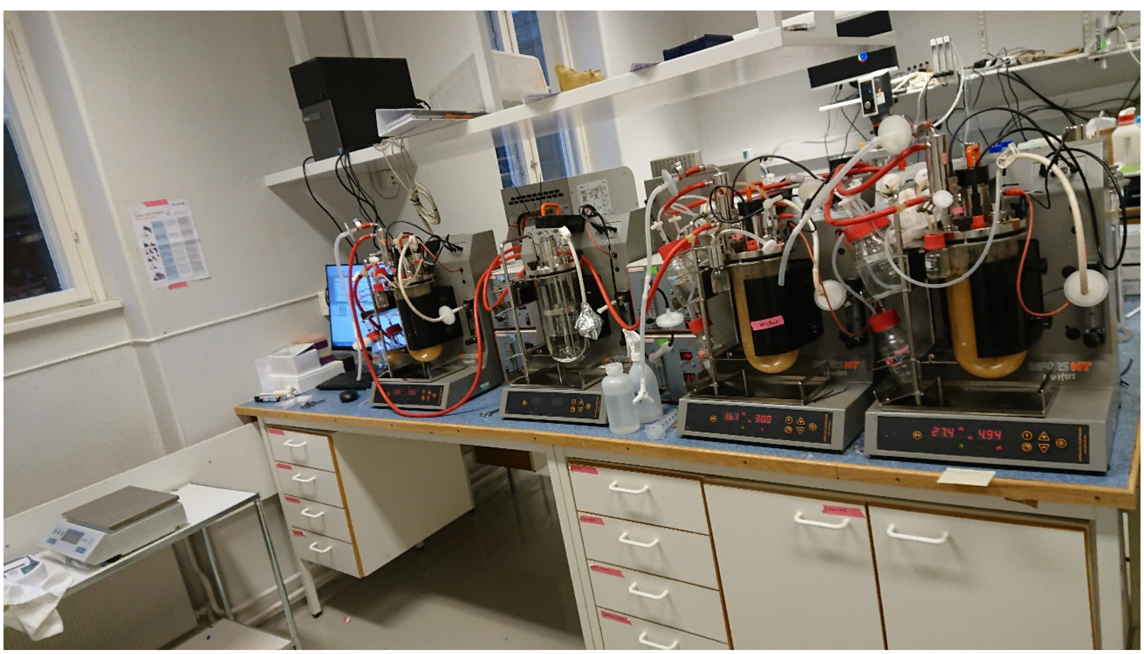
Topics for master theses at the research group for

Bioprocess Technology and Biorefining (BioRef)

Web: https://www.nmbu.no/en/faculty/kbm/research/groups/btb

March 2021

NMBU Biorefinery laboratory



Fermentation laboratory

Biogas

Contact persons: Svein Horn, svein.horn@nmbu.no,

Concerns regarding global warming, fossil fuel depletion and energy security have resulted in a wide interest in renewable and environmentally friendly fuels production such as biogas and bioethanol. Biogas is produced from different organic waste materials using microorganisms under anaerobic conditions. Biogas contains mainly methane and CO2, which can be either directly combusted to generate heat and electricity or further upgraded to natural gas quality to be used as a vehicle fuel or stored in a natural gas grid for later use. The remaining organic residue (i.e. digestate) after biogas production can be used as a biofertilizer.

There has been technological developments for the production of biogas and bioethanol from lignocellulosic biomass, including agricultural and forestry waste products (straw, stalks, bagasse, woods etc), because of their abundance and environmental sustainability. In a biorefinery setting, the sugars will be extracted prior to biogas process for the production of bioethanol and other products, while the remaining lignin-rich fraction will be used for biogas production. However, the complex compositional and structural features of lignocellulosic biomass makes it generally resistance to biological process. Therefore, an additional pretreatment step (including physical, chemical and/or biological methods) is needed to reduce biomass recalcitrance and facilitates subsequent biological processes (enzymatic hydrolysis, fermentation and biogas processes).

Within the field of biogas technology, Master's students will have an opportunity to conduct their thesis on one or more of the following topics:

1) Evaluation of biogas potential of lignocellulosic biomass pretreated with different techniques such as chemical, physical and/or biological (enzymes)

2) Investigating the effects of hydrogen addition in anaerobic digestions

Biogas laboratory

Enzymology and Fermentation

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Are you interested in discovering new enzymes and learn how to clone, purify and produce them in bioreactors?

