A Modeling Approach to Direct Interspecies Electron Transfer Process in Anaerobic Transformation of Ethanol to Methane

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Abstract

Recent studies have shown that direct interspecies electron transfer (DIET) plays an important part in contributing to methane production from anaerobic digestion. However, so far anaerobic digestion models that have been proposed only consider two pathways for methane production, namely acetoclastic methanogenesis, and hydrogenotrophic methanogenesis via indirect interspecies hydrogen transfer, which lacks an effective way for incorporating DIET into this paradigm. In this work, a new mathematical model is specifically developed to describe DIET process in anaerobic digestion through introducing extracellular electron transfer as a new pathway for methane production, taking anaerobic transformation of ethanol to methane as an example. The developed model was able to successfully predict experimental data on methane dynamics under different experimental conditions, supporting the validity of the developed model. Modeling predictions clearly demonstrated that DIET plays an important role in contributing to overall methane production (up to 33%) and conductive material (i.e., carbon cloth) addition would significantly promote DIET through increasing ethanol conversion rate and methane production rate. The model developed in this work will potentially enhance our current understanding on syntrophic metabolism via DIET.

Keywords: Direct interspecies electron transfer (DIET); anaerobic digestion; ethanol; methane production; syntrophy; mathematical model.

Introduction

Anaerobic conversion of organic matter to methane has been widely recognized as an efficient technology for simultaneous waste treatment and energy generation (Batstone and Virdis 2014; Choi et al. 2013; Holm-Nielsen et al. 2009; Khan et al. 2016; Liu et al. 2011; Mackie and Bryant 1995; Nasir et al. 2012; Tada et al. 2006; Vandevoorde and Verstraete 1987). Anaerobic digestion usually undergoes several steps: disintegration, hydrolysis, fermentation and methanogenesis (Chan et al. 2009). Among them, syntrophic metabolism of fermentation products exists as a crucial step in anaerobic digestion, where an interaction must be maintained in balance between reducing power produced by fermentative bacteria and electron sink provided by methanogenic archaea (Shen et al. 2016). Disruptions in the syntrophic associations between bacteria and methanogens can result in system instabilities during anaerobic digestion. Therefore, effective interspecies electron transfer is critical in anaerobic digestion process.

Previous studies of syntrophic electron exchange in methanogenic systems have focused on indirect interspecies electron transfer, in which hydrogen or formate serves as the electron carriers between microbial species in anaerobic soils and sediments (Conrad 1999; Großkopf et al. 1998). In fact, recent studies have demonstrated direct interspecies electron transfer (DIET) between cells would be an alternative way and also metabolically advantageous transfer mode in methane production compared to interspecies electron transfer through hydrogen or formate (Rotaru et al. 2014b; Storck et al. 2015). DIET has been observed in co-cultures of *Geobacter* species (Chen et al. 2014b; Summers et al. 2010) and in co-cultures of *Geobacter metallireducens* and *Methanosaeta*/*Methanosarcina* species (Chen et al. 2014a; Liu et al. 2012; Rotaru et al. 2014a; Rotaru et al. 2014b). The potential of

interspecies hydrogen/formate transfer mode has been excluded due to the incapacity of hydrogen/formate uptake by these species. Further, much direct evidence also suggested the predominance of DIET over interspecies hydrogen/formate transfer in anaerobic brewery digesters (Morita et al. 2011; Rotaru et al. 2014b; Shrestha et al. 2014; Zhao et al. 2016; Zhao et al. 2015a).

DIET can be accelerated through addition of non-biological conductive materials. For example, granular activated carbon (Liu et al. 2012), biochar (Chen et al. 2014b) and carbon cloth (Chen et al. 2014a) have been found to be able to stimulate DIET in defined co-cultures, but not to promote metabolism of co-cultures performing interspecies hydrogen/formate transfer. Promotion of microbial attachment on these materials were excluded as the syntrophic metabolism was not enhanced with poorly conductive carbon cloth (Chen et al. 2014a). Further study has demonstrated the promotion effect of these conductive materials during long-term operation of up-flow anaerobic sludge reactors (Zhao et al. 2015b). Essentially, addition of conductive materials can short the initial adaptation time of DIET, allowing the distant syntrophic partners to attach to conductive materials for cell-to-cell bio-electrical transfers to be possible.

Mathematical modeling of DIET in conversion of organic matter to methane is of great value to understand of mechanisms involved in this system and to optimize its further applications (Liu et al. 2015a). The well-established Anaerobic Digestion Model No.1 (ADM1) has been widely applied to describe methane production process (Batstone et al. 2002). It contains multiple processes to simulate biochemical reactions within anaerobic digestion, namely disintegration, hydrolysis, acidogenesis, acetogenesis, acetoclastic and hydrogenotrophic methanogenesis, further with several extensions developed to describe processes such as homoacetogenesis (Ni et al. 2011), denitrification (Tugtas et al. 2006; Tugtas et al. 2010) and sulfate reduction (Liu et al. 2015b; Liu et al. 2015c). However, none of these currently available model structures specifically considered the important role of DIET on methane production.

The aim of this work is to propose a new model to understand and describe DIET process between functional microorganisms as well as the effects of conductive materials (taking carbon cloth as an example) on DIET through integrating extracellular electron transfer as a new pathway for methane production. The validity of the model is verified through comparison of model predictions and experimental data on methane generation profiles of four independent studies under different conditions. The developed model in this work will potentially enhance our current understanding on DIET.

