Multidimensional modelling to investigate interspecies hydrogen transfer in anaerobic biofilms

D.J. Batstone\textsuperscript{a},*, C. Picioreanu\textsuperscript{b}, M.C.M van Loosdrecht\textsuperscript{b}

\textsuperscript{a}AWMC, The University of Queensland, 4067 Australia
\textsuperscript{b}Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC, Delft, The Netherlands

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ABSTRACT

Anaerobic digestion is a multistep process, mediated by a functionally and phylogenetically diverse microbial population. One of the crucial steps is oxidation of organic acids, with electron transfer via hydrogen or formate from acetogenic bacteria to methanogens. This syntrophic microbiological process is strongly restricted by a thermodynamic limitation on the allowable hydrogen or formate concentration. In order to study this process in more detail, we developed an individual-based biofilm model which enables to describe the processes at a microbial resolution. The biochemical model is the ADM1, implemented in a multidimensional domain. With this model, we evaluated three important issues for the syntrophic relationship: (i) Is there a fundamental difference in using hydrogen or formate as electron carrier? (ii) Does a thermodynamic-based inhibition function produced substantially different results from an empirical function? and; (iii) Does the physical co-location of acetogens and methanogens follow directly from a general model. Hydrogen or formate as electron carrier had no substantial impact on model results. Standard inhibition functions or thermodynamic inhibition function gave similar results at larger substrate field grid sizes (>10\textmu m), but at smaller grid sizes, the thermodynamic-based function reduced the number of cells with long interspecies distances (>2.5\textmu m). Therefore, a very fine grid resolution is needed to reflect differences between the thermodynamic function, and a more generic inhibition form. The co-location of syntrophic bacteria was well predicted without a need to assume a microbiological based mechanism (e.g., through chemotaxis) of biofilm formation.

1. Introduction

Anaerobic degradation is conversion without an external electron acceptor of complex organics to the most oxidised and most reduced forms of carbon—carbon dioxide and methane, respectively. It is a multistep process, with a number of interdependent steps (Gujer and Zehnder, 1983). These are, in order:

(a) Extracellular hydrolysis of complex particulates to associated monomers (sugars and amino acids).
(b) Fermentation or acidogenesis of sugars and amino acids to simple volatile fatty acids and alcohols.
(c) Syntrophic acetogenesis, converting alcohols and volatile fatty acids into acetate and hydrogen or formate. This process is obligately syntrophic with methanogens that utilise the produced hydrogen or formate to produce methane.
(d) Aceticlastic methanogenesis, to form methane from acetate.

Phylogeny and physiology of microbes mediating these steps is very well understood, but mechanisms of
interaction between the different groups is less well understood, important for reactor operation, and of scientific interest.

An important interaction exists between the syntrophic partners simultaneously degrading higher organic acids to acetate and an electron acceptor product (either hydrogen or formate), and the methanogens that convert the electron acceptor product to methane (Gujer and Zehnder, 1983). The model compound for this reaction is propionate, as oxidation of this compound yields the least free energy. The basic reactions using either hydrogen or formate are shown in Table 1. The reactions are normalised to one mole propionate conversion, and formate concentration is set to be at equilibrium with hydrogen.

As can be seen in Table 1, even at the assumed environmental conditions, the available energy per mole propionate for each partner is very low. The free energy is negative (i.e., the reaction is favourable) in a narrow region of hydrogen concentrations between $10^{-3}$ and $10^{-6}$ bar H$_2$ (or 0.17 times this level as M formate). Therefore, increased hydrogen concentration inhibits acetogenesis by causing unfavourable thermodynamic conditions.

Because of the narrow region of hydrogen or formate concentrations, as well as other reasons discussed below, the microbes in the combined acetogenic reaction (methanogen and acetogen) are often observed as a physical co-culture within anaerobic biofilms (Harmsen et al., 1996). Microscopic observation of the co-culture has been assisted by new tools such as fluorescence in situ hybridisation (FISH) and confocal microscopy (Batstone et al., 2004; Harmsen et al., 1996; Sekiguchi et al., 1999).

