ECN-E--07-045

Co-production of bioethanol, lactic acid, electricity and heat from lignocellulosic biomass

Public report EET project K01116

J.H. Reith

J.A.M. de Bont

(editors)

SEPTEMBER 2007

Acknowledgement

In the Netherlands a 4.5-year R&D project (2002-2006) has been performed with the central objective to develop technologies for the use of lignocellulose as a raw material for bioethanol and lactic acid production. The project was a co-operative effort of the industries Royal Nedalco, Purac biochem and Shell Global Solutions International and the R&D institutes ECN, Agrotechnology & Food Innovations, Wageningen University Dept. of Agrotechnology and Food Sciences, TNO Science and Industry and TNO Quality of Life (formerly TNO Environment, Energy and Process Innovation and TNO Nutrition and Food Research). The project was a follow-up of two EET Kiem projects i.e. KIEM 20117 GFV/WKK-procédé (2000-2001) and KIEM 01006 Thermozym (2001-2002). The project was coordinated by ECN.

This report was edited by J.H. Reith and J.A.M. de Bont. The contributing authors and their affiliations are:

- J.H. Reith, A.R. Boersma J.R. Pels, H. den Uil, M. Mozaffarian, J.F. Kuijvenhoven, E. van Zessen, ECN Biomass, Coal and Environmental Research
- W.T.A.M. de Laat, C. Meijer, H. Niessen, J.A.M. de Bont, Royal Nedalco BV
- M. Jansen, D. Visser, Purac biochem BV
- J.P. Haan, Shell Global Solutions International BV:
- R.R. Bakker, R.H.W. Maas, R. Weusthuis, Agrotechnology and Food Innovations BV:
- M.A. Kabel, H.A. Schols, Wageningen University, Food Chemistry Group.
- H.H. Beeftink, Wageningen University, Food and Bioprocess Engineering Group.
- J. Zeevalkink, G. Bos, P. Boer-Meulman TNO Science and Industry (formerly TNO-MEP)
- J van Groenestijn, H. van Buijsen, D. Binnema, M. van der Maarel, G. Klip, TNO Quality of Life (formerly TNO-MEP and TNO Nutrition and Food Research)

Subcontract work in the project was performed by

- Pneumapress Corporation, USA;
- Lettinga Associates Foundation, Wageningen;
- Nutrienten Management Institute, Wageningen.

The project was supported with a grant of the Dutch Programme EET (Economy, Ecology, Technology) a joint initiative of the Ministries of Economic Affairs, Education, Culture and Sciences and of Housing, Spatial Planning and the Environment under contract number EETK01116/4800001262. The programme is run by the EET Programme Office, SenterNovem.

Abstract

Introduction and purpose of the work

Large-scale CO_2 abatement is a very important issue in our society. Many options are open to reach this goal including the bioconversion of biomass into either energy carriers or bulk chemicals. In this respect, bioethanol and lactic acid are excellent candidates as liquid fuel and bulk chemical, respectively. As for the biomass to be used as feedstock, potential interference with human consumption should be avoided. Hence, lignocellulosic biomass is the preferred option for future large scale processes.

Bioethanol can be applied directly or in the form of ETBE in blends with petrol; lactic acid is a renewable alternative for petrochemical solvents and for production of polylactic acid (PLA) to replace petrochemical packaging materials and other synthetic materials. The preparation of fermentable sugars from lignocellulose is a major challenge for both bioethanol and lactic acid production and requires integral optimization of the trajectory from feedstock through fermentation to product recovery.

The above issues have been addressed in the Netherlands in a 4.5-year R&D project (2002-2006) by a consortium of industries and R&D institutes in the framework of the EET-program. The overall project objective was to develop and evaluate technologies for the use of lignocellulose as feedstock for bioethanol and lactic acid production. Wheat straw was selected as the model feedstock. Major R&D themes in the project were:

- Physical/chemical pretreatment for mobilization of (hemi)cellulose from the lignocellulose matrix. Both mild acid pressurized hot water and mild alkaline pretreatment were studied;
- Optimization of enzymatic cellulose hydrolysis with commercially available enzymes;
- Use of lignocellulose hydrolysates for ethanol and lactic acid fermentation including optimization of the fermentation process setup and process conditions;
- Combined Heat and Power (CHP) generation from fermentation residues including evaluation of the potential utilisation of ashes as secondary building material or fertilizer;
- System modelling and evaluation and integral plant design including economic evaluation;
- Ecologic evaluation in the form of a screening LCA;
- Specification and formulation of bioethanol in blends with petrol.

Approach

The project consisted mostly of experimental work with additional modelling and desk studies. The preparation of fermentable sugars from lignocellulose is a major challenge for both bioethanol and lactic acid production. It requires integral optimization of the trajectory from feedstock through fermentation, as addressed in this project. Pretreatment, enzymatic hydrolysis and ethanol and lactic acid fermentation were studied on laboratory and on pilot scale. Thermal conversion of fermentation residues for CHP generation was assessed via fuel analysis and lab scale combustion experiments. Furthermore the potential utilisation of ashes as building material or fertilizer was evaluated. The developed processes were modelled and evaluated in MS Excel and used as input for conceptual plant designs using Aspen Plus. A screening Life Cycle Analysis (well-to-tank) using SimaPro software was performed for ethanol production from straw as compared to gasoline and ethanol from starch or sugars. A quantitative estimate was made of the well-to-wheel CO_2 equivalents (CO_2 eq.) savings of cellulose ethanol in comparison to alternatives.

Results

Achievements were reached in various areas of the project:

- The development of a robust protocol for feedstock quality assessment, know-how on straw logistics and economics, and on its milling
- Technologies were developed for mild acid pretreatment in pressurized water and for mild alkaline pretreatment.
- Commercial enzyme preparations were tested for their ability to hydrolyze (hemi-)cellulose in the various pretreated straw samples. The relatively best enzyme was selected and used in further work.
- Industrial cellulases with optimal performance for hydrolysis of pretreated wheat straw and in-process viscosity control were characterized in detail for their functioning under process conditions. Model studies provided insight in cellulose-xylan interactions.
- Bioethanol production was developed at lab scale with specific attention being paid to the effect of toxic compounds on the fermenting yeast. Protocols were obtained for bioethanol production from wheat straw hydrolysates.
- Lactic acid production was developed at lab scale with attention being paid to the effect of oxygen on the fermenting fungus
- Bioethanol production was performed at pilot scale as based on the lab scale protocols. The feasibility of producing bioethanol from wheat straw was demonstrated. Several litres of straw ethanol were produced.
- The quality of the bioethanol resulting from the pilot-scale experiment was assessed and it was characterized with respect to application in fuels.
- An integrated model for enzymatic hydrolysis and C5 and C6 co-fermentation was developed. Simultaneous Saccharification and Co-Fermentation was selected as optimal for both bioethanol and lactic acid fermentation.
- Fluidized bed combustion was experimentally confirmed as a suitable technique for CHP generation from fermentation residues with good process stability and no detectable bed agglomeration. Utilisation of ashes and other mineral residues in building material or fertilizer was considered feasible.
- Overall, the optimization steps in the project have led to a concept for the production of ethanol from straw, based on mild acid pretreatment.
- Detailed conceptual designs for large-scale production plants (including economic evaluation) were made for both bioethanol and lactic acid production. The designs were adapted to the possible utilisation of all residue streams. In the designs heat integration and water recycle were optimized.
- The screening LCA indicated improved environmental performance for bioethanol from straw as compared to alternatives. Importantly, points for potential improvements in the production chain were uncovered.

Conclusions

The work has demonstrated the feasibility of producing either bioethanol or lactic acid from wheat straw. This statement holds both for the technological aspects, for the economics of the processes, and for their ecological impacts. These results have been laid down in detail in this report. It is recommended to continue research and development activities within The Netherlands in the area of the bioproduction of fuels and chemicals from lignocellulosic biomass. The field presents interesting opportunities in fighting human-related CO_2 production and it furthermore is situated at the forefront of scientific developments.

Contents

List of tables

List of figures

1. Introduction

J.H. Reith (ECN) and J.A.M. de Bont (Royal Nedalco BV.)

Large-scale CO_2 abatement is an important issue in our society. Many options are open to reach this goal including the bioconversion of biomass in either energy carriers or in bulk chemicals. In this respect, bioethanol and lactic acid are excellent candidates as liquid fuel and bulk chemical, respectively. As for the biomass to be used as feedstock, potential interference with human consumption should be avoided. Hence, lignocellulosic biomass is the preferred option for future large scale processes.

Bioethanol can be applied directly or in the form of ETBE in blends with petrol; lactic acid is a renewable alternative for petrochemical solvents and for production of polylactic acid (PLA) to replace petrochemical packaging materials –such as PET– and other synthetic materials. The industrial use of lignocellulose as a source of sugars for ethanol or lactic acid fermentation still requires considerable R&D. Ongoing efforts have thusfar not resulted in a technology applicable at industrial scale.The preparation of fermentable sugars from lignocellulose is a major challenge for both bioethanol and lactic acid production and requires integral optimization of the trajectory from feedstock through fermentation to product recovery.

The above issues have been addressed in the Netherlands in a 4-year R&D project (2002-2006) by a consortium of industries and R&D institutes in the framework of the EET-program. The overall project objective was to develop and evaluate technologies for the use of lignocellulose as feedstock for bioethanol and lactic acid production. Wheat straw was selected as the model feedstock. Major R&D themes in the project were:

- Physical/chemical pretreatment for mobilization of (hemi)cellulose from the lignocellulose matrix. Both mild acid pressurized hot water and mild alkaline pretreatment were studied;
- Optimization of enzymatic cellulose hydrolysis with commercially available enzymes;
- Use of lignocellulose hydrolysates for ethanol and lactic acid fermentation including optimization of the fermentation process setup and process conditions;
- Combined Heat and Power (CHP) generation from fermentation residues including evaluation of the potential utilisation of ashes as secondary building material or fertilizer;
- System modelling and evaluation and the generation of integral plant designs for large scale production of bioethanol and lactic acid from straw including economic evaluation;
- Ecological evaluation in the form of a screening LCA;
- Specification and formulation of bioethanol in blends with petrol.

The major results, conclusions and recommendations from the project are presented in this report.

2. Feedstock

R.R. Bakker (A&F), E. van Zessen and J.H. Reith (ECN)

A preliminary analysis was performed to establish the costs for collection, storage and transport of wheat straw in the Netherlands. The analysis indicated that costs for straw would range from 38.5 – 57 €/ton (on dry matter basis), representing a “best-case” and a “base-case” scenario, respectively. It should be noted that the costs are based on current state of the art technology for straw collection, storage and transportation in both scenarios. Depending on future technology

Figure 2.1 *Straw harvest in the Netherlands*

development further improvements to the straw collection/storage/transportation chain could be implemented that would lead to further decline in delivered straw costs.

In order to prepare larger amounts of milled straw for pilot-scale fermentation tests at A&F and TNO, approx. 50 kg of baled wheat straw was milled with a large knife-mill at A&F. The straw throughput and energy requirements were measured in order to gain a better understanding in energy use for milling, the primary pretreatment method in the entire straw-to-ethanol/lactic acid production chain. Results indicated that the total energy requirement was on average 19.3 kWh/ton (dry matter straw basis) with an average throughput of 169 kg/h. The measured energy requirement was well within the range that was earlier reported in literature for wheat straw (10.8 - 51.6 kWh/ton), barley straw (13.6 - 53.0 kWh/ton) at comparable dry matter contents (~92%). The measured energy requirement for wheat straw was however lower than energy requirements given for switchgrass (23 - 63 kWh/ton) and higher than given for corn stover (7 - 22 kWh/ton). The results of the milling tests were supplied to other partners in the project, for system evaluation purposes.

To obtain insight in the composition of straw in the Netherlands, biochemical analyses were carried out on straw samples taken from Dutch farmer's fields. The samples, 12 in total, were all analysed by using standard laboratory procedures by two partners in the project, A&F and ECN. The analytical procedures include determination of extractives, lignin, polysaccharides, and ash. The samples of wheat straw were taken from various farmers' fields in the Netherlands in 2003 and 2004, and include samples from the larger batch of wheat straw that was acquired by A&F, provided to other partners and used for all experimental work in the project. The straw samples did not undergo any physical or chemical treatment prior to analysis, except for size reduction which is done for analytical purposes. After analysis, the measured values were compared to two biochemical analyses of wheat straw in other countries which were available from Internet databases. Results from the analyses showed that both cellulose and hemicellulose content in Dutch wheat straw exhibit a significant variability (cellulose 29 - 39 weight%, wt%; hemicellulose 20.6-26.8 wt%; on dry matter basis) and that lignin content, which averages 22.1 wt%, can range from 19.4 to 26.6 wt%. In total, wheat straw contains on average 57 wt% of total carbohydrates, or 61 wt% on a dry, ash free basis (Table 2.1)

Table 2.1 *Average biochemical composition of 12 wheat straw samples in the Netherlands*

		EET Wheat straw (n = 12)		
Measured values				
		Average	Min	Max
Extractives	(wt%)	9.2	5.0	17.7
Total lignin	(wt%)	22.1	19.4	26.6
Uronic acids	(wt%)	2.4	1.3	3.5
Rhamnose	(wt%)	0.4	0.0	1.0
Arabinose	(wt%)	1.9	1.6	2.4
Xylose	(wt%)	19.7	16.8	22.4
Mannose	(wt%)	0.6	0.0	1.7
Galactose	(wt%)	1.0	0.4	1.5
Glucose	(wt%)	33.4	28.8	39.2
Ash	(wt%)	7.5	4.6	9.5
Calculated values		Wheat straw EET (NL)		
		Average	Min	Max
Total carbohydrate	(wt%)	57.0	51.4	66.1
Total lignin	(wt%)	22.1	19.4	26.6
Total hemi-cellulose	(wt%)	23.2	20.6	26.8
Cellulose	(wt%)	33.4	28.8	39.2
Hemi-cellulose (ash free)	(wt% daf)	25.1	22.3	29.1
Cellulose (ash free)	(wt% daf)	36.2	31.0	42.7
Total hemicellulose + cellulose (ash free)	(wt% daf)	61.2	55.3	71.4

wt% = weight %; wt%daf = wt% on dry ash free basis

Data also showed that the composition of Dutch wheat straw is largely similar to the reported composition in the two other countries, Denmark and the USA, with the exception of lignin: lignin content of Dutch wheat straw is approximately 30% higher. In addition, the reported ash content of the American wheat straw (10.2 wt%) however is remarkably high, which could be related to mechanized harvest and handling practices (Table 2.2).

