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TECHNO-ECONOMIC ASPECTS OF ON-SITE CELLULASE PRODUCTION

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On-site cellulase production for lignocellulosic ethanol production based on SO_2-impregnated steam pretreatment followed by simultaneous saccharification and fermentation was investigated from a techno-economic aspect using Aspen Plus and Aspen Icarus softwares. The enzyme fermentation was assumed to operate batch-wise with a cycle time of 100 hours. The base case included sixteen 343 m^3 aerated fermentors arranged in four lines operating according to a merry-go-round pattern. Besides the base case, three cases, with improved productivities, were investigated. The cost of the on-site enzyme production was estimated to range between 6.7-16.5 Eurocent/L ethanol. The cost of carbon source was not included in the total production cost, since the pretreated material was produced in the process.

Keywords: Process simulation, Cellulase fermentation, On-site, Ethanol production, Economics

Introduction

The second generation fuel-ethanol production has not been demonstrated on full-scale so far, although some pilot plants already exist in Europe and North-America. The lignocellulosic ethanol production is the most complex technology compared to sugar- and starch-based processes, which are already well-known and mature. Due to their complex structure the lignocellulosic feedstock require pretreatment prior to the cellulose hydrolysis and ethanol fermentation, that adds one more step to the process.

One alternative of cellulose hydrolysis is the enzymatic way. Although substantial improvements have been made in the last decades, the cost of enzyme is still a major problem in the enzymatic process. In this study on-site cellulase fermentation was modeled and economic evaluation for the enzyme production was conducted.

Materials and Methods

The simulation software

The process was modeled by Aspen Plus flow-sheeting software (Aspen Tech Inc, Cambridge, MA, USA) capable to solve mass and energy balances. It is a powerful tool in comparing different process configurations in terms of efficiency, energy demand or – coupled with Aspen Icarus Process Evaluator (Aspen Tech Inc, Cambridge, MA, USA) – production cost. The later software is able to evaluate the process economics, nevertheless in our case it was used for sizing and estimating the capital investment. The built-in databases of Aspen Plus did not contain all the chemical components e.g. the ones of wood such as cellulose, lignin etc. They were obtained from the biomass databank of NREL.

Economic evaluation

Before performing economic evaluation the process equipments had to be sized. Most of them were sized manually on Excel worksheets except the heat exchanger which was sized by Icarus using the report file from Aspen Plus containing the results of material and energy balances. The manual sizing was also based on Aspen Plus simulation data.

The fixed capital investment – both the direct and indirect costs – was estimated by Icarus, where equipments not present in the Aspen Plus flowsheet, such as pumps, compressors and additional vessels were also included. The built-in database of Icarus was used for cost estimation of all the process components except the fermentors where modifications were made introducing factors to obtain the costs given by a Swedish supplier. The fermentors, however, were cost-estimated as stainless-steel (SS 304) storage tanks and their agitators as well as cooling coils were added separately. The annual fixed capital investment was calculated by use of an annuity factor of 0.110, corresponding to 15-year life of the plant, 7% interest rate, linear

deprecation and zero scrap-value. The reference year was 2008 and 8000 working hours per year were assumed.

The working capital investment was calculated according to the recommendation of Peters and Timmerhaus [1]. To obtain its annual representation the working capital was multiplied by the interest rate.

Table 1 summarizes the specific costs employed in the operating cost estimation.

Table 1: Cost used in the evaluation

Chemicals, nutrients	
Soy-meal (48% protein)	0,16 €/kg
$(NH_4)_2SO_4$	0,10 €/kg
KH_2PO_4	0,10 €/kg
$FeSO_4*7H_2O$	0,11 €/kg
NH_3 (25%)	0,22 €/kg
cc. H_2SO_4	0,05 €/kg
Defoamer	2,15 €/kg
Utilities	
Electricity	48,4 €/MWh
Cooling water	0,02 €/m3
Other costs	
Insurance	1% of fixed capital
Maintenance	2% of fixed capital
By-product credit	
CO_2	3,2 €/t

Base case description

The enzyme production step was based on literature data [2,3], and the process step was implemented in an Aspen Plus model including all major process steps shown in *Fig. 1* described in detail in a previous study [4]. The ethanol plant was assumed to be located in Sweden, with the capacity to process 200 000 dry tons of spruce annually.