### 1.1. Hydrogen vs. formate as electron carrier

As shown in Table 1, formate is thermodynamically equivalent to hydrogen at a ratio formate/H$_2$ of 0.173 M bar$^{-1}$, or 222 M H$_2$ with H$_2$ solubility of 0.00078 M bar$^{-1}$ at 25°C and a bicarbonate concentration of 0.1 M. In addition, acetogens can produce either hydrogen or formate, and most methanogens that can utilise hydrogen can also utilise formate (Boone et al., 1993). Formate is also in an extracellular enzyme-catalysed equilibrium with hydrogen (Thiele and Zeikus, 1988). Therefore, from a reaction engineering and thermodynamic point of view, there is no difference between hydrogen and formate. There are, however, three physicochemical key differences:

(i) Hydrogen has a higher diffusivity than formate.

(ii) Formate is more soluble than hydrogen.

(iii) Formic acid is a strong acid compared to carbon-dioxide.

Point (iii) is not particularly important, as the concentration of formate in a healthy reactor is very low. However, the solubility and diffusivity issues are critical in determining optimal methanogen/acetogen interspecies distance and electron transfer. With the higher solubility, but lower diffusivity, it is possible to achieve greater concentration gradients with formate, and therefore operate at higher interspecies distances. Boone et al. (1989) calculated that a H$_2$ producer could only exert an influence on local H$_2$ concentrations within 10 μm of its surface. A recent review (de Bok et al., 2004) has speculated that hydrogen transfer is promoted at low interspecies distances, while formate transfer is promoted at longer interspecies distances. However, at equilibrium (0.1 M HCO$_3$), the molar concentration of formate is 222 times that of hydrogen, while the diffusivity is 1/50th. Since flux is linear in both concentration gradient and diffusivity, the advantage should always lie with formate. This reflects the consensus from the experimental articles referred to in de Bok et al. (2004). One aspect not fully addressed is the rate of hydrogen or formate consumption. If consumption rate is extremely low, the concentration gradient becomes much flatter, small differences in diffusivity are no longer important, and there is no competitive pressure for species to co-locate.

#### 1.2. Thermodynamic vs. generalised inhibition

Inhibition of propionate utilising acetogens by increased hydrogen levels is due to the thermodynamic limits of the reaction. That is, changes in hydrogen concentration influence the free energy of the overall oxidation reaction, which in turn influences the overall favourability of the reaction. Models that include the thermodynamics explicitly to describe hydrogen inhibition of acetogenesis have been proposed (Hoh and Cord Ruwisch, 1996). A generalised thermodynamic regulation function for the rate of propionate-utilising acetogens is:

$$I = \max \left( 0, \left( 1 - \frac{K}{K} \right) \right),$$  \hspace{1cm} (1)
where \( I \) is the inhibition term, to be multiplied by the base Monod term kinetics in respect with propionate, \( K \) the equilibrium constant (\( K = \exp(-\Delta G^\circ/RT) \)), and \( K' \) reaction quotient. For propionate oxidation to acetate, hydrogen, and bicarbonate, \( \Delta G^\circ = +76.5 \text{ kJ mol}^{-1} \), and

\[
K' = \frac{[\text{H}_2][\text{Acetate}][\text{HCO}_3]}{[\text{Propionate}]},
\]

where the numerators in \( K' \) are the concentrations of products of reaction (M), and denominators are the reactants (M). \([\text{HCO}_3]\) is the bicarbonate concentration.

In anaerobic biofilms, the syntrophic colonies are relatively large, at >50 \( \mu \text{m} \), compared to a microbial size of \( \sim 1 \mu \text{m} \), and located away from the bulk liquid (Harmsen et al., 1996). Therefore, the microbes will not experience bulk hydrogen or formate concentrations directly. To include impacts such as diffusion, a generalised inhibition function may be better suited to describe inhibition with an apparent inhibition parameter (Batstone et al., 2002). The most commonly used generalised model for inhibition is non-competitive inhibition (Pavlostathis and Giraldo-Gomez, 1991). The form of this rate inhibition function is:

\[
I = \frac{1}{1 + S_0/K_I},
\]

where \( S_0 \) is the concentration of the inhibitor in the bulk liquid, and \( K_I \) is the apparent inhibitor concentration at which the rate is 50% of the uninhibited rate.

