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# HYPE

## *High Efficiency Consolidated Bioprocess Technology for Lignocellulose Ethanol*

Volume 1, Issue 1

## Project overview

Fuels from lignocellulose biomass have a high potential to reduce GHG emissions, and hence are an important means to fulfil road transport $CO_2$ emissions targets. They can be a reliable fuel source, which can gradually reduce the dependence on oil imports. Advanced conversion technologies are, however, needed to produce ethanol from a wider range of resources, including lignocellulosic biomass.


The main challenges for improving the hydrolysis and fermentation technologies of lignocellulosic biomass are:

* Improved enzyme performance and reduction of enzyme costs
* Higher yield of saccharification and co-fermentation of C5 and C6 sugars
* High density hydrolysis and fermentation for reduction of investment costs
* Reduced overall process time for hydrolysis and fermentation

This project will approach these challenges by developing a new process concept which integrates the most relevant achievements in the field. The overall aim is to reduce the production costs of ehanol and to accelerate the implementation of new second generation biofuels from lignocellulosic raw materials by overcoming the identified key bottle-necks, presently hindering commercialization.

## Objectives

The goal of the project is through a combined approach to develop a novel continuous consolidated bioprocess concept for the hydrolysis and fermentation of lignocellulosic feedstocks. The technologies will improve the competitiveness of second generation fuel production processes and thus enable future secure and sustainable energy supply for the transport sector.

The concept of combining added thermostable enzymes and consolidated bioprocessing will enable a high feedstock adaptation as well as a significant reduction in process time and cost combined with a full processing of all the carbohydrates. After optimizing the individual process stages, the novel consolidated bioprocessing technology will be tested in laboratory and pilot scales.

### Inside this issue:

## Feedstock pre-treatment

Within Europe, straw is presently one of the most relevant raw materials and was chosen as the first reference biomass for bioethanol production in this project. Other raw materials studied include the corn stover, reed canary grass and willow.

Pretreatments disrupt the plant cell wall and improve enzymatic access to the polysaccharides. Wheat straw and corn stover have been pretreated in an optimized process in the the large pilot scale (50 kg/h) of Inbicon. The liquid and solid fractions of the pretreated straw have been analyzed in detail. The continuous steam pretreatment of corn stover and corn cobs has also been optimized. In the optimized process, the same high ethanol yield can be obtained as for wheat straw (> 200 l ethanol/ton biomass dry matter). Other pretreatment techniques are being developed by BioGold.

Maize *(Zea mays L.)*

## Hydrolysis & fermentation

The high cost of enzymes is still considered a key barrier to the economic production of cellulosic ethanol. Within the HYPE project, various approaches have been taken to decrease the enzyme costs.

Thermostable enzymes offer potential benefits in the hydrolysis of lignocellulosic substrates. Preparations of thermophilic enzymes have been produced for further characterization and testing by ROAL and VTT.

The optimal mixtures of thermostable enzymes have been designed for the liquefaction stage, as well as for the combined liquefaction and saccharification stages.

The composition of the liquefying enzyme preparation consists of five thermostable enzymes. The conditions for the liquefaction at increased temperature combined with saccharification at lower temperature have been optimized at the Universities of Helsinki and Copenhagen.

Saccharification with enzymes produced commercially or by the consolidated organism, *Fusarium oxysporum,* has been compared and the amount of added enzymes has been minimized. The ethanol production characteristics of *F. oxysporum* have been determined on solid and liquid substrates at the National University of Athens. Processive cellobiohydrolase enzymes are the key components of fungal cellulase systems, studied at the University of Tartu.

## Implementation and evaluation in pilot plant

Design of pretreatment equipment for advanced bioprocessing has been started at Inbicon. The reactor system for the consolidated bioprocess has been initiated by the development of a vacuum stripper with the capacity to recover ethanol during fermentation at low temperatures by HOLM Biosystemer. The Inbicon demonstration facility will be used as the base case and comparison for the feasibility study of the developed consolidated bioprocess.

