Fermentative Conversion of Hydrolyzed Douglas fir Biomass into Isobutanol

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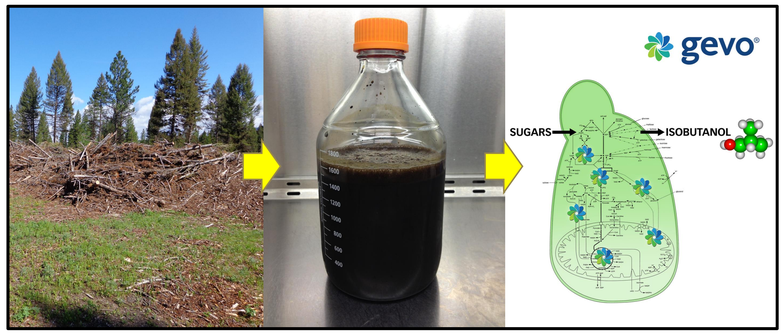


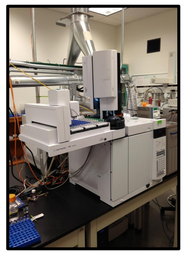
Introduction

Gevo has developed fermentation and process technology to convert biomass sugars to isobutanol and further into renewable jet fuel through chemical processing. Gevo will use GIFT®, Gevo Integrated Fermentation Technology, to produce isobutanol at high productivity, titer, and yield using a yeast biocatalyst adapted to hydrolyzate. The goal of this project will be to produce isobutanol according to a specification developed by Gevo that ensures the isobutanol will be converted into renewable biojet using existing Gevo technology. The specific tasks of this project are: (1) Characterize representative samples of pretreated woody biomass (Douglas fir) for fermentation; (2) Adapt yeast biocatalysts to pretreated biomass hydrolyzates; (3) Produce isobutanol in a 1L batch fermentation from pretreated biomass sugars using an 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 isobutanol in a 20L GIFT® fermentation from pretreated biomass; (8) Produce isobutanol using GIFT® fermentations at demonstration scale. Convert the lignocellulosic isobutanol to biojet for further testing.

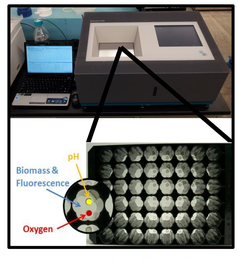
Methods

Characterize representative samples of pretreated Douglas fir hydrolyzate for fermentation

Analytical Analysis

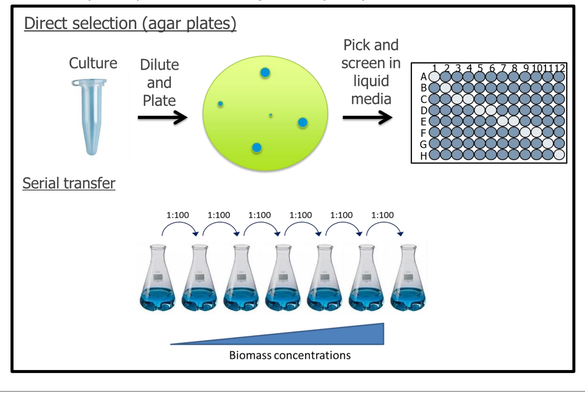


High-throughput Screening

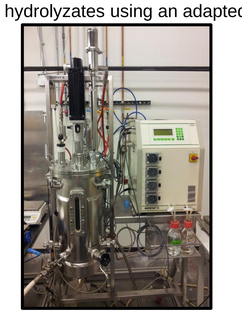


Fermentations

Adapt yeast biocatalyst to pretreated Douglas fir hydrolyzate



Produce isobutanol in fermentations from pretreated Douglas fir hydrolyzates using an adapted yeast biocatalyst



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.

