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REPORT ON PAH DEGRADATION

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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 PAH remediation, SYMBIOREM explores the natural ability of native marine sediment microbiome as well as biostimulation and bioaugmentation strategies to degrade PAH from the marine sediment.

This deliverable provides the background information on the definition of Polycyclic aromatic hydrocarbons (PAHs) and the treatment methods for the sediments. In addition, a description of the methodology (Section 1.2) is given including PAH characterization of the Baltic Sea sediment, the PAH spiking method and the configuration of the anaerobic batch tests. The background concentrations of the collected sediment from the Baltic Sea were low and four different PAHs (Fluoranthene, Benzo(b,k)fluoranthene, Indeno(1,2,3-cd)pyrene, and Benzo(g,h,i)perylene which were found as the dominant types in the Baltic Sea sediment in the literature were spiked to the sediment. The experimental strategy involved biostimulation to trigger anaerobic PAH degradation by microbial consortia in the marine sediment. Glucose (carbon source) and/or Na_2SO_4 (sulfate source) were added to batch bioreactors and the experiments lasted 120 days in total. The results of the experiments (i.e., PAH degradation, carbon consumption, sulfate consumption, phosphorus and ammonium release from the sediments) are presented in Section 1.3. Briefly, the highest PAH degradation (Fluoranthene degradation as 34.65%) was observed with the control batch bioreactors showing that biostimulation strategy did not work well. So, new experiments are planned for the next term to enhance PAH degradation efficiency in the Baltic Sea sediment (Section 1.4).

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1 INTRODUCTION

1.1 Introduction of the experiment

Polycyclic aromatic hydrocarbons (PAHs) constitute a subset of total petroleum hydrocarbons known for their carcinogenic and mutagenic properties. Many studies worldwide, have reported and quantified the accumulation of PAHs in marine sediments which include the Baltic Sea (Biselli et al., 2005; Josefsson. 2022). Therefore, the remediation of PAH pollution in the Baltic Sea is of paramount importance.

In recent years, biostimulation and bioaugmentation have become prevalent bioremediation strategies. Biostimulation involves supplementing nutrients (e.g., nitrogen, phosphorus, and potassium) (Masy et al., 2016) or compost, organic wastes, etc., to enhance the activities of indigenous microbial populations (Qiao et al., 2014). Anaerobic bioremediation, providing the potential of microorganisms in the absence of oxygen, offers a promising solution to the persistent PAH contamination in marine environments.

Therefore, to achieve bioremediation of PAH pollutants in the Baltic Sea sediments, biostimulation using anaerobic batch reactors has been chosen. Our research aims to comprehend and enhance their capacity for degrading PAH contamination.

1.2 Material and methods

The sediments were collected from the Baltic Sea area on 31st of March 2023, Stockholm (N 58°46.5769', E 17°26.1202'). The background values for the 16 PAHs in sediments are presented in Table 1. Due to the relatively low concentrations of PAHs obtained from the sediments, we spiked four PAHs with the highest reported concentrations for the Baltic Sea in the latest literature as the experimental targets-Fluoranthene, Benzo(b,k)fluoranthene, Indeno(1,2,3-cd)pyrene, and Benzo(g,h,i)perylene (Josefsson. 2022). These selected PAHs were procured from Sigma-Aldrich (Germany), dissolved in acetone, and then added to uncontaminated sediment to achieve an initial PAH concentration of 0.5 mg/kg dry sediment. Subsequently, the contaminated sediment was mixed and placed under a fume hood for one week to evaporate the solvent (Bianco et al., 2020).

Table 1 Characterization of 16 PAH in the collected Baltic Sea sediment.

PAH	VALUE (MG/KG TS)
Benzo(a)anthracene	0.024
Chrysene	0.029
Benzo(b,k)fluoranthene	0.17
Benzo(a)pyrene	0.037

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Indeno(1,2,3-cd)pyrene	0.13
Dibenzo(a,h)anthracene	0.04
Naphtalene	<0,020
Acenaphthylene	<0,020
Acenaphthene	<0,020
Fluorene	<0,020
Phenanthrene	0.024
Anthracene	<0,020
Fluoranthene	0.056
Pyrene	0.039
Benzo(g,h,i)perylene	0.024

A series of batch experiments were conducted in triplicate using 120-mL serum bottles. An equal amount of 4 g total solid PAH-contaminated sediment was dispensed in 80 mL synthetic seawater, prepared with NaCl (6.5 psu, the average salinity of the Baltic Sea water). As additional carbon sources and nutrients for investigating the biostimulation effects of PAH degradation, 1 g/L glucose (carbon source) and 1 g/L Na_2SO_4 (sulfate source) were selected. The specific information for each set is detailed in Table 2. The experimental bottles were sealed with septa and aluminum caps, and each bottle was flushed with nitrogen gas for a minimum of 5 min to establish anaerobic conditions. Throughout the 120-day bioreactor operation, the PAH concentration in the sediment, as well as sulfate, PO_4-P, NH_4-N, pH, and glucose concentrations in the bioreactors, were monitored on each sampling day.

