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Calcium phosphate granules recovered from black water treatment: A sustainable substitute for mined phosphorus in soil fertilization

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ABSTRACT

Phosphate is essential for food production. However, phosphate rock, which is the main natural source, is becoming worse in quality due to the depletion of the reserves and contamination with heavy metals and radioactive elements. Marketable phosphate contains more than 13 wt% of phosphorus (P), but run-of-the-mine phosphate rock is of lower grade (8 to 11 wt% of P). In this study, calcium phosphate granules (CaP granules) produced during anaerobic treatment of vacuum collected black water (feces and urine, BW) were assessed for their potential as a substitute for phosphate rock. The quality assessment was based on elemental composition (macro nutrients and heavy metals), crystallography and morphology analyses, dissolution tests, and quantification of micropollutants. CaP granules contained 10 wt% of P, from which 35% was dissolved within 5 min in citric acid and 85% in H_2SO_4. The incineration of the CaP granules increased the P content to 15 wt%, by eliminating the remaining organics (29%), pathogens, and organic micropollutants. Heavy metals (Cu, Zn, Cr, Ni, Cd, As, and Pb) and organic micropollutants in CaP granules were below the Dutch and European regulatory limits for direct fertilizer application. Moreover, incinerated CaP granules complied with the requirements for use in the fertilizer industry. At the current process conditions, CaP granules produced from BW can potentially replace 12% of the phosphate rock used in agriculture as fertilizer.

1. Introduction

Phosphorus (P) is a key nutrient in crop fertilization and in food production for humankind (Cooper et al., 2011). Sedimentary phosphate rock, which is the major source of P for crop fertilization, is finite. Along with the depletion of these reserves, the quality of phosphate rock is becoming an important issue, due to contamination with heavy metals and radioactive elements (Aydin et al., 2010; Cooper et al., 2011; Kratz et al., 2016). Marketable phosphate rock contains more than 13 wt% of P, but run-of-the-mine phosphate rock is of lower grade (8 to 11 wt% of P) (Abouzeid, 2008). Thus, the scarcity and reduced quality of phosphate rock will become a risk for food security.

Considering the global consumption of mineral P as fertilizer (14 Mt $year^{-1}$ in 2005), 21% of the P (3 Mt $year^{-1}$) is lost to the environment as human excreta (Cordell et al., 2009; Elser and Bennett, 2011). The current use of P from phosphate rock can be considered as linear, as at the present date, no recoverable P containing product can economically replace phosphate rock for the production of fertilizer (Desmidt et al., 2015; Nättorp et al., 2017). This is caused by low economic efficiency of the current P recovery technologies and centralized sewage collection, which lead to heavy metals contamination of the recovered products (Desmidt et al., 2015; Houhou et al., 2009; Rule et al., 2006; Schipper et al., 2001). Therefore, the substitution of phosphate rock by recovered P requires special attention for economic and safety aspects, besides the recovery efficiency, the concentration of P, and the percentage of bioavailable phosphate (PO_4^{3-}).

Current technologies for P recovery focus on chemical precipitation of struvite ($MgNH_4PO_4{\cdot}6H_2O$) and calcium phosphate ($Ca_x(PO_4)_y$) in for example: anaerobic effluent, P rich side stream of biological phosphorus removal plants; filtrate from sewage sludge (SS) dewatering; and leachate from acidified sewage sludge ash (SSA) (Desmidt et al., 2015). $Ca_x(PO_4)_y$ can replace phosphate rock in

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conventional fertilizer production, but struvite cannot due to the formation of nitrous gasses from ammonium during the production process (Schipper et al., 2001). Both $Ca_x(PO_4)_y$ and struvite can be directly used as fertilizer, but when produced from waste streams the potential presence of heavy metals, organic micropollutants, and pathogens raises concerns. In some European countries, SS and compost could be used as direct organic fertilizer or as replacement of phosphate rock for production of water-soluble phosphate via incineration, but the high concentration of heavy metals generally measured in sewage sludge limits their applicability (Desmidt et al., 2015; European Commission, 2019; European Parliament, 1986).

Source separation of household streams enables the anaerobic treatment of vacuum collected black water (feces and urine, BW) (Graaff et al., 2010). Vacuum collection (0.25 to 1 L water per flush) of BW leads to high concentrations of P, nitrogen (N), and organic matter (0.15 to 0.30 gP L^{-1}, 1 to 1.4 gNH_4^+ L^{-1} and 10 to 15 gCOD L^{-1}, respectively), and therefore, allows for recovery of energy and nutrients (Graaff et al., 2011). Previously, Tervahauta et al. (2014) described the treatment of concentrated BW in an up-flow anaerobic sludge bed (UASB) reactor, where both methane (CH_4) and calcium phosphate granules (CaP granules) could be recovered. Cunha et al. (2018a) stimulated the formation and growth of CaP granules by adding Ca^{2+} at the bottom of the UASB reactor. The P recovery as CaP granules increased from 2% to at least 31% while removing 89% of the P in BW. Recent results combining UASB and gas-lift technologies (UASB-GL reactor) showed an increased P content in the harvested CaP granules (from 5.6 to 7.8 wt%) (Cunha et al., 2019b).

In this study, we evaluate the potential of CaP granules as a direct fertilizer. The granules were harvested from our UASB-GL reactor during the treatment of concentrated source separated BW. Firstly, the crystal and morphological properties of CaP granules were analysed via X-ray diffraction (XRD) and scanning electron microscope (SEM) coupled with energy dispersive X-ray (EDX). Then, the concentration of heavy metals and organic micropollutants were analytically assessed. Finally, the potential bioavailability of P in CaP granules is examined by dissolution tests in citric acid, distilled water, and sequential extraction with $NaHCO_3$, NaOH, and H_2SO_4.

2. Materials and methods

2.1. Source and preparation of CaP granules

CaP granules were produced in the 45 L UASB-GL reactor reported by Cunha et al. (2019b), which was fed with concentrated BW collected in a Neighborhood with 32 houses in Sneek (The Netherlands). The design of the UASB-GL reactor was based on a combination between a UASB reactor and a gas-lift reactor with a concentric tube for mixing. The UASB-GL reactor operated for 300 days with an organic loading rate (OLR) of 0.8 ± 0.4 gCOD $L^{-1} d^{-1}$ and a hydraulic retention time (HRT) of 23.8 ± 12.3 days, at 25 °C. Between days 189 and 300, the weekly analyses showed consistent concentrations of volatile fatty acids (VFA) below 5 mg L^{-1} in the effluent, a total COD removal of 92 ± 4%, and a VSS and TSS removal of 93 ± 8% and 92 ± 8%, respectively. The inoculum (20 L) originated from another reactor treating BW at the same OLR and temperature for more than 4 years. The monitoring time (300 days) was slightly higher than the measured solids retention time (SRT) in the UASB-GL reactor (290 days), but it may have not been sufficient for fully adaptation of the sludge bed solids to the mixing conditions (Cunha et al., 2019b). Nevertheless, the sludge bed analysis showed a consistent elemental composition and solids concentration and distribution between days 189 and 300, as presented in Fig. C1 and C2 in appendix C. Thus, considering the stable efficiency, inoculum origin, and sludge bed analyses we assume that between days 189 and 300 the reactor operated at steady state. Moreover, the influent P and PO_4^{3-} (soluble) loadings were 12 ± 4 mgP_{Total} $L^{-1} d^{-1}$ and 3 ± 1 $mgPO_4^{3-}$-P $L^{-1} d^{-1}$ of which 90 ± 4% and 88 ± 6% were retained in the reactor, respectively. For this study, sludge containing CaP granules was taken from the bottom of the reactor on operation days 167, 204, 258, and 300 and stored at 4 °C before being subjected to the preparation procedure.

The preparation procedure consisted of flotation and size separation to separate CaP granules (> 0.4 mm diameter) from flocculent sludge after sampling on the UASB-GL reactor. The flotation was based on the ideal internal mixing in the UASB-GL reactor. The ideal conditions were not achieved due to the concentration of suspended solids at the bottom of the reactor. The consequent increase in sludge bed viscosity lowered the magnitude of the buoyancy force and shear rate. The mixing is essential to reduce the thickness of the outer biofilm, increasing the P content in CaP granules. Firstly, 60 to 80 ml of sludge containing CaP granules were placed in a vertical column (Fig. A1 in the appendices). Then, paper filtered effluent from the UASB-GL reactor was used as mobile phase to fluidize the particles in the column at 76 m h^{-1} for 3 min. Less dense inorganic and organic particles were washed out at the top of the column and were collected. Then, the collected washout fraction was centrifuged at 3273 g for 10 min, and the resulting pellet was dried at room temperature before further analysis. This fraction is named “organic fraction” for simplification and contains flocculent sludge and residuals from the outer biofilm around the CaP granules. The remaining particles in the column were further subjected to a size separation using laboratory mesh sieves. Particles larger than 0.4 mm diameter were thoroughly washed with the same mobile phase (effluent), dried at room temperature, and stored for further analyses. These particles (> 0.4 mm diameter) are referred to as CaP granules. A fraction of CaP granules from days 167 and 258 was subjected to 550 °C for 2 h to evaluate the effect of incineration as post-treatment for CaP granules.

2.2. Crystallinity and morphology

XRD analyses of the harvested CaP granules and organic fractions were performed through a Bruker D8 Advance with a diffractometer of 280 mm measurement radius using Cu radiation, Linear PSD 3° detector opening, divergence slit at 0.58°, and a soller slit at 2.5°. The degree of crystallinity was measured by Rietveld pattern fitting using the software TOPAS (Bruker). The R-weighted pattern was kept below 10 for all modeled spectra. The Lorentzian and Gaussian component convolutions of the identified phase structures of hydroxyapatite (HAP, COD 9,002,216 P_63/m) and calcite (COD 9,016,706 R_−3_c) were used for model fitting. The morphology of CaP granules and cross-section elemental mapping were obtained by using a scanning electron microscope (SEM) JEOL JSM-6480LV coupled with NORAN Systems SIX energy dispersive X-Ray (EDX) from Thermo Scientific (USA).

