Final publishable summary report

Biolyfe Project

EXECUTIVE SUMMARY

Biolyfe is a project, supported by EC in the FP7 and coordinated by Biochemtex, aiming to demonstrate an innovative technology for the production of 2nd generation bioethanol on a 40.000 ton/step scale.

During the Biolyfe project, the entire partnership worked to achieve the major project goal: realize and operate the DEMO plant in Crescentino (Vercelli province-Piedmont region). Thanks to the strong effort putted in each task all the targets have been achieved.

The Biolyfe project reached the targets set demonstrating the feasibility of the 2nd generation biothenol production from non-food biomass.

The raw materials chosen and tested as feedstock for the Biolyfe project fulfil several socioeconomic and environmental criteria: contribution to reductions in GHG, energy efficiency, availability and price, as well as not competing with food use. Among the other, Wheat straw, Fiber Sorghum and Arundo donax resulted the most suitable due to the productivity in terms of high ethanol/hectare yield. More than 25 ha of Arundo and 25 ha of Sorgum have been dedicated to specific trials and monitoring.

The pretreatment technology is the first gate-process of the second generation bioethanol production and a key step for the success of the use of lignocellulosic materials. Biochemtex developed the PROESA® technology which is a mild process able to treat no food 2° generation feedstock.

Novozymes worked to improve their enzymes for the Biolyfe project and improve the C5-C6 accessibility, reaching the commercial product Cellic CTec3. With this new cocktail, significant improvement have been achieved for Biolyfe technological results

Lund University and ENEA have worked to improve the fermentabilty of the biomass, optimizing C5-C6 transformation to bioethanol hence integrating process steps and yield. The resulting beer is further purified up to more than 99% by standard distillation and molecular sieves technology.

The ground-breaking event for the demo plant took place on the 12th of April 2011 in Crescentino, in the Vercelli province (Piedmont region). The plant has been inaugurated the 9th, October, 2013; the demo works continued during 2013 with continuous operation and constant optimization and implementation of the production. The ethanol production cover 40.000 ton/y of 2nd generation ethanol with a biomass storage volume, at the plant, of 14.000 m^3 for fresh Arundo and 11.200 m^3 for dry biomass (wheat straw) the whole supply chain was studied and implemented; and demo plant production of lignin ranges from 150.000 to 180.000 tons per year.

The ethanol produced has been tested in 4 VOLVO V50 flex fuel, supplied with E85. Within 150 km from the demo plant a gas station is fueling cars with E85 and E10.

PROJECT CONTEXT AND OBJECTIVES

European Union is strongly committed to a reduction of environmental impact and contribution to anti-climate change policies. This is strongly related with the latest petroleum crisis and the connected environmental impact.

One of the most important instrument in the European legislation is the *integrated climate and energy change package* which includes Directive 2009/28/EC[1] regarding the promotion of the use of energy from renewable sources and fixes specific targets: 20% of the total energy consumption must be produced from renewable sources in 2020, 20% reduction of GHG emissions in 2020, 20% energy saving compared to projections for 2020 and 10% minimum binding target for biofuels in the transport fuel mix in 2020. Biolyfe project target this 10% demonstrating the possibility to produce sustainable 2nd generation bioethanol. Bioethanol is an interesting fossil substituted as the market is expected to grow to almost 15 million cubic meters in the EU by 2020.

According to the different production processes and feedstock, there are two types of ethanol: First-generation ethanol (1G) and Second-generation ethanol (2G). The 1G ethanol is conventionally the result of sugars or wheat/corn starch fermentation and can be considered as a valid substitute to fossil fuels. Nevertheless use of this traditional feedstock can compete with food market. The need for different non-food substrates has lead scientists and technologists to look for new feedstock in order to optimize the process in terms of economical, social and environmental impact.

The second generation ethanol, is a good alternative option because it can be produced by raw materials that do not have a negative impact on environment and agricultural activities. In fact agricultural residues (wheat straw and corn stover are the most attractive) and energy crops (e.g. Arundo donax, switch grass, miscanthus, and short rotation woody crops) can be suitable for ethanol production.

On energy base, considering an energy content of 21.06 MJ/lt for bioethanol and 32.48 MJ/lt for petrol, to comply with the EU-blending target of 10% energy content, a 15.4% v/v blend (E15) will be sufficient.

In this context, the Biolyfe project aims to demonstrate the 2nd generation technology, covering the gap with 1st generation and fitting with European Union targets and expectation for the near-future in terms of biofuels production and environmental impact.

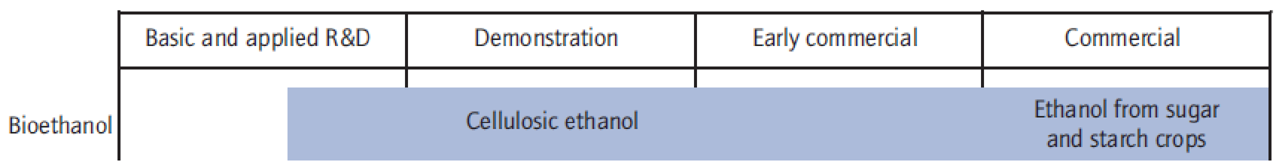


Figure 1 - Commercial status of first and second generation bio-ethanol

(*Biofuels for Transport – Technology Roadmap, IEA 2011)*

The Biolyfe project aims to develop widely applicable technologies that could be exploited broadly by European industry in production of 2nd generation bioethanol.

[1] 2009/28/EC Renewable Energy Directive

To perform this task, the overall goal of the EC-FP7 Biolyfe project was to develop technologies allowing increased and more economical utilization of already chosen renewable lignocellulosic raw material. In order to achieve this objective, in Biolyfe an *industrial demonstration unit* has been realized for the production of 40.000 ton/step of 2nd generation ethanol.

The industrial demonstration unit is needed to prove the technology development in a pilot plant scale, on larger scale and define operating conditions optimizing parameters and collect information and data for further scale up. Biolyfe DEMO focuses on proving the entire crop-to-ethanol value chain on larger scale, generating all the data needed for the design of industrial plants.

The tasks performed in the project address:

- investigation of the energy crops in relation to pre-treatment, viscosity reduction and enzyme hydrolysis;
- optimisation of pre-treatment conditions for optimum hydrolysis;
- enzymatic hydrolysis in a viscosity reducing step of pretreated material;
- efficient hydrolysis of the chosen pretreated lignocellulosic raw materials;
- saccharification of sugars to ethanol with a yield of 75%;
- minimization of the enzyme loading, approaching the desired load;
- efficient integration of hydrolysis and fermentation processes at industrial demonstration scale;
- testing and optimization at full industrial demo scale (10 ton/h biomass input and 2.5 ton_{EtOH}/h, i.e.20.000 ton_{EtOH}/y) of the complete plant: the entire lignocellulosic bioethanol production chain;
- environmental, social and economic assessment;
- market assessment.

Targeting a reduction in cultivation costs, by specific agronomical solutions, will leads to reducing ethanol price. This investigation is carried out for those energy crops that are expected to be the most promising for Europe in future 2nd gen bioethanol production. Biolyfe aims to set-up, test and prove at full demonstration scale the biomass supply and logistics.

From a technological point of view Biolyfe would tests solutions to increase accessibility of the cellulose surface to enzymes that ensures a better hydrolysis of the cellulose component, with the use of the most advanced current biomass pre-treatment methodology. Reduced costs due to high biomass consistency process, obtained by novel enzymes and fermentation development, resulting in high ethanol concentrations and reduced volumes of liquid streams in the fermentation residues and in the production plant.

Reduced operative and capital costs, in prevision of future industrial facilities, due to the study of all operative conditions in the demonstration plant is the synthesis of all the goals set in Biolyfe project.

MAIN SCIENTIFIC AND TECHNOLOGICAL RESULTS

Most relevant results on biomass selection and use

The raw materials chosen and tested as feedstock for the Biolyfe project fulfil several socioeconomic and environmental criteria: contribution to reductions in GHG, energy efficiency, availability and price, as well as not competing with food use.

Based on previous scientific and agricultural research and considering also Italian pedo-climatic conditions, tests on 2^{nd} generation biomass have been carried out in Biolyfe project. Among the other, Wheat straw, Fiber Sorghum and Arundo Donax resulted the most suitable due to the productivity in terms of high ethanol/hectare yield.

Forewords

The analysis of the most suitable biomass for second generation bioethanol production has been lead by AGRICONSULTING. A limited number of species have been previously selected by a bibliographic survey, among them fiber sorghum, Arundo donax, Miscanthus and Switchgrass have been identified as the most promising crops already cultivated in the Crescentino area (VC). Agricultural residues have been considered as well.

Most relevant biomass to be cultivated/recovered in the area of interest

Although there is not a strictly “sustainability” limitation for the distance (as in the case of power generation) between the biomass production site and the plant, a supply basin of 70 km was taken into account.

The use of land in this basin identified the areas in which diversification of production was reasonable. In particular the areas cultivated with:

- winter cereals and maize;
- winter cereals, maize and rice.

This choice comes out from a market analysis done by AGRI: cereals plays a key role in the supply basin, but not all the species have the same market trend and the same profitability. They have to be considered as commodities, liable to the world market trend. Actual and future cereals price seems not to be able to sustain farmers activity, also in the case of constancy of the production costs.

For both situation (rice excluded or included), a specific methodology developed by AGRI was adopted. The method allows, by means of filters, to exclude (for each municipality) small fields, where agriculture is a secondary activity. Basis are the years 2006-2009. Further on, a reasonable and prudential quota (based on previous experiences and studies) of the land suitable to be diversified was applied. The main focus was on winter cereals and maize. This results in the data shown in figure 2.

The maximum average density of the diversification in the supply basin is of 3,5% (rice excluded). Taking into account the average surface of non-cultivated land (2006-2009), the quota of diversified land actually used for traditional crops is of 0,1%. The analysis shows that energy crops can be a valid alternative both for the land not cultivated and the surface actually used for traditional crops.

As for dedicated crops, between the candidate species, Arundo donax was chose as main crop, while fiber sorghum was chose as a second possibility. After the demonstration activity, sorghum was excluded because of the yield is too much addicted to season weather (rainfalls).

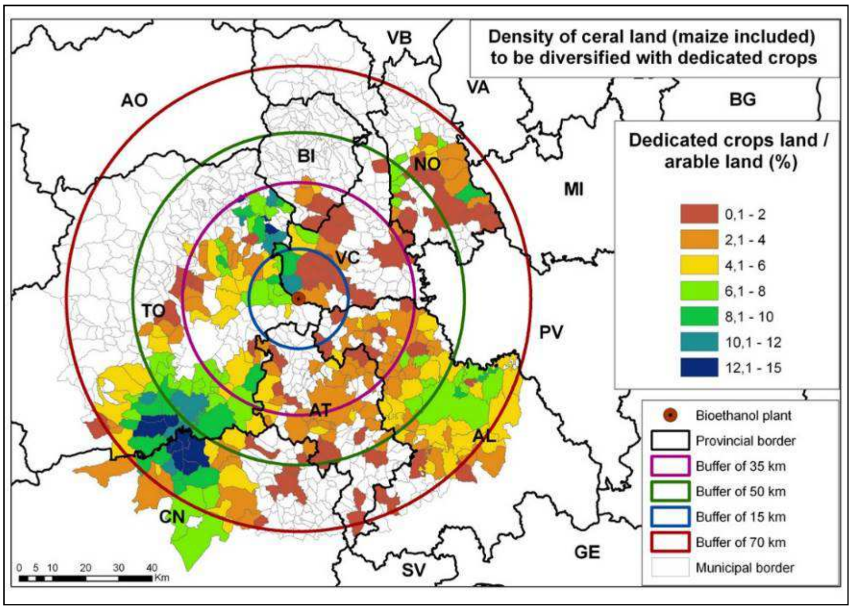


Figure 2 - Results of the methodology in the supply basin

The production cost of the two crops were compared: 1.160 €/ha*y for sorghum and 500 €/ha*y for Arundo. The gross margin for the farmers is related to the biomass price that the bioethanol plant intends to pay. A projection was done: for farmers a gross income of 300-400 €/ha was, in 2010, the minimum to move them to diversification. Arundo is the most profitable crop, also in the case of a yield of 20 t dm/ha of dry matter, while the price of Sorghum able to guarantee an interesting gross margin is always higher, and the yield should be over 20 t/ha of dry matter (dm).

As for agricultural residues (cereal straw, rice included), a specific analysis of the production and a market analysis was done.

For winter cereals straw the whole average surface (at municipal level) cultivated in the 70 km radius from bioethanol plant between 2009 and 2012 was 89.400 ha, that means, for at least 3 t/ha (moisture content less than 15%), a production of 268.200 t/y. The harvesting period is June-July. Straw is usually harvested by contractors in large square bales (typical size 50-100 cm x 80-120 cm, with an adjustable length of 70-240 cm or more) and stored in shed or in piles covered with plastic material. The destination of the product is for animal feeding and animal litter. As for the use as litter, the demand varies with the weather of the season, and in particular with the rainfalls level: if the year is particularly wet, the consumption of straw increases as well as the demand. This has strong effects on the market price. The market is controlled by traders, which concentrate the local offer.