Materials and Methods

Model development

The model proposed in this study incorporates DIET into syntrophic metabolism of fermentation products through introduction of electrons as a new component in ADM1, which can well predict DIET process with relatively simple model structure and limited number of model parameters. Such simplification will not only reduce model calibration efforts but also ensure the model to be easily integrated with existing anaerobic digestion models for more comprehensive simulations, and in turn make the DIET model more practically applicable. Specifically, *Mred* (the reduced mediators) and *Mox*

(the oxidized mediators), defined as respective reduced and oxidized extracellular electron mediators, are considered as state variables in the developed model. The electron transfer is modeled by a recirculation loop between *Mred* and *Mox* (*Mred* ⇌ *Mox* + e^-), i.e., an increase in *Mred* being balanced by a decrease in *Mox* and vice versa, with the total amount of mediators (C_{tot}) to be constant ($S_{Mred} + S_{Mox} = C_{tot}$).

Taking anaerobic transformation of ethanol to methane as an example, ethanol is first oxidized to acetate, with four electrons produced through reduction of *Mox* to *Mred* (Equation 1).

$$CH_3CH_2OH + H_2O + 4Mox \rightarrow CH_3COOH + 4H^+ + 4Mred \quad (1)$$

Acetoclastic methanogens then utilize acetate for methane production (Equation 2)

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2)$$

Meanwhile, methane is produced by acetoclastic methanogens via DIET using electrons generated from ethanol oxidation (Equation 3).

$$CO_2 + 8Mred + 8H^+ \rightarrow CH_4 + 2H_2O + 8Mox \quad (3)$$

In addition, methanogenesis can be carried out in the presence of hydrogenotrophic methanogens through indirect interspecies hydrogen transfer route (Equations 4 and 5)

$$2H^+ + 2Mred \rightarrow H_2 + 2Mox \quad (4)$$

$$CO_2 + 8Mred + 4H_2 \rightarrow CH_4 + 2H_2O + 8Mox \quad (5)$$

The detailed kinetics and stoichiometry of the developed model are listed in Table 1. The Michaelis-Menten equation is used to describe kinetics of these biological reaction rates. Each reaction rate is described as a function of the concentration of substrates (i.e., ethanol, acetate, *Mox*, *Mred* or hydrogen) involved in the reaction. An example kinetics equation of syntrophic metabolism between ethanol degradation (Equation 1) and DIET methane production (Equation 3) is provided as below, i.e., Equations 6 and 7, respectively. Microbial growth and decay are also considered in the model. Model components and parameter values are showed in Tables 2 and 3.

$$r1 = k_1 \frac{S_{eth}}{S_{eth}+K_{eth}} \frac{S_{Mox}}{S_{Mox}+K_{Mox}} X_{eth} \quad (6)$$

$$r3 = k_3 \frac{S_{Mred}}{S_{Mred}+K_{Mred}} X_{ac} \quad (7)$$

where K_{eth}, K_{Mox}, and K_{Mred} are the half saturation constants for ethanol, *Mox* and *Mred*, and $k_1$and k_3 are the maximum specific uptake rates of Equations 1 and 3, respectively. S_{eth} is the ethanol concentration, while S_{Mox} and S_{Mred} are the concentrations of the oxidized and reduced mediators involved in direct electron transfer. X_{eth} and X_{ac} are the biomass concentrations of ethanol-metabolizing bacteria and acetoclastic methanogens, respectively.

The DIET model was formulated and implemented in AQUASIM 2.1d (Reichert 1998). This program offers a flexible definition of the kinetic model, flow scheme, and process control strategies; it also provides support for graphic display of the support of the simulation results, corresponding experimental data, and communication with spreadsheet programs (Reichert 1998). In order to integrate the differential equations of the model, as a first step, the partial differential equations are discretized in space. Then the spatially discretized partial differential equations together with the ordinary differential equations and the algebraic equations are integrated numerically in time with the algorithm which is based on the implicit variable-step variable-order Gear integration technique.

Experimental data for model evaluation

Experimental data of four case studies under different conditions studying DIET during biotransformation of ethanol to methane were applied to exam the model prediction capacities.

Case I (Rotaru et al. 2014b): Ethanol-adapted *Geobacter metallireducens* (ethanol-metabolizing microorganisms) and acetate-grown *Methanosaeta harundinacea* (acetoclastic methanogens, incapacity of using hydrogen/formate for methane production) were grown separately with their own specific medium in the anaerobic pressure tube, prior to co-cultivation. Co-culture batch test was carried out with *Geobacter metallireducens* (0.5 mL) and *Methanosaeta harundinacea* (1 mL) grown in a 50-mL fresh water medium containing 20 mM ethanol and CO_2. Samples were taken regularly with N_2:CO_2 flushed hypodermic syringe for analyzing the dynamics of ethanol, acetate and methane during 85-day cultivation. More details on system operation and measurement can be found at Rotaru et al. (2014b).

Case II (Rotaru et al. 2014a): Ethanol-adapted *Geobacter metallireducens* and acetate-grown *Methanosaeta barkeri* (also acetoclastic methanogens, incapacity of using hydrogen/formate for methane production) were also cultivated separately with their own specific medium, prior to initiating co-cultures. Co-culture cultivation was initiated with the inoculum of *Geobacter metallireducens* (0.5 mL) and the inoculum of *Methanosaeta harundinacea* (0.5 mL) added in 9-mL modified DSM 120 medium containing 20 mM ethanol, with CO_2 as the only electron acceptor. Then, 10% inoculum of initiated co-cultures was transferred to 45-ml medium to start batch test. Samples were taken regularly to analyze ethanol, acetate and methane during 31 days. More details on system operation and measurement can be found at Rotaru et al. (2014a).

