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Phosphorus release from sewage sludge and digestate driven by biological sulfate reduction: effect of feed sulfate concentration and thermal hydrolysis†

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Phosphorus recovery from waste streams stands out as a strategic practice to ensure phosphorus availability to future generations. The release of phosphate mediated by biological sulfate reduction is an interesting bioprocess for phosphorus recovery from sewage sludge in wastewater treatment plants in which chemical phosphorus recovery is foreseen. This study investigates the effect of biological sulfate reduction at different feed sulfate concentrations (up to 8000 mg L⁻¹) on the anaerobic phosphate release from both sewage sludge and digestate as well as the impact of sulfate addition on energy recovery from the sludge via biomethane production. During anaerobic digestion, up to 62.3% of the phosphate initially present in the sludge as iron(III) phosphate was released with 8000 mg L⁻¹ feed sulfate. However, biomethane production was significantly reduced (>40%) when sulfate was added at concentrations above 100 mg L⁻¹. The use of thermal hydrolysis on the sludge digestate was found to be an effective strategy for phosphorus recovery from the sludge without compromising the biomethane production during anaerobic digestion. A phosphate release from iron(III) phosphate of up to 48.7% was obtained when adding 4000 mg L⁻¹ sulfate to the digestate previously hydrolyzed for 2 hours. Finally, the implementation potential of the proposed strategy in full-scale wastewater treatment plants is discussed.

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Water impact

This study holds crucial implications for water sustainability by introducing a novel approach to phosphorus release from sewage sludge. Biological sulfate reduction offers a pathway to mitigate phosphorus scarcity, ensuring its future availability. The findings underscore the potential for environmentally conscious wastewater treatment, with an optimized strategy balancing phosphorus recovery and energy production, contributing to more sustainable water management systems.

1 Introduction

In the modern paradigm of circular economy, the recovery of waste materials has become of utmost importance to reduce the depletion of natural resources and environmental pollution. In this context, the wastewater treatment plant (WWTP) can be considered as a source of valuable materials and energy to be recovered rather than discarded along with the effluent.¹ Among the primary materials potentially

recoverable within a WWTP, phosphorus (P) has been addressed as a scarce resource due to the finite availability and uneven geopolitical distribution of phosphate rock reserves.² P is an essential element to secure food production to the ever-growing human population in the next future through the use of fertilizers in agriculture. The recovery and reuse of P from waste flows can help pursuing several sustainable development goals, including SDG2 (zero hunger), SDG6 (clean water and sanitation), SDG11 (sustainable cities and communities), and SDG13 (climate action). Moreover, improper P management and release in WWTPs can lead to serious environmental problems such as eutrophication. For this reason, P discharge in EU countries is regulated by European and national legislations, limiting the concentrations in treated water to 1–2 mg L⁻¹ total P. Therefore, modern WWTPs are designed to remove P through chemical or biological methods, the first being the most

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widespread in Europe because of its high effectiveness, easy implementation, and lower capital expenditure.^{3,4}

Chemical P removal in WWTPs is based on the addition of multivalent metal (*e.g.*, iron, calcium, magnesium, and aluminium) salts, which act as precipitators of phosphate ions (PO_4^{3-}) before or after the biological stage, resulting in P recovery typically ranging from 65% to 99%.⁵ As a result of chemical P precipitation, primary and/or secondary sludge is enriched in P and other metals and can be considered as a valuable pool of these elements. However, P separation from the complex sludge matrix is not an easy task and several chemical methods applicable to sewage sludge and/or sludge ashes have been proposed and applied in the past years, including wet leaching followed by struvite precipitation, magnetic vivianite separation, sludge melt gasification, white P recovery, and thermochemical sodium sulfate process.⁴ Alternatively, P could be retained in the sludge to be used as fertilizer after stabilization.⁶

Until now, biological methods for P recovery have been poorly explored. In this context, biological sulfate reduction (BSR) is a well-known bioprocess that may be exploited for P recovery from wastewater or sludge. BSR proceeds under anoxic conditions and consists in the reduction of sulfate ions (SO_4^{2-}) to sulfide species by microbes known as sulfate-reducing bacteria (SRB), which typically use organic carbon as energy source, which also defines the process stoichiometry.⁷ Sulfide can exist in water as hydrogen sulfide (H_2S), bisulfide (HS^-), or monosulfide (S^{2-}) depending on pH. Sulfide species have the capacity to form insoluble compounds with metals, which can be easily precipitated.^{8,9} In general, metal sulfides exhibit lower solubility than their phosphate and hydroxide counterparts, leading to a more efficient precipitation and higher stability over a wider pH range.¹⁰ For instance, ferric sulfide (Fe_2S_3 , $\text{pK}_{\text{sp}} = 85.0$) is much less soluble than ferric phosphate ($\text{Fe}_3(\text{PO}_4)_2$, $\text{pK}_{\text{sp}} = 36.0$). Previous experiences showed that sulfide addition was capable of mobilizing phosphate ions from ferric precipitates in sediment systems,¹¹ chemical sludge from a drinking water plant,¹² sewage sludge,¹³ and sludge digestate.¹⁴ Therefore, sulfide seems to act as an efficient PO_4^{3-} extractor by reducing Fe(III) phosphates and forming highly insoluble Fe(II) sulfide precipitates.

Such studies have mainly focused on direct sulfide addition to the P-rich sludge matrix,^{12–14} while PO_4^{3-} release from sludge *via* BSR co-occurring with anaerobic digestion (AD) remains poorly explored.¹⁵ Coupling BSR and AD in the same unit may simplify the sludge streamline of a WWTP where BSR-mediated P recovery is implemented, also considering that PO_4^{3-} release from microbial biomass and organic matter (OM) is commonly observed in WWTP digesters.^{16,17} On the other hand, increasing sulfide levels in the anaerobic digester may cause the inhibition of fermentative and methanogenic microorganisms, thereby negatively affecting AD.¹⁸ In this case, recovering P from the sewage digestate might be a suitable solution, although a dedicated BSR-mediated P recovery stage involving digestate

hydrolysis to release additional organic carbon should be foreseen. For a correct implementation of the BSR-mediated P recovery, it is therefore fundamental to investigate different scenarios, *i.e.*, concomitant AD–BSR and post-digestion BSR, and identify the potential advantages and limitations of each process configuration.

In view of the above, the present study primarily investigates BSR-mediated P release from chemically pretreated waste activated sludge and thermally hydrolysed digestate in laboratory-scale digesters. At the same time, the effect of the feed SO_4^{2-} concentration on the BSR and AD processes was also evaluated. To this end, experiments were run at different feed SO_4^{2-} concentrations to investigate how these would affect the PO_4^{3-} release and biomethane production from the sludge during concomitant AD–BSR as well as the PO_4^{3-} release during post-digestion BSR operated on untreated or thermally hydrolysed sludge digestate. Finally, based on the obtained results, insights for future process implementation and upscaling are provided.

