# Technoeconomic Study on Steam Explosion Application in Biomass Processing

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#### Abstract

This work is based on the data collected during trials of a continuous steam explosion (SE) plant, with a treatment capacity of about 350 kg/h, including the biomass fractionation section. The energy and water consumption, equipment cost, and manpower needed to run this plant have been used as the base case for a techno-economic evaluation of productive plants. Three processing plant configurations have been considered: (I) SE pretreatment only; (II) SE followed by the hemicellulose extraction; (III) SE followed by the sequential hemicellulose and lignin extractions. The biomass treatment cost has been evaluated as a function of the plant scale. For each configuration, variable and fixed cost breakdown has been detailed in the case of a 50,000 t/y plant.

**Index Entries:** Steam explosion; economic evaluation; scale up; biomass processing.

#### Introduction

Among the numerous processes of biomass treatment, steam explosion (SE) has been the object of great interest since the 1980s, when steam-exploded lignocellulosic materials were considered as a polysaccharide source for ethanol production (1). This method is still very popular, and has achieved important goals such as increased overall yield by using catalysts in the pretreatment step and detoxification (2-6). The debate about the best pretreatment system is still open; moreover, there is continuous development of new methods. The possibility of using SE in ethanol production has somewhat obscured its potential use for pulp paper and board production,

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90 Zimbardi et al.

for which the process was originally developed (7,8). After many publications about technical and economical viability, the set up of a SE-based mill producing pulp paper from straw has recently been announced in Oregon. It is argued that if SE technology spreads in this field, the equipment would be cheaper because of the scale economy and competition; as well, the system's performances would be improved. In this scenario, the use of SE for ethanol production will also gain a competitive edge with respect to the other available pretreatments. As regards the utilization of SE as productive technology, there is a lack of information about the needs breakdowns in terms of energy and chemicals. These data are basic requirements of scaling up simulations and their experimental attainment has been the object of the first part of this work. The SE can be used to obtain several products by changing the treatment conditions and the recovery of the biomass components, so it is worthwhile to study different plant configurations. In the second part of this article, we report on a scale-up study of a medium size plant operating for the biomass fractionation. The work is aimed at combining laboratory results with industrial needs.

#### Methods

At the ENEA's research center in Trisaia (South Italy) a continuous SE pilot plant capable of treating up to  $350\,\mathrm{kg/h}$  of biomass, at 50% humidity, has been operating for about 7 yr. In addition to the SE machinery, it is operating a fractionation section by which the biomass can be separated into three streams. This section is based on the aqueous extraction of hemicellulose and lignin carried out in thermostated slurry tanks of  $4~\mathrm{m}^3$ ; the filtration is carried out by countercurrent, multistage belt filters, typical of the pulp and paper industry. The plant is composed of  $38~\mathrm{main}$  machines (including pumps, conveyers, stirrers).

The first plant stream is hemicellulose, available both as aqueous solution at 4% DM and as concentrated solution at about 50% DM. The second liquid stream is a basic lignin solution at 2-4% DM, that can be concentrated up to 50%. The third product stream is a wet solid residue rich in cellulose.

This pilot plant has recently been equipped with devices to measure the consumption of electricity; steam; water; and chemicals. The object of the experimental work has been to obtain the biomass treatment cost in the following cases:

- Case I: production of just steam exploded biomass;
- Case II: production of an aqueous hemicellulose stream and a lignincellulose solid;
- Case III: production of aqueous solutions of hemicellulose and lignin and one cellulose rich solid.

The plant was operated three times for 7 h each time. The optimal plant settings were determined in previous (not reported) work, but the SE-treatment conditions must be adapted on a case by case basis depending upon

the end use of the products. The steady conditions were reached 1 h after the start up in Case I, 1 h in Case II, and 2 h in Case III. The data relevant to each case were collected only in their steady state. The non-steady states, such as the time intervals and tank filling time, were ignored. Wheat straw was used as the starting biomass.

