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Impact of Recycling Stillage on Conversion of Dilute Sulfuric Acid Pretreated Corn Stover to Ethanol

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Objective

Assess impact of process water recycle on process performance

Goal: Achieve 25% process water recycle

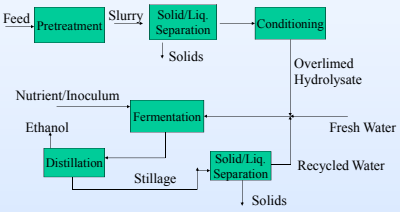
with no negative impact on process performance

Introduction

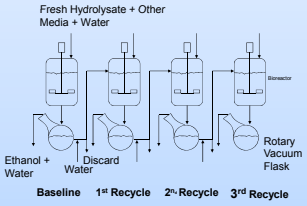
- Large quantities of water needed in biomass-to-ethanol process (Approx. 6 Gal /Gal of EtOH produced)
- Recycle waste water reduces fresh water requirements and downstream treatment needs
- Consequence of recycling is accumulation of non-metabolizable compounds that may inhibit fermentation
- Knowledge of inhibitors and how to minimize their effect on fermentation performance is key
- The amount and the nature of inhibiting compounds depends on:
  - Raw material
  - Prehydrolysis & enzymatic hydrolysis procedure
  - Extent of recirculation

Materials and Methods

Process Configuration



Experimental Procedure



Triplicate runs performed at each condition

Hydrolysate: Overlimed pretreated corn stover liquor
- Pretreated at 30.0% total solids (TS) loading (34.2% after flash step)
- Adjusted to 15% to 20% TS loading
- Initial component concentrations (g/L): Glucose: 33.6, Xylose: 81.3, Acetic acid: 16.1, HMF: 3.3, and Furfural: 4.1
- OL Liquor supplemented with glucose to 55 and 74 g/L, respectively at 15% and 20% TS

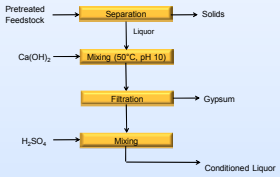
Strain: *Zymomonas* mobilis 8b
Growth Media: Rich Medium (RM) (10 g/L Yeast Extract + 2 g/L KH_2PO_4) plus hydrolysate
Fermentation: BioStatQ with 340 mL working volume, pH = 5.8, Temperature = 35°C, Initial OD = 1 @600nm

Distillation: In rotary evaporator, replacing evaporation with DI water afterward
Recycle ratio (RR): 10% and 25%, defined as the amount of stillage water recycled
Quality Control: Fermentations done in triplicate, with pure sugar control
Procedure: Three recycles were done at each condition to achieve a steady state concentration of acetic acid
Total of 12 sets of fermentation performed, each set included 4 fermentation

Experimental Setup

For each Condition:
- Total of 4 Fermentors; Three with recycling of stillage at one condition in triplicate. One for pure sugar control with Acetate
- A shake flask control to check for culture viability
- Initial OD =1 @600nm
- Initial OD =4 @600nm to evaluate initial cell concentration

Conditioning Method: Overliming (OL) Process

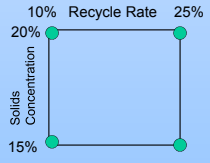


Analytical

- Sugar concentrations measured by HPLC using the Shodex SP0810 carbohydrate column (55°C, 0.6 mL/min, water mobile phase, RI detection) and ethanol concentration measured using BioRad HPX-85
- Liquor densities measured using an Anton-Paar densitometer

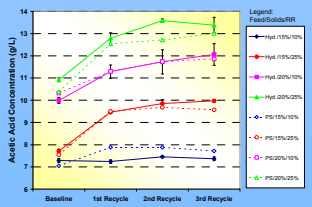
Experimental Design

2-factor, 2-level factorial design
3 to 4 iterations required to achieve steady state concentrations

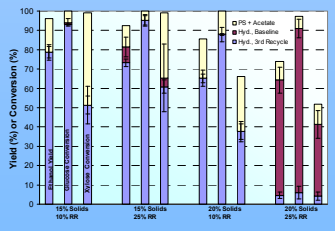


Results

Achieving Steady State



Performance Results



Issues:

- Acetate inhibition of *Zymomonas:*
  - At lower pH is major problem
  - At higher pH as acetate concentration builds up inhibition becomes an issue
- Other inhibitors may become an issue

Summary of the Results

- With 15% Solids loading & 25% RR there was little impact
  - Ethanol Yield: 75%
  - Glucose & Xylose Utilization: 95% & 60%
- With 20% Solids loading & 10% RR some impact was noticed
  - Ethanol Yield: 65%
  - Glucose & Xylose Utilization: 85% & 37%
- With 20% Solids loading & 25% RR, impact was very significant
  - Ethanol Yield: 10%
- High acetate at 20% solids level in pure sugar did not affect performance
- Under 20% solids & 25% RR conditions in pure sugar,
  - Ethanol yield: 75%
  - % Gluc & Xyl utilization: 95% & 50%
- Increasing initial cell mass improved pure sugar control but not in hydrolysate process
  - Confirms other inhibitors besides acetic acid have effect on the process

Conclusions

- Higher yield and sugar utilization in pure sugar compared to hydrolysate shows:
  - Other inhibitors besides acetic acid are producing negative impact on the process
- With 20% Solids loading and 25% RR, impact was very significant
  - Ethanol Yield: 10%

Acknowledgements

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