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March 10, 2019

Dear Editorial Board,

On behalf of my co-author, I am submitting the enclosed manuscript entitled “Thermodynamic analysis of the pathway for ethanol production from cellobiose in *Clostridium thermocellum*” for publication in *Metabolic Engineering* Journal. This paper describes the impact of increasing ethanol concentration on the thermodynamic landscape of *Clostridium thermocellum* and plausible resolution strategies. The manuscript has been approved for submission by all the authors. This work has not been submitted for publication nor has it been published in whole or part, elsewhere.

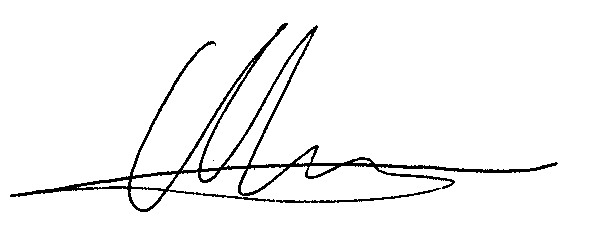
We believe that this work would be of interest to researchers in the metabolic engineering community, as it provides a workflow to identify thermodynamic bottlenecks under high ethanol concentrations by using time-varying metabolomic datasets of *C. thermocellum* grown with and without added ethanol. We found that the thermodynamic bottleneck was distributed across multiple reactions due to increase in NADH and sugar phosphate pools.

We also observed that the thermodynamic bottlenecks can be resolved by modifying the cofactor specificity of one or multiple reactions such as NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, ATP-dependent phosphofructokinase, and NADPH dependent aldehyde dehydrogenase.

We believe that Drs Ross Carlson, Elad Noor, Chris Henry, and Richard Sparling (contact information on the following page) are eminently qualified to review the submitted manuscript.

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Sincerely,



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