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Original Research

Predicting the potential of sludge dewatering liquors to recover nutrients as struvite biominerals

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ABSTRACT

Phosphorus and nutrient recovery from wastewater as mineral salts can support local replenishment of fertilisers and reduce mining, contributing to the circular economy. Wastewater and related streams are rich in nutrients, however; there is need to develop bio-based processes to recover them. This study investigates the fractions of phosphorus (P) used by *Brevibacterium antiquum* to form struvite biominerals (bio-struvite) in wastewater sludge dewatering liquors. After 72h of incubation, 25.6 mg P/L were recovered as bio-struvite from 12.4 mg P/L organic plus condensed P and 13.2 mg P/L of ortho-phosphate. The potential of sludge dewatering liquors to recover nutrients as struvite was investigated by characterising ten types of sludge liquors (originating from primary, secondary sludge, feed to anaerobic digester and digestate, from 3 types of wastewater treatment plants) for their P fractions together with other parameters relevant for *B. antiquum* growth. Results indicated that liquors obtained from primary sludge, feed to anaerobic digesters and digestate were the most suitable to produce bio-struvite, as these were found to frequently have a high content of organic and condensed P, between to 276–732 mg P/L. Liquors, from all the investigated sites, presented a higher potential for bio-struvite production than with conventional struvite precipitation. This study demonstrated that *B. antiquum* could convert organic and condensed P into bio-struvite, and this opens up a completely new way to recover forms of phosphorus that are not typically available for nutrient recovery in a single process.

1. Introduction

Phosphorus (P) is a non-replaceable nutrient essential for all living organisms. Application of nutrient rich fertilizers in agricultural practices has enabled the increase of crop yields with P being critical to secure food production [1]. However, useable sources of P are limited and have an asymmetric world distribution. Five countries are responsible for 80% of the world production of rock phosphate through mining and three countries own >80% of the reserves [2].

Phosphorus recovery from wastewater is expected to contribute to filling this gap in supply [3]. Liquors from dewatering sludge have been pointed out as the wastewater stream with better potential for P recovery [4]. Sludge dewatering liquors have high concentrations of ortho-phosphate (PO_4-P) (up to 167 mg P/L) while making up a small fraction of the main stream of treatment flow (1–2% v/v) [5,6]. As much as 30% of the total phosphorus load can be lost to the sludge dewatering liquors [7] add to the P load further down the treatment works. The removal of P from sludge dewatering liquors has the potential to be more economical compared with its removal from mainstream processes, and thus the wastewater treatment plant (WWTP) can be operated with lower operational costs and extended capacity.

Phosphorus recovery from sludge dewatering liquors can be accomplished through precipitation of PO_4-P minerals such as hydroxyapatite or, more common, struvite, a salt of magnesium, ammonium, and phosphate ($MgNH_4PO_4.6H_2O$). Conventional chemical struvite requires magnesium dosing and pHs >8.5 that are often accomplished with chemicals, or CO_2 stripping [8]. Commercially available processes need a source of liquors with PO_4-P >100 mg P/L to remain economically viable [9].

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Selected bacteria as *Brevibacterium antiquum* were shown to mediate the biological formation of struvite (bio-struvite) when incubated in sterile sludge dewatering liquors with 31 mg P/L, without the addition of other chemicals [10]. Naturally occurred bio-struvite has been observed associating with various organic substances in a state of biodegradation as in kidney stones, guano deposits, manures, animal excreta and sediments rich in organic remains [11]. At WWTP, the pH, temperature and availability of nutrients make it possible to apply microorganisms for bio-struvite production [10,12]. Mechanisms for bio-struvite production have been investigated and have shown that *B. antiquum* uses biologically controlled mineralisation to produce bio-struvite intracellularly, where its internal chemistry supports the supersaturation of Mg, NH_4 and P to precipitate bio-struvite [13]. This suggested the possibility of a process to recover nutrients from low PO_4-P wastewater streams without the requirement for chemical additives. Where current chemical struvite recovery cannot recover phosphorus efficiently or economically [14]. However, the underlying bio-struvite which forms of P are used by the certain bacteria to produce the bio-struvite are not yet known.

Phosphorus in wastewater exists in a complex variety of forms that can be difficult to separate and quantify [15]. The most accepted definitions distinguish P in: readily reactive P, organic P and condensed P, according to their reactivity with ammonium molybdate and susceptibility to acid hydrolysis [16,17]. Readily reactive P includes dissolved inorganic phosphate-ion forms and can include loosely adsorbed ortho-phosphate ions complexes (i.e.: PO_4-P). This is the only form of P available for the precipitation of chemical struvite. The organic P fraction in wastewater includes substances such as DNA, adenosine triphosphate (ATP) and phospholipids. This fraction has been reported to make up 14–21% of the total P in sludge collected from enhanced biological phosphorus removal (EBPR) processes treating domestic wastewater [18]. Substances in the organic P fraction are resistant to hydrolysis when subjected to acid conditions. A strong acid oxidant, such as perchloric acid, is required to free the PO_4-P ions [16]. On the other hand, substances in the condensed P fraction can be hydrolysed in acid conditions at temperatures between 100 and 121 °C [17]. This fraction includes condensed inorganic polyphosphates (polyP) accumulated by bacteria in EBPR, and biological nutrients removal (BNR) processes [19]. Given the acid conditions used, a fraction of adsorbed PO_4-P can also be solubilised and add to the amount quantified as condensed P [16].

Sludge dewatering liquors can be sourced from a wide variety of sludges, with expected differences in their P fractions, particularly if different P removal technologies were applied. Sludge from secondary treatment such as EBPR/BNR processes can have 300 mg polyP-P/g VSS, well above 30 mg P/g VSS in conventional activated sludge [20]. However, there is very limited information regarding the characterisation of different forms of P in sludge dewatering liquors.

This study identified the fractions P used by *B. antiquum* to promote bio-struvite formation in sludge dewatering liquors. This information, together with parameters relevant for *B. antiquum* growth, were matched against the characterisation of ten types of sludge dewatering liquors to estimate their potential to recover nutrients as bio-struvite.

2. Materials and methods

2.1. Materials

Brevibacterium antiquum, strain DSM 21545, was obtained from a commercial culture collection (German Resource Centre for Biological Material, Brunswick, Germany). Pure bacteria starter cultures were prepared by growing *B. antiquum* in sterile synthetic media (4 g/L of yeast extract, 2 g/L of magnesium sulphate heptahydrate, and 2 g/L of di-potassium hydrogen phosphate) at 20–22 °C under agitation at 150 rpm (Stuart SSL1, Fisher Scientific, Loughborough, UK) for 4 days.