Rice straw is harvested in large square bales too. The average 2009-2012 surface in the basin was 181.500 ha. With a yield of 5 t/ha (moisture content less than 15%), in the supply basin around 907.500 t of rice straw are produced every year. Rice straw is not widely used for animal litter because it can cause pedal diseases to animals: if available, stockmen prefer to pay more to get wheat straw. The harvesting period could be in September (after grain harvesting) or in Spring. Spring harvest allows a partial degradation of the straw in order to obtain a more suitable material for animal litter, while for the use in the Biolyfe plant, September harvesting is suggested, collecting a non-degraded material.

Arundo and fiber sorghum at demonstration level

As for Arundo, more than 28 ha were used for demonstration. Two phases have to be distinguished:

- propagative material production;

- biomass production.

Propagative material production (one year cycle) consists in: soil preparation through ploughing (depth 25 cm), harrowing, chemical weeding, transplanting of rhizomes with specific genome (no GMO are used; distance 1.1 x 0,3 m; 30.300 rhizomes/ha), plant growth, mechanical weeding (no selective chemical product exists), rhizomes chains (50 cm) uproot. These operations are done in nursery fields. Than rhizomes chains are transported to the processing plant where they are cut to obtain 8-9 rhizomes per chain to be transplanted for biomass production. One hectare of nursery produce material for 15 ha in biomass production. The project allowed to model, on the base of the requested quantity of biomass, the needed surface of nursery and the location of the processing plant.

Biomass production (10 years cycle) consists in: soil preparation through ripping (if necessary) ploughing (depth 30 cm), harrowing, chemical weeding, fertilization if in case, rhizomes transplanting (distance 1 x 1 m; 10.000 rhizomes/ha), mechanical weeding, irrigation (if necessary but only for the 1st or 1st and 2nd year), rhizomes transplanting, harvesting (in case of the demonstration monitored with a GPS apparatus) every one-two year, in function of the obtained yield and the supply management strategy. At the end of the cycle in nursery or in a production field a chemical weeding and an uproot removed the crop.

Although for soil preparation (plough, harrow, etc.) and harvesting (forage harvesting) common equipment were used, for rhizomes transplanting, rhizomes chains uproot and processing into rhizomes for biomass production, the study of prototypes was needed. During the project, starting from existing equipment with very low working capacity (e.g., transplanting 0,12 ha/h, uproot of rhizomes chains 940 rhizomes chains/h, rhizomes chains processing 2.000 rhizomes/h, high use of manpower), several improvements were reached, and new prototypes, quite near to be commercialized, were developed.

The fuel consumption of common operations are in line with those reported in bibliography, while for the new equipment more on-field tests should be need to confirm the gathered data. Also the crop growth needs more time to get correct information: e.g. following the bibliography data Arundo has the yields reported in Table 1. During the demonstration, at the 2nd year the effective yield was around 5 t dm/ha; probably at the 6th year the yield would be in line with bibliography data, but more time is needed to check this.

Table 1: Hypotesis of yields in dry matter of Arundo

Age of plants (years)	Yield (t dm/ha)
0	0
1	2
2	10
5	25
7	25
8	20
10	10

Arundo is harvested almost all the year (except in Summer and when weather conditions do not allow to enter the fields. Thus, storage period is very short (1-2 months). The best way of storage in these conditions seemed the use of silo-bags at the border field (weight 2 t ar/m; Figure 3). Inside temperature and MC (Moisture Content) were monitored. A specific equipment unpack the silo-bags. Also storage under roof can be done, depending on the available space.

Figure 3 - Silo-bag equipment

As for sorghum, sowed in April, operation consists in soil preparation (ploughing, chemical weeding, harrowing, rolling), fertilization, precision sowing, irrigation (for biomass production farmers tend to reduce or eliminate this operation to save money) and harvesting.

In case of low growth of the crop, a delay in harvesting allowed to wait for September rainfalls but, of course, the natural drying did not occur, and bales have more than 50% in MC (moisture content). Anyway round-bales storage was done in pile: covered with a specific paper called “Walky®” already used in forestry and covered with plastic but mould was noted. The product was unusable till the first month of storage.

This suggested to eliminate fiber sorghum from the suitable dedicated crops or, at least, to use it to diversify the supply.

Contracts and biomass acceptance procedures

The supply chain of the Biolyfe bioethanol plant is mainly based on two sources:

- dedicated crops (Arundo donax; a secondary opportunity such as fiber Sorghum could be considered but the risks reported above have to be taken into account);
- agricultural residues (cereal straw).

As for Arundo, the contract of cultivation created an exclusive relationship between CTXI and the farmers.

The acceptance procedures ensure:

- the congruence with the bioethanol plant specifics: MC should not exceed 80% and contaminants should be lower 1% in weight; proper measurements are set.
- the congruence with the ISCC-EU certification system, in order to apply for the fees planned in the cultivation contract;

MC measurement, associated to the yield of a field, provides the application of the “Yield fee” to the farmer, and payments of biomass are based on flat rates (fixed, surface, irrigation and sustain to soil preparation fees).

Straw is considered as a standard product, but the average MC has not to be higher than the 14%. Straw is supplied by traders (e.g. wheat straw) or by large farmers/consortiums (rice straw) under common commercial relationships. As for acceptance procedures, a visual check (of contaminants and/or moulds proliferation) remained the main issue.

In case of straw delivering by traders, particular efforts have to be planned in order to trace the origin of biomass, following the adopted certification system (in case of Biolyfe plant the ISCC-EU system).

Certification systems under the RED directive

The outputs of the facility are:

- bioethanol, used as biofuel in transport;
- lignin: a by-product recognized as biomass and converted into power in a dedicated plant of the facility.

Thus, the following items were considered for biofuels and bioliquids:

- the promulgation of the Decree of the Italian Ministry of Environment (issued jointly with the Ministry of the Economical Development and the Ministry of Agriculture) of 23th January 2012 (published on 7th February 2012), that sets up the National Sustainability System of Biofuels and Bioliquids,
- the ISCC-EU voluntary certification systems recognized by the Commission and adopted by the Bioethanol plant;

As for power production from biomass, the Decree of the Ministry of Agriculture of 2nd March 2010 was taken into account, but not described.

Although CTXI chose the ISCC-EU, both the systems, based on the RED Directive, were described, as well as the actors of the chain and the related duties.

The basis of the certification are the “mass balance” and the “GHG saving”: these data have to be certified, updated at every change of the owner of the biomass and have to follow the lot to the final destination.

Analysis with a GIS/GPS tool of the supply chain

The set up of a supply strategy requires to optimize the logistic in order to limit the cost of delivering and follow the plant supply plan. A good support is offered by the GIS/GPS technologies, able to give a global view of the available resources. The GPS punctual data were implemented in a system able to minimize the cost of the supply in function of road typology and type of vehicle. The system “Optima®" (a tool created by AGRI) implemented the GPS data of the fields contractualized by CTXI at May 2013. Basically, “Optima” individuated four sectors: near the Biolyfe plant, at North, at North-East and at South East of the facility. Fields are mainly those used for intensive crops.

The easiest solution to transport the harvested biomass is to use the same dumpers that followed the forage harvester. Nevertheless, the GIS analysis showed how this is not possible for all the sectors and cultivated fields. Not all the fields in the sectors are unified enough to justify the creation of a storage yard (Figure 3). Such areas should need efforts to increase the cultivated surface; at the moment the best solution seems to be the storage in silo-bags at the field border. A good action should be to convince operators to install a GPS apparatus on their equipment, able to send the position of working in real time to the facility supply management and automate the recording of the information. Finally, the creation of a dedicated system able to record the state of the fields (age of crop, harvested/not harvested, etc.), fitting the existing system of the authority controls in agriculture was suggested.

Basis to improve the certification systems with a dedicated web-based tool.

Following the Italian normative and the ISCC-EU systems for the biomass certification, AGRI modelized biomass traceability systems, creating the fundamentals of the Web-based tool. The system was developed in the past by AGRI to manage and certify the supply of biomass power plant. Anyway, with the proper adjustments, the basis can be used also for the biomass used to produce bioethanol.

The traceability system model consists in two components:

- A document management system, consisting of several documents, each one essential and uniquely linked to the others by a specific Unique Identification Code of the chain;
- A computer application: Web-based tool (Agritrack®) which has the role of storing the documental system information, and consequently: verify the integrity of stored data and verify the traceability of biomass, through specific reports aiming monitor the relationships with the operators of industry and the state of supply to the industrial operator.

From the operational standpoint, the system allows to manage and acquire all the necessary information of various types of biomass, such as:

- Dedicated crops
- Biomass from forest management (this is not the case of Crescentino plant)
- Farm residues
- Residues of agro-industrial activity

Moreover the system:

- traces all movements of biomass, from the producer to the factory, under the terms by the current legislation;
- manages and monitors contracts with different suppliers;
- monitors the flow of input material at the factory.
- creates specific reports about the status of conferring biomass at the industrial operator.

In addition to these basic functions, the system also can manage:

- the amount of biomass and energy input;
- the percentage of biomass linked to specific incentive tools (if in case)
- the cost of biomass energy vector (bioethanol);

Bioethanol production technology advances

Pre-treatment

The pretreatment technology is the first step where cellulose is modified and made ready to enzyme modification improving accessibility and hydrolyzability of the carbohydrate polymers. After the scouting of all the biomass pretreatment technologies, Biochemtex has selected the most promising process and implemented it; a pilot plant operating in a continuous mode has been built and is currently operated at Biochemtex facility in Rivalta Scrivia.

Pretreated material, or viscosity reduced material were sent to partners in order to carry on research.

After the pretreatment of the biomass, the aim of this stage is to liquefy the solid content stream in order to guarantee a constant and continuous flow of the material into the fermenter. This section allows a complete mixing and adequate retention time for the first enzymatic liquefaction of complex cellulose and hemicellulose to simpler oligomer chain (C5 – C6) prepared for an efficient downstream conversion to ethanol.

Effective biomass pretreatment

The production of concentrated hydrolyzates is a key step in C6/C5 sugars biorefineries. In particular, to obtain high ethanol levels in the fermentation broth, high concentrations of sugars in the hydrolyzates are necessary. This implies that concentrated biomass suspensions with solids loading higher than 20-25% must be processed. To produce concentrated syrups, biomass pretreatment and hydrolysis were deeply investigated in the present project and the relevant processes improved. The biomass selected for the process optimization has been *Arundo donax*. It contains significant percentages of C5 sugars and this implies the importance of selecting pretreatment conditions that minimize the degradation of pentoses along with an effective biomass destructuration. At this scope, two processes were considered: one step steam-explosion at mild thermal conditions along with small amounts of acid catalysts (ACSEP carried out by using the ENEA batch plant); Biochemtex proprietary technology PROESA®.

High gravity hydrolysis of biomass

The process of concentrated slurries containing at least 20% solids is called high gravity hydrolysis. It has the advantage to produce higher concentration of the final product, thus reducing the distillation costs, the bioreactors capacity and the amount of waste waters. On the other side, the challenges of high gravity hydrolysis are the high viscosities that limits the mass transfer and results in a poor mixing; furthermore, it increases the inhibition by end-products. Concerning the fermentation, it often implies a high concentration of microbial inhibitors, an osmotic stress due to high solute concentration, and the toxic effect of ethanol (synergistic inhibition). As a result, the process yields are typically low.

The main contribution of the ENEA work in the project has been to increase the basic knowledge on the hydrolysis process, using the innovative enzymatic blends provided by Novozymes, testing reactor and mixing geometry, and evaluating process strategy (SSF, SHF, hybrid process).

One possible strategy to avoid the high viscosity is the fed-batch feeding of biomass. Among the various feeding options, discontinuous feeding of biomass and enzymes, as shown in Figure 2, ensures, in principle, that the same specific activity is maintained during the process for a more prolonged time. Some tests demonstrated that this strategy produced a net relative increase of the process yields (figure 4).

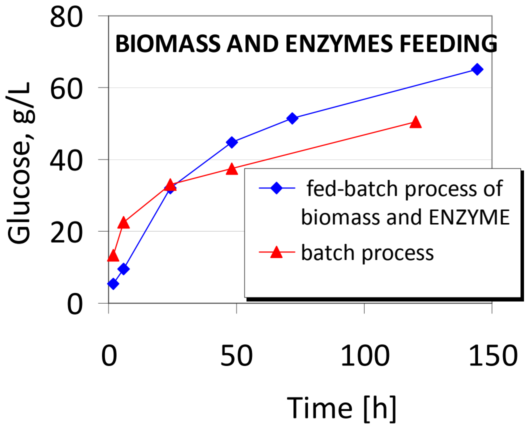


Figure 4 - Effect of the bioreactor feeding strategy during the hydrolysis of *Arundo* fiber with CTEC2.

The composition of the enzymatic cocktail play an important role in determining the process efficiency. Several enzymatic cocktails were provided by Novozymes through the project and tested in the ENEA's labs. In particular, CTEC2 showed a sharp improvement in all the specific properties with respect to the previous cocktails. The overall result is the reduction of the effect of cellobiose inhibition and increase of the process yields. Also the xylanase acitivity is very important as recent studies in the literature have demonstrated that even small amounts of xyloooligomers (1.4 mg/mL) can inhibit the cellulase activity. After CTEC2, another blend, namely NS22140, was prepared by Novozymes containing an higher amount of xylanase. Figure 5 shows the effect of combing an optimized feeding strategy with the use of an enzymatic cocktail containing xylanase. The results indicate a net relative increase of the process yields.

