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

Authors: Ryan Spiller, Holly Rohrer, Yat-Chen Chou, Ryan Davis, Min Zhang, Nancy Dowe

Abstract
To achieve the economic goal of producing biofuels that can compete with petroleum-based products, we increased titters of 2,3-butandiol (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) into a feedstock process. Several materials and feed strategies were evaluated to determine the best titer and economic feasibility. Titters 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 productivity yields. 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 titters 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 high rate of hydrolysis promoted co-utilization of the sugars. We reached 150 g/L BDO using concentrated DDR liquors under trouble and continuous feeding strategies. The clarified liquors showed high titters, 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 KH2PO4, 50 g/L glucose) in a non baffled shake flask for eight hours in an incubator at 30°C and 180 rpm.
• The seed was grown in a 500 mL BioStat-Q plus fermentor with operating conditions: RMG 10%, 300 mL of working volume, an agitation of 500 rpm, a temperature of 30°C, pH of 5.8 titrating with 4N KOH, and an air flow rate of 40 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 fermentor.
• 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.

Economic Analysis of DMR Whole Slurry Feeds vs DDR Clarified Liquor
- 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/L enzyme loading.
- 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.

Continuous Feed Results in 125 g/L of BDO Titter
- 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.

Conclusion
We have developed a scalable fermentation to produce 2,3-butandiol (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 titter and were successful in hitting our 125 g/L BDO titter goal using concentrated liquor.

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