2.1.1. Sludge dewatering liquors

Different types of sludges, from primary sedimentation tanks, secondary clarifiers feed to anaerobic digester (AD) and digestate, were collected from three full-scale WWTP. The sampled WWTPs had distinct strategies to manage P: WWTP1 had a biological nutrient removal (BNR) process, WWTP2 had no phosphorus removal processes (NPR) and WWTP3 had chemical phosphorus removal (CPR) with activated sludge as secondary treatment and ferric chloride addition to the primary effluent for. In both WWTPs BNR and CPR, primary and secondary sludges were pre-thickened separately to 7–8% solids content and stabilized in anaerobic digesters with imported sludge from nearby municipal WWTP (approx. 40% v/v, in both sites). The digested sludge was stored in holding tanks, from 10 to 27 days before dewatering from typical 7% solids to 22% solids in horizontal centrifuge-decanters. Cationic polymer, anti-scaling and antifoam additives were used to aid the centrifugation process. For this study, primary sludge was collected from the feed to the pre-digestion thickening centrifuges, and the secondary sludge was collected from the feed line to the pre-digestion belt thickeners. Feed sludge to AD was collected directly from the feed line. Digestate was collected from the outlet line of AD in the BNR site, and after the post-digestion holding tanks in the CPR site. The NPR site (200,000 PE) had no specific processes for phosphorus removal. The primary settlers were de-sludge manually and the primary effluent was split between the activated sludge process and trickling filters (50% v/v). The primary and the secondary sludges were mixed in a pre-thickening weir tank. From the pre-thickening weir tank, the sludge was stored in holding tanks until fed to the anaerobic digester. Samples were collected in the sludge line of the primary settler tanks and both from the feed to AD and digestate. The sludge sampling points for each one of the WWTPs are summarised in Table 1, that varied due to accessibility of the sludge sampling points for each WWTPs.

The same dewatering procedure was applied to all the collected sludges, in order to obtain the respective dewatering liquors. The collected sludges were centrifuged in 50 mL disposable centrifuge tubes (Sanyo MSE Falcon 6/300 centrifuge, 300 G, 5 min). The supernatant was collected and stored under refrigerated conditions (<4 °C) for further analysis and testing, while the caked sludge pellet was discarded.

2.2. Bacteria cultivation in sludge dewatering liquors

B. antiquum was added to autoclaved (121 °C, 20 min) sludge dewatering liquors from the starter culture and incubated at room temperature (20–22 °C), under agitation at 150 rpm (Stuart SSL1, Fisher Scientific, Loughborough, UK) for 4 days. Control tests, not inoculated with *B. antiquum*, were also prepared.

The pH of the sludge liquors used was adjusted before autoclaving to ensure that the sterile sludge liquors keep a pH between 7.3 and 7.7. The pH of some sludge liquors was found to increase to pH values > 9 during autoclaving, which is not representative of the pH observed for sludge liquors [12]. To minimize the addition of PO_4, NH_4, and Mg^{2+} from the starter culture to the sludge dewatering liquors, the bacterial cells were separated from the synthetic media by centrifugation (2400g, 5 min, Sanyo MSE Falcon 6/300 centrifuge), and then re-suspended to the original volume with sterile 0.9% NaCl solution. Inoculation of sludge liquors was done with 1 volume of re-suspended bacteria to 10 vol of sludge liquors.

Table 1
Sludge collection points for the three WWTP investigated.

	WWTP1 500,000 PE Biological nutrient removal (BNR)	WWTP2 200,000 PE No phosphorus removal processes (NPR)	WWTP3 700,000 PE Chemical phosphorus removal (CPR)
Sludge collection points	-Primary sediment. tank -Secondary clarifier -Digestate	-Primary sediment. tank -Feed to AD -Digestate	-Primary sediment. tank -Secondary clarifier -Feed to AD -Digestate

PE = population equivalent.

2.3. Investigation of the sources of phosphorus for biological struvite formation

To follow the fate of the various phosphorus fractions during the growth of *B. antiquum* in sludge liquors, an experiment was setup with sacrificial bottles in quintuplicate (Fig. 1). Each bottle was prepared with 50 mL of sludge liquors obtained from digestate from BNR site. To maximize the potential for struvite production, the sludge liquors were supplemented with magnesium making phosphate the limiting ion for struvite formation. Magnesium sulphate was added to a final concentration of 52 mg Mg^{2+}/L using a 290 g/L solution of magnesium sulphate heptahydrate (Fischer BioReagents, Loughborough, UK).

During sampling, each test bottle was divided into aliquots: 10 mL of sample were saved from quantifying pH and total phosphorus (TP). The remaining volume was mixed with 5 mL of 2000 g/L sucrose solution in a 50 mL tube and centrifuged (Sanyo MSE Falcon 6/300 centrifuge, 2400g, 10 min) using density differences to separate bio-struvite from the sample (Fig. 1). The bio-struvite content was quantified by measuring the phosphate content of the pellet after solubilisation with 0.05M HCl.

The supernatant was collected together with 3.5 ± 1.0 mL of the sucrose layer, placed in a clean 50 mL tube and centrifuged again (Sanyo MSE Falcon 6/300 centrifuge, 2400g, 10 min) (Fig. 1). The supernatant was collected to quantify the condensed phosphorus and PO_4-P. Total dissolved phosphorus was quantified from this supernatant at the beginning and at the end of 72 h of incubation. The biomass pelletized in the second centrifugation step was saved and kept frozen for total cellular phosphorus analysis (Fig. 1).

2.4. Quantification of condensed and organic phosphorus

The Cond-P was converted to PO_4-P using a modified APHA 4500-P.B.2 method to quantify acid-hydrolysable phosphorus [16]. The Standard Method, APHA 4500-P.B.2 [16] was modified to reduce health and safety risks. Each sample (250 μL) was added to 4.65 mL of de-ionized water, mixed, and then acidified with 100 μL of sulphuric and nitric acid solution (75 mL 96% sulphuric acid mixed with 150 mL of de-ionized water and then supplemented with 1 mL of conc. 68% nitric acid) and allowed to digest for 30 min at 120 °C in a preheated thermoreactor (Spectroquant TR620, Merck-Millipore, Watford, UK). After digestion, the mixture was neutralized with 150 μL of 6M aqueous sodium hydroxide solution. This modified method was validated and calibrated against set nine filtered sludge liquors samples spiked with 5 levels of sodium hexametaphosphate (Fisher Scientific, Loughborough, UK). The validation results clearly showed a good correlation between the amount of PO_4-P measured in function of hexametaphosphate (21.6–127.3 mg P/L) with r^2 that varied between 0.986 and 0.998. The average slope and standard deviation was 0.302 ± 0.023 mg P/mg hexametaphosphate, representing a recovery of 94.6% of the hexametaphosphate as PO_4-P. Organic phosphorus (Org-P) was calculated by subtracting the Cond-P from the TP, taking in consideration the concentration of PO_4-P before the acid hydrolysis.