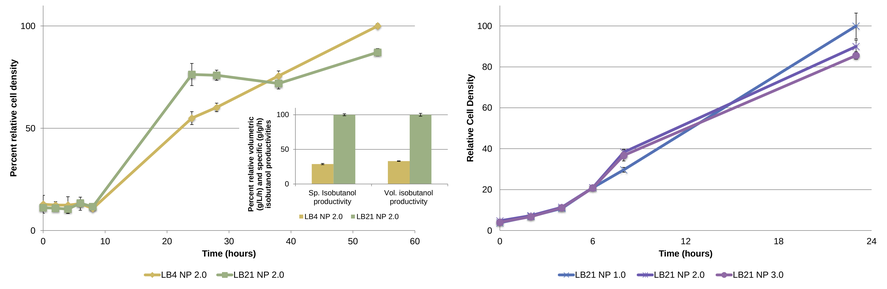


Figure 1. Growth curves, volumetric isobutanol productivity and specific isobutanol productivity of LB4 and LB21 (LB4 evolved in FS-10 SPORL) in 100% v/v FS-10 unwashed mild bisulfite solids (UMBS) hydrolyzate supplemented with nutrient package (NP) 2.0 (left) and growth curves of LB21 in 40% v/v Cosmo Hemlock reject fibers hydrolyzate supplemented with NP 1.0, 2.0 or 3.0 (right). Growth experiments were carried out under high aeration conditions using shake flasks incubated at 33°C. Cell density was measured using a spectrophotometer. Error bars represent standard deviation.

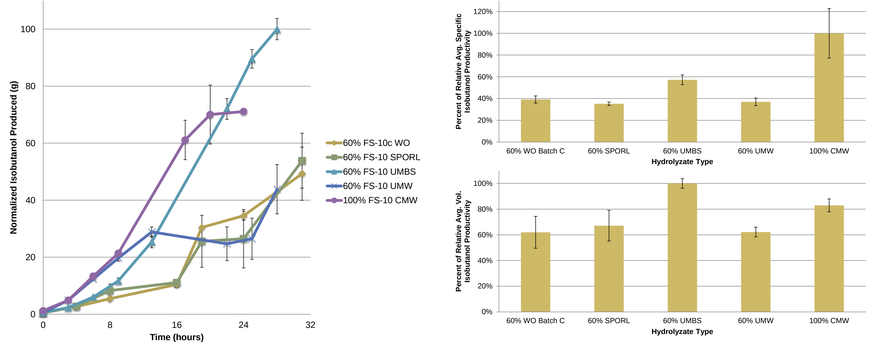


Figure 2. Isobutanol production of LB4 in: FS-10c WO, FS-10 SPORL, UMBS and UMW and LB22 in FS-10 CMW (left) and specific (right top)/volumetric (right down) isobutanol productivity. Data was combined from multiple 1L GIFT® experiments. All hydrolyzates were clarified to remove solids and all were supplemented with nutrient package 1.0. Mock medium contained equal amounts of corresponding sugars, acetate, and supplements. 100% (v/v) hydrolyzate is equal to approximately 30-36% equivalent solids for all biomass materials. Fermentation was carried out at 33°C and pH controlled. Isobutanol levels were determined by GC analysis and cell density was measured using a spectrophotometer. Error bars represent the standard deviation.