The pre-treated material stream was divided into a major stream fed directly to the simultaneous saccharification and fermentation (SSF) step and a minor stream (7.5% in the base case) led to the *Trichoderma* fermentation where the enzyme amount required by SSF assumed to be 15 FPU/g WIS (filter paper unit/g water insoluble solid) was obtained. The whole broth could be added to SSF since it was carried out at 37 °C and above 35 °C the growth of mycelia is entirely inhibited. Using the whole culture had several advantages: i) no additional separation was needed, which decreased the cost; ii) the enzymes adsorbed on the surface of the lignin and the cells as well as the ones trapped in the cytoplasm could also be utilized.

All the sugars present in the fermentation medium were taken into account in anhydro equivalent i.e. the polymer and monomer sugars in the pretreated material and the carbohydrate content of the soy-meal (26%) were assumed to be consumed entirely. The yields were the same for the hexosans and pentosans *(Table 2).* It must be mentioned that the fermentation whose results were used in the model was carried out on sulphite-pulp [2]. In order to apply these data key-assumptions had to be made: the lignin content did not affect the enzyme production, which was concluded in the same article, furthermore the monomer sugars present in the medium did not result in catabolite repression.

The base case included 16 aerated agitated fermentors, each 343 m^3 in volume, arranged in four lines. The working volume was 72% of the total one.

Cooling was performed by use of cooling coils. The fermentors operated in atmospheric pressure, and were not pressure-rated for steam sterilization. The pre-treated material and the makeup water coming entirely from the evaporation step were considered sterile, hence only cleaning-in- place was applied in the tanks. The cost of nutrient sterilization was assumed to be negligible.

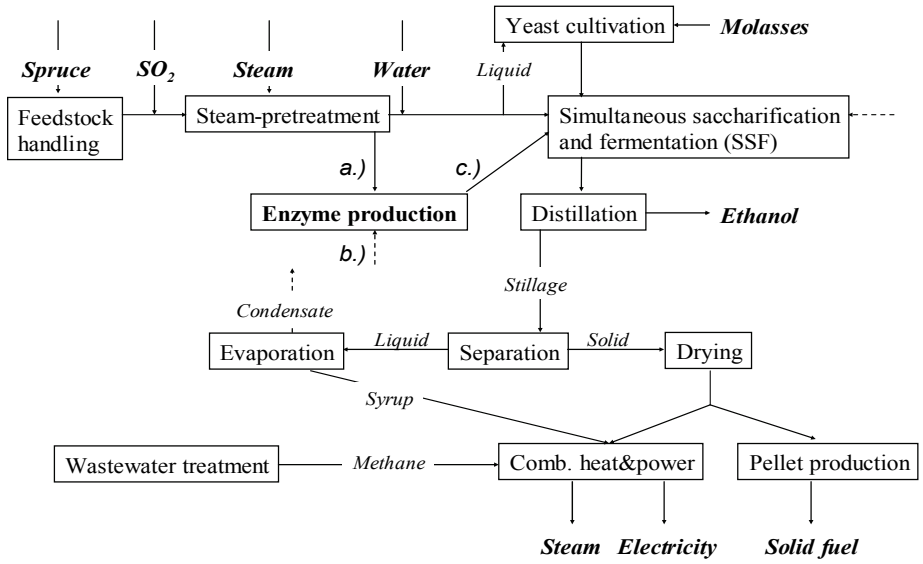

Figure 1: Boundary conditions of the modelled wood-to-ethanol process
(*a.* steam pre-treated spruce slurry, *b.* condensate recycled to enzyme fermentation, *c.* fermentation broth)

Table 2: The features of *T. reesei* MCG-77 fermentation

Temperature	30	°C	[2]
pH	6		[2]
Fermentation time	90	h	[2]
Cycle time	100	h	[5]
Aeration rate	0.5	VVM [1]	[2]
Power to the broth	0.5	kW/m^3	[5]
Mycelium yield	0.27	g/g CH	[3]
Soluble protein yield	0.26	g/g CH	[3]
Activity yield	185	FPU/g CH	[2]
Specific activity	0.71	FPU/mg protein	*
CH concentration [2]	2	%	[2]
Productivity	61	FPU/(l*h)	[2]

