Tervahauta et al. (2014), the accumulation of P in the UASB reactor (56 and 51%, respectively) was higher than the previously reported by Graaff et al. (2010) (39%), all under similar operational BW treatment conditions. This is likely due to changes in Ca and  $\text{HCO}_3^-$  concentrations in BW after changes in quality of drinking water.  $\text{HCO}_3^-$  concentration in BW during this study and in Tervahauta et al. (2014) was  $1.9 \pm 0.8$  and  $3.6 \pm 1.1 \text{ g L}^{-1}$ , while it was  $5.6 \pm 2.8 \text{ g L}^{-1}$  prior to the optimization of softening of drinking water (Graaff et al., 2010; Mulder et al., 2014). The soluble Ca in BW during this study and in Tervahauta et al. (2014) was  $48 \pm 11$  and  $62 \pm 7.4 \text{ mg L}^{-1}$ , respectively, while in Graaff et al. (2010) was  $39 \pm 18 \text{ mg L}^{-1}$ . The higher Ca in Tervahauta et al. (2014) is perhaps due to the lower average  $\text{PO}_4$  concentration compared to this study ( $49 \pm 9$  and  $57 \pm 12 \text{ mgPO}_4\text{-P L}^{-1}$ , respectively), which decreased CaP precipitation. Due to the lower  $\text{HCO}_3^-$  concentration and consequent increase in free Ca, during this study and in Tervahauta et al. (2014), CaP precipitation was favoured and possibly led to CaP granulation.



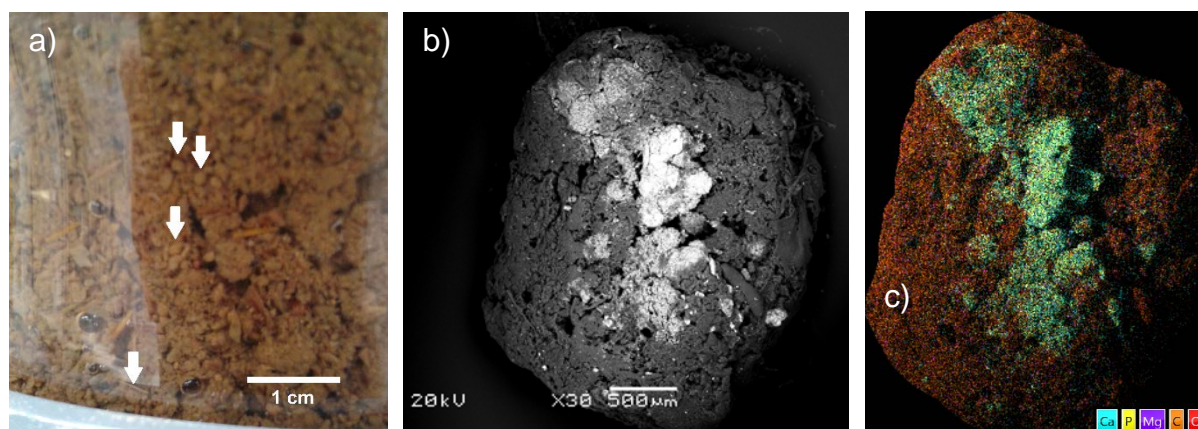
**Figure 2.** Correlation between soluble Ca and  $\text{HCO}_3^-$  in the influent BW and  $\text{PO}_4$  removal, during the monitoring period (260 days).

### Granule formation and characterization

A gradual formation of granules was observed approximately after 100 days at the lower part of the sludge bed (Figure 3a). A backscattered SEM representation of a CaP granule from a sample taken at 5 cm from the bottom of the reactor is shown in Figure 3b. The inorganic rich core (whitish) is surrounded by an organic outer layer (dark grey), supporting the formation of granules from an inorganic seed particle (Cunha et al., 2016). According to the EDX analysis on the sampled granule (Figure 3b), P and Ca were the dominant elements in the centre (Figure 3c). This indicates the presence of CaP in the core of the granules as previously reported by Tervahauta et al. (2014). Visualization of Mg containing precipitates was also possible, but with lower intensity compared to Ca precipitates. The P, Ca and Mg contents of the densest fraction in the separation column were  $61 \pm 8$ ,  $81 \pm 15$  and  $19 \pm 2 \text{ mg g}^{-1} \text{ TSS}$ , respectively. Moreover, the ash content of this fraction was  $35 \pm 6\%$ . Both P and ash contents were higher in Tervahauta et al. (2014), perhaps due to the longer operation time (988 days) compared to this study (260 days). This is because formation and growth of CaP crystals is greatly time dependent (Gajjeraman et al., 2007). Nevertheless, the P content