We develop novel fish feed by integrating bioprocessing of non-food biomass and bioprocessing technologies to exploit woody biomass, such as spruce/birch trees and cultivated brown macroalgae as sustainable supply of high-quality proteins in animal diets.

Seaweed

Pre-processing techniques such as enzymatic hydrolysis of macroalgae is required to release fermentable sugars. After enzymatic hydrolysis, special yeast strains are cultivated on the macroalgae hydrolysates to produce single cell proteins in bioreactors. The commercial importance of macroalgae has been increasing over the past decade, but only few commercial enzymes are available for macroalgae hydrolysis, and they are prohibitively expensive.

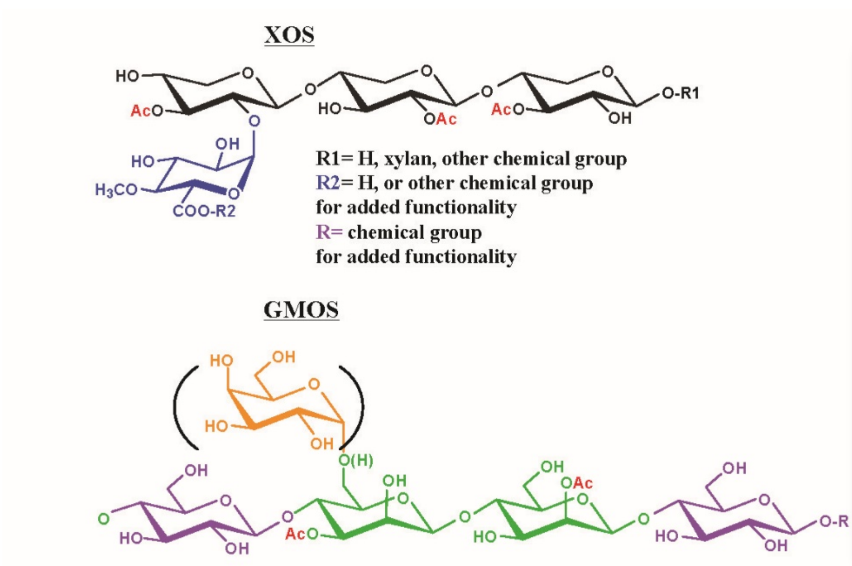
The objective of this work is to screen and characterize novel enzymes for efficient hydrolysis of seaweed. This may also involve production of enzymes and single cell proteins in fermenters. We offer:

- MSc thesis position in a multidisciplinary group with modern facilities
- Challenging projects, which gives you the possibility to learn many exciting techniques:
  - Cloning and expression of recombinant proteins
  - Protein purification
  - Enzymatic activity measurements
  - Advanced analytics (HPLC, MS etc.)
  - Bioreactor experiments

Woody Biomass

The majority of terrestrial biomass resource in a future bio-economy will be plant based material (lignocellulosic biomass), which is recalcitrant and challenging to process. Enzymatic depolymerization of polysaccharides in lignocellulosic biomass is a key technology for future biorefineries and is currently the subject of extensive research. Hemicelluloses are major constituents of non-edible feedstocks relevant to biorefineries.

Softwoods like Norwegian spruce contains about 25% gluco(galacto)mannan (GMOS) and about 10% (arabino)xylan (XOS) (Fig.1). These polymers are largely inaccessible when entangled in the plant cell wall. Xylan is highly abundant in several other relevant feedstocks like hardwoods (birch/aspen) and straw (wheat, barley etc.), which contain ~30% xylan. Notably, there are striking similarities in the hemicellulose structures in edible sources like wheat and non-edible sources like forest biomass.