Table 2 Anaerobic batch experiment conditions.

GROUPS	CARBON ADDITION	SULFATE ADDITION
Control	--	--
Glc addition	1 g/L glucose	--
Glc addition+sulfate addition	1 g/L glucose	1 g/L Na_2SO_4

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1.3 Experimental results

As shown in Figure 1, the maximum removal for Fluoranthene, Benzo(b,k)fluoranthene, Indeno(1,2,3-cd)pyrene, and Benzo(g,h,i)perylene in the anaerobic bioreactors over the 120-day operation were 34.65%, 7.96%, 17.95%, and 8.25%, respectively. All maximum removal of PAHs were observed in the group without additional carbon sources and sulfate (control group), indicating that the supplementary carbon sources and sulfate additions inhibited the degradation of PAHs. For instance, on day 120, the treatment group initially supplemented with 1 g/L glucose and 1 g/L Na_2SO_4 displayed only 19.80% degradation of Fluoranthene and 0.44% of Benzo(b,k)fluoranthene, with no degradation observed for the other two PAHs, Indeno(1,2,3-cd)pyrene, and Benzo(g,h,i)perylene. It is noteworthy that none of the treatment groups achieved a satisfactory degradation rate for the targeted 4 PAHs, especially concerning the high-molecular-weight PAHs (Benzo(b,k)fluoranthene, Indeno(1,2,3-cd)pyrene, and Benzo(g,h,i)perylene).

