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**Dossier**

*Second and Third Generation Biofuels: Towards Sustainbility and Competitiveness*
Seconde et troisième génération de biocarburants : développement durable et compétitivité

# The NILE Project – Advances in the Conversion of Lignocellulosic Materials into Ethanol

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**Résumé** — **Le projet NILE et la conversion des matériaux lignocellulosiques en éthanol** — Le projet NILE, acronyme de "*New Improvements for Lignocellulosic Ethanol*", était un projet européen (2005-2010) consacré à la conversion des matières premières lignocellulosiques en éthanol. Ses principaux objectifs étaient de concevoir de nouvelles enzymes adaptées à l'hydrolyse de la cellulose en glucose et de nouvelles souches de levure capables de convertir efficacement tous les sucres présents dans la lignocellulose en éthanol. Une autre partie du projet consistait à tester ces nouveaux systèmes dans une installation pilote et à évaluer les impacts environnementaux et socio-économiques de la production et utilisation à grande échelle d'éthanol lignocellulosique.

Deux matières premières modèles (l'épicéa et la paille de blé) prétraitées de façon semblable, ont été étudiées. Différentes approches ont été tentées pour améliorer la saccharification de ces matières premières, par exemple, la recherche de nouvelles enzymes efficaces ou l'ingénierie d'enzymes. Plusieurs stratégies d'ingénierie génétique ont été utilisées pour obtenir des souches stables de *Saccharomyces cerevisiae* capables de fermenter le xylose et l'arabinose, et de tolérer les composés toxiques présents dans les hydrolysats lignocellulosiques. L'installation pilote pouvait traiter 2 tonnes de matières sèches par jour, et l'hydrolyse et la fermentation pouvaient être menées successivement ou simultanément. Un modèle global intégrant la chaîne d'approvisionnement en matière première a servi à évaluer les performances économiques et environnementales de la production d'éthanol lignocellulosique.

L'évolution dirigée d'une enzyme du cocktail cellulolytique produit par le champignon *Trichoderma reesei*, et la modification de la composition de ce cocktail améliorent l'hydrolyse enzymatique des matières premières prétraitées. Cependant, ces résultats n'ont pu être reproduits à grande échelle.

Le rendement de conversion et la productivité spécifique en éthanol ont été sensiblement augmentés grâce à l'ingénierie métabolique des souches de levure et au développement d'un procédé optimal de fermentation. Les essais en pilote ont confirmé le bon comportement de ces souches de levure en conditions industrielles ainsi que la possibilité d'utiliser les résidus riches en lignine comme combustible. Le coût de production de l'éthanol et le bilan des émissions de gaz à effet de serre étaient très dépendants des sources d'énergie utilisées.

D'un point de vue plus global, les résultats ont montré que l'optimisation du procédé nécessite de codévelopper toutes les étapes de façon intégrée et de valider les améliorations dans une installation pilote, afin notamment de pouvoir comparer différentes configurations et d'en déterminer les effets sur l'économie du procédé et ses impacts environnementaux.

***Abstract — The NILE Project – Advances in the Conversion of Lignocellulosic Materials into Ethanol*** *— NILE ("New Improvements for Lignocellulosic Ethanol") was an integrated European project (2005-2010) devoted to the conversion of lignocellulosic raw materials to ethanol. The main objectives were to design novel enzymes suitable for the hydrolysis of cellulose to glucose and new yeast strains able to efficiently converting all the sugars present in lignocellulose into ethanol. The project also included testing these new developments in an integrated pilot plant and evaluating the environmental and socio-economic impacts of implementing lignocellulosic ethanol on a large scale.*

*Two model raw materials – spruce and wheat straw – both preconditioned with similar pretreatments, were used. Several approaches were explored to improve the saccharification of these pretreated raw materials such as searching for new efficient enzymes and enzyme engineering. Various genetic engineering methods were applied to obtain stable xylose- and arabinose-fermenting Saccharomyces cerevisiae strains that tolerate the toxic compounds present in lignocellulosic hydrolysates. The pilot plant was able to treat 2 tons of dry matter per day, and hydrolysis and fermentation could be run successively or simultaneously. A global model integrating the supply chain was used to assess the performance of lignocellulosic ethanol from an economical and environmental perspective. It was found that directed evolution of a specific enzyme of the cellulolytic cocktail produced by the industrial fungus, Trichoderma reesei, and modification of the composition of this cocktail led to improvements of the enzymatic hydrolysis of pretreated raw material. These results, however, were difficult to reproduce at a large scale.*

*A substantial increase in the ethanol conversion yield and in specific ethanol productivity was obtained through a combination of metabolic engineering of yeast strains and fermentation process development. Pilot trials confirmed the good behaviour of the yeast strains in industrial conditions as well as the suitability of lignin residues as fuels. The ethanol cost and the greenhouse gas emissions were highly dependent on the supply chain but the best performing supply chains showed environmental and economic benefits.*

*From a global standpoint, the results showed the necessity for an optimal integration of the process to co-develop all the steps of the process and to test the improvements in a flexible pilot plant, thus allowing the comparison of various configurations and their economic and environmental impacts to be determined.*

## FOREWORD

The project NILE was completed on March 2010. This article is a picture of the results obtained at the end of the project and does not include the advances made on the development of cellulosic ethanol since that time.

## INTRODUCTION

Among the various renewable energy sources, biomass is an option particularly suitable for the production of transportation fuels, *i.e.* biofuels. Biofuels are especially attractive when they can be produced from lignocellulosic raw materials (second generation biofuels) because of a high reduction in global greenhouse gas emissions and reduced competition for land needed to produce food and feed. However, the production processes of biofuels from lignocellulosic feedstocks have to become less costly if a future commercialization is to be successful. The production of second generation bioethanol is being investigated worldwide, and demonstration projects are about to be implemented. These processes generally include four main steps:

- a physical-chemical pretreatment aiming at breaking up the structure of the raw material so that its constituent polymers (cellulose, hemicellulose and lignin) are made accessible to depolymerization agents;
- an enzymatic hydrolysis of cellulose (and possibly hemicellulose) to monomeric sugars;
- the conversion of these sugars to ethanol by fermentation;

– the separation of ethanol from the fermentation broth by distillation generally followed by a final dehydration to give an engine-compatible fuel.