2.3. Chemical analysis

Quantitative elemental composition (P, Ca, Mg, K, Na, S, Fe, Cu, Zn, Al, Cr, Ni, Cd, As, and Pb) of CaP granules and organic fractions were measured with inductively coupled plasma – optical emission spectroscopy (Perkin Elmer Optima 5300 DV, ICP-OES) after an HNO_3 (69%) digestion using microwave induced heating (Milestone Ethos Easy) at 148 °C for 15 min. The concentration of total solids (TS) was determined by the weight difference between the sample dried at 105 °C for 12 h and the raw sample (Clesceri et al., 1998). The volatile suspended solids (VSS) content was determined by the weight difference between the sample dried at 105 °C for 12 h and the sample incinerated at 550 °C for 2 h (Clesceri et al., 1998).

Micropollutants listed in Table B1 in the appendices were analyzed with liquid chromatography - tandem mass spectrometry (LC-MS/MS) after a solid/liquid extraction using 100 mg of solid sample and 2 ml of solvent solution. The solvent solution contained 5 M methanol, 200 mM formic acid, 15 mM ammonia, and 1 mM oxalic acid. The extraction mixture was shaken at 100 rpm for 60 min in a shaking plate and then

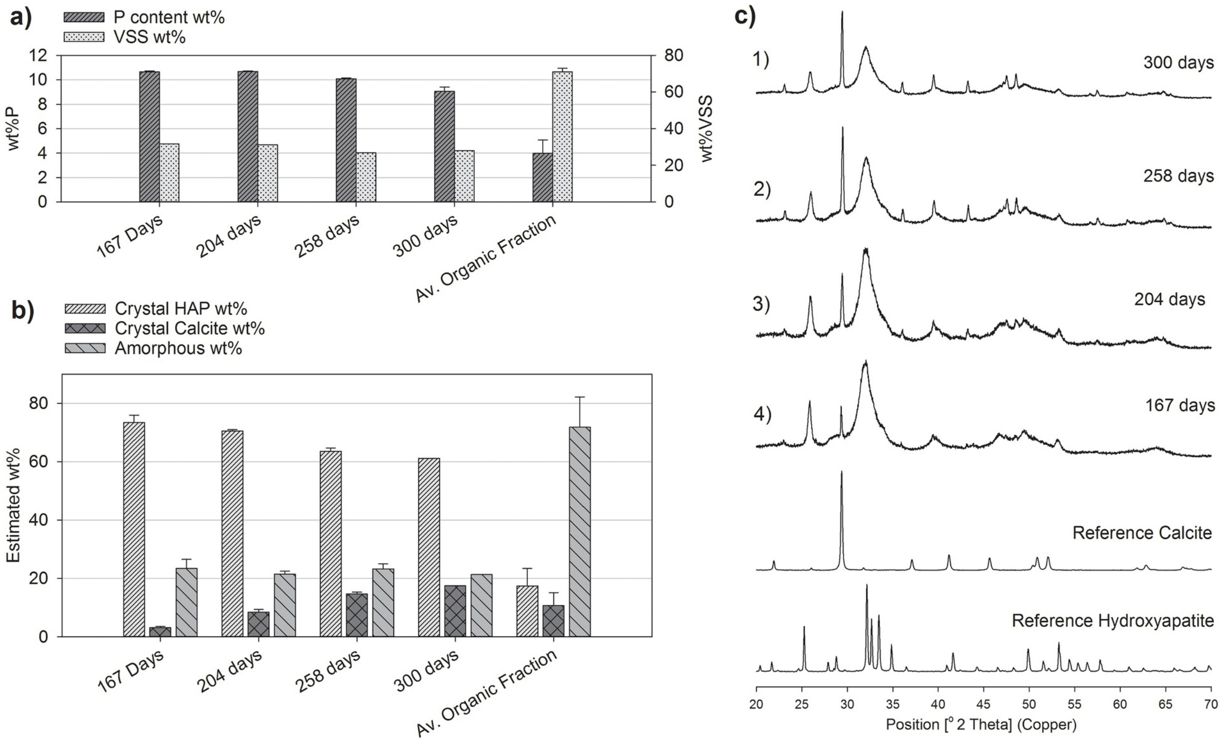

Fig. 1. P and VSS contents of CaP granules taken on operation days 167, 204, 258, and 300 and flocculent organic fraction (averaged organic fractions) (a). Estimated mass percentage of hydroxyapatite, calcite, and amorphous composites, based on Rietveld pattern fitting for the same samples (b). XRD spectrum of the four CaP granules samples and hydroxyapatite and calcite references (c).

centrifuged at 3273 g for 15 min. The supernatant was divided into two vials per sample (each 450 µL). In one vial, a standard solution containing all the analyzed compounds (Table B2 in the appendices) was added to verify the measurements during the analytical process. In both vials, an internal standard solution (Table B3 in the appendices) was added. Then, the micropollutants were analyzed in an Agilent LC-MS/MS system consisting of Agilent infinity 1260 LC-system (degasser, binary pump, auto sampler with cooled tray, and column oven) and Agilent 6420 triple Quadrupole MS with electrospray ionization (ESI). The compounds were separated in an Agilent Zorbax RRHD Eclipse Plus C18 column (50 × 2 mm and 1.8 µm particle size). The mobile phase was acetonitrile and water with NH_4HCO_2. The micropollutants were measured in multiple reaction monitoring (MRM) in positive and negative mode. The standard solution in Table B3 in the appendices was used for quantification.

2.4. Bioavailability

P dissolution test with 2% citric acid was adapted from the standard protocol 4.1.6.1 in the method book II of the Association of German Agricultural Research Institutes (VDLUFA, 1995). Before the dissolution tests, all the samples were thoroughly ground with a mortar and pestle. The variation in surface area after grinding was neglected. The experiments were conducted for 30 min in 50 ml volumetric flasks under continuous shaking at 100 rpm. For each test, 0.5 g of ground sample was dispersed in distilled water as reference control and in 2% citric acid solution, which was prepared using citric acid monohydrate (Sigma Aldrich). After 30 min, the mixed solution was filtered with 0.45 µm glass fiber filters (Cronus PTFE filter) and immediately prepared for further analysis with ion chromatography (Metrohm 761 Compact) for cations (Ca^{2+}, NH_4^+, and Mg^{2+}) and PO_4^{3-} and with ICP-OES for elements (P, Ca, Mg, Fe, and Al). The experiments were done for all aforementioned samples of CaP granules, as well as commercial hydroxyapatite (Sigma Aldrich) and struvite obtained from mixing MgO and urine.

2.5. Sequential extraction

PO_4^{3-} was extracted from 0.5 g of ground CaP granules, which were suspended sequentially in three solutions. The solutions contained 0.5 M of $NaHCO_3$, 0.1 M NaOH, and 1 M H_2SO_4, to measure P bound to organics, to Al or Fe, and other inorganic material, respectively. Ground CaP granules were firstly placed in a 50 mL falcon tube containing 30 mL of $NaHCO_3$ solution for 16 h at 100 rpm. Then, the mixture was centrifuged at 3273 g for 10 min. The supernatant was filtered with a paper filter with a pore size of 3 µm (Whatmann blue ribbon filters). The solids retained on the filter were mixed with the pellet of the centrifugation by rinsing with the next solution (NaOH) to a final mixture volume of 30 mL in a 50 mL tube. The new mixture was mixed for 16 h at 100 rpm for PO_4^{3-} extraction and afterward centrifuged as mentioned before. The supernatant was paper filtered as in the previous step and the solids on the filter were mixed with the pellet using H_2SO_4 solution to a final mixture volume of 30 mL. The mixture was once more mixed at 100 rpm for PO_4^{3-} extraction and centrifuged at 3273 g for 15 min. Then, the supernatant was paper filtered. PO_4^{3-} (soluble) and total P were determined in the paper filtrated supernatant after each extraction step using IC and ICP-OES, respectively. The mass balance was calculated considering the percentage of P in CaP granules dissolved during each extraction.

3. Results

3.1. $Ca_x(PO_4)_y$ content and crystallinity

The separation procedure (flotation at 76 m h^{-1} plus sieving of particle > 0.4 mm diameter) increased the P content of CaP granules from 7.8 ± 0.6 wt% to 10.1 ± 0.8 wt% (Fig. 1a). The average P content in CaP granules was 23 ± 2 wt% as P_2O_5. The P in the separated fraction of CaP granules represented about 75 ± 4% of the total P in the sludge taken from the bottom of the reactor. The remaining 25 ± 1% of P was in the residual sludge (organic fraction). The P content in the CaP granules decreases slightly over the operation time (from 10.6 ± 0.1 wt% on day 167 to 9.1 ± 0.3 wt% on day 300). The average Ca/P molar ratio was 1.9 ± 0.3, while the theoretical Ca/P molar ratio of HAP ($Ca_{10}(PO_4)_6OH_2$) is 1.67. The calculated co-precipitation of Ca as $CaCO_3$ and organic compounds represented, on average, 14 ± 3% of the Ca in CaP granules. Mg, S, K, and Na contents in the CaP granules were 0.35 ± 0.06, 0.29 ± 0.03, 0.11 ± 0.02 and 0.28 ± 0.03 wt%, respectively. The VSS content in CaP granules was on average 29 ± 2 wt% (Fig. 1a). According to Rietveld pattern fitting, the degree of crystallinity in CaP granules was 77 ± 2%, from which 67 ± 6% and 11 ± 6% were HAP and calcite, respectively (Fig. 1b). The estimated average content of amorphous composites in the CaP granules was 22 ± 1%. Apart from the inorganic components in the CaP granules, an organic fraction was present as a more flocculent matter. This organic fraction sampled on operation days 167, 204, 258, and 300 contained a P and VSS contents of 4.0 ± 1.1 and 71 ± 2 wt%, respectively.

Peak identification showed that HAP and calcite were the dominant crystal phases in the CaP granules (Fig. 1 from 1 to 4). Moreover, SEM-EDX analysis showed that P, Ca, and O were the most abundant elements in the CaP granules (Fig. 2c). The $Ca_x(PO_4)_y$ enrichment in CaP granules was occurring via agglomeration of crystal units (Fig. 2d). This growth mechanism of CaP granules was consistent with the one described in Cunha et al. (2018b).

