



Nitrogen and sulfur requirements for *Clostridium thermocellum* and *Caldicellulosiruptor bescii* on cellulosic substrates in minimal nutrient media[☆]



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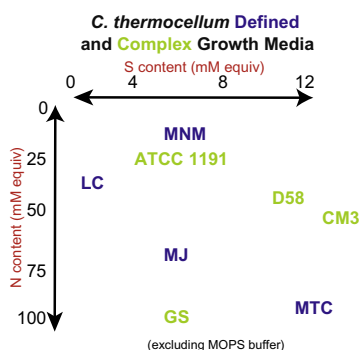
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HIGHLIGHTS

- ▶ NH_4^+ , urea and glutamine are nitrogen sources for cellulose-grown *C. thermocellum*.
- ▶ Urea (8.3 mM) is sufficient for the fermentation of 0.4% cellulose.
- ▶ Both bacteria use sulfate, but produce more oxidized fermentation products.
- ▶ Co-cultures grown with both cellulose and xylan were stable with sulfate.
- ▶ *C. thermocellum* grew on defined, minimal medium without reducing ethanol yield.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 1 August 2012

Received in revised form 8 October 2012

Accepted 1 December 2012

Available online 27 December 2012

Keywords:

Clostridium thermocellum

Caldicellulosiruptor bescii

Nitrogen source

Sulfur source

Defined growth medium

ABSTRACT

Growth media for cellulolytic *Clostridium thermocellum* ATCC 27405 and *Caldicellulosiruptor bescii* bacteria usually contain excess nutrients that would increase costs for consolidated bioprocessing for biofuel production and create a waste stream with nitrogen, sulfur and phosphate. *C. thermocellum* was grown on crystalline cellulose with varying concentrations of nitrogen and sulfur compounds, and growth rate and ethanol production response curves were determined. Both bacteria assimilated sulfate in the presence of ascorbate reductant, increasing the ratio of oxidized to reduced fermentation products. From these results, a low ionic strength, defined minimal nutrient medium with decreased nitrogen, sulfur, phosphate and vitamin supplements was developed for the fermentation of cellobiose, cellulose and acid-pretreated *Populus*. Carbon and electron balance calculations indicate the unidentified residual fermentation products must include highly reduced molecules. Both bacterial populations were maintained in co-cultures with substrates containing cellulose and xylan in defined medium with sulfate and basal vitamin supplements.

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[☆] This manuscript has been authored by UT-Battelle, LLC, under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes.

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