*calculated

[1] Air volume/working volume/minute

[2] Carbohydrate concentration given in anhydro equivalent

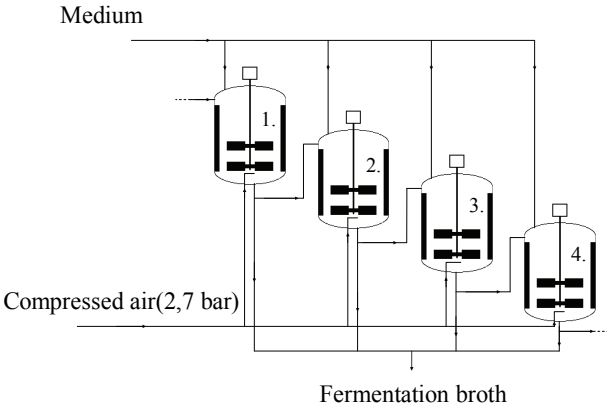

Figure 2: Schematic flowsheet of Kornuta process (1 line – 4 fermentors)

The four fermentors in a given line followed the same schedule, however they started being shifted in 25 hour intervals. At 25 hour 10% of the culture in the first vessel was transferred to the second one and used as inoculum (the second one gave inoculum to the third one etc.). The culture was at its peak growth and the cellulase concentration was low enough, hence fast sugar formation i.e. catabolite repression in the second vessel could be avoided. The fermentation lasted for 90 hours and was followed by a 10 hour harvesting, cleaning, charging period giving a 100 hour cycle time. After 100 hours from the start of the first fermentation the fourth vessel was ready to transfer inoculum to the first one closing the line to a loop *(Fig. 2)*. This operation pattern was referred as "Kornuta merry-go-round" [5].

In case of contamination inoculum could be transferred from a vessel in another line and both lines could continue uninterrupted. The air supply was provided by compressors, one for each line. The four lines had a common medium preparation vessel that received the pretreated material, the makeup water and the nutrients whose concentrations were the following: 0.5% soy-meal, 0.15% $(NH_4)_2SO_4$, 0.07% KH_2PO_4, 0.001% $FeSO_4 \cdot 7H_2O$. The outlet stream before being fed to the fermentors was cooled down to 30 °C in a heat exchanger. The system contained 16 inlet and 16 outlet pumps.

Other investigated cases

Besides the base case (A) three hypothetical cases with improved productivities were investigated *(Table 3)*. In case B the activity yield was enhanced by 50%, which also connoted 1.5-fold productivity. In case C the carbohydrate content (CH) was increased to 4% and the same yield with doubled productivity was assumed. In case D both parameters was enhanced, which resulted in tripled productivity.

Table 3: Modified parameters in the various cases

	Base case (A)	Enhanced yield (B)	Enhanced CH conc. (C)	Enhanced yield, CH (D)
Activity y., FPU/g CH	185	278 (1,5x)	185	278 (1,5x)
CH conc.	2%	2%	4% (2x)	4% (2x)
Prod., FPU/(l*h)	61	92 (1,5x)	122 (2x)	183 (3x)

Results and Discussion

While according to the model the *Trichoderma* fermentation consumed all the sugars being fed, it did not alter the amount of other substances (lignin, inhibitors etc.). The water consumption declined monotonous from A to D, whereas the other components had two levels.

Table 4: Component flows entering and departing the enzyme fermentation

Flow, kg/h	**A**	**B**	**C**	**D**
In				
Hexosans	782	535	782	535
Pentosans	15	11	15	11
Hexoses	352	241	352	241
Pentoses	63	43	63	43
Lignin	439	300	439	300
Water	55814	38167	27031	18485
Produced				
Enzyme	304	208	304	208
Mycelium	317	217	317	217
CO_2	712	487	712	487

They were higher in scenario A/C and lower at B/D *(Table 4)*. It can be due to the two activity yields applied which determined the carbon source demand as well as the product formation.

The total capital investment, i.e. the sum of fixed and working capitals varied in a range between 16 and 34 M€ which multiplied by the annuity factor gave the annual capital cost of 1.8–3.7 M€/year *(Table 5)*.

Table 5: Total capital investment and the annual costs in M€

	A	B	C	D
Total capital investment, M€	34	25	19	16
Costs, M€/year				
Capital	3.72 (41%)	2.74 (43%)	2.06 (41%)	1.75 (46%)
Chemicals, nutrients	0.49 (5%)	0.34 (5%)	0.44 (9%)	0.30 (8%)
Utilities	3.89 (43%)	2.62 (41%)	1.96 (39%)	1.28 (34%)
Other costs	1.02 (11%)	0.75 (11%)	0.56 (11%)	0.48 (12%)
By-product credit, M€/year				
CO_2	-0.02	-0.01	-0.02	-0.01
Total, M€/year	**9.09**	**6.44**	**5.00**	**3.81**

Besides the capital the utilities namely the electricity used by agitators, compressors, pumps was found the other largest contributor in production cost. The cost of cooling water was negligible. The carbon-dioxide credits were two order smaller than the costs. The sum of chemicals, nutrients and other costs were estimated not being more than 20% of the total. It must be pointed out, that the cost of carbon source was not included in either the annual or the specific enzyme production cost, since the pre-treated material was produced in the process.

