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A state-of-the-art steam explosion unit at the Norwegian University of Life Sciences - solubilization of carbohydrates, formation of inhibitors and analyses of complex samples.

Cost FP0901 Current needs in Biorefinery analytics

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A state-of-the-art steam explosion unit at the Norwegian University of Life Sciences - solubilization of carbohydrates, formation of inhibitors and analyses of complex samples.

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Introduction

Efficient enzymatic or microbial degradation of lignocellulosic biomass necessitates some kind of pretreatment to make the carbohydrates more accessible. Steam explosion (SE) is known to be one of the most efficient and environmentaly benign pretreatment methods. The severity of the pretreatment is determined by the residence time and the temperature in the high pressure reactor. Generally, harsher pretreatment conditions make the sugars in the biomass more accessible for hydrolysis. However, harsh conditions also lead to monosaccharide decomposition producing compounds such as HMF and furfural, which are potential inhibitors for microorganisms. Thus, optimal SE conditions will be a compromise between carbohydrate accessibility and inhibitor formation. The Norwegian University of Life Sciences has recently installed a highly flexible and high throughput SE facility on campus (Fig. 1) that is currently being used to study conversion of a variety of biomasses. Here we present some data on oat straw. Globally about 11 million tons oat straw is available, which could potentially yield 2.8 GL bioethanol.
The large analytical need associated with using a high-throughput SE unit has prompted the build up of a new analytical platform which makes efficient monitoring of different pretreatment conditions possible. A few examples of the analytical tools that we use are shown below.

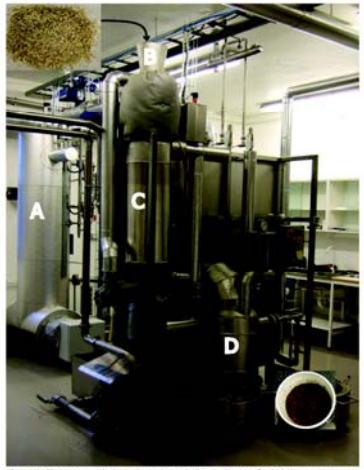


Figure 1. The steam explosion unit; A; Steam boiler, B; Biomass feed, C; Pressure reactor, D; Flash tank with removable bucket

Methods

SE pretreatment . Pretreatment was carried out in a new steam explosion unit designed and built by the Norwegian company Cambi AS. The biomass was treated in a 20 L pressure reactor where the pressure was kept constant by automatically feeding steam from a steam boiler. Treatment was ended by a fast pressure drop into a flash tank (see Fig. 1).
Enzyme treatment.
50 g/L SE treated oat straw was hydrolysed with of Novozym 188 and Celluclast (20 FPU/g DM, Novozymes) using the following conditions:
50 g DM/L, pH 6.0,T=55°C.
Carbohydrate and inhibitor analysis. Analyses were conducted using a Dionex Ultimate 3000 system set up with a Metacarb 87H (4.6x250mm Agilent), at 65°C, 0.2mL/min 5mM H_2SO_4 with serial RI (glucose, xylose, acetate, ethanol) and UV (285 nm) detection (HMF and furfural).

Results and discussion

Furfural and HMF are thermal degradation products of C5 and C6 sugars, respectively. Fig. 2 shows the concentration of these compounds in oat straw exposed to different SE conditions. It is evident that furfural and HMF start to accumulate at temperatures above 200°C.

While HMF concentrations increase with higher temperatures, furfural reaches a maximum concentration at 210°C.

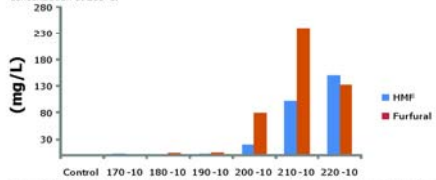


Figure 2. Inhibitor concentrations in 50 g/L slurries of non-washed SE-treated oat straw. Steam explosion conditions (T, in °C, and residence time, in minutes) are printed on the x-axis. Control = untreated oat straw.

