Title:	Developing Bioproduction Strategies from N2: CO2 Gaseous Feedstock Using Clostridium Ljungdahlii as a Model Organism
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Abstract:	Developing C1 gaseous feedstock (such as CO 2 , CO, and CH 4) utilizing biotechnologies is desired to circumvent the increasing carbon emissions associated with the existing naphtha- based production platforms. Bioproduction from CO 2 has gained substantial attention as one of the possible solutions to reduce carbon emissions and prevent climate change. Gas fermentation and microbial electrosynthesis are considered promising multi-carbon chemical production biotechnologies. However, these rely on ammonia-derived salts for their nitrogen requirements. These salts are primarily obtained through the energy and carbon emission- intensive Haber-Bosch process. Considering the potential of these technologies to contribute to transforming the fossil-based chemical synthesis industry, just circumventing the ammonia- derived salts can further reduce large amounts of CO 2 emissions associated with ammonia production. In this context, bioproduction from N 2 and CO 2 gaseous feedstocks can offer a desired solution. However, low production titer and rates remain a key challenge with both enriched mixed and pure microbial cultures. Thus, my thesis work focused on understanding and developing strategies for the enhancement of bioproduction from gaseous N 2 and CO 2 feedstocks using Clostridium ljungdahlii, a well-known CO 2 -fixing acetogen capable of also fixing N 2 . The key strategies include Adaptive laboratory evolution (ALE), and in-silico metabolic engineering. C. ljungdahlii was selected because of its known genome-scale metabolic model, the availability of well-established metabolic engineering tools and its prior utilization as a biocatalyst in both gas fermentation and microbial electrosynthesis processes. The wild-type strain produced 70 mg/L acetic acid at the 5 mg/L/day rate under comepltely gases carbon, nitrogen and energy sources (N 2 :CO 2 :H 2). After 30 culture transfers in the ALE experiments with only gaseous substrates, there was a >40% increase in the product titer (~110 mg/L) produced at 18 mg/L/day rate. Though the product titer did not increase much in subsequent culture transfers, the growth and production rates increased considerbaly after 30 th transfer. Limited use of gaseous substrates could be the possible reason for limited growth and production. A reasonable increase in production was achieved with the ALE strategy; however, further understanding of the key substrates essential for growth is required to maximize the growth and production. Reduced ferredoxin was found to be a limiting factor in biomass generation, according to a flux balance analysis. Hence, improved bioproduction may be achieved by increasing flux through reduced ferredoxin, for instance, by using easily metabolized energy sources.
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