The on-site cellulase production reduced the produced ethanol amount providing the same feedstock utilization, since the carbohydrates were consumed partially by the enzyme fermentation. The ethanol plant using commercial enzyme produced 59 563 m^3 ethanol per year, whereas the base case (A) and case C merely 55 000 m^3. The cases B/D with enhanced activity yield produced more ethanol (56 441 m^3/year), since less pre-treated material was needed for the cellulase fermentation.

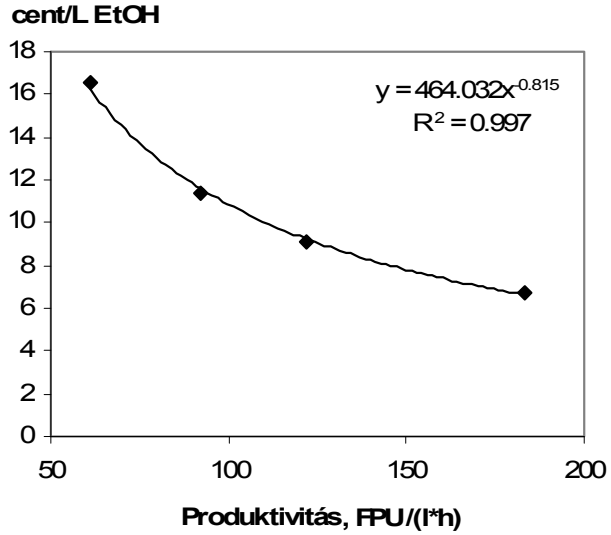

Figure 3: Specific enzyme cost as a function of productivity

By increasing the productivity the specific enzyme cost reduced monotonously. The fitted curve was close to hyperbola having the index of 0.8 *(Fig. 3)*. The breakdown of specific enzyme cost also shows that the main contributors were the capital and the utilities *(Fig. 4)*. In the base case 16.5 Eurocent/L EtOH was found. At tripled productivity (D) the specific enzyme cost was 6.7 Eurocent/L (41% of the base case).

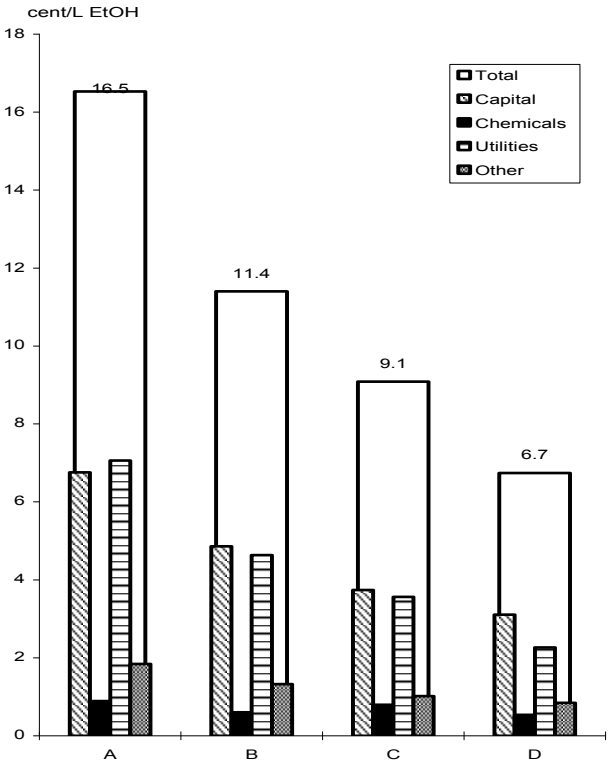

Figure 4: Breakdown of specific enzyme cost (produced ethanol: 55 000 m^3/year at A/C, 56 441 m^3/year at B/D)

Summary

The total cost of on-site cellulase production (diminished with cost of carbon source) was estimated to range between 6.7–16.5 Eurocent/L ethanol for the four investigated scenarios. Capital investment and electricity were found the main contributors.

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