Fig. 3 shows the concentration of glucose, xylose and acetate before and after enzymatic hydrolysis. The highest release of glucose (and acetate) in SE-treated material occurred in the pretreatment range from 200-220°C; the highest release of xylose occurred at lower T (200°C), probably due to extensive decomposition at higher T. After 24 h of hydrolysis maximum xylose release was still found in the straw fraction treated at 200°C. Maximum glucose release was found for material pretreated at 210 and 220°C.

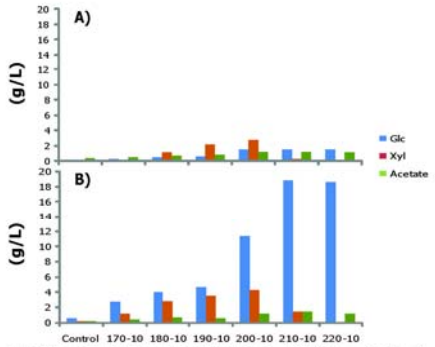


Figure 3. Released Glucose (Glc), Xylose (Xyl) and Acetate before (A) and after (B) 24 hours of hydrolysis with Celluclast and Novozyme 188. The X-axis is the same as described in the legend of Fig. 2.

A good analytical platform is a prerequisite for this type of studies. In addition to the 'catch all' workhorse methods presented in Figs. 2 and 3, this platform includes the following complementary methods for analysis of substrates and products (solid- and water soluble fractions):
1. Organic acids (Dionex Ionpac AS11, suppressed conductivity detection).
2. Monosaccharide composition (Seaman hydrolysis, CarboPac PA1 column, pulsed amperometric detection).
3. Phenolic compounds and furaldehydes by high throughput UPLC screening (reversed phase columns, UV and CAD detection).
4. UPLC high throughput carbohydrate (HILIC with CAD) analysis will complement CarboPac PA1 analyses.
Methods 1 and 2 are run simultaneously on a Dionex ICS3000 dual system. Methods 3 and 4 utilize the power of UPLC.

Concluding remarks

The new SE unit at Ås enables rapid screening of numerous SE conditions. Combined with appropriate analytical tools, this enables us to rapidly define optimal saccharification (and fermentation) processes for a wide range of lignocellulosic materials, as illustrated by the results for oat straw presented above.

Acknowledgement

We gratefully acknowledge funding from The Research Council of Norway grant nr. 190877/S60. We would like to thank CAMBI AS for constructing the SE unit and developing SOPs for its use.