*Inbicon biomass refinery demonstration plant*

# Project partners:

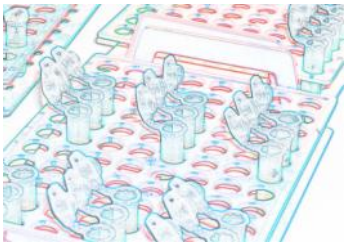
## University of Helsinki, Finland

The activities at UH, Department of Food and Environmental Sciences, focus on biosciences related to the conversion and upgrading of lignocellulosic polymers; *i.e.* cellulose, hemicelluloses and lignin. A special emphasis is on the enzymology of the major carbohydrates, especially on basic knowledge on the structure and disassembly of the substrate, interactions of biocatalysts and substrates in the heterogenous matrix and on the potential of biological systems for exploiting these important renewable raw materials. One of the focus areas is the identification of factors restricting the hydrolysis of lignocellulosic substrates to platform sugars. The chemistry laboratories are equipped with modern instrumentation (HPLCs, GCs, GC-MS, HPAEC-PAD, LC-MS). The University of Helsinki (Prof. Liisa Viikari) acts as the coordinator of the project.

*" We will either find a way, or make one" ~ Hannibal Barca*

## VTT, Finland

VTT, Technical Research Centre of Finland has pioneered in various aspects of the enzymatic cellulose hydrolysis, the structure-function studies on industrially important enzymes and has been developing the enzymatic hydrolysis processes up to pilot scale. Novel cellulases from various sources have been cloned, characterized and engineered.



VTT has versatile bioprocessing facilities, from numerous research fermentors up to well equipped pilot facility consisting of fermentors with volume up to 1.5 $m^3$ and the required downstream processing equipment.

VTT has strong research groups related to various fields of biotechnology, and the ones involved in HYPE project are Bioprocessing studying enzymatic hydrolysis and Cell Factory concentrating on fermentation technology. The major aims of VTT studies are to evaluate and develop optimal enzyme mixtures for efficient hydrolysis of project materials, to reveal and analyse the bottlenecks of hydrolysis mechanisms and to study fermentation of process materials utilizing among other organisms VTT's yeast utilizing pentose sugars.

## Roal Oy, Finland

Roal Oy is an industrial enzyme manufacturer having a modern, recently expanded, production facility combined with a long-term biotechnical research on plant fibre modifying enzymes. During the years, Roal has developed high-producing proprietary *Trichoderma* mutant strains with tailored strain backgrounds for cost-effective production of industrial enzymes. Roal produces enzymes for several different industrial applications, *e.g.* baking, food, technical and feed industries. Over 90 % of the products are exported worldwide.

Roal was established in 1991 as a joint venture by former Finnish Alko Ltd and German Röhm GmbH. Both companies had a decades-long history in producing industrial enzymes. Today Roal is owned by Altia and Associated British Foods (ABF, UK). Majority of the enzyme products are marketed and distributed in co-operation with AB Enzymes.

## University of Copenhagen, Denmark

The research objective of the group at Forest and Landscape, University of Copenhagen is to improve understanding of biomass recalcitrance, the influence of the plant cell wall structure/composition and enzyme-substrate interaction with the intention of improving the efficiency and cost competiveness of enzymatic processing of biomass into biofuels. This work is done in close collaboration with industry and one of the key areas has been the development and application of technologies for high solids enzymatic hydrolysis and fermentation.

The role in HYPE is to test and optimize the use of thermostable enzymes for the high temperature liquefaction and saccharification of pretreated biomasses with special emphasis on using high solids conditions (above 20% initial water insoluble solids). Another task is the evaluation of the various biomass (wheat straw, corn stover, reed canary grass and willow) and pretreatment methods used in the HYPE project.

*"Everything that depends on the action of nature is by nature as good as it can be"*
*~ Aristoteles*

## Holm Christensen Biosystemer ApS, Denmark

Biosystemer is a small independent family owned development company formed in 1976. Holm will participate in the project by designing and constructing new equipment for the continuous consolidated bioprocess and the ethanol removal system.

Conversion of fermentable sugars to ethanol is an energy efficient process, more than 90% of the energy of the sugars is transferred to ethanol in the beer. Unfortunately recovery of the ethanol from the beer requires a substantial amount of energy.

The work of Holm aims at reducing the energy cost of the recovery process by introducing a novel diabatic vacuum distillation process with a potential reduction of 10-40% compared with Inbicons base case comprising of adiabatic vacuum process with direct steam injection. The highest reduction is achieved when vapour compression or heat pump systems are employed for the energy input.

