Contract N° 019882

NILE

New Improvements for Lignocellulosic Ethanol

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Priority name: Sustainable Energy Systems

Publishable final activity report

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Project co-ordinator organisation name: Institut Français du Pétrole (IFP)

Publishable final activity report

Introduction - Project outline

The overall objective of NILE was to develop cost effective production of clean bioethanol from lignocellulosic biomass (LCB), enabling its use as a transport biofuel. NILE has investigated and evaluated new systems and technologies for an efficient conversion of lignocellulose to bioethanol. Selected systems have been verified using a unique and fully integrated pilot plant providing reliable data for global socio-economic and environmental assessments and for the design of a future demonstration unit.

The activities of NILE aimed at overcoming critical hurdles in process development. Key issues were:

- decreasing the cost of enzymatic hydrolysis of lignocellulose to fermentable sugars using new engineered enzyme systems;
- removing current intrinsic limitations in the conversion of fermentable sugars to ethanol by i) constructing inhibitor-tolerant pentose-fermenting industrial yeast strains, and ii) combining fermentation and saccharification;
- validating the engineered enzyme systems and yeast strains in a fully integrated pilot plant using softwood as a model feedstock. Validation includes all process steps and recycling of process streams. Wheat straw was considered as an alternative model feedstock;
- analysing socio-economic and global environmental impacts of the production and use of bioethanol from LCB based on the new data obtained;
- dissemination and training of target groups.

By its integrated structure, NILE has supported the development of research activities in close connection with industrial processes, their validation, their technical and socio-economic assessments, their dissemination as well as training activities. This development ensured a real industrial and societal impact of the developed technology thus strengthening European competitiveness.

NILE has lasted 4.5 years, from October 2005 to March 2010. It has been accepted for funding by the European Commission within the 6th Framework Programme. The planned total budget was 12.6 M€ (7.7 M€ funded by the EC). The project succeeded in mobilising the critical mass of end-users and world-leading expertise necessary to improve the whole bioethanol from LCB chain. 22 partners from 12 countries participated to the project. The project consortium was the following:

Participant category	Participant name	Participant short name	Country
Research Center	Institut Français du Pétrole	IFP	F
Industry	ENI	ET	I
Industry	Roal Oy	Roal	FIN
Industry	SAF-ISIS (SI)	SI	F
Industry	SEKAB BioFuels & Chemicals AB	Sekab	S
SME	DIREVO Industrial Biotechnology GmbH	Direvo	D
SME	Sekab E-Technology	Etek	S
SME	Granit Recherche Développement	GRANIT	CH
Other*	BioAlcohol Fuel Foundation	BAFF	S

Other	European Renewable Energy Centres Agency	EUREC	B
Research Center	Centre National de la Recherche Scientifique	CNRS	F
Research Center	Centro Ricerche Fiat	CRF	I
Research Center	Institut National de la Recherche Agronomique	INRA	F
Research Center	Latvian State Institute of Wood Chemistry	IWC	LV
Research Center	Technical Research Centre of Finland	VTT	FIN
University	Swiss Federal Institute of Technology Zürich	ETHZ	CH
University	Johann-Wolfgang Goethe-Universitaet Frankfurt am Main	JWGUF	D
University	Imperial College of Science, Technology and Medicine	ICSTM	UK
University	Universidade Nova de Lisboa/Faculdade de Ciências e Tecnologia	UNL	P
University	Lunds Universitet	ULUND	S
University	The Weizmann Institute of Science	WI	IL
Industry	Mondi Business Paper Services AG	Mondi	A

* Other = Foundation and Association

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Work performed and main results obtained

1. Enzymatic hydrolysis

Introduction

The NILE project aimed to slash the cost of enzymatic hydrolysis, which was estimated to account for 30-50% of the cost of the complete ethanol production process.

Trichoderma reesei is one of the best known fungi for producing cellulases (enzymes that break up long cellulose molecules successively into smaller molecules and ultimately glucose, which can be fermented to ethanol). It secretes eight major enzymes that display three types of activity: two cellobiohydrolyases, liberating cellobiose, a dimer of glucose, from the ends of the long cellulose molecule, five endoglucanases that attack cellulose at random points in the molecule and one ß-glucosidase, splitting cellobiose to glucose. The extent to which *T. reesei* produces and secretes these enzymes depends on the material that the fungus is grown on. Part of the challenge is also to help *T. reesei*'s cellulolytic enzymes reaching the cellulose, which, in typical lignocellulosic material, is surrounded by other polymers, like lignin and hemicellulose. *T. reesei* does not naturally produce all the enzymes required to weaken this enclosing structure, but can be engineered to produce some of them.

Before the NILE project, work to improve *T. reesei*'s performance focused on creating new strains by inducing random genetic mutations or by targeted genetic modification with more copies of useful genes and more active systems in the cell governing the production of cellulases.

WP1 was active during the 4.5 years of NILE. Contractors implicated in the WP were VTT, INRA, WI, Roal, SAF-ISIS, CNRS and IFP, which fulfilled the function of WP leader.

Identifying new efficient cellulases and helper enzymes

Genome-mining of the *T. reesei* genome in NILE led to the identification of a high number of genes encoding uncharacterised enzymes, though the number was relatively small compared to other fungi. Strikingly, some classes of hemicellulases are missing and no ligninase is expressed. Some of these “missing” enzymes were searched in some species of *Aspergillus* and other *Trichoderma* related fungi and were tested for their suitability as “helper enzymes” for hydrolysis.

Additionally, *T. reesei* was grown on pure cellulose, pretreated wheat straw and spruce, and expression of each cellulase and hemicellulase gene was monitored using DNA transcriptome microarrays. The purpose was to determine which of these genes were specifically expressed on complex technical substrates compared to pure cellulose. Such genes were characterised as they could represent an important limiting step in the lignocellulosic biomass hydrolysis reaction.

In total, 38 new enzymes that might play a role in the degradation of lignocellulosic substrates were found. Biochemical data were obtained for 13 enzymes that were identified as relevant for hydrolysis efficiency. In addition, the effect of 10 other enzymes that had been identified, classified and successfully cloned in work before NILE, but whose efficacy was not known, was investigated.

Identifying and enhancing by directed evolution the enzymes whose activity limits the overall rate of hydrolysis

Analysis performed in NILE has shown one of the cellobiohydrolase, CBH2, to be the enzyme that limits the rate at which cellulose can be hydrolysed (Figure 1.1). Using “directed evolution”, two CBH2 variants were engineered. One featured twice the activity of the wild type enzyme (Figure 1.2). Its performance was confirmed in laboratory scale hydrolysis tests.

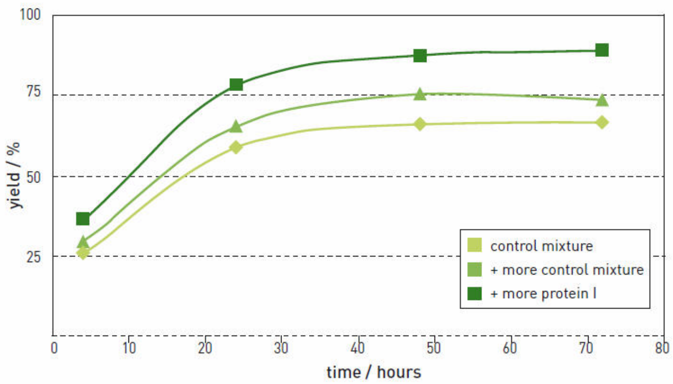


Figure 1.1: *Effect of the addition of protein CBH2 on hydrolysis of steam-exploded spruce compared to greater quantities of the control mixture.*

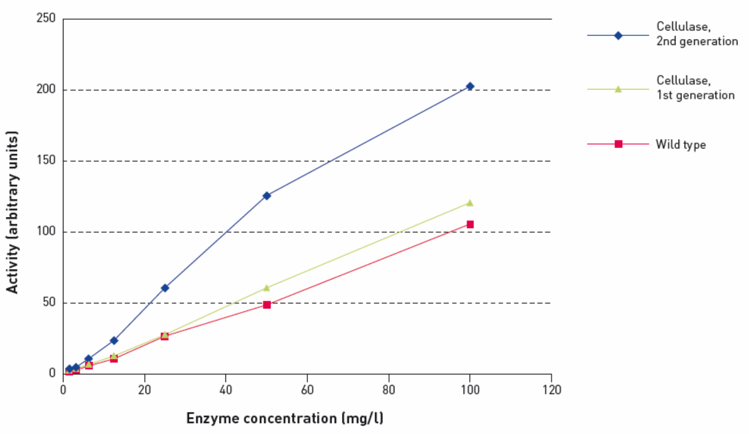


Figure 1.2: *Directed evolution" has led to the discovery of cellulases with twice the activity of wild type cellulases. Hydrolysis with the 2nd generation variant dosed at 100 mg cellulase/l yields the same glucose levels as the wild-type cellulase dosed at 200 mg/l..*

Constructing new bi- and multi-functional enzymes that improve the enzyme efficiency on lignocellulosic substrates.

We also aimed to improve the hydrolysis rate by building new artificial cellulases made up of two smaller enzymes that had the potential to act synergistically (and so faster) than if they each acted independently in two separate reactions. Combining bacterial DNA with fungal DNA, the enzymes NILE developed were "chimeric". Genes that code for the building of chimeric enzymes have been designed for *T. reesei*. Different constructions involving bacterial cohesins and dockerins, and fungal proteins have been designed. The role of the bacterial components (Figure 1.3) was to "glue" the enzymes together in an ordered manner. Attempts were made to induce *T. reesei* to express such enzymes, but this proved difficult: there is a yet unidentified factor that prevents functional expression of the bacterial components in *T. reesei*. Directly fused *T. reesei* cellulases, without any bacterial component have been investigated in the last months of the project with promising results: of the 3 constructs tested, all display the dual activity and one is more efficient than the two enzymes taken alone.

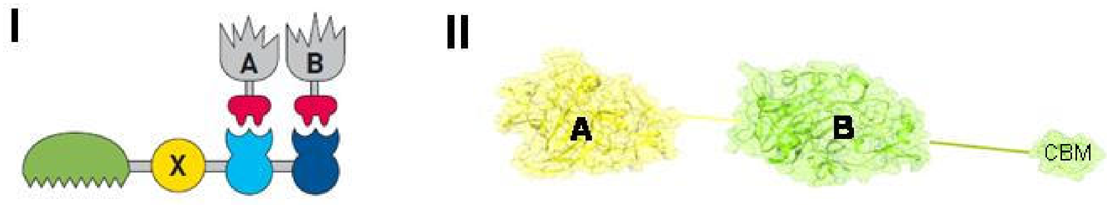


Figure 1.3: *The two strategies used for construction of bifunctional enzymes: (I): bacterial cellulosome approach and (II) direct genetic fusion.*

Improving T. reesei expression system

Using two UV mutagenesis steps and innovative screening methods, we developed four *T. reesei* strains with a capacity to secrete greater quantities of enzymes than a reference strain. These enzymes are as efficient at hydrolysing lignocellulosic materials as those secreted by the reference strain (Figure 1.4).

