

# Anaerobic Fermentation of Lignocellulosic Biomass to Produce Biofuels

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## Abstract

A substantial and underused resource for the creation of sugars and biofuels is lignocellulosic biomass. Economically, this method is difficult because of the structural complexity of lignocellulosic biomass and the requirement for several pretreatment and enzymatic stages for sugar release. Here, we describe a unique method for employing the anaerobic fungus isolate strain C1A to directly convert lignocellulosic biomass to sugars and biofuels in a single container without the use of exogenous enzymes. In this method, the strain C1A's saccharolytic and fermentative activities are promptly inhibited and decoupled, causing a buildup of the sugar monomers glucose and xylose in the culture medium. *Escherichia coli* strain K011 then turns the generated sugars—along with the fungal hyphal lysate—into ethanol. The high ethanol yield was attained as a result of strains K011 completely converting sugars to ethanol, strain C1A accumulating ethanol as a minor fermentation end product during its initial growth phase, and strain K011 possibly converting unidentified substrates in strain C1A's hyphal lysate to ethanol. An innovative, adaptable, and exogenous enzyme-free method for producing direct biofuel from lignocellulosic biomass is presented in this paper. It makes use of a hitherto underutilised class of organisms called anaerobic fungus.

**Keywords:** Saccharolytic • Fermentation • Lignocellulosic biomass • Cellulases • Proteolytic enzymes • Cytosolically

## Introduction

Future sustainable energy landscape scenarios are known to be indispensable without the production of biofuels from lignocellulosic biomass. Given its accessibility, affordability, and high energy content, lignocellulosic biomass provides a significantly untapped source for the manufacture of biofuels. However, lignocellulosic biofuels now make up a very small portion of the total output of renewable or even biofuel-based energy. The most popular method for producing biological biofuels from lignocellulosic

biomass makes use of a variety of purified enzymes to liberate monomeric sugars from pretreatment biomass. The generated sugars are then converted to biofuels by a special sugar-metabolizer. Numerous facets of this strategy have been thoroughly examined, with the help of substantial funding sources in the public, private, and public-private partnership sectors. Despite the fact that plant genetic engineering has made great progress, the rate of discovery and characterization of pretreatment methods and enzymes is still relatively rapid. This is primarily caused by the high cost of cellulases, hemicellulases, and accessory enzymes needed to break down structurally complex substrates, the high cost and operational complexity of pretreatment methods needed to improve enzyme access to lignocellulosic biomass, and the operational complexity of the process required by differences in optimal temperatures and/or redox requirements at various stages of the process and the frequent formation of inhibitor. A viable alternative approach for breaking down lignocellulosic biomass uses microorganisms rather than pure enzyme mixtures since it might solve many of the issues with exogenous enzyme-based processes. Eliminating the need for enzymes, avoiding harsh plant biomass pretreatments, and consolidating processes might result in significant cost reductions.

Undoubtedly, the identification and use of strong and invasive biomass-degrading anaerobes is necessary for the success of such a strategy, as is the design of a solid and stable platform for the optimal distribution of lignocellulosic substrate used between microbial growth, extracellular enzyme production, and desired end products. One of the most effective, but mostly unrecognised, anaerobic biomass degraders are members of the anaerobic gut fungus. An extensive range of cell-bound and cell-free cellulolytic, hemicellulolytic, glycolytic, and proteolytic enzymes are produced by anaerobic gut fungus in the rumen, hindgut, and faeces of ruminant and non-ruminant herbivorous mammals and reptile herbivores. It has been demonstrated that axenic cultures of anaerobic fungi may break down a sizeable portion of plant biomass substrates in simple conditions. Lactate, formate, acetate, and hydrogen are the main fermentation byproducts created during the anaerobic fungi's metabolism of sugars produced during biomass saccharification. A little amount of ethanol is also frequently created cytosolically from acetyl-CoA via the aldehyde dehydrogenase/alcohol dehydrogenase enzyme system. This quantity is typically 0.02 g/g to 0.1 g/g substrate metabolised. Anaerobic fungi are prospective producers of biofuel from lignocellulosic biomass due to their effective biomass-degradation abilities. However, their use in axenic monocultures is prohibited by the fact that acids predominate over alcohols as fermentation byproducts.

## Conclusion

In this paper, we provide a unique method for producing biofuel from lignocellulosic biomass. The method uses the anaerobic fungus isolate strain C1A to directly convert lignocellulosic biomass to sugars and biofuels in a single container without the use of foreign enzymes. In this method, fungal saccharolytic and fermentative metabolism are decoupled using straightforward physiological adjustments, resulting in a buildup of sugars that might later be converted to biofuels. On a variety of lignocellulosic biomass substrates, including moderately alkaline pretreatment maize stover, we show that this method is feasible. Anaerobic fungi are a promising alternative for producing low cost ethanol from lignocellulosic biomass because of the possible cost reductions, input and output flexibility, and operational consolidation.