Numerous obstacles to the development of lignocellulosic ethanol production processes still exist (Himmel *et al.*, 2007; Margeot *et al.*, 2009), including the definition of a low-energy consuming and efficient pretreatment, the high cost of the enzymatic hydrolysis, the efficient conversion of pentoses and hexoses to ethanol in the possible presence of toxic compounds generated by the pretreatment step, the quality and use of lignin and the need for an optimal process integration that minimizes water and energy demands. Most of these problems, except pretreatment, have been addressed in the framework of a 5-year European project, the acronym of which was NILE (New Improvements for Lignocellulosic Ethanol) and which was completed in March 2010. In this project, two raw materials have been considered, an agricultural residue, wheat straw, and a soft wood, spruce. This article presents some of the approaches investigated in NILE to decrease the hydrolysis cost, to improve the ethanol conversion and the process integration. Results on the up-scaling in a pilot plant as well as on economic and environmental assessments of lignocellulosic ethanol are also presented.

### Pretreatment of Lignocellulosic Feedstocks

Physical-chemical pretreatment of lignocellulosic biomass is a prerequisite to an efficient action of hydrolytic enzymes, although it does not have a depolymerization effect itself. Many technologies have been investigated including steam explosion, Liquid Hot Water (LHW), Ammonia Fiber EXpansion (AFEX), dilute acid cooking, alkaline cooking, organosolv extraction, etc. It can generate some compounds (furanics and phenolics), thereafter named inhibitors, which may be toxic to yeasts. In NILE, steam explosion and dilute acid cooking (one or two-steps) were used (Sekab technology).

## 1 ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSIC FEEDSTOCKS

Cellulosic substrates are recalcitrant to degradation, thus high concentrations of enzymes are required (Himmel *et al.*, 2007). Although recent advances have been achieved, the enzymatic hydrolysis step still needs further improvement. *Trichoderma reesei* is a filamentous fungus which is already exploited at an industrial scale for its capacity to secrete high concentrations of cellulases, the enzymes responsible for the depolymerization by hydrolysis of cellulose chains to the constituent monomer, glucose. *T. reesei* secretes nine major cellulolytic enzymes that exhibit 3 types of activities leading to a complete hydrolysis of cellulose chains:

– 2 cellobiohydrolases (CBH) that attack cellulose at its reducing (CBH1) or non-reducing (CBH2) chain ends, liberating cellobiose, a dimer of glucose;
– 5 endoglucanases (EG) that attack cellulose at random points of cellulose, especially at the amorphous zones of cellulose;
– 2 ß-glucosidases (BGL), splitting cellobiose to glucose.

Besides cellulases, *T. reesei* is also able to secrete hemicellulases such as xylanases. Because hemicelluloses are composed of various hexoses and pentoses, and contain side chains, they need a diverse range of hemicellulases for their degradation. However, they are more easily degraded than cellulose by chemical agents because of lower chain lengths and a different structure (cellulose in plant cell wall is in the form of crystalline microfibrils). The release of the complete genome sequence of *T. reesei* confirmed the presence of other genes that may possibly be involved in lignocellulosic biomass degradation, *e.g.* hemicellulases, but the total number of these genes and their diversity were low compared to those present in the genomes of other biomass-degrading micro-organisms (Martinez *et al.*, 2008).

One of the approaches used to improve the enzymatic hydrolysis was to identify new efficient cellulases and "helper" enzymes that could positively complement the enzymatic cocktail produced by *T. reesei*. Helper enzymes are enzymes which could weaken the structure surrounding cellulose, mainly composed of lignin and hemicelluloses, and thus facilitate the action of cellulases (Berlin *et al.*, 2005). Genome-mining of the *T. reesei* genome showed that some classes of hemicellulases and ligninases were missing. Some of these missing enzymes were identified in species of closely-related fungi and selected as possible helper enzymes. Additionally, a whole-gene expression study (transcriptome) of *T. reesei* was carried out to determine the genes specifically expressed when the fungus is grown on lignocellulosic raw materials compared to pure cellulose. In total, 38 new enzymes that might play a role in the degradation of lignocellulosic substrates were found. This list included cellulases hemicellulases and helper proteins and fused enzymes. Biochemical data were obtained for 13 enzymes that were identified as relevant for hydrolysis efficiency. The efficiency of the enzymes was assessed on real substrates: wheat straw or softwood pretreated by steam explosion in dilute acid conditions. The results of complementation showed that there was no dramatic effect of any of the enzymes tested on the hydrolysis rate. Actually, the pretreatment used was very

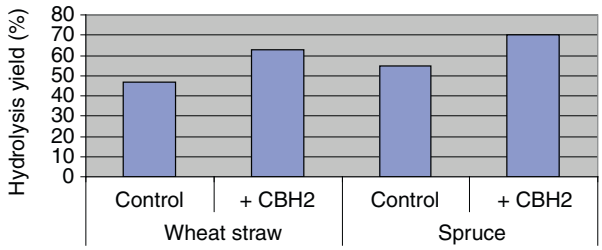


Figure 1

Effect of an enrichment in CBH2 enzyme of the *T. reesei* enzyme mixture on the hydrolysis yield of pretreated wheat straw and spruce. The hydrolysis yield was calculated after 24 h. The same total amount of enzymes was used in the control and in the CBH2 assays. In the CBH2 assay, 25% of the total amount was replaced by pure CBH2. The substrates were pretreated by steam explosion in the presence of diluted $H_2SO_4$.

efficient in hydrolyzing hemicelluloses and breaking up the lignocellulosic structure so that the effect of helper enzymes was very low.

Another approach was to determine which enzyme in the enzymatic cocktail produced by *T. reesei* was the limiting step in the hydrolysis and then to improve it.

Figure 1 shows the positive effect of the addition of CBH2 on the hydrolysis yields of pretreated wheat straw and spruce. Addition of CBH1 led to a weaker effect while no effect was observed with EG.

Having identified that CBH2 was the limiting step, the specific activity of CBH2 was improved using directed evolution. This technique used combinatory technologies to create hundreds of thousands variants of the target gene which were then selected by high throughput screening using appropriate rapid activity tests. New CBH2 variants were created with twice the specific activity of the wild type *T. reesei* CBH2 gene (*Tab. 1*). The two most promising CBH2 variants were expressed in *T. reesei*.