3.2. Heavy metals

The metal present at the highest concentration in CaP granules was Fe (1099 mg kg^{-1} of total solids (TS)) followed by Al (304 mg kg^{-1} of TS), Zn (183 mg kg^{-1} of TS) and Cu (47 mg kg^{-1} of TS). All the other metals (Cr, Ni, Cd, As, and Pb) were below the Cu concentration. Moreover, the concentration of heavy metals (Cu, Zn, Cr, Ni, Cd, As, and Pb) in CaP granules were below the regulatory limits for application as inorganic and organic fertilizer within The Netherlands and Europe (Table 1). For instance, Cd and Ni often limit the application of SSA and phosphate rock, but in CaP granules their concentrations were 8 and 4 times lower, respectively, than the European regulatory limit (Table 1 and 2).

Furthermore, the overall heavy metal content was lower in CaP granules than in the separated organic fraction, consisting of dispersed sludge and the remains of the detached biofilm from the CaP granules. Table 1 reports the averaged concentration of heavy metals for CaP granules and the respective organic fractions taken at different operation days, as aforementioned. The separated organic fraction from the CaP granules could not be used as fertilizer in The Netherlands, due to the relatively high Zn content (Table 1). However, concerning European legislation, it complies with the regulation for application as organic fertilizer, regarding heavy metals (European Commission, 2019).

The organic content in CaP granules (~ 29 wt%) limits the direct replacement of phosphate rock in the fertilizer industry. In the thermal processing route, the organic content reduces the particle strength during the sintering of milled solids containing P, decreasing the downstream production capacity. In the wet processing route, the organics release unwanted fumes (Abouzeid, 2008; Schipper et al., 2001). Therefore, the characteristics of incinerated CaP granules were assessed and compared with other products used as inorganic fertilizer and as raw material for P fertilizer production (Table 2). All the regulated heavy metal concentrations were below the limits for the application of incinerated CaP granules as inorganic fertilizer. Moreover, the heavy metal content in incinerated CaP granules was similar to or lower than phosphate rock and triple superphosphate (TSP). Additionally, the

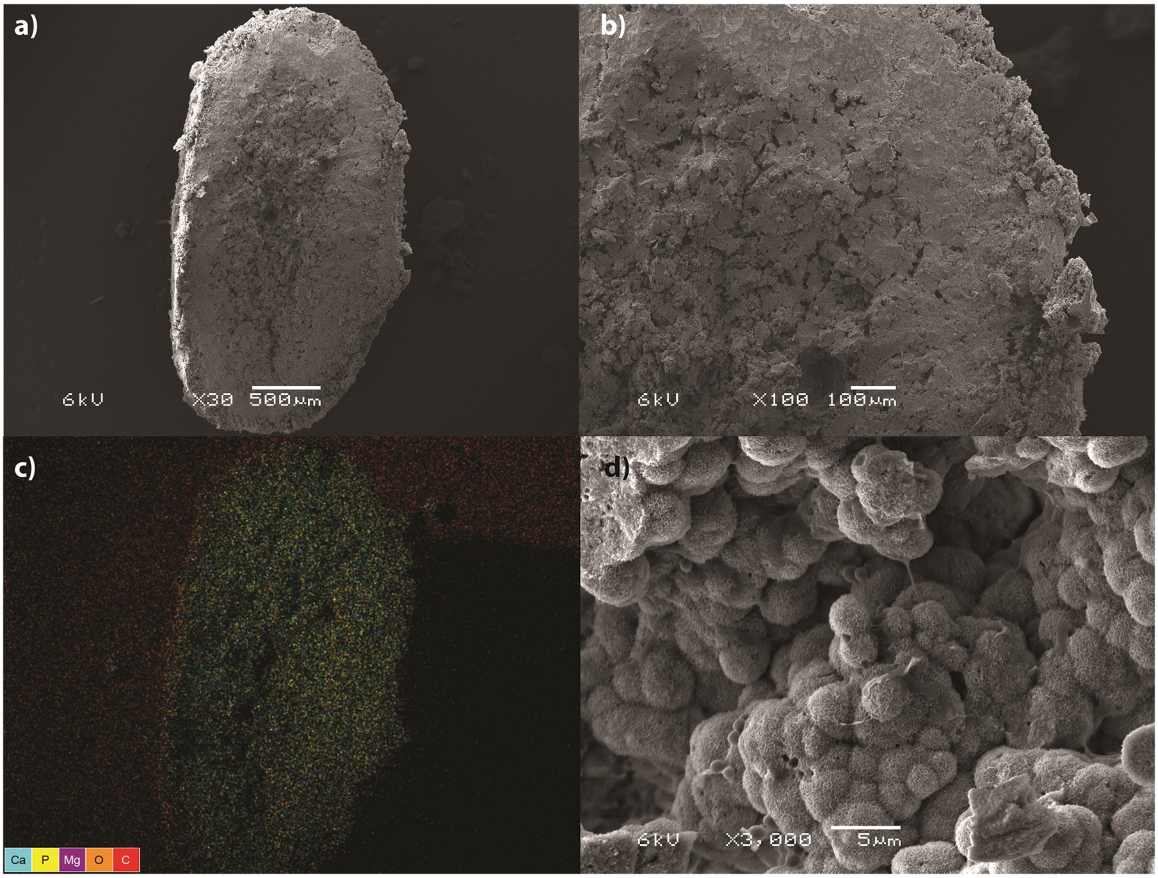

Fig. 2. SEM illustration of the cross-section of a representative CaP granule at different magnifications (a, b, and d) and EDX elemental mapping of the granule cross-section (c). Note that the greenish color results from the simultaneous presence of Ca (cyan) and P (yellow).

Table 1

Heavy-metals assessment (mg kg^{-1} of total solids (TS)) for CaP granules and organic fraction and lower regulatory limits for application as both inorganic and organic fertilizer in The Netherlands (NL) and Europe (EU), according to the revised fertilizer regulation, and for application of sewage sludge in Europe (EU) and the criteria for Eco-Label based on JRC/IPTS (2011).

$mg\ kg_{TS}^{-1}$	CaP Granules $N = 8$	*sd*	Organic Fraction $N = 8$	*sd*	Ƒ $\frac{[Organic\ fraction]}{[CaP\ granules]}$ $\frac{mg\ kg_{TS}^{-1}}{mg\ kg_{TS}^{-1}}$	Fertilizer (NL) [1]	Fertilizer (EU) [2]	Sewage Sludge (EU86/278) [3]	Eco-label Fertilizer [4]
Fe	1099	*175*	1648	*356*	**1.5**	n.a	n.a	n.a	n.a
Cu	47	*5*	214	*14*	**4.5**	75	n.a.	1000 - 1750	100
Zn	183	*31*	780	*59*	**4.3**	300	n.a.	2500 - 4000	400
Al	304	*60*	868	*138*	**2.9**	n.a	n.a.	n.a	n.a
Cr	39	*6*	59	*24*	**1.5**	75	2 (Cr VI)	n.a	100
Ni	13	*3*	24	*2*	**1.9**	30	50	300 - 400	50
Cd	0.18	*0.02*	0.70	*0.04*	**3.5**	1.25	1.5	20 - 40	1.5
As	0.20	*0.00*	0.63	*0.05*	**3.1**	15	60	n.a	n.a
Pb	1.18	*0.28*	4.75	*0.42*	**3.6**	100	120	750 - 1200	120

[1] Ehlert et al. (2013);.
[2] European Commission (2016);.
[3] European Commission (1986);.
[4] Quintero et al. (2015) n.a.: not applicable.

Table 2

Comparison of P and heavy-metal contents in CaP granules after incineration at 550 °C for 2 h with other recovered products, phosphate rock, triple superphosphate, and the requirements for fertilizer industry.

		CaP Granules (550 °C) (this study)	*sd*	Struvite (Urine) [1]	Sewage Sludge Ash [2]	Phosphate Rock [3]	Triple Superphosphate (TSP) [3]	Requirements for Fertilizer Industry [4]
P	wt%	15	*1*	12	9	13	20	> 11
P_2O_5	wt%	34	*2*	27	21	30	45	> 25
Fe	$mg\ kg_{TS}^{-1}$	1577	*390*	480	58,500	2253	2160	< 10,000
Cu		73	*10*	90	767	31	22	< 500
Zn		274	*69*	225	2330	426	390	< 1000
Al		443	*100*	n.a.	67,200	2642	2818	n.a.
Cr		57	*11*	< 2	159	148	171	n.a.
Ni		16	*2*	2	73	41	27	n.a.
Cd		0.23	*0.02*	< 2	2	23	24	n.a.
As		0.25	*0.07*	< 11	11	14	8	n.a.
Pb		1.65	*0.07*	< 22	123	4	4	n.a.
Sr		n.d		n.a.	n.a.	1057	909	n.a.
U		n.d		n.a.	n.a.	141	125	n.a.

n.d. - not determined; n.a. – not available / not applicable;.
[1] Gell et al. (2011);.
[2] Herzel et al. (2016);.
[3] Kratz et al. (2016);.
[4] Schipper et al. (2001).

incinerated CaP granules, which were free of organic compounds, complied with the requirements as a raw material for the fertilizer industry, including the P content which was on average 35 wt% as P_2O_5.

3.3. Organic micropollutants

Pharmaceuticals, personal care, and household products were detected in both the CaP granules and the respective separated organic fractions (Table 3). In the granules, Cetirizine, which is an antihistamine for the treatment of allergies, was found at the highest concentration of 2.5 mg kg^{-1} of TS, followed by Doxycycline, Ciprofloxacin, Oxazepam, and Azithromycin which were found in the range of 0.2 to 0.5 mg kg^{-1} of TS. Azithromycin, Ciprofloxacin, and Doxycycline were the antibiotics found at the highest concentrations in CaP granules and organic fractions. Azithromycin was, on average, 4 times more concentrated in the organic fraction (0.9 mg kg^{-1} of TS) than in the CaP granules (0.2 mg kg^{-1} of TS). Carbamazepine and Gabapentin (both antiepileptics) were also found in the CaP granules and the respective organic fractions. Ibuprofen, which is a common anti-inflammatory drug, was only measurable in one sample of the CaP granules (56 μg kg^{-1} of TS), while it was found to be more concentrated in the organic fraction (131 μg kg^{-1} of TS). Diclofenac, which is another anti-inflammatory drug, was less concentrated and evenly distributed between the CaP granules and the organic fraction (7 to 8 μg kg^{-1} of TS). Other compounds were measured but were below the detection limit (Table B4 in the appendix). Reported concentration ranges for measured micropollutants in BW, SS, and animal manure are also given in Table 3. Ciprofloxacin, Oxytetracyclin, and Tetracycline were detected at relatively high levels in SS and animal manure, when compared to the CaP granules.

