# 1 Publishable summary

## 1.1 Project context and objectives

Production of fuel ethanol from lignocellulose has been shown to have a significantly more positive net energy and $CO_2$ balance than ethanol produced from grain or sugar beet. Fuel ethanol is currently made by large scale yeast fermentation of sugars originating from various annual crops (sugar cane, corn, wheat, barley). Especially, second generation lignocellulosic waste raw materials offer an alternative for being abundant and not competing with food and feed production. For converting lignocellulosic biomass into sugars, enzymatic hydrolysis is presently considered as the most promising technology. This is due to the higher yields, generation of lower amounts of inhibitory compounds and the high potential for improvement, typical of biotechnologies. The enzymatic hydrolysis technology is, however, not yet used in industrial scale, although there are several operational lignocellulose to ethanol pilot and demonstration units all over the world, including European countries (Spain, Sweden, Denmark, Italy, Finland).

The overall aim of the HYPE project is to accelerate the implementation of new second generation biofuels from lignocellulosic raw materials by overcoming the identified key bottlenecks presently hindering the commercialization. The goal of the project is through a combined approach to develop a novel consolidated bioprocess concept for the hydrolysis and fermentation of lignocellulosic feedstocks. In the new process concept, previously developed efficient hydrothermal pretreatment technology will be applied to obtain a high consistency raw material stream, which will be prehydrolyzed at high temperature by thermostable enzymes. In the consolidated bioprocess, the amount of externally added enzymes is expectedly significantly reduced by exploiting an organism, capable of producing a set of lignocellulose hydrolyzing enzymes. Additional cost reductions are expected to be provided by introduction of a simultaneous ethanol fermentation and recovery process. After optimizing the individual process stages, the novel consolidated bioprocessing technology will be tested in laboratory and pilot scales.

## 1.2 Work performed and main results

Straw is presently one of the most relevant raw materials, not competing with food production and was chosen as the first reference biomass for bioethanol production in this project. Other raw materials studied include corn stover, reed canary grass and willow. The previously developed IBUS technology is the main method of pretreatment. Pretreatment of various raw materials has been optimized by Inbicon and BioGold. High consistency operation throughout the process is a main target, allowing high product concentration and potentially decreasing the ethanol production costs.

The cost of enzymes is a key target for improvements and considered a barrier to economic production of cellulosic ethanol. Thermostable enzymes offer potential benefits in the hydrolysis of lignocellulosic substrates. Optimized preparations of thermophilic enzymes, produced by ROAL, have been designed for the liquefaction stage, as well as to the combined liquefaction and saccharification stages using purified thermostable and reference enzymes from *Trichoderma reesei*. The conditions for the liquefaction at increased temperature combined with saccharification at lower temperature have been optimized at the Universities of Helsinki and Copenhagen. In the consolidated process concept, the amount of added enzymes could be minimized in the saccharification stage with enzymes produced by the consolidated organism, *Fusarium oxysporum* at NTUA.

Several factors have been recognized to restrict the enzymatic hydrolysis of lignocellulosic substrates. The most important technical process parameter restricting the hydrolysis is the overall substrate consistency level which is required for improved process economy and efficiency. It has been observed, however, that the high dry matter consistency in hydrolysis decreases the hydrolysis rate due to several factors, including end product inhibition of enzymes, limited diffusion and less efficient mixing. The main approach taken in the HYPE project is to carry out efficient liquefaction at the early stage of the hydrolysis, and optimizing the enzyme preparation for high consistency hydrolysis. Other factors constraining the enzyme performance include the crystallinity and recalcitrance of cellulose and the presence of lignin and hemicelluloses limiting the hydrolysis rate. This has resulted in further targets for improving the enzyme mixtures. In addition, methods for the quantitative determination of the processivity of CBHs acting on cellulose have been developed at UT, increasing understanding on the hydrolytic and synergistic mechanisms of cellulases.

The ethanol yield could be increased by improved fermentation of all carbohydrates. For this, two organisms, a xylose fermenting yeast and *F. oxysporum* have been tested on pretreated straw. The fungal strain has the further advantage of being able to produce hydrolytic enzymes. The use of this organism has, however, also some technical limitations.

Design and rebuilding the pretreatment equipment in the Inbicon pilot plant for consolidated bioprocessing has been accomplished to minimize the water to dry matter ratio. A series of large scale pilot tests with wheat straw has been conducted to characterize the recovery of hemicelluloses as a function of severity in the slurry system. Hot water pretreatment technology has been developed at Biogold. In addition, a reactor system for the consolidated bioprocess has been initiated by development of diabatic vacuum stripping system to recover ethanol during fermentation at Holm. The work with feasibility models has been started. The process is based on the configuration of Inbicon's demonstration plant in Kalundborg, and the process is modeled in an in-house developed model. All major flows, calculated based on a number of process parameters, have already been calculated, based on experience at the Inbicon pilot facilities.

## 1.3 Expected final results and their potential impact

The generic technologies to be developed target at major reductions of ethanol production costs. These technologies include high consistency consolidated process technology, efficient pretreatment technologies, new enzymes for liquefaction and saccharification allowing lower needed dosages, higher yield in ethanol production, as well as decreased ethanol distillation costs. The process concepts are modifications of the present SSF and SHF processes. The overall process concept will ultimately lead to decreased energy demand and increased energy integration. These basic improvements will lead to both decreased capital and operating costs. For the technical issues the question is about the time frame for implementation. The non-technical issues will expectedly not affect the impacts. The national and EU energy policies are based on decisions driven by the Kyoto agreement and climate change. The increased demand for biofuels requires 2$^{nd}$ generation biofuel production technologies and the stake-holders are committed to this target.

A special feature of this project is that it addresses the key issues of the bioethanol 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 2$^{nd}$ 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. The developed process concept will be modeled and technically most feasible process stages 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 European security of energy supply thus reducing oil dependency and help sustainable rural economic development. Europe has a leading position in the production of biodiesel whereas the production of bioethanol is still low as compared to North America and Brazil.