The steam explosion unit 'explodes' cell wall material

Steam boiler, biomass feed, pressure reactor, flash tank,

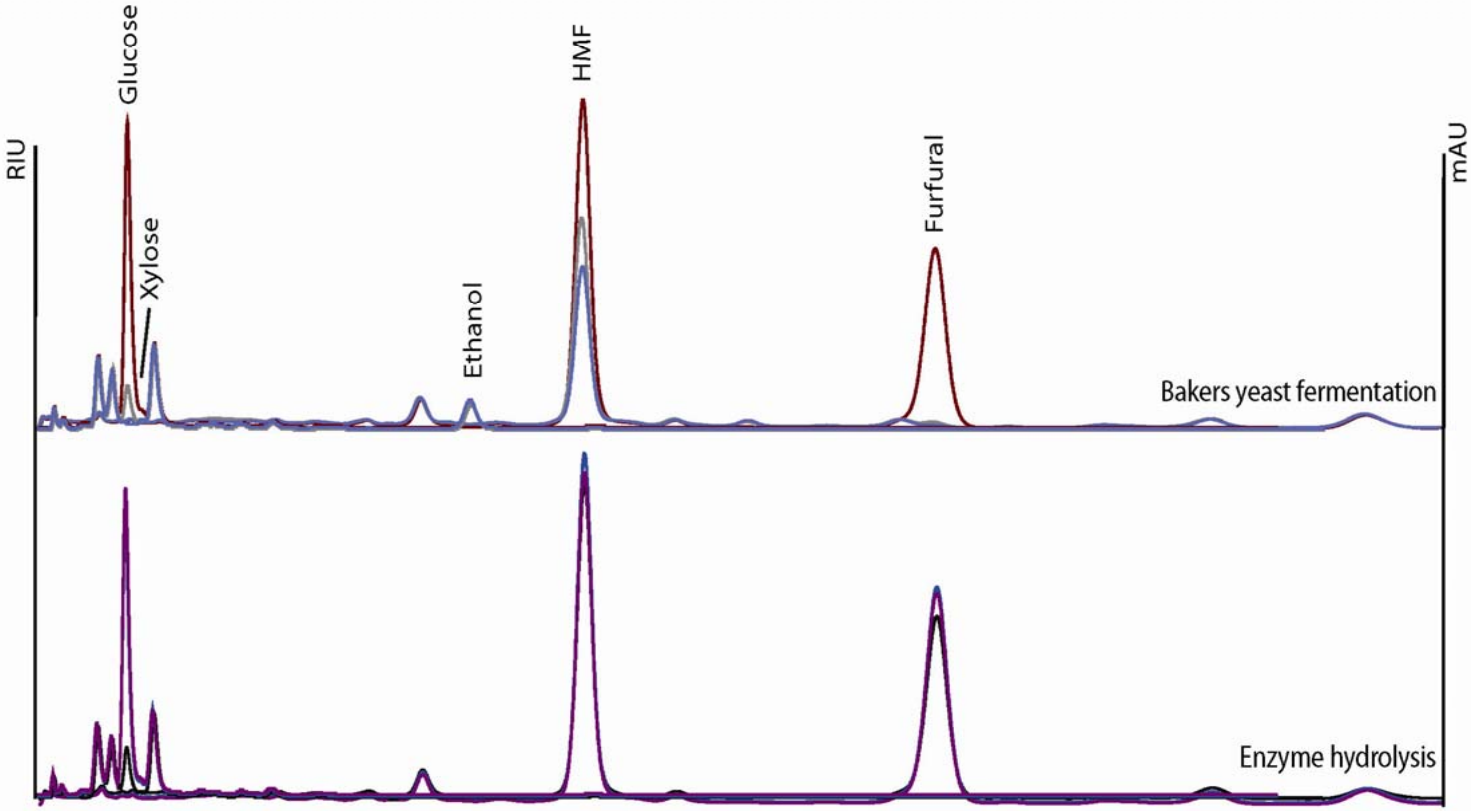
Sample types and number of samples

- Pretreatment (SE), hydrolysis and fermentation yields; **soluble** part and **solid** part
  - Solubilized material analyzed directly
  - Residual solids are chemically degraded (e.g. Acid hydrolysis), then component analysis by HPLC
  - Experimental design with combinations of the above evolve very fast into a **large number of samples**.

An up to date flexible Liquid Chromatography toolbox

Dual setup, rapid method switching. A wide range of detectors, 2 EDs, RI, CAD, 3 UVs, Conductivity, several interchangeable between systems, serial/paralell set-up, improve annotations/observe more peaks. Offline MS

