Multidimensional modelling of anaerobic granules

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Abstract A multispecies, two- and three-dimensional model was developed, based on a previously published planar biofilm model, and the biochemical structure of the ADM1. Several soluble substrates diffuse and react in the granule. Local pH is calculated from acid-base equilibria and charge balance. The model uses individual-based representation of biomass particles within the granule (biofilm), and describes spreading by an iterative pushing technique. The overall computational domain consists of one granule, and is divided into a grid with Cartesian coordinates. The number of grid elements does not limit the number of biomass particles, and it is not necessary to use grid-spreading techniques, such as cellular automata, which result in Cartesian artefacts. The model represents both microscopic and macroscopic features in granule structure, previously observed using *in-situ* molecular techniques, and can be effectively used to interpret these results.

Keywords 3D model; granulation; multispecies; ADM1; anaerobic digestion; methanogenic granules

Water Science & Technology **Vol 52 No 1-2 pp 501–507** © IWA Publishing 2005

Introduction

Development of a strong, consistently sized anaerobic granular sludge bed is a key component in successful operation of high-rate anaerobic digesters. The process of anaerobic granulation is still not well understood despite extensive research into granulation mechanisms, microbial ecology, and significant contributing factors. Theories of granulation include the influence of: (a) surface activity, (b) relative substrate kinetics, (c) interactions and spatial requirements of obligate syntrophic groups, and (d) impact of a number of environmental factors such as pH, temperature, and intermediate concentrations. In particular, formation of a layered structure has been proposed, based on the relative kinetic rates of the different steps in anaerobic digestion (AD). AD is a multistep process, with (in order), extracellular hydrolysis, acidogenesis, acetogenesis, and hydrogenotrophic and aceticlastic methanogenesis.

Recently, models have been published of anaerobic granules (spherical biofilms), with multiple functional biological groups, and variation in a single dimension (depth into the granule) (see Batstone *et al.*, 2004 for an example, and review of 1D models). These are valuable in assessing the impact of relative kinetics in the different steps in anaerobic digestion. However, they do not allow in-depth assessment of microscopic spatial distribution, as the concentrations are still lumped over the section area and, consequently, concentration gradients of both chemical and microbial species are one-dimensional. It is also much more difficult to interpret complicated factors such as attachment and detachment, or interactions that occur at a microscopic level.

Multidimensional models are exciting tools for evaluating existing kinetically structured models of anaerobic digestion, and interpreting results obtained from advanced microbial ecology analysis (using molecular methods). Multidimensional modelling (2 or 3 spatial dimensions) has been historically difficult because of the large numbers of state variables required, a lack of effective biomass spreading mechanisms, and highly efficient numerical procedures for both time stepping, and solution of differential-algebraic equations. These

problems have been largely solved, and multidimensional modelling has been effectively applied mostly for evaluation of flat biofilms (Picioreanu *et al.*, 2004). In this paper, we will present a multidimensional model of anaerobic granulation, produced by adapting the methods of Picioreanu *et al.* (2004) and Kreft *et al.* (2001) to anaerobic granules, and the commonly used ADM1 structured model (Batstone *et al.*, 2002).

Methodology

A multidimensional, multispecies model has a number of important components. There is inadequate space here to discuss the methods fully, but the basic approach is based on the method of Picioreanu *et al.* (2004), with special considerations for anaerobic granules (spherical biofilms). The principal components are shown in Figure 1, and detailed below. A specific pushing mechanism was used to describe interaction of the growing and dividing biomass particles leading to biofilm spreading. Biochemical model structure and parameters were taken directly from the ADM1, except that uptake rates and decay rates were uniformly multiplied by a factor of ten, as for Batstone *et al.* (2004). The model was solved using ordinary personal computer equipment.

