SYNTHETIC CARBON FIXATION FOR IMPROVED MICROBIAL FERMENTATION YIELDS

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Microbial fermentation is a well-established conversion technology to utilize our cheap and abundant renewable carbohydrate resources for the production of fuels and chemicals. However, these processes are inherently limited by the decarboxylation reactions occurring during traditional fermentations (glycolysis) leading to low feedstock conversion and product yield. In general, a maximum of two-thirds of the carbon contained in the feedstock can be recovered in useful fermentation products, the rest being lost as undesirable CO_2. With recent advances in synthetic biology, the evolved CO_2 can be utilized as a carbon feedstock by native or engineered organisms, avoiding the loss of CO_2 and increasing overall yield of fuels and chemicals.

A promising CO_2 utilization route is the Wood-Ljungdahl pathway as it is the most efficient pathway for non-phototrophic CO_2 fixation. In this pathway, two molecules of CO_2 are reduced with 8 reducing equivalents to form one molecule of acetyl-CoA, a primordial biological building block for cellular components. The acetyl-CoA molecule can be used in the various downstream pathways to produce the desired products. Through theoretical modeling, the increased product yield when coupling glycolysis to the Wood-Ljungdahl pathway has been shown.

In order to validate this concept, five microorganisms which natively contain the Wood-Ljungdahl pathway were grown in the presence of carbohydrates and exogenous gases, and were shown to have improved product mass yields. Using carbon tracing experiments, we demonstrated gaseous carbon uptake was continuous and independent of the presence of higher-energy carbohydrate substrates. Therefore, this type of anaerobic, non-photosynthetic mixotrophic fermentation can be used to overcome some of the challenges associated with the current production of biofuels via traditional fermentations.

To utilize mixotrophic fermentation for the successful production of biobutanol in an industrially relevant strain, *Clostridium acetobutylicum* was engineered to reduce CO_2 by the heterologous expression of two key enzymes used in autotrophic CO_2 fixation. The CO dehydrogenase (CODH) and acetyl-CoA synthase (ACS) are two essential proteins of the Wood-Ljungdahl pathway and form a bifunctional heterotetrameric complex. The CODH/ACS enzyme can reversibly catalyze CO_2 to CO, effectively enabling a biological water-gas shift reaction at ambient temperatures and pressures. Functional expression of the *C. carboxidivorans* CODH/ACS complex was demonstrated in the solventogen *C. acetobutylicum*, and the strain exhibited both CO_2 reduction and CO oxidation activities. The CODH reactions were studied using isotopic labeling to verify that CO was a direct product of CO_2 reduction and vice versa. The CODH enzyme was hypothesized to use a native *C. acetobutylicum* ferredoxin as the electron carrier for CO_2 reduction. Heterologous CODH activity depended on actively growing cells and required the addition of nickel, which is inserted into CODH without the need to express the native Ni insertase protein. Increasing CO concentrations in the gas phase inhibited CODH activity and altered the metabolite profile of the CODH-expressing cells. This work provides the foundation for engineering a complete and functional Wood-Ljungdahl pathway in non-native host organisms, thereby allowing the application of mixotrophic fermentation to an industrially relevant organism to achieve 100% feedstock conversion.