Fermentation process development for *Streptomyces venezualae* as a platform organism for production of branched fuel compounds

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Introduction

Biosynthesis of highly branched short and long-chain hydrocarbons would enable production of biofuels with desirable and tunable properties, including compression ignition fuels with low freezing points. Polyketide synthase (PKS) pathways are a promising route towards production of such compounds; engineering of PKS pathways favors a native host- this work therefore focuses on development of *Streptomyces venezualae* ATCC 10712 as a platform organism. While *S. venezualae* is well characterized for industrial production of antibiotics, currently available protocols for high density fermentation make use of rich media and high-purity dextrose. The viability of *Streptomyces* as a platform organism for large-scale cellulosic biofuel production is therefore currently unknown. Development of protocols for high density fed-batch fermentation of *S. venezualae* with cellulosic sugar feed in minimal medium is necessary to evaluate the viability of this strain as a production organism for fuels and commodity chemicals.

Growth of S. venezualae is observed across variable cellulosic feedstocks and pretreatment methods

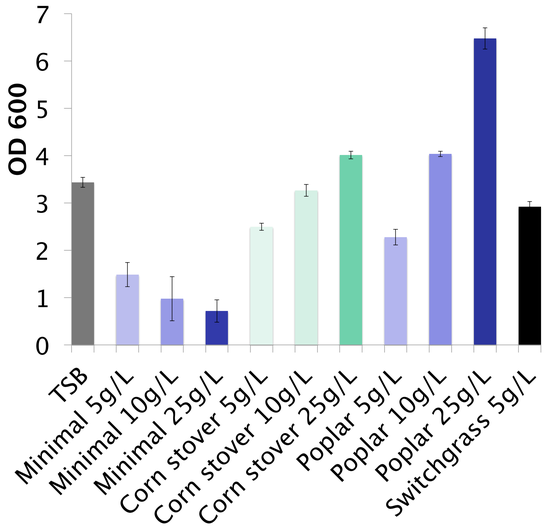

Fig. 1: Optical density of *S. venezuealae* cultures grown for 24 hours on minimal media supplemented with varying concentrations of glucose and hydrolysate. Captions indicate initial glucose concentration.

Materials and Methods

Hydrolysate production: Three hydrolysates were tested in shake flask fermentations: alkali-pretreated corn stover, steam explosion-pretreated poplar, and IL-pretreated switchgrass. Alkali-pretreated corn stover was used as feed in subsequent fed-batch fermentations.

Shake flask fermentation: Cultivation in 50ml shake flasks was used to determine fermentation suitability across a range of pretreatment methods, feedstocks, and initial sugar concentrations. Minimal cultivation medium as described by Kieser et al[1] was supplemented with either pure glucose or cellulosic hydrolysates, with initial glucose concentrations ranging from 5-25g/L.

Fed-batch fermentation: To achieve higher culture densities, fed batch fermentation was tested in 2L fermenters with controlled pH and DO. Batch phase media contained minimal media supplemented with glucose and xylose in the same proportions as the corn stover hydrolysate. Corn stover hydrolysate feed containing 35g/L total sugars was initiated upon exhaustion of initial glucose and continued until the fermentation reached its full 2L volume.