The measurement of electricity consumption in Case I was carried out by considering 11 machines, including the local biomass handling machines (straw chopper, belts); flow counters measured the steam and water consumption. In Case II, five additional items (two stirrers, two pumps, and one belt filter) and eight additional items in Case III (two stirrers, four pumps, one belt, and one conveyer) were included. The heat content of hot exploded biomass was sufficient to keep the temperature of the hemicellulose extraction tank around the optimal process temperature of 60°C. Instead, steam was used in Case III to warm the lignin slurry, as lignin extraction had to be carried out at around 90°C. The cooling water for the biomass feeder and belt machines was measured separately.

The collected data were used to carry out a technoeconomic evaluation for a medium size plant able to fractionate  $50,000\,t/y~(25,000\,t_{DM}/y)$  of biomass, assuming 334 working days, which corresponds to a scaling factor of 17.8.

The scale-up cost was based on pilot-plant costs and the specific consumption data. The equipment purchasing cost (PC, the cost of the basic machinery) was evaluated by using the so-called Lang factors that allow researchers to estimate the cost of common items in the chemical industry on the basis of the relationship:

$$c = c_R \cdot s^n$$

where  $c_R$  is the reference cost, s is the scaling factor, and n is the Lang factor (9). This method is useful in the chemical industry, as it provides a bridge between bench scale and larger plants; however, one has to be aware that the obtained costs can be flawed by errors of  $\pm 30\%$ . The other direct fixed costs (DFC), including income, were calculated as PC percentage. Depreciation, maintenance, insurance, and operating costs were evaluated as DFC quota. Depreciation was assumed linear with time, with a plant life of 15 years and a dismissal value of 10%. Other operating costs were based on the Italian market.

It was assumed 12 workers per shift and 3 supervisors would operate the plant (10). The manpower cost was used as a reference value in evaluating the cost of administration and process monitoring (chemical analyses). The cost of steam generation was based on the use of methane as fuel.

The consumption of water, steam, and chemicals were evaluated as proportional to the treated biomass, because they are related to the intrinsic process chemistry. The electricity consumption was calculated by assuming a direct proportionality between the treated biomass for the biomass feeding section, and an exponential factor of 0.5 for the other machinery. The technoeconomic analysis stops with the production of liquid streams

92 Zimbardi et al.

of hemicellulose and lignin, and a wet-cellulose-rich solid for which data were experimentally obtained; detailed hypotheses about treatment and uses of such streams are reported in the literature (11–14).

The product streams were analyzed for their chemical composition. The ash content of the raw and exploded materials was determined by sample combustion at 600°C (ASTM-1106, modified). The exploded materials were extracted, rinsed twice for 10 min with water at 65°C and at solid-liquid ratio of 10%. The extracted fraction was analyzed directly by HPLC to determine the carbohydrate monomers. The oligomer content was determined by calculating the difference in total sugar after hydrolysis with  $H_2SO_4$  (1% at 121°C for 1 h) and the initial monomer content. Sugar degradation was accounted for by introducing experimental coefficients. The solid residue was delignified twice with NaOH [1.5% (w/v) at 90°C for 15 minutes and solid-liquid ratio equal to 8%]. Lignin was recovered from the alkaline phase by precipitation at pH 2 with  $H_2SO_4$ . Acid-soluble lignin was determined by UV spectrophotometry at 205 nm, assuming an extinction coefficient of 110 dm<sup>3</sup>/g-cm (TAPPI Useful Methods 250). The residue was hydrolyzed following the Klason method to determine the residual lignin in the fibers and the carbohydrate content (TAPPI T13 m-54, modified). All the reported data refer to the solids dried at 105°C and, for the carbohydrates, to the polysaccharide form (15).