3.4. Bioavailability

In a 2% citric acid solution, 35 ± 5% of the P in the CaP granules dissolved within 30 min (Fig. 3), which was similar to struvite produced from urine (33 ± 3%) and higher than powder HAP (14%) under the

Table 3

Average concentration of micropollutants in CaP granules and separate organic fractions (μg kg^{-1} of total solids (TS) and μg kg^{-1} of volatile solids (VS)) for all samples taken. N refers to the number of samples where each specie was detected. The complete list of analyzed compounds and recoveries are in Table B4 and B.5 of the appendix. Reference concentration ranges of measured compounds for black water (BW) and sewage sludge (SS) and animal manure.

Description	Type	N	CaP Granules μg kg$_{TS}^{-1}$	*sd*	CaP Granules μg kg$_{VS}^{-1}$	*sd*	N	Organic Fraction μg kg$_{TS}^{-1}$	*sd*	Organic fraction μg kg$_{VS}^{-1}$	*sd*	Black water μg L^{-1} [1]	Sewage Sludge μg kg^{-1}	Animal Manure[a] μg kg^{-1}
Cetirizine	Antihistamine	*5*	**2502.31**	240.78	**8379.00**	488.92	*5*	**4185.52**	181.37	**5968.32**	407.60			
Doxycycline	Antibiotic	*5*	**460.01**	27.83	**1547.49**	148.20	*5*	**376.60**	52.85	**536.73**	78.17		966–1780[5]	550–3100[6,b]
Ciprofloxacin	Antibiotic	*5*	**352.64**	157.37	**1156.96**	450.07	*5*	**373.65**	153.32	**536.94**	232.40	0 – 500	1600–11,000[4,5]	300–3000[7]
Oxazepam	Sedative	*5*	**218.94**	30.56	**736.51**	115.19	*5*	**548.64**	49.03	**781.49**	70.27	3 – 45		
Azithromycin	Antibiotic	*5*	**198.06**	118.75	**665.27**	379.93	*5*	**932.44**	329.95	**1324.27**	460.81		50–1220[2,5]	
Metformin	Antidiabetic	*5*	62.74	20.06	214.41	82.70	*5*	70.25	19.20	100.21	28.54	3000 – 8000	300–450[5]	
Ibuprofen	Anti-inflammatory	*1*	55.59	–	178.29	–	*5*	130.65	59.84	184.57	81.38	25 – 125	10–600[2,3]	
Benzalkonium Chloride C12	Biocide	*5*	43.66	13.82	144.14	36.99	*5*	160.71	35.44	229.20	53.18	16	n.d.–120[4]	
Metoprolol	B-blocker	*5*	39.48	6.36	133.24	27.03	*5*	141.80	26.26	201.34	31.65	200 – 550	130–170[3]	
Tetracycline	Antibiotic	*5*	28.46	28.63	90.06	88.69	*5*	11.89	5.23	17.04	7.86		< 8–27,90[3,5]	n.d.–1600[6,7]
Paracetamol	Analgesic	*4*	24.16	10.83	81.53	33.57	*5*	15.50	6.09	21.99	8.39	2000 – 10,000		
Caffeine	Stimulant	*5*	13.20	6.04	43.73	18.79	*5*	19.10	13.04	27.58	19.25		55–1600[3]	
Diclofenac	Anti-inflammatory	*5*	7.59	3.20	25.84	12.13	*5*	6.90	1.74	9.85	2.54	0 – 15	100–350[2,3]	
Benzalkonium Chloride C14	Biocide	*4*	4.75	2.00	15.38	5.93	*5*	16.00	2.93	22.82	4.44		n.d.–120[4]	
Carbamazepine	Antiepileptic	*5*	4.19	2.81	13.70	8.63	*5*	7.45	5.67	10.75	8.38		8–550[2,3]	
Ofloxacin	Antibiotic	*5*	3.18	2.12	10.29	6.49	*4*	1.53	0.57	2.21	0.84		n.d.–200[2]	
Gabapentine	Antiepileptic	*5*	2.38	1.54	7.96	4.99	*5*	1.57	1.05	2.27	1.56			
Oxytetracyclin	Antibiotic	*5*	1.64	0.43	5.50	1.40	*2*	1.95	0.14	2.83	0.25		60–114[5]	9700–170,000[7]
Sulfapyridine	Antibiotic	*1*	1.33	–	4.27	–	*5*	2.28	0.88	3.28	1.34			
Clindamycin	Antibiotic	*2*	0.65	0.06	2.06	0.20	*2*	0.62	0.07	0.90	0.11			
Hydrochlorothiazide	Antidiabetic	*5*	< 50	–	–	–	*5*	88.73	17.76	125.89	21.66	100 - 600	n.d.–390[2,3]	
Clarithromycin	Antibiotic	*2*	0.23	0.04	0.71	0.12	*2*	0.61	0.47	0.89	0.68		n.d.–350[4,5]	

[1] (Butkovskyi et al., 2015);.

[2] (Radjenović et al., 2009);.

[3] (Malmborg and Magnér, 2015);.

[4] (Östman et al., 2017);.

[5] (McClellan and Halden, 2010);.

[6] (Jones-Lepp and Stevens, 2007);.

[7] (Hu et al., 2008);.

[a] reference for swine and chicken manure;.

[b] for Doxytetracycline; n.d. - not detected;.

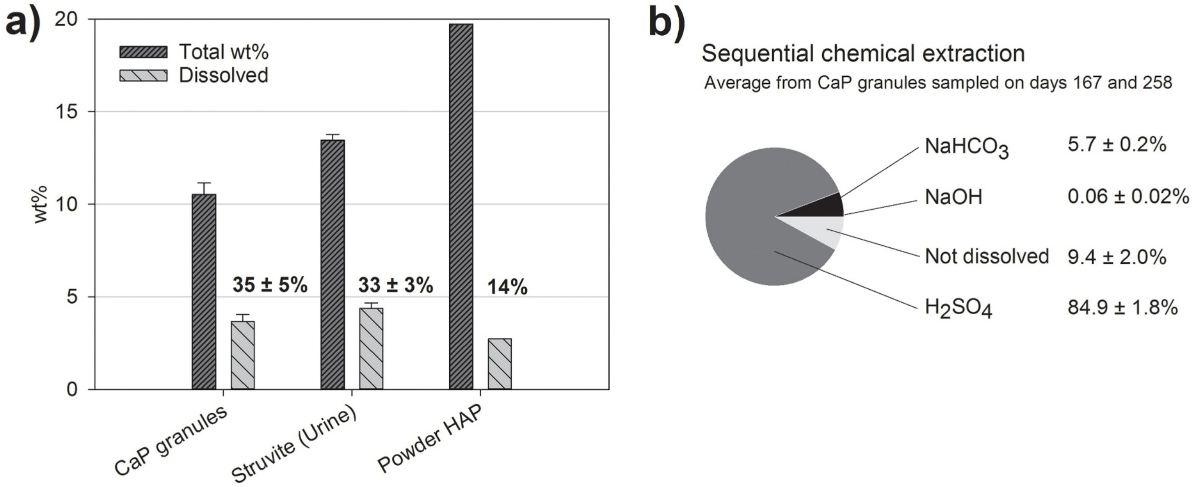

Fig. 3. Dissolution test for P in the ground CaP granules (average from the CaP granules taken on operation days 167, 204, 258 and 300), ground struvite produced from urine and powder hydroxyapatite (HAP), using a 2% citric acid solution according to the standard method VDLUFA (1995) (a). Sequential chemical extraction of PO_4^{3-} from the CaP granules taken on operation days 167 and 258, using $NaHCO_3$ for organically bound P, NaOH for Al and Fe bound P, and H_2SO_4 for dissolution of another inorganic P (b).

same conditions. In distilled water, the P dissolution was below 2% for the CaP granules, struvite, and HAP. The sequential extraction showed that most P (85 ± 2%) was dissolved in acidic conditions (H_2SO_4), while only 0.1% and 6% were soluble in strong-alkaline (NaOH) and weak-alkaline ($NaHCO_3$) conditions, respectively.

4. Discussion

4.1. Compositional and crystallographic characteristics of CaP granules

The concentrations of macro elements in the CaP granules were relatively stable, although a large variation in the influent (BW) concentrations of organic matter (from 3 to 42 $gCOD_{Total}\ L^{-1}$), bicarbonate (from 0.8 to 3.6 $gHCO_3^{-}\ L^{-1}$), and nutrients (from 94 to 480 $mgP_{Total}\ L^{-1}$, 76 to 558 $mgCa_{Total}\ L^{-1}$ and 21 to 201 $mgMg_{Total}\ L^{-1}$) were observed during the operation of the UASB-GL reactor as described in Cunha et al. (2019b). The average P content in the CaP granules taken on operation days 167, 204, 258, and 300 showed a decrease of 14% (Fig. 1), due to an increase in bulk pH from 7.4 on operation day 220 to 7.7 on day 300, caused by an increase in HCO_3^{-}, and consequently, more $CaCO_3$ precipitation (Cunha et al., 2019b). The robustness of the process regarding the compositional standardization of P content in the CaP granules was dependent on bulk pH (Cunha et al., 2019a, 2018b).