Case III (Chen et al. 2014a): Similarly, ethanol-adapted *Geobacter metallireducens* and acetate-grown *Methanosaeta barkeri* were cultivated separately as mentioned in *Case II*. Before batch tests, cotton cloth (control, without conductive materials) or carbon cloth (experimental, with conductive materials) strips of 0.1 – 0.2 g per tube in the culture medium were autoclaved in pressure tubes under a N_2:CO_2 atmosphere for 30 min. After that, 5% inoculum of initiated co-cultures (*Geobacter metallireducens* and *Methanosaeta barkeri*) was added to each cloth present tube containing 10 mM ethanol as only electron donor. In both tests, samples were taken regularly to analyze ethanol and methane during 30 days. More details on system operation and measurement can be found at Chen et al. (2014a).

Case IV (Zhao et al. 2015b): Two identical 1-L up-flow anaerobic sludge reactors, one packed with carbon cloth pieces (1 × 1 cm^2 per piece, 2.5 g in total) and one in the absence of carbon cloth as control after 10-d start-up, were continuously operated. The seed sludge was collected from an anaerobic digester at a municipal wastewater treatment plant. 0.5 L sludge was added into each reactor at the beginning. A synthetic wastewater (4110 mg COD/L) with ethanol as carbon source was used as the influent for both reactors. The initial hydraulic retention time (HRT) were 24 hours then decreased to 18 hours after 14-d operation. Effluent acetate and ethanol were sampled and analyzed daily. Methane production rate was calculated through measuring gas volume in the gas collection bag and methane concentration in the bag every 8 h. More details on system operation and measurement can be found in Zhao et al. (2015b).

Testing the model prediction ability

The developed model contains 15 kinetic and stoichiometric parameters (Table 3). Among them, 12 parameter values have been well established previously. Therefore, literature reported values were applied on them. The rest 3 parameters, maximum ethanol uptake rate (k_1), maximum acetate uptake rate (k_2), and maximum reaction rate of DIET methanogenesis (k_3), are the key parameters relating to DIET process and thus calibrated with experimental data.

For *Cases I*, *II* and *III*, Equations 4 and 5 were not considered in the model. The reasons are (Shrestha et al. 2013; Summers et al. 2010): (1) evidence has shown that *Geobacter metallireducens* is incapable of releasing electrons generated from ethanol oxidation as hydrogen or formate, but able to transfer these electrons to *Methanosaeta* species; (2) *Methanosaeta* species are acetoclastic methanogens and not able to use hydrogen/formate for methane production. Thus, it should be noted that conventional ADM1 models only consider acetoclastic and hydrogenotrophic methanogenesis will fail in describing DIET process.

C_{tot} (the sum of S_{Mox} and S_{Mred}) was set with a value of 0.01 mmol/L and S_{Mox} =S_{Mred} at the initial stage based on previously reported literature (Pan et al. 2013). This is acceptable as the absolute value of C_{tot} is not critical for model simulation calibration and prediction. For Cases I to III with pure cultures, the initial concentrations of microbes were set based on experimental measurements. For Case IV, the initial biomass concentrations were set based on convergence simulation of the continuous-flow reactor. Such approach will not affect k values (Ni et al. 2015).

Parameter estimation were carried out by minimizing the sum of squares from the deviations between the experimental data and model predictions, using the secant function in AQUASIM 2.1d (Reichert 1998). The best-fit parameter values (k_1, k_2 and k_3) for each case are summarized in Table 4.

Results

Model evaluation using experimental data from Case I

Firstly, the proposed model is tested to predict the experimental results of *Case I*. The simulated ethanol, acetate and methane results with the developed model are shown in Figure 1, together with experimental data. At the beginning 40 d, both ethanol oxidation and methane production rates were relatively low, showing a lag phase likely due to the microbial adaption. After initial adaption of *Geobacter* and *Methanosaeta* species to DIET, both rates increased significantly in the following period, indicating the coupling of ethanol oxidation and methane production via DIET. Eventually, ca. 1.66 mmol methane was produced from ca. 1.15 mmol ethanol, further confirming the presence of DIET since theoretical methane production from only acetoclastic pathway should be the same amount as initial ethanol concentration. The developed model well predicted these trends. The good match between model predictions and measured data confirmed that the established model captures the dynamics of ethanol, acetate and methane in the presence of DIET. The calibrated parameter values providing the best model fittings with the experimental results are summarized in Table 4.

Model evaluation using experimental data from Case II

The experimental data of *Case II* were then applied to exam the established DIET model for the prediction capacity on ethanol, acetate and methane dynamics. The obtained values of the estimated parameters (k_1, k_2 and k_3) using experimental data from this case are summarized in Table 4. Substrate dynamics (Figure 2) were similar to *Case I*, except for the much shorter initial lag phase (i.e., 7 vs. 40 days), due to the better adaption of co-cultures in ethanol metabolism. The model reasonably predicted these substrate trends, with higher reaction rates (k values) than those of *Case I* (Table 4) likely because of better syntrophic metabolism that an interaction is maintained in balance between reducing power produced by ethanol-metabolizing bacteria and electron sink provided by acetoclastic methanogens. However, there were differences between model predictions and experimental measured results on methane on day 26 and 31, likely caused by the unexpected experimental errors as per mass balance.