The developers of the generalised IWA Anaerobic Digestion Model No. 1, ADM1 (Batstone et al., 2002) used the above arguments to justify the use of non-competitive inhibition over thermodynamic inhibition. This has been subsequently criticised (Kleerebezem and van Loosdrecht, 2004). The dependency of the two inhibition functions (1) and (3) on \( \text{H}_2 \) partial pressure is shown in Fig. 1.

No one has yet shown whether a generic non-competitive or thermodynamic function is best suited to describing micro-scale thermodynamic inhibition in a lumped parameter model. Other considerations in selection between a thermodynamic and non-competitive inhibition function include:

(i) a non-competitive function requires an estimate of an inhibition coefficient.
(ii) the thermodynamic equation is non-linear, and discontinuous at \( \Delta G' = 0 \), which makes a numerical model solution difficult (even with continuous approximations).

The situation where the use of a thermodynamic inhibition is most justified is in individual-based modelling of biofilms (Picioreanu et al., 2004a). Because microorganisms are modelled on an individual level, the hydrogen concentration used in the model is that experienced at the cell surface.

2. Research problem and approach

Mathematical modelling of anaerobic biofilms, and in particular, granules, has been used to clarify and validate (or disprove) conceptual models (Batstone et al., 2004; Buffiere et al., 1995). These models have all been one dimensional in space, and therefore only give basic knowledge of spatial distribution of microbial populations along the granule radius. Multidimensional modelling, i.e., two- and three-dimensional spatial representations of solute and microbial distributions, has been used very effectively in investigating aerobic biofilms (Picioreanu et al., 1998, 2004a). These techniques would be very effective in addressing some of the research questions related to syntrophic interactions in anaerobic biofilms (Picioreanu et al., 2004b, 2005). In this paper, we apply the widely used kinetic model ADM1 (Batstone et al., 2002) to a multidimensional domain, in simulation of anaerobic granules to address the following research questions:

(a) Whether hydrogen or formate is the principal electron carrier and whether this is important.
(b) Whether a thermodynamic inhibition function produces significantly different results in comparison with a more universal inhibition function.
(c) Whether experimental observations of the physical syntrophic co-location of acetogens and methanogens can be justified by basic principles, or whether a physiological mechanism, such as cell signalling is required.

3. Methods

3.1. Model implementation

Implementation overview: The basic approach is based on the method of Picioreanu et al. (2004a), with special considerations for anaerobic granules (spherical biofilms). This has the following main components (see also Fig. 2):

(i) A field of concentrations of soluble compounds, based on a Cartesian grid. The soluble compounds are dissolved substrates and products involved in ADM1. This is the solution domain for all substrate components, as well as pH. We used a square (in 2-d) or cubic (in 3-d) substrate field.
A set of biomass particles in different states. Each biomass particle (cell) is a separate differential state, represented by its mass (and size), position, and type (species). Biomass particles are effectively independent of substrate field, and each substrate grid element can contain multiple particles.

Only 2-d modelling was considered, and each biomass particle is effectively a cylinder with length the same as the width of a substrate grid element. Additional aspects for 3-d modelling, as well as comparison with a 3rd party 1-d biofilm model are presented in Picioreanu et al. (2005). Biochemical uptake rates, and pH were calculated according to the ADM1 (Batstone et al., 2002). The model was written in C++ and executed on consumer (Pentium IV) personal computers. The high-resolution simulations shown here with non-competitive inhibition required approximately 4 days to simulate 20 days growth on a 2.7 GHz Intel Pentium Celeron.

**Domain and domain boundaries:** The model is based around a single granule, with fixed bulk liquid concentrations (Fig. 2). Assuming this granule is representative, model results can be scaled up to represent the entire reactor. Although the bulk liquid is not modelled here, the overall substrate conversion/production rates for the granule can be calculated. Addition of a bulk liquid model is technically possible (Picioreanu et al., 2004b). However, it increases the numerical stiffness of the model such that a new solution framework would be required. Also for computational convenience, the granule is modelled with an upper size limit, and all biomass growing...
above this size is sheared off. Results are presented here at a moment in time just after granule growth to this size limit (approx. 20 days).