According to XRD analyses, HAP was the dominant crystal phase in the CaP granules, with an average of 67 wt%. However, the calculated content of HAP based on the P content in CaP granules is 55 ± 4 wt%, which is significantly lower than the percentage obtained by Rietveld pattern fitting (XRD). The difference can be explained by the nanocrystalline properties of the crystal HAP in the CaP granules (Gajjeraman et al., 2007). The HAP nanocrystals in the CaP granules widen the expected peaks located between positions 30° and 35° in the XRD spectrum (Fig. 1 from 1 to 4 and the reference spectrum of HAP). This led to an overestimation of the HAP content in the modelled spectrum by approximately 12%. A much lower deviation was observed for $CaCO_3$ (from the modelled 11 wt% to the calculated 14 wt%). Nevertheless, the low content (< 0.5 wt%) of Mg, K, Al, and Fe indicates that almost no Mg/K-struvite and aluminum and iron phosphates were present. This is further supported by sequential chemical extraction (Fig. 3), which shows that only 6% of the P in the CaP granules was organically bound and 0.1% was connected to Fe or Al. The latter was supported by the residual Fe (0.1 wt%) and Al (0.03 wt %) contents found in the CaP granules. Regarding Fe, weekly influent (< 15 mg L^{-1}) and effluent (< 7 mg L^{-1}) measurements of the UASB-GL reactor showed an accumulation of Fe above 2.3 mg L^{-1} of treated BW. Regarding Al, the concentration in the influent and effluent was always below the limit of quantification (> 7 mg/L). Part of the organics in the CaP granules originate from extracellular biopolymers (EPS) as shown in Fig. 2 and in Cunha et al. (2019a). EPS are known to significantly contribute to particle aggregation (El-Mamouni et al., 1998; Schmidt and Ahring, 1994), which is a key step in the production of larger CaP granules. The SEM analysis of a CaP granule cross-section (Fig. 2) suggests that the growth of CaP granules was occurring via agglomeration of spherical units (approximately 2 μm diameter), which were likely composed of HAP nanocrystals with approximately 5 nm diameter (Gajjeraman et al., 2007; Tao et al., 2007). Hypothetically, the HAP nanocrystals are involved in a hydration layer containing both $Ca_x(PO_4)_y$ and $CaCO_3$ amorphous phases (Bian et al., 2012; Rokidi et al., 2011). The agglomeration of crystal units was then mediated by surface to surface contact between crystal units or by contact with EPS in the granule core. With the maturation, the compactness, particle density, and settling velocity of the CaP granules increase, leading to the concentration of mature granules at the bottom of the reactor, where they can be harvested.

4.2. Hazardous compounds in the CaP granules

The application of nutrients recovered from wastewater is often prohibited due to the presence of heavy metals, micropollutants, and pathogens. Regarding heavy metals, the separated organic fraction contained overall a higher concentration than the CaP granules, due to adsorption onto organic compounds, such as humic substances and solid organic matter (Amir et al., 2005; Dong et al., 2013; Kobielska et al., 2018). As shown in Table 1, Cu and Zn had the highest affinity for the organic fraction (*f* of 4.5 and 4.3, respectively), which is in line with the findings of Amir et al. (2005) and Dong et al. (2013). The lower affinity observed for Cr and Ni was explained by association with Fe, which had similar selectivity to the organic fraction as Cr and Ni (Dong et al., 2013). The heavy metal content in the CaP granules might still be associated with the organic matter since 29% of the CaP granules weight was organic (Fig. 1). Incineration of CaP granules reduces the organic content, but the heavy metals remain in the ash as indicated by the higher concentration of heavy metals in the incinerated CaP granules compared to the raw granules (Table 1 and 2). Thus, the shear force applied to the CaP granules by the internal mixing in the UASB-GL reactor is essential to reduce the heavy metal content in CaP granules by washing off organics (outer biofilm). Cd and As were at very low levels for both the CaP granules and the organic fraction when compared to phosphate rock. Pb was higher than Cd and As, but still far below the regulatory limit of 100 to 120 mg kg^{-1} of TS.

The micropollutants in CaP granules originate directly from human excretion and/or disposal of unused medicines, disinfectants, and cleaning products into the toilet. Thus, the variety and concentration of micropollutants were dependent on anthropological aspects at the collection of BW. The concentration of certain organic micropollutants in the organic fraction (Table 3) were in line with the previous quantification of micropollutants in BW sludge from Butkovskyi et al. (2017). During anaerobic digestion, micropollutants can be removed from the liquid phase by biodegradation (micropollutants are co-metabolized by microorganisms) and by adsorption and absorption (micropollutants are immobilized within the solid matter) (Alvarino et al., 2018). Sorption of micropollutants in anaerobic sludge was found to be minor (Butkovskyi et al., 2015), but for certain micropollutants, such as Doxycycline, Ciprofloxacin, Cetirizine, and Azithromycin, sorption (both adsorption and absorption depending on hydrophobicity and ionization) was observed (Table 3). Cetirizine and Azithromycin, which are both lipophilic or hydrophobic (logP of 0.87 and 2.44, respectively), were more concentrated in the organic fraction than in the CaP granules. This was supported by the approximated concentration of Cetirizine and Azithromycin in the CaP granules and the organic fraction when based on volatile solids as shown in Table 3; 8.4 ± 0.5 and 7.0 ± 0.4 μg kg^{-1} of VS for Cetirizine and 0.7 ± 0.4 and 1.3 ± 0.5 μg kg^{-1} of VS for Azithromycin in CaP granules and organic fraction, respectively.

On the other hand, Doxycycline and Ciprofloxacin, which are both hydrophilic (logP of −3.29 and −0.85, respectively) and negatively charged at pH 7.4 (gross charge distribution of −0.79 and −0.02, respectively), were found in similar concentrations in the CaP granules and the organic fractions. Most likely, hydrophilic compounds adsorbed to both the CaP granules and the organic fraction by electrostatic interactions of the ionic groups (Alvarino et al., 2018). At pH below 8.5, HAP is positively charged (Bell et al., 1973). Lipophilic (hydrophobic) compounds were likely absorbed into the lipophilic microbial cell membrane and lipids in the flocculent sludge, inducing a higher concentration for the organic fraction than for the CaP granules (Alvarino et al., 2018). Both mechanisms (adsorption and absorption) are of concern regarding the incorporation of micropollutants in the CaP granules, because 29% of the CaP granules mass is still organic.

The level of micropollutants in CaP granules was relatively lower compared to reference ranges for SS and animal manure (Table 3). Animal manure is recycled on land for centuries, and human excreta or “night soil” was also recycled on land until the development of permanent settlements (Lofrano and Brown, 2010). Then, the implementation of sewers drove the wastewater discharge to surface water instead of agriculture land (Ashley et al., 2011). Enabling the efficient recycling of nutrients and organic matter is crucial for the future sustainability of food production. The sum concentration of organic micropollutants measured in the CaP granules was 4 ± 1 mg kg^{-1} of TS, from which 26% were antibiotics. The toxicity of these organic micropollutants even at very low levels is unknown. Moreover, the formation of unknown by-products from biotransformation and a combination of pollutants raises uncertainty regarding health safety. Industrial micropollutants, such as polychlorinated biphenyls (PCB), polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polycyclic aromatic hydrocarbons (PAH) were not expected to be present in CaP granules produced from BW. The proposed regulation limits only the PAH to < 6 mg kg^{-1} of TS (European Commission, 2019).

Gell et al. (2011) measured a reduction of colony forming units (CFU) from 36,200 CFU g^{-1} in the influent BW to 100 CFU g^{-1} of wet UASB sludge formed at 35 °C, which is lower than the proposed European regulatory limit of 1000 CFU g^{-1} (European Commission, 2019). However, generally, the average removal of pathogens, such as *E. coli* spp., *Salmonella* spp., and *Enterococcus* spp., during mesophilic treatment is only 2 log, which is not sufficient to ensure safety (Avery et al., 2014; Horan et al., 2004). Moreover, the unknown microbial speciation and the ascending concerns with the spread of antibiotic resistant genes (ARG) during wastewater treatment introduces uncertainty and calls for further research. By incineration of the CaP granules, pathogens and measured micropollutants will likely be disintegrated (Abouzeid, 2008). Then, the only pollutants remaining in the incinerated CaP granules are the heavy metals, which are below the regulatory limits.

4.3. Process feasibility and usability of the recovered CaP granules

The BW treatment scheme in source separation sanitation comprises three steps: anaerobic digestion, nitrogen removal, and P recovery (Graaf and Hell, 2014). P recovery was achieved by adding Mg^{2+} in the third reactor to induce struvite precipitation. As an alternative, CaP granulation combines the anaerobic treatment and P recovery in a single reactor, reducing the capital expenditures (CAPEX) by 32% (1.08€ $person^{-1}$ $year^{-1}$) based on the model described in Roefs et al. (2017) for 1200 PE and 30 years lifetime. Regarding the operational expenditures (OPEX), both alternatives require similar operational settings but a different dosage of supplements. For struvite precipitation, both base (NaOH) to increase the pH and Mg^{2+} are necessary to precipitate struvite, while for CaP granulation only Ca^{2+} is needed. According to Roefs et al. (2017), the cost associated with the addition of MgO and NaOH for struvite precipitation is 2.43 € $person^{-1}$ $year^{-1}$. Considering the scenario (1200 PE) as reported in Roefs et al. (2017), the cost for adding Ca^{2+} ($CaCl_2$) is 1.04€ $person^{-1}$ $year^{-1}$ based on a required addition of 250 $mgCa^{2+}$ L^{-1}_{BW} and a market price for $CaCl_2$ (77%) of 0.4€ kg^{-1} (Cunha et al., 2018a; Stevén, 2020).

The total cost reduction by implementing CaP granualtion is 2.47€ $person^{-1}$ $year^{-1}$ over a lifetime CAPEX of 30 years. However, for locations with tap water with greater hardness than in the north of the Netherlands (> 53 $mgCa^{2+}$ L^{-1}) less Ca^{2+} is required to form CaP granules, and therefore, a lower cost is associated with Ca^{2+} addition. Moreover, the production capacity of CaP granules is 0.25 kgP $person^{-1}$ $year^{-1}$ (57% of the P in BW), while as struvite is only 0.22 kgP $person^{-1}$ $year^{-1}$ (48% of the P in BW), considering an excretion rate of 0.44 kgP $person^{-1}$ $year^{-1}$ (Cunha et al., 2019b; Graaff et al., 2011). Regarding the P removal capacity, the total P concentration in the final effluent is approximately 20 mg L^{-1} for both alternatives, CaP granulation and struvite precipitation (Cunha et al., 2018a; Graaf and Hell, 2014). Thus, CaP granulation is a more cost-effective and efficient alternative for P recovery in source separated sanitation. Additionally, recovery of P as $Ca_x(PO_4)_y$ enables the formulation of fertilizers with a more specific N:P ratio, in contrast to struvite

($NH_4MgPO_4•6H_2O$). Also, it allows the blending of CaP granules with Phosphate Rock in the fertilizer industry for the production of water soluble phosphate (Schipper et al., 2001).