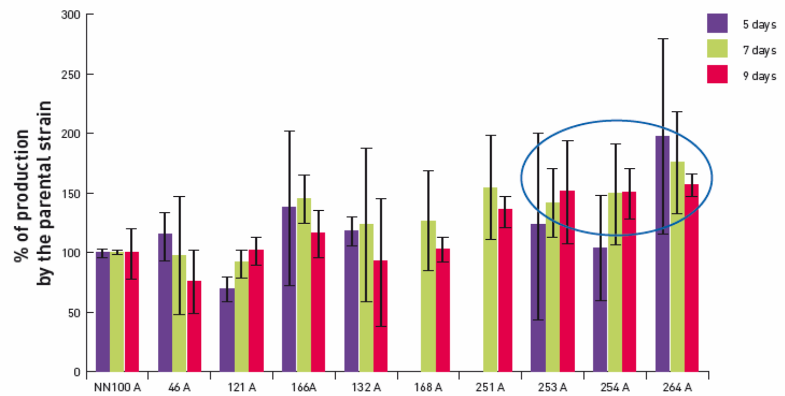


Figure 1.4: *Production of cellulases by nine mutagenized clones of T. reesei at intervals of 5, 7 and 9 days of culture as a percentage of production by wild T. reesei (NN100 A). Strains 253A-264A appear to secrete more enzymes, with production reaching 150% of the wild strain after at least one interval.*

Evaluating enzyme mixtures in hydrolysis of the two NILE substrates and producing the best ones for pilot scale tests

A total of 36 enzyme constructs were expressed or purified in sufficient amounts so that their hydrolysis properties on pretreated wheat straw and spruce could be assessed. Relative proportions of the main, already characterised components of the cocktails were also explored.

To the best of our knowledge based on literature, the work carried out during NILE led to the most exhaustive and systematic exploration of *T. reesei* enzyme catalogue to date. Besides reviewing the *T. reesei* enzymes, we explored the possibility to engineer some of them and tested cellulases from other organisms. Combination of the best enzymes characterised by WP1 allows us predicting a significant decrease in enzymatic loading or increase in glucose yield compared to a reference enzyme mix.

Thanks to the early identification of CBH2 as the limiting step of the hydrolysis reaction (besides the well-known β-glucosidase limitation), the components for three cocktails were produced at a large scale in order to test them at pilot scale in WP3:

- A reference mix, with optimised β-glucosidase content.
- A first enhanced cocktail consisting in the reference mix enriched in wild-type CBH2.
- A second enhanced cocktail consisting in the reference mix enriched in improved variants of CBH2.

Conclusions and impact

Work in WP1 yielded several scientific results with significant impact on the research community: three articles have already been published while one is currently under review and seven others are planned. The work on directed evolution on enzymes CBH2 resulted in one patent filing, while one to three patents are considered regarding bifunctional enzymes. These results may contribute to provide keys for either enzyme or ethanol producers to enhance cellulase cocktails from *Trichoderma*, focus research on promising topics, and decrease cost of lignocellulosic biomass hydrolysis.

We estimate that combination of the best improvements discovered in WP1 could lead to a 2-fold reduction in enzyme loading for enzymatic hydrolysis in the right process conditions. If we include increase and optimisation of the β-glucosidase activity (which is known to be a very critical parameter that can greatly contribute to decreasing cellulase loading[1]), we may predict a 4-fold potential loading reduction compared to commercial cellulases available at the beginning of the project (which had a low beta-glucosidase activity) . The original NILE target was a 5-fold cost reduction. Our conclusion is that we underestimated the difficulty of enhancing hydrolysis reaction beyond β-glucosidase

[1] Ayrinhac et al, submitted for publication in Org. Proc. Res. Dev.

optimisation, and, at least in the case of wheat straw, we dealt with relatively well pretreated substrates on which improvements with helper enzymes could not be easily obtained.
Beyond NILE, WP1 partners will continue collaboration and focus on better characterisation of the enhanced CBH2 enzymes, as well as a better exploration of the potential of bi-functional enzymes.

2. The fermentation of the products of hydrolysis

Part of NILE's work aims at developing yeast strains and fermentation technology that result in improved fermentation of all sugars in lignocellulose hydrolysates ("hydrolysate": the product of hydrolysis). The challenge is that lignocellulose hydrolysates contain several sugars, which *Saccharomyces cerevisiae* (baker's yeast) does not easily and not naturally ferment to ethanol. These include xylose and arabinose. In addition, the hydrolysate also contains a number of compounds, formed during pretreatment and hydrolysis that inhibit the fermentation of the sugars (Figure 2.1).

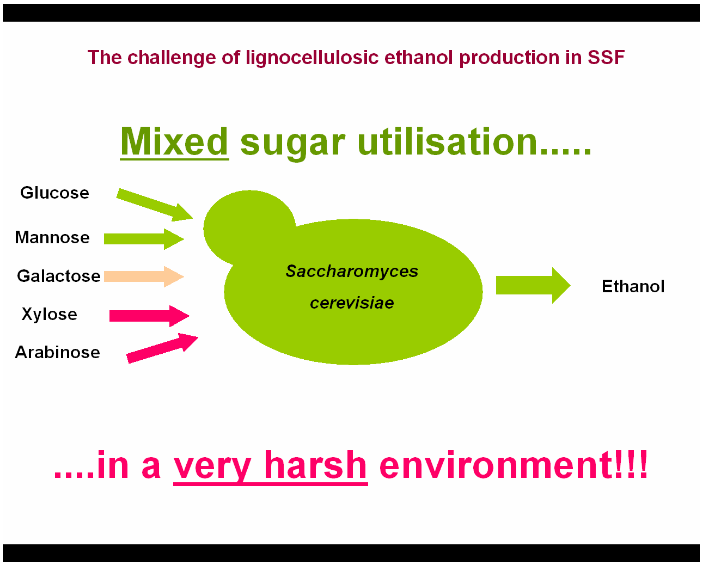


Figure 2.1 *- Fermentation of lignocellulose hydrolysate exposes S. cerevisiae to harsh conditions.*

For fermentation to occur, the pentoses must first be brought into the cell. Channels through the cell membrane ("transporters") enable this to happen. NILE researchers have cloned and expressed different pentose transporters. *Saccharomyces cerevisiae* expressing the xylose transporter GXF1 from *Candida intermedia* and growing in a low concentration of xylose grew more rapidly and reached higher cell density than a control. Figure 2.2 shows the location of the transporters. Another strain of *S. cerevisiae* expressing the AraT transporter from the fungus *Candida arabinofermentans* was found to grow to approximately 10x higher concentration in arabinose than *S. cerevisiae* that lacked the transporter.

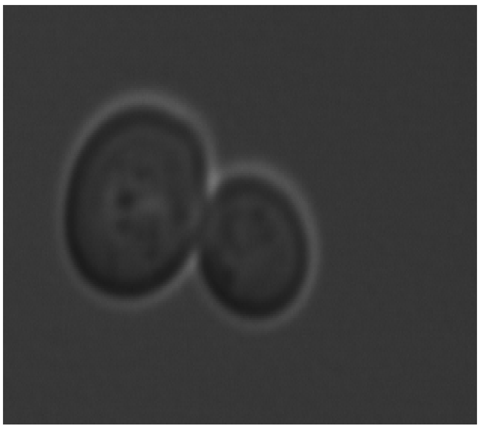
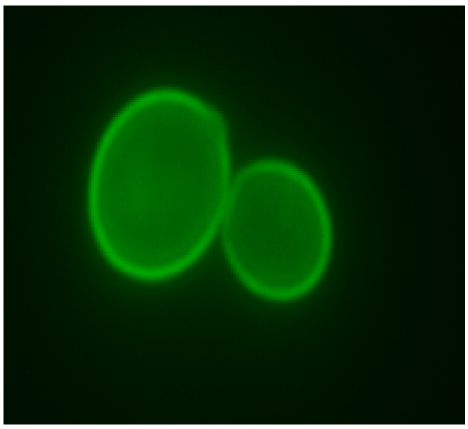



Figure 2.2. *Location of pentose transporter in the membrane of the yeast cell*

Once inside the cell, enzymes work on the pentoses to convert them to ethanol. In NILE codon optimization, *i.e.* using the genetic code of baker's yeast *S. cerevisiae,* was shown to significantly improve ethanolic arabinose fermentation (Figure 2.3).

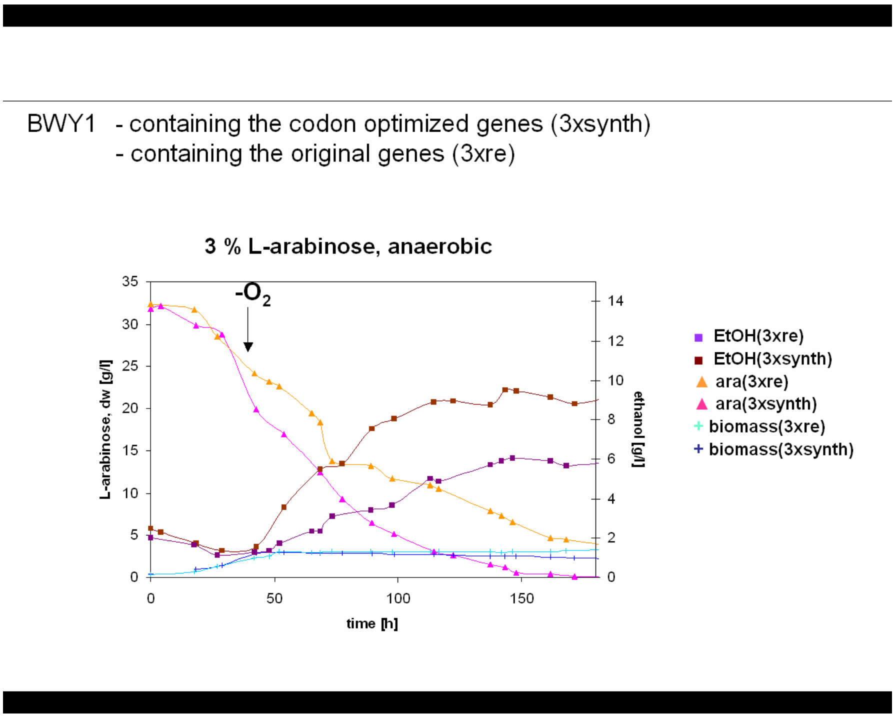


***Figure 2.3** Improved ethanol production with codon optimised genes developed in NILE*

With site-directed mutagenesis, i. e. exchanging an amino acid in an enzyme, NILE researchers created a new enzyme, K270R, that results in quicker xylose fermentation, and almost reached the project's yield target of 0.4 grams ethanol per gram of total sugars, *i.e.* glucose and xylose (Figure 2.4).The theoretical yield is 0.51. Currently operating industrial processes, which ferment only hexoses because no other sugars are present, reach 0.45-0.50.

