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ORIGINAL PAPER

Biological recovery of phosphorus from waste activated sludge via alkaline fermentation and struvite biomineralization by *Brevibacterium antiquum*

Sevil Coşgun · Büşra Kara · Büşra Kunt · Ceren Hür · Neslihan Semerci

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Abstract Struvite biomineralization is a promising method for phosphorus recovery from wastewater treatment plant streams, and the growth of responsible microorganisms in mixed cultures is one of the most critical points for applying this process in pilot and full-scale. This study aimed to investigate the growth and bio-struvite production of *Brevibacterium antiquum* in mixed sludge culture. Alkaline fermentation was applied at different pH conditions to enhance the phosphorus content of sludge for an efficient recovery, and pH 8 was determined as the most feasible considering the phosphorus release and sludge characteristics. Growth optimization studies showed that NaCl's presence decreases the growth rate of *Brevibacterium antiquum* and bio-struvite production. At the same time, pH in the range of 6.8–8.2 did not alter the growth significantly. In addition, studies showed the ability of *Brevibacterium antiquum* in unsterilized fermented sludge centrate to grow and recover the phosphorus as struvite. Thus, our results indicated the potential of struvite biomineralization in full-scale wastewater treatment plants.

Keywords Biomineralization · *Brevibacterium antiquum* · Struvite · Phosphorus recovery · Alkaline fermentation

Abbreviations

TP	Total phosphorus
COD	Chemical oxygen demand
MLVSS	Mixed liquor volatile suspended solids
MLSS	Mixed liquor suspended solids
VFA	Volatile fatty acid
OD	Optical density
FT-IR	Fourier-transform infrared spectroscopy
SEM–EDS	Scanning electron microscopy with energy dispersive spectroscopy
AP	Apatite phosphorus
NAIP	Non-apatite inorganic phosphorus
AHP	Acid-hydrolyzable phosphorus
Org-P	Organic

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Introduction

Phosphorus is an essential element for all living organisms; used as a fertilizer in agriculture and has no alternative in ensuring food safety. However, phosphorus has irreversible sources, and it is expected that the world will face phosphorus scarcity and thence to failure of food security in the coming centuries (Ye et al. 2016); therefore, phosphorus recovery has come to the fore in recent years. In sync, phosphorus is one of the major pollutants in wastewater; most municipal wastewater treatment plants have employed activated sludge process for the pollutants, including phosphorus (Scholz 2006), by producing vast amounts of excess sludge. Although a challenging waste, excess sludge is a secondary phosphorus source; therefore, it is an auspicious alternative for recovery.

Grover et al. (1997) have introduced struvite biomineralization, and later, Soares et al. (2014) studied this process for wastewater and the dewatering sludge liquors. Due to its advantages over chemical precipitation, biomineralization is a promising alternative. Conventional struvite precipitation processes require a pH > 8.5 and, accordingly, high consumption of chemicals or CO_2 along with being applicable in phosphorus concentrations more than 100 mg/L to be cost-effective (Simoes et al. 2020). Plus, chemical struvite precipitation requires a complex reactor design due to struvite nucleation and crystallization steps. Low turbulence attributes small crystal sizes due to lower dissipation; while the higher can break more giant crystals; moreover, the presence of ions such as Ca^{2+}, Fe^{2+}, and NO_3^- can significantly reduce the crystal size (Krishnamoorthy et al. 2021). In the study of Li et al. (2021b), the struvite crystals sizes were around 100 µm in lab-scale trials in a conical flask. Tarragó et al. (2016) has achieved a crystal size of 314 µm with a continuous air-lift reactor combined with a settler, while larger crystal sizes can also be found in the literature with different configurations. Nevertheless, Leng et al. (2020) reported struvite crystals > 300 µm from their biomineralization studies.

Brevibacterium antiquum (will be mentioned as *B. antiquum* in the rest of the paper) was isolated from permafrost sediment samples by Gavrish et al. (2004) and reported to be capable of struvite biomineralization (Soares et al. 2014). *B. antiquum* is a gram-positive, halotolerant, and aerobic bacterium with a rod/coccoid shape and 0.6-1 µm cell size; it can grow in the temperature range of 7–37 °C and pH range as 5.5–10 (Leng et al. 2020). Furthermore, *B. antiquum* follows the "Biologically Controlled Mineralization" (Simoes et al. 2018a), which is defined as the formation of crystals with high content and uniform size and shapes, resulting from the physiological activities (Chen et al. 2019a); for instance, they can make specific arrangements in isolated compartments to control the solution composition within the membrane-bound lipid vesicles (Leng and Soares 2021). Furthermore, the study of Simoes et al. (2018a) showed the ability of *B. antiquum* to use organic phosphorus and polyphosphates in addition to reactive phosphorus, and this finding gives an advantage over chemical methods and other microbial species. Besides, Simoes et al. (2018b) reported that the relatively high growth rate of *B. antiquum* (3.44/d) compared to most common species in WWTP (such as heterotrophic denitrifies with 3.7/d) may allow the bacterium to compete in treatment plants. All these findings indicate the potential of *B. antiquum* in real stream applications.

