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Full Length Article

Experimental testing of a spatiotemporal metabolic model for carbon monoxide fermentation with *Clostridium autoethanogenum*

Jin Chen[a], James Daniell[b], Derek Griffin[b], Xueliang Li[b], Michael A. Henson[a,*]

[a] *Department of Chemical Engineering and Institute for Applied Life Sciences, University of Massachusetts, Amherst, MA 01003, USA*
[b] *LanzaTech Inc., Skokie, IL 60077, USA*

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ABSTRACT

Gas fermentation is an attractive route to produce alternative fuels and chemicals from non-food feedstocks, such as waste gas streams from steel mills and synthesis gas produced from agricultural residues through gasification. An improved strain of *Clostridium autoethanogenum*, an acetogenic bacteria, was developed by LanzaTech and shows high potential in production of ethanol and 2,3-butanediol from industry waste gas (mainly CO/CO_2) via gas fermentation. In this study, a spatiotemporal metabolic model was formulated and evaluated using steady-state CO fermentation data collected from a laboratory-scale bubble column reactor. The spatiotemporal model was comprised of a genome-scale reconstruction of *Clostridium autoethanogenum* metabolism and multiphase convection-dispersion equations that govern transport of CO, secreted byproducts and biomass. The model provided good agreement with measured ethanol, acetate and biomass concentrations obtained at a single gas flow rate. Then to obtain satisfactory steady-state predictions at three gas flow rates, the upper bound of the proton exchange flux in the genome-scale reconstruction was correlated with the gas flow rate as an indirect means to account for the effects of acetate secretion on extracellular pH. We believe the modeling method established by this work has strong potential to facilitate commercial-scale design of gas fermentation processes for production of biofuel and biochemicals.

1. Introduction

A large proportion of alternative energy and commodity chemicals are currently produced from renewable crop feedstocks which may exacerbate the worldwide food shortage [1]. Thus, the development of new microbial process technologies to utilize non-food, but abundant resources, such as agricultural residues or industrial waste gases is needed. Gas fermentation has emerged as one of the most promising routes for converting industrial waste gases (consisting mainly of CO, CO_2 and/or N_2) and synthesis gas (mainly CO and H_2) into renewable liquid fuels and chemicals by specialized bacteria [2,3]. *Clostridium autoethaogenum*, an acetogenic anaerobic bacterium, has been found to be effective and robust in fermenting CO into ethanol and acetate through the Wood-Ljungdahi pathway [4–10]. However, the yield of ethanol in the wild-type strain is low compared with that of acetate (*e.g.*, 0.2 g/l for ethanol and 3.5 g/l for acetate [10]). Using an iterative selection process, researchers at LanzaTech developed an improved strain of *C. autoethanogenum* [11,12] which provides a substantially increased biofuel yield [12].

Despite the encouraging performance of *C. autoethanogenum*, only a limited number of studies that focus on the technical and energetic feasibility of gas fermentation using this acetogen have been published, possibly due to the difficulties of operating in an anaerobic reaction environment. Most published studies on gas fermentation were performed using bench-scale, continuous stirred tank reactors (CSTRs) [13–15]. Due to the need for high agitation rates to achieve good gas-liquid mass transfer, CTSRs are not economically feasible for large-scale production. By contrast, bubble column reactors exhibit good heat and mass transfer efficiencies with lower operation costs due to mixing from gas sparging configurations [16,17]. Therefore, bubble column technology is promising for commercial-scale gas fermentation. Compared to CSTRs, bubble column operations are more difficult to optimize due to spatial variations across the column which can lead to different growth environments as a function of column position. The main aim of the present study is to investigate the effects of operating conditions, most notably the feed gas flow rate, on cellular growth and product

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* Corresponding author at: Life Science Laboratories Building, University of Massachusetts, 240 Thatcher Way, 413-545-3481, USA.

E-mail addresses: jchen3@umass.edu (J. Chen), James.Daniell@lanzatech.com (J. Daniell), Derek.Griffin@lanzatech.com (D. Griffin), Bruce.Li@lanzatech.com (X. Li), mhenson@umass.edu (M.A. Henson).