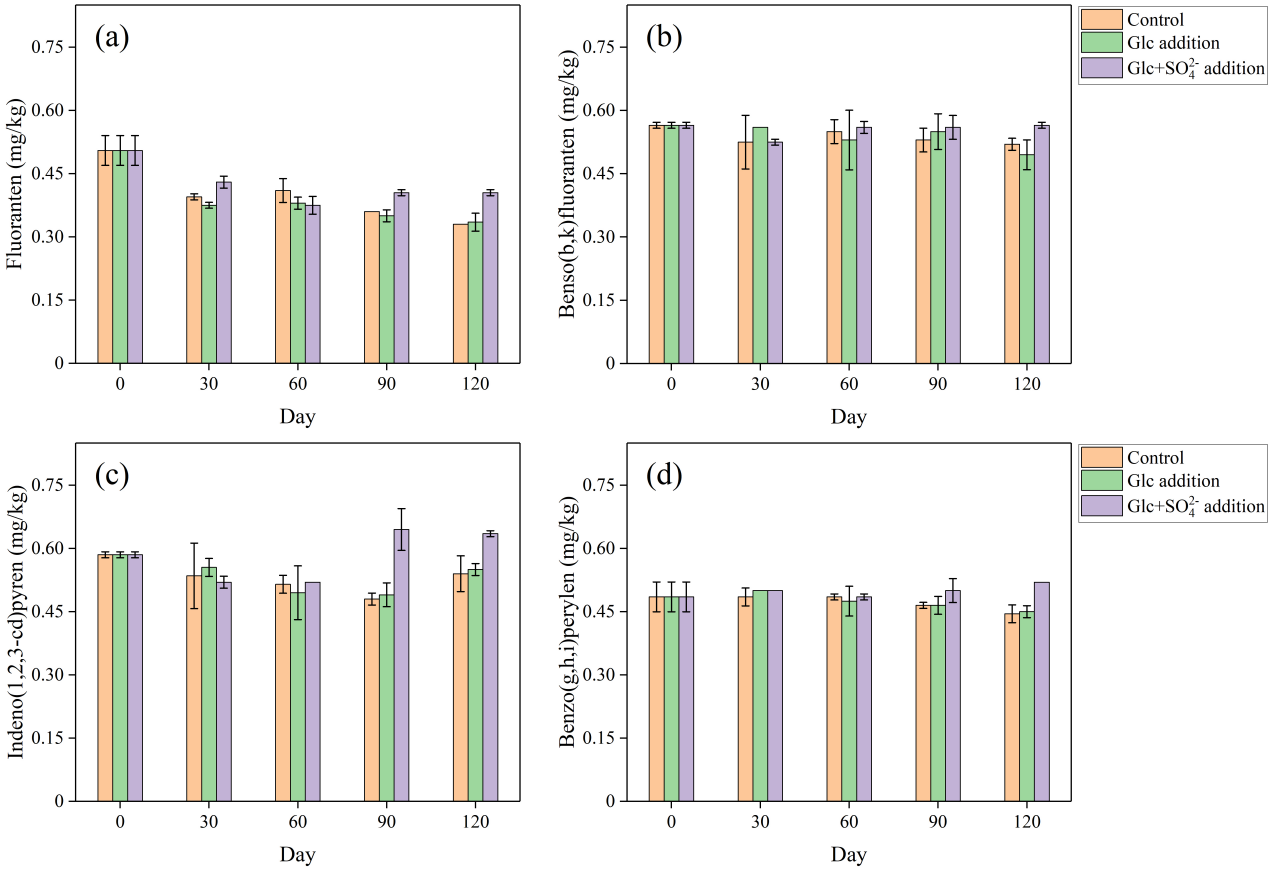

Figure 1 Variations of the degradation of aimed 4 PAHs under different conditions during 120-day anaerobic operation: (a) Fluoranthene; (b) Benzo(b,k)fluoranthene (c) Indeno(1,2,3-cd)pyrene rage salinity of the Baltic Sea wate; (d) Benzo(g,h,i)perylene.(Glc addition: glucose addition)

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Concentrations of SO_4^{2-}, PO_4-P, and NH_4-N in the liquid were also monitored. As depicted in Figure 2a, the Na_2SO_4 in the sulfate-addition group was consumed, decreasing from the initial concentration of 1066.42 mg/L to 702.06 mg/L. In contrast to the results of PAH degradation, the treatment groups supplemented with additional carbon sources and Na_2SO_4 exhibited optimal P release (Figure 2b). By Day 120, the PO_4-P concentration was 10.34 mg/L, significantly higher than the control (7.77 mg/L) and the group with only additional carbon sources (7.50 mg/L). The release of NH_4-N displayed a first increase followed by a decline trend (Figure 2c), which may be related to microbial anaerobic activity, and further investigation is required to reveal the underlying mechanisms.