Activated sludge is a potential source of phosphorus for struvite biomineralization. However, only a small fraction is found in soluble form; therefore, pre-release is required for efficient recovery. Anaerobic fermentation is a well-proven method for phosphorus release. Overall, four major steps (solubilization, hydrolysis, acidogenesis, and methanogenesis) were involved in the process (Luo et al. 2018; Xu et al. 2018); solubilization and hydrolysis include breaking down the organics into small molecules and contributing to the solubilization of polyphosphates and organic phosphorus (Chen et al. 2019b; Hu et al. 2019; Wu et al. 2017). For enhanced fermentation, the initial dissolution step must be accelerated due to the complex matrix of activated sludge containing several organic and inorganic matters, various microorganisms and, cemented and flocculated structures via extracellular polymeric substances (Luo et al. 2018); and alkaline pretreatment is an efficient, cost-effective, and feasible method for this purpose. Several studies have indicated that alkaline pretreatment accelerates the hydrolysis of sludge (Luo et al. 2018). Results of the Chen et al. (2007) showed a significant release of phosphorus during alkaline fermentation. The study of He et al. (2016) demonstrated the dissolution of non-apatite phosphorus (bound with oxides

and hydroxides of Al, Fe, Mg, and Mn) under alkaline conditions, in addition to the release of phosphorus due to sludge solubilization. Wu et al. (2017) had studied phosphorus release through fermentation with acid and alkaline pretreatments and obtained 71.4% higher phosphorus solubilization with alkaline fermentation than the neutral.

One of the most critical factors determining the fate of biomineralization in full-scale applications is applicability in real streams due to the wide variety of potential inhibitors in wastewater as well as the potentially competitive microbial species found in wastewater treatment plants. The idea behind this study is to determine optimum fermentation conditions for better release of phosphorus from excess sludge and to investigate the survival and bio-struvite production of *B. antiquum* in unsterilized fermented sludge centrate.

Materials and methods

Sludge source and characterization

Sludge and dewatering reject waters were obtained from Atakoy Enhanced Biological Wastewater Treatment Plant, Istanbul, Turkey. Characteristics of the raw sludge used in this study are shown in Table 1.

Table 1 Characteristics of raw sludge (mean and standard error)

	mg/L		mg/L
sCOD	256.81 (±82.98)	TP	217.9 (±20.76)
tCOD	14,903.1 (±2157.35)	Organic-P & AHP	15.9 (±4.99)
pCOD	13,998.5 (±2009.21)	Particulate-P	191.1 (±20.19)
MLSS	14,963.2 (±1532.39)	PO_4-P	21.0 (±3.31)
MLVSS	8832.7 (±857.30)	NH_4-N	24.8 (±3.90)

MLSS mixed liquor suspended solids, *MLVSS* mixed liquor volatile solids, *sCOD* soluble chemical oxygen demand, *tCOD* total chemical oxygen demand, *pCOD* particulate chemical oxygen demand, *TP* total phosphorus, *Organic-P* organic phosphorus (aqueous), *AHP* acid-hydrolysable phosphorus, *PO_4-P* orthophosphate, *NH_4–N* ammonia–nitrogen

Alkaline fermentation

Low mesophilic (30 °C) alkaline fermentation studies were conducted at pH 8, 9, and 10 to solubilize the phosphorus in waste activated sludge (Figure S1). During the fermentation, sludge samples were collected, and the pH of the reactor was adjusted daily to maintain a constant internal pH. After each sampling, N_2 gas was purged to keep the anaerobic condition. In addition, an outline pipe was placed in the water as a precaution against uncontrolled CH_4 production. The raw sludge had variable characteristics for each batch; therefore, ammonia–nitrogen (NH_4–N) and VFA concentrations were normalized to the initial mixed liquor suspended solid concentration ($MLSS_{in}$) and the initial mixed liquor volatile suspended solid concentration ($MLVSS_{in}$). Sludge fractionation during alkaline fermentation was studied according to the SMT protocol proposed by Pardo et al. (2004). In addition, TP, PO_4^{3-}, NH_4^+, COD, and MLVSS were determined on Standard Methods (American Public Health et al. 2005). During the fermentation, sludge solubilization and phosphorus release were quantified as shown in Eq. (1):

$$\text{Release}(\%) = \frac{(\text{soluble COD, P - soluble COD, P}_{\text{initial}})}{\text{particulate COD, P}_{\text{initial}}} \times 100 \quad (1)$$

where pCOD is determined by subtracting sCOD from tCOD and particulate phosphorus by subtracting soluble phosphorus from the particulate.