Table 2.2 *Biochemical composition of wheat straw from the Netherlands, Denmark and the USA*

		Wheat straw	Wheat straw*	Wheat straw**
Calculated values		EET (NL)	Phyllis (DK)	DOE (USA)
		Average	Average	Average
Total carbohydrate	(wt%)	57.0	57.8	54.9
Total lignin	(wt%)	22.1	15.1	16.9
Total hemi-cellulose	(wt%)	23.2	24.6	19.2
Total cellulose	(wt%)	33.4	33.2	32.2
Hemi-cellulose (ash free)	(wt% daf)	25.1	26.7	21.4
Cellulose (ash free)	(wt% daf)	36.2	36.0	35.9
Total hemicellulose + cellulose (ash free)	(wt% daf)	61.2	62.7	57.3

wt% = weight %; wt%daf = wt% on dry ash free basis. * Database Phyllis record # 1092; http://www.ecn.nl/phyllis/
** DOE database # 154 http://www1.eere.energy.gov/biomass/feedstock_databases.html /

A draft analytical protocol was developed for determination of the fermentability and economic value of specific feedstocks for bioethanol and lactic acid fermentation. Basic procedures and parameters include: Sampling & sample preparation, determination of total solids and ash, analysis of full mineral composition, determination of extractives and monomeric sugars. Complementary analyses were selected for: determination of starch, calorific value fermentation residue, and enzymatic hydrolysis yield. The developed protocol is based on proven protocols by NREL, ASTM and TAPPI and protocols in use at A&F and ECN. Further development is recommended with respect to weighing of the various elements of the protocol, in relation to requirements of the production process. Furthermore validation of the protocol is recommended for a variety of feedstock and feedstock batches.

3. Production of ethanol and lactic acid from wheat straw using a mild acid thermal pretreatment

Jan Zeevalkink, Gijs Bos, Petra de Boer-Meulman, Johan van Groenestijn and Hugo van Buijsen (TNO Science and Industry and TNO Quality of Life)

In a mild acid thermal pretreatment process the wheat straw is treated under mild conditions (pH = 2 to 4, T = 180 – 190^{o}C). The goal of this process is to make the cellulose in the biomass more accessible. During this pretreatment and in the following hydrolysis, the wheat straw is converted into a mixture of monosaccharides (wheat straw hydrolysates). These monosaccharides are the feedstock for the production of ethanol and lactic acid. In this project pretreatment and hydrolysis were tested under different reaction conditions, to optimize the final sugar production. Finally fermentation experiments were performed, to investigate the effects of the pretreatment and hydrolysis processes on the fermentation process (Figure 3.1)

The following subjects were experimentally investigated:
- Particle size reduction of the wheat straw to produce a slurry
- Selection of type and amount of acid for the mild acid thermal pretreatment
- Reaction conditions for the mild acid thermal pretreatment
- Effects of inhibitors during hydrolysis and fermentation
- Separate hydrolysis and fermentation (SHF) of pretreated wheat straw
- Simultaneous saccharification and fermentation (SSF)

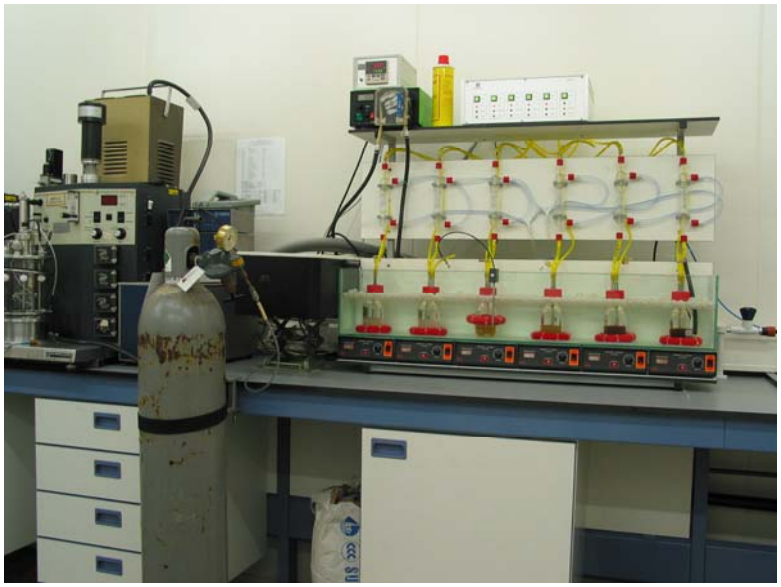

Figure 3.1 *Biological Activity Monitor (BAM). This system was used by project partners Royal Nedalco, TNO and A&F for fermentation research*

The experiments gave the following results:
- Slurries with a dry weight content up to 12 wt% are suitable for this process. This limitation is caused by the mixing behavior of the straw slurry.
- These slurries can be produced based on a dry particle size reduction method that produces particles from 2 – 3 mm.
- During the mild acid thermal treatment a pH of 2 is optimal for the sugar production. However, a pH of 4 also gives satisfactory results and minimizes the acid consumption as well as the gypsum production in case sulphuric acid and lime are used.

- Sulphuric acid is the best acid for the pretreatment process as this acid is relatively cheap and is effective in reducing the pH.
- Ethanol can be produced from the thermal/mild acid pretreated wheat straw in a 12 % dry weight slurry using SHF or SSF and *Saccharomyces cerevisiae.*
- SHF takes 24 hours for hydrolysis and 12 hours for fermentation (at pH 4.5); with an enzyme addition (GC220) of 128 g per 1000 g dry weight of wheat straw (1336 µl/100 ml slurry). The resulting ethanol concentration is 18 g/l.
- SSF at 32˚C with pre-hydrolysis takes 6 hours for the pre-hydrolysis and 48 hours for fermentation (at pH 5), while the enzyme addition is 1250 µl/100 ml slurry. The ethanol concentration reached is 15 g/l.
- The above implies that SHF leads to a higher volumetric ethanol production rate and higher ethanol concentrations. On the other hand, the enzyme consumption is slightly higher for SHF, which is an additional cost factor. At both optimum procedures, the lactic acid production is low.
- Furfural and acetic acid are the most important inhibitors produced during the thermal/ mild acid pretreatment. These inhibitors decrease the fermentation rate to levels known in molasses fermentation, which rate is economically viable as well.
- In the fermentation, furfural can be fully converted into the less toxic furfurylalcohol. The yeast uses this reduction preferentially to glucose reduction into glycerol.
- Thermal/acid pretreated wheat straw should be diluted 50% with water to obtain a fast conversion of glucose and xylose into lactic acid by *Rhizopus oryzae.*
- In lactic acid fermentations, the formation of large mycelium clusters should be prevented and the growth of small pellets should be encouraged. This guarantees the proper oxygen supply required for the production of lactic acid.

Figure 3.2 *Picture of large scale batch pretreatment experiments in a 25 liter autoclave*

Figure 3.3 *Large scale test with a pump to test the production of hydrolysate*

The results on ethanol production can be used for a pilot plant project. Most of the attention needed will be for pumping and producing more concentrated slurries. The level of inhibitors in 12% slurries is sufficiently low to allow an increase to 25% slurries, because at these concentrations no inhibition problems in ethanol fermentations are expected. Besides that, there is room to adapt the yeasts to inhibitors, which was not explored in this project. Using these 25% slurries, SSF will be more attractive than SHF. The thermal mild acid pretreatment can be used for other fermentation processes as well. Actions have been taken to acquire projects in this field. The way to produce lactic acid from wheat straw is now given: disperse growth is important and should be further optimized. Higher slurry concentrations should be tried as well. *Rhizopus oryzae* with a low pH optimum may be interesting for lactic acid fermentations combined with in situ product recovery of undissociated lactic acid.

4. Mild alkaline pretreatment and fermentation

R.R. Bakker, R.H.W. Maas and R. Weusthuis (A&F)

At A&F research was performed on alkaline pretreatment and fermentation of lignocellulosic biomass. In summary, $Ca(OH)_2$ (lime) pretreatment is a promising pretreatment route to enhance enzymatic hydrolysis of lignocellulose and can be characterized by high enzymatic degradability and no significant delignification or xylan degradation (Table 4.1, Figure 4.1).

Table 4.1 *Composition of untreated and lime-pre-treated wheat straw*

	Composition [%]						
	Rha	Ara	Xyl	Man	Gal	Glc	Lignin
Wheat straw, untreated	0.3	2.2	19.8	1.1	1.5	33.3	21.8
Lime-pre-treated wheat straw, washed solids	n.d.	2.0	18.9	0.0	0.0	32.8	23.5
Lime-pre-treated wheat straw, supernatant	n.d.	0.1	0.1	0.0	0.0	0.0	n.d.

Rha: Rhamnose; Ara: Arabinose; Xyl: Xylose; Man: Mannose; Gal: Galactose; Glc: Glucose.

During ethanol fermentation of lime-pretreated biomass, the primary fermentation inhibitor of concern is acetic acid, especially at low pH. In addition, lime pretreatment was easily scaled up to pilot-scale (200 L) for larger scale ethanol fermentations, and lime pretreatment could also be integrated with Simultaneous Saccharification and Fermentation (SSF) to lactic acid.

Both acid- and alkaline-pretreated wheat straw was successfully used as substrate for SSF in the available bioreactor at A&F, and at dry matter concentrations up to 20%, without severe difficulties due to high initial viscosity of the substrate.

The fermentability of all substrates at the selected process conditions was good, which was indicated by ethanol and glucose concentrations in the substrate throughout the test. Production of lactic acid as a byproduct during SSF however needs further study. In addition, enzymatic hydrolysis of pretreated wheat straw needs further optimization, in particular with regard to xylan-to-xylose conversion, as only 23% of xylan in the pre-treated straw was converted to (monomeric) xylose.

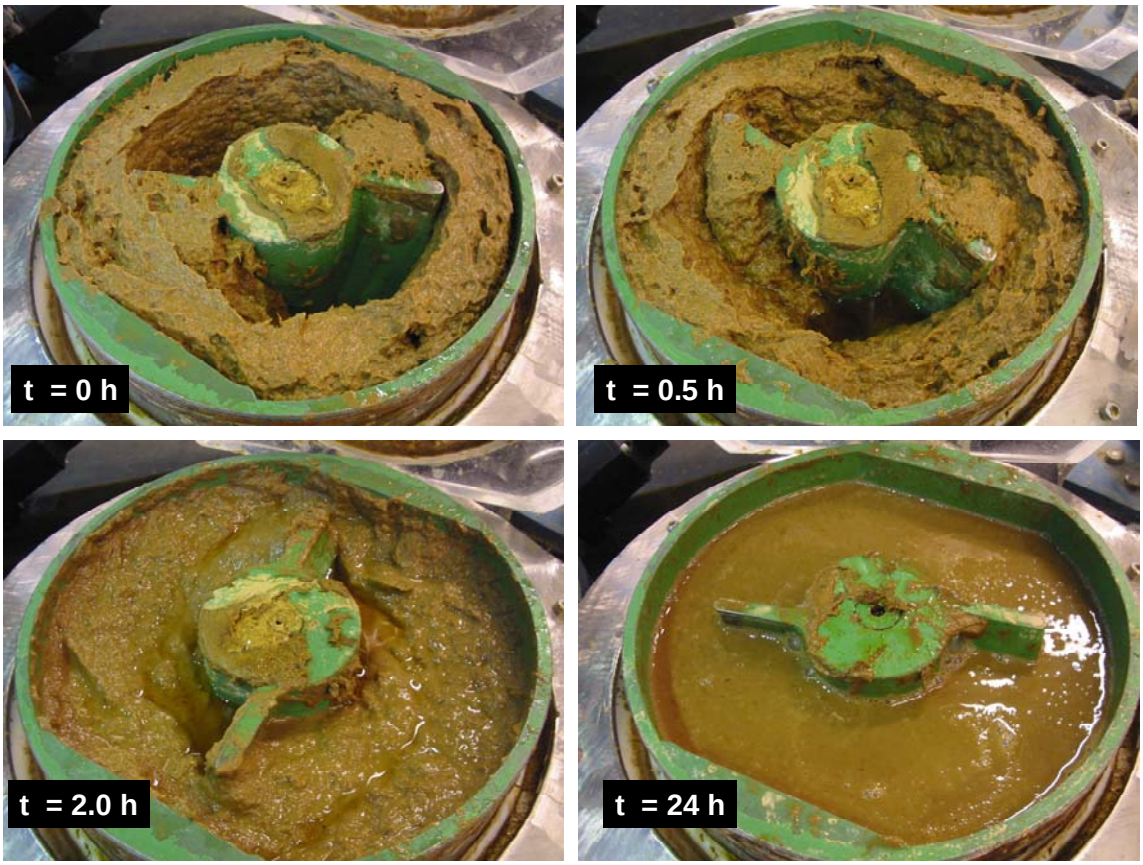

Figure 4.1 *View of pulp reactor during enzymatic hydrolysis at t = 0, 0.5, 2 and 24 h after adding enzymes*

Experiments with a thermotolerant Bacillus (*Bacillus coagulans* DSM 2314) clearly demonstrated the potential of using alkaline pretreated wheat straw in SSF-type fermentation to lactic acid. Automatic pH control in lactic acid fermentation was realized by feeding alkaline pretreated wheat straw for extended periods, without the need for an additional source of alkali and with no large fluctuations in pH. In general, no sugar accumulation occurred, indicating good fermentability of the substrate into a final relatively optically pure lactic acid concentration of 42 g/L.