Including other engineered enzymes and helper enzymes, a total of 36 enzymes were produced in sufficient amounts so that their hydrolysis properties on steam exploded wheat straw and spruce could be assessed. The highest beneficial effect could be observed on pretreated softwood using one of the engineered CBH2 where a 20% increase of the hydrolysis yield was obtained. Attempts to decrease the enzyme load were also positive since a similar hydrolysis yield could be obtained with a decrease of the enzyme load of around 50%.

TABLE 1

Hydrolysis activity on wheat straw using wild type and engineered CBH2 enzymes (arbitrary units) (Koch *et al.*, 2009)

| Wild type | 1st generation CBH2 | 2nd generation CBH2 |
|---|---|---|
| 100 | 120 | 200 |

1st and 2nd generation genes correspond to one and two runs of evolution and selection.

## 2 CO-FERMENTATION OF LIGNOCELLULOSE DERIVED HEXOSE AND PENTOSE SUGARS

The complete hydrolysis of lignocellulose generates monosaccharides with six carbon atoms, hexoses (C6), and monosaccharides with five carbon atoms, pentoses (C5). Glucose, mannose and galactose constitute the C6 sugars, while xylose and arabinose make up the C5 sugars. It was early established for non-engineered strains (Olsson and Hahn-Hägerdal, 1993) and recently confirmed for engineered strains (Lau *et al.*, 2010) that baker's yeast *Saccharomyces cerevisiae* is the organism of choice for lignocellulose fermentation. This is primarily due to the extraordinary robustness and inhibitor tolerance of this organism. However, in the lignocellulose context one major drawback of *S. cerevisiae* is that it is unable to ferment pentose sugars and that it rather poorly ferments galactose. A large number of metabolic engineering strategies have been explored to make *S. cerevisiae* pentose fermenting (Hahn-Hägerdal *et al.*, 2007) as well as to improve its galactose utilization (Ostergaard *et al.*, 2000; Bro *et al.*, 2005). Based on these investigations the NILE project took a systems biotechnology (Lee S.Y. *et al.*, 2005) approach to improve concomitant C6 and C5 fermentation of lignocellulose. This included protein and metabolic engineering (Bailey, 1991) of individual cellular reactions, evolutionary adaptation/engineering (Sauer, 2001) of whole cells for lignocellulose substrates as well as the development of novel fermentation protocols (Olofsson *et al.*, 2008a) to improve the efficiency of co-fermentation of all lignocellulose derived sugars in non-detoxified hydrolysates.

In *S. cerevisiae*, C5 sugars are transported into the cell *via* hexose transporters, the Hxt family, which generally have a several orders of magnitude lower affinity for C5 sugars than for C6 sugars (Kötter and Ciriacy, 1993; Lee W. *et al.*, 2002; Saloheimo *et al.*, 2007). The yeast *Candida intermedia* has previously been found to transport xylose *via* an efficient glucose/xylose facilitator, Gxf1 (Leandro *et al.*, 2006). When the *GXF1* gene encoding Gxf1 was expressed in a xylose utilizing strain of *S. cerevisiae*, growth at low sugar concentration was improved (Runquist *et al.*, 2009a). It was also found that Gxf1 provided superior xylose transport compared with other published xylose transporters (Runquist *et al.*,

2009b), which was ascribed to it, being selected based on biochemical properties rather on sequence similarity. In the final stages of NILE, Gxf1 was also expressed in an inhibitor tolerant industrial xylose utilizing strain with the aim to investigate its influence on xylose utilization in Simultaneous Saccharification and Co-Fermentation (SSCF) of lignocellulose (Fonseca *et al*., 2011).

For arabinose, heterologous transporters from the yeasts *Pichia stipitis* (Boles and Keller, 2006) and *Candida arabinofermentans* (Fonseca *et al*., unpublished data) were assessed in arabinose-utilizing strains of *S. cerevisiae*. Similar to the Gxf1 transporter the arabinose transporters were found to improve growth on arabinose at low sugar concentrations (*Fig. 2*).

A major effort of the NILE project was to engineer the initial galactose, xylose and arabinose pathways in *S. cerevisiae*. When the homologous gene *PGM2* encoding the Leloir pathway enzyme phosphoglucomutase was overexpressed in *S. cerevisiae*, it did not only improve anaerobic growth and ethanolic galactose fermentation (Garcia Sanchez *et al*., 2010a), but also anaerobic growth and ethanolic xylose fermentation as well as galactose and xylose co-fermentation (Garcia Sanchez *et al*., 2010b). Ethanolic xylose fermentation was also significantly improved in a *S. cerevisiae* strain expressing a mutant xylose reductase enzyme obtained by protein engineering (Bengtsson *et al*., 2009). Similarly, codon optimization of the genes encoding the bacterial arabinose pathway significantly increased both the ethanol yield and the specific ethanol productivity in arabinose fermentation with engineered *S. cerevisiae* (Wiedemann and Boles, 2008). Co-utilization of xylose and arabinose was achieved when an engineered industrial strain of *S. cerevisiae* harboring the fungal xylose and the bacterial arabinose utilizing pathways (Karhumaa *et al*., 2006) was evolutionary engineered (Garcia Sanchez *et al*., 2010c). The increased xylose and arabinose co-utilization could be related to improved transport of both sugars as well as increased levels of the fungal xylose utilizing enzymes.

Evolutionary adaptation (or engineering) which consists in improving phenotypes by iterative cycles of variation and selection, *e.g.* by increasing exposure to lignocellulose-derived inhibitors was applied to an existing industrial xylose utilizing strain (Wahlbom *et al*., 2003). The evolved strain fermented lignocellulose-containing media faster than the parental strain (Heer and Sauer, 2008), which could later be related to overexpression of two genes encoding furfural reducing activity (Heer *et al*., 2009).

