Scale-Up of One-Pot Ionic Liquid Pretreatment and Fermentation of Sorghum for Bisabolene

U.S. DEPARTMENT OF ENERGY
Office of Science

Rebecca Wong[1], Jipeng Yan[2], Ling Liang[2], Fabrice Masson[2], Qian He[2], and Ning Sun[2]
[1]Grinnell College, [2]Advanced Biofuels Process Demonstration Unit, Lawrence Berkeley National Laboratory

BERKELEY LAB
WORKFORCE DEVELOPMENT & EDUCATION

Abstract

In order to meet increasing energy demands while reducing greenhouse gas emissions, more sustainable and readily available sources of energy will be necessary. Such sources of energy should not only be renewable but also scalable and cost-effective in order to compete with current, non-renewable energy sources. By converting agricultural residues, such as those from sorghum, through deconstruction and fermentation processes involving a novel, Joint BioEnergy Institute (JBEI)-patented process, sourcing sustainable, economical, and scalable renewable energy will become realizable. One-pot ionic liquid (IL) pretreatment offers the advantages of (1) using a singular pot for IL pretreatment and saccharification and (2) requiring no washing of pretreated biomass, resulting in reduced processing time, energy, and expense due to inputs and washing water disposal. The use of cholinium lysine ([Ch][Lys]) IL additionally results in a more environmentally-friendly process due to its low toxicity and reactivity as well as biodegradability. Previous studies by JBEI suggest that the process was effective in liberating sugars (>90 percent sugar yield) at small-scale for switchgrass. After scaling up the process to 2L reactors using sorghum agricultural residue at high solids loading (30 percent), glucose yields of 72.29 percent and xylose yields of 62.59 percent were obtained.

Introduction

- ***Finding advanced biofuels to phase out fossil fuels***
- Bisabolene – precursor to bisabolane, an alternative fuel compound that has been proposed as a diesel replacement
  - Favorable fuel properties such as lower freezing point, greater molecular stability under high pressure, reduced premature ignition, increased energy density, and higher octane number compared to D2 Diesel fossil fuels
- ***Production of bisabolene:***

Pretreatment → Enzymatic Hydrolysis → Fermentation → Analysis

- ***Developing a novel pathway for pretreatment of biomass***
- One-pot ionic liquid (IL) pretreatment – singular pot for pretreatment and saccharification, requires no washing of material to remove IL
  - Potentially more scalable and affordable pretreatment option
- Fermentation – utilize high solid loading (30% compared to 10-15%)
  - Increased sugars and inhibitors from increased biomass loading
- ***Goals:***
- Verify production of bisabolene
- Determine effectiveness of one-pot IL pretreatment in liberating sugars
- Analyze impact of high solid loading on fermentation processes