Growth optimization for *Brevibacterium antiquum*

Brevibacterium antiquum (DSM 21545) was purchased as freeze-dried cultures from the German Resource Centre for Biological Material (Braunschweig, Germany) and grown in R2A 830 agar medium (Table 2) at 28°C. Colonies on agar plates were identified by 16S rRNA Sanger sequencing and inoculated into B41 liquid medium in duplicate. Crystals were collected to be analyzed by FT-IR and SEM–EDS.

Bacterial growth was assessed by optical density (OD) at a wavelength of 650 nm by Shimadzu UV-2450 spectrophotometer in duplicate, and the maximum growth rate was determined according to Eq. 2.

$$\ln [X] = \ln [Xo] + \mu \cdot t \quad (2)$$

Table 2 R2A 830 (The German Resource Centre for Biological Material) and B41 (Simoes et al. 2018a, 2018b) mediums

R2A 830		B41	
Yeast extract	0.5 g	Yeast extract	4 g
Proteose Peptone (Difco no. 3)	0.5 g	$MgSO_4 \times 7\ H_2O$	2 g
Casamino acids	0.5 g	K_2HPO_4	2 g
Glucose	0.5 g	Distilled water	1000 ml
Soluble starch	0.5 g		
Na-pyruvate	0.3 g		
K_2HPO_4	0.3 g		
$MgSO_4 \times 7\ H_2O$	0.05 g		
Agar	15 g		
Distilled water	1000 ml		

Table 3 Characteristics of fermented sludge centrate

Fermented sludge centrate	1	2
Mg	105.47 mg/L	–
PO4-P	95.57 mg/L	134.55 mg/L
NH4-N	210 mg/L	350 mg/L
MLSS	860 mg/L	–
MLVSS	500 mg/L	–
COD	5013.25 mg/L	4355.5 mg/L
pH	7.7	7.7
Acetic acid	1306.36 mg COD/L	1311.49 mg COD/L

Growth optimizations were carried out in B41 liquid mediums in duplicate. Different pH conditions (6.78, 7.2, 7.65 and 8.3) and NaCl (0.5%, 1%, 2.5% and 5%) concentrations were studied to achieve maximum growth rate and bio-struvite recovery (Figure S2). The bacterial growth was evaluated by following optical density (OD) at a wavelength of 650 nm.

Struvite biomineralization from fermented sludge centrates

Following the synthetic medium, biomass samples were harvested at 3000 rpm for 10 min, and the bacterial residue was inoculated into unsterilized centrates of two parallel fermentation reactors (Table 3). Samples were mixed at 150 rpm at room temperature for ten days, and PO_4^{3-}, NH_4^+, COD, MLSS, MLVSS, and VFA's are monitored daily according to Standard Methods (American Public Health et al. 2005). After centrifugation at 6000 rpm for 5 min, residues of collected samples were dissolved in 0.2 N HCl solution, PO_4^{3-} and NH_4^+ concentrations were measured to estimate the bio-struvite production. Crystals were also collected to be analyzed by FT-IR and SEM–EDS. Next-generation sequencing metagenomic analyses were outsourced to monitor changes in the microbial population during incubation through mixed sludge samples collected on the first and tenth days of incubation.

Results and discussion

Sludge solubilization and VFA production during alkaline fermentation

Sludge solubilization is the first step of the anaerobic fermentation, as well as being the rate-limiting process (Xu et al. 2018). Therefore, the release of COD was monitored to follow the sludge solubility during fermentation batches and is shown in Fig. 1. As reported by several researchers (Ma et al. 2016, 2019; Wu et al. 2017), increasing pH enhanced the COD release and sludge solubilization, most likely due to the degradation of extracellular polymeric substances matrix and solubilization of proteins and polysaccharides (Ma et al. 2016; Shi et al. 2021). On the first day of anaerobic fermentation, 9.58% COD release was obtained with pH 10, while sludge solubilities were around 5.21% and 4% for pH 9 and pH 8. Several