In NILE, systems biotechnology approach fermentation strategies were developed with the aim to ferment all lignocellulose sugars without prior detoxification of the substrate, *i.e.* in non-detoxified lignocellulose

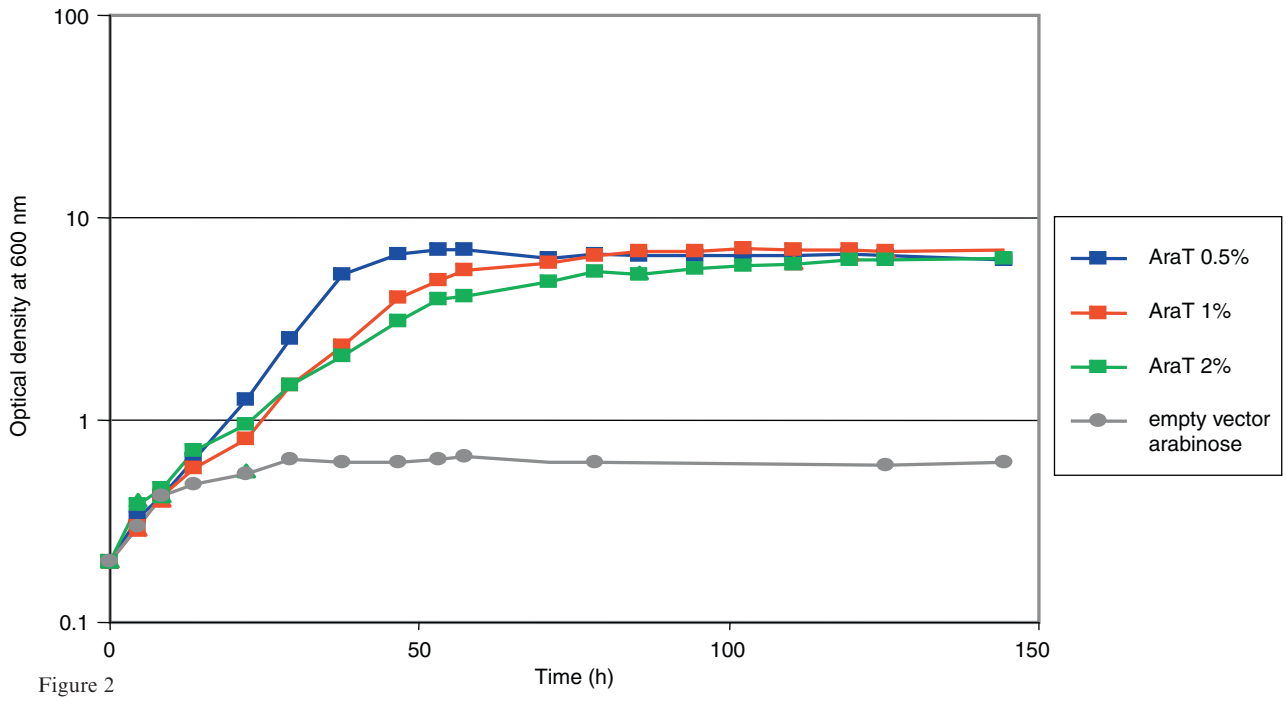


Figure 2

Growth (measured as optical density at 600 nm *vs* time) of an engineered *S. cerevisiae* hxt null strain (Reifenberger *et al*., 1997) expressing the *P. stipitis* arabinose transporter AraT (Boles and Keller, 2006). % corresponds to the initial arabinose concentration.

hydrolysates. Strategies were designed taking into account the accumulated knowledge on the regulation and control of lignocellulose sugar uptake and catabolism in engineered *S. cerevisiae* (Hahn-Hägerdal *et al.*, 2007) to promote:

– high conversion in the enzymatic hydrolysis,
– high conversion of pentose sugars, and
– to develop robust yeast strains tolerant towards inhibitors in lignocellulose hydrolyzates.

The NILE feedstock – wheat straw and spruce – represented different challenges. Wheat straw contains a significant amount of xylose, which can only be converted with yeast engineered for xylose utilization. Spruce, on the other hand, generates a highly inhibitory hydrolysate, which requires a much more robust yeast strain. Based on extensive previous experience (Olofsson et al., 2008a) the principal process approach was Simultaneous Saccharification and Fermentation (SSF), *i.e.* a process in which the enzymatic hydrolysis takes place together with the fermentation. Since C5 sugars, primarily xylose, were converted together with C6 sugars, the term SSCF (Simultaneous Saccharification and Co-Fermentation) is sometimes considered more appropriate.

The implementation of carefully designed fermentation strategies significantly increased the overall ethanol yield and the conversion of xylose both for wheat straw and for spruce. The influence of fermentation strategy on overall ethanol yield (*Fig. 3*) and on xylose conversion (*Fig. 4*) is illustrated for steam pretreated wheat straw. Generally, the ethanol yield decreased with increasing WIS (Water Insoluble Solids) content. Similarly, a fed-batch protocol proved superior to a batch protocol.

It had previously been observed that it was crucially important to adapt yeast strains to hydrolyzate inhibitors (Alkasrawi *et al.*, 2006). Therefore, a cultivation protocol for the preparation of the yeast inoculum was developed (Alkasrawi *et al.*, 2006) and stringently applied throughout the development of novel fermentation strategies. Similarly, it was observed that fed-batch substrate addition benefited yeast performance when the lignocellulose raw material had been severely pretreated (Olofsson *et al.*, 2008b).

At a WIS content of 7%, an ethanol yield of 0.4 g/g and a final titer of 35 g/L were obtained using xylose fermenting yeast in a fed-batch process (*Fig. 3*). For spruce, a value of 0.4 g/g was reached also at 10% WIS. Higher yields require a more complete xylose fermentation for wheat straw, whereas for spruce a more complete hydrolysis is primarily needed.

The choice of operating temperature in SSF is a compromise between the cellulolytic enzymes optimally operating around 50°C and the yeast optimally operating around 30°C. Nevertheless, for the overall process performance and at the expense of the rate of enzymatic hydrolysis it was found beneficial to reduce the process temperature for certain yeast strains (Olofsson *et al.*, 2008b).

