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# Development of phosphate-accumulating microbial community in a sequencing batch reactor (SBR)

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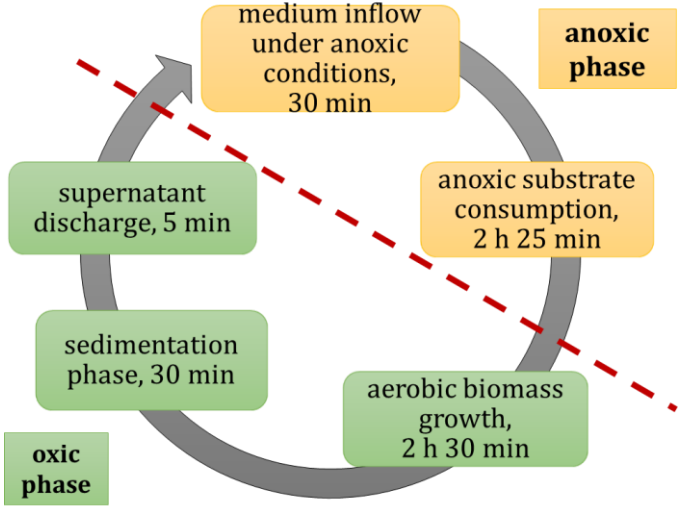
**Motivation and Aim:** The most promising modern technologies for phosphorus removal from wastewater are based on biological treatment using the activated sludge forming in continuous or sequencing batch (SBR) bioreactors. Ability to remove phosphorus is the result of activity of phosphate-accumulating organisms (PAO), a physiological group of microorganisms capable of cyclic phosphate consumption-release and phosphorus accumulation as polyphosphates in the oxic/anoxic cultivation stages.

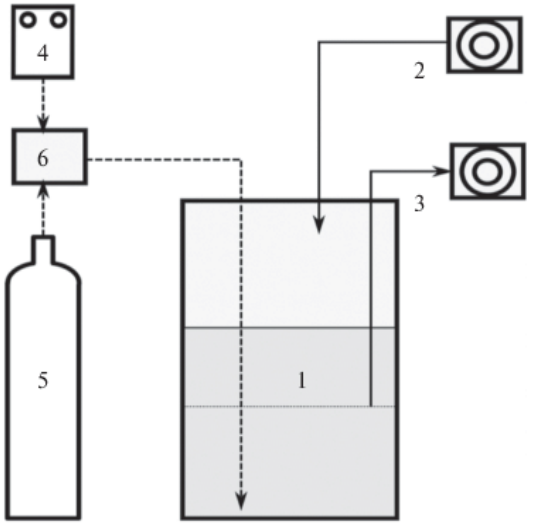
**The goal of the present work** was to obtain a PAO-enriched microbial community with high biomass density, to investigate the dynamics of cell phosphates using Raman scattering spectroscopy (RS spectroscopy), and to determine the taxonomic position of the community and the dominant PAOs.

## Laboratory sequencing batch reactor (SBR)




Standard SBR operation cycle

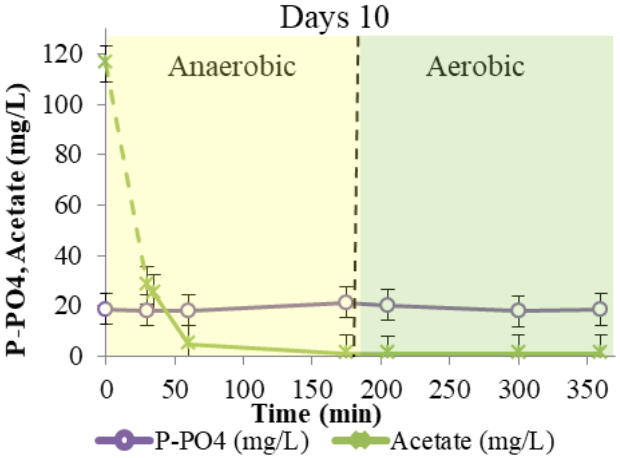
| Cultivation conditions | |
|---|---|
| pH/Cultivation temperature | 7,5-8,2 / 18°C |
| Average time of biomass retention | 17.5 days |
| Hydraulic retention time | 15 h. |
| Carbon and energy source | $CH_3COONa$ |



