

Independent Projects in Biology, Environmental Science and Soil Science, 30 hp (A1E or A2E – Magister or Master) Biology and Soil Science

NB! A1E can be written in Swedish or English, A2E must be written in English.

If you are interested in any of the suggested projects or just want more information please contact the supervisor.

Role of sulfide in regulation of the anaerobic digestion of lipids: Implications for increasing biogas production in municipal wastewater treatment plants

Mains subject: Biology and Environmental Science

Contact: Bettina Müller, bettina.mulle@slu.se

For project description see below.

Characterization of mannoside binding to a cyanobacterial carbohydrate-binding protein by NMR spectroscopy

Main subject: Biology or Chemistry

Contact: Gustav Nestor, gustav.nestor@slu.se

For project description see below.

Unravel the competition between methane-producing microorganisms in biogas systems

Main subject: Biology

Contact: Maria Westerholm@slu.se

For project description see below.

Microbial consortia in rewetted peatlands

Main subject: Biology or Soil Science

Contact: Bettina.Muller@slu.se, or Sabine Jordan@slu.se (Dept of Soil and Environment)

For project description see below.

Purification and properties of artificially matured [Fe-Fe] hydrogenases from syntrophic acetate oxidation bacteria

Main subject: Biology

Contact: Bettina.Muller@slu.se, or Livia.Meszaros@kemi.uu.se (Dept of Chemistry, Uppsala University)

For project description see below.

PROJECT DESCRIPTIONS

Role of sulfide in regulation of the anaerobic digestion of lipids: Implications for increasing biogas production in municipal wastewater treatment plants.

Background

Anaerobic digestion (AD) of lipid-rich wastes with sewage sludge enables a utilization of the excess capacity at existing municipal anaerobic digesters for biogas production, contributing to an energy efficient wastewater treatment process. However, lipids tend to degrade slowly during AD causing process disturbances, associated with accumulation of their intermediate degradation products (long chain fatty acids, LCFA) and the resulting microbial toxicity. In a laboratory trial, we observed that an increase of the sulfide level in a digester treating municipal sewage sludge resulted in a faster conversion of LCFA to biogas, by favoring growth of the LCFA-degrading microorganisms. To our knowledge, this was the first time that such a connection between sulfide level, growth of LCFA-degrading microorganisms, and LCFA turnover kinetics was reported, which provided evidences for potential regulatory role of sulfide in degradation of lipids.

In the next part of the project, we studied the degree and kinetics of the degradation of saturated and unsaturated LCFA at different sulfide levels in six laboratory anaerobic digesters, treating primary and secondary sewage sludge. In particular, the microbial community response to saturated and unsaturated LCFA loads was studied in relation to sulfide level in the digesters. The outcomes of this study are found in the following publication:

Literature

Shakeri Yekta S, Liu T, Axelsson Bjerg M, Šafarič L, Karlsson L, Björn A, Schnürer A (2019). Sulfide level in municipal sludge digesters affects microbial community response to long-chain fatty acid loads. *Biotechnology for Biofuels*, 12, 259 (Link)

Aim

The aim of the proposed master's thesis is to further assess the differences in microbial metabolic functions during anaerobic digestion of saturated and unsaturated LCFA at different sulfide levels. For this purpose, the overall project includes the following steps:

- 1) To perform DNA purification for Nanopore and Illumina sequencing of selected samples
- 2) Perform Nanopore sequencing
- 3) Perform protein and peptide purification for Ms/Ms analysis
- 4) Apply MAFIN (a bioinformatics pipeline) for assembly, binning and annotation
- 5) Analyze and evaluate retrieved data by using MaxQuant and Perseus software

Complementary information

The candidate may only perform some of the steps. Which steps the project will include, depends on the study program of the candidate. The project is suitable for master degree programs related to Bioinformatics, Molecular Biology, Biochemistry and Microbiology.

The project will be performed at SLU, Department of Molecular Sciences, SLU, Uppsala and supervised by Bettina Müller (Bettina.Muller@slu.se) and Sepehr Shakeri Yekta (sepehr.shakeri.yekta@liu.se) from Linköping University, Department of Thematic Studies-Environmental Changes. Please contact us when you are interested in a master thesis.

Characterization of mannoside binding to a cyanobacterial carbohydrate-binding protein by NMR spectroscopy

Cyanovirin-N (CV-N) is an antiviral lectin (a carbohydrate-binding protein) from the cyanobacterium *Nostoc ellipsosporum*. CV-N binds to oligosaccharides composed of mannose sugar residues (mannosides), which are present on the surface of several viruses, such as HIV, Ebola, and influenza virus. For example, the HIV-1 envelope glycoprotein gp120 is heavily glycosylated with mannosides. These sugars can serve as targets for antiviral compounds, which bind to the glycans and interfere with the viral entry into the target cell. CV-N has shown antiviral activity against HIV type I and II and other enveloped viruses like Ebola and influenza at nanomolar concentrations. At present, CV-N is tested for therapeutic use as topical applications in microbicides. The characterization of CV-N/mannoside interaction is important for the development of new microbicides, but also for the general understanding of lectin/mannoside interactions, which may be used as a target to block viral infection when no vaccine is available.