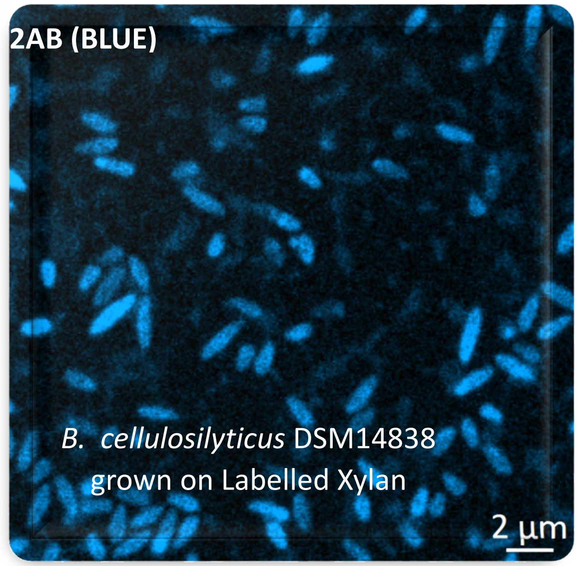


Fig. 1. Examples of oligosaccharides derived from xylans (XOS) and gluco(galacto)mannan (GMOS). The saccharide constituents are: xylose (black), glucose (magenta), 4-Methyl-Glucuronic acid (blue), galactose (orange), arabinose (turquoise) and acetyl (Ac, red).

An ongoing project in our lab aims to producing several low cost and highly defined oligosaccharides (for examples see Fig.1) from non-edible (high abundance) sources. In addition, it seeks to identify key structural determinants for their utilization by commensal bacteria in both the gastro intestinal (GI) tracts of humans and livestock.

β-mannans are the major constituents of hemicellulose and are widely distributed throughout the plant tissues. Despite Firmicutes is the most abundant bacterial phylum in the gut, relatively little is known on the metabolic strategies adopted by the members of this phylum to utilize manno-oligosaccharides that are indigestible by the human host. Depolymerization of β-mannans requires a plethora of carbohydrate active enzymes for the hydrolysis of the backbone and removal of side-chain substituents. We have investigated a Firmicute bacterium and identified the genes coding for proteins involved in manno-oligosaccharides transport and degradation. The Master students will be actively included in spinoff projects of this ongoing research project. We offer:

- MSc thesis position in a multidisciplinary group with modern facilities
- A challenging project, which gives you the possibility to learn many exciting techniques:
  - Cloning and expression of recombinant proteins
  - Protein purification
  - Enzymatic activity measurements
  - Advanced analytics (HPLC, MS etc.)
  - Bioreactor experiments
- This project is in close collaboration with groups in Gotenburg and Helsinki

<u>Enzyme characterization</u>: (some examples, we are working with a large number of enzymes related to hemicellulose degradation, several in collaboration with the PEP group)

<u>**Project title**: *Characterization, Structure and Application of a Mannobiose Epimerase from a Firmicute Bacterium*. (this is also in collaboration with dairy industry, Tine)</u>

Enzymatic epimerization is an important modification for carbohydrates to acquire diverse functions attributable to their stereoisomers. Epimerases are isomerases that catalyze the inversion of the configuration around an asymmetric centre of substrates. We have identified a gene encoding an epimerase in the genome of a Firmicute gut bacterium and confirmed that the recombinant enzyme has a role in the metabolism of β-mannans. The aim of this Master project is to functionally characterize the epimerase and determine the crystal structure. The enzyme has a great potential in environmentally friendly industrial application. This includes the biosynthesis of mannose, that has a remarkable commercial value) by enzymatic epimerization of cello-oligosaccharides.

<u>**Project title:** *Biochemical and Structural Characterization of an Intracellular Mannosidase from a Firmicute Bacterium*.</u>

β-Mannosidases are important constituent of the mannan-degrading enzyme apparatus. These enzymes hydrolyses the non-reducing end of manno-oligosaccharides to release mannoses. In addition, they are relevant for many industrial applications, such as production of feed, for pulp/paper industries as well as the production of biofuel. The aim of this work is to obtain a better insight into the thermostability and catalytic efficiency of a mannosidase, identified in a Firmicute gut symbiont, as these characteristics constitute a powerful tool for improving the enzymatic conversion of mannan through synergistic action with other mannan-degrading enzymes. X-ray crystallography will be crucial to identify key structural determinants and gain more insights into the basis of the catalytic activity.

What is behind the biotechnology for biofuels & chemicals?

Do you want…

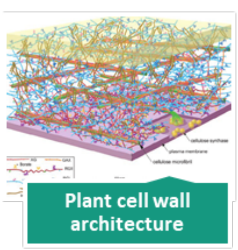
- to be part of the Norwegian bioeconomy?
- to learn how to make biofuel from lignocellulosic biomass?