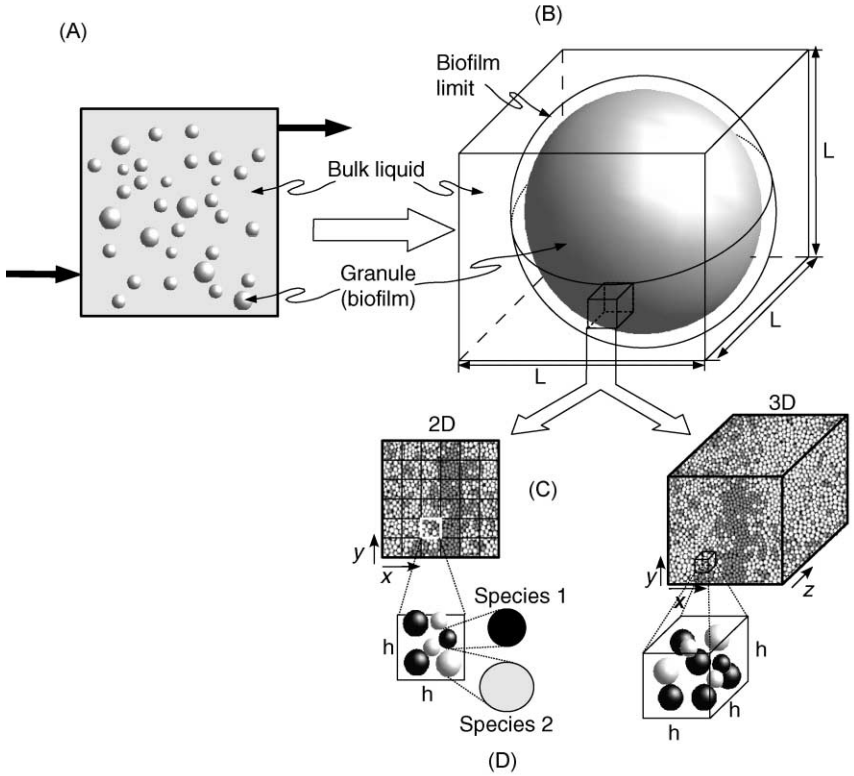

Figure 1 Model biofilm system, including: (A) full reactor, with a number of granules, (B) a representative granule, which is the computational domain for the multispecies, multidimensional model, (C) the 2D and 3D grid elements, with area h × h, and volume h × h × h, respectively, and (D) individual biomass particles, of different possible biomass types. All biomass particles within a single grid element experience the same substrate concentrations, and the biomass concentrations within a grid element are calculated by the mass and number of individual biomass particles within the element volume

Domain

We considered modelling of an entire completely mixed reactor, with the sludge represented by a single representative granule. This granule is contained within a computational domain, with growth in a $n \times m \times p$ Cartesian grid for 3D modelling, or $n \times m$ grid for 2D modelling (where n, m, and p are $< \sim 400$ grid nodes each). While the granule size is not fixed, it is practically restricted to this domain. The Cartesian volume or area consists of: (a) the granule, with specific equations for diffusion of solutes, solute reaction, biomass growth and biomass spreading, and (b) the fully mixed bulk liquid with normal reaction and growth equations (Batstone *et al.*, 2002). In the simulations shown here, the bulk liquid has fixed substrate concentrations, though we have built the model to also consider dynamic liquid mass balances. The modelled granule has an upper size limit (600 μm in the granule below). All biomass growing above this size is sheared off. This is the simplest representation of shear, and causes some problems in modelling surface growth as detailed below.

Representation of particulate (biomass) component states

The two basic methods of representing biomass in multidimensional domains are grid-based, and particle-based. In grid-based models, each grid element consists of a single, homogeneous biomass composition (generally one type). Spreading of the biomass is described by cellular automata (Picioreanu *et al.*, 1998). In particle-based models, biomass particles are represented individually, and a grid element may contain an arbitrary number of different biomass particles, with a fixed total volume (Picioreanu *et al.*, 2004). The grid-based method is simpler, and computationally quicker, but causes Cartesian artefacts. The individual-based method is much better, though computationally slower, because the spreading is more complex. The later method has been used here.