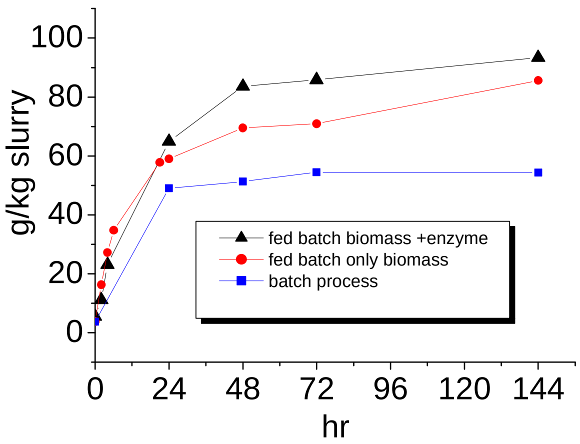


Figure 5 - Effect of the bioreactor feeding strategy during the hydrolysis of Arundo fiber with NS22140.

Another determining factor is the mixing geometry when high DM solutions are used. Figure 6 displays four main bioreactor geometries used at laboratory level to investigate the biomass hydrolysis.

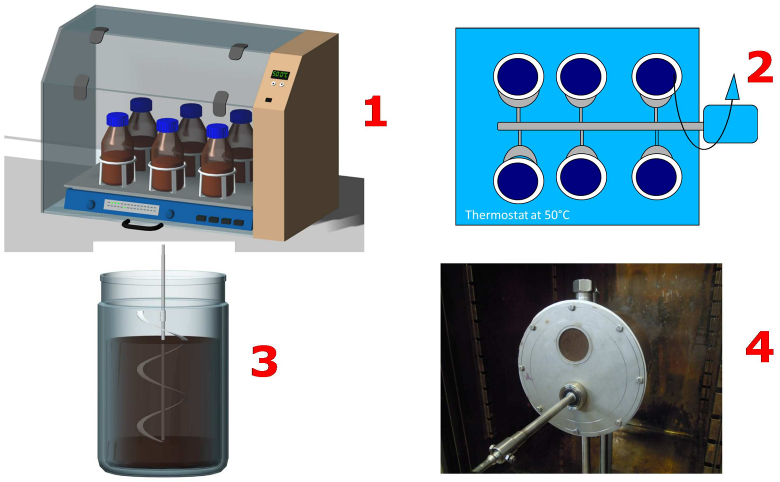


Figure 6 - Four main geometries that can be used for bioconversion processes

In the upper part of figure 6 are shown:

- the orbital shaken flasks (1) typically used for a wide survey of various process conditions (i.e. pH, temperature, dosages),
- the gravimetric mixing (2) obtained for instance by anchoring the flasks to a rotating motor axis,
- vertical (3) stirrer bioreactor
- horizontal (4) stirred bioreactors.

An additional variable in these two last cases is the impeller geometry.

Figure 7 compares some results obtained by using different mixing options. The data indicate wide differences in the process yields. Optimizing the bioreactor geometry and the mixing condition for the viscosity reduction step has been one of the main objective of the Biolyfe project.

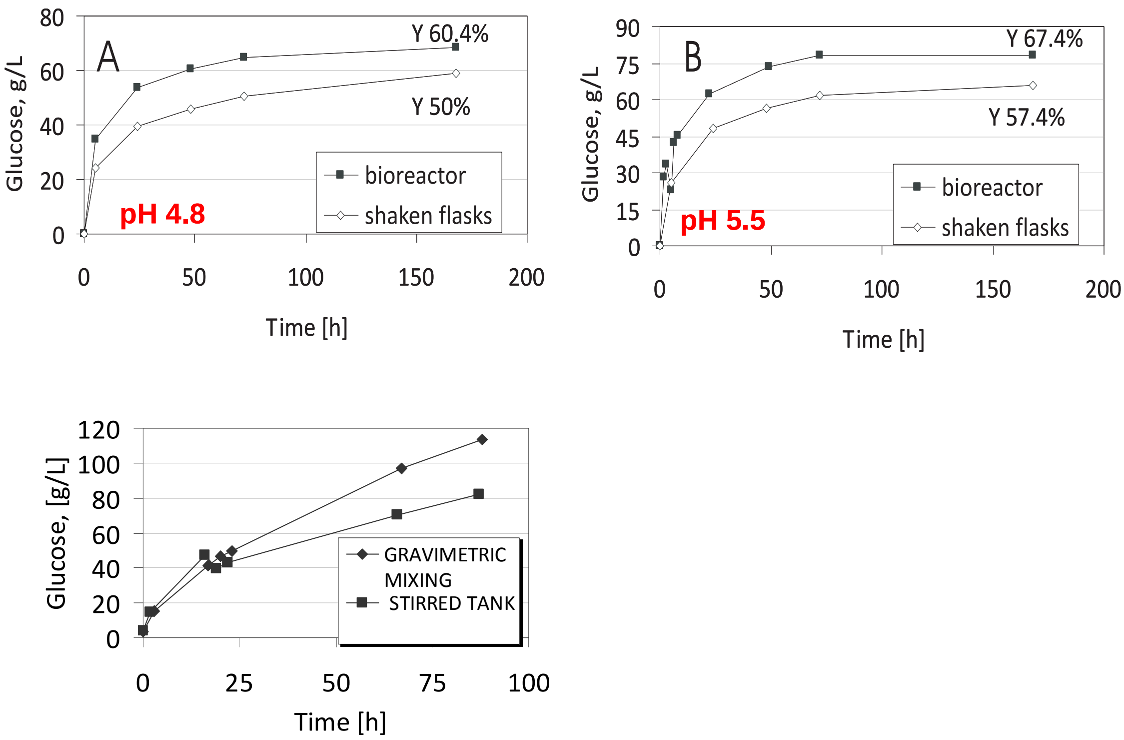


Figure 7 - Effect of various mixing geometry during the hydrolysis

Other important factors in the hydrolysis at high DM content are the inhibition due to the substrate (i.e. unspecific enzymes adsorption) and the inhibition due to the high concentration of products. In this regard, glucose itself could be a source of inhibition. This could be very important in a process scheme consisting for instance in using an hydrolyzate stream as suspending medium to hydrolyse fresh biomass (Figure 8).

Figure 9 displays the glucose yield at increasing the glucose concentration in the hydrolyzates in two setups: hydrolysis of a fresh substrate at 15% solid-liquid ratio (pink line) and hydrolysis at 30% solids. CTEC3 was used in both tests. The results indicate that: glucose was only slightly inhibiting toward the hydrolysis of fresh biomass. Higher glucose concentration reduced the hydrolysis yield by 18%. Therefore, this could be used as a threshold level for starting the subsequent fermentation step. At higher solids loading, namely 30%, inhibition due to the substrate is most likely the prevalent source of inhibition and the glucan yields were unaffected by the glucose concentration. This last finding confirms the opportunity of using an optimized fed batch strategy.

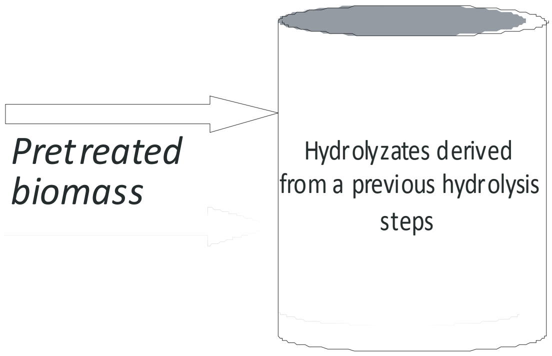


Figure 8 - Generic scheme of a fed batch process

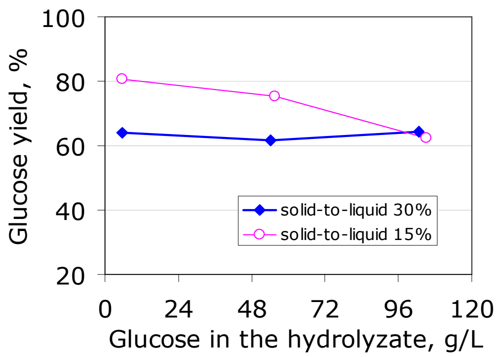


Figure 9 - glucose yield with CTEC3 as a function of soluble glucose in the hydrolyzate at two different solid/liquid ratio.

Enzymes cocktail development

As regard the enzyme selection and use, at the beginning of the Biolyfe project, Novozymes best commercial enzyme product for conversion of biomass was Cellic CTec; whereas the next generation enzyme Cellic CTec2 was already defined and under upscaling for large scale production.

The general vision of Novozymes partner is to produce enzyme products that minimises the use of chemicals, energy and costly hardware for all possible applications of enzymes from detergents over feed and food application to bioenergy. Within the area of biomass conversion, this meant, that Novozymes vision was to develop enzyme cocktails that enabled a pretreatment technology that used no added chemicals, operated at moderate pressure and temperature levels and had a modest construction cost. The Biolyfe project was a perfect fit for realization of commercial scale biomass conversion at sustainable premises that would eventually turn out to be the outcome of the project. Although containing increased hemicellulytic activities compared to Cellic CTec, the Cellic CTec2 was not the enzyme product that could realize this vision and it was the role of Novozymes in the Biolyfe project to develop such and enzyme product, fitting the PROESA® process, and eventually be launched as Cellic CTec3.

Biomass conversion enzyme products Cellic CTec and Cellic CTec 2 were produced without a clear vision of the industrial technological development, since the industry did not yet exist at the time of their launch to the market.

During the course of Biolyfe, Novozymes has shared three enzyme preparations with the partners. First, Cellic Ctec was sampled as state-of-the art at the outset of the project, secondly, the improved enzyme product Cellic Ctec2 was shared and third a tailor-made custom blend was sampled to the partners (Biochemtex and the academic partners ENEA and ULUND) who conducted hydrolysis experiments. There was a critical interaction between Novozymes and these partners and simultaneously feedback on how the change to the cocktail of enzymes changed the optimum process lay-out and operating conditions.

Novozymes Cellic products are expressed by use of the *Trichoderma Reesei* fungi. The enzyme development that led to CTec3 has focussed on eliminating the disadvantages identified

Since Biochemtex, from the beginning of the project, had already zoomed in on a pretreatment technology that did not use any addition of mineral acids, it was clear that more hemi-cellulytic activities were needed in order to improve the release of xylose from the pretreated biomasses wheat-straw and Arundo. It is critical optimise the xylose release to make it available for xylose fermentation.

Based on NZ background information, three hemicellulases were produced in kilogram quantities in Novozymes' pilot facility. The enzyme batches were characterised in terms of enzyme activity, content and purity and then tested in labscale experiments at Novozymes. The three enzymes were then tested at Biochemtex and it was found that all three gave a significant improvement in the release of xylose during hydrolysis.

A custom-made blend of Ctec2 and hemicellulases was prepared in kilogram quantity and the sample was shared among ENEA, ULUND and Biochemtex for further tests.

As described elsewhere (see ENEA, ULUND and Biochemtex), all data pointed at a substantial improvement of supplementing Ctec2 with extra hemicellulases activity. This information was used to guide the design of the next generation of commercial product in the Cellic range and as can be seen from figure 10, there was a profound benefit from going from CTec2 to CTec3 on PROESA® pretreated wheat straw.

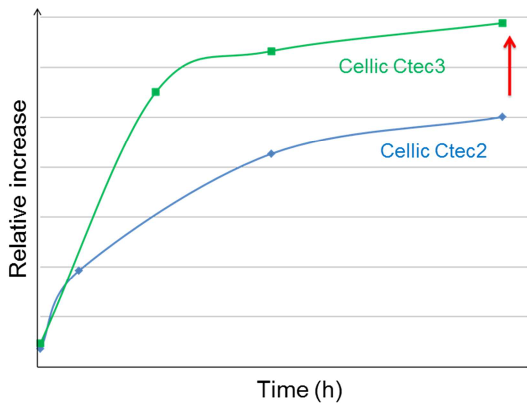


Figure 10 - Improvement in xylose yield at iso-dosage

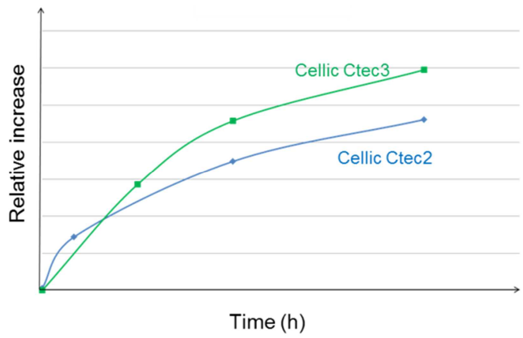


Figure 11 - Improvement in glucose yield at iso-dosage

Process impact: The increased hemicellulases activities in CTec3 is one of the most Biolyfe and PROESA® specific improvement.

