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Large Scale Biomass Production of Obligate Anaerobes for Simultaneous Transcriptomics, Proteomics, Metabolomics, and Lipidomics Analysis

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In order to rapidly determine stress response pathways in anaerobes induced we need to produce biomass for simultaneous analyses using the latest techniques in transcriptomics, proteomics, metabolomics, and lipidomics. To accomplish this, batch cultures of 30 liters of stressed vs. non-stressed Desulfovibrio vulgaris as biological replicates in triplicates are needed ensure that all the analyses will be performed on cells of same condition. Various technical improvements and adaptations were made for the large-scale production and distribution of biomass exposed to a variety of stressors, such as oxygen, salt, nitrate, nitrite, and temperature. Harvesting of D. vulgaris through high-speed centrifugation proved to be the most efficient and uniform method of collecting cells for the various types of analyses vs. filtration. Because of the rapidly changing nature of DNA and short half-life of mRNA, D. vulgaris cultures needed to be immediately cooled to 5°C during biomass sampling. As a result, a fast sample cooling system was invented to chill 300 ml of biomass culture from 30°C to 5°C in less than 3 minutes at a flow rate of 1.8ml/sec. For the oxygen stress of D. vulgaris, culture vessels were fitted with HPLC three valve delivery caps and porous teflon tubing filled with beads for sparging of the cultures with different types of gases (O & N) at various concentrations in a controlled. Because of the concomitant analysis by several labs, rigorous quality control measures were used to insure the quality and sterility of biomass from each time point in a production run, e.g. direct cell counts, optical density, pH, plate streaks, phospholipid fatty acid (PLFA) analysis, and protein assays. In addition, advanced FTIR spectromicroscopy profiling were used to study gross biomolecule changes and to determine optimal sampling times. QA/QC procedure were developed and documented to track every step in production from experiment inception to final analyses, including all chemicals, procedures, and technicians. Data is immediately uploaded to a database that is shared by all investigators (http://vimss.lbl.gov).