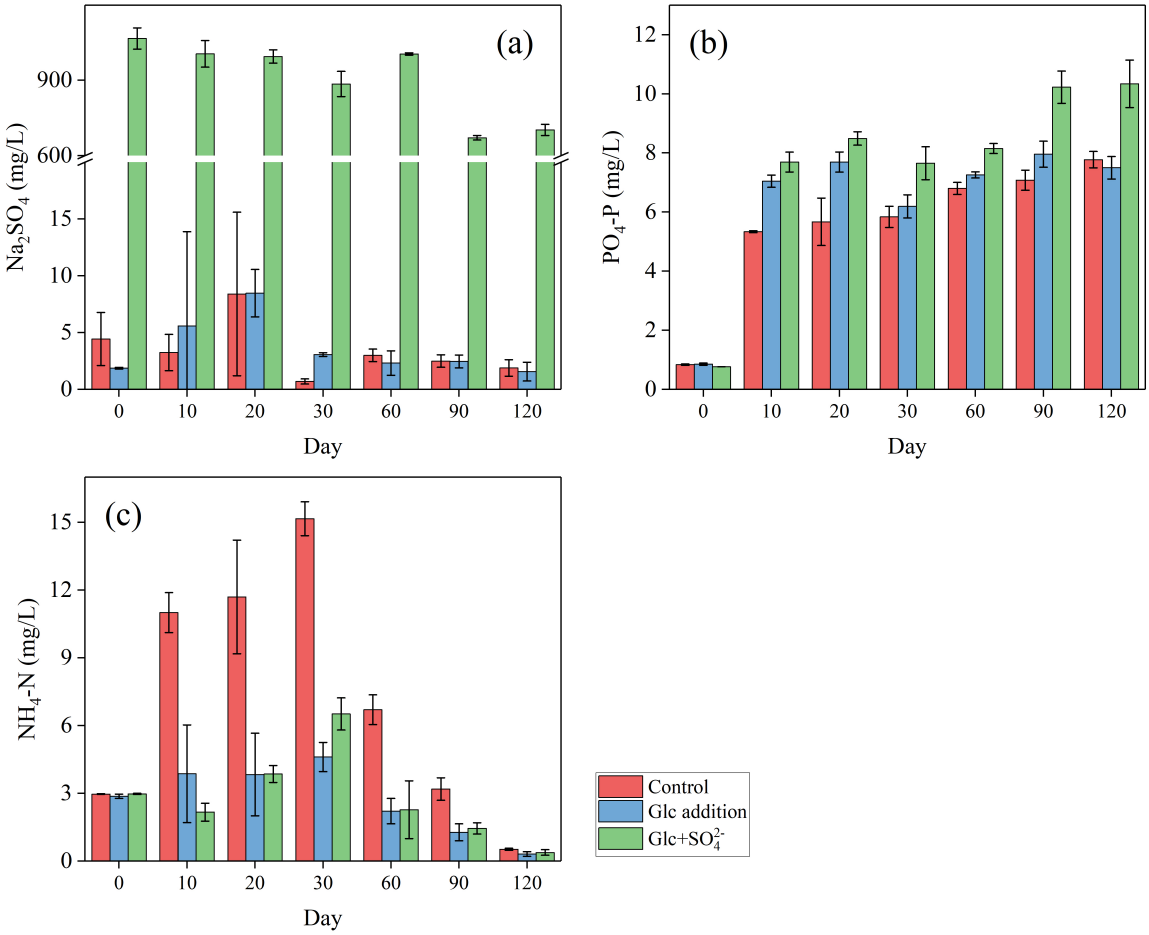

Figure 2 Variations of (a) Na_2SO_4, (b) PO_4-P, and (c) NH_4-N under different conditions during 120-day anaerobic operation (Glc addition: glucose addition).

The pH results indicate that the pH in the control group remained overall stable during the 120-day experiments, fluctuating from 7.8±0.1 initially to 7.4±0.1 (Figure 3a). In contrast, the pH in the glucose addition systems decreased from 7.8±0.1 to 5.6±0.1 by day 3, after which it maintained a relatively stable level. This decrease may be attributed to acidification caused by glucose consumption, as confirmed by the glucose consumption results in Figure 3b. Over 71% of glucose experienced rapid depletion in the initial 3 days, with the consumption rate exceeding 96% by day 10.

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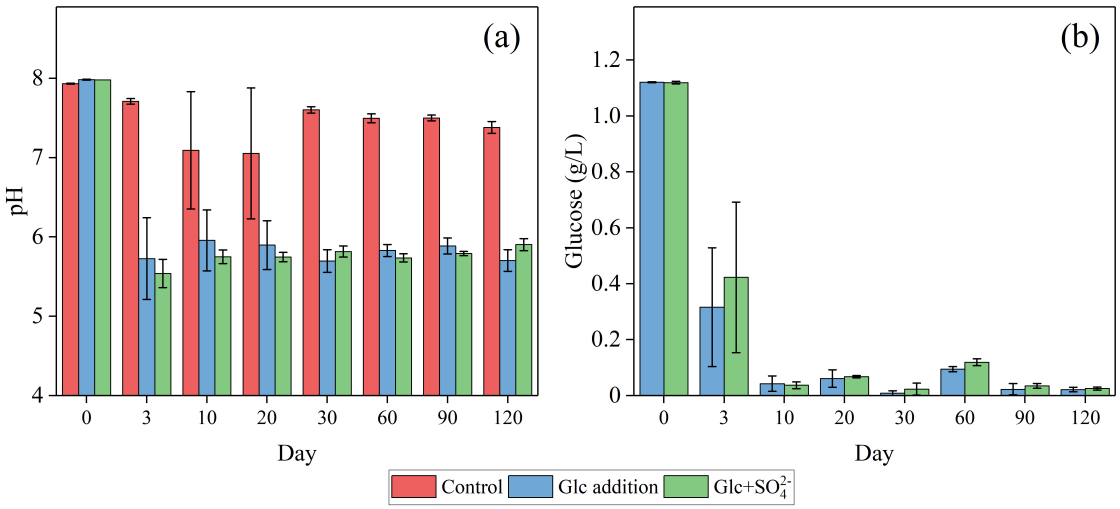

Figure 3 Variations of (a) pH and (b) glucose consumption under different conditions during 120-day anaerobic operation (Glc addition: glucose addition).

