Continuous Enzymatic Hydrolysis Development for Improved Saccharification Performance

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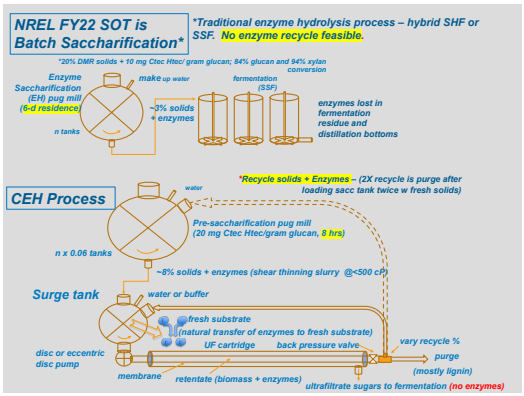


Figure 1. Process Diagram of existing SOT assumptions (batch) versus the Continuous Enzymatic Hydrolysis (CEH) concept

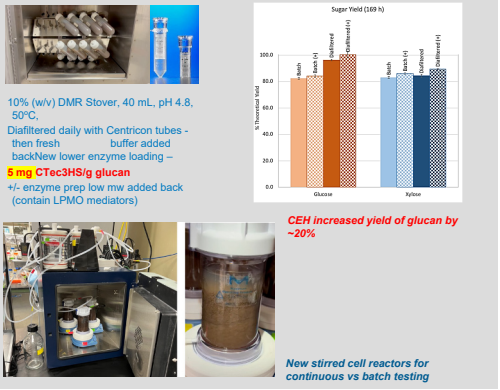


Figure 2. Bench Scale CEH testing demonstrates 20% improvement with lower enzyme loadings.

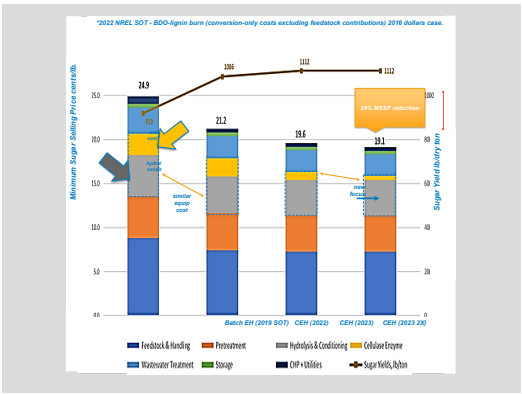


Figure 3. Projected impacts on MSSP ~24%

Background

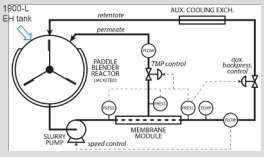
The Continuous Enzymatic Hydrolysis Development (CEHD) project aims to reduce the cost and commercialization risks of Gen2 biorefinery sugar/lignin/ethanol production through development of a deployable continuous enzymatic hydrolysis process. Recent changes in the technical landscape of commercial enzymatic hydrolysis of Gen2 pretreated biomass dictate that the existing hybrid SSF approach be reconsidered. Most importantly, the current practice of "finishing hydrolysis" in SSF must be abandoned due to the fact that new cellulase/hemicellulose formulations from Novozymes, now the sole supplier of commercial Gen2 enzymes in North America, are now not rated for SSF (see NZ CTec3HS product bulletin) We have recently developed bench scale CEH tools to optimize saccharification of DMR pretreated biomass where, unlike SSF with yeast or *Zymomonas*, the pH, temperature, oxygen tension, LPMO mediator concentration, and/or removal of end-product inhibitors can be precisely controlled. In scale up, the goal is to use existing commercial cross flow ceramic membrane filtration external loops coupled to enzymatic hydrolysis (EH) reactors. Pretreated biomass solids and enzymes are retained for reaction while solubilized product sugars are removed in situ, with high extents of conversion and longer enzyme lifetimes achieved through a series of reactor-membrane unit stages. The CEHD project is focused on advancing CEH as a transformational, process-intensified, lower-cost method for producing soluble clarified biomass sugars and insoluble lignin-rich streams.

FY21 Assembled the PDU CEH skid

Single-stage UF system using a 30-L surge tank, one Porex 1.2 m, 0.05 µm **PVDF** membrane, and an integrated pumped external recycle loop.

CEH skid system was fed pre-saccharified DMR slurry (8% w/v) transferred from the 1900-L horizontal paddle blender.

Novel vessel level measurement method (gas law) implemented and validated.



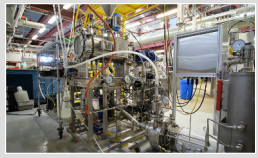


Figure 4. Scale up progress and membrane possibilities

CEH Goal

- *Reduce the CAPEX and OPEX of batch conventional enzyme hydrolysis EH and post hydrolysis solid-liquid separation using continuous EH (CEH) technology.*
- *Process risk reduction in an era of changing fermentation modalities.*

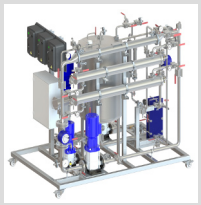
Questions to be Answered

- *Can CEH serve as a disruptive improvement to SAF production via intermediates (ethanol, BDO, etc) from biomass sugars?*
- *Can the enhanced performance from CEH offset the cost of its deployment?*
- *Can CEH enable a nascent cellulosic sugar production technology model?*

Impact - Key Take-Aways for Commercial Implication – CEH versus EH

- *Largely similar CAPEX for batch EH and CEH*
  CEH requires different equipment. EH requires more blender (pug) mills and CEH requires UF units.
- *Largely similar OPEX*
  CEH requires more electricity for evaporation to concentrate the more dilute sugars produced.
  EH requires greater enzyme usage.
- *Higher electricity costs of CEH are offset by eliminating (problematic) polyelectrolyte flocculant used for EH.*
- *Batch EH TEA is dominated by equipment, enzymes, and post hydrolysis solid-liquid separation (flocculation) for lignin separation.*
- *CEH TEA dominated by equipment, evaporation, and higher power requirements.*

NREL mini skid: (1x120 cm) ~ 0.5 m² tubular monochannel membrane =~25 L/h
ALSYS PolyPilot skid: (3x120 cm) ~ 1.5 m² tubular multichannel = ~500 L/h
Commercial UF: (48x330 cm) ~72 m² tubular multichannel >1000 L/h (Polypilot)
Commercial RO: (400 plus) multichannel membranes ~500,000 L/h



**Polypilot 200*

Tangshan UF system for SWRO pre-treatment. UF membranes housed in the T-Rack system, treating 110,000 m³/day of seawater.

Conclusions:
20% sugar release improvement at lower enzyme loading

This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office. The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.

Symposium For Biofuels and Chemicals
Portland, OR
4/30/2023-5/3/2023
NREL/PO-2700-86093