

STI Title: Bioreactor Development for CO₂-Based In Situ Resource Utilization Manufacturing

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Abstract:

Sustainable long-duration manned missions on both the Moon and Mars will require in situ resource utilization (ISRU). Carbon dioxide (CO₂) has great potential as a harvestable resource, making up 95% of the atmosphere on Mars and being produced as respiratory waste in spacecraft and future planetary habitats. Through ISRU, biomanufacturing has the capability to produce a near limitless array of products from local space resources, which include pharmaceuticals, bioplastics, chemical feedstocks, and industrial enzymes. Here, a CO₂-based ISRU recombinant protein bioreactor and associated biomanufacturing organisms were designed to produce a highly stable carbonic anhydrase (CA). Initial work characterized candidate organisms for growth on acetate and formic acid, carbon substrates that can be synthesized via electrochemical conversion of CO₂. To improve growth on the CO₂ producing substrate formic acid and for direct integration of ISRU CO₂, a synthetic Calvin-Benson-Bassam cycle was designed for use in *Cyberlindnera jadinii* and *Escherichia coli*. Genetic modifications in *E. coli* will be facilitated by a tailored CRISPR/Cas9 and λ red recombineering two-vector system. For expression of CA, a blue light regulated T7 promoter was employed for dynamic and small molecule free induction. Efficient bioproduction through a fed-batch exponential feeding strategy was determined via mass balance calculations from ISRU substrates to biomass and CA yield. Flux balance analysis was used to model ISRU substrate metabolism and metabolic pathway engineering in candidate organisms under cultivation strategy conditions for both metabolism reconstruction and pathway design optimization. Finally, a small-scale, disposable bag bioreactor concept for use in the NASA Bioculture System infrastructure was designed to enable CO₂-based CA production in reduced-gravity environments.