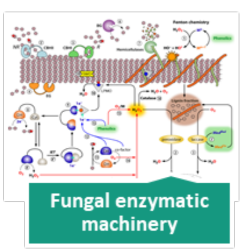
Would you like to find out more about these issues? Then you might want to take a master's thesis in the PEP/BioRef groups. Our research focuses on identifying enzymes that are important in nature to degrade lignocellulosic materials such as wood and straw. Such enzymes could be utilized in applied settings to increase the efficiency of bioprocesses to generate bio-based fuels and chemicals.

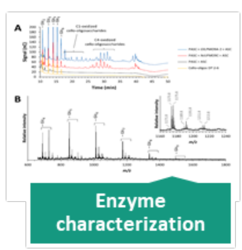
What we offer:

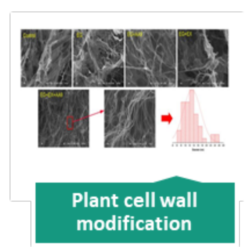
You will get the chance to learn a set of methods useful in later career: enzyme selection, cloning techniques, a wide range of biochemical assays, biomass processing and cutting-edge analytical tools such as HPLC and mass spectrometry.

With us you can learn about:









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Oleaginous fungi

In cooperation with REALTEK

Contact persons: Volha Shapaval, volha.shapaval@nmbu.no, Svein Horn, svein.horn@nmbu.no

Biotransformation of animal fat rest materials by Mucor fungi – lipid accumulation and lipase activity

In Europe, approximately 16 million tonnes of animal fat by-products are processed annually by fat processors and renderers. The total amount of animal fat rest materials is expected to increase continuously, since consumers and health authorities in Europe increasingly focus on low saturated fat diets. Upgrading fatty acid composition of animal fat rest material is attracting a huge interest from industry.

A new technology for upgrading animal fat is fermentation by oleaginous fungi, which are able to convert hydrophobic substrates such as vegetable oils and animal fat into higher value single cell oils that are rich in polyunsaturated fatty acids (PUFAs). Certain filamentous fungi referred to as oleaginous, have the ability to accumulate up to 85% (w/w) lipid as a storage compound of the biomass. The oleaginous fungi accumulate lipids primarily as triacylglycerols (TAGs), which are generally considered as storage lipids. Fatty acids of TAGs are very similar to fatty acids in plant oils, where saturated and monounsaturated fatty acids dominate, and some ω6- and ω3-PUFAs are present.

The main focus of the master thesis is to study the biotransformation of animal fat containing saturated fatty acids rich triacylglycerols (SAT-TAGs) into polyunsaturated fatty acids rich triacylglycerols (PUFA-TAGs) by oleaginous Mucor fungi. The work will involve running fungal cultivations in microtiter plates and shake flasks. Biomass production, lipid production and lipase activity in Mucor cells will be monitored using GC, FTIR spectroscopy, and other analytical techniques

Biorefining:

Project title: ***Production of biochemicals using carbohydrates as a basic compound, for the next generation biorefinery.*** **A spin off project of WoodPrebiotics (above), this will in part be connected to collaboration with industry, Borregaard.**

Xylan and mannan from woody biomass will serve as core compounds to generate novel biochemicals using cross coupling chemistry. The project will involve the combined use of enzyme technology, advanced analytical and purification methods, and different types of chemistry related to cross coupling of carbonyl and carboxyl groups. This MSc is a collaboration with the Chemistry section (Prof. Yngve Stenstrøm).

Contact persons: Bjørge Westereng, bjorge.westereng@nmbu.no, tlf. +4767232575

Prebiotics and gut microbiota

Do you want to learn about prebiotics? We are working on exploring novel prebiotics and tracing them in microbial communities (we work on many different substrates, like Human milk oligosaccharides, Fructooligosaccharides (FOS), various dietary fibers). Such a project involves testing of novel prebiotics in fermentations. E.g. It could be to run fermentations with mannan from spruce (similar to many food stabilizers) using a microbial community from obese people and see if you can alter the community towards beneficial bacteria in this model too. The project will utilize small scale custom designed fermenters to allow small volume-testing of substrates.