The specific aim of this project is to investigate the binding of different mutants of CV-N to certain mannosides by using nuclear magnetic resonance (NMR) spectroscopy. The mutants will be expressed in *E. coli* with a medium that contains sources of ^{15}N and/or ^{13}C , which is a requirement for the NMR analysis. Proteins will be purified with ion-exchange chromatography, size-exclusion chromatography and dialysis, and then subject to NMR analysis. Mannosides will be labelled with ^{13}C to facilitate the analysis and new NMR experiments that are designed for such compounds will be utilized.

Skills that will be developed within this project:

- Expression of isotopically labelled proteins in *E. coli*
- Protein purification
- Protein and carbohydrate analysis by NMR spectroscopy

Unravel the competition between methane-producing microorganisms in biogas systems

We are looking for a highly motivated student who is interested in joining our group to investigate the competition between different methane-producing microorganisms in order to find ways to predict biogas production rates.

Biogas production is a waste-to-energy technology with outstanding climate, environmental and societal benefits. Biogas is produced when organic materials are broken down by microorganisms in an anaerobic environment that proceeds in a series of steps divided into hydrolysis, acidogenesis, anaerobic oxidation and methanogenesis. The last and methane-producing step is extremely important for efficient biogas production. This is also the step that easily gets restricted by toxic compounds or during changes in process operation. A restricted methanogenic step will cause severe process disturbance and decrease the biogas production.

In this project we aim to study the competition between different microorganisms that perform the methane-producing step. Two main pathways for biogas production are acetoclastic methanogenesis (performed by acetate-utilizing methanogens) and syntrophic acetate oxidation (performed by acetate-utilizing bacteria and H₂-utilizing methanogens). Cultivation studies with these two groups of methane-producers will be set up and the impact on the methane production rate by ammonia (a toxic compound formed in the degradation of proteins) and temperature will be investigated. The interplay of the microorganisms will be followed by molecular approaches. The result from the study can be used to predict consequences on methane production rates in biogas processes operating at different conditions.

You will acquire skills in:

1. Anaerobic cultivation techniques
2. Analytical analyses using high-performance liquid chromatography (HPLC), gas chromatography (GC), H₂-measurement
3. Molecular techniques including DNA extraction, agarose gel electrophoresis, quantitative PCR (qPCR), RNA extraction, conversion to complementary DNA (cDNA)
4. Performance of a design of experiment approach.

Complementary information

The project is suitable for master degree programs related to Bioinformatics, Molecular Biology, Biochemistry and Microbiology and will be performed at SLU, Department of Molecular Sciences, SLU, Uppsala. Please contact Maria Westerholm (Maria.Westerholm@slu.se) if you are interested in this project.

Purification and properties of artificially matured [Fe-Fe] hydrogenases from syntrophic acetate oxidation bacteria

Background

The reversible conversion of protons and electrons into molecular hydrogen represents the simplest possible redox reaction in nature, and provides potential in supporting a future society free from fossil fuels. Molecular hydrogen can be used as a renewable fuel, as chemical feedstock to produce methane, methanol, and other hydrocarbons, or in industrial processes such as the Haber-Bosch process for producing ammonium fertilizers. At present hydrogen is mostly produced non-renewably from steam reformation of fossil fuels. The only current efficient renewable way is the electrolysis of water, for which rare noble metals such as platinum need to be used as catalysts.

However, nature is capable of catalyzing hydrogen conversion reversible and efficiently by employing enzyme catalysts, so-called HYDROGENASES, under ambient temperatures and pressures, using one of the most abundant metals on earth, iron. [Fe-Fe] hydrogenases are the champions in frequencies H₂ production with reported turnover of 10,000 per sec and occur both in prokaryotes and eukaryotes (single cellular algae). The biosynthesis requires a complex insertion machinery for incorporating the redox active [Fe-S] clusters and the [2Fe] subsite what impedes cloning, modifications and mechanistic studies. However, [Fe-Fe] hydrogenases have been successfully overexpressed recombinantly in *Escherichia coli* as pro-enzymes in high yields and with high purity. Then, the active enzymes have been produced by adding an artificially produced inorganic [2Fe] active site variant circumventing any species-specific maturation machineries.

[Fe-Fe] hydrogenases are the engine of so-called syntrophic consortia, where one species lives from the hydrogen, which has been produced by another species. In syntrophic acetate oxidizing bacteria, acetate is oxidized to hydrogen, which is consumed by methane producing archaea. Thus, syntrophic [Fe-Fe] hydrogenases might be potential candidates for future bio-hydrogen production.

Project description

Within the 30-credit degree project corresponding to 20 weeks of education, the candidate will investigate the suitability of overexpressing syntrophic [Fe-Fe] hydrogenases in *E. coli* and reconstitute and compare their activities by adding an inorganic [2Fe] active site mimic. These include cloning of the hydrogenase genes using a well-established pET vector system; overexpression in *E. coli*, purification with metal-affinity chromatography, anaerobic work and different spectroscopical methods (UV-Vis and EPR spectroscopy) The candidate student will acquire fundamental understanding of experimental design in molecular biology and obtain competence in laboratory work, data handling, data analysis and manuscript preparation. The candidate is expected to write and present the master thesis in English.