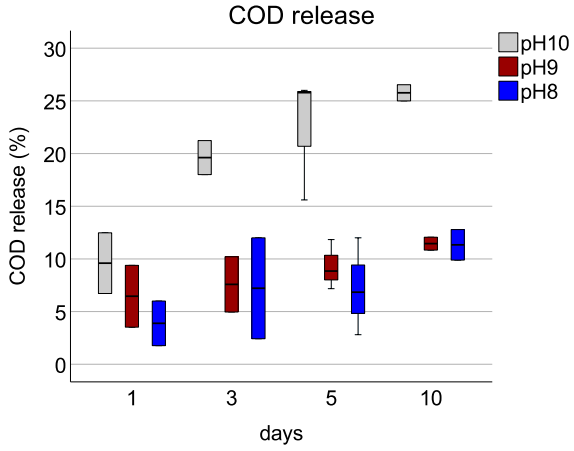

Fig. 1 COD release during alkaline fermentation

studies in the literature report lower and higher sludge solubilizations for the same pH conditions. While our one-day pH 10 fermentation results were similar to the findings of Chen et al. (2021); Ma et al. (2019), Ye et al. (2020), and some other studies had achieved significantly higher COD release with a pH of 10 at 35 °C. Yuan et al. (2011) stated that temperature affects fermentation in different ways, changing microorganisms' growth rate, yield, mortality, and reaction pathways. Therefore, the lower temperature might be the cause of the lower release. On the other hand, COD concentrations were observed to decrease on the last days of fermentation in some batches; this result might indicate that even pH 10 is not alkaline enough to inhibit methanogens' activity completely.

Production of VFAs by anaerobic sludge fermentation can make reasonable use of sludge for organic matter for heterotrophic bacteria in WWTP, so it is common in sludge-related research. Several studies in the literature have shown that alkaline conditions might significantly promote the VFAs production during fermentation (Ma et al. 2019; Wu et al. 2017) by increasing the rate of hydrolysis, thereby increasing soluble substrates for acidification, and inhibiting the activity of methanogens enzymes with higher pH (Ma et al. 2016). In our study, we also monitored the VFA production. pH 10, similar to the sludge solubilization, achieved the highest VFA accumulation. Following the fifth day, VFAs concentration reached a plateau, and further reduction was observed in pH 10 and pH 9 fermentation; this might result from the methane production as discussed in the previous section (Figure S3).

Nutrient release under different fermentation conditions

The effect of different pH conditions on nutrient release during anaerobic fermentation is summarized in Fig. 2. Due to the hydrolysis of nitrogenous organic matter, like proteins and DNA (Chen et al. 2019b), ammonium concentration increases during anaerobic fermentation. As observed in sludge solubilization, the highest ammonia release was achieved by the pH 10 (21.21 $mgNH_4$-N/$gMLSS_{initial}$), while pH 9 and pH 8 rose the ammonia content of sludge from around 1 $mgNH_4$-N/$gMLSS_{initial}$ to 11.47 and 13.67 $mgNH_4$-N/$gMLSS_{initial}$, respectively. It is noteworthy that fermentations were studied three times for both pH 9 and pH 8, and similar results were obtained in general with the superiority of pH 8 over pH 9 in ammonia release. It may be explained by the struvite precipitation under the more alkaline condition, while at pH 10 higher sludge solubilization results with the higher ammonia increase even if some portion is precipitated. Chen et al. (2019b) have reported similar results; while pH 10 has significantly higher ammonia releases, pH 9 and pH 8 have almost the same values during the fermentation, and higher releases were achieved through pH 8 than pH 9 fermentation, occasionally.

As in ammonia solubilization, the highest phosphorus release occurred in pH 10 fermentation studies (Fig. 2b); in line with the reports of several researchers (Chen et al. 2007; He et al. 2016; Ye et al. 2020); alkaline conditions promote the phosphorus and ammonia release during sludge fermentation. On the contrary, Wu et al. (2017) proposed that alkaline pH causes lower release than neutral due to phosphorus precipitation. In our study, while 36.4% release occurred on the first day of pH 10 fermentation, 27.4% and 28% releases were observed at pH 9 and pH 8, respectively. Although, as kept in ammonia, phosphorus release was higher under the pH 8 than pH 9, the results indicated the struvite precipitation again under more alkaline conditions, while higher sludge solubilization in the pH 10 fermentation compensates the phosphorus loss.

The phosphorus releases were observed to increase until the fifth day of fermentation; at pH 10, pH 9, and pH 8 fermentations, the releases reached 48.2%, 38.6%, and 39.9%, respectively. This increase was followed by stabilization and further reduction of phosphorus, most likely due to precipitation under alkaline conditions; similar behavior of phosphorus has been reported in the literature by several researchers, such as Li et al. (2021a).

