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## Motivation

Synthetic flocculants are often applied in coagulation/flocculation process to trigger agglomeration of destabilized particles. A major concern associated with synthetic flocculants is their environmental impact. Promising natural alternatives are extracellular polymeric substances (EPS), due to their ability to bind cations and organics. EPS-derived bioflocculants can be produced whilst treating wastewater by open mixed microbial cultures, thereby offering an environmentally friendly and cost-effective way for synthesis.

## Technological challenge

EPS are secreted by microorganisms in their natural environment for several purposes such as adhering to the surfaces, storing nutrients, and retaining water. They contain polysaccharides and proteins as main components. EPS production can be stimulated by environmental stress (Fig 1). It can be achieved with single or mixed carbon sources, pure or mixed microbial cultures and under sterile or non-sterile conditions.<sup>1</sup> Depending on the purity, EPS can be applied in several fields such as food, health, and water treatment.<sup>2</sup>

In this research, EPS production will be achieved by utilizing wastewater as a nutrient source. Mixed microbial communities will be employed for EPS production under non-sterile conditions (Fig 2, 3). This is accompanied by several challenges including:

- Long-term operation of EPS producing membrane bioreactors
- Extraction and characterization of produced EPS
- Application of EPS as natural flocculant and understanding the flocculation mechanism of EPS
- Investigating the changes in the microbial community during the EPS production process

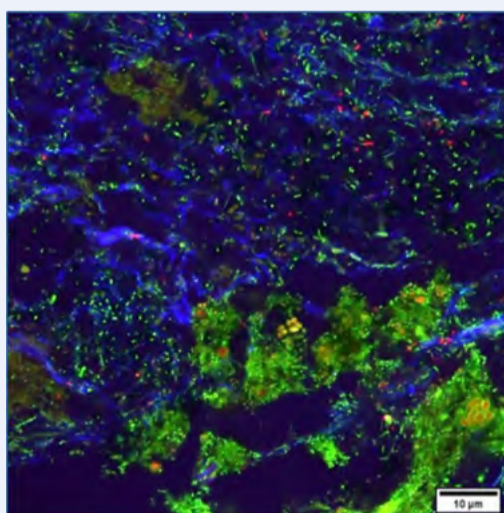


Figure 1. CLSM image of mixed liquor sample taken from EPS-producing bioreactor run under nitrogen limitation. The sample was stained with fluorescent dyes to show individual components of EPS; Sypro Orange (green, proteins), Calcofluor white (blue, polysaccharides), Syto 63 (red, DNA).

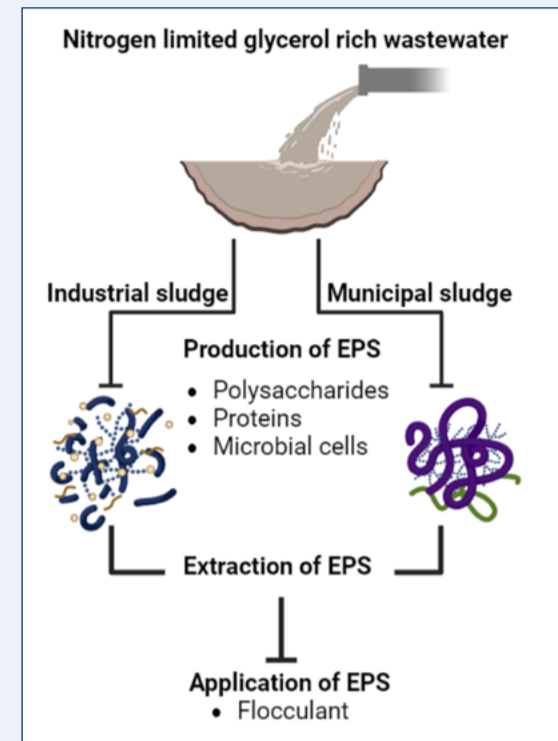


Figure 2. The experimental approach. Two different bioreactors are inoculated with aerobic sludge samples originating from different sources (industrial and municipal sludge). Reactors are fed with nitrogen limited synthetic wastewater containing glycerol as carbon source. Produced EPS are extracted and applied as natural flocculant.

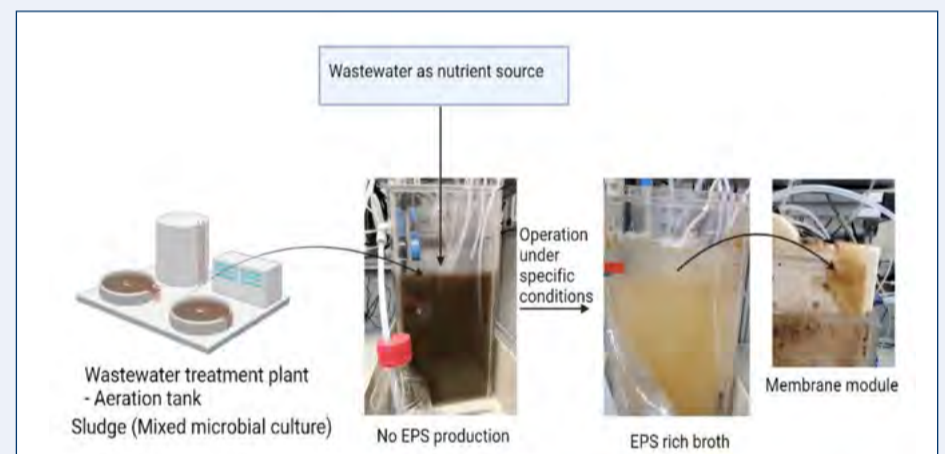


Figure 3. Changes of membrane bioreactor inoculated with aerobic mixed microbial culture during EPS production process. EPS production starts under specific process conditions (nitrogen limitation coupled with short solid retention times). During the production mixed liquor becomes viscous as a sign of presence of EPS.

## Research goals

This project aims to:

- Produce EPS from wastewater and understand the effect of wastewater type on yield and characteristics of EPS in long term operation
- Apply EPS produced from various type of wastewater as natural flocculant
- Understand the microbial community dynamics during the EPS production

## References

- [1] Ajao V., Bruning H., Rijnaarts H., Temmink H., Chemical Engineering Journal (2018), 622-632
- [2] Ajao V., Fokking R., Leermakers F., Bruning H., Rijnaarts H., Temmink H., Journal of Colloidal and Interface Science (2021), 533-544



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