COMETHA

Industrial scale pre-commercial plant
for second generation lignocellulosic ethanol

Summary of activities

August 2015

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The final goal of COMETHA is the construction and operation of an integrated pre-commercial industrial second generation cellulosic bioethanol plant.

During the first 18 months the consortium has worked to achieve the following major goals:

- definition of the feasibility and permitting procedure;
- evaluation of the performances of the main sections of demo plant and of the possibilities for their scale up;
- selection and development of high performance enzyme cocktail;
- definition of the basis for obtaining an integrated assessment for the process;
- elaboration of dissemination activities concerning the project.

Definition of the feasibility study and permitting procedure

A pre-feasibiltiy study for the plant was initially done on an industrial area in Veneto Region (Italy), which is a Site of National Interest requiring an action from the Italian State, in terms of high risk for the environment, extension of the area concerned, quantity and dangerousness of pollutants. Biochemtex studied and visited the site, finding a compromised environmental situation, result of demolition operations held with total non-compliance of the basic standards of safety and environment protection, and a complex legal situation (ongoing lawsuits for environmental damage). As a consequence of this, the site was excluded from the choice and a long and expensive work of evaluation of new possibilities in Europe was started.

Biochemtex then visited a number of other industrial sites and evaluated the best solution also in term of biomass supply chain. The choice on the location of the plant is now in its final evaluation phase. Once this phase will be concluded, the permitting procedures for the chosen location will be started.

Performances and scale up possibilities of the main sections for the pre-commercial plant

The main sections of the demo plant are:

- pretreatment;
- viscosity reduction/enzymatic hydrolysis;
- co-fermentation;

their performance has been investigated in order to find the best engineering solution for the pre-commercial plant construction.

The flexibility of the pretreatment technology to different biomasses was demonstrated, also thanks to an extensive work of testing of new biomasses at pilot and demo scale. Process stability was demonstrated both for *Arundo donax* and wheat straw. The material exiting this section was also analyzed for subsequent hydrolysis with enzymes.

The complex biomass structure was completely deconstructed, with a demonstrated increase in enzyme accessibility. Moreover, the design of this section was optimized and an even flow rate both in charging and discharging zones was guaranteed. Scale up of the pretreatment section could include different steps to regulate the biomass bulk density and moisture content, so two or more lines working in parallel could be necessary.

The performance of the viscosity reduction/enzymatic hydrolysis section is mainly related to the particular characteristics of the feed (accessibility to enzymes, density, fibers size, etc.), the product (de-structured, in terms of low chains length and high porosity), and to the development enzymes.

This last point was obtained thanks to the fruitful collaboration of Biochemtex with Novozymes.

The optimization and process stability of the whole section were also increased. Scale up choices for this section will likely be a single reactor for viscosity reduction, using the residence time validated through the operation of the demo plant in Crescentino.

The performance of the co-fermentation section is manly related to the microorganisms (MOs) used. In the case of the demo plant a strain of *Saccharomyces cerevisiae* able to ferment both C5 and C6 sugars was used, with high ethanol tolerance. Evaluation criteria for the selection of MOs are performance in term of sugar conversion yield and alcohol tolerance, industrial scale development and production, classification level determined by the risk assessment. Good performances have been obtained with the studied microorganism, with particular attention on glucose and xylose assimilation and ethanol production. MO is still in development in order to increase tolerance to inhibitors and xylose consumption velocity. Performance improvements of this section were done also on propagation and on sterilization and cleaning methods (clean-in-place (CIP) techniques).

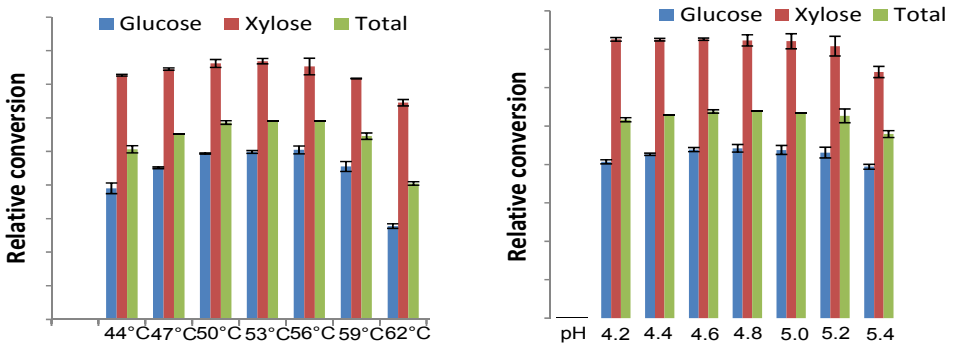
Finally, the **performance of secondary units** (WWT, distillation, lignin separation, power plant, general services) was evaluated. The results confirmed the feasibility of complete process integration, that is fundamental for minimizing energy consumption and maximizing heat recovery of the process itself.

Selection and development of a high performance enzyme cocktail

Novozymes is developing new and more efficient enzymes compared to current benchmark enzymes. This will be achieved by focusing specifically on the needs of the 2nd generation technology and the feedstock to be used. Wheat straw, one of the main feedstock of this kind of biorefinery, has been the main model feedstock used in the enzyme development work.