Volatile fatty acids (VFA) were produced in the glucose-fed systems, and their accumulation increased over time. In systems without sulfate addition, acetic and butyric acids were the dominant VFAs. The concentrations of acetic acid and butyric acid detected in the glucose-fed group were 402.27 mg/L and 243.07 mg/L, respectively (Figure 4a). When sulfate was added to the system, acetic and butyric acids initially dominated the produced VFAs. However, starting from day 90, all VFAs were converted into acetic acid. The concentrations of acetic acid detected on day 90 and day 120 were 824.13 mg/L and 684.35 mg/L, respectively.

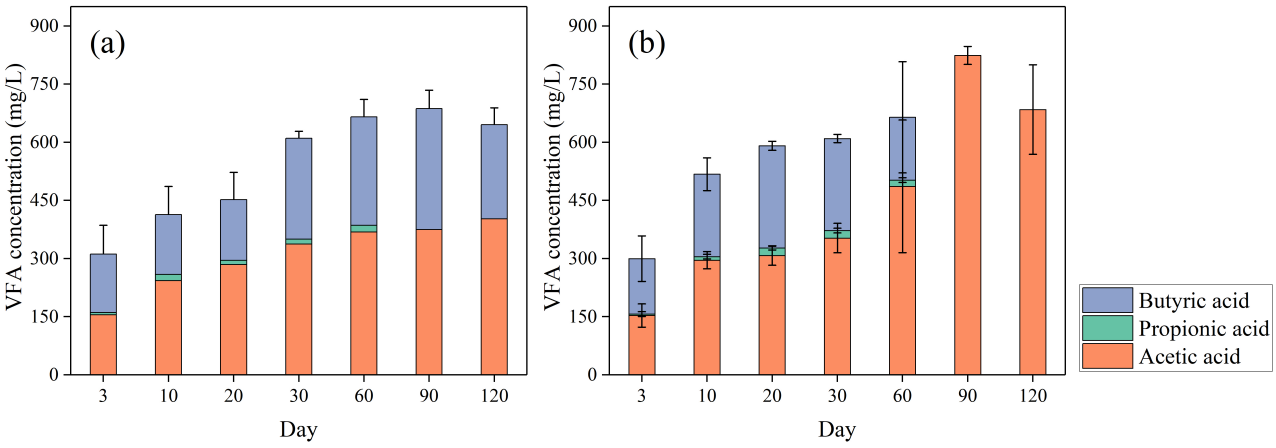

Figure 4 VFA production under different conditions during 120-day anaerobic operation (Glc addition: glucose addition) (a) Glc addition; and (b) Glc+SO_4^{2-}

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1.4 Future experiment on PAH bioremediation

In summary, the PAH degradation results obtained in this experiment are below the targeted level (60%). The anaerobic bioreactor operation over 120 days indicates only a certain degree of degradation for low-molecular-weight PAH (maximum 34.65% for Fluoranthene), and anaerobic biostimulation with additional carbon and sulfate addition inhibits the degradation of PAHs. The underlying reaction mechanisms involved in this process need further exploration and explanation. Bioinformatics analysis can further reveal potential microbial activities and metabolic processes.

Simultaneously, a bioaugmentation strategy should be considered to enhance anaerobic PAH degradation, especially for high-molecular-weight and difficult-to-degrade PAHs such as Benzo(b,k)fluoranthene, Indeno(1,2,3-cd)pyrene, and Benzo(g,h,i)perylene. We are planning to conduct new anaerobic experiments adopting bioaugmentation strategies using three species purchased from DMSZ (German Collection of Microorganisms and Cell Cultures GmbH): *Crucibulum laeve* (DSM 8451), *Glutamicibacter protophormiae* (DSM 20168), and *Cytobacillus firmus* (DSM 12). Subsequently, we will initiate the next round of batch tests focusing more on the degradation of high-molecular-weight PAHs. Furthermore, aerobic degradation of PAH might be also investigated which can be coupled with bioleaching step (Task 4.2) These strategies will allow us how to the design of the cascade bioreactor system in Task 4.3.

2 CONCLUSIONS

The current experimental results indicate that the use of anaerobic reactors alone yields not satisfied PAH degradation. To further enhance the degradation of targeted PAHs, especially high-molecular-weight PAHs, aerobic degradation of PAHs and the bioaugmentation strategies with anaerobic conditions should be designed and implemented. The metabolic pathways involved in PAH degradation and microbial activities in aerobic/anaerobic reactors should also be subjected to further analysis.

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