The general replacement of enzyme components to more active and thermo-stable alternatives (e.g. seen in figure 10,11) in CTec3 and in a more optimal proportions (e.g. figure 12) has been an overarching theme during the enzyme development.

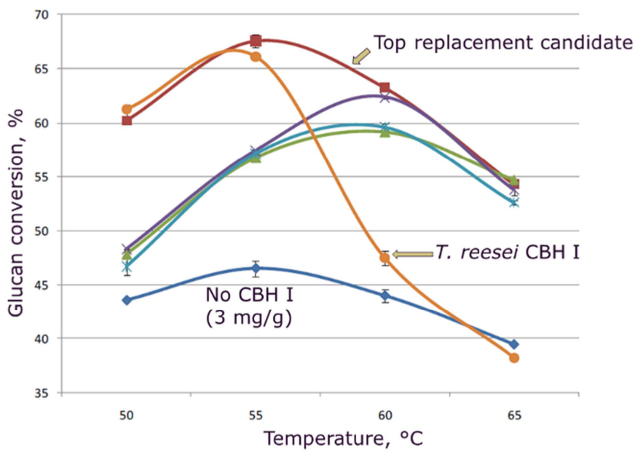


Figure 12 - Shows performance of regular *T.reesei* CBH1 compared to a more thermo-stable alternative candidate for CTec3

A relevant impact on the project has been the development of a more cost-efficient enzymes, that directionally moves the process optimum towards higher conversion, higher solid levels, lower pretreatment severity and possible shorter residence time and the increased thermal stability allows for operation at high temperature dedicated hydrolysis conditions before shifting to fermentation.

The delivery of enzyme product to the demonstration phase of the Biolyfe project required full-scale production of enzymes although the up scaling effort as such was outside of the project.

The production of CTec3 involved several improvements to the resulting cocktail of enzymes apart from the before mentioned increased level of hemicellulases activity which was the focus in Biolyfe. The scaling up for commercial enzyme production took place in Denmark. First, several weeks of experimentation was performed in the pilot plant in Bagsvaerd Denmark before production commenced at Novozymes' factory in facility in Kalundborg .

All the permits, quality control measures, certification etc. were performed as described by Novozymes standard operating procedures.

Production of enzyme supply for the Crescentino demo plant was carried out during months of January-March 2013 by successful enzyme fermentation in two batches (denoted VDNI-1002 and VDNI-1005). Both batches fulfilled all specification requirements yielding an activity of >1700 BHU(2). The enzyme was delivered to demo plant. Also all following batches in 2013 were produced on-spec without any single failed batch.

Within the framework of the Biolyfe project, the parties managed to simultaneously develop and optimize a new enzyme and process technology, by rapid exchange of enzyme samples and feedback, always ensuring that latest learnings and improvements where acknowledged between the parties and could help adjust the directions of the work. This resulted in worlds first commercial scale cellulosic ethanol production facility running on the most cost-efficient enzyme Novozymes have produced and now is producing in commercial quantities.

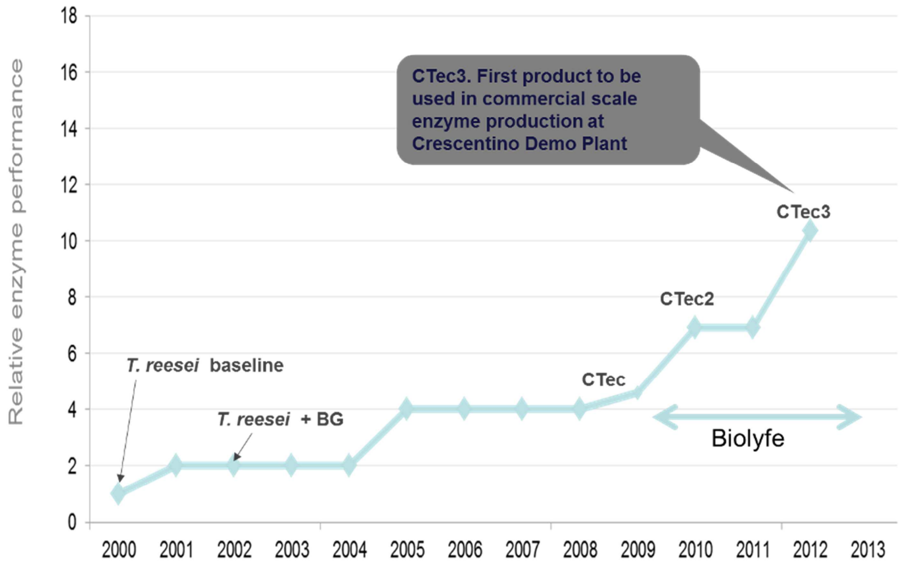


Figure 13 - Cost-efficiency of Novozymes biomass degrading enzymes

Fermentation

The fermentation step is central in any lignocellulose to ethanol process. In Biolyfe fermentation technology was addressed by the partners in work package 3, with the overall aim to reach an improvement of the total ethanol yield starting from the selected biomass. The fermentation is closely connected to both pretreatment, due to the formation of possible inhibitors, and the enzymatic hydrolysis for the formation of fermentable sugars. The fermentation may directly interact with the hydrolysis in SSF processes by its removal of sugars, thereby relieving feed-back inhibition of the enzymes. The Biolyfe process concept is a hybrid process, consisting of a viscosity reduction, an enzymatic hydrolysis and an SSF step. The work on fermentation technology was carried out in collaboration between Novozymes, Biochemtex, ENEA and University of Lund.

The objectives were:

- Assessment of tolerance of a pentose fermenting yeast in relevant hydrolyzates and inhibitor studies.
- SSF studies at high WIS contents with Biolyfe pretreated material
- Development of improved hybrid SHF/SSF processes

The experimental work on fermentation was mainly carried out in lab-scale (Fig 14). A special bioreactor was used in the study of rheology during enzymatic hydrolysis, a main feature of which was the ability to measure torque on-line. In this way, the change in viscosity during the hydrolysis could be studied.

Figure 14 - Fermentors used in lab-scale experiments (ULUND). Left: Reactor for high-WIS hydrolysis experiments with torque measurement. Right: Standard bioreactor for SSF experiments

The main feed-stock used in Biolyfe was Arundo donax (Fig 15) and the liquid fraction of the pretreated material was first evaluated with respect to its inhibitory properties using shake flask fermentations, and then the performance of the slurry and/or fibers were evaluated in simultaneous saccharification and fermentation (SSF).

Figure 15 - Fresh Arundo donax (left) and pretreated material (right)

The tests on the liquid fraction showed from pretreatment developed by Biochemtex gave a relatively inhibitor free material, with the main exception of acetic acid, which is inherent to the material itself. The pretreatment gave a fiber material, which was slightly more difficult to hydrolyze (Fig. 16).

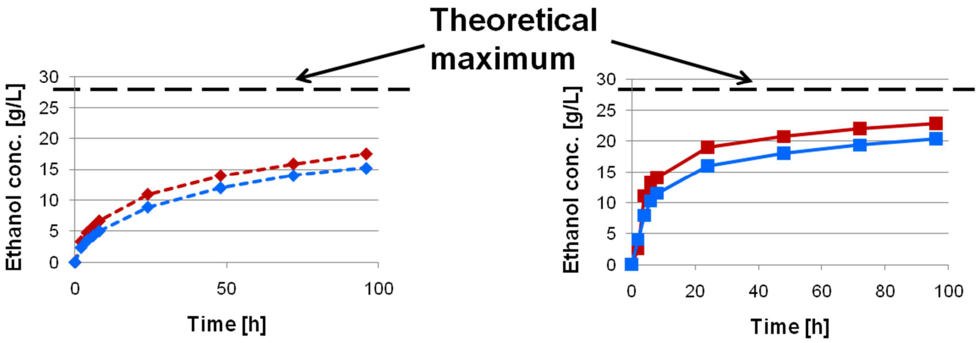


Figure 16 - Ethanol profiles during SSF at 10% WIS on Arundo Donax pretreated with (red) or without (blue) acids at basic dosing of enzyme blend used (left) and with five times that dosing (right). A non-pentose fermenting yeast strain was used in these tests.

The difference in digestibility between acid-free or acid catalysed pretreated fibers is to be expected, and the acid free pretreatment has other benefits in the design of the pretreatment reactor. Furthermore, the liquid fraction produced in acid catalysed pretreatment was found to be more inhibitory. Enzyme development was directed towards improving the hydrolysis yield. Significant improvements in SSF were found during the course of Biolyfe due to better enzymes (Table 2).

Table 2 - Improved ethanol yield in standard SSF assessment at 10% WIS using the same enzyme base loading and yeast concentration (ULUND).

Reference enzyme mixture		Improved enzyme mixture	Change in ethanol yield
Cellic CTec (+ HTec)	→	Cellic CTec2	No significant increase
Cellic CTec2	→	Intermediate enzyme blend	~ 15 % increase
Intermediate enzyme blend	→	Cellic CTec 3	~ 8 % increase
Overall increase			**~ 24 %**

Most of the pentoses in the material are solubilized in the pretreatment, and thus pentose fermentation should be developed for whole slurry fermentation in SSF. When pretreatment liquid was included in SSF, the final ethanol titre after 96 hour increased (Fig. 17), due to fermentation of the solubilized oligosaccharides in the liquid. There was no remaining xylose in the medium at this WIS loading. However, analyses showed glucan still remaining in the material.

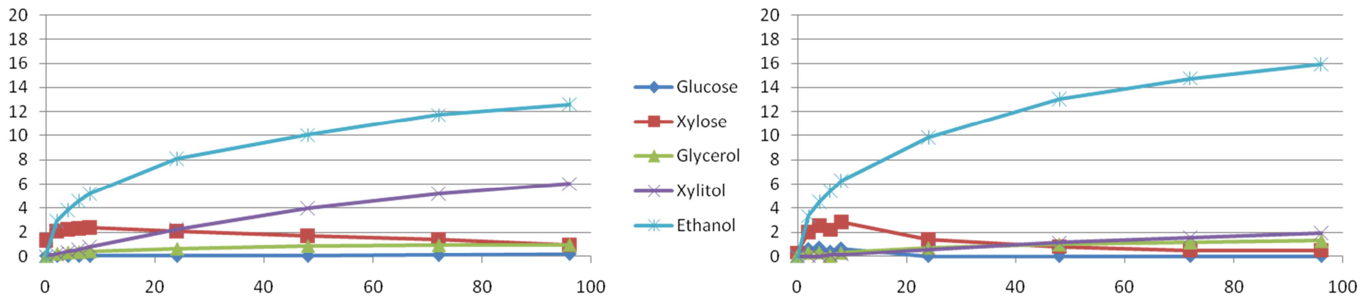


Figure 17 - Concentration profiles during standard SSF experiments using CTec2 with the pentose fermenting strain TMB3400, on Biolyfe pretreated Arundo fiber (A) and slurry (B) material at 10 % WIS content and a temperature of 34 C.

Since the novel enzyme blends work better at higher temperatures, the results point towards process concepts working at higher temperatures, e.g. either SHF, hybrid processes or high temperature SSF. Obviously, these processes also have to work at much higher WIS loadings in order to reach acceptable ethanol concentrations.

Power consumption for mixing needs to be considered in the high WIS processes, and this was studied in Biolyfe. The material was shown to lose its fiber network strength very rapidly (at least up to 20 % WIS content). After only a few hours of hydrolysis the needed torque (and power input) was shown to be rather independent of the starting WIS concentration (Figure 18).

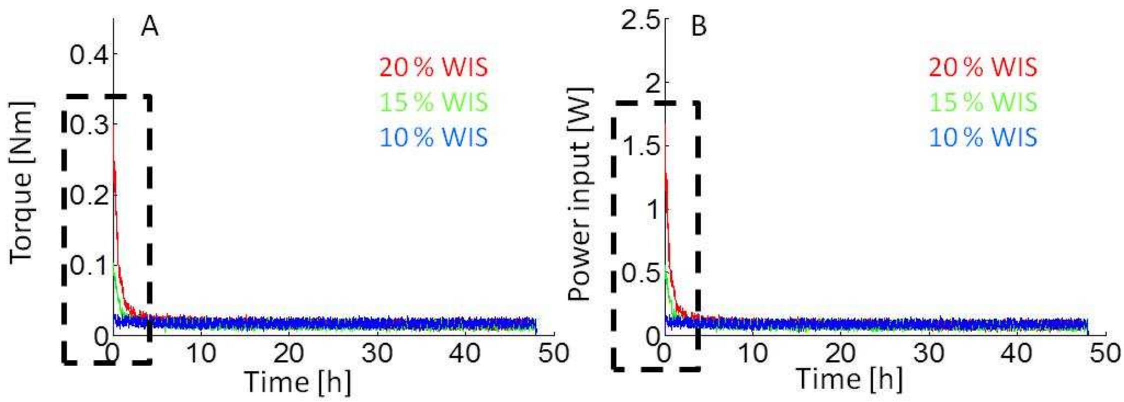


Figure 18 - Torque (A) and power input requirements (B) during enzymatic hydrolysis/viscosity reduction of arundo donax at different WIS contents. The dotted box higlightes the first few hours were the difference between the materials can be seen.