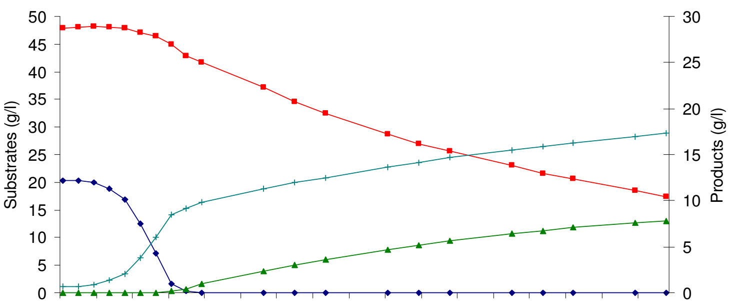


***Figure 2.4a**: Ethanol production from glucose and xylose with S. cerevisiae expressing the natural xylose reductase enzyme.*

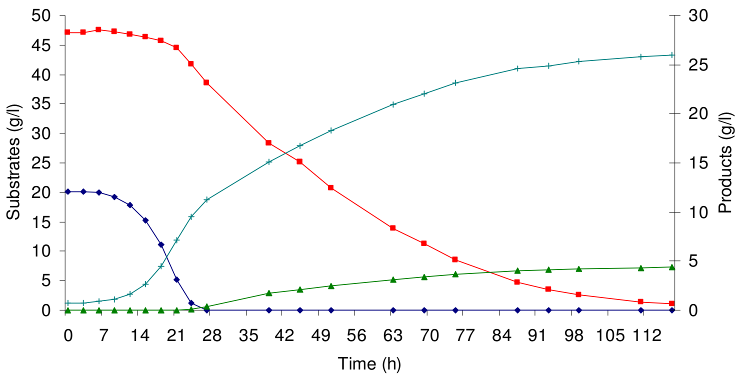


***Figure 2.4b:** S. cerevisiae expressing the NILE-engineered xylose reductase enzyme K270R produces much more ethanol than S. cerevisiae expressing the natural enzyme (Figure 2.4a). Xylose is consumed faster and ethanol is produced faster.*

NILE improved *S. cerevisiae*'s tolerance to inhibitors through "evolutionary adaptation", which was achieved by letting the strain grow in sequential batches in media containing inhibitors. After several hundred generations, clones were isolated from the population and tested for their resistance. They were found to have dramatically shortened lag phases if grown under high furfural concentrations (furfural is an inhibitor. Most importantly, they proved to ferment hydrolysate-containing media faster than the parent strain (Figure 2.5). The reason for the improved growth behaviour of the evolved strain was found to be an over-expression of a specific gene coding for an enzyme that destroys furfural.

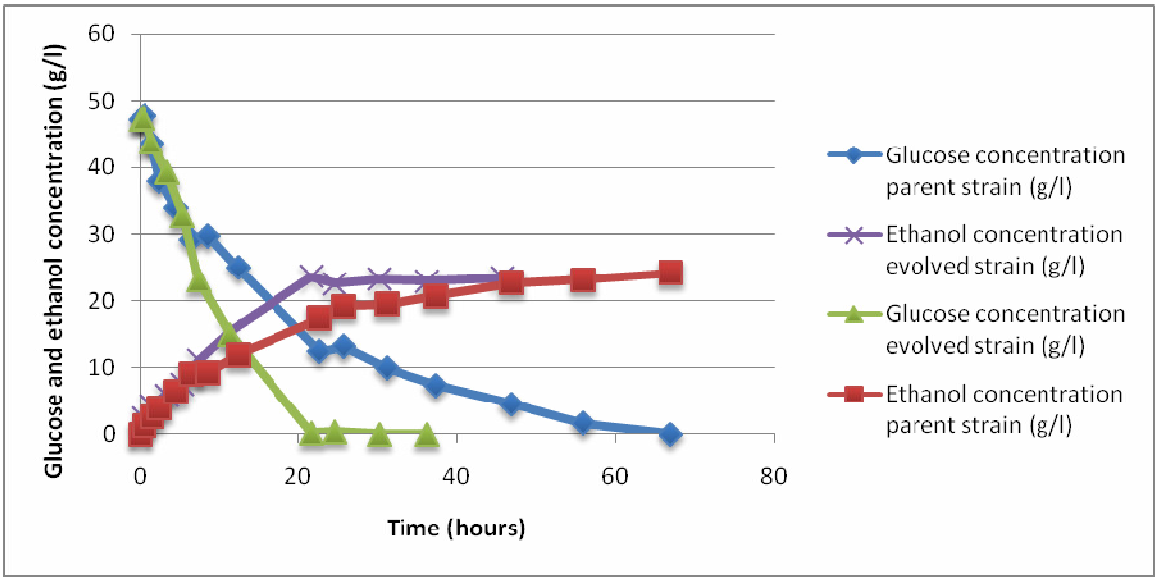


Figure 2.5: *Growth performance of parent and evolved strain in lignocellulosic hydrolysate.under anaerobic conditions. The evolved strain was used in NILE pilot plant trials (see next chapter).*

NILE also developed fermentation strategies for the fermentation of all sugars in non-detoxified lignocellulose hydrolysates. The challenges relate to obtaining a high conversion in the enzymatic hydrolysis, a high conversion of pentose sugars, and obtaining a robustness of the yeast towards inhibitors in the hydrolysates.

The feedstocks in NILE– wheat straw and spruce – represent different challenges. The wheat straw contains more xylose, which is difficult to convert, whereas the spruce material is more inhibitory to the yeast and therefore requires a higher robustness of the yeast strain.
The principal process approach chosen was simultaneous saccharification and fermentation (SSF), *i.e.* a process in which the enzymatic hydrolysis takes place together with the fermentation. Since pentose sugars, primarily xylose, were converted together with the hexoses, the term SSCF (simultaneous saccharification and co-fermentation) is more appropriate.

Significant increases in the overall ethanol yield and the conversion of xylose in SSCF were obtained in NILE, for both wheat straw and spruce feedstock. The ethanol yield typically decreases with increasing WIS content, and yields should therefore always be reported together with the WIS (Water Insoluble Solids) content. The progress in NILE in terms of increase of the overall ethanol yield and xylose conversion is clearly illustrated by Figures 2.6 and 2.7.

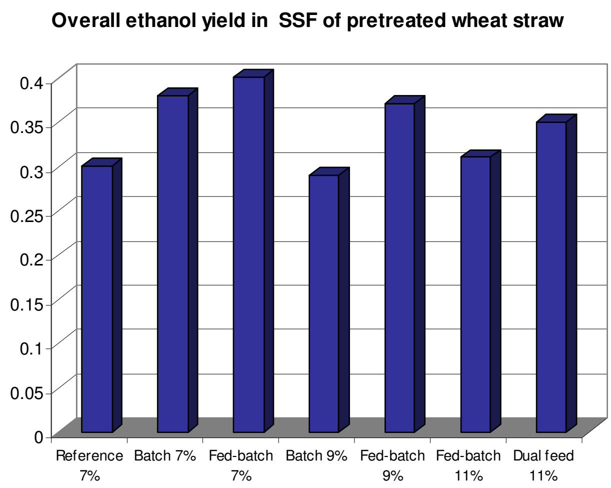


Figure 2.6 *Process improvement in SSF of pretreated wheat straw. The reference experiment represents the ethanol yield using a non-xylose fermenting yeast in a batch SSF using 7%WIS. All other experiments are made with the xylose fermenting yeast TMB3400.*

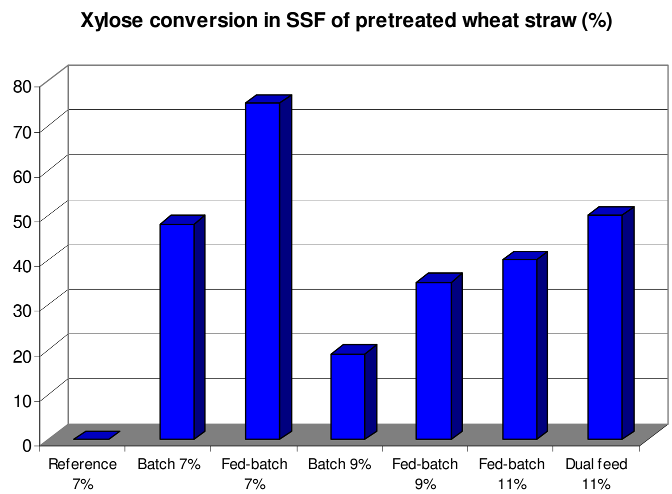


***Figure 2.7**. Xylose conversion in SSF of pretreated wheat straw. The reference experiment represents the ethanol yield using a non-xylose fermenting yeast in a batch SSF using 7%WIS. All other experiments are made with the xylose fermenting yeast TMB3400.*

The basis for the obtained improvements has been:

a) Adaptation of yeast strain towards hydrolysate inhibitors.
This was found highly important for the performance in SSF, and a cultivation protocol for the preparation of the yeast was therefore developed and successfully applied during NILE. Additionally, fed-batch substrate addition was found beneficial for the case of severely pretreated materials.

b) Optimization of the SSF temperature in relation to the yeast strain used.
It was found that lowering the temperature somewhat may be beneficial for the overall process depending on yeast strain, despite a lower rate of enzymatic hydrolysis.

c) Fed-batch addition of substrate
Fed-batch addition of substrate in SSCF was found to improve xylose conversion in the case of the xylose-rich wheat straw material. Fed-batch addition of substrate is furthermore a means of overcoming rheological problems, since the viscosity of the slurry can be kept at low enough values to

allow mixing. This allows a higher final ethanol titer, which is important for the overall process economy.

d) Process optimization giving improved xylose to glucose concentration during SSCF.
A critical factor for efficient co-fermentation in SSF is the ratio between xylose and glucose in the process. In order to optimize this ratio, enzyme feeding strategies were developed, and the principle was proven using pretreated spruce material. This material is particularly challenging since the glucose to xylose ratio is high already in the pretreated slurry. The conversion of xylose in an SSF at 10% WIS was in the best case increased from about 40% conversion to about 80% xylose conversion.

e) Dual feeding in SSCF
A combination of fed-batch addition of substrate and enzyme was found beneficial. This was demonstrated for pretreated wheat straw material. In the case of combining enzyme feed with substrate feed a xylose conversion of about 50% could be reached at 11% WIS. This value should be compared to about 40% with only substrate feeding. A batch process is not even possible due to the high viscosity of the pretreated wheat straw.

At a WIS content of 7%, an ethanol yield of 0.4 g/g was obtained using xylose fermenting yeasts in a fed-batch process. For spruce hydrolysate, a value of 0.4 g/g was reached also at 10% WIS. The target value of the ethanol yield in the fermentation set at the beginning of the project was 0.42 g/g. To reach this value a more complete xylose conversion is needed in terms of pretreated wheat straw, whereas for spruce a more complete hydrolysis is primarily needed.

3. Process technology

***Figure 3.1**: The Örnsköldsvik pilot plant operated by SEKAB E-Technology has used soft wood as raw material in NILE project*

Objective

The overall aim of WP 3 in NILE has been to investigate the full potential of new yeasts and enzymes developed by the NILE partners in WP1 and WP2. Evaluations have been performed both in laboratory scale and at near industrial conditions in two pilot plants using either softwood or wheat straw as raw materials. Verifying yeasts and enzymes at near industrial conditions is key to determine their robustness and possible application in the evolving cellulosic ethanol industry.

The final production cost of the cellulosic ethanol is what finally will make the process viable or not. Finding process bottlenecks and investigate different scenarios of integration are important to reduce the overall costs. In WP 3, process models based on wheat straw and soft wood as raw materials are tools that have been developed for these purposes and will serve as a guide for further experimental work. Finally, combustion and granulation properties of

***Figure 3.2**: The Saf-Isis pretreatment unit*

the lignin rich hydrolysis residues have been evaluated. Due to its energy content, lignin plays an important role in determining the overall economic viability of bioethanol process. Lignin application in non-energy end-uses has also being assessed within WP 3.

