Fermentative Conversion of Hydrolyzed Douglas Fir Biomass into Isobutanol

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Introduction

Gevo has developed fermentation and process technology to convert biomass sugars to isobutanol and further into renewable jet fuel through chemical processing. Gevo has developed GIFT®, Gevo Integrated Fermentation Technology, to produce isobutanol at high productivity, titer, and yield using a yeast biocatalyst adapted to hydrolyzate. This project leverages Gevo's extensive R&D for an isobutanol producing yeast from starch and other sugar based feedstocks. The goal of this project is to produce isobutanol according to a specification developed by Gevo that ensures the isobutanol will be converted into renewable biojet using existing Gevo technology. Quantities of about 1,000 gallons of biojet will be prepared and validated as suitable jet fuel blend stock using ASTM's fit for purpose testing protocol and input from stakeholders. The specific tasks of this project are: (1) Characterize toxicity of a representative sample of pre-treated woody biomass (Douglas Fir) for fermentation; (2) Adapt yeast biocatalyst to pretreated biomass hydrolyzate; (3) Produce isobutanol in a 1L batch fermentation from pretreated biomass sugars using the adapted yeast biocatalyst; (4) Economic assessment of wood to isobutanol, jet; (5) Produce isobutanol in a 1L GIFT® fermentation from pretreated biomass sugars using the adapted yeast biocatalyst; (6) Analysis of isobutanol to close the mass balance and determine potential low-level impurities; (7) Produce ≥2,000 gallons isobutanol from GIFT® fermentations at 40,000 L demonstration scale. (8) Convert lignocellulosic isobutanol to ≥ 1,000 gallons biojet for further testing.

Methods

Characterize representative samples of pretreated Douglas fir hydrolyzate for fermentation

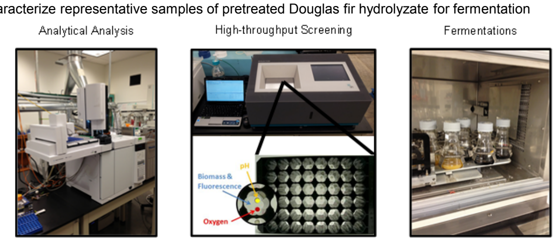


Figure 1. As new pretreated biomass samples are received, Gevo hydrolyzes these and measures resultant sugars by HPLC. Then, the hydrolyzates are characterized for yeast biocatalyst growth and fermentation in an appropriate scale.

Adapt yeast biocatalyst to pretreated Douglas fir hydrolyzate

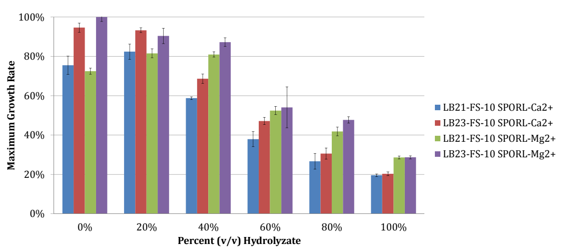


Figure 2. Normalized maximum growth rate of LB 21 and LB 23 strains in various percentages of SPORL-Ca2+ or SPROL Mg2+ hydrolysate

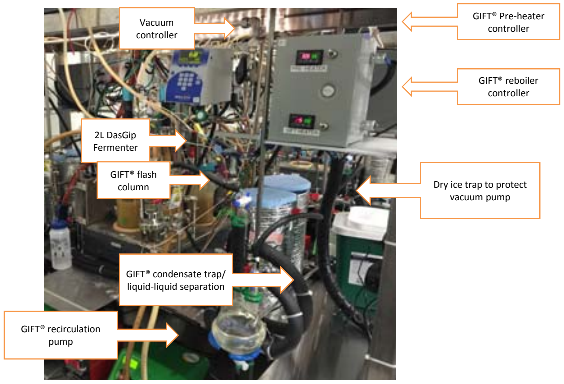


Figure 3. Image and specifications of a 2L DasGip nominal volume bench scale fermenter coupled with a lab scale GIFT® unit.