Co-consumption of Glucose and Xylose is Observed During Fed-batch Fermentation

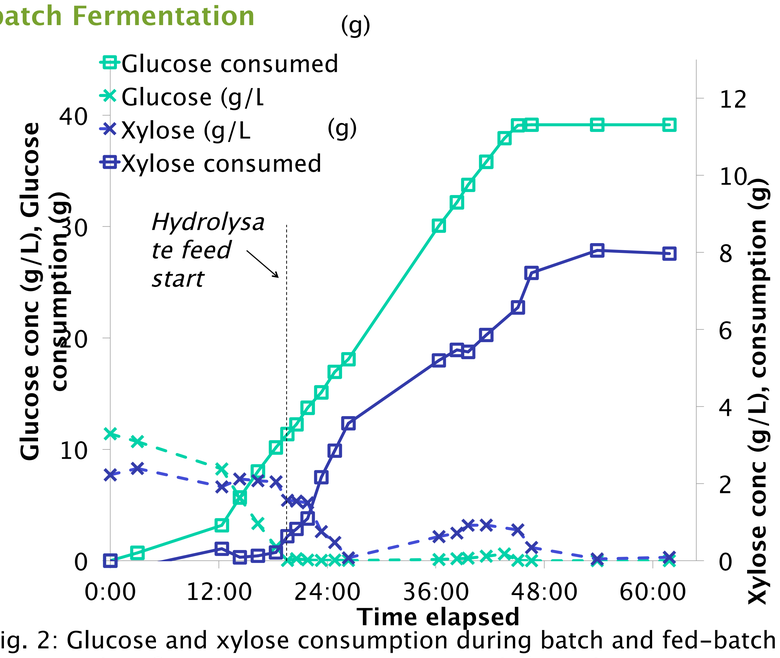

Fig. 2: Glucose and xylose consumption during batch and fed-batch fermentation. Sugars are consumed sequentially during the batch phase, with co-consumption observed following depletion of residual xylose during the fed-batch phase.

1. Kieser, Tobias. *Practical streptomyces genetics*. John Innes Foundation, 2000.

Hydrolysate Fermentation Imposes a Metabolic Cost

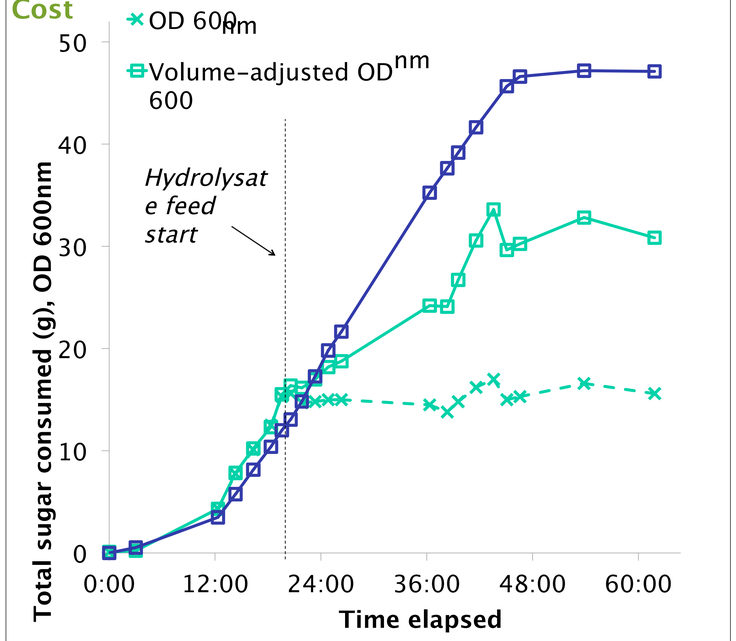

Fig. 3: Optical density and sugar consumption during batch and fed-batch fermentation. Volume-adjusted OD 600 (expressed as OD*L) is used to approximate total biomass production.

Summary/Conclusion:

- Fed-batch fermentation of *S. venezualae* on hydrolysate in 2L fermenters achieved up to a dry cell weight of 9g/L.
- Co-consumption of glucose and xylose is observed during fed-batch fermentation, with a clear preference for glucose over xylose during batch phase.
- Hydrolysate fermentation exacts a metabolic cost on fed-batch fermentation, reducing biomass yield as a function of sugar consumption. Further optimization of pretreatment methods may be necessary to mitigate this effect.
- *S. venezualae* grows readily across a range of pretreatments and biomass sources. Growth with hydrolysates exceeded growth in minimal media alone, indicating that further gains in productivity may be achieved via optimization of minimal media.

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