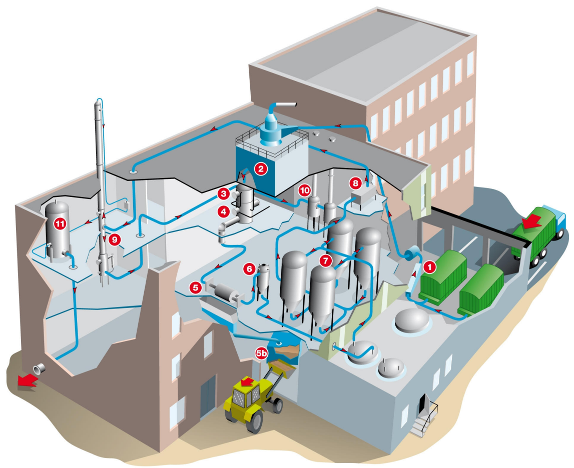


***Figure 3.3**: Cut away diagram of the cellulosic ethanol pilot plant in Örnsköldsvik, Sweden*

Initial results

During the first years of the project, pilot-scale tests with commercially available yeasts and enzymes were performed in the two pilot plants with either softwood or wheat straw as raw materials. In the Örnsköldsvik pilot plant, hydrolysis and fermentation were carried out in separate vessels (Separate Hydrolysis and Fermentation) as well as simultaneously in the same vessel (Simultaneous Saccharification and Fermentation). In the Örnsköldsvik pilot trials, all process steps were included in continuous mode; from intake of raw material to final distillation of ethanol. Pre-treatment conditions that combined good operation with high enzymatic degradability were identified.

In the pilot unit using wheat straw, commercially available enzymes and yeast were evaluated with the separate hydrolysis and fermentation (SHF) process concept. This pilot unit is not continuous, which complicates the transfer of material between the unit operations. The process steps from grinding to fermentation were included in the trials. The end concentration of ethanol reached 4% (v/v), the yields were relatively low mainly due to the pre-treatment conditions used in the large scale reactor. Based on a study comparing slurries from the large scale reactor with slurries from a smaller reactor, it was decided to carry out the coming evaluation of project specific yeast and enzymes with slurry produced in the smaller reactor in laboratory scale.

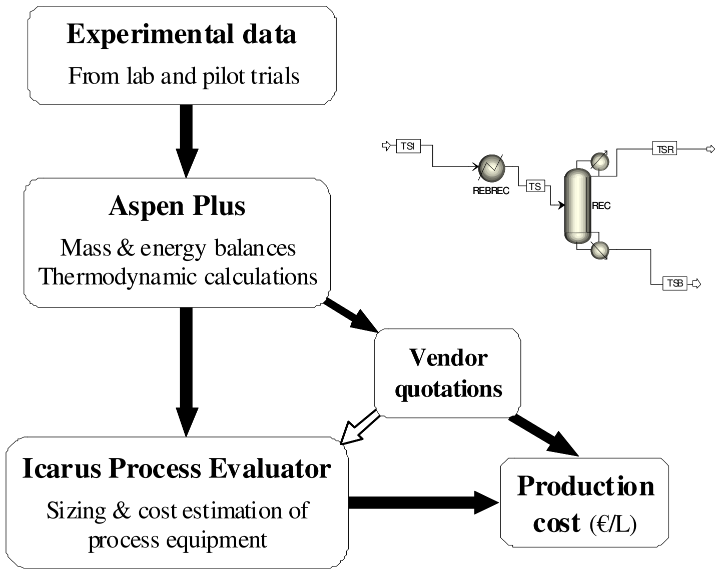


***Figure 3.4:** Schematic procedure for techno-economic assessment*

In the initial pilot plant trials (and lab scale tests) lignin hydrolysis residue, LHR, from both soft wood and wheat straw was produced and distributed to the partners in WP 3 evaluating this co-product. Analyses of the LHR indicated promising results in terms of fuel and granule formation properties. However, the high ash content in wheat straw residue could be a complication, and if not reduced it can cause sintering problems in boilers used for combustion of the LHR.

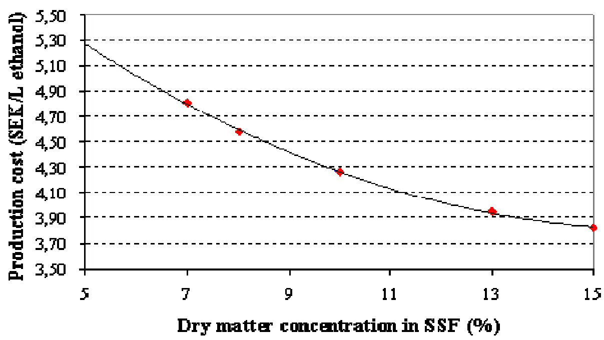


***Figure 3.5**: Ethanol production cost as a function of the dry matter concentration in simultaneous saccharification and fermentation.*

Ethanol production from biomass has not yet been demonstrated on commercial scale. Studies on the economic viability must therefore be based on data from lab scale or in best case pilot or demo scale units. It is important to perform technical economic evaluation based on data for the actual process design, taking into consideration all process steps, since changing the conditions in one step will affect other parts of the process. In order to do that, a commercial flowsheeting program, Aspen Plus, was utilized to perform detailed material and energy balances. For cost estimations ICARUS IPE was used in addition to Aspen. In the first year of the project preliminary models for both softwood and wheat straw were developed. The models were based on process data obtained in the NILE project, and from vendor quotations. One example of a study carried out in the first period of the project is the influence of the dry matter content on the final ethanol production cost for the simultaneous saccharification and fermentation process with steam pretreated spruce (Figure 3.5). The use of a flow sheeting program makes it possible to perform sensitivity analysis, e.g. how the conditions in one process step or how integration with other systems, e.g. as heat and power plant, affect the whole process in terms of energy efficiency and production cost.

Final Results

A series of key experiments in the NILE project took place in the Örnsköldsvik pilot plant between March and June 2009. Two different NILE developed enzymes and one co-fermenting yeast strain were evaluated. Moreover, a three week stability test was also carried out. During this period, the plant was kept in continuous operation with locked pretreatment parameters to verify and improve the internal operation of the plant. During the stability test, commercial yeast and enzymes were used.

From a logistic point of view, the trials were successful and during the stability test, a new record of continuous operation was achieved, 21 days. Prior to the NILE trials the yeast cultivation unit had been improved to reduce infections during the cultivation of yeast. The pilot plant had also been prepared for implementing genetically modified yeast. These efforts resulted in a successful cultivation of a co-fermenting yeast strain developed in WP2. The co-fermenting yeast strain grew at similar rate as ordinary commercial baker's yeast. When used in a following SSF batch the yeast reduced the xylose concentration. The pentose consumption could not be correlated to an increased ethanol yield, due to the low level of pentoses available in spruce wood.

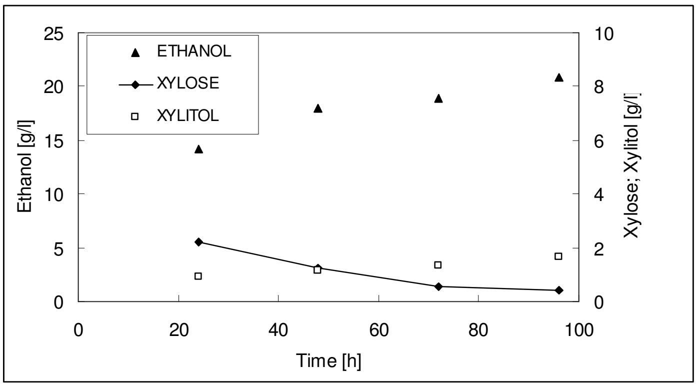


Figure 3.6*: Ethanol-, xylose- and xylitiol concentrations in one of the SSF-tests in the Örnsköldsvik pilot plant trials*

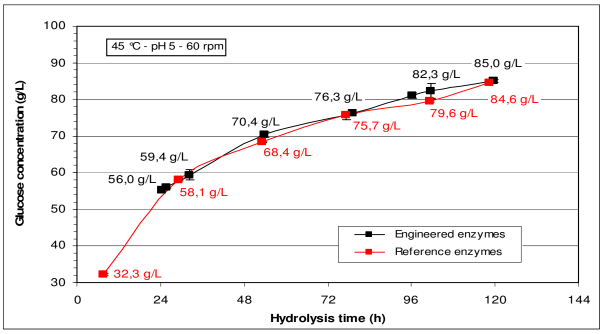


Figure 3.7: *20%WIS hydrolysis (800g DW, 2L capacity reactor), with Reference and "2D-CBH2" enriched mix, at 5 mg/gDM (eq. 10 mg/g cellulose) on wheat straw*

The NILE developed enzyme mixes were found to hydrolyse the pretreated spruce chips equally as efficiently as today commercial enzymes. By further adapting the pretreament conditions in the pilot plant to the enzymes and vice versa, the prospect for higher lignocellulose conversion is good. The potential for improvement was demonstrated in the autumn 2009 in pilot trials carried out outside the NILE framework.

Evaluation of NILE yeast and enzymes on wheat straw were carried out in laboratory scale. The pretreatment was carried out in a small scale reactor and the hydrolysis and fermentation experiments in lab scale. The pentose rich liquid fraction was separated from the solid and the NILE enzymes were added to the solids at a 20 % solid concentration. A commercial enzyme was used as a reference. After 70 h of hydrolysis, no difference was detected between the NILE enzymes and the commercial reference. The overall conversion yield was 70 %. In the fermentation, a significant increase in ethanol yield was achieved with the co-fermenting yeast strain in comparison with a reference strain. About 50 % of available xylose was consumed.

Lignin hydrolysis residue, LHR, produced from both hydrolysis of softwood and wheat straw was transformed to pellets in a pellet mill. The bulk densities and mechanical properties of these pellets met the standards of commercial solid biofuel pellets. Softwood-derived LHRs have a higher energy density than pellets of pure softwood and they also have a higher peak of heat release rate. Straw-derived LHR is less energy dense than softwood derived LHR, the peak of heat release rate is close to that of pelletised softwood. Even though nitrogen and sulphur levels in the LHR granules met the technical standards for chemically treated biomass (CEN/TC335), they formed more NO_x and SO_2 during combustion than softwood granules. Supplementary measures to reduce emissions should therefore be taken when designing combustion systems for LHR pellets.

Figure 3.8*: Softwood –derived LHR pellets*

The composition and performance of purified lignins produced from the NILE LHR were compared to commercially available lignins. It was found that the alkali- and ethanol soluble fractions are quite similar to soda lignins from straw and kraft lignins from softwood respectively. They have the potential to be utilised as sustainable alternatives to phenol and phenolic resin, laminates, foundry sands and as dispersants etc. A process model with focus on the lignin separation has also shown that lignin separation could be economically viable at lignin prices above 250-350 Euro/ton lignin. However, more experimental work is needed to confirm these results.

The process models for both wheat straw and soft wood were updated with experimental results from 2009. In the straw model, the effect of decreased enzyme load achieved in WP 1 gave rise to a reduced production cost of ethanol of 30%. On site production of enzymes was also simulated and that process solution reduced the production cost of ethanol with 6%. Other studies carried out were comparisons between pellet production and generation of electricity from lignin residue, sensitivity analysis of the effect of plant size on production cost. Finally, pentose fermentation was included in one of the cases modelled for wheat straw.

Figure 3.9*: NILE ethanol from the Örnsköldsivk pilot plant*

For the work carried out with the soft wood model the focus was on the utilization of the residue streams and on-site enzyme production, in order to study their impact on the overall process economy. The calculated production costs of ethanol varied between 4.00 and 5.50 Swedish kronor per litre (0.38-0.50 €/L) according to the stillage processing. This production cost is highly dependent on the assumptions made and, like the straw model, the softwood model was mainly used for comparison of different process configurations and for sensitivity analyses. It was shown that replacing final evaporation of the stillage stream with a biological waste water system with biogas production resulted in both an improved overall energy efficiency and a lower production cost of ethanol. The implementation of district heating enables utilization of low energy residual heat from the process, which improves the energy efficiency and lowers the ethanol production cost. However, the need for district heating limits the options for plant locations since there must be a demand for heat. The scenario with on-site enzyme production resulted in slightly lower minimum ethanol selling price compared to the scenario with purchased enzymes.