The 3D biofilm structure is therefore represented by a collection of non-overlapping hard spheres of biomass, also called biomass particles. Each spherical particle contains only one type of active biomass (out of a total of the 8 different biomass states in the ADM1). For this paper, biomass particles are pure biomass, but we have also started representing the inert fraction, as a fraction of the total particle. The density of the sphere is fixed to the biomass density ($\rho = 180\,\mathrm{kg\,m^{-3}}$ here), and its growth related to the biological growth equations in Batstone *et al.* (2002). Below a given size (1×10^{-13} gCOD), the sphere is assumed to disappear. Above a certain size (1.5×10^{-11} gCOD), it splits into two other spheres. One of the best aspects of these representations is that "pushing" from growth can be represented easily without the artefacts that commonly influence other growth models such as cellular automata.

Representation of soluble component states

Within the granule, the transport of soluble chemical species by diffusion is assumed to balance with reactions (partial differential diffusion-reaction mass balances). Solution of the algebraic equations of acid-base equilibria and charge balance to calculate pH and ionic compounds is also done within this framework.

Results and discussion

The basic results of granule growth are shown here without inerts modeling, and with a fixed biofilm upper radius. The new model was verified against Aquasim 2.1d (Reichert, 1994), using identical inputs and initial conditions in planar and spherical geometries. The results indicated good agreement between the two models, except in two cases. First, at the edge of the granule the multidimensional model produces a variable biomass density, which is beyond the scope of the Aquasim 1D model. Second, the hydrogen

concentrations are most influenced by the flux between individuals in the biofilm. Figure 2 shows evolution over time of the granule simulated in Aquasim and the 2D multispecies model. Biomass growth in a 2D model, with a mostly organic acid feed (40 mgCOD L^{-1} all substrates except propionate and acetate; 100 mgCOD L^{-1}, and hydrogen 4×10^{-5} mgCOD L^{-1}) is shown in Figure 3. The granule formation essentially follows a number of growth phases:

0 to 5 days: Nucleation, and homogeneous growth

5 to 20 days: Fully active, with growth in radial clumps

More than 20 days: Only outer 150 μm active, change towards fixed granule structure.

The final granule structure depends strongly on the bulk substrate concentrations. The granule shown above had an outer layer of 50 μm, with mostly clumps of acidogens, and propionate users. The inner region of 100 μm consisted of areas occupied by hydrogen utilisers and consumers, growing syntrophically, as well as acetate utilisers, growing in clumps.

The growth of syntrophic acetogens (propionate, and butyrate utilisers) is of particular interest. When growing near the edge of the granule, these microbes can grow without the presence of hydrogenotrophic methanogens (see Figure 4, centre), as hydrogen can be wasted to the bulk. When growing in the centre, they only grow in the presence of hydrogen utilisers. When the bulk is high in sugars, there is an outer layer of acidogens, and acetogenic groups can only grow in syntrophic groups near the centre of the granule. This is in agreement with the theoretical work of Boone *et al.* (1989), and matches our microscopic observations, as well as those of Harmsen *et al.* (1996). By calculating hydrogen concentrations, and hydrogen fluxes, we can also observe the main directions of hydrogen flux. As shown in Figure 5, when a low bulk hydrogen is assumed (4×10^{-5} mgCOD L^{-1}), together with a high glucose concentration (100 mg L^{-1}), most of the flux from the acidogenic region is to the bulk. All of the hydrogen produced by acetogens is consumed by hydrogen utilisers.