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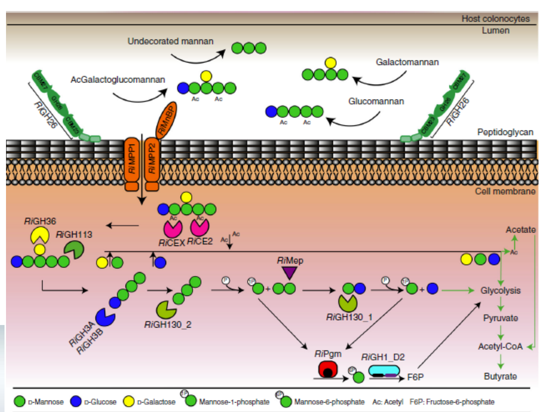

ARTICLE

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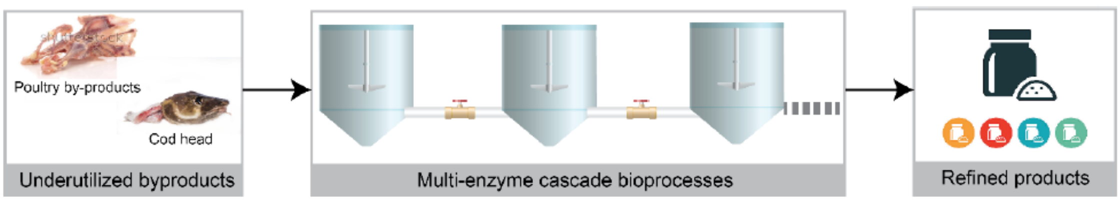
The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β-mannans

Sabina Leanti La Rosa[1], Maria Louise Leth[2], Leszek Michalak[1], Morten Ejby Hansen[2], Nicholas A. Pudlo[3], Robert Glowacki[3], Gabriel Pereira[3], Christopher T. Workman[2], Magnus Ø. Arntzen[1], Phillip B. Pope[1], Eric C. Martens[3], Maher Abou Hachem[2] & Bjørge Westereng[1]

Multistep biotechnological processing of animal and marine by-products

Enzymatic protein hydrolysis (EPH) is gaining significant attention as a versatile processing technology of animal and marine by-products. During the EPH process, enzymes are added to by-products to facilitate breakdown of proteins to smaller peptides and amino acids. This is followed by downstream recovery of three crude fractions: peptides, lipids and mineral-rich sediment. However, by-products from animal and marine origin are not limited to three components only, but consist a multitude of different proteins and other biological molecules. Therefore, there is a need for processing technology where the whole range of components existing in these complex by-products are refined in an optimal way. In this master project we will systematically investigate if multistep biotechnological reactions can be developed to obtain multiple products (e.g. myofibrillar proteins and collagen) from poultry and cod by-products. The candidate will be linked to an ongoing research project comprising several industry partners.



This assignment is suitable for students in biotechnology, biochemistry, and analytical chemistry.

You will be experienced in:

- Biotechnology
- Bioprocessing
- Analytical chemistry
- Chemometrics

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Nofima

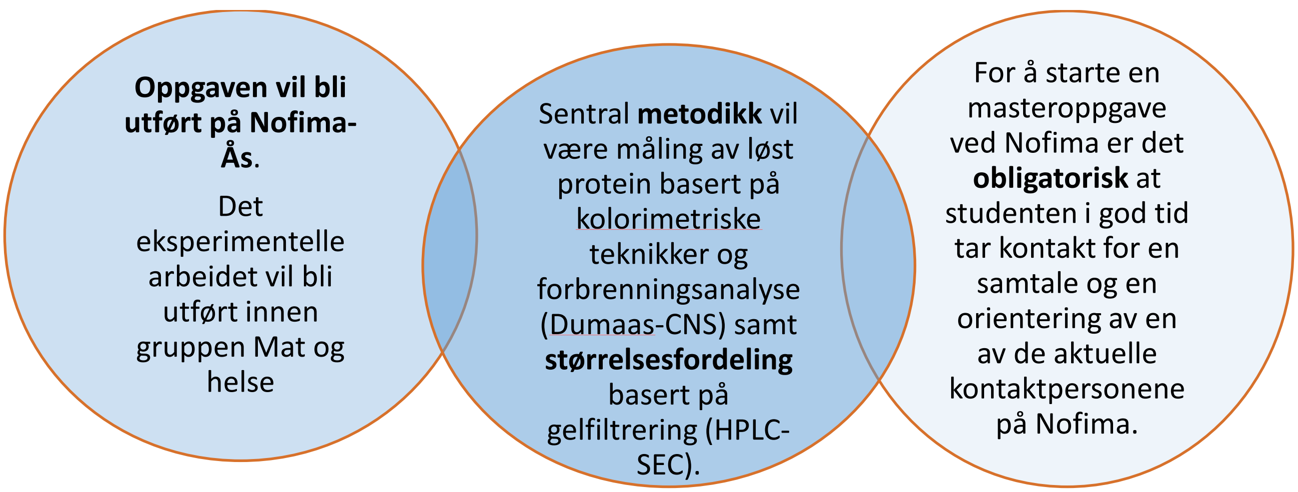
Makroalger som matingrediens

Hvor godt kan tang og tare fordøyes?

