

UNDERSTANDING THE INHIBITION EFFECT OF ACETIC ACID ON ETHANOL PRODUCTION VIA *S. CEREVISIAE* 424A(LNH-ST)

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Goals:

- Characterize the mechanism(s) of inhibition for acetic acid in the genetically modified *S. cerevisiae*.

Statement of Problem:

With increasing environmental concerns and the projection of reaching peak petroleum supply within the next few decades, fuel ethanol derived from cellulosic biomass has emerged as an environmentally friendly alternative to fossil fuels. The major fermentable sugars released from the processing of the biomass are glucose and xylose. The primary processing steps for the release of xylose from the biomass, pretreatment and enzyme hydrolysis, also produce or liberate compounds, such as acetic acid, which can inhibit the subsequent fermentation. It has been shown that acetic acid can act as a fermentation inhibitor thus lowering the production of ethanol. For ethanol production to be economically competitive with petroleum, these inhibition effects need to be minimized. To do this, the mechanism(s) of inhibition needs to be better understood.

The organism of interest is the genetically modified *S. cerevisiae* (424A(LNH-ST)) strain developed at Purdue University. This strain is capable of fermenting both glucose and xylose to ethanol.

Current Activities:

Multiple inhibition mechanisms for acetic acid have been proposed, including cytoplasmic acidification, inhibition of key metabolic enzymes, and a reduction in ATP levels of the organism. Some of these mechanisms have been studied using *S. cerevisiae* strains that ferment glucose only; however, they need to be studied for the genetically modified organism of interest.

Preliminary studies have shown xylose fermentation to be more sensitive to acetic acid than glucose fermentation when using *S. cerevisiae* (424A(LNH-ST)). Further investigation is required to explain this difference.

To learn more about the effects of acetic acid, the activity of major metabolic enzymes will be assayed and inhibition constants will be calculated.

Upon better understanding of the inhibition effect of acetic acid, further engineering of *S. cerevisiae* (424A(LNH-ST)) can be completed to minimize the inhibition.