Model evaluation using experimental data from Case III

The established model were further tested with experimental data of ethanol and methane from *Case III*, in both absence and presence of conductive carbon cloth, respectively (Figure 3). Both ethanol oxidation and methane production rates were enhanced significantly (Figure 3b) as the addition of carbon cloth intensified the DIET. The model outputs matched the experimental data well. k_1 (maximum ethanol uptake rate) and k_3 (maximum reaction rate of DIET methanogenesis) in the experimental batch were ca. 1.5 times and 4 times higher than those values in control batch without carbon cloth addition (Table 4). k_2 (maximum acetate uptake rate) was not increased as conductive materials only enhance syntrophic extracellular electron transfer (i.e., Equations 1 and 3), rather than Equation 2. These results indicated that the developed model is capable of predicting enhanced DIET with the presence of conductive materials.

Model evaluation using experimental data from Case IV

In addition, the experimental data from up-flow anaerobic sludge reactors in both absence and presence of conductive carbon cloth were applied to evaluate the established model (Figure 4). Similar to *Case III*, k_1 (maximum ethanol uptake rate) and k_3 (maximum reaction rate of DIET methanogenesis) in the experimental reactor were both increased significantly compared to those in control reactor without carbon cloth addition (Table 4), in agreement with the observation of enhanced DIET, i.e., higher ethanol consumption (lower effluent ethanol concentrations) and methane production rates, in the experimental reactor packed with carbon cloth. Enhanced DIET would further lead to the increase in the abundance of acetoclastic methanogens, in turn resulting in higher acetate consumption (lower acetate concentrations in the effluent) despite of the unchanged k_2 (maximum acetate uptake rate). The good match between model predictions and measured data further suggested that the developed model can also be used to predict DIET in mix-culture conditions.

Discussion

Anaerobic digestion is the most widely applied and effective strategy to recover bioenergy of methane from organic waste (Appels et al. 2011). Ethanol, an organic compound that is commonly

detected in wastewater discharged from chemical units, brewery factories, or pharmaceutical factories with a substantially high concentration (Kaksonen et al. 2003; Nagpal et al. 2000), can be efficiently treated with anaerobic digestion (Agler et al. 2008). Increasing evidence has shown that cell-to-cell electron transfer via DIET is a metabolically better pathway in terms of high energy conservation than that of interspecies hydrogen/formate transfer in conversion of methane from ethanol (Morita et al. 2011; Rotaru et al. 2014b; Shrestha et al. 2014; Zhao et al. 2016; Zhao et al. 2015a). However, current mathematical models for predicting methane production in anaerobic digestion (i.e., ADM1 and its extensions) only consider acetoclastic and hydrogenotrophic methanogenesis pathways (Batstone et al. 2002), which would fail to describe the methane dynamics in the presence of DIET.

In this study, a new mathematical model describing DIET process in anaerobic methane production is developed, which is of great importance for better understanding and modeling the rate/extent of methane conversion during anaerobic digestion. The key feature of the developed model is the introduction of electron transfer concept through a pool of extracellular electron mediators. Model validity was confirmed using four independent case study reports (Chen et al. 2014a; Rotaru et al. 2014a; Rotaru et al. 2014b; Zhao et al. 2015b). The set of best-fit parameter (k_1, k_2 and k_3) values are summarized in Table 4 and they vary in a relative small range (i.e., less than one order magnitude in the absence of carbon cloth). Values of k_2 (maximum acetate uptake rate) and k_3 (maximum reaction rate of DIET methanogenesis) are comparable, further confirming the important role of DIET. The obtained model parameter values were robust to predict DIET under different experimental conditions (i.e., co-cultures or mixed-cultures, batch cultivation or digester operation, as well as the absence or presence of conductive materials), indicating the wide applicability of the model. Despite the concentration dynamics of *Mred* and *Mox* were not directly validated due to the lacking of analytical method (Rotaru et al. 2014b), our current approach that assumes a value of C_{tot} (the sum of S_{Mox} and S_{Mred}) based on literature report has been verified in previous study (Pan et al. 2013). Also, the verification based on experimentally obtained concentrations of ethanol, acetate and CH_4 has indeed indicated that our new model can reasonably describe DIET, as the modeling results match well with experimental dynamics that acetoclastic methanogens not only convert the acetate produced from ethanol to methane but also consume the additional electrons available from the conversion of ethanol to acetate for methane production.

Effective interspecies electron transfer in the syntrophic associations between bacteria and methanogens is crucial to the efficient function of methanogens (Morita et al. 2011). Since *Methanosaeta* species, capable of DIET, are ubiquitous and abundant in a wide range of methanogenic environments, promoting interspecies electron transfer to *Methanosaeta* via DIET can enhance methane production during anaerobic digestion. As model predicted in Cases *III* and *IV*, conductive carbon cloth addition significantly improve k_1 (maximum ethanol uptake rate) and k_3 (maximum reaction rate for DIET methanogenesis), leading to better methanogenic performance. Better syntrophic metabolism when DIET was enhanced also resulted in a favorable condition for the growth of acetoclastic methanogens despite of the same k_2 (maximum acetate uptake rate). Since the main objective in this study is to validate our model applicability under different conditions using experimental data batch or continuous tests, and with or without carbon cloth addition, the effect of

carbon cloth concentration on k values was not included in the current model. But it can be easily incorporated in future work if studying the impact of its concentration is the main goal.

It should be noted that *Mred* and *Mox* are two lumped parameters used in the model. In reality, DIET is a syntrophic metabolism which electrons flow from cell to cell without being shuttled by mediators. The use of such two lumped parameters reduces the complexity of the model.