4. Socio-economic and environmental impacts and development strategy

Objectives

The socio-economic and environmental impacts and development strategy work package examined the likely economic and environmental performance of cellulosic ethanol production. To do this, work was undertaken in five themes: supply-chain cost modelling, lifecycle analysis, an evaluation of stakeholders and barriers to development, an assessment of the path to market, and a case study on the attractiveness of incorporating the production of lignocellulosic ethanol into an existing paper mill. A summary of each of these themes is presented below.

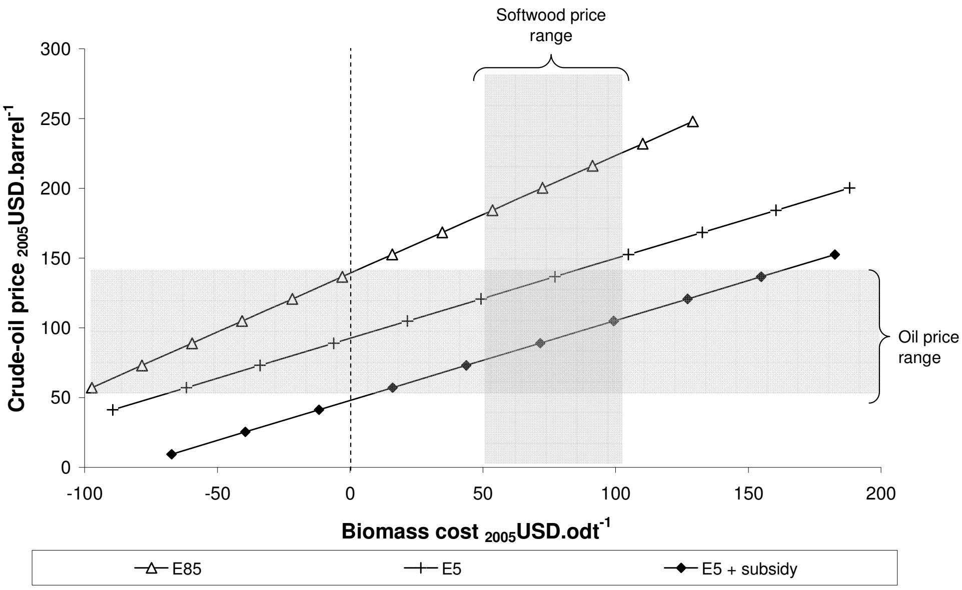
Supply-chain cost modelling

The supply-chain cost modeling was led by Imperial College who developed a spreadsheet based tool incorporating macro driven sensitivity analysis to compare supply-chains. The inputs to the model were two fold: firstly, descriptions of the conversion plant and process (mass balance, capital cost, plant capacity and learning rate); and secondly, descriptions of the supply chain context (feedstock prices, ethanol value, finance package). Two measures of supply-chain cost performance were used: *Net Present Value* (NPV) and the *Levelised Cost per Litre*; both were calculated from a discounted cash flow analysis of supply-chain costs and revenues.

Results from the modelled case-studies identified that the relationship between the cost of feedstocks and the value obtained for ethanol is particularly important to determining commercial viability. This relationship is shown explicitly in the figures below for an illustrative base-case supply-chain configuration in which softwood is converted into ethanol using an enzymatic hydrolysis process. A single point one of the lines corresponds to the oil and biomass prices at which this particular supply-chain would breakeven (i.e. NPV=0); typical oil and biomass prices range are indicated by the shaded areas. For the example shown it can be seen that the E5 and *E5 + subsidy* chains break even at moderate oil and biomass prices, but that the E85 chain would require either very low feedstock prices or very high oil prices. The three lines on the figure correspond to three ethanol pricing methods: *E85* – assumes that the ethanol is valued on the basis of its energy content; *E5* – assumes that ethanol is valued on the basis of the volume of gasoline displaced; *E5 + subsidy* – assumes the ethanol receives an additional subsidy relative to gasoline.

The results of the modelling suggested that ethanol produced from softwood could be cost competitive with gasoline, but that production from straw would generally be less competitive, owing to the smaller proportion of easily fermentable pentose sugars. For the same reason, the benefit of introducing pentose fermentation would be greatest for the straw processes. The commercial attractiveness, however, is by no means certain. The most important factors affecting commercial viability are the cost of feedstocks – primarily determined by location and existing markets – and the value obtained for ethanol – primarily determined by the oil price and policy incentives. Both of these factors are highly uncertain. It is also apparent that supply-chain design will play a vital role in determining whether lignocellulosic ethanol production is commercially viable. The importance of feedstock supply highlights the need for location specific assessments of feedstock availability and price. Similarly, the role of subsidies and policy incentives in creating and sustaining the ethanol market highlights the importance of political engagement, and the need to include political risks in investment appraisal

***Figure 4.1**: Breakeven oil and feedstock prices for base-case softwood chains (Spruce-EH(Np)-C(25)*



Life cycle assessment

Work to examine the life-cycle impacts of cellulosic ethanol production was led by ENI. The aim was to assess the environmental impacts of ethanol produced from softwood or straw and blended with gasoline, and to identify the most favourable environmental scenario. Additional work was undertaken to compare earlier Life Cycle Assessment (LCA) models with the methodologies outlined in the European Renewable Energy Sources Directive: 8/20097 CE.

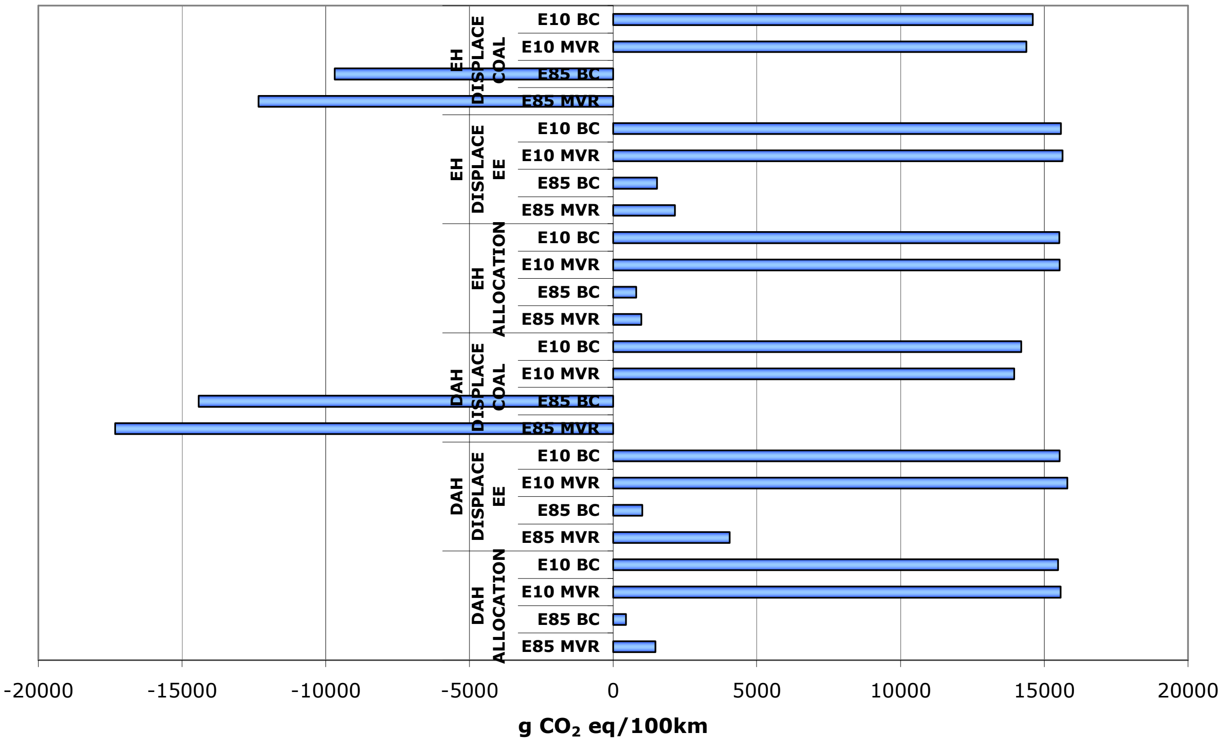
The LCA model described the whole biofuel chain across the complete life cycle. The functional unit (FU) assumed was 100 kilometres driven by a Euro 4 vehicle. Two impact categories were considered: the greenhouse effect and the depletion of non-renewable resources, respectively in terms of grams of CO_2 equivalent and kilograms of Sb equivalent. Once developed, the model was used to calculate the effect of the following variables:

- percentage ethanol blend (E10, E85)
- process technology (SSF, SHF, dilute acid hydrolysis)
- by-product allocation method
- technology for energy recovery (use of process vapour as such or after its mechanical vapour recompression (MVR))

In this way, over 24 chains were defined and compared. The figure below shows illustrative results for the greenhouse gas emissions from ethanol production from wood in Sweden.

In the later stage of the project the LCA studies carried out within the project were compared with the evaluations reported in annex V of the 28/20097 CE RES directive and with the studies published by CONCAWE/EUCAR.

Figure 4.2: LCA study located in Sweden, results: greenhouse effect



Evaluation of stakeholders and barriers to development

The work to examine the role of different stakeholders and potential barriers to development was undertaken by BAFF, E-Tech and SEKAB B&C.

<u>*Report on Barriers*</u>

Ethanol is a new fuel on the European market (Sweden excluded), therefore there are numerous of areas where problems, difficulties or misunderstandings occur which have been referred to as *barriers to the market introduction of cellulosic ethanol*. Awareness of these barriers is key to enabling the market introduction of ethanol happen on a broad scale, in Europe and worldwide. This study focused principally on the non technical barriers to introducing ethanol. Areas such as political impediments, market breakthrough barriers, legislation, regulations and standards, awareness and education and practical problems have been all considered. The report is based on interviews with key people from the different organisations and projects involved in both the Swedish ethanol market and the development of ethanol production technologies. The barriers identified are as follows:

- *Political impediments:* The biofuel market is defined by political decisions, which means that the initiative is really in the hands of the EU and the national governments of the member states. When scaling up of the cellulosic ethanol technology to a commercial scale the financing situation is also much more complex and challenging than for previous development phases.
- M*arket breakthrough barriers:* Market breakthrough in the EU requires: suitable vehicles of different models, refuelling infrastructure, and that the price of the ethanol fuels must be competitive to the traditional oil based fuels. All of this has been the case in Sweden for some years now, which is a great example of the large scale possibilities of ethanol as a way towards sustainable transports. Evidence from Sweden in 2008 when the E85 market diminished by 70% two months after a price shift, shows that price is the main factor that

motivates the customers in the end. Legislation and incentives must therefore be installed if the EU want sustainable biofuels.

- *Regulations, classifications and standards:* The basic problem is that almost all legislation is based on ethanol being an intoxicant. The rules for import, handling and sales are adapted to ethanol for industrial use but demand multiple permits from different governmental authorities, this creates barriers to trade. One solution would be to classify the ethanol different depending on its intended use, but several legislation areas would have to be reformed and this is a long process. Demands and criteria on biofuel sustainability may also create a barrier to trade. There is a need of a global system if we want to have a more global market of biofuels.
- *Awareness and education:* Ethanol is the target of intensive lobbying, as many groups involved have opposing interests. It's important to bring this conflict into daylight, as it affects the media, the debate and the public opinion.