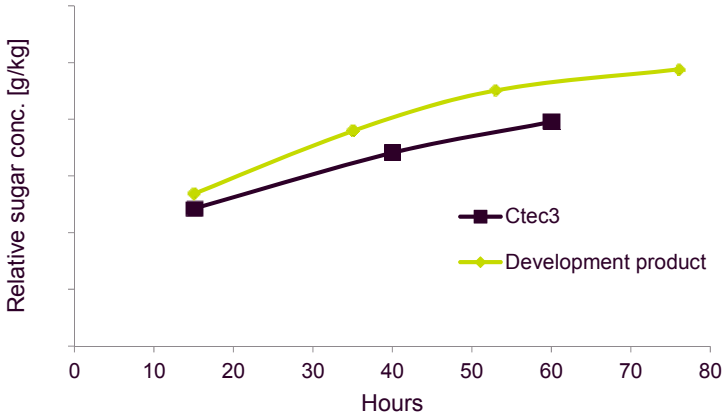
A screening and process optimization effort was undertaken to identify individual enzyme molecules with superior performance under the conditions imposed by the technology. In addition, Novozymes will leverage on their highly efficient enzyme expression systems and develop fungal strains which can produce relatively large quantities of experimental enzyme cocktails. Biochemtex will evaluate the performance in its pilot facility in Rivalta (Italy). The iterative approach including enzyme development and continuous feedback from scale-up trials has proven to be efficient for the development of the hydrolysis step.

Based on previous screening work, initial enzyme cocktail optimization was made using mixture design setup. The performance of the enzyme mixtures was evaluated in hydrolysis experiments on the real substrate produced at Biochemtex pilot plant. The best performing mixture was chosen for further evaluation and optimization of process conditions. The new cocktails have wide temperature and pH optimum and are thus robust to process perturbations which is necessary for industrial operations.



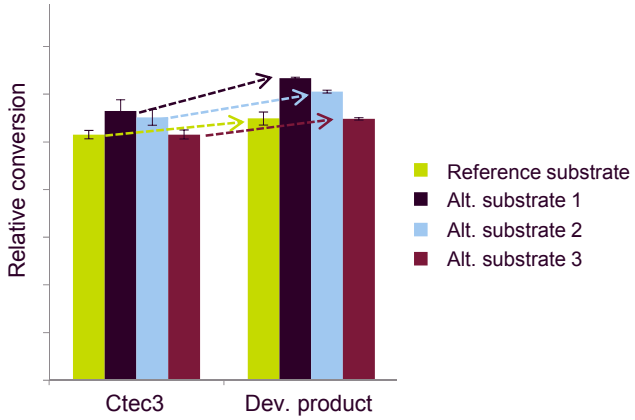
Temperature and pH profiles evaluated in hydrolysis of pretreated substrate from Biochemtex pilot plant.

Further performance evaluations were carried out under the optimal, process relevant hydrolysis conditions. The results from the performance evaluations clearly indicate improved performance over the benchmark enzyme product, Ctec3. Candidate cocktails were produced in kg scale and sampled for testing in the pilot plant. The best performing cocktail was then selected as the main candidate for future work.



Hydrolysis performance of the new enzyme cocktail and benchmark enzyme in the pilot plant

More tests were done studying the response of substrates from different pretreatment conditions to the new enzyme cocktail. The improvement in conversion over benchmark cocktail is more pronounced for some of the new substrates showing a synergetic effect by co-development of enzymes and pretreatment.



Conversion in performance evaluation of Ctec3 and new enzyme cocktail on four differently pretreated materials.

The results highlight the importance of an iterative pretreatment and enzyme development where any changes in pretreatment are considered in enzyme development and pretreatment development accounts for what gives the best potential for further enzyme development.

Integrated process assessment

Imperial College of London is working on developing a framework for assessing the environmental sustainability of COMETHA. The same framework will be used to investigate alternative configurations of the ethanol supply chain. The results of the analysis will provide the foundations for a strategic assessment of environmental benefits and risks associated with lignocellulosic ethanol deployment in Europe.

The methodologies that will be applied to carry out the environmental assessment were identified:

- the conventional LCA will be structured in accordance with the standards ISO 14040 and 14044;
- the water assessment will employ the Water Footprint Assessment methodology prepared by the Water Footprint Network;
- the land use assessment will apply the causal-descriptive ILUC model produced by E4tech (2010).

The key features of the supply chain (input/outputs and geographical locations) were identified, employing a GIS approach to identify areas potentially suitable for the cultivation of giant reed. Moreover data gathering was started, consisting in the collection of supply chain data (in collaboration with Biochemtex) and the collection of study area data including land use/land cover maps, and soil and hydrological data.
Future work will be focused on producing a detailed flow diagram of the ethanol supply chain that will provide the understanding and some of the data needed for the impact assessments.
The results of this work package will help to identify areas where technology and supply chains should be further improved, as well as any risks that could be associated with different fuel chain configurations. A strategic analysis will be conducted to put the results of this work package into perspective with regards to the identification of potential areas for improvements in the supply chain, as well as the impacts of anticipated future supply chain developments and the understanding of environmental benefits/risks associated with wider deployment of lingo-cellulosic ethanol.

Communication and dissemination

ETA Florence worked on communication, dissemination and exploitation activities, which are important to maximize the impact of a project and deliver its results.
In line with the principle that Europe's future economic growth will have to come from innovative products, services and business models, the communication strategy of COMETHA will pursue the following goals, included in a "Plan for dissemination and exploitation" (approved by all partners in February 2015):

- keeping the scientific and industrial community informed about the achievements in the construction and operation of the flagship plant;
- showing the role and importance of the technological cooperation among international partners to achieve a progress beyond the state of the art;
- describing the positive outcomes that will be generated by the construction and operation of the flagship plant;
- demonstrating that industrial scale ligno-cellulosic ethanol production is feasible with today's technologies and know-how;
- informing about the environmental sustainability aspects of ligno-cellulosic ethanol;
- building a stronger political consensus about the role and importance of advanced biofuels.

A number of target audience groups were identified, considering the large size of the project and its high scientific and technological relevance.