COLLEGE OF ENGINEERING

Chemical, Biological & Environmental Engineering

TRILLIUM FIBERFUELS, INC.

Scale-up Production of a Novel Xylose Isomerase (XI) Enzyme in Fed-Batch Fermentation

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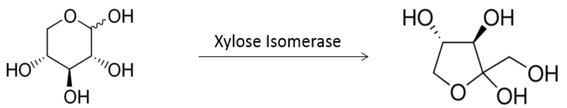
Abstract

Cellulosic ethanol yields could increase 20-40 % with the production of a novel xylose isomerize (XI). This enzyme was produced by genetically engineered *Escherichia coli* cells in a 2 L fed-batch bioreactor. Cells were grown in batch mode from < 0.1 to a maximum cell density of 2.8 ± 0.5 g/L. Cell growth was linear in all batch experiments, suggesting substrate limitations. A maximum linear growth rate of 0.16 g/h was achieved with 25 g/L batch glucose concentration. Cells were induced to make intracellular XI entering fed-batch mode. Fed-batch mode began when batch glucose had been depleted and ended after 24 h when the reactor volume reached 2 L. XI activity increased during and after fed-batch. A maximum activity of 7.2 µmol/g-min and volumetric activity of 0.21 µmol/L-min was achieved after 72 h using 25 g/L batch and 105 g/L fed-batch glucose concentrations.

Introduction

A xylose isomerase (XI) is an enzyme that converts xylose to xylulose, which is an important reaction in ethanol production from plant biomass.



Xylose is the second most abundant sugar in biomass, but it cannot be fermented into ethanol by brewing yeast. The product of the XI reaction can be fermented into ethanol, but current XI enzymes are incompatible with fermentation. The XI enzyme discovered by Trillium FiberFuels is active at fermentation pH (5.5-6.0), while previous XI enzymes are not. Fermentation with the novel XI enzyme could increase ethanol yields by 20-40% shown in Figure 1.

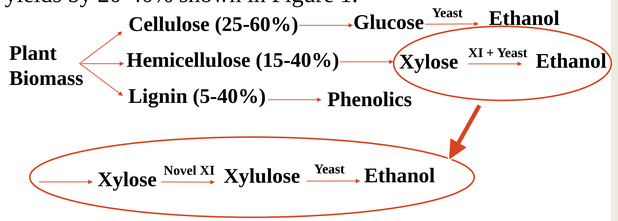
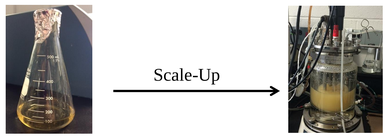


Figure 1. The fermentation process of plant biomass with the use of XI.

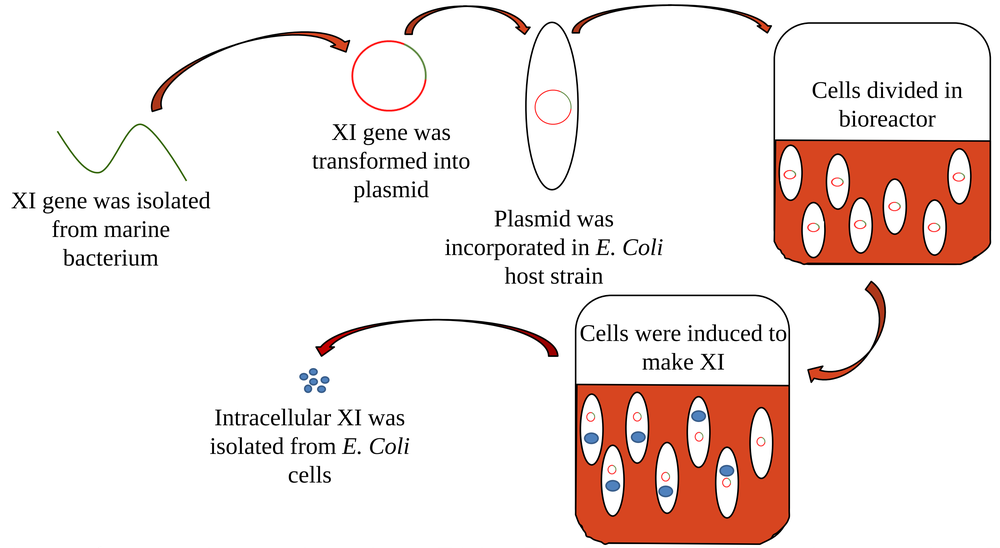
Opportunity



500 mL Shake Flask

3L Fed-Batch Bioreactor

Methods



XI was isolated from a marine bacteria species by Trillium Fiber Fuels and transformed into recombinant *E. coli* cells (Rosetta-gami (DE3) (Novagen) containing pET-24(a) with cloned FpXI). Production of XI was carried out in a 3 L fed-batch bioreactor. High cell density was achieved in batch mode before moving onto fed-batch. Cells were induced using IPTG at the start of fed-batch mode which turns on the XI gene. Glucose was supplied at a constant rate during fed-batch mode. Cells were harvested and lysed to release the intracellular XI product. Crude XI was used to catalyze the isomerization of xylose to xylulose. XI productivity was quantified by measuring xylulose formation.