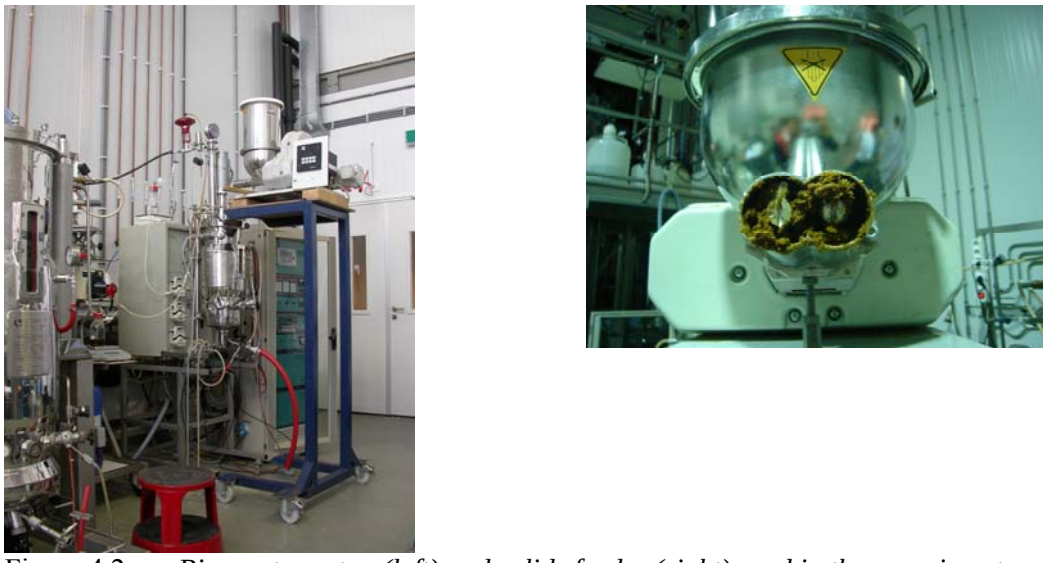

Figure 4.2 *Bioreactor setup (left) and solids feeder (right) used in the experiments.*

In addition to SSF-type fermentation to ethanol and lactic acid, experimental work was carried out to study the ability of the filamentous fungus *Rhizopus oryzae* to convert the pentose sugar xylose into lactic acid. Results indicated that *R. oryzae* is able to convert xylose by the two step reduction and oxidation route into lactate.

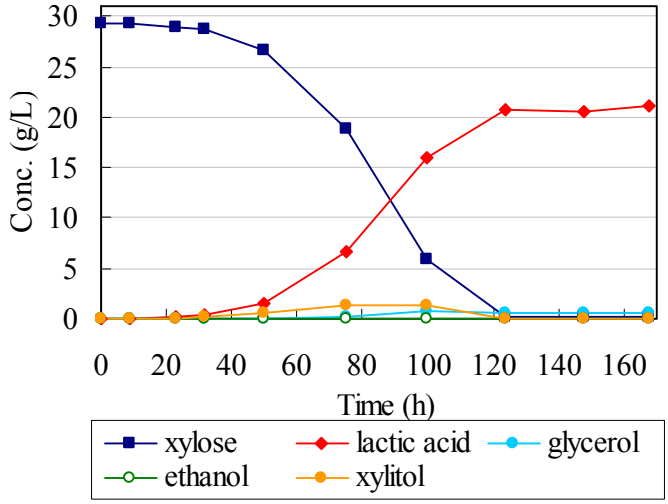

Figure 4.3 *Typical conversion of xylose by R.. oryzae CBS 147.22. Fungal biomass production was 1.3 g/L. Experiments were performed in baffled flasks and were aerobically agitated at 37°C. CaCO3 was used to neutralize the produced lactic acid and maintain pH. Substrates and products were determined by HPLC analysis.*

Finally, samples obtained from various origins from a sugar production process were screened for C5-fermenting microorganisms by using a standard protocol for screening and isolation. Isolates with the highest production of ethanol and lactic acid concentrations were tested for substrate and product tolerance and tolerance to inhibitors (such as acetic acid) that are potentially present in lignocellulosic hydrolysates.

5. Modelling ethanol production from cellulose

H.H. Beeftink (Wageningen University, Food and Bioprocess Engineering Group)

Research on ethanol production from cellulose was performed by René Drissen (01-04-2003 – 10-09-2004) and Marieke Willemsen (01-10-2004 – 01-11-2004). The main results are presented here.

A generic model for glucose production from various cellulose sources by a commercial cellulase complex.
Kinetics of cellulose hydrolysis by Cellubrix were described mathematically, with Avicel and wheat straw as substrates. As a simplification, three reactions were assumed: direct glucose formation and indirect glucose formation via cellobiose. Hydrolysis was found not to involve any soluble oligomers apart from low amounts of cellobiose. Phenomena included were substrate limitation, enzyme adsorption, glucose inhibition, temperature dependency, and enzyme inactivation. In addition, substrate heterogeneity was described by a recalcitrance constant. Model parameters refer to enzyme characteristics on one hand, and substrate-specific characteristics on the other. Quantitative model development was done on basis of Avicel hydrolysis. In order to describe wheat-straw hydrolysis, wheat-straw specific parameter values

were measured. Updating the pertinent parameters for wheat straw yielded a satisfactory description of wheat straw hydrolysis, thus underlining the generic potential of the model.

Quantitative evaluation of separate hydrolysis and fermentation vs. simultaneous saccharification and fermentation.
The hydrolysis model was extended with enzyme inhibition by ethanol. Inhibition by ethanol of direct glucose formation from ethanol and on cellobiose formation from cellulose was described with a first-order inhibition constant: K_i = 95 g/l. The impact of ethanol on cellobiose hydrolysis was found to be negligible. This model extension is essential for a description of simultaneous saccharification and fermentation, SSF. It was shown that yeast has no negative impact on the activity of cellulase. It was further shown that, with a relatively small amount of yeast, inhibitory glucose accumulation in a SSF-type process can be avoided. The SSF type process proved to be superior to the SHF type process, both with respect to ethanol yield per gram glucose (0.41 for SSF vs. 0.35 for SHF) and ethanol production rate, being 30% higher for an SSF type process.

6. Enzymatic hydrolysis

M.A. Kabel, H.A. Schols (Wageningen University, Food Chemistry Group), D. Binnema, M. van der Maarel and G. Klip (TNO Quality of Life)

Commercial enzyme preparations are potentially effective for processing lignocellulosic biomass feedstocks in order to obtain bioethanol. In plant cell walls cellulose fibrils occur in close association with xylans (monocotyls) or xyloglucans (dicotyls). The enzymatic conversion of cellulose/xylans is a complex process involving the concerted action of exo/endocellulases and cellobiases yielding glucose and xylanases yielding xylooligomers and xylose.

An overview of commonly measured cellulase-, cellobiase-, xylanase, and xylobiase-activity of fourteen commercially available enzyme preparations from several suppliers is presented in Table 6.1.

In addition to these main standard-activities, the enzymatic ‘side’-activities, needed to shave off side-chains like arabinosyl residues, glucuronic acid residues and O-acetyl groups from the xylan-backbone, were analysed as well.

In addition to these standardized tests the enzyme-efficiency of degrading native substrates was studied. Grass and wheat bran were fractionated into a water insoluble fraction (WUS), which was free of oligosaccharides and starch. Additionally, cellulose- and xylan-rich fractions were prepared by alkaline extraction of the WUS and were enzymatically digested. Hereby, the capability of cellulose and xylan conversion of the commercial enzyme preparations tested was measured. The results obtained showed that there was a large difference in the performance of the fourteen enzyme samples. Comparing all results, it was concluded that the choice of an enzyme preparation is more dependent on the characteristics of the substrate rather than on standard enzyme-activities measured.

Table 6.1 *Cellulase, cellobiase, xylanase, xylosidase activities (U/ml) and protein concentration (mg protein/ ml) of commercial enzyme preparations*

Enzyme sample	Supplier	FPU (U/ml)[1]	Cellobiase (U/ml)[2]	Xylanase (U/ml)[3]	Xylosidase (U/ml)[7]	Protein (mg/ml)	
						Bradford[4]	Pierce[5]
Cellubrix	Novozymes (Bagsvaerd, Denmark)	56	136	107	<5	43	122
Novozymes 188	Novozymes	<5	1116	31	<5	57	168
Cellulase 2000L	Rhodia-Danisco (Vinay, France)	10	nd[6]	568	<5	7	48
Rohament CL	Rohm-AB Enzymes (Rajamäki, Finland)	51	28	261	11	44	152
Viscostar 150L	Dyadic (Jupiter, USA)	33	111	3074	52	40	163
Bio-feed beta L	Novozymes	<5	12	184	<5	8	29
Energex L	Novozymes	<5	19	5	<5	28	222
Ultraflo L	Novozymes	<5	20	469	10	18	32
Viscozyme L	Novozymes	<5	23	5	<5	27	219
Cellulyve 50L	Lyven (Colombelles, France)	24	Nd[6]	54	<5	34	105
GC 440	Genencor-Danisco (Rochester, USA)	<5	70	272	35	29	118
GC 880	Genencor	<5	86	396	45	43	162
Spezyme CP	Genencor	49	Nd[6]	455	10	41	135
GC 220	Genencor	116	215	677	<5	64	211

[1] Filter paper Units determined according to IUPAC method; pH 5.0 and 50 °C
[2] determined according to IUPAC method with cellobiose; pH 5.0 and 50 °C
[3] determined with the Xylazyme AX method of Megazyme; pH 5.0, 50°C
[4] determined by using the Bradford-assay
[5] determined by using the Pierce-BCA-assay
[6] nd is not determined
[7] determined by the release of 1 µmol 4-nitro-phenol per minute per ml of enzyme from 4-nitrophenyl ß-D-xylopyranoside, pH 5.0 and 50°C

The effect of commercial available enzyme preparations on both alkaline and heat/mild acid pretreated wheat straw was studied. Of the available commercial enzyme preparations preparation GC220 appeared suitable for degradation of cellulose and xylan after pretreatment. Results also showed that GC 220 can lower the initial viscosity of these substrates as required for optimal fermentation (The stability of this GC220 enzyme preparation during storage at 4 °C was checked. The cellulose activity decreased in two years from 109 FPU to 70 FPU. Also, the cellobiase and xylanase activity decreased considerably. Another important aspect for industrial use of enzyme preparations is the variation from batch to batch. Both standard as well as 'side' activities of three batches of GC220 were analyzed. The cellulose activity of these three batches was rather similar, although the xylanase and β-xylosidase activities varied.

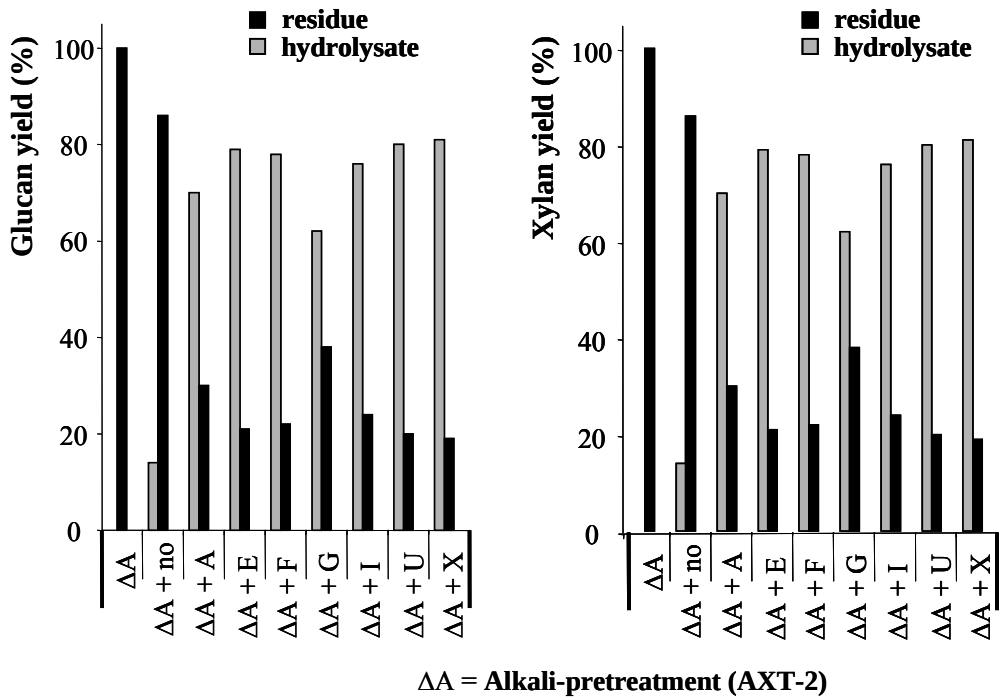

Figure 6.1 *Enzymatic degradation of alkali pre-treated wheat straw (AXT-2) by using various commercial enzyme preparations (see Table 6.1). A=Cellubrix; E=Rohament CL; F=BioethanolXL+CMC; G=Viscostar 150L; I=Rapidase Liq+; U=Spezyme; X=GC220. Per incubation 100 mg of substrate (dry weight) in 10 ml of 25 mM NaOAc buffer (pH 5) and 37 μl of enzyme was mixed, and incubated for 24h at 40°C. The amount of sugars in the remaining residues were analysed*

In order to understand better which enzyme activities were most important after a heat/acid pretreatment of wheat straw, the effect of process conditions used on the liquor and residue composition was studied. Hereto, the pretreatment conditions were expressed in a 'combined severity R_0'-factor'. The higher the combined severity factor (R_0') the more xylan was released from the wheat straw, but the more xylan decomposed and furfural formation occurred. The percentage of residual xylan present after pretreatment appeared to be a good indicator for cellulose degradability or bioethanol production: cellulose degradation by using commercial enzymes was higher at higher severities corresponding to a lower amount of residual xylan.

The xylan release and degradation was studied in more detail by using HPSEC (High-Performance Size-Exclusion Chromatography) and MALDI-TOF mass spectrometry (Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry). The more severe the treatment the more (acetylated) xylans with a Degree of Polymerization (DP) lower than 9 were analysed. The presence of (acetylated) xylans with a Degree of Polymerization (DP) of 9-25 increased slightly from low to medium severity. The quantification of the DP-distribution of the (acetylated) xylans released proved to be a good tool to predict cellulose degradability.

The affinity of xylan to cellulose is an important aspect of many industrial processes, e.g. production of cellulose, paper making and bioethanol production. However, little is known about the adsorption of structurally different xylans to cellulose. Therefore, the adsorption of various xylans to bacterial cellulose (BC) was studied. Also, the relationship between xylan size and adsorption was analysed. BC was used as cellulosic material, because of its high specific surface area and homogeneous structure.

In general, unsubstituted linear xylan parts favoured adsorption to BC. The presence of arabinosyl and O-acetyl substituents to xylan decreased the adsorption of xylan to BC considerably. Removing substituents resulted in higher amounts of adsorbed material. Most likely, increasing the number of unsubstituted xylosyl residues induced the formation of xylan-xylan interactions, which contributed to adsorption to BC. Schematic models are proposed showing the adsorption of structurally different xylans to BC.

