symbiorem

REPORT ON PHOSPHORUS AND METAL RECOVERY

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20.06.2024

PROJECT INFORMATION

Project Acronym	SYMBIOREM
Project Title	Symbiotic, circular bioremediation systems and biotechnology solutions for improved environmental, economic and social sustainability in pollution control
Grant Number	101060361
Project Coordinators	Dr. Leire Ruiz Rubio, Dr. José Luis Vilas Vilela, Dr. Mónica Loyo-Menoyo University of the Basque Country - UPV/EHU
Project Duration	1 September 2022 - 31 August 2026

DELIVERABLE INFORMATION

Deliverable No.	D.4.2.
Dissemination Level [1]	PU
Work Package	WP4
Task	4.2
Lead Beneficiary	KTH
Contributing beneficiary(ies)	UNIBO
Due date of deliverable	June 2024
Actual submission date	20.06.2024

[1] PU = Public
PP = Restricted to other programme participants (including the Commission Services)
RE = Restricted to a group specified by the consortium (including the Commission Services)
CO = Confidential, only for members of the consortium (including the Commission Services)

DOCUMENT HISTORY

V	Date	Beneficiary	Author/Reviewer
1	20.06.2024	KTH-UNIBO	Fengyi Zhu, Ece Kendir Cakmak, Zeynep Cetecioglu Gurol, Chen Chen, Maria Cuertero Botia, Frederico Marques Penha, Sritama Mukherjee, Ulrica Edlund, Giorgia Palladino, Federica D'Amico, Elena Radaelli, Silvia Turroni, Marco Candela
2	21.06.2024	GAIKER	Pilar Brettes

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EXECUTIVE SUMMARY

The overall objective of SYMBIOREM is to improve the effectiveness, sustainability, circularity and cost-efficiency of bioremediation and revitalization strategies for soils, sediments, surface water and groundwater. In terms of sediments, SYMBIOREM develops an integrated remediation and valorization technology for nutrient and metals-rich marine sediments: a cascade bioreactor system that remediates PAH while also recovering phosphorus and metals from highly polluted marine sediments. For phosphorus and metal recovery, SYMBIOREM explores if the biological approaches that can be used effectively and environmentally friendly way.

This deliverable starts with giving technical information about experimental design and results for anaerobic batch bioreactors for phosphorus release from the Baltic Sea sediment with three different operational conditions: carbon addition, nitrogen addition, and sulfur addition. Later, bioleaching experiments and their results for metal release from the Baltic Sea sediment using pure bioleaching bacteria and iron and sulfur oxidizing bacteria enriched from the Baltic Sea sediment are discussed. Reagentless acidification method using polyaniline arrays for supporting bioleaching experiments is also mentioned. Phosphorus recovery from liquid media after being released from marine sediment is being investigated through various simulations and experiments. Finally, methodology for metal recovery from the liquid media after bioleaching and its potential application is mentioned.

Briefly, the results showed that current anaerobic batch bioreactors were able to release phosphorus from the Baltic Sea sediment and their potential in continuous bioreactor operation should be investigated. In terms of bioleaching, metals such as iron, aluminum as well as magnesium and phosphorus can be released from the Baltic Sea sediment especially when natural sediment microorganisms are employed for bioleaching. However, longer retention time should be investigated for better leaching efficiencies. Acidification of bioleaching samples to set the initial pH as 4.5 with polyaniline arrays had also outstanding potential to reduce environmental effects and economic costs when compared to chemical acid addition. Phosphorus recovery simulations and precipitation experiments showed that pH adjustment (to pH 9.22) and cooling experiments showed solid formation. However, struvite precipitation yield was lower. So, kinetics of crystallization of struvite needs to be better investigated to achieve better yields. For metal recovery, synthesized membranes were able to capture target metals, however; their efficiencies can be improved.

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1 Introduction

1.1 Phosphorus Release from the Baltic Sea Sediment

1.1.1 The Effect of Carbon Source on Phosphorus Release

The aim of this set was to investigate phosphorus (P) recovery from the Baltic Sea sediment using anaerobic batch reactors and to explore the effects of different carbon (C) sources on the P release process.

Methodology for anaerobic batch experiments

In this set, four different carbon sources (acetic acid, propionic acid, butyric acid, and glucose) were tested for their ability to stimulate P release from marine sediments under anaerobic conditions. Sediment samples (16 g) were placed in 120 mL serum bottles and treated with various concentrations of the carbon sources (0, 0.5, 1, 2.5, 5, and 10 g/L). The bottles were sealed, flushed with nitrogen, and incubated at room temperature for 15 days with daily mixing. Sampling was conducted on multiple days (day 0, 1, 2, 3, 6, 9, 12, 15), and analyses included measuring PO_4-P, pH, carbon source consumption, and microbial community composition to assess P release and microbial activity. PO_4-P was analyzed by Murphey and Riley method [1] and cuvette tests (LCK 303 Hach Lange, Germany) using DR 3900 Hach Lange spectrophotometer. pH was monitored with Mettler Toledo FiveEasyTM pH bench meter, FE20. Volatile fatty acids were measured with gas chromatography (GC) (Agilent Intuvo 9000) equipped with CP-Sil 5 CB column (25 m × 0.32 mm × 5 µm, Agilent) and a flame ionization detector. Glucose was determined by the dinitrosalicylic acid (DNS)[2] method.

Microbial characterization: 16S rRNA amplicon sequencing and data processing

DNA extraction of the samples was done with Qiagen DNeasy PowerSoil Pro Kits. Library preparation for a total of 344 samples was performed following the Illumina 16S Metagenomic Sequencing Library Preparation (Illumina, San Diego, CA, USA). The V3–V4 hypervariable region of the 16S rRNA gene was amplified via PCR in a final volume of 25 µL with 25 ng microbial DNA, 2X KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland), and 200 nmol/L of 341F and 785R primers with added Illumina adapter overhang sequences[3]. The PCR thermocycle consisted of 3 min at 95°C, 25 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C, and a final 5-min step at 72°C[4]. PCR products were purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) and indexed libraries were prepared by

[1] Cho, YH., Nielsen, S.S. (2017). Phosphorus Determination by Murphy-Riley Method. In: Food Analysis Laboratory Manual. Food Science Text Series. Springer, Cham. https://doi.org/10.1007/978-3-319-44127-6_17

[2] McKee, L.S. (2017). Measuring Enzyme Kinetics of Glycoside Hydrolases Using the 3,5-Dinitrosalicylic Acid Assay. In: Abbott, D., Lammerts van Bueren, A. (eds) Protein-Carbohydrate Interactions. Methods in Molecular Biology, vol 1588. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-6899-2_3

[3] Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic acids research, 41(1), e1. https://doi.org/10.1093/nar/gks808

[4] Turroni, S., Fiori, J., Rampelli, S., Schnorr, S. L., Consolandi, C., Barone, M., & Candela, M. (2016). Fecal metabolome of the Hadza hunter-gatherers: a host-microbiome integrative view. *Scientific reports*, *6*(1), 32826.

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limited-cycle PCR with Nextera technology (Illumina). Indexed libraries were subsequently cleaned-up as described above and then quantified using the Qubit 3.0 fluorimeter (Invitrogen, Waltham, MA, USA), normalized to 4 nM and pooled. The sample pool was denatured with 0.2 N NaOH and diluted to a final concentration of 4.5 pM with a 20% PhiX control. Sequencing was performed on an Illumina MiSeq platform using a 2 × 250 bp paired-end protocol, according to the manufacturer's instructions (Illumina).

Raw sequences were processed using a pipeline combining PANDAseq[5] and QIIME 2[6]. After length and quality filtering, reads were binned into amplicon sequence variants (ASVs) using DADA2[7]. This resulted in 4287.650 ± 2410.315 (mean ± standard deviation) reads per sample and a total number of 16116 ASVs. Only 7 samples resulted to have a number of reads <1000, thus they were excluded in the rarefaction step before subsequent analysis (namely, AcA59, BuA194, Gl261, PrA89, PrA137, PrA149 and PrA161).

Taxonomy was assigned via the VSEARCH algorithm[8] using the SILVA database (138.1 SSURef NR99) as a reference. PICRUSt2 with default parameters was used to predict metagenome functions (KEGG orthology database) based on the ASVs identified in our dataset[9]. Processed reads for 16S rRNA gene sequencing are openly available in European Nucleotide Archive (ENA), reference number PRJEB74909.

Results and discussions

The experiment aimed to evaluate P release from marine sediments stimulated by different C loadings in systems fed with acetic acid, propionic acid, butyric acid, and glucose. As shown in Figure 1, all C sources enhanced P release under anaerobic conditions, with the highest concentrations observed for glucose (6.44 mg/L), followed by propionic acid (5.98 mg/L), butyric acid (5.76 mg/L), and acetic acid (4.99 mg/L). The initial rapid P release occurred within the first 3 days, reaching over 67% of the maximum release. Propionic acid and butyric acid-fed systems significantly outperformed the acetic acid-fed system in P release. The optimal P release concentration was found to be 1 g/L for each C source. However, excessive C input inhibited P release, with high glucose concentrations leading to a decrease in dissolved P over time due to pH drop and metal ion interactions during fermentation. Statistical analyses confirmed these findings, showing significant differences between C concentrations and their impact on P release.

[5] A.P. Masella, A.K. Bartram, J.M. Truszkowski, D.G. Brown, J.D. Neufeld PANDAseq: PAired-eND Assembler for Illumina sequences
[6]Bolyen, E., Rideout, J.R., Dillon, M.R. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**, 852–857 (2019). https://doi.org/10.1038/s41587-019-0209-9
[7] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. HolmesDADA2: high-resolution sample inference from Illumina amplicon data
[8] Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016 Oct 18;4:e2584. doi: 10.7717/peerj.2584. PMID: 27781170; PMCID: PMC5075697.
[9] G.M. Douglas, V.J. Maffei, J.R. Zaneveld, S.N. Yurgel, J.R. Brown, C.M. Taylor, C. Huttenhower, M.G.I. Langille, PICRUSt2 for prediction of metagenome functions, Nat. Biotechnol. 38(6) (2020) 685-688.

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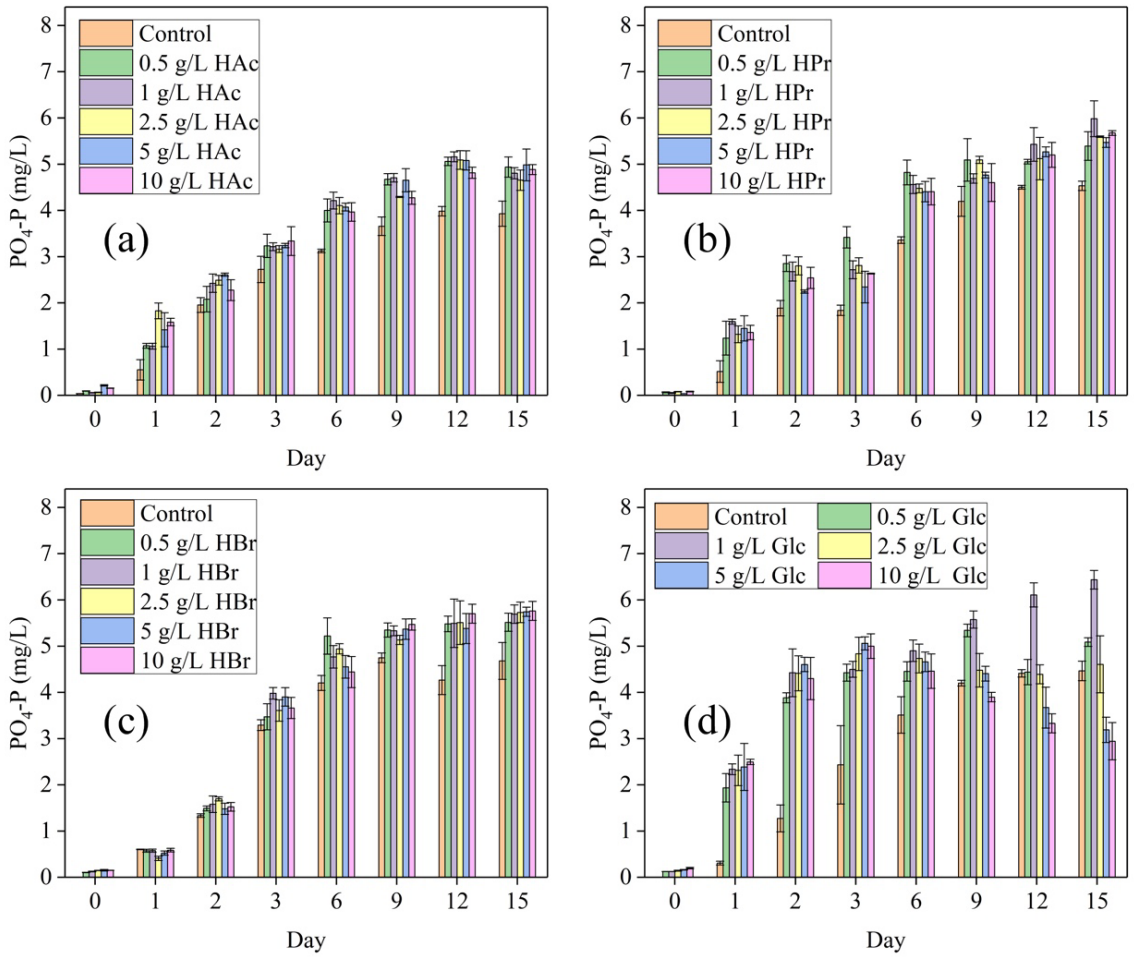

Figure 1 PO_4-P release performance of sediment under different concentrations with four types of carbons-fed systems: (a) acetic acid-, (b) propionic acid-, (c) butyric acid- and (d) glucose fed system. (HAc: acetic acid, HPr: propionic acid, HBr: butyric acid, Glc: glucose).

The experiment results also revealed significant microbial community changes under different C dosing groups, particularly in glucose-fed systems (Figure 2). Initially the microbial structure at family level was dominated by *Desulfosarcinaceae* and *Ilumatobacteraceae*, the communities shifted to be dominated by *Clostridiaceae*, *Fusobacteriaceae*, and *Psychromonadaceae*, especially *Clostridiaceae*. The abundance of *Clostridiaceae* increased notably with higher glucose concentrations. Despite different C sources inducing significant microbial changes, varying concentrations of the same C source did not always lead to community structure changes, except for propionic acid and glucose.

In VFA-fed systems, correlation analysis showed a positive link between C conversion and P release in VFA-fed systems but a negative one in glucose-fed systems. High C concentrations, especially easily utilized ones, led to fermentative bacteria proliferation, potentially inhibiting organic matter decomposition and P release. Sulfate-reducing bacteria (SRB) significantly contributed to sediment P cycling, maintaining around 10% of total microbial relative abundance. SRB facilitated P release via anaerobic respiration and sulfate reduction. In low C-fed groups, sulfate consumption exceeded 95%, but it was below 60% in no

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extra C or high C addition groups. High glucose concentrations negatively impacted SRB abundance and sulfate reduction, thus inhibiting P release, explaining why excessive C addition did not enhance P release.

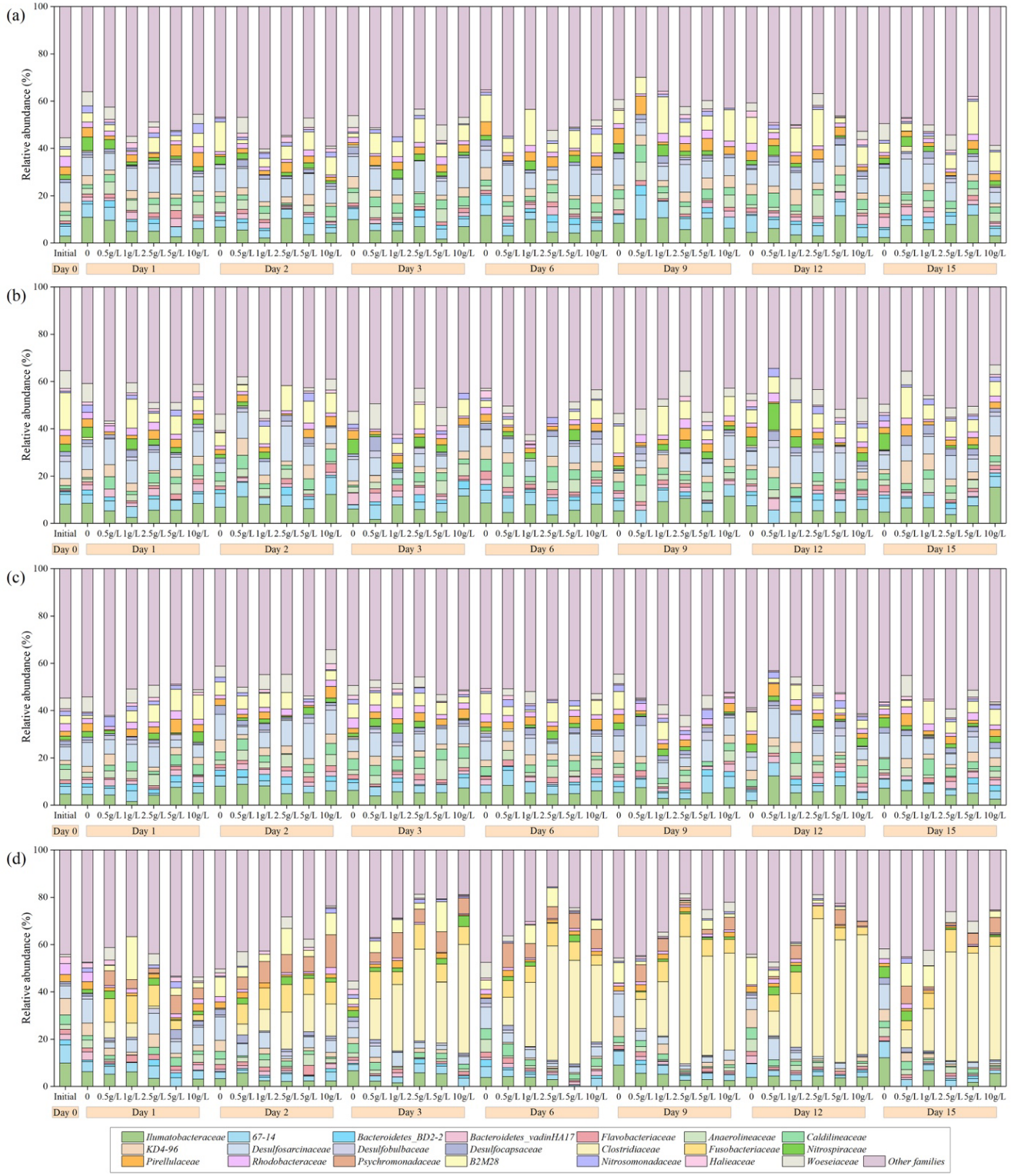

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Figure 2 Compositional structures of microbial communities at family level under different concentrations with four types of carbons-fed systems: (a) acetic acid-, (b) propionic acid-, (c) butyric acid- and (d) glucose fed system.