Et argument for humant konsum av tang og tare et tilsynelatende høyt innhold av **protein** med en ernæringsmessig gunstig aminosyreprofil. En forutsetning for ernæringsnytten av tang er at proteindelen er tilgjengelig og **fordøybar** etter inntak. Med fordøybarhet menes her i hvilken grad proteinet brytes ned til peptider i fordøyelsen (mage tynntarm), og ikke den eventuelle fermenteringen av protein som vil foregå i tykktarmen med hjelp av tarmbakterier. Et viktig aspekt rundt dette vil være koblet til i hvilken form algen spises og **prosesseringen** fram mot det (tørking, frysing, varmebehandling, oppmaling, forvelling, etc). Et sideaspekt med dette er at prosesseringen vil kunne redusere/vaske bort uønskede komponenter som bland annet salt og iod.

Utgangsmateriale vil være tørket eller tørket og finmalt tare som kombineres i en resept med konvensjonelt mel. **Prosessering** mot mat vil være varmebehandling (grøt, ekstrudering) og baking. Algebaserte matprodukter eller ingrediensen vil så bli behandlet i et *in vitro* system med definerte enzymer for å simulere human fordøyelse. Som måleparameter for fordøyelighet vil vi sette opp et system for å måle nedbrytningen av protein i form av øket løselighet og dannelse av peptider.

Oppgaven vil være knyttet opp mot det nasjonale NFR prosjektet: The Norwegian Seaweed Platform ledet av NTNU



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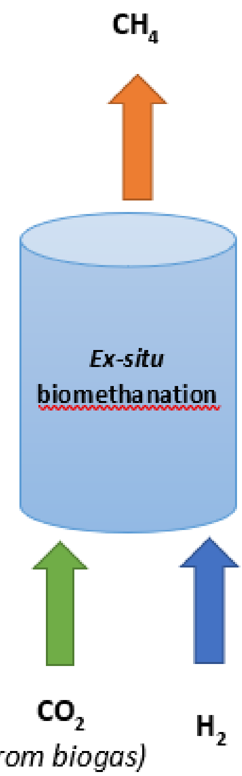
Kontaktperson NMBU: Prof. Svein Horn.

Nofima

Ex-situ biomethanation as a method for biogas upgrading

Biogas is a mixture of methane (CH_4), carbon dioxide (CO_2) and small quantities of other gases produced by anaerobic digestion (AD) of organic matter in an oxygen-free environment. The CO_2 in biogas occupies a significant part of its volume (from 20 up to 45%) representing no energetic use. In this context, the bioconversion of CO_2 (present in biogas) to CH_4 is gaining attention. The *ex-situ* biomethanation is one of methods where CO_2 can be biologically reduced to CH_4 by hydrogenotrophic methanogens upon hydrogen (H_2) addition. This method is one of examples of Power-to-Gas concept that enhances the synergy between renewable energies. This project is going to investigate different process variables that can influence the process efficiency. The work in this project will be linked to ongoing activities in the large research project Bio4Fuels, where both NMBU and NIBIO are partners.



This assignment is suitable for students in biotechnology, biochemistry, microbiology, bioprocess technology and analytical chemistry.