Simulation of particulate biomass states: Biomass was represented as individual non-overlapping spheres (cylinders in 2-d), rather than as square or cube grid elements. If biomass is represented using grid elements, each grid element contains a single, homogeneous biomass type (or no biomass), and spreading of the biomass above a critical concentration is described by a cellular automata approach (Picioreanu et al., 1998). If biomass is represented as individual spherical particles, a grid element can contain a number of spherical biomass particles, and the concentration of each biomass type is the total mass divided by the grid element volume (Picioreanu et al., 2004a). The number of biomass particles is therefore independent of the geometry of the grid, and avoids the grid orientation artefacts typical to cellular automata. At each time step, each biomass particle will grow according to a rate equation based on mass balances. When a biomass particle has grown to a maximum size, it will split into two equally sized daughter particles. The location of all biomass particles is then recalculated iteratively such that the biomass particles do not overlap, and distance moved from the previous time step is minimised (Kreft et al., 2001). However, the main disadvantage of such an individual-based model is that this particle shoving iteration can be the major computational component given that an average simulation contains above $2 \times 10^6$ biomass particles near the end of simulation.

Simulation of soluble components: Within each Cartesian grid element, there are reactions involving solutes according to the ADM1, and solutes transport in and out by diffusion (Fick’s law) with neighbouring elements. Because the grid elements are small, so are diffusion distances. Therefore, the characteristic time of biomass growth is much higher (i.e., in the order of hours) than that for solutes to reach a steady state (i.e., seconds to minutes). Thus, the mass balance involving reaction and diffusion can be assumed to be at steady state each time when a biomass growth step is performed (Picioreanu et al., 2000). The second-order partial differential equations for solute mass balance can then be discretised on the Cartesian grid ($i,j$) and the spatial solute concentration distribution, found by solving the implicit set of algebraic equations. The acid–base equilibria equations are also solved within this framework, to calculate the spatial distribution of pH within the granule. A typical simulation using high-resolution meshing has $513 \times 513$ grid elements, with 12 chemical components, and 7 ionic acids or bases. Therefore, there are $\sim 5 \times 10^6$ implicit algebraic equations to be solved simultaneously. To address this, a multigrid solution is used, as explained in Picioreanu et al. (2004b). Each kinetic equation (Monod, non-competitive, thermodynamic inhibition, pH inhibition, etc.) has a related derivative that is combined according to the Chain rule and sum of derivatives for the purpose of numerical solution of algebraics. This derivative calculation implies that terms that are highly non-linear, or discontinuous (e.g., a thermodynamic inhibition function), cause difficulty in solution, a normal limitation of Newton–Gaussian solvers amplified greatly by the number of implicit variables.

3.2. Configuration for simulation

All biochemical kinetics and stoichiometry were from the ADM1 (Tables 3.1 and 3.2 with mesophilic high-rate parameter values from Tables 6.1 and 6.2 from Batstone et al. (2002)), except that decay and maximum uptake rate were increased by a factor of 10, as previously discussed (Batstone et al., 2004). The following additional physico-chemical and computational parameters were used:

- Diffusivities were for solutes in clear water, as for Batstone et al. (2004), corrected to 307 K.
- Bulk concentrations of: monosaccharides—40 or 100 g COD m$^{-3}$; butyrate—40 g COD m$^{-3}$; propionate and acetate—100 g COD m$^{-3}$; H$_2$ and CH$_4$—$10^{-5}$ g COD m$^{-3}$; bicarbonate ($S^{(b)}_H$)—0.1 M; ammonium ($S^{(a)}_N$)—0.01 M; cations to obtain pH 7.
- Density of all biomass particles of 180 kg COD m$^{-3}$ (Batstone et al., 2004).
- Maximum and minimum individual biomass particle size (diameter) of 2.3 and 0.23 μm, respectively.
- Initial condition is a random seed granule of 30 μm, 50 each of all 7 microbial types, with individual particle diameters half the maximum.
- Total domain size of 2.67 times the maximum granule diameter (i.e., 800 or 1600 μm).
- Time step of 0.125 h.