2.5. Analytical methods

Bacterial counts were taken with flow cytometry using a live/dead cells staining method that provides the number of cells with intact membrane [12]. The concentrations of total phosphorus, chemical oxygen demand, chloride and sulphate ions, were measured using cell test kits according to the manufacturer instructions (Merck-Millipore, Watford, UK). Total suspended solids (TSS), and alkalinity were measured following the standard methods [16]. The pH was measured with a Fisherbrand hydrous

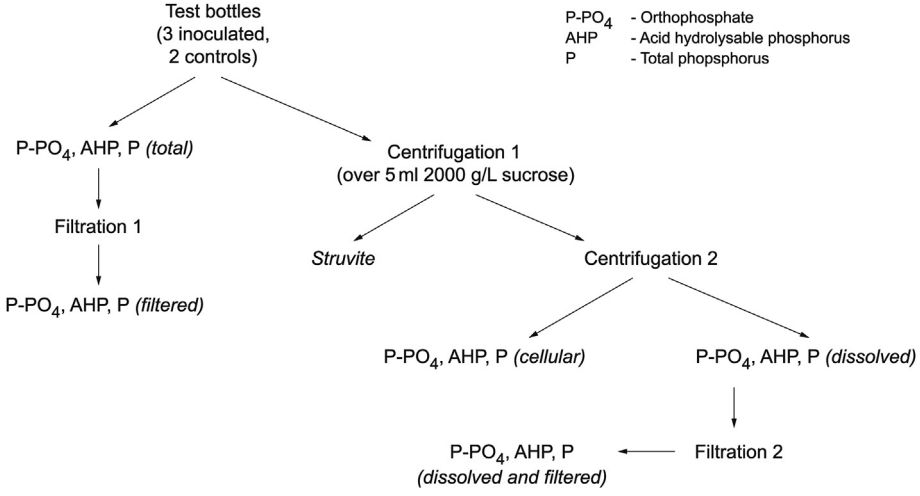

Fig. 1. Flow diagram for sampling and analysis procedures to distinguish sources of phosphorus used in bio-struvite production.

300 pH meter (Fisher Scientific, Loughborough, UK) immediately after sampling. Magnesium was analysed using an atomic absorption spectrophotometer equipped with an air/acetylene burner system (AAnalyst 800, PerkinElmer Ltd, Beaconsfield, UK). Ammonium (NH_4-N), nitrite (NO_2-N), oxidized nitrogen (ON), and phosphate (PO_4-P) were measured using a SmartChem 200 automated discrete analyser and the recommended reagents that adapt colorimetric standard methods (Labmedics, Abingdon, UK). Precipitates morphologies were analysed using environmental scanning electron microscopy (ESEM), coupled with energy dispersive spectroscopy (EDS) to return elemental contributions. Aluminium, arsenic, boron, cadmium, calcium, chromium, copper, iron, lead, mercury, nickel, potassium, selenium, and zinc content of the sludge liquors was assessed by an external lab (Scientific Laboratories Limited, Manchester, UK) using inductively coupled plasma optical emission spectrometry (ICP-OES). Values presented to mean and standard error of duplicate or triplicate tests unless stated otherwise in details.

3. Results and discussion

3.1. Phosphorus fractions used by B. antiquum to produce bio-struvite in sludge dewatering liquors

Liquors collected from the BNR site were used to study which fractions of phosphorus that were used by *B. antiquum* to produce bio-struvite. The sludge dewatering liquors used were analysed for typical quality parameters (Table 2). The concentration of PO_4-P was 49.5 mg/L, ammonium was 889 mg N/L and pH was 7.7 (Table 2). Total phosphorus (65.5 mg P/L), magnesium (18.6 mg/L) and COD (308 mg/L) were low and the used liquors were considered low strength for BNR sludge dewatering liquors (Table 2).

When incubating *B. antiquum* in the BNR sludge dewatering liquors, the cell counts increased from 1.7×10^8 to 2.7×10^8 cells/mL after 72h of incubation, indicating that growth was limited (Fig. 2). This was likely caused by the low amount of carbon available in the sludge dewatering liquors (302 mg COD/L, Table 2). [26]; demonstrated *B. antiquum* requires a readily available carbon source to grow. The concentration of phosphorus per cell decreased from 35 to 20 fg P/cell, after 72h of incubation. Microorganisms isolated from the aerobic section of EBPR process have been reported to contain up to 60 fg P/cell. Cells with >11 fg P/cell have been categorised as being P-accumulators [25]. *B. antiquum* has been reported to accumulate intracellular inorganic phosphate when grown in synthetic media with 71–356 mg PO_4-P/L and high availability of carbon source [27]. The results obtained in this study show similar capabilities of *B. antiquum* to accumulate P intracellularly in sludge dewatering liquors.

The various P fractions were measured during the incubation of *B. antiquum* in the sludge dewatering liquors during the 72h of incubation. Total phosphorus was 59.5 mg P/L, and it maintained constant during the experiment, in both inoculated experiments and controls. Phosphate (PO_4-P) decreased from 28.6 mg PO_4-P/L to 15.4 mg PO_4-P/L after 72h (Fig. 3). Bio-struvite production increased from 11.5 to 25.6 mg P/L from 42 to 72h of incubation (Fig. 3), also similar to previous results [12]. The concentrations of PO_4-P was 33.4 ± 2.3 mg P/L in the controls throughout the incubation period, and no struvite crystals were observed.

The concentration of Org-P was 28.0 mg P/L at the start of the incubation period, but it increased to 33.0 mg P/L at 8h of incubation and then it decreased from 42-72h to 17.2 mg P/L (Fig. 3). The concentration of Cond-P was 3.4 mg P/L at the start of the incubation period but increased to 7.5 mg P/L at 42h of incubation and then it decreased to <1 mg P/L between 56 and 72h of incubation (Fig. 3). The concentration of Cond-P was constant at 3 mg P/L in the non-inoculated controls. The combined organic and Cond-P fraction decreased by 14.4 mg P/L (from 33.0 mg P/L to 18.5 mg P/L, between 42 and 72h). This matched an increase of 14.1 mg P/L in bio-struvite between 56 and 72h (from 11.5 to 25.6 mg P/L). These results suggest that Org-P and Cond-P were released from TP, in the first 42h of incubation and used for bio-struvite production as PO_4-P, after that (Fig. 3). A mass balance of the P fractions highlights the decrease of Org-P and Cond-throughout the incubation period contributing with approximately half the P recovered in bio-struvite (12.4 mg P/L) (Fig. 4) and the remaining P originated from PO_4-P (13.2 mg P/L). The recovered precipitates were confirmed to be bio-struvite via energy dispersive spectroscopy with Mg:P of near 1:1 (Fig. 5) and presented sizes up to 500 μm and the typical orthorhombic structure, expected of struvite (Figs. 4 and 5).

