Lower-cost cellulosic ethanol production using cellobiose fermenting yeast *Clavispora* NRRL Y-50464

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ABSTRACT

For ethanol production from cellulosic materials, there are generally two major steps needed including enzymatic hydrolysis to break down biomass sugars and microbial fermentation to convert available simple sugars into ethanol. It often requires two different kinds of microorganisms since ethanologenic microbes usually do not producing cellulolytic enzymes while the enzyme producing microorganisms are commonly unable to produce ethanol. Genetic engineering efforts for improved enzyme production of ethanologenic agent have been taken but outcomes are yet unsatisfactory. High enzyme cost remains as a significant challenge for sustainable cellulosic ethanol production. ARS scientists discovered and developed a new yeast strain *Clavispora* NRRL Y-50464 that is able to produce sufficient innate β-glucosidase to digest cellobiose and produce ethanol. Beta-glucosidase is a key enzyme component in cellulase for decomposition of lignocellulosic biomass materials. Elimination of the addition of external β-glucosidase would reduce the enzyme cost for cellulosic ethanol production using simulteneous saccharification and fermentation (SSF). We isolated and characterized three forms of β-glucosidase, BGL1, BGL2, and BGL3, from *Clavispora* NRRL Y-50464 that confirmed its dual function of cellobiose digest and ethanol production capability. We demonstrated its highest conversion rate of 0.088 g/L/h with an ethanol production of 32 g/L ethanol from 20% solids loading of corn stover in 48 h, applying cellulase alone without addition of β-glucosidase by SSF. Using a pure commercial available cellulose, it produced 40.44 g/L ethanol within 72 h that reached an industrial standard. The desirable characteristics of this US patented yeast strain including tolerance to inhibitors, fast growth rate, β-glucosidase producing capability, and quick ethanol conversion rate, made it a strong candidate for lower-cost cellulosic ethanol production using SSF.

Keywords: beta-glucosidase, cellobiose fermentation, cellulosic ethanol, simulteneous saccharification and fermentation

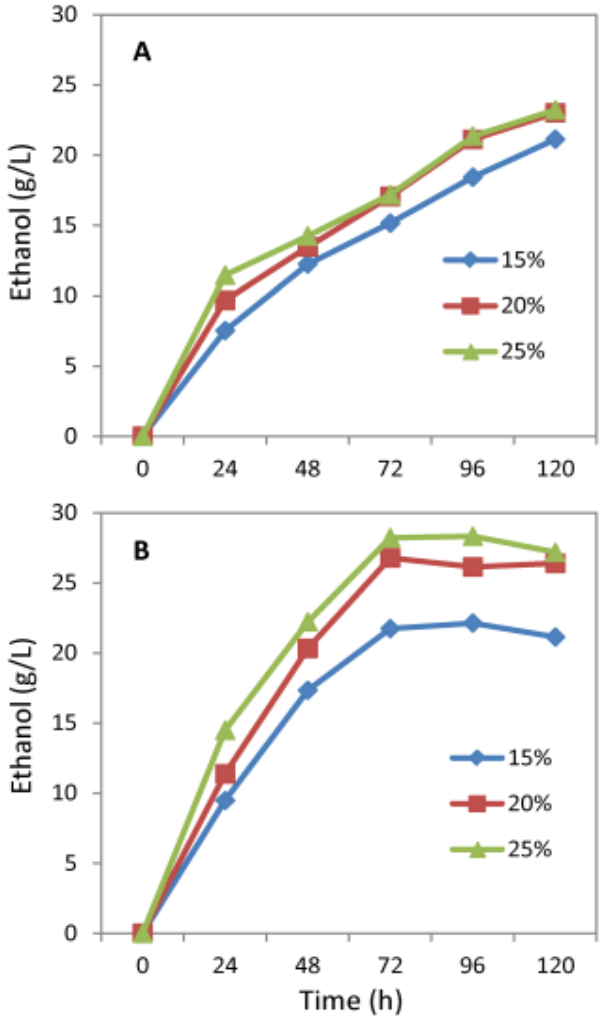

Figure 1. Comparison of cellulosic ethanol production from corn stover pretreated by conventional method (A) and additional lignin removal procedure (B) at feedstock input from 15, 20, and 25 % solids loading levels using Clavispora NRRL Y-50464 in a bottled SSF. Fermentation was carried out using a simultaneous saccharification and

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fermentation process without addition of supplementary β-glucosidase. Variation of replicated ethanol productions for all time points ranged from 0.2 to 1.4%.

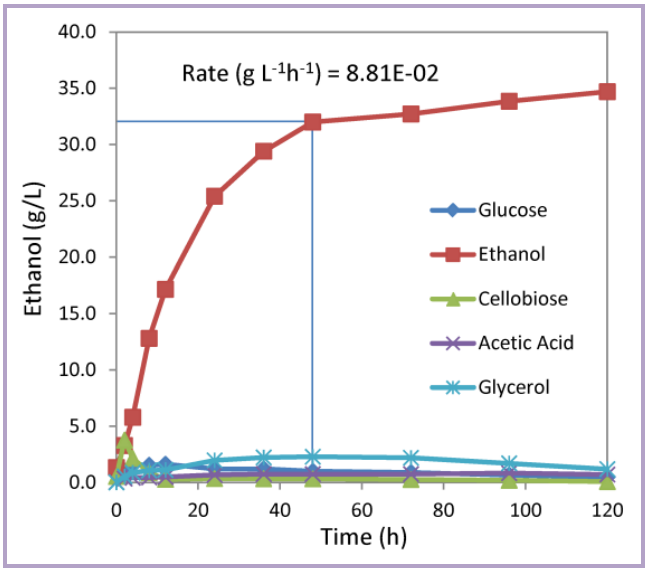

Figure 2. Cellulosic ethanol production using *Clavispora* NRRL Y-50464 fromcellulose of pretreated corn stover with a 20%solids loading (equivalent to 7.57 % cellulose) by simultaneous saccharification and fermentation using 2-L bioreactors. Values of ethanol production are means of three replications with a standard deviation of 0.05 g/L at 48 h and ranged from 0.02 to 0.24 g/L for all time points.

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