A high temperature enzymatic hydrolysis step should increase the enzymatic hydrolysis, especially with novel temperature stable enzyme blends. However, end-product inhibition – of various kinds – could still hinder the enzymatic hydrolysis at long process times. Furthermore, high glucose concentrations are known to hamper the xylose consumption by yeasts, which may favour the SSCF design, in particular if a total process time constraint is added. This thus needs to be experimentally investigated and various process configurations were compared in Biolyfe (Fig 19). It was shown a high temperature SHF step for 48 hours resulted in higher overall yields than either a SSCF or a shorter SHF step.

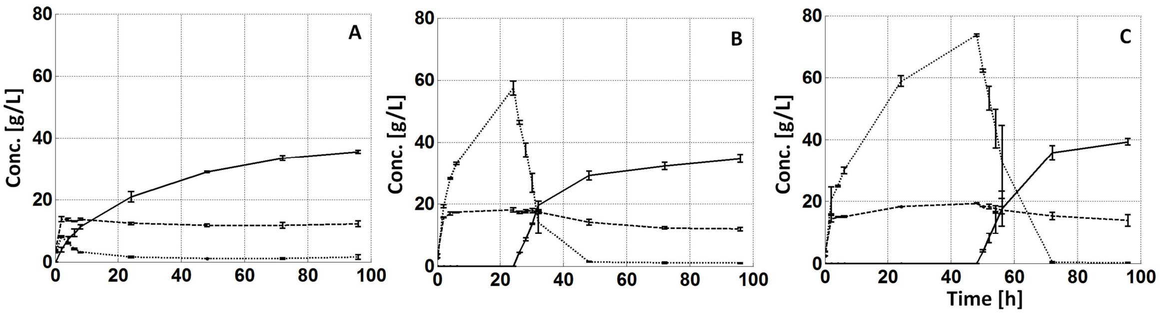


Figure 19 - Sugars (glucose and xylose) and ethanol concentration profiles during three different process options. A) standard SSCF at 34 ˚C, B) pre-hydrolysis at 45 ˚C for 24 hours followed by a 72 hours SSCF, C) pre-hydrolysis at 45 ˚C for 48 hours followed by a 48 hours SSCF. pH was kept at 5.0 throughout the experiments.

Acetic acid was identified as the main inhibitor in the Arundo donax hydrolyzate in Biolyfe. The inhibition by weak acids on fermentation can normally be reduced by increasing the pH, since it is the undissociated form of the acid that causes inhibition. It is furthermore known that pentose fermentation is more affected than glucose fermentation, the exact reason for which, however, is not known.

Shake flask fermentations confirmed that especially xylose consumption was greatly enhanced when increasing the pH (Table 3), and that the effect of the pH increase was stronger at the high acetic acid concentration. However, the less inhibiting conditions provided by the increased pH was furthermore shown to decrease the ethanol yield (Table 3) in favour of glycerol production and cell growth. The pH thus needs to be suitably tuned, with respect to the physiological status of the yeast cells. Further work in the factory process optimization will take place on this point.

Table 3 - Summary of ethanol yields and xylose consumptions obtained in shake flask fermentations. Yields are based on the final (48 hour) values.

	Acetic acid 4 g/L		
pH	**5.0**	**5.5**	**6.0**
Ethanol Yield (g/g)*	0.44 ±0.01	0.43 ±0.00	0.40 ±0.02
Ethanol Yield (%)	85.5 ±1.2	83.8 ±0.6	78.5 ±3.6
Consumed xylose (%)	27.4 ±1.9	38.7 ±2.5	50.2 ±1.2

	Acetic acid 8 g/L		
pH	**5.0**	**5.5**	**6.0**
Ethanol Yield (g/g)*	0.43 ±0.03	0.43 ±0.01	0.42 ±0.01
Ethanol Yield (%)	84.9 ±5.4	84.5 ±1.9	82.3 ±0.9
Consumed xylose (%)	10.7 ±1.2	37.0 ±0.6	48.0 ±2.0

*Based on consumed sugar

In summary, the fermentation development within Biolyfe has shown that:

- The Biolyfe process gives pretreated material, which is less inhibiting than acid catalyzed pretreated materials in fermentation.
- Rapid viscosity reduction was achieved with Biolyfe pretreated material, which facilitates further processing, e.g. fermentation/SSCF
- A 25 % increase in final ethanol concentration was achieved in standard SSF assessments using the novel enzyme blends developed.
- The overall ethanol yield in SSCF is improved by an initial high temperature SHF step
- Tuning of pH during fermentation was identified as an important process parameter for xylose conversion and by-product yields.

OPTIMIZATION OF HYBRID SSF

SSF processes consume sugars in the hydrolyzates thus favouring the further hydrolysis of cellulose. However biomass suspensions with high DM content have high viscosities and high temperature would better assist mixing and product liquefaction. In the Biolyfe project a strategy consisting in a biomass liquefaction followed by SSF has been pursued. Some measurements of the Biochemtex products liquefaction time were done in the ENEA's labs. In particular, off-line measurements of the viscosity were done by using a plate rotational rheometer and consistency indexes were estimated by the mathematical analysis of the results. The data in Figure 20 indicate that liquefaction of the Biochemtex pretreated product occurred in 3 hr from the maximum loading (done in 1.5 hr). Once the material is loaded, the hydrolysis extent was only 32% of the maximum achievable in larger time.

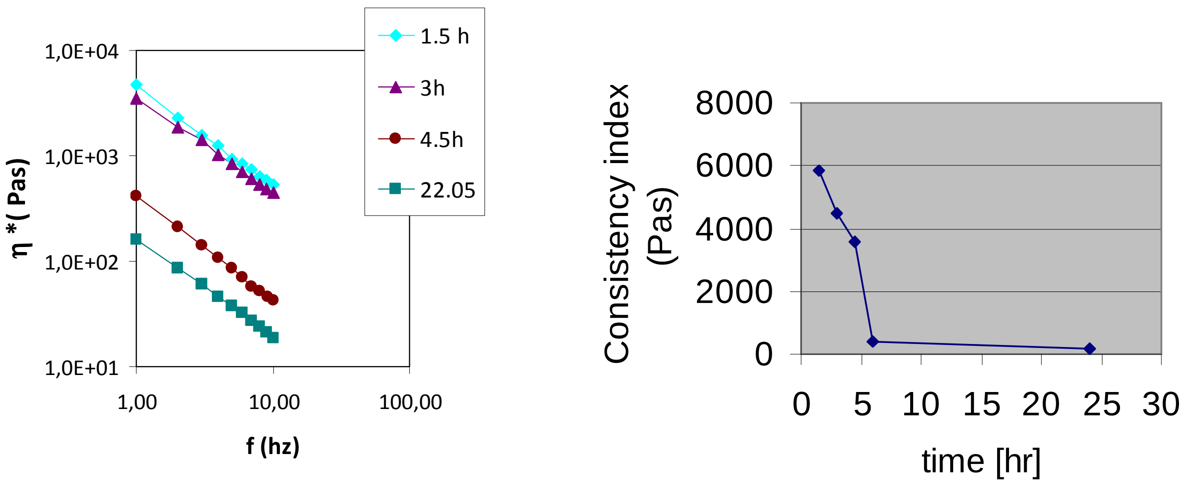


Figure 20 - Viscosity curves during the hydrolysis of pretreated *Arundo donax* (left). Consistency indexes obtained by fitting the viscosity curves with suitable functions

After the liquefaction threshold has been achieved, various reaction time for prehydrolysis can be selected. Longer hydrolysis time would make the process more similar to an SHF. On the contrary, switching from hydrolysis to fermentation after a short liquefaction time, would make the hybrid process more similar to a pure SSF. In addition, short pre-hydrolysis times have the advantage of promoting the xylose uptake during the fermentation of mixed hydrolyzates with suitable microrganisms. In order to conjugate the need for high temperatures of the hydrolysis step, with the optimal conditions for fermentation, temperature modulation during the SSF process was explored.

This strategy could offer the advantage of discontinuously accelerate the hydrolysis while avoiding sugars accumulation. Furthermore, the intermittent increase of the temperature could be an interesting approach for fermentation with co-coltures. In fact, the high temperature step ensures the temporary deactivation of the cells in the broth and could enable subsequent fermentations of carbohydrates (i.e. C5) by sequentially feeding different yeasts. In the present project ENEA investigated the effect of an intermittent step-wise increase of temperature along with fed-batch inoculation during the fermentation of *Arundo donax* sugars. In particular, temperature was periodically increased to 50° to improve the cellulose conversion, then the hydrolyzates were inoculated, with fed-batch mode (yeast and nutrients) when temperature diminished to 32-37°C. Figures 21 and 22 show typical trends of the yeasts activity during SSF with and without temperature modulation.

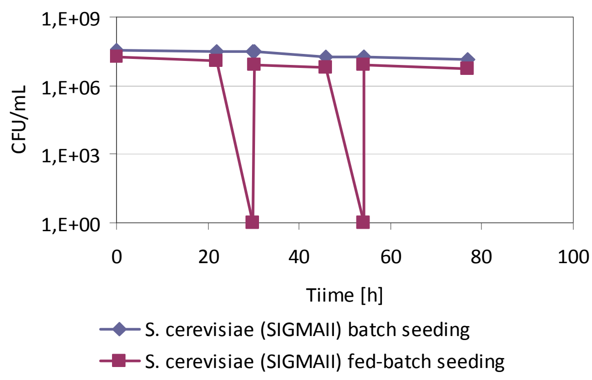


Figure 21 - *S. cerevisiae* cells accessibility during SSF with the temperature modulation and controlled, fermentation carried out at constant temperature and with a batch inoculation.

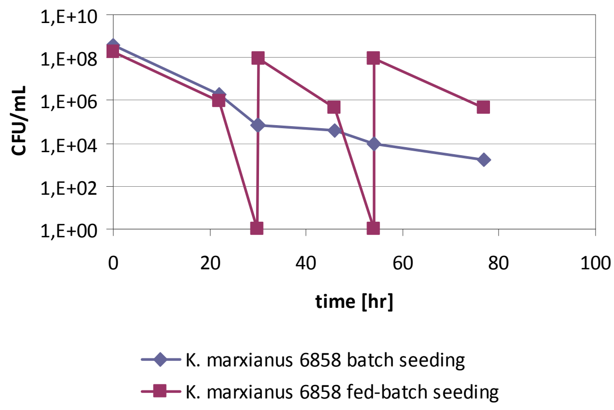


Figure 22 - *K. marxianus* 6858 cells accessibility during SSF with the temperature modulation, followed by a fed batch inoculation (square symbols) and control fermentation, carried out at constant temperature and with a batch inoculation.

The data indicate that *S. cerevisiae* strains had higher ethanol productivities and process yields compared to *K. marxianus* strains. This finding was also confirmed by the *K. marxianus* cells accessibility.

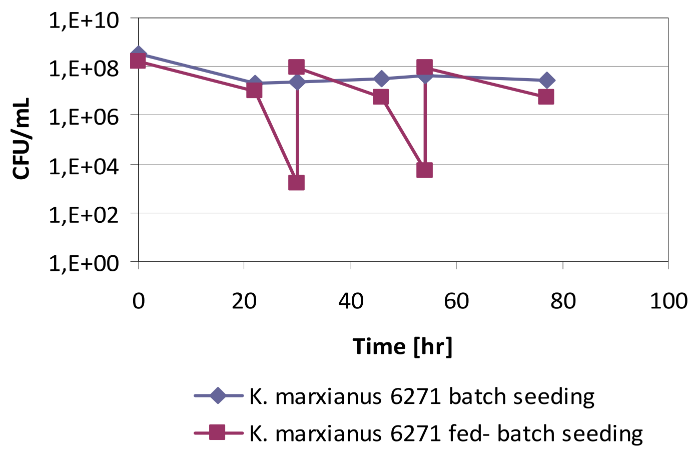


Figure 23 - *K. marxianus* 6271 cells accessibility during SSF with the temperature modulation, followed by a fed batch inoculation (square symbols) and control fermentation, carried out at constant temperature and with a batch inoculation.

Table 4 lists relevant results obtained with various yeasts, for different temperatures and different inoculation strategies. The data are sorted by the overall glucose, estimated as the sum of the residual glucose + glucose converted to ethanol (ethanol/0.51).

Table 4 - List of relevant fermentation tests of *Arundo donax* liquefied by Biochemtex. Effect of various SSF strategies: **B** - batch process; **FB1 -** 2 hydrolysis steps of 8 hr each; **FB2** - 2 hydrolysis steps of 6 hr each; **FB3** - 2 hydrolysis step of 5 hr each.