Methodology

Results and Discussion

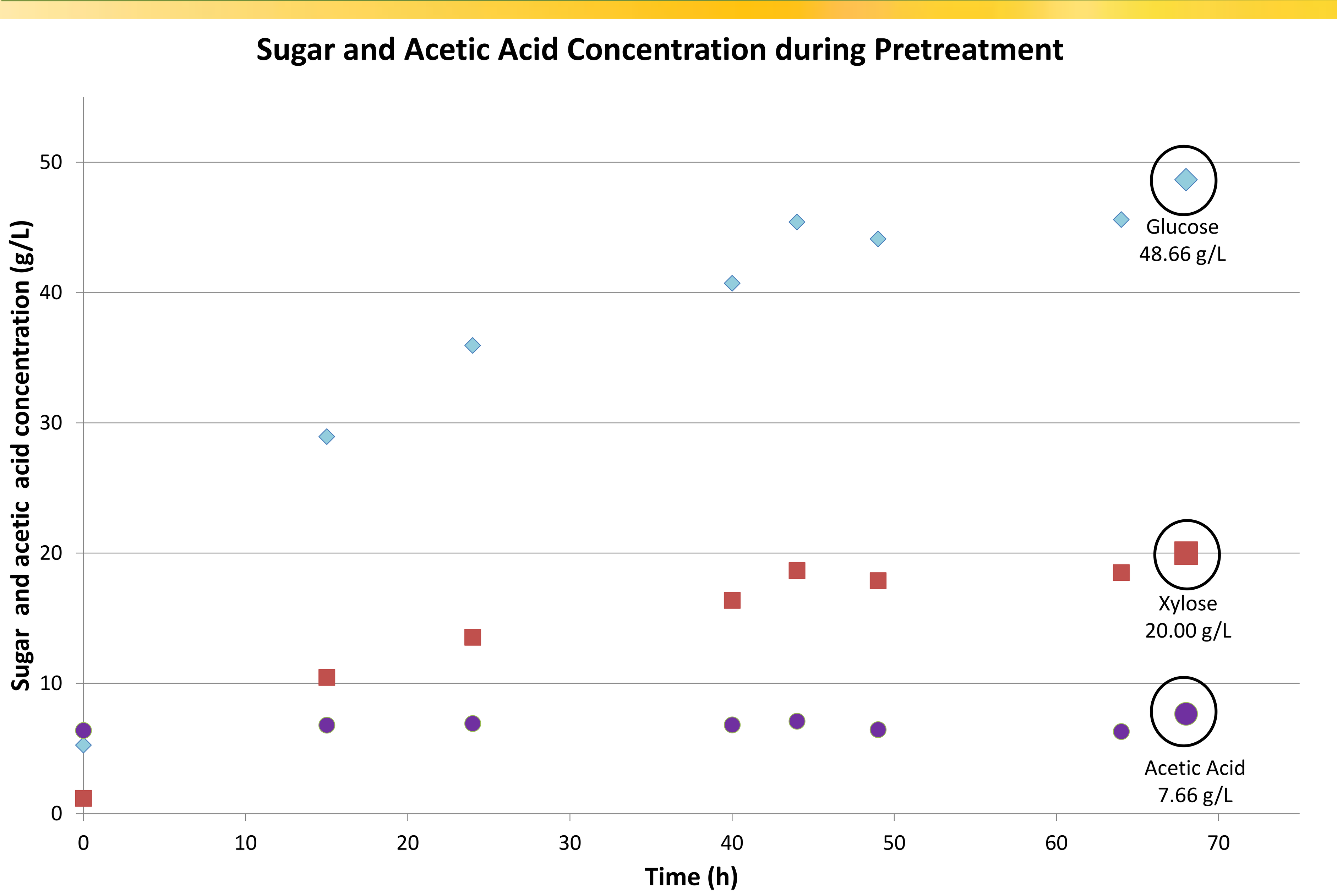

Figure 1 Trends in sugar (glucose and xylose) and inhibitor (acetic acid) concentrations during pretreatment process (Thermo Fisher Scientific Ultimate 3000 UHPLC utilizing Bio-Rad organic acid column)

- Comparable concentrations of sugars found in separate experiment performed in 1L Parr reactors by JBEI (Fig. 1)
  - 46 ± 7 g/L glucose (JBEI)
  - 22 ± 2 g/L xylose (JBEI)
- Growth of *Rhodosporidium toruloides* verified in one reactor (Fig. 2, 3)
  - Unable to quantify growth due to solids loading
- Verified bisabolene yields
  - Inability to quantify due to inaccurate readings

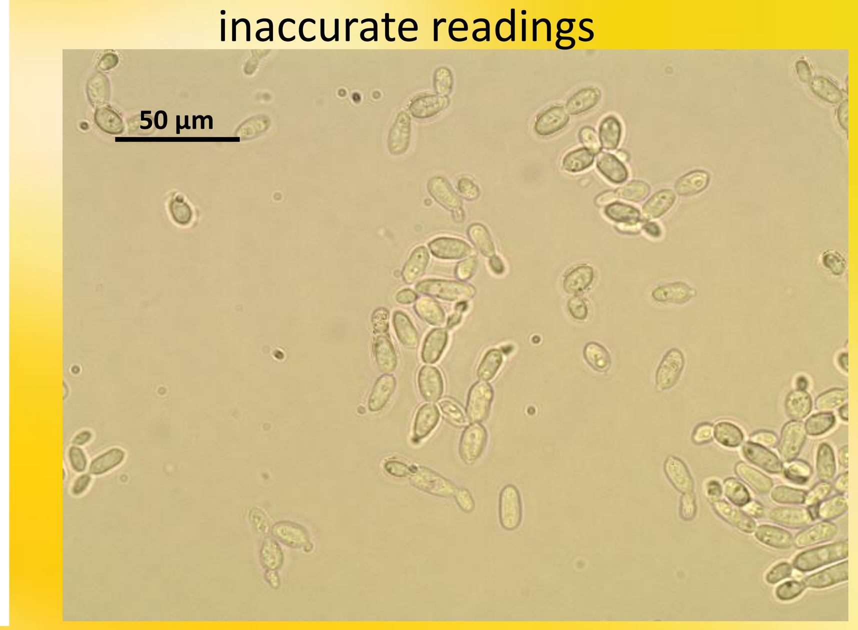

Figure 2 *Rhodosporidium toruloides* microscopic image (100X) (Leica DM4 B Microscope)