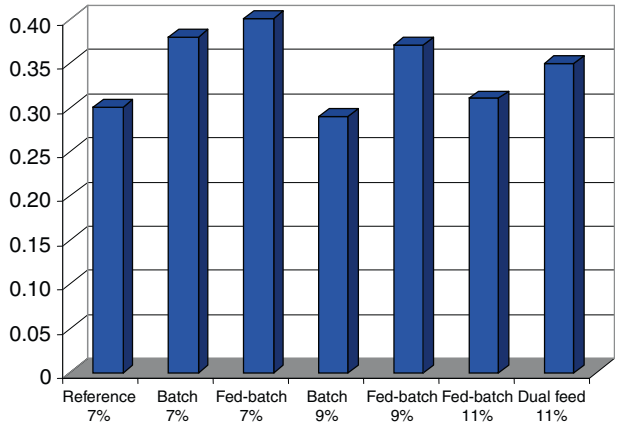


Figure 3

Overall ethanol yield (w/w) in SSF of steam exploded wheat straw. The reference represents the ethanol yield using a non-xylose fermenting yeast in a batch SSF using 7% WIS (Water Insoluble Solids). Subsequent experiments were performed with a xylose fermenting *S. cerevisiae* strain (Wahlbom *et al.*, 2003).

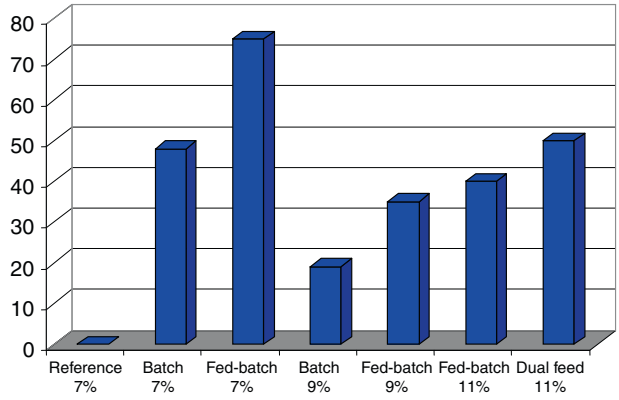


Figure 4

Xylose conversion in SSF of steam exploded wheat straw (in %). The reference represents the ethanol yield using a non-xylose fermenting yeast in a batch SSF using 7% WIS. Subsequent experiments were performed with a xylose fermenting *S. cerevisiae* strain (Wahlbom *et al.*, 2003) in batch or fed-batch SSF using 7 to 11% WIS (in dual feed, enzyme was also progressively introduced).

Fed-batch addition of substrate in SSCF was found to improve xylose conversion of the xylose-rich wheat straw material (Olofsson *et al*., 2008b). Fed-batch addition of substrate is also a mean of overcoming rheological problems, since the viscosity of the slurry can be kept low enough to allow mixing (Olofsson *et al*., 2008a). Additionally this results in higher final ethanol titers, which improves the overall process economy (Galbe *et al*., 2007).

The ratio of xylose and glucose is critical for the efficient co-fermentation in SSF. Enzyme feeding strategies were developed to optimize the sugar ratio, and the principle was proven using pretreated spruce material (Olofsson *et al*., 2010b). This material is particularly challenging since the glucose to xylose ratio is high already in the pretreated slurry. Using enzyme feeding the xylose conversion could be increased from about 40% to about 80% in an SSF at 10% WIS.

The fed-batch strategy was extended to include a combination of fed-batch addition of substrate and fed-batch addition enzyme. The benefit of the dual feed was demonstrated for pretreated wheat straw at 11% WIS (*Fig. 3, 4*). The xylose conversion increased to about 50% compared to about 40% when only substrate was fed (Olofsson *et al*., 2010a). Due to the high viscosity of the pretreated wheat straw a batch process is not even possible.

NILE generated several engineered industrial *S. cerevisiae* strains co-utilizing C6 and C5 lignocellulose sugars. The ultimate aim of NILE was to assess the performance of these strains in the SEKAB pilot-plant (see below). However, due to the time constraints of the project only one evolutionary engineered strain (Heer and Sauer, 2008) was assessed there.

## 3 PROCESS DESIGN

Ethanol production from biomass has not yet been demonstrated on commercial scale. Studies on the economic viability of the technology must therefore be based on data from pilot or demo scale units. For this case, the Ethanol Pilot in Örnsköldsvik, Sweden, was used in the NILE project. The Pilot plant is very flexible with two continuous flows through pretreatment reactors with possibilities to operate down to very low pH ($> 1.5$) and high temperature ($< 230°C$). For enzyme hydrolysis and fermentation, five 10 000 litres tank reactors were used. During the pilot tests, both SSF (Simultaneous Saccharification and Fermentation) and SHF (Separate Hydrolysis and Fermentation) were used. The plant which is operated in continuous mode (24 h per day / 7 days per week) by SEKAB E-Technology, is a complete process with equipment to cultivate GMM (Genetically Modified Microorganism) yeast, dewatering of Lignin Hydrolysate Residue (LHR), distillation of ethanol and scrubbing of vent gases. All process water is collected and sent to biological treatment to clean the water and produce biogas (*Fig. 5*).

In technical economic evaluation of the process, all three products, ethanol, solid lignin fuel and biogas from stillage, have to be taken into consideration. Changing the conditions in one process step will affect other parts of the process. SSF, for example, gives higher content of elements such as nitrogen and alkali in the LHR, compared to SHF. Moreover, 40-50% of the energy content in the feedstock ends up in the solid lignin fuel – a fine particle material dewatered to 50% in a membrane filter press.

The use and value of the lignin as a fuel have been evaluated by the Institute of Wood Chemistry in Riga. Some of the new findings include that the energy consumption required for pellet production is significantly lower for softwood LHR than for softwood sawdust: 0.11-0.34 kWh/kg and 0.46 kWh/kg, respectively. This is explained by the higher elasticity of softwood sawdust, due to the presence of non destroyed morphological structure in the wood cell wall.

The energy required for pellet production was found to increase with Klason lignin content in the LHR samples (*Fig. 6*). The lowest specific energy consumption was observed for LHR from wheat straw and was correlated with the lowest Klason lignin content and the presence of 20% of low molecular water-soluble compounds (predominantly carbohydrates) in the LHR from wheat straw (Andersone *et al*., 2009).

It was calculated, based on earlier studies that the energy consumed for sawdust pelletizing reached 9% of energy produced from softwood pellets combustion. For the investigated LHR, this value varies in the range 2.3-5.8%, which makes a big difference in a production plant, if the lignin should be pelletized. Besides, the durability (DU) which is the amount of the feedstock that becomes pellets and are not recycled as fines, is better for lignin pellets.