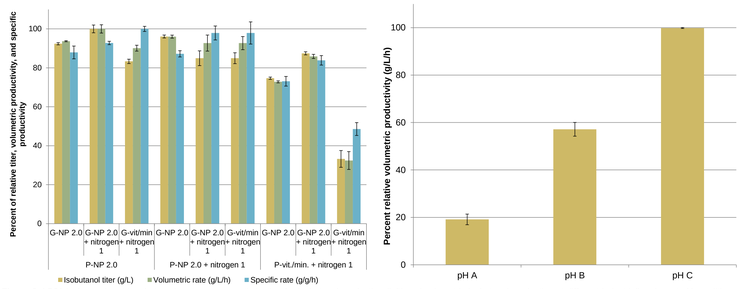


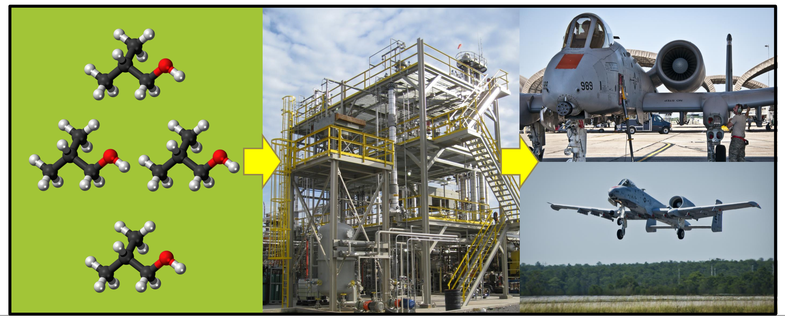
Figure 3. LB21 isobutanol metrics during nitrogen source optimization for isobutanol production (left) and volumetric isobutanol production at different industrially relevant pH conditions in 85% v/v FS-10 UMBS (right). On the **left:** LB21 was grown (G) under high aeration conditions for 23 hours in shake flasks containing NP 2.0, NP 2.0 + nitrogen 1, or vit/min + nitrogen 1. After 23 hours of growth, production (P) was started with LB21 by transferring 1:4 volumetrically into shake flasks containing NP 2.0, NP 2.0 + nitrogen 1, or vit/min + nitrogen 1. All permutation of growth and production were tested for the different nitrogen media. All media contained the same buffering agent and fermentation occurred under low aeration conditions for 24 hours at 33°C. On the **right:** to test industrially relevant pH conditions, a 1:4 volumetric transfer from the 40% v/v FS-10 UMBS hydrolyzate into 100% v/v FS-10 UMBS hydrolyzate at pH A, B or C was carried out to yield an 85% v/v FS-10 UMBS hydrolyzate media. All media contained the same buffering package and NP 2.0, growth was carried out at 33°C and cell density was measured using a spectrophotometer. Error bars represent the standard deviation.

Table 2. Impurity profile of isobutanol produced in FS-10 CMW mock media and 100% v/v FS-10 CMW hydrolyzate. Materials were analyzed by gas chromatography

	FS-10 CMW Mock Media (Weight %)	FS-10 CMW Hydrolyzate (Weight %)
Methanol	0	0
Ethanol	3.0±0.2	3.9±0.7
Acetone	0	0
Isopropanol	0	0
1-Propanol	0.1	0.1
Isobutyraldehyde	0.2	0.1±0.1
Isobutanol	76.8±0.2	75.6±1.0
3-Methyl-1-Butanol	1.1±0.1	1.0±0.1
2-Methyl-1-Butanol	0.6	0.5
2-Phenylethanol	0.1	0.1
All Unknowns	0.1	0.2±0.1
Density	82.0±0.2	81.6±0.3
Water (Weight %)	18.1±0.2	18.6±0.4
Water (Volume %)	21.4±0.5	21.8±0.3

Conclusions

Research and development is proceeding according to the NARA project plan. Characterization of both hemlock and Douglas fir pretreated material is ongoing and the adaptation program continues to provide biocatalysts with better performance than LB4. Biocatalysts from the adaptation program perform better in the new hydrolyzate being tested. In order to obtain the best biocatalyst possible and identify the best conditions for a scaled-up fermentation, a single feedstock and pretreatment process is crucial. Focus on producing improved biocatalysts and subsequently determine the optimum parameters for fermentations and scale-up is ongoing so that we may achieve the final goal of producing 1,000 gallons of biojet from lignocellulosic biomass. Batch fermentations at the 1L scale using FS-10 feedstock indicate that isobutanol production in hydrolyzate is slower but final titers are similar to the control media containing no hydrolyzate. Process optimization will continue in order to find the optimum parameters for isobutanol production.



NARA

Northwest Advanced Renewables Alliance

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