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formation during the continuous cultivation of *C. autoethanogenum* by combining conventional bubble column theory with advanced genome-scale metabolic modeling.

Model simulation is a powerful tool for understanding and optimizing fermentation processes. Recently, great effort has been put into the understanding of acetogens on both molecular and process levels [3]. Genome sequences are now available for several model acetogens, including *C. autoethanogenum* [18–20], *C. ljungdahlii* [21] and *Moorella thermoacetica* [22]. Furthermore, genome-scale models (GEMs) based on extensive reaction networks of central metabolism for *C. autoethanogenum* [10], *C. ljungdahlii* [23] and *M. thermoacetica* [24] have been developed and analyzed. These models provide promising avenues for rational strain design to enhance production of target metabolites [25]. Moreover, steady-state [26] and dynamic [27–30] flux balance analysis (FBA) techniques based on GEMs have become dominant tools for simulating microbial metabolism and fermentation processes. With the construction of a GEM for *C. autoethanogenum* by LanzaTech and collaborators at the University of Auckland, the detailed simulation of *C. autoethanogenum* metabolism in bubble column reactors is now feasible.

In previous work [31,32], UMass developed a spatiotemporal metabolic modeling framework to simulate both spatial and temporal profiles of liquid- and gas-phase metabolites for acetogen growth in bubble column reactors. The model for syngas fermentation involved the integration of a published GEM of *C. ljungdahlii* metabolism [23] with uptake kinetics for dissolved CO and H_2 and reaction-convection-dispersion equations for gas and liquid transport. In the current study, we applied this modeling methodology to CO fermentation with the industrially relevant acetogen *C. autoethanogenum* and compared model predictions to steady-state data collected from a laboratory-scale bubble column reactor. The remainder of this paper is organized as follows. First, the bubble column reactor model is described with an emphasis on novel features compared to our previous publications. Then the experimental data obtained at three different feed gas flow rates and the three steady-state operating points used for model testing are discussed. Finally, model-data comparisons are presented along with a discussion of the key model features needed to obtain satisfactory predictions over a range of gas flow rates.

2. Methodology

2.1. Fermentation process modeling

The spatiotemporal metabolic modeling framework previously developed by UMass [31,32] was used to formulate a bubble column model for CO fermentation with *C. autoethanogenum* as the microbial catalyst. Compared to our previous studies, novel features of the current model include utilization of a GEM for an industrial strain of *C. autoethanogenum,* counter-current flow of liquid and gas within the column, recycle of the liquid stream and direct comparison of model predictions to experimental data. The model accounted for CO and CO_2 gas-liquid mass transfer, cellular consumption of CO and CO_2, and cellular production of biomass, ethanol, acetate, 2,3-butanediol, lactate and CO_2. The bubble column reactor configuration used for modeling and experiments is depicted in Fig. 1. The gas and liquid streams flowed counter currently with gas and liquid introduced into the bottom and top of the column, respectively. The liquid stream removed from the bottom was split into the product stream and a recycle stream to which fresh media was added. The gas feed stream consisted of CO and CO_2 as carbon sources and N_2 as an inert component. As discussed in previous publications [31,32], the model equations were formulated assuming gas phase transport involved only convection while liquid phase transport involved convection and axial dispersion. This simple representation allowed gas-liquid mass transfer coefficients, gas and liquid velocities, and gas and liquid volume fractions to be treated as constant across the column. Furthermore, liquid-phase concentrations were assumed to be independent of column position due to the low reaction rates compared with high convective mass transfer caused by the high liquid recycle rate.

For dissolved CO, the mass balance had the form of a reaction-convection-dispersion equation,

$$\frac{\partial c_{l,CO}(z,t)}{\partial t} = v_{CO}c_X + \frac{k_L a_{CO}(c^*_{l.CO} - c_{l.CO})}{\varepsilon_l} - \frac{u_l}{\varepsilon_l}\frac{\partial c_{l,CO}}{\partial z} + D_l\frac{\partial^2 c_{l,CO}}{\partial z^2} \tag{1}$$

$$u_l c_{l,CO}(H,t) - \varepsilon_l D_l \frac{\partial c_{l,CO}}{\partial z}(H,t) = u_l c_{l,CO}(0,t)Q$$