The characteristics of the studied hydrolysed lignin (Arshanitsa *et al*., 2009) are summarized in Table 2.

Regarding combustion characteristics of LHR, the amount of heat produced during the first 80 minutes of the combustion of both LHR pellets, using commercial boiler (40 kW), exceeded the heat produced by commercial softwood pellets because of a higher content of wood carbohydrates which is the major source of volatiles (Arshanitsa *et al*., 2009). The higher heat production by LHR during the last 45 minutes of the combustion

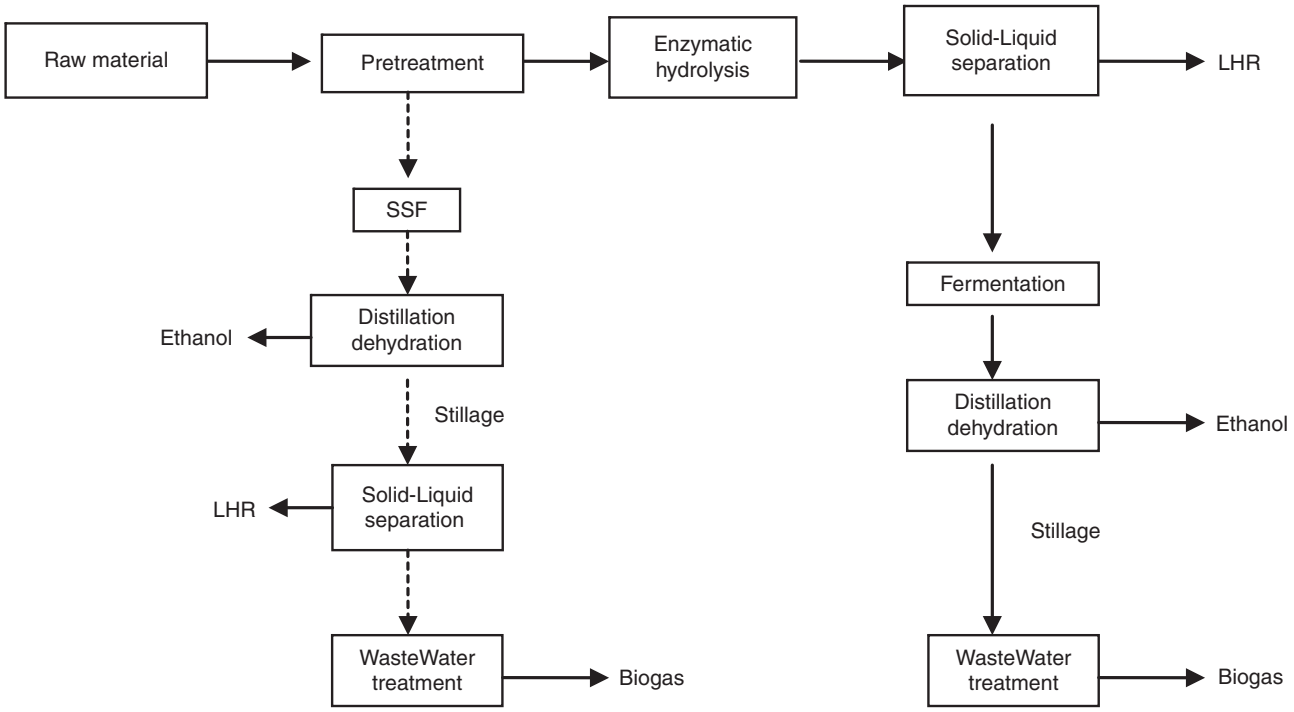


Figure 5

General flow sheet of a process of ethanol production from lignocellulosic biomass considering separate hydrolysis and fermentation (unbroken arrows) or simultaneous saccharification and fermentation (dotted arrows). SSF: Simultaneous Saccharification and Fermentation; LHR: solid Lignin Hydrolysate Residue.

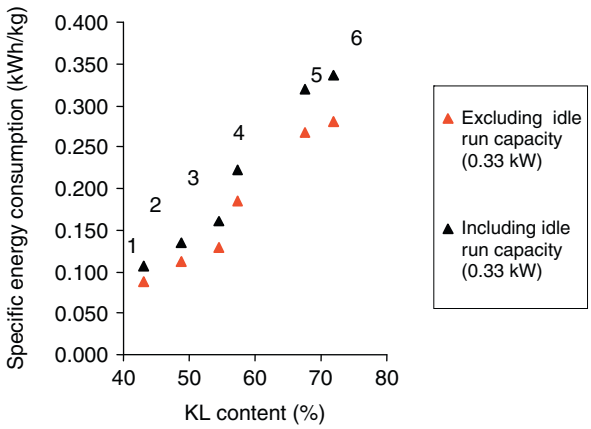


Figure 6

Specific energy consumption needed for the pelletizing of investigated LHR (Lignin Hydrolysate Residue) using a laboratory pellet mill. KL: Klason Lignin.

test (80-125 min) is correlated with accumulation of carbonaceous residues in which combustion proceeds at a lower rate and makes this stage more prolonged in comparison with wood. The latter can be explained by an increase in char formation that extends the total combustion process in the case of LHR softwood and LHR wheat straw in comparison with reference softwood pellets. Under the experimental conditions, the total heat outputs were similar for all tested biofuels (~3 kWh/kg). Taking into account the delay in time of the char combustion step relatively to volatiles burnout step, the impact of char combustion to the total heat output could be used to control the combustion process. It means that prolongation of LHR softwood and LHR wheat straw pellets combustion in this regime will enhance the specific heat power output.

Pelletised LHR take a middle position between coal and wood. For coal, the stage of non volatized carbon combustion is the main process, whereas, for wood the stage of volatiles combustion is the dominant one. These two stages differ significantly by mechanism. The combustion of volatiles proceeds in the upper zone and is mainly regulated by secondary air supply. Char combustion proceeds in the lower part of the combustion

TABLE 2
Characteristics of pelletized biofuels

| Characteristic | Original material | | |
|---|---|---|---|
| | Softwood | Lignin from softwood | Lignin from wheat straw |
| Water content (%) | 6.4 | 6.5 | 6.0 |
| Ash content on DM (%) | 0.55 | 0.40 | 6.4 |
| Lower Heating Value (LHV) (kWh/kg*) | 4.8 | 5.4 | 4.8 |
| Pellet diameter (mm) | 6.0 | 6.0 | 6.0 |
| Bulk density (kg/m$^3$) | 690 | 740 | 730 |
| Durability (DU) (%) | 96.0 | 97.5 | 98.8 |

* Dry matter.

chamber. The latter is connected with a direct carbon oxidation in the bulk char layer and is needed in the regulation of primary air supply for complete char combustion with high heat output and low carbon monoxide emission. By adapting combustion regimes (regulation of primary and secondary air) for LHR, additional heat output could be provided.

