FINAL REPORT FOR ÅFORSK FOUNDATION PROJECT 19-508
Engineering *Bacillus subtilis* for simultaneous treatment of municipal and industrial waste water

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To improve the performance of biological wastewater treatment- and resource recovery processes, bioaugmentation is an interesting approach. Bioaugmentation can involve adding specific microorganisms into a microbial community (MC) to enhance the capacity of the MC for transforming specific contaminants or produce specific products. In this project, we report using Gram-positive bacterium, *Bacillus subtilis* for bio-augmentation of nitrogen removal and electrofermentation processes. We focus on the ability of *B. subtilis* to grow in reject water and its effect on the indigenous microbial community. Reject water is generated when anaerobically treated sewage sludge is dewatered. It contains high concentrations of ammonium, which can account for 25% of the total nitrogen load to a wastewater treatment plant. *B. subtilis* was co-cultured with the microbial communities present in both raw reject waters, which we refer to as ammonium-enriched wastewater, and reject water treated with nitritation (ammonia oxidation to nitrite) and anammox, which we refer to as nitrate-enriched wastewater. The reject water was amended with glucose (1%), which served as carbon source during the experiments. In anaerobic conditions, the indigenous microbial community bio-augmented with *B. subtilis* was able to rapidly remove nitrate from wastewater. In these conditions, *B. subtilis* overexpressed nitrogen assimilatory and respiratory genes including NasD, NasE, NarG, NarH, and NarI, which arguably accounted for the observed boost in denitrification. Next, several experiments were carried out with bioelectrochemical systems to determine if *B. subtilis* could contribute to denitrification in the cathode compartment of such a treatment process and if fermentation of glucose was affected. We attempted to use the the ammonium- and nitrate-enriched wastewater samples bio-augmented with *B. subtilis* in the cathodic compartment of bioelectrochemical systems (BES) operated in anaerobic condition. *B. subtilis* only had low relative abundance in the microbial community, but bio-augmentation promoted the growth of *Clostridium butyricum* and *C. beijerinckii*, which became the dominant species. Both bio-augmentation with *B. subtilis* and electrical current from the cathode in the BES promoted butyrate production during fermentation of glucose. A concentration of 3.4 g/L butyrate was reached with a combination of cathodic current and bio-augmentation in ammonium-enriched wastewater. With nitrate-enriched wastewater, the BES effectively removed nitrate reaching 3.2 mg/L after 48 h. In addition, 3.9 g/L butyrate was produced. In this project, we propose that bio-augmentation of wastewater with *B. subtilis* in combination with bioelectrochemical processes could both boost denitrification in nitrate-containing wastewater and enable commercial production of butyrate from carbohydrate- containing wastewater, e.g. dairy industry discharges. The proposed bio-augmented electrofermentation method in our BES shifted microbial metabolism towards nitrate reduction relevant for wastewater treatment, coupled to butyrate production. This project opens up interesting venues for making wastewater treatment more effective energetically and economically.

Articles published with the ÅForsk Foundation support by grant 19-508 (mentioned in acknowledgements with grant number)

- Rahimi, S., Modin, O., Roshanzamir, F., Neissi, A., Alam, S. S., Seelbinder, B., ... & Mijakovic, I. (2020). Co-culturing Bacillus subtilis and wastewater microbial community in a bio-electrochemical system enhances denitrification and butyrate formation. Chemical Engineering Journal, 125437.
- Rahimi, S., Modin, O., & Mijakovic, I. (2020). Technologies for biological removal and recovery of nitrogen from wastewater. Biotechnology Advances, 107570.