Results

Table 1. Sugar and inhibitor concentrations in FS-10 feedstocks from different pretreatment methods. Compositional analysis was determined using high performance liquid chromatography (HPLC) at Gevo. (n.d. = not detected)

	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Mannose (g/L)	Acetate (g/L)	HMF (g/L)	Furfural (g/L)
FS-10 Wet Oxidation (WO) Hydrolyzate (Pretreatment Batch A)	44.76	6.61	4.18	6.00	16.84	7.94	2.42	0.56
FS-10 Wet Oxidation Hydrolyzate (Pretreatment Batch B)	67.22	4.57	2.37	n.d.	9.04	3.90	n.d.	n.d.
FS-10 Wet Oxidation Hydrolyzate (Pretreatment Batch C)	54.79	12.01	5.38	4.43	9.59	7.99	3.53	0.26
FS-10 SPORL Hydrolyzate	62.74	6.84	5.07	n.d.	11.83	0.62	n.d.	n.d.
FS-10 Mild Bisulfite SSL	7.83	7.32	5.29	2.21	18.06	3.61	n.d.	n.d.
FS-10 Mild Bisulfite Solids Hydrolyzate	63.88	3.77	2.07	0.68	7.52	1.65	n.d.	n.d.
FS-10 Unwashed Mild Bisulfite Solids (UMBS) Hydrolyzate	84.22	10.14	5.26	2.02	20.93	3.44	n.d.	n.d.
FS-10 Unconcentrated Milled Wood (UMW) Hydrolyzate	39.84	7.61	1.87	2.04	11.40	0.74	n.d.	n.d.
FS-10 Concentrated Milled Wood (CMW) Hydrolyzate	61.84	9.21	0.46	1.36	12.76	0.77	n.d.	n.d.
Cosmo Hemlock Reject Fibers Hydrolyzate	111.35	2.02	0.15	1.45	0.79	0.27	n.d.	n.d.
FS-10 SPORL-Mg2+ Pretreated Concentrate	282.63	44.05	18.25	7.18	75.28	10.39	n.d.	n.d.
FS-20 Andritz Trial 35 min.	66.86	5.70	0.75	n.a.	n.a.	1.16	n.d.	n.d.
FS-20 Andritz Trial 45 min.	92.15	2.64	1.81	1.76	4.41	0.57	n.d.	n.d.
FS-20 ZeaChem Pretreatment	102	pending	pending	pending	pending	pending	pending	pending
Evaporated FS-20 ZeaChem Pretreatment	156	pending	pending	pending	pending	pending	pending	pending

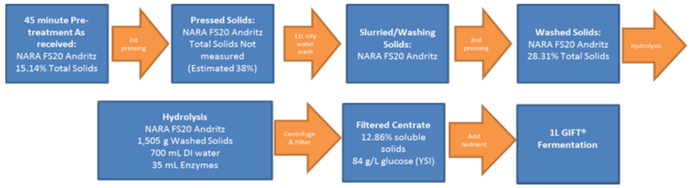


Figure 4. Block flow of Andritz material hydrolysis process

Enzyme Hydrolysis Results from latest three FS-20 Pretreated Feedstocks tested at Gevo:

FS-20 Andritz 35-Minute (Green Bucket):
22.28% solids at end of hydrolysis
72 g/L Free glucose at end of hydrolysis
51.25% glucan (w/w)
222.8 g/L biomass * 0.5125 g glucan/g biomass = 114.19 g glucan/L
114.19 g glucan/L * 1.11 = 126.75 theoretical glucose
72 g/L glucose / 126.75 theoretical = **57% yield**

FS-20 Andritz 45-Minute (Orange Bucket):
21.71% solids at end of hydrolysis
106 g/L Free glucose at end of hydrolysis
49.58% glucan (w/w)
217.1 g/L biomass * 0.4958 g glucan/ g biomass = 107.58 g glucan /L
107.58 g glucan/L *1.11 = 119.42 theoretical glucose
106 g/L glucose / 119.42 theoretical = **88.7% yield**

FS-20 ZeaChem
18.7% solids at end of hydrolysis
102 g/L Free glucose at end of hydrolysis
47.33% glucan (w/w)
187 g/L biomass * 0.4733 g glucan/ g biomass = 88.5 g glucan /L
88.5 g glucan/L *1.11 = 98.24 theoretical glucose
102 g/L glucose / 98.24 theoretical = **104% yield**

All were hydrolyzed using Novozymes CTec3 and HTec3 at 0.08% and 0.008% enzyme loading, respectively.