## 4 ECONOMIC AND ENVIRONMENTAL IMPACTS

The future success of lignocellulosic ethanol technology is closely tied to developments in other major economic sectors – energy, forestry and agriculture. The extent of interactions between these sectors and the nascent second generation ethanol sector varies, depending on how supply-chains are configured and the level of competition for resources that is anticipated. Uncertainties about the policy environment will also affect investors' appetite for risk and will influence what practical steps are taken on the path to market. Within the NILE project, the future environmental and economic performance was examined using an integrated cost and GHG (Greenhouse gas) model. This model used descriptions of alternative conversion processes together with other supply-chain parameters (feedstock and ethanol prices, finance package, and GHG emission factors) to enable the rapid comparison of different process concepts at the supply-chain level (Slade *et al*., 2009a,b).

### 4.1 Supply-Chain Cost Performance

Two of the most important factors affecting the commercial viability of lignocellulosic ethanol are the cost of feedstocks and the value obtained for ethanol. Both of these factors are highly uncertain: feedstock prices are affected by both location and existing markets, whereas the value obtained for ethanol is determined by the oil price and policy incentives. Despite this uncertainty, if market conditions were favourable (and by no means improbable), ethanol produced from softwood and sold as a low percentage blend with gasoline could be cost competitive without requiring subsidy (Slade, 2009c). Production from straw would generally be less competitive unless the pentose sugar fraction – more abundant in straw than softwood – could be valorised. Illustrative supply-chain costs are shown in Figure 7.

### 4.2 Greenhouse Gas Emissions Performance

Quantifying the greenhouse gas (GHG) performance of cellulosic ethanol faces similar challenges than quantifying the emissions from conventional biofuels: there are limited empirical data, significant methodological variation, and results cannot easily be divorced from subjective interpretation (Margeot *et al*., 2009). Work within NILE focussed on identifying the factors which had the greatest influence on supply-chain GHG emissions and identifying the areas of greatest uncertainty.

The affect of geographic location on the estimation and interpretation of GHG emissions performance was one of the factors considered. Figure 8 shows the comparison of enzymatic and dilute acid processes located in Northern Sweden with an average European base-case. In the base-case scenario, the dilute acid process gives rise to lower GHG emissions than the enzymatic process, whereas if the same supply-chains are located in Northern Sweden the merit order is reversed. In this case, the difference can be attributed to variations in the carbon intensity of softwood and electricity production: notably, the carbon intensity of softwood production in this area of Sweden is greater than the European average owing to resource scarcity.

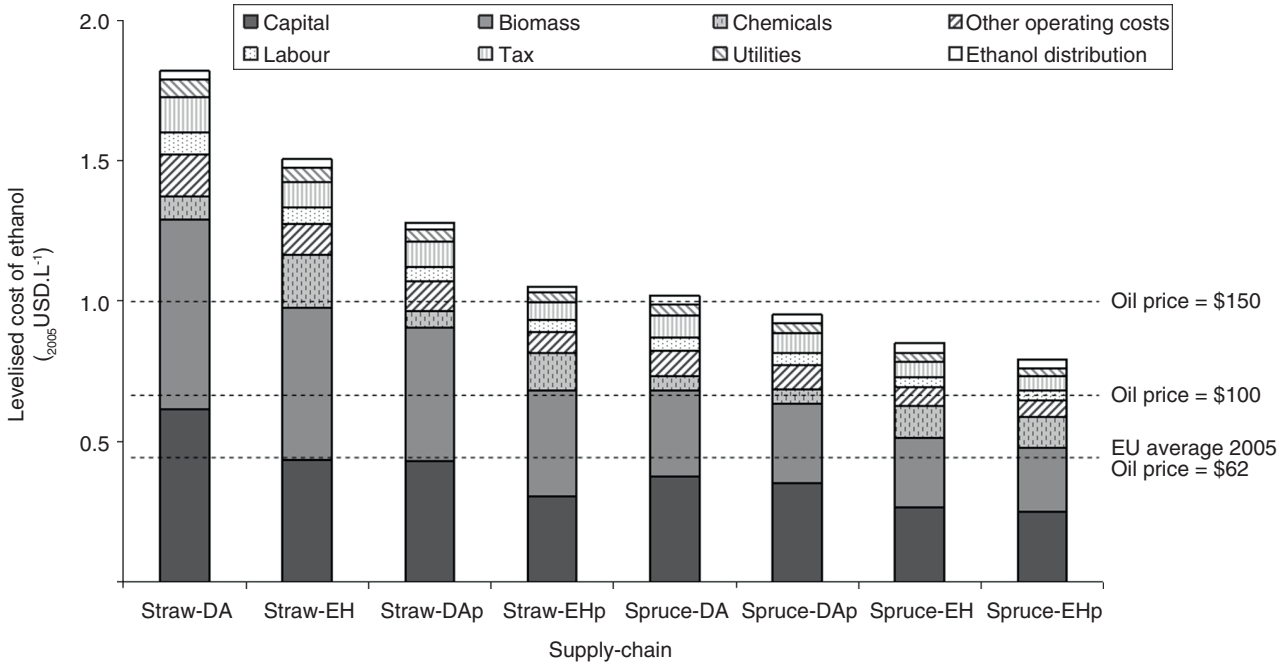


Figure 7

Levelised cost of ethanol production: a comparison of supply-chain concepts (adapted from Slade *et al.*, 2009a). DA: Dilute Acid process. DAp: Dilute Acid process including pentose fermentation. EH: Enzymatic Hydrolysis. EHp Enzymatic Hydrolysis including pentose fermentation.