1.1.2 The Effect of Nitrogen Source on Phosphorus Release

The aim of this set was to Investigate P release under different C/N ratios using NH_4-N as the N source, acetic acid and glucose as the C sources and explore the microbial and enzymatic mechanisms involved in P release across various NH_4-N loading conditions.

Methodology for anaerobic batch experiments

Batch experiments were conducted in 120 mL serum bottles to study the effect of different carbon to nitrogen (C/N) ratios on P release from sediment under anaerobic conditions. Acetic acid and glucose were used as carbon sources. Groups were set up with no NH_4-N addition, and low (C/N=100), medium (C/N=50), and high (C/N=10) NH_4-N addition. Each system involved 168 bottles, operated at 20°C with daily mixing, and sampled over 24 days. Measurements included PO_4-P, NH_4-N, pH, carbon source concentrations, alkaline phosphatase activity, and microbial community structure.

Microbial characterization: 16S rRNA amplicon sequencing and data processing

Library preparation and data processing were performed as described in paragraph 1.1.1. on a total of 150 samples. The sequencing raw data has been archived in the Sequence Read Archive (National Center for Biotechnology Information, US) under the BioProject accession number PRJNA1030998.

Results and discussions

The dissolved PO_4-P released from sediments in acetic acid- and glucose-fed systems under varying NH_4-N loadings is depicted in Figure 3(a) and 3(b). Maximum PO_4-P release was 4.07 mg/L on day 24 for acetic acid-fed and 7.14 mg/L on day 18 for glucose-fed systems without additional NH_4-N. Within the first 6 days, PO_4-P concentrations rose markedly to 3.41 mg/L (acetic acid-fed) and 6.55 mg/L (glucose-fed) without extra NH_4-N addition. Despite a general upward trend in P release over time, the final increases were modest, at 19.35% and 8.26%. Moreover, higher NH_4-N dosing significantly inhibited P decomposition ($p<0.05$). As NH_4-N levels increased from low (COD/N=100) to high (COD/N=10), PO_4-P concentrations in the acetic acid-fed system dropped from 3.62 mg/L to 2.95 mg/L over 24 days, and in the glucose-fed system from 6.32 mg/L to 5.50 mg/L by day 18.

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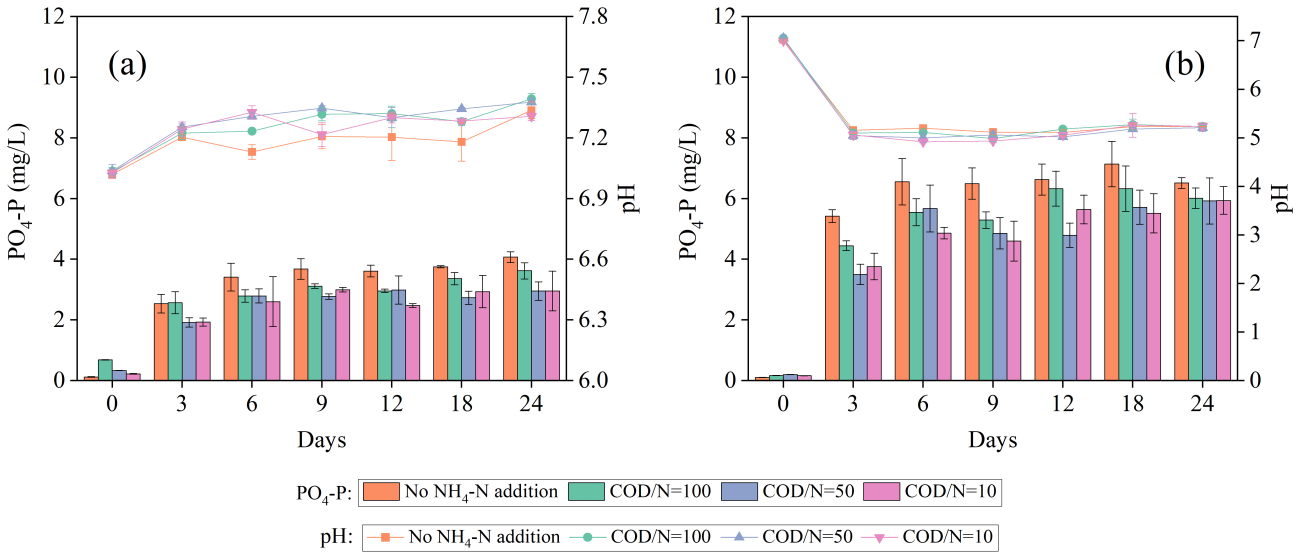

Figure 3 Variations of PO_4-P and pH: (a) in the acetic acid-fed system; (b) in the glucose-fed system during the 24-day anaerobic operation under different COD/N loading

The variations in alkaline phosphatase (AP) activity are depicted in Figure. 4. In the acetic acid-fed system, AP activity initially increased, peaking at 1046.97 mg/(kg·h) on day 12 from an initial 885.90 mg/(kg·h) in the no NH_4-N addition group, before declining to 860.66 mg/(kg·h) by day 24. Conversely, in the glucose-fed system, AP activity decreased from 838.11 mg/(kg·h) on day 0 to 490.67 mg/(kg·h), 447.86 mg/(kg·h), 584.45 mg/(kg·h), and 354.75 mg/(kg·h) on day 6 for the no NH_4-N addition, COD/N=100, COD/N=50, and COD/N=10 groups, respectively. However, the enzyme activity in the no NH_4-N addition group later increased to 967.79 mg/(kg·h) on day 18, surpassing the initial value.

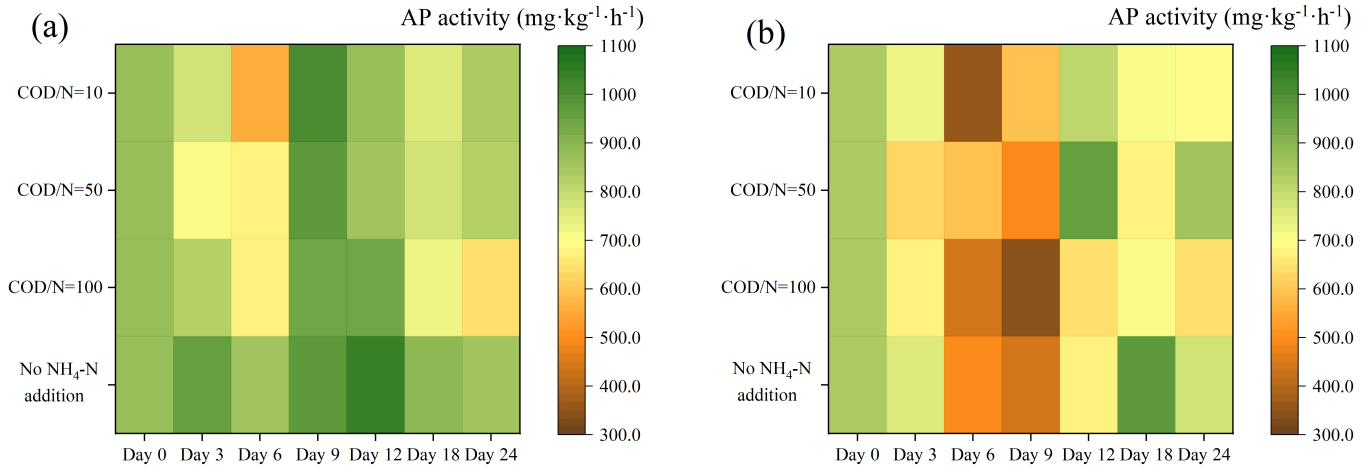

Figure 4 Variations of alkaline phosphatase activity of marine sediment during the 24-day anaerobic operation under different COD/N loading: (a) acetic acid-fed system; (b) glucose-fed system

As shown in Figure 5 (a) and (b), the initial sediment was primarily composed of *Proteobacteria* (21.4% in acetic acid-fed and 32.0% in glucose-fed systems), Planctomycetota (16.0% and 13.5%), and *Chloroflexi*

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(9.9% and 11.8%). Proteobacteria dominated in both systems, playing roles in C, N, and S cycling. *Planctomycetota* participated in ammonia and carbohydrate metabolism. In the acetic acid-fed system without extra NH_4-N addition, *Chloroflexi* increased from 9.9% to 15.6% by day 24. In the glucose-fed system, the abundances of Proteobacteria, *Planctomycetota*, and *Chloroflexi* declined from 32.0% to 24.9%, 13.5% to 8.4%, and 11.8% to 6.3%, respectively, over 24 days. This suggests that organic C utilization influences phylum proportions, with acetic acid favoring *Chloroflexi* due to low acetate consumption and rapid glucose consumption inhibiting organic-decomposing phyla. Initially, *Pirellulaceae* was the most abundant family at 12.2% (acetic acid-fed) and 8.2% (glucose-fed) (Figure 5c and 5d). In the glucose-fed system, *Clostridiaceae* increased to 57.4% by day 9 ($p<0.001$), while *Desulfosarcinaceae* and *Anaerolineaceae* decreased ($p<0.01$). Alpha diversity was higher in glucose-fed groups without NH_4-N until about day 12 ($p<0.08$). Over time, specific taxa changes were noted under different COD/N loading in the glucose-fed system.

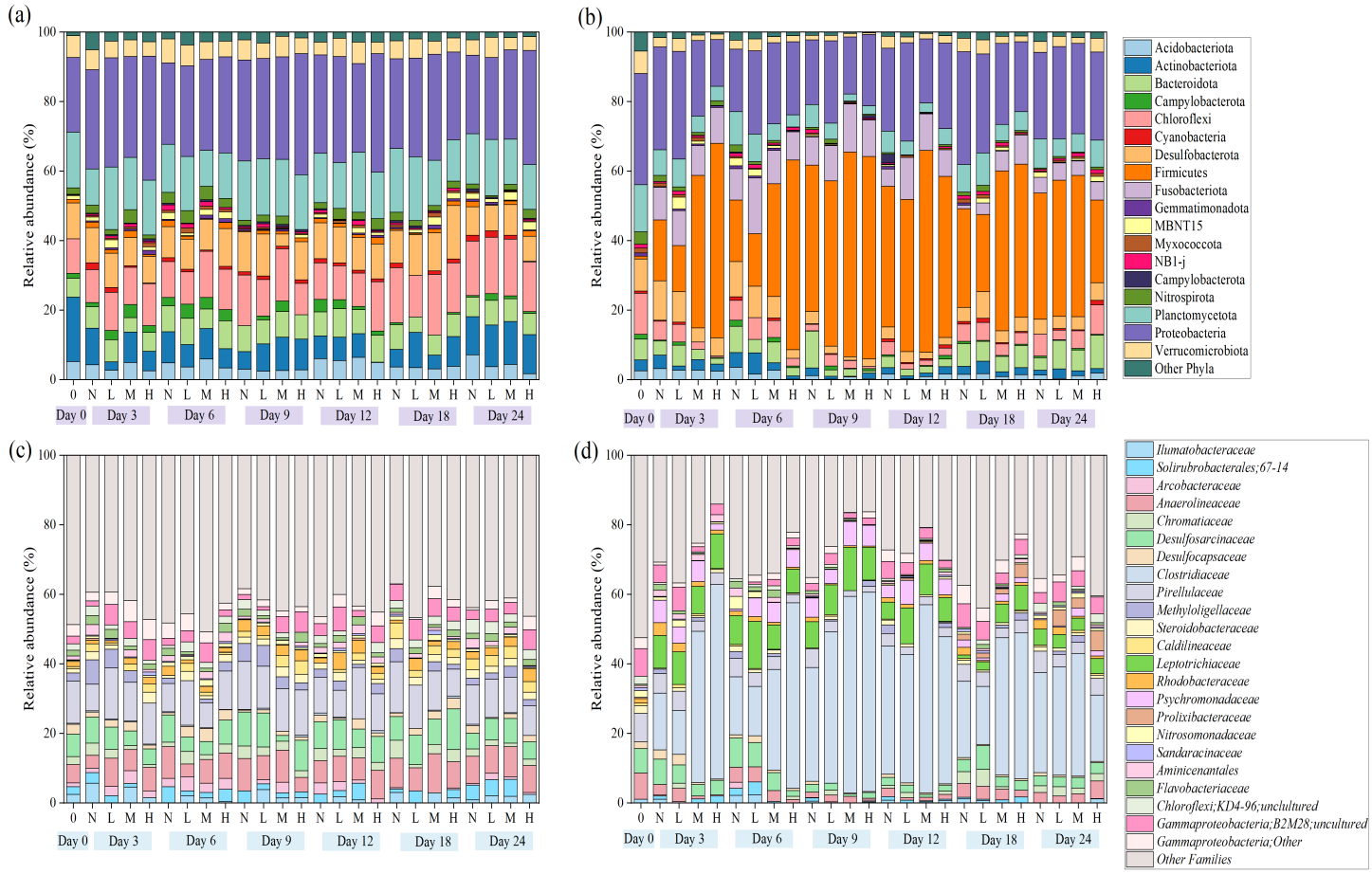

Figure 5 Compositional structure of microbial communities during the 24-day anaerobic operation under different COD/N loading at the phylum level: (a) in the acetic acid-fed system; (b) in the glucose-fed system; and at the family level: (c) in the acetic acid-fed system; (d) in the glucose-fed system (N, no extra NH_4-N addition; L, COD/N=100; M, COD/N=50; H, COD/N=10)

This part of the work was published in Science of The Total Environment March 2024 with the title "Phosphorus mining from marine sediments adapting different carbon/nitrogen strategies driven by anaerobic reactors: The exploration of potential mechanism and microbial activities (https://doi.org/10.1016/j.scitotenv.2024.169902). More detailed information can be found in this paper.

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1.1.3 The Effect of Sulfate on Phosphorus Release

The aim of this set was to investigate the effect of sulfate addition on promoting activity of sulfate reducing bacteria and eventually, the effect on the phosphorus release.

Methodology for anaerobic batch experiments

Due to previous batch experiments and microbial analysis confirming the high presence of sulfate-reducing bacteria (SRB) in our marine sediment and their crucial role in promoting P release, the current study was designed similarly. In this batch, 1 g/L glucose was used as the carbon source. The experimental groups included control, no extra sulfate addition (S0), 1 mM sulfate addition (S1), 2 mM sulfate addition (S2), 4 mM sulfate addition (S4), and 8 mM sulfate addition (S8). Each group was maintained at 20 °C with daily mixing and sampled over 36 days. Measurements included PO_4-P, NH_4-N, sulfate, pH, carbon source concentrations, and microbial community structure.

Results and discussions

The current experimental results, shown in Figure 6, indicate that the addition of sulfate promoted P release. On day 36, PO_4-P release in the S8 group increased from 5.26 mg/L (S0 group) to 6.41 mg/L. The added sulfate was gradually consumed over the time of the anaerobic operation, with concentrations in the S8 group decreasing from 1018 mg/L initially to 496 mg/L. Sequencing data is currently being analyzed. The response of SRB and other microbes to sulfate addition and the mechanisms promoting P release will be investigated once the 16S sequencing data is available.