2 Materials and methods

2.1 Source and preparation of sewage sludge

The sewage sludge used for inoculum and experimental tests was collected from a municipal WWTP located in Nola (Campania region, Italy), characterized by a conventional pre-denitrification (Ludzack–Ettinger) system as biological phase. The sludge was collected from the pre-thickening stage, placed ahead of AD, at two different time points immediately before starting the experiments. The composition of the sludge substrates used during the experimental activity was quite homogeneous and characterized by the following values: pH 7.3 ± 0.1 , total solids (TS) $33 \pm 7 \text{ g L}^{-1}$, volatile solids (VS) $22 \pm 2 \text{ g L}^{-1}$, total COD (tCOD) $29953 \pm 77 \text{ mg L}^{-1}$, soluble COD (sCOD) $742 \pm 235 \text{ mg L}^{-1}$, sulfate (SO_4^{2-}) $37 \pm 23 \text{ mg L}^{-1}$, and phosphate (PO_4^{3-}) $214 \pm 107 \text{ mg L}^{-1}$. The sludge was spiked with an extra 1 g L^{-1} P, supplemented as iron phosphate ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$, Sigma-Aldrich, USA), to simulate the occurrence of a chemical P precipitation in the wastewater streamline generating the sludge. Such a P concentration was chosen considering a PO_4^{3-} concentration in the wastewater entering the WWTP of $10 \text{ mg P per L}^{19}$ and considering that the sludge flow rate is approximately 1% of the influent wastewater flow.¹⁷ In the hypothesis that all influent PO_4^{3-} precipitates with the added iron during chemical precipitation, the P concentration in the sludge would be 100 times higher (*i.e.*, 1 g L^{-1}) than in the influent wastewater.

2.2 Experimental design

All experiments were performed under batch conditions at laboratory scale in 500 mL SIMAX® borosilicate glass bottles (VWR, USA). Each bottle was filled with 300 mL of pre-thickened sludge and SO_4^{2-} was dosed at different concentrations (as reported in the following sections) using sodium sulfate (Na_2SO_4 , ITW Reagents, Italy). All bottles were sealed with caps allowing both gas and liquid sampling and



placed in a thermostatic bath at 35 °C, as described by Morello *et al.*²⁰ Before sealing, the bottle content was flushed with argon for 1 min to ensure anaerobic conditions. All experiments were performed in triplicate. Values reported without a standard deviation are average values. A comprehensive overview of all experiments performed is provided in Table 1. A maximum duration of 21 days was selected for the experiments, as the typical hydraulic retention time for AD of sewage sludge in municipal WWTPs is between 16 and 25 days.²¹

2.2.1 Phosphate release during anaerobic digestion.

Anaerobic tests were performed with FePO₄-spiked sewage sludge at different concentrations of feed SO₄²⁻: 0, 2000, 4000, and 8000 mg L⁻¹ (experiment 1). Monitoring of PO₄³⁻ release and SO₄²⁻ reduction was stopped after 15 days as no further variation was observed. A preliminary test (test 1) was performed to assess the impact of the feed SO₄²⁻ concentration on biomethane production. For this test, SO₄²⁻ was added to the bottles at concentrations of 0, 100, 500, 2000, and 4000 mg L⁻¹ and the CH₄ production was monitored for 21 days. The test allowed better evaluation of the temporal distribution of PO₄³⁻ release and SO₄²⁻ reduction and, thus, fine-tuning the design of experiment 1.

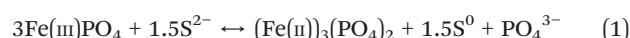
2.2.2 Phosphate release from sewage sludge digestate. To obtain enough digestate to run the experiments, 5 L of sewage sludge were digested in 2 L glass bottles (similar to those described in section 2.2) for 30 days. The obtained digestate had the following composition: pH 7.7 ± 0.3, TS 29 ± 2 g L⁻¹, VS 15 ± 2 g L⁻¹, tCOD 20 658 ± 825 mg L⁻¹, sCOD 197 ± 14 mg L⁻¹, SO₄²⁻ 95 ± 11 mg L⁻¹, and PO₄³⁻ 449 ± 21 mg L⁻¹.

First, anaerobic tests were performed with a similar setup to the AD tests with sewage sludge (except that digestate was used instead of sludge) at feed SO₄²⁻ concentrations of 0, 2000, 4000, and 6000 mg L⁻¹ (experiment 2). In a following experiment (experiment 3), the digestate was first subjected to thermal hydrolysis (TH) with the aim to enhance COD bioavailability during the BSR stage. Anaerobic bottles were placed in an oven at a temperature of 90 °C for a limited duration. Afterwards, the experiment was run at 35 °C with the same feed SO₄²⁻ concentrations as in experiment 2. A preliminary test (test 2) was performed with digestate to assess the best duration of the hydrolysis treatment. 500 mL glass bottles were filled with 40 mL of

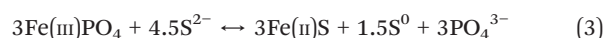
digestate, then closed without tightening the caps, and kept in the oven for different durations, *i.e.*, 0, 1, 2, and 4 h. At the end of the treatment, both the tCOD and sCOD were measured to evaluate the effectiveness of hydrolysis on the release of OM from the digestate. Once the optimal duration was identified based on the amount of solubilized COD and process costs, the digestate was added to the test bottles, hydrolyzed, and supplemented with different SO₄²⁻ concentrations (as for experiment 2) to evaluate PO₄³⁻ release.

2.3 Calculations

The mass ratio of P-PO₄³⁻ released to S-SO₄²⁻ reduced (P/S) for each bottle during the experiments was calculated based on the linear trends of the PO₄³⁻ release and SO₄²⁻ reduction curves and was indicated as P/S_{linear}. The stoichiometric P/S ratio (P/S_{st}) was calculated hypothesizing that (1) all reduced SO₄²⁻ was converted to S²⁻, and (2) all generated S²⁻ reacted with FePO₄, resulting in Fe³⁺ reduction to Fe²⁺ and release of PO₄³⁻ (eqn (1)) followed by Fe²⁺ sequestration as FeS (eqn (2)):¹⁴



Overall, PO₄³⁻ release occurring from iron interactions with sulfide can be expressed as the sum of eqn (1) and (2):



The ratio of COD consumed to SO₄²⁻ reduced (COD/SO₄²⁻) was calculated based on the linear trends of SO₄²⁻ reduction curves based on the available data (days 8–13).