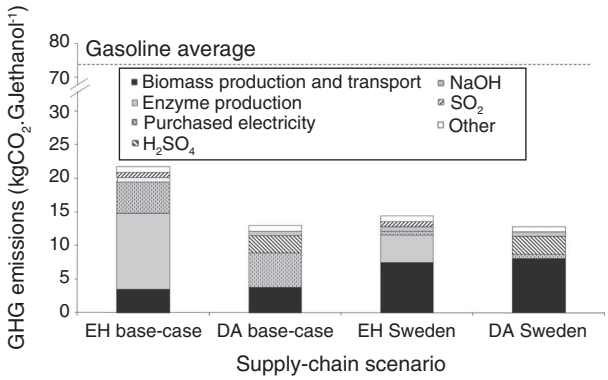


Figure 8

Greenhouse gas emissions from enzymatic and dilute acid supply-chains. DA: Dilute Acid process; EH: Enzymatic Hydrolysis process.

For the supply chains considered within the NILE project, producing and using cellulosic ethanol instead of gasoline were found to reduce GHG emissions but, as the examples in Figure 7 illustrate, it is generally more expensive. If the additional expenses were to be met by a public subsidy, and this were to be justified solely on the basis of the GHG savings, what value would need to be given to the GHG savings? This was one of the questions that the integrated cost and GHG model was used to explore. One scenario, derived from the softwood enzymatic base-case, is shown in Figure 9. This scenario assumed a constant price of biomass and the model was used to calculate the carbon price at which the supply-chain would break-even for a range of different oil prices and assuming either first-plant or *N*th plant finance. It can be seen that the oil price is negatively correlated with the carbon price and that changes in the price of oil have proportionately greater impact than changes in the price of carbon. For this scenario, it is apparent that in order to break-even, the first plant shown in the figure would require an oil price ~40 USD higher than the *N*th plant, or a carbon price in excess of 200 USD.tonne$^{-1}$.

A comparison of the supply-chains described in Figure 7 is shown in Figure 10. As expected the more cost competitive chains require a lower cost of carbon in order to break-even but all chains display a relatively shallow gradient, suggesting that their commercial viability will be relatively insensitive to the carbon price compared to the oil price. For example, if the oil price would be

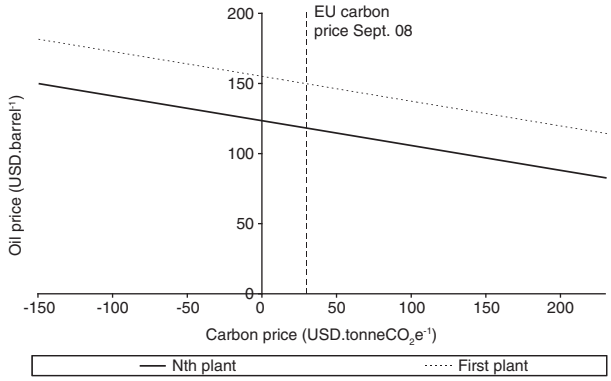


Figure 9

Break-even carbon and oil prices for a stand-alone softwood to ethanol plant. The example shown is for the spruce enzymatic hydrolysis supply-chain assuming: a plant capacity of 25 odt.h$^{-1}$ (oven-dry tonne), constant mid-range feedstock GHG emissions and price, and emissions from electricity equal to the EU27 average. All fossil carbon emissions are allocated to ethanol and residual solid fuel in proportion to their energy content.

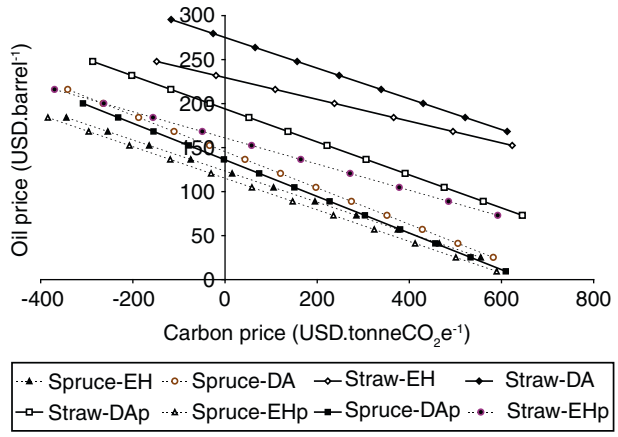


Figure 10

Break-even carbon and oil prices for a range of supply-chain scenario. All chains shown assume a plant capacity of 25 odt.h$^{-1}$, a constant mid-range feedstock price, emissions from electricity equal to the EU27 average, and *N*th plant finance. The softwood chains assume a mid-range embodied carbon content for feedstocks; the straw chains assume a low embodied carbon content. All fossil carbon emissions are allocated to ethanol and residual solid fuel in proportion to their energy content.

$\sim$125 USD.barrel$^{-1}$ the spruce enzymatic hydrolysis process would break even without a carbon subsidy but the spruce dilute acid hydrolysis process would require a carbon price around 125 USD.tonne$^{-1}$. One consequence of this finding is that cellulosic ethanol, although a reasonably efficient way of reducing GHG emissions, appears cost inefficient in terms of the cost per tonne of carbon saved. Yet, the volatility in oil and commodity prices that have occurred in the period 2006-2012 highlights the risk of taking such a static view of the market. A relatively small increase in the price of oil relative to the price of biomass could change this picture entirely. Over the long term, the relative economics of using biomass for co-firing or small-scale heat production compared to ethanol may play a more important role in determining commercial viability of cellulosic ethanol than the price of carbon. It should also be borne in mind that these competing uses of biomass provide energy services that attract different price premiums and may be more or less easily substituted with alternatives.

## CONCLUSION

The work carried out in the NILE project examined several crucial issues from the process standpoint: an exhaustive exploration of enzymes, engineering of yeasts, pilot-tests at a representative scale, paving the way to the definition of a competitive process. However, commercialising lignocellulosic ethanol requires progress from existing small-scale demonstration plant to large industrial installations. Each step along this path needs to be sufficiently attractive to persuade developers and investors that the technology is an opportunity worth pursuing. Although the future market is to a certain extent speculative, many of the organisations and people agents who might be expected to play a role along the development path – *e.g.* technology developers, feedstock suppliers, potential investors, government agencies, etc. – are already in place. In addition to research and development progresses, the decisions that these people take will, at least in the short term, determine the path to market.

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