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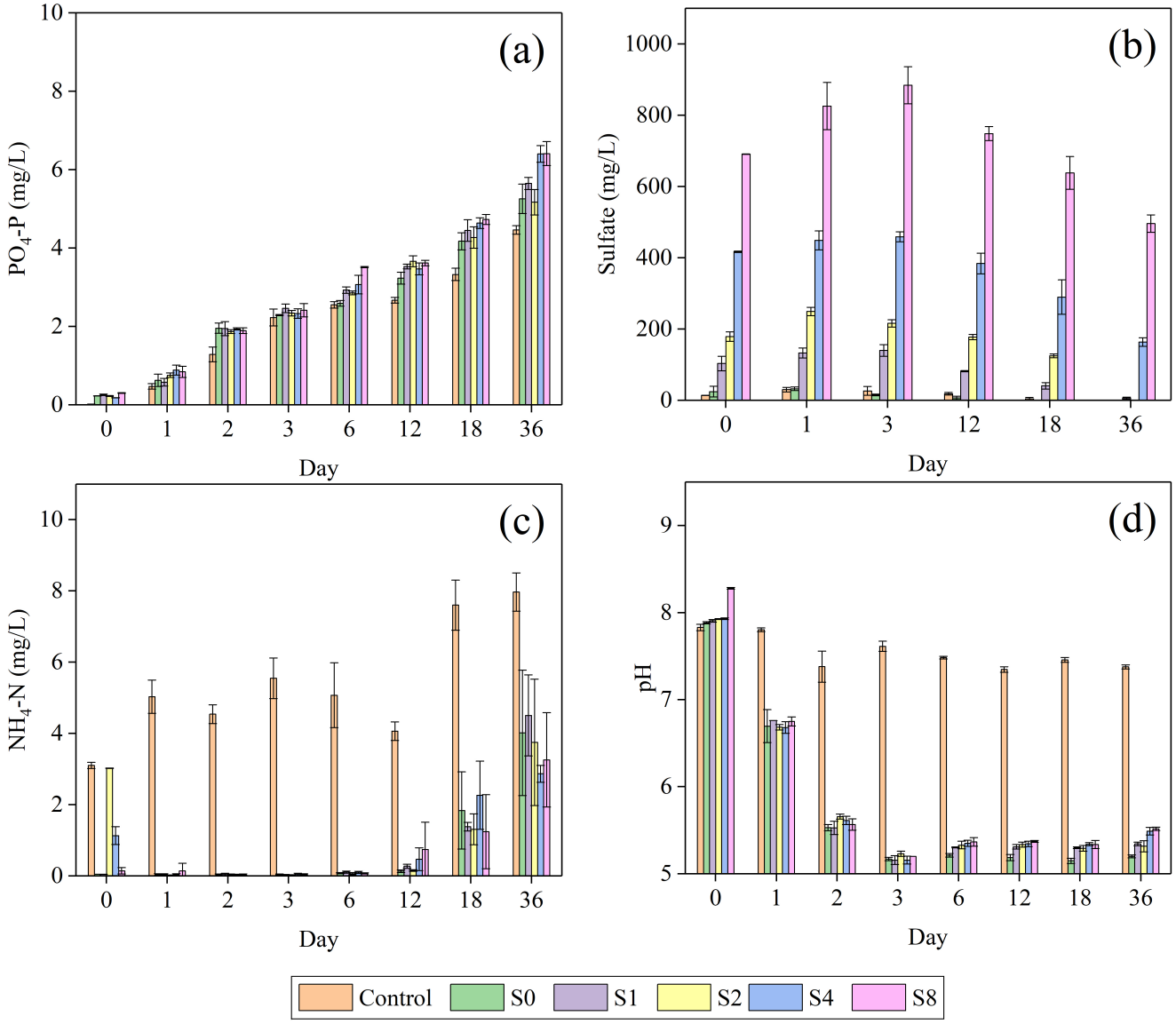

Figure 6 Variations of (a) PO_4-P, (b) sulfate, (c) NH_4-N and (d) pH during the 24-day anaerobic operation under sulfate addition conditions

1.2 Metal Release from the Baltic Sea Sediment

Initially, the anoxic marine sediment collected from the Baltic Sea was initially characterized in terms of metal ions and other elements. The samples were sent to an external laboratory (*Eurofins*) for detailed characterization. The results are provided in Table 1.

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Table 1 Characteristics of the collected Baltic Sea Sediment

Element	Concentration [mg/kg TS]
Aluminium (Al)	44 000
Antimony (Sb)	< 9.8
Arsenic (As)	< 25
Barium (Ba)	200
Beryllium (Be)	< 2.5
Lead (Pb)	38
Boron (B)	51
Phosphorus (P)	900
Iron (Fe)	39 000
Cadmium (Cd)	< 0.98
Calcium (Ca)	6 500
Potassium (K)	15 000
Cobalt (Co)	14
Copper (Cu)	33
Chromium (Cr)	64
Lithium (Li)	41
Magnesium (Mg)	15 000
Manganese (Mn)	610
Molybdenum (Mo)	< 9.8
Sodium (Na)	13 000
Nickel (Ni)	32
Selenium (Se)	< 4.9
Silver (Ag)	< 2.5
Strontium (Sr)	65
Sulfur (S)	8 300
Thallium (Tl)	< 0.98
Tin (Sn)	5.4
Titanium (Ti)	2 200
Uranium (U)	4.3
Vanadium (V)	85

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Zinc (Zn)	160

As can be seen in the Table 1, some of the abundant elements are Al, Fe, K, Mg, P, S and Ti. Based on the EU Commission's Critical Raw Material List, they are classified as follows: [10]

Al: Non critical, high economic importance, low supply risk

Fe: Non critical, high economic importance, low supply risk

P: Critical, high economic importance, high supply risk

K: Non critical, high economic importance, low supply risk

Mg: Critical, high economic importance, high supply risk

S: Non critical, high economic importance, low supply risk

Ti: Critical, high economic importance, high supply risk

On the other hand, some of the metals such as Li and Co are not very abundant in the collected sediment, although they are critical raw materials with high economic importance.

1.2.1 Bioleaching experiments with *Acidithiobacillus thiooxidans*

The aim of this set was to investigate the use of *Acidithiobacillus thiooxidans* as a well-known bioleaching bacterium to release metals from the Baltic Sea sediment. Furthermore, this set was also aimed to understand the effect of microbial acclimation on the bioleaching efficiency.

Methodology for bioleaching experiments:

Acidithiobacillus thiooxidans (DSM 504) was purchased from DSMZ. The cultivation medium (Medium 35) was prepared according to DSMZ[11] and autoclaved prior to use. The culture suspension was mixed with (1:10 v/v) the medium and incubated at 30ºC for one week in growth tubes. After 3 consecutive steps (weekly cultivation), the cultures were incubated in bigger flasks for one more week to provide enough culture for the experiments.

This set included 3 bioleaching cycles which lasted 18 days per cycle. The first cycle of the bioleaching experiments was conducted at four different conditions: Set 1: pure culture+sulfur, Set 2: sulfur, Set 3: pure culture. Set 4: control (no sulfur/pure culture). Since initial pH is important for bioleaching experiments, two different initial pH (4.5 and 7.0) were tested in this set. After 1st cycle, 5% (v/v) of the sediment from

[10] Micuda, Zoran & Salhofer, Stefan & Beigl, Peter & Tran, Chung & Jandric, Aleksander. (2018). Exploration of the material distribution of complex components in waste electrical and electronic equipment. Global Nest Journal. 20. 725-736. 10.30955/gnj.002672.

[11] https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium35.pdf

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the 1st cycle was added to the new flasks with fresh sediment to run 2nd cycle. For instance, 15 mL sediment for the Set 1-2 were taken and it was introduced to the new flasks including 275 mL fresh sediment and sulfur. For the Set 3-4, 15 mL sediment was taken, and it was introduced to the new flasks including only fresh sediment. 2nd cycle had the same conditions (Set 1-4) and pH values (4.5 and 7.0) as in the 1st cycle. Afterwards, a 3rd cycle was run which included 5%(v/v) and 10% of the sediment from the 2nd cycle. However, in this set, the initial pH values were 4.5. 3rd cycle was operated to observe the effect of inoculum ratio to bioleaching efficiency.

All the experiments were set up at 500 mL flasks with a working volume of 300 mL. Total solids (TS) content of the sediment was adjusted to 5% (decreased from 18%) by adding 6 psu saline water that shows Baltic Sea water conditions. 3 g sulfur was added to the flasks (Set 1 and Set 2). The flasks were covered with cotton plug to provide aeration and they were replaced in an incubator at 30ºC with 120 rpm. Additional air was not provided to the flasks. pH was monitored every 3 days. Samples were taken every 6 days. They were centrifuged and supernatant was filtered (0.2 and 0.45 um) to measure metals and anions (PO_4 and SO_4). Metal measurements were done with Inductively Coupled Plasma (ICP) and anion measurements were done with Ion Chromatography (IC). Samples for microbial community analysis were kept in -20ºC and DNA extraction of the samples was done with Qiagen DNeasy PowerSoil Pro Kits.

Results and discussion:

One of the main indicators for oxidation of sulfur in the bioleaching experiment is the pH decrease due to production of sulfuric acid. Figure 7 shows the pH change during the bioleaching experiments. Generally, it was concluded that as sulfur was introduced to the flasks, pH values were decreased to around 1.5 at the end of each cycle. On the other hand, pH values were not lower than 3.0 in the absence of sulfur. It was also concluded that the presence of pure culture did not affect the pH change too much and showed a very similar trend with flasks only had sulfur. Initial pH also affected the rate of pH change in the flasks including sulfur (A+S) at the first 6-9 days, however; the values were similar after 9 days. The results of the 1st cycle and 2nd cycle were very similar. At 3rd cycle, higher inoculum ratio showed a slower pH decrease with A+S samples, however, the rate was same between S (5-10% inoculum ratio) samples.

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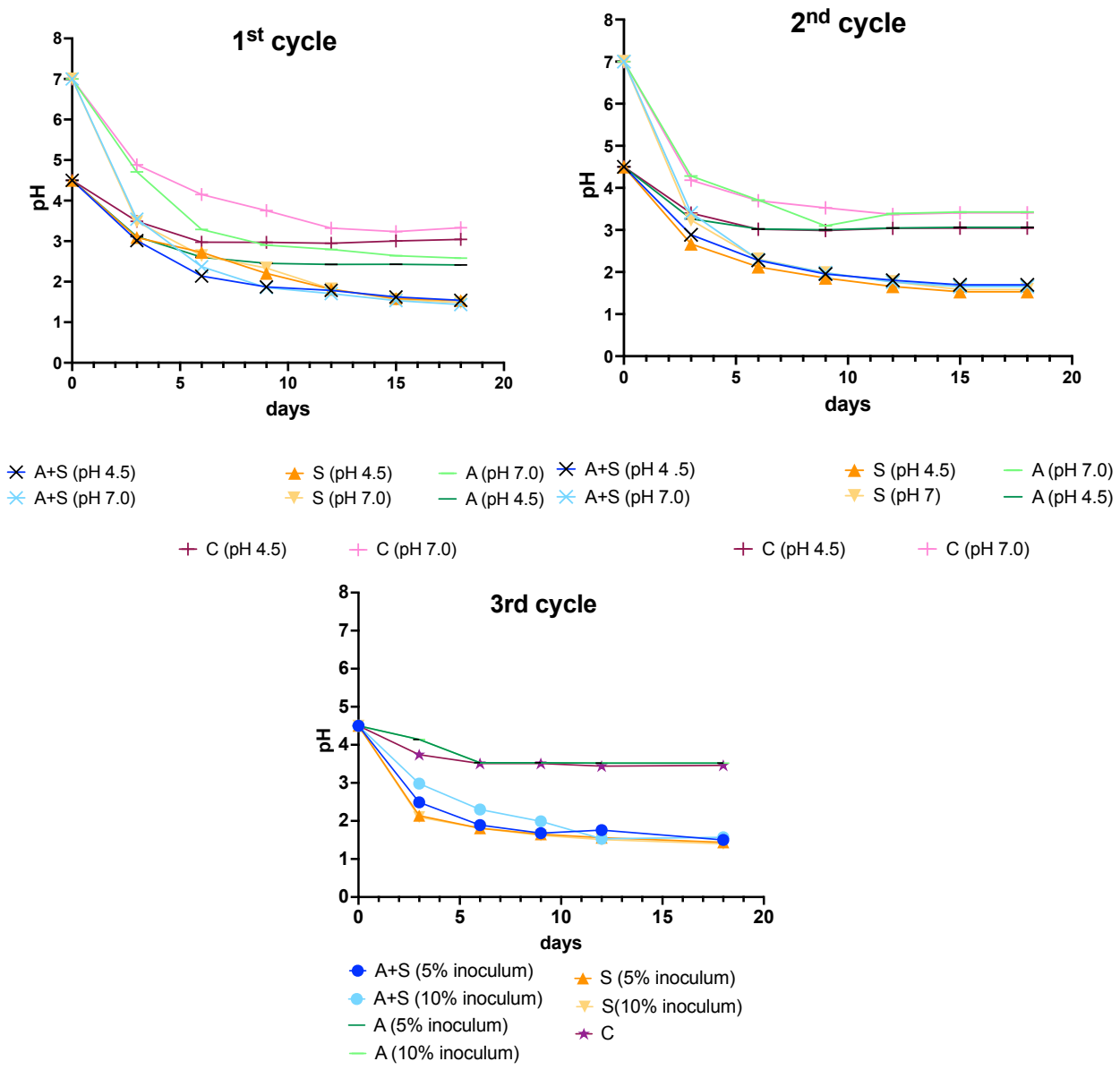

Figure 7 pH change during bioleaching experiments (*A+S: Acidithiobacillus thiooxidans+sulfur*, S: Sulfur, A: *Acidithiobacillus thiooxidans, C: Control)*

The metal concentrations in the liquid at the end of each cycle were shown in Figure 8-10. In the first cycle, the highest Fe concentration was observed as 770 mg/L for A+S (pH 4.5) with around 40% bioleaching efficiency (released % from the sediment). S (pH 4.5-7.0) also showed higher Fe concentrations. The flasks without sulfur addition showed comparatively lower Fe concentrations at the end of the 1st cycle as well as the 2nd and the 3rd cycle. In the second cycle, Fe concentration in A+S (pH 4.5) lowered and the highest Fe release was observed with S (pH 4.5) sample. In the 3rd cycle, it was observed that inoculum increase did not affect significantly Fe concentration in S (pH 4.5) samples. However, Fe concentration decreased with A+S (pH 4.5) samples as inoculum ratio increased. Highest Al concentrations were observed as 460-470 mg/L in all the cycles and sulfur addition positively improved Al release from the marine sediment. The trends between cycles as well as the samples were similar with the Fe concentrations.

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As K and Mg concentrations were analyzed, it was observed that at the results after the 1st cycle and the 2nd cycle were different and concentrations were decreased more than half after the 2nd cycle. However, the results of the 2nd cycle and 3rd cycle were similar. Moreover, unlike Fe and Al, sulfur addition did not improve significantly the release from the sediment for these elements. Apart from these elements, sulfur concentrations were also measured in the liquid medium. A preliminary mass balance calculations showed that all the sulfur was not consumed during bioleaching when sulfur added externally. However, for the samples without sulfur addition, sulfur in the sediment was consumed.