The following factorial conditions were used to evaluate their impact on syntrophic association:

1. Bulk liquid monosaccharide concentrations 40 vs. 100 g COD m$^{-3}$.
2. Maximum granule diameter of 300 μm vs. 600 μm.
3. Substrate grid element of 12.4 vs. 129 elements in 1600 μm domain vs. 3.1 vs. 3.1 μm (high resolution, 513 × 513 elements in a 1600 μm domain).
4. Propionate utilises weak non-competitive inhibition ($K_{ih} = 3.5 \times 10^{-3}$ g COD m$^{-3}$) vs. strong non-competitive ($K_{ih} = 3.5 \times 10^{-2}$ g COD m$^{-3}$), vs. thermodynamic. See Fig. 1 for comparison of these functions.
5. Formate vs. hydrogen as electron carrier. All $K_S$ and $K_I$ values were multiplied by 222 M HCOO$^{-}$MH$_2$ (the equilibrium ration of HCOO$^{-}$ and H$_2$) and a diffusivity of 7.0 $\times$ 10$^{-4}$ m$^2$ d$^{-1}$ (for HCOO$^{-}$) instead of $4.0 \times 10^{-4}$ m$^2$ d$^{-1}$ (for H$_2$) was used. This treats formate as having the same pH, which causes a slight error in pH prediction, caused by the increased pKs of bicarbonate, as compared to formate.

Simulations followed the granule development over 20 days, after which the granule was mature, and had a diameter of 600 μm (i.e., reached the maximum allowable biofilm limit).

3.3. Analysis of simulation outputs

Three criteria were used for assessment of simulations: (i) visual aspect, (ii) interspecies distance, and (iii) propionate cluster size. A visually rendered distribution of cells gives
In order to give a quantitative comparison, two different measures were used. It is generally agreed that interspecies distance between acetogen and methanogen is critical (Boone et al., 1989). Therefore, the distribution of distances between a propionate consuming biomass particle and the nearest hydrogen utilising methanogen was assessed. The other measure used was cluster size, or how many other propionate utilisers had their centres located within four radiiuses of the target cell (Fig. 3). The use of four radii merely reflects subjective visual criteria of a small cluster.

Lack of nutrients leads to shrinking biomass particles, when decay exceeds growth. In order to eliminate smaller, decaying cells, of which there can be a large number, with very little impact on biochemical reactions, only cells above half the maximum mass were considered. For interspecies distances, a 70% confidence level was used to assess distribution width (i.e., 70% of the particles are between the upper and lower limits). For cluster size analysis, the whole distribution was assessed.

4. Results

4.1. Factors influencing interspecies electron transfer

As discussed in the next section, the most useful simulations for comparing interspecies distances were those with high monosaccharide concentration and large granule size limit. Images of cell distribution within the granules for the different conditions are shown in Fig. 4, and the quantitative results are shown in Fig. 5. Changing the random seed had an impact on gross structure (radial positioning of colonies), but no impact on the main factors assessed here (axial position, interspecies distances, and microbial yields). From the factors evaluated by model simulations, the grid resolution used for...
The spatial distribution of substrates had the largest impact (see Figs. 4 and 5), with visible differences, significant interspecies distance differences, and cluster size difference. The only other factor that had a significant impact on interspecies factors (visible, interspecies distance, propionate cluster) was the use of thermodynamic-based inhibition function at high resolution (but not low resolution). High resolution is needed to represent properly the impact of thermodynamic inhibition as compared to non-competitive inhibition. Strong non-competitive vs. weak non-competitive inhibition and hydrogen vs. formate for electron transfer both have no impact on interspecies distance distribution. While it had no impact on interspecies distance, weak vs. strong non-competitive inhibition had an impact on syntroph cell yield as shown in Fig. 5b.

The differences are most effectively shown by the interspecies distance distribution (Fig. 5a). All distance distributions are log-normal, with the following major factors:

(i) Low resolution leads to a very long tail in the distribution, with some propionate utilisers at long distances (>5 μm) from the nearest hydrogen utilisers.
(ii) High resolution causes a narrower distribution.
(iii) With a low grid resolution, there are no major differences between thermodynamic inhibition, and either non-competitive inhibition.
(iv) At a high grid resolution, thermodynamic inhibition causes a very narrow distribution (<2.5 μm).