The possible existence of different groups of ethanol-metabolizing bacteria and methanogens were not specifically considered for model simplification, which were lumped together in our model. This assumption can be revised later when more information about kinetics of different ethanol-metabolizing and methanogenic microorganisms becomes available. Also, disintegration, hydrolysis and acidogenesis were not presented in the current model as this study focuses on DIET during anaerobic transformation of ethanol to methane. The impact of biofilm matrix and diffusion on DIET was not considered in this study as all experimental systems are under completely mixed conditions. In fact, it has been reported that the biofilm thickness can affect extracellular electron transfer process (Renslow et al. 2013; Strycharz et al. 2011). This is expected because the current produced via diffusion-based extracellular electron transfer consists entirely of electrons delivered to the electrode and half of the electrons produced and subsequently accepted by mediators are lost to the bulk solution. Also, the conductivity of the biofilm can influence the transmission of electrons through a biofilm matrix (Strycharz-Glaven et al. 2011), as it reduces the requirement for high mediator concentrations and limits mediator losses (Renslow et al. 2013). Our simplification over the complicated reaction-diffusion-electrochemical approach (Storck et al. 2015) can well predict DIET with relatively simple model structure and limited number of model parameters, which can make the implementation, application, and comprehension of the model easier. However, these processes are readily to be incorporated into our model to describe substrate and methane dynamics in complex systems.

Conclusion

In this study, a new mathematical model is proposed to describe DIET process in anaerobic transformation of ethanol to methane. The proposed model was successfully used to reproduce experimental results from four independent cases with different conditions and indicated the wide applicability of the proposed model. Modeling results indicated that DIET plays an important role in contributing to methane production during anaerobic digestion, i.e., comparable k_2 (maximum acetate uptake rate) and k_3 (maximum reaction rate for DIET methanogenesis), and the addition of conductive carbon cloth would significantly improve k_1 (maximum ethanol uptake rate) and k_3 (maximum reaction rate for DIET methanogenesis), leading to better the methanogenic performance. This DIET model can be further incorporated into well-established ADM1 model to provide insights on process design and optimization during anaerobic digestion of ethanol wastewater.

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References

Agler MT, Garcia ML, Lee ES, Schlicher M, Angenent LT (2008) Thermophilic anaerobic digestion to increase the net energy balance of corn grain ethanol. Environ Sci Technol 42:6723-6729.

Appels L, Lauwers J, Degrève J, Helsen L, Lievens B, Willems K, Van Impe J, Dewil R (2011) Anaerobic digestion in global bio-energy production: potential and research challenges. Renew Sustainable Energy Rev 15:4295-4301.

Batstone DJ, Keller J, Angelidaki I, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA (2002) The IWA Anaerobic Digestion Model No 1(ADM 1). Water Sci Technol 45:65-73.

Batstone DJ, Virdis B (2014) The role of anaerobic digestion in the emerging energy economy. Curr Opin Biotechnol 27:142-149.

Chan YJ, Chong MF, Law CL, Hassell DG (2009) A review on anaerobic–aerobic treatment of industrial and municipal wastewater. Chem Eng J 155:1-18.

Chen S, Rotaru A-E, Liu F, Philips J, Woodard TL, Nevin KP, Lovley DR (2014a) Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures. Bioresour Technol 173:82-86.

Chen S, Rotaru A-E, Shrestha PM, Malvankar NS, Liu F, Fan W, Nevin KP, Lovley DR (2014b) Promoting interspecies electron transfer with biochar. Sci Rep 4:5019.

Choi W-H, Shin C-H, Son S-M, Ghorpade PA, Kim J-J, Park J-Y (2013) Anaerobic treatment of palm oil mill effluent using combined high-rate anaerobic reactors. Bioresour Technol 141:138-144.

Conrad R (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiol Ecol 28:193-202.

Großkopf R, Janssen PH, Liesack W (1998) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol 64:960-969.

Holm-Nielsen JB, Al Seadi T, Oleskowicz-Popiel P (2009) The future of anaerobic digestion and biogas utilization. Bioresour Technol 100:5478-5484.

Kaksonen AH, Franzmann PD, Puhakka JA (2003) Performance and ethanol oxidation kinetics of a sulfate-reducing fluidized-bed reactor treating acidic metal-containing wastewater. Biodegradation 14:207-217.

Khan MA, Ngo HH, Guo WS, Liu YW, Zhou JL, Zhang J, Liang S, Ni BJ, Zhang XB, Wang J (2016) Comparing the value of bioproducts from different stages of anaerobic membrane bioreactors. Bioresour Technol 214:816-825.

Liu F, Rotaru A-E, Shrestha PM, Malvankar NS, Nevin KP, Lovley DR (2012) Promoting direct interspecies electron transfer with activated carbon. Energy Environ Sci 5:8982-8989.

Liu Y, Wang Q, Zhang Y, Ni B-J (2015a) Zero valent iron significantly enhances methane production from waste activated sludge by improving biochemical methane potential rather than hydrolysis rate. Sci Rep 5:8263.

Liu Y, Zhang Y, Ni B-J (2015b) Evaluating enhanced sulfate reduction and optimized volatile fatty acids (VFA) composition in anaerobic reactor by Fe (III) addition. Environ Sci Technol 49:2123-2131.

Liu Y, Zhang Y, Ni B-J (2015c) Zero valent iron simultaneously enhances methane production and sulfate reduction in anaerobic granular sludge reactors. Water Res 75:292-300.

Liu Y, Zhang Y, Quan X, Chen S, Zhao H (2011) Applying an electric field in a built-in zero valent iron–anaerobic reactor for enhancement of sludge granulation. Water Res 45:1258-1266.

Mackie RI, Bryant MP (1995) Anaerobic digestion of cattle waste at mesophilic and thermophilic temperatures. Appl Microbiol Biotechnol 43:346-350.