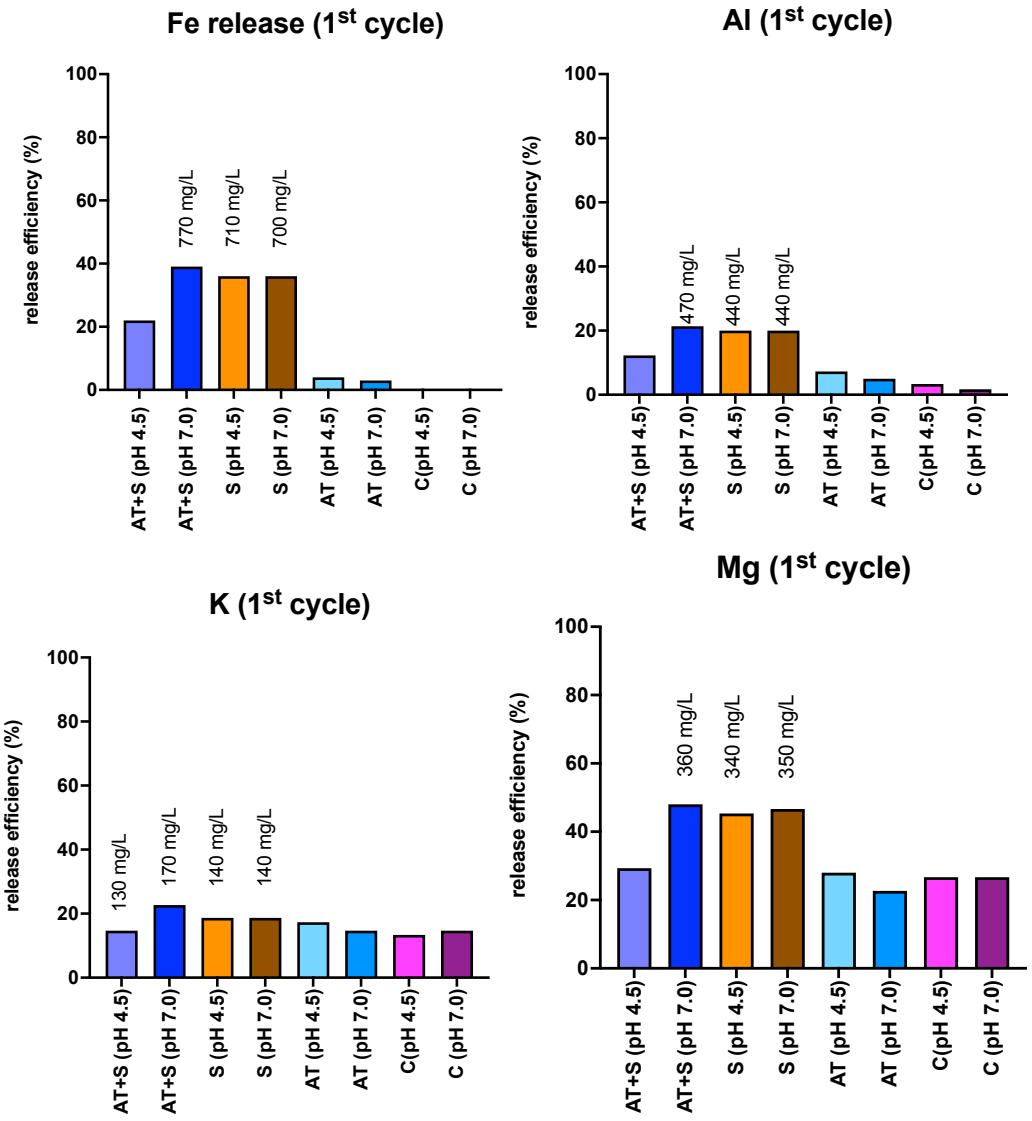

Figure 8 Metal concentrations at the end of 1st cycle

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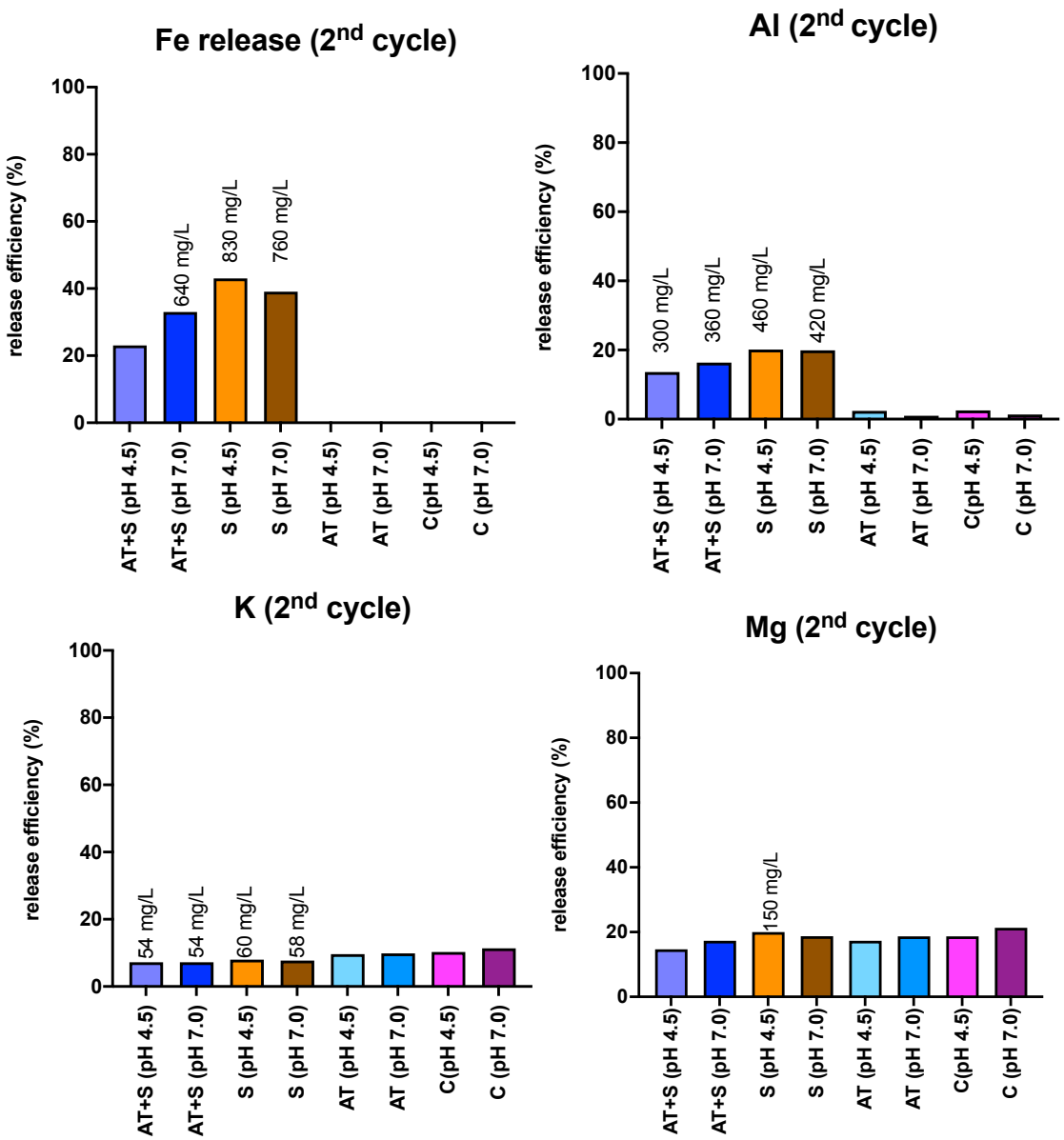

Figure 9 Metal concentrations at the end of 2nd cycle

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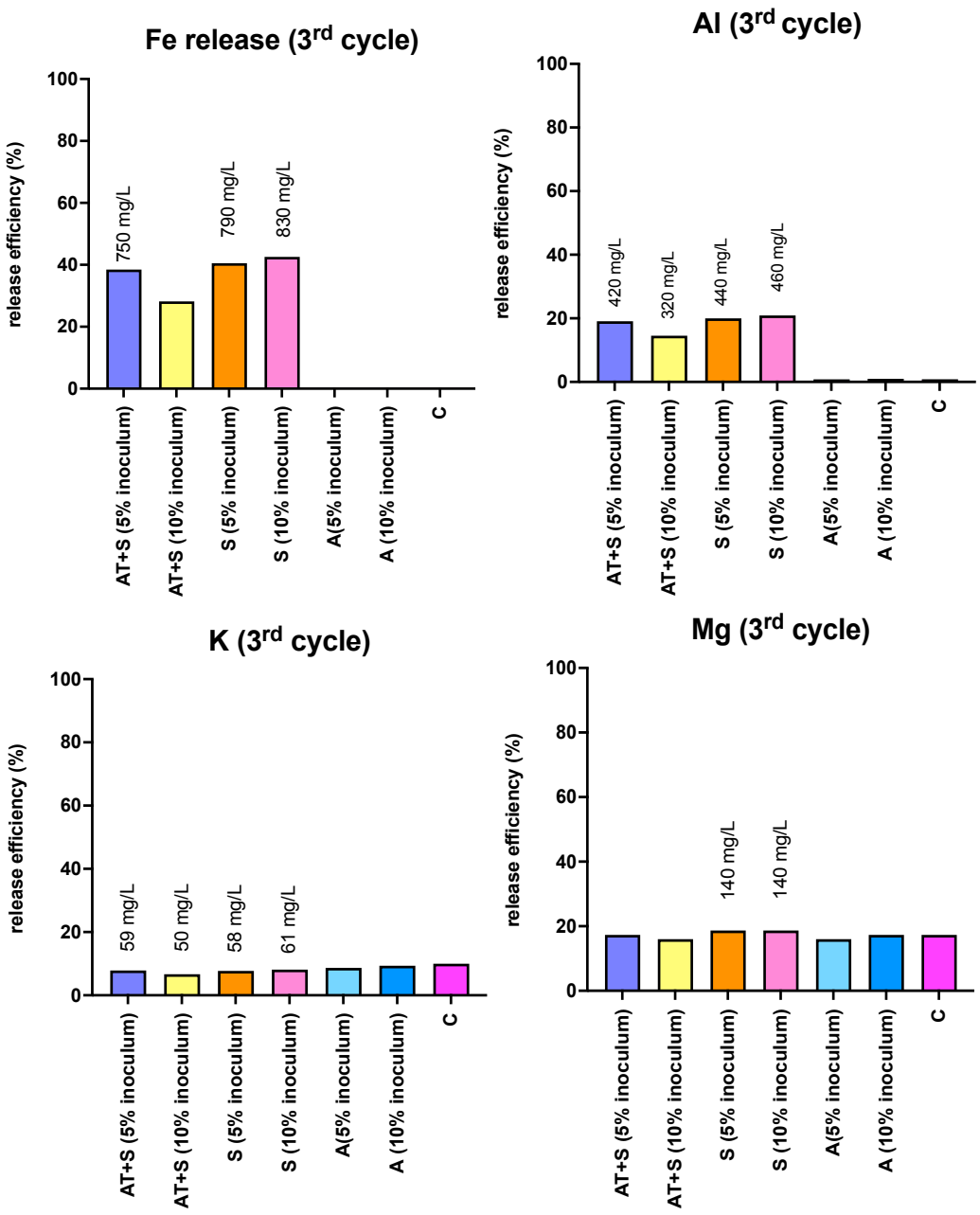

Figure 10 Metal concentrations at the end of 3rd cycle

More detailed characterization was done for the 1st cycle of the samples (Figure 11) and the analysis is onging for other cycles. The results of Al and Fe concentrations showed there is an increasing trend from the beginning of the experiments for the samples with sulfur addition (A+S and S) until day 18. It was concluded that there would be more release after 18 days and may reach a stationary phase afterwards. PO_4-P concentrations were higher for A+S samples at the beginning due to presence of phosphorus in the inoculum medium, however they were dropeed to low levels until day 6. PO_4-P concentrations started to increase after day 6, probably due to lower pH values. The highest PO_4-P concentration was achieved as 14 mg/L. SO_4 concentrations showed an increase trend until the end of the experiments for A+S and S

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samples, and the rate of SO_4 increased after day 18. These results showed that the retention time should be increased to observe better metal and phosphorus release as well as sulfur oxidation.

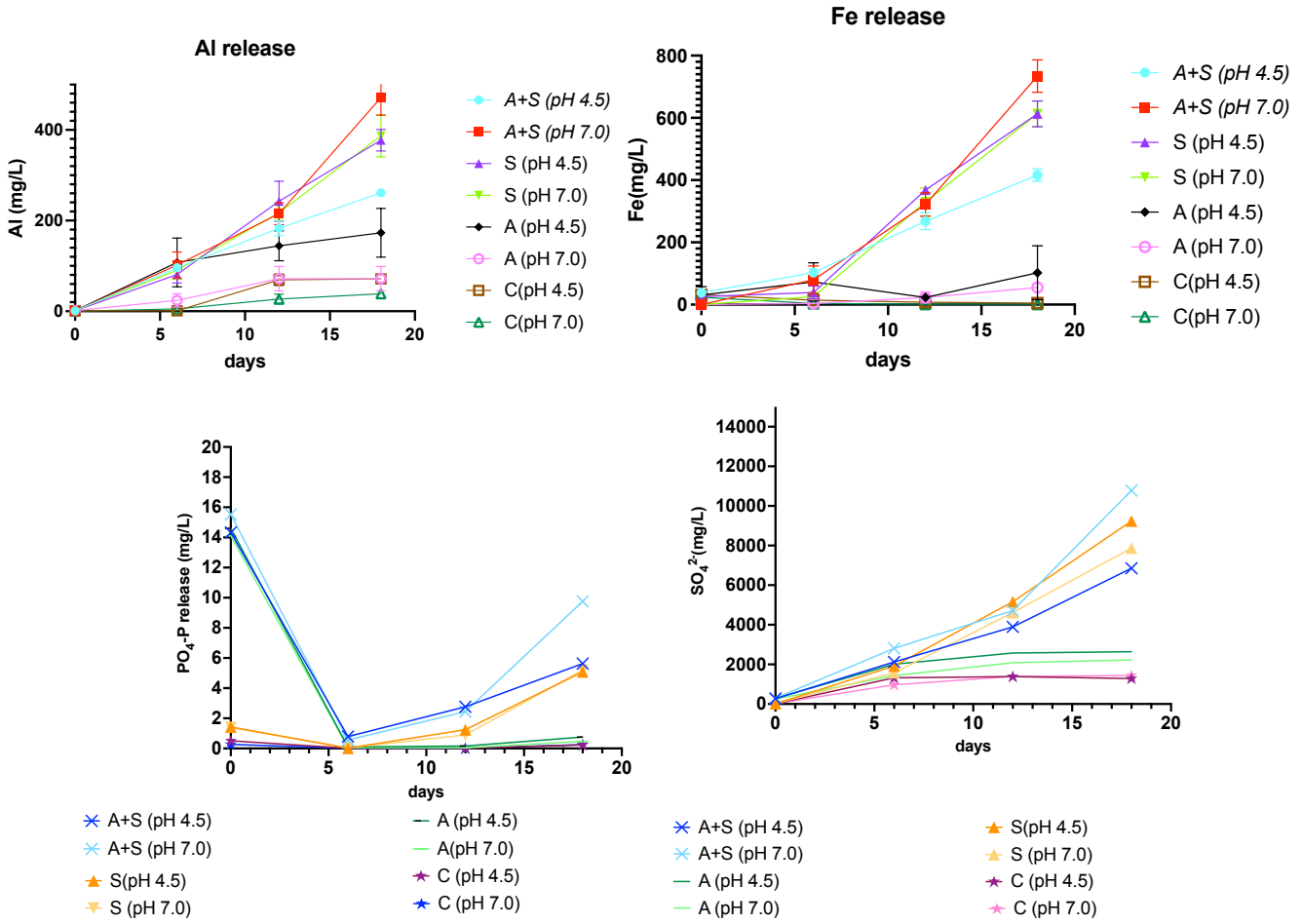

Figure 11 Al, Fe, PO_4-P and SO_4 concentrations in the flasks (1st cycle)

1.2.2 Bioleaching experiments with *Acidithiobacillus Ferrooxidans*

The aim of this set was to investigate the use of *Acidithiobacillus ferrooxidans* as a well-known bioleaching bacterium to release metals from the Baltic Sea sediment.

Cultivation of the A. ferrooxidans

A.ferrooxidans was purchased from DSMZ (DSM 583) and it was cultivated according to instructions by DSMZ (Medium 882) and autoclaved before use. In order to grow *A. ferrooxidans,* 1 mL of suspension was added to the growth tubes including 9 mL of medium. The tubes were also diluted 10-100 times. Later, the tubes were incubated at 30°C without mixing. The cultures were reinoculated weekly by transferring 1 mL of each culture to 9 mL fresh medium and this procedure was repeated twice. Later, the volume of the cultures was increased up to 400 mL by keeping inoculum ratio as 10 % (v/v) at 30°C with mixing. Growth of *A. ferrooxidans* was monitored using OD_{600}.

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Bioleaching experiments

The experiments were conducted in batch bioreactors set up in 250 mL Erlenmeyer flasks with a working volume of 150 mL. TS of the sediment were adjusted to 10% and 5% by adding water. Both Fe2+ (0-10 g/L) and S (0-5 g/L) were added as energy sources. Initial pH was set to 2.0, 4.5 and 7.0. Inoculum ratio was set as 7.5% The flasks were incubated at room temperature at 180 rpm for 18 days. Each run was done in duplicate. Fe and Al were chosen as the target metals based on the characterization data and results of the previous set (Section 1.2.1) concentrations were measured by ICP after centrifuging the samples and filtering the supernatant.

Results and discussion

pH values of the bioleaching experiment (Figure 12) with *A. ferrooxidans* showed a sharp pH decrease for the samples which had initially pH as 4.5 and 7.0. On the other hand, for the samples with an initial pH of 2.0 increased at the beginning. After 18 days, the highest pH values were observed as 3.25 and 3.26 without sulfur/iron addition. Lowest pH value was observed as 1.75 with sulfur and iron addition.