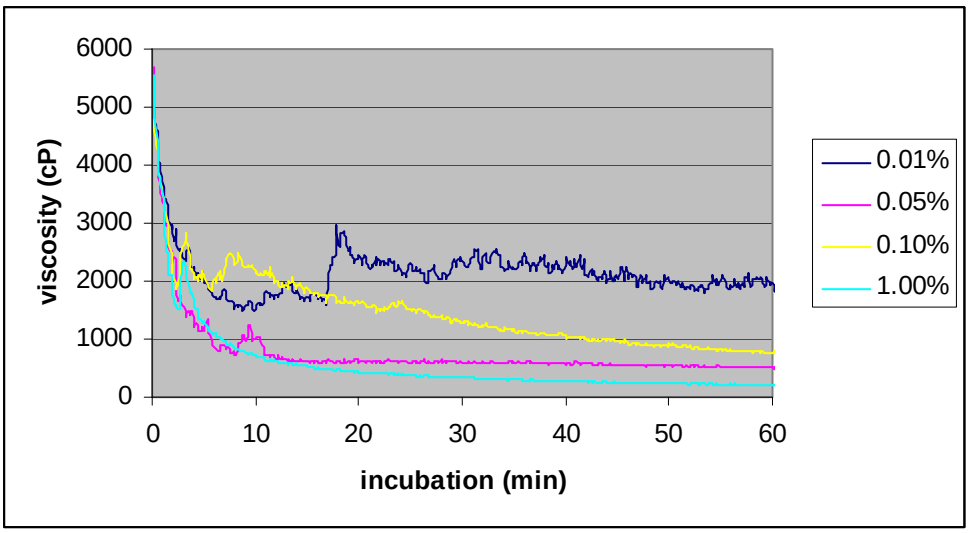

Figure 6.2 *Decrease of viscosity in an incubation of alkaline pre-treated wheat straw with GC220 in concentrations of 0.01%, 0.05%, 0.10% and 1.00%*

Various state-of-the-art techniques were shown to be of use in the identification of structural features of degraded and solubilised xylans (e.g. arabinosyl and acetyl substitution). These techniques included High-Performance Anion Exchange Chromatography (HPAEC), High-Performance Size-Exclusion Chromatography (HPSEC), and two types of Mass Spectrometry (MS). In addition to these analytical techniques a new method was developed, mainly useful for the identification of oligosaccharides as released during enzymatic saccharification. Hereto, various plant polysaccharide derived mono- and oligosaccharides were derivatized with the fluorescent 9-aminopyrene-1,4,6-trisulfonate (APTS) and subjected to capillary electrophoresis (CE) in combination with laser induced fluorescence (LIF) detection. CE-LIF was suitable for mol-based quantification of various APTS-monosaccharides. CE-LIF of APTS-oligosaccharides showed high resolutions, while analysis times were at maximum 15 minutes. The coupling of CE to electrospray-iontrap mass spectrometery (MS) with online UV detection showed to be a powerful technique in the identification of APTS-oligosaccharides. For the first time, various APTS-xylo-oligosaccharides, having either no, O-acetyl, arabinosyl or xylosyl substitutions at varying positions, were identified by using CE-LIF and CE-MS.

In conclusion, the overall efficiency of the feedstock pretreatment and enzymatic saccharification is depending on a good balance between a low inhibition of the final fermentation and good substrate degradability. The latter is a combination of degrading both physical and chemical features of the feedstock with minor increase of inhibiting compounds like acetic acid and furfural, enabling enzymes to reach the cellulose and hemicellulose structures. The complexity of the xylan structures, however depending on the feedstock used, should be considered when optimising enzyme-cocktail. The ‘side’ activities are of high relevance in order to degrade the xylans completely. Therefore, it is recommended for further research to analyse also experimental enzyme preparations enriched in ‘side’ activities, next to the commercial available ones as well.

7. Ethanol fermentation of hydrolysates

W.T.A.M. de Laat, C. Meijer, H. Niessen and J.A.M. de Bont (Royal Nedalco BV)

Fermentability studies have been carried out using hydrolysates either from wheat bran or wheat straw. Wheat bran contains limited amounts of cellulose whereas wheat straw is rich in cellulose. After scaling down the fermentation test set up, the optimum pH was determined and appeared to be around pH 4.2. From this study it is demonstrated to be possible to ferment hydrolysates at volumetric productivities > 2 g/L/h at an ethanol yield > 0.46 g ethanol /g sugar, showing that the objective set in the project could be met.

During the project a breakthrough was reported by researchers at BIRD Engineering and at Delft University of Technology[1]. A xylose fermenting yeast was developed and the organism fortunately was made available for experimentation within the present project. Using this yeast, a yield of 250 Litre of ethanol /ton of wheat straw (dry matter basis) was demonstrated whereas normally only 180 L/ton of wheat straw was found. The scale up of the pretreatment in batch autoclaves seems to be difficult and scale up experiments for the pretreatment are proposed. Experiments aiming at the effect of inhibitors towards the yeast revealed that commercialisation of cellulosic ethanol is not prevented by these by-products of hydrolysate preparation.

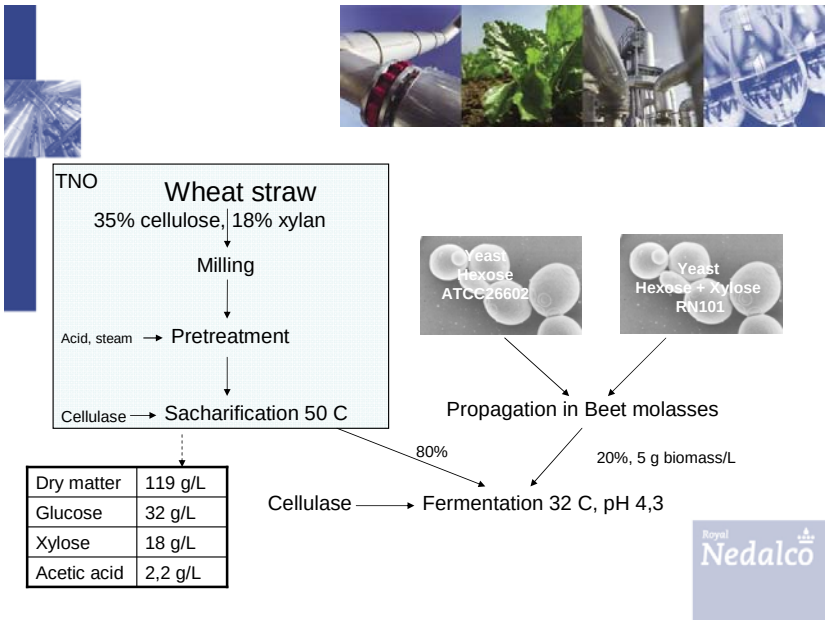

Figure 7.1 *Experimental setup for comparing a hexose + xylose yeast strain RN101 with a traditional hexose strain*

Enzymes represent a very important aspect for the economics of producing cellulose ethanol. Data obtained from 2 enzyme-producing companies were analyzed. It was calculated that the real cost of enzymes in the production process is only 0.015 dollar per gallon of ethanol. This observation opens new approaches in designing a process if the enzymes are produced on site of the ethanol-producing facility.

Several of the results obtained by Nedalco and other partners have been incorporated in the conceptual design of a large-scale ethanol plant based on wheat straw. Nedalco has given input to this design that was carried out by ECN and is presented in section 12 of this report.

[1] M. Kuyper et al, 2005. Evolutionary engineering of mixed-sugar utilization by a xylose fermenting Saccharomyces cerevisiae strain. FEMS Yeast Research 5 (2005): 925-934.

8. Lactic acid fermentation

M. Jansen and D. Visser (Purac biochem BV)

Work on the fermentation of wheat straw hydrolysates to lactic acid was carried out by Purac in close collaboration with A&F and TNO. The main objective of this research was to identify specifications that the hydrolysates should fulfill to allow successful and economical fermentation to lactic acid. The results of this research were used as input for the system evaluation and conceptual plant design performed by ECN (section 13), as well as for the lactic acid fermentation experiments with *Bacillus coagulans* 2314 carried out by A&F (section 4).

The main conclusion from the experimental work is that it is technically feasible to produce lactic acid from wheat straw hydrolysates, provided sufficient care is taken to minimize the presence of toxic components such as furans. This eliminates the need for additional upstream processing for removal of these components. The lactic acid strains used in these studies were reasonably tolerant to acetic acid, furfural and hydroxymethyl furfural.

The main conclusion from the economic evaluation (presented in section 13) is that the use of wheat straw as an alternative carbohydrate source is currently not economically feasible. A manufacturing cost price of 0.19 €/kg fermentable sugar was derived. Currently, this is lower than the price for white sugar, but the quality of this wheat straw derived sugar (impurities still present) will lead to a higher cost price for lactic acid. Therefore, significant improvements are required to achieve an economical process on the following items (order of importance):

- pretreatment (higher dry matter content, higher yield of fermentable sugar production)
- enzymatic hydrolysis (lower cost price, higher stability)
- fermentation sections (recycle loops).

The Down Stream Processing (DSP) of lactic acid was not studied here, but definitely needs to be considered in further research. However, near future commercialization is not anticipated.

9. Thermal conversion of fermentation residue

A.R. Boersma (ECN)

The cellulose and hemicellulose fractions of lignocellulose are largely consumed during fermentative production of bioethanol and lactic acid. The residue after fermentation of the pretreated and hydrolyzed wheat straw represents a significant part of the energy input and can serve as a fuel to provide the process with heat and power in a combined heat and power (CHP) plant. The residue consists of lignin, unhydrolyzed sugar polymers and oligomers, minerals from the feedstock and added process chemicals. Currently, experience related to the combustion of this type of wheat straw fermentation residue and insight in a suitable conversion technology for CHP are lacking.

The objective is to provide information about the possibilities of thermal conversion by means of

- fuel analysis;
- selection of thermal conversion technology and flue gas cleaning section (desk study);
- performance of combustion experiments (bench scale);
- evaluation of the possibility of co-firing the fermentation residues in a coal-fired power plant (desk study).

For ethanol fermentation of wheat straw, two process alternatives have been studied in this project: a) the mild acid route (sulphuric acid pretreatment) and b) the mild alkaline route

(calcium hydroxide pretreatment). For lactic acid fermentation, the wheat straw was alkaline pretreated.

Experimental fermentation residues were supplied by project partners and residue compositions were calculated from the process designs. Compared to the feedstock wheat straw, fermentation residues contain relatively high ash concentrations and relatively high concentrations of sulphur and calcium due to additions in the upstream processes. It is expected that the combustion behavior will resemble the behavior of the original feedstock wheat straw, rather than wood. The high sulphur content will result in significant SO_2 concentrations in the flue gas after combustion. Based on the analyses and modeled residue compositions, there is an expected risk of smelt induced bed agglomeration. The risk on sinter induced bed agglomeration is less than for straw, because of the partial removal of potassium in the upstream processes. A fouling risk is present. A knocking mechanism (as commonly used in coal fired boilers) is recommended.

Three combustion experiments have been performed with: a) acid pretreatment route ethanol fermentation residue (SSF-D), b) alkaline pretreatment route ethanol fermentation residue (SSF-E) and c) alkaline pretreated lactic acid fermentation residue (SSF-4). The dried fermentation residues were combusted in a laboratory-scale bubbling fluidized bed system (BFB; bed material: sand) of 5 kW(th). The experiments (temperature: 850°C, duration: 4-7 hours) showed no agglomeration and no extensive coating has been detected on the bed material that may indicate enhanced agglomeration risk. For a more profound assessment, longer experiments are necessary. The flue gas measurements show that SO_2 and HCl concentrations are higher than the emission limits. Part of the sulphur is released in the flue gas as SO_2, but a large fraction of the sulphur is bound to the ash due to the presence of calcium. With respect to fouling, high ash deposition rates are observed, but the ash is not sticky.

Figure 9.1 *Experimental set-up*

Circulating fluidized bed combustion ($200MW_{th,\ input}$) is selected as preferred conversion technology due to its suitability for moist fuels with a small particle size. The technology

(including gas cleaning) is considered as state of the art and it is expected that it has the lowest technological risks, although no industrial experience exists with wheat straw residues from ethanol or lactic acid fermentation. In the future, gasification may be a viable option due to its higher electrical efficiency, but this technology is currently still under development. Since the fermentation residues are considered as waste and contain significant amounts of sulphur, legislation for waste incineration applies. This implies that the gas cleaning has to include a flue gas desulphurization unit (wet or dry), an ElectroStatic Precipitator (ESP) for particulates removal, HCl removal system (in case of a dry Flue Gas Desulfurization system, FGD) and a de-NO_x system to meet the emissions limits.

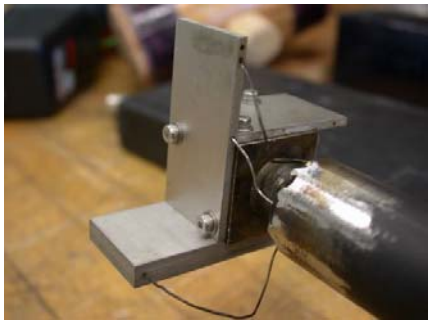

Figure 9.2 *Deposition probe before experiment*

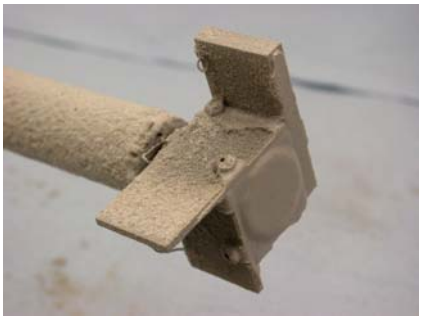

Figure 9.3 *Deposition probe after 7 hours of experiment*

Using a predictive linear co-firing model, the conclusion is drawn that there is no limitation for co-firing fermentation residues in a pulverized coal fired power plant up to 20 wt%, which is the targeted co-firing percentage in the long term. The model however does not include other specific criteria, which can only be experimentally verified with representative samples in co-firing tests. An extensive model and/or co-firing experiments (lab and full scale) will be required to determine more accurately the maximum co-firing percentage and with more detailed information about the used coal and the installation in which the co-firing of the fermentation residue is foreseen. Whether power plants will accept the fuel, depends on e.g. type of installation, permit, the used coal (blend), the composition of other secondary fuels and the price of the fermentation residue.

10. Utilization of mineral residues

J.R. Pels (ECN)

The remaining ashes from combined heat and power (CHP) generation from fermentation residues form a considerable waste stream and a bulk solution must be found. Another substantial part of the minerals is removed from the process in the form of a minerals concentrate.