## National Technical University of Athens (NTUA), Greece

National Technical University of Athens, BIOtechMASS Unit, School of Chemical Engineering is focused on development of new biotechnical tools; enzymes and micro-organisms, for the production of second generation biofuels, bio-based polymers and chemicals and is active in a broad range of related scientific fields. BIOtechMASS has extensive expertise in discovery of novel enzyme activities for modification or degradation of plant cell wall material, development of new-enzyme processes for production of high added value products, saccharification and fermentation of agricultural wastes and metabolic engineering for the effective fermentation of glucose and xylose. These skills have already successfully contributed to tailoring bioresources for specific bio-processes. The major contribution to the HYPE project is ethanol production from various pretreated raw materials (pretreated wheat straw, sorghum) using the *Fusarium oxysporum* consolidated system.

*A bioreactor containing F. oxysporum*

## University of Tartu, Estonia

The University of Tartu (UT) was founded in 1632 by the Swedish king Gustavus Adolphus. UT is Estonia´s leading centre of research and training.

In the HYPE project, the main task of the UT cellulase group is the detailed characterization of cellulases and identification of the "bottlenecks" in the hydrolysis process. Novel methods for measuring the values of catalytic constants, processivity, and inhibition constants for cellulases have also been developed. Furthermore, the mechanism of rate retardation in cellobiohydrolase (CBH) catalyzed cellulose hydrolysis, endo-exo synergism and limitations of the processivity of CBHs by substrate have been enlightened. Thus, the HYPE project has contributed in the increase in our understanding on the basic molecular mechanisms of the complex process of cellulose degradation.

Cellulase group: Front from the left, docent Priit Väljamäe and MSc student Riin Velleste. Back from the left, lecturer Hele Teugjas and PhD students Jürgen Jalak and Mihhail Kurašin.

## BioGold, Estonia

BioGold Ltd was established in 2006 based on Estonian private capital. BioGold is a small engineering company that specializes in laboratory and chemical process equipment and control system design and installation, as well as project management and consulting. BioGold is experienced both within the biofuels and power engineering and technology. It has participated in many national and international projects on these topics. It was one of the first companies in Estonia to believe in second generation biofuels to be technically possible.

BioGold's engineering staff is equipped with a variety of backgrounds and skills. The company has its own laboratory facilities, including different biomass pre-treatment reactors, as well as other pre-treatment facilities.

Through HYPE project, BioGold hopes to promote the second generation biofuels field, which in turn can help to provide better services to the Estonian and European Industry. BioGold will exploit the results in designing and building up commercial scale. bioethanol plant in Estonia based on local lignocellulosic biomass (straw).

## Inbicon A/S, Denmark

Inbicon is a fully owned subsidiary of DONG Energy, Denmark's largest energy group. Inbicon aims to be the world leader in the development of sustainable biomass refineries distinguished by our pre-treatment technologies and energy-integration systems.

Inbicon will continue improving of its pre-treatment concepts based on enzymatic decomposition of biomass and waste, demonstrate the practicality of our technologies on a commercial scale, and partner with investors, developers, and current ethanol producers to plan and build cellulosic ethanol production around the world. Inbicon also seeks to collaborate with other technology developers whose concepts can be successfully integrated to advance ethanol as a profitable alternative to oil-based fuels.In HYPE Inbicon is responsible of the raw material processing, bioconversion technology, as well as energy and ethanol production.

# Impacts

A special feature of this project is that it addresses the key issues of the ethanol production process, including the pretreatment, liquefaction, saccharification, fermentation, ethanol recovery and distillation, and overall energy integration. Overcoming these technological barriers would improve the technical and economical feasibility of 2nd generation bioethanol production. The raw material base represents relevant raw materials already accessible in many countries, which do not compete with food production. A model to test the process parameters and alternative solutions will be used as a basis of process design.

*"It's better to know some questions than all of the answers" ¨~ James Thurbert*

The developed process concept will be demonstrated in pilot scale. The development of biofuels in the transport sector has a strategic impact on key environmental issues, such as climate change and global warming, and on local pollution in compliance with the Kyoto commitment. It will also enhance the European security of energy supply by reducing the oil dependency and help sustainable rural development.

**Project partners on the map**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