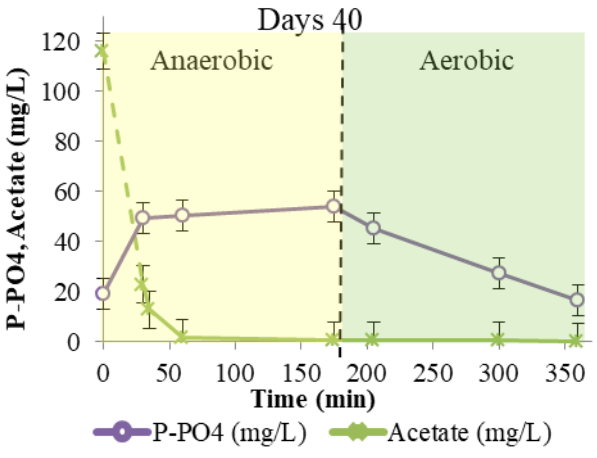

Schematic representation of the SBR: bioreactor (1), pump for medium supply (2), pump for culture removal (3), air compressor (4), dinitrogen reservoir (5), gas supply regulation system (6).

# Analysis of the dynamics of phosphates in the medium

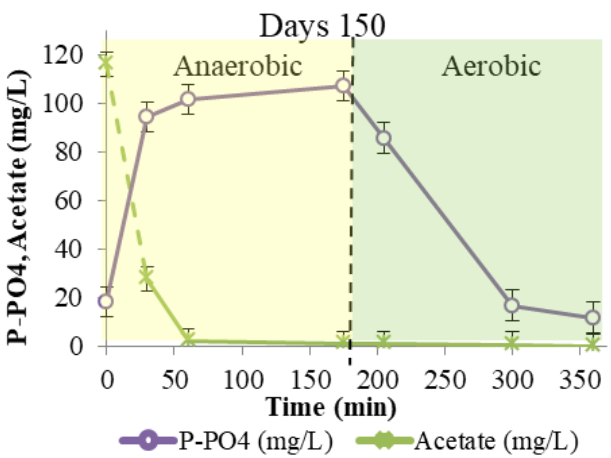
Throughout the cultivation period, the organic substrate (acetate) was completely consumed by the microbial community during the anaerobic phase of each SBR cycle.




During the first 10 days, no phosphorus consumption or release was reliably detected.




Subsequent cultivation resulted in increased amount of phosphorus released during the anaerobic phase (its concentration was 50 – 60 mg $P\text{-}PO_4/L$)

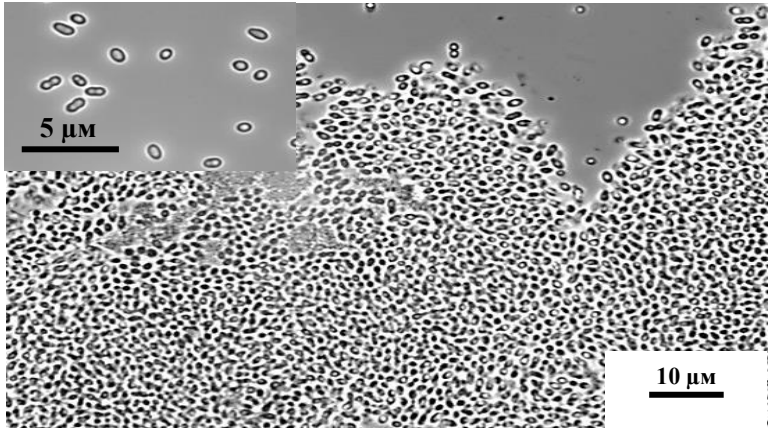



By day 150 of cultivation, the maximum phosphate concentration in the medium during the anaerobic phase was 100–110 mg $P\text{-}PO_4/L$