observed in the CaP granules is relatively higher compared to other types of biomass granular sludge, ranging from 20 to 36 mgP g<sup>-1</sup>TSS (D'Abzac et al., 2010; Yu et al., 2001; Langerak et al., 1998). According to the XRD analysis (results not shown) hydroxyapatite (HAp) and carbonated HAp are the dominant phases in the granules. This is in line with the analysis previously reported by Tervahauta et al. (2014).

CaP granules produced from BW have lower heavy metals content than sewage sludge ash and BioP sludge ash (Tervahauta, Weijden, et al., 2014). For instance, faeces represents only 10% of the concentration of heavy metals in centralized sewage, and they are mainly from human excretion (Tervahauta, Rani, et al., 2014). Thus, for resource recovery proposes source separated BW is a better option. The heavy metals content of rock phosphate largely depends on the location of extraction and ore type (Aydin et al., 2010). Yet, the worsening quality and depletion of rock phosphate threat its use, resulting in reduction of commercial ore deposits (Koppelaar and Weikard, 2013). Recovered products from wastewater streams, such as CaP granules and struvite, are then possible substitutes for rock phosphate. However, risks with other potential micropollutants and resistant bacterial genes need further investigation, especially in CaP granules due to the residual organic content (Graaff, Vieno, et al., 2011; Butkovskiy et al., 2016).

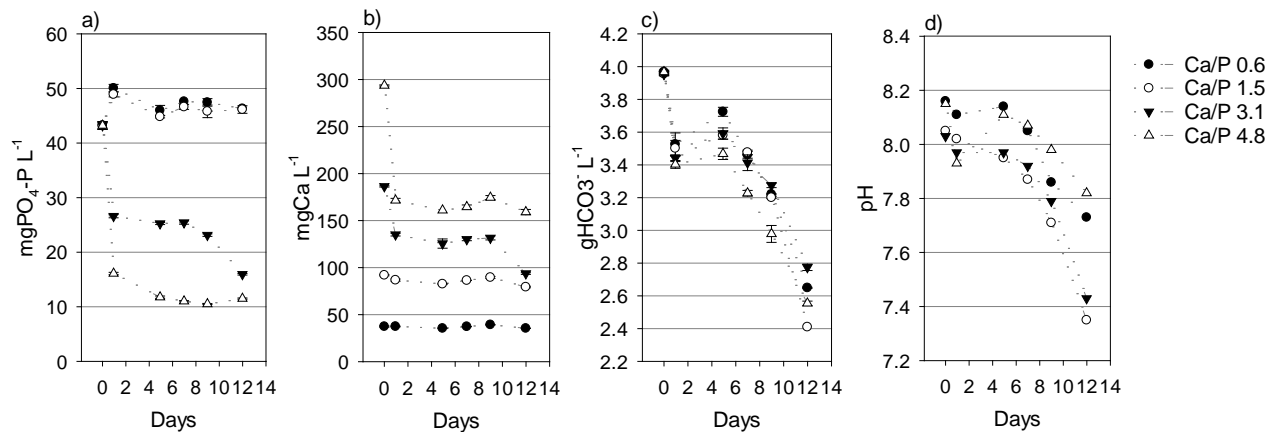


**Figure 3.** Image of the sludge bed on day 93 (a), corresponding to the start of granulation. Arrows indicate CaP granules in the sludge bed. Backscattered SEM image of a representative CaP granule cross-section (b), and the respective elemental distribution acquired with EDX (c).