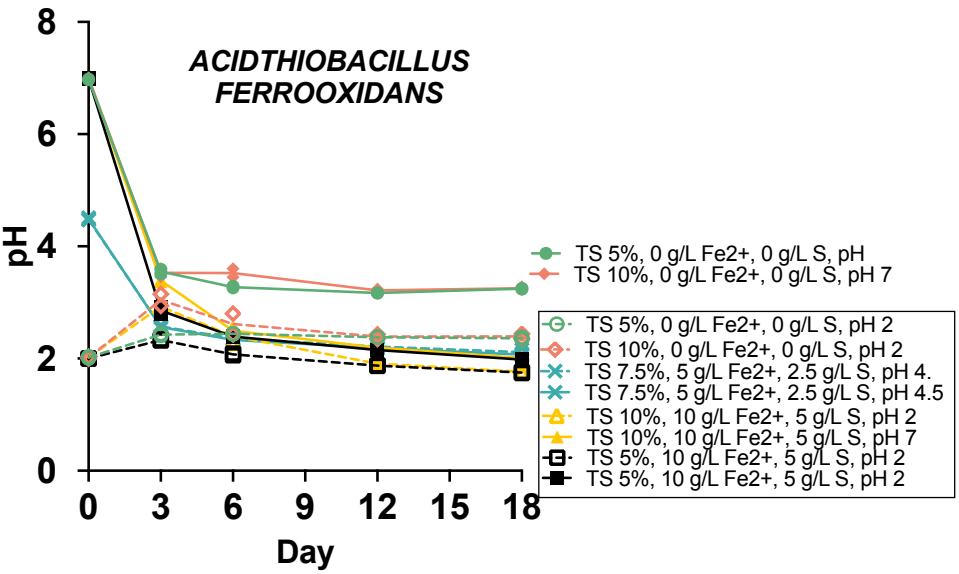

Figure 12 pH changes during bioleaching experiments with A. ferrooxidans

In this set, Al and Fe concentrations were measured during bioleaching experiments and the results at the end of the experiment (day 18) were given in Figure 13. Fe precipitation was observed on day 0 for the samples having an initial pH 7.0 and iron addition conditions. As pH was set as 2.0 at the beginning (day 0), Fe leaching was observed due to acid addition. The highest Fe solubilization was observed as 205 mg/ at 5% TS, with no added Fe^{2+}/S and initial pH 2.0. It was obvious that external Fe addition did not improve Fe release from the sediment. The highest Al solubilization was observed as 621 mg/L with 10% TS, 10 g/L Fe^{2+}, 5 g/L S and initial pH 2.0.

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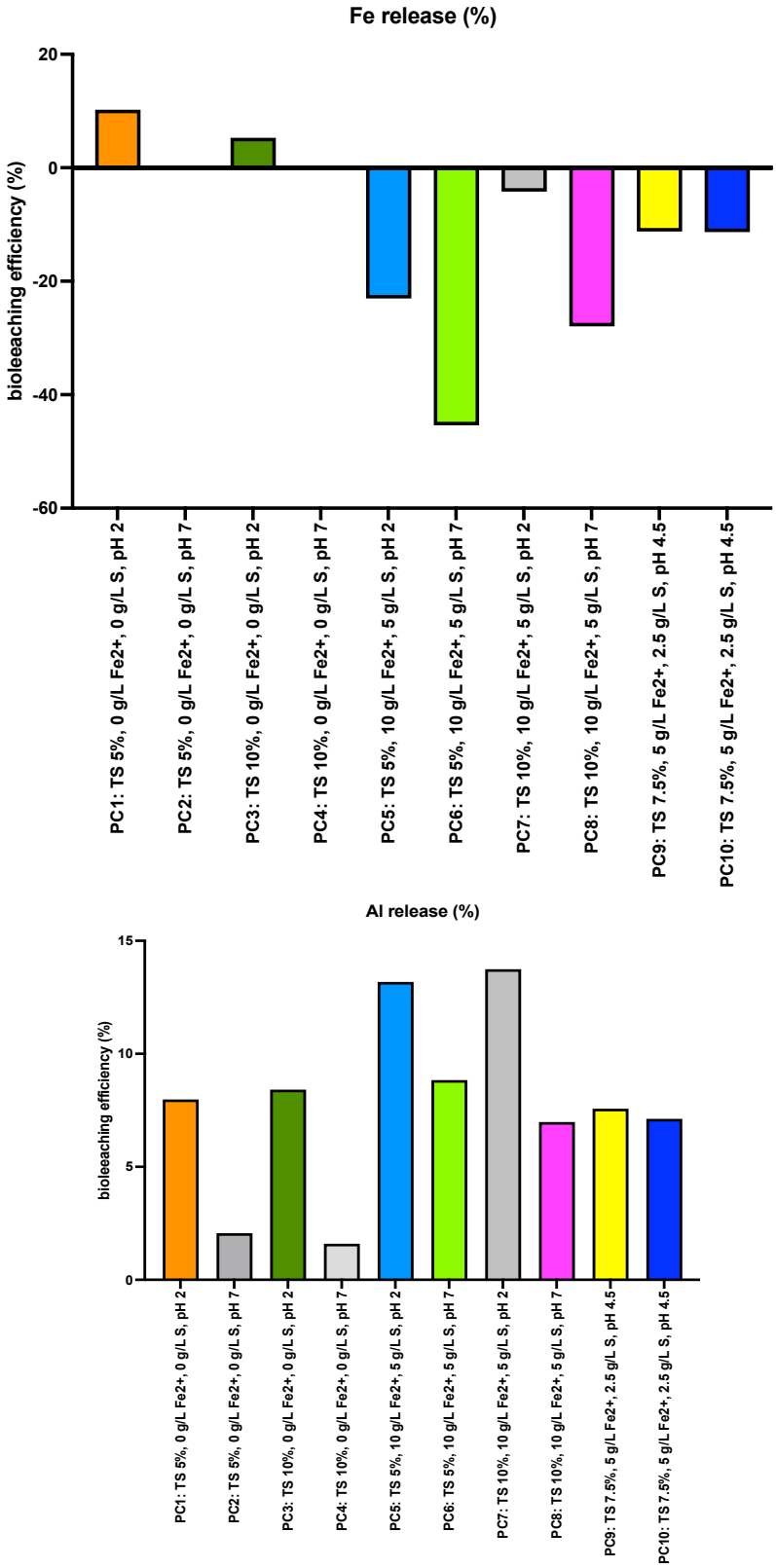

Figure 13 Fe and Al Bioleaching efficiency (%) with A.ferrooxidans

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1.2.3 Bioleaching experiments with Sediment Enriched Iron Oxidizing Bacteria

The aim of this set was to investigate the use of iron oxidizing bacteria enriched from the Baltic Sea sediment to release metals from the sediment.

Enrichment stage

300 mL of the sediment (5% TS was) added to a 500 mL Erlenmeyer flask with 20 g/L $FeSO_4$ x $7H_2O$ and the mixture is incubated at room temperature with 180 rpm. The pH decreased was monitored until it reached to 2.5. Later, 30 mL of the enriched sediment was added to 270 ml fresh sediment (5%) with 20 g/L $FeSO_4$ x $7H_2O$. 4 consecutive cycles were run for a total of 18 days.

Bioleaching experiments

The experiments were conducted in batch bioreactors set up in 250 mL Erlenmeyer flasks with a working volume of 150 mL. TS of the sediment were adjusted to 10% and 5% by adding water. Fe2+ (0-20 g/L) was added as energy source. Initial pH was set to 2.0, 4.5 and 7.0. Inoculum ratio was set as 7.5% The flasks were incubated at room temperature at 180 rpm for 12 days. Each run was done in duplicate. Fe and Al concentrations were measured by ICP after centrifuging the samples and filtering the supernatant.

Results and discussion

Figure 14 shows the pH values during bioleaching with enriched iron oxidizing bacteria. pH values showed a sharp decrease for the flasks with initial pH values of 7.0 or 4.5. On the other hand, flasks with an initial pH of 2.0 slightly increased until day 3. The lowest final pH was observed with 20 g/L Fe^{2+} and an initial pH of 2.0.

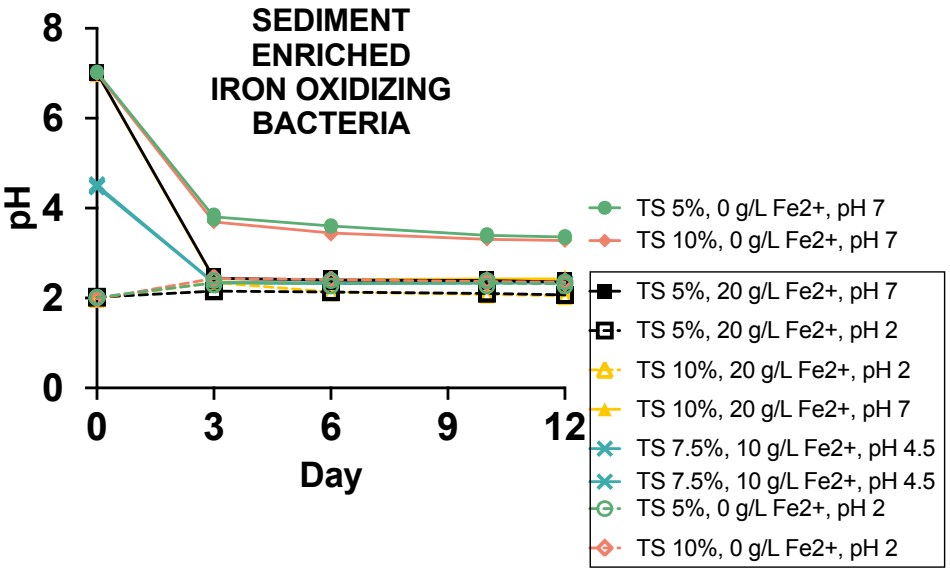

Figure 14 pH changes during bioleaching experiments with sediment enriched iron oxidizing bacteria

Figure 15 depicts the Fe and Al bioleaching efficiencies. Negative Fe leaching efficiency means that iron precipitated as the solid fraction. More precipitation was observed with higher initial pH and lower TS

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content (5%). At initial pH 2.0, some initial chemical leaching was observed due to acid addition. At the end of the experiments, the highest iron solubilization was achieved as 207 mg/L with the initial pH of 2.0, with 5% TS and no added Fe^{2+}. The highest Al leaching was observed as 400 mg/L with 10% TS, 20 g/L added Fe^{2+} and initial pH 2.0. As the TS%, Al concentration was 232 mg/L.

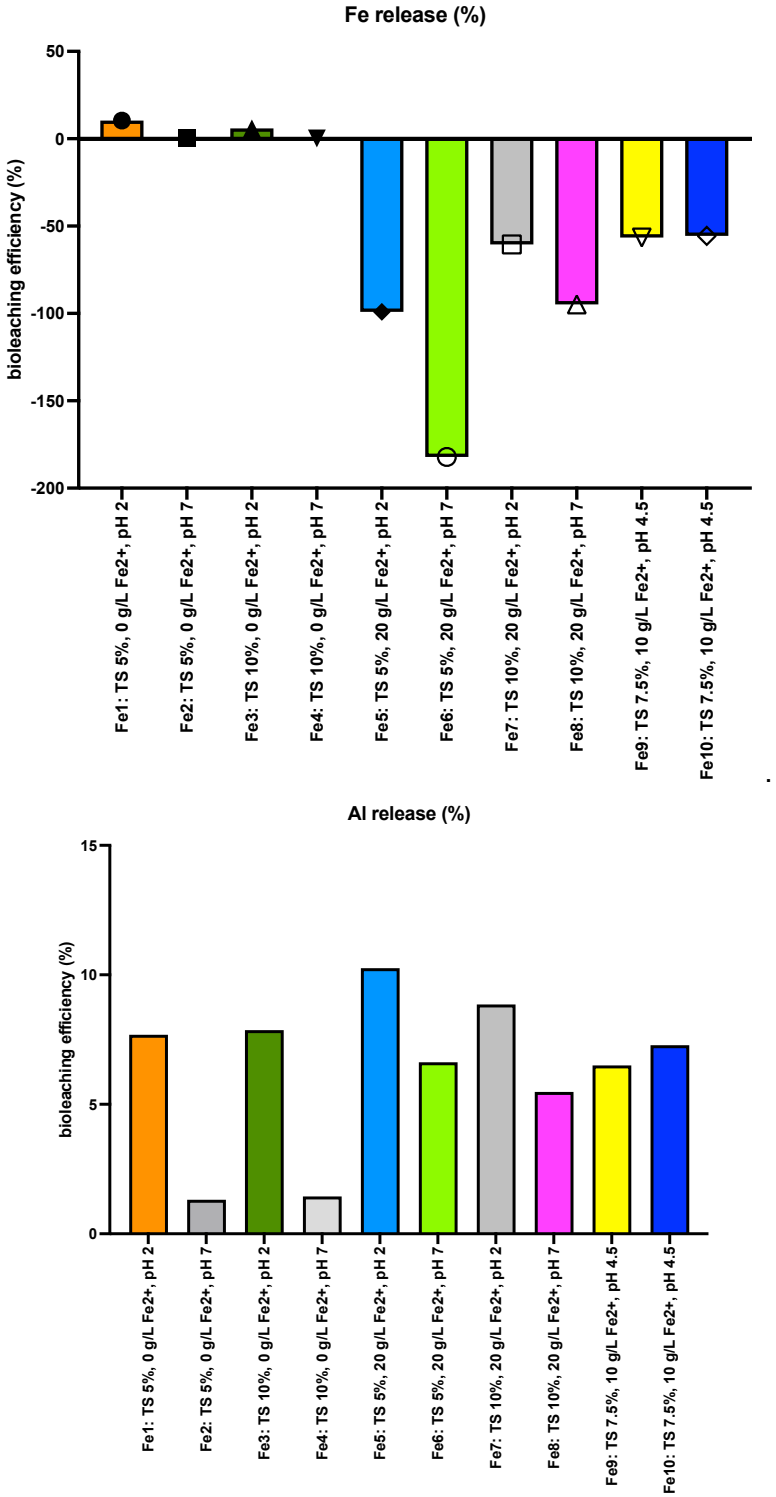

Figure 15 Fe and Al Bioleaching efficiency (%) with enriched iron oxidizing bacteria

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1.2.4 Bioleaching experiments with Sediment Enriched Iron and Sulfur Oxidizing Bacteria

The aim of this set was to investigate the use of iron and sulfur oxidizing bacteria together for bioleaching experiments which were enriched from the Baltic Sea sediment.

Enrichment stage

The enrichment of sulfur oxidizing bacteria was based on adding elemental sulfur to the sediment promoting the growth of sulfur oxidizers. Briefly, 300 mL sediment (5% TS) was added to an Erlenmeyer flask with 10 g/L elemental sulfur and incubated at room temperature with 180 rpm. The pH change was monitored until 2.0. Later, 30 mL of the sediment was transferred to 270 mL fresh sediment (5%) with 10 g/L added sulfur. Enrichment was done in 8 cycles for 55 days. For the enrichment of iron oxidizing bacteria, 300 mL sediment (5% TS) containing 20 g/L $FeSO_4$ x $7H_2O$ incubated at room temperature with 180 rpm. As the pH reached 2.5, 30 mL of the enriched sediment was added to 270 mL Leathen medium with the following composition: 0.45 g/L $(NH_4)_2SO_4$, 0.15 g/L K_2HPO_4, 0.05 g/L KCl, 0.5 g/L $MgSO_4$ x $7H_2O$ and 0.01 g/L $Ca(NO_3)_2$ x $2H_2O$, along with 20 g/L $FeSO_4$ x $7H_2O$ and incubated. The enrichment was done in 5 cycles for 32 days.

Bioleaching experiments

The experiments were conducted in batch bioreactors set up in 250 mL Erlenmeyer flasks with a working volume of 150 mL. TS of the sediment were adjusted to 10% and 5% by adding water. Both Fe2+ (0-10 g/L) and S (0-5 g/L) were added as energy sources. Initial pH was set to 2.0, 4.5 and 7.0. Inoculum ratio was set as 7.5% (sulfur oxidizing bacteria: 3.5% and iron oxidizing bacteria:3.5%). The flasks were incubated at room temperature at 180 rpm for 18 days. Each run was done in duplicate. Fe and Al concentrations were measured by ICP after centrifuging the samples and filtering the supernatant.

Results and discussion

Figure 16 shows the pH changes in the bioleaching experiment with enriched iron and sulfur oxidizing bacteria as inoculum. The flasks with initial pH of 7.0 or 4.5 showed a sharp decrease until day 3 and the pH of the flasks with initial pH of 2.0 increased slightly. The flasks with added Fe^{2+} and S and an initial pH of 2.0 had the lowest pH at 1.53-1.54 after 18 days.

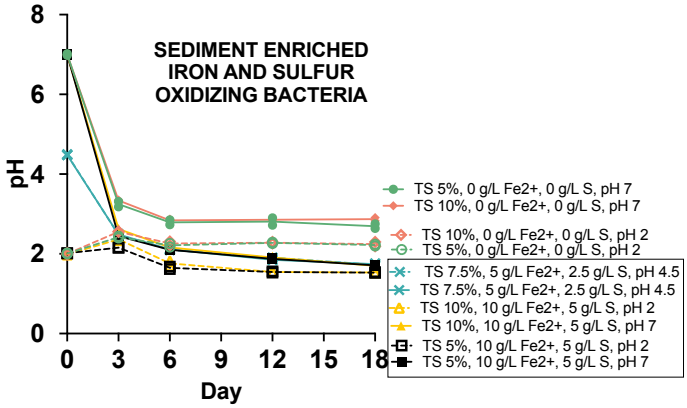

Figure 16 pH changes during bioleaching experiments with sediment enriched iron and sulfur oxidizing bacteria

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Figure 17 shows the bioleaching efficiencies of Fe and Al. As in the previous sets, precipitation was also observed in this set. The highest dissolved iron concentration was 2332 mg/L with 7.5% TS, 5 g/L Fe^{2+}, 2.5 g/L S and initial pH 4.5. In terms of Al, highest concentration was achieved with 10 g/L Fe^{2+} and 5 g/L S and an initial pH of 2.0 and the values were 413 mg/L and 810 mg/L for 5% TS and 10% TS, respectively. In this set PO_4 release was also observed as 11.63 mg/L at 5% TS, 10 g/L Fe2+, 5 g/L S and initial pH 2 corresponding to a phosphate concentration of 11.63 mg/L. Moreover, SO_4 values indicated that there was an increase in the SO_4 concentration when S was added to the flasks showing the oxidation of sulfur.