Microrganisms	Process Type	Yeast inoculation	T [°C]	Ethanol (%wt)	Overall glucose [g/L]
S. cerevisiae (M861)	SHF	B	32°C	3,5	68
S. cerevisiae (SIGMA II)	HSSF	B	32°C	3,3	66
K marxianus k6858	H SSF	FB 1	32-50°C	1,4	74
S. cerevisiae (SIGMA II)	HSSF	FB 1	37-50°C	3,8	75
S. cerevisiae (SIGMA II)	HSSF	FB 3	37-50°C	3,8	75
S. cerevisiae (SIGMA II)	HSSF	B	37°C	3,7	75
K marxianus k6858	H SSF	B	32°C	1,6	77
S. cerevisiae (SIGMA II)	HSSF	FB 3	37-50°C	4,2	84
K. marxianus k6271	H SSF	B	32°C	4,4	85
S. cerevisiae (SIGMA II)	HSSF	B	37°C	4,2	90
S. cerevisiae (SIGMA II)	H SSF	B	32°C	4,6	91
K. marxianus k6271	H SSF	FB 2	32-50°C	4,7	91
S. cerevisiae (M861)	H SSF	B	32°C	4,8	92
S. cerevisiae (M861)	H SSF	FB 2	32-50°C	4,7	93
S. cerevisiae (SIGMA II)	H SSF	FB 2	32-50°C	5,0	98

The highest ethanol yield was 82% of the theoretical (corresponding to a concentration of 5 wt%) obtained under the same experimental conditions of enzyme dosage and pH, the process run in SHF mode yielded 3.5 wt% ethanol.

Concerning the enzymes dosage, the results in Table 3 indicates that the process yields obtained by using a lower enzymes loading were lower. This implies that even if hybrid SSF, produces an higher ethanol yields, with respect to a pure SHF process, hydrolysis of residual cellulose represents the bottle neck of the process. This indicate the importance of fine tuning the pre-liquefaction step to increase the efficiency of hydrolysis at low enzymatic ratio.

Demo realization

Crescentino plant construction was followed by a team of engineers that followed each part of the construction from installation of equipment to piping and civil works, each step was followed according to internal procedures in order to keep work on time and solve critical situations with low impact on the schedule.

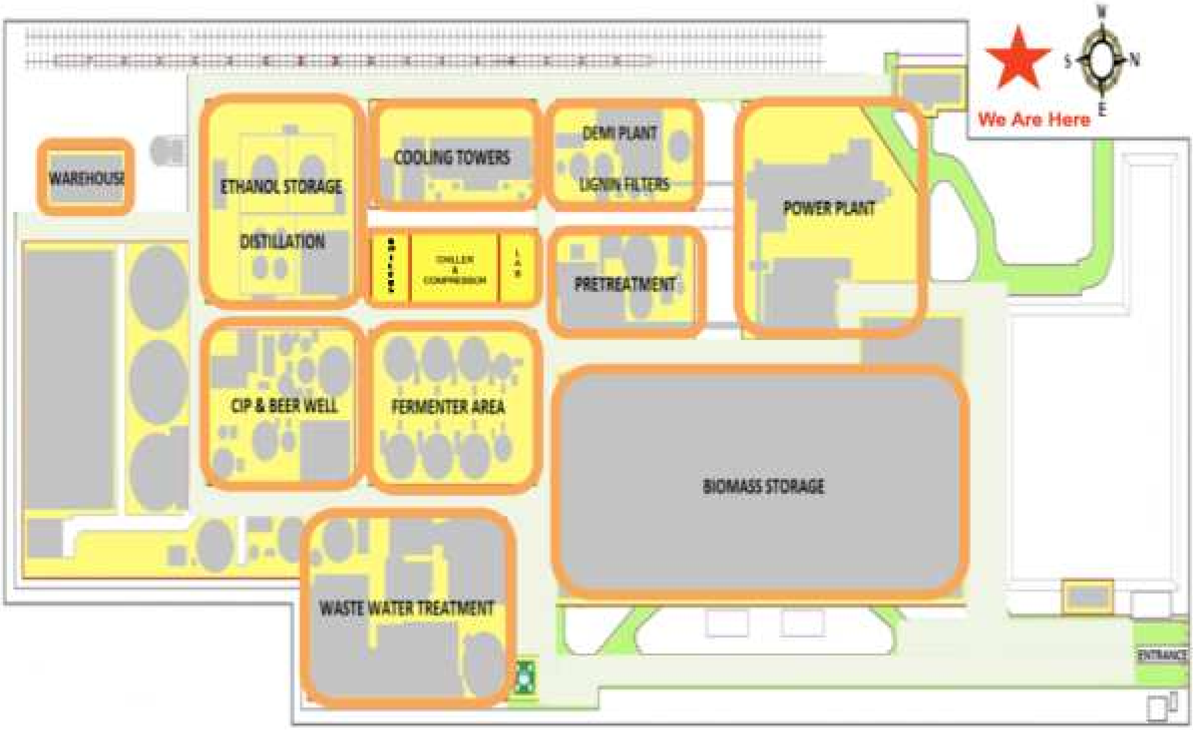


Figure 24 – Demo layout

The Site in Numbers

- Footprint of 15 ha
- Biomass storage capacity: 27.000 Mt
- 13 MW green electricity from lignin
- 100% water recycle: zero water discharge
- 100 operators on staff

<u>Construction time line</u>

The ground-breaking event for the demo plant took place on the 12th of April 2011 in Crescentino, in the Vercelli province (Piedmont region).

The image below shows the mayor and the Ghisolfi family (Mr. Vittorio and Mr. Guido Ghisolfi in the photo) inaugurating the area dedicated to the construction of the plant.

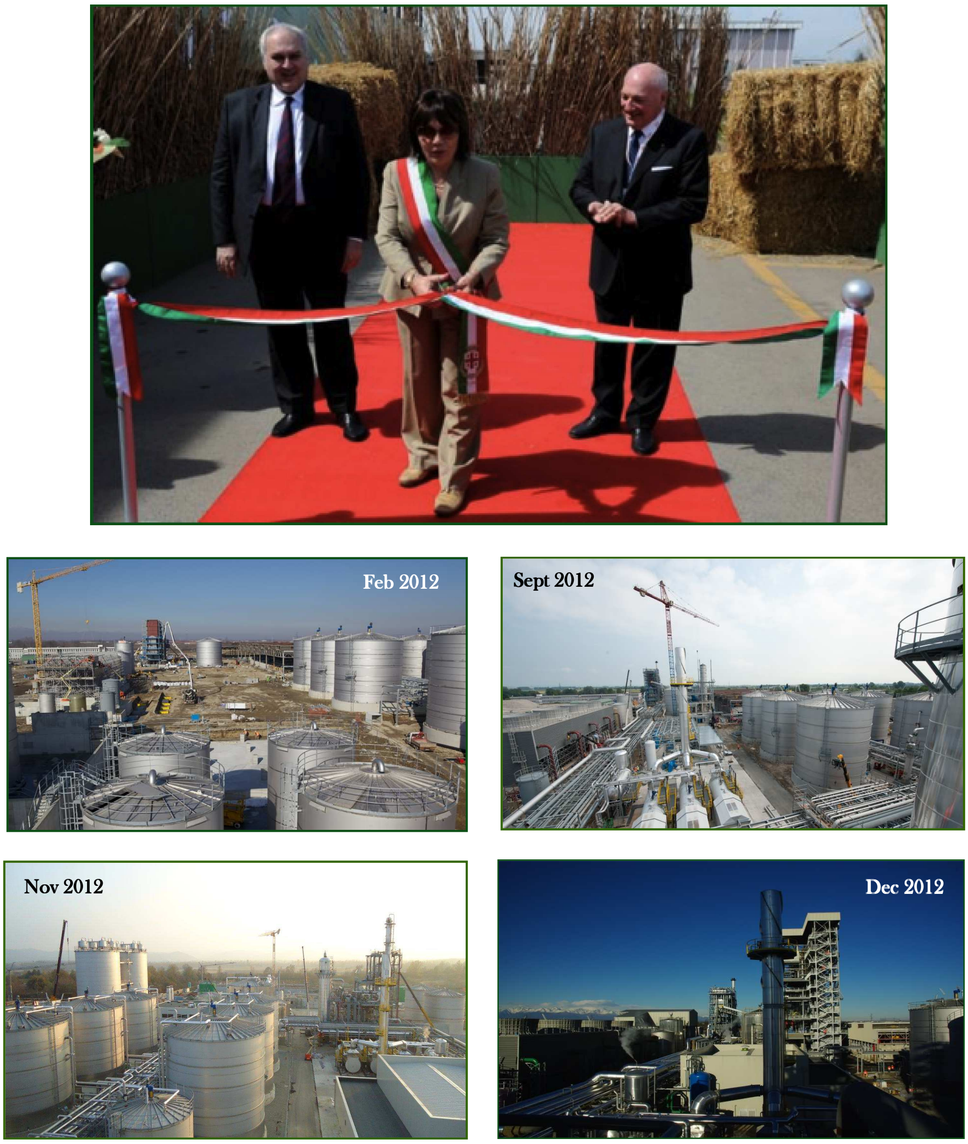


Figure 25 – DEMO built up and inauguration

Demo plant start up and operation run were accomplished. Actually the plant is able to work according to diluted Arundo case. This set up foresees both the higher amount of dry feedstock fed to the plant and the higher presence of water in the process; for this reason, the equipment designed to process this case are able to manage all the other cases, by a size point of view, moreover several technical solutions (e.g. recirculation, buffer tank, process integration) have been adopted, to guarantee a continuous ethanol production rate also at low production rate. The product: Bioethanol is produced according to specific ranges and sold outside with all the required certification.

Information needed for the successful scale-up to commercial scale, necessitated the construction and operation of this demonstration plant in Europe. Today, demo plant allows deriving the most of the information needed. The optimal performances of flagship facilities depend for a significant degree also of commercial, not technical, requirements, therefore an optimum design is the result of a careful consideration of the range of conditions over which the commercial plant will have to operate and the critical criteria to be met. As a consequence of the demo plant operation, through the realization of the scaled up project is expected to deliver market-leading cost of cellulosic ethanol without subsidies, helping speed widespread adoption of renewable biofuel.

Sugar solution, due to its purity and high concentration, can be used in chemical and biological processes to produce, for example, diesel substituting fuels or chemicals. Internal development and partnership with other companies are giving very interesting results that in short times will allow to fully exploit the biorefinery concept inherent PROESA® technology

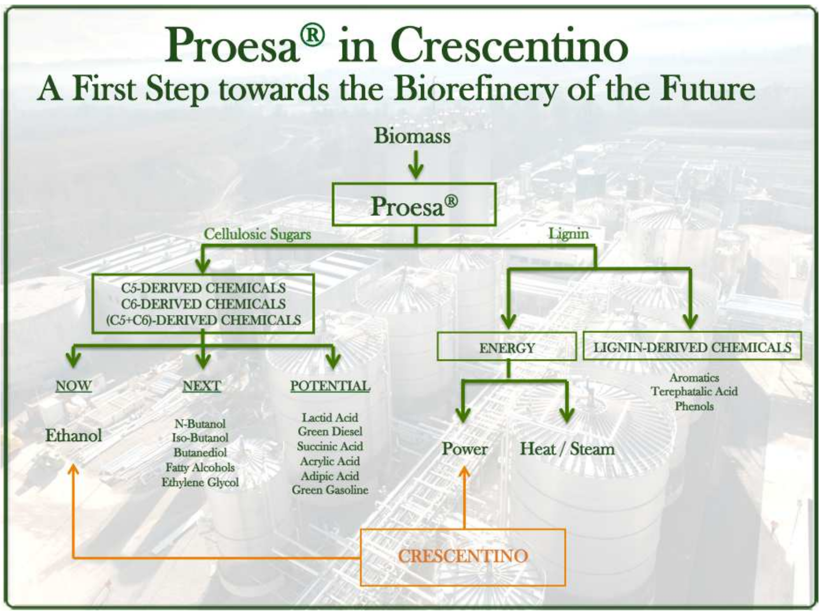


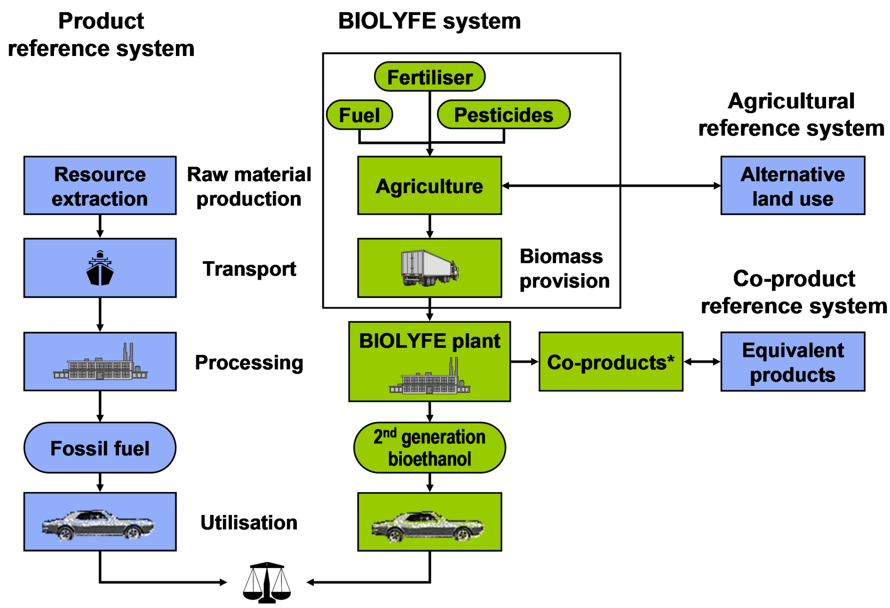
Figure 26 – Biorefinery schema

Sustainability Assessment

The provision of sustainably produced fuels from renewable resources is an important social goal and is also reflected in EU policy. Important objectives include reducing greenhouse gas emissions, reducing the dependency on fossil fuels and generating sources of income in rural areas. Biofuels currently on the market are predominantly produced from crops that can also be utilised as foodstuffs. Their life cycle greenhouse gas emissions are generally lower than those of fossil fuels are, but partially still substantial. Moreover, awareness is increasing that these fuels may be accompanied by some considerable drawbacks, because of both the competition with foodstuffs and the negative impacts on other environmental factors such as eutrophication and acidification.