Results

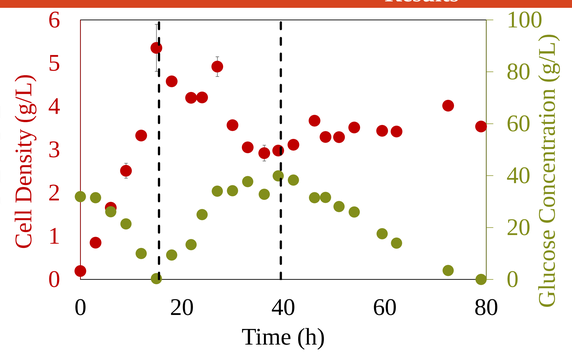


Figure 2. The cell and glucose concentration during fed-batch run. Batch mode occurred for 15 h and fed-batch for 24 h.

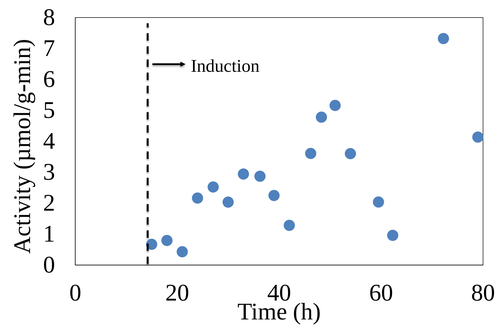


Figure 3. XI activity during the fed-batch run. Cells were induced with IPTG when fed-batch mode was started.

The fed-batch run results are shown in Figure 2 with input parameters shown in Table 1. A maximum cell concentration of 2.8 ± 0.5 g/L was achieved at the end of batch mode.

Table 1. Input parameters for XI production in a 3 L fed-batch bioreactor.

	Batch (1L)	Fed-Batch (1L)
Glucose (g/L)	25	105
Temp. (°C)	30	25
pH	7	7

Cells were grown in batch mode for 15 h to increase cell density. Cells were induced with IPTG to make product after batch mode and fed-batch medium was fed at a constant rate for 24 h. Product results are shown as specific enzyme activity in Figure 3. A maximum enzyme activity of 7.2 µmol/g-min was achieved 57 h after induction.

Results

Five bioreactor runs were completed, one batch (B1) and four fed-batch (FB1-FB4). Input parameters for each run are shown in Table 2. Runs were compared on metrics shown in Figure 4.

Run	Glucose conc. (g/L)	
	Batch	Fed-batch
B1	15	-
FB1	15	150
FB2	25	105
FB3	45	85
FB4	45	65

Table 2. Summary of input variables for the batch (B1) and fed-batch runs (FB1-FB4)

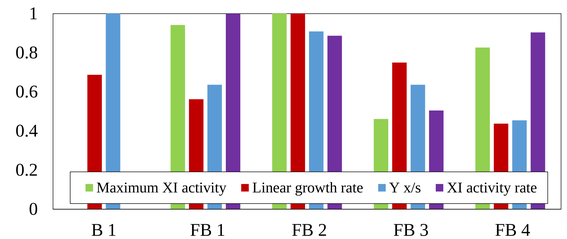


Figure 4. **(a)** Comparison of maximum XI activity, XI production rate, linear cell growth rate, and growth and production yield coefficient, $Y_{X/S,}$ across four fed-batch runs (FB1-FB4) and a batch run (B1). Values are normalized to the maximum value in each category: 7.3 µmol/g-min maximum XI activity, 0.14 µmol/g-min-h XI activity rate, 0.16 g/h linear cell growth rate, and 0.12 g cell/g glucose $Y_{X/S}$. **(b)** Cell and glucose concentrations for B1 showing linear cell growth.

Cell growth was linear in all batch runs (B1 shown in Figure 5a as example), suggesting oxygen to be a limiting substrate. A maximum volumetric activity of 0.21 µmol/L-min occurred during FB2 at 72 h, shown in Figure 5b.

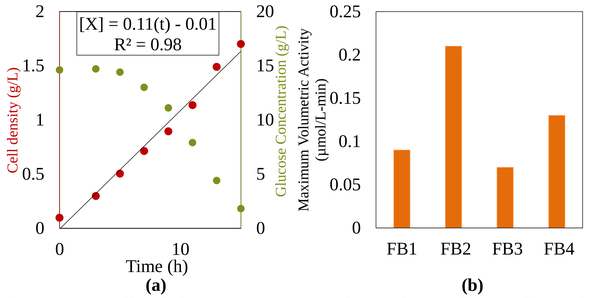


Figure 4. **(a)** Cell and glucose concentrations for B1 showing linear cell growth. **(b)** The maximum volumetric activity of runs FB1-FB4.

Acknowledgements

Curtis Lajoie, Steve Potochnik, Jakob Townsend, Lynda Bradley, Josh Kitner, Eric Eichenbaum, Dr. Philip Harding

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