$$_R/(Q_R + Q_M),\quad \frac{\partial c_{l,CO}}{\partial z}(0,t) = 0,\ \ c_{l,CO}(z,0) = c_{l0,CO}$$

where: $c_{l,CO}$ and$c^*_{l.CO}$ represent the CO concentration and the CO saturation concentration, respectively, in the liquid phase (mmol/l); v_{co} is the CO uptake rate (mmol/gDW/h) which is a function of CO concentration based on the kinetic model equation (see Eq. (10)); c_X is biomass concentration (g/l); $k_L a_{CO}$ is CO volumetric mass transfer coefficient (1/h); ε_l is the liquid phase volume fraction; u_l is liquid superficial velocity (m/h), which is calculated from the liquid recycle flow rate and represents the mass transfer by advection; D_l is the dispersion coefficient (m^2/h); and z represents the position in the column (m), which takes values from 0 to H. A Danckwerts boundary condition was imposed at the top of the reactor ($z = H$) due to the introduction of liquid, while a zero slope boundary condition was applied at the bottom ($z = 0$) of the reactor. Here Q_R and Q_M are the liquid recycle rate (L/h) and the media feeding rate (L/h), respectively. The initial dissolved CO concentration $c_{l0,CO}$ (mmol/l) was calculated from the initial CO gas phase concentration using Henry's law. The dissolved CO_2 mass balance had an analogous form,

$$\frac{\partial c_{l,CO_2}(z,t)}{\partial t} = v_{CO_2}c_X + \frac{k_L a_{CO_2}(c^*_{l.CO_2} - c_{l.CO_2})}{\varepsilon_l} - \frac{u_l}{\varepsilon_l}\frac{\partial c_{l,CO_2}}{\partial z} + D_l\frac{\partial^2 c_{l,CO_2}}{\partial z^2} \tag{2}$$

$$u_l c_{l,CO_2}(H,t) - \varepsilon_l D_l \frac{\partial c_{l,CO_2}}{\partial z}(hH,t) = u_l c_{l,CO_2}(0,t)Q$$

$$_R/(Q_R + Q_M),\quad \frac{\partial c_{l,CO_2}}{\partial z}(0,t) = 0,\ \ c_{l,CO_2}(z,0) = c_{l0,CO_2}$$

For gas-phase CO and CO_2, the mass balances had the form,

$$\frac{\partial c_{g,CO}(z,t)}{\partial t} = \frac{-k_L a_{CO}(c^*_{l,CO} - c_{l,CO})}{\varepsilon_g} - \frac{u_g}{\varepsilon_g}\frac{\partial c_{g,CO}}{\partial z} \tag{3}$$

$$c_{g,CO}(0,t) = c_{gf,CO},\ \ c_{g,CO}(z,0) = c_{g0,CO} = \frac{P_{CO}(z)}{RT} = y_{CO}\frac{P_0 + \rho g(H-z)\varepsilon_l}{RT}$$

$$\frac{\partial c_{g,CO_2}(z,t)}{\partial t} = \frac{-k_L a_{CO_2}(c^*_{l,CO_2} - c_{l,CO_2})}{\varepsilon_g} - \frac{u_g}{\varepsilon_g}\frac{\partial c_{g,CO_2}}{\partial z} \tag{4}$$

$$c_{g,CO_2}(0,t) = c_{gf,CO_2},\ \ c_{g,CO_2}(z,0) = c_{g0,CO_2} = \frac{P_{CO_2}(z)}{RT} = y_{CO_2}\frac{P_0 + \rho g(H-z)\varepsilon_l}{RT}$$

where: $c_{g,CO}$ and c_{g,CO_2} are the gas phase concentrations (mmol/l) of CO and CO_2; ε_g is termed the gas holdup which is the gas phase volume fraction, where $\varepsilon_g + \varepsilon_l = 1$; u_g is the gas superficial velocity (m/h); the partial pressures P_{CO} and P_{CO2} were calculated using the mole fraction (y_{CO} and y_{CO_2}) of gas phase CO and CO_2; $c_{gf,CO}$ and $c_{gf,CO_2}$are the CO and CO_2 concentrations (mmol/l) in the feed