As shown by the average microbial concentrations in the granule (high resolution data in Fig. 5b), while a

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**Fig. 5** – (a) Median ( ), upper and lower 70% confidence limits for H2 and propionate utiliser interspecies distances, and (b) average concentration of propionate and hydrogen utilisers in the granule field (low resolution corrected for geometry). Low inhibition \( K_{I,\text{H}_2} = 3.5 \times 10^{-5} \text{ g COD m}^{-3} \), high inhibition \( K_{I,\text{H}_2} = 3.5 \times 10^{-4} \text{ g COD m}^{-3} \), low resolution 129 x 129 grid points, high resolution 513 x 513 grid points.
thermodynamic inhibition function causes a narrow distance distribution, it is actually more favourable for growth than strong non-competitive inhibition, with a higher cell yield for both syntrophic partners.

Visually, a strong difference can be distinguished between simulations in which the concentration fields were computed with high and low resolution (Fig. 4a and b; see resolution squares), with much larger clumps of propionate and hydrogen utilisers at low resolution. This is reflected in the cluster size analysis. The low resolution clusters were much larger, with 10–20% of cells in clusters above 8 cells at low resolution compared with 5–8% of cells in clusters above 8 cells at high resolution. Microscopic observations with FISH (Harmsen et al., 1996) indicate that the high resolution model indeed reflects reality better.

4.2. Other observations

Influence of other factors on results: The current model generated artefacts associated with low monosaccharide in the bulk liquid (factor a), and small maximum granule diameter (factor b). Problems caused by low monosaccharide in the bulk are related to the simplistic bulk model (i.e., constant concentration). The low bulk H₂ concentrations caused excessive growth of propionate utilisers on the surface of the granule, as they could treat the bulk as an infinite sink (i.e., a model limitation). In contrast, high monosaccharide concentrations allowed the formation of a surface layer of acidogens that effectively isolated syntrophic and aceticlastic microbes from bulk concentrations. The outer layer of acidogens simulated the bulk as a limited (rather than infinite) source and sink of volatile fatty acids (VFA), hydrogen, and inorganic carbon. Likewise, a high (constant) bulk H₂ concentration caused excessive growth of H₂ utilisers on the granule surface (see below). The need for a better model of the bulk liquid is further addressed in the discussion. A maximum granule size of 300 μm simply did not allow for full development of the structural elements observed in more mature granules.

Granule structure: A number of other observations could be made, without direct relationship to the interspecies distance issue. The different simulation scenarios had no impact on structure except for the different syntrophic distances. Structure was always an outer layer of acidogens of approximately 100 μm, followed by an inert zone, and then aceticlastic core. The acidogenic layer was a moving front, and the “inert” zone behind the acidogenic layer was caused by the relative growth rates of acidogens compared to other microbial groups.

Hydrogen sinks: The two major sinks for hydrogen were the bulk (caused by low bulk hydrogen concentrations) and syntrophic zones (see Fig. 6). This conflicts with the concept that syntrophic zones allow higher than bulk (or local area)

![Fig. 6 – Entire granule structure, showing hydrogen flux arrows. The granule is grown under low resolution, and weak inhibition. See Fig. 4 for rendering method. Colours are acidogens (red), aceticlastic methanogens (yellow), butyrate/valerate oxidisers (cyan), propionate utilisers (green), and hydrogenotrophic methanogens (dark blue).](image-url)
hydrogen concentrations (Boone et al., 1989). Increasing bulk hydrogen concentration artificially by changing the boundary settings (to $1 \times 10^{-5}$ gCODm$^{-3}$) caused massive surface growth of hydrogen-utilising methanogens, which is unrealistic. This issue is related to treating the bulk as an infinite source/sink of compounds, and is addressed in the discussion.

Thermodynamic inhibition function: The discontinuous and non-linear nature of the thermodynamic inhibition function caused difficulties in model solution as discussed in Section 3, even with a continuous approximation, such that solution required approximately 10 times as long with this function.

