

**INCREASED BIOHYDROGEN PRODUCTION BY ADDING REDUCED EXTRACELLULAR
ELECTRON SHUTTLING COMPOUNDS (HYDROQUINONES)**

Xiaofeng Ye*

Xinyu Zhang

Eberhard Morgenroth

Kevin T. Finneran

Civil and Environmental Engineering
University of Illinois, Urbana-Champaign
4162 NCEL, 205 N. Mathews Ave.

Urbana, IL 61801

Voice: 217-333-8121

Fax: 217-333-6968

ye3@illinois.edu

Bio-H₂ is an energy carrier that leads to zero greenhouse gas emission. However, the yield and the rate of fermentative hydrogen production are limited, which decreases its potential in the global fuel market. We have utilized extracellular hydroquinones (reducing equivalents that can cycle between oxidized and reduced states) to alter electron and carbon distribution in the fermentative microorganism, *Clostridium beijerinckii*. H₂ yield increased by 20% along with a 5-fold increase of hydrogen production rate when 250 μM anthrahydroquinone disulfonate (AH₂QDS) was amended to growing cells. Free energy calculations demonstrate that this pathway is only favorable when the concentration of hydroquinone:quinone is greater than 95% present as the hydroquinone. Low concentration sulfide added to maintain the greater than 99% hydroquinone does increase hydrogen yield, but the biomass growth lag time increases proportionately to the sulfide concentration. Finally, continuously transferred cells (often referred to as “degenerate”) generate more hydrogen via this pathway than cells grown from spores (the industry standard method). A chemostat was used to increase bio-hydrogen production, and hydroquinones have increased H₂ production rates and yield by 1.5 to 2 times compared to glucose-alone operation. Metabolic modeling based on free energy has been used to identify how hydroquinones shift fermentation products.

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