Morita M, Malvankar NS, Franks AE, Summers ZM, Giloteaux L, Rotaru AE, Rotaru C, Lovley DR (2011) Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. MBio 2:e00159-11.

Nagpal S, Chuichulcherm S, Livingston A, Peeva L (2000) Ethanol utilization by sulfate-reducing bacteria: An experimental and modeling study. Biotechnol Bioeng 70:533-543.

Nasir IM, Ghazi TIM, Omar R (2012) Production of biogas from solid organic wastes through anaerobic digestion: a review. Appl Microbiol Biotechnol 95:321-329.

Ni B-J, Batstone D, Zhao B-H, Yu H-Q (2015) Microbial Internal Storage Alters the Carbon Transformation in Dynamic Anaerobic Fermentation. Environ Sci Technol 49:9159-9167.

Ni B-J, Liu H, Nie Y-Q, Zeng RJ, Du GC, Chen J, Yu H-Q (2011) Coupling glucose fermentation and homoacetogenesis for elevated acetate production: experimental and mathematical approaches. Biotechnol Bioeng 108:345-353.

Pan Y, Ni B-J, Yuan Z (2013) Modeling electron competition among nitrogen oxides reduction and N2O accumulation in denitrification. Environ Sci Technol 47:11083-11091.

Reichert P (1998) AQUASIM 2.0—user manual. Swiss Federal Institute for Environmental Science and Technology Dubendorf, Switzerland

Renslow R, Babauta J, Kuprat A, Schenk J, Ivory C, Fredrickson J, Beyenal H (2013) Modeling biofilms with dual extracellular electron transfer mechanisms. PCCP 15:19262-19283.

Rotaru A-E, Shrestha PM, Liu F, Markovaite B, Chen S, Nevin KP, Lovley DR (2014a) Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina barkeri. Appl Environ Microbiol 80:4599-4605.

Rotaru A-E, Shrestha PM, Liu F, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin KP, Lovley DR (2014b) A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. Energy Environ Sci 7:408-415.

Shen L, Zhao Q, Wu X, Li X, Li Q, Wang Y (2016) Interspecies electron transfer in syntrophic methanogenic consortia: From cultures to bioreactors. Renew Sustainable Energy Rev 54:1358-1367.

Shrestha PM, Malvankar NS, Werner JJ, Franks AE, Elena-Rotaru A, Shrestha M, Liu F, Nevin KP, Angenent LT, Lovley DR (2014) Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment. Bioresour Technol 174:306-310.

Shrestha PM, Rotaru A-E, Summers ZM, Shrestha M, Liu F, Lovley DR (2013) Transcriptomic and genetic analysis of direct interspecies electron transfer. Appl Environ Microbiol 79:2397-2404.

Storck T, Virdis B, Batstone DJ (2015) Modelling extracellular limitations for mediated versus direct interspecies electron transfer. ISME J 10:621-631.

Strycharz SM, Malanoski AP, Snider RM, Yi H, Lovley DR, Tender LM (2011) Application of cyclic voltammetry to investigate enhanced catalytic current generation by biofilm-modified anodes of Geobacter sulfurreducens strain DL1 vs. variant strain KN400. Energy Environ Sci 4:896-913.

Strycharz-Glaven SM, Snider RM, Guiseppi-Elie A, Tender LM (2011) On the electrical conductivity of microbial nanowires and biofilms. Energy Environ Sci 4:4366-4379.

Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR (2010) Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science 330:1413-1415.

Tada C, Tsukahara K, Sawayama S (2006) Illumination enhances methane production from thermophilic anaerobic digestion. Appl Microbiol Biotechnol 71:363-368.

Tugtas AE, Tezel U, Pavlostathis SG (2006) An extension of the Anaerobic Digestion Model No. 1 to include the effect of nitrate reduction processes. Water Sci Technol 54:41-50.

Tugtas AE, Tezel U, Pavlostathis SG (2010) A comprehensive model of simultaneous denitrification and methanogenic fermentation processes. Biotechnol Bioeng 105:98-108.

Vandevoorde L, Verstraete W (1987) Anaerobic solid state fermentation of cellulosic substrates with possible application to cellulase production. Appl Microbiol Biotechnol 26:479-484.

Zhao Z, Zhang Y, Quan X, Zhao H (2016) Evaluation on direct interspecies electron transfer in anaerobic sludge digestion of microbial electrolysis cell. Bioresour Technol 200:235-244.

Zhao Z, Zhang Y, Wang L, Quan X (2015a) Potential for direct interspecies electron transfer in an electric-anaerobic system to increase methane production from sludge digestion. Sci Rep 5:11094.

Zhao Z, Zhang Y, Woodard T, Nevin K, Lovley D (2015b) Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. Bioresour Technol 191:140-145.

Table Captions

Table 1. Stoichiometric Matrix and Process Kinetic Rate Equations for the Biological Reaction Model

Table 2. Definition of Components in the Model

Table 3. Kinetic Parameter Values of the Developed Model for Case IV

Table 4. Best-Fit Parameters with 95% Confidence Intervals Describing DIET in Four Case studies

Figure Captions

Figure 1. Model evaluation with experimental data on ethanol, acetate and methane from batch test in *Case I* (Rotaru et al. 2014b).

Figure 2. Model evaluation with experimental data on ethanol, acetate and methane from batch test in *Case II* (Rotaru et al. 2014a).

Figure 3. Model evaluation with experimental data on ethanol and methane from batch tests in *Case III* (Chen et al. 2014a): (a) in the absence of conductive carbon cloth; (b) in the presence of conductive carbon cloth.