As such, sludge liquors with high content in Org-P and Cond-P are expected to contribute to the formation of bio-struvite. *B. antiquum* has been shown to be able to utilise casein as carbon source [13], which belongs to the family of phosphoproteins. This corroborates the fact *B. antiquum* can hydrolyse complex forms of organics containing P. To our knowledge, no other nutrient recovery process can make use of these forms of P without additional steps to promote their hydrolysis to phosphate. However, there is a lack of information about the Org-P and Cond-P in sludge liquors, including different approaches to P removal.

3.2. Characterisation of liquors prepared from different types of wastewater sludge

Sludge dewatering liquors from sludge collected from primary sedimentation tanks, secondary clarifiers, feed to AD and digestate, from three full-scale WWTP were analysed for their P fractions and other wastewater quality parameters in order to assess the potential of each sludge liquors type for the application of a bio-struvite process and compare with chemical struvite recovery methods (Table 3).

Ortho-phosphate content varied from <0.3 mg PO_4-P/L in liquors produced from CPR feed to AD, to a maximum of 112.5 mg PO_4-P/L, in liquors obtained from NPR feed to AD. These match values reported for digested sludge liquors, which ranged from 0.6 to 167 mg PO_4-P/L [5,6]. Only one of the liquors had an ortho-phosphate >100 mg PO_4-P/L, which is the minimum amount necessary for the economic recovery of struvite using conventional chemical precipitation processes [9]. Hence, based on PO_4-P

Table 2
Characteristics of the sludge dewatering liquors collected from a BNR WWTP1 and comparison with values found in literature.

Parameter	BNR sludge dewatering liquors	Range in literature	References
pH	7.7	7.2–7.9	[21,22]
Total phosphorus (mg P/L)	65.5 ± 2.1	79–169	[23]
Ortho-phosphate (mg P/L)	49.5 ± 7.7	43–169	[21,23]
Magnesium (mg/L)	18.6 ± 0.4	24–110	(Thomas, 2007 [23];
Ammonium (mg N/L)	888.6 ± 33.8	355–1170	[21,23]
Chemical oxygen demand (mg/L)	302.0 ± 1.4	308–1762	[24]; Thomas, 2007)

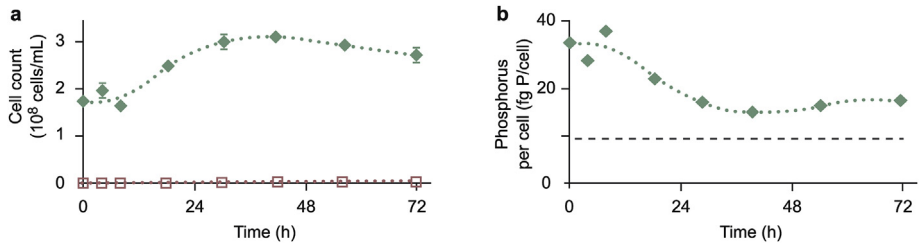

Fig. 2. *B. antiquum* cell count (a) and phosphorus content per cell (b) when incubated in sludge dewatering liquors at room temperature for 72h. Inoculated experiments (◆, closed symbols), non-inoculated controls (□, open symbols); high P accumulation reference value (11 fg P/cell) (dashed line) for EBPR microorganisms with high phosphorus accumulation [25].

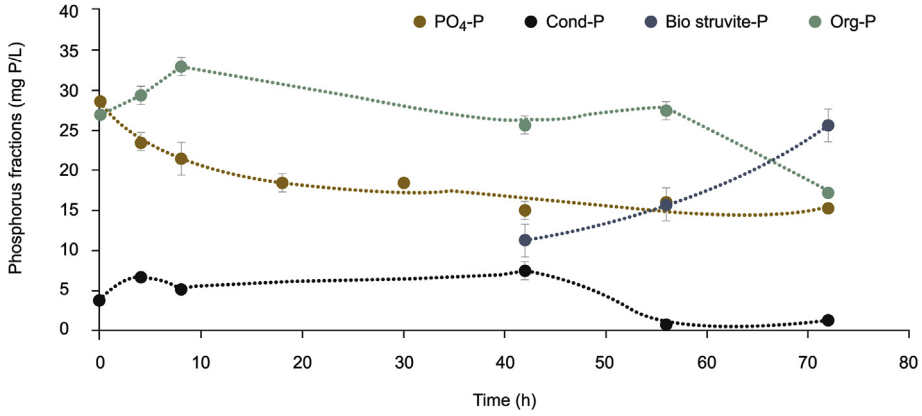

Fig. 3. Changes in P fractions when growing *B. antiquum* in sludge liquors from a BNR site, at room temperature for 72h. Errors bars show standard deviation of triplicate experiments.

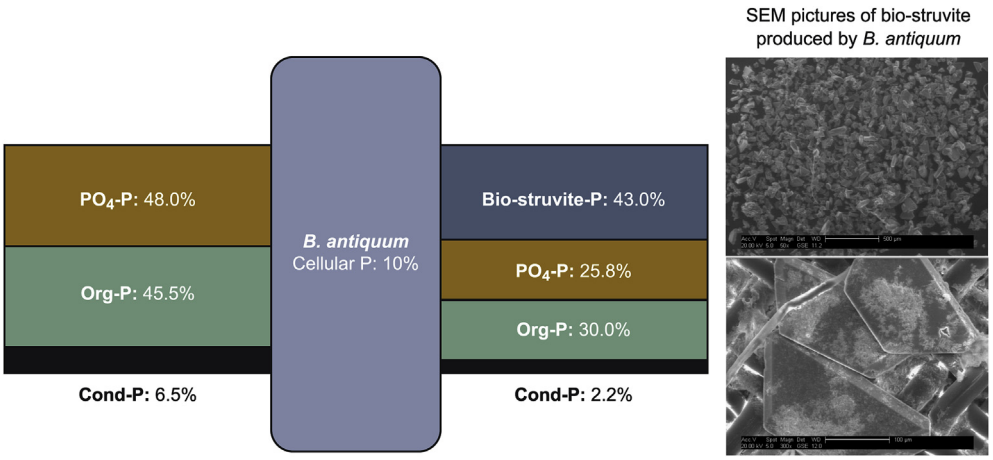

Fig. 4. Proportion in percentage, of phosphorus fractions in sludge dewatering liquors (left side) and after incubating *B. antiquum* for 72h with the formation of bio-struvite (right side) and scanning electron microscope (SEM) pictures of the bio-struvite produced showing the orthorhombic crystal structure.