Combustion ashes may be used directly or indirectly (as a raw material) for the production of fertilizer and building materials. The materials selected for evaluation constituted:

- fly ashes and bottom ash from fluidized bed combustion of fermentation residues of ethanol production (SSF-D and SSF-E) and lactic acid production (SSF-4); see section 9;
- modelled fly ashes from ethanol production using mild acid pretreatment;
- modelled mineral concentrate from ethanol production using mild acid pretreatment.

Fly ash was collected from the lab-scale combustion tests (see section 9) in filters during the last 6 hours of the experiment. The fly ash was collected at 200°C in four batches. Bottom ash was

collected after the experiment. Fly ashes were analysed for their chemical composition and subjected to Scanning Electron Microscopy (SEM).

Modelled fly ashes represented three different cases of ethanol production in a large-scale facility. All cases include acid pretreatment and hydrolysis, but with different levels of mineral removal upstream from combustion, where 0%, 40% and 75% of the soluble salts (limited to K, Na and Cl) that were originally present in the wheat straw have been removed from the fermentation residue. Fly ashes that result from these three cases are identified as A, B and C, respectively. The salt is removed in the form of a concentrated fluid containing K, Na and Cl. This stream is identified as "mineral concentrate".

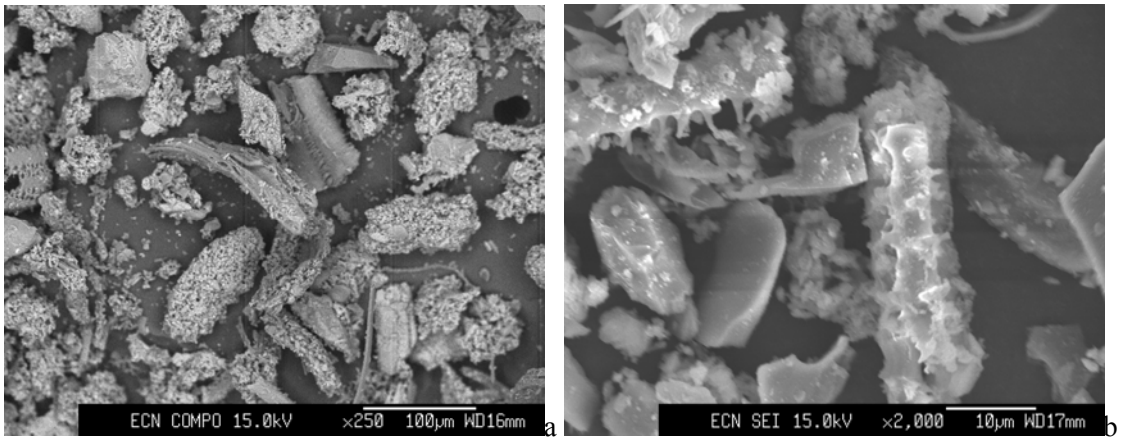

Figure 10.1*SEM picture of fly ash particles from fluidized bed combustion of wheat straw fermentation residue SSF-E (a) and a close-up of silica skeletons (b)*

Utilization as building material

The fly ashes (real and modelled) from combustion of wheat straw fermentation residue have been tested for compliance to the criteria of the EN-450 and of the Dutch Building Materials Decree (DBMD). The general conclusion is that ashes from combustion of fermentation residues can – in principle – be used as building materials, but they have limited options:

- The fly ashes from SSF-D and SSF-E do not comply with category 1 limits of the DBMD. They may comply with category 2 limits (restricted use) but that is not an attractive option. Direct utilization as bulk building materials is unlikely or impossible. Chloride, sulphate, molybdenum and selenium are the critical components.
- The fly ashes from SSF-D and SSF-E do not comply with the criteria of the EN-450 and are not suitable for utilization as filler in cement in the same way as powder coal fly ash is used currently. Chloride is the most critical component; sulphate is likely to be a problem as well.
- With the current knowledge of the up-scaled process, it is unlikely that optimisation of hydrolysis and salt removal will eventually result in fly ashes that comply with the DBMD or EN-450.
- Fly ashes from wheat straw residues appear to be attractive for application in special cement products or as raw material for building products, where chloride content is of less importance.
- Bottom ashes from up-scaled installation will probably comply with the category 1 limits of the DBMD and are likely to be useable in applications like road construction.
- More accurate assessment of the potential of the ashes for utilization in building materials will require larger samples that are more representative for full scale.

Utilization as fertilizer

In the evaluation of direct utilization as fertilizer, the agricultural value of the materials is calculated based on the available nutrients. This results in a minimum dosage for use on land in kg per hectare. Also, for each material, a maximum allowable dosage is calculated, based on the

content of contaminants. When the maximum allowed dosage exceeds the minimum required dosage the material can potentially be used as fertilizer.

Table 10.1 *Summary of agricultural value of products*

Product	nutrient	comparable fertilizer	expected problems	overall assessment (on farmland)	minimum dosage in kg ha^{-1} (dry)
SSF-D fly ash	P, K, S and Ca	unknown	low nutrient content, low availability of P, dusting	-	3500-4000
SSF-E fly ash	S and Ca	gypsum ($CaSO_4$)	dusting	+/-	400-450
model fly ash A	K, S and Ca	potassium sulphate (K_2SO_4)	dusting	+	600-1200
model fly ash B	K, S and Ca	potassium sulphate (K_2SO_4)	dusting	+	900
model fly ash C	K, Mg, S and C	"patentkali" (K_2SO_4 + $MgSO_4$)	dusting	+	1900
mineral concentrate	K	potassium sulphate (K_2SO_4) potassium chloride (KCl)	dry matter content?	+	100

The general conclusion is that ashes from combustion of fermentation residues may be used as fertilizer, but they have limited options:

- Potassium is the key element and the most attractive component for application as fertilizer. Fly ashes that contain a reasonable amount of K are likely to be permitted as fertilizer. These are ashes resulting from a process without removal of soluble minerals. Those fly ashes can have a small positive value.
- Fly ashes of residues SSF-D and SSF-E, as well as model ash C (which result from a process with substantial removal of soluble minerals) have limited agricultural value and will not be permitted to be used as fertilizer.
- Materials with $CaSO_4$ as primary mineral may be used as fertilizer, but $CaSO_4$ is not a valuable fertilizer.
- The mineral concentrate has the best options for utilization as a fertilizer, in particular when available with low water content.

There appear to be two strategies with regard to removal of soluble salts upstream from the combustion system that lead to utilization of the fly ashes either as fertilizer or in building products:

1. Minimal removal of soluble minerals upstream from the combustion will result in fly ashes that have a potential use as fertilizer, but it almost prevents utilization as building material.
2. Maximising the removal of soluble minerals will disable the utilization of fly ashes as fertilizers, but improves the changes for utilization as building materials. The mineral concentrate that is produced in this case is an attractive fertilizers product.

11. Fuel-ethanol: specifications, logistics

J.P. Haan (Shell Global Solutions International BV)

Ethanol/gasoline mixtures have the tendency to phase separate when the water content has reached a certain level. We tested the influence of ethanol and water content in ethanol/gasoline/water mixtures on phase separation. Experiments were done either by cooling a certain mixture and observing the temperature of haze formation (first sign of phase separation) or cooling mixtures to a fixed temperature and analyzing the separate liquid phases.

The use of dry fuel-ethanol, containing a maximum of 0.2-0.3 % water, should in principle circumvent the undesired phase separation phenomenon. Furthermore to lower the risk of phase separation of ethanol/gasoline blends by water, a minimum of 4-5 % ethanol in the blend should be maintained.

Equipment that transports or stores fuel-ethanol or ethanol/gasoline blends should be cleaned and dried prior to a first use of fuel ethanol (neat or blends with gasoline). This equipment should then be reserved for the mentioned fuels.

Pipeline operators are generally very hesitant to transport fuel-ethanol by means of multi-product pipelines. Reasoning behind this is the risk of dirt and water pick up by fuel-ethanol. A dedicated pipeline could serve as a solution, but the economics of such a solution will determine whether this option is viable (special case in e.g. in Brazil or USA).

Large batches of fermentation ethanol (originating from wheat straw feedstock) were worked up and concentrated to above 90 % wt ethanol by means of distillation (no dehydratation). In general the distilled products were relatively clean. The acid pre-treated products contained however too much methanol, but this may be removed in practice in continuous distillation process.

More reason for concern is the acid level of one of the ethanol products originating from an acid pre-treated feedstock. It is unclear why the acid contaminant level of this particular product is much higher than a comparable distilled product from the same production route (i.e. similar acid pretreatment). However it shows that the contaminant level of a fuel-ethanol needs to be examined on a regular basis.

12. System evaluation and conceptual design of a straw to bioethanol plant

J.F. Kuijvenhoven, H. den Uil, E. Deurwaarder, J.H. Reith (ECN), W.T.A.M. de Laat and H. Niessen (Royal Nedalco BV)

Models for co-production of bioethanol, electricity and heat from wheat straw for both mild alkaline and thermal mild acid pretreatment were developed in MS Excel and used to generate mass and heat balances and estimates of investments and operational costs. The models were subjected to sensitivity analyses to identify major process sensitivities and design parameters in a system evaluation. The results and the developed block scheme (Figure 12.1) served as a starting point for the conceptual plant design.

The objectives of the conceptual plant design were:
- to design an integrated plant for the conversion of wheat straw to bioethanol, electricity and heat in which the project results are combined;
- to develop solutions for heat integration and internal water recycling;
- to optimize utilization of waste streams including ashes, CO_2 and waste water;
- to estimate investments and production costs in a large-scale production plant;
- to identify points for attention in the design as input for further R&D.

Additional objectives were to prove the process concepts of zero waste water effluent and energy self-sufficiency in a large-scale bioethanol production plant using wheat straw as feedstock.

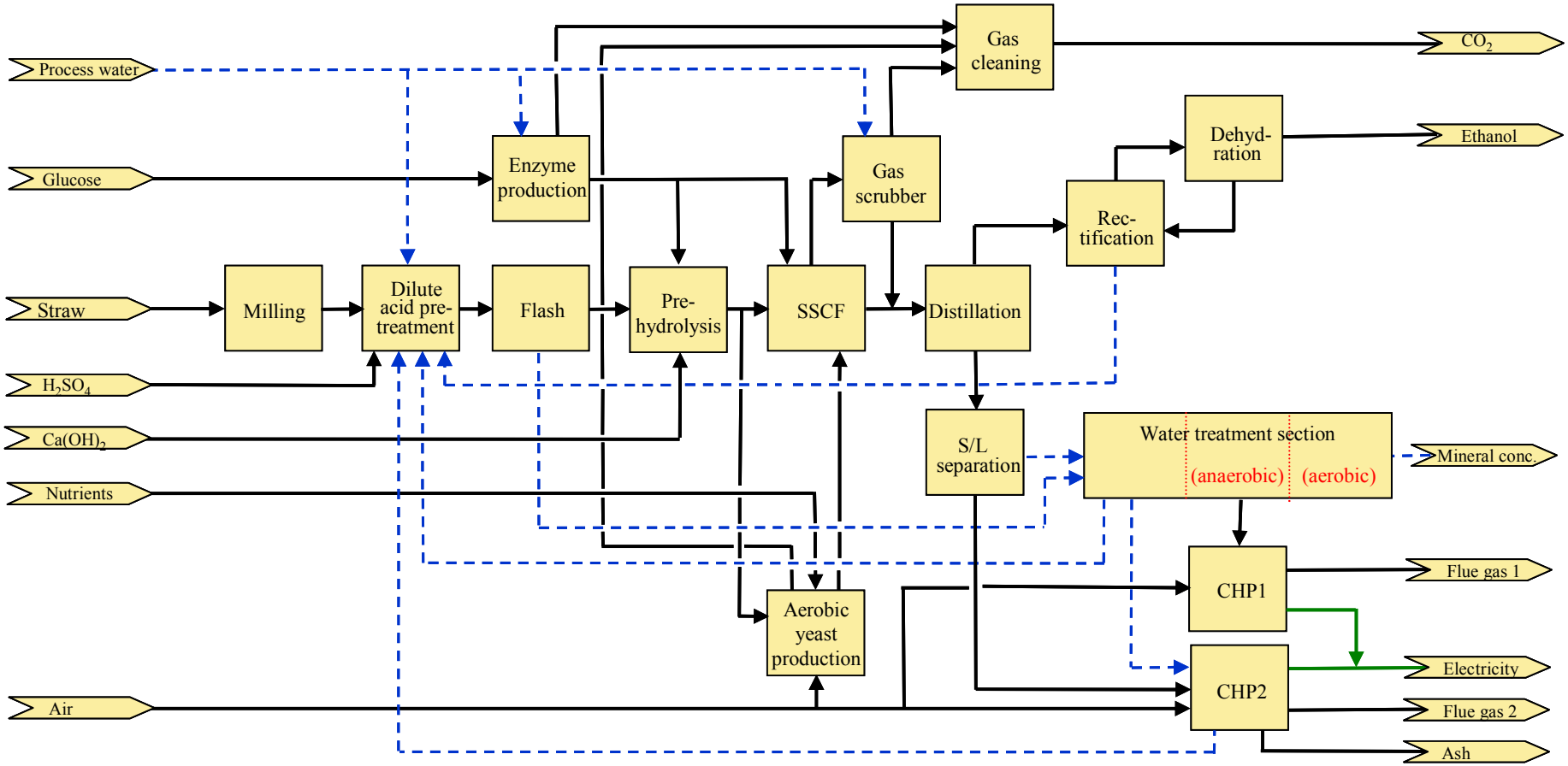

Figure 12.1 *Straw to bioethanol process block scheme*

The designed plant processes 773 kton/y (11% moisture) of wheat straw and has a production capacity of 190 million Litres of bioethanol (99.6 wt%) per year with 8,000 hours operating time, on-site enzyme production and an assumed plant life of 15 years. Thermal mild acid treatment was selected as pretreatment method. The feedstock wheat straw is fed to the system at 25% dry weight.

The focus in the conceptual design was on heat integration and process water recycling. Detailed mass and energy balances were created in Aspen Plus and the effects of recycling of water and soluble minerals on the variety of process steps and composition of waste streams were modelled and verified with experimental and literature data. Not all components which are needed to model the process were available in the standard Aspen plus database. To overcome this obstacle, the standard process model database was expanded with self–defined components for which the data were taken from literature or modelled with the use of properties of similar components. Thus an improved, extended component database was created.