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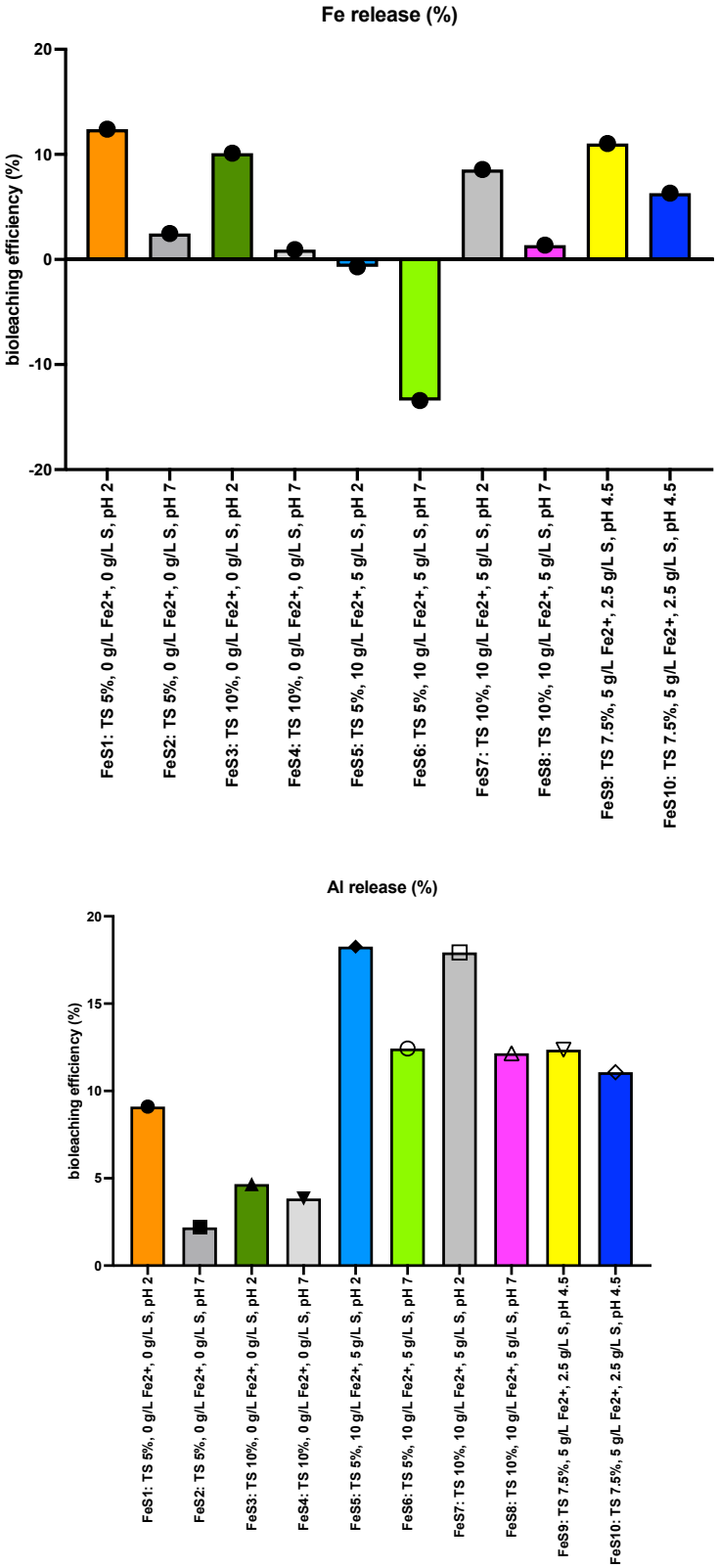

Figure 17 Fe and Al Bioleaching efficiency (%) with enriched iron and sulfur oxidizing bacteria

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1.3 PANI Acidification

The aim of this subtask is to acidify sediment- water mixture to reach initial pH as 4.5 for bioleaching experiments without need for any chemicals. Polyaniline (PANI), a conductive polymer renowned for its electrochemical properties, functions as an effective electrochemical proton pump. In this study, a reagentless electroanalytical method utilizing a PANI mesh-array to regulate the acidification process of sediment samples is introduced. The PANI mesh was synthesized electrochemically onto stainless steel meshes, which were utilized as the working electrode for proton delivery within a 40 ml cell. The related electrochemical cell setup for sample acidifying is shown in Figure 18.

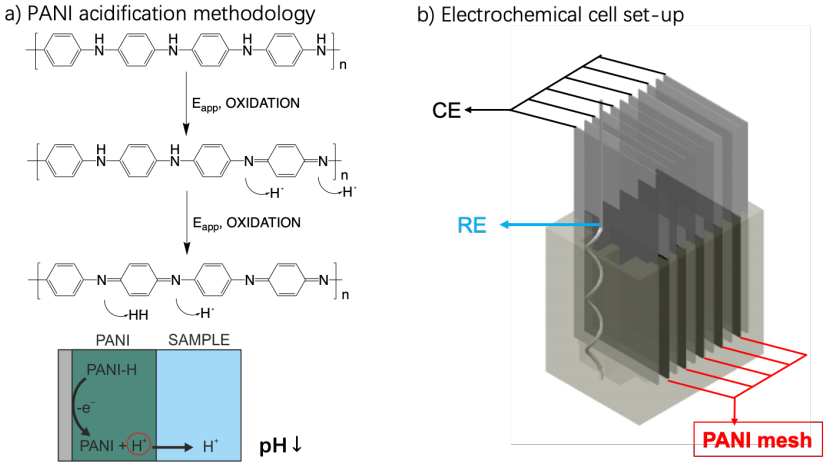

Figure 18 a) the PANI acidification methodology, the electrochemical set up for the PANI acidification system. Ag/AgCl wire is used as the reference electrode (RE), screen printed carbon electrodes are used as the counter electrodes (CE), working electrodes (WE) are the PANI coated meshes.

A constant potential (0.4V) was applied on PANI meshes to deliver proton into the sample solution. By increasing the area of PANI mesh by increasing the number of meshes in the system, the final pH of the sample can be controlled in a lower range (below pH 4). By employing different numbers of PANI meshes, the final pH of the sediment sample can be controlled. In the experimental setup, employing three PANI meshes allowed to well adjust the final pH of the sediment sample to around 4.50. This was achieved while maintaining a constant potential of 0.4 V applied to the PANI array for a duration of 1200 s. To ensure prolonged functionality and usability of the PANI mesh, it can be regenerated in an acidic solution. This feature significantly extends the lifetime of the PANI mesh, enabling multiple uses for efficient proton delivery.

Bioleaching experiments combined with PANI Acidification

In this part, bioleaching experiments were designed to observe the difference between PANI and acid addition at the beginning of the experiments on metal and phosphorus release efficiency. The experiments were conducted in 100 mL glass Erlenmeyer flasks with a working volume of 40 mL. The TS content of the sediment in the flasks was set as 5% by adding saline water (6 psu). 0.4 g Sulfur was added to the flasks to promote sulfur oxidizing bacteria in the sediment and to observe their effect on the bioleaching performance. The control sets were prepared without addition of sulfur. Initial pH of the one set of bioleaching samples was reduced to pH 4.5 with PANI. On the contrary, HCl was added to the other set of bioleaching samples to achieve initial pH of 4.5. The flasks were shaken on an orbital shaker at 180

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rpm for 18 days. During the experiments, pH, PO_4-P and SO_4 were measured. Metal concentrations will be also measured.

Figure 19 shows pH change in the flasks starting with an initial pH 4.5. The pH results showed that initial pH adjustment with PANI or acid addition coupled with sulfur addition promoted the pH decrease. At the end of the experiments, pH was decreased to 1.39 and 1.42 for PANI acidified and acid added samples, respectively. On the other hand, the lowest pH values were 2.60 and 3.02 for PANI acidified and acid added samples without sulfur addition. According to PO_4-P results, at the end of day 18, the maximum PO_4-P concentration was reached as 4.4 mg/L with acid assisted bioleaching. On the other hand, the concentration was 3.6 mg/L PANI assisted bioleaching.

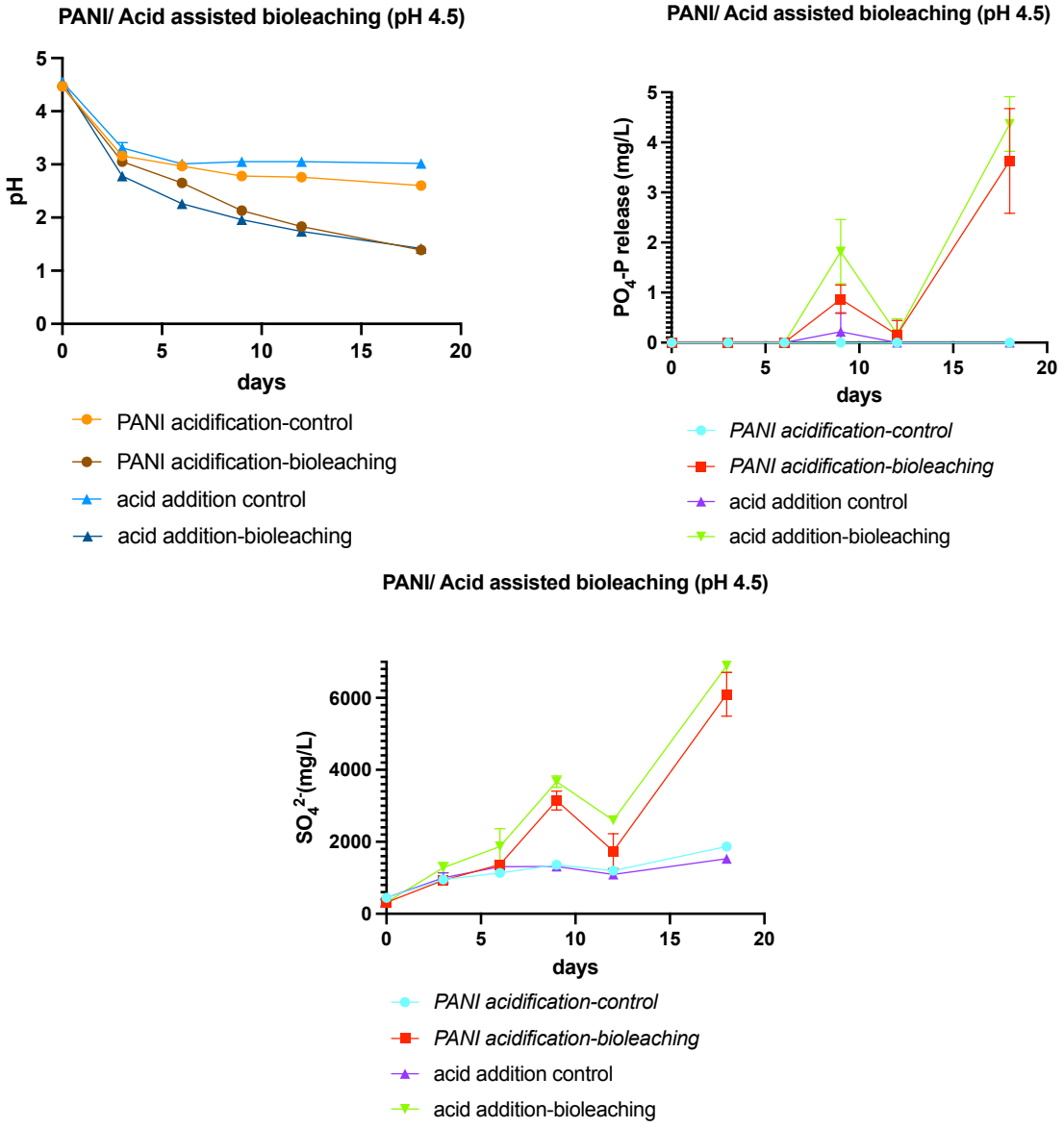

Figure 19 PANI Acidification coupled bioleaching results

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1.4 Phosphorus Recovery Experiments

The aim of this part is to provide technical background including simulations and experiments for phosphorus recovery potential from the marine sediment after phosphorus is retained in the liquid after anaerobic bioreactor operation/bioleaching.

1.4.1 Simulation

Methodology

Thermodynamic simulations were carried out using free open-source software PHREEQC (PHREEQC for Windows version 3, USGS). The simulation is carried out based on composition of solution obtained through characterization of the fermentation effluent (base case). Simulations were performed to evaluate the effects of different parameter on the supersaturation index (SI) of struvite, as a measure of struvite's tendency to precipitate. Struvite was chosen due to the presence of P and NH_4^+ in solution, both ions that comprise the struvite molecule and that need to be removed from the solution. Parameters varied were pH (7.5 – 10.0), concentration (based on 50 wt% of solution water removal), temperature (-20 °C to 100 °C), presence of impurities (Al) and addition of reagents ($MgCl_2$ and NH_4OH).

Results and discussions

The results obtained from all the characterization processes are summarized in Table 2

Table 2 Initial solution characterization

Parameters	Base solution
pH	7.45
Temperature (°C)	20
Phosphorus (PO_4^{3-}) (ppm)	71
Nitrogen (NH_4^+) (ppm)	74
Magnesium (Mg^{2+}) (ppm)	105
Aluminium (Al) (ppm)	3

Based on the results obtained the molar ratio of base solution is calculated. The molar ratio of P: N: Mg is 1: 2.31: 1.88.

The thermodynamic simulation was carried out in PHREEEQC software based primarily on simulation plan from. Base solution was simulated with the data from Table 2. The solution has supersaturation index value (SI) of 0.60, which confirms that solution is undersaturated at this stage for precipitation of struvite. The results obtained are summarized below.

Effect of pH

The effect of pH was simulated using the solution parameters from Table 1 and varying pH from 7.5 to 10. The results in the form SI vs pH graph are shown in Figure 20.

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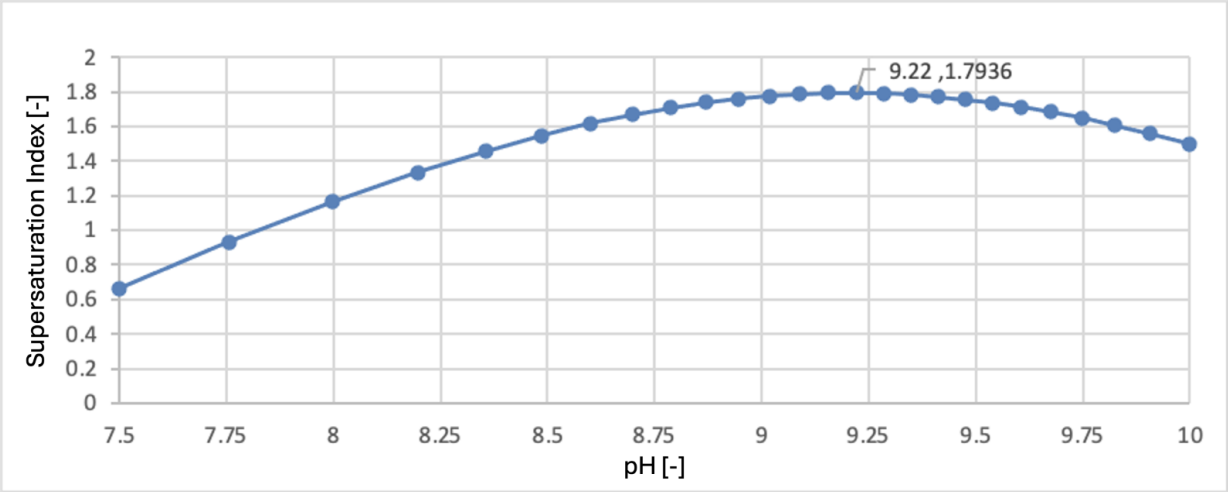

Figure 20 Effect of pH on the supersaturation index of struvite

It can be seen that with increase in pH, the supersaturation index increases until a maximum value of approximately 1.79 at pH 9.22, after which it starts to steadily decline. In principle, higher SI value implicates higher driving force for crystal nucleation, which can affect how fast struvite crystallization happens and the yield of struvite. The yield of struvite increases until maximum yield at pH of 9.22. The simulation can also predict the amount of struvite that can precipitate. The yield obtained between standard solution and pH 9.22 solution varies from 0.0004 to 0.00228 moles, which removes almost all phosphorous present the solution at the start of the simulation. This highlights the high efficiency of P removal possibilities just by changing the pH. It is noteworthy that the simulations consider thermodynamics in equilibrium, disregarding kinetics.

Effect of impurities

The bioreactor product contains impurities like Al^{3+} in trace amounts. The ICP measured Al content is about 3 mg/L which in terms of moles about 0.0001 moles. Simulation varied moles of Al in solution up to 0.001 moles (10 times the solution content). The results show that Al concentration has negligible impact on SI values, from 1.80 to 1.76 with concentration increase from 0.0001 to 0.001, respectively (Figure 21).