<u>Report of Stakeholders</u>

This report is a compilation and description of stakeholders that BioAlcohol Fuel Foundation, BAFF and SEKAB have identified. The report is not restricted to specific regions or technologies and includes different types of organisations from research institutes to private companies. Information on each stakeholder includes: country of origin, website, technology and plans to scale up the technology to industrial scale. A total of 122 stakeholders were identified between month 25(October 2007) to month 46 (July 2009).

High level conclusions that can be drawn from the stakeholders list include:

- 77 of the 122 companies in this list are based in the USA, 21 in Europe (particularly in Denmark, UK and the Netherlands) and 12 in Asia (including Australia and NewZealand).
- Many projects in USA supported under the DOE programs launched in 2007-08 are in the planning, negotiation, financing and construction phase.
- All over the world, but particularly in China and Japan, pilot plants have been built for R&D. The results obtained, however, have not been published. Hence, it is very difficult to know and evaluate how far they have come and how close to a viable industrial process they are.

The path to market for cellulosic ethanol and contribution to EU policy objectives

Work on this task was led by Imperial College and considered three interrelated questions: the path to market for cellulosic ethanol, the contribution of policy to the successful development of the technology, and the potential contribution of cellulosic ethanol to discrete policy objectives. From this work the following recommendations for policy makers were proposed.

- *Targeted support for research and development should be increased.* Europe is currently perceived as far less attractive a destination for the early stage development of biofuels projects than the US.
- *Technologies that are candidates for policy support should be prioritised on the basis of efficient resource and energy use.*
- *Existing market support should be maintained.*
- *The "cost per tonne of carbon saved" metric should be used with caution to compare the merits of dissimilar technologies.* This metric is frequently used to compare technology options for the purpose of policy formation but is sensitive to changes in relative price. There is an implicit assumption in the use of this metric that relative prices remain static and this deserves to be questioned. Currently, gasoline is valued at a premium to biomass and coal that reflects its greater versatility as a fuel, but what is less certain is how this premium might change if supply constraints were to increase.
- *Investigate how policy uncertainty can be mitigated as an investment risk.* EU policy towards biofuels is predicated on the benefits that increased biofuel use will provide. There are significant areas of uncertainty, and, taking the ongoing debate around land-use-change as an example, there is little prospect that this uncertainty will be resolved. Keeping policy under

constant review is a pragmatic response but acts as a disincentive to investment and increases the cost of the investments that are made.
- *Address consequential impacts at the policy rather than project level.* Consequential impacts such as land-use-change are uncertain but are not necessarily affected by the choice of conversion technology. It therefore makes sense to address these at the policy level, including some form of periodic and systematic impact assessment.

Commercialising lignocellulosic ethanol requires progress from existing small-scale demonstration plant to large industrial installations. Interviews with companies and other stakeholders revealed the following best practice guidelines for demonstration and scale-up:
- Prioritise access to feedstocks
- Be aware of the logistics needed to handle raw material and by-products
- Develop the technology with a full scale plant in mind
- Be conservative with scale-up, ensure that lessons are learnt at small scale and employed at large scale
- Value hands on experience as source of competitive advantage
- Integrate demonstration projects with other facilities to reduce the cost and lower the risk
- Pursue strategic partnerships, to pool knowledge, mitigate risk, but also to demonstrate credibility to investors and future customers who might be wary of working with a small company
- Apply reasonable measures to get public funding, including choosing the most favourable policy regime

The attractiveness of incorporating the production of lignocellulosic ethanol into an existing paper mill: a case study by Mondi Business Paper.

Mondi's case-study focused on a pulp mill in Austria. It described the raw material supply chain, evaluating how it may be affected by increased demand for raw materials, and assessed the commercial attractiveness of lignocellulosic ethanol from the perspective of a paper industry incumbent. Conclusions from the study included the following points:
- A significant increase of wood consumption for energetic use has been forecast for Austria until 2020. According to all studies a high demand in sawn-wood will always be a pre-condition for a high energy wood mobilization. Because of the very limited price elasticity it is likely that a shortage of wood will occur resulting in significantly higher wood costs than in the past.
- Energy consumption from harvesting wood only represents 1.1 – 5.6 % of the energy content of the fresh wood. Energy consumption and emissions from transports can be significant, if long transport distances on road would be assumed, if however transport by railway is possible they not necessarily are of a critical magnitude.
- Under a scenario of limited raw-material availability, bioethanol production based on the same raw-material as pulp and paper production, might not be the most attractive type of biofuel generation to be combined with pulp and paper industry. Yet, other types of biofuel production, such as pre-extraction of chips for hemicelluloses, gasification of residual biomass or black liquor, enzymatic treatment of chips or pulp, which make use of by-products of the pulp and paper process, might offer more synergies.
- As long as the growth rate for paper consumption is at a level, which allows to further increase production, and margins are attractive in comparison with biofuels production, it can be assumed, that investments in additional paper production capacity will have a higher focus for the industry. This is especially relevant if bioethanol production is based on the same raw-material as paper production and does not make use of by-products of the raw-material supply chain or the pulp and paper production process

5. Evaluation of lignocellulosic ethanol for automotive applications

Introduction about the use of bioethanol as automotive fuel

The definition of the future scenario of EU fuels distribution with a target scenario at 2020 and 2030 is a dynamic task influenced by several factors, technical, economical, social and political ones. It is a matter of fact that oil based fuels will play an important role in the incoming decades, while the electrification process of the vehicle will progressively develop through the first stop&start system moving to mild-hybrid and going up to full-hybrid plug-in vehicles.
At the same time, EU priorities on reducing dependency from oil import, on reducing global carbon footprint of transportation sector and in enhancing economy based on local energy resources have pushed during these last years the definition of several recommendations/actions to promote the production and use of alternative fuels, both for transportation and energy sector.
Starting from the EU Directive 2003/30/CE of 08.05.2003 which included a progressive introduction of biofuels in the Member States up to an energy equivalent amount by 5.75% at the 2010 time horizon, several publications have been followed in order to indicate the right practice to move towards a less oil dependent scenario. These have been implemented mainly in the two following papers: the “Biomass action plan” (COM(2005) n°628 of 07.12.2005) and the “EU strategy for biofuels” (COM(2006) n°34 of 08.02.2006), also followed by “Biofuels in the EU – a vision for 2030 and beyond” (ref. EUR22066 of 14.03.2006) where general strategies to support the implementation of biofuels (up to 25% in 2030) are given clearly indicating the benefit coming from the contribution represented by biofuels.

In December 2008 European Parliament seals climate change package which set for the European Union climate targets by 2020: a 20% reduction in greenhouse gas emission, a 20% improvement in energy efficiency and a 20% share for renewables in the European Union energy mix. Moreover the European Parliament and the Council in April 2009 established Directive 2009/30 to lay down the result of climate package that must be achieved by every Member State; National authorities have to adapt their laws to meet the Directive goals, but are free to define the way their own action plan.
The Directive is focusing on improving energy efficiency and promoting technological development for production of energy from renewable sources and as regards the specification of petrol, diesel and gas-oil and introducing a mechanism to monitor and reduce greenhouse gas emissions.
In addition of the general targets, in the European Directive is provided sector reduction: for transportation one, the request is a 10 % target for energy from renewable sources. It would be appropriate and achievable objectives, and such a framework that includes mandatory targets should also provide the business of European Community with the long-term stability to make rational, sustainable investments in the renewable energy sector which are capable of reducing dependence on imported fossil fuels and boosting the use of new energy technologies.
Moreover the need for energy efficiency in transport sector is imperative because a mandatory percentage target for energy from renewable sources is likely to become increasingly difficult to achieve sustainably if overall demand for energy for transport continues to rise.

Thanks to the role that Internal Combustion Engines will continue to play in the next decades, the benefit due to GHG reduction associated to the production/distribution pathway (the so called “Well to Tank” contribution) of 2nd generation biofuels will be completed by the contribution coming from the evolution of vehicle powertrains. With regard to this aspect, spark ignited engines (normally used with bioethanol) will provide in the incoming period the most important contribution to CO2 emission reduction thanks to their technological evolution: in fact, most of car manufacturers are developing new engine platform with lower cylinder displacement (the so called “downsizing”) to reduce losses of energy due to internal frictions, and coupled with turbocharger to enhance the availability of engine torque at lower revolution speed. This configuration, combined with the adaptation of the engine transmission gear box, enable the engine to run at lower speeds and with higher thermal efficiencies. In addition to this new approach, a second significant contribution will be represented by the increasing use of Variable Valve Actuation system that, avoiding the use of the throttle valve on the spark ignited engine, will drastically reduce the energy spent to transfer the intake charge into the engine and the exhaust out of the engine. Globally the expected reduction in CO_2 emissions is close to 20% compared to a standard gasoline naturally aspirated engine.

In this context bioethanol is an extremely interesting fuel which, thanks to its properties, especially in terms of knocking resistance and high latent heat of vaporization, perfectly meets the requirement of such a kind of new engine platforms. So, the use of high bioethanol blends, such as E85, is beneficial from the point of view of CO_2 tailpipe emissions (the so called "Tank to Wheel" side) both for the contribution (-3,5% compared to gasoline) coming from its chemical composition and from the potential optimization of the engine compression ratio to achieve an additional increase in thermal efficiency that, compared to gasoline, can result in a contribution by 2-3 points.

A wide availability of E85 based on sustainable 2nd generation processes could promote, also in EU, a progressive diffusion of the so called FFVs (Flex Fuel Vehicles) that have been developed during the last decade in Brazil, where the wide availability of sugar cane cultivations together with the political actions have created the conditions for sustainability even on 1st generation process. Research & development activities needed to support bioethanol production processes based on lignocellulosic biomass are, in this context, a mandatory step to let industries decide for investments in such a sector.

In the meantime, a wider use of bioethanol is also possible via its direct incorporation in the gasoline fuel resulting in low ethanol content blend: current EN 228 gasoline specification allows the direct incorporation of oxygenated compounds up to 2.7% O_2 by weight in the fuel, resulting in a 5% by volume ethanol content or a 15% ETBE (Ethyl Tertiary Butyl Ether) composed by 47% by weight from ethanol. This standard will be modified by 2014 in order to increase the maximum oxygen content to 3.5% allowing the incorporation of 10% by volume bioethanol in the fuel without any specific label at the pump. For this reason, most of the vehicles now in production in EU are not only compatible with E5 but also with E10, a fuel that requires just few modifications to the engine/vehicle components. With this approach, of course, most of the global effect in GHG reduction is in charge of the Well to Tank balance, but the most important aspect is the leverage effect that can be exploited on a large number of standard gasoline vehicles that are already and will be available on the roads.

Description of the experimental activities
Taking into account the specific issues related to the use of both low and high ethanol content blends**,** the experimental activity has been split between IFP and CRF in order to cover both the aspects related to the impact of the blends on engine parts (deposit formation, fouling, etc.) and those related to the bioethanol potential in terms of fuel conversion efficiency, pollutants formation reduction and engine performance.
At the end of the project performance and emissions of the vehicle have been measured using 1st generation and 2nd generation process bioethanol in order to demonstrate that engine calibration could not change between the two fuels.