5. Discussion

5.1. Interspecies distance

The simulations here indicate that the main mechanism for formation of syntrophic communities is the high uptake rate and high affinity of hydrogen utilisers for hydrogen, as well as low conversion rate of propionate, rather than simply hydrogen inhibition. Additionally, in the current simulations, overall, the syntrophic colonies are major sinks for hydrogen, including the acidogenic region. These conclusions are dependent on the kinetic parameters used, especially for hydrogen utilisers, which have largely been sourced from lumped parameter experiments. However, these are the default ADM1 parameters, and are based on both structured model fitting to experimental data (Siegrist et al., 2002), as well as measurements from pure cultures of methanogens (Pavlostathis and Giraldo-Gomez, 1991). These driving forces for low interspecies distances mean that the actual form of electron carrier (formate vs. hydrogen) has a lesser impact than discussed in the literature, as indicated by our results. There is therefore no need to distinguish between hydrogen and formate for modelling purposes.

The large difference in interspecies distance distributions between low resolution and high resolution is reasonable, since at low resolution, all cells in a $12.4 \mu m \times 12.4 \mu m$ grid element experience the same hydrogen concentration. There is therefore no driving force for closer interspecies distances, apart from cells being forced together by displacement. When the $H_2$ concentration field is computed with high resolution, there is an obvious driving force (the concentration gradients), as the grid element is $3.1 \times 3.1 \mu m$, and there is therefore an advantage of clustering at distances on this order of magnitude.

5.2. Inhibition function

The results indicate that when both the substrate grid and biomass particle size are at a microbial level $<5 \mu m$, using a thermodynamic inhibition function becomes significantly different from non-competitive inhibition. Below this resolution, including to the point of a fully lumped parameter mixed tank model, there is no inherent justification in using a fundamental thermodynamic model over a more generic model. When modelling at the cellular level, however, the thermodynamic function gives different results, and provides an elegant way to implement thermodynamic regulation; however, the numerical problems need to be alleviated.

5.3. Acidogenic layer

The moving acidogenic front has been observed experimentally by Batstone et al. (2004). It could not be replicated using a 1-d model in that paper, and it was hypothesised that the outer layer, followed by an inner inert layer was produced by pH inhibition of the inner acetogens/methanogens. The results here have shown that instead it is caused by the difference in growth rates between acidogens and acetogens/methanogens. The layer observed in Batstone et al. (2004) was only $30 \mu m$, and this is related to the maximum uptake rate ($k_{ua}$) and decay rate ($k_{dec}$). It is important to note that in mixed systems, these two parameters are strongly correlated for parameter estimation. When estimating biofilm active depth, they are not correlated. Biofilm modelling, together with real measurements of biomass activity depth, therefore offers the possibility to independently estimate decay and maximum uptake (or growth) rates. Both have already needed adjustment from the default ADM1 set simply to achieve a realistic biofilm (Batstone et al., 2004).

5.4. Model limitations

The biggest limitation of the current model for a realistic reactor system is that bulk liquid was not modelled, although in terms of mass balance equations this was possible for simpler model cases (Picioreanu et al., 2004b). Because of this, the bulk liquid is an infinite source or sink of hydrogen and other substrates. In the results presented here, this is circumvented by developing an outer layer of acidogens that acts as a limited source of hydrogen, propionate, acetate, and other components. In order to model a system that has (for example) only propionate feed, a proper bulk model would be required. To do this, a solver with an adaptive time step size is needed in the solution of the non-steady-state balance of solutes in the bulk liquid. Future model development will focus on this aspect.

6. Conclusions

The model predicted that formate as electron carrier (high concentration, low diffusivity) produced the same interspecies distances as hydrogen as electron carrier (low concentration, high diffusivity). The main factor that impacted interspecies distances between hydrogen producer, and hydrogen consumer was the resolution of the substrate field in the model. A fine resolution (3$\mu m$ grid element width) caused a narrower interspecies distance distribution. Using thermodynamic inhibition as compared to non-competitive inhibition only had an impact at a fine grid resolution. Syntrophic co-location of hydrogen producers and consumers was clearly observed in the model, and reflected well real-world observations. However, we believe this is caused by the relative high uptake rates of hydrogen consumers, rather than just the sensitivity of hydrogen producers to hydrogen concentrations. Increased inhibition constants had no impact...
on interspecies distance distribution, but decreased overall yield of hydrogen producers and consumers. Animations of the simulations and hydrogen profiles are given in supporting material. Alternatively, they can be found at http://www.biofilms.bt.tudelft.nl/material/index.html.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2006.06.014.

REFERENCES


