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# Evaluating the Feasibility of Higher Titers and Yields for 2,3-butanediol Production to Achieve the Biofuel Industry Economic Goal of Under $3.50 GGE

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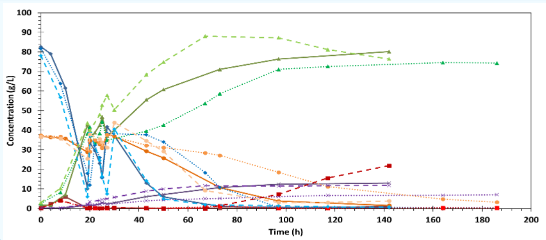
## Abstract

To achieve the economic goal of producing biofuels that can compete with petroleum-based products, we increased titers of 2,3-butanediol (BDO) production. BDO has many advantages over ethanol; including lower toxicity, higher heat of capacity, and is a precursor to various valuable applications. To produce BDO for fuel upgrading, we have genetically modified Zymomonas mobilis (YC-1). Several materials and feed strategies were evaluated to determine the best titer and economic feasibility. Titers from DMR corn stover in a batch process were in the range of 70-80 g/L. The fully hydrolyzed material was easy to work with but suffered from dilution issues. We pushed the concentration of solids to above 25% w/w and were met with numerous challenges. This included an initial sugar concentration above 150 g/L, the high solid concentration altered the oxygen mass transfer rate and affected which products were produced, and the fermentation conditions were not optimal for the enzyme. Despite these challenges, our current results yielded 100 g/L of upgradeable products (70 g/L BDO and 30 g/L acetoin) and 83% fermentation process yield. However, the poor enzymatic hydrolysis yield of 64%, unconverted xylose, and the high acetoin levels warrant further exploration for optimization. Using unhydrolyzed solids as the feed material, we achieved titers in the range of 75-95 g/L BDO. The raw solids feed, though operationally more challenging to work with, proved to be promising as the rate of hydrolysis promoted co-utilization of the sugars. We reached 150 g/L BDO using concentrated DDR liquors under boules and continuous feeding strategies. The clarified liquors showed high titers, high productivity, high yield, and high sugar utilization with little drawback operationally. The target goal of under $3.50 GGE was achieved in several feeding strategies. Scale-up feasibility has also been demonstrated at the 100L scale using both whole slurry and liquor hydrolysates.


## Materials and Methods

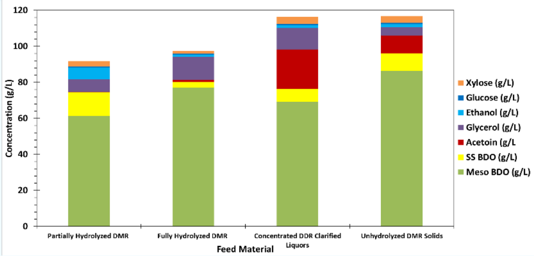
- An integrated strain of Zymomonas mobilis was used. The revive was grown in 16 mL of RMG 5% (10 g/L Yeast Extract, 2 g/L $KH_2PO_4$, 50 g/L glucose) in a non-baffled shake flask for eight hours in an incubator at 30ºC and 180 rpm.
- The seed was grown in a 500 mL BioStat-Q plus fermenter with operating conditions: RMG 10%, 300 mL of working volume, an agitation of 500 rpm, a temperature of 30ºC, pH of 5.8 titrating with 4N KOH, and an air flow rate of 100 ccm using sparge rings. The culture was grown under these conditions for 22 hours until the glucose concentration reached 40 – 50 g/L remaining in the fermenter.
- The fermenters were the same as the seed except for an agitation of 700 rpm and an air flow rate of 40 ccm using an overlay ring. The concentration of initial sugars varied for each experiment but was based on the efficiency of enzymatic hydrolysis and solids loading.

## DMR Whole Slurry Fermentation Fed With Various Materials




Fed batch fermentation products using hydrolyzed DMR whole slurry. Line Type (Feed Material): Solid (Fully Hydrolyzed DMR), Dotted (Partially Hydrolyzed DMR), Dashed (Clarified DDR Liquor)

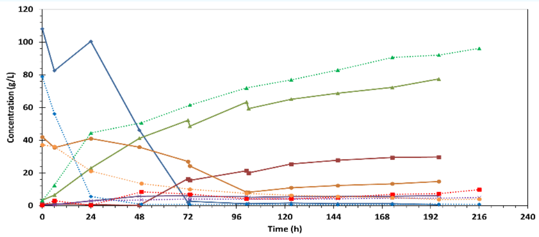
- The overall productivity of the partially hydrolyzed feed was the lowest at 0.4 g/L/h. The raw solids were next at 0.49 g/L/H, with fully hydrolyzed at 0.57 g/L/h, and clarified liquors at 0.7 g/L/h
- Enzymatic hydrolysis of the raw solids to glucose and xylose during fermentation was lower compared to the hydrolyzed DMR whole slurry before fermentation
- Glucose and xylose utilization was over 90% for every condition
- The fully hydrolyzed material was easy to work with but suffered from dilution issues, unhydrolyzed solids were operationally challenging but promoted co-utilization of sugars, and the clarified liquors were easy to work with and produced high titer, yield, and productivity




