7. Development of a LC-MS/MS-Based Cell Density Assay for Assessing Microbial Growth on Complex Solid Biomass Substrates

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC’s approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC researchers provide enabling technologies in characterization, ‘omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion.

The use of cellulolytic microbes to deconstruct plant-derived lignocellulosic biomass attempts to address the increased global demand for renewable transportation fuels, ultimately through the production of ethanol. One such microbe, the anaerobic thermophile Clostridium thermocellum, is an ideal candidate for further study due to its cellulolytic and ethanologenic capabilities. Though C. thermocellum is naturally efficient at degrading complex plant biomass, current efforts aim to enhance this microbe’s catalytic capacity by better understanding the unique enzymatic system it employs. To this end, extensive work using soluble and insoluble model substrates like cellobiose and crystalline cellulose have been used to study various aspects of cellulose utilization. However, lignocellulosic substrates like switchgrass (SWG) have received considerably less attention due in part to difficulties in measuring seemingly routine fermentation metrics such as the C. thermocellum growth dynamics, substrate conversion per unit cell and product yield/titer—all of which are industrially relevant and generally require knowledge of the amount of catalyst (i.e., microbes) present at a given time. Unfortunately, complex solid biomass substrates like SWG often preclude accurate cell count determination both by visual- and protein-based counting assays—the latter of which can be skewed by residual biomass-derived proteins. To address this problem, we developed a novel, mass spectrometry-based proteomic cell density assay that employs a quantitative metric called Matched-Ion inTensity (MIT). This assay capitalizes on proteomic’s ability to uniquely assign peptides to specific organisms/species while concurrently providing protein abundance information useful for organism quantitation. Initial work examining a dilution series of C. thermocellum on a constant amount of SWG demonstrated the power of this method whereby total organism MIT (tMIT) provided an almost perfect match to the C. thermocellum dilution series, regardless of interfering SWG-derived proteins or small molecules. Applied to real-world samples, tMIT specifically and accurately tracked cell density across both simple (cellobiose or Avicel) and complex SWG carbon sources, thereby alleviating the above-mentioned analytical challenge. Furthermore, tMIT shows promise in accurately determining organism ratios in simple co-cultures and could become a necessary tool in the field of community proteomics.

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