# Recirculation of Cellulolytic Enzymes in an Ultrafiltration Membrane Reactor *

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In recent years the enzymatic hydrolysis of lignocellulosic materials has become a subject of intensified interest because the hydrolysate can serve as a raw material for fermentatively produced chemicals.[1] However, available cellulolytic enzyme systems have a relatively low specific activity, about a hundred times lower than comparable amylolytic enzymes.[2] Therefore, high enzyme concentrations have to be used in order to achieve a reasonable rate and degree of hydrolysis, which in turn makes enzyme recycling an economic necessity in such a process.

Enzyme recycling has been reported to be possible by adsorption onto fresh substrate,[3] immobilization to a solid matrix, [4] as well as with the use of ultrafiltration methods.[5] Cellulolytic enzyme systems consist of at least three different activities which have been reported to adsorb with different strengths to cellulose.[6] It will therefore be difficult to obtain a complete recovery of the whole enzyme complex using adsorption alone. Immobilization of enzymes to solid matrices, plus the utilization of macromolecular or solid substrates, result in poor enzyme efficiency due to the introduction of diffusion limitation and steric hindrance. Ultrafiltration, on the other hand, allows complete recovery of the enzymes without the problems accompanying immobilization, provided that the right membrane is selected.

Cellulose hydrolysis in ultrafiltration membrane reactors has so far only been carried out with pure cellulose substrates.[5]

The present communication describes hydrolysis studied, with emphasis on enzyme recovery, in a membrane reactor using pretreated lignocellulosic material from a fast growing species of sallow (Salix Q082) at concentrations of 100 g/l dry weight. The fraction >1 mm from the lignocellulosic material, prepared by milling, was pretreated with 4 % NaOH (g/g) for 2 h at 160 °C.

The cellulolytic enzymes used were the following: 10 g/l cellulase from *Trichoderma reesei*, SP 122, with an *endo*-glucanase activity of 157 mg RS/ml min measured with CMC as substrate,[7] and 10 g/l cellobiase from *Aspergillus niger*, NOVOZYME 188, with a β-glucosidase activity of 45 μmol *p*-nitrophenol/g min using *p*-nitrophenyl-β-*D*-glucopyranoside as substrate.[8] Both enzymes were generously supplied by NOVO A/S, Copenhagen, Denmark.

A cylindrical ultrafiltration membrane reactor with a working volume of approximately 250 ml was equipped with a polyamide membrane, BM 100, molecular weight cut off 10 000 (Berghof GmbH, Tübingen, West Germany). The reactor was operated continuously by replacing the permeate stream with buffer solution from a 5l pressure feed container by means of an automatic level controll.

Semicontinuous hydrolyses were carried out in

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Table 1. Consecutive semicontinuous enzymatic hydrolyses experiments in an ultrafiltration membrane reactor utilising *one* initial addition of cellulolytic enzymes. 0.1 M acetate buffer, pH 4.8, 40 °C, dilution rate 1.0 $h^{-1}$, stirring 500 rpm.

| Exp. | Substrate added (g) | | | | ΣS | Σ *RS* in [b] permeate (g) | Yield $\frac{\Sigma RS}{\Sigma S}$ | Productivity g RS/g enz. | Initial conversion rate g RS/g enz. h |
|---|---|---|---|---|---|---|---|---|---|
| I | 25[a] | 2.35 | 2.35 | 2.35 | 32.0 | 30.1 | 0.94 | 12.1 | 0.58 |
| II | 16.5[a] | 2.3 | 2.3 | 2.3 | 23.4 | 18.7 | 0.80 | 7.5 | 0.44 |
| III | 16.5[a] | 2.3 | 2.3 | 2.3 | 23.4 | 15.4 | 0.66 | 6.2 | 0.28 |
| Total | | | | | 78.8 | 64.2 | 0.81 | 25.8 | |

[a] Initial substrate addition in each experiment. Subsequent additions are intended to keep the dry weight in the reactor constant (see also text). [b] *RS*=reducing sugars measured by the DNS method (9).

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the ultrafiltration membrane reactor in order to study how much the productivity of the enzyme in terms of g sugar/g enzyme could be improved. In each experiment, three intermittent additions of fresh substrate were made, after which the hydrolysis was left to proceed until no more soluble sugars were produced. The amount of substrate to be added was estimated from the product concentration in the permeate, with the intention of keeping the dry weight in the reactor at a level of 100 g/l. The amount of reducing sugars in the permeate volume was derived from

$$\Sigma RS\,(\mathrm{g}) = \int_0^v (\mathrm{glucose} + \mathrm{cellobiose})\,\mathrm{d}v$$

Table 1 summarizes three consecutive experiments where a total of 78.8 g substrate were hydrolyzed using only the initially added enzymes. 81 % of the substrate was recovered as soluble sugars in the permeate, giving an enzyme productivity of 25.8 g sugar/g enzyme. In a parallel batch hydrolysis experiment under equivalent conditions, only 4.7 g sugar/g enzyme was obtained. Thus, the productivity of the enzymes could be increased more than 5 times by their recovery in an ultrafiltration membrane reactor.

However, the initial conversion rates, measured during the first hour of each experiment, declined 25 % with each experiment (Table 1), indicating that a fraction of the enzymes became successively inactivated. This could be due to operational conditions such as pH, temperature and mechanical stress.

In order to estimate the influence of these different factors, the enzyme activities were measured as a function of time under hydrolysis conditions, in the absence of substrate, with and without stirring. With stirring, 8 % of the *endo*-glucanase and 10 % of the β-glucosidase activity were lost over a 6 day period, compared to an unstirred reference where 6 and 3 %, respectively, were lost. It was therefore assumed that the adsorption of enzymes to the non-degradable fraction of the substrate could account for the decrease in hydrolysis rate.

*Table 2.* Soluble enzyme activity after completed hydrolysis, % of initial activity.

| | I | II | III |
|---|---|---|---|
| *endo*-Glucanase | 22 | 18 | 9 |
| β-glucosidase | 74 | 59 | 44 |

This was investigated by measuring soluble enzyme activity at the end of each of the three experiments (Table 2). After the first experiment, when digestion was complete, soluble *endo*-glucanase activity was reduced by 78 % and soluble β-glucosidase activity by 26 %. The fact that hydrolysis took place with the recycled enzyme during the second and third experiments indicates that the adsorbed enzymes were desorbed when fresh substrate was added – however, not completely. When undigested substrate accumulates in the reactor more enzyme seems to be adsorbed to it (Table 2). This points out the importance of relating the pretreatment of lignocellulosic materials not only to hydrolyzability, but also to its influence on a possible recovery of the enzymes.

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