### Results

For this experiment, about 4.8 t of straw (3.4 DM) were processed in 21 h of plant operation. The SE treatment conditions were 200°C for 3 min, which corresponds to a severity factor of 3.24 ( $\log R_{\rm o}$ ) (16,17). The flow sheet of a complete fractionation (Case III) is reported in Fig. 1, the streams are normalized to the treatment of 100 kg<sub>DM</sub>/h of biomass. Table 1 shows the composition of the three product streams. Table 2 reports the specific consumption rates of steam, electricity, water, and chemicals as obtained by running the SE plant.

The cost breakdown for the scaled plant in each of the three operational cases is reported in Table 3. The specific cost of the SE biomass processing is reported as a function of the plant scale in Fig. 2. The data are expressed in Euros and refer only to the treatment costs and do not account for any selling costs.

It is worthwhile to point out that the data reported in this work refer only to one SE treatment condition, while it is possible to change the amount and quality of each stream by tuning the process severity. Low severities, around  $\log R_{\circ}$  3, are used to produce animal feed and pulp for paper production; higher severities, around  $\log R_{\circ}$  4, are best suited to the larger disruption required for lignocellulosic to be used as a substrate in bioconversion process.

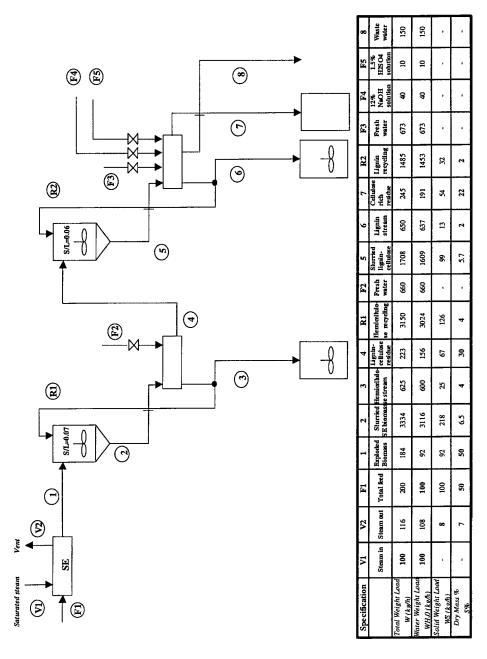


Fig. 1. Steam explosion plant scheme. The table shows the mass flows for 100 kg/h of dry biomass fed.

Percent Compositions of the Process Stream and Recoveries Obtained by SE and Fractionation Table 1

|   | le Total                      | 92.0                           | (%)                           |            |                   |             |               | 93.7                      |             | 1   |          |
|---|-------------------------------|--------------------------------|-------------------------------|------------|-------------------|-------------|---------------|---------------------------|-------------|-----|----------|
|   | e Solid<br>) residue          | 54.0                           | Single component recovery (%) | 20.7       | 0.9               | 86.5        | 15.3          | 42.6                      |             |     |          |
|   | Caustic phase (extraction II) | 12.7                           |                               |            | l                 |             |               | 51.1                      |             | 1   |          |
|   | Aq. Phase (extraction I)      | 25.3                           | S                             | 47.4       | 53.1              | 3.8         | 46.0          | I                         | 1           | 1   |          |
|   | Solid<br>residue              |                                |                               | 9.6        | 0.3               | 63.0        | 0.2           | 18.5                      | 1           | 1   | 70       |
| * | Caustic phase (extraction II) | Mass recovery <sup>a</sup> (%) | Stream composition (% DM)     | I          | I                 | I           | I             | 94.5                      | I           | I   | ια<br>ια |
|   | Aq. Phase (extraction I)      |                                |                               | 47.0       | 5.9               | 5.9         | 1.2           | I                         | 1           | 1   | 30.0     |
|   | Straw                         | X                              |                               | 23.1       | 2.6               | 36.2        | 9.0           | 21.6                      | 6.7         | 7.6 | 16       |
|   |                               |                                |                               | $Xylose^b$ | $A$ rabinose $^b$ | $Glucose^b$ | $Galactose^b$ | ${ m Lignin}^{arepsilon}$ | Extractives | Ash | 7        |

<sup>a</sup>Dry matter at 105°C. <sup>b</sup>Expressed as condensed (polymeric) form. <sup>c</sup>Klason lignin, both acid insoluble and soluble.