The direct use of CaP granules as fertilizer for food production is not compatible with the European regulation (EU) 2019/1009, which was recently approved to include waste streams as a source for fertilizing products (European Commission, 2019). Raw materials originated from human fecal matter were excluded from the list of Component Material Categories (CMC). However, considering chemical, biological, and physical properties, CaP granules could be a candidate for application as P rich solid organic fertilizer, since they comply with the regulatory limits for heavy metals, organic micropollutants, and pathogens. Yet, as highlighted in the paragraph 19 of the (EU) 2019/1009 (European Commission, 2019), mono-incineration is a potential post-treatment to enable the use of CaP granules as fertilizer for food production. The incinerated CaP granules (15 wt% of P or 35 wt% of P_2O_5) are free of pathogens and the heavy metal content was below the regulatory limit, as shown in Table 2 and 3.

The cost reduction obtained by implementation of CaP granulation overweighs the cost for production and incineration of CaP granules. The implementation of CaP granulation enables a cost reduction of 2.47€ $person^{-1}$ $year^{-1}$ in The Netherlands for P recovery during the treatment of source separated BW. The combined cost for mono-incineration and acid leaching of SSA is estimated at 0.3 to 1.7€ $person^{-1}$ $year^{-1}$ (Nättorp et al., 2017). Acid leaching of SSA is required to reduce the heavy metals content before P recovery. In contrast to SSA, chemical leaching is not needed for the CaP granules, due to the already low heavy metals content. The cost for mono-incineration of CaP granules can, therefore, be achieved under 0.3€ $person^{-1}$ $year^{-1}$. Yet, when mono-incineration is the established route for sludge disposal, which is the case for some plants in The Netherlands, Germany, and Switzerland, incineration of CaP granules can be realized without additional costs (Herzel et al., 2016). The current price for phosphate rock mined in Morocco, which is the main source of P in Europe, is 0.72€ kg^{-1} of P (Nättorp et al., 2017). However, due to market volatility, the price of phosphate rock can increase as it has previously occurred in 2008 with prices as high as 4€ kg^{-1} of P (Ridder et al., 2012). Thus, the incinerated CaP granules are a competitive product to replace part of the phosphate rock input in agriculture. For non-agricultural applications, such as gardening and landscaping, CaP granules could be used as direct fertilizer without the need for incineration.

Considering the production capacity (0.25 kgP $person^{-1}$ $year^{-1}$), CaP granules could replace 12% the worldwide annual consumption of mined phosphate rock per capita (Cordell et al., 2009; Ott and Rechberger, 2012). However, the estimated global annual loss of phosphorus to the environment within agricultural activities (~ 15 Mt P $year^{-1}$) is higher than the P input as phosphate rock (14 Mt P $year^{-1}$), and only 8 and 17% of the P harvested as crops is actually consumed by humans with meat and vegetable-based diets, respectively (Cordell et al., 2009). For the EU27, the accumulation of P in the agricultural sector is ~ 65% of P imported for crop production (Jedelhauser and Binder, 2015; van Dijk et al., 2016). Thus, besides the recovery of P from waste streams, the use efficiency of P in agricultural land is crucial to reduce the need for mined phosphate rock. Improved P use efficiency could increase the capacity of the CaP granules in replacing phosphate rock in agriculture.

5. Conclusions

In this study, CaP granules produced during anaerobic treatment of BW were assessed for chemical composition, structure, and crystallography. The objective was to evaluate their potential as an alternative to finite phosphate rock. CaP granules contained 10 wt% of P (or 23 wt % of P_2O_5), corresponding to 55 wt% of HAP. Through incineration, the remaining organic material (29 wt%) was removed, increasing the P content to 15 wt% (or 34 wt% of P_2O_5). Heavy metal (Cu, Zn, Cr, Ni, Cd, As, and Pb) quantities were below the European and Dutch regulatory limits. The total mass of micropollutants in CaP granules was only 4 mg kg^{-1} of TS, from which 26% were antibiotics. Considering the revised European regulation for fertilizing products, CaP granules can potentially be directly used as P rich solid organic fertilizer in agriculture. Dissolution tests showed that 35% of the P in CaP granules was released within 5 min in citric acid, while for phosphate rock only 14% of the P was released. Moreover, incinerated CaP granules can directly replace phosphate rock in the fertilizer industry.

CRediT authorship contribution statement

Jorge Ricardo Cunha: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - original draft. **Chris Schott:** Methodology, Formal analysis, Formal analysis, Investigation, Writing - original draft. **Renata D. van der Weijden:** Software, Validation, Writing - review & editing, Supervision. **Lucía Hernández Leal:** Validation, Writing - review & editing, Supervision, Project administration. **Grietje Zeeman:** Conceptualization, Validation, Writing - review & editing, Supervision, Funding acquisition. **Cees Buisman:** Conceptualization, Validation, Writing - review & editing, Supervision, Funding acquisition.

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.

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Appendices A. Scheme for separation.

Fig. A1

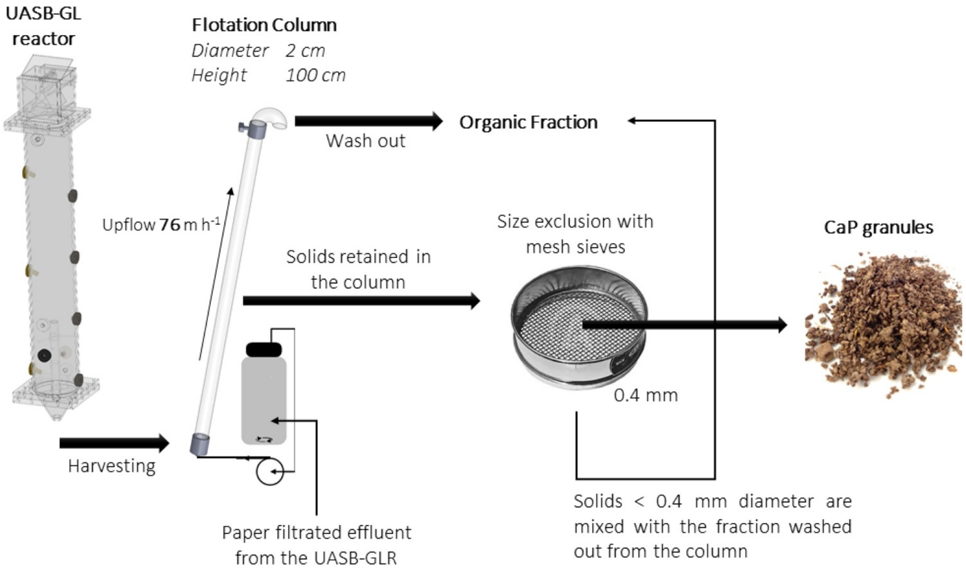

Fig. A1. Schematic representation of the process for separation of CaP granules from the organic fraction.

Appendices B. Micropollutants

Table B1, B2, B3, B4, B5

Table B1
Micropollutants analyzed and respective code and limit of detected (LOQ).

Name	Code	LOQ (ng g^{-1})
Acesulfame	ACSUL	5
Amoxicillin	AMOX	2
Ampicillin	AMP	2
Aspirin	ASA	20
Azithromycin	AZI	2
Benzalkonium Chloride C12	BaC12	2
Benzalkonium Chloride C14	BaC14	2
Benzylparaben	BzPB	5
Butylparaben	BuPB	5
Caffeine	CAF	1
Carbamazepine	CBZ	0.2
Cefotaxime	CFT	5
Cetirizine	CETRZ	20
Ciprofloxacin	CFX	0.5
Clarithromycin	CLAR	0.2
Clindamycin	CM	0.5
Diclofenac	DFN	0.5
Diethyltoluamide	DEET	1
Dimetridazole	DM	5
Doxicycline	DOX	2
Erythromycin	ERYT	5
Ethylparaben	EtPB	10
Flumequine	FLUMEQ	1
Gabapentine	GAPE	0.5
Hydrochlorothiazide	HCTZ	50
Ibuprofen	IBPF	20
Ketoprofen	KTPF	2
Lincomycin	LINCOM	1
MCPA	MCPA	10
Mecoprop	MCPP	10
Metformin	METF	5
Methylparaben	MePB	10
Metoprolol	METPL	2
Naproxen	NAPR	2
Ofloxacin	OFX	0.5
Oxazepam	OXA	1

(*continued on next page*)

Table B1 (*continued*)

Name	Code	LOQ (ng g^{-1})
Oxytetracyclin	OTETR	1
Paracetamol	PARA	10
Penicillin G	PENG	1
Penicillin V	PENV	2
Propylparaben	PrPB	5
Sucralose	SUCRAL	5
Sulfachloropyridazine	SUCLOP	0.5
Sulfadoxin	SUDOX	0.5
Sulfamethazine	SULFAM	1
Sulfapyridine	SULPYR	1
Sulfomethoxazole	SMO	1
Tetracycline	TETR	1
Tilmicosin	TILMIC	5
Tilosin	TYLOS	1
Triclocarban	TCC	1
Triclosan	TCS	20
Trimethoprim	TRIM	2

Table B2
Standard solution used for recovery and calibration dilutions D1, D2, D3, D4, D5 corresponding to 0, 20, 40, 80, 160, and 320 times dilution, respectively.