2.4 Analytical methods

TS, VS, and tCOD were measured on unfiltered sludge and digestate samples following the standard methods.²² sCOD and ionic concentrations were measured on the liquid fraction of the samples obtained after filtration through Minisart 0.45 µm syringe filters (Sartorius, Germany). sCOD in filtered liquid samples was measured as tCOD. Ionic concentrations, *i.e.*, PO₄³⁻, SO₄²⁻, and thiosulfate (S₂O₃²⁻), were measured by ion chromatography as described by Di

Table 1 List of the experiments (E) and preliminary tests (T) performed on sewage sludge and digestate

Experiment	Objective	Substrate	Feed SO ₄ ²⁻ (mg L ⁻¹)	Added PO ₄ ³⁻ (mg L ⁻¹)	Duration	Monitored parameters
T1	Effect of initial SO ₄ ²⁻ concentration on CH ₄ production	Sludge	0, 100, 500, 2000, 4000	1000	21 d	CH ₄ , SO ₄ ²⁻ , PO ₄ ³⁻
E1	PO ₄ ³⁻ release during AD coupled to BSR	Sludge	0, 2000, 4000, 8000	1000	15 d	SO ₄ ²⁻ , PO ₄ ³⁻
E2	PO ₄ ³⁻ release during BSR of digestate	Digestate	0, 2000, 4000, 6000	1000	21 d	SO ₄ ²⁻ , PO ₄ ³⁻
T2	sCOD release from digestate after thermal hydrolysis at 90 °C	Digestate	0	0	0, 1, 2, 4 h	sCOD, tCOD
E3	PO ₄ ³⁻ release during BSR of hydrolyzed digestate	Digestate	0, 2000, 4000, 6000	1000	21 d	SO ₄ ²⁻ , PO ₄ ³⁻ , sCOD, tCOD



Capua *et al.*²³ The pH was measured in unfiltered samples using an InoLab level 2 pH meter (WTW, Germany) with a Polyplast pH electrode (Hamilton, USA). A TCN 115 laboratory oven (Argo Lab, Italy) used for TS and VS analysis was also used for the TH of digestate.

2.5 Statistical analysis

The statistical evaluation of the data was performed based on one-way analysis of variance (ANOVA) with the data analysis tool of Excel 365 (Microsoft, USA) with the aim to compare the PO_4^{3-} concentrations released from the different anaerobic bottles. The significant difference was considered at 95% ($p \leq 0.05$).

3 Results and discussion

3.1 Phosphate release during anaerobic digestion of sewage sludge

The first experiment confirmed that PO_4^{3-} was effectively released from sewage sludge containing FePO_4 during AD (Fig. 1). Test 1 revealed that PO_4^{3-} release and SO_4^{2-} reduction at feed SO_4^{2-} concentrations up to 4000 mg L^{-1} mainly occurred

within the first 7 days and that no considerable difference ($p > 0.05$) with the reference condition (*i.e.*, 0 mg L^{-1} feed sulfate) in terms of PO_4^{3-} released was observed up to 500 mg L^{-1} feed SO_4^{2-} (Fig. S1†). Therefore, during experiment 1 the bottles were monitored for 15 days with a more frequent sampling and a higher range of feed SO_4^{2-} concentrations (2000–8000 mg L^{-1}) was investigated (Table 1). The experiment confirmed that PO_4^{3-} was mostly released within the first 7–10 days at all tested feed SO_4^{2-} concentrations. After 15 days, $1911 \pm 199 \text{ mg L}^{-1}$ PO_4^{3-} , corresponding to 62.3% of the PO_4^{3-} initially present as FePO_4 in the sludge (*i.e.*, 1 g P per L), was released at the highest feed SO_4^{2-} concentration of 8000 mg L^{-1} . As expected, less PO_4^{3-} was released at lower initial SO_4^{2-} concentrations. Specifically, 1590 ± 29 and $821 \pm 15 \text{ mg L}^{-1}$ PO_4^{3-} were released at 4000 and 2000 mg L^{-1} feed SO_4^{2-} , respectively. Only $452 \pm 52 \text{ mg L}^{-1}$ PO_4^{3-} was released from the sludge with no feed SO_4^{2-} . High PO_4^{3-} release from sewage sludge during AD can be observed even in WWTPs where no P removal/recovery strategy is implemented. For instance, Ferraro *et al.*¹⁷ observed a PO_4^{3-} concentration as high as 436 mg P per L in the digestate centrate produced within a conventional WWTP based on a modified Ludzack–Ettinger scheme. However, it is evident that the SO_4^{2-} addition promoted PO_4^{3-} release from the FePO_4 used to mimic chemical P precipitation in the mainstream treatment.

The link between PO_4^{3-} release and SO_4^{2-} reduction is evidenced by the trend of SO_4^{2-} concentration during the experiment. Under all conditions, SO_4^{2-} was consumed within the first 7–10 days, matching with the trends of PO_4^{3-} release. No SO_4^{2-} was present after 10 days except at 8000 mg L^{-1} feed SO_4^{2-} . However, SO_4^{2-} reduction stopped at day 10 even under this condition, likely indicating that the primary electron donor (*i.e.*, rapidly biodegradable OM) fuelling SO_4^{2-} reduction was consumed. The SO_4^{2-} reduction rate was similar at 4000–8000 mg L^{-1} feed SO_4^{2-} (683–718 $\text{mg L}^{-1} \text{ d}^{-1}$), while it was about half at 2000 mg L^{-1} (Table 2). Despite the increase of SO_4^{2-} reduction rate at higher initial SO_4^{2-} levels, the g of released PO_4^{3-} per g of reduced SO_4^{2-} did not increase accordingly but remained quite stable. The $\text{P/S}_{\text{linear}}$ ratio calculated under the different operating conditions was in the range of 0.27–0.32 (Table 2). The P/S_{st} ratio calculated according to eqn (3) was 0.64, *i.e.*, about twice the $\text{P/S}_{\text{linear}}$ ratio.

It should be noted that the calculation for P/S_{st} was based on a number of hypotheses, including the complete reaction of the generated S^{2-} with iron phosphate to free PO_4^{3-} in the liquid phase. However, the share of S^{2-} that effectively reacts with iron is not 100%, as other sulfide precipitates can be formed, *e.g.*, by sulfide reaction with other metals.²⁴ This can explain the lower P/S ratios observed in comparison to the one calculated stoichiometrically.