Table 2
Biomass Processing Data Obtained from Operating the ENEA's Steam Explosion Pilot Plant Having a Capacity of  $175 \text{ kg}_{DM}/h$  (350 kg/h as Wet Biomass)

|  | Case I | Case II | Case III |
|--|--------|---------|----------|
| Steam for SE, <sup>a</sup> kg/kg <sub>DM</sub>     | 1.3    | 1.3     | 1.3      |
| Steam for thermostatation, kg/kgdm                 | 0      | 0       | 0.25     |
| Electricity, kWh/kg <sub>DM</sub>                  | 0.31   | 0.39    | 0.47     |
| Water, kg/kgdm                                     | 1.9    | 8.5     | 14.2     |
| Cooling water, kg/kgdm                             | 5.7    | 14.3    | 22.8     |
| NaOH kg/kg <sub>DM</sub>                           | 0      | 0       | 0.05     |
| H <sub>2</sub> SO <sub>4</sub> kg/kg <sub>DM</sub> | 0      | 0       | 0.0015   |

<sup>&</sup>lt;sup>a</sup>At optimized SE procedure, with a value of  $1 \text{ kg/kg}_{DM}$  was measured.

<sup>&</sup>lt;sup>d</sup>For biomass feeder, and belt filters.

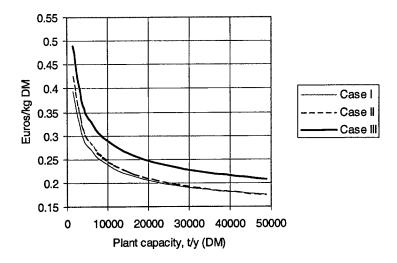


Fig. 2. Dependence of the specific biomass processing cost on the plant scale.

## **Discussion**

The mass loss occurring under the SE conditions is rather difficult to ascertain. By thermogravimetric measurements (TGA) it could be assumed that there is no appreciable pyrolytic loss with temperature and time, however the steam is expected to boost hydrolytic reactions. Ibrahim and Glasser (18) report that for the batch apparatus a mass loss between 16.9% and 24.4% occurs when logRo values range from 3.70 to 4.54, and attribute the loss to the flushing out of solid fibers through the gas outlet of the cyclone. In the continuous system, an almost complete recovery of the solid

<sup>&</sup>lt;sup>b</sup>The biomass feeder alone contributed to 0.13 kWh/kg<sub>DM</sub>.

<sup>&</sup>lt;sup>c</sup>For raw material umidification to 50%, steam production, and extractions.

Table 3 Breakdown Cost for a Biomass Pretreatment Plant Based on the Steam Explosion Having a Capacity of 25.000 t/y (DM) Expressed in Milion Euros