Improvements and applications

Most of the work done by us so far has been in verifying the model, improving a number of features, including the spreading model and algebraic solution for soluble chemical species. As such, we have mainly been working with small (600 μm width) 2D and 3D domains, as computational time is much less intensive with these. However, many

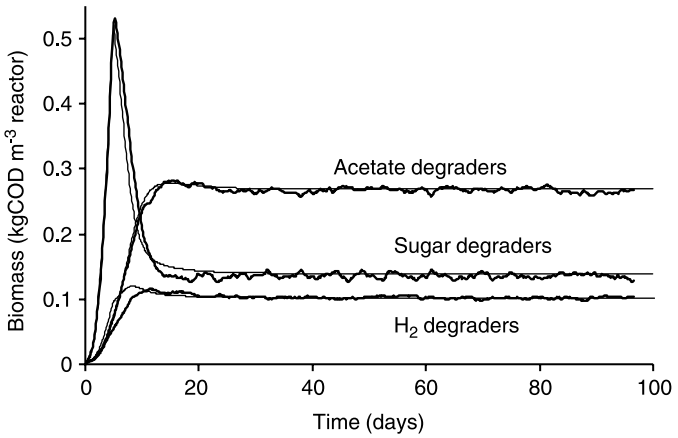

Figure 2 Evolution over time of different biomass types in the Aquasim 2.1d biofilm model (thin line), and multidimensional model (heavy line) for a planar biofilm with upper size limit of 140 μm

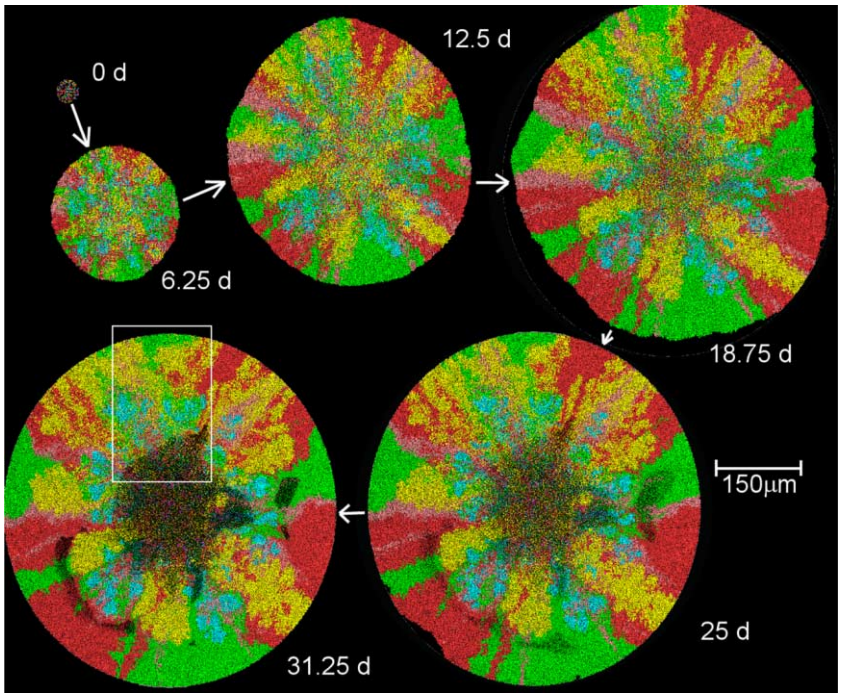

Figure 3 Growth of a granule (600 μm upper diameter) over 31 days on mostly organic acid feed, with different microbial types in different shades. The area in the centre after 25 days is mostly unoccupied due to biomass decay. The highlighted region at 31.25 days refers to Figure 4 (Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from http://www.iwaponline.com/wst.)

features cannot be observed in small granules, including inactive regions, venting, and historical growth elements. A major part of our future work will involve long simulations of big granules, as well as addition of inert biomass.