Alternative biogenic fuels are therefore currently being developed to increase benefits and reduce drawbacks. A very promising option here is the conversion of lignocellulosic (woody and fibrous) biomass to bioethanol. A consortium formed for this purpose worked in a common project named Biolyfe (“Second generation bioethanol process: demonstration scale for the step of lignocellulosic hydrolysis and fermentation”). The project developed technologies allowing an increased and economically viable utilisation of the lignocellulosic feedstock for the production of second generation bioethanol. In order to achieve this objective, the Biolyfe project focuses on hydrolysis and fermentation steps. Biolyfe started in January 2010 and lasts for 3 years. The project is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-239204). In the Biolyfe framework was launched what is currently the world's largest lignocellulose-bioethanol facility in Crescentino, Italy.

An integrated sustainability assessment of the realisation of 2nd generation ethanol biorefineries in Europe in the period 2014-2020 is also performed as part of this project. It analyses whether a future, large-scale dissemination of the Biolyfe 2nd generation ethanol process using mature technology provides benefits from environmental, economic and social perspectives compared to the use of existing biofuels or fossil fuels. This comprehensive analysis investigates scenarios, which model various possible future implementations of this technology and its whole life cycle integration, from the provision of raw materials to the energy utilisation of the bioethanol, and a variety of optimisation options (fig. 27).



* if applicable (depending on assessed scenario)

Figure 27 -Schematic overview of the life cycle of Biolyfe bioethanol compared to the reference system fossil fuel.

Assessment approach

This study supplements established assessment methodologies such as the environmental life cycle assessment (LCA) and cost-benefit analysis from a business perspective by innovative approaches to cover and integrate all sustainability-related aspects of future Biolyfe biorefineries. In respect to the environment, LCA methodology primarily covers global and regional impacts but is still under development regarding local and site-specific impacts. To still provide reliable decision support, it is extended by a new qualitative, life cycle based assessment of local aspects termed life cycle environmental impact assessment (LC-EIA), which uses methods originating from environmental impact assessment (EIA). Furthermore, a SWOT analysis (strengths, weaknesses, opportunities, threats) qualitatively examines all sustainability aspects not covered by environmental and economic assessment. Besides several particular aspects, the SWOT analysis focusses in this study on social impacts and competition about biomass. The used innovative approach for an integrated sustainability assessment includes harmonisation of settings for all individual assessments beforehand and a later joint evaluation of results using multi-dimensional comparison metrics and a structured transparent discussion. *This way, the integrated sustainability assessments helps decision makers to manage complexity instead of hiding it*. The application of this innovative assessment approach proved useful to provide balanced and specific recommendations. These relate not only to the bioethanol process itself but also to its integration into a whole life cycle and even to its potential role in a competitive future bio-economy taking into account risks of shifting burdens from one sustainability aspect to another.

Biolyfe bioethanol: Some principal results

General results: Biolyfe bioethanol can contribute to environmental benefits in terms of climate change, saving non-renewable energy resources and photochemical ozone formation (summer smog). However, other impacts such as acidification or nutrient input into ecosystems must be taken into account – like for first generation biofuels, too. Unlike European first generation biofuels, Biolyfe bioethanol can be produced from perennial crops, which can be cultivated with relatively low local environmental impacts especially on soil and fauna. Land use competition may occur, in particular if cultivated biomass such as Arundo or other energy crops are used as a feedstock but, in contrast to first generation biofuels, this risk can and should be minimised within the Biolyfe supply chain by exploitation of agricultural residues (e.g. wheat straw) where feasible and combination of their use with possible dedicated crop cultivation – preferentially on idle (abandoned) land. According to Biochemtex calculations, from a market prospective, Biolyfe bioethanol production costs are more competitive than first generation biofuels, and depending on oil price, they can even aim at competing with conventional fossil fuels. Thus, Biolyfe bioethanol can also contribute to farmers' income and generate permanent jobs in both industry and agriculture.

Biolyfe bioethanol presents an option for helping to achieve the sustainability goals of climate protection, energy security and promoting rural development. Advantages in these aspects can be seen in comparison to fossil fuels – as it is the case for many other biofuels. In many cases, nevertheless, Biolyfe bioethanol can also show important advantages compared to other biofuels under certain conditions as discussed in the following paragraphs. Please note that quantitative results presented in those paragraphs are greatly influenced by the agreed methods used, boundary conditions and technology development depicted in the scenarios. Thus, comparisons are only valid within the same framework of setting, which are uniformly applied to all scenarios within this study.

Specific technological aspects: There are some important differences between Biolyfe bioethanol and established bioethanol types in the specific characteristics of their impacts. First, there are obviously large differences in the adopted technology: 2nd generation biofuel technology is less tested to date and is associated with high capital costs even economical assessment shows high profitability for future plants. However, it does provide an innovation gain for society and is more readily accepted (currently at least) by society than traditional biofuels. This is, because agricultural residues such as straw can be used as feedstock, whereas 1st generation ethanol is based on cultivated crops, which can compete with food. Nevertheless it is noteworthy to mention, that concerning innovation, this assessments based on assumptions for full mature technology. In contrast, today the development is at the stage of an industrial demonstration plant. Therefore and because of the innovation gain and, second, because Biolyfe bioethanol can help to achieve the sustainability goals of climate protection, energy security and promoting rural development specified above, this technology should continue to be tested on large scale and optimised further. The Biolyfe bioethanol facility itself is characterised by being capable of energy self-sufficient operation utilising Arundo and wheat straw.

The second general difference relates to feedstocks. Because feedstocks, which have not previously been used, or only on a relatively small scale, are employed in Biolyfe bioethanol facilities, the infrastructure and logistics paths, including for storage, still have rooms for optimisation. Moreover, acceptance among farmers for cultivating innovative perennial cultures such as Arundo donax (giant reed) is still low and may require incentives such as coverage of risks to develop. However, perennials also provide benefits in terms of lower agricultural expenditures and lower local environmental impacts on soil and fauna. Additionally, these cultures generally yield more ethanol per hectare in combination with the applied Biolyfe ethanol second generation technology than first generation biofuels.

Altogether, this means that there is a tendency for some remarkable benefits in terms of the feedstock supply compared to most established biofuels, which contrasts on the other hand with conversion requiring higher expenditures.

Arundo cultivation on existing managed farmland may be promising, because under suitable conditions land use efficiencies can be achieved that are greater than for the majority of established, first generation biofuels. Per hectare, Arundo-based Biolyfe ethanol, can typically save e.g. 140 GJ of non-renewable energy and 8 t of GHG, whereas wheat grain based ethanol accounts for roundabout 50 GJ and 3 t of GHG and rape seed biodiesel 40 GJ and 2 t GHG (Errore. L'origine riferimento non è stata trovata.28). At the same time, cultivation conditions for Arundo have a considerably lower impact on the soil. However, here it is essential to consider the respective local conditions, because among other things, the Arundo yield depends greatly on water availability. Thus, there is a certain danger of water mismanagement with regard to nature and the population in areas of low water availability unless cultivation practise is focussed on avoiding irrigation, as it is the case for the integrated agricultural strategy of Biochemtex for the 2nd generation bioethanol plant in Crescentino.

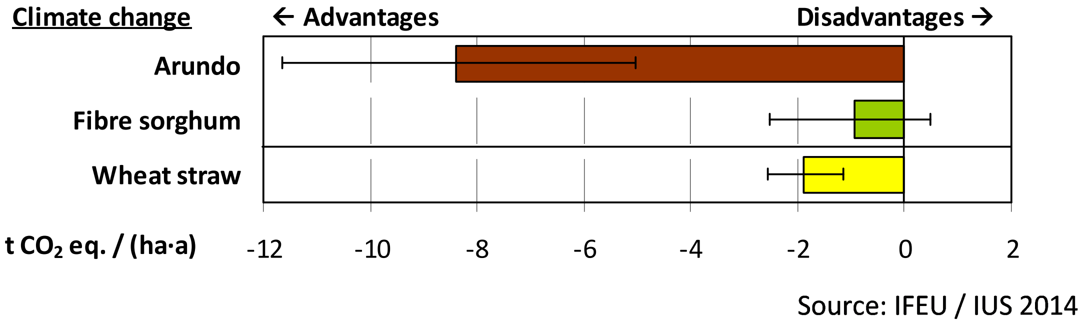


Figure 28 – Greenhouse gas emission savings per hectare and year due to production and use of Biolyfe bioethanol instead of gasoline. The feedstocks *Arundo donax* and fibre sorghum require agricultural land, whereas wheat straw is harvested from already cultivated land.

Costs: The economic analysis by Biochemtex reveals that Biolyfe bioethanol produced from wheat straw and Arundo can be cheaper in future than bioethanol produced from fibre sorghum (**Errore. L'origine riferimento non è stata trovata.**29) and cheaper than most established biofuels of today.

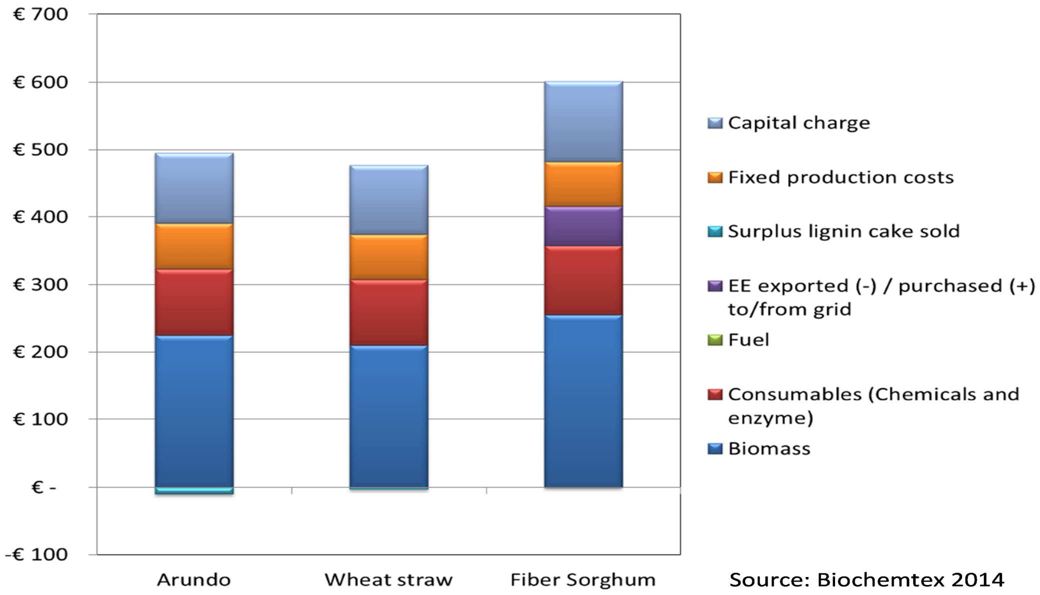


Figure 29 - Comparison of the results of total production costs in € / t ethanol for different feedstocks (*Arundo donax*, wheat straw and fibre sorghum) under standard conditions at a scale of 100 000 t per year. EE: electric energy

Conclusive evaluation and integration in the overall context

From an overall environmental perspective, the provision and utilisation of Biolyfe bioethanol produced from wheat straw and, subordinately, Arundo, in particular from idle land, provides more benefits in some aspects, such as climate change, compared to the majority of established biofuels. In other aspects, it is comparable or associated with only minor drawbacks.

The economic analysis presented here reveals that Biolyfe bioethanol produced from wheat straw and Arundo is also cheaper than bioethanol produced from fibre sorghum and more competitive compared to established biofuels in future. Similar to other biofuels, Biolyfe bioethanol will depend on political support for a certain transition period.

If biofuels can be produced from by-products such as straw or from energy crops on idle (abandoned) land, they basically do not compete with foodstuffs or fodder. Whenever dedicated crops from currently managed land are considered, it is advisable to extend this study with additional land use studies specific for the respective geographical situation. Politically, it is required to establish a higher-level biomass and land use strategy to manage competition and local planning based on it in order to take the site-specific benefits and drawbacks of various energy crops into consideration.

In technological terms, further optimisation is still possible as the Crescentino plant is a demonstration unit. This bioethanol facility, which uses the innovative technology, represents a very important step in this direction. Its successful operation will open up opportunities for additional facility construction projects and logistic concepts and can induce positive societal effects in terms of innovations.