To better understand phosphorus behavior during alkaline fermentation, fractionation was studied for pH 10, pH 9, and pH 8 fermentations (Fig. 2c). As a result of sludge solubilization, organic phosphorus is released from the solid phase into the surrounding solution during the fermentation; Wu et al. (2017) propose converting organic phosphorus to inorganic form by anaerobic bacteria during fermentation. Non-apatite inorganic phosphorus (bound to aluminum, iron, and manganese oxides and hydroxides) was one of the dominant solid phosphorus

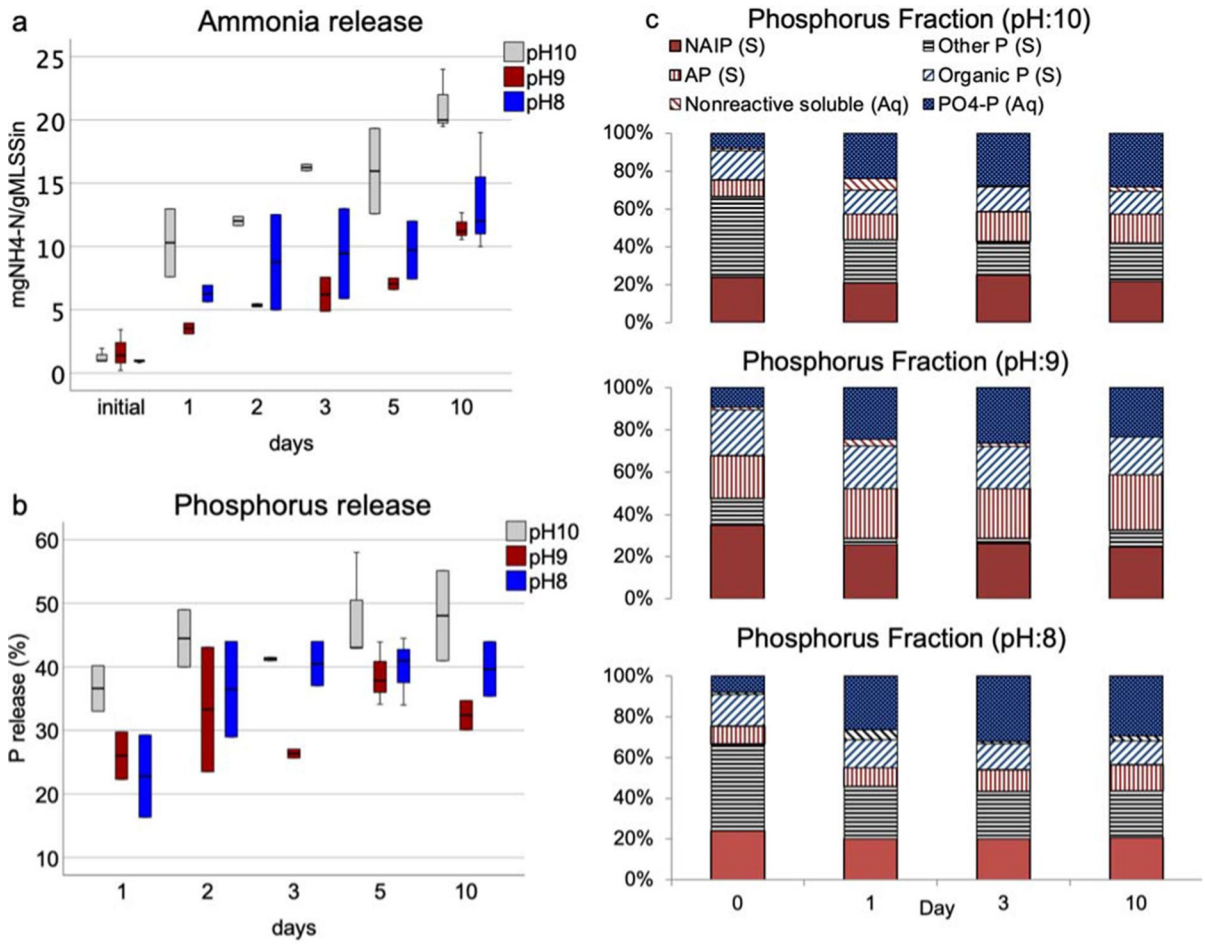

Fig. 2 Ammonia concentrations (**a**) and phosphorus releases (**b**) during alkaline fermentation and changes in phosphorus fractions (**c**). *AP* apatite phosphorus in solid phase, *NAIP* non-apatite inorganic phosphorus in solid phase, nonreactive soluble phosphorus: acid-hydrolyzable phosphorus and organic phosphorus in the liquid phase, *Org-P* organic phosphorus in the solid phase, PO_4-*P* orthophosphate, *Other P* phosphorus in solid-phase cannot be determined my SMT protocol)

types with the 26.4–39.1% presence, even though there is no chemical phosphorus removal in the source treatment plant. However, similar NAIP contents were reported in the literature for biological treatment plants, such as the study of Li et al. (2021a). Fermentation also solubilized the non-apatite phosphorus in addition to organic phosphorus; however, the decrease was observed only in the beginning. Several researchers report that NAIP is reduced under alkaline conditions due to replacing PO_4^{3-} ions with OH^- (He et al. 2016; Li et al. 2021a; Xu et al. 2015), so it is most probably not an outcome of fermentation. On the other hand, apatite phosphorus, which is defined as bound to calcium but can also include magnesium and iron forms (Pokhrel et al. 2018), also increased, and results indicated the phosphorus precipitation again during fermentation.