Figure 4. Model evaluation with experimental data on effluent ethanol, acetate and methane production from continuous reactor operation in *Case IV* (Zhao et al. 2015b): (a) without carbon cloth addition; (b) with carbon cloth addition. HRT was decreased from 24 h to 18 h on day 14.

Figure

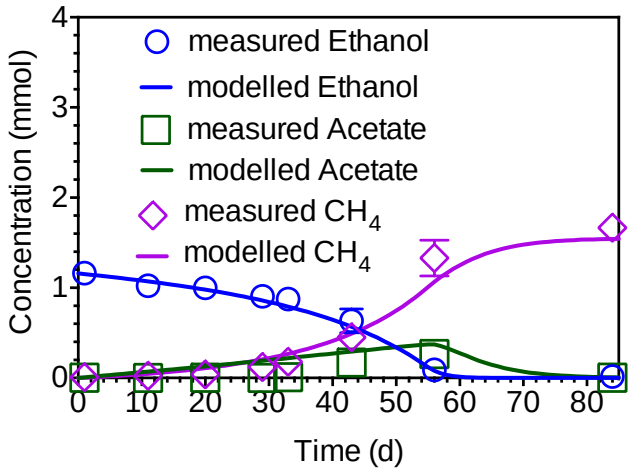

Figure 1. Model evaluation with experimental data on ethanol, acetate and methane from batch test in *Case I* (Rotaru et al. 2014b).

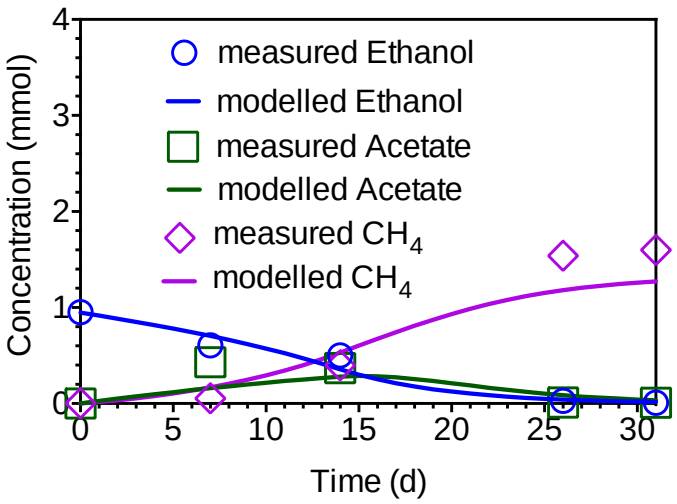

Figure 2. Model evaluation with experimental data on ethanol, acetate and methane from batch test in *Case II* (Rotaru et al. 2014a).

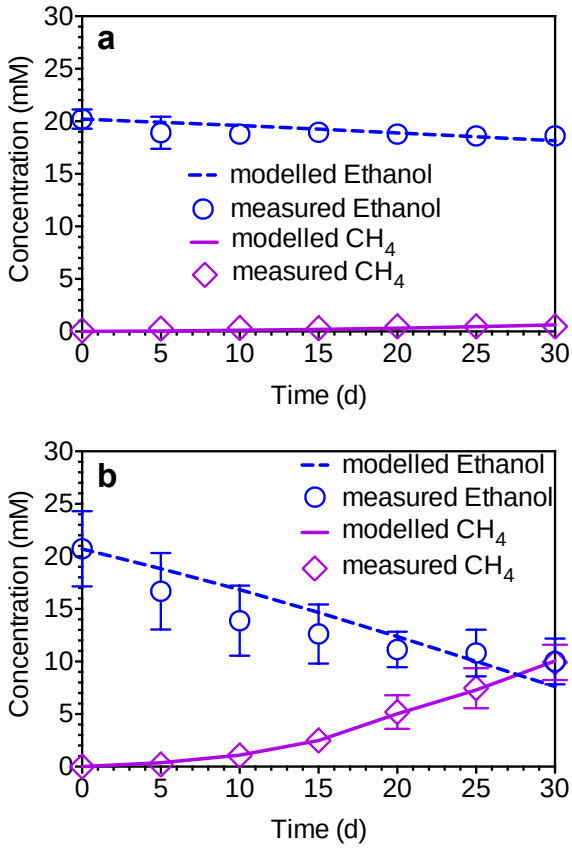

Figure 3. Model evaluation with experimental data on ethanol and methane from batch tests in *Case III* (Chen et al. 2014a): (a) in the absence of conductive carbon cloth; (b) in the presence of conductive carbon cloth.

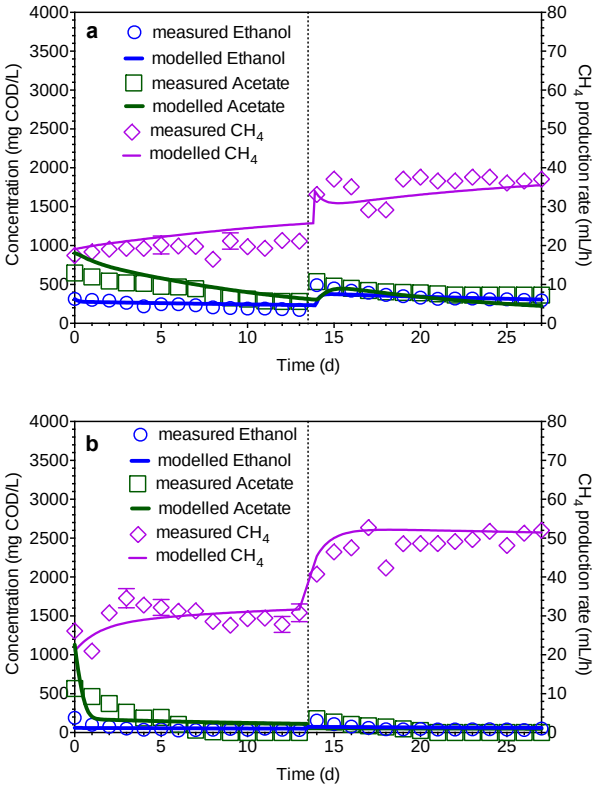

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Table

Table 1. Stoichiometric Matrix and Process Kinetic Rate Equations for the Biological Reaction Model