content, none of the sludge liquors tested was a good candidate for P recovery using chemical struvite precipitation. Total phosphorus in the liquors varied by as much as 2 orders of magnitude, from 1.1 mg P/L in the CPR secondary sludge liquors, up to 767.5 mg P/L in the BNR digestate (Table 3). These values widen the ranges found in literature (4–200 mg P/L) [28,29]. In all sites, digestate sludge liquors had >200 mg P/L TP. This was three times more than the TP in the liquors obtained from the full-scale site with 65.5 mg P/L (Table 3), through dewatering by centrifugation. This difference is thought to be related to the use polymers in the full-scale dewatering processes to increase sludge dewaterability [30]. On the other side, the concentration of PO_4-P was similar in both prepared liquors in this study (35.0 mg P/L) and liquors collected at full-scale site (49.5 mg PO_4-P/L). This suggests that the dewatering process had an impact on the content and speciation of phosphorus as more Org-P and Cond-P was present in the liquors prepared without polymers. Consequently, the type dewatering process, as well as the chemicals added, are expected to impact the fractions of phosphorus available for bio-struvite formation.

Organic phosphorus in the digestate sludge liquors from the NPR and BNR sites were up to >100 mg P/L. Hence, these liquors have a considerable potential for bio-struvite formation. Liquors

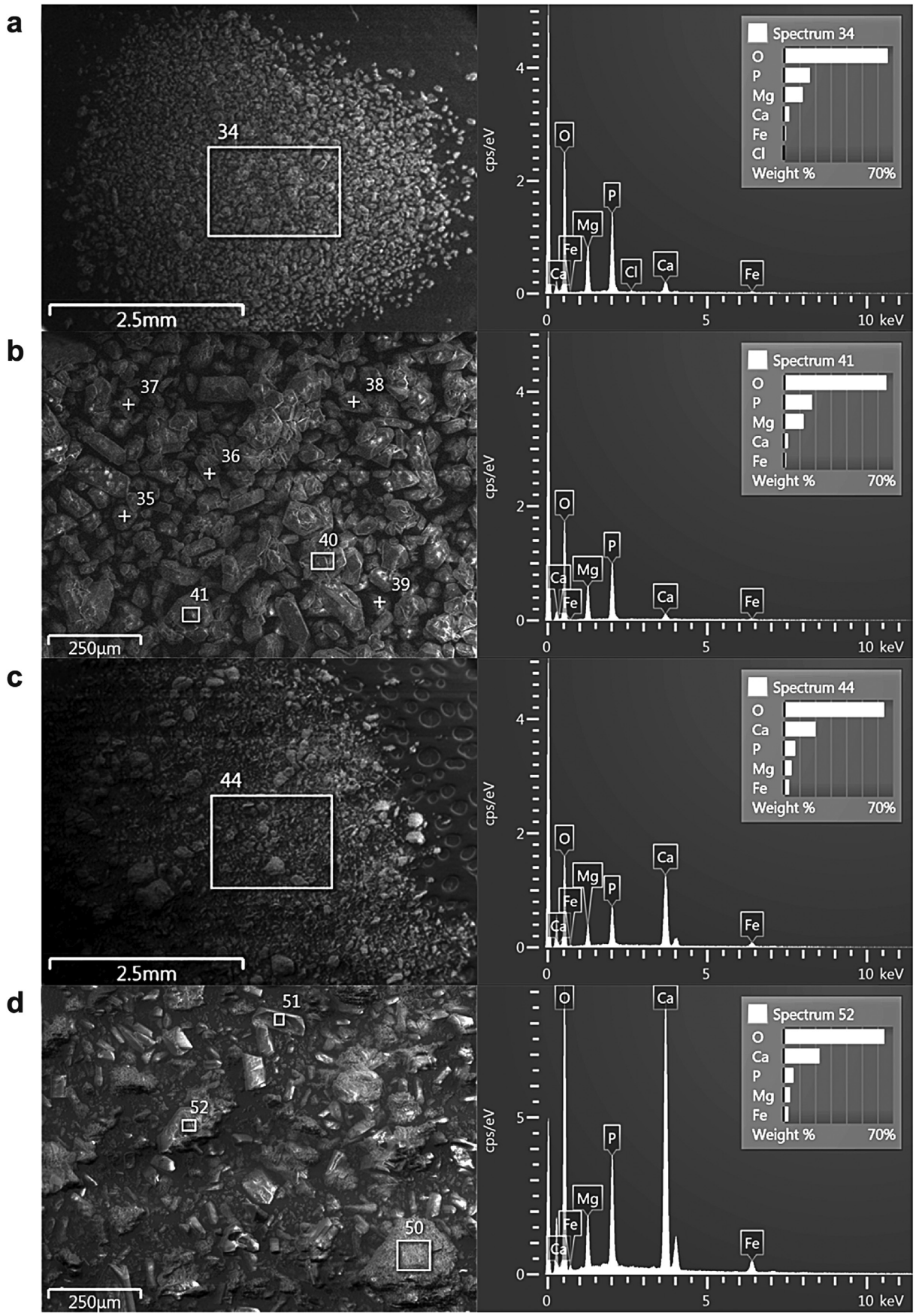

Fig. 5. Environmental electron scanning microscope photos, and energy-dispersive X-ray (EDX) spectra of selected areas highlighted in the photos, of bio-struvite: (a) 18x magnification, and (b) 100x magnification. And of chemical struvite: (c) 18x magnification, and (d) 100x magnification.

from the primary sludge of all sites, from the NPR digester feed sludge, and from the CPR digested sludge, also presented an opportunity for the recovery of Org-P. In these liquors, 5 of 14 samples were classed as having 10–30 mg P/L of Org-P. In contrast, low amounts of Org-P (<10 mg P/L) were found in the primary sludge liquors from the NPR site, and in all secondary sludge liquors tested.

Low amounts of Org-P (<10 mg P/L) were found in the primary sludge liquors from the NPR site, and in all secondary sludge liquors tested. Thus, secondary sludge liquors presented the lower potential for recovery bio-struvite via conversion of organic phosphorus.

