Quantitative analysis of tetrahydrofolate metabolites from the acetogen *Clostridium autoethanogenum*

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Tetrahydrofolate (THF) metabolites (Methenyl-THF; Methylene-THF; Methyl-THF) are the spinal cord of autotrophic growth by the Wood-Ljungdahl pathway found in acetogens[1,2]. Despite their importance, no method is available to measure intracellular concentrations of THF intermediates; most likely because of their rapid degradation in the presence of oxygen[1]. Hence, we developed a method for sampling anaerobic cultures to identify and quantify THF metabolites. Culture were injected into an anoxic serum bottle containing anoxic acetonitrile. The samples were then filtered inside the anaerobic chamber and concentrated prior to LC-MS analysis. LC-MS analysis was performed using a Dionex Ultimate 3000 coupled to a QTRAP operated in positive ion mode. Chromatographic separation was achieved using a Phenomenex Gemini-NX C18 column. Comparison of oxic versus anoxic sampling revealed that THFs can only be measured if sampled anoxically. THFs quantification also showed a similar decreasing trend at high biomass, a trend shared by acetyl-CoA, the end product of the WL pathway. Although quantification of THFs is likely only reliable using labelled standards, the method developed here is useful for relative quantification of THFs levels across different growth conditions.

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