

IMPROVING BIOCHEMICAL YIELDS WITH MIXOFERM

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Acetyl-CoA is a primary hub for metabolism and is the building block for most biochemicals of interest. However, the yields for biochemicals derived from acetyl-CoA are inherently limited because of the decarboxylation of pyruvate to acetyl-CoA which releases CO₂. To overcome this limitation, White Dog Labs (WDL) developed a fermentation technology called MixoFerm™ (also known as anaerobic, non-photosynthetic mixotrophy). This technology uses microorganisms capable of concurrently utilizing both organic (e.g., sugars) and inorganic (e.g., CO₂) substrates. Using MixoFerm, CO₂ can be fixed back into acetyl-CoA and thus improve biochemical yields (g product/g substrate consumed). Here, we demonstrate simultaneous utilization of both fructose and syngas by *Clostridium ljungdahlii* and *Clostridium autoethanogenum*. We next engineered *C. ljungdahlii* to produce the non-native metabolite acetone at a yield 35% greater than the theoretical maximum acetone yield without mixotrophy. Finally, we designed and generated a strain of *C. ljungdahlii* capable of consuming glucose, which the wild-type strain is unable to do. With the ability to improve biochemical yields, MixoFerm™ is a robust and flexible platform technology to improve process economics and product life-cycle analysis