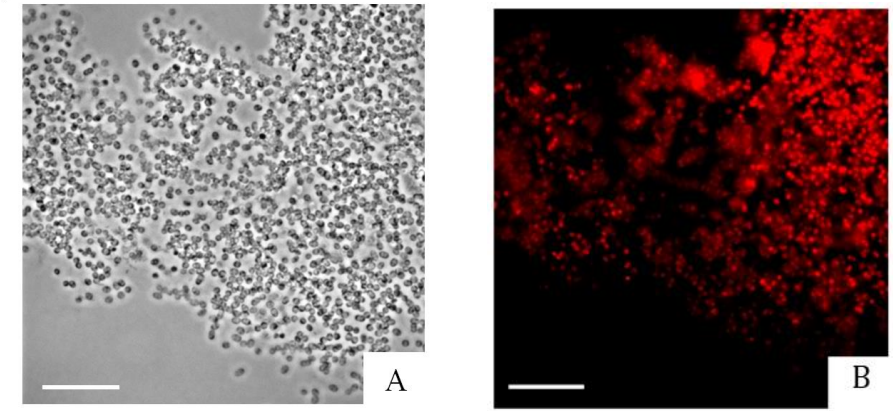
| Days | 10 | 40 | 150 |
|---|---|---|---|
| **Efficiency of P-removal** | 25,5±3,5 | 34,7±3,5 | 63,5±3,5 |
| **P-mol/C-mol** | 0,002±0,05 | 0,27±0,05 | **0,72±0,05** |
| **P-removal, mg/L** | 6,4±1,6 | 8,7±1,6 | 13±1,6 |
| **biomass concentration, g DB/L** | 3,1±0,3 | 3,2±0,3 | 3,5±0,3 |

Phosphorus content increased to **16.5 ± 0.15%** of the dry ash-free biomass matter, which was much higher than phosphorus content in the **biomass of most microorganism (1–2.5%)**

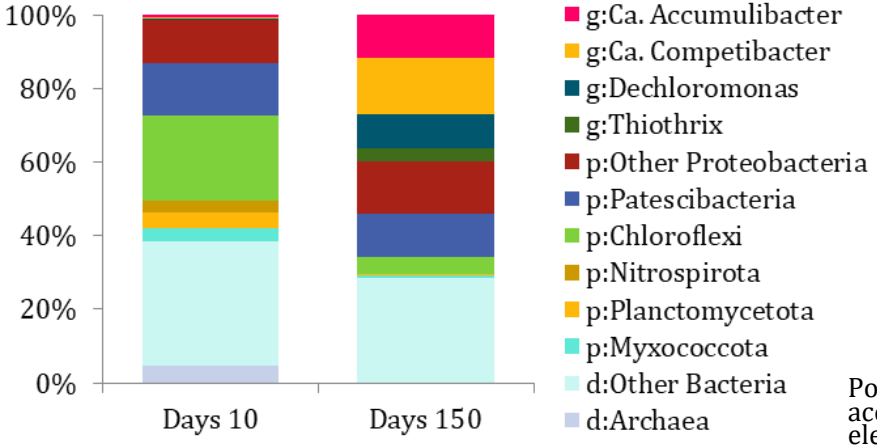
# SBR microbial community



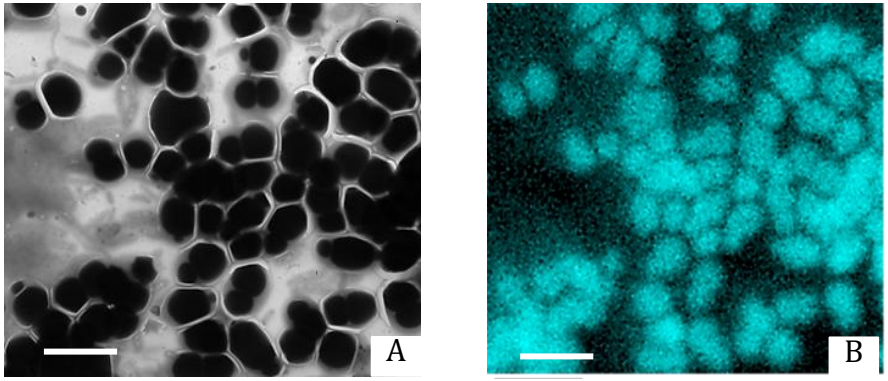

Cells with inclusions in the bioreactor phosphate-accumulating microbial community: individual cells and cell aggregates.