In an iterative way the process design was shaped and adapted to a zero wastewater effluent plant that generates two useful side products: a mineral concentrate (K, Cl), which can potentially be used as fertilizer, and ashes from the CHP section that can be used as building material (see section 10). The purified water is recycled to the process (Figure 12.2). Removal of 40% of the minerals was chosen in the base case producing a mineral concentrate which does have fertilizer value, as discussed in section 10.

Detailed flow sheets, indicating all equipment, were created. An economic evaluation was performed to estimate plant investment costs and the production cost and minimum selling price for bioethanol from straw.

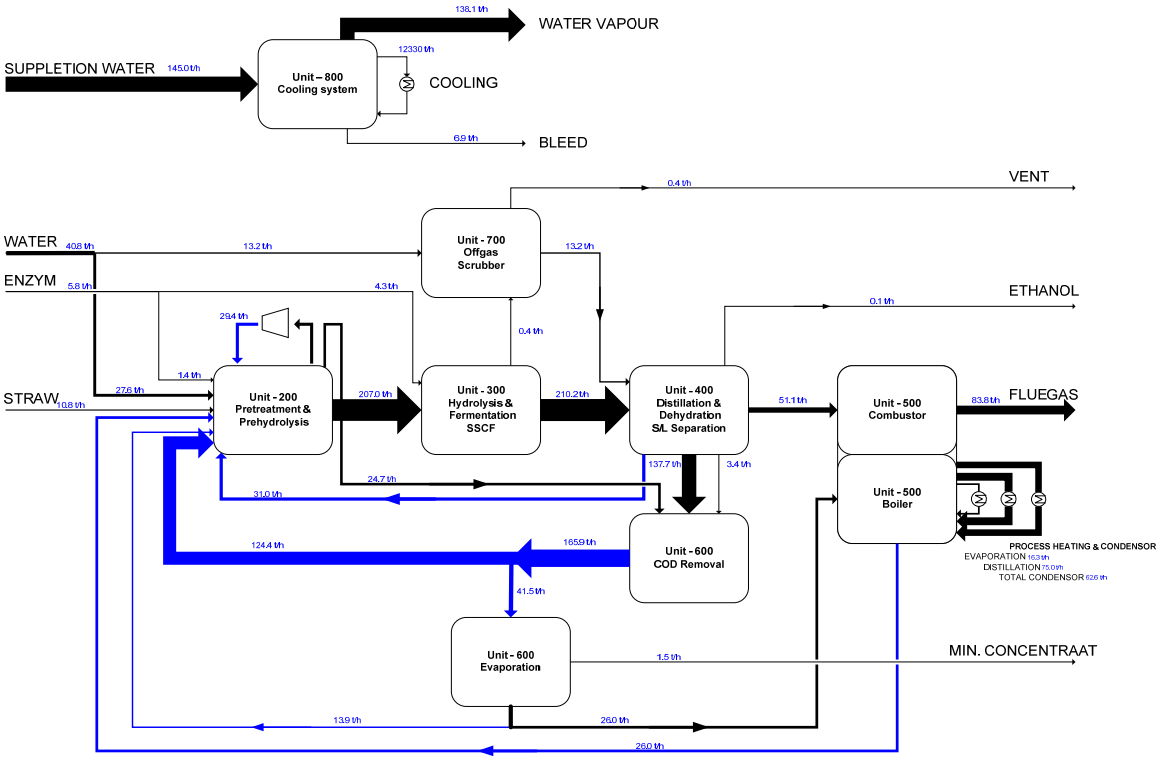

Figure 12.2 *Water flow (t/h) in process (black arrows) and recycle streams (blue arrows). The thickness of the arrows indicates the relative amount of water*

The developed process concept converts straw into fuel grade ethanol, electricity and heat and does not make use of external energy sources. Via thermal mild acid pretreatment and enzymatic (pre)hydrolysis sugars are produced from hemi-cellulose and cellulose, the major

components of straw. Both the C_5 and C_6 sugars produced are co-fermented to ethanol by a genetically modified yeast strain. The ethanol is subsequently purified by distillation, rectification and dehydration. A residue, that contains mainly lignin, minerals and unhydrolyzed sugar polymers and oligomers, is dewatered and combusted in a CHP unit to generate heat for the production process and electricity (31.8 MW_e). The effluent is treated by anaerobic digestion to yield biogas, which is used to produce additional electricity (7.5 MW_e). An electricity surplus of 25.0 MW_e is available for export to the grid. The energy efficiency of the process is 36.5% to ethanol and 6.6% to net electricity, overall 43% on LHV_{ar} basis.

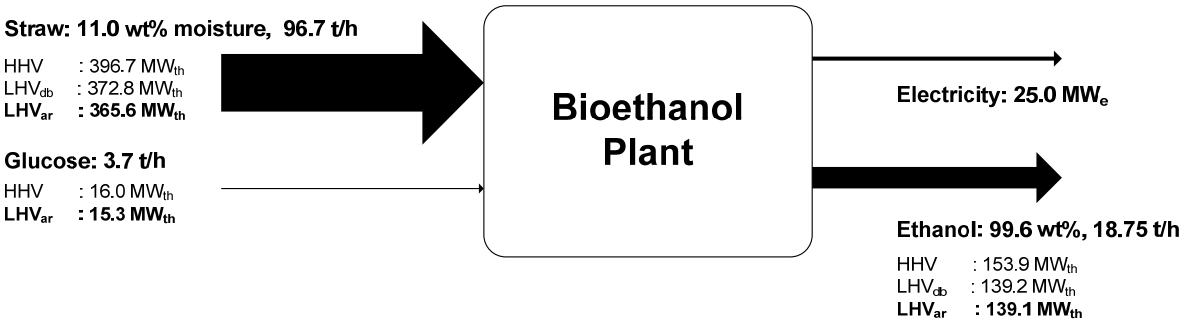

Figure 12.3 *Main Inputs & Outputs of the straw to ethanol plant*

The total capital investment (CAPEX) is estimated at 319 M€, with the largest share (47%) for the CHP unit including a dedicated flue gas cleaning section (Figure 12.4).

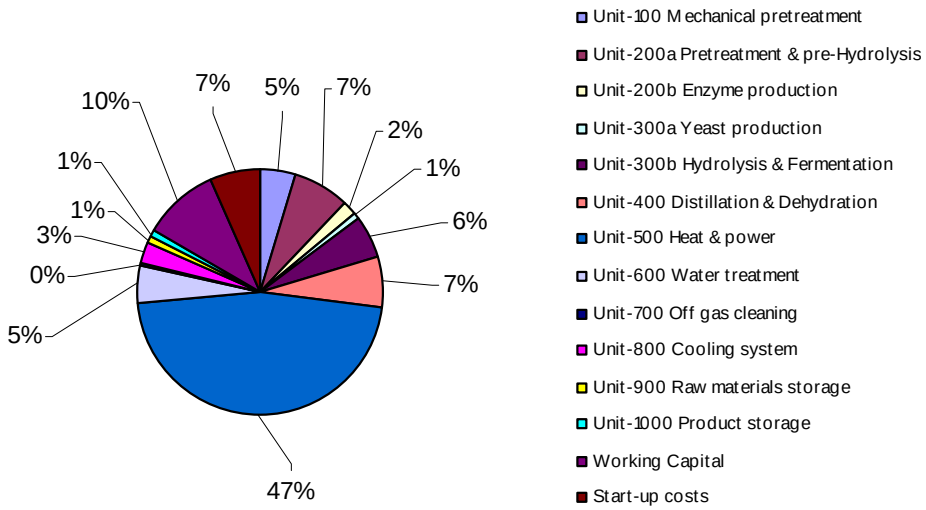

Figure 12.4 *Distribution of investment costs (CAPEX)*

The ethanol production cost at straw feedstock costs of 38.50 € /ton dry weight was estimated at 0.52 €/L, including benefits from net electricity delivered to the grid (0.04 €/L). The capital costs are the largest cost driver (33%) followed by the straw feedstock costs (25%) as shown in Figure 12.5. The minimum ethanol selling price for an IRR of 15% was estimated at 0.75 €/L.

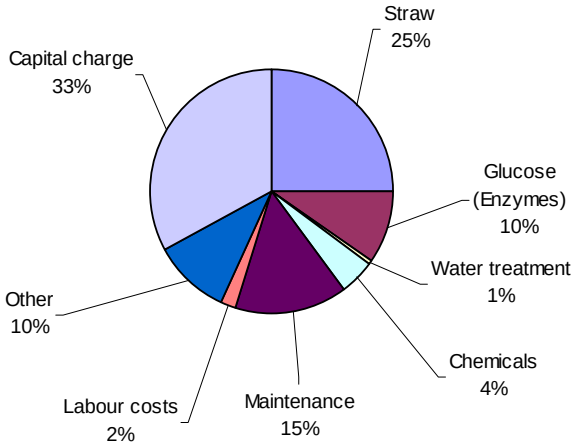

Figure 12.5 *Distribution of production costs*

The work shows that it is possible to design and operate the plant in a way that useful co-products are created without affecting process conditions in a negative manner. Design of a "zero effluent" plant without wastewater effluent is indeed possible (see Figure 12.2). Compared to a non-integrated process a reduction by 60% in water consumption from 19 to 7.8 L/L ethanol is attainable. The remaining water consumption is used for the largest part in the cooling towers (6.1 L/L ethanol). This suggests that the use of air-cooling could be considered to further reduce water consumption. Water also leaves the plant in the flue gas from the CHP unit. To obtain a process without any net water consumption, recovery of water from the solid residue that is fed to the CHP unit or flue gas is necessary. However the environmental benefits of such further reductions should be balanced against diminishing "green" electricity production.

13. System evaluation and conceptual design of a straw to lactic acid production plant

J.F. Kuijvenhoven, H. den Uil (ECN), M. Jansen, D. Visser (Purac biochem), R.R. Bakker, R. Maas and R. Weusthuis (A&F)

A system evaluation and a detailed conceptual plant design were performed for lactic acid production from wheat straw. The process block scheme resulting from the system evaluation (Figure 13.1) was updated to accommodate new ideas and experimental results in close collaboration with A&F and Purac biochem. The objective was to generate a first conceptual design of a facility producing lactic acid, steam and electricity from straw.

The pretreatment method used in the design is the mild alkaline method. The process can fulfil its own energy needs. The Down Stream Processing (DSP) section of Purac, in which the lactic acid solution is processed to pure lactic acid, derivatives and by-products, was left out of the scope the design. A special feature of the process is the re-use of alkaline pretreated wheat straw as a medium for pH control in the successive Simultaneous Saccharification and Co-Fermentation (SSCF) task, without any regeneration.

A detailed process block scheme and a Gannt diagram (not shown) for the batch processes were created. The process was modeled with the aid of Aspen Plus flowsheeting software to obtain mass and energy balances. The necessary equipment for the process sections and the costs were estimated by means of an Excel model.

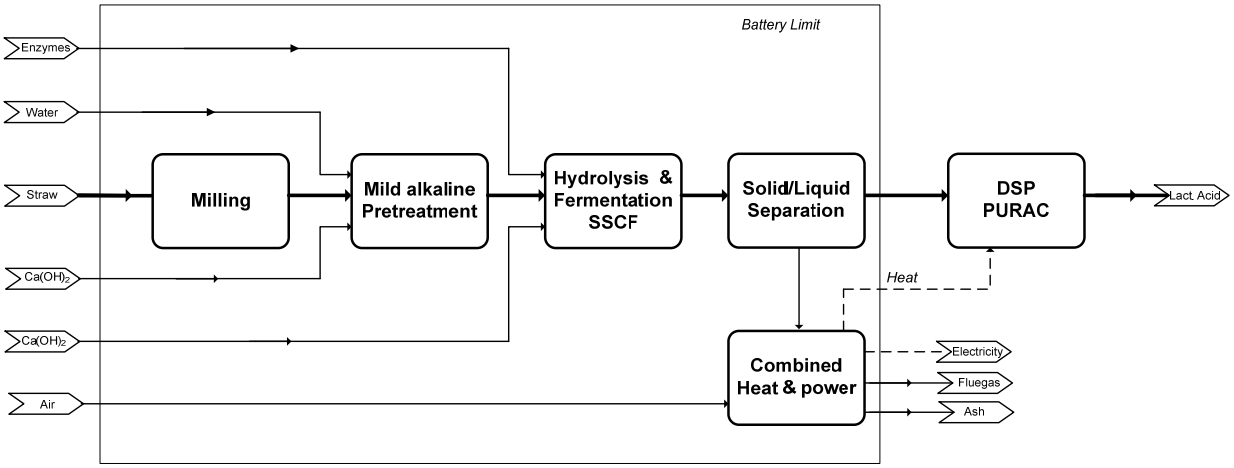

Figure 13.1 *Simplified block scheme of lactic acid production from straw feedstock*

The plant processes 227 kton/y of straw and produces 1187 kton/y LA solution (95.4 kton/y @ 8 wt%), 0.16 PJ/y of green electricity to the grid and 208 kton/y of steam for the DSP. Hot water is recycled from the DSP. The total investment costs (CAPEX) for the facility are estimated at 127 MEuro. The production costs of LA in the solution are estimated at 0.44 Euro/kg_{LA}, including benefits from delivered steam value (0.04 Euro/kg_{LA}) and net electricity delivered to the grid (0.04 Euro/kg_{LA}).

During hydrolysis of the hemicellulose and cellulose 114 kton/y of fermentable sugars are produced. The calculated production costs of these sugars amount to 0.19 €/kg_{Sugars}, including benefits from delivered steam value (0.04 €/kg_{Sugars}) and net electricity delivered to the grid (0.03 €/kg_{Sugars}). As discussed in section 8, this is lower than the current price for white sugar, but the quality of this wheat straw derived sugar (impurities still present) is expected to lead to a higher cost price for lactic acid compared to current production costs.