Code	Standard μg/ml	D1 μg/ml	D2 μg/ml	D3 μg/ml	D4 μg/ml	D4 μg/ml	D5 μg/ml
ACSUL	**0.259**	0.0	5.2	10.4	20.7	41.4	82.9
AMOX	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
AMP	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
ASA	**0.129**	0.0	2.6	5.2	10.3	20.6	41.2
AZI	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
BAC (75%C12,25% C14)	**0.133**	0.0	2.7	5.3	10.7	21.3	42.6
BuPB	**0.127**	0.0	2.5	5.1	10.1	20.3	40.5
BzPB	**0.131**	0.0	2.6	5.2	10.4	20.9	41.8
CAF	**0.132**	0.0	2.6	5.3	10.6	21.1	42.2
CBZ	**0.133**	0.0	2.7	5.3	10.7	21.3	42.6
CETRZ	**0.133**	0.0	2.7	5.3	10.7	21.3	42.6
CFT	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
CFX	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
CLAR	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
CM	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
DEET	**0.132**	0.0	2.6	5.3	10.6	21.1	42.2
DFN	**0.131**	0.0	2.6	5.2	10.5	21.0	41.9
DM	**0.133**	0.0	2.7	5.3	10.7	21.3	42.6
DOX	**0.134**	0.0	2.7	5.3	10.7	21.4	42.7
ERYT	**0.133**	0.0	2.7	5.3	10.7	21.3	42.6
EtPB	**0.126**	0.0	2.5	5.0	10.1	20.2	40.3
FLUMEQ	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
GAPE	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
HCTZ	**0.128**	0.0	2.6	5.1	10.2	20.5	41.0
IBPF	**0.129**	0.0	2.6	5.2	10.3	20.7	41.3
KTPF	**0.126**	0.0	2.5	5.1	10.1	20.2	40.4
LINCOM	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
MCPA	**0.127**	0.0	2.5	5.1	10.2	20.3	40.7
MCPP	**0.133**	0.0	2.7	5.3	10.6	21.3	42.6
MePB	**0.129**	0.0	2.6	5.2	10.3	20.6	41.2
METF	**0.133**	0.0	2.7	5.3	10.7	21.3	42.6
MTPL	**0.132**	0.0	2.6	5.3	10.6	21.1	42.3
NAPR	**0.131**	0.0	2.6	5.2	10.5	21.0	41.9
OFX	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
OTETR	**0.134**	0.0	2.7	5.3	10.7	21.4	42.7
OXA	**0.133**	0.0	2.7	5.3	10.7	21.3	42.6
PARA	**0.132**	0.0	2.6	5.3	10.6	21.2	42.4
PENG	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
PENV	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
PrPB	**0.128**	0.0	2.6	5.1	10.2	20.5	41.0
SMO	**0.133**	0.0	2.7	5.3	10.7	21.4	42.7
SUCLOP	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
SUCRAL	**0.264**	0.0	5.3	10.6	21.1	42.3	84.6
SUDOX	**0.134**	0.0	2.7	5.3	10.7	21.4	42.7
SULFAM	**0.133**	0.0	2.7	5.3	10.7	21.4	42.7
SULPYR	**0.134**	0.0	2.7	5.3	10.7	21.4	42.7
TCC	**0.130**	0.0	2.6	5.2	10.4	20.7	41.4
TCS	**0.264**	0.0	5.3	10.6	21.1	42.3	84.6
TETR	**0.134**	0.0	2.7	5.3	10.7	21.4	42.7
TILMIC	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7
TRIM	**0.134**	0.0	2.7	5.3	10.7	21.4	42.8
TYLOS	**0.133**	0.0	2.7	5.3	10.7	21.3	42.7

Table B3
Internal standard solution and calibration dilutions D1, D2, D3, D4, D5 corresponding to 0, 20, 40, 80, 160, and 320 times dilution, respectively.

Code	Internal Standard μg/ml	D1 μg/ml	D2 μg/ml	D3 μg/ml	D4 μg/ml	D4 μg/ml	D5 μg/ml
TRIM-D9	**0.366**	18.3	18.3	18.3	18.3	18.3	18.3
DIA	**0.366**	18.3	18.3	18.3	18.3	18.3	18.3
FNPF	**1.827**	91.3	91.3	91.3	91.3	91.3	91.3
AT-D7	**0.366**	18.3	18.3	18.3	18.3	18.3	18.3
CFX-D8	**0.366**	18.3	18.3	18.3	18.3	18.3	18.3
SUDOX-D3	**0.366**	18.3	18.3	18.3	18.3	18.3	18.3

Table B4
Complete list of results obtained for each sample of organic fraction and CaP granules and also the blank measurement.

			118 org	167 org	204 org	258 org	300 org	118 CaP Granules	167 CaP Granules	204 CaP Granules	258 CaP Granules	300 CaP Granules	Blank
Amoxicillin	AMOX	ng/g	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Paracetamol	PARA	ng/g	12	11	12	26	17	< 10	21	39	24	13	< 10
Trimethoprim	TRIM	ng/g	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Sucralose	SUCRAL	ng/g	163	156	177	138	178	80	119	108	116	108	< 5
Sulfamethazine	SULFAM	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Azithromycin	AZI	ng/g	596	635	1263	1283	886	64	170	391	181	184	< 2
Clindamycin	CM	ng/g	0.7	0.6	< 0.5	< 0.5	< 0.5	< 0.5	0.6	0.7	< 0.5	< 0.5	< 0.5
Sulfachloropyridazine	SUCLOP	ng/g	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Sulfadoxin	SUDOX	ng/g	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Sulfomethoxazole	SMO	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Tilosin	TYLOS	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Penicillin G	PENG	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Cetirizine	CETRZ	ng/g	4371	4394	4058	4020	4085	2464	2764	2731	2336	2216	< 20
Penicillin V	PENV	ng/g	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Clarithromycin	CLAR	ng/g	0.9	0.3	< 0.2	< 0.2	< 0.2	0.3	0.2	< 0.2	< 0.2	< 0.2	< 0.2
Carbamazepine	CBZ	ng/g	17.0	7.3	6.3	3.8	2.8	8.7	3.9	4.3	3.1	0.9	< 0.2
Ketoprofen	KTPF	ng/g	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Naproxen	NAPR	ng/g	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Ibuprofen	IBPF	ng/g	89	52	186	137	189	< 20	< 20	56	< 20	< 20	< 20
Metformin	METF	ng/g	62	102	53	61	74	34	61	65	90	64	< 5
Dimetridazole	DM	ng/g	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Gabapentine	GAPE	ng/g	3.3	1.7	1.1	1.3	0.5	1.4	1.7	4.9	2.7	1.2	< 0.5
Sulfapyridine	SULPYR	ng/g	3.4	2.8	2.3	1.7	1.2	< 1	< 1	1.3	< 1	< 1	< 1
Lincomycin	LINCOM	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Caffeine	CAF	ng/g	23.2	38.7	11.3	18.0	4.3	8.9	21.1	15.3	5.6	15.1	< 1
Ampicillin	AMP	ng/g	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Ofloxacin	OFX	ng/g	0.8	2.2	1.5	1.5	< 0.5	5.5	4.8	3.7	1.5	0.5	< 0.5
Ciprofloxacin	CFX	ng/g	599.3	439.7	352.3	268.2	208.8	577.2	411.2	369.3	201.6	203.9	< 0.5
Oxytetracyclin	OTETR	ng/g	< 1	2.1	1.9	< 1	< 1	1.5	1.4	2.4	1.7	1.3	< 1
Cefotaxime	CFT	ng/g	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Tetracycline	TETR	ng/g	20.2	12.7	10.5	6.2	9.8	77.6	29.9	14.1	11.5	9.2	< 1
Metoprolol	METPL	ng/g	123	138	141	121	186	35	41	36	36	50	< 2
Tilmicosin	TILMIC	ng/g	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Doxicycline	DOX	ng/g	422	407	324	314	415	501	436	440	447	477	< 2
Erythromycin	ERYT	ng/g	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Oxazepam	OXA	ng/g	540	601	514	491	597	190	215	264	232	194	< 1
Flumequine	FLUMEQ	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Diethyltoluamide	DEET	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Benzalkonium Chloride C12	BaC12	ng/g	212	114	168	159	150	47	55	58	27	32	< 2
Diclofenac	DFN	ng/g	6.4	6.1	8.8	8.5	4.6	3.9	7.4	9.8	11.7	5.2	< 0.5
Benzalkonium Chloride C14	BaC14	ng/g	21	14	16	13	16	4	5	7	2	< 2	< 2
Acesulfame	ACSUL	ng/g	1746	835	3196	1374	2646	179	370	293	299	325	< 5
Hydrochlorothiazide	HCTZ	ng/g	88	66	82	93	115	< 50	< 50	< 50	< 50	< 50	< 50
MCPA	MCPA	ng/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Methylparaben	MePB	ng/g	< 10	< 10	12	< 10	< 10	< 10	< 10	20	< 10	< 10	< 10
Mecoprop	MCPP	ng/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Ethylparaben	EtPB	ng/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Propylparaben	PrPB	ng/g	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Aspirin	ASA	ng/g	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20
Benzylparaben	BzPB	ng/g	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Butylparaben	BuPB	ng/g	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Triclocarban	TCC	ng/g	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Triclosan	TCS	ng/g	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20

Table B5
Recovery rates for each measurement.