Taking the phosphorus release and fractionation results into account, as well as the reduced sludge filterability as fermentation continues (Figure S4), 3-day fermentation at pH 8 was chosen for the rest of the study. Phosphorus release was 40.6% on average on the third-day fermentation for pH 8, similar to pH 10, and did not alter onwards. Therefore, fermentation studies were carried out accordingly for the next step.

Table 4 Maximum growth rates of Brevibacterium antiquum with different pH conditions and NaCl concentrations

pH	6.78		7.20	7.65	8.2
Maximum growth rate (h^{-1})	0.18		0.22	0.18	0.20
P removal (%)	17.68		27.28	29.81	35.16
P removal (%) in control experiments	4.98		9.28	11.01	23.08
NaCl	0%	0.5%	1%	2.5%	5%
Maximum growth rate (h^{-1})	0.25	0.17	0.09	0.09	0.05
P removal (%)	27.57	22.44	0.34	0	0

Growth optimization of *Brevibacterium antiquum*

Brevibacterium antiquum was grown on agar plates in the first stage of biomineralization studies, and Sanger sequencing of the 16S rRNA genes revealed *B. antiquum* with 100% sequence similarity (Table S1). Following the agar plates, colonies were grown in the B41 liquid medium; crystals were collected, and SEM–EDS (Figure S5) analysis indicated the presence of struvite in the precipitate.

NaCl and pH optimizations were carried out to achieve the maximum growth of *B.antiquum.* NaCl optimization was also critical and might be advantageous for the study's primary aim; for applying *B. antiquum* to unsterilized sludge centrate, salt might be a barrier to promoting the *B. antiquum* growth while inhibiting other microorganisms. During the pH optimization, control experiments with the same incubation conditions without *Brevibacterium antiquum* inoculation were carried out to avoid any interference of chemical struvite precipitation; phosphorus removals were obtained by subtracting the removal in control experiments from the ones with the inoculant. As shown in Table 4, maximum growth was achieved without NaCl supplementation and around neutral pH levels; however, the pH changes did not significantly alter the growth. The results were similar to Simoes et al. (2018a); the maximum growth rate was achieved under almost neutral pH values. However, other findings in our study were poles apart from the previous report by Simoes et al. (2018b); the maximum growth rate was achieved without any NaCl supplementation as 0.25 h^{-1} (0.21 h^{-1} on average for the exponential phase) and further decreased with increasing NaCl concentrations. On the contrary, Simoes et al. (2018b) reported the presence of NaCl up to 3% made the growth rate of *Brevibacterium antiquum* increase. Meanwhile, Leng et al. (2020) and Leng and Soares (2021) revised the NaCl optimum as 0.5%. Besides, Smirnov et al. (2005) also reported the reduced growth rate by the presence of NaCl. The presence of NaCl inhibits most bacteria due to the osmotic stress; in addition, Na^+ can interfere with enzyme- and protein-synthesizing activities (Nagata et al. 1995; Pham et al. 2017). Even though *Brevibacterium* strains are known to be halotolerant with mechanisms to overcome salt stress, such as producing osmoprotectants (Pham et al. 2017), there is a gap in knowledge about the inhibitory mechanism of NaCl on *B. antiquum,* and further biochemical studies are needed to clarify. However, there is a strong commonality in the literature and our findings; NaCl reduces phosphorus recovery as bio-struvite as NaCl increases the solubility of struvite (Leng et al. 2020). As shown in Table 4, there was almost no phosphorus removal under relatively high NaCl concentrations. Therefore, *Brevibacterium antiquum* was inoculated to fermented sludge centrate without NaCl supplementation.

Phosphorus recovery from fermented sludge centrate as bio-struvite

Following the growth in synthetic media, *Brevibacterium antiquum* was inoculated into unsterilized fermented sludge centrate. Nutrient removal and recovery were monitored daily and are presented in Fig. 3. As shown, on the second day of incubation, the NH_4-N of concentration in the centrate increased. Similar results were reported by Leng and Soares (2021), with a higher rise within the first day of the incubation. Since *Brevibacterium antiquum* utilizes proteins as a carbon source (Leng et al. 2020), NH_4^+ release might result from protein biodegradation.