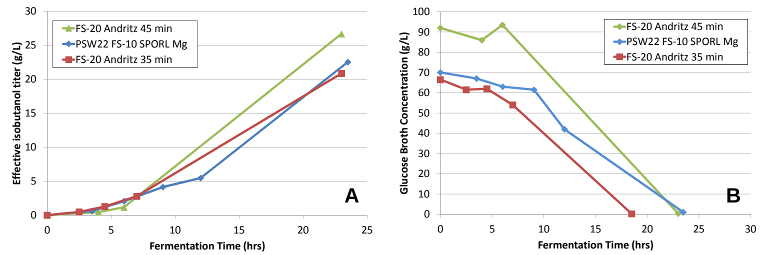


Figure 5. (A) Total effective isobutanol produced in GIFT® fermentations using FS-20 Andritz 45 min, FS-10 SPORL Mg, and FS-20 Andritz 35 min material as a substrate. (B) Broth glucose concentration over the course of the fermentation using FS-20 Andritz 45 min, FS-10 SPORL Mg, and FS-20 Andritz 35 min material as a substrate. Note that inefficient (low yield) hydrolysis generated hydrolyzates that contained at most 90 g/L glucose (as a representative sugar). GIFT® fermentations are most effective when sugar concentrations are 150 g/L or greater. This way, effective isobutanol titers can reach up to 100 g/L iBuOH or greater. Here, sugar exhaustion is the reason for 20-25 g/L iBuOH effective titers.

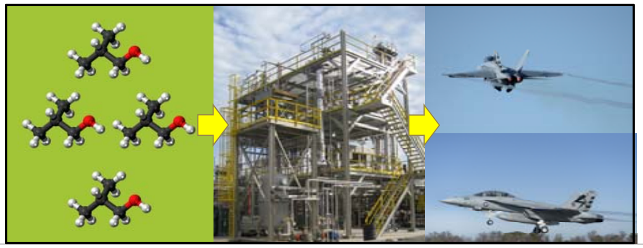
Figure 6. Hydrolyzed material as received from Andritz FS-20 trial (A) and processing steps at Gevo. (B) Fruit and wine press used to press as-received solids, which were also washed with 1 volume of water equal to the pressate. (C) DasGip 2L vessel fitted with 3 marine style impellers and electrically-controlled heating block used to perform enzymatic saccharification. (D) 1L nominal volume RotoVap rotary evaporator used to concentrate hydrolyzate up to sugar levels of ~ 150 g/L.

1,000 Gallon Bio-Jet Demonstration Path Forward

- Hydrolysis of pretreated feedstock at Gevo has been optimized. Lower solids (12% w/w) hydrolysis of FS-20 ZeaChem material resulted in increased hydrolysis yield from <80% to >90%.
- Evaporation and concentration of ZeaChem hydrolyzate increased fermentable sugar to >150 g/L, a concentration that is suitable for isobutanol fermentation using GIFT®.
- 1L GIFT® fermentations will be carried out to validate the current nutrient package, fermentation process, and yeast performance in concentrated ZeaChem material

Conclusions

Work continues to proceed according to the project plan. Optimization of fermentation conditions and characterization of fermentation performance has reached a level that can enable further scale up to demonstration scale. A yeast biocatalyst that is suitable for demonstration scale has been identified and characterized in 1L GIFT® fermentations in representative hydrolysate material. A nutrient package has been optimized that is ready to implement at the demonstration scale. Currently, fermentation performance meets or exceeds target volumetric rates and yields needed to produce 1,000 gallons of IPK. Further process optimization would be required for commercialization. The adaptation program has continued to provide improved biocatalyst strains and has recently been used to adapt Gevo's second generation biocatalyst to hydrolyzate. Gevo will continue to provide input to refine the process model. Process development work will continue in order to support the 1,000 gallon IPK demonstration task.



NARA
Northwest Advanced Renewables Alliance

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