Although the maximum $\text{P/S}_{\text{linear}}$ value was obtained at the highest feed SO_4^{2-} concentration of 8000 mg L^{-1} , this would result in a too high residual SO_4^{2-} level in the sludge

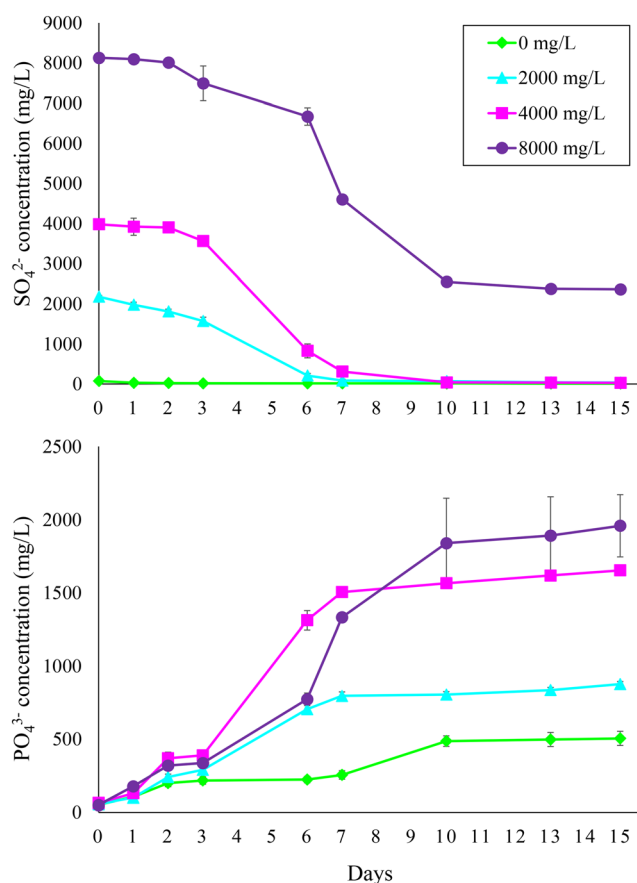


Fig. 1 Sulfate reduction and phosphate release during anaerobic digestion of sewage sludge at different feed sulfate concentrations (*i.e.*, 0, 2000, 4000, and 8000 mg L^{-1}).



Table 2 Ratios of P-PO_4^{3-} released to S-SO_4^{2-} reduced ($\text{P/S}_{\text{linear}}$) based on the linear trends of the PO_4^{3-} release and SO_4^{2-} reduction curves obtained with sewage sludge (E1) and hydrolyzed digestate (E3)

Experiment	Feed SO_4^{2-} concentration (mg L^{-1})	Linear trend period (d)	$\text{P/S}_{\text{linear}}$	SO_4^{2-} reduction rates ($\text{mg L}^{-1} \text{d}^{-1}$)
E1	2000	2–7	0.32 ± 0.04	345 ± 16
	4000	2–7	0.31 ± 0.01	718 ± 11
	8000	2–10	0.27 ± 0.06	683 ± 5
E3	2000	8–21	0.38 ± 0.01	146 ± 2
	4000	8–21	0.32 ± 0.05	216 ± 56
	6000	8–21	0.33 ± 0.04	266 ± 29

at the end of AD due to the lack of available OM to complete the BSR process (Fig. 1). Moreover, as the supplementation of SO_4^{2-} represents a cost for the proposed P recovery strategy, optimizing the SO_4^{2-} dosage is necessary to make the process profitable. Besides, the main issue with coupling AD to BSR for P recovery is related to the impact of the generated sulfide on methane production, which is outlined in the following section.

3.2 Effect of the feed sulfate concentration on methane production from sewage sludge

Fig. 2 illustrates the specific methane production obtained during test 1 at the different feed SO_4^{2-} concentrations. The baseline methane production represented by the reactor with no feed SO_4^{2-} amounted to $160 \pm 12 \text{ NL CH}_4$ per kg VS_{in} . The methane production decreased at increasing feed SO_4^{2-} concentrations, reaching 145 ± 8 , 94 ± 12 , 64 ± 6 , and $26 \pm 15 \text{ NL CH}_4$ per kg VS_{in} at 100, 500, 2000, and 4000 mg L^{-1} feed SO_4^{2-} , respectively. These results indicate that CH_4 production was decreased by 9%, 41%, 60%, and 84% at 100, 500, 2000, and 4000 mg L^{-1} feed SO_4^{2-} (Fig. 2). Therefore, due to inhibition of methanogenic activity, coupling BSR to AD imposes a trade-off between PO_4^{3-} release and CH_4 production from sewage sludge. A smarter approach might be to perform BSR on the sludge digestate in order to

separate the CH_4 production from the P recovery stage, as discussed in the next section.

3.3 Phosphate release from sludge digestate via biological sulfate reduction

Fig. 3 illustrates the PO_4^{3-} release and SO_4^{2-} reduction trends obtained with sludge digestate (experiment 2). SO_4^{2-} concentrations only slightly decreased compared to the initial levels under all operating conditions, with the highest SO_4^{2-} reduction of 40% achieved at 2000 mg L^{-1} feed sulfate. The lower SO_4^{2-} consumption compared to that observed with undigested sludge led to little PO_4^{3-} release. Indeed, the PO_4^{3-} released from the digestate after 21 days was very similar ($p > 0.05$) under all tested feed SO_4^{2-} concentrations

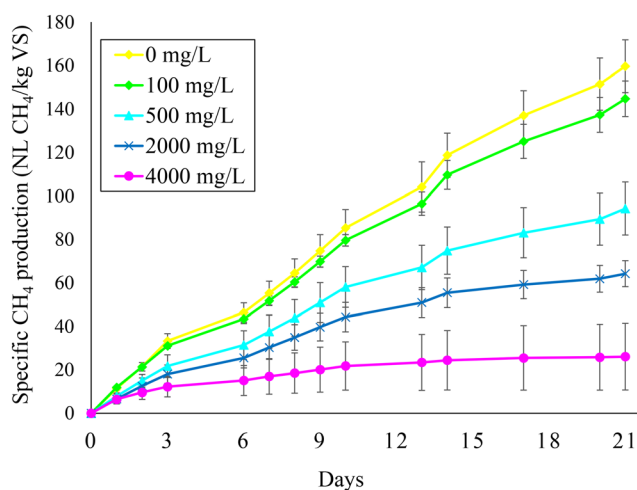
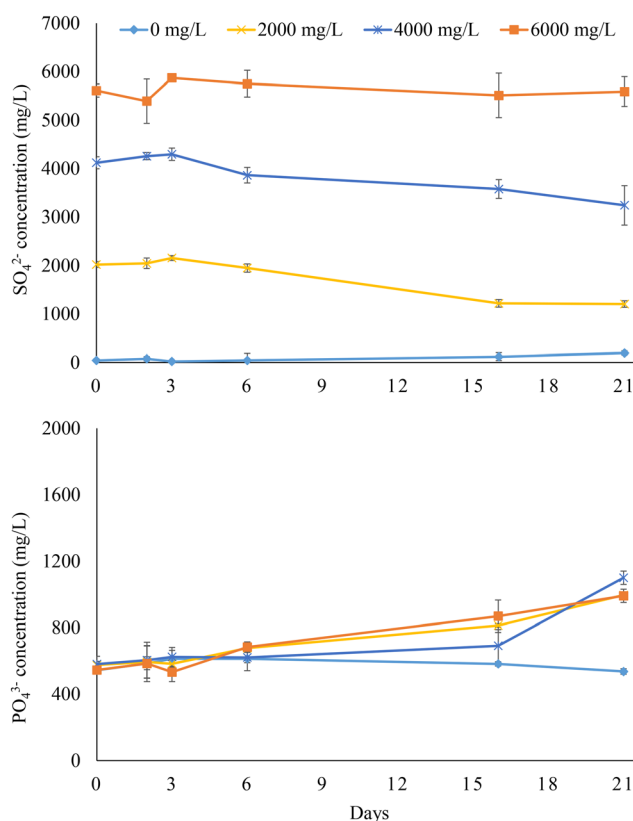
**Fig. 2** Methane production during anaerobic digestion at different feed sulfate concentrations (i.e., 0, 100, 500, 2000, and 4000 mg L^{-1}).**Fig. 3** Sulfate reduction and phosphate release in the anaerobic bottles fed with sludge digestate at different feed sulfate concentrations (i.e., 0, 2000, 4000, and 6000 mg L^{-1}).