### The potential recovery CaP granules with Ca addition

Relatively high concentrations of soluble PO<sub>4</sub> ( $59.5 \pm 6.9$  mgP L<sup>-1</sup>, representing approximately 31% of the total P in BW) is present in the UASB reactor effluent, although soluble Ca ( $33 \pm 5.9$  mg L<sup>-1</sup>) is still available. This is due to the thermodynamic equilibrium. The Ca/P molar ratio in BW is only 0.65, indicating that Ca is limiting for CaP precipitation, such as amorphous CaP, octacalcium phosphate (OCP) and HAp, for which Ca/P molar ratio varies from 1.35 to 1.67 (Habraken et al., 2013). Geochemical modeling of treated BW showed that more soluble CaP species are under saturated (SI < 1), such as amorphous CaP (0.17) and OCP (0.02); both phases are precursors of the most stable phase HAp (Habraken et al., 2013). Therefore, some preliminary tests were done to investigate the effect of addition of Ca in treated BW (Figure 4). P precipitation was observed for Ca/P molar ratios of 3.1 and 4.8. For instance, at Ca/P molar ratio of 3.1 the concentration of PO<sub>4</sub> decreased 63% in 12 days, yet co-precipitation of CaCO<sub>3</sub> was observed (approximately 36% of the Ca precipitated as CaCO<sub>3</sub>). The absence of P precipitation at Ca/P molar ratio of 1.5 can be explained by the presence of humic substances in treated BW ( $89 \pm 34$  mgC L<sup>-1</sup>). This is because humic substances decrease the activity of free Ca and PO<sub>4</sub> by complexation (Song et al., 2006). Both HCO<sub>3</sub><sup>-</sup> and pH decreased in all columns due to equilibration with atmospheric CO<sub>2</sub>. According to Figure 4, addition of 300 mgCa L<sup>-1</sup> (Ca/P molar ratio of 3) in BW could potentially

increase the accumulation of P to 88%, considering the P concentrations in the effluent and the soluble Ca in BW. This would enhance the formation and growth of CaP seed particles, increasing the CaP granulation rate. Thus, addition of Ca directly in the UASB reactor will need to be tested further to estimate the optimal Ca loading for P recovery as CaP granules. Simultaneously, COD removal and methanization rate should be maintained at the required levels. Furthermore, the composition and structure of CaP granules depend on the process configuration. So, ongoing research is focusing on the formation and growth rate of CaP granules, and on the process hydrodynamics for selective separation and harvesting of CaP granules.



**Figure 5.** Variation of soluble  $\text{PO}_4$  (a) and Ca (b),  $\text{HCO}_3^-$  (c) and pH (d) in treated BW, over a period of 12 days in 1 L recirculation columns, for different Ca/P molar ratios: 0.6 (no Ca addition), 1.5, 3.1 and 4.8.

## CONCLUSIONS

P accumulation in the UASB reactor (56% in this study) is significantly dependent on the  $\text{HCO}_3^-$  and Ca concentrations in BW. When  $\text{HCO}_3^-$  was below  $2 \text{ g L}^{-1}$ , soluble  $\text{PO}_4$  decreased during BW treatment, due to precipitation of hydrolysed and adsorbed P. For  $\text{HCO}_3^-$  values above  $2 \text{ g L}^{-1}$ , soluble  $\text{PO}_4$  increased. This is because  $\text{HCO}_3^-$  decreases the ion activity of free Ca, reducing CaP precipitation and consequently, P accumulation. Most likely, because  $\text{HCO}_3^-$  in BW was relatively low in this study ( $1.9 \text{ g L}^{-1}$ ), CaP granulation was observed after 100 days of operation. Formed CaP particles were colonized by microorganisms, which developed an outer biofilm around these. CaP precipitation, incorporation of external particles and attachment of organics are the possible growth mechanisms. After 260 days, 4.4% of the total P fed was found as CaP granules in the sludge bed. Addition of Ca increases the P accumulation and, therefore, possibly enhances the granulation rate. Efficient BW treatment was achieved in the presence of CaP granules. At  $25^\circ\text{C}$ , more than 80% of the total COD was removed, of which 60% converted into  $\text{CH}_4$ , indicating that CaP granulation is not at the expense of  $\text{CH}_4$  production.



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