Ammonia and dilute acid pretreatment of grass silage, a versatile biorefinery feedstock

Ville Pihlajaniemi[1] • Simo Ellilä[1] • Piritta Niemi[1] • Matti Siika-aho[1] • Sakari Poikkimäki[1] • Marketta Rinne[2] • Raija Lantto[1]
[1)]VTT Technical Research Centre of Finland Ltd; [2)]Natural Resources Institute Finland (Luke), Jokioinen, Finland

Introduction

Grass crops are highly competitive in productivity, rich in protein and there is an estimated surplus production capacity of 20 Mton (DM) in Europe (Mandl 2010). Grass can be ensiled by existing technology for providing a year-round supply of silage for green biorefining into innovative biochemicals and feed applications.

We previously compared dilute acid steam explosion and ammonia soaking as pretreatments for hydrolysis of grass silage (Niemi *et al*., 2016). Now we take a closer look at the processes and their suitability for producing fungal protein with the prominent filamentous fungus *Paecilomyces variotii* (Alaviuhkola *et al.*, 1975).

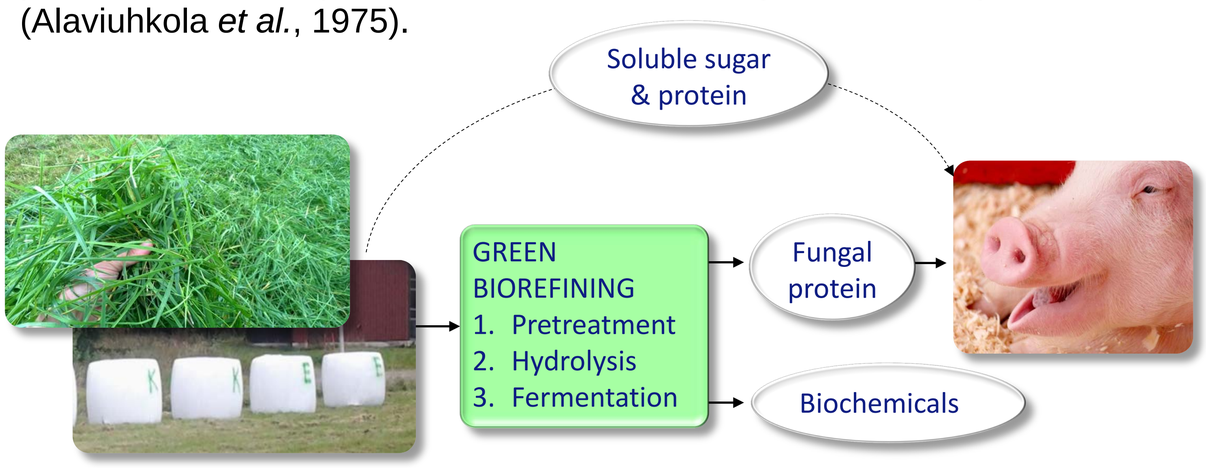

Figure 1. *Grass silage as a versatile feedstock*

Single reactor ammonia process

Scale-up of the ammonia process was demonstrated as consecutive ammonia soaking, ammonia evaporation and hydrolysis performed in a single reactor at 20% DM. Ammonia was efficiently recovered by evaporation and the solids were hydrolyzed, showing 70% sugar yield. Residual ammonia was synergistically utilized as a nitrogen source for fungal protein production.

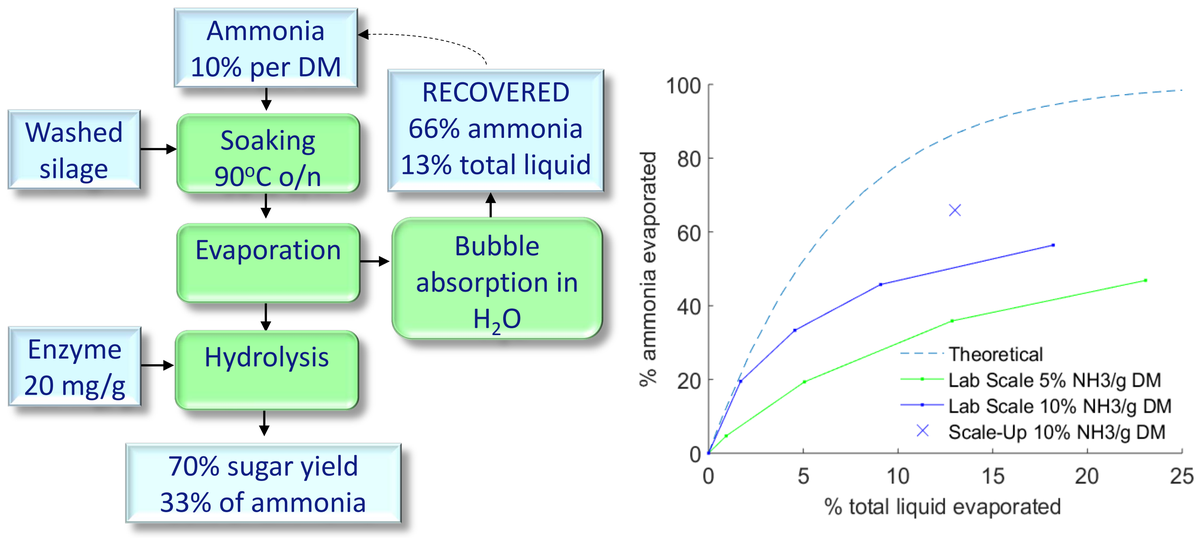

Figure 2. *Single reactor NH_3-process and ammonia recovery by evaporation.*

Hyrolysis of steam exploded silage

Hydrolysis of steam exploded silage was investigated as a function of enzyme dosage (E), time (t) and solids concentration (c). The hydrolysis yields were expressed with an empirical model, modified from Pihlajaniemi *et al*., (2015), $R^2 = 0.97$.