Table 3 Soluble and total chemical oxygen demand (sCOD and tCOD, respectively) as well as pH characteristics of sewage sludge, sludge digestate, and hydrolyzed sludge digestate prior to performing the biological sulfate reduction experiments (*i.e.*, experiments 2 and 3)

	Sewage sludge	Sludge digestate	Hydrolyzed digestate
tCOD (mg L ⁻¹)	30 030 ± 350	20 658 ± 825	21 013 ± 1563
sCOD (mg L ⁻¹)	977 ± 11	197 ± 14	2077 ± 360
pH	7.3 ± 0.1	7.7 ± 0.3	7.9 ± 0.1

(2000–6000 mg L⁻¹), as it ranged from 420 to 519 mg L⁻¹. This means that a maximum of 16.9% of the initial PO₄³⁻ linked to Fe(III) was released in the aqueous phase. No significant variation of the PO₄³⁻ concentration (579 ± 30 mg L⁻¹) was observed in the experiment with no added SO₄²⁻, confirming that BSR was the main driver for PO₄³⁻ release.

The digestate was characterized by a tCOD of 20 658 ± 825 mg L⁻¹ and an sCOD of 197 ± 14 mg L⁻¹, being respectively 31% and 80% lower than those of the untreated sludge (Table 3). AD proceeds through the conversion of biodegradable OM into biogas, leaving the least biodegradable organic fraction as a residue. The lower sCOD, which was likely linked to the lower content of the more rapidly biodegradable OM fraction, compared to the untreated sludge explains the limited SO₄²⁻ reduction in the bottles fed with sludge digestate, indicating that biodegradable OM is the primary energy source fuelling the BSR process. For successful P recovery *via* BSR from sludge digestate, strategies enhancing COD solubilization and bioavailability are thus necessary, as illustrated in the following section.

3.4 Impact of thermal hydrolysis on phosphate release from digestate *via* biological sulfate reduction

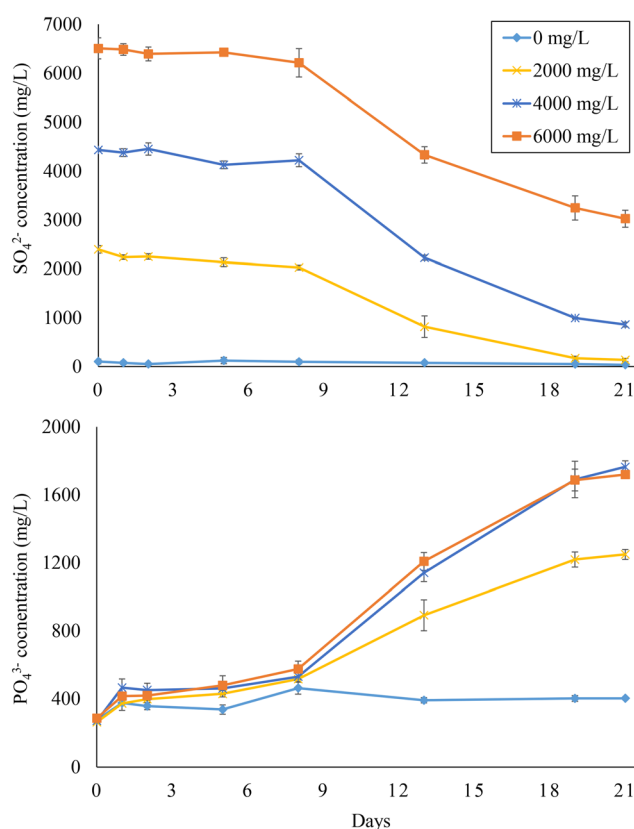
TH can represent a solution to increase the amount of sCOD in the sludge digestate and enhance PO₄³⁻ release through BSR. Table S1† shows how sCOD increased with time when applying a 90 °C TH to the sludge digestate (test 2). After 1 h of hydrolysis, sCOD increased by 6.4 times, reaching 1829 ± 152 mg L⁻¹, with the ratio of sCOD to tCOD (sCOD/tCOD) increasing from 1.4% to 9.0%. Prolonging the hydrolysis time further promoted COD solubilization, although sCOD values observed at 2 and 4 h were quite similar. Indeed, sCOD was 4143 ± 40 mg L⁻¹ at 2 h and 4910 ± 57 mg L⁻¹ at 4 h, resulting in sCOD/tCOD ratios of 20.4% and 24.2%, respectively.

Although slightly better results were obtained after 4 h of hydrolysis in terms of sCOD increase, a duration of 2 h was selected to hydrolyze the sludge digestate in experiment 3 based on lower energy costs and shorter treatment times. Therefore, prior to experiment 3 the reactors used for the tests with different SO₄²⁻ concentrations were hydrolyzed for 2 h, resulting in a sCOD concentration of 2077 ± 360 mg L⁻¹. The COD concentrations of untreated, digested, and hydrolyzed sludge are listed in Table 3. The sCOD concentration obtained after 2 h of hydrolysis was lower than that observed in test 2 due to the higher amount of sludge in

the bottles (300 mL) compared to that of the preliminary test (40 mL).