The concentrations of Cond-P also varied considerably for all sludge liquors, except liquors from secondary sludge. In digestate sludge liquors, the Cond-P varied by 2 orders of magnitude, from <10 mg P/L to >100 mg P/L (Table 3). As in the case of the Org-P, the digestate sludge liquors had a considerable potential for bio-struvite production through the metabolisation of Cond-P fraction, not typically recovered in existing processes. In AD feed sludge liquors, 4 of 5 samples had a Cond-P between 10 and 100 mg P/L. The content of Cond-P in secondary sludge liquors was <10 mg P/L for all samples.

Because of the presence of phosphorus accumulating organisms in the BNR sites [20], more Cond-P was expected to be found in these liquors, in particular, for secondary and digestate sludge liquors. The lack of evidence for a higher content in Cond-P in liquors from the BNR site, relative to liquors from the other sites, might be the result of the BNR Cond-P remaining in the sludge, i.e., with solid fraction.

Beyond phosphorus, struvite formation requires magnesium and ammonium at the same molar amounts as phosphate. The NPR, and CPR digestate sludge liquors had the highest average magnesium concentrations at 45.8, and 53.5 mg Mg^{2+}/L, respectively. Accounting for all liquors, the content of magnesium varied between 15 and 56 mg Mg^{2+}/L. Published values show that magnesium in sludge liquors can range from 3 to 170 mg Mg^{2+}/L (Thomas, 2007). Magnesium was the limiting ion for struvite production in 7 out of the 28 samples of sludge liquors tested. Magnesium limitation was observed more often in BNR digested sludge liquors, and in NPR digester feed sludge liquors.

Moreover a Mg:PO_4 molar ratio above 1.3:1 is frequently recommended as necessary to trigger the precipitation, or to achieve sufficient rates of phosphorus recovery [4,31,32]. Adding magnesium based chemicals to liquors to produce struvite can represent up to 75% of the costs of the processes [9].

The need for a molar ratio of Mg:P higher than 1:1 has not been addressed yet in the bio-struvite process. As *B. antiquum* can make use fractions of P beyond the soluble PO_4-P used in conventional chemical precipitation, magnesium can end up limiting the recoverability of the Org-P and Cond-P in a bio-struvite process. This situation was identified for 7 of 18 samples where magnesium was the limiting ion for chemical struvite precipitation. In these 7 samples, conventional struvite precipitation could potentially recover 0.4–46.6 mg PO_4-P/L, while a bio-struvite process could potentially recover 35.7–61.2 mg P/L, without adding magnesium.

Ammonium was present in excess concentrations relative to P and magnesium, in liquors from primary sludge, AD feed sludge, and digestate (17.1–888.6 mg NH_4-N/L). Ammonium was the limiting ion in liquors obtained from secondary sludge, for both the CPR and BNR sites (<4.3 mg NH_4-N/L). This further reinforces the secondary sludge liquors as having the least potential for struvite formation.

Previous research has shown that a carbon source is required to promote the growth of *B. antiquum* [13,26]. The growth rate was increased 3 fold, from 0.9 to 3.4 1/d, when the COD of digested sludge liquors (<500 mg COD/L) was supplemented with 562–1686 mg COD/L as acetate [26]. The amount of COD in the liquors here investigated varied with the type of sludge. The liquors with the highest COD were the AD feed sludge (3334–7710 mg COD/L), followed by primary sludge (32–6667 mg COD/L), digestate (189–2442 mg COD/L), and secondary sludge (<25.0–105 mg COD/L). As such, the liquors from AD feed sludge and primary sludge have sufficient COD to sustain the growth of *B. antiquum,* whilst the liquors from digested sludge and secondary sludge were likely to limit its growth rate and hence bio-struvite production.

Previous studies have also highlighted the importance of managing the pH. The production of bio-struvite, and *B. antiquum* cell growth were reduced at pH values < 7.3, and >8.3. In liquors from digested sludge, the degassing of CO_2 was seen to increase the pH to values outside the optimal growth range for *B. antiquum* [12]. For the liquors tested here, the pH decreased along the series: digestate sludge liquors (7.4–8.0), secondary sludge liquors (7.0–7.3), primary sludge liquors (5.1–7.2), and AD feed sludge liquors (5.0–6.6). A CO_2 degassing case-by-case assessment might be necessary to show that *B. antiquum* remains viable and grows.

An increase in calcium content of 100 mg Ca^{2+}/L has been associated with a decrease in the growth rate of *B. antiquum* [26]. The content of calcium in digested sludge liquors was varied greatly between 68.0 and 580.0 mg/L (Table 3). The lowest amounts were found in secondary sludge liquors, from 81.0 to 94.0 mg Ca^{2+}/L. Primary sludge liquors from the BNR site had a content of calcium close to what was found in digested sludge liquors 110.0–150.0 mg Ca^{2+}/L. The AD feed sludge liquors from the CPR and NPR sites had the highest values found going from 290.0 to 580.0 mg Ca^{2+}/L. The CPR primary sludge liquors were also high with 300.0 and 450.0 mg Ca^{2+}/L, whilst in the NPR site the calcium content varied from 95.0 to 370.0 mg Ca^{2+}/L (Table 3).

Chloride, sulphate, boron, and aluminium, cadmium, chromium, copper, lead, mercury, selenium, and zinc where also monitored (Table 3) as these might impact the quality and purity of the products recovered. The impacts of these parameters on the formation of bio-struvite, and on the growth of *B. antiquum*, are not yet known.

3.3. Sludge liquors potential for bio-struvite production

A semi-quantitative/qualitative assessment of the potential for use with *B. antiquum* to produce bio-struvite was made of each type of sludge liquors characterized (Table 4). The production potential in percentage was calculated taking into consideration the characteristics of the sludge liquors measured, as described in Table 3, against the optimal characteristics for nutrient recovery for both chemical and bio-struvite. The most evident outcome is that none of the liquors investigated were suitable for P recovery with a conventional chemical struvite process. This is because the PO_4-P content in all liquors was below the 100 mg P/L recommended as necessary for economic recovery. Production potential were $\leq$60% (Table 4) based on measured liquor characteristics compared to optimal yield characteristics for chemical struvite (Le Corre et al.,. 2009). All liquor types showed a higher potential for bio-struvite production, $\geq$ 55% (Table 4). This reflects both the opportunity to recover organic and condensed phosphorus, and also the potential for *B. antiquum* to be used in liquors with less than 100 mg P/L.