Current production and experience, mainly focused on Brazilian market, relies on conventional naturally aspirated engines where the main technical progress during this last decade has been represented by the development of the so called "flex fuel" control systems. These systems have been conceived to recognize the ethanol content in the blend (going from 20% by volume up to 100%) and to automatically adapt the engine control parameters (fuel metering and spark advance regulation). In this way fuel combustion is always optimized but the base architecture of the engine is the original one designed for gasoline operations.

The interest in NILE activities is to evaluate bioethanol with regard to turbocharged / downsized engines as, under these configurations, the optimisation of the engine compression ratio could be done based on bioethanol properties and, when operating with gasoline, knocking can be avoided reducing the boosting pressure at the intake. This means to be in measure to take advantage of the high octane number and high latent heat of vaporization of bioethanol introducing a new generation of engines really tuned to provide higher thermal efficiency when running on high ethanol blends.

Tests at IFP have been focused on the evaluation of the fouling phenomena: for this purpose a normalised test has been done both with standard gasoline as reference fuel and with bioethanol blends, E10 and E85. The fouling cycle is based on the repetition of a single, simple cycle; after running-in (new engine only) and checking, the engine is operated for a period of 60 hours under cyclic conditions, simulating stop-go operation, with the inlet valves pegged to prevent rotation. The ability of a fuel formulation to influence deposit formation on the inlet valves is determined by measuring the mass of the deposit on the engine parts.

Activities at CRF have been devoted to the evaluation of bioethanol on a downsized turbocharged engine that has been adapted to the scope of the project by modifying some engine components in order to ensure their material compatibility to ethanol. Engine has been calibrated at the engine test bench and after installed into a prototype FIAT Grande Punto vehicle to perform the assessment of bioethanol blends from the point of view of performance, driveability, fuel economy and emissions both on the NEDC driving cycle and under real use conditions.
Tests have been also completed using two FFV vehicles, a FORD Focus and a FIAT Grande Punto currently available on the Brazilian market, in order to evaluate bioethanol behaviour also on current production vehicles and to better understand any potential influence of the bioethanol production processes on combustion behaviour.

The ethanol used in the fuel mixture was produced by SEKAB and was based on lignocellulose material derived from sulphite pulp mill.

Results and discussion

Measurements on engine component deposits have given interesting results showing that formation of deposits on the cold part of the engine such as intake valves could be sensitive to the latent heat of vaporisation of the blend and to the fuel composition.
Using E85 gasoline heavy hydrocarbons are replaced by alcohol and the overall deposit mass shows a significant reduction; with E10, on the contrary, the increase of the blend vapour pressure induces a more difficult evaporation of heavier hydrocarbons that condensate on the cold intake valve (see Figure 5.1).

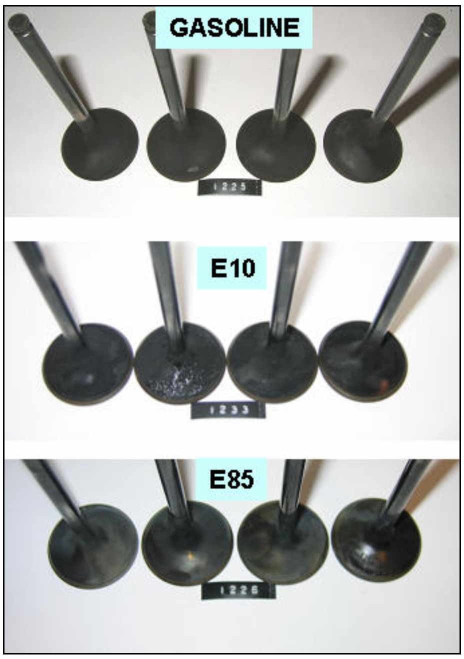


***Figure 5.1**: Deposits on the intake valves*

The behaviour of the "low" blend E10 has pointed out the importance of controlling the vapour pressure of the final blend: actually the E10 obtained with a splash blending operation (diluting E85 with standard gasoline) has probably lead to a higher volatility that has caused an increase of deposits, coming from the heavy aromatic side of the E10 blend, on the "cold" intake valves, enhanced also by the more important cooling effect of the ethanol fraction evaporating.
On the other side, deposits on hottest parts such as piston tops and cylinder head show a linear reduction with ethanol content increase.
Globally, tests have shown a significant benefit coming from ethanol incorporation in reducing deposits on engine parts, illustrated in Figure 5.2, where the pictures of the cylinder head after the reference test and the measures of the deposit are represented.

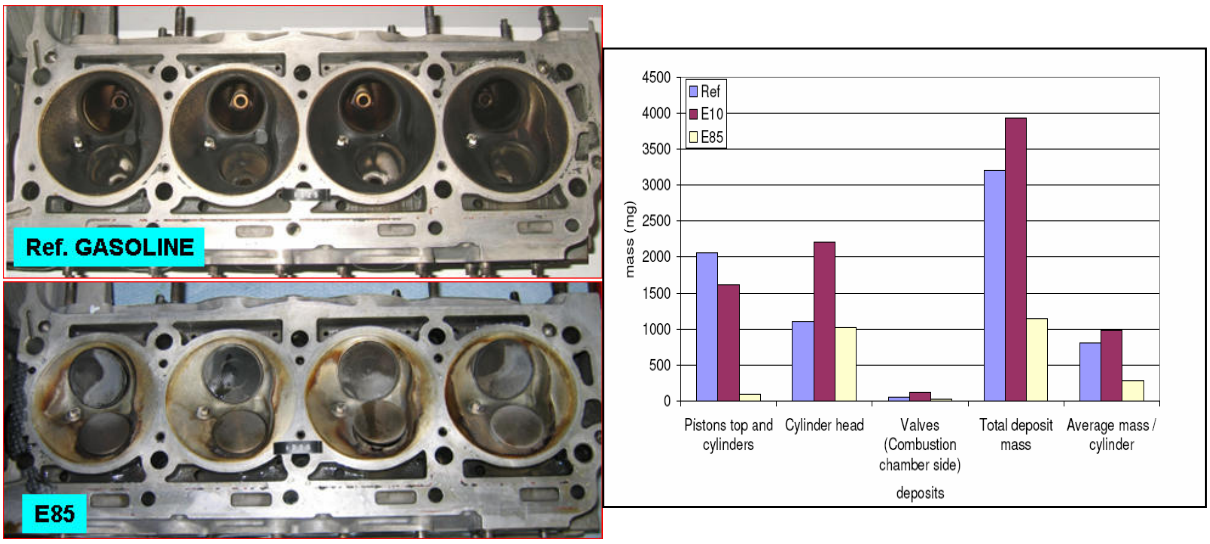


***Figure 5.2**: Comparison of deposits on test engine*

This behaviour is mainly due to the chemical composition of the fuel, ethanol being completely aromatic free and having a cleaning effect due to its highly polar molecules.

Tests at CRF have been carried out on a prototype version of the 1.4 turbocharged gasoline engine (see Figure 5.3) that has been adapted in order to guarantee the right dimensioning of the injection system components and the compatibility to "high" ethanol blends, such as E85.

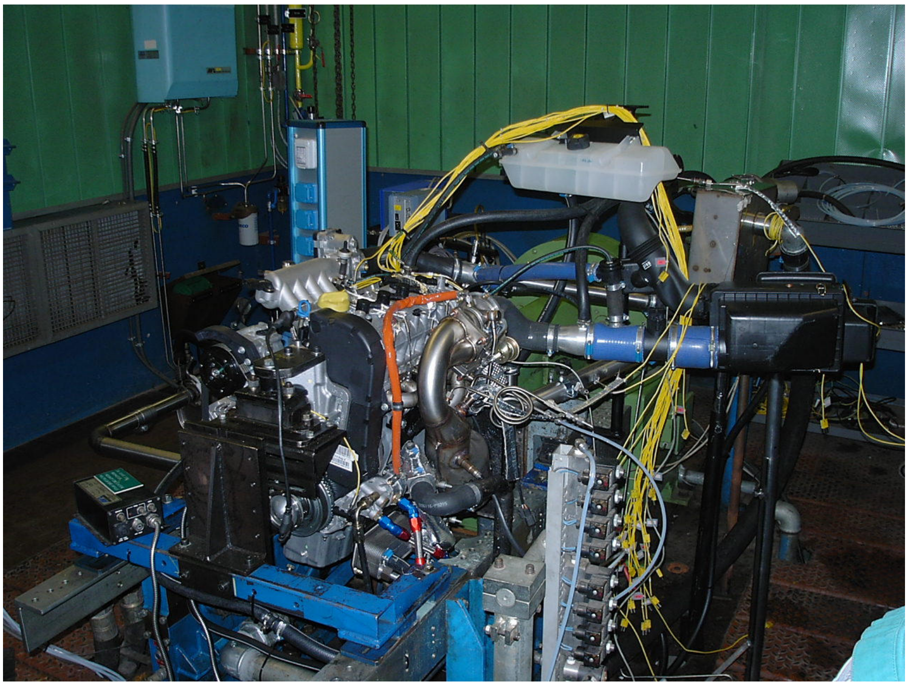


***Figure 5.3**: Prototype turbocharged engine at the engine test bench*

The characterization of the engine under full load conditions is quite impressive as the combination of the high octane number with the high latent heat of vaporization allows to a more efficient engine regulation, completely operated under stoichiometric conditions, with even a slight increase in maximum performance (see Figure 5.4)

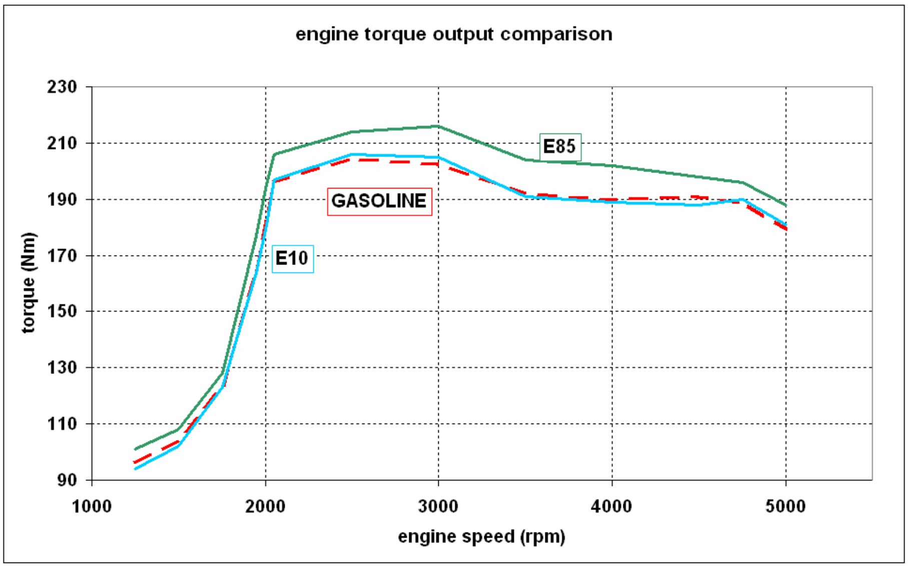


***Figure 5.4**: Potential of bioethanol blend on engine performance*

This behaviour has a double effect: on the one hand the fuel economy of the vehicle in real use conditions, and especially at higher load/speed where the standard gasoline regulation requires a rich mixture to avoid knocking and contains the temperatures at the exhaust, and, on the other hand, the stoichiometric conditions performed when running with the E85 blend results in maintaining the optimum conversion efficiency of the 3-way catalyst, thus ensuring the simultaneous reduction of the pollutant emissions.