The SO₄²⁻ reduction and PO₄³⁻ release curves of experiment 3 are depicted in Fig. 4. Within the 21 days of the experiment, SO₄²⁻ was completely reduced when a feed SO₄²⁻ concentration of 2000 mg L⁻¹ was used, while around 3500 mg L⁻¹ SO₄²⁻ were consumed at 4000 and 6000 mg L⁻¹ feed sulfate. At these SO₄²⁻ concentrations, PO₄³⁻ concentration reached about 1800 mg L⁻¹ at the end of the experiment, yielding between 1400 and 1500 mg L⁻¹ PO₄³⁻ released. This indicates that up to 48.7% of the PO₄³⁻ initially associated with Fe(III) could be released.

P/S_{linear} values (days 8–21) were in the range of 0.32–0.38, close to the ratios observed in experiment 1, while SO₄²⁻ reduction rates ranged from 146 ± 2 to 266 ± 29 mg L⁻¹ d⁻¹ (Table 2). Both SO₄²⁻ reduction and PO₄³⁻ release started after 8 days (lag phase) from the start of the experiment (Fig. 4), which contrasts with the trends observed with

**Fig. 4** Sulfate reduction and phosphate release in bottles fed with hydrolyzed (2 h) sludge digestate at different feed sulfate concentrations (*i.e.*, 0, 2000, 4000, and 6000 mg L⁻¹).

sewage sludge where no lag phase was observed (Fig. 1). Also, the SO_4^{2-} reduction rates were lower than those observed during experiment 1 (Table 2). Most probably, SRB were heat-shocked during the thermal treatment, resulting in the initial lag phase and slower SO_4^{2-} reduction. However, the thermal treatment worked as an efficient strategy for PO_4^{3-} release based on the P/S_{linear} values and final yields. At the end of the BSR tests, no tCOD consumption was observed at 0 mg L^{-1} feed SO_4^{2-} , while 40–53% of the initial tCOD was consumed at feed SO_4^{2-} concentrations of 2000–4000 mg L^{-1} (Table S2†). The $\text{COD}/\text{SO}_4^{2-}$ ratios decreased from 2.4 ± 0.0 to 1.2 ± 0.4 when increasing the feed SO_4^{2-} concentration from 2000 to 6000 mg L^{-1} . This trend suggests that COD consumption was more effectively driven towards BSR at high SO_4^{2-} concentrations when concomitant OM-consuming bioprocesses such as AD are inhibited.

3.5 Practical considerations

This study demonstrates that BSR can be an effective strategy to promote P recovery from sewage sludge in WWTPs performing chemical P precipitation. Based on the obtained results, the most suitable configuration of the sludge streamline would foresee the typical pre-thickening and AD stages followed by TH (e.g., at 90 °C for 2 h) and SO_4^{2-} addition (e.g., 4000 mg L^{-1}) prior to a second anaerobic stage for BSR (Fig. 5). In such a scheme, the formation of iron(II) sulfide and PO_4^{3-} release would occur in the completely mixed BSR reactor, while the precipitate separation from the P-rich liquid phase would occur in the subsequent dewatering stage. The added SO_4^{2-} could originate from chemicals such as Na_2SO_4 , being widely produced worldwide also as a by-product of

hydrochloric acid production and other chemical processes. As an alternative, SO_4^{2-} might be generated on site through biogas desulfurization in anoxic biofilters fed with nitrified wastewater^{25,26} or in aerobic bioreactors fed with the sulfide-rich solution produced by chemical scrubbing. Both strategies would provide an inexpensive source for SO_4^{2-} supply. When selecting the initial SO_4^{2-} concentration, it is important to consider and limit the potential increase of free sulfide levels in the digestate. Free sulfide may cause corrosion to metallic installations of the plant.²⁷ To prevent this, residual SO_4^{2-} levels should be minimized and the sulfide-sink capacity of the digestate evaluated. pH plays an important role in the emission of gaseous H_2S .²⁸ Maintaining a slightly alkaline pH during BSR would prevent H_2S emission while sustaining the biological process.

Dewatering can be performed afterwards to separate the solid fraction from the phosphate-rich supernatant to be used for P recovery. This last step could be performed by adopting one of the existing technologies, including crystallization and electrochemical precipitation systems, which mainly target the generation of P-rich precipitates (e.g., struvite and hydroxyapatite) to be used for fertilizing purposes and selected based on current market requirements.³ As an alternative to crystallization, the PO_4^{3-} content of the liquid fraction of the digestate could be directly applied to soil in the context of treated water reuse in agriculture, which is being advocated also by the recent EU regulations (Regulation 2020/741). However, it should be noted that P speciation in liquid and solid phase influences its uptake by plants, as PO_4^{3-} is more readily available than struvite-P, the latter being slowly released to the soil. The use of crystal P can be advantageous to avoid overfeeding

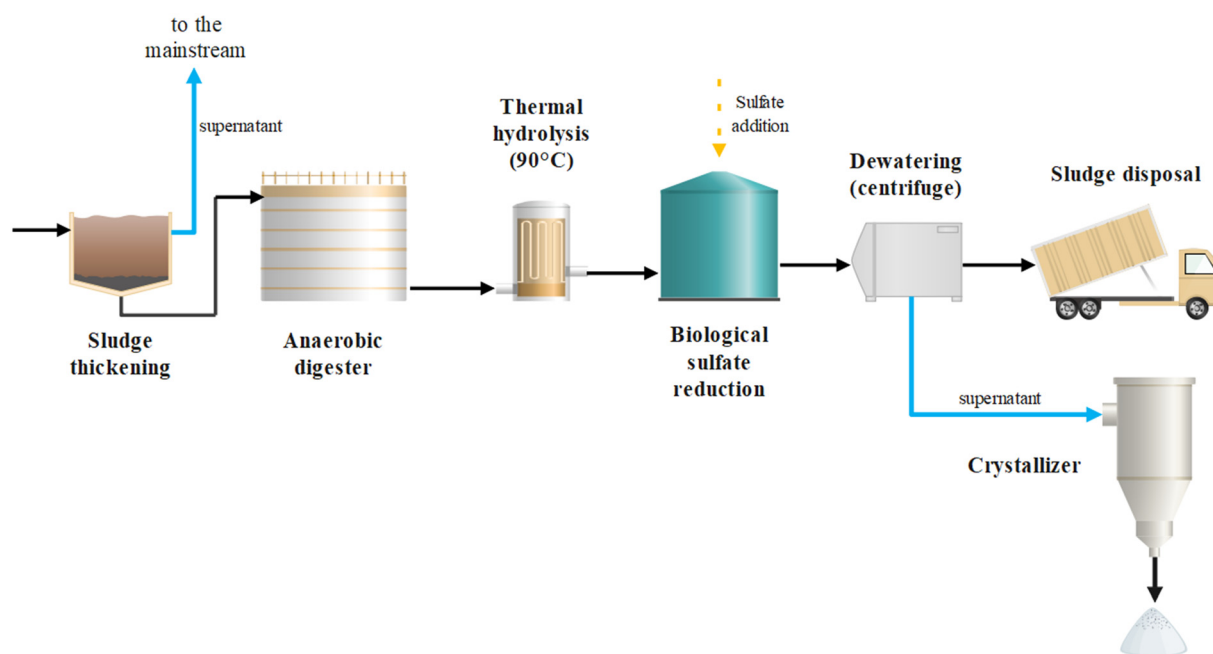


Fig. 5 Layout of a WWTP sludge streamline integrating anaerobic digestion, thermal hydrolysis, and biological sulfate reduction combining energy and P recovery.



phenomena and leaching of nutrients into the aquifers. Moreover, solid fertilizers are easier to handle and transport, which is an important factor to enhance their utilization considering the still limited acceptance of farmers towards the use of waste-derived fertilizers.