*Ca*. Accumulibacter as part of the microbial community of aggregates: A – phase contrast, B – analysis of microbial aggregates by FISH cells hybridized with the PAO651 probe (Zeiss 43 light filter). The scale ruler is 20μm.

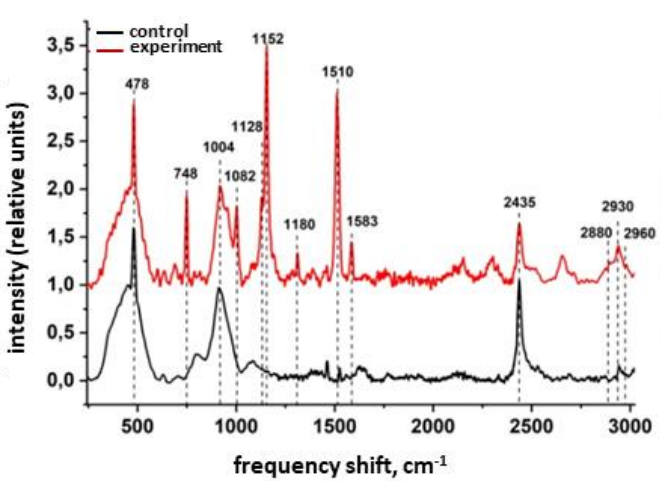


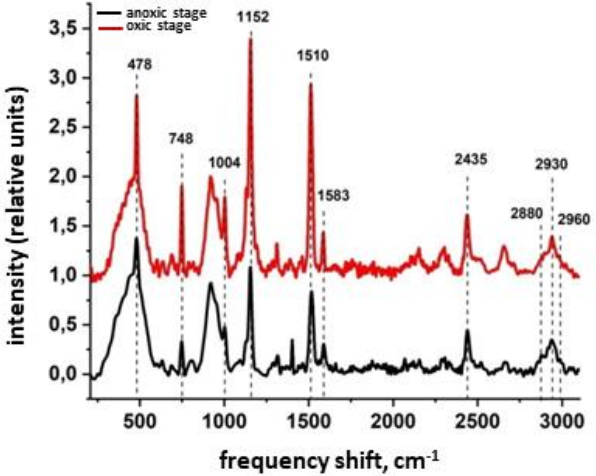

Taxonomic composition of the SBR microbial community




Pointwise analysis of the elemental composition of bacterial cells in the phosphate-accumulating community determined by mapping with X-ray microanalysis: A – electron micrograph of the cells; B – phosphorus distribution in the cells, with phosphor-rich granules indicated by arrows. The scale ruler is 5μm.

# Research of intracellular polyphosphates by Raman spectroscopy




| Peak position, $cm^{-1}$ | Characterization |
|---|---|
| 478 | O–P–O vibrations |
| 748 | Vibrations of PO phosphate groups |
| 1004 | Vibration of carbonate ion $CO_3$ ($^{2-}$) or $CH_3$ |
| 1082 | $PO_4$ vibration, F2 band |
| 1128 | C–H deformations, $PO_4^{3-}v_3$ vibrations |
| 1152 | Vibrations of the C–C bond, antisymmetric $PO_2$ extensions |
| 1180 | C–H deformations |
| 1440 | N=N extensions |
| 1510 | Vibrations of the C=C bond |
| 1583 | Amide I vibrations |
| 2435 | Vibrations and extension of the P–OH bond |
| 2880, 2930, 2960 | Proteins and lipids, C–H groups of fatty acids, proteins, or water |

*Jillavenkatesa and Condrate, 1998; Zhang and Silva, 2010; Penkov, 2021; Frost et al., 2014

**Conclusion:** Thus, during the operation of the bioreactor, there was a change in the taxonomic composition of activated sludge. By 150 days of cultivation in SBR, a stable functioning phosphate-accumulating microbial community was obtained with a high accumulation of phosphorus in the biomass and accumulation and release of phosphates typical of the PAO phenotype in the aerobic and anaerobic phases of the SBR cycle. The main representative of the PAO in the community was *Ca*. accumulibacter.