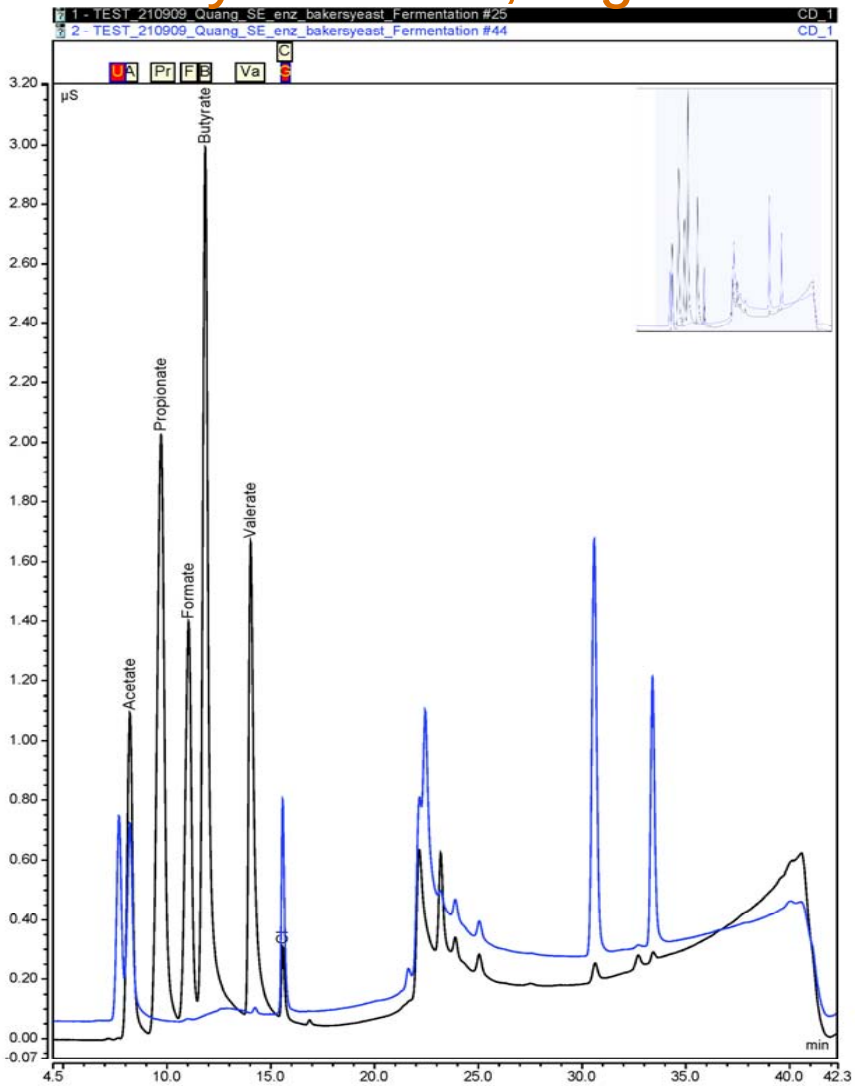
Screening effects of enzyme hydrolysis and fermentation



Time course enzyme hydrolysis (0, 4, 24h) and fermentation (0, 24, 72h) of SE 220 -15.
Chromatograms 6-60 min

Ethanol formation, furfural removal, slow HMF reduction, glucose dissappear, disturbing peaks

Complementary methods, organic acids



Example with Ionpac, high capacity 'insensitive' to matrix components.
Work fine with fermentations, enzyme hydrolysis, other complex smpl matrices

Methods

- Metacarb87H 'Catch most' screening;mono-/disacch, ethanol, acetate, 5-HMF and furfural (60min run,5mM H_2SO_4), RI+UV.
- Metacarb87P (column made for sugar analyses, ok separation of most mono-/di-saccharides (35min, water/acqueous buffers), RI.
- CarboPac columns (PA1 and PA200) for mono comp./complex oligosaccharide analyses on ICS3000 (HPAEC), PAD
- BEH Amide (2.1x50mm and 2.1x150mm), runtime 2-20 min HTP or HiRes (HILIC mode), UPLC-CAD, complement HPAEC separation at other pH's
- IonPac AS11H (organic acids C1-5) High sensitivity/resolution, (IEC), suppressed conductivity
- UPLC methods (to be implemented)
  - monosaccharide decomposition products (furaldehydes)
  - Phenolic compounds (ferulic acid, p-coumaric acid, coniferyl alcohol etc) on high throughput C18 UHPLC.

Thank you for your attention, on behalf of

- The PEP (protein engineering and proteomics) group
- http://pep.umb.no/pepwiki.php?title=PEPFront