We believe the model has a number of applications, including advanced interpretation of structures. Using molecular methods, with confocal microscopy, one can directly observe model structure, using generalised molecular probes for the major groups. We can therefore directly relate observed granule structure to simulated structure. There is

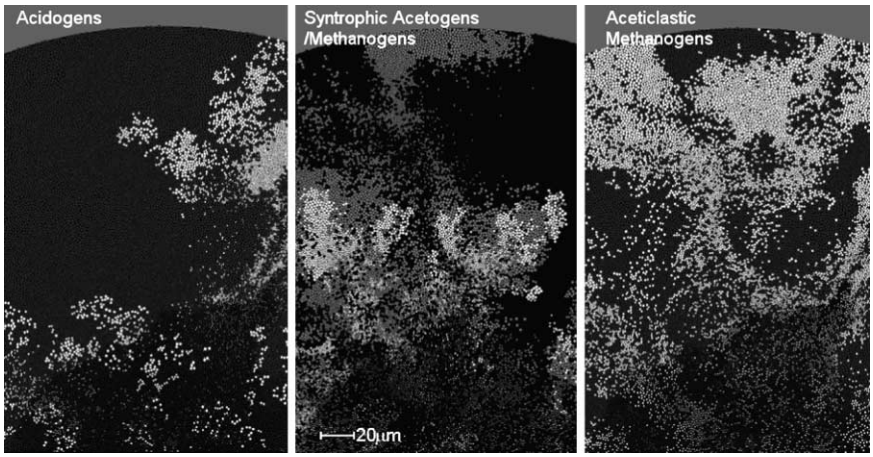

Figure 4 Highlighted area from Figure 3, showing: (a) acidogens (white) left, (b) acetogens (grey) and hydrogenotrophic methanogens (white); centre, and (c) aceticlasts right

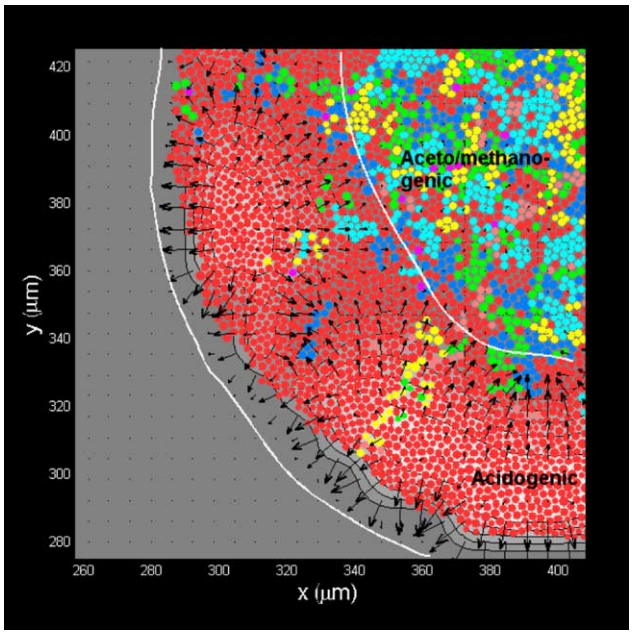

Figure 5 Hydrogen flux (arrows) in a small (200 μm upper limit) granule, fed mostly sucrose (Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from http://www.iwaponline.com/wst.)

also the possibility for better parameter estimation, as the active regions are highly sensitive to decay parameters, which are generally highly correlated with uptake and half saturation rate in mixed systems. This can be seen here, where uptake and decay rates needed to be multiplied by a factor of 10 from the base ADM1 set to observe a realistic structure. The impact on a mixed reactor would not be observed, as these parameters are correlated, but in a biofilm, the parameters are effectively decoupled.

As an overall, reactor simulation tool, a multidimensional model is just as effective as a one-dimensional model, and is not recommended. However, for interpreting theory, and microscopic observations, the multidimensional model is highly effective, and can provide unique insights.

Conclusions

The developed model is a very powerful tool for evaluating granulation theory, and for assessment of field and experimental observation. It identifies observed structural characteristics at both the macroscopic (>0.1 mm) scale and microscopic (<0.1 mm) scale. The greatest strength is at microscopic scale, where previously developed 1D models cannot predict microbial interactions, including syntrophy, and competition for space and substrate effectively.

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