POTENTIAL PROJECT IMPACT

The construction of demo plant will accelerate the deployment of sustainable technologies aiming to facilitate the development of energy technologies towards cost effectiveness for a more sustainable energy economy for Europe and ensuring that European industry can compete successfully on the global stage. Furthermore, it will provide reasonable basis for ensuring the complete value chain including the supply chain of sustainable biomass resources to the plant and it will be the further and essential step towards process cost reduction.

Achievement of commercial availability for advanced biofuels allowing competitiveness with fossil fuels at the prevailing economic and regulatory market conditions;

1. demonstration activities in order to develop and optimize every step of the process for bio-ethanol production;
2. Providing a worldwide transition to a low carbon economy by increasing environmental sustainability in biofuels production in order to minimize energy demand and cost;
3. Increasing of bioenergy sources in order to strengthen EU leadership position for renewable transport fuels on the world market scenario;

The impact of the Biolyfe results will be a driving force in the context of biorefineries. Demo unit could represent the basis for each biorefinery that will use lignocellulosic material, with the possibility of increasing the competitiveness of the biorefinery itself and provides an industrial pathway aiming at lower production costs and increased productivity: this makes the Biolyfe project a factor of high dissemination quality in economic terms.

Biolyfe dissemination activities

Within the Biolyfe project several activities were implemented in order to widely disseminate Biolyfe activities and results among important stakeholders and the public in Europe as well as to actively promote the exchanges between RTD experts, stakeholders and key actors on large-scale realization of second generation ethanol production and use.

CREATION OF A FUNCTIONING INFRASTRUCTURE

In the framework of the Biolyfe project efforts were undertaken to set-up a functioning infrastructure in order to demonstrate the distribution and use of 2nd generation bioethanol under real operating conditions. This infrastructure includes the installation of a fuel distribution system in the vicinity of the ethanol production site as well as the on-road operation of Flex-Fuel vehicles (FFV).

Based on experience gained by ETA (sub-contractor) within the EU-funded project BEST (Bioethanol for Sustainable Transport) on the implementation of a FFV fleet operating with E85 fuel in La Spezia, a manual was elaborated on necessary authorisation procedures and permits for the blending, storage, set up of the E85 pump, as well as the distribution of the ethanol fuel. CXTI and WIP identified Alfa Centauro Immobiliare Srl in Tortona, Italy as a partner for the installation of the E10/E85 pump. Alfa Centauro operates several gas stations in the Tortona region and one of their stations in Tortona was selected for the installation of the fuel pump. After signing an agreement of cooperation between WIP and Alfa Centauro, WIP and ETA performed all necessary tasks (including the purchase of equipment) related to the installation of the E10/E85 fuel pump together with Alfa Centauro.

A specific training for the staff of the fuel station in Tortona was held in May 2012. The aim of the training was to assist the staff on the operation and maintenance of the E10/E85 fuel pump, as well as the origin of the fuel, its specifications and relevant legislation. The training was held in Italian language and was implemented by ETA. The training manual on the operation and maintenance of the fuel pump was elaborated by WIP.

On 7th June 2012, the official opening of the new fuel station took place in Tortona, Italy. The opening of the fuel station was an important step towards the comprehensive concept 'from the field to the wheel', thus publicly demonstrating the feasibility of a real alternative to traditional fossil fuels.

The ceremony was opened by the Mayor of Tortona, Mr. Massimo Berruti, and the Research & Development Director of Mossi & Ghisolfi Group, Mr. Dario Giordano. Fuelling options for FFV were demonstrated for two types of blended gasoline: E10 (a mix of gasoline with 10% of bioethanol) and E85 (a mix of gasoline with 85% bioethanol).

Figure 30 - Official opening of the new E10/E85 fuel station in Tortona, Italy

Figure 31 - Donation of Flexi-Fuel vehicle to the city of Tortona, Italy

During the inauguration ceremony, Mossi&Ghisolfi Group donated a Flexi-Fuel vehicle (designed to run on various blends of gasoline with ethanol) to the city of Tortona. Other three Flexi-Fuel cars are used by the Group to advertise the product and demonstrate the benefits of PROESA® technology for the production of second generation bioethanol.

Figure 32 - Flexi-Fuel vehicle at the new fuel station

On the occasion of the opening of the fuel station Mr. Dario Giordano stated that 'Sustainable chemistry is the future of Mossi&Ghisolfi Group and a real opportunity for economic revitalization of the country' - 'Today, thanks to research we have an economically and socially sustainable alternative to oil. We should firmly believe and bet on the Italian green economy focusing on research to provide sustainable solutions'.

Finally, based on the experiences gained with the set-up of the infrastructure for the distribution system in the vicinity of the Crescentino ethanol production site, an infrastructure and distribution concept was developed for a future large scale second generation bioethanol production unit with a capacity of about 200.000 tons of ethanol per year.

SHARING THE KNOWLEDGE AND THE STATE OF THE ART

The world's first commercial-scale plant for the production of bioethanol from non-food biomass sources was officially inaugurated with a public event on 9 October 2013 in Crescentino, Italy. The Crescentino (Vercelli) bio-refinery is owned by Beta Renewables, a joint venture between Biochemtex, Mossi Ghisolfi Group engineering company, American fund TPG (Texas Pacific Group), and the Danish company Novozymes, a world leader in bio-innovation.

In order to involve and inform leading experts about the progress achieved in the framework of the Biolyfe project, three conferences have been organized. The first Biolyfe conference was organised on the occasion of the 19th European Biomass Conference and Exhibition (6-10 June 2011, Berlin, Germany). This workshop focused on the sustainable production and sourcing of lignocellulosic feedstocks and attracted about 60 stakeholders from all over Europe. The second Biolyfe conference was organised on the occasion of the 21st European Biomass Conference and Exhibition (3-7 June 2013, Copenhagen, Denmark). The goal of this conference was to provide an insight into second generation bioethanol production.

The final Biolyfe conference was organised as stand-alone event on 4th December 2013 in Brussels. The conference attracted over 30 participants from all over Europe.

In addition, Agriconsulting, with the support of Biochemtex, organized a crop demonstration workshop in Turin on 15 November 2013 with the title "Dedicated crops for 2nd generation bio-ethanol production: opportunities and prospects for the local agriculture people and operators".

Handbook

A Biolyfe Handbook on 2nd generation bioethanol production processes was finalised and printed in December 2013 (available on website) in two Parts:

- Part I includes a general comprehensive overview on Lignocellulosic Ethanol Process and Demonstration.
- Part II of the handbook includes a detailed description of the demonstration plant in Crescentino, presenting the world's largest demo plant ready to be transferred all over the world.

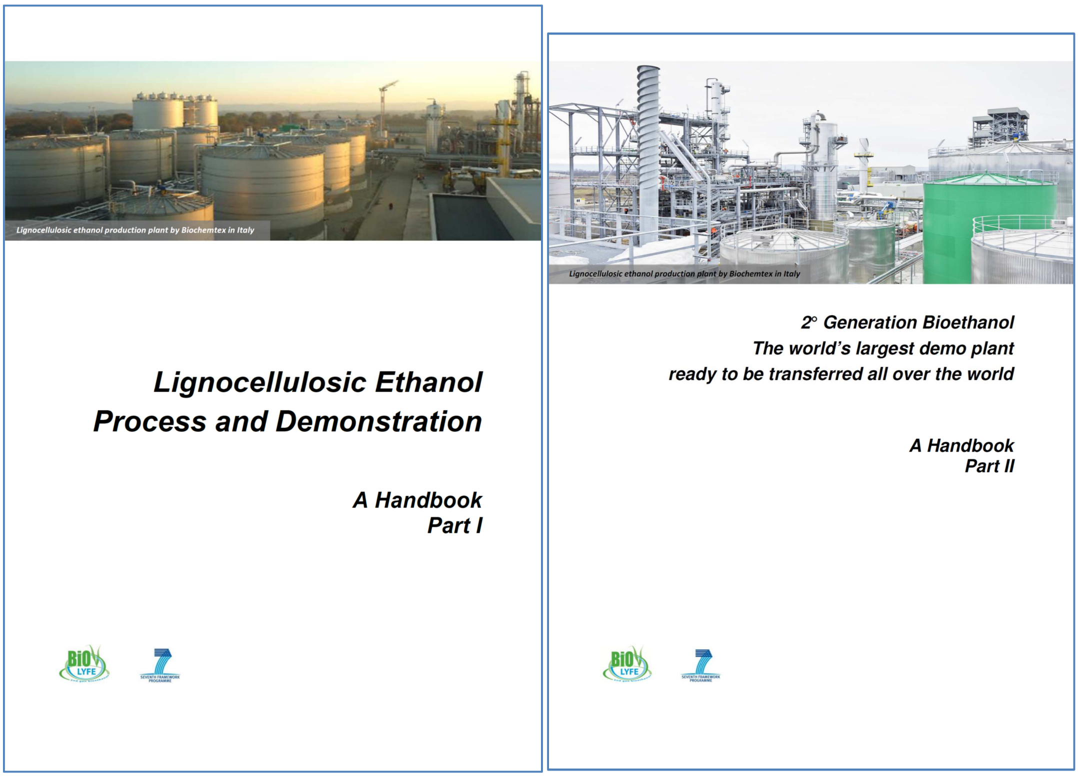


Figure 33 - Biolyfe handbook – Part I and Part II (December 2013)

Website

In March 2010, the Biolyfe project website was established at www.Biolyfe.eu. This project website was continuously up-dated to present project activities and results, and will remain operational for at least three years after the end of the project. Further dissemination material included the Biolyfe logo, project flyers and a project brochure.

Figure 34 - *Biolyfe homepage (December 2013)*

In February 2010, the Biolyfe stakeholder database with more than 1,400 contacts was established building on existing WIP databases. The database was continuously updated and contains more than 2,300 contacts in December 2013. In order to keep second generation bioethanol stakeholders updated on Biolyfe project activities and results, a **quarterly Biolyfe newsletter** was produced and sent via email to stakeholder database contacts. (actually all available in the website)

RECOMMENDATIONS FROM THE PROJECT EXPERIENCE

The following recommendations were deduced from the conclusions taking into account all perspectives on environmental and economic sustainability.

They are presented for each group of stakeholders.

Policy makers

Policy makers assume a particularly important role when framing future options and in conflict management. Because new technologies such as 2nd generation biorefineries will increase the demand for biomass, even if non-food, the competition between bio-based materials, chemicals, fuels and energy, fodder and nature conservation centred around biomass or land use represents one of our most important social challenges.

This must be actively managed with clear objectives. Among the others, several specifically measures can be highlighted:

- In the mid- to long-term, national and European **biomass allocation and land use plans** should be compiled.
- **Regional planning**, which comprises project planning guidelines, should be based on this premise. This framework should also rule out the cultivation of cultures that are unsuited to the local conditions. For example, whether greater water demand represents an environmental burden or not, or the quantity of agricultural residues that can be extracted without impairing soil fertility, depends on the location. Moreover, regional planning is also important because market participants with individual high biomass demand and large market power are created with the aid of public funding, and may be additionally created by establishing biorefineries. Interaction of biomass on other markets can be managed by appropriate planning.
- Following an initial phase necessary to establish the technology, **support for biorefineries** should be oriented around the reductions in environmental impacts.

As an additional measure independent of biomass and land use competition, we recommend:

- Before mature industrial facilities are established on a large scale a **regulatory framework**, which will ensure sustainable production, should be defined. It should comprise a sustainability analysis for each specifically planned large facility.

Companies

- **Biomass selection** for the bioethanol process should be based on sustainability criteria. Of the biomass types investigated here, wheat straw should be preferentially adopted, then Arundo produced on idle land and only subordinately Arundo produced on managed farmland. Fibre sorghum should be avoided as main feedstock because it does not reach the environmental and economic performance for this specific location and conditions.
- The extent to which bioethanol facilities can be designed to provide greater **flexibility** for processing a variety of lignocellulosic **raw materials** needs to be investigated. A variety of different feedstock can then be exploited, which can ensure greater operational security, but also presents additional options for sustainable biomass provision through feedstock diversification.
- The bioethanol facility should be optimised as far as possible in terms of **energy efficiency**. The aim should at least be bioethanol production without purchasing additional electricity or fossil fuels. Otherwise, the results of the life cycle assessment may in part be substantially degraded.

- Companies have to actively **build up trust of society** and local communities. An indispensable part will be the development of credible strategies to avoid negative impacts.

Farmers

- **Shifting to perennial crops**, requires to ensure the market availability on medium long time.
- Farmers should not risk **long term fertility of soils** by extracting too much straw for short term income generation.
- **Cooperation could help as damper for farmer risk**.
- **Reduction of fertiliser demand**. Especially the use of nitrogen fertilisers to produce agricultural feedstocks generally contributes significantly to the results of life cycle assessments for biofuels. Appropriate optimisation of farming practices and breeding towards low nitrogen content in the harvested biomass as shown for Arundo in field trials can considerably improve the environmental impacts of bio-based products through reduction of fertiliser demand.

Future improvement

Further progresses could be envisaged in consideration of the experience gained in operating the demo

- **Recycling nutrients from fermentation residues**.

Efficient production processes: 'enzymes'. Further improvement in enzymes production and use will positively affect the LCA balance.

- **Energy efficient production processes: 'purification'**.
- **Reduction of nitrogen use in agriculture**.
- **Process integration of the whole biorefinery**.