Variable Process	S_{CH4} COD	S_{eth} COD	S_{ac} COD	S_{h2} COD	S_{Mox} mM	S_{Mred} mM	X_{eth} COD	X_{ac} COD	X_{h2} COD	X_I COD	Kinetics rate expressions
1. Ethanol degradation		-1	$-\frac{2(1-Y_{eth})}{3}$		$-\frac{1-Y_{eth}}{24}$	$\frac{1-Y_{eth}}{24}$	Y_{eth}				$k_1 \frac{S_{eth}}{S_{eth}+K_{eth}} \frac{S_{Mox}}{S_{Mox}+K_{Mox}} X_{eth}$
2. Acetoclastic methanogenesis	$1-Y_{ace}$		-1					Y_{ace}			$k_2 \frac{S_{ac}}{S_{ac}+K_{ace}} X_{ac}$
3. Methanogenesis via DIET	$1-Y_{ace}$				$\frac{1}{8}$	$-\frac{1}{8}$		Y_{ace}			$k_3 \frac{S_{Mred}}{S_{Mred}+K_{Mred}} X_{ac}$
4. Hydrogen production				$1-Y_{eth}$	$\frac{1}{8}$	$-\frac{1}{8}$	Y_{eth}				$k_1 \frac{S_{Mred}}{S_{Mred}+K_{Mred}} X_{eth}$
5. Hydrogenotrophic methanogenesis	$1-Y_{h2}$			-1					Y_{h2}		$k_{hydro\_ch4} \frac{S_{h2}}{S_{h2}+K_{h2}} X_{h2}$
6. Decay of ethanol-metabolizing bacteria					-1		-1			f_I	$k_{dec} X_{eth}$
7. Decay of acetoclastic methanogens								-1		f_I	$k_{dec} X_{ac}$
8. Decay of hydrogenotrophic methanogens									-1	f_I	$k_{dec} X_{h2}$

Table 2. Definition of Components in the Model

Number	Component	Definition	Unit
1	S_{eth}	Ethanol	g COD m^{-3}
2	S_{ac}	Acetate	g COD m^{-3}
3	S_{Mox}	Reduced mediator	mM
4	S_{Mred}	Oxidized mediator	mM
5	S_{CH4}	Methane	g COD m^{-3}
6	S_{h2}	Hydrogen	g COD m^{-3}
7	X_{eth}	Ethanol-metabolising bacteria	g COD m^{-3}
8	X_{ac}	Acetoclastic methanogens	g COD m^{-3}
9	X_{h2}	Hydrogenotrophic methanogens	g COD m^{-3}
10	X_I	Inert, non-biodegradable organics	g COD m^{-3}

Table 3. Kinetic Parameter Values of the Developed Model for Case IV

Parameter	Definition	Value	Unit	Source
k_1	Maximum reaction rate of Process 1	0.37	g COD/(g COD × h)	(1)
K_{eth}	Half saturation constant for S_{eth}	300	g COD/m^3	(2)
k_2	Maximum reaction rate of Process 2	0.97	g COD/(g COD × h)	(1)
K_{ace}	Half saturation constant for S_{ac}	150	g COD/m^3	(2)
k_3	Maximum reaction rate of Process 3	0.43	g COD/(g COD × h)	(1)
k_{hydro_ch4}	Maximum reaction rate of Process 5	1.68	g COD/(g COD × h)	(2)
K_{h2}	Half saturation constant for S_{h2}	0.018	g COD/m^3	(2)
K_{Mox}	Half saturation constant for S_{Mox}	0.0001	mmol/L	(3)
K_{Mred}	Half saturation constant for S_{Mred}	0.001	mmol/L	(3)
C_{tot}	The sum of S_{Mox} and S_{Mred}	0.01	mmol/L	(3)
k_{dec}	Decay rate	0.005	g COD/(g COD × h)	(2)
Y_{eth}	Yield coefficient for X_{eth}	0.06	g COD/g COD	(2)
Y_{ace}	Yield coefficient X_{ac}	0.05	g COD/g COD	(2)
Y_{h2}	Yield coefficient for X_{h2}	0.06	g COD/g COD	(2)
f_I	Fraction of X_I in biomass decay	0.10	g COD/g COD	(2)
Sources: (1) This study; (2) Batstone et al., 2002; (3) Pan et al., 2013.				

Table 4. Best-Fit Parameters with 95% Confidence Intervals Describing DIET in Four Case studies

	Case I	Case II	Case III [a]	Case III [p]	Case IV [a]	Case IV [p]
k_1	0.72±0.08	0.81±0.14	0.30±0.03	0.46±0.04	0.37±0.06	1.70±0.11
k_2	4.80±0.01	6.90±0.20	0.99±0.02	---	0.97±0.05	---
k_3	0.40±0.05	0.68±0.10	0.48±0.01	1.91±0.02	0.43±0.09	4.51±0.30

[a] indicates in the absence of conductive carbon cloth while [p] means in the presence of conductive carbon cloth.