The techno-economic convenience of such a proposed P recovery strategy should be assessed through pilot- and full-scale trials, as especially the hydrolysis step seems to be affected by the applied scale and may significantly impact the overall costs of the process. Taboada *et al.*²⁹ report an investment cost for the TH unit ranging from €1 to 2 million for WWTPs with size from 0.1 to 1 million population equivalent (PE). Considering an electricity cost of €0.12 per kW h and the reported electricity demand of 10 kW h m⁻³,²⁹ the operational costs for the TH unit would be €1.2 per m³ of digestate (~€0.01 per m³ of influent wastewater). This cost would be lower for the TH process considered in this work as it is performed at ambient pressure, and the required temperature of 90 °C is much lower than that typically applied for sludge solubilization prior to AD (>150 °C). The cost of Na₂SO₄ addition would be around €0.4 per m³ of digestate by considering a market price of €109 per ton and an initial SO₄²⁻ concentration of 4000 mg L⁻¹. The total cost for patented P crystallization technologies ranges between €6 and 10 per kg of P recovered,³⁰ while the application of chemical and biological scrubbers for SO₄²⁻ generation would cost €0.05–3.3 per kg S (corresponding to €0.7–4.3 per m³ of digestate at 4000 mg L⁻¹ initial SO₄²⁻).³¹ The costs for implementing a P recovery strategy at a WWTP can be counteracted by savings, *e.g.*, due to reduced P back-flow with the digester supernatant resulting in a lower demand of iron precipitants in the mainstream treatment, and revenues from sale of the produced P-rich products.

Implementing a TH stage after the AD process can also provide further solid reduction and enhance dewatering.³² Therefore, this approach will help to minimize the volumes of sewage sludge exiting the municipal WWTPs, which is highly topical considering the increasing amount of sewage sludge produced by wastewater treatment.^{33–35}

4 Conclusions

P recovery from sewage sludge *via* BSR showed promising results when applied to sludge generated in WWTPs where chemical P precipitation is implemented. The obtained results indicate P recovery from sludge digestate as the most promising process configuration, as it enables keeping AD and BSR separate and avoids affecting the biomethane potential of the sewage sludge due to sulfide inhibition. However, since most of the biodegradable OM is consumed during AD, a 2 h TH at 90 °C was shown to increase the sCOD of the sludge digestate from 197 to 2077 mg L⁻¹ and to ensure almost 50% of PO₄³⁻ release from FePO₄ when 4000 mg L⁻¹ SO₄²⁻ was fed prior to BSR. Based on these results, pilot- and full-scale trials are recommended for a more appropriate cost evaluation taking into account the costs for the

implementation of the hydrolysis and BSR units, the savings in terms of reduced sludge disposal and improved energy recovery, and the revenues from the commercialization of solid P-rich fertilizer products.

Data availability

Data for this article, including tables and figures, are available in the open access repository at the link <https://www.iris.unina.it> or at the section “publications” of the webpage of the corresponding author: <https://www.docenti.unina.it/stefano.papirio>.

Conflicts of interest

There are no conflicts to declare.

References

- 1 N. Di Costanzo, A. Cesaro, F. Di Capua and G. Esposito, Exploiting the Nutrient Potential of Anaerobically Digested Sewage Sludge: A Review, *Energies*, 2021, **14**, 8149.
- 2 R. W. Scholz, A. E. Ulrich, M. Eilittä and A. Roy, Sustainable use of phosphorus: A finite resource, *Sci. Total Environ.*, 2013, **461–462**, 799–803.
- 3 F. Di Capua, S. de Sario, A. Ferraro, A. Petrella, M. Race, F. Pirozzi, U. Fratino and D. Spasiano, Phosphorous removal and recovery from urban wastewater, *Sci. Total Environ.*, 2022, **823**, 153750.
- 4 J. Uzkurt Kaljunen, R. A. Al-Juboori, W. Khunjar, A. Mikola and G. Wells, Phosphorus recovery alternatives for sludge from chemical phosphorus removal processes – Technology comparison and system limitations, *Sustainable Mater. Technol.*, 2022, **34**, e00514.
- 5 A. Magri, M. Carreras-Sempere, C. Biel and J. Colprim, Recovery of Phosphorus from Waste Water Profiting from Biological Nitrogen Treatment: Upstream, Concomitant or Downstream Precipitation Alternatives, *Agronomy*, 2020, **10**, 1039.
- 6 F. Carraturo, A. Siciliano, A. Giordano, F. Di Capua, F. Barone, E. Casaletta, F. Cicotti, M. Guida and F. Adani, Ecotoxicological assessment of waste-derived organic fertilizers and long-term monitoring of fertilized soils using a multi-matrix and multi-species approach, *Sci. Total Environ.*, 2024, **912**, 169341.
- 7 S. Papirio, G. Esposito and F. Pirozzi, Biological inverse fluidized-bed reactors for the treatment of low pH- and sulphate-containing wastewaters under different COD/SO₄²⁻ conditions, *Environ. Technol.*, 2013, **34**, 1141–1149.
- 8 G. Esposito, A. Veeken, J. Weijma and P. N. L. Lens, Use of biogenic sulfide for ZnS precipitation, *Sep. Purif. Technol.*, 2006, **51**, 31–39.
- 9 D. K. Villa-Gomez, S. Papirio, E. D. van Hullebusch, F. Farges, S. Nikitenko, H. Kramer and P. N. L. Lens, Influence of sulfide concentration and macronutrients on the characteristics of metal precipitates relevant to metal recovery in bioreactors, *Bioresour. Technol.*, 2012, **110**, 26–34.