| DIRECT FIXED COST  | Expression   | Case I  | Case II   | Case III  |  |
|--|--|---|---|---|--|
| Total Plant Direct Cost, TPDC  |  |   |   |   |  |
| Purchasing cost, PC<br>Installation<br>Piping  | 0.4PC<br>0.35PC  | 4.649<br>1.860<br>1.627   | 5.241<br>2.096<br>1.834   | 5.437<br>2.175<br>1.903   |  |
| Instruments<br>Electricity supplying<br>Buildings<br>Place and Adaptation  | 0.3PC<br>0.15PC<br>0.35PC<br>0.15PC                              | 1.395<br>0.697<br>1.627<br>0.697  | 1.572<br>0.786<br>1.834<br>0.786  | 1.631<br>0.816<br>1.903<br>0.816  |  |
| Total Total Plant Indirect Cost, TPIC  | 2.7PC  | 12.553  | 14.151  | 14.680  |  |
| Plant Enginneering Building Engineering Total Total Plant Cost, TPC (TPCD+TPIC) Income Total Direct Fixed Cost   | 0.15TPDC<br>0.10TPDC<br>0.25TPDC<br>3.375PC<br>0.17PC<br>3.545PC | 1.883<br>1.255<br>3.138<br>15.692<br>0.790<br>16.482                          | 2.123<br>1.415<br>3.538<br>17.689<br>0.891<br>18.580                          | 2.202<br>1.468<br>3.670<br>18.351<br>0.924<br>19.275                          |  |
| OPERATIONAL COST   | Expression   | Case I  | Case II   | Case III  |  |
| Cost related to DFC  |  |   |   |   |  |
| Devaluation <sup>a</sup> Maintenenace Insurance Plant Management Total Cost related to manpower  | DFC(1-f)/n<br>0.02DFC<br>0.005DFC<br>0.03DFC<br>0.122DFC         | 0.989<br>0.330<br>0.082<br>0.494<br>1.895                                     | 0.836<br>0.372<br>0.093<br>0.557<br>1.858                                     | 0.867<br>0.385<br>0.096<br>0.578<br>1.927                                     |  |
| Direct Manpower <sup>b</sup> Benefits Supervision Labor equipment Chemical Analyses Administration Total Raw material (straw) <sup>c</sup> Fuel (methane) <sup>d</sup> Chemicals | MP<br>0.4MP<br>0.4MP<br>0.1MP<br>0.15MP<br>0.6MP<br>2.65MP       | 0.661<br>0.264<br>0.264<br>0.066<br>0.099<br>0.397<br>1.752<br>1.317<br>0.625 | 0.661<br>0.264<br>0.264<br>0.066<br>0.099<br>0.397<br>1.752<br>1.317<br>0.625 | 0.661<br>0.264<br>0.264<br>0.066<br>0.099<br>0.397<br>1.752<br>1.317<br>0.625 |  |
| NaOH <sup>e</sup><br>H <sub>2</sub> SO <sub>4</sub> (98%) <sup>f</sup><br>Total<br><i>Utilities</i>  |  | 0<br>0<br>0   | 0<br>0<br>0   | 0.461<br>0.004<br>0.465   |  |
| Electricity <sup>g</sup> Water <sup>h</sup> Steam (thermoregulation) <sup>i</sup> Total  |  | 0.518<br>0.033<br>0.550   | 0.571<br>0.146<br>0.716   | 0.627<br>0.232<br>0.052<br>0.912  |  |
| Waste management <sup>l</sup> Total Operational Cost SPECIFIC COST, Euros/kg <sub>DM</sub>   |  | 4.822<br>0.193  | 4.951<br>0.198  | 0.165<br>5.846<br>0.234   |  |

<sup>&</sup>quot;n= years of plant activity; set as 15; f = plant value at dismissal set as 10%.

 $<sup>{}^{</sup>b}$ Twelve workers on shift, with individual gross wage of 20 Euros/h.

<sup>°0.043</sup> Euros/kg. d0.284 Euros/m3.

<sup>60.36</sup> Euros/kg.

f0.10 Euros/kg. g0.12 Euros/kWh.  $^{h}0.67 \text{ Euros/m}^{3}$ . <sup>i</sup>0.013 Euros/kg. <sup>1</sup>6 Euros/m<sup>3</sup>.

fibers is achieved and only volatile compounds escape. In our case, an 8% mass loss has been estimated; this is an average value obtained from several measurements of feed weighting and from the analytical determinations of dry matter and ash contents in the product streams. The volatiles are condensed and conveyed to aerobic biological treatment; in a larger plant the distillation of this stream and the recovery of chemicals, such as furfural, could be considered.

The hemicellulose-rich stream is composed of 60% (DM) sugars, both in monomeric and oligomeric forms. The undetermined portion (40% DM) is a complex mix of extractives, ash, solubilized lignin, and degradation products. 51% of the lignin is recovered in the basic stream and 43% is found in the solid residue where cellulose accounts for the 63% of the mass.