14. Life Cycle Analysis of bioethanol from straw

M. Mozaffarian (ECN)

The goals of the Life Cycle Analysis (LCA) are to assess the environmental impacts of bioethanol production from wheat straw, and to compare these with gasoline and first generation bioethanol production from sugar beet and wheat.[2]

A "simplified" LCA was carried out according to ISO 14040 standards. The bioethanol process is considered as a black box, only inputs and outputs are considered. Bioethanol is chosen as the "end point" of the study. The LCA has therefore a "cradle-to-gate" character, with the gate being the fuel pumping station where bioethanol is delivered to vehicles. In other words, the LCA is equivalent to a "well-to-tank" (WTT) analysis. Also an estimate has been made for use of ethanol as transportation fuel equivalent to a "well-to-wheels" (WTW) analysis. Data from 1990 to 2010 and beyond has been used, with the Netherlands as the geographic region. The functional unit for this study is 1 MJ of bioethanol for use as transportation fuel (on LHV basis). As modelling tool the package SimaPro, developed by PRé Consultants, has been used. The data used for the inventory analysis are all (with the exception of data for glucose) based on the Ecoinvent database, available in the SimaPro software. When possible, data for the Dutch

[2] The LCA data for ethanol from sugar beet and wheat are based on Malça, J., Santos, C., Freire, F.: Life Cycle Assessment of bioethanol from sugar beet and wheat-comparison with gasoline. 9th International Chemical Engineering Conference, September 21-23, 2005

conditions are used. Using the CML[3] impact assessment method, the following environmental impact categories were considered: depletion of abiotic resources, global warming, ozone layer depletion, human toxicity, fresh water aquatic ecotoxicity, marine aquatic ecotoxicity, terrestrial ecotoxicity, photochemical oxidation, acidification, and eutrophication.

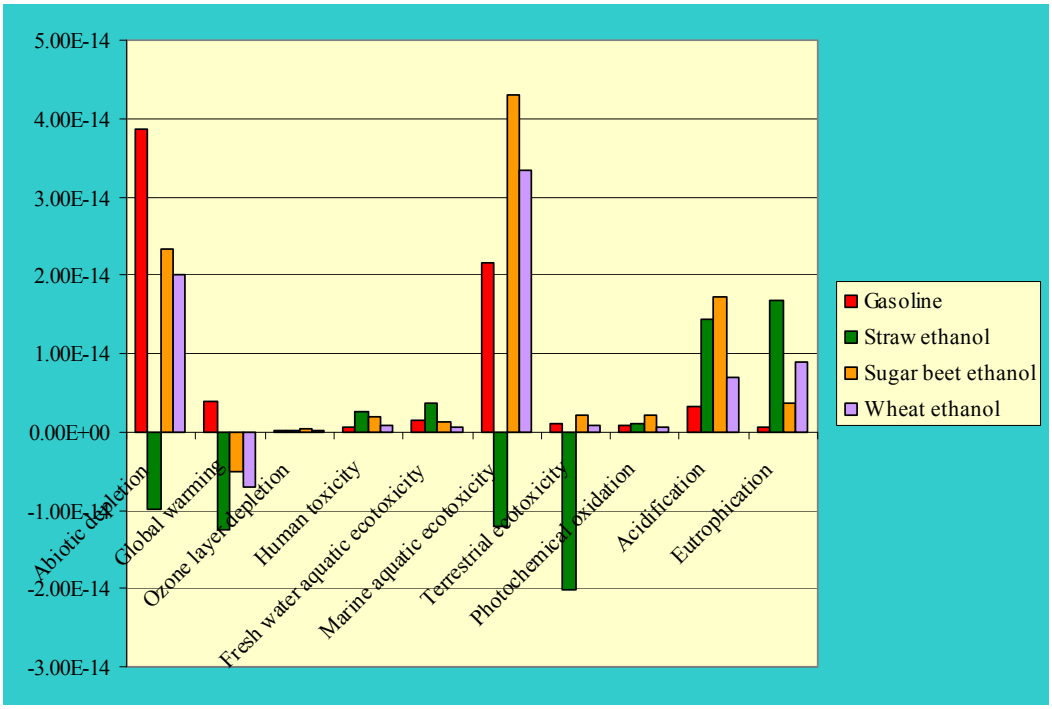

Figure 14.1 *Environmental impacts of straw ethanol, alternative ethanol routes (based on sugar beet / wheat), and gasoline (normalisation results)*

The LCA results show that straw-to-ethanol has a better environmental performance with respect to abiotic depletion and global warming, as compared to gasoline. Gasoline has an advantage in the environmental impact categories acidification and eutrophication. While straw-to-ethanol has advantages in the impact categories marine aquatic and terrestrial ecotoxicity, it has a slightly worse performance with respect to human toxicity and fresh water aquatic ecotoxicity. After normalisation of the results based on total European emissions "Western Europe, 1995", the significant impacts (Figure 14.1) were on marine aquatic ecotoxicity, abiotic depletion, terrestrial ecotoxicity and global warming (all with advantage for bioethanol), followed by acidification and eutrophication (with advantage for gasoline).

The avoided amount of fossil-based electricity production by heat & power generation from non-fermentable biomass fractions has a large impact on the overall environmental performance of the process, especially in the impact categories marine aquatic ecotoxicity, abiotic depletion, and global warming. This is also the main reason for the better environmental performance of the straw-to-ethanol process, compared to the sugar beet and wheat ethanol routes, because the energy requirement of the latter cases is usually fulfilled by external fossil-based fuels. Optimisation of the heat and power production and consumption can result in a better environmental performance of the straw-to-ethanol process. This can, among others, be achieved by more profound heat integration within the process, and by using the more efficient future CHP technologies, based on biomass gasification, instead of the applied biomass combustion, based on the currently best available technology.

[3] Centrum voor Milieuwetenschappen Leiden (CML); Leiden University, Institute of Environmental Sciences (CML)

Disposal of residues in controlled inorganic landfills has negligible environmental impacts in the straw-to-ethanol process over the first 100 years (short-term emissions). However, as the environmental impacts of disposal in the long term (> 100 years) could be important, application of residues in building materials and/or fertilisers is recommended.

Flue gases in the straw-to-ethanol process are the major contributors to the impact categories photochemical oxidation and acidification, and important contributors (after straw) to the impact category eutrophication. Reduction of the NO_x/SO_x/CO/VOC emissions in the flue gases will therefore lead to lower environmental impacts in these categories.

Straw production and use have a saving effect only in the categories global warming and terrestrial ecotoxicity. In all other categories it has a negative impact, mainly due to the production and application of fertilisers, polyethylene synthesis (for baling), production and transport of diesel fuel, production of agricultural machinery, agricultural activities, and transport. Improvements in the agricultural(-related) activities are therefore required to reduce the negative environmental impacts of straw production and use on the straw-to-ethanol process. Variation of the straw transport distance between 20 and 100 km showed that even at a distance of 100 km, the straw transport will have a lower environmental impact in each impact category compared to the effect of avoided fossil-based electricity, straw, glucose, and flue gases.

Table 14.1 *WTW GHG emissions of bioethanol processes and gasoline as well as GHG saving potential of bioethanol routes*

GHG emissions		Gasoline	Sugar beet ethanol	Wheat ethanol	Straw ethanol (base case allocation)	Straw ethanol (18% allocation)
WTT	[g CO_2-eq/MJ]	17.8	-24	-33.4	-59.1	-42.4
TTW	[g CO_2-eq/MJ]	75.2	72.2	72.2	72.2	72.2
WTW	[g CO_2-eq/MJ]	93.0	48.2	38.8	13.1	29.8
Saving	[%]		-48%	-58%	-86%	-68%
- WTT	[kt CO_2-eq/yr]				308	241
- TTW	[kt CO_2-eq/yr]				12	12
- WTW	[kt CO_2-eq/yr]				320	253

Based on the "well-to-wheels" estimate a GHG emission saving of 48% for sugar beet, 58% for wheat, and 86% for straw ethanol are calculated. Based on the assumed annual plant capacity of 150,000 tonnes bioethanol (ca. 4 PJ), the annual WTW GHG saving potential of the plant, compared to gasoline, is 320 ktonnes CO_2-eq. Doubling of the economic allocation factor for straw from 9% (base case) to 18% has a large effect on the environmental performance of the process. It will result in a GHG saving of 68%, which is 18% points lower than the base case (86%). The annual GHG saving potential of the straw-to-ethanol plant is with 253 ktonnes CO_2-eq., 67 ktonnes lower than the base case (320 ktonnes CO_2-eq).

For future work it is recommended to update the LCA of the straw-to-ethanol process with straw data applicable for the situation in the Netherlands and to make a detailed analysis of the impact of straw removal on nutrients and fertilisation.

15. Project management and communication

J.A.M. de Bont (Royal Nedalco BV) and J.H. Reith (ECN)

During the project approx. 40 meetings were held for presentations and discussion of results, dedicated thematic meetings and strategic meetings to discuss overall progress and issues relating to the R&D program, finances and/or external cooperations and contacts.

Approximately half way the project, the consortium decided to shift the focus in the experimental R&D from fermentation to the more upstream processes physical-chemical pretreatment and enzymatic (pre)hydrolysis because these steps are most critical. In this context it was also decided to limit the C5 selection work. Overall the cooperation in the project occurred in a constructive manner.

The dedicated project website http://bemz.ecn.nl/index.html proved a suitable instrument for internal and external project communication. Other communication / dissemination activities included:

- A press release was given out at the occasion of the successful production on pilot scale of the first Litres of ethanol from wheat straw in July 2005.
- 7 publications thusfar in peer reviewed scientific journals. Several manuscripts are in preparation.
- Various other publications in conference proceedings, technical and more general media.
- Approx. 20 presentations about project results in international conferences and workshops a.o. in the framework of the EU Network of Excellence Bioenergy, task group Biofuels.
- Input in a scientific mission to Brazil (industries and academia).
- Several meetings were organized with invited lectures and discussions with distinguished international experts in the field.

A list of publications and other communication activities related to the project is presented in section 17. It is expected that more publications will be generated about project results during 2007 and 2008. The project website will remain "on line" until further notice.

16. Achievements and recommendations

J.H. Reith (ECN) and J.A.M. de Bont (Royal Nedalco BV)

Major achievements from the project are highlighted below. Per item recommendations are given.

Standardized analytical methods and experimental protocols
Harmonized/standardised methods have been developed and they have been tested for measuring and reporting of enzymatic activities as well as for feedstock analyses.

Costs, logistics and composition of feedstock
The project results provide an in depth analysis of costs and logistic aspects of wheat straw as feedstock in The Netherlands. Good know-how was obtained concerning the composition of wheat straw including its variation over time. For quality assessment of raw materials a robust, draft protocol has been developed and partly validated for the quality assessment of (new) raw materials for the production of either ethanol or lactic acid. The protocol uses standard analytical methods and procedures.

Extensive validation of the protocols is recommended.

Wheat straw has been studied with respect to raw materials availability and choice. A technical and economic evaluation has been performed resulting in a comprehensive overview of the suitability of wheat straw as a substrate for the production of cellulosic ethanol or lactic acid.

A broadened selection of suitable feedstock is recommended. Also the development of multi-product biorefinery strategies for existing or new crops is recommended.

Pretreatment processes developed
Processes have been developed both at lab scale and up to pilot scale for
- mild acid pretreatment in pressurized water for bioethanol production,
- mild alkaline pretreatment for lactic acid production.

Technologies were developed for handling of slurries with high dry matter percentages of up to 25 w/w %. The knowledge obtained allowed for the design of a pretreatment reactor system capable of handling such high dry matter levels.

In the developed technology and know how for mild alkaline pretreatment of wheat straw for lactic acid fermentation, the use of process chemicals *i.e*. $Ca(OH)_2$ is specifically tailored for integration with current industrial lactic acid production and Down Stream Processing.

For both the mild acid and the alkaline route, generation of a more detailed process/reactor design is recommended and scaling up of the technology should be done to at least Process Development Unit (PDU) or pilot plant level.

Enzymatic (pre)hydrolysis
An integrated model for enzymatic cellulose hydrolysis and fermentation was developed. Based on the model Simultaneous Saccharification and Co-Fermentation (SSCF) was selected as optimal for both bioethanol and lactic acid fermentation. The predictions from the model were confirmed experimentally.

Enzymatic (pre-)hydrolysis and enzymatic reduction of viscosity both are vital to the overall process. Industrially-available cellulases were selected with optimal performance both for hydrolysis of pretreated wheat straw and for reduction of viscosity. Selected enzymes were characterized in more detail. As a result, a detailed overview was obtained of the technical and economic feasibility of commercially available cellulases for effective production of hydrolysates from pretreated wheat straw for ethanol and lactic acid fermentation.

The R&D has further provided insight and knowledge on the requirement and assessment of specific enzyme activity (e.g. xylanase) activity in cellulase cocktails tailored for wheat straw.

Model studies provided insight and knowledge of mechanisms that may play a role in industrial processes such as the shielding of cellulose by adsorption of xylan oligomers or lignin that hinder enzymatic hydrolysis.

Enzyme companies are currently very active in optimizing the performance of industrial enzymes. It is recommended to have intimate interaction with such a company in order to be able to deal with state-of-the-art enzyme preparations.

Ethanol and lactic acid fermentation
Technology and know how for ethanol and lactic acid fermentation from straw have been developed. Experiments were performed both on lab and pilot scale. At lab scale, ample knowledge was obtained on process parameters. An important aspect was the minimization of the formation of inhibitors. As a result, potential problems with inhibitors were brought under

control, even at high dry matter concentrations and extensive water recycle. At pilot scale, several litres of straw ethanol were produced as based on the experience gained with the lab-scale experiments. The bioethanol produced was characterized with respect to application in fuels.

It is recommended to further investigate upscaling and process optimization with special attention for the abatement of infections that may occur in the fermentation of (specifically) lignocellulose hydrolysates when pH-values are higher than customary in the industry (e.g. at high acetic acid concentrations).

Ethanol fuel blends
Specification and logistics requirements of fuel ethanol blends were evaluated. Know how has been generated on the composition and fuel characteristics of bioethanol produced from wheat straw. Furthermore, know how has been generated on the distribution and logistic aspects of ethanol fuel blends.

Dewatering of fermentation residue / stillage fraction
The potential of Pneumapress® pressure filter technology for dewatering of fermentation residues (stillage fraction) prior to CHP generation has been experimentally confirmed on lab scale.

It is recommended to scale up and test Pneumapress® dewatering at pilot scale in a prolonged test set. Manufacturer Pneumapress has a skid mounted pilot unit with 1 m^2 filtration area available for on-site testing. Further optimization of the already high dry matter percentages of the filter cakes e.g. by optimization of filter media pore size is recommended.

Combined Heat and Power generation
Fluidized bed combustion was experimentally confirmed as a suitable technique for CHP generation from fermentation residues. Insight was generated in fuel performance of these highly specific residues with respect to agglomeration and fouling risks and the potential for co-firing in coal fired power stations. A first order conceptual design was created for a dedicated CHP unit including flue gas cleaning section for inclusion in the conceptual integral plant design and the economic evaluation.

Utilisation of ashes and mineral concentrate
Know how has been generated about utilisation and ecological and economic valorization of ashes and mineral concentrate in building material or fertilizer. The knowledge obtained includes recommendations to optimize the utilization potential by modification of upstream process conditions.