- 10 J. F. Blais, Z. Djedidi, R. B. Cheikh, R. D. Tyagi and G. Mercier, Metals Precipitation from Effluents: Review, *Pract. Period. Hazard., Toxic, Radioact. Waste Manage.*, 2008, **12**, 135–149.
- 11 E. E. Roden and J. W. Edmonds, Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe (III) oxide reduction versus iron-sulfide formation, *Arch. Hydrobiol.*, 1997, **139**, 347–378.
- 12 E. Mejia Likosova, J. Keller, R. A. Rozendal, Y. Poussade and S. Freguia, Understanding colloidal FeSx formation from iron phosphate precipitation sludge for optimal phosphorus recovery, *J. Colloid Interface Sci.*, 2013, **403**, 16–21.
- 13 F. Kato, H. Kitakoji, K. Oshita, M. Takaoka, N. Takeda and T. Matsumoto, Extraction efficiency of phosphate from pre-coagulated sludge with NaHS, *Water Sci. Technol.*, 2006, **54**, 119–129.
- 14 P. Wilfert, J. Meerdink, B. Degaga, H. Temmink, L. Korving, G. J. Witkamp, K. Goubitz and M. C. M. van Loosdrecht, Sulfide induced phosphate release from iron phosphates and its potential for phosphate recovery, *Water Res.*, 2020, **171**, 115389.
- 15 C. Lippens and J. De Vrieze, Exploiting the unwanted: Sulphate reduction enables phosphate recovery from energy-rich sludge during anaerobic digestion, *Water Res.*, 2019, **163**, 114859.
- 16 W. Yao, C.-X. Yang, Y. Lu, Y.-Y. Lu, S.-X. Wang, B.-C. Huang and R.-C. Jin, Enhancing phosphorus release from sewage sludge via anaerobic treatment: State-of-art progress and future challenges, *Chem. Eng. J.*, 2024, **483**, 149346.
- 17 A. Ferraro, S. de Sario, A. Attanasio, F. Di Capua, A. Gorgoglione, U. Fratino, M. C. Mascolo, F. Pirozzi, G. Trancone and D. Spasiano, Phosphorus recovery as struvite and hydroxyapatite from the liquid fraction of municipal sewage sludge with limited magnesium addition, *J. Environ. Qual.*, 2023, **52**, 584–595.
- 18 F. Di Capua, D. Spasiano, A. Giordano, F. Adani, U. Fratino, F. Pirozzi and G. Esposito, High-solid anaerobic digestion of sewage sludge: challenges and opportunities, *Appl. Energy*, 2020, **278**, 115608.
- 19 N. Y. Mezenner and A. Bensmaili, Kinetics and thermodynamic study of phosphate adsorption on iron hydroxide-eggshell waste, *Chem. Eng. J.*, 2009, **147**, 87–96.
- 20 R. Morello, F. Di Capua, L. Pontoni, S. Papirio, D. Spasiano, U. Fratino, F. Pirozzi and G. Esposito, Microaerobic Digestion of Low-Biodegradable Sewage Sludge: Effect of Air Dosing in Batch Reactors, *Sustainability*, 2021, **13**, 9869.
- 21 N. Bachmann, *Sustainable biogas production in municipal wastewater treatment plants*, Technical report, IEA Bioenergy, 2015.
- 22 APHA, *Standard Methods for the Examination of Water & Wastewater*, American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), Washington, DC, 21st edn, 2005.
- 23 F. Di Capua, M. C. Mascolo, F. Pirozzi and G. Esposito, Simultaneous denitrification, phosphorus recovery and low sulfate production in a recirculated pyrite-packed biofilter (RPPB), *Chemosphere*, 2020, **255**, 126977.
- 24 A. E. Lewis, Review of Metal Sulphide Precipitation, *Hydrometallurgy*, 2010, **104**, 222–234.
- 25 R. Khanongnuch, F. Di Capua, A. M. Lakaniemi, E. R. Rene and P. N. L. Lens, H₂S removal and microbial community composition in an anoxic biotrickling filter under autotrophic and mixotrophic conditions, *J. Hazard. Mater.*, 2019, **367**, 397–406.
- 26 R. Khanongnuch, F. Di Capua, A. Lakaniemi, E. R. Rene and P. N. L. Lens, Transient-state operation of an anoxic biotrickling filter for H₂S removal, *J. Hazard. Mater.*, 2019, **377**, 42–51.
- 27 N. Di Costanzo, F. Di Capua, A. Cesaro, F. Carraturo, M. Salamone, M. Guida, G. Esposito and A. Giordano, Headspace micro-oxygenation as a strategy for efficient biogas desulfurization and biomethane generation in a centralized sewage sludge digestion plant, *Biomass Bioenergy*, 2024, **183**, 107151.
- 28 D. Rathnayake, K. C. Bal Krishna, G. Kastl and A. Sathasivan, The role of pH on sewer corrosion processes and control methods: A review, *Sci. Total Environ.*, 2021, **782**, 146616.
- 29 A. Taboada-Santos, J. M. Lema and M. Carballa, Energetic and economic assessment of sludge thermal hydrolysis in novel wastewater treatment plant configurations, *Waste Manage.*, 2019, **92**, 30–38.
- 30 L. Egle, H. Rechberger, J. Krampe and M. Zessner, Phosphorus recovery from municipal wastewater: An integrated comparative technological, environmental and economic assessment of P recovery technologies, *Sci. Total Environ.*, 2016, **571**, 522–542.
- 31 M. Arnold, *Reduction and monitoring of biogas trace compounds*, Espoo, 2009.
- 32 C. Cai, C. Hu, W. Yang, Y. Hua, L. Li, D. Yang and X. Dai, Sustainable disposal of excess sludge: Post-thermal hydrolysis for anaerobically digested sludge, *J. Cleaner Prod.*, 2021, **321**, 128893.
- 33 R. Morello, F. Di Capua, G. Esposito, F. Pirozzi, U. Fratino and D. Spasiano, Sludge minimization in mainstream wastewater treatment: Mechanisms, strategies, technologies, and current development, *J. Environ. Manage.*, 2022, **319**, 115756.
- 34 R. Morello, F. Di Capua, E. Sahinkaya, G. Esposito, F. Pirozzi, U. Fratino and D. Spasiano, Operational strategies enhancing sewage sludge minimization in a combined integrated fixed-film activated sludge – oxic settling anaerobic system, *J. Environ. Manage.*, 2023, **345**, 118808.
- 35 R. Morello, F. Di Capua, Ç. Kalkan Aktan, T. Yilmaz, G. Esposito, F. Pirozzi, U. Fratino, D. Spasiano and E. Sahinkaya, Unravelling the impact of oxic-settling-anaerobic cycle implementation and solid retention time on sludge generation, membrane operation, and contaminant removal in membrane bioreactors, *Chem. Eng. J.*, 2024, **496**, 153800.