Primary sludge liquors stand out as the liquors with more potential for growing *B. antiquum* and recover P as bio-struvite. Secondary and digestate feed sludge liquors showed specific challenges. Secondary sludge liquors were not suitable due to the low carbon source available and P content. Anaerobic digestion feed sludge liquors need to be investigated case by case, as these have the potential to reach too high pH values. The combination of the liquors from digestate sludge and primary sludge has the potential to address magnesium, carbon source, and pH limitations and it

Table 3
Characterisation of sludge dewatering liquors from sludge collected from primary sedimentation tanks, secondary clarifiers, feed to AD and digestate, from three full-scale WWTP with different phosphorus removal.

Site	Liquors sludge source	Total phosphorus (mg P/L)	Organic phosphorus (mg P/L)	Condensed phosphorus (mg P/L)	Ortho-phosphate (mg P/L)	Ammonium (mg N/L)	Dissolved Chemical Oxygen Demand (mg/L)	Total suspended solids (mg/L)	Dissolved oxidisable nitrogen (mg N/L)	Dissolved nitrite (mg N/L)	Alkalinity (mg $CaCO_3$/L)	pH	Magnesium (mg/L)	Calcium (mg/L)	Chloride (mg/L)	Sulphate (mg/L)	Aluminium (mg/L)	Arsenic (mg/L)	Boron (mg/L)	Chromium (mg/L)	Copper (mg/L)	Iron (mg/L)	Lead (mg/L)	Nickel (mg/L)	Potassium (mg/L)	Zinc (mg/L)
BNR	Primary	30.2 ± 1.6	10 to 30	<10	2.5 ± 0.0	64.4	770 ± 23	858 ± 25	<0.1	<0.1	610 ± 14	6.9	33	130	126	84	<0.02	<0.02	0.32	<0.01	0.01	16	<0.03	<0.01	46	<0.01
		19.6 ± 0.6	<10	<10	9.8 ± 0.2	28.3 ± 2.0	228 ± 13	383 ± 3	<0.1	<0.1	377 ± 15	6.7	27	110	113	73	<0.02	<0.02	0.26	<0.01	<0.01	3.4	<0.03	<0.01	30	<0.01
		92.0 ± 2.8	10 to 30	10 to 30	65.7 ± 2.2	45.5 ± 5.0	420	1409 ± 97	<0.1	<0.1	583 ± 10	6.6	40	150	150	47	0.94	<0.02	0.41	<0.01	<0.01	2.7	<0.03	0.02	63	0.18
	Secondary	46.0 ± 1.0	<10	<10	35.2 ± 0.0	<4.0	105 ± 3	117 ± 6	2.1	<0.6	248 ± 8	7	32	81	150	144	<0.02	<0.02	0.26	<0.01	<0.01	0.1	<0.03	<0.01	50	<0.01
		19.0 ± 0.0	<10	<10	11.1 ± 1.2	<4.0	<25	52 ± 23	1.2 ± 0.1	<0.1	213 ± 6	7.2	26	88	120	122	<0.02	<0.02	0.22	<0.01	<0.01	<0.01	<0.03	<0.01	36	<0.01
		39.9 ± 0.1	<10	<10	33.8 ± 0.3	2.4 ± 0.7	<25	213 ± 13	0.6 ± 0.3	<0.6	224 ± 1	7.1	28	90	264	138	<0.02	<0.02	0.37	<0.01	<0.01	0.1	<0.03	<0.01	50	0.01
	Digested	70.8 ± 2.0	10 to 30	30 to 100	8.5 ± 0.1	861.6	639 ± 119	2787 ± 71	<0.1	<0.1	4250 ± 0	7.9	33	130	168	90	2.00	0.03	0.15	<0.01	0.08	8.9	0.07	0.06	170	0.52
		87.2	30 to 100	<10	55.3 ± 1.5	713.2 ± 19.5	629 ± 15	1208 ± 106	<0.1	<0.1	3403 ± 6	8	32	120	132	43	0.71	<0.02	0.08	<0.01	0.07	8.8	<0.03	0.04	87	0.12
		767.5 ± 60.1	>100	>100	35.0 ± 2.0	713.4 ± 33.5	555	21617 ± 424	<0.1	<0.1	5883 ± 64	7.8	15	68	188	28	0.21	0.04	0.15	<0.01	<0.01	0.76	<0.03	0.04	170	0.09
NPR	Primary	134.0	<10	30 to 100	63.0 ± 0.1	193.6	4296 ± 356	2073 ± 35	<0.1	<0.1	913 ± 38	5.2	38	370	132	78	0.49	<0.02	0.15	<0.01	0.06	16	<0.03	0.02	73	0.15
		11.7 ± 1.4	<10	<10	12.8 ± 0.2	31.0 ± 0.7	32 ± 1	243 ± 3	<0.1	<0.1	377 ± 6	7.2	18	95	80	45	<0.02	<0.02	0.07	<0.01	0.02	0.44	<0.03	<0.01	31	<0.01
		21.8 ± 0.3	<10	<10	15.4 ± 0.5	17.3 ± 2.4	230	344 ± 15	<0.1	<0.1	422 ± 6	7.2	15	100	316	34	0.04	<0.02	0.10	<0.01	0.02	0.32	<0.03	<0.01	35	<0.01
	Digester feed	96.3 ± 1.3	10 to 30	10 to 30	50.2 ± 0.1	167.2	3335 ± 155	1773 ± 72	<0.1	<0.1	820 ± 10	5.4	34	320	144	78	1.20	<0.02	0.18	<0.01	0.15	24	0.04	0.03	66	3.3
		87.6 ± 1.7	30 to 100	<10	51.4 ± 2.2	189.9 ± 11.1	7710	4783 ± 370	<0.1	<0.1	1090 ± 10	5	47	580	158	95	0.14	<0.02	0.18	<0.01	0.03	110	<0.03	0.05	80	0.23
		216.0 ± 65.0	30 to 100	30 to 100	112.5 ± 4.3	287.1 ± 63.4	5397	3757 ± 261	<0.1	<0.1	1113 ± 33	5.2	56	450	368	15	0.13	<0.02	0.20	<0.01	0.03	4.9	<0.03	0.04	96	0.24
	Digested	59.7 ± 2.9	10 to 30	10 to 30	27.5 ± 1.2	551	189 ± 93	1297 ± 18	1.02	<0.6	2700 ± 10	7.7	28	150	216	78	0.14	<0.02	0.08	<0.01	0.05	0.58	<0.03	0.02	78	0.06
		18.1 ± 1.3	10 to 30	<10	<0.3	612.1 ± 12.9	290 ± 40	1557 ± 95	<0.1	<0.1	3377 ± 15	7.8	34	110	166	22	1.10	<0.02	0.08	<0.01	0.11	9.5	<0.03	0.04	85	0.13
		360.5 ± 7.8	>100	>100	3.4 ± 0.4	554.5 ± 14.9	508	7717 ± 317	<0.1	<0.1	4160 ± 10	7.6	33	150	352	25	0.31	0.03	0.06	<0.01	0.03	3.3	<0.03	0.04	100	0.07
CPR	Primary	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		73.5 ± 2.3	30 to 100	<10	11.3 ± 2.3	211.2 ± 6.4	3752 ± 62	3763 ± 156	<0.1	<0.1	1323 ± 29	5.9	36	300	172	39	<0.02	<0.02	0.66	<0.01	<0.01	21	<0.03	0.02	110	<0.01
		186.0 ± 22.6	10 to 30	>100	46.6 ± 6.3	113.9 ± 6.9	6668	3583 ± 148	<0.1	<0.1	1037 ± 15	5.1	48	460	476	17	0.12	<0.02	0.76	<0.01	0.02	29	<0.03	0.05	100	0.20
	Secondary	1.7 ± 0.1	<10	<10	0.5 ± 0.0	<4.0	82 ± 1	60 ± 3	2.6	1.2	233 ± 6	7.3	18	87	204	114	<0.02	<0.02	0.50	<0.01	<0.01	0.06	<0.03	<0.01	40	<0.01
		1.1 ± 0.1	<10	<10	0.4 ± 0.1	4.3 ± 0.1	<25	46 ± 8	7.7 ± 0.4	0.2	247 ± 64	7.1	17	85	144	72	<0.02	<0.02	0.33	<0.01	0.01	0.01	<0.03	<0.01	36	<0.01
		2.2 ± 0.1	<10	<10	0.1 ± 0.0	2.3 ± 0.4	36	147 ± 20	2.8 ± 0.4	<0.6	223 ± 8	7.3	20	94	332	97	<0.02	<0.02	0.43	<0.01	0.02	0.05	<0.03	<0.01	55	<0.01
	Digester feed	78.9 ± 1.2	10 to 30	30 to 100	0.7 ± 0.0	264.3	5238 ± 153	5185 ± 191	<0.1	<0.1	1410 ± 113	5.3	51	340	276	132	<0.02	0.03	0.40	<0.01	0.02	250	<0.03	0.21	130	1.00
		59.5 ± 0.5	30 to 100	10 to 30	<0.3	510.6 ± 24.7	5325 ± 149	6773 ± 32	<0.1	<0.1	2487 ± 35	6.6	56	290	184	66	<0.02	0.03	0.48	<0.01	<0.01	120	<0.03	0.10	170	<0.01
		_	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Digested	54.4 ± 1.1	10 to 30	10 to 30	21.8 ± 0.4	750.4	680 ± 265	1495 ± 156	<0.1	<0.1	3840 ± 0	7.8	30	140	402	126	0.29	0.04	0.59	<0.01	0.04	2.1	<0.03	0.06	160	0.09
		36.0 ± 2.4	30 to 100	<10	<0.3	829.6 ± 42.3	2442 ± 38	7717 ± 74	<0.1	<0.1	4483 ± 61	7.4	46	150	400	24	1.90	0.05	1.20	0.01	0.11	61	0.12	0.11	310	0.50
		276.5 ± 101.1	<10	>100	0.4 ± 0.1	832.0 ± 43.6	523	16993 ± 203	<0.1	<0.1	6820 ± 35	7.7	46	82	870	29	0.10	0.10	1.50	<0.01	0.01	2.2	<0.03	0.10	460	0.08