Moving to the vehicle side, the prototype was based on a FIAT Grande Punto, originally equipped with a 1.4 8v naturally aspirated engine; the engine has been removed and replaced with the prototype turbocharged one (Figure 5.5)

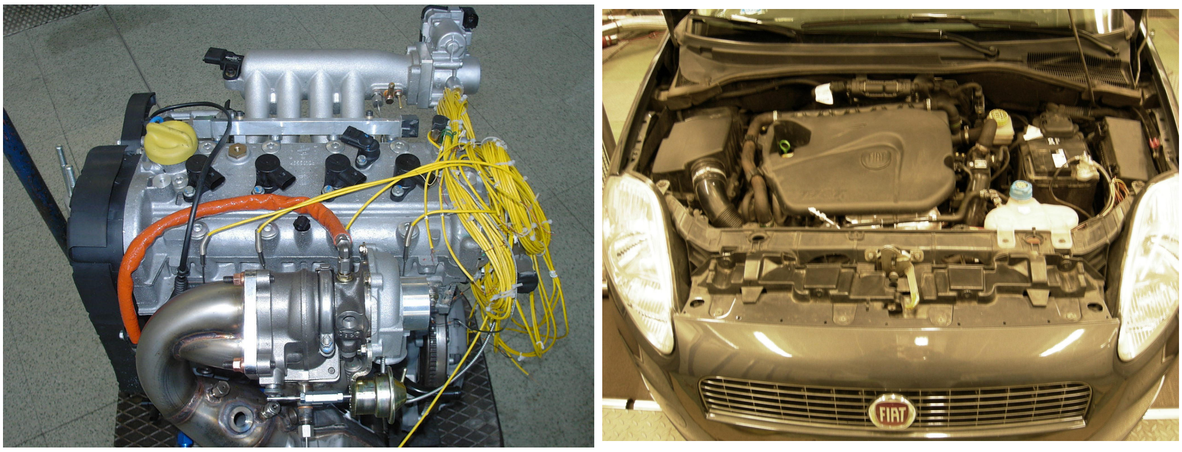


Figure 5.5:*Prototype engine before and after installation into the vehicle*

Measures obtained on vehicle have confirmed engine test bench data: vehicle top speed (related to maximum power output) raises from 193 kph to 196 kph and acceleration tests, mostly dependent on the torque output slope, have shown a significant benefit in terms of vehicle "fun to drive" (acceleration, progression); data are shown in Figure 5.6.

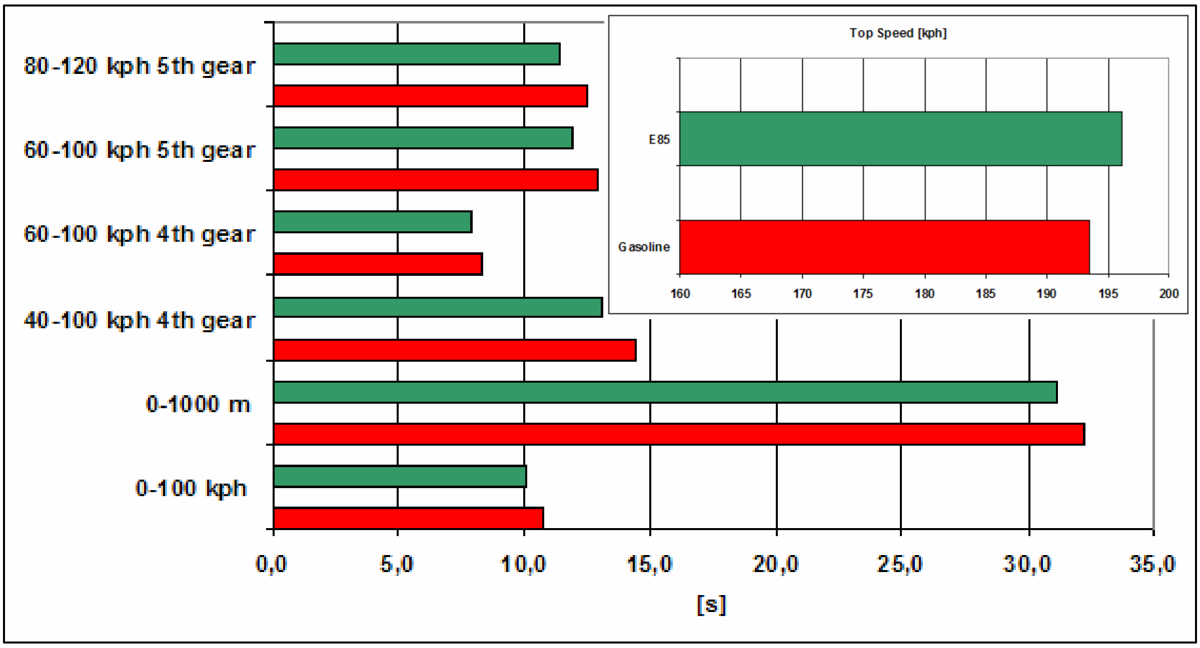
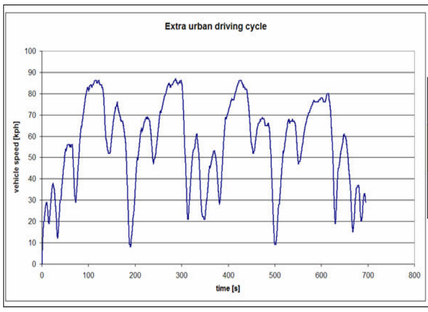


Figure 5.6: *E85 performance compared to reference gasoline*

Fuel economy has been evaluated under real use test conditions: because of the possible optimization of the spark advance timing due to the knocking resistance of bioethanol, high ethanol content blend such as E85 shows a benefit in terms of energy efficiency that can reach 4-5% in extra urban driving conditions with high transient operations, being even more important compared to the standard conditions of the NEDC homologation cycle (see Figure 5.7)



	Gasoline	E85
MJ/100km	226	215
l/100km	6.8	10.0

4% saving on energy basis

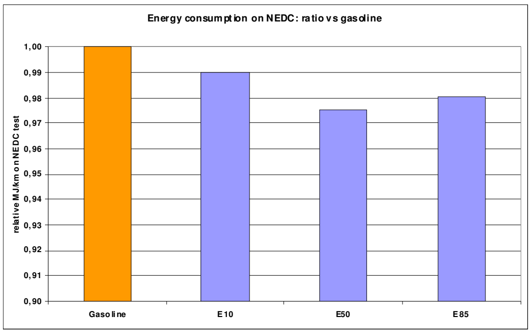


***Figure 5.7**: Influence of bioethanol on energetic fuel consumption*

Evaluation of aldehydes on NEDC and under cold start conditions (- 7°C) has shown a direct dependency only of acetaldehyde from ethanol content, while formaldehyde was not affected (if the methanol content in the fuel is negligible). Despite this dependency, the absolute value of the emissions results is very low.

6. Dissemination and training

NILE project brochures 1 (Jan 2008[1]) and 2 (March 2010[2]) illustrate the results achieved by the middle and end of the project respectively.
Two workshops for biomass specialists were organised, at the European Biomass Conference held at Valencia (Spain) in 2008, and at the World Bioenergy/Clean Vehicles and Fuels Conference held at Stockholm in 2009.
Lectures on biofuels (two per year) were given at the Biomass specialization of the European Master on Renewable Energy at the University of Zaragoza (Spain) in 2007, 2008 and 2009. A one day course on biofuels was given in 2008 and 2009 at the IFP School. A 2-hour teaching material was prepared for the European Master in Renewable Energy
In June 2008, NILE held a dinner debate at the European Parliament, at the time that sustainability criteria for biofuels were being discussed, bringing the project to the attention of MEPs and people interested in biofuels in Brussels.
A total of 4 newsletters and 5 press releases have been issued.

Main lessons drawn from NILE

Among the different approaches aiming at improving the enzymatic hydrolysis of lignocellulosic raw materials, some proved to be successful such as the optimisation of the composition of the enzyme mixture produced by *Trichoderma reesei* (for instance, increasing the proportion of CBHII) and the engineering of CBHII by directed evolution. Some other options look promising but have to be further investigated: the construction and expression of fused cellulases displaying a higher activity than the mixture of the individual ones, and the possibility of increasing the concentrations of cellulases secreted by random mutagenesis of *T. reesei* strains. The project has also shown that there was no significant effect of the addition of helper enzymes on the hydrolysis of wheat straw or softwood previously steam exploded in diluted acid conditions. Although the pretreatment was not studied in NILE, it appears that the potential improvements of enzymes were greatly dependent upon the efficacy of the pretreatment. Another observation is that the materials to be hydrolyzed were quite different when they were prepared at a PDU and a pilot scale showing that pretreatment up-scaling is a crucial issue. Other important parameters in the enzymatic hydrolysis are some operating conditions such as the initial WIS concentration and the agitation, which, in addition to a less efficient pretreatment, have lowered the improvements of enzymes when they have been used at a large scale.
As already reported, significant progresses have been obtained regarding the conversion of pentoses, both xylose and arabinose, to ethanol by engineered *S. cerevisiae* strains and the final yield obtained

[1] http://www.nile-bioethanol.org/doc/NILE_brochure_v6.pdf
[2] http://www.nile-bioethanol.org/doc/NILE_brochure_2010_new.pdf

were very close to the objectives. The efforts of the WP2 partners eventually resulted in four new industrial strains: one highly tolerant to toxic compounds, one with a new xylose transporter, one with the xylose isomerase system and one able to co-utilize arabinose and xylose. In addition, it was clearly shown that exploiting the full capacities of the new strains requires an adapted fermentation strategy, especially for SSCF. Because of the procedure required to obtain agreement for using genetically engineered microorganisms in the pilot plant, the selection of the yeast strain for pilot trials had to be made early in the programme. Even though the results obtained with the TMB3400-FT3 strain were a good xylose consumption and sugars to ethanol conversion yield, better performances should be achieved using the new available strains combined with the optimal fermentation strategy. In addition, these new results should also positively impact the costs calculated using the models developed in NILE thanks to a higher productivity, a higher conversion yield and a lower enzyme loading.
The pilot trials on softwood were carried out according to the initial planning without any technical interruption. The global conversion yields were lower than expected, mainly because of the pretreatment efficacy and an underestimate of the learning curve to switch from two step acid hydrolysis to acid pretreatment and enzymatic hydrolysis. Continued work after the NILE- project has verified results from lab and PDU- units. In addition to the observations already reported, the results showed that, in the conditions used, SSF was a good option. High amounts of lignin hydrolysate residues (LHR) could be obtained for a detailed characterization which generally confirmed their suitability as fuels. The necessity for an optimal integration of the process was also demonstrated using the process models developed.
In WP4, it was shown that profitable production and GHG savings require optimised supply-chains, integrated facilities and a favourable market. Feedstock / product markets and GHG factors are location specific and generalisation is risky. The path to market is considered speculative by stakeholders and the next step is technology validation and demonstration in production scale.
Although ethanol is already commonly used as a fuel in spark ignited engines, complementary tests were carried out aiming at a better knowledge on the use of ethanol (E10 or E85) in downsized engines and in engines coupled with turbocharger. Except emissions of acetaldehyde, ethanol was beneficial in terms of deposits in engines, engine performances, driveability and emissions of regulated and non regulated pollutants. The project also confirmed that cellulose-based ethanol can with normal distillation and dewatering meet the specifications from the fuel sector.