$$Y = (Y_{max} - \beta_1 c)\left(\frac{\beta_2 E}{\beta_2 E+1}\right)\left(\frac{\beta_3 t}{\beta_3 t+1}\right)\left(\frac{\beta_4 E\sqrt{t}}{\beta_4 E\sqrt{t}+1}\right)$$

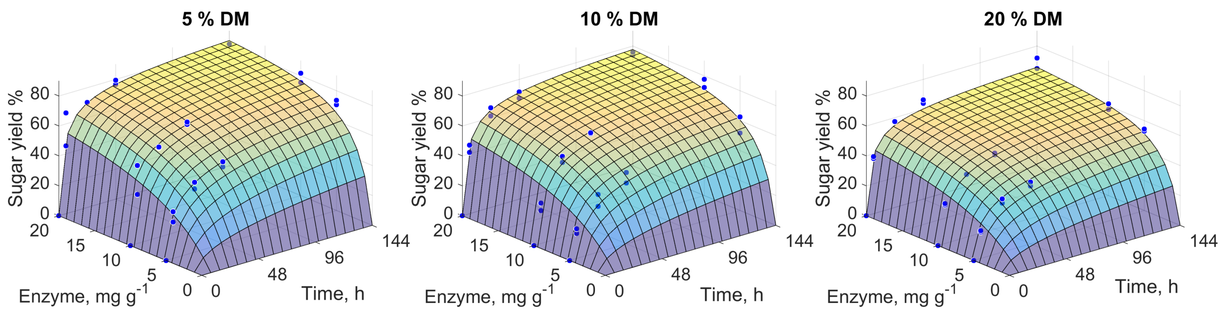

Figure 3. *Hydrolysis yields and the model response surfaces.*

Fungal protein production

Hydrolysates of the pretreated silage were converted into fungal protein by *Paecilomyces variotii*. Ammonia could be used as a nitrogen source, reaching a similar protein content but a lower biomass yield compared to organic nitrogen. The hydrolysates showed an inhibitory effect, particularly affecting growth rate.

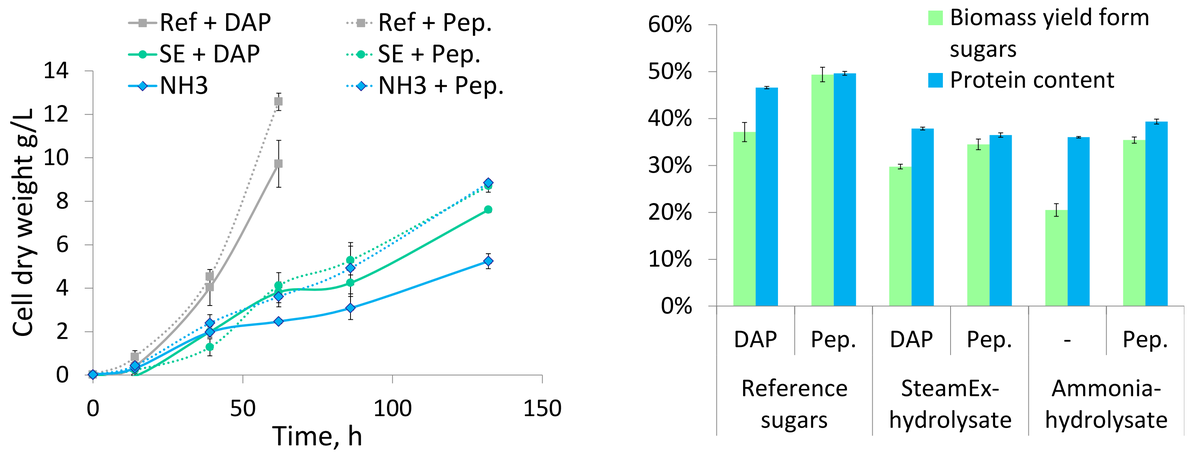

Figure 4. Paecilomyces *growth curves & yields with hydrolysates from steam explosion (SE) and ammonia soaking (NH_3) compared to pure sugars (Ref). Comparison of organic nitrogen source (Peptone) to ammonia (DAP).*

Methods

Steam Explosion: In 10 L pressure reactor at 195 °C for 15 min, with 1% H_2SO_4 per DM, followed by rapid pressure release into receiver tank. **Single reactor ammonia process:** In 4 L reactor with a scraping mixer element and an oil-jacket heating (Lödige). **Lab scale ammonia treatment:** NH_3 soaking in 500 ml Schott bottles in convection oven. Ammonia recovered with laboratory distillation equipment and determined by titration. **Hydrolysis conditions:** Flashzyme Plus (cellulase product kindly provided by Roal Oy, Rajamäki, Finland), 45 °C, pH 5. ***Paecilomyces* cultivations:** Performed in flask scale with industrial strain VTT D-75018, 200 rpm, 30°C, with 25 g/l total sugar and 10 g/l nitrogen source. Nitrogen was quantified from lyophilized washed cell mass using GC and multiplied by 6.25 to yield total protein.

Conclusions

- Silage hydrolysates are applicable for fungal protein production
- Efficient ammonia recovery was demonstrated in a single reactor process
- Synergistic utilization of ammonia as nitrogen source was shown in the production of fungal protein
- The effects of hydrolysis conditions on sugar yield were modeled

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Contacts
Ville Pihlajaniemi
Tel. +358 40 143 9379
ville.pihlajaniemi@vtt.fi