At the end of the batches, crystals were collected, and FT-IR and SEM–EDS analyses showed K, NH_4^+, and PO_4^{3-} ions and metal–oxygen bonds in the precipitates, indicating that they were struvite. (Figure

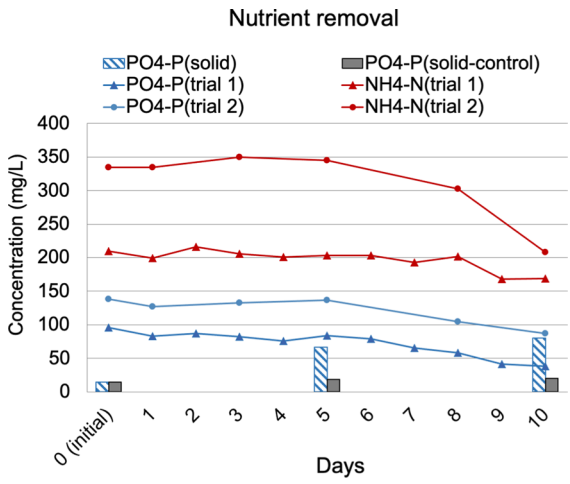

Fig. 3 Phosphorus and ammonia removals from fermented sludge centrates through struvite biomineralization

S6 and S7). As shown in Fig. 3, 60.1% phosphorus removal and 63.5% recovery (as observed from solid-phase phosphorus accumulation) were achieved at day ten for Trial 1. On the other hand, the phosphorus removal was 37% for Trial 2. Although the removals in Trial 1 and 2 were quite different in percentages, PO_4-P in solutions decreased 57.4 mg/L and 51 mg/L for Trial 1 and 2, respectively. Meanwhile, there was only 5.45% phosphorus recovery during the control experiment (without bacterial inoculum).

To be noted, the phosphorus removals from fermented sludge centrate were higher than from synthetic media in terms of percentage, which seems like an unexpected result. However, the higher removal in fermented sludge samples was related to lower initial phosphorus values than B41 media (Tables 2 and 3). Differences were also observed when compared with the literature. For example, Simoes et al. (2018b) achieved 51.7% phosphorus recovery after ten days of incubation with sterile sludge dewatering liquor. On the other hand, phosphorus removal was reported as 71% after three days of incubation in the study of Leng and Soares (2021). The differences can be related to different bacterial concentrations in studies; therefore, there is a need for further studies to standardize the removals in struvite biomineralization research. With all these uncertainties, this result nevertheless demonstrates the potential of struvite biomineralization by *Brevibacterium antiquum* for phosphorus recovery from non-sterile sludge and wastewater streams; however, the relatively low recovery rate indicates the need for process optimization.

Next-generation sequencing metagenomic analyses were applied to monitor the behavior of *Brevibacterium antiquum* in mixed sludge culture; sludge samples on the first and last day of incubation were analyzed in this context. Initially, the most abundant species was *Simplicispira piscis.* The genus *Simplicispira* has already been reported in sewage and activated sludge; however, as an aerobe, *S. piscis* was unexpected to be dominant in an anaerobic fermentation culture (Hyun et al. 2015). Another aerobe, *Flavobacterium ardleyense,* was the second dominant type; as a member of genus *Flavobacterium,* which is found in again activated sludge (Park et al. 2007) and known as a hydrolyzing and fermenting bacteria (Zhang et al. 2017). Fermented sludge samples were collected on the fourth day of fermentation; therefore, the dominance of aerobic species might be explained by the small growth rate of anaerobic bacteria; four days may not be enough to outlast members of the aerobic activated sludge community. Another abundant bacteria was *Fermentimonas caenicola* (with a former name as *Lascolabacillus massiliensis*), which is facultatively anaerobic and with an optimum temperature of 37 °C and pH range of 6.5 and 8.5; and isolated from the human gut (Beye et al. 2018); therefore, very abundant *F. caenicola* in fermented sludge was not an unexpected result. There are also species like *Macellibacteroides fermentans* and *Tissierella creatinophila* found in anaerobic wastewater treatment plant units (Harms et al. 1998; Jabari et al. 2012).

The initial abundance of *Brevibacterium antiquum* was 1.5% after inoculation and increased to 15% after incubation. The other species that grew during incubation were *Flavobacterium ardleyense*, *Acinetobacter marinus,* and *Gelidibacter mesophilus,* belonging to the genera reported to be found in wastewater treatment plants (Carr et al. 2003; Kim et al. 2017; Park et al. 2006, 2007). The class *Flavobacteria* was reported in anaerobic digesters (Wong et al. 2013; Yang et al. 2014). González et al. (2008) had shown that the members of the genus such as *Flavobacterium johnsoniae, Flavobacterium psychrophilum,* can also consume carbohydrates as an energy source in line with the paper of Zhang et al. (2017), which proposes the genus

Flavobacterium utilizes carbohydrates, lipids, and proteins. Therefore, there is a possibility that *Flavobacterium ardleyens* and *Brevibacterium antiquum* can dominance a microbial community in different wastewater treatment plant streams, which are rich in both substrates. However, further studies are needed to get a deeper understanding of the community behavior for struvite biomineralization in larger scale applications (Fig. 4).