The steam consumption ranges from 1.0 to 1.3 kg/kg<sub>DM</sub> (Table 2) and these data are in agreement with those reported by Jollez, Chornet, and Overend who studied the effect of explosion procedures on steam consumption and measured a steam consumption of 1–4 kg/kg<sub>DM</sub> while operating a 4 t<sub>DM</sub>/h digester. In the scaling analysis, a specific steam consumption of 1 kg/kg<sub>DM</sub> was assumed. The electricity consumption is rather high, from 0.31 kWh/kg<sub>DM</sub> in Case I to 0.47 kWh/kg<sub>DM</sub> in Case III. In all cases, 0.13 kWh/kg<sub>DM</sub> are imputed to biomass feeding in the pressurized reactor. In the scaling analysis, the electricity consumption of the feeder has been kept constant (restrictive assumption), while for the other machinery an exponential factor of 0.5 has been used. In the latter calculation the obtained values of 0.044, 0.062, and 0.080 kWh/kg<sub>DM</sub> (respectively, Case I, Case II, and Case III) are in agreement with the data reported for the analogous operations of washing and screening in paper industry.

Cooling water was 1.5–3 times the process water; in the scaled plant it has been assumed that this stream will be reintroduced in the process, either to carry out the aqueous extractions or recycled as cooler. Heat recovery and warm water can also be obtained from volatile condensation, but this option has not been included in the scaling analysis, because of lack of experimental data.

Only for complete fractionation (Case III) are chemicals required to delignify the biomass. The neutralization of the residual NaOH in the solids with H<sub>2</sub>SO<sub>4</sub> generates a slightly concentrated Na<sub>2</sub>SO<sub>4</sub> solution (4 mM).

As shown in Table 3, the purchasing costs (PC) play a key role. They have been based on real pilot plant cost and scaled by using a Lang factor of 0.6 for the patented SE equipment (feeder and digester), while for the other machinery we have used a Lang factor of 0.4, because they are more commonly available items (tanks, belts, pump) (9).

The purchasing cost in Case III is only 15% higher than in Case I. This datum alone highlights the influence of the SE equipment cost on the economic analysis. Indeed, the difference in final specific treatment costs retain almost the same original shift: in Case I it is 0.193 Euros/kg<sub>DM</sub>, while in Case III it is 0.234 Euros/kg<sub>DM</sub>; corresponding to a difference of 17%. The plant depreciation is the most important single item as it accounts for 15%

of the specific cost in Case I and 21% in Case III. Total energy consumption (fuel and electricity) weighs upon the cost from 24% (Case I) to 22% (Case III).

In the process economics, the raw material price must be considered. In the Italian market, straw is purchased at  $0.05~\rm Euros/kg$ , which would correspond to about 20% of the production cost. The cheapest available lignocellulosic material is waste paper, but its market and supply is unreliable; moreover, the price can range from  $0.035~\rm Euros/kg$  to the gratuitousness, depending on the selection quality. Scale economy could help to get cheaper treatment. As can be inferred from Table 3, a plant with a capacity below  $10,000~\rm t/y$  would not be considered in any case. By doubling the plant size from  $25,000~\rm to~50,000~\rm t/y$  the specific treatment cost could be 10% lower, but in this case biomass supply could be a serious problem, at least in the Italian framework.

The hemicellulose-rich stream can be used as a substrate for enzyme production after detoxification (19). Lignin is mainly used  $in\ situ$  as fuel; alternatively, its potential as a fine chemical source can be exploited. For example, many researchers have verified the possibility of using it in polymeric intermediates (18). As is widely reported in the literature, the use of just SE biomass is not recommended for ethanol production, because of the degradation products that can inhibit bioconversion (20–24). Converting only the cellulose rich residue to ethanol results in a simpler process. Moreover, the ethanol yield benefits by delignification as a strong inverse trend with the lignin content is reported (25); in fact, the insoluble lignin hinders the cellulose accessibility by enzymes and irreversibly binds and inactivates cellulases. The slight difference of treatment cost between the products obtained in Cases III and I would suggest the viability of carrying out delignification in an ethanol production process.