	118 org	167 org	204 org	258 org	300 org	118 CaP Granules	167 CaP Granules	204 CaP Granules	258 CaP Granules	300 CaP Granules	Blank
PARA	79%	78%	74%	76%	70%	87%	86%	87%	87%	92%	98%
TRIM	64%	69%	71%	67%	62%	81%	78%	75%	96%	91%	99%
SUCRAL	2%	17%	14%	6%	5%	24%	1%	16%	12%	13%	102%
SULFAM	45%	52%	45%	44%	40%	74%	67%	72%	75%	68%	97%
AZI	155%	171%	107%	172%	139%	177%	178%	146%	158%	159%	103%
CM	50%	55%	51%	52%	53%	84%	75%	74%	82%	78%	94%
SUCLOP	27%	29%	24%	16%	21%	47%	32%	37%	41%	42%	104%
SUDOX	74%	78%	72%	70%	74%	79%	81%	82%	82%	80%	100%
SMO	35%	35%	34%	33%	31%	55%	40%	51%	51%	41%	88%
TYLOS	113%	114%	110%	109%	116%	110%	114%	117%	124%	121%	105%
PENG	79%	84%	84%	84%	88%	82%	96%	94%	108%	85%	106%
CETRZ	45%	93%	33%	84%	49%	30%	47%	106%	34%	98%	108%
PENV	102%	88%	104%	83%	77%	79%	81%	80%	96%	82%	111%
CLAR	111%	99%	100%	93%	100%	102%	97%	92%	100%	106%	96%
CBZ	77%	84%	79%	83%	82%	91%	86%	88%	87%	78%	95%
KTPF	81%	82%	74%	70%	74%	68%	68%	85%	82%	57%	102%
NAPR	61%	59%	51%	47%	47%	38%	35%	58%	51%	26%	94%
IBPF	89%	91%	87%	95%	105%	102%	87%	87%	93%	78%	97%
METF	45%	27%	50%	55%	38%	47%	39%	33%	53%	57%	95%
DM	89%	79%	72%	73%	77%	91%	89%	82%	87%	84%	96%
GAPE	90%	88%	81%	91%	80%	90%	95%	89%	84%	90%	95%
SULPYR	108%	99%	95%	100%	104%	100%	106%	104%	97%	96%	102%
LINCOM	91%	100%	108%	89%	96%	95%	96%	93%	104%	102%	86%
CAF	87%	81%	90%	84%	96%	84%	100%	102%	90%	88%	101%
AMP	47%	44%	42%	41%	52%	66%	53%	53%	54%	54%	94%
OFX	38%	45%	38%	38%	42%	60%	49%	61%	60%	72%	95%
CFX	23%	10%	0%	22%	28%	34%	21%	35%	58%	25%	92%
OTETR	49%	48%	37%	40%	40%	67%	52%	55%	62%	60%	78%
CFT	32%	24%	30%	24%	33%	60%	37%	33%	50%	51%	128%
TETR	52%	53%	39%	51%	49%	69%	60%	68%	73%	70%	82%
METPL	74%	66%	70%	55%	58%	80%	90%	76%	85%	81%	94%
TILMIC	172%	154%	162%	186%	187%	194%	223%	205%	189%	194%	104%
DOX	30%	33%	57%	54%	54%	82%	62%	72%	66%	56%	78%
ERYT	1619%	1566%	1608%	1455%	1471%	1627%	1913%	2026%	2478%	2569%	116%
OXA	75%	74%	71%	64%	71%	80%	74%	83%	89%	69%	97%
FLUMEQ	89%	90%	87%	85%	85%	89%	89%	89%	89%	85%	91%
DEET	95%	95%	96%	93%	95%	95%	94%	96%	97%	96%	100%
BaC12	74%	80%	72%	70%	82%	79%	83%	82%	78%	77%	81%
DFN	93%	89%	87%	80%	84%	85%	79%	89%	90%	70%	95%
BaC14	28%	37%	23%	25%	37%	29%	28%	31%	32%	19%	45%
ACSUL	63%	127%	120%	186%	119%	117%	107%	114%	110%	107%	102%
HCTZ	112%	94%	73%	86%	117%	117%	96%	89%	74%	55%	82%
MCPA	120%	93%	120%	135%	176%	75%	130%	96%	121%	130%	75%
MePB	195%	166%	161%	182%	178%	184%	176%	184%	200%	159%	107%
MCPP	157%	110%	105%	133%	131%	89%	105%	97%	92%	143%	125%
EtPB	115%	152%	148%	147%	155%	166%	124%	114%	110%	131%	93%
PrPB	140%	97%	117%	130%	120%	109%	120%	115%	139%	145%	96%
ASA	118%	121%	150%	113%	127%	134%	147%	127%	143%	110%	115%
BzPB	106%	126%	118%	131%	136%	119%	109%	137%	143%	118%	86%
BuPB	118%	94%	112%	106%	124%	123%	102%	110%	106%	132%	89%
TCC	71%	74%	71%	74%	68%	87%	84%	87%	82%	68%	77%
TCS	97%	59%	101%	74%	106%	105%	89%	86%	80%	58%	88%

Appendices C. Sludge bed analyses

Fig. C1, C2

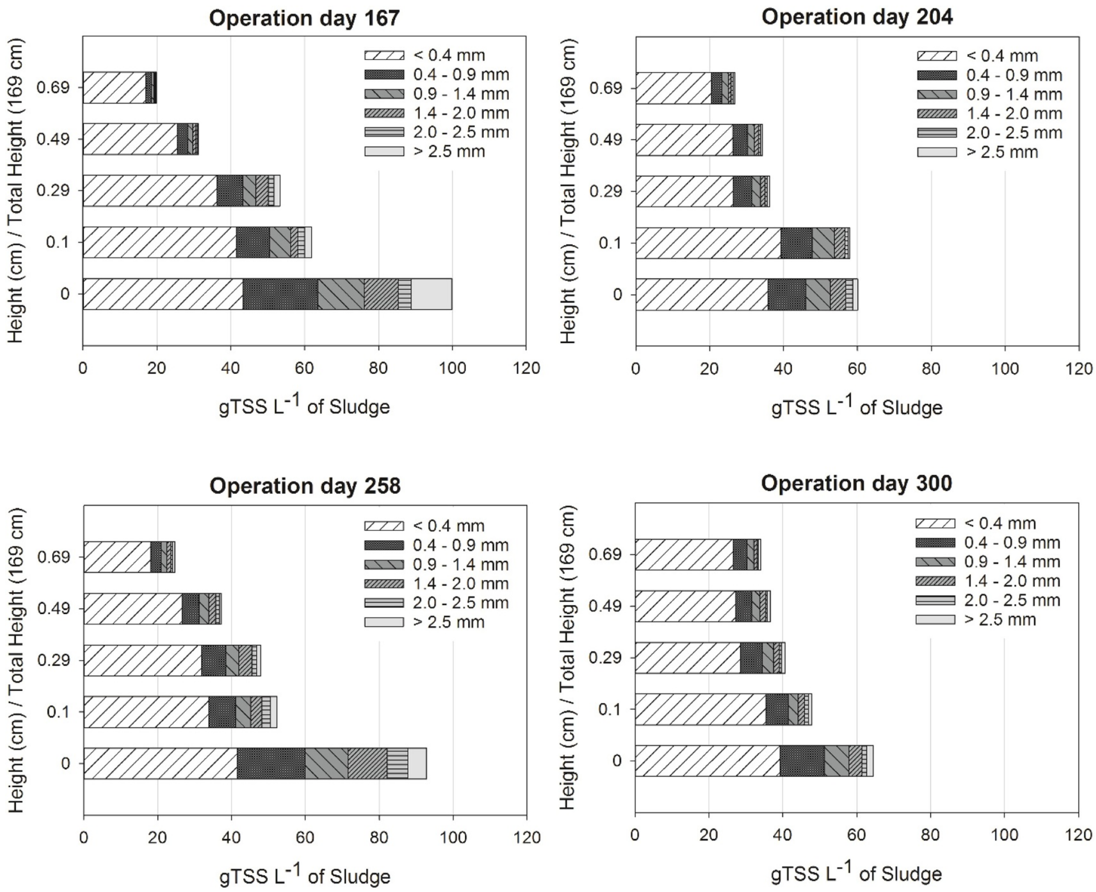

Fig. C1. Vertical distribution of TSS concentrations categorized in six size fractions (< 0.4 mm, 0.4 – 0.9 mm, 0.9 – 1.4 mm, 1.4 – 2 mm, 2 – 2.5 mm, and > 2.5 mm) from sludge taken from the UASB-GLR (45 L) on operation days 167, 204, 258, and 300.

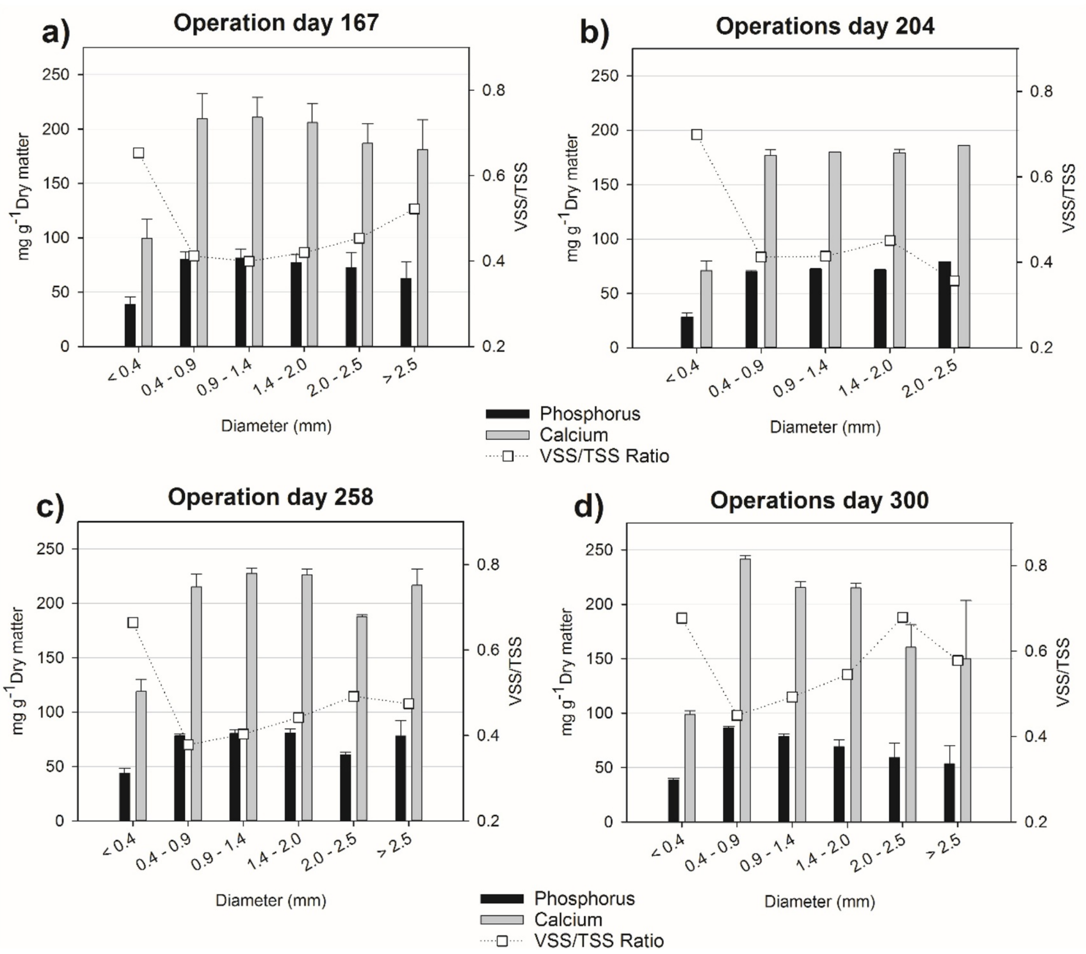

Fig. C2. P and Ca contents and VSS/TSS ratio of different sized particles from the bottom of the UASB-GLR at operation days 167 (a), 204 (b), 258 (c), and 300 (d).

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