Conclusion

In the scope of the study, sludge fermentations in the range of pH 8 and 10 were employed. Even though higher pH promoted phosphorus release, no remarkable increase was observed between pH 10 and pH 8; therefore, pH 8 was chosen as optimum for the study. Prior to the application of struvite biomineralization via *Brevibacterium antiquum* for the phosphorus recovery from fermented sludge centrate,

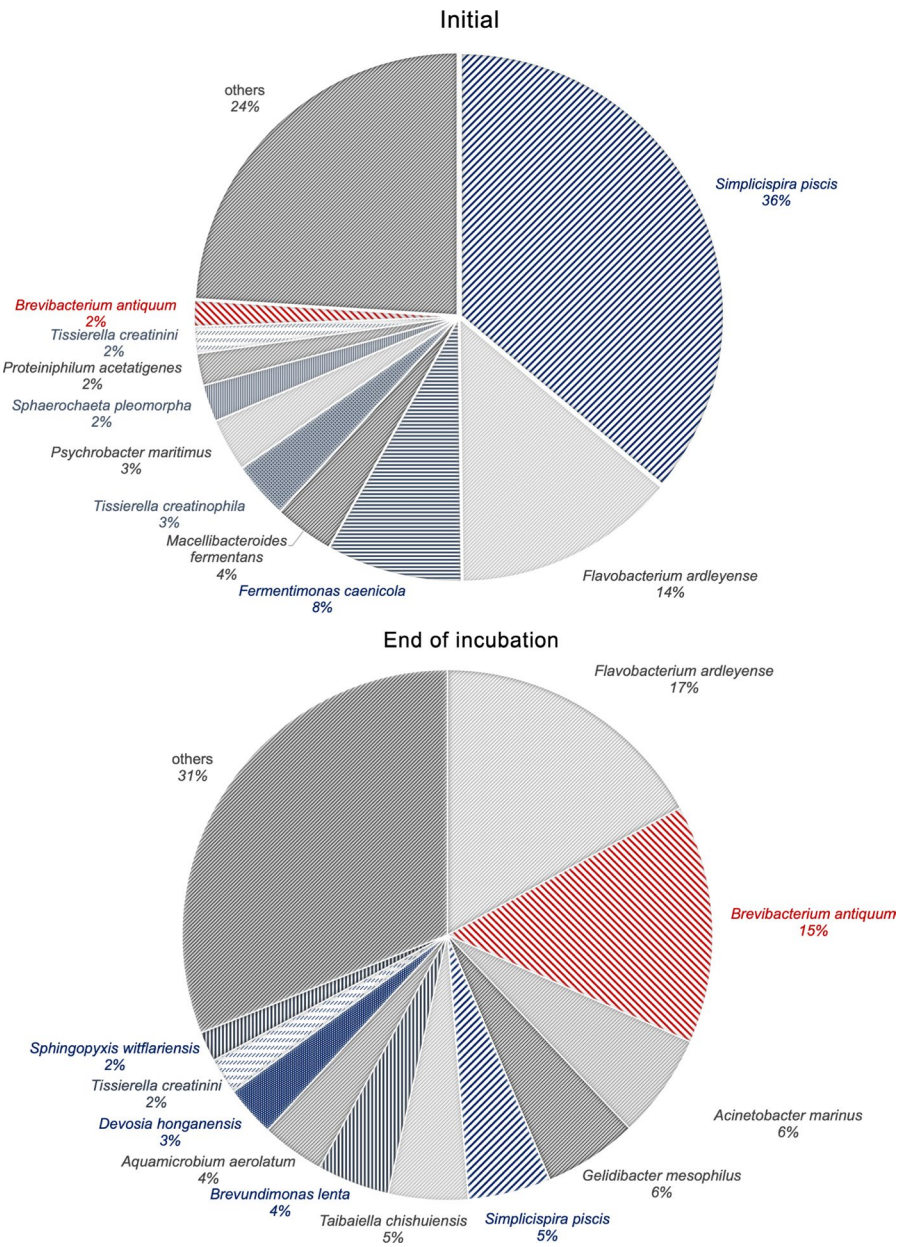

Fig. 4 Microbial community before and after ten-day incubation

growth optimizations were studied, and neutral pH levels were determined as optimum. In addition, both the growth of *Brevibacterium antiquum* and struvite formation were reduced by the presence of NaCl. Finally, application of *Brevibacterium antiquum* into the unsterilized fermented sludge centrate showed the biological struvite mineralization was achieved, while metagenomic analyses showed the ability of *Brevibacterium antiquum* to grow this mixed culture. Overall, the results demonstrated the potential of *Brevibacterium antiquum* for biostruvite recovery applications in wastewater streams, although further work is required to clarify and optimize the process.

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Data availability Data will be available on request: sevil.cn@gmail.com.

Declarations

Conflict of interest There is no conflict of interests to declare.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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