Trametes versicolor as biodegrader and biocatalyst when using lignocellulose for ethanol production

Marie Holmgren

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Fakultetsopponent: Professor Mohammad Taherzadeh,
Swedish Centre for Resource Recovery, University of Borås, Borås, Sweden.

Umeå Plant Science Centre, Department of Plant Physiology
901 87 Umeå
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Author
Marie Holmgren

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Abstract
Energy consumption has increased rapidly during the last century due to population growth and greater industrialization. Lignocellulosic-based biofuels are being developed as alternatives to fossil fuels. For many years the question of how 5-carbon sugars in biomass are utilized in nature has been a vexed one. A patent by Sellstedt and Holmgren (2005) showed increased ethanol production, compared with fermentation using only *Saccharomyces cerevisiae*, through the use of a fungal mix from a degraded wood sample found in a forest.

The aim of the work presented in this thesis was to find a fungus that naturally utilizes 5-carbon sugars and metabolizes these sugars to ethanol; elucidate the capacity of this fungus to utilize 5-carbon and 6-carbon sugars simultaneously; assess the ability of the fungus to metabolize the inhibitors produced when lignocellulose is used; and find out whether this fungus could be used for biological pretreatment of lignocellulose to replace industrially produced enzymes.

The results showed that the fungal mix grew well on glucose, xylose, hemicellulose and cellulose. In addition, we were able to identify the fungi present, by using PCR-amplification and sequencing of DNA, as *Chalara parvispora*, *Xylaria sp* and *Trametes hirsuta/Trametes versicolor*. In a reconstitution study, the fungi so identified were shown to produce an amount of ethanol equal to that of the fungal mix. We were also able to show that *C. parvispora* could produce ethanol from xylose.

T. versicolor could be grown in culture, under hypoxic conditions, with various mixtures of hexoses and xylose and with xylose alone. After 354 h of culture we found very strong correlations between ethanol fermentation (alcohol dehydrogenase activity and ethanol production), sugar consumption and xylose catabolism (xylose reductase, xylitol dehydrogenase and xylulokinase activities) in the cultures. In a medium containing a 1:1 glucose/xylose ratio, the efficiency of fermentation of total sugars into ethanol was 80 %.

A variety of inhibitors are formed during pretreatment procedures; they include, for example, phenolics, levulinic acid, HMF and furfural. These inhibitors were used in this study in order to reveal their effects on the growth of cells as well as on sugar utilization, enzyme activities and ethanol production by the white-rot fungus *T. versicolor*. The inhibitors had a positive effect on fresh weight, the largest increase being observed with the inhibitor furfural. *T. versicolor* metabolized all the inhibitors during 15 days of experimentation.

It is known that fungi can degrade cellulose, hemicellulose and lignin through a series of enzymatic reactions. Is it possible to eliminate chemical pretreatment and instead use a biological pretreatment? If *T. versicolor* could serve as both a biodegrader and a biocatalyst it would lead to reductions in the costs of ethanol production and lower costs for pretreatments for other renewable fuels too. Experiments with different pretreatments applied to *Salix viminalis* and *Populus tremula* were conducted with and without fungi, as well as with enzymes, to evaluate whether *T. versicolor* was suitable as a biodegrader. The results showed that *T. versicolor* was able to degrade lignocellulose to glucose, and thus is suitable as a biodegrader and in addition has xylanase and beta-glucosidase enzymes that are related to similar enzymes in other fungi.

Keywords
Lignocellulose, 5-carbon, 6-carbon, *Trametes versicolor*, xylose reductase, xylitol dehydrogenase, xylulokinase, phenolics, levulinic acid, HMF, furfural, biodegrader, biocatalyst, pretreatment, xylanase, beta-glucosidase.

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