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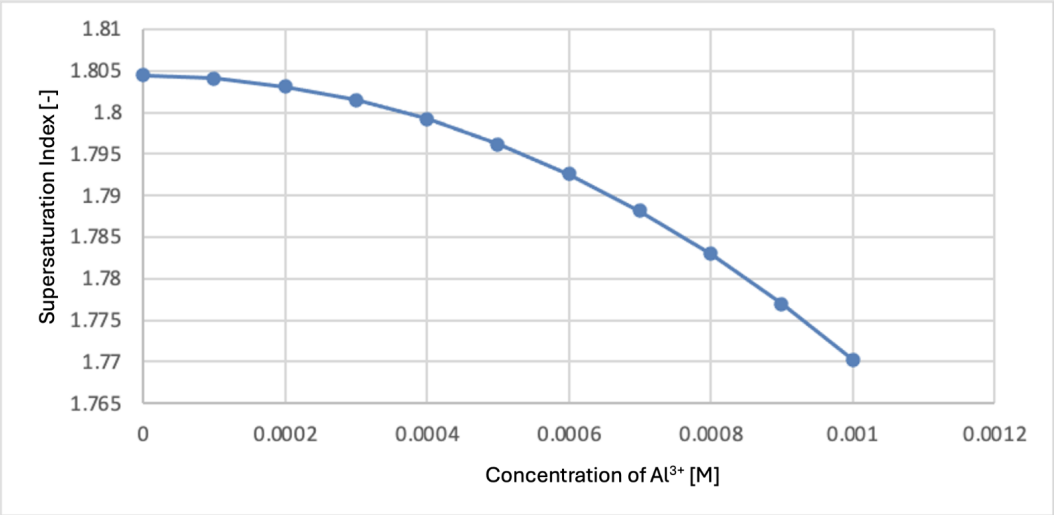

Figure 21 Effect of Al ions concentration on the supersaturation index of struvite.

Evaporation

Simulations were performed by heating the base solution to 100 °C, followed by the removal of 50 % of the water (Figure 22). It can be observed that the heating of the solution causes SI to become negative. This prevents struvite precipitation, since the solution becomes highly undersaturated (SI < 0). However, the water removal increases the SI value highlighting the increase in concentration of ions in the solution, as expected. The concentrated solution was simulated for cooling process by lowering temperature to 20 °C. SI for struvite after evaporation and cooling was found to be 1.18 and 2.42, for natural (7.45) and adjusted (9.22) pH.

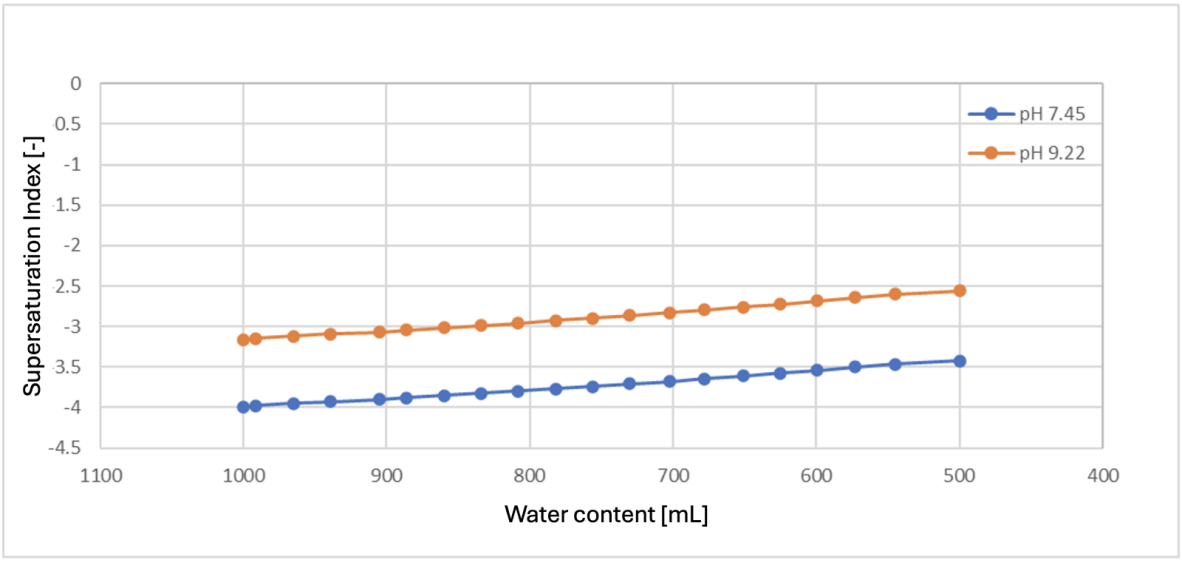

Figure 22 Effect of water removal on the supersaturation index of struvite.

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Temperature effect on struvite formation

The temperature effect was assessed by changing the temperature from 100 °C to -20 °C while keeping volume and pH at constants. The results are presented in Figure 23. It can be observed that the SI value increases with decrease in temperature, from 100 °C to -20 °C), from -4.01 to 3.55 in case of pH 7.45 and from -3.19 to 5.16 in case of pH 9.22. Despite the great increase in the SI with the cooling, the results from these simulations are, however, inaccurate due to the software's inability to simulate the formation of ice throughout the temperature change.

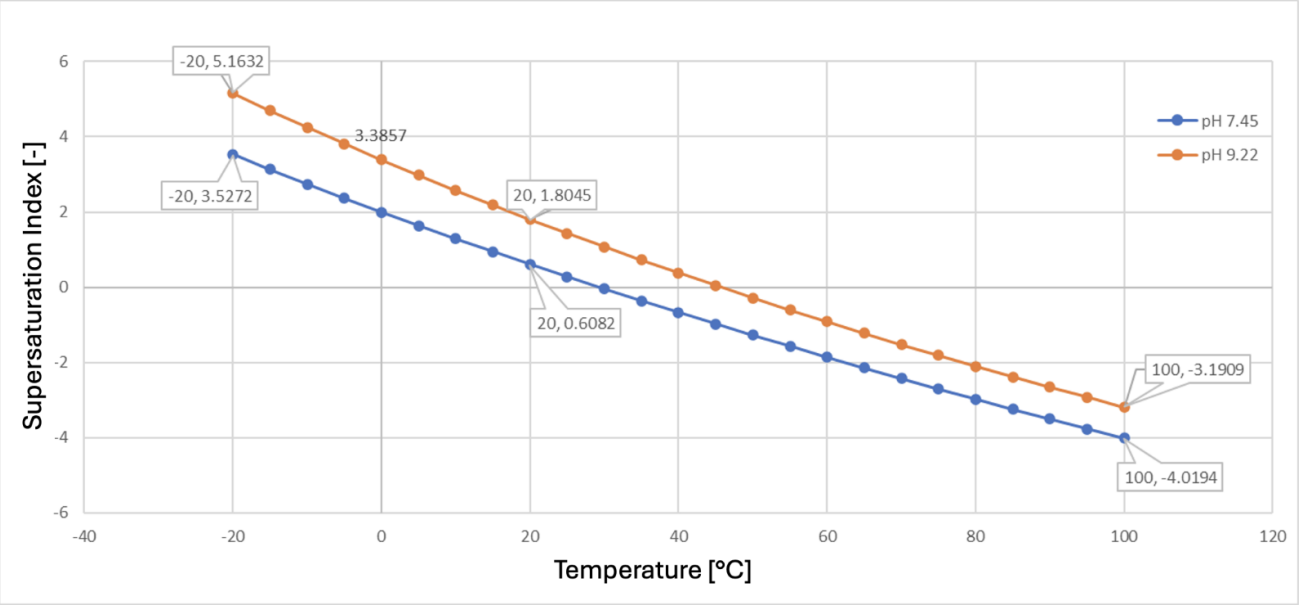

Figure 23 Effect of temperature on the supersaturation index of struvite.

Addition of NH_4OH

The base solution has molar ratio of P:N: Mg is 1:2.31:1.88. The simulations on different molar ratio of P:N is increased from 1:2.31 to 1:2.5, 1:3, 1:3.5 and 1:4 through addition NH_4OH. The results are shown in Figure 24. From the graph it is observed that addition of NH_4OH showed huge increase in SI values of simulations with base pH 7.45 solution and evaporation case with pH 7.45. increasing SI values from 0.6 and 1.18 to 2 and 2.60 when P: N ratio increased from 1:2.31 to 1:4. This SI increase is result of increase in pH of solution with the addition of NH_4OH. In the case of pH 9.22, the increase in SI values is relatively low as SI increases from 1.8 to 1.98 and 2.4 to 2.56 when P: N ratio increased from 1:2.31 to 1:4. The minimal increase SI is due to the already alkaline nature of pH adjusted solution as compared to standard.

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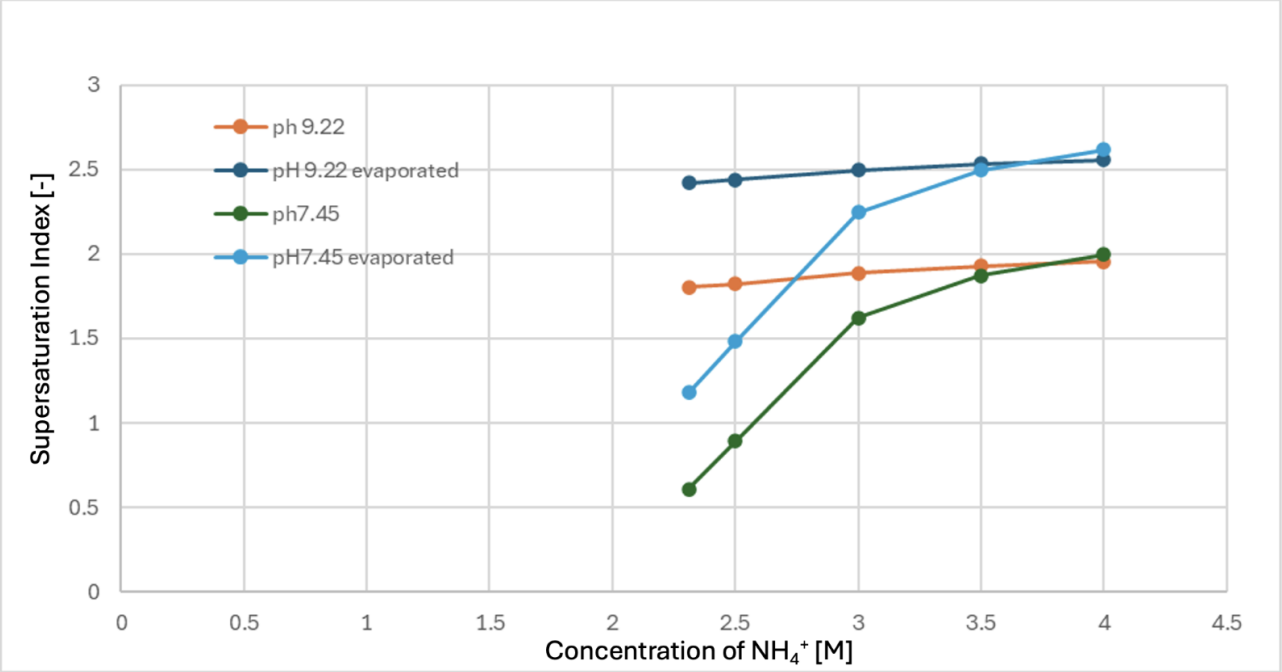

Figure 24 Effect of ammonium ions on the supersaturation index of struvite.

Addition of $MgCl_2$

The molar ratio of P:Mg was simulated from 1:1.88 to 1:2, 1:2.5, 1:3 and 1:3.5 through addition MgCl2. From Figure 25, it is observed that addition of $MgCl_2$ results in decreasing in SI value with increasing in P:Mg ratio. In case of base case pH 7.45 and 9.22, the SI decreases slightly from 0.60 to 0.56 and 1.82 to 1.80 respectively with molar ratio increase, while for the concentration after the evaporation step, the SI decreases from 1.18 to 1.10 and 2.42 to 2.39 respectively. The concentration of Mg2+ ions have little to no impact on SI due to the already high P: Mg molar ratio of the initial solution. This is aligned with the literature which suggests that the increase in Mg molar ratio above 2 shows negative impact on SI values.

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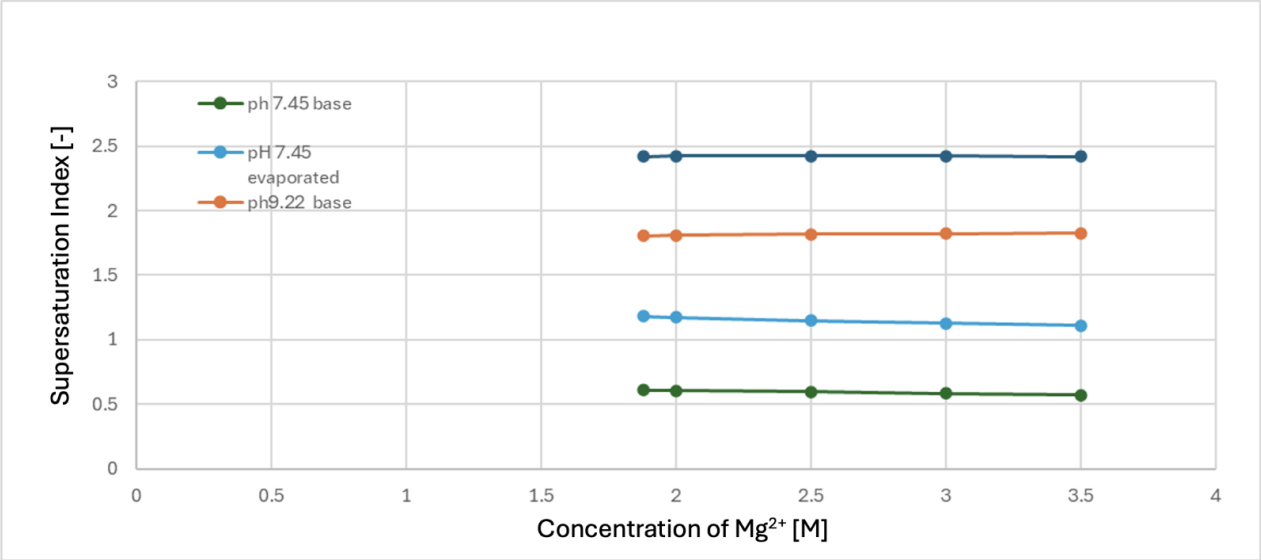

Figure 25 Effect of magnesium ions on the supersaturation index of struvite.

1.4.2 Struvite precipitation

Methodology

The same conditions from the thermodynamic simulations were performed experimentally to try and validate the data. Experiments performed were: pH adjustment from 7.45 to 9.22, evaporation of 50 % of the water in both pH and cooling crystallization from 20 °C to 7 °C with pH 9.22. Solid samples were characterized using X-ray diffraction and optical microscopy. Liquid samples were characterized via ICP-OES. PO4-P and NH4-N were analyzed using Hach kits.

Results and discussions

Table 3 shows the results for P, N and Mg content, as well as the experimental and simulated yield of struvite formation to compare the efficiency of P removal and solid formation.

Table 3 Solution composition during experimental analysis.

Content	PO_4-P (mg/L)	NH_4-N (mg/L)	Mg^{2+} (mg/L)	Experimental yield (g)	Simulated yield (g)
Initial solution – pH 7.45	68.7	75.0	105.0		
pH-adjusted 9.22	31.0	47.0	72.4	0.126	0.496
Evap. – pH 7.45 – Concentrate	124.2	69.2	195.6	0.011	0.149

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Evap. – pH 7.45 – Condensate	0.2	74.8	0.001		
Evap. – pH 9.22 – Concentrate	110.4	39.4	183.6	0.074	0.550
Evap. – pH 9.22 – Condensate	0.05	98.0	0.003		
Cooling – pH 9.22	21.0	34.0	60.0	0.158	0.520

It can be seen that as result of evaporation process, concentration of P and Mg in the solution are almost doubled. However, low concentration of NH_4^+ ions was verified in the concentrate. This is a result of evaporation of ammonia during the heating process because of its volatile nature. This in turn decreased the molecular ratio of P:N and reduces the yield of struvite crystallization by lowering the supersaturation of solution. Both the pH-adjusted and the cooling crystallization showed a decrease in the concentration of P, N and Mg, which matched the higher yield of solids obtained in these scenarios. It can be seen that efficiency of struvite removal is much lower than that predicted through PREEQC simulations. The high efficiency of predicted through PHREEQC is inherently inaccurate due to its limitations regarding simulation of thermodynamic equilibrium and not considering reaction kinetics which has significant impact on crystallization/precipitation processes. In case of the concentrated samples, lower precipitation efficiency is partly a result of loss of large amounts ammonia ions during the evaporation process.
Figure 26 shows the XRD analysis of sample obtained from pH adjustment. Most of the peaks match the peaks from the standard struvite sample, identifying the precipitate as struvite. Figure 24 (b) shows an optical microscopy of the crystals, displaying typical struvite needle-like morphology.