BNR, site with biological nutrients removal; NPR, site without phosphorus removal technologies; CPR, site with chemical phosphorus removal. Cadmium (<0.01 mg/L), mercury (<0.01 mg/L) and selenium (<0.04 mg/L) were below the detection limit for all samples. Values detail the mean ± standard deviation when replicate measurements were taken.

Table 4
Qualitative assessment of the potential application of the bio-struvite process for different types of sludge liquors. This was calculated taking in consideration the characteristics of the sludge liquors measured, as described in Table 3, against the optimal characteristics for nutrient recovery for both chemical and bio-struvite. The optimal sludge characteristics gave a total of 16 points for chemical struvite (total of 4 parameters considered) and 28 points for bio-struvite (total of 7 parameters considered).

Sludge source	Site type	Phosphate	Magnesium	Ammonium	pH	Chemical struvite production potential	Phosphorus	Magnesium	Ammonium	COD	pH	Alkalinity	Calcium	Bio-struvite production potential
Primary	BNR	−	−	+	−	56%	+	+	+	+	−	+	+	71%
	NPR	−	−	+	−	56%	+	+	+	−	−	+	−	64%
	CPR	− −	+	+	− −	50%	+ +	+ +	+	+ +	−	+	−	79%
Secondary	BNR	−	+	−	−	44%	+	+	−	− −	+	+	+	64%
	CPR	− −	−	+	−	50%	− −	−	+	− −	+	+	+	57%
AD feed	NPR	−	−	+	− −	50%	+ +	−	+	+ +	−	−	−	57%
	CPR	− −	+	+	− −	50%	+ +	+ +	+	+ +	−	−	−	75%
Digestate	BNR	−	−	+	−	56%	+ +	−	+	−	+	−	+	71%
	NPR	− −	+	+	−	56%	+	−	+	− −	+	−	+	61%
	CPR	− −	+	+	−	56%	+ +	+	+	−	+	−	+	71%

Qualitative and numerical quantification used in calculation of the production potential: + + Always suitable = 4; + Variable and not always suitable = 3; − Variable and mostly not suitable = 2; − − Not suitable = 1.

should be further investigated to validate the estimates here formulated.

4. Conclusions

B. antiquum can use >50% of the organic and condensed phosphorus fractions to produce bio-struvite. This opens the opportunity to recover two more phosphorus fractions that were not available for recovery before. Different types of sludge liquors were found to frequently have a high content of organic and condensed phosphorus, between to 276–732 mg P/L in sludge liquors from digestates.

Liquors, from all the investigated sites presented a higher potential for bio-struvite production and phosphorus recovery than with conventional struvite precipitation. But none the liquors were found to have all the conditions suitable for bio-struvite formation. Combining primary sludge with digested sludge was suggested to mitigate carbon source limitation without increasing the calcium content of the combined liquors.

Bio-struvite produced by *B. antiquum* enables the recovery of phosphorus that is not available for recovery using current chemical processes. This widens the range of sludge dewatering liquors that can potentially be used for phosphorus recovery.

Declaration of competing interest

The author declares no competing interests or conflict of interest.

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