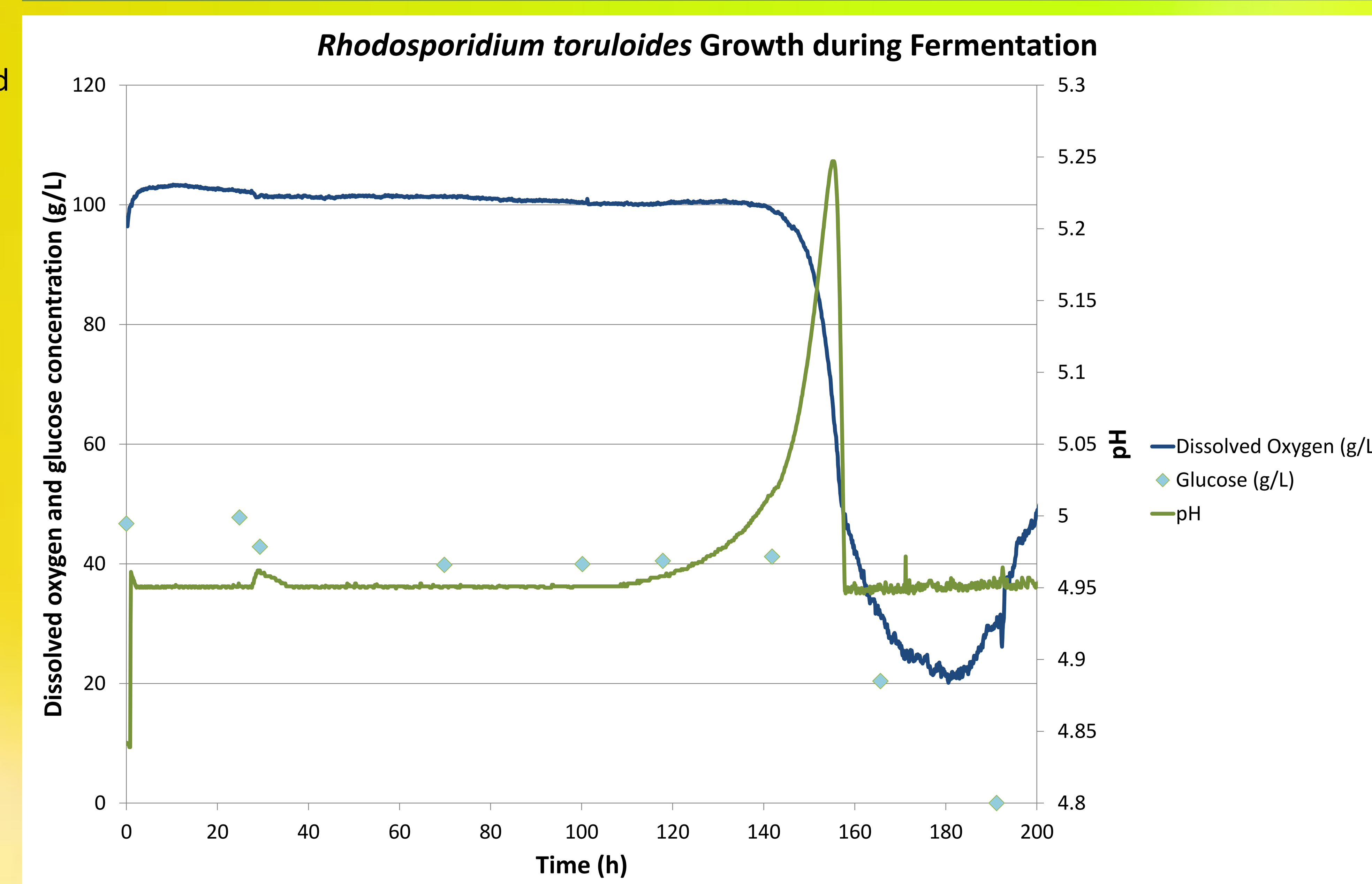

Figure 3 Growth of *Rhodosporidium toruloides* shown by variations in dissolved oxygen, pH, and glucose (Thermo Fisher Scientific Gallery Plus Automated Photometric Analyzer and Sartorius Bioreactors)

Conclusions and Future Work

- Verified production of bisabolene but unable to quantify
- Glucose yields of 72.29%, xylose yields of 62.59%
- High solid appears to lower viability of *Rhodosporidium toruloides*
- ***Challenges:***
  - Recalibrate instrument to accurately measure bisabolene
  - Lower glucose yields than predicted with long growth time (more than 100 hours)
  - Low success rate (three out of four 2L reactors failed to grow)

- Optimization of experiments to determine why *Rhodosporidium toruloides* did not grow
  - Filtration (0%, 50%, 100%) – high solid loading inhibition
  - Yeast Extract and Peptones (YEP) addition – lack of nutrients
  - Additional dodecane addition – product feedback inhibition
  - pH (5, 7) – growth conditions
  - Inoculation (Yeast Extract, Peptones, Dextrose (YPD), 50% filtered hydrolysate, none) – adaptability to media

This work was supported in part by the U.S. Department of Energy, Office of Science, and Office of Workforce Development for Teachers and Scientists (WDTS) under the Science Undergraduate Laboratory Internships Program (SULI). I would like to thank my mentors Dr. Jipeng Yan and Dr. Ning Sun for mentorship and advice, the SULI coordinators and organizers, as well as the entire SULI cohort. A special thanks to Ana Nuñez Castrejon, Lupe Aguirre, Alex Koegel, Fabrice Masson, and Dr. Jipeng Yan for usage of their photographs.