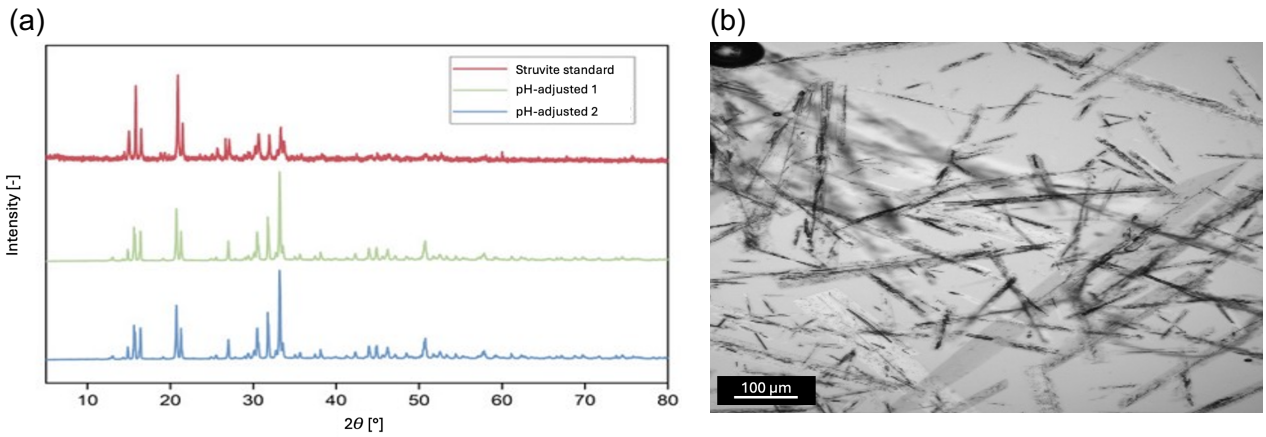

Figure 26 (a) XRD analysis for struvite identification (b) optical microscopy of crystals obtained from the pH adjustment experiment

Thermodynamic simulations can be used to find optimal parameters for struvite precipitation considering specific characteristics of the solutions. Results for pH-adjustment, evaporation and cooling all showed promising results in terms of increase in the supersaturation index for struvite. Addition of ammonium and magnesium containing reagents, on the other hand, showed little to no effect on the struvite SI.

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Data from the simulations was used to guide experimental work. Concentration step with evaporation showed an increase in concentration of P and Mg, as expected, yet NH_4^+ did not follow that trend and was found to evaporate along with the water, hence hindering the formation of struvite. Only pH adjusted and cooling experiments showed solid formation. Despite the formation of struvite, the yield was not higher than 30 %. Kinetics of crystallization of struvite needs to be better investigated.

1.5 Metal Recovery Experiments

The aim of this part to investigate metal recoveries from liquid media after bioleaching occurs. Adsorption membranes are needed as a post-treatment after bioleaching to capture released heavy metal ions and metalloids. Herein, two different functional adsorbent materials were developed and tested, both biobased. The first adsorbent type was prepared by sulfonation of alkali lignin (recovered from canola straw in an in-house biorefinery process) and the second was prepared by carboxylation of microfibrillated cellulose (recovered from green algae according to our previously developed process)[12] producing polymer materials with high surface anionic charge.
Each adsorbent was integrated as a sandwiched layer within a cellulose matrix. Each membrane was produced in three steps: i) adding 250 mL of a cellulose pulp dispersion (pre-beaten to avoid large agglomerates) to a glass-sintered funnel (diameter of 10 cm) followed by suction filtration until a semi-solid surface is achieved, ii) adding 50 mL of an aqueous dispersion of the adsorbent onto the never-dried cellulose layer, and iii) adding another 250 mL of the cellulose pulp dispersion followed by suction filtration until a semi-solid surface is achieved. The resulting three-layered membrane was then gently removed from the funnel and allowed to dry to constant weight under pressure. Five replicates of each membrane were produced.

Recovery experiments

Four bioleached water samples from the previous experiments (A+S pH 4.5, A+S pH 7.0, S pH 4.5 and S pH 7.0) were received for metal removal experiments. 50 ml of each sample were passed through each membrane without any external pressure (Figure 27). Clear filtrate without any visible particle/fiber was collected and stored in centrifuge tubes for further analysis with ICP-OES.

As per the quantitative results that were obtained for water samples before and after membrane treatment, the residual metal concentrations in water are tabulated. 32 elements were analyzed by ICP-OES to have a comprehensive look at the membrane activity (Table 4). Among them it can be observed that, broadly, both the membranes are able to scavenge in the range of 50-80% of most of the elements present in water. However, ML(lignin membrane) performed better than MC (cellulose membrane) in the given conditions. Elements highlighted in blue are of interest for recovery in view of their environmental and economic values.

[12] Georgouvelas D, Abdelhamid HN, Li J, Edlund U, Mathew AP. All-cellulose functional membranes for water treatment: Adsorption of metal ions and catalytic decolorization of dyes. Carbohydr Polym. 2021 Jul 15;264:118044. doi: 10.1016/j.carbpol.2021.118044. Epub 2021 Apr 7. PMID: 33910746.

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Future experiments are planned for improving the recovery efficiency by changing the working parameters such as pH, and so on.

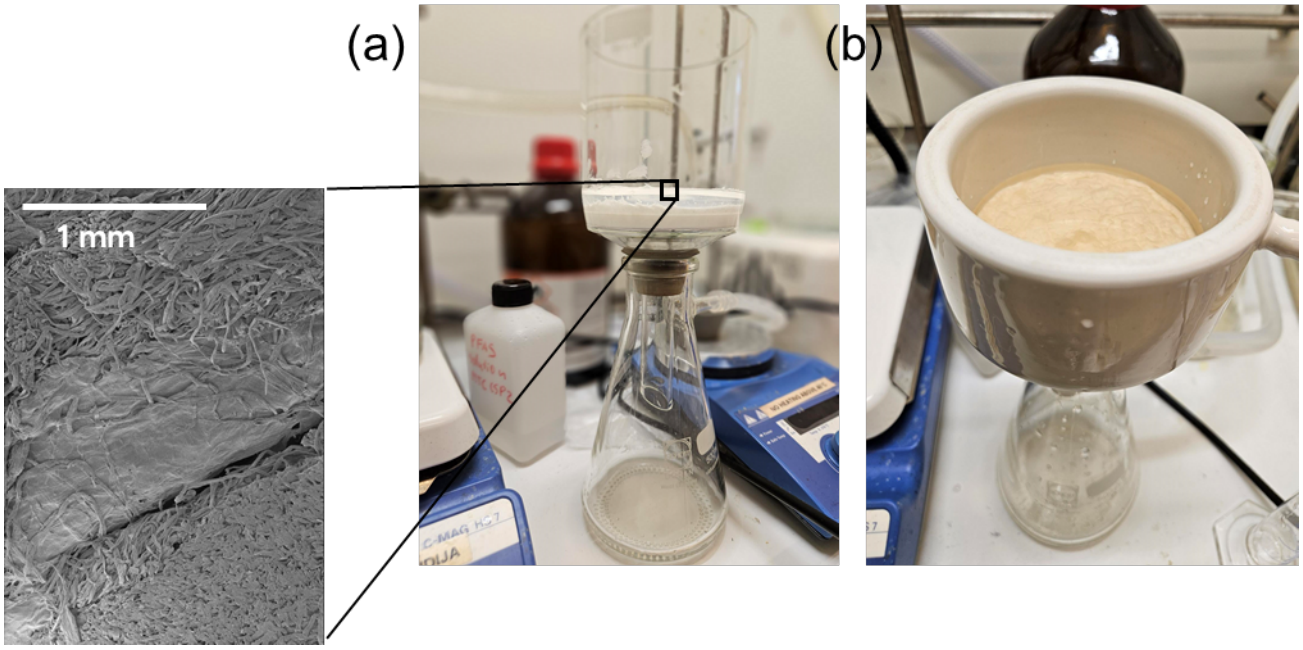

Figure 27 (a) Sandwiched membrane produced using functionalized cellulose showing fibrillar structure in Scanning Electron Microscopy image (inset); (b) as-received bioleached water samples being passed through the membrane under gravity.

Table 4 Concentrations of the elements before and after membrane treatment (mg/L)

	A+S (pH 4.5)	After ML	After MC	A+S (pH 7.0)	After ML	After MC	S (pH 4.5)	After ML	After MC	S (pH 7.0)	After ML	After MC
Na	2800	2100	2300	3200	960	2700	3100	990	2900	3200	780	2500
K	110	49	52	170	47	59	140	43	48	140	30	45
Ca	130	110	84	210	77	96	220	110	85	230	70	92
Fe	420	250	280	770	330	500	710	430	530	700	230	430
Mg	220	100	100	360	110	130	340	110	120	350	92	120
Mn	11	4,6	4,8	18	5	5,6	18	5,5	5,8	18	4	5,5

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	A+S (pH 4.5)	After ML	After MC	A+S (pH 7.0)	After ML	After MC	S (pH 4.5)	After ML	After MC	S (pH 7.0)	After ML	After MC
Al	270	160	180	470	200	290	440	250	310	440	140	260
Sb	0,00031	0,00048	0,00047	0,00055	0,00059	0,0013	0,00068	0,00073	0,00073	0,00059	0,00053	0,002
As	0,084	0,035	0,037	0,16	0,041	0,049	0,14	0,043	0,047	0,14	0,028	0,042
Ba	0,12	0,07	0,098	0,12	0,094	0,12	0,12	0,092	0,13	0,12	0,081	0,11
Be	0,026	0,011	0,011	0,032	0,011	0,014	0,054	0,012	0,014	0,044	0,0097	0,014
Pb	0,9	0,34	0,46	1,5	0,45	0,83	1,6	0,71	0,98	1,7	0,31	0,82
B	1	0,31	0,34	1,3	0,29	0,38	1,3	0,32	0,38	1,3	0,25	0,37
P	21	14	16	31	15	22	19	13	16	19	6,9	13
Cd	0,019	0,013	0,014	0,029	0,015	0,02	0,03	0,02	0,024	0,031	0,012	0,021
Si	200	82	86	230	63	83	230	73	87	240	55	77
Co	0,24	0,17	0,19	0,39	0,2	0,31	0,38	0,27	0,33	0,39	0,084	0,28
Cu	0,64	0,42	0,38	1	0,29	0,45	1	0,43	0,38	1	0,24	0,39
Cr	0,44	0,19	0,19	0,82	0,23	0,27	0,72	0,23	0,25	0,71	0,16	0,22
Li	0,58	0,38	0,41	0,99	0,44	0,66	0,91	0,57	0,71	0,88	0,31	0,58
Mo	0,0039	0,00044	0,0013	0,0068	0,00098	0,0017	0,0056	0,00057	0,0043	0,0052	0,00059	0,0018
Ni	0,59	0,25	0,25	0,97	0,26	0,3	0,94	0,29	0,3	0,98	0,21	0,28
Se	0,097	0,031	0,033	0,17	0,036	0,044	0,18	0,043	0,048	0,18	0,032	0,045

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	A+S (pH 4.5)	After ML	After MC	A+S (pH 7.0)	After ML	After MC	S (pH 4.5)	After ML	After MC	S (pH 7.0)	After ML	After MC
Ag	0,0018	0,0013	0,0015	0,0016	0,00088	0,0012	0,0019	0,0012	0,0015	0,002	0,00078	0,0014
Sr	1,6	0,87	0,86	2,5	0,82	0,97	2,6	1,1	1	2,7	0,67	0,97
S	2100	1500	1600	3800	1700	2600	3300	2200	2600	3300	1100	2100
Tl	0,0021	0,0013	0,0015	0,0041	0,0016	0,0025	0,0031	0,0018	0,0023	0,0031	0,00096	0,0019
Sn	<0,0010	0,0064	0,0051	0,0019	0,077	0,011	<0,0010	0,0075	0,0055	<0,0010	0,075	0,0063
Ti	0,38	0,25	0,28	1,4	0,7	1	0,73	0,48	0,61	0,6	0,22	0,41
U	0,092	0,053	0,061	0,16	0,064	0,1	0,16	0,094	0,13	0,17	0,055	0,1
V	0,29	0,12	0,13	0,73	0,21	0,25	0,7	0,23	0,25	0,61	0,13	0,19
Zn	3,5	1,6	1,5	5,7	1,8	1,7	5,6	1,8	1,8	5,7	1,4	1,7

2 CONCLUSIONS

Task 4.2 was devoted to investigate phosphorus and metal release from the Baltic Sea sediment to the liquid media through biological applications including anaerobic bioreactors and bioleaching. This task also focused on the recovery of phosphorus and metals from the liquid media. All the information gathered from this task will be upgraded and used in the next task which aims to design a cascade bioreactor system. The system will include operational units for phosphorus and metal release from the sediment to the liquid media (water) and simultaneous recovery of phosphorus and metals.

The main conclusions and future directions for improving the technologies are summarized in the table below:

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Table 5: Main observations and future directions

	EXPERIMENT	CONCLUSIONS	FUTURE DIRECTIONS
ANAEROBIC PHOSPHORUS RELEASE EXPERIMENTS	Effect of carbon addition on phosphorus release	Highest concentrations observed for glucose (6.44 mg/L), followed by propionic acid (5.98 mg/L). Excessive C inhibited P release. Optimal concentration is 1 g C/L. Significant microbial community changes occurred. Sulfate reducing bacteria were abundant in the sediment (10%) and promoted P release.	
	Effect of nitrogen addition on phosphorus release	Maximum PO_4-P release was 4.07 mg/L on day 24 for acetic acid-fed and 7.14 mg/L on day 18 for glucose-fed systems without additional NH_4-N. High NH_4-N dosing significantly inhibited P release. Sulfate reducing bacteria and phosphorus accumulating organisms decreased that suppressed P release.	*Testing carbon addition with continuous experiments* *Exploring the abundance of SRBs and PAOs in continuous operation*
	Effect of sulfate addition on phosphorus release	Maximum PO4-P release was 6.41 mg/L with 8 mM sulfate addition.	

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BIOLEACHING EXPERIMENTS	Experiments with <u>*A.thiooxidans*</u>	*A.thiooxidans* with sulfur addition did not show improved metal release when compared to sulfur addition. Maximum Fe concentration was achieved as 830 mg/L after 3rd cycle. Maximum Al concentration was achieved as 470 mg/L and the values were similar within sets and cycles. Release of Mg and K were lower in the 2nd and 3rd cycle. P release was also observed as 14 mg PO_4.P/L (after 1st cycle).	*Testing bioleaching with longer retention time to improve the efficiency* *-Focusing sulfur oxidizing bacteria instead of iron oxidizing bacteria* *Increasing TS to achieve higher metal concentration in the liquid* *Testing continuous operation*
	Experiments with *A.ferrooxidans*	Fe precipitation was observed when iron was added led to negative bioleaching efficiency. Maximum Fe concentration was 220 mg/L at 5% TS, no added Fe^{2+}/S, pH 2.0 Maximum Al solubilization was 621 mg/L with 10% TS, 10 g/L Fe^{2+}, 5 g/L S, pH 2.0	
	Experiments with enriched iron oxidizing bacteria	Fe precipitation and negative bioleaching efficiency was also observed. Maximum Fe concentration was 207 mg/L at 5% TS, no added Fe^{2+}, pH 2.0.	

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		Maximum Al concentration was observed as 400 mg/L at 10% TS, 20 g/L Fe^{2+}, pH 2.0.	
	Experiments with enriched iron and sulfur oxidizing bacteria	Maximum Fe concentration was 232 mg/L at 7.5% TS, 5 g/L Fe^{2+}, 2.5 g/L S. pH 4.5. Maximum Al concentration was 810 mg/L 10 at 10% TS, 10 g/L Fe^{2+}, 5 g/L S, pH 2.0. P release was observed as 11.6 mg PO_4-P mg/L.	
	Experiments with PANI acidified sediment samples	Maximum PO_4-P release was observed as 4.4 mg/L with acid assisted bioleaching. For PANI assisted bioleaching, it was 3.6 mg/L.	
PHOSPHORUS RECOVERY EXPERIMENTS	Effect of pH	The yield of struvite increased until pH was 9.22 with a yield 0.00228.	*Better understanding for kinetics*
	Effect of impurities	Al has negligible impact on supersaturation index (SI).	
	Effect of evaporation	Heating caused negative SI and water removal increased SI. SI was 1.18 and 2.42 for pH 7.45 and pH 9.22.	
	Effect of temperature	High temperatures led to low SI. SI was 3.55 and 5.16 for natural pH and pH 9.22.	

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	Addition of NH_4OH	The addition of NH_4OH increased Si. At pH 7.45, SI was 2.6 when P:N ratio is 1.4. At pH 9.22, it was 2.4.	
	Addition of $MgCl_2$	$MgCl_2$ addition decreased SI as Mg:P ratio increased.	
	Struvite precipitation	The simulation yields were higher than the experimental yields due to not considering reaction kinetics. Concentrated samples via evaporation resulted in low yields due to ammonia loss.	
METAL RECOVERY EXPERIMENTS	Membrane adsorption	Lignin membrane performed better than cellulose membrane. The membrane efficiency was 50-80% for most of the elements.	*Testing recovery efficiencies at different pH and characterization of membranes.*

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