



Techno-economic analysis of corn stover fungal fermentation to ethanol



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HIGHLIGHTS

- We examine near-term, mid-term and long-term economics of lignocellulosic ethanol by fungal fermentation pathways.
- Recombinant *S. cerevisiae* provides the most attractive process economics with the ethanol cost of \$2.51/gallon.
- Co-producing organic acids can improve the process economics and the ethanol cost could be reduced to \$2.22/gallon.
- The opportunity for fungal fermentation exists for lignocellulosic ethanol production.
- Fungal process economics can be improved by genetic engineering techniques.

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ABSTRACT

Researchers at the Pacific Northwest National Laboratory (PNNL) perform fungal research and development activities to support the goal of promoting renewable energy production as set by the U.S. Department of Energy (DOE). This techno-economic analysis assesses the process economics of ethanol production from lignocellulosic feedstock by fungi to identify promising opportunities, and the research needed to exploit them. Based on literature derived data, four different ethanologen strains are considered in this study: native and recombinant *Saccharomyces cerevisiae*, the natural pentose-fermenting yeast, *Pichia stipitis* and the filamentous fungus *Fusarium oxysporum*. In addition, filamentous fungi are applied in multi-organism and consolidated process configurations. Organism performance and technology readiness are categorized as near-term (<5 years), mid-term (5–10 years), and long-term (>10 years) process deployment. Processes classified as near-term could reasonably be developed in this shorter time frame, as suggested by recent literature. Mid-term technology process models are based on published lab-scale experimental data. Yields near the theoretical limit are classified as long-term technology goals. Among the four ethanologen strains, recombinant *S. cerevisiae* provides the most attractive process economics as defined by the lowest Minimum Ethanol Selling Price (MESP). This also falls in a range of the model analysis results suggested by literature based on different feedstock and organisms. Moreover, the analysis of mid-term and long-term processes shows improved profitability, revenue and process economics when co-producing chemicals on-site is applied, resulting in 1.98\$/gallon of ethanol from a mid-term process scenario. The results of the analysis suggest that the opportunity for fungal fermentation exists for lignocellulosic ethanol production.

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1. Introduction

The Renewable Fuel Standard (RFS) set a target of 36 billion gallons per year of renewable fuel by 2022, of which 21 billion gallons is

to be produced from cellulosic biomass [1]. One aspect of reaching this goal is to produce ethanol as a primary transportation fuel from non-food or cellulosic sources including wood and agricultural residues. Among crop residues available in the United States, corn stover is the most abundant [2], and can be used as a raw material for ethanol production through either thermochemical or biochemical processing. Thermochemically, ethanol is produced through thermal degradation of the feedstock followed by subsequent catalytic chemical routes [3]. Biochemically, ethanol is produced through fermentation of pretreated biomass, such as corn stover, by organisms such as yeast, filamentous fungi, or bacteria [4]. The biochemical route is attractive because corn ethanol technology is already well established and corn stover is readily available.

Abbreviations: CAFI, Consortium for Applied Fundamentals and Innovation; CRS, Congress Research Service Reports; DOE, Department of Energy; GAL, Gallon; IRR, Internal Rate of Return; LHV, Low Heating Value; MESP, Minimum Ethanol Selling Price; MYPP, Multi-Year Program Plan; NREL, National Renewable Energy Laboratory; PNNL, Pacific Northwest National Laboratory; RFS, Renewable Fuel Standard; TCI, Total Capital Investment; TDC, Total Direct Cost; TEA, Techno-economic Analysis; VVM, Volume per Volume per Minute; XI, Xylose Isomerase.

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This study investigates several fungal fermentation routes to produce ethanol from corn stover. Conversion parameters are derived from published research on those organisms [4]. As the first step in developing the techno-economic analysis (TEA), a process flow sheet is designed to establish mass and energy balances. Practical economic assumptions subsequently made before using the discounted cash flow method to determine product selling price. Based on the TEA potential, process pathways were identified and categorized by their maturity and possible developmental timeframe: near-term (1–5 years), mid-term (5–10 years), and long-term (10 years or longer). Fungal research opportunities are identified with the goals of improving the process economics and developing potentially commercial processes.

Each process model includes feedstock pretreatment, on-site enzyme production, saccharification, fermentation, ethanol purification, steam production and electricity generation. Sensitivity analysis is conducted on the mode of fermentation by altering the dedicated ethanol production organism. Additionally, alternative process configurations such as consolidated bioprocessing and co-production of chemicals on-site are also investigated by a sensitivity study.