It is recommended to produce larger amounts of ashes for nutrient availability tests with plants, tests on leaching behaviour and other dedicated tests on practical application, in co-operation with potential buyers.

Conceptual designs for large-scale ethanol and lactic acid production from straw
Conceptual designs for large-scale production plants including economic evaluation have been generated. Designs have been produced for the co-production of bioethanol and electricity and the co-production of lactic acid, electricity and steam from wheat straw. Both designs were adapted to the utilisation of all residue streams, optimal heat/energy integration and integration of water recycle systems.

The conceptual plant design for bioethanol production from wheat straw is an integral design for a zero liquid waste water effluent plant with wheat straw as feedstock. The plant is

autonomous for the supply of electricity and heat including delivery of surplus green electricity to the grid.

The conceptual straw-to-ethanol design forms a considerable upgrade of the international state-of-the-art, i.e. the NREL design[4]. New elements vis-a-vis the NREL design include:

- high degree of heat integration in the pre-treatment reactor design
- minerals problems are solved in the present design
- more detailed distillation section
- the current design includes on-site enzyme production
- the design shows that a zero waste water effluent plant is indeed possible
- extension of the NREL-bioethanol concept to a plant producing lactic acid from lignocellulosics

For both designs -especially the straw to ethanol design- the generation of a technical design is recommended. Also it is recommended to construct and operate an R&D pilot plant.

LCA results

The performed screening LCA provides insight in the environmental aspects of the well-to-tank production chain. The LCA indicates improved environmental performance for bio-ethanol from straw as compared to gasoline and first generation ethanol alternatives.

In order to identify points for potential improvement, a detailed improvement analysis and follow-up is recommended.

Knowledge generation and dissemination

In terms of knowledge generation and dissemination, an overview of major issues and developments in the field has been generated that is both excellent and up-to-date. It includes a multitude of contacts and networks, including direct links to distinguished parties in the international R&D sector (including EU-level) and industry. A substantial number of publications, presentations and conference papers has been delivered in a wide range of subjects and topics. Interestingly, three individual project participants have made a transfer from R&D to industry (Royal Nedalco and DSM Life Science).

It is recommended to carry on with the (inter)national contacts. It obviously is recommended to finalize the preparation of publications/ presentations that are still in preparation. They will contribute to the further exposure of the project results in the international setting.

Overall conclusion and recommendation

Overall, the optimization steps in the project have led to concepts for the production of bioethanol and lactic acid from straw. Two alternative pretreatment routes were considered. For bioethanol, the mild acid route has been studied in more detail, whereas the alkaline route was investigated especially in the production of lactic acid.

Upscaling to the level of a Process Development Unit (PDU) or a pilot plant is strongly recommended with the active involvement of current consortium partners and one or more industrial partners, possibly in a multi client project. Involving the current consortium will have the benefit that the generated expertise and knowledge are utilised and maintained.

[4] •A. Aden et al., Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis for Corn Stover, NREL, Golden, CO, Report TP-510-32438, June 2002. http://www.osti.gov/energycitations/product.biblio.jsp?osti_id=15001119

17. Publications

1. J.H. Reith, H. den Uil, H. van Veen, W.T.A.M. de Laat, J.J. Niessen, E. de Jong, H.W. Elbersen, R.Weusthuis, J.P. van Dijken and L. Raamsdonk. 2002. Co-production of bioethanol, electricity and heat from biomass residues. Proceedings of the 12th European Conference on Biomass for Energy, Industry and Climate Protection, 17 -21 June 2002, Amsterdam. Pp. 1118 - 1123.
2. Openbare samenvatting EET K 01116, 2002.
3. "Op weg naar hernieuwbare brandstoffen en groene chemicaliën. Ethanol en melkzuur uit plantaardige biomassa" Artikel in EET Nieuwsbrief, september 2002.
4. Openbare presentatie project EET K 01116, 19 november 2002, KIT, Amsterdam.
5. E. de Jong, R.R. Bakker, H.W. Elbersen, R.A. Weusthuis, R.H.W. Maas, J.H. Reith, H. den Uil, R. van Ree, 2003. Perspectives for Bioethanol Production in the Netherlands: feedstock selection and pretreatment options. Poster presented at the 25th Symposium on Biotechnology for Fuels and chemicals, Breckenridge, Colorado, 2003.
6. Project website: http://bemz.ecn.nl/index.html
7. J.H. Reith et al, 2003. Co-production of cellulosic bioethanol, power and heat; technical, economic and ecological feasibility (including R&D issues). Poster presented at the 4th European Motor Biofuels Forum, 24-26 November 2003, Berlin, Germany.
8. Otto (2004). Preparation of lactic acid from a pentose-containing substrate. US2004/0203122A1.
9. J.A.M. de Bont, 2005. Presentation at BFC net Symposium, 19 and 20 January 2005, Steyr, Austria.
10. "Op een houtje rijden". Publicatie in De Ingenieur, 13 mei 2005.
11. R.R. Bakker, R. H.W. Maas, M.A. Kabel, H.A. Schols, R.A. Weusthuis, and E. de Jong Hydrolysis & Fermentation of Lime-pre-treated Biomass, 2005. Poster presented at the World Congress on Industrial Biotechnology and Bioprocessing, Florida, April 2005.
12. Persbericht: Wageningse onderzoekers produceren eerste liters bioethanol uit stro". 1-7-2005. / Press release Wageningen UR: 01-07-2005.
13. Bermgras in de tank, Chemvakblad, 2005.
14. R.H. W. Maas, Oral presentation at 5th Biomass PhD day, September 2005, Wageningen University and Research Centre, Wageningen, the Netherlands.
15. R.R. Bakker, 2005. Hydrolysis & Fermentation of Lime-pre-treated Biomass. Oral presentation at the 26th Symposium on Biotechnology for Fuels and chemicals, Denver, Colorado, United States, 2005.
16. Bakker R.R., Elbersen H.W. (2005) Managing ash content and quality in herbaceous biomass: an analysis from plant to product. In: Proceedings 14th European Biomass Conference. 17-21 October 2005, Paris.
17. J.H. Reith, 2005. Presentation at BioEnergy NoE workshop Ethanol from Lignocellulose, October 2005, Reims, France.
18. E. de Jong, Hydrolysis & Fermentation of Lime-pre-treated Biomass. Oral presentation at the American Chemical Society, Hawaii, United States, December, 2005.
19. Maas, R.H.W., R.R. Bakker, G. Eggink, R. Weusthuis, Production of lactic acid from xylose by *Rhizopus oryzae*, poster presented at:
    - 27th Symposium on Biotechnology for Fuels and Chemicals, May 2005, Denver, Colorado, USA.
    - 2nd Symposium Graduate Research School Biotechnological Sciences Delft Leiden, November 2006, Delft, the Netherlands.
    - 11th Netherlands Biotechnology Congress, March 2006, Ede, the Netherlands.
20. R.R. Bakker, Status of Cellulosic Ethanol, Oral Presentation Gave netwerkdag, Utrecht, 18 april 2006.
21. Maas, R.H.W., R.R. Bakker, G. Eggink, R. Weusthuis, Lactic acid production from xylose by the fungus *Rhizopus oryzae*, Applied Microbiology and Biotechnology Vol. 72, Number 5, 2006, pp. 861-868

22. Marieke Willemsen et al. Quantitative evaluation of separate hydrolysis and fermentation vs. simultaneous saccharification and fermentation.
23. J.A.M. de Bont, 2006. Presentation 28th Symposium on Biotechnology for Fuels and Chemicals. Nashville, USA. April 30 - May 03, 2006.
24. J.H. Reith, 2006. Presentation at BioEnergy NoE workshop Bioethanol from Wood, July 3-4, 2006, Graz, Austria.
25. Groenestijn J.W. van, Binnema D.J., Efficient conversion of starch and cellulose from co-products of food industry and agriculture to ethanol, 2. European bioethanol technology meeting, Detmold, 25-26 April 2006.
26. Kabel, M.A., Van der Maarel, M.J.E.C., Klip, G., Voragen, A.G.J. & Schols, H.A. (2006). Standard assays do not predict the efficiency of commercial cellulase preparations towards plant materials. *Biotechnology and Bioengineering*, *93*(1), 56-63.
27. Kabel, M.A., Van den Borne, H., Vincken, J.-P., Voragen, A.G.J. & Schols, H.A. Structural differences of xylans affect their interaction with cellulose. *Carbohydrate Polymers (2006).*
28. Kabel, M.A., Heijnis, W.H., Bakx, E.J., Kuijpers, R., Voragen, A.G.J. & Schols, H.A. (2006b). Capillary electrophoresis fingerprinting, quantification and MS-identification of various APTS-derivatized oligomers derived from plant polysaccharides. *Journal of Chromatography A*, 1137, 119-126.
29. J.F. Kuijvenhoven, Process Design for an Integrated Lignocellulose to Bioethanol Production Plant, Oral presentation at Netherlands Process technology Symposium NPS 6, Veldhoven, 24rd & 25th of October 2006
30. J.F. Kuijvenhoven, Process Design for an Integral (ligno) Cellulose to bioethanol production plant, IDP NR 032-06, Delft Ingenious Design, TU Delft, July 2006
31. J.F. Kuijvenhoven, Design of a plant for the production of ethanol from wheat straw, Oral presentation at the Bioenergy Network of Excellence (NoE) Annual Researchers' Meeting, Helsinki, October 2006.
32. Kabel, M.A., Bos, G., Zeevalking, J., Voragen, A.G.J. & Schols, H.A. 2007. Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. *Bioresource Technology* Volume 98, Issue 10, July 2007, Pp 2034-2042.
33. J.H. Reith, 2007. Presentation at Conference BioRefintec 2007, 24-25 Apr, 2007 Amsterdam, The Netherlands.
34. J.H. Reith, 2007. Presentation at European Conference Biomass for Energy and industry and climate protection, Berlin, 7th to 11th May 2007.
35. A.R. Boersma, 2007. Presentation at European Conference Biomass for Energy and industry and climate protection, Berlin, 7th to 11th May 2007.
36. M. Mozaffarian, 2007. Poster at European Conference Biomass for Energy and industry and climate protection, Berlin, 7th to 11th May 2007.
37. Drissen, R.E.T., Maas, R.H.W., Maarel, M.J., Van Der, Kabel, M.A., Schols, H.A., Tramper, J., Beeftink, H.H., 2007. A generic model for glucose production from various cellulose sources by a commercial cellulase complex. Biocatalysis and Biotransformation, Volume 25, Issue 6 November 2007 , pp. 419 - 429

Manuscripts in preparation

38. Bakker R. R., R.H.W. Maas, M. A. Kabel, H.A. Schols, R. A. Weusthuis, and E. de Jong Lime pretreatment of wheat straw to enhance hydrolysis & fermentation to ethanol.
39. Maas, R.H.W., R.R. Bakker, M.A. Kabel, E. de Jong, R.A. Weusthuis, Conversion of lime treated wheat straw into ethanol: evaluation of separate hydrolysis and fermentation and simultaneous saccharification and fermentation.
40. Maas, R.H.W., R.R. Bakker, M. Jansen, D. Visser, E. de Jong, G. Eggink, R. Weusthuis, An integrated approach of simultaneous saccharification and fermentation of lime treated wheat straw to L(+)-lactic acid by a thermotolerant *Bacillus coagulans*.
41. Maas, R.H.W., M.A. Kabel, H.A. Schols, G. Eggink, R.A. Weusthuis, Utilisation and fermentation of carbohydrate polysaccharides by fungal *Rhizopus oryzae* strains.

42. Ronald H.W. Maas, Robert, R. Bakker, Arjen R. Boersma, Iemke Bisschops, Jan R. Pels, Ed de Jong, Ruud A. Weusthuis and Hans Reith. Pilot-Scale Conversion of Lime-Treated Wheat Straw into Bioethanol: Quality Assessment of Bioethanol and Valorization of Side Streams by Anaerobic Digestion and Combustion.
43. Kuijvenhoven, J.F. et al. Conceptual design of a large-scale straw to ethanol production plant.

18. Abbreviations

ASTM	American Society for Testing and Materials (ASTM) http://www.astm.org/
BAM	Biological Activity Monitor
BC	Bacterial Cellulose
BFB	Bubbling Fluidized Bed
C-5, C5	Designates sugars containing 5 C(arbon) atoms (xylose, arabinose)
C-6, C6	Designates sugars containing 6 C(arbon) atoms (e.g. glucose, mannose)
CAPEX	Capital Expenditure (= total capital investment)
CE	Capillary Electrophoresis
CFB	Circulating Fluidized Bed
CHP	Combined Heat and Power generation
CO_2-eq.	CO_2 - equivalents
DBMD	Dutch Building Materials Decree (“Bouwstoffenbesluit”)
DP	Degree of Polymerization
DSP	Down Stream Processing
EET	Economy, Ecology and Technology Program
EGSB	Expanded Granular Sludge Bed system
EN-450	European Standard 450
ESP	ElectroStatic Precipitator
ETBE	Ethyl Tert-Butyl Ether
FGD	Flue Gas Desulfurization
FPU	Filter Paper UnitS (a unit for quantifying enzyme activity)
GHG	Green House Gas
HPAEC	High-Performance Anion Exchange Chromatography
HPLC	High-Performance Liquid Chromatography
HPSEC	High-Performance Size-Exclusion Chromatography
IRR	Internal Rate of Return
ISO	International Organization for Standardization
kWh	kiloWatthour
LA	Lactic Acid
LCA	Life Cycle Analysis
LHV	Lower Heating Value
LIF	Laser Induced Fluorescence
MALDI-TOF	Matrix-assisted laser desorption/ionisation-time of flight (mass spectrometry)
MJ	Mega Joule = 10^6 Joules
MS	Mass Spectrometry
MW	Mega Watt = 10^6 Watts
NREL	National Renewable Energy Laboratory, USA.
PDU	Process Development Unit
PET	Poly Ethene Terephtalate
PLA	Poly Lactic Acid
R&D	Research and Development
SEM	Scanning Electron Microscopy
SHF	Separate Hydrolysis and Fermentation

S/L	Solid/Liquid separation
SSCF	Simultaneous Saccharification and Co-Fermentation
SSF	Simultaneous Saccharification and Fermentation
TAPPI	Technical Association of the Pulp and Paper Industry http://www.tappi.org/
VOC	Volatile Organic Compounds
WTT	“Well-To-Tank”
WTW	“Well-To-Wheel”