Depending on interests learn:

- To operate biogas reactors
- Monitor biogas activity
- Microbiology of biogas reactors
- Modelling and theoretical calculations
- Analytical chemistry

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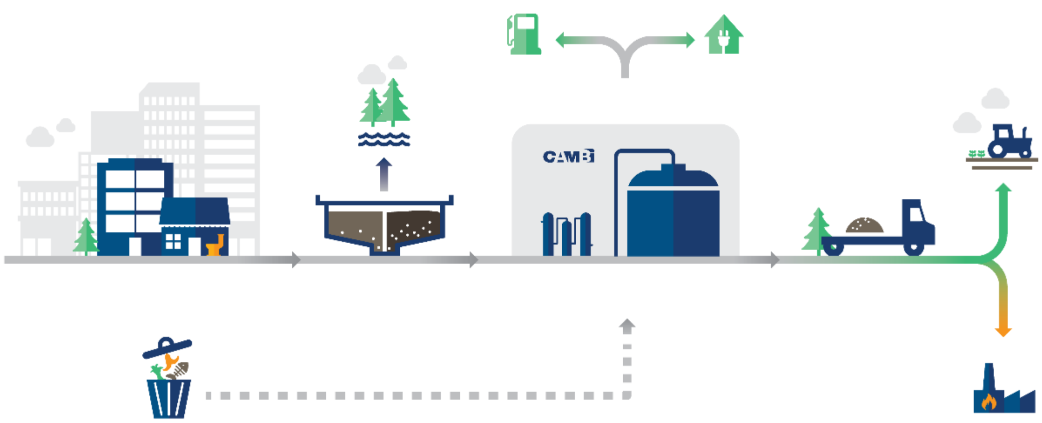
NIBIO

Pushing the limits for anaerobic digestion with thermal hydrolysis

Anaerobic digestion is widely used for the stabilization and valorization of sewage sludge and production of renewable energy. The digested sludge is degraded in CSTR-type digesters where organic material is converted to biogas and the resulted digestate is less odorous and carries less pathogens.

Cambi's thermal hydrolysis process (THP) accelerates the anaerobic digestion process, removes odors, secures a pathogen free final product, and improves the dewaterability of the digestate. Together, these benefits reduce the sludge treatment's carbon footprint. More about thermal hydrolysis can be found here: https://www.cambi.com/what-we-do/thermal-hydrolysis/

With 67 thermal hydrolysis plants in 22 countries, Cambi is the market leader and the most experienced supplier of thermal hydrolysis. Cambi has had close collaboration with NMBU and NIBIO on THP-AD research over the years and invested in test facilities at the biogas laboratory. The lab today has two pilot THP units which allows for further research on THP's impact and potential for improving anaerobic digestion.



Although THP is a well-established technology, knowledge on how the process impacts anaerobic digestion is scares in some areas. The three following projects aim to address some of the outstanding questions with great commercial importance:

1) Municipal anaerobic digestion systems are typically designed for approximately 20 days retention time. Facilities with thermal hydrolysis pre-treatment have been designed for 15 days. But are even shorter retention times possible? Only very few studies have investigated how THP influences the limits for hydraulic retention time in anaerobic digestion. Numerous batch studies have been performed, but these does not allow for the selection of a specialized rapid growing microbial community for the digestion. Hence, any kinetics data extracted from these studies will be biased towards the starting microbial community and modelling using these data generally severely underestimates THP's potential for reducing HRT. In this master project the effects of HRT on AD performance and digestate characteristics will be studied both on THP-treated and non-treated sewage sludge. The project aims to challenge the current practice of 15 days retention time and have potential to fundamentally influence the design of future anaerobic digestion systems with thermal hydrolysis.

2) During THP some recalcitrant organic compounds are formed. This may give brown color to treated wastewater which is undesirable for e.g., wastewater reclamation facilities. It is commonly hypothesized that this color is linked to the temperature during THP (~165°C) which facilitates the Maillard reaction. The Maillard reaction is well known from food sciences, where the chemical reaction between amino acids and reducing sugars gives browned food its distinctive flavor. However, being a highly temperature dependent reaction, reducing the temperature during thermal hydrolysis to 150°C or 140°C could limit the formation and types of Maillard products. This master project aims to study how THP temperature influences sludge characteristics and especially the quantity and quality of Maillard products in different sewage sludges. The study may involve lab experiment and analysis as well as full-scale data collection and plant survey.
3) Anaerobic digestion of agro-waste for biogas production is a growing market in particularly Europe. However, anaerobic digestion of straw and manure is only partial, and much of the energy in the substrates are not converted to biogas in conventional digestion even at relatively long HRTs. This result in an energy rich, high carbon, residual fiber fraction. Thermal hydrolysis has the potential of making more of this energy available for conversion to biogas. The aim of this master project is investigating a more novel configuration of THP between two digestion steps, and how THP application on the fiber fraction of digestate influences the biogas production in a second stage digester.

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