Final concentrations for DMR whole slurry fermentation with different feed material

Legend for line graph colors: Blue (Glucose), Orange (xylose), Green (BDO), Red (acetoin), Purple (glycerol)

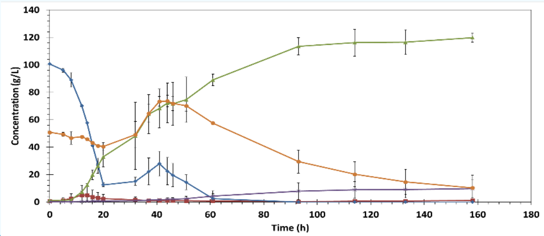
## DMR Whole Slurry Fed With Unhydrolyzed DMR Solids




Fed Batch fermentation products in DMR whole Slurry. Line Type: Solid (25% solids), Dotted (Unhydrolyzed Feed)

- The batch phase started at 19% total solids, and an additional 7% total solids was added between the two feeds
- The unhydrolyzed solids feed strategy benefited from less dilution being added but the efficiency of the enzymes was not optimized during fermentation conditions

## Continuous Feed Results in 125 g/L of BDO Titer




Continuous feed fermentation products in clarified DDR liquor

- The highest titers can be achieved using clarified liquors
- The feed material can be concentrated to over 600 g/L total sugars allowing for less dilution
- After glucose has been fully consumed the agitation speed should be reduced to 350 rpm, reducing the production of acetoin.

## Economic Analysis of DMR Whole Slurry Feeds vs DDR Clarified Liquor

| | Whole Slurry Batch | | | Whole Slurry SSF | | | Clarified Sugars Fed-Batch | |
|---|---|---|---|---|---|---|---|---|
| **Case** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **Minimum Fuel Selling Price ($/GGE)** | **$3.74** | **$3.65** | **$4.15** | **$3.30** | **$3.82** | **$4.91** | **$3.28** | **$3.34** |
| Sorbitol Cost (cents/GGE) | 17.6 | 8.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 9.3 |
| A400 Enzyme Production Cost (Cents/GGE) | 52.8 | 52.8 | 61.8 | 49.4 | 95.0 | 188.8 | 45.1 | 45.1 |
| BDO Titer (g/L fermentation effluent) | 97.6 | 97.6 | 82.6 | 102.8 | 99.2 | 92.8 | 142.4 | 151.9 |
| Sorbitol Loading (g/L fermentation volume) | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| Enzyme Loading (mg/g cellulose to sacch) | 10 | 10 | 10 | 10 | 20 | 40 | 10 | 10 |
| Fuel Yield (GGE/ U.S. ton feed) | 36.23 | 36.23 | 30.94 | 38.73 | 38.54 | 38.24 | 42.43 | 42.43 |
| Evap % loss of BDO (cases 1-6) or sugars (cases 7-8) | 15.1% | 15.1% | 13.7% | 14.1% | 14.4% | 15.1% | 0.0% | 0.0% |
| NG usage-A810 High solids Boiler (kg/hr) | 0 | 0 | 0 | 0 | 0 | 0 | 800 | 500 |

MFSP and other key TEA results for BDO fermentation scenarios evaluated

- The lowest MFSP cases are Clarified Sugar Fed-Batch at 140 g/L BDO target titer, as well as the whole slurry SSF case at 10 mg/g enzyme loading
- The added cost for 2 g/L sorbitol is not justified if it can only achieve a 10 g/L higher BDO titer, adding a net $0.06/GGE for the 150 g/L case with sorbitol addition

## Conclusion

We have developed a scalable fermentation to produce 2,3-butanediol (BDO) from biomass sugars. The process uses DMR corn stover and enzymes to release fermentable sugars. The sugars are converted by the recombinant biocatalyst *Zymomonas mobilis*, to produce BDO which is a versatile, upgradeable chemical for sustainable aviation fuels, tires, and adhesives. Key to the economics is producing a high titer from the fermentation which reduces the amount of water to remove in downstream separations and upgrading. We pushed the concentration of whole slurry solids to above 25% w/w solids with the goal of 100 g/L BDO titer and were successful in hitting our 125 g/L BDO titer goal using concentrated liquor.

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