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### References

- 1. Wyman, C.E. (1994), Bioresour. Tech. 50, 3-16.
- 2. Ogier, J. C., Ballerini, D., Leygue, J. P., Rigal, L., and Pourquiè, J. (1999), Oil & Gas Science and Technology-Revue de l'IFP 54, 67–94.
- 3. Tsuda, M., Aoyama, M., and Cho, N.-S. (1998), Bioresour. Technol. 64, 241-243.
- 4. Eklund, R., Galbe, M., and Zacchi, G. (1995), Bioresour. Technol. 52, 225-229.
- 5. Wu, M.M., Chang, K., Gregg, D.J., Boussaid, A., Beatson, R.P., and Saddler, J.N. (1999), *Appl. Biochem. Biotechnol.* **77–79**, 47–54.
- 6. Schwald, W., Samaridge, T., Chan, M., Breuil, C., and Saddler, J.N. (1987), in *Enzyme Systems for Lignocellulosic Degradation*, Coughlan, M. P., ed., Elsevier, London, pp.231-242.

- 7. Kokta, B.V., Ahmed A., and Esdade. B. (1993), J. of Wood Chem. and Tech. 13, 213-236.
- 8. Wallis, F. A. and Wearne, R. H. (1992), Carbohydrate Polymers 17, 103–110.
- 9. Holland, F.A., Watson, F.A., and Wilkinson, J.K. (1979), *How to Estimate Capital Cost*. Modern Cost Engineering:Methods and Data, McGraw Hill, NY.
- 10. Marchessault, R.H. (1988), in *Proceedings of the International Workshop on Steam Explosion Technique*, Focher B. and Marzetti A., eds. Gordon and Breach, pp. 391–400.
- 11. von Sivers, M. and Zacchi, G. (1995), Bioresour. Tech. 51, 43-52.
- 12. Avellar, B. K. and Glasser, W. G. (1998), Biomass and Bioenergy 14, 205-218.
- 13. Grado, S. C. and Chandra, M, Jeva (1998), Biomass and Bioenergy 15, 115-124.
- 14. Gregg, D. and Saddler, J.N. (1995), Biomass and Bioenergy 9, 287-302.
- 15. Zimbardi, F., Viggiano, D., Nanna, F., Demichele, M., Cuna, D., and Cardinale, G. (1999), Appl. Biochem. Biotechnol. 77–79, 117–125.
- Abatzoglou, N., Chornet, E., and Belkacemi, K. (1992), Chem. Eng. Science 47, 1109–1122.
- 17. Abatzoglou, N., Koeberle, P., Chornet, E., Overend, R., and Koukios, E. (1990), *Am. J. Chem. Eng.* **68**, 627–638.
- 18. Ibrahim, M, Glasser, W.G. (1999), Bioresour. Tech., 70, 181–192.
- 19. Palmquist, E., Hahn-Hagerdal, B., Szengyel, Z., Zacchi, G. & Reczey, K. (1995), Enzyme Microb. Technol. 20, 286–293.
- 20. Larsson, S., Reimann, A., Nilvebrant, N., and Jonsson, L. (1999), *Appl. Biochem. Biotechnol.* 77, 91–103.
- 21. van Zyl, C., Prior, B. A., and du Preez, J. C. (1991), Enzyme Microb. Technol. 13, 82–86.
- 22. Tran, A. V. and Chambers, R. P. (1985), Biotechnol. Lett. 7, 841–846.
- 23. Sanchez, B. and Bautista, J. (1988), *Enzyme Microb. Technol.* **10**, 315–318.
- 24. Oliveira, S. C., Paiva, T. C., Visconti, A. E., Giudici, R. (1998), *Appl. Biochem Biotech.* **74.** 161–172.
- 25. Todd, B., Ehrman, C. I., Adney, W. S., Thomas, S. R., and Rimmel, M. E. (1997), *Appl. Biochem. Biotechnol.* **62**, 99–104.