1.1. Fungal ethanologen organisms

1.1.1. Yeast

Wild type *S. cerevisiae* is available commercially and used in the corn ethanol industry, but does not ferment five-carbon sugars. Two approaches to improve ethanol productivity by *S. cerevisiae* are addressed [4]: addition of the enzyme Xylose Isomerase (XI) and using recombinant DNA technology to incorporate five-carbon utilization capabilities. Xylose isomerase, also known as glucose isomerase, is widely used for the production of high fructose corn syrup. This enzyme can be used to isomerize xylose to xylulose, which is fermentable to ethanol by *S. cerevisiae*. When reaction equilibrium is reached, xylose isomerization to xylulose is less than 20%, which may cause low ethanol productivity [4–8]. Thus, simultaneous fermentation and isomerization (SFIX) has been applied to consume the xylulose as it is produced and pull the isomerization reaction toward the production of ethanol [4]. Alternatively, xylose isomerase can be expressed heterologously in genetically engineered *S. cerevisiae* strains [4–8]. Yet another approach to xylose

utilization is to incorporate the genes for xylose reductase and xylitol dehydrogenase into the *S. cerevisiae* genome [4].

Natural yeast strains without genetic alterations that utilize five-carbon sugars to produce ethanol do exist, such as *Candida shehatae*, *Pachysolen tannophilus*, *Pichia stipitis* (also known as *Scheffersomyces stipitis*) and *Kluyveromyces marxianus* [9]. Very often these strains have low ethanol tolerance and require microaerobic conditions for efficient ethanol production. In large scale production processes such conditions may be difficult to control and prove to be energy intensive [10,11]. However, omission of genetic alterations may prove to be a faster route to a market ready process if the organism is capable of sufficient ethanol production.

1.1.2. Filamentous fungi

Other organisms considered in this analysis include filamentous fungi that can naturally ferment glucose and xylose as well as minor sugar constituents of lignocellulose, such as arabinose, galactose and mannose, to ethanol. Species belonging to the genera *Fusarium*, *Aspergillus*, *Rhizopus*, *Monilia*, *Neurospora* and *Paecilomyces* all have these capabilities [9]; extensive research has been done on *F. oxysporum*. Filamentous fungi have a distinctive advantage in cellulosic fuel production because of their enzyme production and natural cellulose degrading capabilities. Therefore, they have recently drawn some attention in the development of consolidated bioprocessing where enzyme production, saccharification and fermentation are all performed in one unit operation [12–14].

This study considers four fungal ethanologen strains that have recently been studied for their ethanol producing ability. They are *S. cerevisiae*, *P. stipitis* (natural pentose fermenting yeast), recombinant *S. cerevisiae* and *F. oxysporum* (filamentous fungus). They possess different characteristics and provide both advantages and disadvantages for ethanol production as summarized in Table 1. In this paper two processing schemes are examined: (1) segregated production of enzymes and ethanol, and (2) simultaneous enzyme and ethanol production.

2. Materials and methods

2.1. Process modeling and process economic evaluation

Process models were developed with CHEMCAD[®] [15], a commercial process simulator capable of calculating mass and energy

Table 1
Advantages and disadvantages of select fungal organisms for ethanol production.

Ethanologen	Advantages	Disadvantages
<i>S. cerevisiae</i> (industrial baker's yeast)	<ul style="list-style-type: none"> – Commercially available for industrial scale processes – High ethanol tolerance – Fast ethanol production rate 	<ul style="list-style-type: none"> – Does not utilize pentoses
<i>S. cerevisiae</i> plus xylose isomerase enzyme	<ul style="list-style-type: none"> – Utilizes xylose (after isomerization to xylulose) to make ethanol – High ethanol tolerance – Fast ethanol production rate 	<ul style="list-style-type: none"> – Expensive xylose isomerase
Natural pentose fermenting yeast (<i>P. stipitis</i>)	<ul style="list-style-type: none"> – Utilizes xylose to make ethanol – Natural strain of yeast 	<ul style="list-style-type: none"> – Microaerobic process (complex operation) – Additional energy consumption for process air compressor
Recombinant <i>S. cerevisiae</i> expressing XI	<ul style="list-style-type: none"> – Utilizes xylose – High ethanol tolerance – Fast ethanol production rate 	<ul style="list-style-type: none"> – Not yet available for industrial scale process – Sequential, not simultaneous sugar utilization (glucose first, then xylose, no arabinose)
Filamentous fungi <i>F. oxysporum</i>	<ul style="list-style-type: none"> – Natural ability to ferment a wide range of carbohydrates including xylose and arabinose to ethanol – Capable of hydrolyzing a wide variety of polysaccharides to simple sugars – Natural strains 	<ul style="list-style-type: none"> – Microaerobic process (complex operation) – Additional energy consumption for process air compressor – Low ethanol-tolerance – Slow ethanol production rate

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